Phosphorus dynamics and submerged aquatic macrophytes in hell kettles

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PHOSPHORUS DYNAMICS AND SUBMERGED AQUATIC MACROPHYTES IN HELL KETTLES

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ABSTRACT

A study was made of the phosphorus ecology of Hell Kettle ponds, a Site of Special Scientific Interest in County Durham, UK, in order to help establish the causes of the temporary "whitening" of *Chara hispida* in summer 1996. *Chara hispida*, the most abundant organism in Croft Kettle, one of the two small calcareous ponds comprising Hell Kettles, was first reported in 1777 and since then its presence has been accounted many times, perhaps being the longest continuous record for a freshwater algal species anywhere in the world.

The aims were to assess the concentration and variability of aqueous N and P as well as a number of other physical and chemical variables on spatial and temporal scales and the response of *Chara hispida* to these changes. Studies focused on Croft Kettle and key aspects included analysis of water chemistry, P sequential fractionation of sediments, tissue N and P contents and surface phosphatase activities of *Chara hispida*. This involved monthly surveys of surface as well as depth profiles of water chemistry during 1999-2001 and many other visits. Data was also collected from Double Kettle and the farm-borehole (representing groundwater) for comparative means.

Croft Kettle was stratified in summer (approximately May to October) with severe deoxygenation in the hypolimnion. Aqueous N and P concentrations were about 150 $\mu g\ L^{-1}$ TN and 15 $\mu g\ L^{-1}$ TP respectively, but showed high within-year and intra-annual variability. Episodic events and autumnal turnover caused only short-term increase in aqueous P concentrations and co-precipitation with $CaCO_3$ was suspected. Depth profile studies of sediments (0-35 cm) for TN and TP content and N:P ratio suggest historical changes in N and P dynamics.

Seasonal changes as well as a high range of tissue N and P contents were observed in *C. hispida* apical tips during this survey, possibly indicating that *C. hispida* is capable of rapid nutrient uptake and storage. Supportive evidence for this hypothesis arose from incubations of *C. hispida* under a series of aqueous P concentrations as well as the seasonal study on phosphatase activity. *C. hispida* apical tips collected from Double Kettle had on average higher tissue N and P contents than those collected from Croft Kettle, probably corresponding to the higher nutrient content of Double Kettle's water.

Possible reasons for aquatic vegetation changes as well as the "whitening" of *C. hispida* during summer 1996 are discussed. Unusually warm summer along with wind-protected shores due to the dense reed vegetation may have resulted to reduced water mixing, light penetration and increased deoxygenation in the hypolimnion and thus stress on *C. hispida*. However, there is no indication of a long-term destabilization of the system indicating that the event of 1996 was only a "temporary instability".

LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	analysis of variance
APA	alkaline phosphatase activity
ASFA	automatic segmented flow analyser
BD	bicarbonate buffered dithionite solution
bis-*p*NPP	bis-*p*-nitrophenyl phosphate
BSAC	British Sub-Aqua Club
DW	deionised water
DIN	dissolved inorganic nitrogen
DMG	3, 3-dimethyl-glutaric acid
DNA	deoxyribonycleic acid
d.wt	dry weight
EA	Environment Agency of England and Wales
EDTA	ethylenediaminetetra-acetic acid
EN	English Nature
FRP	filtrable reactive phosphorus
FOP	filtrable organic phosphorus
FTP	filtrable total phosphorus
g	gravitational force
GIS	geographical information systems
HEPES	(N-[2-Hydroxyethyl]piperazine-N-[2-ethanesulphonic acid])
Km	Michaelis constant
Ks	apparent half-saturation constant for enzymatic activity
MU	4-methylumbelliferone
MUP	4-methylumbelliferyl phosphate
NCC	Nature Conservancy Council
NERC	Natural Environment Research Council
NIES	National Institute for Environmental Studies of Japan
NRA	National Rivers Authority
NVC	National Vegetation Classification
NWA	Northumbrian Water Authority
PAR	photosynthetically active radiation (400-700 nm)
P*i*	phosphate (any form of orthophosphate)
PDE	phosphodiester
PDEase	phosphodiesterase
PME	phosphomonoester

PMEase	phosphomonoesterase
*p*NP	p-nitrophenol
*p*NPP	p-nitrophenyl phosphate
PP	particulate phosphorus
SD	standard deviation
SE	standard error
SSSI	Site of Special Scientific Interest
TIN	total inorganic nitrogen
TP	total phosphorus
WTW	Wissenschaftliche-Technische Werkstätten
w/v	weight/volume

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Dr M.G. Kelly (Bowburn Consultancy) and G. Cummins made a report on diatoms and pollen in the sediments respectively and a summary of this work is included here. Dr J. Lamont-Black (Civil Engineering, University of Newcastle-upon-Tyne) showed me some of his analytical data for the ponds and groundwater (the well) and Dr I.M. Head (Fossil Fuels and Environmental Geochemistry, University of Newcastle) sent interesting papers about a population of the bacterium *Achromatium* from Croft Kettle.

Most of all I would like to thank my parents for their unconditional support.

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CHAPTER 1 Introduction

1.1 Preamble

This project originated from the observation that many shoots of *Chara hispida* turned white in the summer of 1996 in Croft Kettle pond, near Darlington, County Durham. *Chara hispida* was first reported in 1777 (Robson, 1777) from one of the two highly calcareous Hell Kettles ponds and has been recorded many times over the past eighty years, probably making it the oldest continuous record of a freshwater alga in the world.

A die-back in the charophyte beds and a decline in size and abundance of phosphorus sensitive algae, such as *Rivularia* sp., *Chaetophora elegans* and *Chaetophora incrassata* (Livingstone & Whitton, 1984; Gibson & Whitton, 1987) was noticed in 1996. B.A. Whitton who has studied the ponds (in particular the algal assemblages) on a frequent but irregular basis since at least 1970, suggested that increased P availability might have influenced the aquatic ecosystem of Croft Kettle.

The subject of study was P dynamics in Hell Kettle ponds with respect to the influence of P on the biota. Data are presented on seasonal changes of nutrients, temperature and oxygen in the surrounding water and sediment and in N and P content of the thallus of *Chara hispida*. This introductory chapter reviews studies concerning the sources and the availability of P to the biota within freshwater bodies and particularly lentic systems. This includes the importance of nutrients fractions and the processes, which mediate changes in various "pools".

1.2 Hell Kettles

Hell Kettles (NZ 281 109, Figure 3.2) is a Site of Special Scientific Interest (SSSI) that consists of a fen and two small calcareous ponds of contrasting character. Both ponds lie on boulder clay over a Magnesian Limestone aquifer and it is the only place in County Durham where open water is directly fed by subterranean springs (Hedley, 1997). It has an abundance of aquatic macrophytes and flowering plants, an unusual community of sessile diatoms and other green and blue-green algae and the plant records go back more than two centuries to Stephen Robson's "British Flora" (1777). A number of people have studied the ponds since then, in particular Nicholson (1925) and Wheeler & Whitton (1971) who published papers on its flora.

One of the ponds, Croft Kettle (the most important of the two, Figure 3.12), punctures the boulder clay and is in direct contact with the aquifer, consequently being spring-fed from it. It has highly calcareous "green" coloured water, is about 7m deep, steep sided and has a luxuriant growth of the stonewort *Chara hispida* (Wheeler & Whitton 1971). The margins are largely comprised of *Phragmites australis* and *Cladium mariscus*. The other pond, Double Kettle, developed its present form from the coalescence of two smaller ponds. It has shallow, saucer-shaped margins, turbid water, a paucity of submerged plants and supports a narrow band of reed swamp, dominated by *Phragmites australis* (Wheeler & Whitton 1971; Hedley, 1997). It receives only surface water, its level is dependent on rainfall, is partially filled-in, and is considered a more dormant feature although the possibility of some groundwater seepage into it from the limestone cannot be dismissed. However, it has probably always been connected to Croft Kettle, either by seepage across the marsh, which lies between them, or by a channel (Hutchinson 1794), which was latterly been converted to a (balancing) pipe in 1971.

1.3 Ponds

A pond can be defined as a limited environment without a continual interchange of population with neighbouring biotopes (Macan, 1973). Traditionally, any study of a pond involves extensive chemical analysis of some dissolved elements and physical factors such as temperature, oxygen, light, pH and conductivity. However, studies often focus on key elements such as N and P since concentrations of elements such as Na, Ca, Mg, K, H, HCO_3, Cl and SO_4 are usually far in excess of the needs of the plants and remain constant in concentration throughout the year. Moreover, many attempts have been made to distinguish a lake from a pond using biological and physical criteria, but there are always exceptions. However, a general rule that could apply is that ponds are less stable than lakes. Heavy rain, one still warm night, the sudden death and decomposition of an algal population, a chance visit by a few ducks are reasons that might change completely the water chemistry and alter the vegetation in a pond. Therefore, it seems likely that the chances of spot analysis detecting such changes are small and continuous records of basic ecological factors are necessary to assess these changes and determine management decisions (Macan, 1973)[1].

[1] Comments in this paragraph all come from Macan's (1973) account on ponds and lakes.

1.4 Nutrients in freshwaters

1.41 Introduction

Nutrients are one of the driving forces behind primary productivity in aquatic ecosystems. Their availability is partly a function of the rates of external and internal inputs and permanent removal within the system by biological, chemical and/or physical processes. The three primary macronutrients are carbon (C), N and P (Rast & Thornton, 1997). Carbon in aquatic ecosystems occurs in a variety of chemical forms, such as dissolved in water as equilibrium products of carbonic acid and in organic compounds as dissolved and particulate (Wetzel & Rich, 1973) and is usually found to be adequately available for primary productivity (Golubic *et al.*, 1979). Occasionally, C may be locally exhausted but it can easily be replenished due to atmospheric inputs (Vrede *et al.*, 1999). Consequently, due to their limited availability and universal biological demand, the two key nutrients affecting the trophic status of aquatic ecosystems are N and P, which share the distinction of being a major growth-limiting nutrient in the global biosphere (Wetzel, 1983; Fox, 1993).

In freshwater ecosystems, P is most commonly in shortest supply and has long been recognised as the most critical nutrient limiting primary productivity (Hutchinson, 1967; Schindler, 1974; Driscoll *et al.*, 1993; Maher & Woo, 1998), especially for lakes in regions of hard and poorly weathered rocks, such as the Canadian Shield (Kilinc & Moss, 2002). However, a number of freshwater ecosystems may show N limitation too, particularly in the tropics (Kessler & Jansson, 1994; Moss *et al.*, 1997; Harris, 1999). Therefore, for its aquatic ecosystem it is important to establish the relative importance of N and P in the eutrophication process in order to evaluate the availability and to establish trends and rates of change (Rast & Thornton, 1997).

Redfield *et al.* (1963) observed that phytoplankton that are neither N nor P limited, typically have a stoichiometric C:N:P relationship of about 106:16:1. As a result, this stoichiometric composition of the plankton biomass has been used as an indicator of the nutrient status, since if one of the two nutrients is present in noticeably smaller concentrations than the Redfield relationship predicts, then there is a high probability that this nutrient is limiting growth. Moustafa *et al.* (1998) suggested that N limitation is most likely to occur when the N:P ratio is <10:1 and P-limitation when N:P is >17. At intermediate N:P ratio (>10 and <17), either N or P may be limiting. However, a number of studies have shown that the N:P ratio in nutrient limited cells is very plastic

and suggested that caution should be taken when predicting nutrient limitation only based on biochemistry (Geider & La Roche, 2002).

1.42 Nitrogen

During the past decades, the supply of N to global terrestrial ecosystems has doubled as a consequence of human activity (Gallaway, 1998) and the negative effects range from impacts to humans, ecosystems and to atmospheric processes (Cowling *et al.*, 1998). Conducts, such as increased fertilisation, changes in land use, increased industrial and domestic wastewater and fossil fuel combustion are some of the reasons behind this increase (Heathwaite *et al.*, 1994). In addition, a steady increase of N loading in freshwater environments has been observed resulting also in high land to sea fluxes. This is mainly due to the high mobility of N in soils and inputs from soils to freshwaters are largely in the form of nitrate (Harris, 1986).

Nitrogen in freshwater bodies is found in several forms and the dynamics of N cycling are complex because of the existence of four dissolved forms of inorganic N (Lampert & Sommer, 1997). The dominant forms in surface waters are dissolved inorganic (DIN), dissolved organic (DON) and particulate N (PN), but only dissolved forms can be directly assimilated by living organisms (Moss, 1998). The pools of DIN include nitrate (NO_3^-), nitrite (NO_2^-), nitrous oxide (N_2O), molecular (N_2) and ammonium (NH_4^+), but most plants can only utilise (assimilate) NO_3^- and NH_4^+. The transport of N through these different forms is dependent on biological uptake and regeneration, through four processes. Three of the processes; fixation, ammonification and nitrification, convert gaseous N into usable DIN forms and denitrification (the fourth process) converts fixed N back to the gaseous state (Moss, 1998). In oligotrophic freshwaters, NO_3^- is the dominant form of DIN because of the rapidity of nitrification and the even distribution of oxygen throughout the basin. In eutrophic waters, both NO_2^- and NH_4^+ may become abundant as decomposition processes in the sediments and in deep water deplete the oxygen, release reduced forms of N (Harris, 1986) and physical events may serve to redistribute DIN at seasonal and storm event scales into the surface waters.

Denitrification, as the only biologically mediated process by which NO_3 is reduced to N_2, has become increasingly important particularly in eutrophic lakes as it ultimately removes N from the water column (Harris, 1999). Denitrification is carried out by heterotrophic anaerobic bacteria such as *Pseudomonas* sp. (Gamble *et al.*, 1977) that use N oxides as terminal electron acceptors to generate energy (ATP) through anaerobic

respiration (Knowles, 1982). Though, in addition to oxygen, a number of other environmental factors such as the concentration of NO_3 and organic C, temperature, microbial numbers and pH, affect the rate and end products of denitrification (Parkin, 1990; Groffman, 1991; Golterman, 1995). Presence of primary producers on the sediment also strongly influences denitrification and higher rates have been observed below macrophyte stands, but lower rates below benthic algae (Seitzinger, 1990). In addition, fauna through bioturbation can increase nitrification and subsequent denitrification. Furthermore, Golterman (1991) found a link between N, P and S cycles in sediments, indicating that N losses could result in stronger fixation of P.

As almost anoxic conditions are required, denitrification in the aquatic environment is restricted to zones of reduced oxygen status, particularly sediments (Seitzinger, 1990). Aquatic sediments present an ideal environment for denitrification, since they are rich in organic matter that upon decomposition releases NH_4, which is then available for nitrification and subsequent denitrification. This is of particular importance in aquatic systems that receive N from pollution sources, since the removal of a portion of that N via denitrification, may help control the degree of eutrophication of the system (Seitzinger, 1990). Wetland areas are ideal sites for denitrification due to their rich in organic matter sediments and stagnant water and can lead to major losses of N (Valiela & Teal, 1978; Howard-Williams, 1985; El-Habr & Golterman, 1990).

1.43 Phosphorus

Phosphorus is the eleventh most abundant mineral in the earth's crust and does not exist in a gaseous state (Stumm & Morgan, 1970). Under natural conditions, P is almost exclusively found in a fully oxidised state with four oxygen atoms in a tetrahedral structure (Reynolds, 1978), best known as phosphate (P*i*), and only very rarely reduced P compounds (i.e. phosphine) are formed (Hutchinson, 1957). In freshwater and marine systems, P*i* is found in a variety of forms such as inorganic ions, inorganic polymers, organic compounds, living micro-organisms and dead detritus (Moss, 1998) and can be separated into dissolved and particulate forms (Broberg & Persson, 1988). The sum of all the forms is called total P and is a measure of the potential fertility of a water body (Moss, 1998). However, only some forms of P are immediately available for plant and algal growth and others may become so through microbial or enzymatic activity.

The dissolved phase includes inorganic P*i*, inorganic polyphosphates and dissolved organic and colloidal P (Broberg & Persson, 1988). Inorganic P*i* is commonly known

as ortho-phosphate and may have one or two hydrogen atoms covalently bonded to the oxygen atoms (PO_4^{3-}, $H_2PO_4^-$, HPO_4^{2-}), dependent on pH (Stumm & Morgan, 1970). Orthophosphate is the principal form in which P is utilised among components of aquatic systems and is considered to be the most readily transported form of P into plant cells (Raghothama, 1999). Inorganic polyphosphates are the result of more than one O-P bonds and the simplest of these is pyrophosphate ($P_2O_7^{(\text{variably charged 1- to 4-})}$), which can be readily utilised by microbes and overall contributes to the total bio-available P pool (Sundareshwar *et al.*, 2001). Dissolved organic P (DOP) could be a significant fraction of dissolved P, particularly when the concentration of inorganic P*i* is low and studies have shown DOP to be predominantly of large molecular weight or colloidal nature. The DOP pool is consisted of a multitude of chemical forms and some potentially bio-available forms are: phosphate monoesters and diesters (such as nucleic acids), sugar phosphates, phospholipids and inositol hexaphosphate (Bentzen & Taylor, 1992; Chróst & Siuda, 2002; Turner *et al.*, 2002). Bentzen & Taylor (1992) suggested that when P is in high demand, plankton appears to also have high affinity for DOP. However, DOP cannot directly be transported into plant cells and becomes bio-available only after transformation by physical, chemical and biological processes (i.e. enzymatic degradation). DOP is an important component in soil leachate and overland flow (Turner & Haygarth, 2000) and can be a critical source of P for aquatic organisms in particular freshwater ecosystems (Whitton, 1991).

Phosphorus particulate phase includes many mineral and amorphous precipitates, such as adsorbed P, organic P precipitates, crystalline minerals and reaction products of metals (Al^{2+}, Fe^{2+}, Mg^{2+} and Ca^{2+}), which have a strong affinity for P*i* anions (Holtan *et al.*, 1988). Chemical and mineralogical analysis of particulate P have shown compounds such as calcite, dolomite, Fe hydroxides, clay minerals and organic matter (Van Esk, 1982). Particulate P forms are an important component of total P and are thought to be biologically unavailable (Reid *et al.*, 1980). Nevertheless, the inorganic and organic dissolved and particulate forms of P undergo continuous transformations (Boström *et al.*, 1988), which influence their bio-availability (Reynolds & Davies, 2001).

The classification of P into 'particulate' and 'dissolved' forms, however is not resolved analytically as chemical species but as operationally defined fractions which usually include various chemical species of a similar general type (Burton, 1973). This operational classification is based on the reactivity of P forms with the molybdenum blue method (Murphy & Riley, 1962) and separation by a 0.45-μm membrane filter

(Goulden, 1972). Filtered fractions capable of forming the molybdenum blue complex under acid conditions are termed reactive (dissolved) and are generally thought to be directly bio-available. Filtered and unfiltered fractions requiring prior treatment, such as oxidation of organic matter by alkaline or acid peroxulphate using autoclave or microwave heating, are termed unreactive and are not directly bio-available. Common fractions which are usually determined include: filtrable reactive P (FRP, or often referred as dissolved reactive P (DRP) or soluble reactive P (SRP)); dissolved unreactive P or organic P (OP); total dissolved P or total filtrable P (FTP); particulate P (PP) and total P (TP) (Haygarth & Sharpley, 1999). However, such classification is only as good as the method determining it, and doubts about the actual size of the dissolved P pool have been raised. The current prevailing idea is that current methods of P determination (such as the molybdenum blue, enzymatic, radiobiological bioassays and gas chromatography), largely overestimate the actual concentration of dissolved P*i* as much as twofold (Baldwin, 1998; Hudson *et al.*, 2000). This inaccuracy has been attributed to the hydrolysis of organic P compounds and displacement of P from colloids (Baldwin, 1998) as well as complexation of molybdate with copper and arsenic ions (Broberg & Pettersson, 1988). Concern has also been raised for the filtering through membranes (Bickford & Willett, 1981) and preservation and storage of samples (Fishman *et al.*, 1986; Lampert *et al.*, 1992; Kotlash & Chessman, 1998) and studies have shown evidence for hydrolysis (Denison *et al.*, 1997) and exchange of particles in the sample container (Maher & Woo, 1998) prior to P determination.

1.44 Eutrophication

Eutrophication involves the enrichment of nutrients (notably N and P) above natural levels, resulting in an increase in the net primary productivity of ecosystems which is usually associated with loss of amenity value and water quality (Moss *et al.*, 1996b). This enrichment has to be coupled to the intrinsic characteristics (e.g. morphometry) of aquatic ecosystems to determine the speed, degree and types of changes (Lau & Lane, 2001). For example, the rate of eutrophication in lotic systems (e.g. rivers) tend to be very different from lentic systems (e.g. lakes), owing to differences in environmental and ecological conditions. In addition, shallow lakes in comparison with deep ones tend to be more prone to eutrophication as they provide more opportunity for contact as well as mixing of the eutrophic zone with the hypolimnion and the sediment, where nutrients are stored (Lau & Lane, 2001).

In view of the problems that eutrophication causes, one important aspect of water quality assessment involves the monitoring of trophic status (Rast & Thornton, 1997).

Monitoring of P concentration in freshwaters is a common practise worldwide and a statutory remit of the British government organisation the Environment Agency (Howell, 1994). It has been suggested that one measurement of P at the spring overturn is enough to estimate the algal biomass, since a strong relationship amongst phytoplankton, chlorophyll *a* and total P has long been confirmed by a number of authors (Dillon & Rigler, 1974; Schindler, 1978; Hecky & Kilham, 1988; Elser *et al.*, 1990; Guildford & Hecky, 2000). However, limnologists have also turned to biological solutions since chemical sampling programmes are often poor predictors of the biological consequences of P enrichment. Some biological methods involve the physiological and biochemical monitoring of aquatic macrophytes (Grasmuck *et al.*, 1995; Schneider *et al.*, 2000) and freshwater algae (Kelly & Whitton, 1995; Steinman, 1998). A review on biological methods by Kelly & Whitton (1998) outlines some of the methods currently in use. In addition, predictive models that are able to provide estimates of baseline nutrient concentrations from microfossil assemblages preserved in sediments have been developed (Lotter *et al.*, 1998; Bennion & Smith, 2000; Bennion *et al.*, 2001). However, as with all floristic based monitoring systems, the communities are subject to a range of parameters, other than P, and probably a combination of both chemical and biological measures is necessary.

1.5 Sources of phosphorus

1.51 Introduction

The quantity of P found in a water body is determined by the climate, the character of the geological substrates, soils of the catchment area, sediments, biotic component and the inflowing water (Hejny & Kvet, 1978). In turn, these properties are determined by basic geological and climatic factors, such as rock type, temperature, wind, precipitation and humidity and by the quality of inflowing water, which in turn is influenced by the character of the catchment area and by its agricultural and silvicultural use and management (Ulehlova & Pribil, 1978). Thus, P containing particles that are supplied and circulate in fresh waters are a mixture of in-washed (allochthonous) inorganic and organic particulates which consist of products of catchment area erosion, litter fall, transport by tributaries, atmospheric precipitation and sewage inflow, and the remains of organisms, which have grown in the water body itself (autochthonous). Allochthonous sources are generally put into two classes; point and non-point (diffuse) sources (Heathwaite *et al.*, 1997). Point sources have a fixed point of discharge, but the composition and rate of discharge can vary markedly and diffuse sources follow a wide

range of routes such as overland flow and throughflow. Hence, four important sources of P containing particles, supplied to fresh waters can be identified (Moss, 1998):

1. Weathering products such as primary or secondary minerals
2. Atmospheric deposition
3. Human point and non-point sources
4. Biologically produced cells of plants, bacteria and animals.

1.52 Weathering products

The main natural sources of P are the igneous, sedimentary and metamorphic rocks (Moss, 1998). Phosphorus forms 0.1% of the rocks that make up the bulk of the earth's crust, its total amount is estimated at 10^{25} g (Golterman, 1975) and geochemically is classified as a trace element (Van Wazer, 1961; Lerman & Imboden, 1995). There are at least 206 identified P minerals (Broberg & Persson, 1988), of which only one family, the apatites, is quantitatively important (Holtan *et al.*, 1988). Thus, natural inorganic P deposits occur primarily as P*i* in the mineral apatite, which is defined as a natural, variously coloured calcium fluoride P*i* ($Ca_5F(PO_4)_3$) with chlorine, hydroxyl and carbonate sometimes replacing the fluoride (Smith, 1990).

Chemical and mechanical weathering, erosion and/or solubilisation are the processes responsible for the transfer of P from the lithosphere to the pedosphere and hydrosphere (Ahl, 1988). Rate of release is slow and is determined by factors such as type of rock, topography, water, oxygen and organisms (Ahl, 1988). For example, the greater permeability and porosity of some limestone types, exposes them to increased weathering potential and so to increased P export (Pentecost & Whitton, 2000), even though they may not be the most concentrated sources of geological P. When released into the environment, P*i* will speciate according to the pH of the surrounding soil and is usually not readily available for uptake in soils. This is because most of it is rapidly immobilised as calcium or iron P*i* and adsorbed to soil particles or incorporated into organic matter. Phosphate is only freely soluble in acid solutions and under reducing conditions (Kpomlekou & Tabatabai, 1994). Hence, the P in the parent rocks solubilised by the weathering process is either incorporated into the hydrological cycle or accumulated in the organic soil layer (Ahl, 1988).

1.53 Atmospheric deposition

Research regarding atmospheric deposition of nutrients to aquatic environments has primarily focused on C, N and sulphur (S), which exist in the atmosphere in vast

quantities, mostly as gases, and their atmospheric composition has largely been altered from human activity (Moss, 1998). Atmospheric deposition may often be a significant source of C, N and S to freshwater bodies, especially in regions with high-energy consumption densities and animal breeding (Lijklema, 1994). On the other hand, the input of atmospheric P to aquatic ecosystems has largely been ignored since the atmospheric P pool constitutes a relatively insignificant part of the P cycle.

Atmospheric P transfer to water bodies takes place through dust and microbial debris by wet and dry deposition (Ahl, 1988). Studies have shown that dry deposition is probably the most important of the two, although it is extremely problematic to measure (Heathwaite *et al.*, 1997). Atmospheric dust originates from three sources; terrestrial airborne, volcanic and cosmic matter (Ahl, 1988). Human activities have significantly increased the terrestrial airborne matter, which makes up the main bulk of atmospheric dust and studies have shown that between 60 to 90% is of anthropogenic origin (Ahl, 1988).

Lijklema (1994) estimated that P atmospheric deposition in Netherlands is of the order of 0.01g m^{-2} y^{-1} and Gibson *et al.* (1995) that P deposition in Ireland is about 0.022 g m^{-2} y^{-1} and globally ranges from 0.005 to over 0.1 g m^{-2} y^{-1}. However, Barica & Armstrong (1971) found out that contribution by snow might supply as much as 80% of the P load in the oligotrophic lakes of western Ontario. Hence, even though the atmospheric deposition of P is of limited significance in most freshwater bodies, its quantitative importance, particularly to upland, pristine, oligotrophic waters cannot be dismissed. Furthermore, the importance of this source increases when the lake to land area ratio increases (Ahl, 1988). Finally, even though the direct input of P to water bodies may be quite low, atmospheric P deposited on terrestrial surfaces may be quickly exported by leaching to water bodies too.

1.54 Point and diffuse sources

Although rock weathering and natural atmospheric precipitation still dominate the major ion composition of the world's fresh waters, human activities now probably have the greatest effect on key nutrient concentrations such as N and P (Moss, 1998). Humans are also thought to be involved to the phenomenon of eutrophication of most aquatic ecosystems, since freshwater bodies, which had existed for thousands of years, showed no sign of enrichment until farming and settlement made a serious impact on the watershed (Lijklema, 1994). Urbanisation, changes in land use, agriculture and use of freshwaters for receiving sewage outfalls are the main factors that increased the total

freshwater P*i* concentration and are usually separated into point and non-point sources (Golterman, 1975).

The primary human point sources include: sewage treatment plants, industrial effluents (particularly from petroleum production and refining, pulp and paper, organic and inorganic chemicals), intensive animal industries, fish farms and irrigation and storm water drains. Sewage treatment plants provide most of the available P to surface waters, which originates from human excretions and the use of industrial products, such as detergents, toothpastes, pharmaceutical and food-treating compounds. Primary treatment in sewage works removes only 10% of the P in wastewater; secondary treatment removes only 30% and the remainder is discharged directly into water bodies (Smith, 1990). Tertiary treatment is required to remove additional P from the water, but due to its high cost is not common practise worldwide.

The primary diffuse sources of P are runoff from land areas, especially areas mined for P*i* deposits, used for sewage sludge application, agricultural areas and urban residential areas. This source includes the leaching and drainage of fertilisers and other soil nutrients, plant material, animal excreta and other debris and the removal of soil particles. Estimates of the P*i* content of the runoff are difficult to make but recent studies have shown that it is mostly of particulate and organic nature (Haygarth & Jarvis, 1997; Turner & Haygarth, 2000). Agriculture is thought to be the main diffuse source of nutrients in freshwater bodies mainly due to changes in the natural use of the land and/or intensive fertilisation (Lijklema, 1994). Firstly, changes in land use of the catchment area and possible removal of its original vegetation cover may cause destruction to the mechanisms that conserved P in the land ecosystem (Moss, 1998). In addition, they may increase the overland runoff especially on soils with a low permeability and/or a high slope and further increase the input of P. For example, livestock could accelerate the P inputs to freshwater systems by either enhancing soil erosion or by producing manure. Alternatively, intensive fertilisation can be a direct source of P, when fertilisers are applied to soils near a water body, especially if the soils have low P retention. Nonetheless, P*i* has low mobility in soils since it is either incorporated into organic matter or absorbed by iron and aluminium oxides and clays, but a saturation point exists. Hence, usually a part of the P applied to the land is being taken up by the vegetation and the surplus either accumulates in the soil or leaches directly into freshwater bodies or into the groundwater (Lijklema, 1994). Then, in regions subject to excessive P loading, P*i* enriched groundwater slowly migrates to surface waters. The extent of this diffuse source is a function of features such as the

loading history, soil adsorption capacity, precipitation and groundwater level and may persist for several decades after the sources are eliminated (Lijklema, 1994).

In the more densely settled parts of the world, point sources are often the major contributor of P to aquatic ecosystems, but not so in rural environments, where diffuse sources are still the main supplier. Point sources are much easier to identify than diffuse sources and early remediation works focused primarily on this aspect of human inputs (Lampert & Sommer, 1997). A number of international conferences were held and agreements taken for reductions on the content of P from point sources and in many countries the P inputs are drastically reduced (Van Liere & Gulati, 1992; Van der Molen *et al.*, 1998). Recently, effort has been put into reducing diffuse sources too (Moss *et al.*, 1996b; Madgwick, 1999; Goetz & Zilberman, 2000). Reducing diffuse sources however, is not an easy task since it depends on a number of factors and a proper understanding and management of the whole catchment area is needed (Johnes, 1999; Moss, 1999). Finally, even though measures have been introduced to reduce external loading to aquatic ecosystems, a decrease in lake water P concentration is not usually observed (Cullen & Forsberg, 1988). The reason is internal loading of P released from the sediment which was created when external loading was high (Søndergaard *et al.*, 1993; Søndergaard *et al.*, 1999).

1.55 Biological production

The quantity of P found in waters of aquatic ecosystems is not only characterised by the geology, external inputs and land use of the catchment but also by its size, shape, storage facilities and biotic component (Ahl, 1988). In a normal, non-polluted situation the concentration of P is usually low compared to the demand and by far the larger part of the nutrients is in the biotic component and photosynthesis is the process that controls dissolved P*i* levels (Fox, 1993). After the death of organisms, stored P is often released in an organic form and a tight coupling between the N:P ratio of the food with the N:P ratio of the release has been observed (Golterman, 1995). Abiotic breakdown of organic and particulate P compounds in waters with neutral or weakly alkaline pH is usually very slow (Shen & Morgan, 1973) and heterotrophic recycling is needed to maintain primary production. Bacterial degradation, transformation and mineralisation of particulate and dissolved organic matter are the key processes determining turnover rates of organic and inorganic P (Fox, 1993) both in the water column and in sediments. However, bacteria are also principal producers as well as decomposers of organic matter, and P that could be released through metabolic reactions, extracellular release

and lyses of cells, is also a critical source in freshwater bodies (Törnblom & Rydin, 1998). Heterotrophic recycling can be distinguished in two major classes.

Phosphorus from phytoplankton and bacteria can be remineralised in hours, days, or even minutes (Harris, 1986) while they are still suspended in the water column. Several studies have shown that up to 50 to 90% of the organic matter synthesised by micro-organisms is mineralised within the epilimnion (Bossard & Uehlinger, 1993). The rapidity of the turnover is facilitated by the fact that no redox changes are necessary for uptake and metabolism and by the fact that release of TP from dead cells is very rapid (Harris, 1986).

In contrast, most aquatic plants die late in autumn and sink to the sediments where they are then mixed. Recycling processes which pass through the sediments may take months, and for some fractions of organic nutrients years or decades (Golterman, 1995). The intensity of macrophyte decomposition depends on factors such as plant species (size, morphological structure and initial chemistry), wave energy, oxygen, temperature, and animal activity (Graneli & Solander 1988). For example, emergent plants have supporting tissue, which is resistant to microbial attack, while submerged plants do not contain much cellulose and are easily mineralised upon death (Twilley *et al.*, 1986). However, release of P by macrophytes does not only take place in the sediments through decomposition of their organic matter but also directly from either healthy or senescing plants (Vanek, 1991), through grazing by invertebrates, fish and waterfowl and interactions with their epiphytes (Graneli & Solander 1988). Of particular importance are the exchange of P with epiphytes since they could be a major part of primary production in many shallow aquatic ecosystems (Wetzel, 1983) and can play an important role in P cycling. A number of studies have shown that epiphytes (especially the adnate component) can derive some portion of their P requirement from macrophytes throughout the macrophyte growing season (Srevenson *et al.*, ; Burkholder & Wetzel, 1989). Finally, in shallow oligotrophic lakes, macrophytes play the dominant role in P dynamics since they are not only the main source of autochthonous detritus as they usually prevail in total biomass but also could act as a nutrient pump, taking up P from the sediment and releasing it to the water column (Graneli & Solander, 1988).

1.6 Phosphorus cycling in lentic systems

1.61 Introduction

The transport of P in lentic systems appears to be controlled by a combination of physical, chemical, atmospheric, biological and human activities. Its cycling however, amongst sediments, aquatic plants and water column is controlling the productivity of lentic systems. An understanding of the size of these pools and the fluxes between them is essential for the assessment and management of the trophic character of freshwater bodies (Lijklema, 1994). The basic processes responsible for the P cycle in a lentic system are (Ohtake *et al.*, 1982):

1. Net PO_4-P uptake by phytoplankton, bacteria and macrophytes.
2. Settling of P in the hypolimnion and/or the sediment surface.
3. Release of PO_4-P from lake sediment.
4. Resuspension of P into the water column.

1.62 Phosphorus acquisition

Bacteria and algae are the only two components of aquatic ecosystems, which depend solely for their nutrition on the transport of dissolved substances from water, through the cell wall/membrane barrier, to the interior of the cell (Jansson, 1988a). Aquatic plants could acquire P either from the water via the leaves or from the substrate via roots too (Graneli & Solander, 1988). An energy-mediated transport process is mostly reported to be the driving force for P uptake via a H^+/P*i* or Na^+/P*i* symport (Ullrich & Glaser, 1982; Reid *et al.*, 2000). A number of dissolved and particulate forms of P may be utilised for growth by bacteria, algae and aquatic plants in lake water, either directly or following physical, chemical and biological transformations. The major form in which primary producers acquire P is as dissolved inorganic P*i*, or commonly called orthophosphate and its transport is generally recognised as the prime direct P uptake mechanism (Jansson, 1988a). Orthophosphate however, occurs usually in minute quantities and Bossard & Uehlinger (1993) found out that it only contributed 13% of the TP in the water of a very large number of lakes in northern Wisconsin, USA. Therefore, it seems that most of the P in freshwaters is in other forms than the soluble reactive one. In response to the low levels of available P and to maintain sufficient cytoplasmic P, organisms have developed highly specialised physiological and biochemical mechanisms to acquire and utilise P*i* from the environment (Raghothama, 1999). For example, algae and bacteria are known to have a remarkable affinity for orthophosphate and a large storage capacity (either as polyphosphate granules or in the

vacuole), which gives rise to the so called "luxury consumption" of P under conditions of high P supply (Raghothama, 1999). However, when all external and internal P reservoirs are depleted, organisms produce phosphatase enzymes which hydrolyse organic and particulate P forms to orthophosphate, in order to compensate for the lack of dissolved inorganic P (Whitton, 1991).

Phosphatases are a broad group of non-specific enzymes that catalyse the hydrolysis of many organic P compounds, such as phosphomonoesters, phosphodiesters, phosphotriesters and P*i* anhydrides, and liberate orthophosphate, that is available for uptake by algal cells (Reichardt, 1971). The majority of reports in the literature are concerned with only two classes of these enzymes: the phosphomonoesterhydrolases (PMEase), which are able to utilise phosphomonoesters (i.e. sugar phosphates) and phosphodiesterhydrolases (PDEase), which are able to utilise phosphodiesters (i.e. nucleic acids). This is because, these P compounds form an important component of organic P in both water and soil and thus their enzymatic catalysis could have an essential function in the P cycling of lentic systems. Some of these enzymes are synthesised and excreted externally to the cytoplasmic membrane (Turner *et al.*, 2001), while others remain in the cell and utilise internal organic P. In ecological studies, however, only the surface phosphatases which are located either on the surface of algal cells or on the cell membrane are of importance and standard bioassays for measurement of phosphatase activity only determine this component. High activity of these two groups of enzymes has been detected in various plants, animals and micro-organisms and indicate that they could play an important role in the P*i* supply during orthophosphate depletion (Jansson *et al.*, 1988b). However, part of the DOP in natural waters is composed of a high molecular weight fraction which is resistant to enzymatic breakdown (Hino, 1989). The bio-availability of organic and particulate P depends on several factors such as particle size, P speciation and geochemical characteristics (Stone & English, 1993) and estimates of the biologically labile portion of DOP range from 10% for up to more than 50% (Chróst & Overbeck, 1987; Hino, 1988; Siuda & Gude, 1994).

Many environmental factors affect phosphatase activity and the most important is pH (Turner *et al.*, 2001). Phosphatases, have maximum hydrolysable capacity at different pH values; hence, the enzymes are classified either as acid or alkaline phosphatases since they show their optimum activities in acid and alkaline ranges, respectively (Jansson *et al.*, 1988b). The majority of ecological studies are concerned with alkaline phosphatase activity (APA) (Berman, 1970; Heath & Cooke, 1975;

Pettersson, 1980; Huang & Hong, 1999) and only a limited number of studies have determined acid phosphatases of lake water and micro-organisms (Jansson *et al.*, 1981; Cotner & Heath, 1988; Lee, 2000). Other factors include chemical elements such as, Ca (Goldman *et al.*, 1990), Al (Bitti *et al.*, 2001), Zn (Jansson *et al.*, 1988b), surface area/volume ratio (Hernández, 1999), light (Hernández *et al.*, 1995) and internal and external P concentration (Whitton, 1991; Christmas & Whitton, 1998; Turner *et al.*, 2001).

In response to P limitation, organisms increase intracellular and enhance secretion of extracellular phosphatases in order to mobilise P from organic compounds (Bieleski, 1973; Natr, 1992). Hence, in many ecological studies, high phosphatase activity is generally thought to indicate condition of P*i* deficiency and the level of alkaline phosphatase activity indicates the degree of eutrophication of lakes (Jansson *et al.*, 1988b). In addition, phosphatase activity has been used as an indicator of P deficiency in algal cultures and natural phytoplankton populations (Whitton, 1991; Kalinowska, 1997). However, there is a lot of controversy on this particular matter. A number of studies have shown that phosphatase activity in lakes is not repressed by soluble reactive P concentrations ranging up to 0.32 mg L^{-1}, but others have shown that even small amounts of orthophosphate in lake water can inhibit phosphatase activity (Reichardt *et al.*, 1967; Siuda *et al.*, 1982; Jamet *et al.*, 1997). Jansson *et al.* (1988b) provides a review of phosphatase activity as a deficiency indicator and conflicting evidence. It appears that organisms produce phosphatase enzymes not only, when they need to acquire P from the environment, but also when P is available in abundance and overall is about the management and strategy of organisms for survival.

Thus, once P enters freshwater ecosystems it will be rapidly taken up by primary producers, particularly during the growing season, when the light driven photosynthesis exerts a demand usually greater than the rate of supply. Its utilisation during the spring to autumn growing season however, depends on the trophic status of the water body. In eutrophic lakes, P concentration usually drops rapidly from winter to spring as the biotic demand exceeds supply once temperature rises. In oligotrophic lakes, the relative decrease in P concentration is less than that in eutrophic lake systems because biotic demand in the summer period is lower. Hence, P concentrations commonly show summer minima and winter maxima. The winter maxima arise due to high surface and subsurface catchment inputs by increased rainfall, low internal turnover by microbial activity due to a decrease in temperature and low photosynthesis rates due to decreased light availability.

1.63 Sedimentation

The wave energy of lakes usually does not keep minerals of larger than clay size suspended for long periods. Large containing P particles that cannot be rapidly fractionated or removed, are then settled on the sediment surface and are not any more available for primary productivity in the trophic zone (Golterman, 1998). The ability of organic and particulate P to remain suspended in turn depends on a number of physical, chemical and biological factors (Stone & English, 1993). Among these factors, some physical properties of great importance include volume, buoyancy, surface area and the surface area/volume ratio (Stone & English, 1993). The <8 μm size fraction of these particles is supposed to be able to remain suspended while larger detritus precipitates (Stone & English, 1993).

However, microorganisms keep P suspended more efficiently than detritus of similar density. Algal rapid reproduction is the primary mechanism to compensate sedimentation out of the photic zone since it takes place every 0.5 – 5 days, which often is fast enough to sustain them in surface water layers (Broberg & Persson, 1988). Sinking rate is also regulated by the physiological vitality of the cells (i.e. dead diatoms show increased sinking rates by a factor of 3-5 compared to live cells) (Broberg & Persson, 1988). Furthermore, some algae may also show reduced sinking rates by being motile, which even creates the possibility of vertical migrations. Thus, sinking rates do differ among species according to their physiological and biochemical properties and vary from zero for cryptomonads, low for green and blue-green algae (due to gas vacuoles) and high for diatoms (Broberg & Persson, 1988). In addition, zooplankton exclusion also increases the residence time of total P and reduces P loss by sedimentation (Bossard & Uehlinger, 1993).

Finally, sedimentation of P does not only depend on physical and biological factors but also on chemical adsorption. An important mechanism mediating the downward flux of P involves its interactions with $CaCO_3$ (Otsuki & Wetzel, 1973; Murphy *et al.*, 1983). This is of particular importance in hardwater lakes, where a number of studies have shown that high rates of P deposition were related to $CaCO_3$ precipitation (Effler & Driscoll, 1985; Wodka *et al.*, 1985; Kleiner, 1988; Gonsiorczyk *et al.*, 1998; Effler *et al.*, 2001). A characteristic example is the hard water Lake Luknajno in Poland, where a co-precipitation of P with $CaCO_3$ was observed, resulting in lower concentrations of dissolved P than the predicted ones (Kufel & Kufel, 1997). In addition, epilimnetic decalcification and thus P co-precipitation can proceed vigorously during summer, because precipitation of $CaCO_3$ is enhanced by increases in water temperature, CO_2

degassing and the photosynthetic activity of phytoplankton (Tefler, 2000). Moreover, presence of nitrate can also induce calcite formation since algal utilisation of nitrate will create a microenvironment around the cell with a pH high enough to enhance calcite formation (Burkholder & Wetzel, 1989). For example, Murphy *et al.* (1983) observed that the development of an *Aphanizomenon* bloom, appeared to be responsible for the removal of P*i* by a co-precipitation reaction with $CaCO_3$. Driscoll *et al.* (1993) conducted a series of laboratory experiments in order to test the effectiveness of this removal and the relation with Ca concentration. Their results suggested that high Ca concentrations (range of Ca concentration was between 160 to 900 mg L^{-1}) efficiently removed PO_4 from the water column but at lower Ca concentrations, the solution became gradually undersaturated with respect to Ca-PO_4 minerals (i.e. apatite ($Ca_5(PO_4)_3.OH$)). Furthermore, an important factor in the co-precipitation of PO_4 with Ca is pH. Driscoll *et al.* (1993) also pointed out that a consistent release of PO_4 and an increase in Ca concentration, was observed with decreasing pH, indicating solubilisation of Ca-P minerals with a subsequent release of PO_4. However, chemical adsorption of P not only take place in lake water but on the sediment surface too (Golterman, 1995). Freshwater sediments have a large P binding capacity mainly because of their high content in clay, silt, Fe and Ca.

Ultimately most of the P will be incorporated into detritus particles and settle on the sediment surface due to gravity and will be unavailable for primary productivity in the trophic zone (Golterman, 1998). At the same time, sedimentation connects lake sediments with organic matter production in the overlying water, in the littoral zone, and in the drainage area (Hartikainen *et al.*, 1996).

1.64 Sediments

Sediments are usually the largest single pool of P in many aquatic systems and it has been suggested that 1 m^2 of surface sediment (10 cm deep) contains 300 times the P content of a 5-m deep overlying water column (Lijklema, 1998). Sediments may act as a source or sink (Kairesalo & Matilainen, 1994), but the currently accepted view is that they act as a net sink, since continuous accumulation of detritus into the sediment surface will leave some P too deep within the substrate to be re-introduced to the water column and it will be permanently removed from biocirculation (Istvánovics & Somlyody, 2001). Although generally true on an annual basis, sediment release (internal loading) can represent a substantial part of seasonal inputs to lakes, particularly those that undergo periodic hypolimnetic anoxia (Istvánovics, 1994). For instance, in Lake Bysjon, Vanek (1991) estimated that the total amount of P transported to, was of

the same order of magnitude as the hypolimnetic release from the bottom sediments. Release from the sediments into the water column involves the volatilisation of sediment-bound P into porewater and then porewater P movement into the overlying water column (Burley *et al.*, 2001). Hence, the P loading of the sediment, due to sedimentation and the related periodical release of P into interstitial and overlying water, determine the fate and concentration of P in many freshwater bodies.

Traditionally P cycling in sediments is thought to be an abiotic process (Prairie *et al.*, 2001), which involves these main mechanisms: precipitation with Ca^{2+}, Mg^{2+} and/or incorporation into precipitating $CaCO_3$ and adsorption of dissolved P mainly onto ferric hydroxide (Fe(OOH)), Al^{2+} and Mn^{4+} which have a strong affinity for P*i* anions (Driscoll *et al.*, 1993; Reimers *et al.*, 1996; Golterman, 1998). Co-precipitation of P with $CaCO_3$ is thought to be the first transport mechanism towards the sediments (particularly in hard water lakes) and is strongly dependent on pH. However, there the presence of even small amounts of Fe(OOH) induces an adsorption mechanism and $CaCO_3$ adsorbs the excess P*i* that cannot be bound to the Fe(OOH) (Golterman, 1988, 1998). For example, Golterman (1988) found out that a waterlogged calcareous soil, containing about 20 % $CaCO_3$ and 0.5 % Fe, had the larger part of P fixed onto Fe(OOH). Even in a hard water lake such as Lake Balaton, where up to 65 % of the sediments consist of carbonates, most of the P was associated with Fe (Lijklema, 1994). However, in lakes with low Fe:P ratio, and/or too high pH (Fe has a stronger adsorption power at lower pH values (Golterman, 1995)), release and binding of P*i* cannot be controlled exclusively by Fe and additional chemical and physical mechanisms such as sorption of P to clay minerals and humic substances have been suggested too (Boström, 1984).

In turn, these abiotic mechanisms are controlled by factors such as redox potential, O_2, SO_4^{2-} and pH (Driscoll *et al.*, 1993; Golterman, 2001). Redox potential is probably the ultimate regulator since it controls P fixation and release in the sediment, by determining the oxidation/reduction state of the metal ions and hence their adsorption/desorption power. When the redox potential falls below 200 mV, Fe^{3+} is rapidly reduced to Fe^{2+} and the associated P is released into the interstitial and overlying water (Borovec & Hejzlar, 2001). Sediments are probably always strongly reducing, except at their surface, where an oxidised microzone several millimetres thick can develop when exposed to oxygenated waters (Hutchinson, 1957). This microzone is usually brown in colour, owing to the presence of Fe(OOH) and when fully developed has a potential of about 500 mV (Hutchinson, 1957). Hence, this oxygen-rich region

leads to the precipitation of Fe-P and acts as a barrier against diffusion from the mud to the water (Hutchinson, 1957). However, in the summer, when the supply of oxygen is reduced mainly due to stagnation and/or stratification, the oxidised microzone tends to become thinner and finally disappears when the potential in the surface layer of the mud lies at about 200 mV (Hutchinson, 1957). Hence, Fe^{2+} and P*i* are released to the overlying water (Gächter & Meyer, 1993). In addition, high concentrations of H_2S in the overlying water and/or the sediment and decrease in pH may increase P*i* release. In lakes in which a considerable amount of H_2S is formed, some ferrous sulphide (Fe-S) may be precipitated removing enough Fe to allow P*i* to remain in solution (Hutchinson, 1957). Furthermore, when lake sediments acidify (e.g. by high CO_2 concentration) part of the P bound to $CaCO_3$ is solubilised and its precipitation depends only at the presence of Fe(OOH) to re-adsorb P*i*. Finally, Golterman (1995) observed that NaCl, $MgCl_2$ and $CaCl_2$ (normally occurring compounds in freshwater) also play an important role and can increase P adsorption onto Fe(OOH) as much as 50%.

Phosphorus bound to reducible forms of Fe and Mn as well as Ca^{2+} and Al^{2+}, is in general a potentially mobile pool of P in sediments. It may be released under oxygen depletion upon reduction of the reactive oxidised species of the metals or in calcareous sediments by the pH/calcium/P reactions. Therefore, maintenance of oxygenated conditions at the sediment-water interface is critical to minimise or eliminate P release to the overlying water (Driscoll *et al.*, 1993). This is of particular importance for shallow lakes where the trophic status is strongly influenced by the internal release of P from lake sediments under anoxic conditions, and its re-suspension during turbulent periods (Kleeberg & Kohl, 1999a).

However, P cycling in sediments is not only an abiotic process but involves biological mediation too. Of primary importance is P assimilation and release by sediment bacteria. Originally, bacterial activity was assumed to play only an indirect role by providing the necessary conditions for redox and pH dependent abiotic sorption/desorption or precipitation/dissolution reactions (Gächter & Meyer, 1993). However, it is now well known that sediment bacteria play an important role in the uptake, storage and release of P by sediments at both aerobic and anaerobic conditions (DeMontigry & Prairie, 1993; Prairie *et al.*, 2001).

Sediment bacteria depend on P as a nutrient and they are able to take it up from organic substrates but also directly from the water. Their main P supply usually is the settling organic and inorganic material from the epilimnion. However, part of their

demand for P may also be met by diffusion of P*i* from the interstitial water and studies have shown that inhibition of sediment bacteria increased the concentration of P*i* in the interstitial water. Furthermore, as bacteria decompose settled organic material, bacterial biomass is produced in the sediment, which is approximately 3 to 5 orders of magnitude higher than in the water but also rapidly decreases within the sediment at greater depth (DeMontigry & Prairie, 1993). Hence, bacterial biomass can also act as an important P reservoir and Gächter & Meyer (1993) found out that P incorporated in sediment bacteria may exceed the yearly deposition of bioavailable P several times, especially in oligotrophic lakes. However, P bound in sediment bacteria may be released through metabolic reactions, extracellular reactions and lyses of bacterial cells in response to changing environmental conditions (e.g. shift towards anoxia) and should therefore be considered as a potentially mobile P pool in sediments (Törnblom & Rydin, 1998). Accordingly, the amount of P in bacteria has been shown to be large enough to explain most of the P-release observed from some lake sediments (Törnblom & Rydin, 1998). This suggestion was supported by studies on poly-P in lake sediments, which have shown that bacterial poly-P constituted between 32 and 50 % of the NaOH-extracted P fraction (Törnblom & Rydin, 1998). However, it seems unlikely that all bacterial P would be realised as P*i* and some of it may be buried as particulate or organic P in the sediment (Gächter & Meyer, 1993). In addition, the inhibition of sediment bacteria, may actually decrease the release of P from sediments to lake water (Törnblom & Rydin, 1998), since microbial activity is the main process of O_2 consumption, causing a shift towards anaerobic conditions and loss of the oxic sediment-water interface (Hartikainen *et al.*, 1996). In summary, bacteria at the sediment/water interface may perform the following functions (DeMontigry & Prairie, 1993): mineralise organic P, take up and store P in their biomass either as dissolved or particulate, release P when environmental conditions are not favourable and finally transfer P from the sediment to the water column or vice versa.

However, bacteria activity is not the only biological process which influences P cycling in sediments but mobilisation of P by rooted macrophytes is an important regulator of P release too as well as sediment related mechanisms influence growth in submerged macrophytes (Barko & Smart, 1986). Aquatic macrophytes dominate the littoral lake zones and form dense mats resulting to a build up of organic matter on the sediment directly below the stands (Graneli & Solander, 1988). In these areas, P release can be increased through rapid mineralisation of organic matter, which is enhanced by oxygen release from roots and low redox conditions at the sediment surface, but also by active uptake of P from the sediment through the roots and release

to the water column (Graneli & Solander, 1988; Kairesalo & Matilainen, 1994). Stephen *et al.* (1997) conducted a series of experiments in order to determine whether macrophyte stands influence P dynamics of sediment and suggested that at their presence release of P from the sediment was overall higher. Though, release of P from cores taken from plant beds of evergreen species did not show any differences (Stephen *et al.*, 1997).

In conclusion, the interactions between P and sediments in aquatic systems are very complex in nature and comprise of both physicochemical and biologically dominated processes such as adsorption/desorption and biological assimilation in biofilms and sediment bacteria (House & Warwick, 1999) and usually take place within the uppermost 5-10 cm of sediments (Reimers *et al.*, 1996). Understanding and quantifying these processes is a major concern to water managers in charge of eutrophication control and restoration of freshwater ecosystems (Davelaar, 1993; Driscoll *et al.*, 1993). Knowledge of the fractional composition of P can help evaluate the potential of a particular sediment to adsorb or release P when conditions, such as pH or dissolved oxygen concentration change (Marsden, 1989; Noges & Kisand, 1999). A number of sequential extraction techniques have been developed in the last decades in order to quantify various forms of sediment P, intending to yield a greater differentiation than simple organic and inorganic P determinations (Psenner & Pucsko, 1988; Penn & Auer, 1997). At present, more than 20 extraction schemes exist (Williams *et al.*, 1971; Hieltjes & Lijklema, 1980; Ruttenberg, 1992; Golterman, 1996) and many of them have been critically reviewed by several authors (Boström, 1984; Psenner *et al.*, 1988; Levy & Schlesinger, 1999). These methods yield operationally defined P fractions or forms, which can indeed provide insight to sediment P composition and fate (Pettersson *et al.*, 1988; Penn & Auer, 1997). However, this implies that P compounds of similar behaviour or reactivity are included in a single fraction. The main fractions (artificial groups of similar compounds) include: P which is dissolved or immediately available, P soluble at low redox potentials, P soluble at high pH, chemically and/or biologically inert P and P which may be dissolved by enzymatic microbial activity (Psenner *et al.*, 1988).

1.65 Resuspension

Sedimentation of organic and particulate matter is an important process in the removal of P from the water column and its deposition on sediments (Section 1.63), but several mechanisms for upwards nutrient transport are also known. Molecular diffusion, which is the basic transport process, is slow and dominates only in anaerobic

conditions in stagnant water bodies (Golosov & Ignatieva, 1999). The disturbance of the bottom by animals may also be important, by turning over sediment and resuspending it in the water column or by irrigating deeper layers with water currents (Stephen *et al.*, 1997). However, temperature and wind are the main two physical factors responsible for resuspending surface sediment particles into surface waters (Hutchinson, 1957).

Temperature or wind induced water movement (turbulence) will resuspend weakly compacted surface sediments, enhance diffusion of dissolved P from the sediments and also transfer nutrient-rich interstitial water to the water column (Bronmark & Hansson, 1998). The latter is mostly enhanced during the autumn cooling, when the upper sediment layer is warmer than the near-bottom water and this enhances pore water transfer across the sediment-water interface by several orders of magnitude greater than that of molecular diffusion (Golosov & Ignatieva, 1999). In addition, extreme turbulence such as that during autumn overturn could also get onto suspension large amounts of particulate P, but these will return to the sediments relatively quickly under the influence of gravity (Bronmark & Hansson, 1998). Hence, the most important export from the sediments is that of orthophosphate and of soluble inorganic P, as it is directly or indirectly available for uptake and remains in the water column for a longer period than particulate forms.

Thus, in fully polymictic lakes, P could be rapidly returned from the sediment and thereby rendering P loss by sedimentation less critical (Jeppesen *et al.*, 1997). In addition, mixing of surface water layers with bottom ones, increases their oxygen content, which enhances mineralisation (Ramm & Scheps, 1997). In contrast, oxygen in water overlying sediments also influences the redox-potential of surface sediments, prevents considerable accumulation of organic matter and ultimately controls internal P-loading (Ramm & Scheps, 1997). However, some water bodies exhibit vertical thermocline profiles, particularly during the summer months, which impede vertical turbulence and consequent return of P to the surface water layers. Hence, subsequent anoxia in deep waters will lead to a build up of P*i*, resulting in strong vertical gradients of P*i* concentrations. Nonetheless, during the period of autumn cooling, P*i* is distributed throughout the lake and fertilises surface waters again. Finally, an important factor that reduces resuspension is the presence of macrophytes. Several studies have shown that submerged macrophytes diminish water exchange between the littoral and pelagial zones and reduce wind induced resuspension by stabilising the sediment (Graneli & Solander, 1988; Janse, 1997).

1.7 Charophytes

1.71 Ecology and distribution

Characeae (charophytes or stoneworts) are anatomically highly developed green algae, which show a worldwide distribution in fresh and brackish waters (Andrews *et al.*, 1984a) and usually inhabit hardwater, oligo/mesotrophic lakes with low P concentrations (Forsberg, 1965b). They often form dense, large beds, referred as meadows (Van den Berg *et al.*, 1998), which show substantial seasonal biomass changes, high accumulation of nutrients and slow decomposition rates during winter (Pereyra & Ramos, 1981). They normally occur within the depth range of 1-14 m, with a district edge zone towards the open water (Blindow, 2000b) but there are reports of individual species growing in damp soil and at depths of over 30 m (Andrews *et al.*, 1984a; Kufel & Kufel, 2002). Nygaard & Sandjensen (1981) pointed out that deep-living populations also have a potentially high turnover of biomass and nutrients and a profound influence on the O_2 and nutrient dynamics of lakes.

Charophytes grow erect above the substratum and the shoot is composed of alternating coenocytic single-celled internodes and multi-cellular nodes, which form lateral branchlets (Andrews *et al.*, 1984a). Growth takes place at the apex and is balanced by death at the base of the plant and lower internodal cells (Andrews *et al.*, 1984a). Rates of photosynthesis and dark respiration are usually higher in the summer, with the former mostly depending on light intensity and the latter on temperature (Nygaard & Sandjensen, 1981). Furthermore, the internodal cells of charophytes have been widely studied (Kwiatkowska & Papiernik, 1999; Henzler & Steudle, 2000; Proseus *et al.*, 2000; Beilby & Shepherd, 2001) due to their giant size and have contributed to the progress of plant membrane biophysics (Tazawa & Shimmen, 2001).

Charophytes are anchored to the sediment by colourless rhizoids, which consist of single or few-celled filaments (Andrews *et al.*, 1984c) and presence or absence depends on growth conditions (Andrews, 1987). In addition, rhizoid development depends on size of substrate particles and most likely factors to be responsible are the photon flux density reaching the lowest node as well as the oxygen concentration of the interstitial spaces (Andrews *et al.*, 1984c). Rhizoid participitation in nutrient uptake is thought to be negligible since biomass of charophyte rhizoids is small in comparison to the above-ground biomass (Kufel & Kufel, 2002). However, Andrews (1987) showed that even though C uptake is negligible, P*i* uptake could be responsible for a substantial amount of the total P*i* taken up by the whole plant in aerobic sediments with high interstitial P*i*

concentrations. Thus, it seems likely that if a well-developed rhizoid system is present and P*i* concentrations in the interstitial water are substantially greater that the open water above, then the rhizoids could play an important role in P*i* uptake which can then be transfered to the shoot (Andrews, 1987).

However, as it was mentioned above, Chara inhabit hard water lakes, with high Ca and bicarbonate concentrations. Hence, the excess of these ions in the lake water has a number of direct or indirect consequences for the well-being of the plants. Firstly, Wetzel & McGregor (1968) showed that a Ca level of 20 mg L^{-1} could reduce the rate of photosynthesis. In addition, lake waters are well buffered by high content of carbonate and bicarbonate, and the maximal pH variation usually observed is within one unit and at the interval of 7.5 to 8.5 (Wetzel, 1960). Furthermore, the alkaline pH and high concentrations of Ca create considerable amounts of $CaCO_3$ and consequent precipitation. Hence, $CaCO_3$ (marl) probably constitutes the main part of the sediments in these lakes but also heavily encrusts the plants. Welch (1952) indicated that $CaCO_3$ composes at about 30% of the dry weight of *Chara* plants and $MgCO_3$ about 2% but (Blindow, 1992a) found $CaCO_3$ concentrations as high as 53% in *Chara tomentosa* from Lake Hornborgasjon, a southern Swedish Ca-rich lake. However, $CaCO_3$ deposition is not only dependent on Ca concentration of water but it has also been claimed (Welch, 1952) that calcification becomes thickest, when clear, calm weather permits rapid accumulation on those parts of the plant exposed to the strongest light. Evidence that encrustation is almost exclusively the result of photosynthesis was provided by Cattaneo & Kalff (1978) who found very little deposition on plastic leaves and it has also been shown that the ratio of calcification to photosynthesis is ~1 at pH 8 and increases with pH (McConnaughey, 1991). Accordingly, the substantial marl encrustation could explain the consistently lower nutrient content of charophytes found in literature, as compared to other submerged macrophytes (Blindow, 1992a). Though, since most of the marl occurs as $CaCO_3$ on the plant's surfaces, it could be easily removed if desired, by sonication or rinsing in dilute acid (Carpenter & Adams, 1977).

Moreover, $CaCO_3$ will also be present in the water and this will affect nutrient availability, since important elements such as P, Fe and trace elements will be precipitated or fixed in forms not assimilable by plants. Consequently, waters of this type have an oligotrophic character, which is indicated by the scarcity of phytoplankton. Additionally, marl encrustation, which is mainly caused by bicarbonate use during photosynthesis, indicates the ability of charophytes to utilise bicarbonate. This has been shown experimentally (Cattaneo & Kalff, 1978) and Simons & Nat (1996) pointed

out that *Chara globularis* and *Nitella translucens*, are relatively good CO_2-users, while strictly hardwater species such as *Chara hispida*, are efficient HCO_3-users.

Finally, charophytes are also supposed to exert allelopathic effects on aquatic macrophytes and on planktonic and epiphytic algae (Kufel & Ozimek, 1994). One clear demonstration of allelopathy is that of *Chara globularis* Braun, which is seldom found with epiphytes (Stevenson *et al.*, 1996). This alga is commonly associated with a "rank, pungent odour" where it occurs in abundance and two biologically active sulphur compounds (a dithiolane and trithiane) were isolated, which inhibited photosynthesis of phytoplankton species and of the epiphytic diatom *Nitzschia palea* (Stevenson *et al.*, 1996). However, many *Chara* species are often overgrown with diatoms and blue-green algae, which indicates that allelopathy may not be common in the Characeae family (Blindow, 1987).

1.72 Relationship with phosphorus

Forsberg (1964; 1965a) demonstrated experimentally that 15-30 $\mu g\ P\ L^{-1}$ in lake water could be potentially toxic for the growth of axenic cultures of *Chara globularis* and *Chara zeylanica*. He also observed luxuriant growth of *Chara* sp. in lakes with TP concentrations usually lower than 20 $\mu g\ L^{-1}$ and concluded that *Chara* sp. are physiologically sensitive to high P concentrations. In addition, he demonstrated that other water environmental factors, such as temperature, light, pH, major and minor chemical elements (with exception of P) seemed to be of secondary importance for the charophytes compared with P concentration. The term "secondary" importance means that these factors could range within very wide limits and yet be tolerated by the charophytes. Supporting evidence was provided by Hough & Putt (1988) who showed that increasing P concentrations did not enhance biomass production in *Chara vulgaris*. Furthermore, recent ecological studies have shown that charophytes in Sweden inhabit freshwater bodies with TP concentrations mostly below or about 20 $\mu g\ L^{-1}$, indicating their very low P requirements (Blindow, 2000b). Accordingly, the diminishing charophyta flora in the human cultivated areas has been explained as a consequence of increasing P concentrations of waters by pollution, which then in some way negatively influences their growth. Hence, charophytes have been used as indicators of water quality as well as means of maintaining good water quality with rich biotic components (Simons & Nat, 1996).

In response to Forsberg's observations, a number of people have studied P toxicity in charophytes and in contrast have shown that they can tolerate P concentrations much

higher than 20-30 μg L^{-1}. First of all, Wetzel & McGregor (1968) demonstrated that increasing P levels over a range of 0-305 μg L^{-1} above those of natural levels of the parent plants resulted in a slight but consistent inhibition of photosynthetic growth, but the response was not nearly as marked as that observed by Forsberg. In addition, Henricson in 1976 repeated Forsberg's experiments and found that at P concentrations between 0 and 2000 μg L^{-1} there was no significant difference in growth rates of axenic *Chara globularis* (Blindow, 1988). Blindow (1988) also found that there was no limitation in growth of *Chara tomentosa* and *Chara hispida* at inorganic P concentrations up to 1000 μg P L^{-1} and observed that charophytes also occurred in lakes with P concentrations far in excess of the 20 μg L^{-1} TP limit (Blindow, 1992a). Finally, Kufel & Ozimek (1994) demonstrated that a wide range of inorganic P concentrations in laboratory experiments enhanced biomass production in *Chara aspera*. They also suggested that *Chara aspera* has a high capacity to absorb and accumulate P and even in lake water enriched with P, it may still remain P limited.

Hence, the controversy in literature concerning the response of *Chara* sp. to elevated concentrations of P in lake water makes the assessment of their ecological role and importance quite difficult. Originally, it was thought that parallel to increasing P concentrations in lake water an increasing phanerogamic vegetation seemed to appear and that *Chara* lakes (usually of oligotrophic character) were a forestage in the development of (eutrophic) *Potamogeton* lakes (Blindow, 1991). However, Blindow (1991) determined that charophytes can be superior competitors than angiosperms and can successfully colonise shallow lakes previously dominated by phytoplankton due to higher biomasses and accumulation of larger amounts of nutrients. Owing to this potential, storage capacity is probably one of the competitive advantages of *Chara* plants and the other is the ability of plants to absorb P rapidly in case of seasonal inputs of P in lake water. Hence, *Chara* may not only act as a P sink but also as a P buffer preventing massive development of planktonic algae (Kufel & Ozimek, 1994). Supporting evidence was provided by Krolikowska (1997) who observed that at stations covered with *Chara*, P, N and chlorophyll *a* concentrations in the water were markedly lower than at stations devoid of plants.

1.8 Aims

As mentioned in Section 1.1, one particular observation led to the present study. Towards the end of the warm summer of 1996, many shoots of *Chara hispida* close to water surface in Croft Kettle had become yellowish and parts of some had turned white. Marren (1998) considered this effect to represent a disaster. Several factors might interact to impair the environment where the *Chara hispida* grows, even if one factor is the immediate cause of the unhealthy state of the alga. Another possibility might be that several factors are potentially harmful individually to *C. hispida* and these factors are additive in inducing the stress response. The possible reasons for this which were considered at the time were:

1. Changes in the amount or quality of the input water.
2. Increased P as a result of long-term eutrophication or perhaps recent events, such as arrival of Canada geese.
3. Rise in temperature and any consequent influence on formation and persistence of a thermocline.
4. Increase or change in higher plant debris entering the pond, leading to decreased oxygen concentration at the bottom of the pond. One possibility is that *Phragmites* may partially have replaced *Cladium* around the pond and that its debris may break down more rapidly.
5. Introduction of a pathogen.
6. Change in trophic structure due to differences in fish stocks.

The main aim of this study was to assess P dynamics in Hell Kettles as well as a number of other physical and chemical parameters and to determine their effect on growth of *C. hispida* especially in Croft Kettle. In order to achieve these, a number of more specific aims were identified:

1. To provide a descriptive account of the ponds, especially those physical and chemical features most relevant to plant growth.
2. To assess the P status of Croft Kettle.
3. To provide a general account of the biology of *C. hispida* in these ponds.
4. To establish the likely cause of the bleaching of *C. hispida* in 1996.

CHAPTER 2 Methods

2.1 Safety

Field visits to the site were made during daylight, usually with two field operatives. Safety equipment for sampling the water without diving included: waders, life vest and wading stick. Membrane dry-suit, aqua-lung equipment, face mask, snorkel, fins, diving gloves and hood were worn whenever snorkelling or diving and these were always performed by two qualified divers and according to the BSAC rules.

2.2 Standard laboratory techniques

All chemicals used were of AnalaR grade. Stock solutions and reagents were made up using deionised water. Glassware and plastics for nutrient analysis were rinsed a couple of times with hot tap water and then with deionised water prior to soaking in 10% DECON (non-anionic surfactant) for at least 24 h. They were then rinsed several times in deionised water and oven-dried at 105 °C for standard glassware and 40 °C for plastics. Volumetric glassware were left to dry at room temperature.

2.3 Sampling programme

Initially the periods between sampling were slightly irregular, but later this developed into a standard programme of monthly samples. This programme included surface water samples taken from nine locations (Figure 3.5): 4 in Croft Kettle; 4 in Double Kettle; and 1 from the nearby borehole (representing groundwater). A standard 'surface' sample was taken 2 m from the edge of the pond at 30 cm depth and measurements were made during the period 1030 – 1200 h moving from Croft to Double Kettle to the borehole (time is shown as GMT). The sample dates for the 'surface' measurements were:

1998 29 Oct, 20 Nov

1999 8 Jan, 25 Feb, 31 Mar, 17 Apr, 7 May, 17 Jun, 20 Jul, 14 Aug, 17 Sep, 21 Oct, 15 Nov, 7 Dec

2000 5 Jan, 2 Feb, 7 Mar, 12 Apr, 7 May, 5 Jun, 7 Jul, 7 Aug, 7 Sep, 12 Oct, 14 Nov, 12 Dec

2001 5 Jan, 17 Feb, 13 Jul, 3 Aug, 8 Sep, 19 Oct

The observed gap in sampling dates in 2001 was due to the foot and mouth outbreak in UK, which restricted access to the ponds. Water was sampled for N and P fractions and part way through this programme; the sampling was extended to total alkalinity, silica (Si) and suspended chlorophyll *a*. Water for Si analysis was collected on the same day as the 'surface' water measurements, but for practical reasons, total alkalinity and suspended chlorophyll *a*, 3-5 days later under the same conditions as the standard 'surface' sampling survey. In addition to the above sampling, a few additional surveys of N and P were made in Croft Kettle during the earlier part of this programme (1998: 14 Nov; 1999: 25 Jan, 1 Feb, 27 Apr, 20 May, 4 Jun, 24 Aug, 24 Sep). The values all fell within the limits of measurements made in the main survey and the data is included in the figures shown but not in the data analysis.

The remainder of the sampling used scuba diving and this was solely on Croft Kettle. The standard sampling programme was carried out on the following dates:

1999 7 May, 25 Jun, 25 Jul, 27 Aug, 30 Sep, 5 Nov, 3 Dec

2000 8 Jan, 8 Feb, 11 Mar, 14 Apr, 14 May, 12 Jun, 18 Jul, 11 Aug, 15 Sep, 24 Oct, 27 Nov, 17, Dec

2001 13 Jul, 3 Aug, 8 Sep, 19 Oct

This survey made use of two ropes suspended in the middle of the pond. The ropes were suspended at a position where the pond was 5 m deep (position indicated in Figure 3.5) and water samples were collected at 1-m intervals from the surface downwards (i.e. 6 positions). Bottles were attached to each line in advance of the survey and opened underwater. One line had a 250-mL glass bottle at each position, while the other line had a 750-mL polypropylene bottle at each position. The glass bottles were used for measurement of dissolved oxygen and the plastic bottles for measurement of N and P. The samples for N and P were divided into three subsamples, so each position generated three samples for analysis. A water sample was also taken above the *Chara hispida* beds at approximately the locations where surface water samples were taken during the monthly sampling programme, but at about 2 m depth. Shoots or sometimes whole plants of *C. hispida* were collected at similar position to the water above the beds, but only from site 2 (Figure 3.5). Sediment samples were collected at 5 m depth, but because of the disturbance involved in sampling, it was decided to collect these at a different position to those where the bottom water was sampled, about 5 m away (Figure 3.5). Seven cores were taken at each sampling occasion and stored at 4 °C in the dark, but only four were used for analysis (Section 2.8).

In addition to this standard programme, a number of intensive 3-dimensional surveys were made also in Croft Kettle on different dates. During summer 2000, an intensive survey of the physical, chemical and vegetation characteristics of Croft Kettle took place, which included the identification of submerged aquatics in Croft Kettle as well as the flowering plants around both ponds. This involved many field visits as well as scuba diving in Croft Kettle once or twice a week. For practical reasons not all the determinants required for the most detailed studies could be done on one day. Even if more divers were available, the size of the pond would restrict what could be done without having a marked influence on some chemical variables (e.g. dissolved oxygen). The features determined were:

1. 3-D map, showing surface dimensions and depth contours at 1-m intervals
2. 2-D map of submerged vegetation
3. 2-D map of fringing vegetation around Croft Kettle (showing mainly distribution of *Phragmites australis* and *Cladium mariscus*)
4. A list of the flowering plants of Hell Kettles ecosystem
5. 3-D distribution of temperature and oxygen in Croft Kettle (on two occasions)

The information regarding the physical mapping was collected at approximately 1-m intersections of a 50 x 50 m grid covering Croft Kettle. Area estimates were made in GIS after preparing maps by interpolating gridpoints. The software programme used for the analysis and graphic representation of the data was arc-view GIS, version 3.2. This provides great flexibility, allowing a paper map to be quickly produced that exactly meets the needs of the user and analysis and modelling become possible.

2.4 Physical and chemical variables measured on site

On each sampling occasion a range of variables were recorded on site, using Wissenschaftliche-Technische Werkstäten (WTW) meters and probes. Temperature, conductivity, pH and dissolved oxygen of water were measured during the 'surface' standard survey, and Eh and pH of sediments during the scuba diving survey for the period between March 2000 and February 2001.

A WTW (model LF 320) meter was used to measure temperature and conductivity in conjunction with a TetraCon 325 probe. Calibration with British Drug House (BDH) standards (36, 1413 $\mu S\ cm^{-1}$) was carried out monthly. The thermistor was calibrated at 5 °C and room temperature. A mercury thermometer was used for reference. Dissolved oxygen concentration was measured using a WTW meter (model OXI 196).

This was calibrated before every sampling occasion on site using a WTW Oxical-S sleeve. Polarisation of the electrode was immediate. Checks for membrane damage or air bubbles trapped under the membrane were made before sampling. The membrane was changed and probe cleaned every six months. pH of water and sediments was measured with a WTW meter (model pH 196) in conjunction with a WTW SenTix electrode (type 41-3). The meter was calibrated in the field using BDH standard buffers (4.0, 7.0 and 9.22).

Eh ("redox potential") of sediments was measured with a WTW pH meter in conjunction with a WTW SenTix ORP electrode. The values recorded here were at 14 mm depth (i.e. the platinum surface was approximately 14 mm below the loose surface of the sediment). pH measurements were made in as similar a position to Eh as possible, but as there was little change in pH with depth, this had little influence of the results. $E_{7.0}$ values were not calculated, since the pH values were so near to pH 7.0 that this made little difference to the results.

In addition, a pair of PAR sensors (SKYE, Sk 280) were used to measure the attenuation of photosynthetically available radiation (PAR, 400-700 nm). These sensors were connected to a SKYE datalogger, to which a Microscribe microcomputer interface was also connected. One light sensor was placed above the water surface and out of the shade, to act as a reference to record any difference in the incident light while the other sensor was either attached to a metal frame attached to a wooden pole graduated at 0.5 m intervals or lowered by scuba-diving. The reference and depth irradiance readings were recorded every 0.5 m intervals and the procedure was repeated until at least four sets of readings were obtained. The attenuation of downwelling light (K_d value) was calculated according to Beer-Lambert's Law of exponential change of light with water depth (Kirk, 1983; Fahnenstiel *et al.*, 2000). A profile of depth versus light was constructed from these data for Croft and Double Kettle on 18 Feb 2000 (1200 h) and Croft Kettle only on 20 Jul 2001 (1300).

Finally, three temperature data-loggers (model Tinytag Plus, Gemini Data Loggers, UK) were placed in Croft Kettle to provide more detailed information on spatial and temporal differences. The temperature data-loggers were first placed in Croft Kettle in December 1999. Two were placed on the bottom, at the south ($\approx$ 5 m depth) and north ($\approx$ 6 m depth) ends respectively and the third was attached to the balancing pipe ($\approx$ 0.5 m depth) at the north of the pond (Figure 3.3). The loggers recorded the mean water temperature every 1-h intervals. At the beginning of March 2000, the probes were

recovered in order to check their condition and then replaced at the same spots after a week. The surface one was taken out in December 2000, the bottom at the west end in January 2001 and the third in February 2001. The temperature loggers where placed back in the pond at July and finally recovered at October 2001. This time they were suspended from a line very close to site 5 (Figure 3.5) where the standard depth profile survey took place. The first one was placed directly above the sediment, the second 1.5 m above and the third, 1.5 m above the second one (≈ 2 m below the water surface).

2.5 Water analysis

2.51 Introduction

All water samples were collected in polypropylene bottles, with the lid closed at the sampling site and were returned to the laboratory on ice in a cool box. Water samples for filtered nutrient analysis were passed through pre-washed (300 mL deionised water passed through the filter directly before use), 47-mm diameter, cellulose acetate membrane filters of 0.45 μm nominal pore size, held in an autoclaveable Nalgene filter tower, under a filtration pressure of < 300 mm Hg. Analysis of samples, with the exception of filtrable total and total N that were stored at -20 °C for up to two months, took place also on the day of sampling. Reagents were made fresh on the day of analysis from stock solutions (see below). The stock solutions were renewed every three months or if notable changes in solutions occurred or otherwise stated. A calibration graph was prepared using a dilution series from the standard solutions, and the mean factor relating concentration to absorbency was determined for the concentration range of interest. Samples with absorbance beyond the linear range of the spectrophotometer (>0.7, Shimadzu UV-150-02 double beam, Turner Designs 10-AU), were re-analysed after dilution with deionised water. Blanks were deionised water and standards were diluted from 1000 mg L^{-1} stock solutions. Detection limits were calculated as three times the standard deviation of the blank sample. Results are expressed as the element, not the ion.

2.52 Nitrogen

The following N fractions were determined directly: nitrate, nitrite, ammonium, filtrable total N and total N. Total inorganic, organic and particulate N were calculated from these direct measurements (Table 2.1). Potassium nitrate (for NO_3-N), sodium nitrite (for NO_2-N) and ammonium chloride (for NH_4-N), supplied by BDH were used for the standard solutions. Stock solutions and reagents are presented in Tables 2.2 and 2.3.

NO_3-N In order to analyse water samples for nitrate the first step was to convert the entire nitrate to nitrite by passing the sample through a cadmium column as described by Stainton *et al.* (1977). The column was packed with freshly activated cadmium with an average grain diameter of approximately 0.5 mm. The cadmium grains were stored in 2 N HCl and activated by rinsing in deionised water, followed by swirling in 2% w/v $CuSO_4$ $5H_2O$. Then, 5 mL of buffer solution, 25 mL of deionised water and 25 mL of filtered sample were mixed and passed through the column approximately 7 mL min^{-1}. The last 25 mL were retained for analysis. Reagents were then added to analyse for nitrite (see below). The amount of nitrate was calculated as the difference between reduced nitrate-nitrite and sample nitrite.

NO_2-N Nitrite may be acidified to nitrous acid, which couples with the addition of two amines to form a pink azo dye. 25 mL of filtered sample, 2.5 mL of buffer and 0.5 mL of sulphanilamide were added to a 100-mL Erlenmeyer flask. After 5 min, 0.5 mL of NNED were added and left for a minimum of 10 min for the colour to develop. The sample was mixed after the addition of each reagent. The sample was read at 543 nm using a spectrophotometer, and was stable for 2 h. Colour development obeys Beer's Law and is linear to within the operating limits of the spectrophotometer (0.0-0.7 absorbance values). The applicable range of this method is 0.1 to 500 $\mu g\ L^{-1}$ NO_2-N. The detection limit was 2 $\mu g\ L^{-1}$ NO_2-N.

NH_4-N Ammonium was determined spectrophotometrically by the indophenol blue method of Solórzano (1969) as described by Stainton *et al.* (1977). Under alkaline conditions, ammonium forms an indophenol blue colour with phenol and hyperchlorite. 1 mL of phenol reagent were added to 25 mL filtered sample, followed by 1 mL nitroprusside and 2.5 mL of oxidising reagent in a fume cupboard. The sample was mixed after the addition of each reagent. The colour was read after a minimum of 1 hour using a spectrophotometer at a wavelength of 640 nm. The colour was stable for up to 24 h. The detection limit was 3 $\mu g\ L^{-1}$ NH_4-N.

TN and FTN Nitrogenous compounds were oxidised to nitrate by heating with an alkaline persulphate solution under pressure. The filtered (for FTN) and unfiltered (for TN) water samples were digested broadly following the alkaline persulphate oxidation method of Johnes & Heathwaite (1992). This method allows for the simultaneous determination of N and P, including dissolved and particulate fractions. However, in this study, it was only used for the determination of FTN and TN. 5 mL of oxidising reagent (Table 2.3) were added to 25 mL of sample in 60 mL autoclavable

polypropylene bottles. The bottles with their screw caps loosened (to prevent implosion), were then autoclaved at 750 mm Hg for 90 min at 120 °C. FTN and TN were then determined as NO_3-N by reduction to nitrite with cadmium, using a SAN^{plus} segmented flow analyser (Section 2.93). The detection limit was 5 μg L^{-1} NO_3-N.

Table 2.1 Nitrogen speciation

Name	Acronym	Filtration	Digestion	N Species
Nitrate	NO_3-N	0.45 μm	None	NO_3
Nitrite	NO_2-N	0.45 μm	None	NO_2^-
Ammonium	NH_4-N	0.45 μm	None	NH_4^+
Total inorganic N	TIN	0.45 μm	None	$NO_3^- + NO_2^- + NH_4^+$
Filtrable total N	FTN	0.45 μm	Alkaline persulphate	dissolved inorganic and organic N
Filtrable organic N	FON	0.45 μm	None	FTN – TIN (< 0.45 μm)
Particulate N	PN	None	None	TN – FTN (> 0.45 μm)
Total N	TN	None	Alkaline persulphate	all N species

Table 2.2 Stock solutions and reagents for analysis of inorganic nitrogen fractions: NO_3-N, NO_2-N and NH_4-N.

Stock solutions	Reagents
Alkaline stock Dissolve 100 g sodium citrate and 5 g NaOH in 250 mL deionised water. Leave to cool and dilute to 500 mL. Store for up to 3 months.	Oxidising Mix 400 mL alkaline stock with 100 mL sodium hypochlorite (12% w/v available chlorine).
Buffer Dissolve 100 g ammonium chloride, 20 g sodium tetraborate and 1 g EDTA into 500 mL deionised water and dilute to 1000 mL. Store for up to 1 month.	Phenol Dissolve 100 g phenol in 1000 mL of 95% ethanol.
Sulphanilamide Dissolve 5 g sulphanilamide in 200 mL of deionised water. Add 100 mL 11.63 M HCL and dilute to 500 mL. Store for up to 1 month.	Sodium nitroprusside Dissolve 1 g sodium nitroprusside in deionised water and dilute to 200 mL.
NNED (N-1-napthylethlenediame dihydrochloride) Dissolve 0.5 g NNED in 500 mL deionised water. Store for up to 1 month in a low actinic glass bottle.	

Table 2.3 Stock solutions and reagents for analysis of organic and particulate N fractions: FTN and TN.

Stock solution	Reagent
NaOH stock Dissolve 150 g NaOH to 500 mL deionised water. Leave to cool and dilute to 1 L. Final concentration 3.75 M NaOH.	Oxidising Add 15 mL NaOH stock to 50 mL deionised water. Dissolve 5 g $K_2S_2O_8$ (potassium persulphate) (gently heat to <40 °C if necessary) and dilute to 100 mL. Reagent was prepared fresh as required.

2.53 Phosphorus

Analysis for P was done following the ascorbic acid method of Eisenreich (1975) after Murphy & Riley (1962). The principle is that ammonium molybdate and antimony potassium tartrate in acid medium react with P to form a heteropoly phosphomolybdic acid complex, which is reduced to intensely coloured molybdenum blue by ascorbic acid. This colour is read spectrophotometrically at 882 nm and is directly proportional to the amount of P present in the water sample. Prior treatment by filtration and/or acid digestion partitions P into distinct fractions, which are estimates of chemical species (Table 2.4). Filtrable reactive P (FRP), filtrable total P (FTP) and total P (TP) were determined directly. Two other fractions, organic P (FOP) and particulate P (PP) were calculated from these determinants. Working standard solutions were prepared by dilution of the stock standard in the range of 1 to 200 $\mu g\ L^{-1}$ for calibration. Stock standard solution (1000 mg L^{-1} PO_4-P) was prepared from anhydrous potassium dihydrogen phosphate (KH_2PO_4), supplied by BDH. Blanks and standards were subject to the same treatment as the water samples. The detection limit was 1 $\mu g\ L^{-1}$ PO_4-P for all fractions. The use of standard additions of a range of 5 to 1000 $\mu g\ L^{-1}$ PO_4-P to the pond and borehole water did not indicate any positive or negative interference. Stock solutions and reagents are presented in Table 2.5.

Table 2.4 Phosphorus speciation

Name	Acronym	Filtration	Digestion	P Species
Filtrable reactive P	FRP	0.45 µm	None	Orthophosphate and colloidal (< 0.45 µm) P
Filtrable total P	FTP	0.45 µm	Acid persulphate	Orthophosphate, colloidal and organic P
Organic P	FOP	0.45 µm	Acid persulphate	FTP - FRP
Particulate P	PP	None	Acid persulphate	TP - FTP
Total P	TP	None	Acid persulphate	all P species

For the analysis of FTP and TP, 5 mL of digestion reagent (Table 2.5) were added to 25 mL samples (filtered or unfiltered) in 100 mL Erlenmeyer flasks. The flasks were covered with aluminium foil and autoclaved at 750 mm Hg for 60 min at 120 °C. The flasks were cooled to room temperature and 5 mL of mixed reagent I added. To determine FRP, 5 mL of mixed reagent II were added to 25 mL samples of filtered water. The blue colour developed and was read after 20 min, on a spectrophotometer at 882 nm, using 10 cm cuvettes. The colour was stable for approximately 2 h.

Table 2.5 Stock solutions and reagents for the analysis of phosphorus fractions: FRP, FTP and TP.

Stock solutions	Reagents
Digestion acid Mix 100 mL H_2SO_4 with 500 mL deionised water, leave to cool, then make up to 1000 mL. Final concentration 1.8 M H_2SO_4. Store in the dark.	Digestion Reagent Add 10 mL digestion acid to 50 mL deionised water. Then, dissolve 6 g potassium persulphate and dilute to 100 mL.
Antimony stock Mix 53.3 mL H_2SO_4 with 500 mL deionised water and leave to cool. Dissolve 0.748 g $K(SbO)C_4H_4O_6$ $\frac{1}{2}H_20$ (antimony potassium tartrate) and dilute to 1000 mL. Filter any precipitate. Store in the dark for up to 3 months at 4 °C.	Mixed reagent I Add 25 mL antimony stock to 25 mL molybdate stock. Then, dissolve 0.2 g ascorbic acid and dilute to 100 mL.
Molybdate stock Dissolve 10.839 g of Na_2MoO_4 $2H_20$ in 500 mL of deionised water and dilute to 1000 mL. Store in the dark for up to 3 months at 4 °C.	Mixed reagent II Mix 25 mL antimony stock, 10 mL digestion acid and 25 mL molybdate stock. Then, dissolve 0.2 g ascorbic acid and dilute to 100 mL.

2.54 Dissolved oxygen

For samples that it was difficult to use the oxygen probe (like the depth profile studies), dissolved oxygen was measured by the azide modification of Winkler's titration method (APHA *et al.*, 1992). 250 mL glass bottles were used, which were filled and stoppered underwater. Analysis took place as soon as the water samples were returned to the laboratory. Two syringe needles were inserted; one to inject the reagents into the bottle; and the other to act as a release pressure valve. After the addition of each reagent the syringes were removed and the bottle was shaken. Finally, 200-mL sample aliquots were titrated with 0.025 M $Na_2S_2O_3$ to a pale straw colour.

2.55 Total alkalinity

Total alkalinity was measured according to the APHA *et al.* (1992) manual. 200 ml of filtered water were used and titrated with 0.1 N HCl at an end-point pH of 4.5 (mostly used for any routine analyses and in the presence of silicates and phosphates). A titration curve was prepared for the standardisation of reagents.

2.56 Silica

Silicate-Si was measured according to the APHA *et al.* (1992) manual with the molybdosilicate method. Ammonium molybdate at pH approximately 1.2 reacts with silica and any phosphate present to produce heteropoly acids. Oxalic acid is then added to destroy the molybdophosphoric acid but not the molybdosilicic acid and the intensity of the yellow colour, which is proportional to the concentration of molybdate-reactive silica and is then measured spectrophotometrically at 410 nm.

2.57 Suspended chlorophyll *a* in water

Chlorophyll *a* concentration of pond water was determined using overnight extraction with cold methanol (Marker, 1995). A measured volume of sample, usually 1 L for Double Kettle and 2 L for Croft Kettle, was filtered through a GF/C glass-fibre filter (47-mm diameter) using a slight vacuum (< 300 mm Hg) to avoid cell damage. The filter paper was left to slightly dry and was folded three times. This was then placed in a snap cap vial, which was previously filled with 20 ml of 100 % methanol (sufficient quantity to cover the filter paper), and the cap sealed. The vial was wrapped with aluminium foil to ensure no light in order to avoid degradation and was left at about 18 to 24 h at 4 °C in the dark. Water was then accurately added to make the solvent up to 90 %. The sample was well mixed using a vortex stirrer and the filter paper removed, squeezing as much of the solvent back into the vial as possible. The solvent was centrifuged in a falcon tube for 10 minutes at 3000 *g*. The clear extract was measured in a Shimadzu UV-150-02 double beam spectrophotometer at 665 nm with a reading at 750 nm to correct for turbidity. A blank of 90 % v/v methanol/water was used in the reference beam of the spectrophotometer. To correct for phaeopigments, 0.1 mL of 0.3 M HCl were added to 10 mL extract, which was then mixed with a glass rod and left for 5 to 10 min. The extract was then neutralised and mixed with the same amount of organic base, comprising 3 mL of 2-phenythylamine made up to 100 mL with 100 % methanol. The extract was then read at 665 nm and 750 nm. To calculate, the corrected, undegraded chlorophyll *a* concentration the following equation was used:

$$\textit{Chlorophyll}\ \text{a concentration}\ (\mu g\ L^{-1}) = \frac{13\,(2.667\ x\ (A_n - A_m))\ x\ v}{d\ x\ V}$$

Where: A_n = pre-acidified 665 reading – pre-acidified 750 reading,
A_m = post-acidified 665 reading - post-acidified 750 reading,
v = volume of extract (ml), d = cell pathlength (cm)
V = volume of sample filtered (L)

2.6 *Chara hispida* studies

2.61 Introduction

C. hispida plants were collected from Croft Kettle at site 2 (Figure 3.5) at 1.5 m depth by scuba diving and from a shallow area (≈30 cm water depth) of Double Kettle from site 1. Several shoots of *C. hispida* were taken from each site on each sampling occasion of the standard scuba diving survey and were transported back in the laboratory in plastic bags on ice in a cool box. *C. hispida* samples were analysed and/or prepared for long-term storage within 24 h of collection. Phosphatase activity was determined broadly following the method of Christmas & Whitton (1998). Nutrient content was determined on a Scalar Autoanalyser after digestion with hydrogen peroxide (H_2O_2) and H_2SO_4 of the dried (105 °C) plant material (Section 2.8). Studies of phosphatase activity and nutrient content were made on terminal (≈2 cm) tips or otherwise stated. This was considered most likely to reflect the recent ambient chemical conditions and in addition, the youngest parts have fewer epiphytes (Andrews *et al.*, 1984a). As it was required to establish the features of *C. hispida* itself rather than the whole community, it was important to obtain material as free of epiphytes as possible. Fry *et al.* (1985) showed that it is possible to remove the majority of the epiphytic population of aquatic vascular plants using a stomacher. In an attempt to follow this method manually, deionised water was added to a sterile plastic bag with 7-10 tips of *C. hispida* and was then gently shaken for approximately two minutes. The water was then filtered through a 0.45 μm GF/C filter, replaced with fresh water and the procedure repeated 2-3 times. The material that was collected on the filter was then carefully scraped and was also analysed for surface phosphatase activity and represented the activity of epiphytes.

2.62 Marl removal

Deposition of $CaCO_3$ (mostly) and $MgCO_3$ on shoots of *C. hispida*, is a common consequence of photosynthesis in hardwaters and undoubtedly contributes to the fresh and dry weight of the plants (Section 1.7). In order to elucidate, the effect that calcification had on the determination of dry weight, *C. hispida* shoots were collected monthly and the marl content was measured. Four to seven tips were washed (as above), blotted on tissue paper, placed into aluminium envelopes and left to dry for a minimum of 24 h in a 105 °C oven. Each individual shoot was weighed to 0.0001 g on a microbalance and the dry weight including marl deposits was determined. Then, each individual *C. hispida* tip was placed into a 40 mL weak HCl acid solution (2%) in a stoppered polypropylene tube and was swirled for 30 sec. The tips were immediately removed from the acid solution, blotted on tissue paper, placed into aluminium envelopes and left to dry for a minimum of 24 h in a 105 °C oven. The dry weight of each individual shoot was estimated again and the difference between the dry weight before and after removal of marl content represented the marl deposition on each tip. The results are expressed as % d.wt $CaCO_3$.

2.63 Phosphatase activity

2.631 Assay medium

Assays were performed in an artificial freshwater medium (Table 2.6) modified from CHU's number 10D medium (Chu, 1942). The modifications to the medium included; halving the original EDTA concentration and removing P and N as they inhibit phosphatase activity (Grainger *et al.*, 1989). The assay medium was obtained by dilution from stock solutions, renewed every 3 months.

Table 2.6 Chemical composition of assay medium.

Chemicals	Conc (mg L^{-1})	Chemicals	Conc (mg L^{-1})
$CaCl_2 .2H_20$	35.830	$ZnSO_4 .7H_20$	0.056
$MgS0_4 .7H_20$	25.000	$MnCl_2 .4H_20$	0.045
$NaHCO_3$	15.850	$NiSO_4 .7H_20$	0.038
KCl	4.280	$CuSO_4 .5H_20$	0.020
$Na_2EDTA .2H_20$	1.670	$CoSO_4 .7H_20$	0.010
$FeCl_3 .6H_20$	1.210	$Na_2Mo0_4 .2H_20$	0.007
H_3B0_3	0.715		

2.632 Substrates

Phosphomonoesterase (PMEase) and phosphosdiesterase (PDEase) were measured using analogue substrates supplied by Sigma Co., U.S.A. PMEase was measured using

two substrates; methylumbelliferyl phosphate (MUP) and paranitrophenyl phosphate disodium salt (*p*NPP). PDEase was measured using bis-paranitrophenyl phosphate sodium salt (bis-*p*NPP).

2.633 Buffers

All chemicals used in the buffer solutions were supplied by Sigma Co., U.S.A. Buffers were dissolved in assay medium and the pH adjusted with 5 M NaOH, the final concentration of the buffer was 50 mM.

Table 2.7 The pH range of buffers used in phosphatase assays.

Buffer	pH range
3, 3-dimethyl-glutaric acid (DMG)	3.0 - 6.5
N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (HEPES)	7.0 - 8.0
Glycine	8.5 – 10.0

2.634 Phosphatase assay

C. hispida tips and epiphytic material were assayed in sterile snap-cap vials containing a final volume of 6.0 ml buffered solution. The vials were placed in a shaking water bath (*ca* 100 strokes min^{-1}) at 25 °C and left for 20 min to equilibrate. The assays were carried out using 5.8 mL of buffered assay medium and 0.2 mL analogue substrate (*p*NPP, bis-*p*NPP and MUP) to give a final concentration of 100 μM. The assay was terminated after 40 min, which was found to be the time on average to give high enough activity for detection without the need for dilution. Termination involved transferring a 2.5 mL assay aliquot to 0.25 mL of terminator in a test tube and mixed. The terminator consisted of 2.5 mM EDTA, 50 mM K_2HPO_4 and 50 mM NaOH. The terminator not only halted the reaction by raising the pH, but also maximised the fluorescence or colour development (Christmas & Whitton, 1998). Fluorescence of MU was read on a Perkin Elmer LS – 3B fluorescence spectrophotometer at 444 nm (emission) under an extinction of 356 nm. The colorimetric products *p*NP and bis-*p*NP were read at 405 nm on a Shimadzu double-beam spectrophotometer, UV-150-02 (Turner Designs 10-AU). Allowance for non-enzymatic hydrolysis and fluorescent impurities were made, by subtracting the absorbance of control (incubations with shoots omitted) and blank (incubations with shoots but substrate omitted) from the final measured value. Only small amounts of chemical hydrolysis were detected for all substrates used. The activity was determined using calibration curves constructed from *p*NP, bis-*p*NP and MU (product) standards in assay medium.

Following analysis, the *C. hispida* tips were removed from the vials and blotted dry on tissue paper. In case of the epiphytes, the assay medium was firstly filtered through pre-weighted 0.45 μm GF/C filters and then the filters were blotted dry. The material was left overnight at room temperature to air dry. The shoots and filters were then placed into aluminium foil envelopes and dried at 105 °C for a minimum of 24 h. The envelopes were then placed in a desiccator and left to cool. The shoots and filters were weighed to 0.0001 g on a microbalance. Enzyme activity was expressed as μmol product released g^{-1} d.wt h^{-1}. Four replicates plus one control and one blank were used for each site and substrate. The detection limits for *p*NPP and bis-*p*NPP were 2 μmol product g^{-1} d.wt h^{-1} and for MUP, 3 μmol MU g^{-1} d.wt h^{-1}. The maximum rate of P*i* uptake (apparent Vmax) and the affinity (apparent Km) were calculated by fitting the experimental data to the Michaelis-Menten hyperbolic function, using the Sigma Plot computer program.

2.64 Analysis of whole *Chara hispida* plants

In September 2000, whole *C. hispida* plants were removed and cut into 10-15 cm sections. All the sections were dried at 105 °C and stored for subsequent analysis of marl and elemental content to assess the distribution of these elements throughout the shoot. As described later, *C. hispida* grew well in Croft Kettle in 1999 and 2000 and the number of sampled shoots was a negligible part of the ecosystem.

2.65 Laboratory experiments

2.651 Introduction

C. hispida apical shoots (20-30 cm) were sampled from a depth of 1.5 m from Croft Kettle, site 2 (Figure 3.5) on 12 August 2001. They were immediately transported to the laboratory in an icebox container filled with pond water. The shoots were thoroughly washed to remove epiphytes. Clean and healthy looking stems, measuring 10-14 cm in length, cut directly below a node, were selected from the freshly collected field material and marked with a different colour thread. The fresh weight (superficial moisture was removed with a paper towel) and length (to the nearest mm) of all shoots were measured as well as the length between each node and side branches. The shoots were then placed into flasks (according to the design of the experiments, see below) and the flasks were closed with a thin layer of cotton wool, tight with an elastic band. Fourteen shoots were also frozen at –80 °C on the day of collection, after dipping them in a container filled with liquid N for 5 min to stop any enzymatic activity and acted as controls.

In addition, about 50 L of pond water from Croft Kettle were also collected on the same day and filtered through 0.45 GF/C filters. The ambient characteristics of the sampled pond water were: 15.6 °C, 3.38 mS cm^{-1}, 7.91 (pH), 7.6 μg L^{-1} (FTP) and 52.5 μg L^{-1} (FTN). Pond water was used for these experiments since artificial lake water or different media can sometimes be deficient in some factor present in the water (Wetzel & McGregor, 1968).

At the end of the experiments, the final fresh weight and the length of each node and branch were measured (including newly formed side branches). The shoots were then dipped to liquid nitrogen and kept at -80 °C until day of analysis. A number of determinads for each shoot were calculated including N&P content, chlorophyll *a* and pigment composition.

2.652 Effect of temperature on *Chara hispida* shoots

2000-mL Erlenmeyer flasks were filled with 1500 mL of filtered pond water and five shoots of *C. hispida* were placed into each flask. The flasks were kept under constant experimental conditions in four growth chambers under 16 h light (40-45 μmol m^{-2} s^{-1}) and 8 h dark regime and were shaken every day. Each chamber was set up at 12, 15, 18 and 21 °C respectively and four flasks were placed into each chamber. Overall, 80 shoots (16 flasks) of *C. hispida* were studied under a series of temperatures. One shoot was removed from each flask (every fortnight) at these dates: 29 August, 14 and 28 September, 15 and 30 October.

2.653 Effect of phosphorus enrichment on *Chara hispida* shoots

1000-mL Erlenmeyer flasks were filled with 700 mL of filtered pond water. They were kept under constant experimental conditions in a growth room; at 15 °C under 16 h light (40-45 μmol m^{-2} s^{-1}) and 8 h dark regime and were also shaken on a daily basis. Two shoots of *C. hispida* were placed in each flask. P*i* was added as Na_2HPO_4 in the Croft Kettle water, resulting in a series of concentrations: 2, 4, 6, 8, 10, 15, 20, 30, 40, 50, 75, 100, 150, 200, 300, 400, 500, 750, 1000, 1500 and 2000 μg L^{-1} PO_4-P. Overall, 42 shoots (21 flasks) of *C. hispida* were studied under a series of P*i* concentrations. After 31 days (12 September), the shoots were removed from the flasks.

2.7 Sediments and cores

2.71 Phosphorus sequential fractionation

Surficial sediments (0-10 cm) were collected monthly by diving, between February of 2000 and 2001. Coring positions are given in Figure 3.5. Seven plexiglass tubes (3.5 cm inner diameter; 18 cm length) were filled on each occasion and the best (intact) four were treated within 48 h of sampling. These tubes were pushed in by hand, capped on top and bottom, transferred back to the laboratory within a pre-chilled cool box and placed in a dark room at 4 °C. On the day of analysis, overlying water, which was 2-3 cm deep, was drained. Cylindrical slices of sediment (0-2, 2-4, 4-7, 7-10 cm) were extruded from the tubes, passed through a 0.2 mm sieve to remove larger particles and transferred into Nalgene, polypropylene, 50-mL centrifuge tubes. Deionised water (5-10 mL) was added to the sediment in order to ensure that the final suspension had the same viscosity and was well mixed. Then, 2 g wet sediment was subsampled and placed into new, Nalgene, polypropylene, 50-mL centrifuge tubes (n=16) for extraction. In addition, three 2 g subsamples were also taken from each sediment section, placed overnight at 105 °C in an oven to dry and the percentage of dry matter from loss in weight was calculated in order to be able to express the results on a dry weight basis.

Phosphorus fractions were estimated using the sequential extraction scheme (Table 2.9) developed by Psenner *et al.* (1984) and Psenner & Pusko (1988). The procedure consists of an initial extraction with deionised water, followed by the treatment with 0.11 M buffered (pH=7) sodium bicarbonate/dithionite (BD) solution, a subsequent extraction with 1 M NaOH, a 0.5 M HCl extraction and a hot extraction with 1 M NaOH. In step one, 50 mL of deionised water were added into each tube. The tubes were placed in a shaking water bath (*ca* 100 strokes min^{-1}) at 25 °C and were shaken for 15 min. The tubes were then centrifuged (15 min at 3000 *g*) and the supernatant carefully decanted from the pellet. In step two, 25 mL BD-reagent were added to the sediment pellet. After 30 min shaking (as above) at 40 °C, the supernatant was collected and another 25 mL BD-reagent were used to wash the sample and extract reabsorbed P*i*. After the second centrifugation the wash solution was added to the first supernatant. In step three, an 16 hour extraction (shaking) with 25 mL of 1 M NaOH was followed by one NaOH wash. In step four, a 0.5 M HCl solution was used. This extraction was also followed by an HCl wash. In the last step, the sediment pellet was incubated with 1 M NaOH at 85 °C for 24 h followed by a NaOH wash.

Following centrifugation, the supernatants of each extraction were filtered through 0.45-μm membrane filters. Phosphate was then determined following the ascorbic acid method of Eisenreich *et al.* (1975, Section 2.52). However, in order to avoid possible interferences from Fe and S (inhibition of the formation of the blue coloured complex and precipitation of the phosphomolybdic acid complex), the extracts were diluted. A series of dilutions were tested and was determined that no dilution for the water extracts was required, x25 for HCl and NaOH extracts and x50 dilution for the BD extracts were sufficient for further P analysis, which followed directly after the end of each extraction. The FRP and FTP fractions were determined for each sediment sample and extraction. Furthermore, in order to test the reproducibility and accuracy of the results four samples of 'control' sediment were analysed alongside, on each occasion, following the same protocol. Five of the initial cores of surface sediment (0-10cm) were mixed together and left to dry at 40 °C. This was achieved by placing the sediment into an oven at 40 °C and dry to constant weight (successive weightings did not differ by more than 1 to 2 mg). The sediment was then left to cool in a desiccator. The sediments were then homogenised and subsamples of 1 g d.wt were used and acted as 'controls'.

Table 2.8 Phosphorus sequential fractionation scheme

Extractable P fraction	Extractant	Reagents		Temp (°C)	Time (h)
Readily available P (water soluble P)	H_20	Deionised water		25	0.25
Reductant soluble P (i.e. Fe-P, Mn-P)	BD	Na-bicarbonate ($NaHCO_3$)	0.11 M	40	0.5
		Na-dithionite ($Na_2S_2O_4$)	0.11 M		
P bound to Al and humic substances	NaOH	NaOH	1 M	25	16
Acid-soluble P (Ca bound P)	HCl	HCl	0.5 M	25	16
Residual P (refractory P)	Hot-NaOH	NaOH	1 M	85	24

2.72 Deep cores

Two full-length sediment cores have been obtained from Croft Kettle, in addition to the small cores taken for nutrient analysis. The second core, used for most of the work was obtained on 12 March 1999; its location is shown in Figure 3.5. A raft was placed in the middle of Croft Kettle and was secured with cross ropes. The depth from the deck to the sediment interface was 5.7 m. A 9.82-m core was obtained, together with

two 40-cm cores of the upper sediments in Perspex tubes. The long core was used in order to assess historical changes of trophic status and land use by looking on diatom (Appendix 1), pollen distribution (Appendix 2) and dating (Appendix 3), while one of the sediment cores in Perspex tubes, which was sliced on 1-cm intervals, was analysed for total N and P.

2.8 Nutrient analysis of plants and sediments

2.81 Introduction

The total N and P content of plant and sediment samples was measured using the digestion technique developed by Houba *et al.* (1989) which can be applied for the determination of Ca, Mg, K, Na, N-total and P-total in both plant tissue and sediment material. Briefly, the material was immersed in a mixture of sulphuric acid, selenium, potassium sulphate and salicylic acid (Table 2.9) overnight before digestion. The salicylic acid formed compounds with NO_3-N present, preventing losses of NO_3-N during the digestion step. Actual digestion was then started with hydrogen peroxide, which oxidised the larger part of the organic matter. Heating decomposed excess H_2O_2 and digestion was completed by concentrated sulphuric acid at elevated temperature with selenium and potassium sulphate acting as a catalyst.

During all analyses, unknown samples were run alongside known standards. These standards included certified reference material of *Chlorella*, pond sediment, pepper bush leaf and tea leaf supplied by the National Institute for Environmental Studies, Japan (NIES) and Community Bureau of Reference, EC. Total N (as NH_4-N) and P (as PO_4-P) were quantified using a SAN^{plus} segmented flow analyser (Skalar Analytical, Breda, The Netherlands). Ca, Mg, Zn, Na, Fe, Cu and Mn were quantified using a Perkin Elmer Optima 3300RL ICP Emission Spectrometer with samples run using a Perkin Elmer AS90 autosampler.

2.82 Digestion

Oven dried material (105 °C, 48 h) was weighed (20 $\pm$ 5 mg) and put into digestion tubes with 2 mL digestion reagent (Table 2.9), ensuring that all material was fully immersed. The tubes were left to stand overnight in a fume cupboard and were then heated for 2 h at 100 °C in a Skalar 5620/40 digestion block (5600 controller). The tubes where then removed and left to cool. Two mL of hydrogen peroxide were then added to each tube, which was heated at 330°C, until the rigorous reaction ceased. The

tubes were removed from the digester and allowed to cool before repeating the process a further two times, or more (until clarification), then the mixture was heated at 330°C for a further 2 h. Once cooled, the clear digest was diluted to 500 mL in deionised water and TN (as NH_4-N) and TP (as PO_4-P) was quantified using the automatic segmented flow analyser (ASFA).

Table 2.9 Reagents for digestion procedure of total N and P content of plant and sediment material.

Reagents	Chemicals		Procedure
Digestion mixture	Sulphuric acid (H_2SO_4)	1 L	Mix Kjeldahl powder with sulphuric acid and stir the solution between 3 to 6 h at 300 °C until the mixture turns colourless
	Kjeldahl powder Containing:	3.5 g	
	Potassium sulphate (K_2SO_4)	3.48 g	
	Selenium (Se)	0.02 g	
Digestion reagent	Salicylic acid (HOC_6H_4COOH)	10.8 g	Dissolve the salicylic acid to the digestion mixture and mix. Solution stable for 24 h
	Digestion mixture	150 mL	
Hydrogen Peroxide	Hydrogen peroxide (30%, H_2O_2)		

2.83 Automated analysis of nitrogen and phosphorus

The ASFA was set with lines for NO_3/NO_2, NH_4 and PO_4 according to Skalar's users manual (1995). Automated segmented flow analysis is a continuous flow method of chemical analysis in which streams of reagents and samples, segmented with air bubbles, to eliminate cross contamination, are pumped through a manifold to undergo treatment such as heating and mixing before entering a flow cell where absorbance is measured spectrophotometrically. The chemical analysis of nitrate and phosphate followed the approximately the same principles as the manual methods for water analysis (Section 2.5). Ammonia was chlorinated to monochloramine, which reacted with salicylate to 5-aminosalicylate. After oxidation and oxidative coupling a green coloured complex was formed. The reaction was catalysed by nitroprusside, and dichloroisocyanurate was used for chlorine donation. The colour of the formed complex was measured at a wavelength of 660 nm. Spectrophotometric absorbance was converted by the San^{plus} software version 6.2 to ppm (mg L^{-1}). For calibration, a set of five standards, of the same chemical matrix as the samples, were also analysed. If the samples could not be analysed immediately, they were kept at -18 °C for up to 2 months. All reagents were made as specified in Tables 2.10-2.12.

Table 2.10 Reagents for automatic analysis of nitrate and nitrite.

Reagent	Chemicals		Procedure
Buffer solution	NH_4Cl	50 g	Dissolve ammonium chloride in ± 800 mL deionised water. Adjust pH to 8.2 with ammonia solution. Fill to 1 L with deionised water, add Brij 35 and mix.
	Ammonia solution (25%) (NH_4OH)	± 1 mL	
	Deionised water	1000 mL	
	Brij 35 (30%) (polyoxyethylene lauryl ether – non-ionic detergent)	3 mL	Solution is stable for 1 week, stored at 4°C when not in use.
Colour reagent	o-Phosphoric acid (85%) (H_3PO_4)	150 mL	Dilute o-phosphoric acid to ± 700 mL deionised water. Add sulphanilamide and α-naphthtylethylene diamine dihydrochloride and dissolve. Fill to 1 L with deionised water and mix.
	Sulphanilamide ($C_6H_8N_2O_2S$)	10 g	
	α-Naphthylethylene di-amine dihydrochloride ($C_{12}H_{16}Cl_2N_2$)	0.5 g	Solution is stable for 1 week, stored in a dark coloured bottle at 4°C, when not in use
	Deionised water	850 mL	
Rinsing liquid	Deionised water / digested deionised water		Matrix of the rinsing liquid is kept the same as that of the sample

Table 2.11 Reagents for automatic analysis of phosphorus.

Reagent	Chemicals		Procedure
Ammonium molybdate solution	H_2SO_4 (97%)	40 mL	Dissolve sulphuric acid in ± 800 mL deionised water. Add ammonium molybdate and dissolve. Fill to 1 L with deionised water, add FFD6 and mix.
	Ammonium molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$)	4.8 g	
	Deionised water	1000 mL	
	FFD6	2 mL	Solution is stable for 1 week, stored at 4°C when not in use.
Potassium antimony tartrate stock	Potassium antimony tartrate ($K(SbO)C_4H_4O_6.0.5H_2O$)	300 mg	Dissolve potassium antimony tartrate in ± 80 mL deionised water. Fill to 100 mL with deionised water and mix.
	Deionised water	100 mL	Solution is stable for 1 month, stored at 4°C when not in use.
Ascorbic acid solution	Ascorbic acid ($C_6H_8O_6$)	4.5 g	Dissolve ascorbic acid in ± 200 mL deionised water. Add stock solution potassium antimony tartrate. Fill to 250 mL with deionised water and mix.
	Stock solution potassium antimony tartrate	5 mL	
	Deionised water	1000 mL	Solution is stable for 1 week, stored at 4°C when not in use.
	FFD6	2 mL	
Rinsing liquid	Deionised water / digested deionised water		Matrix of the rinsing liquid was kept the same as that of the sample.

Table 2.12 Reagents for automatic analysis of ammonia.

Reagent	Chemicals	Procedure
Buffer solution	Potassium sodium tartrate 33 g ($C_4H_4O_6KNa.4H_2O$) Sodium citrate 24 g ($C_6H_5O_7Na_3.2H_2O$) Deionised water 1000 mL Brij 35 (30%) 3 mL	Dissolve potassium sodium tartrate in ± 800 mL deionised water. Add sodium citrate and dissolve. Fill to 1 L with deionised water, add Brij 35 and mix. Check pH and adjust if necessary with hydrochloric acid to 5.2 ± 0.1. Solution is stable for 1 week, stored at 4°C when not in use.
Sodium salicylate solution	NaOH 25 g Sodium salicylate ($C_7H_5NaO_3$) 80 g Deionised water 1000 mL	Dissolve sodium hydroxide in ± 50 mL deionised water. Add ± 800 mL deionised water. Add sodium salicylate. Fill to 1 L with deionised water and mix. Store in a dark coloured bottle. Solution is stable for 1 week, stored at 4°C when not in use.
Sodium nitroprusside solution	Sodium nitroprusside 1.0 g ($Na_2[Fe(CN)_5NO].2H_2O$) Deionised water 1000 mL	Dissolve sodium nitroprusside in ± 800 ml deionised water. Fill to 1 L with deionised water and mix. Store in a dark coloured bottle. Solution is stable for 1 week, stored at 4°C when not in use.
Sodium dichloroiso-cyanurate solution	Sodium dichloroiso-cyanurate 2.0 g ($C_3N_3O_3Cl_2Na.2H_2O$) Deionised water 1000 mL	Dissolve sodium dichlorisocyanurate in ± 800 mL distilled water. Fill to 1 litre with distilled water and mix. Solution is stable for 1 week, stored at 4°C when not in use.
Rinsing liquid	Deionised water / digested deionised water	Matrix of the rinsing liquid was kept same as that of the sample

2.9 Computing and statistics

Pentium computers with Windows 98/2000 operating systems were used throughout the study period. Word processing was performed using Word 2000 and data handling using Excel 2000. Graphs were produced using Sigmaplot for Windows v6.0. Linear and non-linear regression equations and best fit lines were calculated with Sigmaplot. The SPSS.PC computer software system v.11.0 was used for statistical analysis. All data were transformed as required to meet the requirements of homogeneity of variance and normality. Analysis of variance (ANOVA) was used to detect differences, which were evaluated with Tukey's honestly significant difference (HSD) tests. Arc-view GIS, version 3.2 was used for mapping and subsequent graphic representation of the data.

CHAPTER 3 History and site description

3.1 History

Hell Kettles (NZ 281 109, Figure 3.2) is a wetland site consisting of two small calcareous ponds (kettles) of contrasting character and an area of fen between the two (Whitton & Gibbons, 1966). Surtees (1823), Longstaffe (1854) and Fordyce (1857) summarize some of the early accounts of the area known as Oxen-Le-Field (field of waters: also Oxney-Field or Oxenhale in older accounts). Hell Kettles were originally four ponds, situated in a field adjoining the highway between Darlington and Croft. Hutchinson (1794) shows the shape and size of the ponds as drawn by Grose in October 1774 (Figure 3.1).

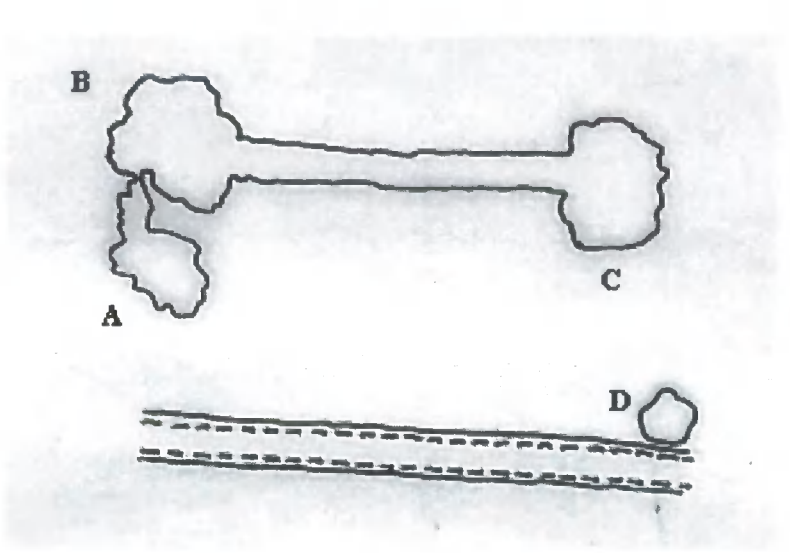

Figure 3.1 Diagrammatic representation of the ponds in 1774 (Hutchinson (1794). A and B are now the Double Kettle and C the Croft Kettle.

The approximate diameters were A and B) 34.5 m; C) 25.5 m; and depths were A) 5.9 m; B) 4.2 m; C) 5.1 m; D) 1.6 m (all values from old accounts have been converted from yards or feet to metres). Longstaffe (1854) reported that "two of them were joined together by natural means and the fourth one was dried out. Thus, presently, they are accounted as two". A record by Clayton (1894) gave a value of 7.3 m depth for one place in Croft Kettle and 6.4 m for one place in the Double Kettle. Further information from old records is summarized in Appendix 4.

Figure 3.2 Aerial view of Hell Kettles in spring 1996. Photo created by D.G. from separate aerial photos obtained by English Nature.

The ponds lie on boulder clay over a Magnesian Limestone aquifer. One of them, Croft Kettle, punctures the clay and is in direct contact with the aquifer and, as a consequence, is spring-fed. It is the only place in Co Durham where open water is directly fed by subterranean springs (Hedley, 1997). The other pond, Double Kettle, is the one formed from the coalescence of two smaller ponds. It is uncertain if it receives any direct input from the underground aquifer, but, if it does, this is only a small contribution. It addition to surface runoff and field drainage, which are highly dependent on rainfall, it has probably always been connected to Croft Kettle by seepage across the fen, by a channel (Hutchinson, 1794) or, in late 1970, by a (balancing) pipe (Wheeler & Whitton, 1971). This pipe rusted after a few years, but had been replaced by 1980.

Even though the older accounts include some highly improbable information, there are also details, which may give insight into the history of the ponds. A few are included here. The most graphic account of the origin of the ponds is that in the Chronicle attributed to Brompton, Abbot of Jerveaux, Yorkshire: "On Christmas day, 1179, a wonderful matter fell out at Oxenhale, viz. that in the land of Lord Hughe, bishop of Duresme, the ground rose up to such a height, that it was equal to the tops of the highest hills, and higher than the spires and towers of the churches, and so remained at that height from nine of the morning till sun-set. But at the setting sun the earth fell in with such a horrid crash, that all who saw that strange mound, and heard its fall, were so amazed, that for very fear many died, for the earth swallowed up that mound; and where it stood was a deep pool" (Fordyce, 1857). However, Brompton did not compile the Chronicle personally and nobody knows who did, but such a chronicle exists and is dated 1328. Supporting evidence of this event comes from Roger Hoveden, who wrote at the end of 12th and beginning of the 13th century (Longstaffe, 1854); "If due allowance is made for a little exaggeration, the story of the old chronicler may be entitled to some consideration". Lord Lyttleton observes that the account is of one pit only, whereas, when he wrote, there were four, with a long canal uniting two of them and a third joined by a short cut. However, there are none records how the pool subsequently became divided into four, if in fact at one time there was only a single pool.

How the ponds got the name Hell Kettles can only be speculated. The records of Bishop Skirlaw, who introduced a land fee in 1389, state that "certain lands called Hell are found". According to Hutchinson (1794), it is not known whether the pools took their name from the land, or the land from the pools. Another hypothesis is that the

words Hell Kettles are derived from halen (salt) and keld (spring), which are correct descriptively and etymologically; since the water in these reservoirs is strongly impregnated with several mineral salts, and is indeed a "spa" water (Manson, 1898).

Several other local legends and a few more extracts from older accounts - some decidedly dubious - are given without much comment here, though aspects are discussed later. The ponds were at one time popularly supposed to be bottomless; and the water in them was asserted to be hot, indicating their infernal origin. The ponds were also supposed to be connected with the Tees by an underground passage. Leland (1545), Camden, and others have perpetuated traditions of a duck, a goose and a cow, passing from these pool to the Tees. According to Bousfield (1881), "in a rich pasture, usually full of sleek fat cattle, are the deep pits called Hell Kettles (Bousfield, 1881)". Manson (1898) reported that the Kettles are in fact springs, surrounded by a thick growth of aquatic plants-sedges and rushes. The remaining comments in this paragraph all come from his account. Surface channels join three of these ponds and the water is carried away by a stell or streamlet, which supplies the neighbouring farms with water, and runs into the Skerne. The fourth and smallest pool is detached and close to the road. The water is drunk by the cattle and does not seem to be at all injurious to them. The waters are of a sulphurous nature, similar to those found at Croft Spa and Dinsdale, a circumstance that indicates their volcanic origin. The water of the large pool is beautifully clear around the margin, discovering a bed lined with vegetation of an exquisite green that indeed merits the appellation of a very pretty little mere, the two conjoined are darker and the smallest quite muddy.

3.2 Geology and hydrology

The ponds are situated on strata, which, in a descending order, are (Manson 1898): Alluvial clay, sand, gravel and clay; Red Sandstone; and Magnesian Limestone. The Red Limestone is a porous and shaley micaceous rock, which holds a very considerable quantity of water in its interstices (Manson, 1898). It is soft and soon weathers, and is not capable of standing much stress or pressure. Cavities of considerable size eventually form in the Magnesian Limestone due to solution and disintegration (Chilton, 1981). More detailed information on the strata comes from work on the 75-m borehole sunk near the adjacent farm by the NWA in 1990 to provide drinking water. Measurements are presented in metres and are converted from values given in feet.

Sandy Clay	0 - 2.7
Sand and Gravel	2.7 - 6.6

Boulder Clay	6.6 - 20
Marl/Sandstone bands	20 - 39
Marl	39 - 75
Magnesian Limestone	75

A number of changes have occurred in the region in recent decades, which potentially might influence the supply of water to the Croft Kettle and perhaps also the local water-table. The following summary is based largely on Hedley (1997). In 1967, a borehole was installed by the NWA near the gauging station at Broken Scar (NZ 256142), some 4.5 km to the north-west of Hell Kettles. The onset of pumping corresponded with a period of very low water in Double Kettle and a gradual decline in the outflow from the pipe supplying the farm with water from Croft Kettle. The owner of the farm (then J.R. Fell) raised concern about the reduced flows at this time (Wheeler & Whitton, 1971) and throughout the 1980s. He commented (letter of 10.8.69 to B.A.Whitton.) that the level of the Double Kettle "never varied anything like as much as it does since the winter of 1967/68".

Different (experimental) rates of pumping from Broken Scar accompanied by monitoring of the farm supply outfall for the whole of 1985, provided strong evidence that an abstraction in excess of 9×10^3 m^3 d^{-1} (2 million gallons per day) reduces throughflow in Croft Kettle, though the relationship is not straightforward, there being a delayed effect. It was suggested that it may also involve subsoil water. The NWA installed a flow gauge in 1987 to provide permanent monitoring of the outflow rate from the pipe.

The NWA installed a borehole at the nearby farm in 1990, solving the farm's water supply problem. This changes also largely solved any problem of water shortage for Croft Kettle, since the only outflow from the pond was then the balancing pipe connecting the two ponds. However, in late September 1990 doubling of abstraction at Broken Scar led to obvious falls in the levels of both ponds and cessation of borehole supply for the farm. This was presumably reversed by reducing the rate of abstraction. Other changes in the region include the installation of a borehole for Darlington Hospital. It has also been speculated that overlying strata adjacent to the site may have been punctured during work in 1996 by Yorkshire Water on the Tees-Wiske crossing, but there is no evidence of actual impact and there is no record of a sustained decline of the head of Magnesian Limestone water that would indicate such an impact.

J. Lamont-Black (pers. comm., Newcastle University) has been investigating groundwater chemistry and flow patterns in the Darlington region, in particular the dissolution of the Permian gypsum strata by groundwater. In order for dissolution to take place, four criteria must be met: evaporite status; undersaturated groundwater; energy to drive water through the system; outlet for water. Piezometric data show head differences across gypsum, with 3-4 m artesian head at the farm borehole near Hell Kettles. Groundwater in the southern part of Darlington contained higher levels of dissolved sulphate from Coal Measures derived pyrite and Permian gypsum (discernable by strontium sulphate ratios). Although Lamont-Black did not comment, presumably new boreholes encourage movement of water and hence dissolution and increased subsidence in the region. In a newspaper article, Radford (1999) reported on a talk by Tony Cooper of The British Geological Survey at the British Association meeting. A band running under Darlington and Ripon is afflicted by sudden, dramatic holes in the ground; there was a collapse near Ure Lodge, Ripon in 1834, which left a hole 20 m deep, and another in 1997, which formed a hole 6 m deep (Cooper, 1998). Subsidence damage in the region had cost £ 1.3 m in the previous 10 years. It was also suggested that the legends and facts of Hell Kettles may have inspired Lewis Carroll to write of the famous hole of Alice in Wonderland.

Recently (March 2002), Lamont-Black compared his and our chemical data on Hell Kettles (pers. comm.). As his comments cannot be summarized easily and he and others are planning publications on the Darlington region, which are expected to be in draft form by next year, most of this information is not integrated here. However, one point is especially relevant. He speculates that the closure of Mainsforth colliery and the cessation of related pumping may have led to a rise in the local water table in the Hell Kettles region; if this occurs, it would negate any effect of removal of water by pumping at Broken Scar. He also explained that the artesian head in the region increases on passing from N-W to S-E.

Two sediment cores (Section 2.83) have been obtained by B. Huntley and associates. One was taken in 1991 and the other on 12 March 1999. The first was used for an undergraduate study (Dodsworth, 1991) and has subsequently been stored at 4 °C. The second (5-m core) has been used for diatom and pollen analysis and the results are presented in Appendices 1 and 2. Correspondence from the Palaeoecology Unit at University College London to the NCC in 1991 indicates that they planned to sample a core, but apparently this never occurred.

3.3 Chemistry

A few chemical measurements were recorded for Double Kettle in 1881 and for both ponds on 5 April 1926 (Nicholson, 1929). Other measurements were made on 4 May 1967, 1 June 1970, 5 May 1971 and 5 October 1971 (Hudson et al., 1971). A marked change in the chemistry of the Double Kettle occurred between 1970 and 1971 as a result of the transfer of water from Double Kettle, with a rise in Ca from 130 to 455 mg L^{-1}. In addition, Northumbrian Water Authority in 1997 and J. Lamont-Black in 1999 (as mentioned previously) determined some chemical parameters of the water in both ponds. All these are summarised in Table 3.1.

3.4 Vegetation

There are sufficient old accounts of the vegetation or individual plant species to make it clear that some species have persisted for a long time, while others have certainly been lost from the site. Robson recorded *Cladium mariscus* and *Chara tomentosa* (now known as *C. hispida*) at Croft Kettle in 1777 and all detailed records subsequently have also listed these. In the case of the latter, this is perhaps the longest continuous record for a freshwater algal species anywhere in the world. According to Page (1905), "Hell Kettles is a remarkable site of large deep ponds surrounded by tall fen and wet grassland and boggy ground overgrown with rushes and sedges. It is a botanical region worthy of note. Here grows *Cladium mariscus* so valuable in the eastern countries for thatching; and among other rarities, *Utricularia vulgaris*, *Viburnum lantana*, *Juncus obtusifolia*, *Carex stricta* and *Chara hispida* all denote the peculiar features of a fen vegetation. In addition, *Iris foetidissima*, a very rare plant, was found in the damp woods adjacent to the ponds."

Table 3.1 Chemical data for Hell Kettles[1]

	1881	1926		1967		1969		1970		1971 (top)		1971(bottom)		1984		1997		1999	
	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D
Alkalinity						244	192									224	198	240	190
Temporary hardness						244	192												
Permanent hardness						1156	96							1560	1400				
Magnesium hardness	30	122	30	148	40	264	92	164	110							97.4	94.4	98	94
Calcium hardness	134	507	130			1136	196	305	130	495	455	510	454	1220	1030	516	490	490	440
Total dissolved solids						2080	584												
Sodium (Na)	13							68	41	32.5	28	31	29					44	41
Potassium (K)								4.5	8	3	4	3	4					4.2	4.5
Silica (as Si)	1.2	3.0	1.8	>6	0.5			4.4	2.8	9.7	4	8.9	4						
Chloride						28	77			36	37	35	37			29.4	28.7	31	30
Sulphate (SO_4-S)	102	372	46			470	63											433	400
Nitrite (NO_2-N)♦										<10	<10	180	<10	< 5	< 5	< 5	< 5		
Nitrate (NO_3-N) ♦					200					5	6	246	5	< 20	< 20	< 40	< 40		
Ammonia (NH_4-N)♦														< 10	< 10	30	40		
Phosphate ($P0_4$-P) ♦										17	5	17	5	13	3	< 5	< 5		
Water Level																			
pH♣						7.9	8.2									7.97	7.99	7.8	8.0
Conductivity♠																2.58	2.51	2.20	2.10

Values for all other elements (parameters) except the ones with a superscript are measured in mg L^{-1} (C = Croft, D = Double).
♠Conductivity is measured in mS cm^{-1}; ♣ pH is measured in pH (-log $[H^+]$) units; ♦Nitrogen fractions and phosphate are measured in μg L^{-1}.

[1] Values in these table were not obtained during this work but are quoted from a number of other sources.

Nicholson (1929) gave a more detailed account of the vegetation and reported that over a score of the plants previously recorded were no longer present. These and other floristic changes are summarized in Section 3.9. Further changes in the vegetation or loss of particular species were reported for 1970 and 1971 by Wheeler & Whitton (1971), including a marked decline in *Cladium* cover. Beds of *Elodea canadensis* with a compact growth form and often an iron oxide deposit occurred in the shallower parts of the Double Kettle in 1970 (B.A. Whitton, pers. comm.). Luxuriant plants of this species occurred in the Croft Kettle in June 1966, but these vanished between February and September 1968, probably largely due to grazing by snails. During diving surveys made in 1970 and 1971, it became clear that small pieces were continually being transported from the Double Kettle by anglers. *Elodea* has not been seen in Croft Kettle from the late 1970s onwards (B.A.Whitton, pers. comm.). For instance, it was not seen during a thorough diving study on 31 May 1998 (D. Hyde, pers. comm.). In addition, there have been a number of further changes at the Hell Kettles site since 1971. Drainage of the surrounding fields and, to a lesser extent, ease of public access and cattle grazing, probably account for changes outside the ponds, but other factors need also to be considered for the ponds.

Because of the heavy grazing by cattle evident in parts of the pond fringe and fen in 1970, part of the fen was fenced off from grazing and trampling by anglers in the winter of 1970/1971. The adjacent field has not been fertilized since the mid-1970s, although grazing cattle may aggregate near the ponds. In 1996, the vegetation was mapped to National Vegetation Classification (NVC) standards, indicating a decrease in the number of species and area cover, but also the reappearance of two to three rare species (e.g. *Eleocharis uniglumis*) (Hedley, 1997). Perhaps these had been overlooked in the 1971 survey (B.A.W., pers. comm.). The surrounding belt of reeds became more prominent during the 1990s (B.A.Whitton, pers. comm.).

The NCC identified Hell Kettles ponds as a SSSI in 1976. The site is of significant conservation value as it has the only good examples of pond and fen communities on the Magnesian Limestone in Co. Durham in conjunction with their historically recorded flora and the only examples of subsidence ponds on Magnesian Limestone in Britain. The site is not only one of the most important wetlands in lowland co. Durham, but this is the only site in N-E. England with *Cladium mariscus*, *Eleocharis uniglumis*, *Blysmus compressus*, *Berula erecta* and *Juncus subnodulosus*. *Chara hispida* also occurred in the pond at Hardwick Hall, Co. Durham, during the 1970s, but by the late 1990s appeared to have disappeared from there (B.A.Whitton, pers. comm.).

3.5 Microorganisms

The large and distinctive bacterium *Achromatium oxaliferum* is frequent in silt around the edge of the pond (Wheeler & Whitton, 1971) and a population from the vicinity of Croft Kettle has been used in studies comparing this and two other populations from northern England (Gray *et al.*, 1999a, b). There were marked differences in the sequences of 16S rRNA genes from the different sites, which was interpreted as evidence of local endemism (Gray *et al.*, 1999a). Six clones from Hell Kettles grouped together and were separated from the clones from two sites in the Lake District. A Hell Kettles population required organic substrates for growth, whereas a Rydal Water one could grow autotrophically using sulphide (Gray *et al.*, 1999b). However, it would be premature to suggest that *A. oxaliferum* is never autotrophic at Hell Kettles.

3.6 Animals

The shining ram's horn snail (*Segmentina nitida*) was recorded from this site in 1970, though this record is open to considerable doubt (K. Pratt to C.J. Spray, letter of 28.5.98). According to Pratt, this species lives in unpolluted, usually calcareous waters in the ponds and drains of grazing marshes, so at least Croft Kettle is a potentially suitable site. Canada geese were seen at the site in 1996 (C.J. Spray, pers. comm.) and either seen or evidence from droppings etc each spring in subsequent years (1997-08: B.A.W.; 1999-03: D.G.).

3.7 Hell Kettles morphometry

As mentioned in Section 3.1, there were originally four ponds. One (D in Figure 3.1) dried out and two (A and B in Figure 3.1) became joined some time between 1774 in the survey by Fordyce and 1857, when Longstaffe published his account. There are surprisingly many old records of dimensions. Fordyce's survey gave these dimensions: the diameters of A and B were 34.5 m and C was 25.5 m; depths were A) 5.9 m; B) 4.2 m; C) 5.1 m; D) 1.6 m (all values from old accounts have been converted from yards or feet to metres, with values corrected to the nearest 10 cm below). A record by J.C. Clayton in 1894 gave a depth of 7.3 m for one place in Croft Kettle and 6.4 m for one place in the Double Kettle. Another record by Abbey and Garbutt gave depths of 5.7 m and 4.5 m for the parts of the Double Kettle corresponding to A and B in Figure 1, and 6.4 m for Croft Kettle. Hudson *et al.* (1971) record a maximum depth of 6.5 m for Croft Kettle.

The present situation is shown in Figures 3.2 (aerial), 3.3 and 3.4 (two versions of outline and key features), 3.5 (sample sites) and 3.6 and 3.7 (depth contours of Croft Kettle). Key statistics are given in Table 3.2. Note that the following maps have been obtained in four different ways and the present study only determined the characteristics of Croft Kettle.

Table 3.2 Morphometric data for Croft Kettle.

Depth (m)	Surface (planimetric) area (m^2)	Water-substratum contact area (m^2)	Volume (m^3)
0-1	190.5	232.5	1065.2
1-2	266.5	303.8	810.5
2-3	139.2	179.6	634.0
3-4	139.2	172.5	496.0
4-5	226.1	246.7	303.4
5-6	155.2	166.2	107.4
6-6.7	41.3	43.8	8.8
Total	1158.7	1345.4	3425.4

The surface level used as reference (0 m) was 30 cm above the upper surface of the outflow pipe shown in Figure 3.3. The pond surface fluctuated within a range of 5 cm for much of spring and summer 2000 and the extreme range was about 10 cm during this study.

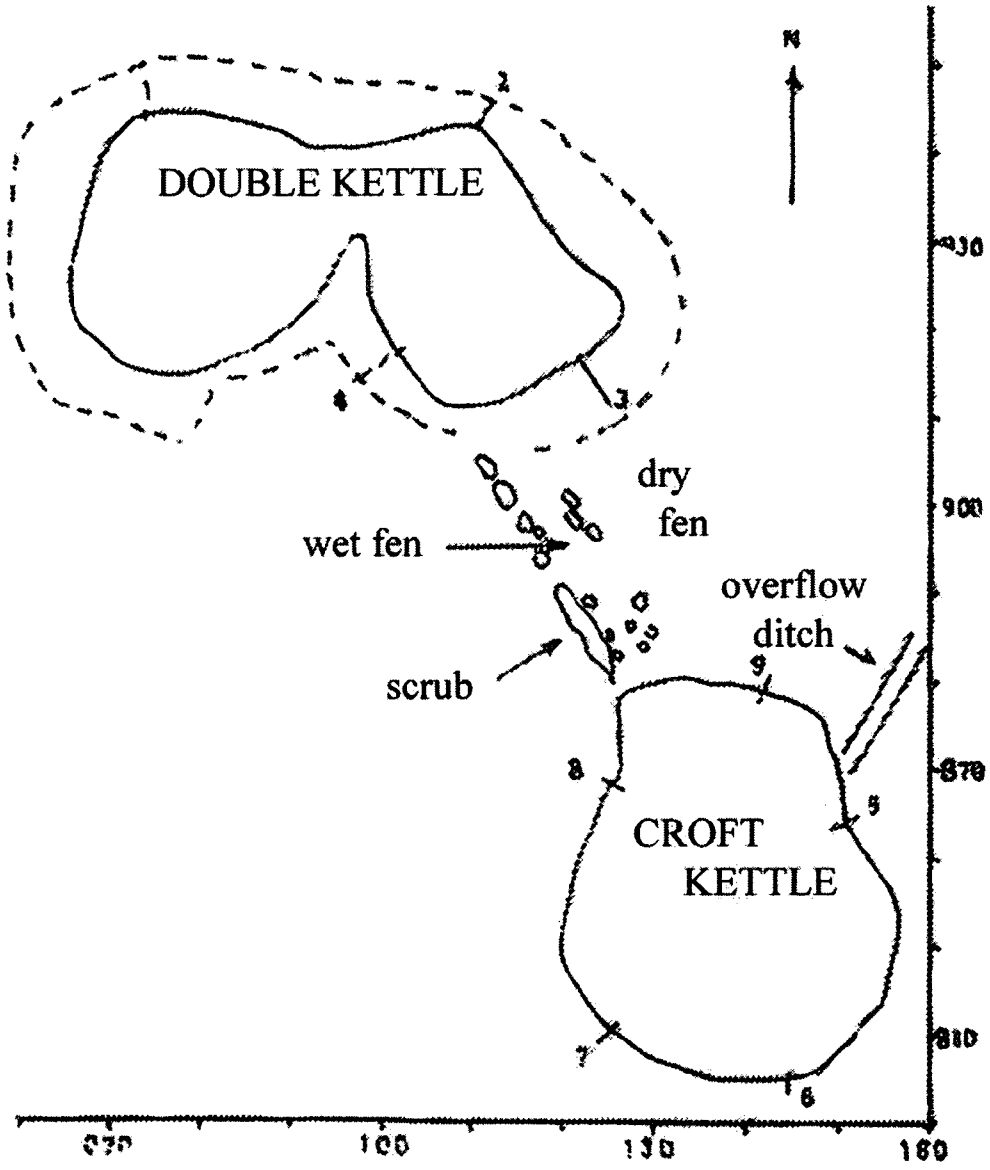

Figure 3.3 Outline map of Hell Kettles given by Wheeler & Whitton (1971) based on a combination of 6-inch maps and a partial survey of the site.

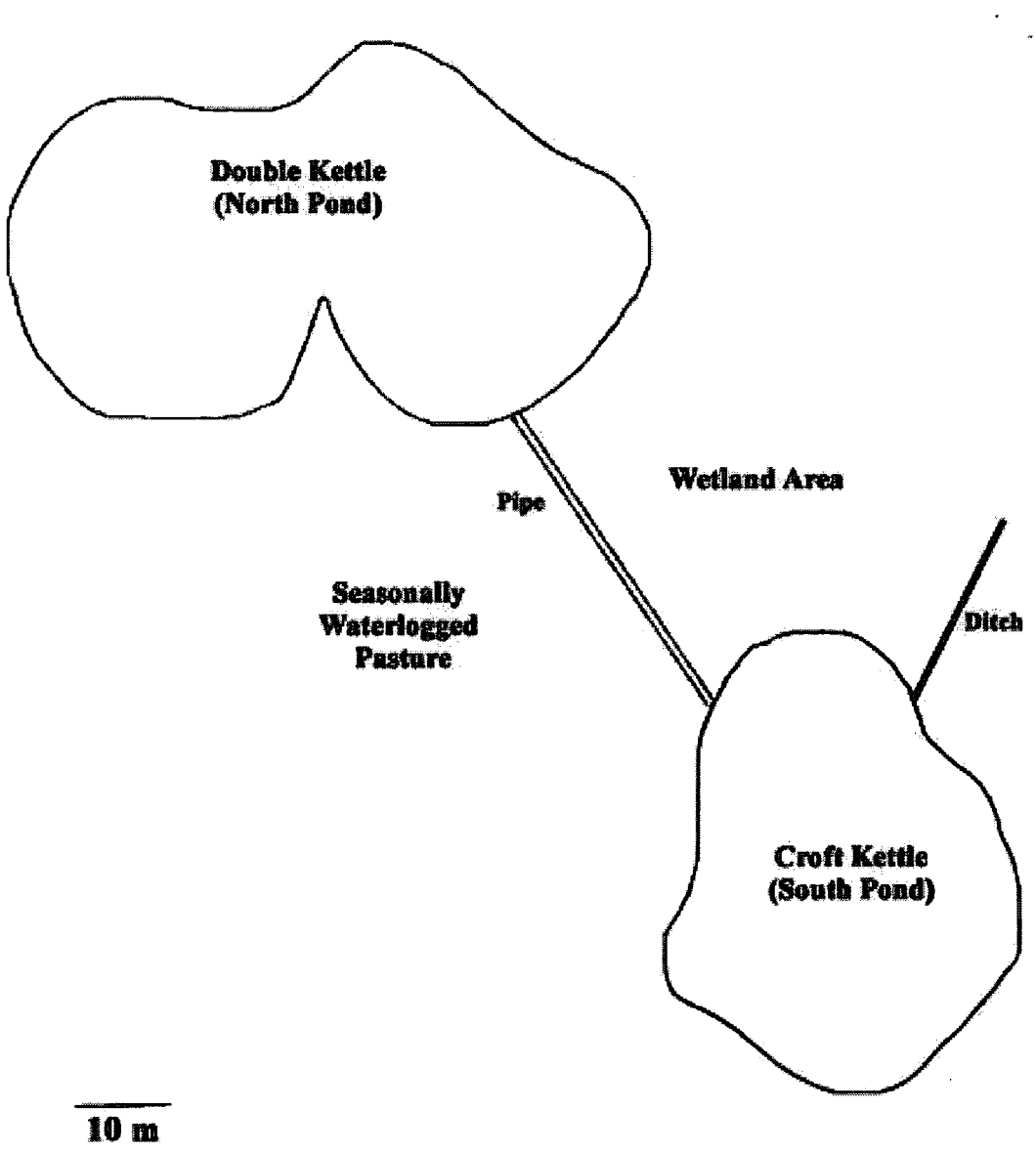

Figure 3.4 Outline map of Hell Kettles in English Nature files, presumably used for vegetation survey in 1996.

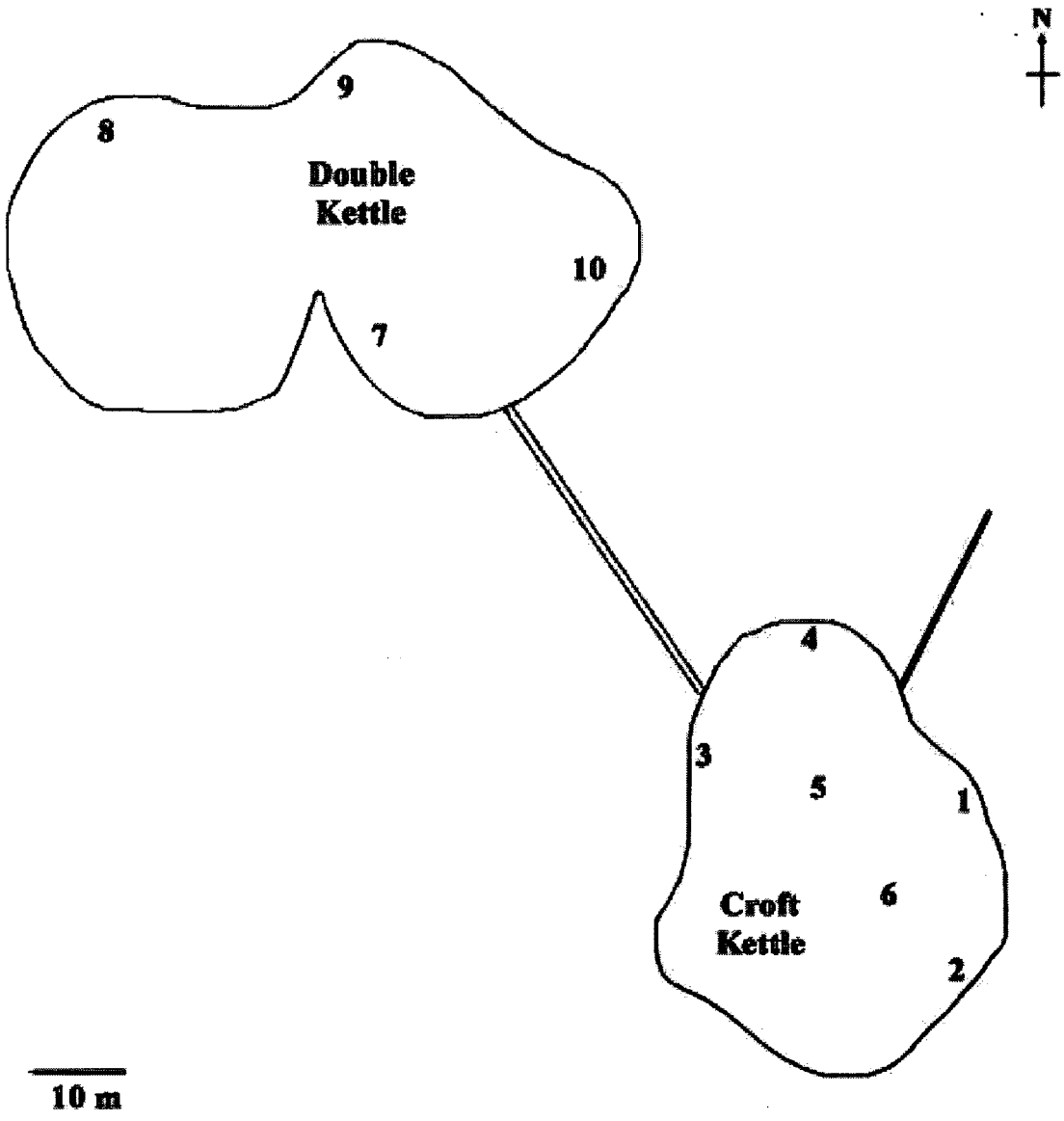

Figure 3.5 Hell Kettles ponds (using Figure 3.4 outline), showing location of the main sampling sites.

Croft Kettle	1,2,3,4	surface water samples
	5	position for vertical sampling; sediment core used for dating, pollen and diatoms was also taken from approximately here
	6	approximate position for routine sediment samples for chemical analysis
Double Kettle	7,8,9,10	surface water samples

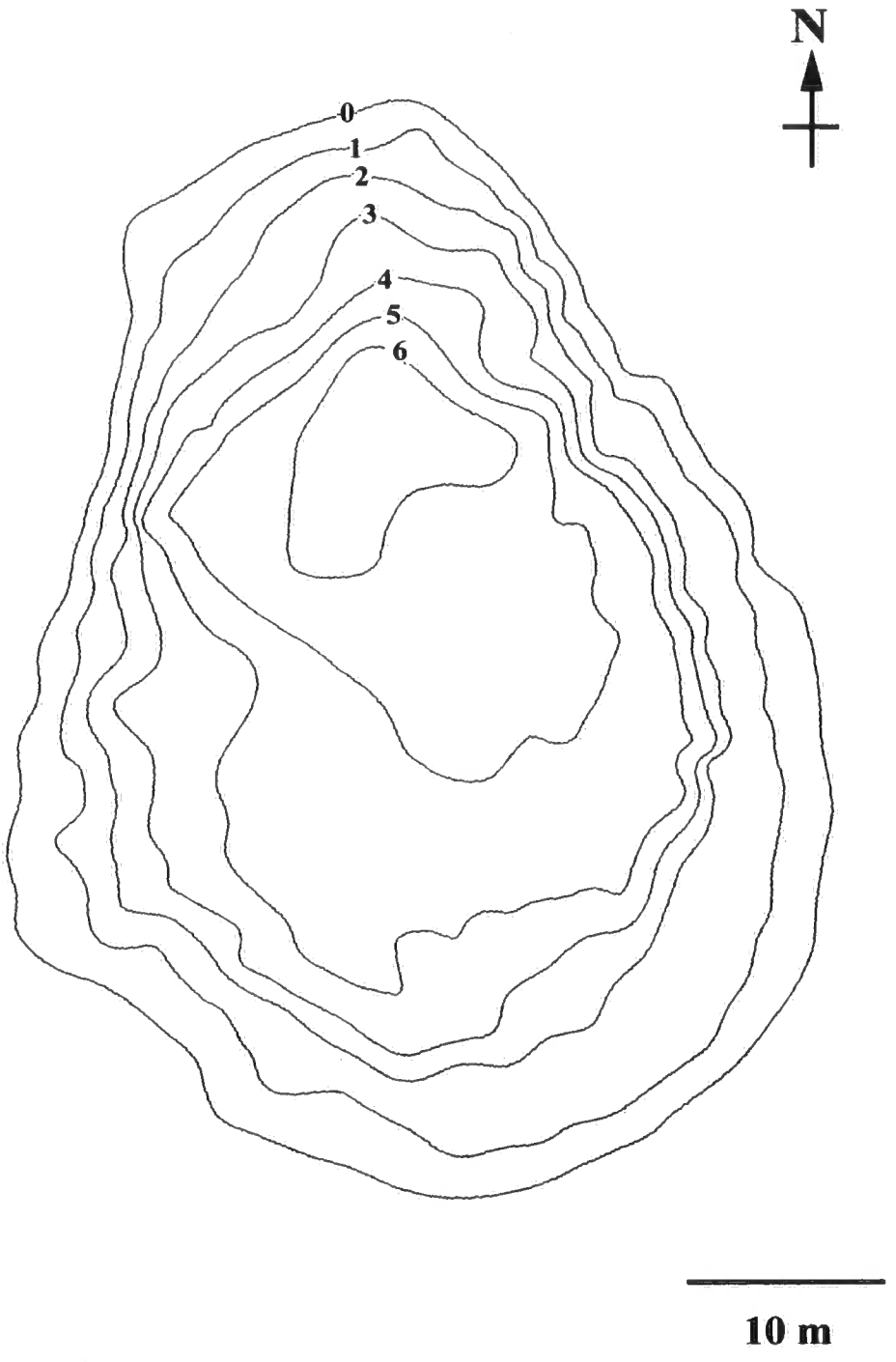

Figure 3.6 Croft Kettle, showing 1-m depth intervals.

Note that this is a different outline from that in Figures 3.3 and 3.4. The outline of the pond is based on points at the edge of the water related to a grid-system of ropes. This is probably the most accurate of the maps available.

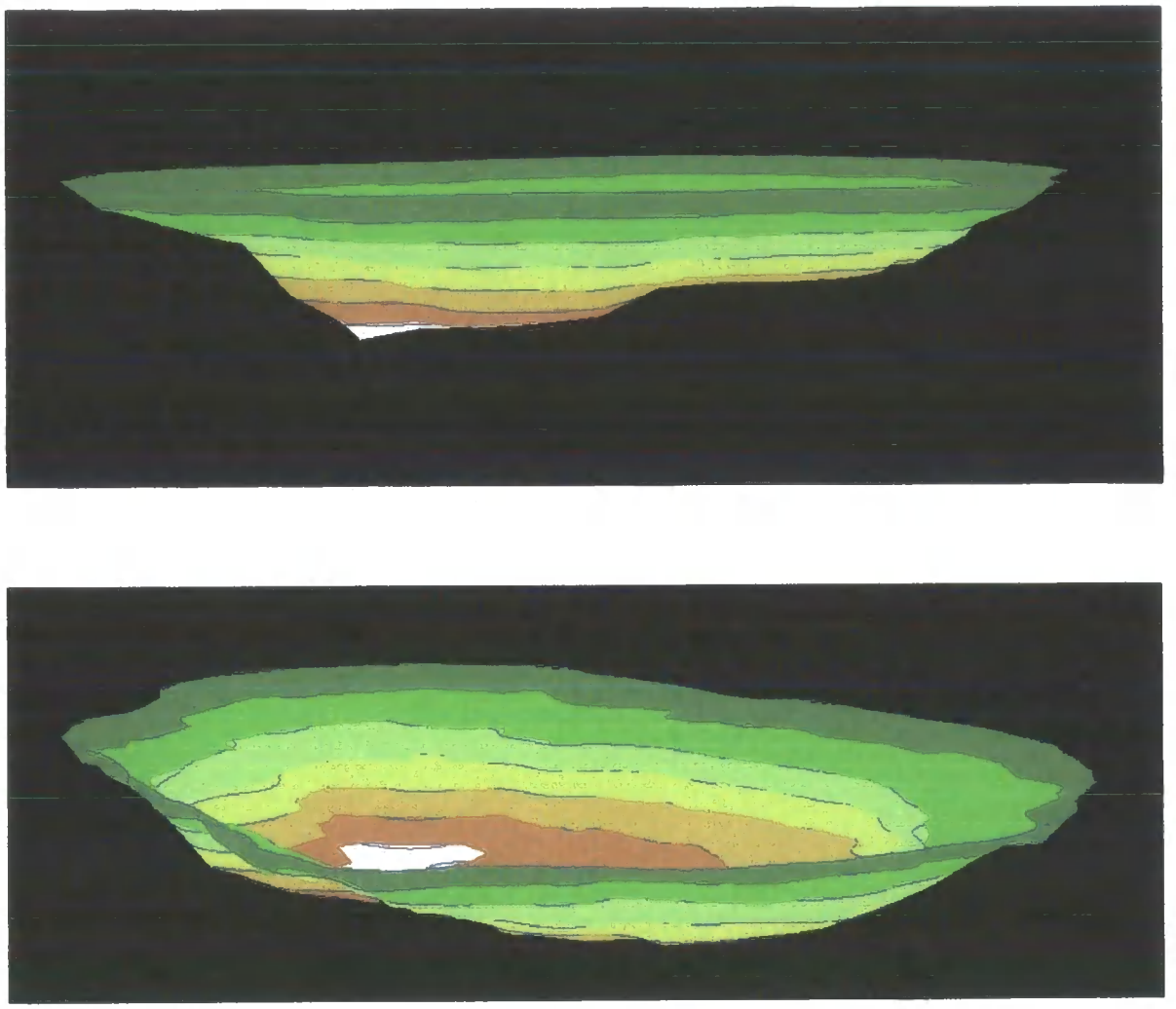

Figure 3.7 Two 3-dimensional views of Croft Kettle, showing 1-m depth intervals. The contours here relate to those shown in Figure 3.6.

3.8 Biological surveys

3.81 Suspended chlorophyll *a*

The concentration of photosynthetic pigments is used extensively to estimate phytoplankton biomass, since chlorophyll *a* constitutes approximately 1 to 2% of the dry weight of planktonic algae. A programme of sampling for suspended chlorophyll *a* at 30 cm depth was commenced in spring 1999. This fraction includes both phytoplankton and any small phototrophs attached to particles as well as cells or filaments detached from epiphyte or sediment communities by wind action or other causes.

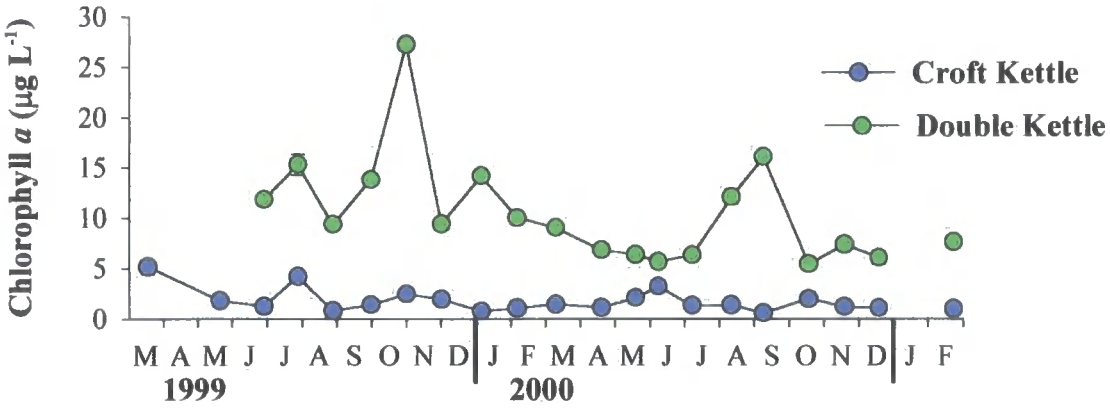

Figure 3.8 Monthly changes in chlorophyll *a* in samples collected from both ponds over the studied period at 30 cm depth. Vertical bars represent ± one SE (n=4) (if not marked, SE was smaller than the circle radius).

Chlorophyll *a* concentration in Croft Kettle was low, with an average of 1.8 ± 0.25 µg L^{-1} (ranged 0.6 to 5.2 µg L^{-1}) and there was little evidence for seasonal variation. The concentrations in Double Kettle were consistently higher, with an average value of 10.6 ± 1.21 µg L^{-1} and a high value in the end of autumn (27.3 ± 0.4 µg L^{-1}). A student's paired samples t-test showed that concentrations in Croft and Double Kettle are significantly different ($p<0.001$). In addition, the depth profile (on meter intervals) of chlorophyll *a* in Croft Kettle, which was determined at 17 March 2000 did not show considerable changes with depth (average=1.6 ± 0.14 µg L^{-1}).

3.82 Past and present vegetation maps of Croft Kettle

The distribution of *Chara hispida* in Croft Kettle as well as of the other submerged macrophytes was mapped in July 2000 (Figure 3.10). *Chara hispida* forms dense, large beds (Figure 3.11) with a district edge zone towards the open water. At the edge of the pond, shoots reach the water surface during summer and there is a clear pattern of white, unhealthy looking shoots deeper in the stands with healthy looking biomass in the upper water layer. The density of *C. hispida* shoots per m^2 was also determined and was estimated as 170 ± 12 shoots m^{-2} (mean ± SE of four 1-m^2 quadrats for the area covered by *C. hispida*). *Chara hispida* was the dominant submerged organism (almost a monoculture) with only a few shoots of *Hippuris vulgaris* (≥50) present within the charophyte bed in the areas indicated in Figure 3.10.

Some botanical changes in addition to the event of the summer of 1996 have taken place in Croft Kettle since 1970. In particular and with respect to the aquatic and submerged plants, *Elodea canadensis* increased and then disappeared. Its spread was probably favoured by re-inoculations due to anglers, while grazing by snails was probably a key factor leading to its loss (B.A. Whitton, pers. comm.). Large snails are apparently absent now (pers. observation & D. Hyde, pers. comm.), so any reintroduction of the plant might lead to its spread again. Hair-forming algae in Croft Kettle have also decreased markedly (pers. observation), apparently in the last few years (B.A. Whitton, pers. comm.). Their success depends on there being a period of the year when P levels are very low (B.A. Whitton, pers. comm.). *Potamogeton pectinatus*, which grew in lower areas of Croft Kettle in 1970, have apparently also disappeared. In addition, recent evidence indicates that the so-called *Chara vulgaris* was mis-identified in 1970 and is actually *C. hispida* (named *C. hispida* thin in Figures 3.9 and 3.10) in a moribund state due presumably to the reducing conditions during the summer. *C. hispida* (thin) distribution has changed and in particular has mostly disappeared from the east site of Croft Kettle and reduced elsewhere. Accordingly, its maximum depth distribution has changed and currently does not exceed 4.5 m, while in 1970 its depth distribution reached 5 m. *C. hispida*'s distribution, independent of the event of summer 1996, has not changed since 1970 and shows a maximum depth distribution of 4-4.2 m, similar to 1970.

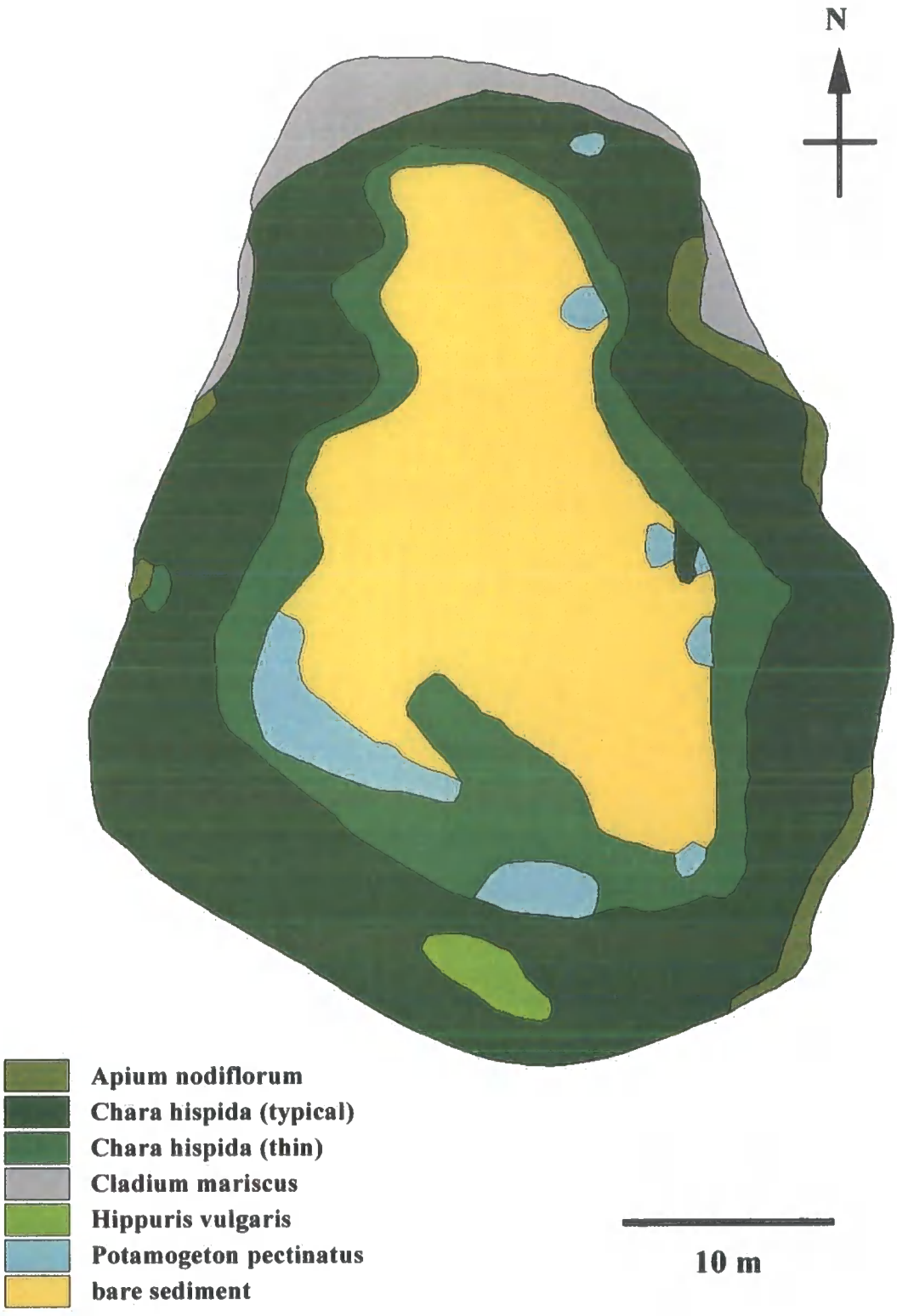

Figure 3.9 Map of vegetation in Croft Kettle, June 1970. Map was copied from Wheeler & Whitton (1971) and was digitally reproduced using Arc-view.

Figure 3.10 Map of vegetation in Croft Kettle, summer 2000.

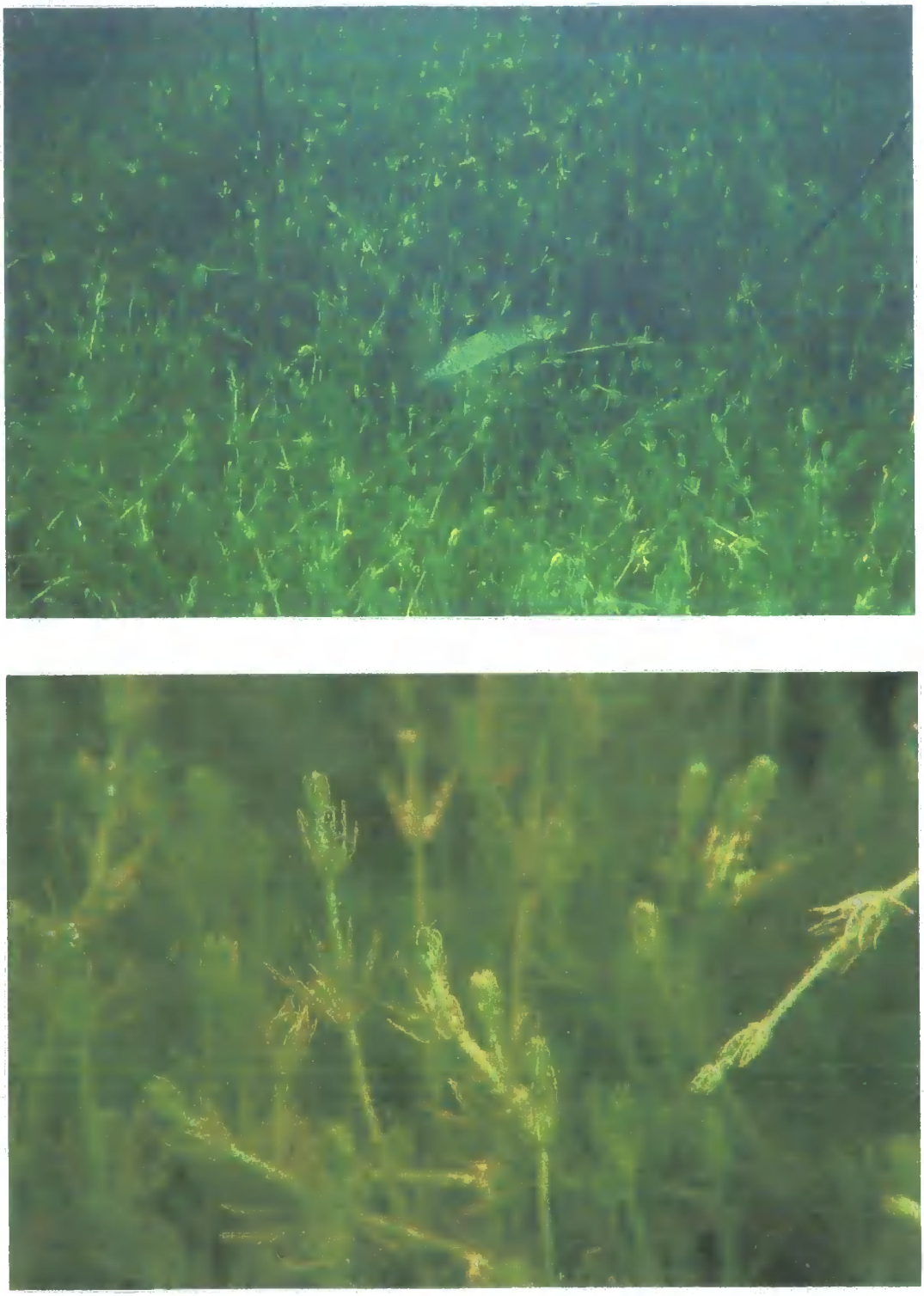

Figure 3.11 *Chara hispida* in Croft Kettle at July 2001. Upper, part of meadow; lower, apical shoots.

Figure 3.12 Croft Kettle. Upper, 1970s; lower, 2001.

CHAPTER 4 Seasonal studies of water chemistry

4.1 Introduction

In order to identify what is responsible for the botanical changes of Hell Kettle ponds (Section 3.9) and particularly Croft Kettle and since little was known of the water chemistry characteristics of these water bodies (Table 3.1), a number of physical and chemical variables were monitored on a monthly basis for most of the study period. Priority, was given to the two key elements affecting the trophic status of freshwater bodies (Section 1.4) and these were N and P. The N:P ratio was also examined in order to establish the relative importance of N and P and evaluate the availability of these nutrients to the biota.

The major objectives were to investigate the seasonal variability of the water chemistry and to establish trends and rates of change over the three-year study period, and to enable reliable determination of nutrient fractions in detail. Studies were made on the two ponds and the borehole water in the farm well. The most detailed studies (three-dimensional) were on Croft Kettle. Overall, this chapter describes the seasonal patterns in physical, physico-chemical and chemical variables and is separated into three sections:

1. Surface water chemistry;
2. Vertical profiles of temperature, oxygen, N and P;
3. N:P ratio of water

4.2 Sites and sampling programme

Water samples were collected and physical and chemical measures were taken monthly (Section 2.3). Spot samples and detailed surveys were also taken on intervening days. The outbreak of "foot and mouth" disease in early 2001, meant that four months elapsed from the sampling on 22 February 2001. In addition, the borehole water could not be sampled during winter due to covering of the pump by the farmer in order to protect it from frost. Surface water measurements were taken from four sites in Croft and four sites in Double Kettle and depth profile studies were made from the middle of Croft Kettle (Section 2.3, Figure 3.5). However, since little variability was shown among sampling sites in each pond, the mean value ± the standard error for its variable at each sampling occasion are shown here.

4.3 Air temperature and precipitation

Atmospheric data were obtained by the meteorological station at Carlton in Cleveland. Figure 4.1 shows monthly changes in air temperature and precipitation during the study period. The mean monthly values (n=28-31) of minimum and maximum measurements are presented for air temperature and the monthly sum for precipitation. Air temperature showed clear seasonal trends while rainfall was much more variable and did not show any clear seasonal patterns.

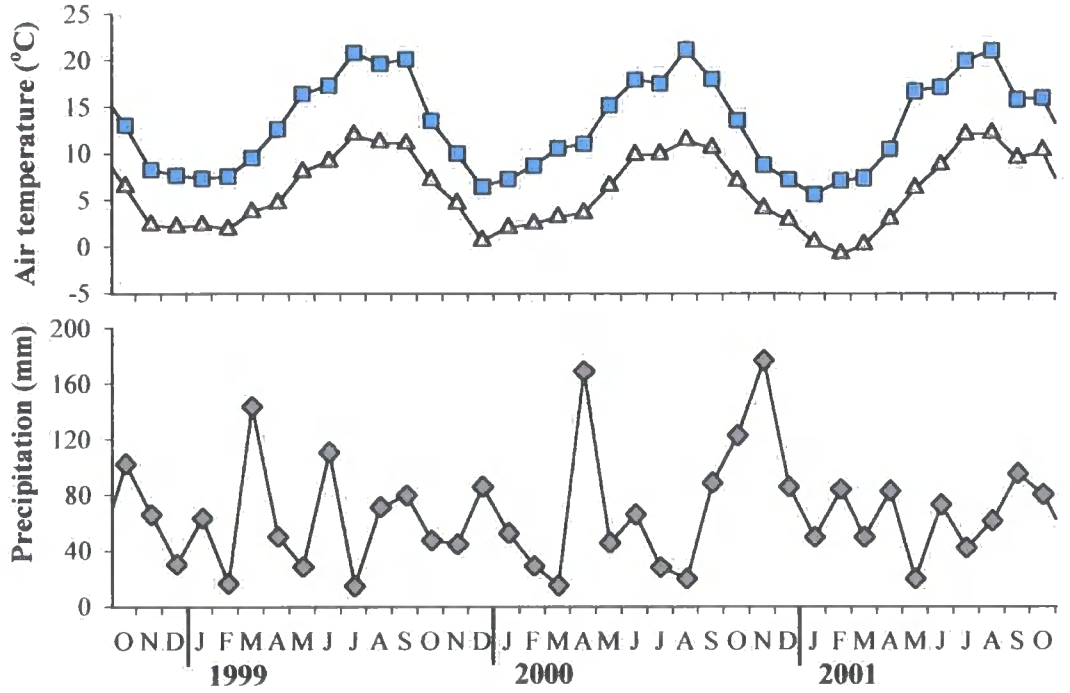

Figure 4.1 Changes in air temperature (■ max, ▲ min) and precipitation with time at Hell Kettles and their catchment between October 1998 and October 2001.

According to these air temperature and precipitation values and the average potential evapotranspiration rates for North-East of England (calculated by the Ministry of Agriculture Fisheries and Food), the turn over time for Croft Kettle could be from one to two years. However, these average figures conceal considerable year-to-year variability and present only a gross estimate since many parameters are needed in order to be able to construct an accurate model.

4.4 Light attenuation

The absorption and attenuation of light by the water column are major factors controlling temperature and potential photosynthesis in lake waters. The rate at which

light decreases with depth, depends upon the amount of light-absorbing dissolved substances and is diagrammed via isopleths following Lambert-Beers' law.

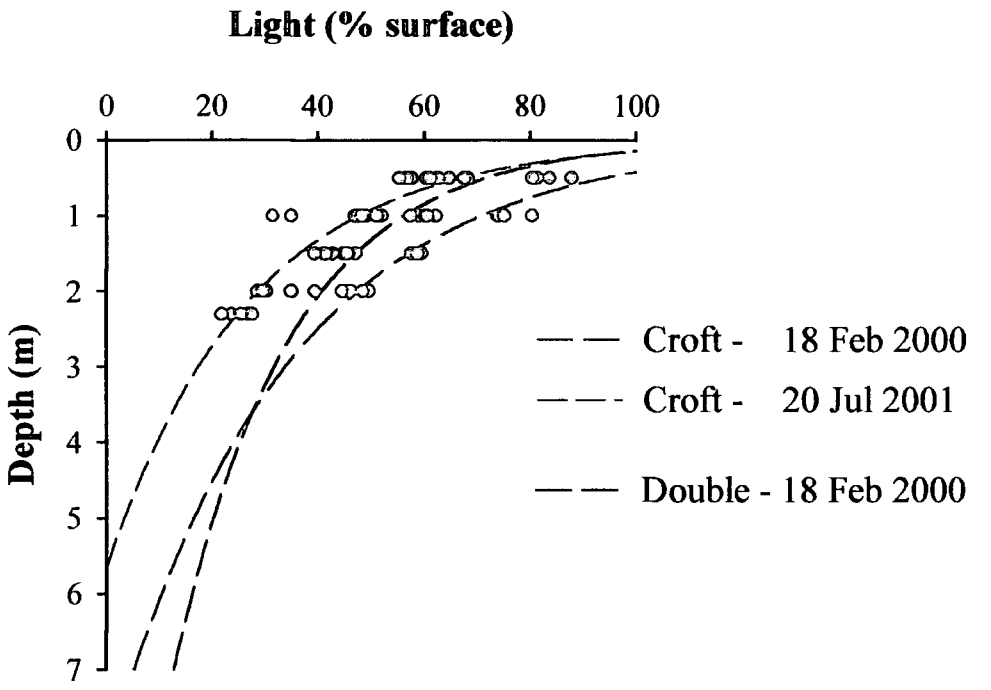

Figure 4.2 Changes in light attenuation with depth in Croft and Double Kettle at 18 Feb 2000 (12.00 h) and 20 Jul 2001 (13.00 h).

Average light attenuations coefficients (K_d) were 0.35 ± 0.058 m^{-1} and 0.55 ± 0.316 for Croft and Double Kettle respectively in February 2000 and 0.77 ± 0.222 m^{-1} in July 2001 in Croft Kettle, indicating that the greatest water clarity occurs in February in Croft Kettle. In addition, the isopleths indicate that the euphotic zone could extend up to the bottom of the pond during uniformity of the water column (absence of thermocline) especially in the winter. However, during the summer and due to existence of a thermocline light could be inhibited and the eutrophic zone could change.

4.4 Physical and chemical variables of surface water

4.41 Introduction

The results for the monthly sampling of surface water are shown in Figures 4.3 to 4.8. Most of the variables for the two ponds and the borehole water have been sampled for more than two years and the data are summarized in Table 5.1.

Table 4.1 Range of physical and chemical values of surface water in Croft and Double Kettle and the borehole.

	Croft Kettle			Double Kettle			borehole		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Air temperature (°C)	9.7	-7.0	29.9						
Precipitation (mm)	67.6	14.8	176.8						
Water temperature (°C)	10.5	4.2	18.7	10.8	3.7	19.7	11.1	9.5	12.7
Conductivity (mS cm^{-1})	2.38	1.14	2.62	2.25	1.46	2.55	1.65	1.27	1.75
pH	7.62	7.30	7.91	7.83	7.40	8.16	7.07	6.95	7.25
O2 (mg L^{-1})	10.7	8.0	12.8	10.4	5.9	13.0	1.2	0.3	3.4
O2 (% saturation)	96	69	128	93	58	116	11	3	31
Alkalinity (mg $CaCO_3$ L^{-1})	226	134	257	179	138	197	283	270	302
Silica (mg SiO_2-Si L^{-1})	6.3	5.3	6.8	2.7	1.0	4.1	5.4	4.9	5.7
TN (μg N L^{-1})	144.3	42.3	595.3	256.4	156.3	582.8	74.6	59.3	94.2
TIN (μg N L^{-1})	42.7	12.0	156.1	69.8	10.4	138.2	39.4	27.1	68.2
TP (μg PO_4-P L^{-1})	15.5	4.7	104.4	31.2	11.3	96.1	5.2	2.2	13.1
FRP (μg PO_4-P L^{-1})	3.6	1.1	13.0	4.1	0.7	13.0	1.4	0.9	2.9

4.42 Temperature

Surface water temperature showed clear seasonal trends in Croft and Double Kettle, with winter minima and summer maxima but showed little variation over time in the borehole water (Figure 4.3). Maximum and minimum temperatures were 18.7 and 4.2, 19.7 and 3.7 and 12.7 and 9.5 °C for Croft-Double Kettle and borehole respectively. The warmest water temperature was recorded in July of 2000 for both ponds. Paired sample t-tests identified significant differences in the mean temperature between both ponds and the borehole water ($P<0.001$) and between Croft and Double ($P<0.01$). Borehole water temperature remained quite stable throughout the study period, especially comparatively to the ponds ones. The little change shown could be attributed to variation in the quantity of water left to run before the measurements were taken since the borehole water temperature should be quite stable as the water originates from a deep underground aquifer (Section 3.2).

4.43 Conductivity

Conductivity of surface water in the ponds and the borehole water ranged from 1.14 to 2.62 mS cm^{-1} and showed little variation with time (Figure 4.3). Conductivity showed higher values in the sequence of Croft>Double>borehole and significant differences in distribution were identified by the Wilcoxon signed-rank test between

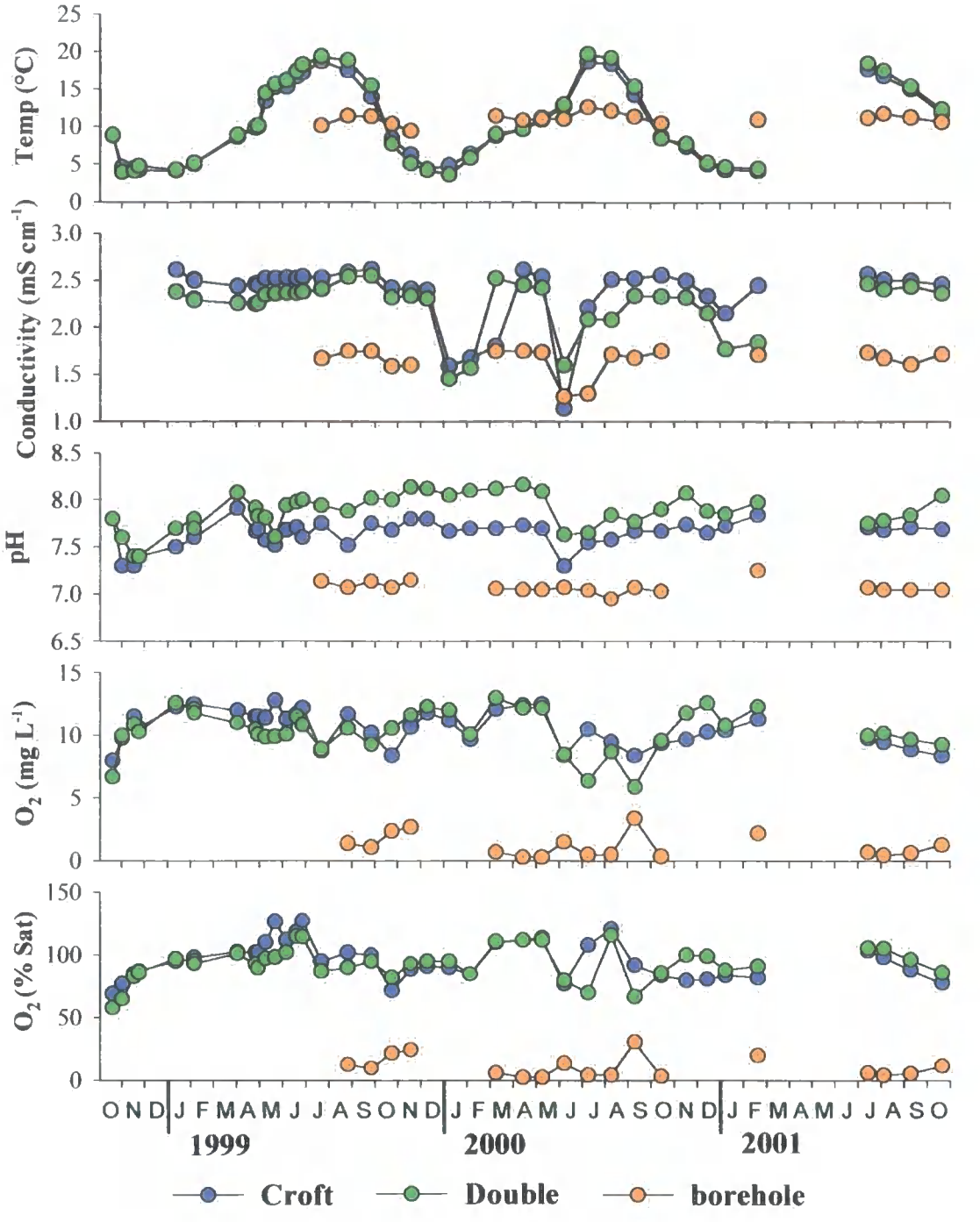

Figure 4.3 Changes in surface water temperature, conductivity, pH and O_2 (as concentration and % saturation) with time in Croft Kettle, Double Kettle and borehole, between October 1998 and October 2001. No data for borehole during part of winter 1999/2000.

both ponds and the borehole water and between Croft and Double Kettle ($P<0.001$). The three periods with a marked drop in conductivity (Jan 1999, Jun 2000 and Jan 2001) of both ponds could be attributed to the fact that sampling took place after a period of high rainfall, when the less dense water is found near the surface.

Accordingly, the low conductivity values observed especially in early Jun 2000 and early January 2001 took place directly after the highest amounts (peaks) of precipitation (170 and 177 mm) during the study period. The lower conductivity of the borehole water in comparison with the ponds ones also indicated that it is derived from deep underground sources.

4.44 pH

The pH of the ponds ranged from 7.3 to 8.16 (diel studies would be needed to establish this) with some indication of a seasonal pattern but with relatively low within-year variability. pH was highest in summer and slightly lower in winter, possibly due to higher photosynthetic rates. The mean value of Croft Kettle was 7.62, slightly less than Double Kettle (7.83), but higher than the groundwater (7.07) and significant differences of the mean ($P<0.001$) were identified between both ponds and the borehole water.

4.45 Dissolved oxygen

Surface dissolved oxygen ranged between 69 and 128 % saturation in Croft Kettle and 58 and 116 % in Double Kettle (Figure 4.3). There was reduction in dissolved oxygen in surface water in autumn of each year in both ponds ($\approx$ 20 % in Croft Kettle, much less in Double Kettle), indicating the breakdown of stratification and the mixing of upper and lower layers of water. Borehole water had low levels of dissolved oxygen and the variation shown was as mentioned above, attributed to the amount of water left to run from the pump immediately prior to sampling, as well as depending on the extent of use by the farmer prior to sampling.

4.46 Total alkalinity

Alkalinity of water is the sum of all the titratable bases, indicating its acid-neutralising capacity, is often correlated with conductivity and is mostly influenced by concentrations of $CaCO_3$ (APHA *et al.*, 1992). The results (Figure 4.4) show a clear distinction between the three sources of water. The mean for Croft Kettle was 226 $\pm$ 4.6 mg L^{-1} $CaCO_3$, considerably less than the borehole water (283 $\pm$ 1.7), but higher than Double Kettle (178 $\pm$ 2.8). Alkalinity in both ponds was lowest on 5 June 2000, after an intensive rain event (Figure 4.1) that led to flooding of the field adjacent to the ponds.

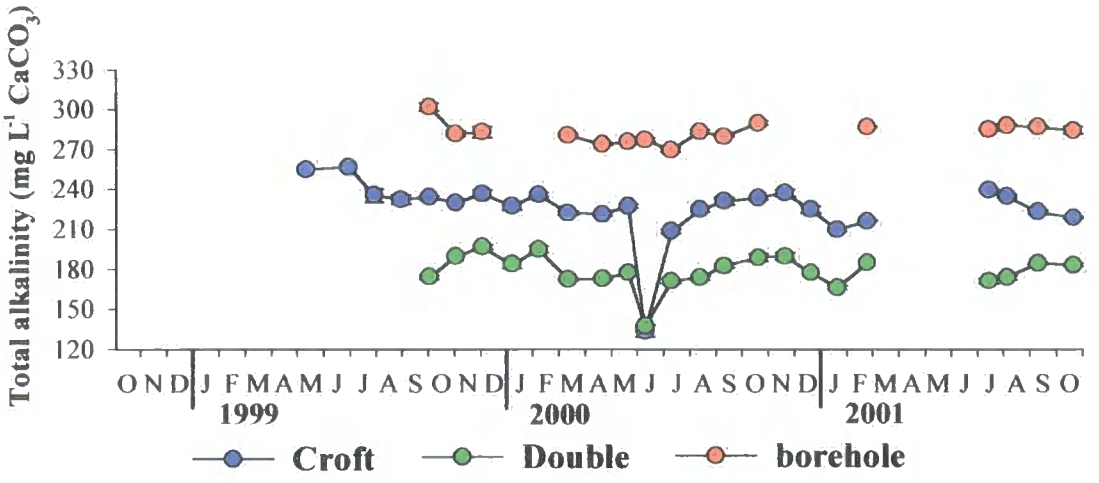

Figure 4.4 Changes in total alkalinity with time in surface water from Croft, Double and borehole between April 1998 and October 2001 (mean ± SE, n=4). Vertical lines represent SE (if not marked, SE was smaller than the circle radius).

4.47 Silica

Apart from its quantitative importance, silica is of immense significance as a major nutrient for diatoms, the dominant phytoplankton organisms of most lakes (Moss, 1998). The results (Figure 4.5) indicate relatively little variation in silicate concentrations in Croft Kettle and borehole water, but considerable changes in Double Kettle (range 1.0 - 4.1 mg L^{-1}).

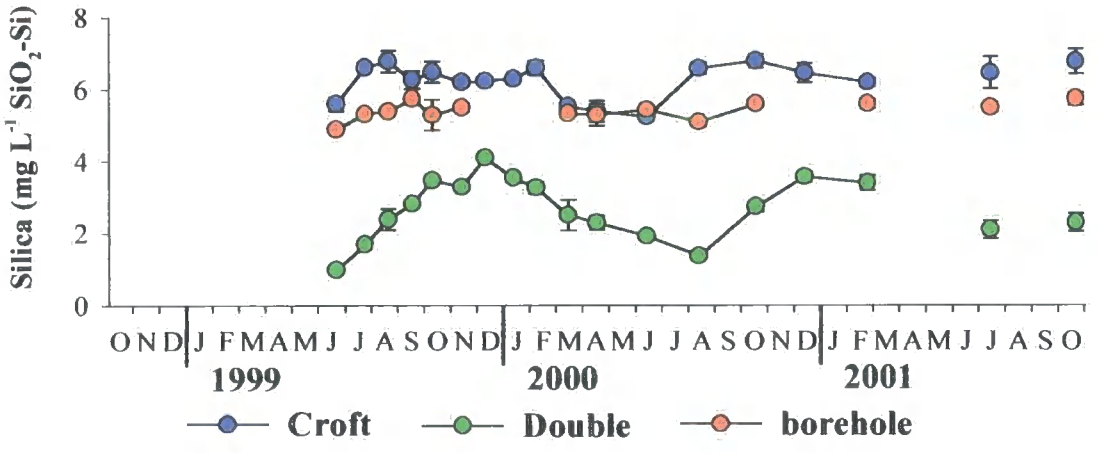

Figure 4.5 Changes in dissolved silica-Si concentrations with time in surface water from Croft, Double and borehole between April 1998 and October 2001 (mean ± SE, n=4).

4.48 Nitrogen

Only inorganic N fractions were determined in the first year of study. Organic N was recorded from January 2000 onwards. Examples of data and general trends from this study are described, and the relationships with air and water temperature, precipitation and dissolved oxygen are mentioned where relevant. Figures 4.6 and 4.7 show changes in the inorganic and organic N fractions of surface water in Croft, Double and borehole.

Mean surface nitrate (NO_3-N) concentrations for the study period were 20.4 ± 2.84 (n=30), 14.2 ± 1.50 (n=30) and 10.4 ± 1.14 (n=22) μg L^{-1} for Double, Croft and borehole respectively and all three sites showed significant mean differences ($P<0.05$) to each other. At Croft Kettle, the highest value was 47.4 μg L^{-1} and the lowest 4.8 μg L^{-1}, compared to 71.6 and 2.9 μg L^{-1} in Double Kettle. In borehole water, NO_3-N concentration was lower than the kettles, the maximum being 20.9 μg L^{-1}. NO_3-N concentration was higher in 2000 than 1999 and the highest values were recorded at 5 June 2000, a day after the summer flood, at both ponds. In addition, NO_3-N distribution above *Chara* in Croft Kettle was not significantly different from the surface one. Seasonal trends were observed at both ponds, with concentrations slightly higher during winter and spring particularly in 2000. However, NO_3-N concentration did not show any marked seasonality and distribution varied considerably. Nitrate was significantly negatively correlated with alkalinity at both ponds ($p<0.001$), but no significant association was observed with water temperature.

Nitrite (NO_2-N) formed the least significant contribution to TIN at all sites and times and was always less than 5 μg L^{-1} and often close to or, below detection limits (2.35). NO_2-N concentrations in Double Kettle were slightly higher than in Croft and in borehole and no seasonal trends were observed. No significant spatial differences were found within Croft, between surface concentration and above *Chara*. Finally, NO_2-N concentration showed significant negative relationships with air and water temperature and positive relationships with NH_4-N and TIN in Double Kettle, negative correlations with conductivity and pH and positive correlations with all the nutrient fractions in Croft Kettle and only weak positive correlations with NH_4-N and TIN in borehole samples.

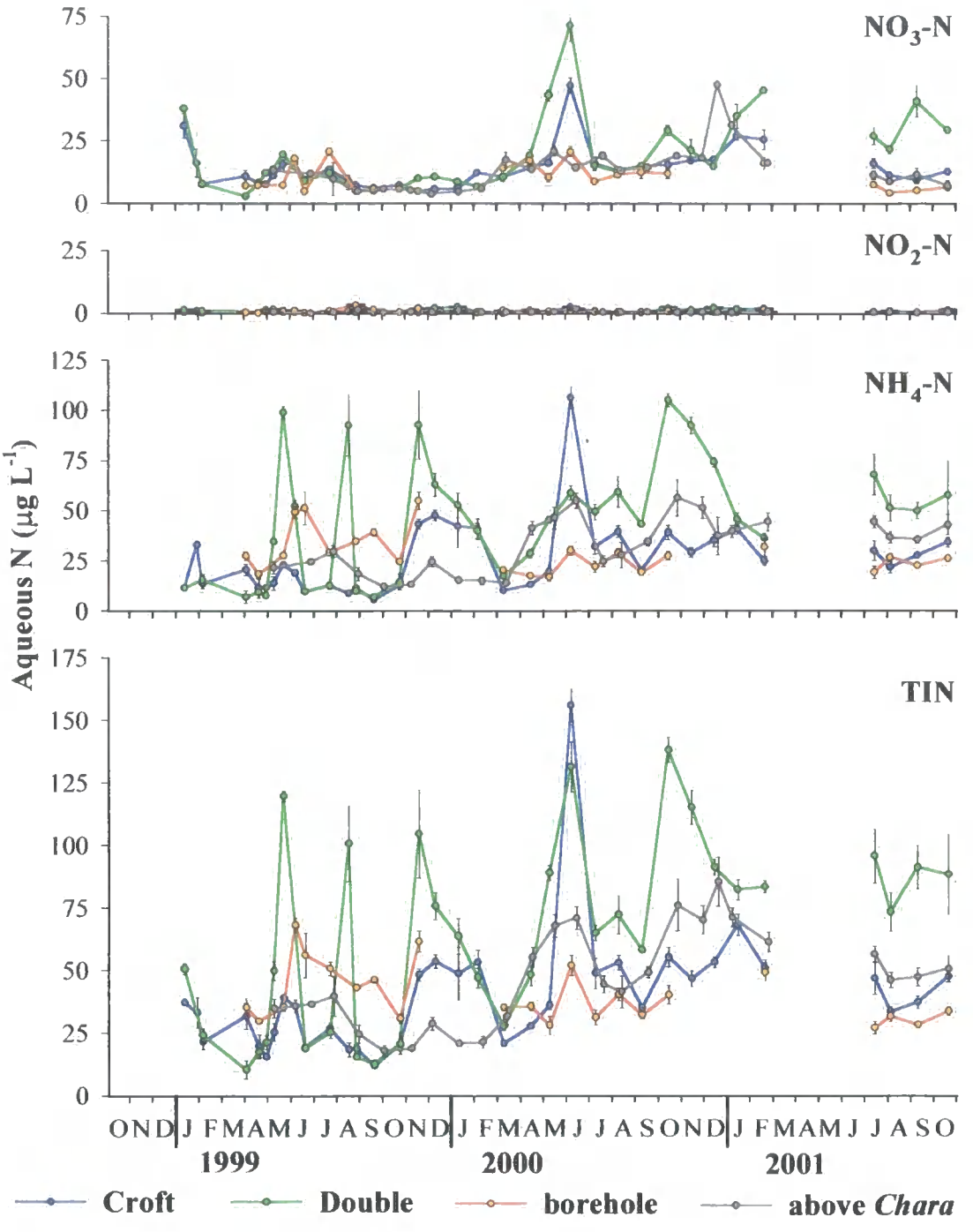

Figure 4.6 Changes in concentration (as µg L^{-1}) of inorganic N fractions with time in water from Croft, Double and borehole, between January 1999 and October 2001 (mean ± SE, n = 4).

Ammonium (NH_4-N) formed the most important contribution to TIN and concentrations exceeded those of nitrate at more than 80% of the sampling occasions at both ponds and the borehole water. Mean surface NH_4-N concentrations were 48.4 ± 5.33 (n=30), 28.6 ± 3.45 (n=30) and 27.9 ± 2.08 (n=22) µg L^{-1} for Double, Croft and borehole respectively. NH_4-N concentration in Double Kettle was significantly

different from Croft ($P<0.05$), but no significant differences amongst both ponds and borehole were found. The highest observed concentration in Croft Kettle was 106.3 ± 5.3 μg L^{-1}, which was recorded directly after the summer flood in June 2000 and was more than twofold higher than any other peak. Furthermore, NH_4-N concentration showed peaks towards the end of autumn at Croft (observed only in 1999) and Double (observed in 1999 and 2000) which could be attributed to the break down of stratification and mixing of hypolimnetic with epilimnetic layers of water. NH_4-N concentration was slightly higher during 2000 than 1999 at Double and considerably higher at Croft. In Croft Kettle, no significant difference was found between NH_4-N concentration directly above the bed of *Chara* and surface water. Finally, NH_4-N showed no discernible seasonal trend and was negatively correlated with conductivity and alkalinity only at Croft Kettle.

Total inorganic N varied significantly among sites and seasons. It ranged from 12.0 to 156.1, 10.4 to 138.2 and 27.1 to 68.2 μg L^{-1} at Croft, Double and borehole respectively, showing the highest values at June 2000, directly after the summer flood. Comparison between sites showed that it was lower in the borehole and higher in Double Kettle. Temporal and spatial variation of TIN is attributed to changes in NH_4-N concentrations, which formed the most important contribution. Significant negative correlations were observed between TIN and pH, conductivity and alkalinity in Croft Kettle and positive with the majority of nutrient fractions.

Organic and particulate N fractions (Figure 4.7) were considerably higher than inorganic N. TN was more than twofold that of TIN on the majority of the sampling occasions at both kettles, but not at the borehole. Mean TN was 144.3 ± 28.08 (n=18), 256.4 ± 22.46 (n=18) and 74.6 ± 2.76 (n=13) μg L^{-1} for Croft, Double and borehole respectively. The highest peak was observed in June 2000 and is again attributed to the summer flood. Concentrations of TN did not show any clear patterns of seasonal variability, although FTN was slightly higher in the summer months in both ponds. FON only contributed a small amount to TN, particularly in Croft and borehole, and PN was the fraction mostly influencing TN. Comparisons between sites showed that Double Kettle's FTN, TN, FON and PN concentrations were significantly different from those of either Croft Kettle or the borehole ($P<0.01$) and also Croft Kettle's from those of the borehole ($P<0.05$). Furthermore, no significant differences on either organic or particulate N fractions were found between surface and above *Chara* sites in Croft Kettle. Finally, significant negative correlations were observed with pH,

conductivity and alkalinity for most of the organic and particulate N fractions at both ponds, but not at the borehole.

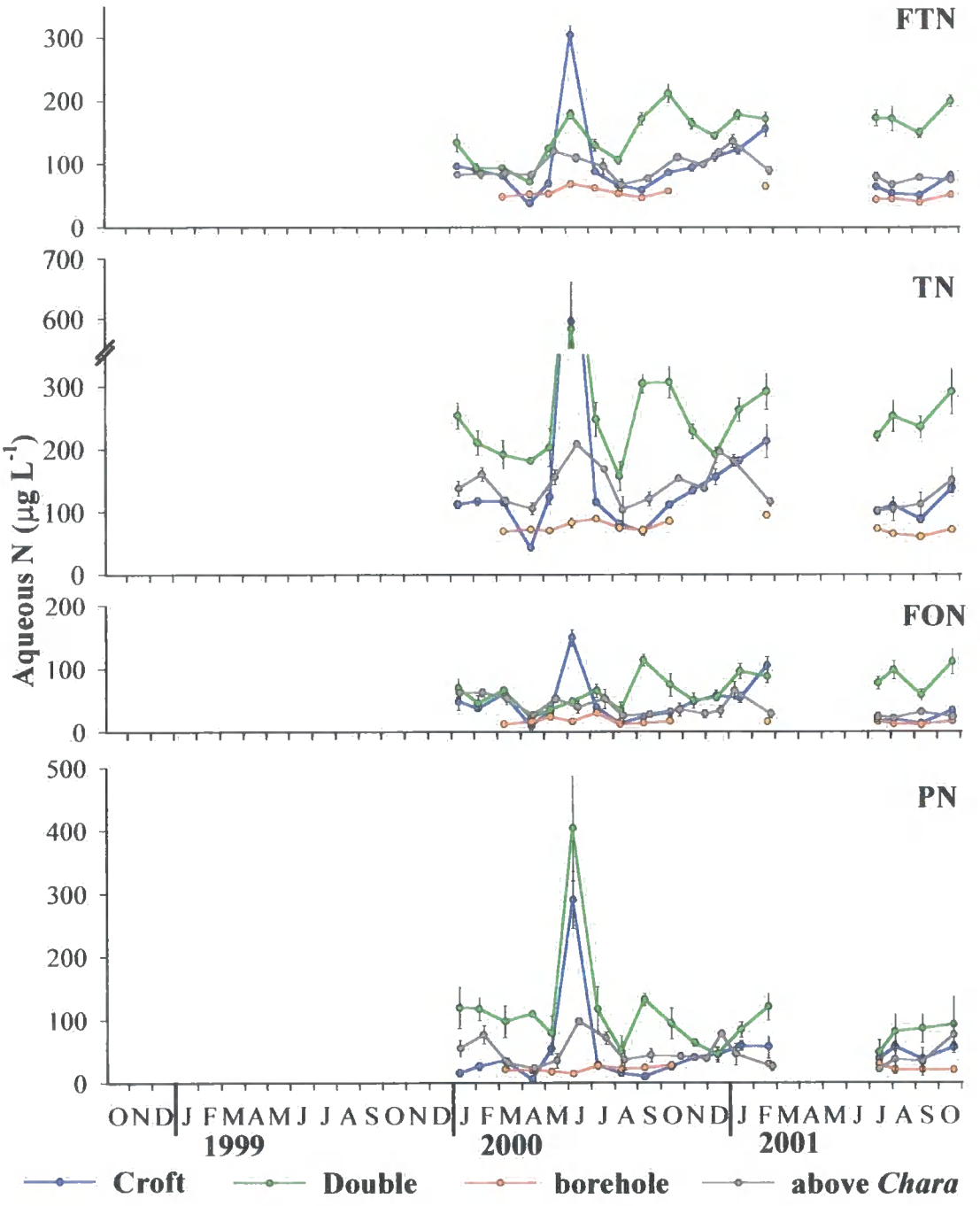

Figure 4.7 Changes in concentration of organic N fractions with time in water from Croft, Double and borehole, between January 2000 and October 2001 (mean ± SE, n = 4).

4.49 Phosphorus

Five P fractions were measured for all samples (Section 2.5), including inorganic and organic dissolved fractions, as well as the contribution from the particulate phase. Figure 4.8 shows the changes in P fractions in surface water in Croft, Double and borehole during the study period. Examples of data and general trends from this study are described, and the relationship with other important variables mentioned where relevant.

Filtrable reactive phosphorus (FRP) concentrations were very low for most of the year and sometimes close to the detection limit. FRP constituted only at about 20-35% of total phosphorus (TP) at all sites and showed a strong positive correlation with it only for Croft Kettle ($r=0.779$, $p<0.001$). FRP showed considerable variation in both ponds, but was quite stable in the borehole water and approximately two orders of magnitude lower when compared with the ponds. Mean surface concentrations of FRP were 3.6 ± 0.43 ($n=33$), 4.1 ± 0.53 ($n=30$) and 1.4 ± 0.10 ($n=19$) $\mu g\ L^{-1}$ at Croft, Double and borehole respectively. In addition, even though FRP concentrations showed considerable differences on different sample dates, no apparent seasonal patterns were observed. At Double Kettle, FRP peaked towards the end of autumn in 1999 and 2000, which can possibly be attributed to the breaking of stratification and mixing of the water column. Higher concentrations of FRP were also observed in Croft Kettle, in the surface water samples and directly above *Chara* in May and June of 1999 and in the surface water in June of 2000 after the flooding of the fields. The observed peaks in May and June of 1999 coincided with problems in P analysis of the borehole water and values for the borehole are not included for this period. These peaks will be discussed later. Paired t-test comparisons identified significant differences only between the ponds and the borehole water, but not between Croft and Double Kettle. Differences in the mean were not also identified between FRP concentration directly above *Chara* and in surface water.

Organic phosphorus (FOP=FTP-FRP) concentrations in surface water were very low for most of the year at all three sites and was the P fraction that showed the least variability. No seasonal trend of FOP concentration was apparent at any of the three sites. Mean surface concentrations of FOP were 4.3 ± 0.73 ($n=33$), 7.9 ± 1.02 ($n=30$) and 1.2 ± 0.25 ($n=19$) $\mu g\ L^{-1}$ at Croft, Double and borehole respectively. Peaks of FOP concentration occurred in summer and autumn of 2000 in both ponds and this can be related either directly (June peak in Croft) or indirectly (October peaks in Croft and

Double) to the summer flood and the subsequent breaking of stratification and mixing of waters which brought the settled organic matter into the epilimnion. In addition, a significant ($P<0.05$) difference in the mean was identified between FOP concentration directly above *Chara* (3.0 ± 0.32 μg L^{-1}, n=25) and in surface water. Finally, FOP constituted only at about 25-30% of TP and showed significant positive correlations with it at all three sites.

Filtrable total phosphorus (FTP) is composed of FRP and FOP and remained at a concentration of less than 20 μg L^{-1} for most of the year at all three sites. FTP is a potential source of P for biota, although FOP requires transformation by enzymes such as phosphatases prior to utilisation (1.6). Double Kettle showed the highest FTP concentrations and no seasonal patterns were observed. FTP's variation was caused either by FRP or by FOP concentrations and is not going to be discussed in detail. FTP was positively correlated with both FRP and FOP at both kettles and only with FOP at the borehole. In general, FTP showed stronger correlations with FOP (highest; $r=0.943$, $p<0.001$ at Double), although FOP only contributed approximately 50% of FTP at Croft and borehole and 65% at Double. FTP was negatively correlated with conductivity, pH, O_2 % saturation and alkalinity, only at Croft Kettle.

Total P is composed of both filtered and particulate P and tended to follow the trend of the dominant component. Surface concentrations at all three sites, were quite low for most of the year and usually remained at concentrations below 20 to 30 μg L^{-1}. Double Kettle showed consistently higher concentrations than Croft and the difference was approximately one order of magnitude higher. Mean surface concentrations were 15.5 ± 3.02 (n=33), 31.2 ± 2.88 (n=30) and 5.2 ± 0.71 (n=19) μg L^{-1} at Croft, Double and borehole respectively. TP reached slightly over 100 μg L^{-1} only in one occasion and that was in the epilimnion of Croft Kettle in June of 2000, after the summer flood, but then declined almost exponentially. Otherwise, TP concentrations were at their highest for the years studied at overturn in autumn. Moreover, concentrations of TP in surface water and directly above *Chara* did not show any significant differences, even though there was a mean difference of 5.7 ± 3.84 μg L^{-1}.

Finally, particulate phosphorus (PP) was always a substantial fraction of the TP (contribution ranged between 60% in Double and 45% in borehole) and followed a similar variation pattern that was also highly correlated (e.g. $r=0.975$, $p<0.001$ at Croft Kettle).

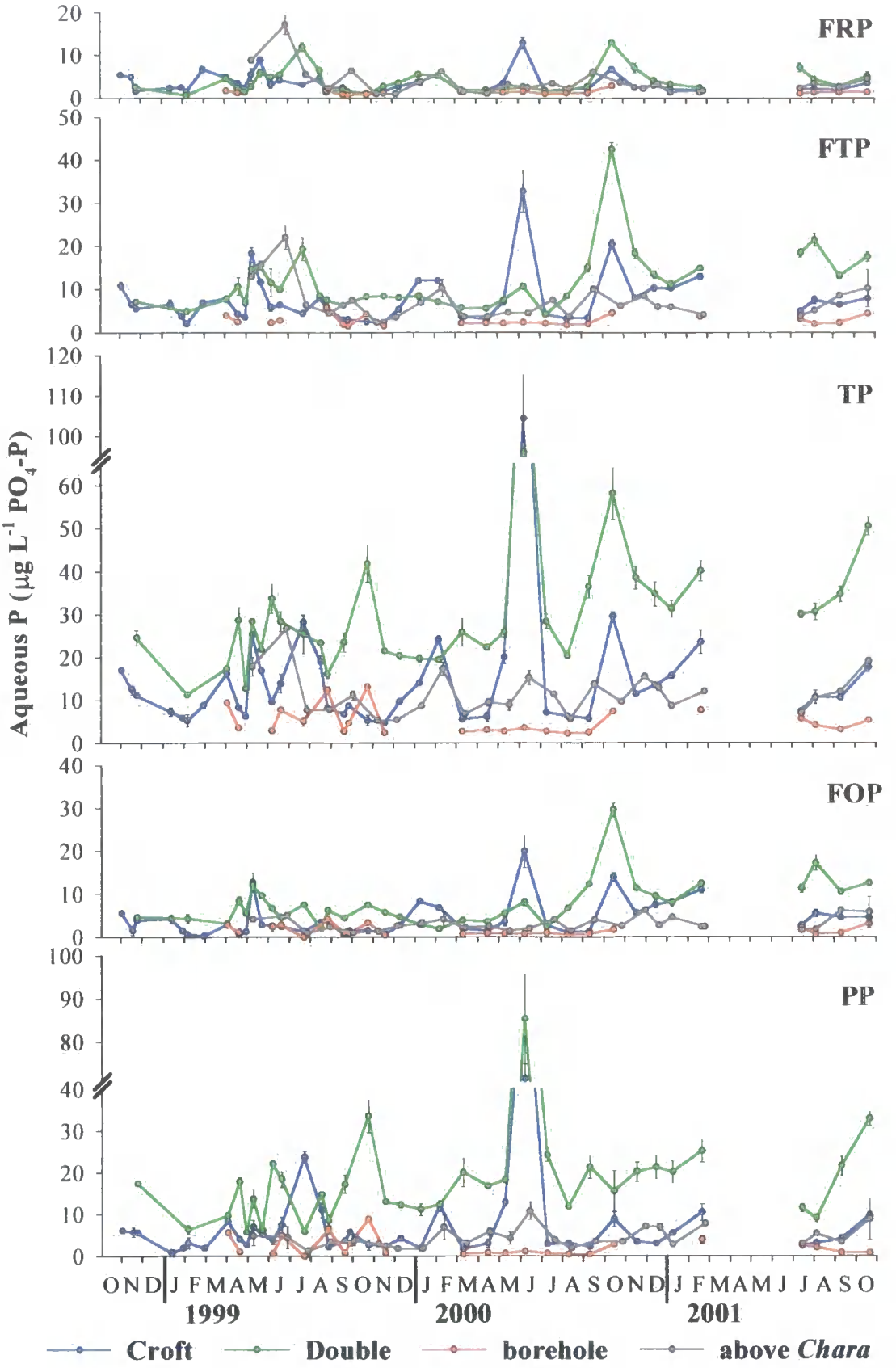

Figure 4.8 Changes in concentration of P fractions with time in Croft, Double and borehole, between October 1998 and October 2001 (mean ± SE, n=4).

4.5 Vertical profiles of temperature, oxygen, nitrogen and phosphorus

4.51 Introduction

The results for the monthly sampling of depth profiles and a number of detailed studies and spot analysis that took place only in Croft Kettle are shown in Figures 4.12-4.18. Sampling dates (Section 2.3) of the depth profile studies were usually a week after the surface ones. Examples of data and general trends from this study are described, and the relationship with other important variables mentioned where relevant.

4.52 Temperature

Temperature data-loggers were placed in the pond at two different occasions (Section 2.4) and water temperature was measured on a 3-dimensional basis. The aims of these studies were to determine the extent, duration and thickness of the thermocline and thereby extent of anoxia. In addition, possible differences of water temperature between the bottom of the north and south site of Croft Kettle, could indicate the position of the incoming groundwater.

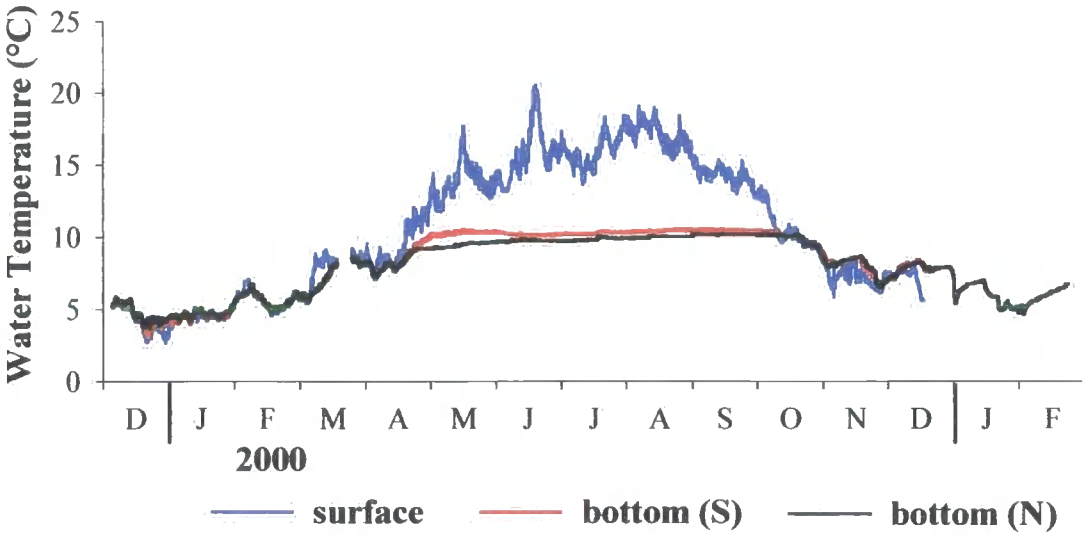

Figure 4.9 Changes in water temperature (on hourly intervals) with time in Croft Kettle between December 1999 and February 2001. Data obtained by three temperature data-loggers (Section 2.4).

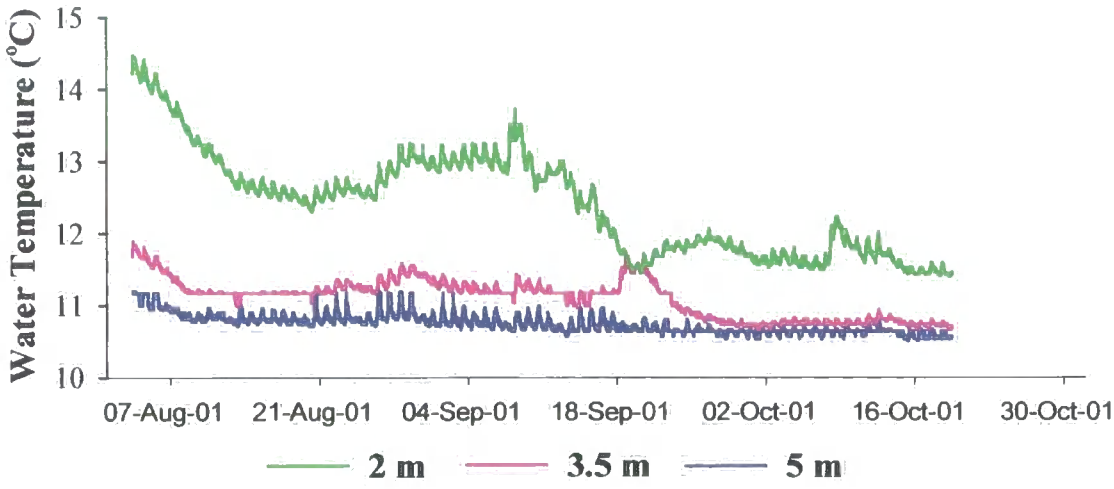

Figure 4.10 Changes in water temperature (on half hour intervals) with time in Croft Kettle between August 2001 and October 2001. Temperature data loggers were placed at different depths (depth from water surface indicated) in the middle of the pond.

Croft Kettle was isothermal at temperatures between 3 and 10 °C in winter and stratified in summer, with surface temperatures up to 20 °C and hypolimnion temperatures just below 10 °C (Figures 4.9, 4.10). The thermocline was located at a depth between 2-3.5 m (Figure 4.9). Stratification lasted from mid-April to end of October in 2000 and for the whole period that the data loggers were placed in the pond in 2001 (August to mid-October). A break of the thermocline took place at 20 September 2001 but did not lead to full mixing of the epilimnion with the hypolimnion and stratification continued. The temperature data-loggers placed on the bottom at the southern and northern extremities of Croft Kettle during 2000 (Figure 4.9) did not show any significant differences in temperature between the two logging locations for most of the year, but south site had slightly higher temperature during stratification. In addition, the detailed survey of 10 August 2000 (Figure 4.11) showed that distinct water temperature layers were formed throughout the pond and that it was not a localised effect at the middle of the pond.

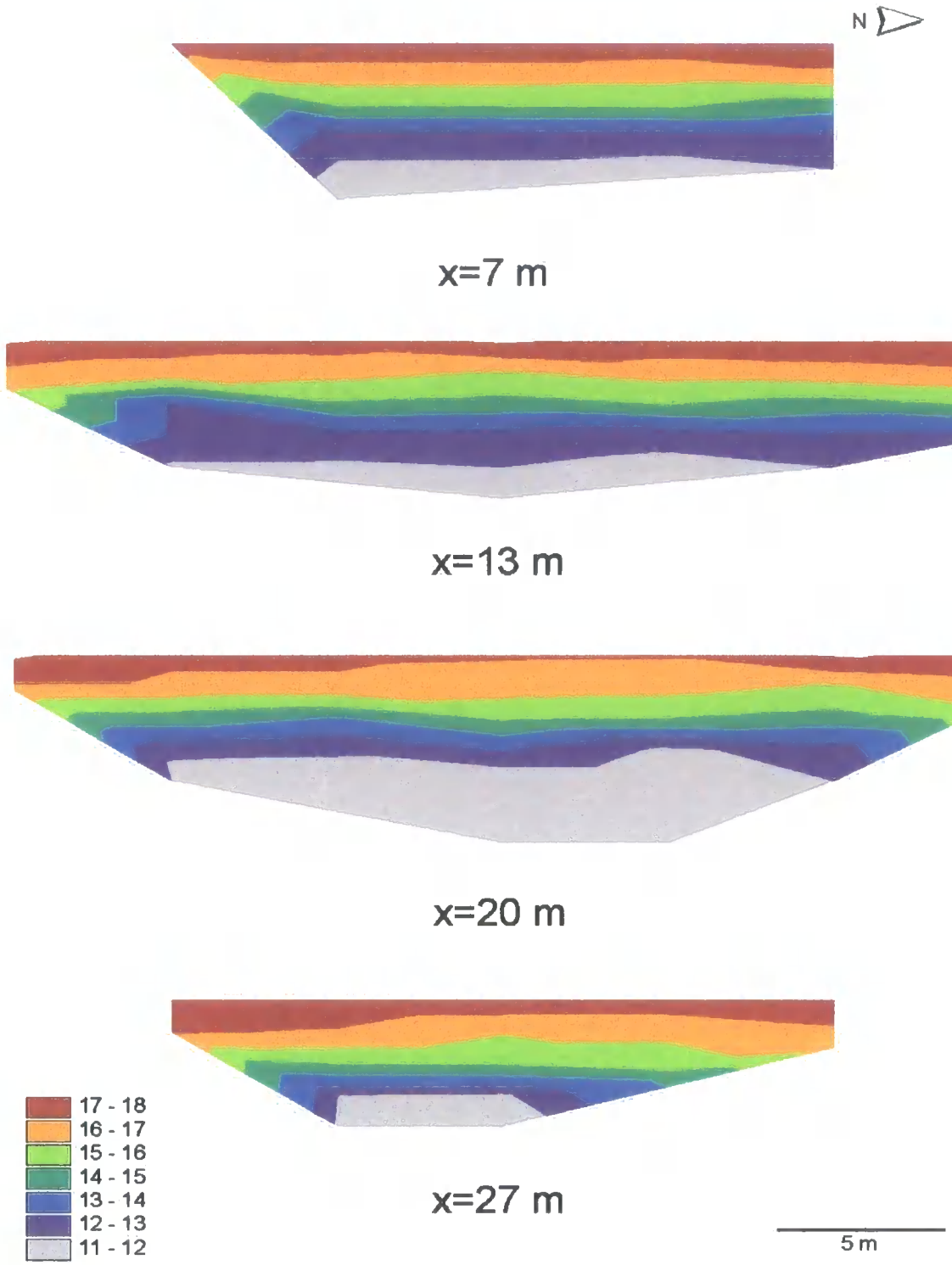

Figure 4.11 Water temperature profiles (°C) of Croft Kettle on 10 August 2000 sampled between 1100 and 1600. The four sections shown, run from south to north. Individual sampling points were made at 5-m horizontal intervals and 1-m vertical intervals.

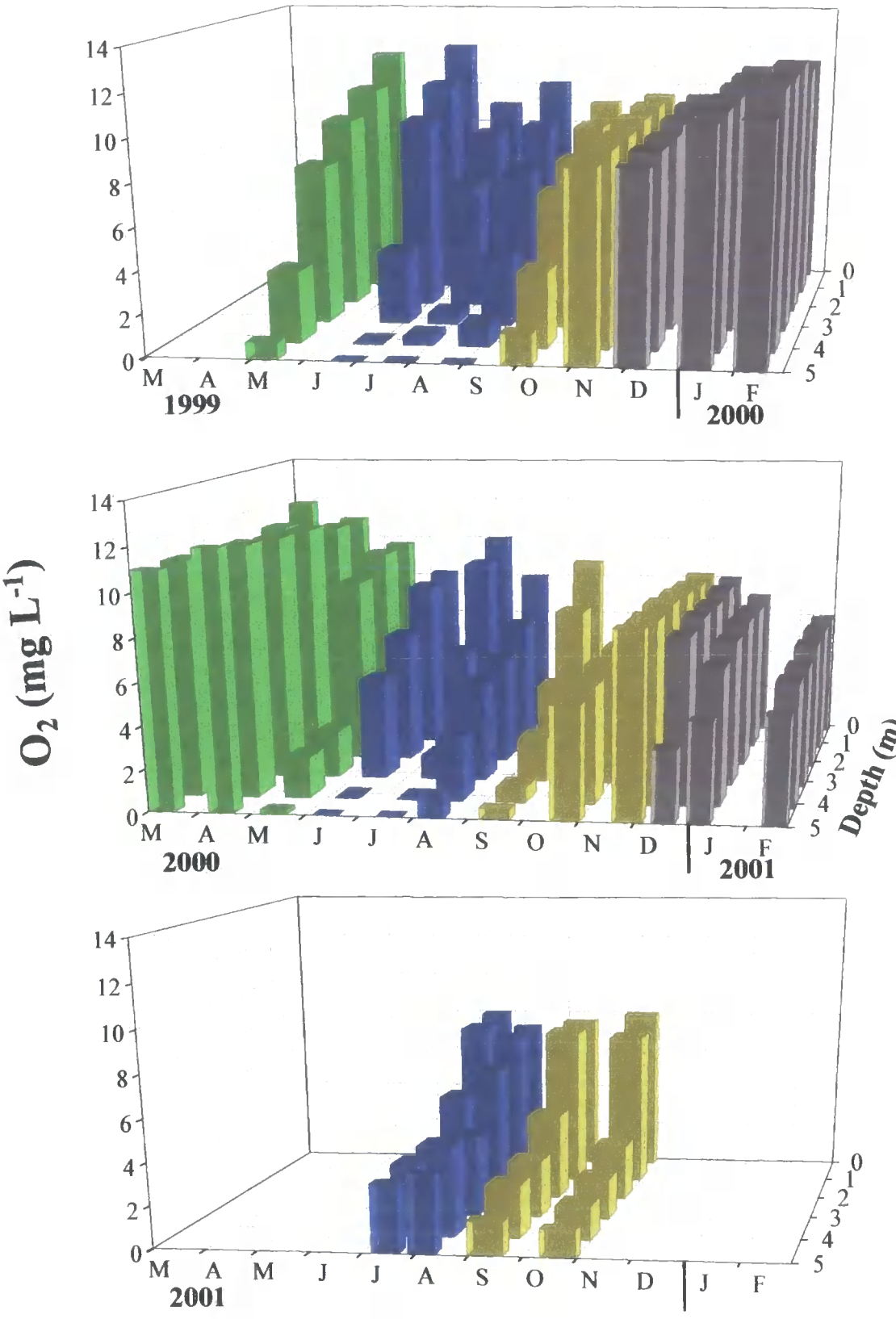

Figure 4.12 Changes in dissolved oxygen concentration with time (monthly) and depth (1-m intervals) in Croft Kettle between May 1999 and October 2001. Colours represent seasons (■-spring, ■-summer, ■-autumn, ■-winter).

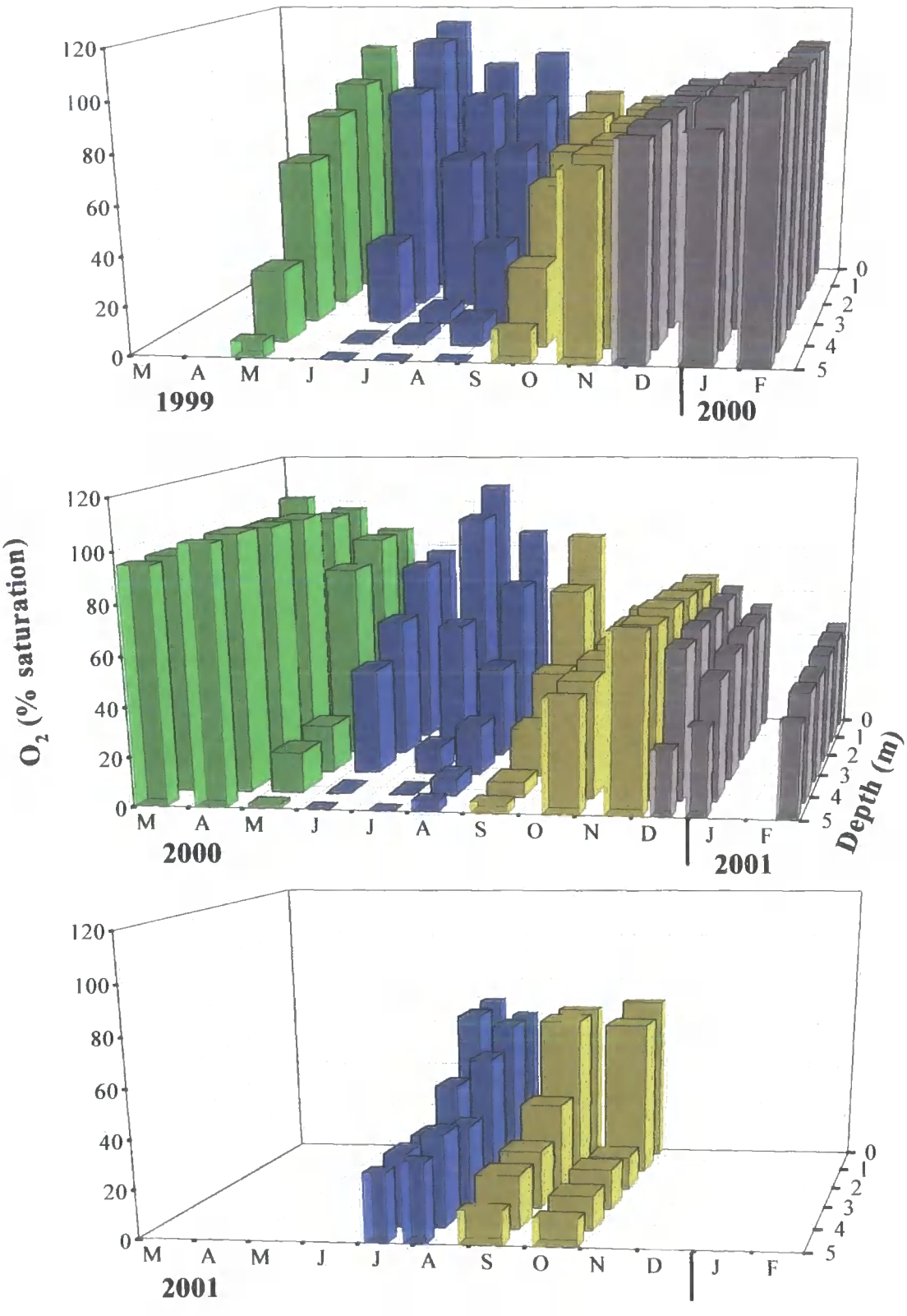

Figure 4.13 Changes in the dissolved oxygen percentage saturation with time (monthly) and depth (1-m intervals) in Croft Kettle between May 1999 and October 2001. Colours represent seasons (■-spring, ■-summer, ■-autumn, ■-winter).

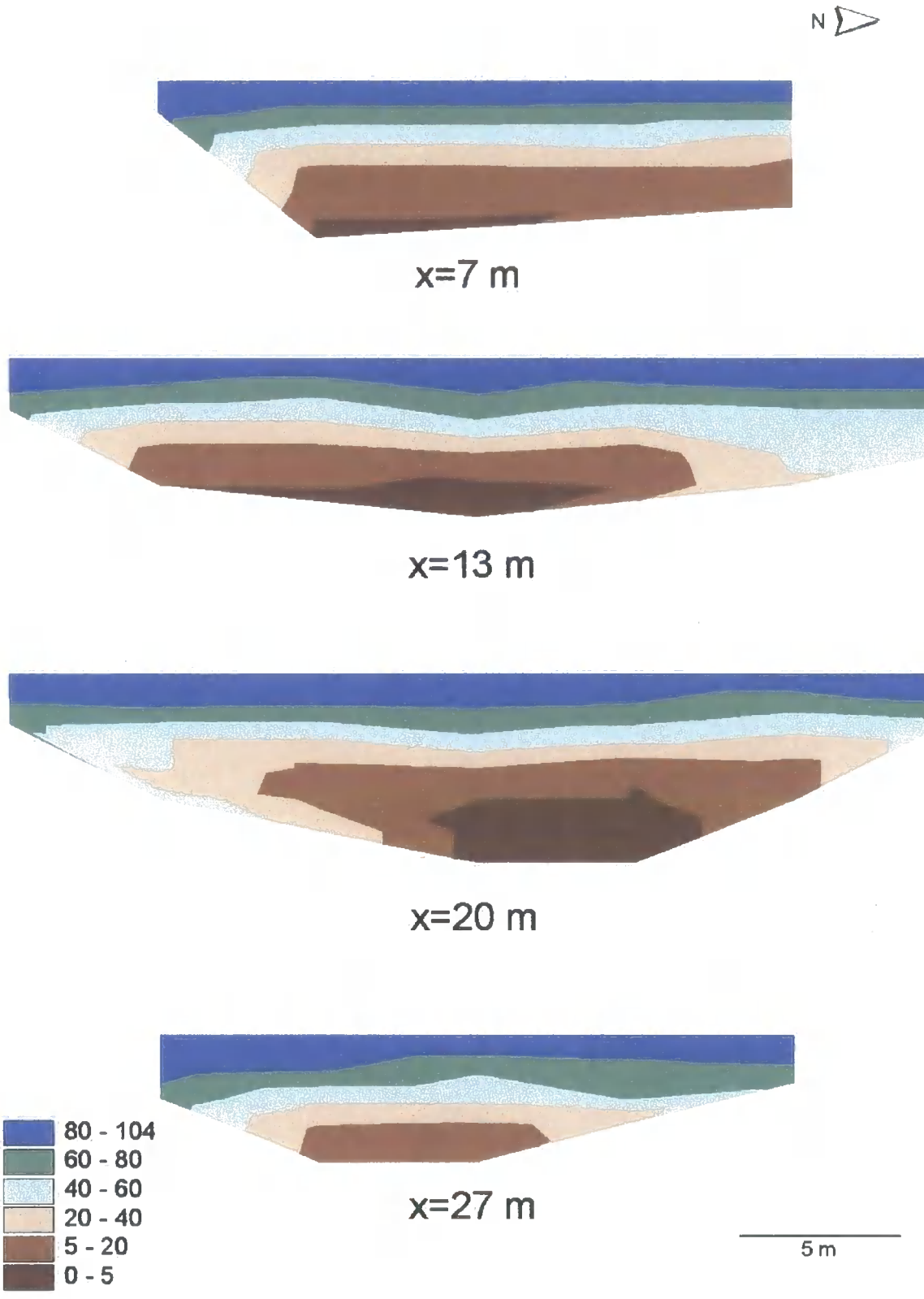

Figure 4.14 Dissolved oxygen profiles (% saturation) of Croft Kettle on 10 August 2000 sampled between 1100 and 1600. For details of sampling, see legend to Figure 4.11.

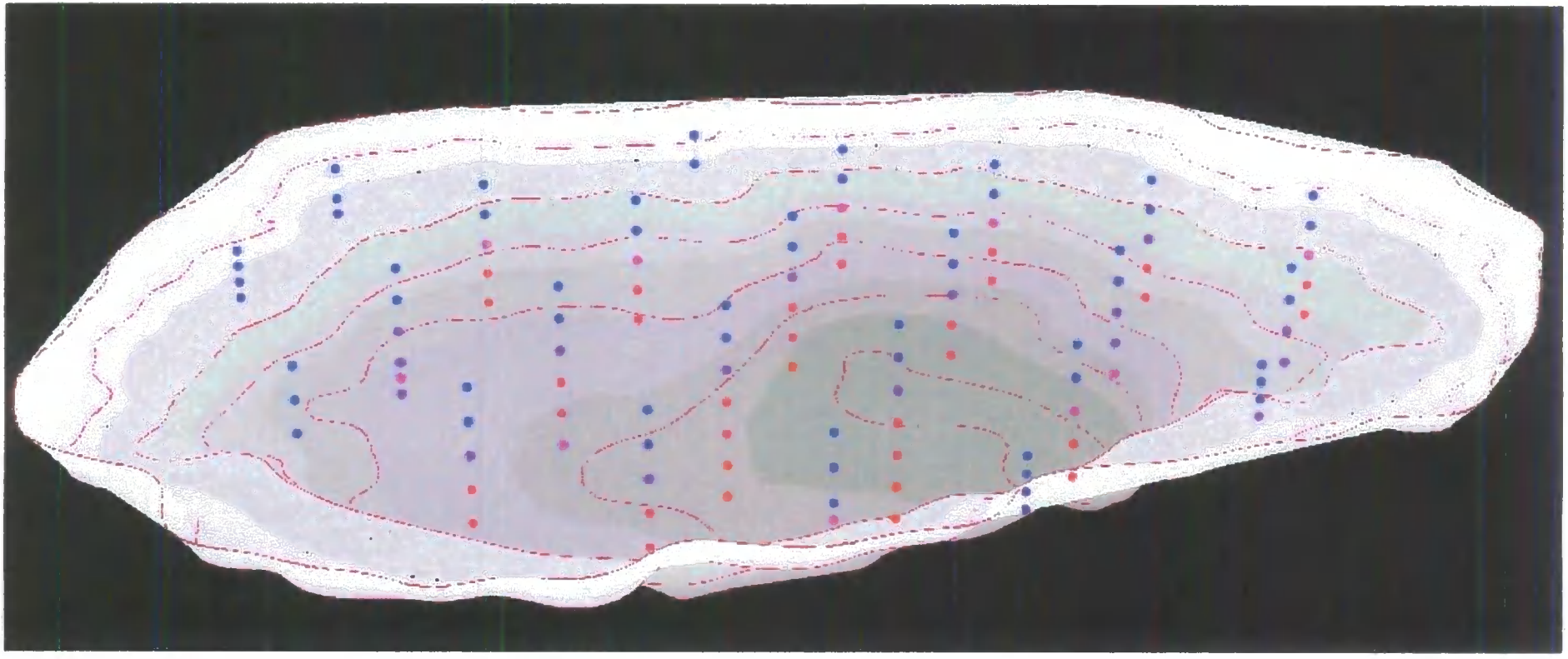

Figure 4.15 A 3-dimensionl diagram of Croft Kettle showing the oxygen concentration (mg L^{-1}) on 17 August 2000 sampled between 1100 and 1600. Individual sampling points were made at 5-m horizontal intervals and 1-m vertical intervals. Dots indicate range of oxygen concentration (● 0-2 mg L^{-1} ● 2-4 ● 4-6 ● 6-8 ● 8-10).

4.53 Dissolved oxygen

The vertical profiles (1-m intervals) of dissolved oxygen concentration were measured in the middle of Croft Kettle (water depth ≈ 5 m) by Winkler titration (2.5). Definite zones of severe deoxygenation, eventually complete anaerobiosis were maintained between late spring and mid autumn in Croft Kettle (Figures 4.12 and 4.13) in 1999 and 2000. A substantial part of the lake volume (below about 3 m) was completely anaerobic or showed severe deoxygenation in summer of 2000 (Figures 4.14 and 4.15). However, the same phenomenon was not observed at the littoral areas, particularly the areas of the pond covered by the charophyte bed, where dissolved oxygen was considerably higher than the pelagic area. Anoxia in the hypolimnion was not observed in summer 2001, when reduced levels of dissolved oxygen were detected for up to almost 1 m from the water surface, but not completely anoxic. In addition, the reduction in dissolved oxygen in February of 2001 coincided with the formation of an ice layer on the pond (Figures 4.12 and 4.13).

4.54 Nitrogen

Figures 4.16 and 4.17 show changes in the mean (n=3) of inorganic and organic N fractions with time and depth (1-m intervals) at the middle of Croft Kettle. In general, the depth profile studies of N fractions of the water column at the middle of the pond showed considerably less variation than the surface ones close to the pond edge. In addition, more and stronger correlations were observed between water column N concentrations and environmental variables, i.e. water temperature showed significant negative correlations with average water column NO_3-N ($P<0.05$) and FON ($P<0.05$) concentrations and positive with average water column NH_4-N ($P<0.01$) concentration.

NO_3-N concentration was quite similar from top to bottom with average water column concentration of 19.5 ± 1.75 μg L^{-1} (n=25), which was higher than the surface one at the edge of the pond (mean difference of at about 5 μg L^{-1}). Paired samples t-tests identified significant differences between surface ($P<0.05$) and above *Chara* ($P<0.01$) water samples. No distinct peaks were observed, particularly in comparison with surface distribution, and seasonal trends were clearer than surface ones, with concentrations higher during winter and lower during summer.

In contrast, NO_2-N and NH_4-N were considerably higher in the hypolimnion than the epilimnion during the summer period and this phenomenon was observed in both 1999 and 2000, but not in 2001. The highest difference between hypolimnion and

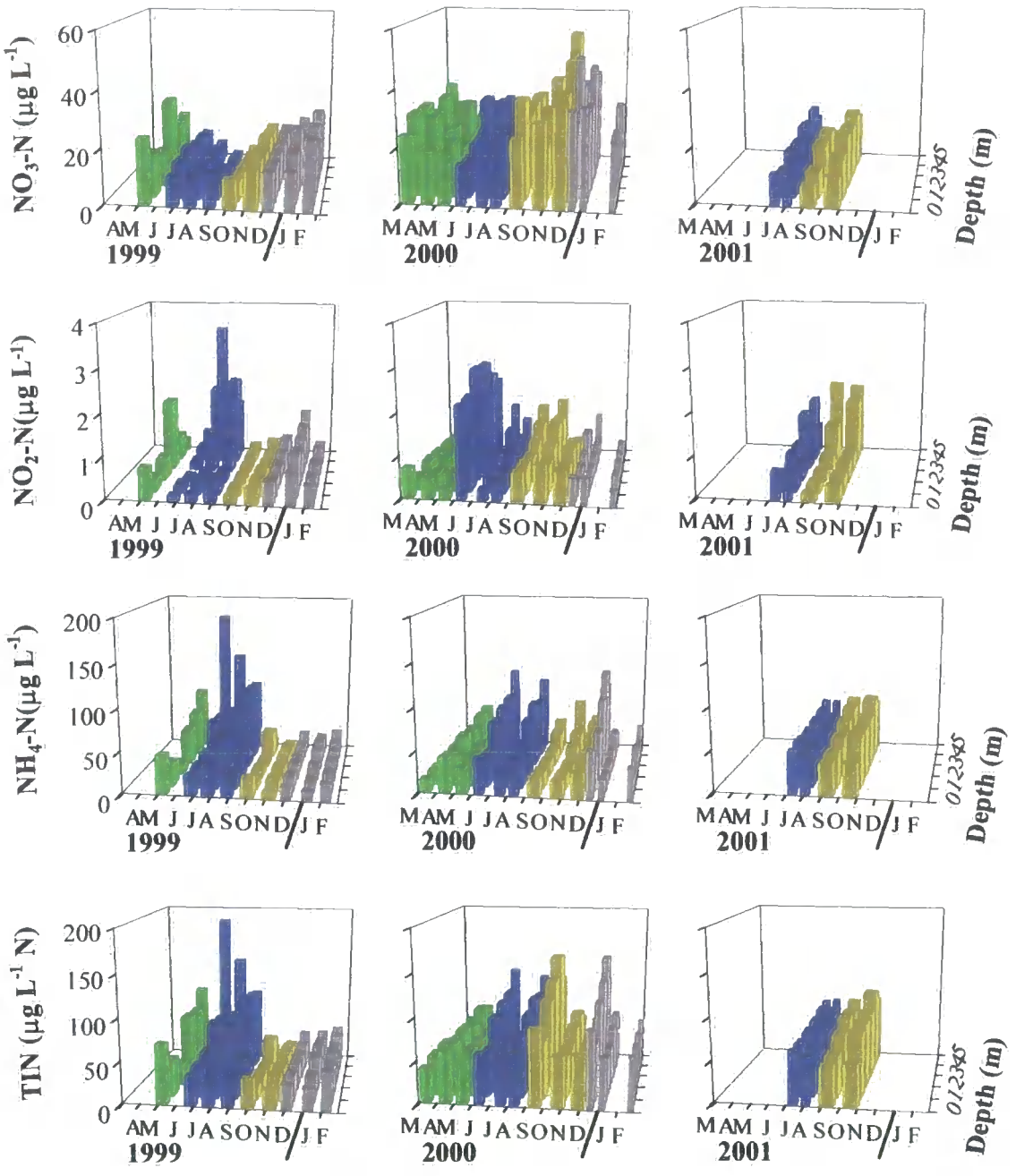

Figure 4.16 Changes in concentrations of inorganic N fractions with time and depth in Croft Kettle, between May 1999 and October 2001 (mean, n = 3). Colours represent seasons (■-spring, ■-summer, ■-autumn, ■-winter).

epilimnion concentration was observed for NH_4-N in June 1999 and was almost tenfold. Average water column concentrations were 0.7 ± 0.08 µg L^{-1} and 39.2 ± 3.82 µg L^{-1} for NO_2-N and NH_4-N respectively, which were also higher than surface ones. However, no significant mean differences were found between surface concentration, 'above *Chara*' and with depth in the middle of the pond for either NO_2-N or NH_4-N, with the exception of the 4th ($P<0.01$, $P<0.05$) and 5th ($P<0.01$, $P<0.05$) metres (p values are for

NO_2-N and NH_4-N respectively). TIN followed similar variation patterns to NH_4-N, which formed its most important contribution.

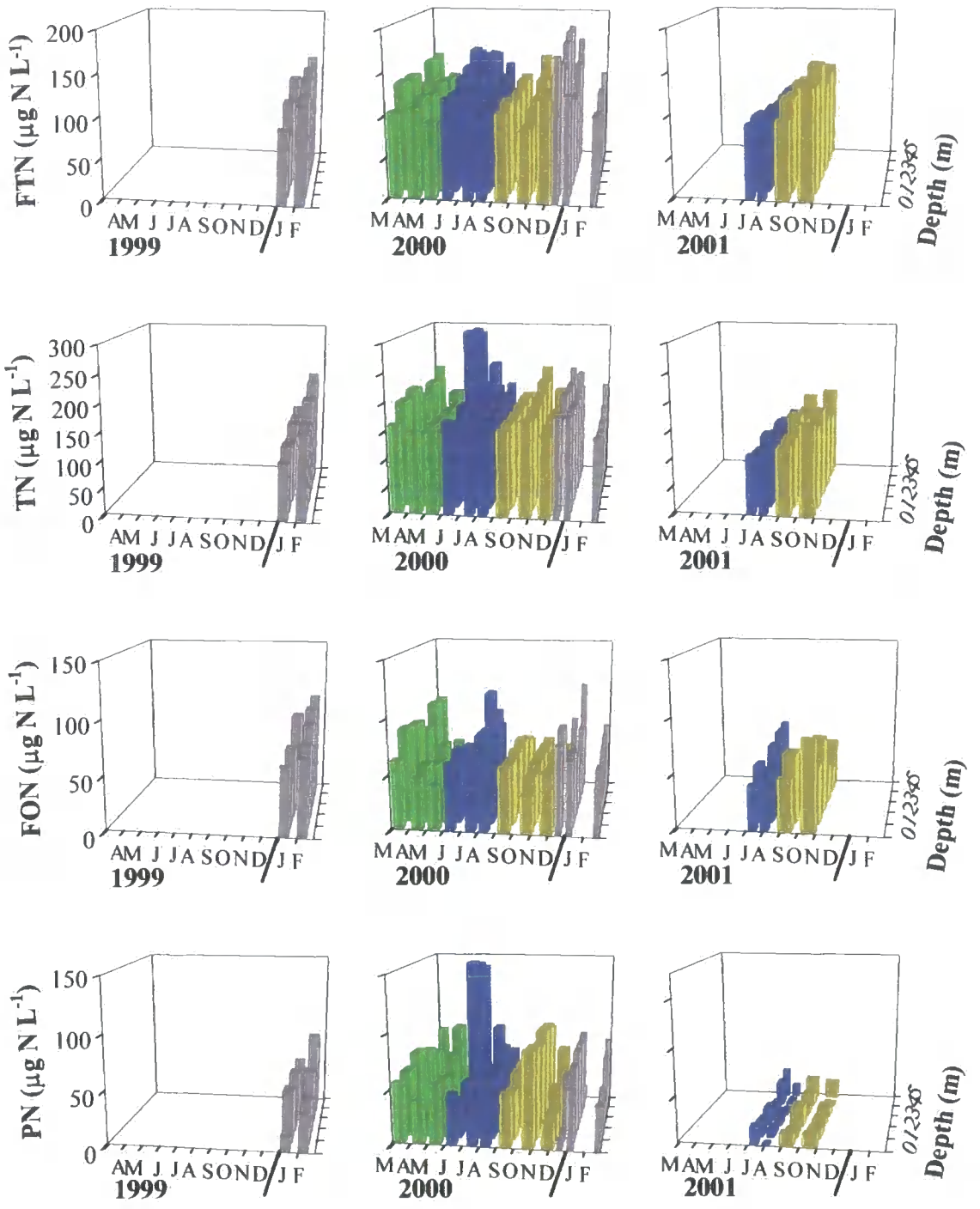

Figure 4.17 Changes in concentration of organic N fractions with depth and time in Croft Kettle, between January 2000 and October 2001 (mean, n = 3). Colours represent seasons (■-spring, ■-summer, ■-autumn, ■-winter).

The depth profile studies of organic and particulate N fractions at the middle of the pond, showed considerably less variation than inorganic N fractions, with almost uniform water column concentrations and no apparent seasonal trends. However, higher hypolimnion concentrations were also recorded at the summer of 2000, which were of particulate nature and can in turn be attributed to the summer flood, which washed a lot of particulate matter into the pond water. The June 2000 sampling occasion for the depth profile study took place at the 12th, a week after the surface one, which originally detected the input of particulate matter into the pond. The fact that no increase was observed at the surface concentration in the middle of the pond is indicative that the particulate matter had already settled in the hypolimnion and is probably the cause of the considerably higher hypolimnion concentrations.

4.55 Phosphorus

As for surface concentrations, five P fractions were measured for all samples for the depth profile studies at the middle of Croft Kettle. Figure 4.18 shows the changes of mean concentrations (n=3) of P fractions with time and depth between April 1999 and October 2001. In summary, P fractions showed slightly different concentrations to surface ones with clearer seasonal trends and considerable less variation. In addition, higher hypolimnion than epilimnion P concentrations were observed during the summer of 1999 and 2000 but not of 2001. Highest differences between hypolimnion and epilimnion concentration were approximately fourfold. Average water column FRP and FTP showed significant ($P<0.05$) positive correlations with water temperature and TP and PP negative with surface dissolved oxygen ($P<0.05$).

Average water column FRP had a concentration of 5.5 ± 0.89 μg L^{-1} which was higher than in surface waters at the edge of the pond and those measured above *Chara*, but it was not found significantly different ($P>0.05$) from paired samples t-tests. The concentrations in the water column were similar from overturn in late October until the start of stratification in May but were not constant and showed seasonal trends. FRP concentration increased in the hypolimnion in May 1999 and June 2000 and remained considerably higher than those measured in the epilimnion, until overturn (September). The highest mean concentration of the water column was recorded at a depth of 5 m and was approximately 30% higher than the other ones. FRP contributed at about 50 % of FTP and 25% of TP.

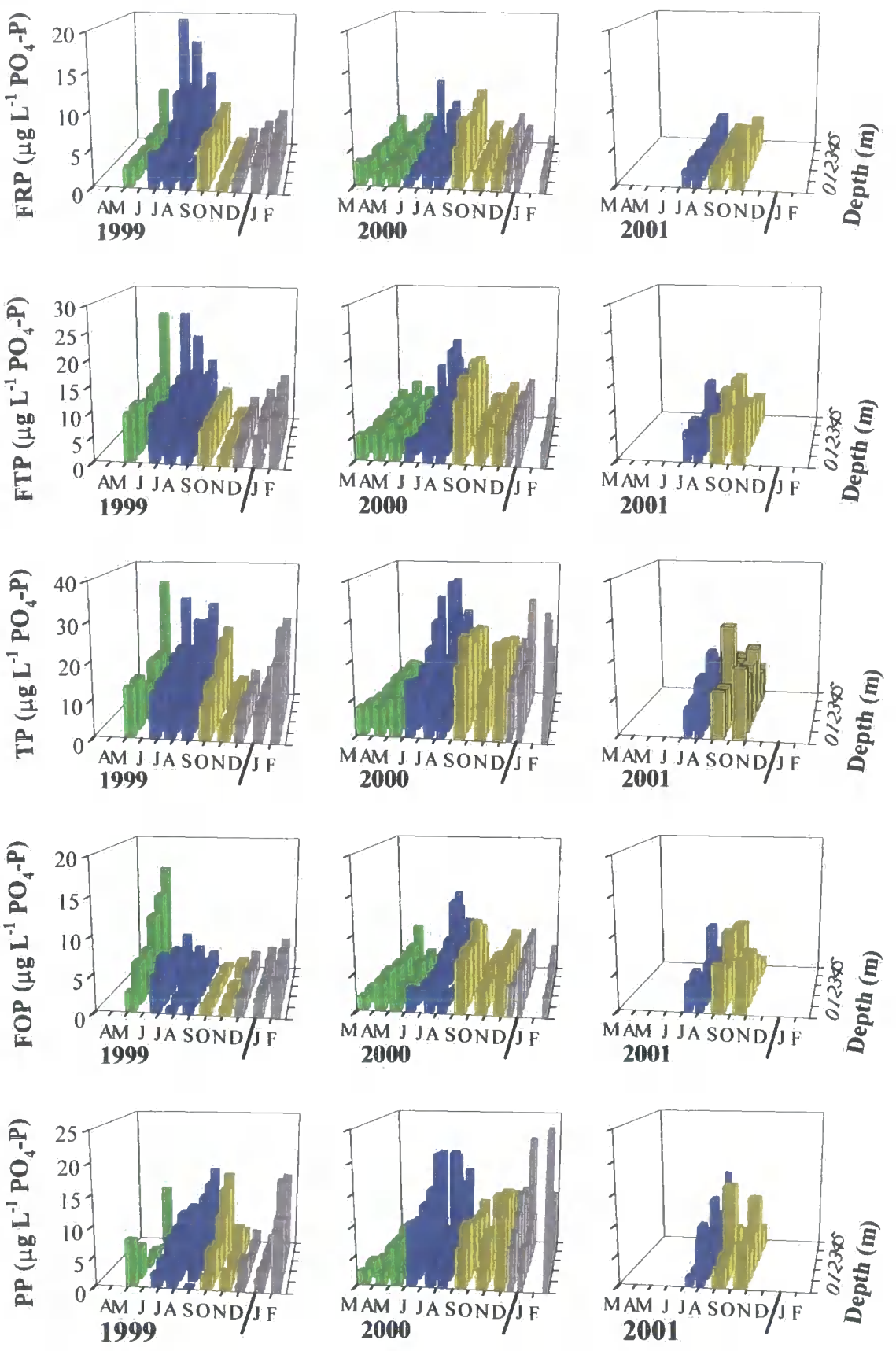

Figure 4.18 Changes in concentration of P fractions with time and depth in Croft Kettle, between May 1999 and October 2001 (mean, n=3).

FOP concentration also followed similar changes over time and depth and formed a similar quantitative contribution to FTP and TP as FRP. However, average water column FOP (3.8 ± 0.37 μg L^{-1}) was lower than that measured in surface waters at the edge of the pond, in contrast to FRP, but it was not found significantly different ($P>0.05$) from paired samples t-tests. Furthermore, the highest mean FOP concentration of the water column was found at a depth of 3 m and not at 5 m as was observed for all the other fractions.

TP and PP concentrations also followed similar changes over time and depth as FRP, FOP and subsequently FTP. Average water column concentrations were 14.2 ± 0.99 μg L^{-1} and 6.7 ± 0.71 for TP and PP respectively, which were similar to mean surface concentrations and higher than those measured above *Chara*. The highest mean water column concentrations were observed at the 5th metre and were 17.6 ± 1.82 μg L^{-1} and 8.1 ± 1.21 for TP and PP respectively. No distinct peaks were observed at the epilimnion in the middle of the pond, indicating that the peak of particulate P which was detected at 5 June 2000, at the surface water close to the edge had already settled into the hypolimnion. Finally, as for surface concentrations, PP was always a substantial fraction of TP and formed its most important contribution.

4.6 N:P ratio

High N:P ratio (approximately >10) is considered indicative of P limitation in freshwaters (Section 1.4). Four ratios; TIN:FRP, TIN:FTP, FTN:FTP and TN:TP were calculated. TIN:FRP is a ratio of dissolved inorganic nutrients, whereas TIN:FTP includes both the inorganic and organic P fractions. FTN:FTP is a ratio of inorganic and organic nutrients (potentially bioavailable) and TN:TP of total nutrients including the particulate phase too. A summary of the data is presented in table 5.2 and Figure 5.18 shows the changes with time between October 1998 and October 2001.

In general, N:P ratio ranged between 1.3 and 57.6 and showed considerable variation with time. Lower N:P ratios were observed in Croft and Double Kettle than in the borehole water and higher during winter than summer months. The second year of study (2000) showed considerably higher N:P ratios than the first year (1999) and this was attributed to higher concentrations of N. The low ratios (<10) may suggest N as well as P limitation at certain times of the year, since the concentrations of both N and P were such that either is likely to be limiting, irrespective of high or low ratios.

Table 4.2 a) Mean nutrient ratios of Croft, Double, borehole and directly above *Chara* sites between October 1998 and October 2001. b) Mean N:P ratios in the middle of Croft Kettle at 1-m intervals .

Site	TIN:FRP			TIN:FTP			FTN:FTP			TN:TP		
a)	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max
Croft	4.9	15.8	33.4	2.7	7.3	21.6	4.2	12.1	21.6	3.8	10.2	21.4
Double	2.1	22.5	51.4	1.3	6.7	15.7	5	13.4	31.5	5.4	7.8	12.8
borehole	10.3	28.3	57.6	7.4	15.7	41.7	11.6	21.9	30.3	11.5	21.7	33.5
above Chara	2	19.3	54.4	1.7	8.7	16.6	7.6	17.2	26.4	7.8	13.4	21.3
b)												
0-m	6.3	21.1	46.7	3.7	9.2	19.4	8.5	18.1	31.4	4.5	13.3	28.1
1-m	2.5	20.2	41	2.1	9.2	22.7	7.2	19.3	37.3	4.5	13.4	29
2-m	2.6	21.6	44.9	2	9.7	29.9	7.3	19.1	39.7	5.9	13.2	34.7
3-m	1.8	20.1	38.3	1.5	9.3	21.5	6.6	17.0	28	6.5	12.7	30.2
4-m	5.7	20.5	40.7	3.7	9.4	22.2	7.3	17.3	27.4	6.5	12.1	28.8
5-m	2.2	18.7	42.3	1.9	8.7	17.2	7.8	16.1	28.3	4.9	12.6	30.9
Mean		19.4			8.7		7.6	16.6			11.9	

TIN:FTP showed the lowest values and TIN:FRP the highest and that was attributed to the substantially low FRP values in the epilimnion of both ponds as well as in the borehole water. Borehole water N:P ratio was higher than the other sites for most of the year and it was the only site that all four nutrient ratios showed mean concentrations of N:P >10. Croft and Double Kettle showed similar N:P ratio, with the exception of TIN:FRP which was significantly ($P<0.01$) higher in Double and this is attributed to the very low FRP concentrations observed in Croft Kettle throughout the year. Moreover, all four mean N:P ratios, were higher in the surface water of Croft Kettle than directly above the charophyte bed. Finally, N:P ratios of the water column at the middle of Croft Kettle were considerably higher than the ratios of the water surface and above *Chara* ones at the edge of the pond and did not show much variation with depth.

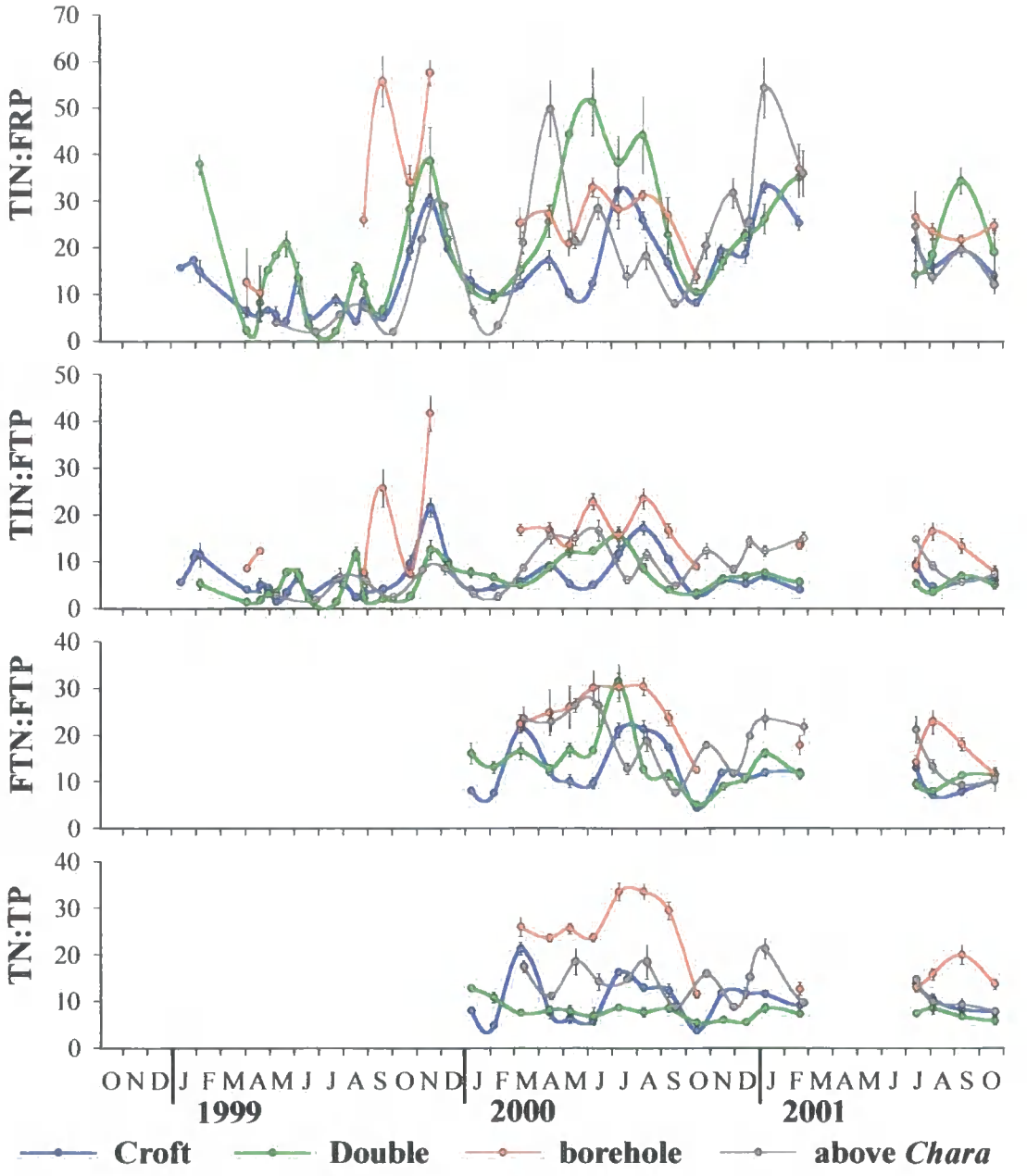

Figure 4.19 Changes in concentration of four N:P ratios with time in Croft, Double, borehole and directly above *Chara* sites between October 1998 and October 2001 (mean ± SE, n=4). Emphasis is given to Croft and Double Kettle, which are indicated with thicker lines.

4.7 Discussion

The variability in water chemistry was examined in samples taken on a monthly basis between October 1998 and October 20001 from Croft, Double and borehole, in addition to more detailed studies in Croft Kettle. Seasonal and spatial trends were evident at both ponds for many of the environmental variables and a number of strong positive and negative correlations were identified. Croft Kettle showed low concentrations of nutrients, particularly in comparison with other freshwater environments (Chapter 7) with some distinct peaks mostly in the summer. Double Kettle showed higher concentrations of inorganic, organic and particulate nutrients, most likely indicating that this pond receives higher surface runoff and field drainage as well as an internal effect from its biota (i.e. disturbance and resuspension of bottom sediments by carp). The borehole water, showed much more stable physicochemical characteristics and substantially lower N and P concentrations with no indication of seasonal variability. This, together with the fact that the O_2 concentration was low ($\approx$10% saturation) indicates that the source of water was from a deep, fairly unpolluted aquifer. Furthermore, external inputs as well as internal cycling of nutrients were evident in Croft Kettle and suspected for Double Kettle. Supporting evidence for internal regeneration of nutrients in Croft Kettle arose from the fact that nutrient concentrations were higher in the hypolimnion than the epilimnion particularly during the summer. In addition, a number of significant positive correlations were found between water temperature and NO_2-N, NH_4-N, FRP and FTP only for the mean water column concentrations and not for the surface measures, possibly indicating release from the water-sediment interface.

Surface water temperature was very similar at both ponds, showed clear seasonal patterns with winter minima and summer maxima (range from 3.7 to 19.7 °C) and was highly correlated with air temperature ($r\approx0.95$ for both sites). In contrast, borehole water showed quite stable temperature of approximately 11 °C. This most likely indicates that input of groundwater does not significantly affect the water temperature of any of the ponds. Moreover, the temperature data-loggers placed on the bottom of the southern and northern extremities of Croft Kettle during 2000 did not show significant differences in temperature between the two sites. The aim of this study was to identify the area of groundwater input. Though, due to the absence of differences a number of possibilities exist; point of groundwater inflow was not close to either of the loggers (inflow was suspected to take place at the southern end of the pond, Section 3.2), therefore a direct change could not be detected; groundwater percolated through the

sediment from a number of places, therefore changes in temperature were uniform and detected by both loggers; and groundwater inputs were comparatively low and did not affect water temperature.

The detailed studies at Croft Kettle revealed that the pond was isothermal in winter and stratified in summer (approximately from mid-May to October), with hypolimnion temperatures just below 10 °C. The thermocline was located between a depth of 2-3.5 m and extended to a considerable area of the pond, but there was no evidence that stratification included the littoral areas too, particularly the areas covered by the charophyte bed. Further evidence for the development of stratification and the localisation of the thermocline came from the depth profile studies of dissolved oxygen. Distinct layers of severe deoxygenation, eventually complete anaerobiosis, were observed in the hypolimnion of Croft Kettle at the water depth between 3 and 5 m approximately from May to October in 1999 and 2000 but not in 2001. Deoxygenation, in 1999, was much more severe than in 2000. This can either be attributed to the slightly higher water temperatures in 1999 as well as to the summer flood, which substantially changed the water characteristics of the pond, even for a short period. Comments cannot be made for the summer of 2001 since data was only obtained from July onwards. Moreover, much higher O_2 concentrations were found within and directly above the charophyte bed (40-60% saturation). However, the dissolved O_2 studies took place during the day (mid-afternoon) and is not known by how much these concentrations drop during the night when respiration reduces O_2 levels even further. In addition, the large differences of oxygen concentration between surface and deeper water within stands of *C. hispida* indicate that this dense vegetation probably reduces water movement and thus the amount of suspended sediment particles in the water. The reduction in dissolved oxygen in surface water in autumn of each year in Double Kettle provides some indirect evidence for the development of stratification in this pond too. Strong winds, even though Croft is well protected by the surrounding *Phragmites* reed, and drop in temperature are probably the main reasons for the autumn overturn.

Croft Kettle has two direct inputs of water, groundwater and precipitation (including surface runoff). On the other hand, Double Kettle receives water from a balancing pipe from Croft Kettle and from precipitation (Section 3.2). Hardness of water, as indicated by alkalinity and conductivity estimates, was higher in groundwater and lower in Double Kettle indicating that Croft Kettle receives overall higher amounts of such groundwater than Double.

The pH of the ponds and the borehole was alkaline with some indication of a seasonal pattern (only in the ponds; though diel studies would be needed to establish this) and with relatively low within-year variability, indicating that frequency of sampling is not of great concern. It was highest in summer and slightly lower in winter, possibly due to higher photosynthetic rates (Section 1.7).

Silica together with N and P are the nutrients that mostly limit primary productivity in freshwaters and silica was also determined initially on a monthly and later bi-monthly basis for a considerable period of the study. Given that silica is largely geologically-derived from weathering reactions, and is not present in significant quantities in either agricultural or sewage effluent, the most likely source is the sediments (Bennion & Smith, 2000). Mean concentrations in Croft, Double and borehole were 6.3 ± 0.12, 2.7 ± 0.2 and 5.4 ± 0.06 $\mu g\ L^{-1}$ respectively and were comparatively higher than those observed in many other temperate lakes (Section 7.2). The results indicate relatively little variation in silicate concentrations in Croft Kettle and borehole water, but considerable changes in Double Kettle (range 1.0 - 4.1 $mg\ L^{-1}$) being at their lowest in spring and summer. This pattern probably emerges because of the close association with diatom growth phases. However, levels were considerably high, particularly in comparison with N and P and silica was never depleted, indicating that silica could not be the limiting nutrient throughout the year.

Both ponds and the borehole water had generally very low N concentrations, particularly in comparison with other freshwater ecosystems (mean TN ranged between 75 and 250 $\mu g\ L^{-1}$ for all sites) with the exception of a few distinct peaks and showed quite markedly intra-annual variability. The low levels of N however were not unexpected for two main reasons; possibly due to high denitrification rates in the adjacent wetland area as well as in the ponds during the summer period when temperature increases and oxygen becomes depleted at the water-sediment interface (Section 1.4); and due to the fact that for at least the last twenty years there was no fertiliser application in the adjacent catchment area surrounding the ponds (as was agreed between English Nature and the owner of the fields, in order to protect the SSSI).

The highest concentrations of N ($\approx$ 600 $\mu g\ L^{-1}$) in both ponds were observed in 5 June 2000, after the flooding of the fields, which washed a lot of particulate mater (i.e. cattle dung) into the water, and were at least an order of magnitude higher than any other observed values. Otherwise, "high" concentrations in the surface water were

mostly observed during the autumn overturn, which transferred accumulated N from the hypolimnion to the epilimnion of both ponds. NH_4-N formed the most significant contribution to TIN with several peaks in Croft and Double Kettle, while PN and ON often contributed similar amounts to TN and were both substantially higher (≈twofold) in Double than Croft Kettle. The depth profile studies of Croft Kettle showed that NH_4-N was much higher towards the bottom during the summer stratification. It is probable that deoxygenation of the hypolimnion caused a release of NH_4-N from the bottom sediments (Section 1.4). In general, the comparatively higher NH_4-N concentrations than NO_3-N indicate that nitrate reduction rates were much higher than nitrification rates in the pond.

Croft Kettle had generally low P concentrations in the surface water (mean TP = 15.5 ± 3.02 μg L^{-1}, range= 4.7- 104.4), which remained low for most of the year with the exception of episodic events (i.e. summer flood) and during autumn overturn. The highest values were recorded during the summer, increasing notably below the oxic–anoxic boundary. The FRP concentrations were low in the epilimnion and lower than those measured in the hypolimnion. This is usually related to the development of deoxygenated conditions to the hypolimnion and release of P from the sediment-water interface (see Section 1.64). What is striking, however, is the fact that epilimnion concentrations remained at a lower order of magnitude than that in the hypolimnion until the autumn overturn. This is indicative of a relatively strong stratification and high water column stability. Furthermore, the peaks which were observed particularly in Croft Kettle, at the 4th of June 2000 and at autumn overturn decreased very quickly, almost exponentially. P concentration dropped fast and low concentrations were observed at the 12 of June during the diving- sampling occasion. This can be attributed to the hard nature of the water with calcium carbonate, which has the ability to precipitate P to the sediment. FOP and PP were very low too, indicating the absence of bulk organic material in the water column. P concentrations, that were also measured above the charophyte bed in Croft Kettle were slightly lower than the surface water but significant differences were found only for FOP ($P<0.05$), possibly indicating (hypothesis) that either FOP was utilised by phosphatase enzymes produced by *Chara* or that allelopathic substances, produced by *Chara* reduced phytoplankton growth close to the bed (see Sections 1.6 and 1.7).

Double Kettle had overall higher TP concentration than Croft Kettle (mean TP = 31.2 ± 2.88 μg L^{-1}, min= 11.3, max= 96.1) and did not show any apparent seasonal

patterns. Higher values were observed at mid-autumn when the mixing of waters occurs and in May, possibly indicating release from the sediment (pure speculation).

Borehole water had generally low P content and showed very little variability. The water samples from the borehole were sometimes contaminated with soil particles as a result of the disturbance caused by removal, and this could be the cause for the variability observed in both N and P fractions. Therefore, it is believed that the reported variability could be due to sampling error. In addition, the water chemistry caused problems in the analysis of P during the May-July period of 1999 and most of the summer results have been removed from this data set. (FRP concentrations were found to be higher than FTP and TP concentrations.) Personal communication with J. Lamont-Black, a geologist from Newcastle University (Section 3.2), who was sampling Croft Kettle and the borehole water at the same time, indicated that there was an unusual contamination of the aquifer, mostly of organic nature. It is believed that this caused a positive interference in the analysis of P, mostly of FRP, which was not subjected to further treatment (digestion) before analysis. The same phenomenon was also observed in Croft Kettle but not in Double for almost the same period and the samples had to be diluted before rational results were obtained. Thus, the increase that was observed both in the hypolimnion and the epilimnion of the pond at that period have not been discussed in detail in this section, since it could either be due to the contamination of the groundwater or due to release from the sediments which reached the epilimnion or analytical error.

Finally, N:P ratio was calculated for each sampling event within the context of total nutrient concentration to determine N and/or P limitation. Four N:P ratios were estimated in order to include inorganic, organic and particulate fractions, since both inorganic and organic fractions can become bioavailable through enzymatic processes. N:P ratio showed considerable variation, but with no apparent seasonal trends. Low N:P ratio (<10) was identified at different times of the year at both ponds mostly in 1999 when N concentrations were overall lower than 2000. However, concentrations of both N and P were such that either is likely to be limiting, irrespective of high or low ratio. The highest N:P ratio was found in the borehole water, possibly indicating that substantial amounts of N are lost probably by denitrification or that P concentrations were increased through internal cycling.

4.8 Summary

1. Croft Kettle was isothermal at temperatures between 3 and 10 °C in winter and stratified in summer (approximately May to October of 1999 to 2001), with surface temperatures up to 20 °C and hypolimnion temperatures just below 10 °C. Double Kettle showed similar surface water temperatures to Croft and stratification was suspected. Borehole had a stable water temperature of approximately 11 °C.
2. Hardness of water, as indicated by alkalinity and conductivity estimates, was higher in groundwater and lower in Double Kettle indicating that Croft Kettle receives overall higher amounts of groundwater than Double.
3. pH at Croft and Double Kettle was alkaline with relatively low within year variability. Borehole water was neutral.
4. Distinct layers of severe deoxygenation, eventually complete anaerobiosis were maintained between late spring and mid autumn in the hypolimnion of Croft Kettle in 1999 and 2000 and suspected for Double Kettle, but the same phenomenon was not observed in 2001. DO in water from the borehole was stable and low.
5. SiO_2-Si showed little variation in Croft and borehole water, but showed considerable inter-annual changes in Double Kettle. However, SiO_2-Si levels were considerably higher than N and P, indicating that it is likely that SiO_2-Si concentrations are not limiting primary productivity.
6. Surface TIN and TP levels in Croft Kettle were usually less than 40 and 15 μg L^{-1}, with the exception of episodic events and during autumnal circulation.
7. Higher values of N and P, particularly NH_4 and FRP, were recorded in the hypolimnion compared with the epilimnion of Croft Kettle during the summer and suspected for Double Kettle due to their increase in concentration after the autumnal circulation.
8. Double Kettle had overall higher N and P concentrations than Croft, particularly of fractions of organic and particulate nature.
9. Borehole water had low N and P content and the variability shown is believed to be due to contamination with soil particles as a result of the disturbance caused by abstraction.
10. High (>10) and low (<10) N:P ratio (see Section 1.4) was identified at different times of the year at both ponds indicating periods of P and N limitation. However, N and P concentrations were such that either is likely to be limiting, irrespective of high or low ratio.

CHAPTER 5 Phosphorus in sediments

5.1 Introduction

The water chemistry of Croft Kettle indicates that the pond has overall low concentrations of inorganic and organic P for most of the year (Section 4.4). However, the formation of a thermocline and the depletion of oxygen from a considerable part of the hypolimnion coincided with higher P concentration in the hypolimnion than the epilimnion during both summers of 1999 and 2000 (Section 4.5). It is generally accepted that hypolimnetic anoxia is strongly associated with dissolved P release from the sediments (Sediment 1.64). Sediments act both as sinks and sources of P in the water, with the dynamics influenced by the forms in which P occurs, the Eh ("redox") potential, the chemistry of the overlying water and other environmental factors such as temperature and oxygen (Section 1.64). In view of this close relationship between development of anoxia and P release from sediments, it was decided to assess whether the observed P increase in the hypolimnion during summer periods and in the whole pond during autumn overturn, was due to P being released from the sediment.

A monthly survey of the depth profile of surface (0-10 cm) sediments over one year was planned. This would reflect the seasonal changes on spatial and temporal scales. A sequential P fractionation scheme was carried out using the method developed by Psenner & Puczko (1988) together with digestions of sediment for total N and P. Sediment cores were taken from a point near that indicated at site 6 (Figure 3.5). The water depth was 5 ± 0.2m. The sediment at this position was devoid of macroflora and infauna. The unvegetated zone was characterised by highly reducing sediments, periodically covered by *Spirogyra* mats.

The objective was to examine the horizontal distribution and temporal variation of P forms in surface sediments of Croft Kettle and to estimate release potentials and P saturation levels. The chapter is separated into three sections:

1. Experimental variation of the techniques used;
2. Temporal and spatial variability of total N and P content and N:P ratio;
3. Temporal and spatial variability of P fractions.

The research reported here is the first to my knowledge to investigate both temporal and vertical variability in sediment P speciation.

5.2 Determination of analytical variation

5.21 Digestions for total nitrogen and phosphorus

To test the precision and reproducibility of the digestion technique, four control (Section 2.7) sediment samples were digested at the end of each digestion run. The N and P content (expressed as μg of N or P per g dry weight of sediment) and the N:P ratio was determined.

The N content of Croft Kettle's surface air dried sediment (control) was 8106 ± 219.7 μg N g^{-1} of d.wt. (mean ± SE), the P content was 772 ± 21.7 μg g^{-1} d.wt and the N:P ratio was 10.6 ± 0.29 (Figure 5.1). Analysis showed considerable analytical variation with the relative standard deviation (RSD) being at the level of 17-18%. However, analysis of P was more variable than for N. N:P ratio showed the highest variance since it is an indirect measure derived from the other two and the effect is additive.

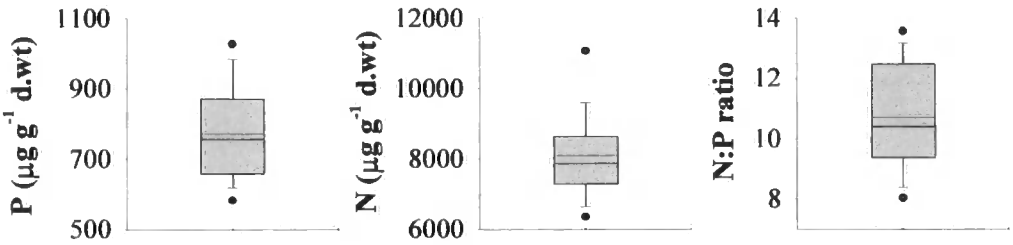

Figure 5.1 Box plots of N and P content and N:P ratio of Croft Kettle control sediments (n=40). The median values are presented as black lines and the mean in blue. The boxes indicate the 25th and 75th percentiles, the error bars indicate the 10th and 90th percentiles and the dots the 5th and 95th percentiles, respectively.

5.22 Extractions of phosphorus fractions

Analysis of control sediments was also introduced for the sequential extraction method on a regular basis. Four replicate samples were analysed simultaneously with the monthly sediment samples between May 2000 to February 2001, giving a total of 40 samples.

Sequential analysis of P fractions in control sediments (Figure 5.2) showed considerable analytical variation, notably for FRP and FTP in H_2O-P. FOP was the P

species with the highest % error, since it is an indirect measurement (FTP-FRP). Average RSD levels for the majority of the fractions and species were between 20 and 30%. The total-P content of the sediment is an indirect estimation and is the sum of all the other fractions. This showed the least variation for FRP, FTP and FOP.

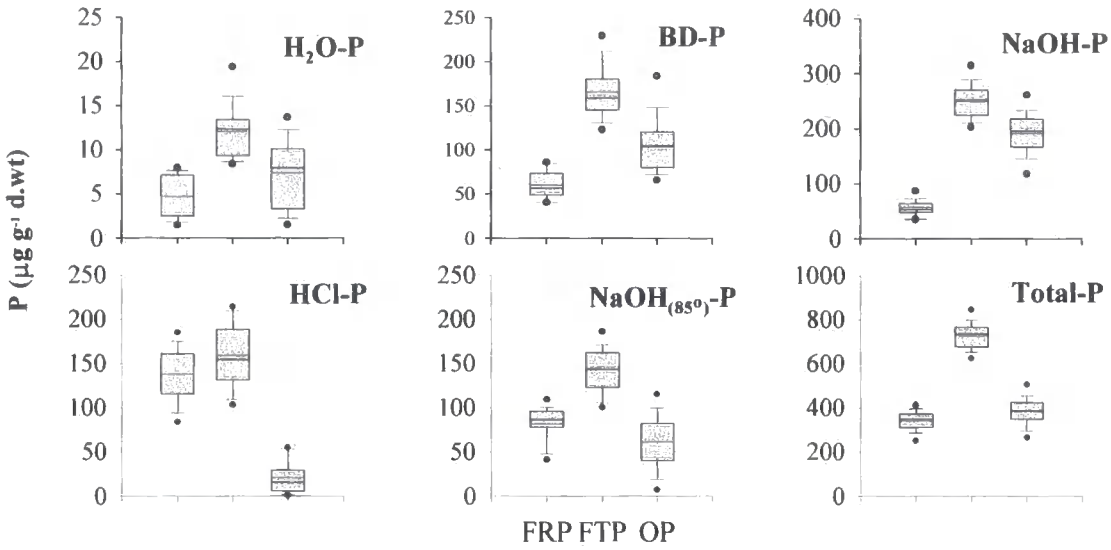

Figure 5.2 Box plots of FRP, FTP and FOP species of all P fractions of Croft Kettle control sediments (n=40). The median values are presented as black lines and the mean ones as blue. The boxes indicate the 25th and 75th percentiles, the error bars indicate the 10th and 90th percentiles and the dots the 5th and 95th percentiles respectively.

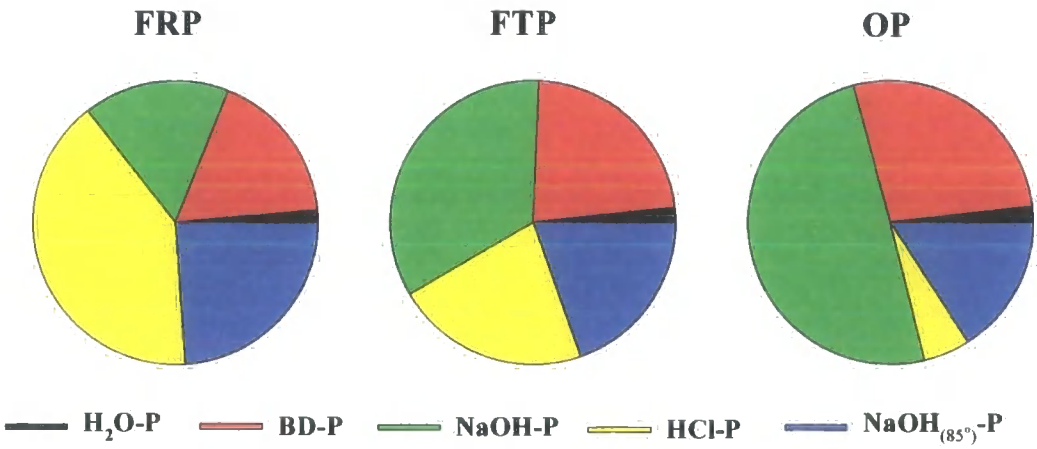

Figure 5.3 Pie charts showing the distribution of P fractions in the control sediments for the three different P species (FRP, FTP, FOP).

H_20-P was the smallest P fraction in the sediment representing 1.4, 1.7, and 1.9% of FRP, FTP and FOP respectively (Figure 5.3). The dominant FRP fractions were HCl-P (40.5%) and $NaOH_{(85°)}$-P (23.9%). FTP fractions were more evenly distributed with

BD-P (22.7%), NaOH-P (34.1%), HCl-P (21.8%) and $NaOH_{(85°)}$-P (19.7%). FOP content was dominated by the NaOH-P (49.6%) and BD-P (27.2%). FOP in the HCl-P fraction was very low (5.4%) indicating that the 0.5 M HCl solution extracts mainly inorganic P.

5.23 Comparison between phosphorus digestions and extractions

The sum of sequential P fractions should equal the total P determined by digestion methods since total digested P is assumed to be less subject to errors than summing up the different fractions measured separately. Total-P was 772 ± 21.7 and 729 ± 10.4 μg g^{-1} d.wt as obtained by digestion and extraction respectively (Figure 5.4). The mean difference (total P minus P fractions) was 5.5% and generally below ± 10% of TP, which is considered an average limit by Psenner & Puczko (1988). In addition, P extractions of control sediments showed overall less variation than sediment digestions but no significant correlation was found between them.

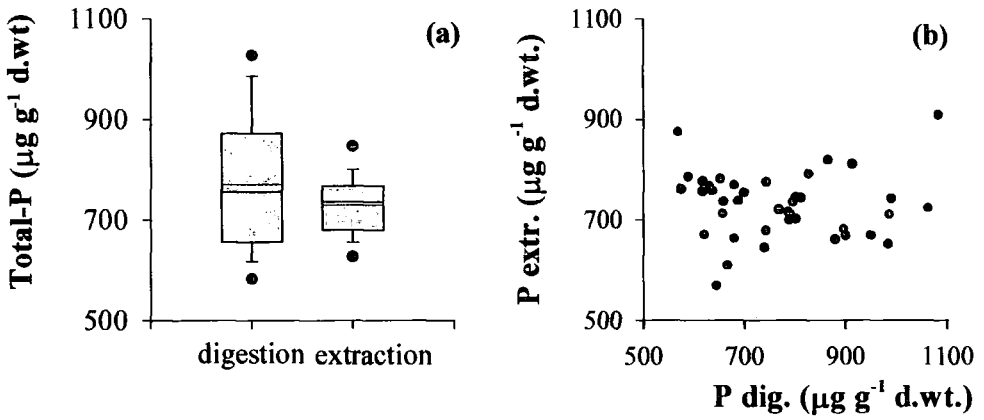

Figure 5.4 The total-P content in control sediments from Croft Kettle measured by Kjeldahl digestion of the sediments and summing up all the P fractions of the sequential extraction; (a) box plots indicating the variance of the methods and (b) the relationship between total-P measured by the two methods.

5.3 Total nitrogen and phosphorus content

The total N and P contents of air dried environmental samples was determined by Kjeldahl digestions on a monthly basis and the N:P ratio was determined. Due to the high variation, three subsamples of each core section (n=4) were analysed which gave 12 samples for each month between March 2000 and February 2001. In total 144

samples per depth section were determined and included analytical (x3) and spatial (x4) replication.

Table 5.1 Annual mean total N and P concentrations and N:P ratio of Croft Kettle surface sediments between March 2000 and February 2001. Total N and P are presented as $\mu g\ g^{-1}$ d.wt.

	Section	Mean	SD	SE	Min	Max
N	0-2 cm	9133	664.7	191.9	8494	10350
	2-4 cm	7819	738.4	213.2	6287	9080
	4-7 cm	7070	827.3	238.8	6190	8526
	7-10 cm	7034	1232.2	355.7	5520	8733
P	0-2 cm	883	80.1	23.1	770	990
	2-4 cm	748	110.1	31.8	580	881
	4-7 cm	657	112.2	32.4	498	845
	7-10 cm	692	136.1	39.3	505	887
N:P	0-2 cm	10.4	0.71	0.20	9.0	11.4
	2-4 cm	10.6	0.76	0.22	9.1	11.7
	4-7 cm	11.0	0.73	0.21	9.5	12.6
	7-10 cm	10.6	1.25	0.36	8.5	13.5

The uppermost section of sediment had the highest content (9133 ± 191.9 and 883 ± 23.1 $\mu g\ g^{-1}$ d.wt) of N and P respectively (Table 5.1). The other sections followed with a descending order according to depth with the exception of the P content of the 7-10 cm section, which was slightly higher (692 ± 39.3) than the 4-7 cm section (657 ± 32.4). The highest difference was found between the first two sections and that was 14.4% (1313 $\mu g\ g^{-1}$ d.wt) and 15.2% (134 $\mu g\ g^{-1}$ d.wt) for N and P respectively. The mean N:P ratio was close to 11 and did not show any significant variation with depth since both N and P content showed relatively similar decrease with depth. In addition, total N and P contents of all sediment sections showed considerable variation throughout the year.

The uppermost sediment section was found to be significantly different from all the other sections for both N and P but not for N:P ratio (Table 5.2). More significant ($P < 0.001$) differences were found between the 0-2 cm section and the two deepest (4-7 and 7-10 cm) for both N and P. The 2-4 cm sediment section, even though significantly different from the 0-2 cm one was only to the $P < 0.01$ level for N and $P < 0.05$ level for P. The 2-4 cm section also failed to show significant differences with the deeper sediment sections. Finally the P values for the last two sections were very close to 1, indicating that these sections have similar N and P contents.

Table 5.2 ANOVA p-statistics from comparisons of N, P and N:P ratio of sediment sections. The probability values are shown at 95 % significance level as *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, ns: not significant.

		0-2 cm	2-4 cm	4-7 cm
N	2-4 cm	**		
	4-7 cm	***	ns	
	7-10 cm	***	ns	ns
P	2-4 cm	*		
	4-7 cm	***	ns	
	7-10 cm	***	ns	ns
N:P	2-4 cm	ns		
	4-7 cm	ns	ns	
	7-10 cm	ns	ns	ns

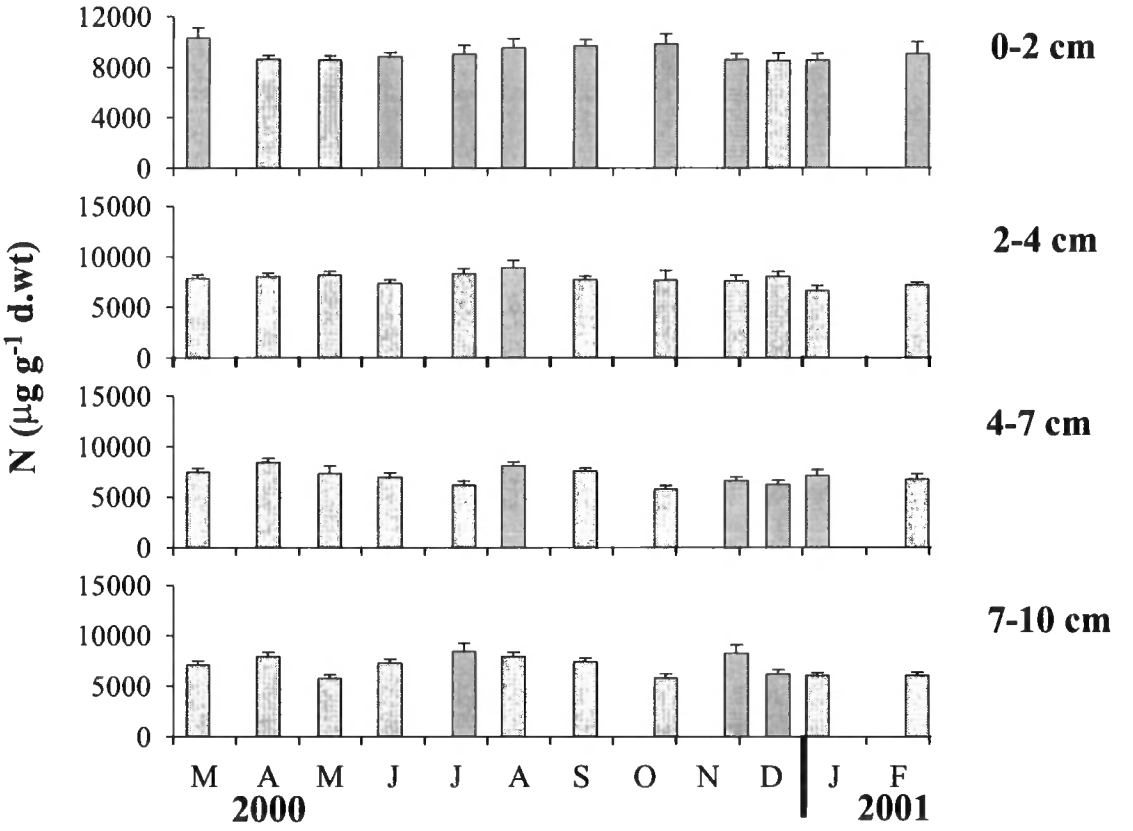

Figure 5.5 Changes in total N with time in surface sediments from Croft Kettle between March 2000 and February 2001. Vertical bars indicate the mean (n=12) and error bars one SE.

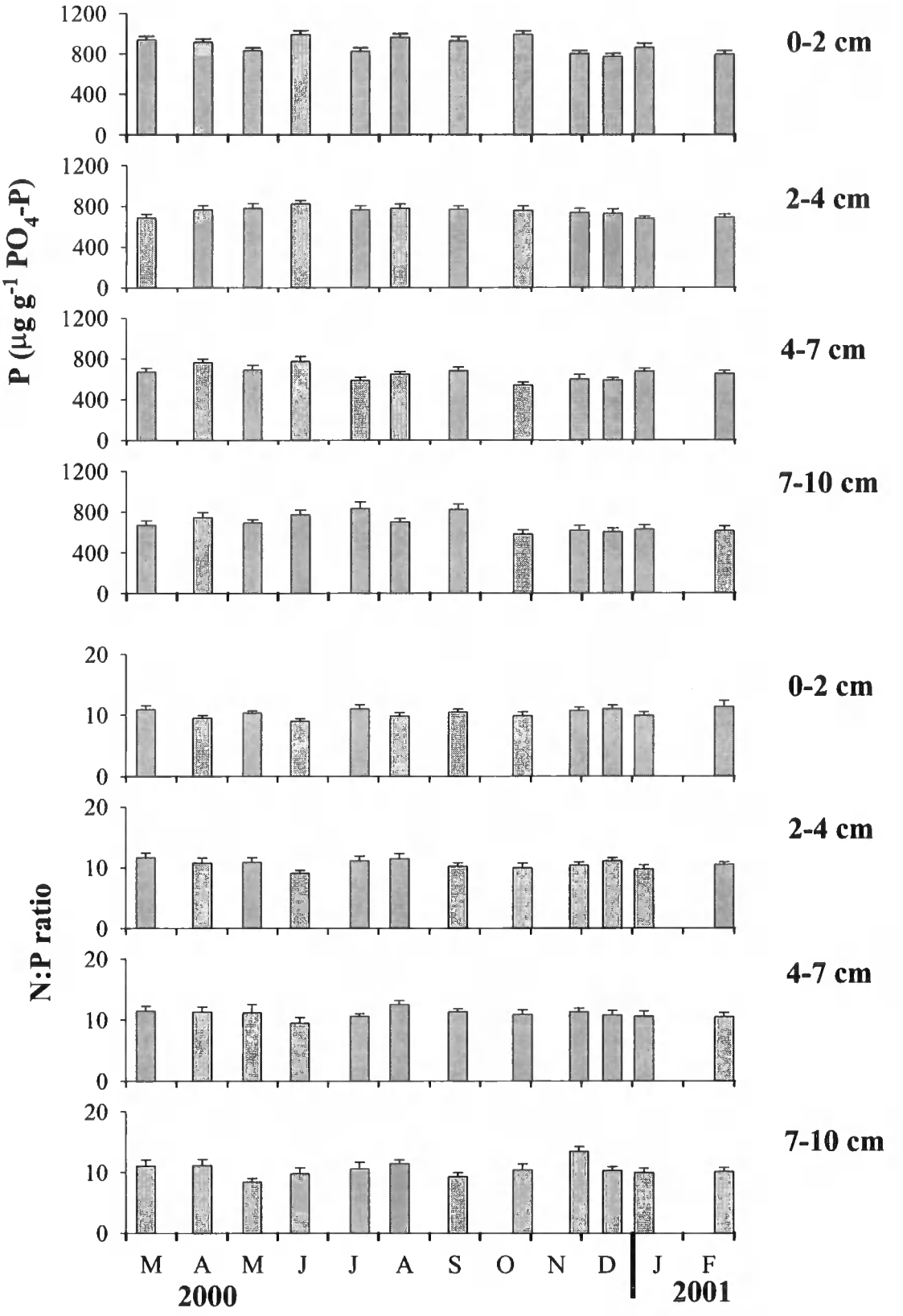

Figure 5.6 Changes in total P and N:P ratio with time in surface sediments from Croft Kettle between March 2000 and February 2001. Vertical bars indicate the mean (n=12) and error bars one SE.

Total N and P content of surface sediments of Croft Kettle varied considerably showing both temporal and spatial variability (Figures 5.5 and 5.6). The highest N content was observed in March 2000 (10350 ± 753.5 μg g^{-1} d.wt) for the 0-2 cm sediment section and the lowest in October 2000 (5797 ± 333.4 μg g^{-1} d.wt) for the 4-7 cm section. The highest P content was observed in June 2000 (991 ± 38.1 μg g^{-1} d.wt) for the 0-2 cm sediment section and the lowest in October 2000 (442 ± 28.2 μg g^{-1} d.wt) for the 4-7 cm section. Overall, winter months showed lower total N and P contents for almost all sediment sections with the exception of the 4-7 cm one, where minima for both total N and P were identified in October 2000. The N:P ratio did not show any considerable monthly variation throughout the year indicating that N and P are following similar patterns of change, and were always lower than Redfield stoichiometry (Section 1.4). The highest value was observed in November 2000 (13.5 ± 0.72) for the 4-7 cm section and the lowest in May 2000 (8.5 ± 0.56) and both were observed in the deepest lying sediment section (7-10 cm).

The highest total N monthly differences were; 1856 μg g^{-1} d.wt (18 %) for the 0-2 cm sediment section, 2292 (26 %) for 2-4 cm, 2662 (32 %) for 4-7 cm and 2663 (32 %) for the deepest sediment section and for total P were 221 μg g^{-1} d.wt (22 %), 145 (18%), 232 (30%) and 258 (31%) respectively. The highest monthly differences for both N and P were mostly obtained by the subtractions of spring or summer values to winter ones. Differences of adjacent observations (months) were at the level of 0-20%. However, as it was shown in Section 5.21, determination of total N and P by the digestion technique showed considerable analytical variation with the relative standard deviation (RSD) being at the level of 17-18%. Therefore, possible monthly differences could be due to analytical error and are not going to be discussed further. In order to reduce part of this drawback, the data are presented on a seasonal basis (Figure 5.7). Comparisons are made in order to test for significant differences of means, possibly indicating seasonal and spatial patterns.

Total N and P contents of Croft Kettle's surface sediments (0-10 cm) showed considerable seasonal variation (Figure 5.7). The pattern, generally followed for both total N and P, indicated a decrease towards the winter period. The two uppermost sediment sections (0-4 cm) showed much less seasonal variation than the other sections, with overall higher total N and P contents (in particular P) during spring and summer than autumn and winter. Total P concentration of sediments overall showed slightly higher seasonal changes than total N concentrations for the two uppermost sediment sections with highest being 13 %, but the deeper lying sediments showed similar

variations in both total N and P concentrations, with highest being 23 %. Differences between successive seasons however, were much smaller. Highest seasonal values were identified in the uppermost sediment section (9364 ± 342.9 μg g^{-1} d.wt for total N in autumn and 924 ± 23.9 μg g^{-1} d.wt for total P in summer). The summer maxima for the surface sediment section coincided with the June 2000 flood, which washed a lot of particulate material into Croft Kettle. N:P ratio variations were on a very small range and did not follow any marked patterns of change. The maximum range of N:P ratio for all sediment sections was between 10.0 ± 0.6 and 11.2 ± 0.15 (always below Redfield stoichiometry).

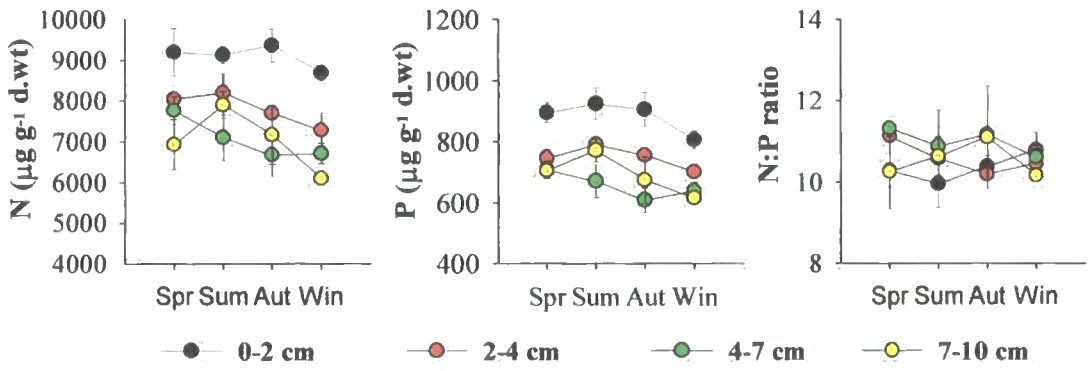

Figure 5.7 Seasonal variation of total N and P and N:P ratio in surface sediments from Croft Kettle between March 2000 and February 2001. Circles show the mean and error bars one SE of 36 separate determinations.

One way analysis of variance (Table 5.3) has shown that there were only a few significant differences for seasonal variation for both N and P and N:P ratio for almost all the sediment sections. Seasonal variation of total P contents showed the most significant differences, followed by total N, while seasonal changes in N:P ratio did not show any significant differences. Significant differences for seasonal changes in total P were identified for all sediment sections and seasons, but the majority and most significant ones ($P<0.001$) were identified only between summer and winter. In particular, in the uppermost sediment section total P content in winter was significantly different from both summer and autumn values. Finally, no significant differences were observed in the two surface sediment sections (0-4 cm) for total N concentrations, but some were shown for the deeper lying ones, with the most significant being between summer and winter ($P<0.001$) in the deepest sediment section.

Table 5.3 ANOVA p-statistics from comparisons of seasonal variation of N, P and N:P ratio of the four sediment sections. The probability values are shown at 95 % significance level as *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, ns: not significant.

	Time	N content			P content			N:P ratio		
		Spr	Sum	Aut	Spr	Sum	Aut	Spr	Sum	Aut
0-2	Spr									
cm	Sum	ns			ns			ns		
	Aut	ns	ns		ns	ns		ns	ns	
	Win	ns	ns	ns	ns	***	**	ns	ns	ns
2-4	Spr									
cm	Sum	ns			ns			ns		
	Aut	ns	ns		ns	ns		ns	ns	
	Win	ns	ns	ns	ns	*	ns	ns	ns	ns
4-7	Spr									
cm	Sum	ns			ns			ns		
	Aut	*	ns		**	ns		ns	ns	
	Win	*	ns	ns	ns	ns	ns	ns	ns	ns
7-10	Spr									
cm	Sum	ns			ns			ns		
	Aut	ns	ns		ns	*		ns	ns	
	Win	ns	***	*	ns	***	ns	ns	ns	ns

5.4 Depth profile of N and P content

A 35 cm perspex sediment core from the middle of Croft Kettle was sliced on 1 cm intervals during the 12 March 1999 visit for the collection of the deep cores. The exact position of the sampling is shown in Figure 3.5. Three subsamples of each slice were analysed for total N and P and the mean ± the standard error are presented below.

All determinads showed considerable variation with depth (Figure 5.8). N content showed a periodical pattern of change with depth, with periods of high concentration (max = 7150 ± 484.3 $\mu g\ g^{-1}$ d.wt for 10-11 cm section) and shorter periods of low concentration (min = 2462 ± 180.4 $\mu g\ g^{-1}$ d.wt for 33-34 cm one). P content generally followed a pattern of increase with depth. The highest P value was observed for the 34-35 cm section and was 1153 ± 92.6 $\mu g\ g^{-1}$ d.wt and the lowest was 637 ± 31.1 $\mu g\ g^{-1}$ d.wt for the 5-6 cm one. Therefore, the highest differences amongst sections were 65 and 45% for N and P respectively. N:P ratio was higher in the first 18 cm of the core and showed a considerable decrease in the later half, mainly following the increase in P content. The asymmetrical pattern of vertical change in N:P ratio probably indicates

that changes in the surrounding landscape and environmental conditions as well as the water chemistry, periodically favoured either N or P sediment deposition.

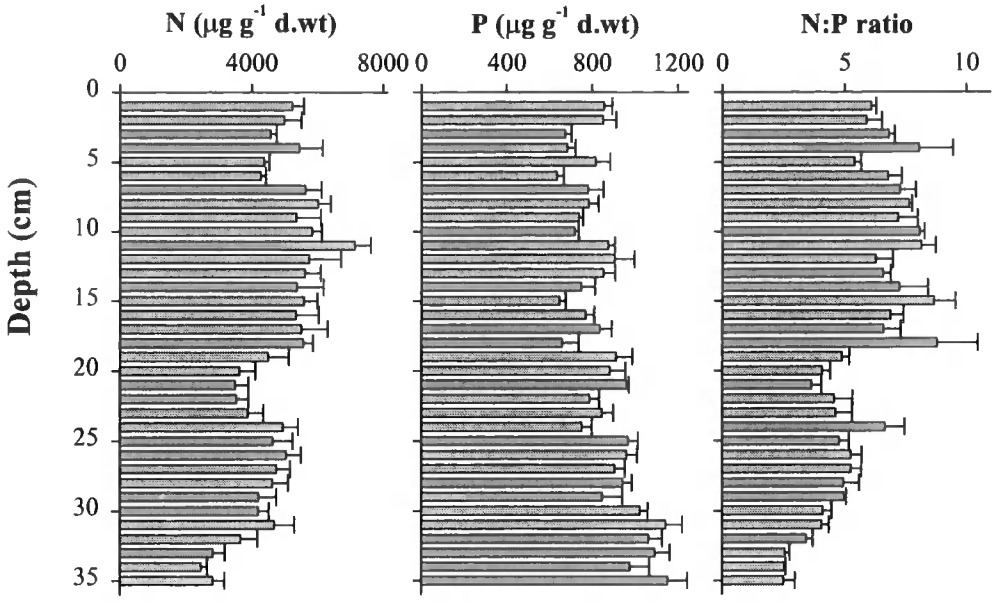

Figure 5.8 Depth profile (every 1 cm interval) of N and P and N:P ratio in a sediment core collected from the middle (site 5, Figure 3.5) of Croft Kettle at 12 March 1999. Vertical bars show the mean and error bars one SE (n=3).

5.5 Redox potential (Eh) and pH

Eh is primarily a measure of ability of sediments to supply or to accept electrons and it is determined by a number of physical, chemical and biochemical reactions in the sediments (i.e. oxygen). Redox potential (Eh) and pH were measured as soon as the cores were brought to the surface, with the electrode introduced into the sediment at ≈14 mm depth.

The results of the monthly survey (Figure 5.9) showed that sediment core samples at -14 mm depth were highly reduced (Eh<-100 mV) throughout the year, suggesting O_2 depletion and presence of mineral electron acceptors (i.e. Fe^{3+}, Mn^{4+}) in the sediment. Seasonal variation in pH and Eh was recorded with the lowest Eh values during summer (min. = -335 ± 2.5 mV in June) and highest during winter (max. = -113 ± 17.1 mV in January). In contrast, pH temporal changes did not follow the same pattern and showed lower values during summer and winter than autumn and spring. In addition, no significant relationship was found between the two. Sediment pH values were close to neutral and slightly lower than water pH.

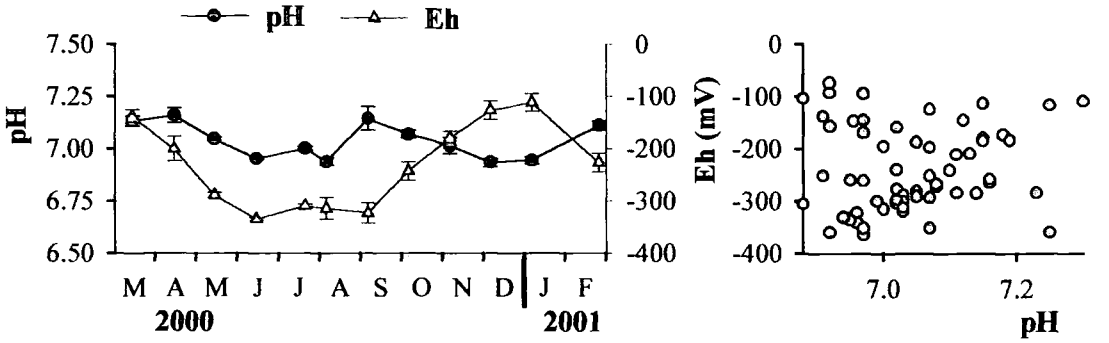

Figure 5.9 Changes in pH and Eh with time of surface sediments from Croft Kettle between Mar 2000 and February 2001 (mean ± one SE of four sediment cores) and their relationship (Figure on the right).

5.6 Sequential phosphorus fractionation

Sediment cores were collected monthly from March 2000 to February 2001 to investigate both vertical and seasonal variations in P fractions and species of the sediment. A sequential chemical extraction (fractionation) method was applied to the collected material to quantify the following P fractions, or forms: H_2O-P; readily available-P (gives a good estimation of immediately available P), BD-P; reductant soluble-P (sorbed-P, Fe and Mn bound-P), NaOH-P; extractable biogenic-P (easily degradable organic-P, humic and Al bound-P), HCl-P; calcium mineral-P (e.g. apatite) and $NaOH_{(85°)}$-P; refractory organic-P. Two P species were determined directly (FRP and FTP) and FOP by the subtraction of FTP-FRP. Average annual vertical sediment profiles of P fractions and species are presented in Table 5.4 and their monthly variation is shown in Figures 5.10-12.

FRP in the 0-2 cm sediment section from Croft Kettle mainly consisted of NaOH-P, HCl-P and $NaOH_{(85°)}$-P which accounted for 25, 31 and 27% of total-FRP respectively. Average concentration of NaOH-P was 83 ± 4.7 (range from 34 to 171 μg g^{-1} d.wt), of HCl-P 100 ± 4.1 (range from 47 to 150 μg g^{-1} d.wt) and of $NaOH_{(85°)}$-P 89 ± 4.4 (range from 48 to 238 μg g^{-1} d.wt). The remainder was mainly BD-P (16%), while H_2O-P only constituted a minor part (>1%). In addition, FRP fractional composition and concentration varied only slightly with increasing depth and was independent of FTP concentration. FTP in the 0-2 cm sediment section from Croft Kettle mainly consisted of NaOH-P which accounted for 44% and the remainder was $NaOH_{(85°)}$-P (23%), HCl-P (18%) and BD-P (13%), while H_20-P only constituted a minor part (2-3%).

Table 5.4 Annual means and descriptive statistics of P fraction data of Croft Kettle's surficial sediments as determined by the sequential P extraction (n= 48).

		FRP					FTP					FOP				
P fractions ($\mu g\ g^{-1}$ d.wt)	Sediment fractions (cm)	Mean	SD	SE	Min	Max	Mean	SD	SE	Min	Max	Mean	SD	SE	Min	Max
H_2O-P	0-2	2.8	3.07	0.44	0.4	13.3	13.4	8.38	1.21	2.4	34.7	10.6	7.32	1.06	1.2	31.7
	2-4	2.9	2.80	0.40	0.6	12.2	13.4	7.63	1.10	3.3	38.1	10.5	6.66	0.96	2.0	33.2
	4-7	3.4	2.68	0.39	0.7	12.7	13.8	7.18	1.04	3.1	26.1	10.5	5.92	0.85	0.4	21.5
	7-10	4.0	3.48	0.50	0.9	17.2	14.6	7.07	1.02	4.1	27.3	10.5	5.57	0.80	2.0	22.3
BD-P	0-2	52.6	19.49	2.81	23.0	95.3	87.2	25.95	3.75	39.8	147.0	34.6	20.86	3.01	2.5	79.8
	2-4	45.0	20.24	2.92	13.9	106.5	72.4	25.35	3.66	28.2	137.9	27.4	16.91	2.44	1.7	60.7
	4-7	39.4	18.54	2.68	14.7	77.5	67.6	23.05	3.33	25.8	118.8	28.2	15.03	2.17	6.4	62.5
	7-10	42.1	23.94	3.46	9.9	107.6	67.6	25.65	3.70	29.0	127.0	25.5	14.77	2.13	1.5	56.5
NaOH-P	0-2	82.7	32.40	4.68	33.9	171.0	297.2	92.63	13.37	94.6	528.2	214.6	77.62	11.20	50.9	428.1
	2-4	62.0	27.98	4.04	25.8	140.1	207.7	65.67	9.48	93.3	332.2	145.6	65.69	9.48	27.0	275.9
	4-7	54.7	21.45	3.10	13.3	103.0	165.8	49.24	7.11	81.2	257.9	111.1	53.93	7.78	7.5	244.1
	7-10	55.7	24.66	3.56	13.2	106.1	150.8	62.61	9.04	71.9	307.6	95.1	65.24	9.42	5.5	294.3
HCl-P	0-2	100.3	28.61	4.13	47.2	150.1	124.8	29.81	4.30	64.3	184.6	24.8	13.25	1.91	1.4	57.7
	2-4	101.3	38.89	5.61	35.5	287.0	120.8	39.76	5.74	45.8	302.1	19.8	15.03	2.17	0.9	84.8
	4-7	95.8	34.91	5.04	24.7	184.2	115.9	36.57	5.28	38.0	211.5	20.1	14.12	2.04	1.2	76.3
	7-10	96.1	31.05	4.48	30.6	155.5	113.0	31.43	4.54	35.2	167.9	17.6	10.74	1.55	1.4	45.7
$NaOH_{(85°)}$-P	0-2	89.0	30.31	4.38	48.4	238.0	159.4	50.16	7.24	69.4	277.7	70.6	41.69	6.02	9.2	153.9
	2-4	84.2	41.54	6.00	30.0	261.7	153.6	63.80	9.21	17.3	305.4	69.9	49.56	7.15	3.9	199.0
	4-7	92.4	47.74	6.89	24.3	205.4	157.2	58.18	8.40	69.4	276.9	64.8	41.23	5.95	8.1	216.0
	7-10	100.3	59.41	8.58	21.2	317.2	169.0	71.48	10.32	57.0	370.2	68.7	46.96	6.78	3.8	220.8
Total-P	0-2	327.4	73.09	10.55	203.6	520.9	682.0	151.89	21.92	426.1	1032.5	354.6	101.85	14.70	187.2	660.5
	2-4	295.5	88.70	12.80	143.2	524.7	567.9	132.97	19.19	279.1	875.5	272.5	87.28	12.60	133.7	522.4
	4-7	285.6	88.63	12.79	154.3	441.5	520.2	105.17	15.18	328.5	725.1	234.6	64.98	9.38	151.4	452.1
	7-10	298.3	110.58	15.96	122.5	569.5	515.0	130.24	18.80	299.4	803.3	216.7	85.70	12.37	105.4	502.7

FTP fractions overall followed a similar pattern of spatial changes with FRP fractions and did not show considerable changes with depth with the exception of BD-P and NaOH-P. Accordingly, the highest differences were found for NaOH-P, which showed a pattern of decrease with depth. The difference between NaOH-P content of the surface sediment section (0-2 cm) and the second one (2-4 cm) was 7% and 12% and 15% for the other sections with decreasing order in depth respectively. Assuming, that NaOH-P represents humic and Al-bound P, these findings indicate that there is much more humic and loosely organic material on the top sediment layer than in deeper ones. On the same time, total-FTP concentration in the surface sediment (total-FTP = 682 ± 21.9 $\mu g\ g^{-1}$ d.wt) was higher than that in deeper-lying sediments. A slight decrease with depth was also observed for the other sediment sections (568 ± 19.2, 520 ± 15.2 and 515 ± 18.8 for 2-4, 4-7 and 7-10 cm sections respectively).

FOP fractions showed very similar depth distribution to FTP, with NaOH-P (range 43.9 to 60.5%) dominating the total-FOP organic fraction and $NaOH_{(85°)}$-P (range 19.9 to 31.7%) following. The values for the 4–10 cm section were 30 to 40% lower than those at the surface.

However, the distribution between reactive (FRP) and non-reactive (FTP) was different almost for each fraction. A large portion (mean = 68%, range between 63 to 73%) of the NaOH-P was present in organic form, while of the reductant soluble P (BD-P) only ≈40% was present in organic form. 80 to 85% of P extracted with hydrochloric acid was reactive. The hot NaOH extracts ($NaOH_{(85°)}$-P) showed a similar distribution of reactive vs. non reactive P (50-50%), however with total FTP being only 50% of the first NaOH extraction. This part represents the refractory P. In addition, the difference between FTP and FRP extracted by NaOH probably indicates that most of the organic P which is not further differentiated is being extracted at this stage.

In order to test the significance of the observed changes with depth, one-way analysis of variance (ANOVA) was used to detect differences, which were evaluated with Tukey's honestly significant difference (HSD) tests (Table 5.5). Few significant differences were found between P fractions and species for horizontal distribution in the surficial sediments of Croft Kettle. The majority of the significant differences were found only for two P fractions and that were BD-P and NaOH-P. BD-FTP surface content was significantly different ($P<0.05$) from all the other sediment sections and the same was observed for NaOH-FRP and NaOH-FTP ($P<0.01$). Significant differences were also found amongst surface BD-FRP, BD-FOP and NaOH-FOP and some of the

deeper layers (mainly 4-7 and 7-10 cm). Total-P (sum of all P fractions) was also affected by these variability and showed significant differences in surface distribution from the deeper sediment layers for both FTP and FOP.

Table 5.5 ANOVA p-statistics from comparisons for depth distribution of all P fractions and species determined by the sequential extraction scheme (as *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, ns: not significant).

P fractions ($\mu g\ g^{-1}$ d.wt)	Sediment Fraction (cm)	FRP			FTP			FOP		
		0-2	2-4	4-7	0-2	2-4	4-7	0-2	2-4	4-7
H_2O-P	0-2									
	2-4	ns			ns			ns		
	4-7	ns	ns		ns	ns		ns	ns	
	7-10	ns	ns	ns	ns	ns	ns	ns	ns	ns
BD-P	0-2									
	2-4	ns			*			ns		
	4-7	**	ns		***	ns		ns	ns	
	7-10	ns	ns	ns	***	ns	ns	*	ns	ns
NaOH-P	0-2									
	2-4	***			***			ns		
	4-7	***	ns		***	*		***	***	
	7-10	***	ns	ns	***	***	ns	***	***	ns
HCl-P	0-2									
	2-4	ns			ns			ns		
	4-7	ns	ns		ns	ns		ns	ns	
	7-10	ns	ns	ns	ns	ns	ns	*	ns	ns
$NaOH_{(85°)}$-P	0-2									
	2-4	ns			ns			ns		
	4-7	ns	ns		ns	ns		ns	ns	
	7-10	ns	ns	ns	ns	ns	ns	ns	ns	ns
Total-P	0-2									
	2-4	ns			***			***		
	4-7	ns	ns		***	ns		***	ns	
	7-10	ns	ns	ns	***	ns	ns	***	**	ns

The water soluble P (1 to 3% of total P) was the only fraction whose behaviour was extremely variable and independent of the sediment section concerned. H_20-FTP showed lower values in spring and summer months and higher in autumn and winter ones regardless of depth. The BD-FRP (reactive) fraction (mainly ferric hydroxide-bound phosphate), which represented between 10 and 20% of total-FRP, showed different distribution in each sediment layer, with considerable temporal variation. The BD-FRP concentration of the sediment surface was highest in January (72 ± 9.9) and February (71 ± 9.0) and lowest in May (29 ± 3.3) at the beginning of Croft Kettle's stratifation. Overall, BD-FRP was higher in winter and BD-FTP was higher in summer

months. The increase in BD-FRP in the winter possibly indicates the presence of an oxic layer on the sediment surface (above 14 mm depth), which favours adsorption of P by the ferric hydroxide-bound fraction.

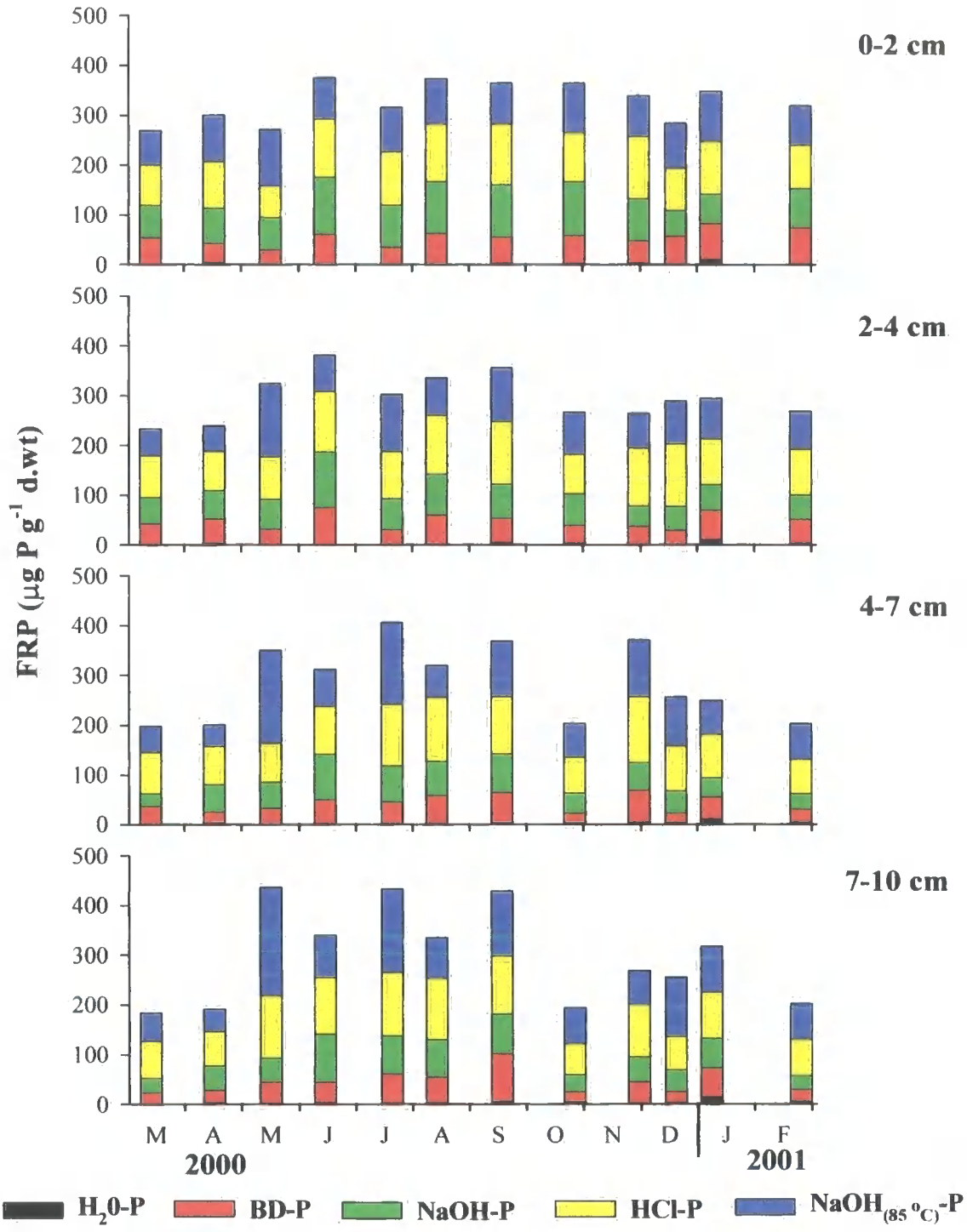

Figure 5.10 Changes in FRP of P fractions with time in surface sediments from Croft Kettle between March 2000 and February 2001. Bars were composed by the mean value of each P fraction from four sediment cores and represent the total P extracted.

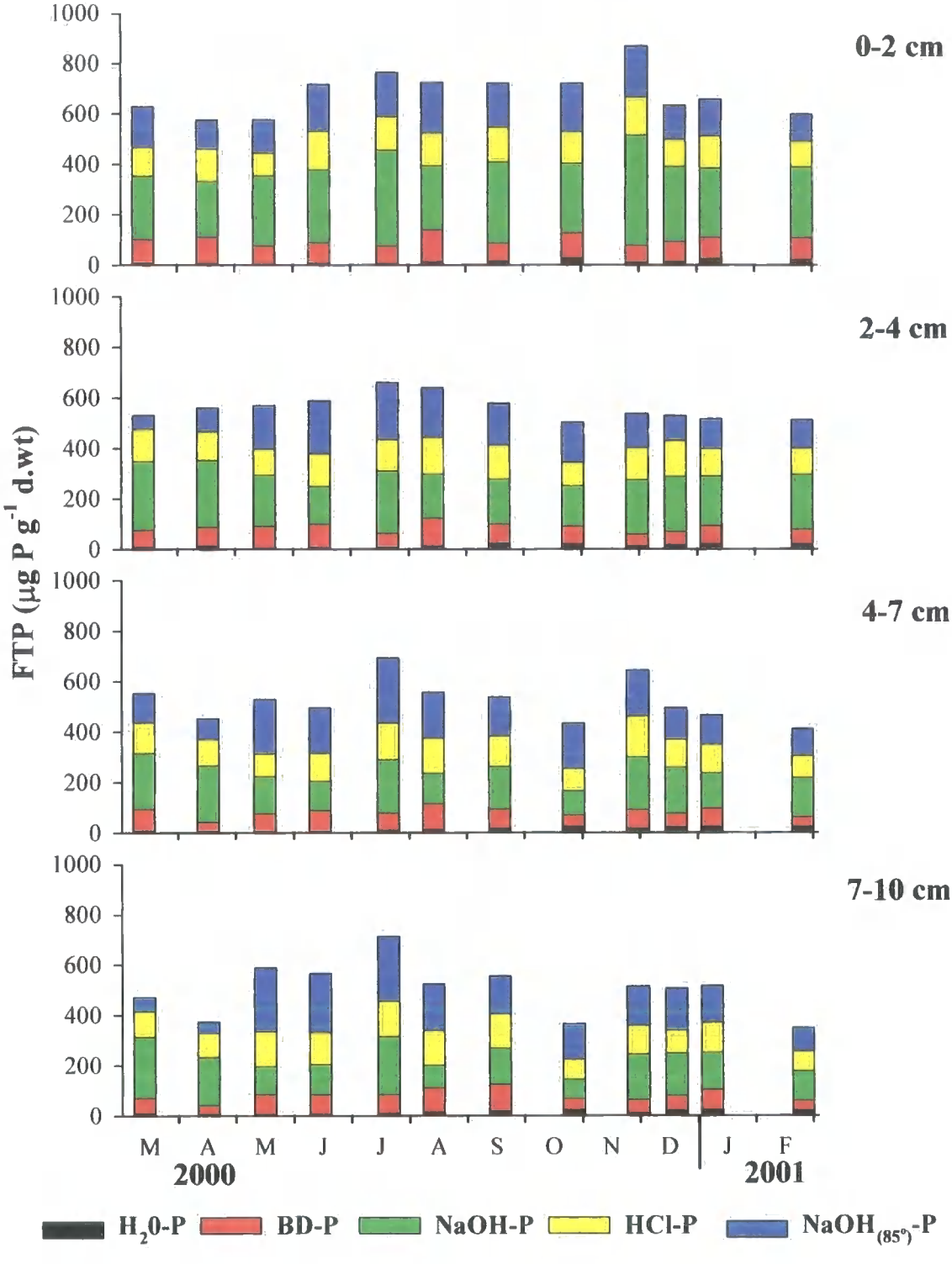

Figure 5.11 Changes in FTP of P fractions with time in surface sediments from Croft Kettle between March 2000 and February 2001. Bars were composed by the mean value of each P fraction from four sediment cores and represent the total P extracted.

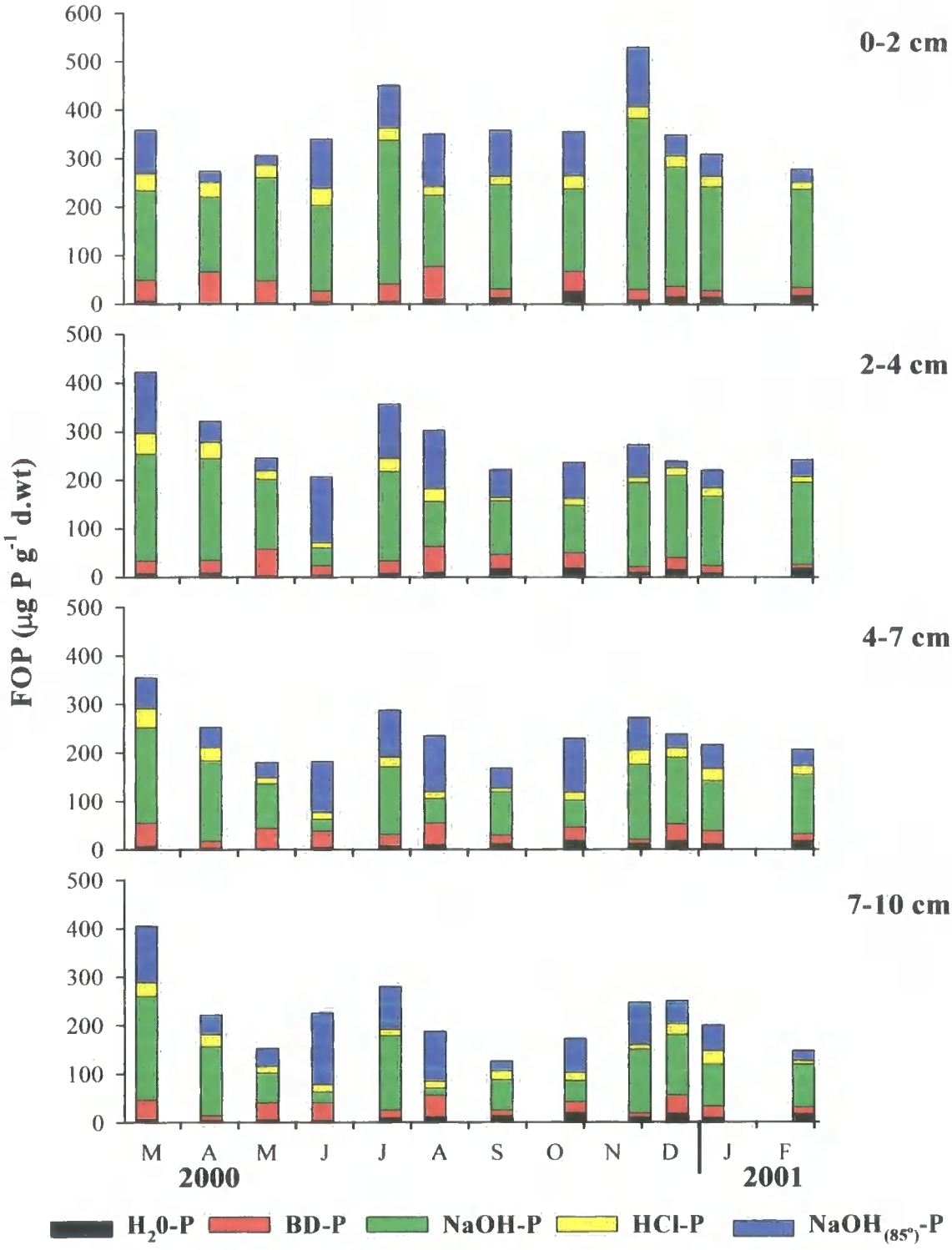

Figure 5.12 Changes in FOP of P fractions with time in surface sediments from Croft Kettle between March 2000 and February 2001. Bars were composed by the mean value of each P fraction from four sediment cores and represent the total P extracted.

NaOH-FTP showed a marked increase at the sediment surface in July and November directly after the June flood event and the October (autumn) turnover, suggesting that these events favoured adsorption of P into this fraction (i.e. higher amount of humic material was deposited to the sediment). NaOH-FOP fraction also showed similar pattern of distribution, indicating that this increase was of organic nature. The calcium-bound phosphate fraction (HCl-P) was the largest inorganic (FRP) fraction. The highest HCl-FRP values were observed in the same season for all sediment sections; in September for the two upper sections (123 ± 13.1 and 126 ± 16.3 μg g^{-1} d.wt for the 0-2 and 2-4 cm sections respectively); and in November for the deeper ones (132 ± 17.0 and 160 ± 8.9 μg g^{-1} d.wt for the 4–10 cm layers). The lowest values appeared at different seasons for the surface (0-4 cm) and deeper (4-10 cm) sediment layers; in May for the surface ones (63 ± 5.9 at 0-2 cm and 79 ± 9.9 μg g^{-1} d.wt at 2–4 cm); and in October for the deeper ones (72 ±15.5 at 4-7 cm and 63 ± 10.7 μg g^{-1} d.wt at 7-10 cm). Finally, the refractory phosphate ($NaOH_{(85°)}$-P) fractions represented 23 to 33% of total-FTP and showed a slight increase with increasing depth. The highest levels were observed in June (258 ± 9.7 μg g^{-1} d.wt) for the 4-7 cm section. The lowest levels were observed for the 2-4 cm section in March (53 ± 3.5 μg g^{-1} d.wt).

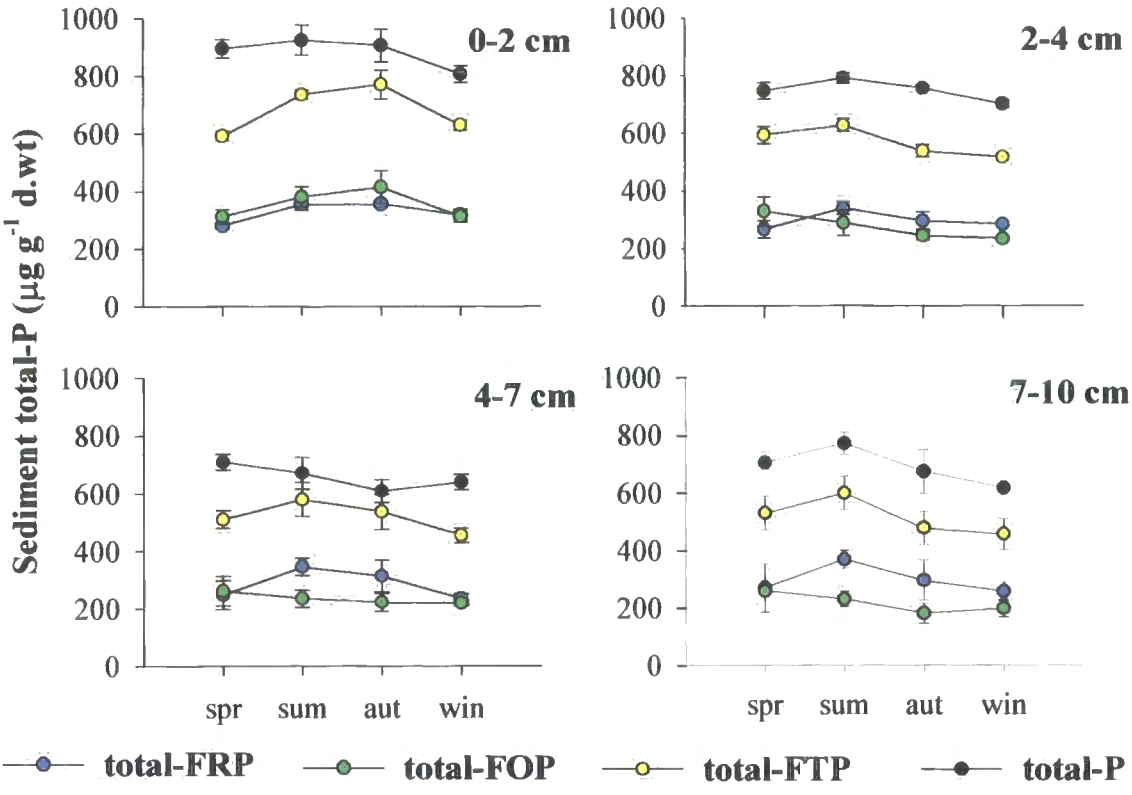

Figure 5.13 Seasonal variation of total P, FRP, FOP and FTP in surface sediments from Croft Kettle between March 2000 and February 2001. Circles show the mean and error bars one SE of 12 separate determinations.

Temporal variation in total-P, as was determined by the sum of all the P fractions, was quite marked at Croft Kettle's surficial sediments (0–10 cm) and different for the four sediment layers and three P species studied (Figures 5.10 to 5.12). The reactive fraction (FRP) represented ≈45% of total-P in the sediment and showed considerable temporal variation particularly in the deeper (4-10 cm) sediment sections. Maximum values were observed in May (435 ± 70.3) and July (432 ± 12.1 μg g^{-1} d.wt) for the deepest sediment layer (7-10 cm). The maximum values (870 ± 58.8 μg g^{-1} d.wt) for total-FTP, occurred in the surface layer in November and in the 2-10 cm ones in July (658 ± 7.6, 692 ± 22.2 and 711 ± 16.3 μg g^{-1} d.wt respectively moving downwards with depth). The FOP fraction represented at most ≈55% of total-P in the sediment. Maxima were observed in July (450 ± 12.7 μg g^{-1} d.wt, after the summer flood) and November (531 ± 47 μg g^{-1} d.wt) for the surface layer. However, most of the temporal changes will not be further discussed, since percentage monthly variation was lower than the analytical variation (Figure 5.2).

Table 5.6 ANOVA p- statistics from comparisons for seasonal variation of total-FRP, total-FTP and total-FOP species of the four sediment sections. The probability values are shown at 95 % significance level as *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, ns: not significant.

	Time	FRP			FTP			FOP		
		Spr	Sum	Aut	Spr	Sum	Aut	Spr	Sum	Aut
0-2 cm	Spr									
	Sum	*			ns			ns		
	Aut	*	ns		*	ns		*	ns	
	Win	ns	ns	ns	ns	ns	ns	ns	ns	*
2-4 cm	Spr									
	Sum	ns			ns			ns		
	Aut	ns	ns		ns	ns		ns	ns	
	Win	ns	ns	ns	ns	ns	ns	*	ns	ns
4-7 cm	Spr									
	Sum	*			ns			ns		
	Aut	ns	ns		ns	ns		ns	ns	
	Win	ns	**	ns	ns	*	ns	ns	ns	ns
7-10	Spr									
cm	Sum	ns			ns			ns		
	Aut	ns	ns		ns	ns		ns	ns	
	Win	ns	ns	ns	ns	*	ns	ns	ns	ns

One way analysis of variance showed few significant seasonal changes in the total-P for reactive (FRP), non-reactive (FTP) and organic (FOP) content of surficial sediments

as were determined by the sequential extraction (Table 5.6). Only the differences observed between spring and summer and spring and autumn were significant for the surface sediment (0-2 cm) for total-FRP, suggesting an increase of dissolved P during summer and autumn. Furthermore, the differences in total-FRP (increase) between spring and summer and (reduction) between summer and winter were found significant for the 4-7 cm sediment section, suggesting higher dissolved P concentrations during the summer. Total-FTP also showed significant differences for almost all the sediment sections (increase from spring to autumn for 0-2 cm and decrease from summer to winter for 4-10 cm). Finally, the only significant changes for total-FOP were identified in the first two sediment layers and that was between spring and autumn and spring and winter for the 0-2 and 2-4 cm sections respectively. These last two observations will be discussed further at Chapter 7.

5.7 Discussion

Sediment digestions for N and P and N:P ratio and a sequential P fractionation scheme according to Psenner & Puczko (1988) were used to explore vertical and temporal changes in Croft Kettle's surface sediments (0-10 cm). The highest total-P (883 ± 23.1 μg g^{-1} d.wt) and total-N (9133 ± 191.9 μg g^{-1} d.wt) concentrations were observed at the uppermost sediment section, and showed significant differences with depth, while N:P ratio (max = 11.0 ± 0.21) did not show any vertical gradients. Total-P content of all sediment sections showed similar patterns of distribution for both techniques. Seasonal changes for total N and P but not for N:P ratio were identified for all sediment layers (0-10 cm) from the digestion technique and for total-FRP, total-FTP and total-FOP as determined from the sequential extraction. Thus, surface sediment P (0-10 cm) must be regarded as highly dynamic, comprising of not only refractory particulate P, but also of a major organic (possible biogenic) fraction, which could eventually be decomposed and released.

It is general request of extraction methods that the sum of fractions should be equal the total P determined by digestion methods since total digested P is assumed to be less subjective to errors than summing up the different fractions measured separately. Total-P measured for each sediment section, by digestion, was 883 ± 23.1, 749 ± 31.8, 657 ± 32.4 and 692 ± 39.3 and by extractions 682 ± 21.9 568 ± 19.2, 520 ± 15.2 and 515 ± 18.8 μg g^{-1} d.wt for 0-2, 2-4, 4-7 and 7-10 cm sections respectively. The mean difference (total P minus P fractions) was 23% (ranged between 20 and 25%), which was much higher than the one for Croft Kettle control sediment (<10%). This is

possibly an indication that either the use of fresh weight for the analysis adds an extra experimental error to the determination of P, or that much more P is extracted when the sediment is first left to dry. In summary, P extractions even though showed lower P concentrations, had less analytical variability than sediment digestions and no significant correlations were found between them. The higher percentage error of the digestion technique could be attributed to the fact that automatic analysis was used for the determination of phosphate and ammonia.

Significant differences were found between profoundal and littoral sediments in Croft Kettle. The N and P contents and N:P ratio of Croft Kettle's surface sediments collected from the middle of the pond were much lower than the cores collected from site 6 (Figure 3.5), which is much closer to the charophyte bed, even though the water column is of similar depth. In addition, N:P ratio of profoundal sediments of Croft Kettle (Figure 5.8) showed lower values than N:P ratio from site 6 as well as significantly lower values than Redfield stoichiometry. This possibly suggests that either the "nature" (composition) of sediments at the middle of the pond was much different or that they suffered more intense anoxia and had much higher denitrification rates. In addition, the depth profiles of N and P and N:P ratio (Figure 5.8) of profoundal sediments showed significant changes with depth indicating possible changes in the surrounding landscape, which have altered the depositional pattern of both N and P in contrasting ways.

Sequential P fractionation of the four sediment sections showed that the highest portion of total P was extracted with NaOH solution (30 to 44% of total-FTP), which extracts P bound to Al oxi-hydroxides and organic matter, with pH being one of the factors governing the ability of these compounds to release P. BD-P fraction (redox labile P) accounted for only 12-13% of total-FTP and the observed maxima of the BD-P fraction at the uppermost sediment section was probably due to higher redox potential at the sediment surface. However, the relative small contribution of P bound to redox labile Fe compounds (BD-P), comparing to the prevailing NaOH-P fraction, suggest that P release caused by oxygen depletion and subsequent drop of redox potential in the sediment, would be less significant than potential changes in pH of the sediment. Most of the other P fractions in all surface sediment sections contributed only a small percentage to total P with the exception of HCl-P (30 to 35% of total-FRP, ≈20% of total-FTP and only 7-10% of total-FOP) and did not show considerable temporal or spatial variation.

Sharp declines with depth for P-content but not as much for percentage relative composition were observed for the NaOH-P species and less for the BD-P ones, suggesting diagenetic solubilisation and subsequent diffusion (release) of P to the overlying water. However, the total-P gradient observed was mostly due to a distinct NaOH-FTP gradient, often being 50 to 100% higher in the surface than in the deeper sediment sections and not due to NaOH-FRP. Therefore, this vertical gradient could be attributed to the presence of a biofilm layer at the sediment surface (biogenic organic P) or to contemporary sedimentation of organic bound P as phytoplankton or macrophyte detritus and subsequent accumulation in the upper sediment and not due to migration of dissolved P. In addition, the presence of a NaOH-FOP gradient also indicates that only a relatively small organic P fraction (approx. 30-50% of the original organic P content of the surface sediment and probably even less of the sedimenting organic material will be permanently deposited in Croft Kettle sediments. Finally, most of the changes in vertical distribution, as indicated by the relative percentage contribution of P fractions were observed between the uppermost sediment section (0-2 cm) and the deeper sections (2-10 cm). This is probably due to stabilised values of pH and redox potential after 2 cm in depth, despite variations in sediment composition and aging.

Finally, no direct evidence of undergoing diagenesis (solubilisation and release to the water column) during the summer stagnation period, which is accompanied with lower Eh values was found, even though some P fractions did show both temporal and vertical variation, especially the decrease observed in total-P concentration of the deeper lying sediment sections. In addition, due to the high experimental variability of the P fractions with the relative standard deviation reaching levels up to 30-40% for FRP and FTP and much higher for FOP, it was very difficult to make conclusions about temporal variation of P fractions in the surficial sediments of Croft Kettle. However, it is implied that some buffer mechanism (dissolution of Fe-P and direct precipitation as Ca-P favoured by the relatively alkaline pH) is controlling release of P to the overlying water, or that the rate of release is not greater than the rate of deposition, approaching a steady state condition, particularly since there is little mixing between the epilimnion and the hypolimnion of the pond during the summer hypolimnetic anoxia.

5.8 Summary

1. N and P contents of surface sediment (0-2 cm) were significantly higher than the deeper layers, in particular 4-10 cm sections.
2. Seasonal changes in total N and P content were identified for all sediment layers.
3. Surface profoundal and littoral sediments in Croft Kettle have different N and P contents and N:P ratio.
4. Vertical distribution of N and P and N:P ratio showed considerable variation, with no apparent trends.
5. The redox potential (Eh) in Croft Kettle, at a depth of 14 mm, was always negative, irrespective of season and varied between -74 and -364 mV. The pH profile did not show either temporal changes or a significant relationship with redox potential.
6. H_20-P extracted with deionised water (readily available P) constituted the smallest fraction (2-3 %) in the sediment with 25% in reactive and 75% in organic form, with no obvious vertical or seasonal trends, between March 2000 to February 2001.
7. BD-P extracted with buffered sodium dithionite reagent (reductant soluble P; Fe-P, Mn-P) constituted about 12-14% of total P of which ≈60% was reactive and 40% organic, with few changes in vertical distribution and no seasonal trends.
8. P extracted with NaOH at 25 °C (P bound to Al and humic substances) constitututed about 30-40% of total P (30% reactive and 70% organic). This was the biggest P fraction in the sediment and there was a clear pattern of change with depth.
9. P extracted with HCl (apatite-phosphate) constituted about 20-25% of total P (85% reactive and 15% organic), indicating that $CaCO_3$ is an important factor in immobilizing P in the sediment in this particular environment.
10. P extracted with hot-NaOH (refractory-residual phosphate) constituted about 25-30% of total-P (50-50% distribution between reactive and organic). This P fraction does not react with the adjacent interstitial water.

CHAPTER 6 Studies on *Chara hispida*

6.1 Introduction

Concentration of nutrients in water, influences species growth and biomass, and increased availability is reflected in the biota, by means of internal contents and physiological responses such as surface phosphatase activity (Section 1.6). In view, of the close relationship between submerged macrophytes (algae) and water chemistry, *Chara hispida* was chosen, as the appropriate organism for studies on seasonal and spatial variation of its nutrient status, due to its presence throughout the year, its abundance and importance (Section 3.9) to Croft Kettle.

The aim was to examine the response of *C. hispida* to changing nutrient concentrations in the water. Tissue N and P content and phosphatase activity of *C. hispida* apical tips in both ponds and their associated epiphytes were determined on a monthly basis over the study period. This would reflect the seasonal changes on spatial and temporal scales. A preliminary investigation of *C. hispida* showed that was capable of expressing phosphatase activity. The data collected over the study period were correlated with aqueous P fractions. This could be of great importance in determining the role of *C. hispida* in nutrient cycling as well as being a benchmark for future monitoring of nutrient status of the ponds and the alga.

Further experimental studies were planned with the aim of investigating growth and physiological responses of *C. hispida*, when incubated under a range of P concentrations and temperatures. Studies on influence of water temperature and aqueous P were decided due to the considerably high summer water temperatures observed in the epilimnion of Croft Kettle and the contrasting literature concerning P toxicity in charophytes (Section 1.7).

6.2 Sampling strategy

Shoots of *C. hispida* were sampled approximately monthly from Croft and Double Kettle, starting in April 1999. However, it proved impossible to collect material between March to June of 2001 due to the foot and mouth outbreak in England. Furthermore, *C. hispida* disappeared from Double Kettle and it was not possible to collect any plants for the remaining of the study period (July to October 2001).

6.3 Carbonate content of apical tips

6.31 Introduction

C. hispida has a peculiarly characteristic brittleness and a roughness to the touch due to the high amount of marl deposits that bears on its surface (Section 1.7). Studies have shown that deposition of marl on charophytes is mostly due to $CaCO_3$ deposition and magnesium carbonate only contributes a small percent (< 5 %, Section 1.7). Hence, $CaCO_3$ deposition on shoots undoubtedly contributes to the fresh and dry weight of the plants. In order to elucidate, the effect that calcification had on the determination of dry weight, *C. hispida* shoots were collected monthly and the $CaCO_3$ content was measured.

6.32 Experimental studies

In order to test if washing up at 2 % HCl solution (as described in Section 2.62) was enough to remove all the $CaCO_3$ deposition from *C. hispida* apical tips and what were the effects of this treatment to the elemental composition of the plants three different concentrations of HCl solution were tested. Elemental composition was determined on tips, which had only been dried (control); on tips which had gone through the phosphatase assay conditions (after assay); and tips that had been washed with 1 %, 2 % and 5 % HCl solution. Seven apical tips were used for each treatment.

5 % HCl solution removed slightly higher amounts of $CaCO_3$ from the other treatments and removed the highest Ca and Mg amount (Figure 6.1). However, % d.wt content of most of the other elements was not higher as would be expected due to the lower dry weights and Mn, Cu and Zn were the only elements that followed that rule. In contrast, Na and N showed lower contents and P and Fe showed similar contents. Overall, elemental concentration after washing with 2 % HCl solution was similar to 5 % wash but higher than 1 % wash. Tips analysed after going through the phosphatase assay conditions showed different elemental compositions from the control ones, indicating that the assay procedure has an effect on the elemental composition of the plants. In addition, Ca and Mg contents were lower than the control ones, possibly indicating that part of the external $CaCO_3$ deposition was removed.

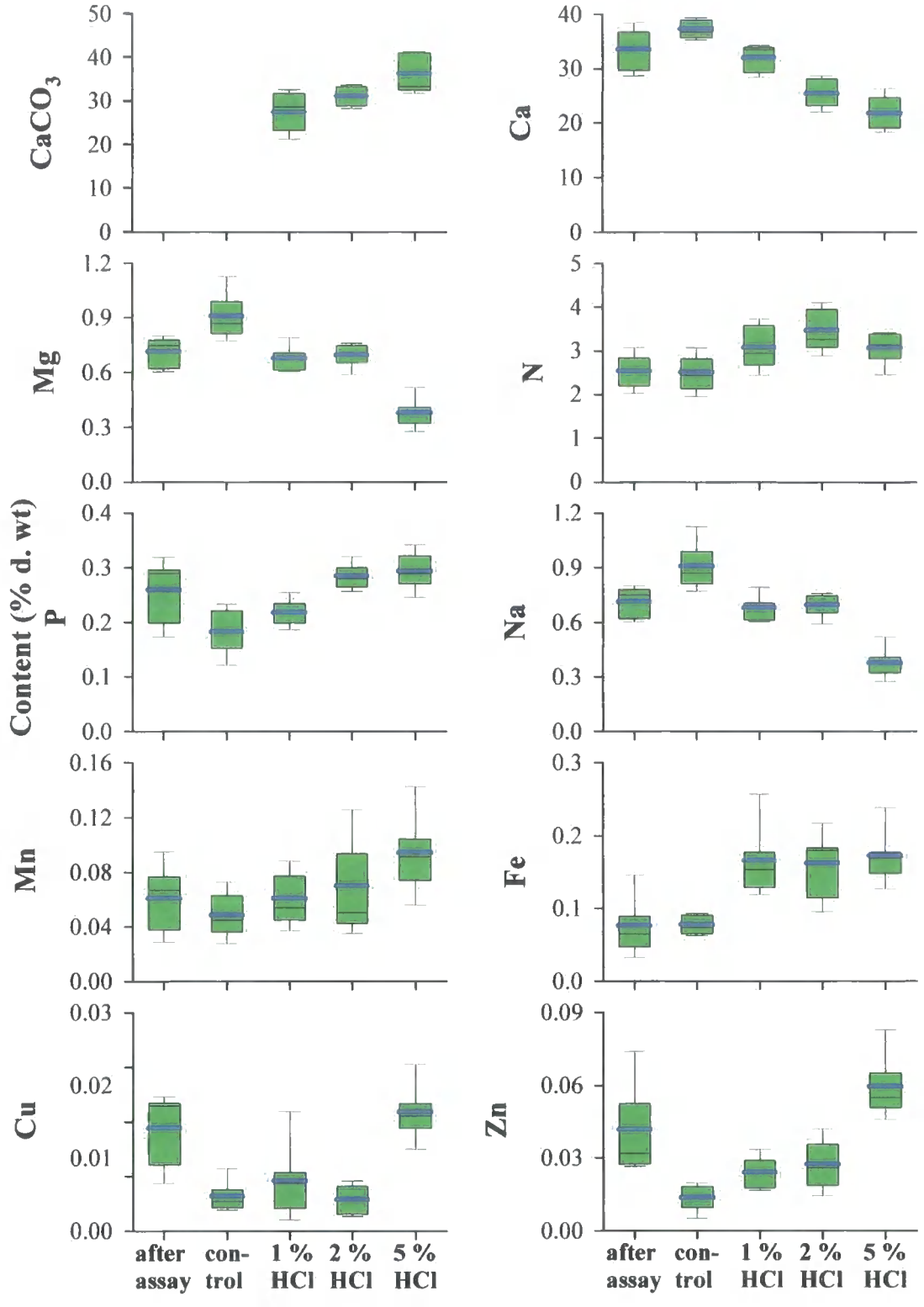

Figure 6.1 Elemental composition of *C. hispida* apical tips from Croft Kettle (n=7); after the phosphatase assay, no treatment (control); and washing with 1, 2 and 5 % HCl solutions. The median values are presented as a black line and the mean ones as blue. The boxes indicate the 25th and 75th percentiles and the error bars indicate the 10th and 90th percentiles.

6.33 Seasonal study

Calcium carbonate content showed considerable seasonal and monthly variation in *C. hispida* apical tips in both ponds (Figure 6.2) with no apparent seasonal trends. Seasonal and monthly distribution also varied between the two ponds. $CaCO_3$ comprised, on average between 35% and 40% of the dry weight of *C. hispida* apical tips even though values as high as 49.2% and 51.7% were recorded in Croft and Double Kettle respectively. Paired samples t-test indicated that $CaCO_3$ content of *C. hispida* shoots in Croft Kettle (mean = 34.4 ± 0.85) was significantly different ($P<0.001$) from the ones in Double Kettle (mean = 40.3 ± 0.87). However, this observation is in contrast with the fact that Ca hardness (Table 3.1) of Croft Kettle water (490 mg L^{-1}) was higher than Double Kettle (440 mg L^{-1}). A possible explanation could be that the location (site 7, Figure 3.5) from where *C. hispida* shoots were collected at Double Kettle (shallow area and plants were very close to the water surface) favoured the process and rate of calcification.

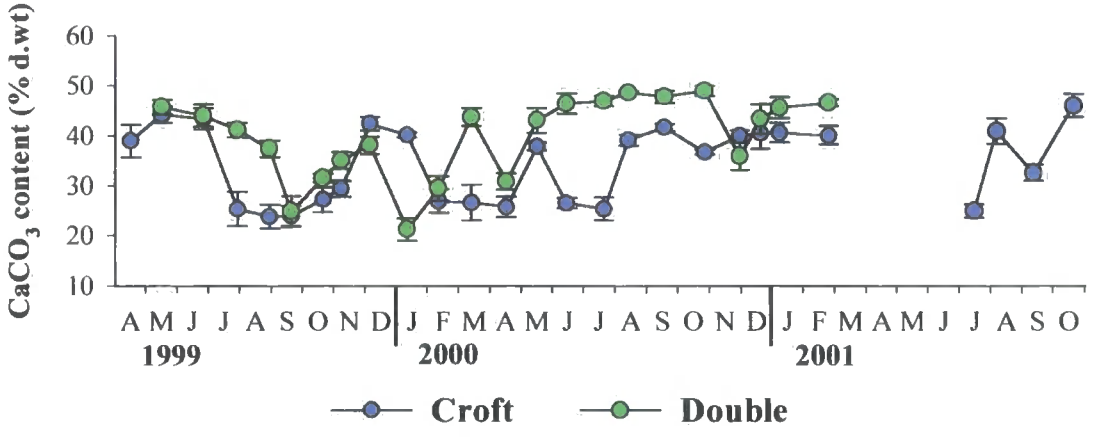

Figure 6.2 Changes of $CaCO_3$ content (as % d.wt) of *C. hispida* apical tips from Croft and Double Kettle with time between April 1999 and October 2001. Vertical bars indicate one SE of the mean (May to February 2001, n=4; July to October 2001, n=7).

6.4 Elemental composition of whole plants

As mentioned in Section 2.6 apical tips were used for most of the seasonal studies on *C. hispida*. However, in order to test how these measured values for only a small part of the shoot reflected concentrations of whole shoots (which show apical growth, Section 1.7), *C. hispida* shoots of approximately 1 m length were collected, sectioned into 10-cm fractions and their elemental composition was determined. In September

2000, a number of shoots were collected from site 2 (Figure 3.5) in Croft Kettle. Eleven shoots of similar morphological characteristics were chosen, cleaned of sediment and debris, washed free of obvious epiphytes, dried at 105 °C oven and ground to homogeneity. Four shoots were analysed for a number of chemical elements and the results are presented in Figure 6.3. Analysis was extended to potassium and sulphur, but the results are not presented since the addition of some of the reagents of the digestion procedure positively interfered with the outcome. Seven more shoots were analysed for N and P after washing with 2 % HCl solution and the results are presented in Figure 6.4.

Table 6.1 Elemental composition (as % d.wt.) of *C. hispida* plants collected from Croft Kettle at September 2001.

	N	Ca	Mg	P	P_i	Zn	Na	Fe	Cu	Mn
Mean	0.860	41.550	0.794	0.093	0.077	0.009	0.572	0.136	0.003	0.032
SD	0.3867	6.8178	0.1476	0.0430	0.0518	0.0047	0.3630	0.0451	0.0018	0.0219
SE	0.0663	1.1692	0.0253	0.0074	0.0089	0.0008	0.0623	0.0077	0.0003	0.0038

Analysis of 1-m shoots of *C. hispida* (Figure 6.3) indicated vertical gradients for most of the chemical elements, with higher contents in the youngest apical part of shoots than the lower parts. This pattern was clear especially for N and P, where there was almost a twofold difference in content between the youngest part and the lowest part (oldest) of the shoot. In contrast, the lowest Ca content was observed in the youngest part and Ca was probably the only element that showed a progressive increase with shoot height (moving from top to bottom). Na and Cu were the only two elements that vertical gradients were not observed and contents were similar from top to bottom. In addition, Fe in particular and Mn showed a two-way distribution. Fe content originally increased with shoot height (moving from top to bottom), then it showed a progressive decrease and finally at the last 20 cm of the shoot increased again. This could be an indication of Fe uptake from the sediment.

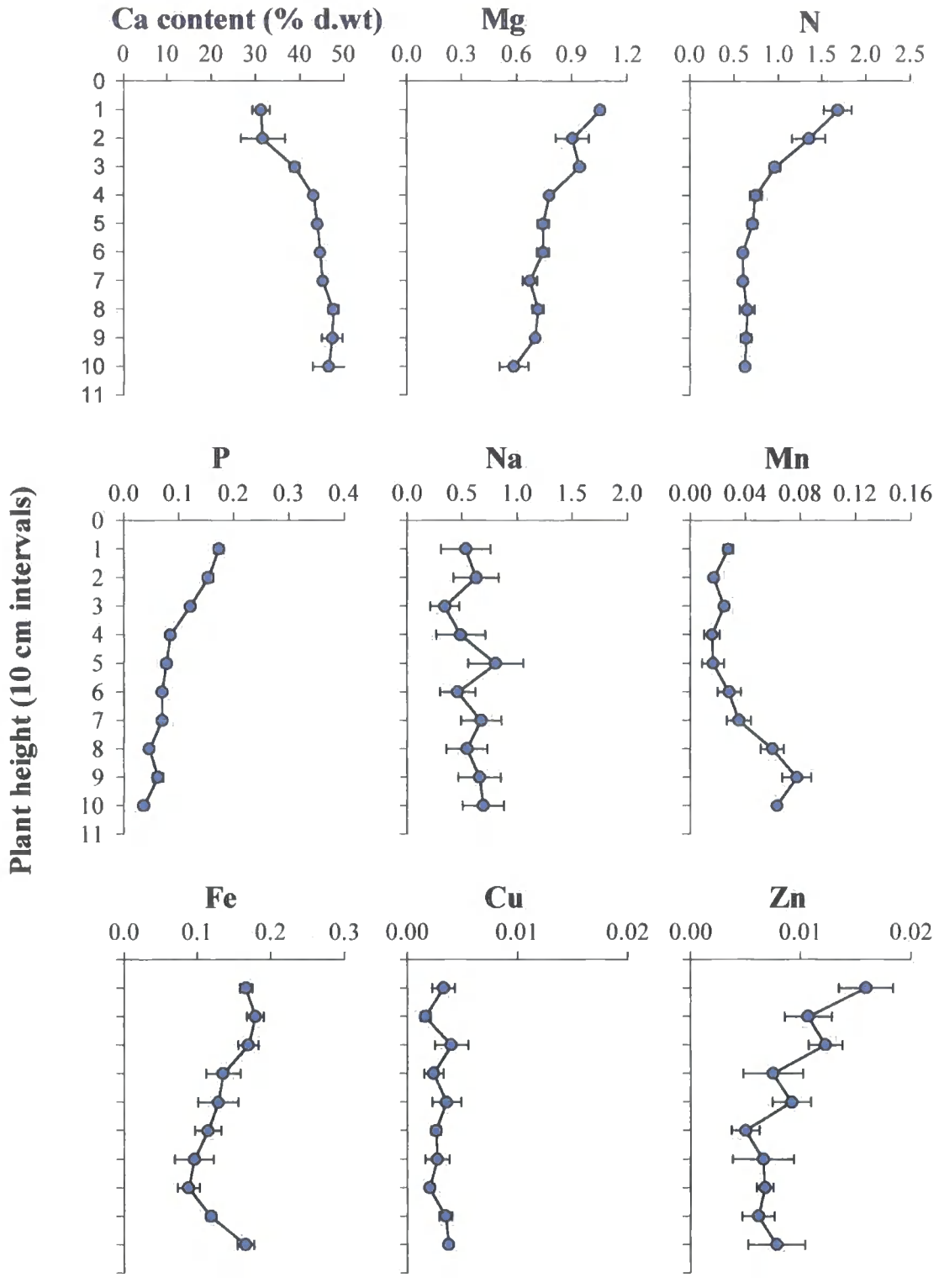

Figure 6.3 Elemental composition of whole *C. hispida* plants sampled from Croft Kettle at September 2000. No correction for $CaCO_3$ deposition took place. Dots represent successive 10-cm high horizontal layers of the shoots, moving from the apice (1: 0-10 cm) to the bottom. Vertical bars indicate ± one SE of the mean (n=4).

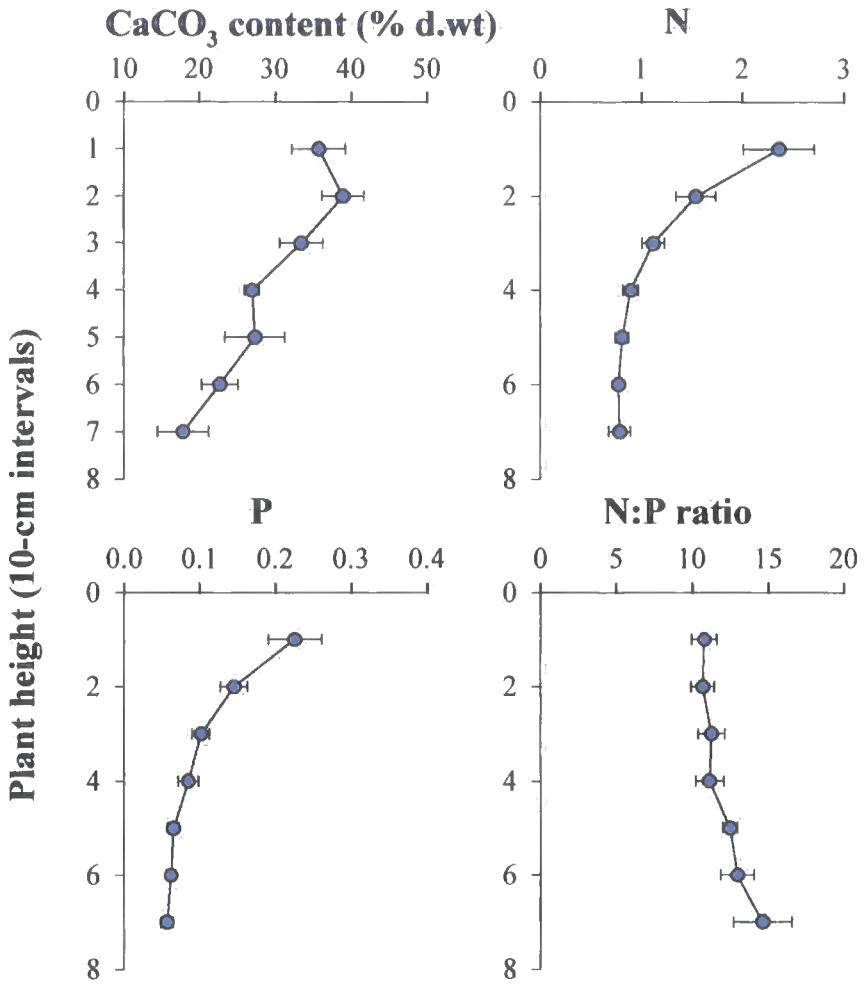

Figure 6.4 Elemental composition of whole *C. hispida* plants from Croft Kettle at September 2000. Plants were washed with 2 % HCl solution before analysis. Dots represent successive 10-cm high vertical layers of the shoots, moving from the apice (1: 0-10 cm) to the bottom. Vertical bars indicate ± one SE of the mean (n=7).

Strong vertical gradients were observed for N and P, with higher contents in the younger apical part of shoots than the lower parts, even after the correction for $CaCO_3$ deposition. Highest contents were observed for the youngest apical part, which showed more than twofold difference from the oldest one. N:P ratio for *C. hispida* shoots averaged 12.0 (range 10.7 to 14.7) and did not show any vertical patterns, indicating that N and P content changes follow the same pattern. $CaCO_3$ removal was much higher in the upper parts of the shoot and accounted for 30-40 % of the dry weight of the plant. The $CaCO_3$ pattern shown here is probably in contrast to the previous observation (Figure 6.3) that Ca content was higher in the oldest parts of the shoot. This could be either due to the fact that washing up did not remove all the $CaCO_3$

deposition in the lower parts, maybe because aging has made it more stable, or that tissue Ca content is much higher in the lower parts.

6.5 Nitrogen and phosphorus content of apical tips

Apical tips of *C. hispida* were digested using the H_2SO_4 method (Section 2.82) and nutrient content was based on N and P as a percentage dry weight. A total of 12 apical shoots were analysed each month for each pond and the mean ± SE are presented below. Uncorrected values (dry weight estimation without accounting for $CaCO_3$ deposition) are also presented as N* and P* for means of comparison but will only be discussed briefly.

Table 6.2 Summary of N and P content (% d.wt) and N:P ratio of *C. hispida* apical tips from Croft and Double Kettle between April 1999 and February 2001. Values not corrected for $CaCO_3$ deposition are presented as N* and P*.

	Tissue content (% d.wt)	Mean	SD	SE	Min	Max
Croft	N*	2.67	1.071	0.206	1.13	4.32
Kettle	N	4.19	1.834	0.353	1.49	7.23
	P*	0.19	0.091	0.017	0.06	0.37
	P	0.30	0.158	0.030	0.07	0.62
	N:P ratio	15.6	3.65	0.70	9.5	22.7
Double	N*	2.79	0.719	0.150	1.14	3.70
Kettle	N	4.76	1.359	0.283	1.83	6.58
	P*	0.22	0.083	0.017	0.07	0.37
	P	0.39	0.162	0.034	0.11	0.73
	N:P ratio	13.7	2.45	0.51	9.2	19.0

Mean tissue N and P contents of *C. hispida* shoots of both ponds showed considerable, but similar temporal and analytical variation. Site to site differences in tissue P concentrations, as indicated by the magnitude of the standard error relative to the mean, were substantial in comparison with those of N. *C. hispida*'s apical tips mean N and N* contents were slightly lower in Croft than Double Kettle as well as mean tissue concentrations of P and P*. In contrast, N:P ratio was higher in Croft (15.6 ± 0.70) than Double Kettle (13.7 ± 0.51). Paired samples t-test showed that P and P* contents and N:P ratio of *C. hispida* apical tips in Croft were significantly different ($P<0.001$) from those growing in Double Kettle. Comparatively, only N was found to be significantly different ($P<0.05$) between *C. hispida* shoots collected from the two ponds.

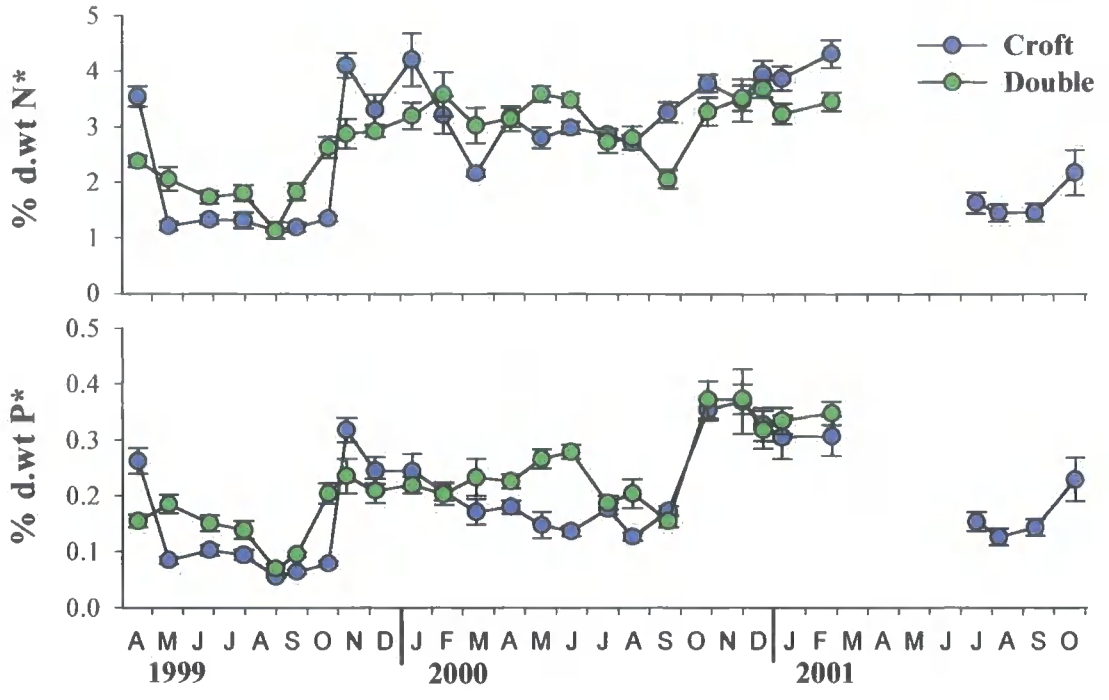

Figure 6.5 Changes in N* and P* content (as % d.wt) of *C. hispida* apical tips from Croft and Double Kettle with time between April 1999 to October 2001. Vertical bars indicate ± one SE of the mean (n=12).

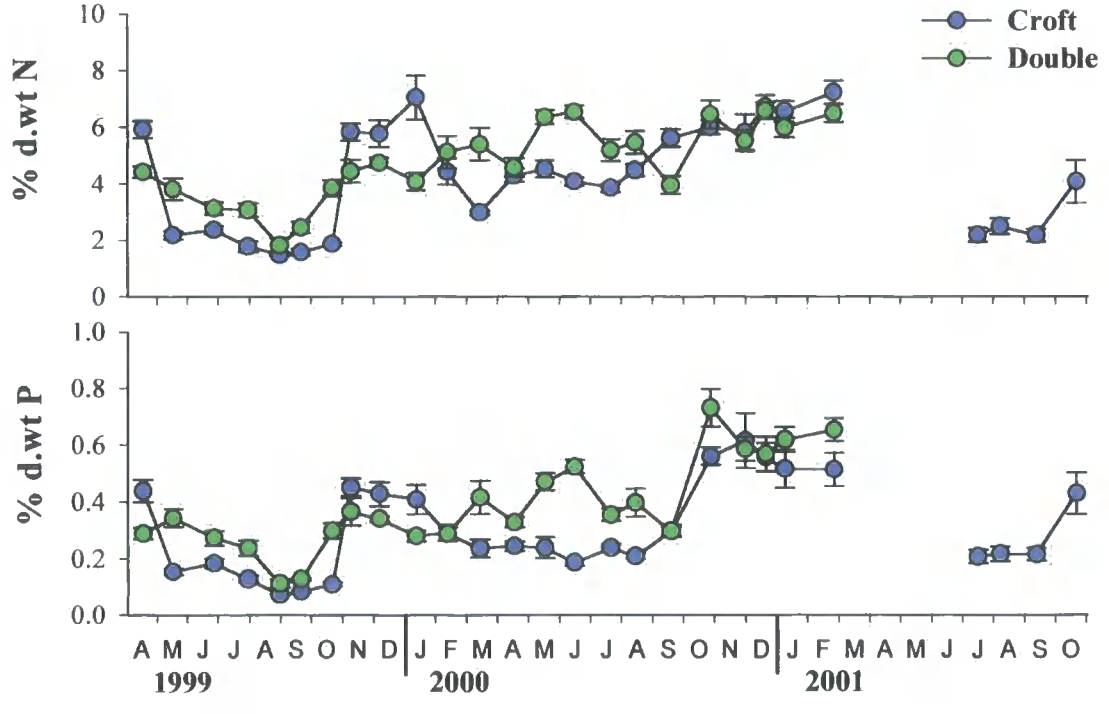

Figure 6.6 Changes in N and P content (as % d.wt) of *Chara hispida* apical tips from Croft and Double Kettle with time between April 1999 to October 2001. Vertical bars indicate ± one SE of the mean (n=12).

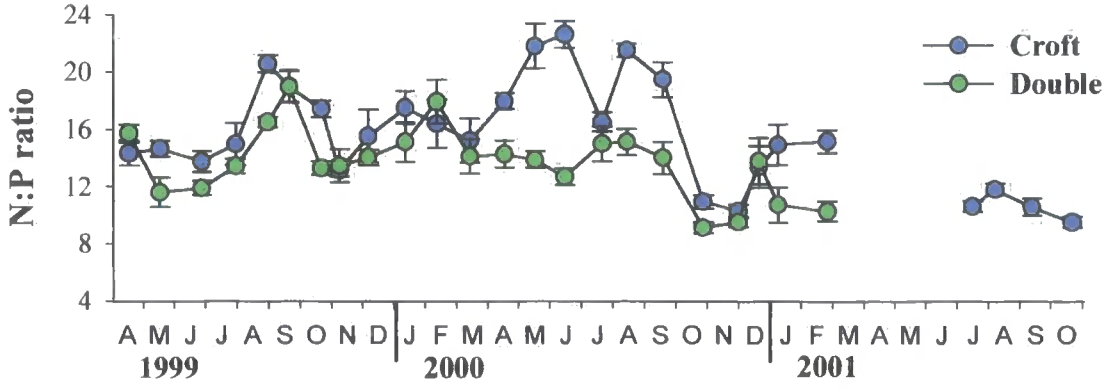

Figure 6.7 Changes of N:P ratio in *C. hispida* apical tips from Croft and Double Kettle with time between April 1998 to October 2001. Vertical bars indicate ± one SE of the mean (n=12).

Clear seasonal patterns were observed for both N and P contents of *C. hispida* apical tips from both ponds, with generally higher concentrations in winter and late autumn, than in summer and early spring (Figure 6.6). In Double Kettle, however the same pattern was not followed in the year 2000 when spring and early summer tissue N and P contents were considerably higher than the winter values. In addition, with the exception of N:P ratio in Croft Kettle all the other variables showed significantly different distribution ($P<0.001$) in the first year of monitoring from those observed during the second year.

Tissue N content of *C. hispida* in Croft Kettle showed maxima in January 2000 (7.05 ± 0.786) and February 2001(7.23 ± 0.407 % d.wt) and subsequently decreased and remained relatively low for the growing season (April to September) in comparison with the winter values. On the other hand, tissue N of *C. hispida* in Double Kettle showed the highest values in June (6.53 ± 0.228 % d.wt) and December (6.58 ± 0.286 % d.wt) of 2001. At Croft Kettle a similar pattern was evident for *C. hispida*'s P content, which showed peaks in November for both years monitored (0.45 ± 0.031 and 0.62 ± 0.097 % d.wt for 1999 and 2000 respectively), but *C. hispida* from Double had again high concentrations on June 2000 (6.54 ± 0.228) as well as late October (6.44 ± 0.496) of 2000. Overall, seasonal changes in the N and P contents of *C. hispida* followed the same pattern of change but with N contents of *C. hispida* apical tips showing slightly higher variability than P contents.

N:P ratio with the exception of spring and early summer of 2000 followed the same seasonal pattern in both ponds, with lower ratio in Double than Croft Kettle (Figure 6.7). Overall, N:P ratio was slightly higher during the summer months (from the beginning until the end of the growing season) and showed a marked decrease after autumn overturn of both years studied.

6.6 Phosphatase activity of apical tips

6.61 Introduction

In view of the close relationship between "surface" phosphatase activities and P limitation of algae (Section 1.6), it was decided to assess whether organisms in Hell Kettles were capable of expressing phosphatase activity. In spite of $CaCO_3$ deposition, *C. hispida* was thought to be the best organism for a seasonal study, as is by far the most abundant organism especially in Croft Kettle and is present throughout the year. The results for a seasonal study of approximately 2-cm apical tips of plants removed from Croft Kettle (site 2, Figure 3.5) and Double Kettle (site 7) are shown here (Figure 5.8). A parallel study (Figure 5.9) was made on epiphytes (mostly *Spirogyra*) collected from the vicinity of the upper part of the *Chara* plant.

6.62 Preliminary studies

Prior to a seasonal study in phosphatase activity of *C. hispida*, a number of experiments were carried out to assess the factors influencing PMEase activity, in order to determine the appropriate assay conditions. Experiments were conducted on shoots of *C. hispida* collected from Croft Kettle, which were kept in a transparent tank filled with 70 L of pond water in the greenhouse of Durham University Botanic Garden. *C. hispida* apical shoots were collected from a depth of 1.5 m from site 2 (Figure 3.5) at 3 March 2001. The tank was kept under a partially controlled temperature of 15 °C under natural daylight duration. With the exception of the factor under investigation, the initial assays were carried out using 100 μM MUP at pH 9.0 at 15 °C for an incubation period of 20 min.

Surface phosphatase activity, is markedly influenced by pH (Section 1.6). In view of this, the influence of pH on phosphatase activity of *C. hispida* was tested over a wide range, in half pH unit increments. The highest rate of activity was observed for pH values above 8 (Figure 6.8). The optimum pH value for the essay was chosen to be 9.5 since at higher pH values (above 10) possible lysis of cells takes place. Time course

experiments were carried out to test the linearity of the phosphatase reaction in order to determine a suitable incubation period for further studies. The rate of hydrolysis of MUP was slightly higher for an incubation period of 40 min than the other incubation times. Therefore, subsequent assays were carried out at this incubation time. Water temperature in Hell Kettle ponds varies seasonally in a range of 4-20 °C. The influence of temperature on phosphatase activity was tested over a similar range. The rate of hydrolysis showed maxima at 20 °C, thus later assays were conducted at this temperature. The influence of substrate concentration was quantified to assess the apparent V_{max} and K_m values of the rate of activity. This would determine the substrate concentration suitable for the seasonal study on *C. hispida*. V_{max} was equal to 5.35 and the K_m to 45.93. Therefore, a concentration of 100 µM was chosen to estimate phosphatase activity under saturated conditions, which is generally greater than the potential concentration of naturally occurring substrates.

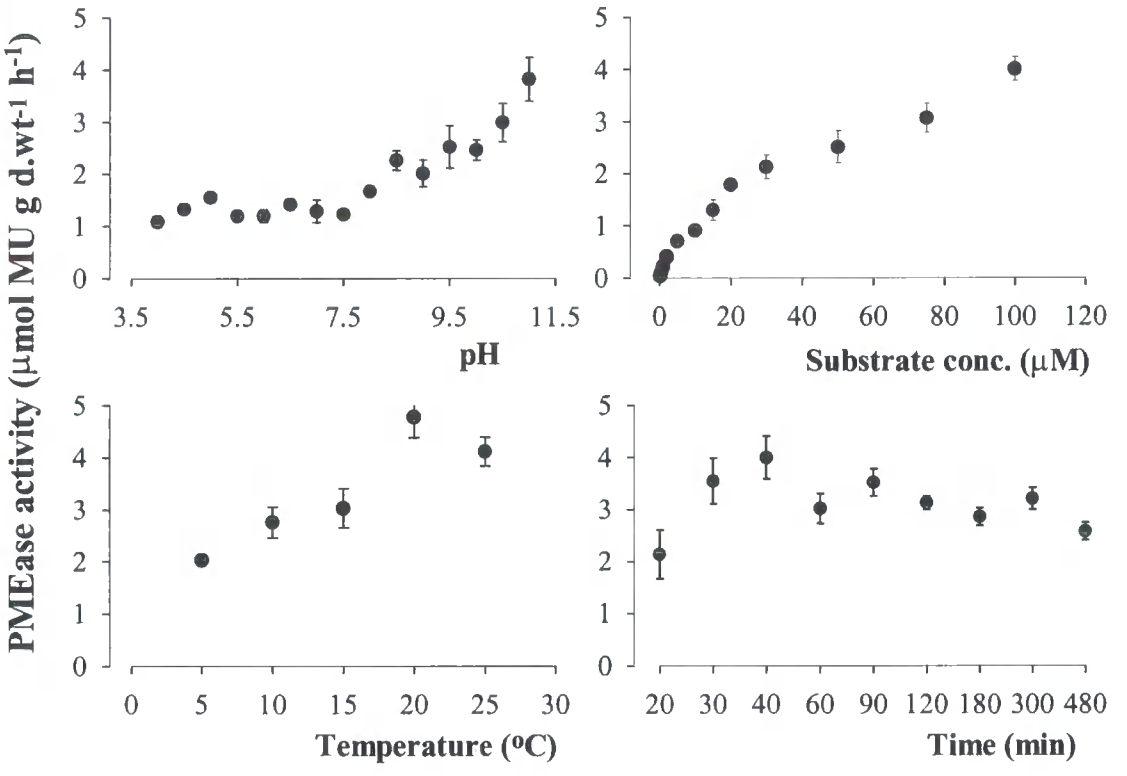

Figure 6.8 Influence of pH, substrate concentration, temperature and assay time in PMEase activity of *C. hispida* apical tips. Vertical bars indicate ± one SE of the mean (n=12).

In addition, phosphatase activity of *C. hispida* tips was quantified under light and dark conditions (n=7 for each treatment). Higher PMEase activity was observed for incubation in the dark (4.67 ± 0.168 µmol MU g $d.wt^{-1}$ h^{-1}) compared to that in light

under a light intensity of 80 μmol $m^{-2}s^{-1}$ PAR (3.54 ± 0.336). This fact could indicate that *C. hispida* is probably a low light adapted alga and that phosphatase enzymes could perform better under these conditions.

Finally, in order to test the effect of epiphytes on phosphatase activity of *C. hispida*, washed (Section 2.6) and unwashed tips (n=7 for each treatment) and epiphytic samples were assayed simultaneously. The washed *C. hispida* shoots showed an average (n=7) PMEase activity of 3.17 ± 0.413, the unwashed ones 4.71 ± 0.516 and the epiphytes 13.25 ± 3.729 μmol g $d.wt^{-1}$ h^{-1}. This indicates that, epiphytes (mostly diatoms), despite showing higher PMEase activity, interfere little quantitatively with the determination of PMEase activity of *C. hispida* tips.

6.63 Monthly survey of phosphatase activity

Phosphomonoesterase (PMEase) and phosphodiesterase (PDEase) activity was determined on a monthly basis on *C. hispida* apical shoots and its associate epiphytes from Croft and Double Kettle between April 1999 and October 2001, using two different substrates for PMEase (*p*NPP and MUP) and one for PDEase (bis-*p*NPP). Dry weights were corrected for $CaCO_3$ deposition. *C. hispida* and its epiphytes showed detectable activity throughout the year at both sites sampled, with the highest activity observed for PMEase and particularly for *p*NPP substrate. PDEase activity was considerably lower than PMEase activity for most of the period studied.

PMEase activity of *C. hispida* apical tips showed clear seasonal patterns in Croft and partially in Double Kettle (Figure 6.9). In general, higher rates of activity were observed in Double than Croft Kettle and the overall seasonal trend was of winter maxima and summer minima with the exception of late spring and early summer of 2000 in Double Kettle. Maximum values were observed in July 2000; 63.3 ± 2.85 μmol MU g^{-1} h^{-1} for MUP and 24.9 ± 2.57 μmol *p*NP g^{-1} h^{-1} for *p*NPP and both were observed in Double Kettle. PDEase activity showed higher temporal variability than PMEase activity, with no apparent seasonal trends and the highest rate was measured in April 1999 (10.9 ± 0.59 μmol bis-*p*NP g^{-1} h^{-1}) and that was again in Double Kettle. In addition, phosphatase activity was overall higher in the second year of study (2000) than the first one (1999).

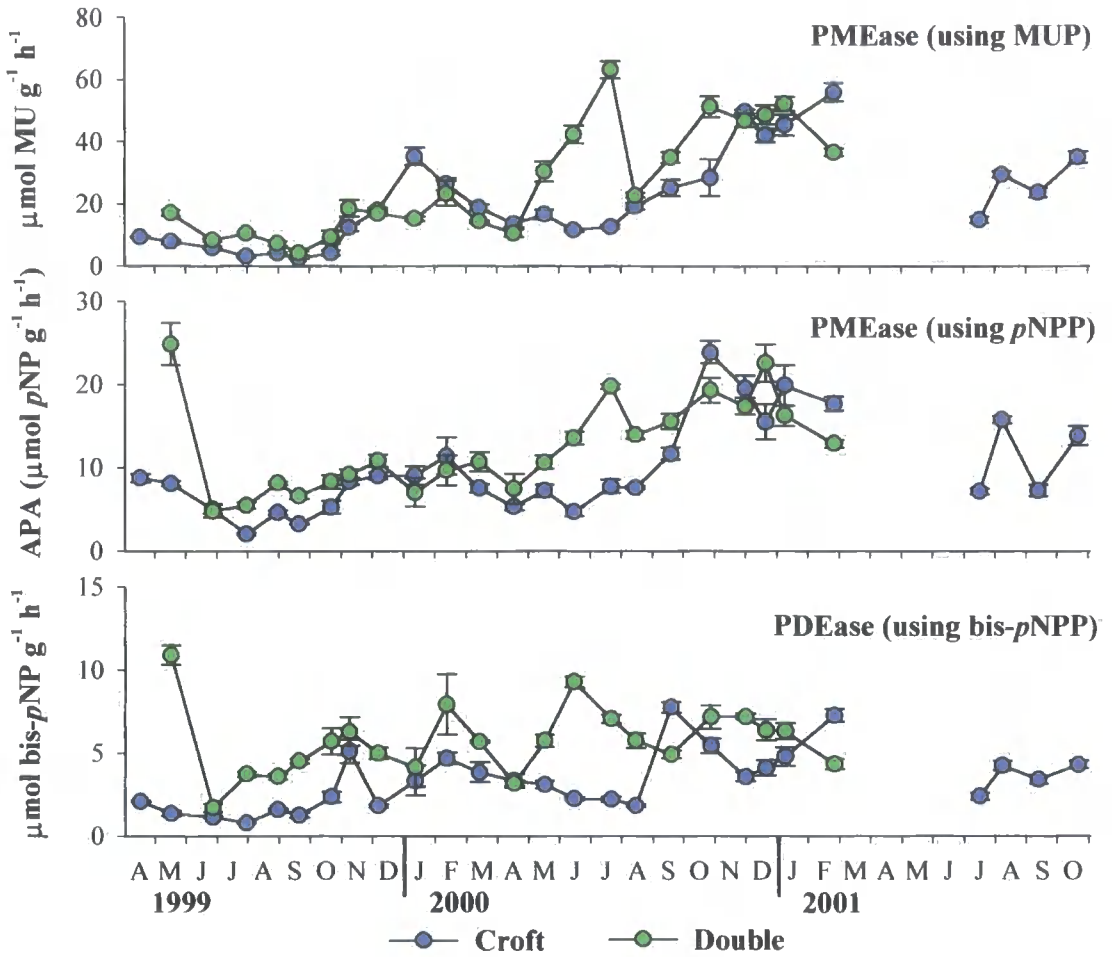

Figure 6.9 Changes in phosphatase activities (using all three substrates) of *C. hispida* apical tips from Croft and Double Kettle with time, between April 1999 to October 2001. Vertical bars indicate ± one SE of the mean (n=4).

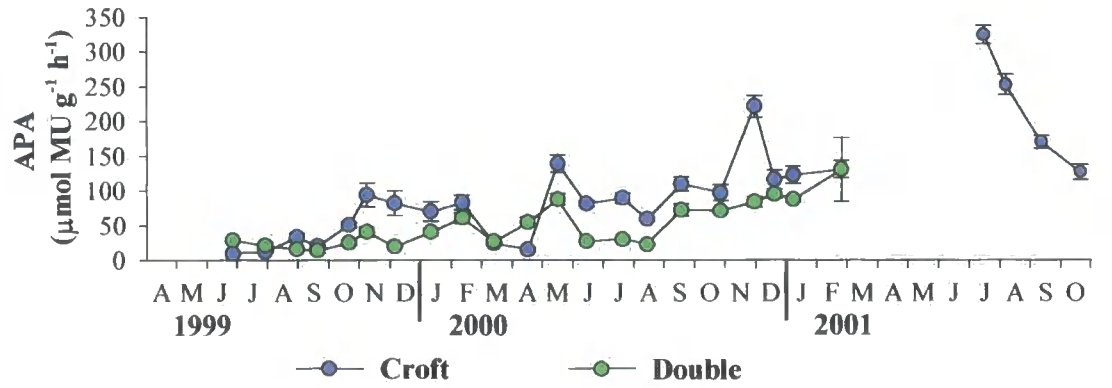

Figure 6.10 Changes in PMEase activities of *C. hispida* epiphytes in Croft and Double Kettle with time between April 1999 to October 2001. Vertical bars indicate ± one SE of the mean (n=4).

Epiphytes (Figure 6.10) showed much higher PMEase activity than *C. hispida*, with the highest rate observed in July of 2001 (324.6 ± 13.65 μmol MU g^{-1} h^{-1}). Overall, considerably higher rates of activity were observed in the summer and early spring of 2000, which coincided with the highest observed biomass of *Spirogyra* on the *C. hispida* beds. In addition, epiphyte activity showed considerable temporal and spatial variability in the period studied. Activity was lower in the first year and progressively increased in the following ones. A number of factors could be accounted for this observed pattern and is probably a combination of lower P concentrations of the water, as well as the increase in biomass of epiphytic *Spirogyra* observed in the last two years of study.

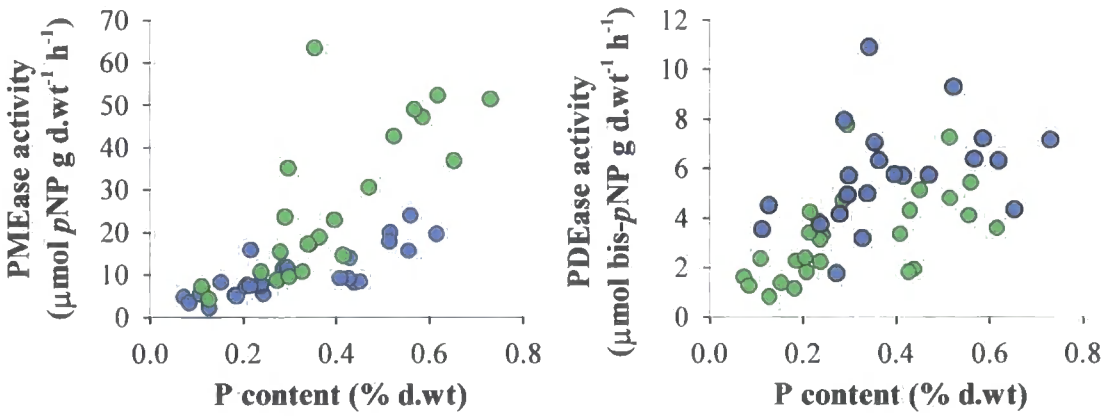

Figure 6.11 Relationships between PMEase and PDEase activity and P content of *C. hispida* shoots for Croft (●) and Double (●) Kettle. Samples were collected monthly, between April 1999 and October 2001.

PMEase and PDEase activity of *C. hispida* apical tips from both ponds showed positive relationships with P content of their tips (Figure 6.11). In addition, the relationships between PMEase and PDEase activity of *C. hispida* apical tips, concentrations of P fractions of water, tissue P contents and N:P ratio were tested at each pond for the studied period (Table 6.3). Significant positive relationships were found between most of the variables in both ponds. However, most of the significant positive correlations between P fractions of water tissue P and phosphatase activity were observed only for Double Kettle. Significant negative relationships were only observed amongst N:P ratio and the other parameters with the exception of the negative relationship found between tissue P and mean FRP concentration of the water column at Croft Kettle. These observations possibly suggest that phosphatase activity is either independent of internal P contents or indicates a constant P limitation and on the same time mostly depends on availability of nutrients in the water.

Table 6.3 Intercorrelation matrix of P fractions of water with phosphatase activity of *C. hispida* apical tips using three different substrates, tissue P contents and N:P ratio. Only significant ($p<0.05$) values are shown.

	Tissue P	Tissue N:P	PMEase (MUP)	PMEase (*p*NPP)	PDEase (bis-*p*NPP)	column FRP	column FOP	column TP	ab. *Chara* FRP	ab. *Chara* FOP	ab. *Chara* TP	surface FRP	surface FOP
Croft Kettle													
Tissue N:P	-0.468	1.00											
PMEase (MUP)	0.784		1.00										
PMEase (*p*NPP)	0.813	-0.493	0.841	1.00									
PDEase (bis-*p*NPP)	0.577		0.701	0.686	1.00								
column FRP	-0.461					1.00							
column FOP							1.00						
column TP						0.499	0.554	1.00					
ab. *Chara* FRP						0.746			1.00				
ab. *Chara* FOP		-0.444	0.401				0.718	0.458		1.00			
ab. *Chara* TP						0.525	0.406	0.581	0.776	0.627	1.00		
surface FRP												1.00	
surface FOP				0.413								0.773	1.00
surface TP												0.925	0.790
Double Kettle													
tissue N:P	-0.770	1.00											
PMEase (MUP)	0.758	-0.471	1.00										
PMEase (pNPP)	0.585	-0.434	0.732	1.00									
PDEase (bis-pNPP)			0.468	0.721	1.00								
surface FRP						1.00							
surface FOP	0.642	-0.685	0.435	0.506		0.508	1.00						
surface TP	0.493	-0.456	0.435		0.399		0.488						

6.7 Growth experiments

6.71 Introduction

Two *ex-situ* experiments were conducted in *C. hispida* apical shoots (10-14 cm in length), in order to test *C. hispida*'s response to a wide range of FRP water concentrations and water temperatures (Section 2.65). The initial and final fresh weight (superficial moisture was removed by a paper towel) and the length (to the nearest mm) of each node and lateral branchlet were measured as well as pigment composition and tissue N and P content. The results on the length of each individual node and lateral branchlet are not presented here since is above the scope of this chapter. Growth was determined as the percentage of relative growth (RG) (the percentage of total increase in weight and length, including lateral branchlets) for the P-enrichment experiment. In addition, the relative growth rate (RGR), which expresses growth in terms of a rate of increase in size per unit of size and allows for more equitable comparison than 'actual' measures was also used for the temperature experiment which involved a time series analysis. The ratio of Length:Weight (L:W, total length : fresh weight) is also presented, indicative of possible physiological changes (Andrews *et al.*, 1984b). Finally, through the measurement of pigment concentration in the shoots it was also hoped to gain evidence of cell breakdown and possible light adaptation (Schagerl & Pichler, 2000).

6.72 Phosphorus enrichment of water

In general, a substantial positive increase in length and weight of all *C. hispida* apical shoots was observed after 31 days of incubation under a wide range of FRP concentrations (Figure 6.12). Enhanced growth was observed for concentrations of P approximately up to 100-150 $\mu g\ L^{-1}$. Total length of *C. hispida* shoots showed higher percentage increase than weight. The range of % total increase in length per flask was between 14.8% (for 1500 $\mu g\ P\ L^{-1}$) and 53.0% (for 75 $\mu g\ P\ L^{-1}$). The initial (26.1 ± 0.45) and final (23.8 ± 0.49) ratio of L:W showed very little variability and was slightly higher after the incubation period for all the P enrichments (Figure 6.13). The slightly increased shoot elongation, in comparison with the increase in biomass, could be attributed to the comparatively low light intensity used in the experiment (Barko & Smart, 1981; Round, 1981).

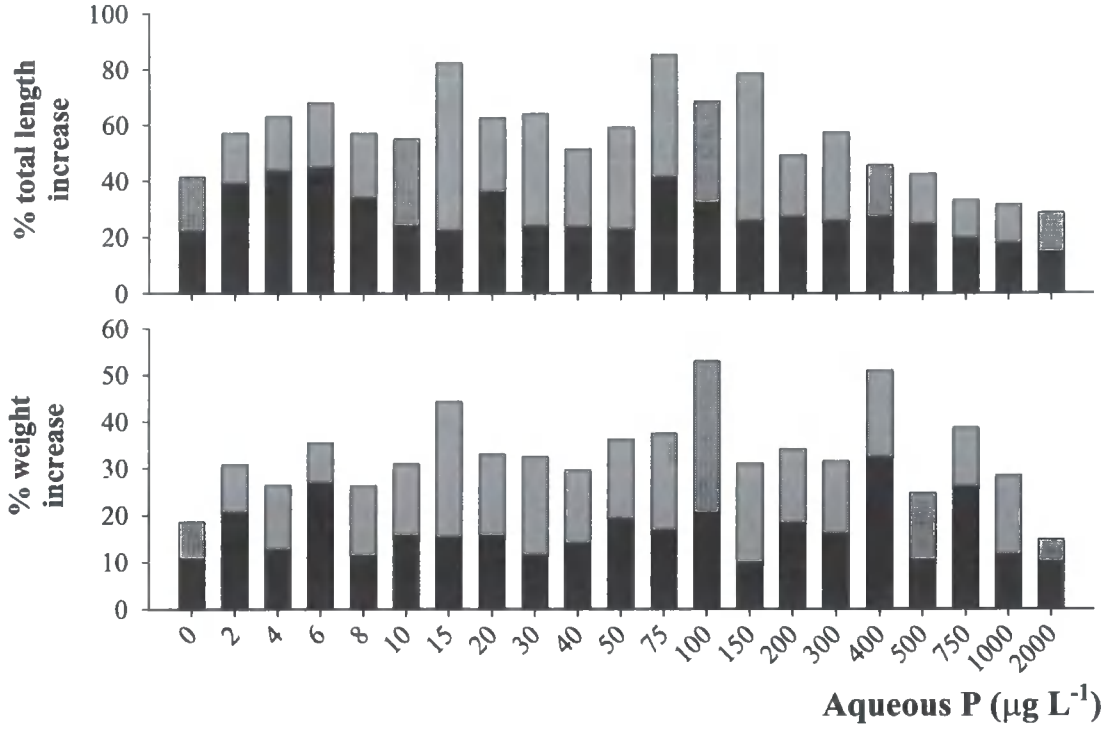

Figure 6.12 The percentage increase of total length and weight of *C. hispida* apical shoots per flask incubated under a wide range of FRP concentrations for 31 days (1st ■ and 2nd ■ shoot).

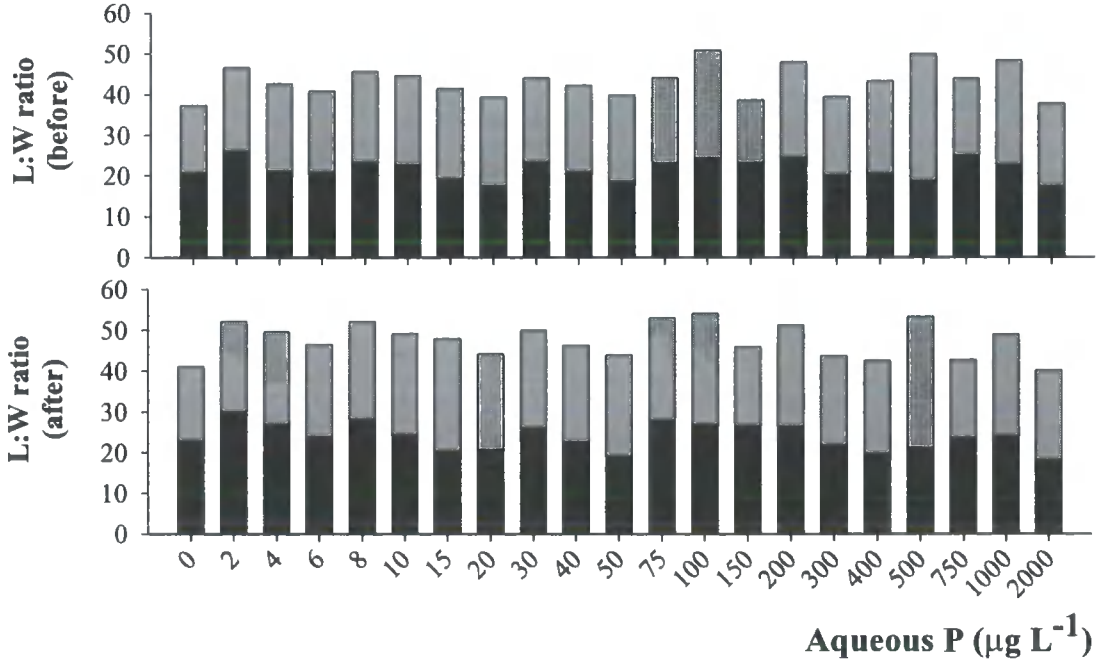

Figure 6.13 The initial (ambient) and final L:W ratio (per flask) of *C. hispida* apical shoots incubated under a wide range of FRP concentrations for 31 days (1st ■ and 2nd ■ shoot).

Chl *a* concentration (Figure 6.14) of *C. hispida* apical shoots after a 31-day incubation in a wide range of P concentrations was in the range of 0.17-0.46 % f.wt. Chl *a* concentration of initial shoots (n=14) was in the range of 0.17-0.36 % f.wt. In general, the results indicate that chl *a* concentration showed an increase with increasing P concentration of the water up to the level of 750 μg L^{-1} but then a progressive decrease was observed for the higher P enrichments. Chl *b* was considerably low in comparison with chl *a*, but followed a similar pattern of change. No significant changes where observed in the C*a* to C*b* quotient for any of the P enrichments. Chl *c* was absent for most of the treatments but showed an increase with increasing P concentration. This could be attributed to increased epiphytic growth at higher P concentrations. Phaeophytin concentrations showed high variation for individual shoots and treatments and no obvious trends with increasing P concentration were observed. In addition, the ratio between OD664 (absorbance at 664 nm) before and OD665 after acidification, for the initial (1.48 ± 0.032) and final (1.41 ± 0.016) shoots did not show significant differences and was considerably close to 1.7 indicating that the shoots were in good physiological condition (Schagerl & Pichler, 2000).

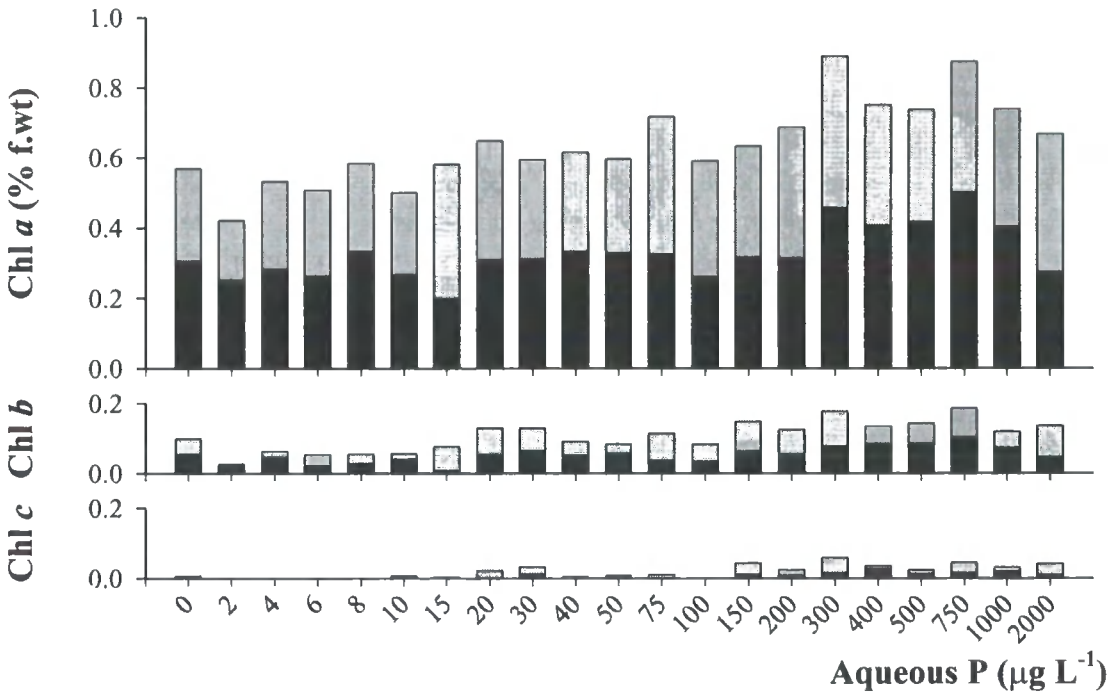

Figure 6.14 Chlorophyll a, b and c and phaeophytin concentrations (expressed as % f.wt) of *C. hispida* shoots incubated (for 31 days) under a wide range of FRP concentrations. Vertical bars represent the total for the two individual shoots placed at each flask (1st ■ and 2nd ■ shoot).

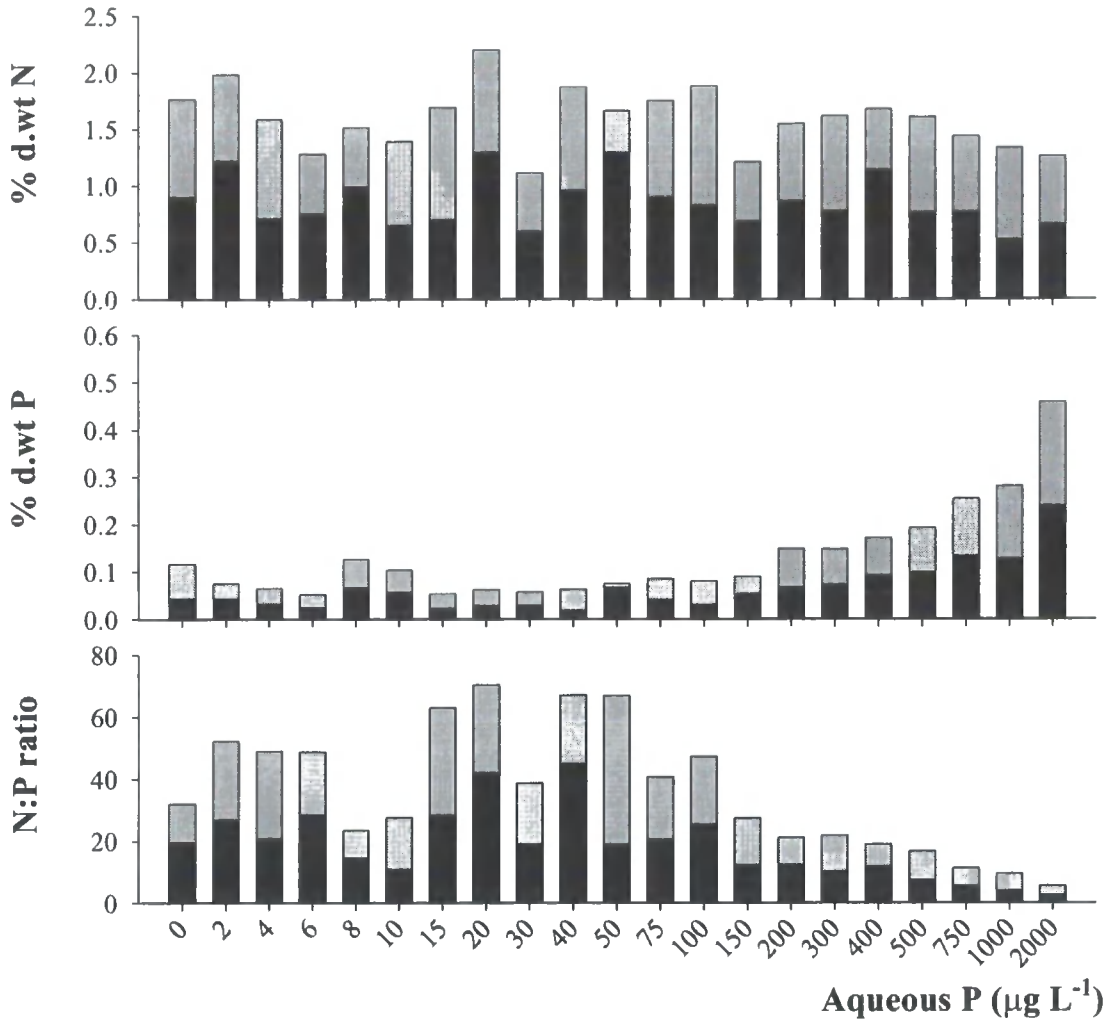

Figure 6.15 Nitrogen and phosphorus content and N:P ratio of *C. hispida* shoots incubated (for 31 days) under a wide range of aqueous FRP concentrations. Vertical bars represent the total for the two individual shoots placed at each flask (1st ■ and 2nd ■ shoot).

Phosphorus concentration of water from each flask was measured at the end of the experiment (31 days) for all flasks. FRP concentration was extremely low for most of the flasks (approximately 1-2 μg P L^{-1}), with the exception of the last two flasks (added P of 1500 and 2000 μg L^{-1} respectively), where FRP concentration was ≈ 12-15 μg L^{-1}. FTP concentration showed a similar distribution pattern with FRP, but with considerably higher values (FTP ≈ 10 μg L^{-1} for all flasks except the last two, where FTP ≈ 170 μg L^{-1}).

Phosphorus content of *C. hispira*'s apical shoots changed substantially with increasing aqueous P concentration (mostly >150 μg L^{-1}) after an incubation of 31 days. The highest P content (0.24 % d.wt P) was observed for the highest aqueous P

enrichment. Low aqueous P enrichments (up to 150 μg L^{-1}) did not affect the P content of *C. hispida* shoots, probably due to the parallel increase in biomass. P enrichment of water however, had only little influence on N contents of *C. hispida* apical shoots, which did not show any obvious pattern of change with increasing P concentration in the water. An exception was the slight decrease, which was observed for aqueous P concentrations higher than 150 μg L^{-1}. N:P ratio followed a similar pattern of change to N, but the decrease was much higher at aqueous P concentrations more than 150 μg L^{-1}.

6.73 Influence of water temperature

In general, a substantial positive increase in length and weight of all *C. hispida* apical shoots was observed under all incubations (Figure 6.16). The best growth was observed at 18 °C and 21 °C. The highest % weight growth of *C. hispida* shoots (24.7 ± 2.25) was observed at 18 °C, but the % length increase was slightly higher at 21 °C (55.9 ± 6.03). However, *C. hispida* shoots at 21 °C, even though showed increased growth approximately at the first four weeks of incubation, growth was substantially reduced for the rest of the period. This pattern was not observed at 18 °C, where the shoots showed a uniform increase in length and weight. The range of % total increase in length for the whole period was between 27.8 ± 2.73% (at 12 °C) and 55.9 ± 6.03% (at 21 °C) and for weight between 12.3 ± 2.14% (at 15 °C) and 24.7 ± 2.25 (at 18 °C). The difference between the highest and lowest observed growth was almost twofold for both length and weight and total length showed higher percentage increase than weight. Relative growth rates showed a completely different pattern of distribution than relative growth. Considerably higher rates of RGR were observed for the first two weeks of incubation and then a constant and gradual decrease was evident. L:W ratio showed very little variability and was slightly higher after the incubation period for three out of the four incubation temperatures (except growth at 21 °C). One-way analysis of variance (Table 6.4) showed significant differences for growth at different water temperatures. Most of the significant differences were observed between growth at 12 and 15 °C to 18 and 21 °C. Finally, significant changes in growth were not observed between 12 and 15 °C and 18 and 21 °C.

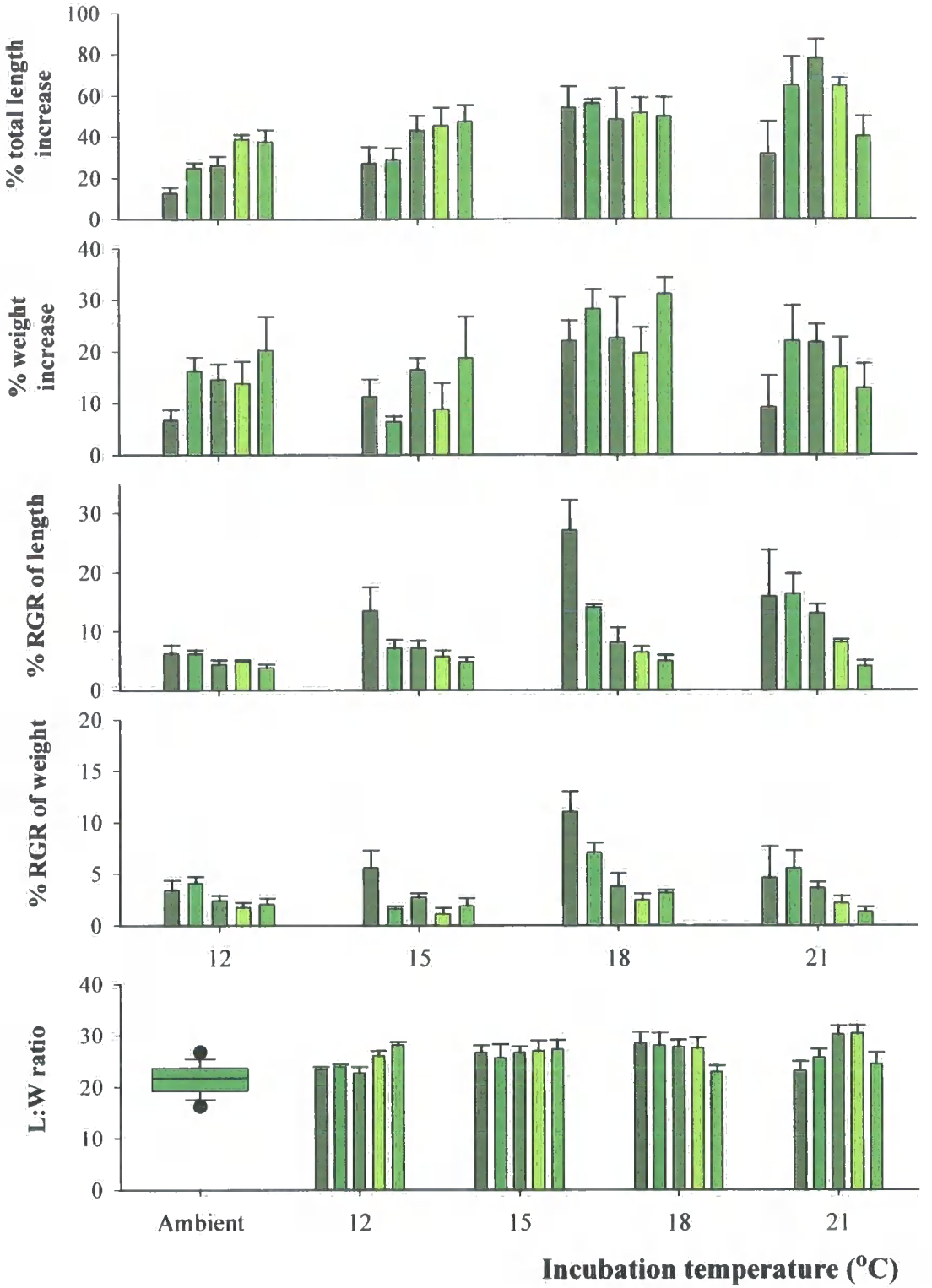

Figure 6.16 The % increase of total length and weight, the % relative growth rate and the initial (ambient) and final L:W ratio of *C. hispida* apical shoots growing under 4 temperatures on a time series. Ambient values indicate *C. hispida*'s characteristics when collected from Croft Kettle. The groups of vertical bars indicate length of incubation time (■2 ■4 ■6 ■8 and ■10 weeks). Vertical lines represent ± one SE of the mean (n=4).

Table 6.4 ANOVA p-statistics from comparisons of % total length to weight growth of *C. hispida* shoots incubated under a series of temperatures for a total of 10 weeks (as *** P < 0.001, ** P < 0.01, * P < 0.05, ns: not significant).

		12 °C	15 °C	18 °C
% total length	15 °C	ns		
increase	18 °C	***	ns	
	21 °C	***	*	ns
% weight increase	15 °C	ns		
	18 °C	**	***	
	21 °C	ns	ns	ns

Table 6.5 ANOVA p-statistics from comparisons of chlorophyll a, b and c and phaeophytin concentrations (% f.wt) of *C. hispida* shoots incubated under a series of temperatures for a total of 10 weeks (as *** P < 0.001, ** P < 0.01, * P < 0.05, ns: not significant).

		Ambient	12 °C	15 °C	18 °C
Chl *a*	12 °C	***			
	15 °C	**	***		
	18 °C	***	ns	***	
	21 °C	***	ns	***	ns
Chl *b*	12 °C	***			
	15 °C	***	**		
	18 °C	***	ns	*	
	21 °C	***	ns	*	ns
Phaeo	12 °C	*			
	15 °C	ns	*		
	18 °C	**	ns	*	
	21 °C	***	ns	**	ns

Chl *a* concentration of initial (ambient) shoots (n=14) was in the range of 0.17-0.36 with an average of 0.279 ± 0.0167 % f.wt and a considerable decrease was observed for all the incubations at all temperatures (Figure 6.17). The highest decrease was observed for *C. hispida* shoots at 21 °C where the average after 10 weeks of incubation was only 0.156 ± 0.0095 % f.wt. In general, the results indicate that chl *a* concentrations were highest in the plants incubated at 15 °C, were low but constant with time for the incubations at 12 and 18 °C and showed a considerable decrease with time at 21 °C. Chl *b* was considerably lower in comparison with chl *a*, followed a similar pattern of change for all the treatments, but was considerably higher than the initial one. Significant changes were also observed in the chl *a* to chl *b* quotient for all the

treatments. Chl *c* was extremely low or absent for all the treatments. Phaeophytin concentrations were slightly higher at 15 °C and showed considerable variation with time and amongst individual shoots. In addition, the ratio between OD664 before and OD665 after acidification, for the initial (1.48 ± 0.032) and final (1.53 ± 0.029) shoots did not show significant differences and was considerably close to 1.7 indicating a good physiological condition of shoots. One-way analysis of variance (Table 6.5) showed significant differences ($P<0.001$) between ambient chl *a*, *b* and *c* and phaeophytin concentrations and concentrations found in shoots after the incubation in a series of water temperatures. Significant differences in pigment content were also found amongst incubations at different temperatures and most of them were observed between growth at 15 °C to 18 and 21 °C.

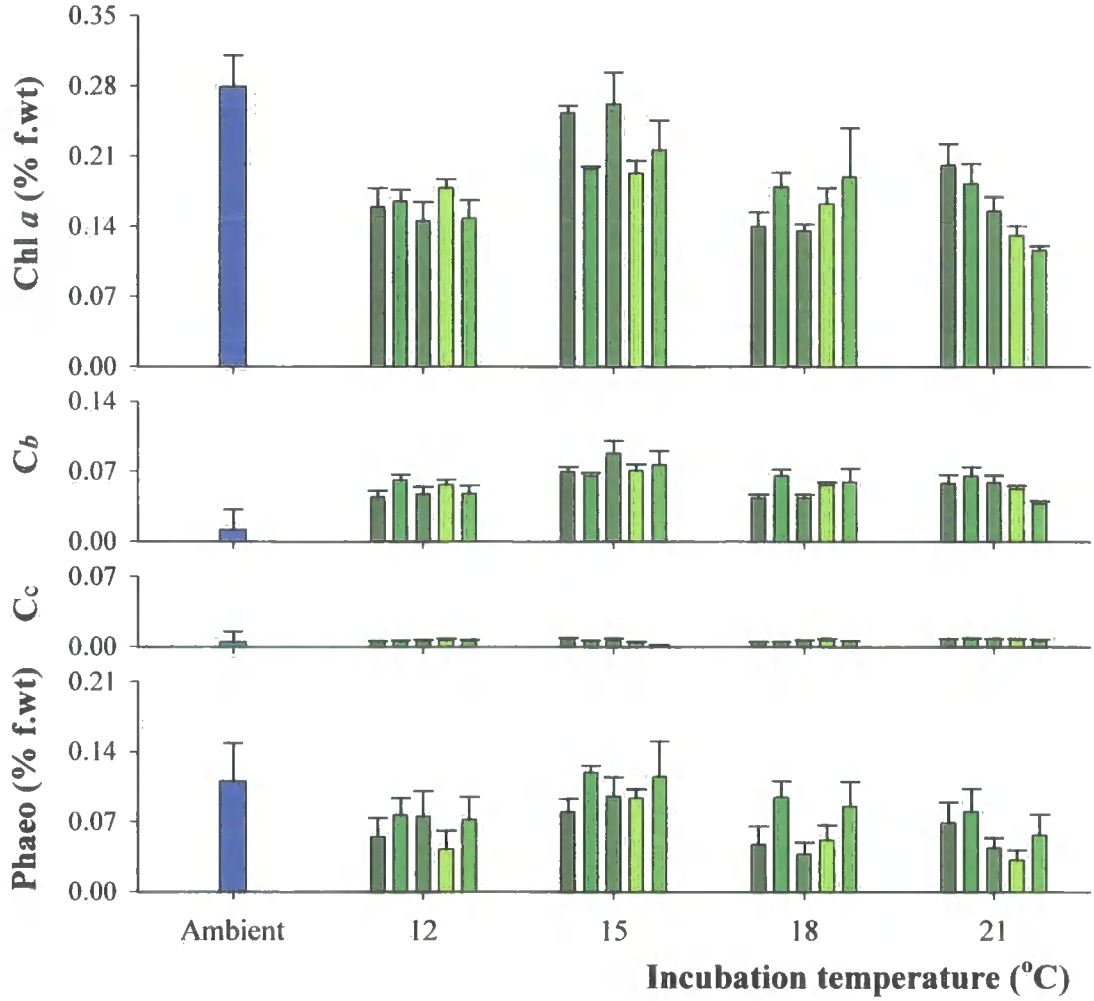

Figure 6.17 Chl *a*, *b* and *c* and phaeophytin concentrations (% f.wt) of *C. hispida* apical shoots growing under four temperatures on a time series. The groups of vertical bars indicate the length of the incubation time (■2 ■4 ■6 ■8 and ■10 weeks). Vertical lines represent ± one SE of the mean (n=4).

6.8 Discussion

Tissue $CaCO_3$ content of *C. hispida* apical tips comprised about 30-40 % of the dry weight and showed spatial and temporal variability, but with no apparent seasonal trends. In addition, the overall higher $CaCO_3$ content of *C. hispida* shoots collected from Double Kettle than Croft Kettle suggests that $CaCO_3$ deposition, is not only a function of hardness and calcium content of water (since Croft Kettle has harder water than Double Kettle). It has been suggested that calcification is also a function of photosynthesis and thus, metabolic activity of the plants as well as being influenced by environmental parameters (Section 1.7). Supporting evidence for this explanation arises from the fact that *C. hispida* shoots from Double Kettle were collected from a settled, shallow part of the pond, very close to the water surface whereas shoots from Croft Kettle were from about 1.5 m deep water.

Treatment with 1, 2 and 5% HCl solutions indicated that 2 % is probably the best concentration to be used for removing $CaCO_3$ deposits from *C. hispida* shoots according to the method described in Section 2.62. Treatment with 1 % HCl solution was probably not strong enough to remove all the $CaCO_3$ deposition, while treatment with 5 % even though removed more $CaCO_3$ than the other solutions indicated possible removal of tissue chemical elements. In addition, vertical gradients of tissue elemental composition were evident for most of the determined elements and particularly for N and P, suggesting that estimation on total N and P content of *C. hispida* plants cannot be based on concentrations of the youngest part of the shoot.

C. hispida apical 2-cm tips showed spatial and temporal changes in both nutrient content and phosphatase activity during the study period. The N and P contents of *C. hispida* apical tips were higher in Double Kettle than Croft Kettle, consistent with increases of nutrients in the water. Clear seasonal patterns were evident for *C. hispida* apical tips, with increased tissue N and P contents over the winter and early spring at both sites, which subsequently decreased and remained relatively constant for the rest of the growing season. However, N and P contents showed unusually high values in the summer of 2000 in Double Kettle and it was the only time in the studied period that the general seasonal trend of winter maxima and summer minima was not followed. The later peak of tissue N and P, especially of May and June 2000 in Double Kettle coincided with the summer flooding of the adjacent fields and with flowering and fruiting of the plants, probably suggesting that N and P were accumulated in shoots during this phase of the life cycle. In addition, tissue N was higher in the second year

of study, which was consistent with the observed increase in N concentration of the water.

N:P ratio for *C. hispida* apical tips averaged 15.6 (range 9.2 to 19) for Croft Kettle and 13.7 (range 9.2 to 19.0) for Double Kettle and was close to Redfield stoichiometry, neither suggesting N nor P limitation. However, during summer N:P ratio was higher than 16:1, possibly indicating that P was the limiting nutrient of growth for most of the growing period. In addition, the marked decrease of N:P ratio observed after autumn overturn of both years also suggests increased availability of P in the water during this particular period and rapid uptake. Supportive evidence for this hypothesis arises from the fact that a sharp decrease in the N:P ratio was observed at Croft Kettle in July 2000, a month after the summer flood. This also indicates the ability of *C. hispida* to absorb P from the water much faster than N possibly by means of phosphatase enzymes or just through diffusion. However, both N and P concentrations in *C. hispida* are probably in excess of critical levels as was determined from the P enrichment experiments, suggesting that even during the growing season these nutrients were available in adequate amounts in the tissues.

PMEase activity of *C. hispida* apical tips from both ponds showed winter maxima and summer minima, but this was not apparent for summer 2000 at Double Kettle. PDEase activity showed higher temporal variability high no apparent seasonal trends and overall was much lower than PMEase actitivity. *C. hispida* apical tips collected from Double Kettle showed overall higher rates of activity than Croft Kettle. Seasonal variation in PMEase or PDEase activity did not show an inverse relationship with P content at any of the ponds. In contrast, PMEase and PDEase activity of *C. hispida* apical tips showed strong positive correlations with P content. Positive correlations were also found between PMEase activity and dissolved aqueous P fractions, although this was not so clear at both ponds for all substrates used. The absence of negative correlations amongst PMEase and PDEase activity and tissue P suggests that phosphatase activity is not a direct response to low tissue P content. Therefore, the current seasonal trend of PMEase and PDEase activity observed in Croft and Double Kettle is against the general hypothesis that phosphatase activity is generally higher when tissue P content is low.

A possible explanation for this could be that rate of phosphatase activity is dependent on substrate availability and on the life cycle of the plants. Supporting evidence arises from the fact that PMEase and PDEase activity showed increased

activities during times of increased nutrient concentrations in the water, such as after autumn overturn and the flooding of the surrounding fields, which washed a lot of dissolved and particulate matter into the ponds (increased availability of substrates). This phenomenon of luxury consumption (i.e. accumulation in tissues in excess of immediate metabolic requirements) has been noted in other macrophytes too.

Evidence for this phenomenon also arises from the growth of *C. hispida* shoots in pond water enriched with FRP. Increased aqueous FRP concentrations enhanced biomass production in *C. hispida* shoots and promoted P accumulation in the tissues. P content of *C. hispida* shoots showed little or no change in aqueous P concentrations up to 150 $\mu g\ L^{-1}$, whereas enrichments higher than that produced a progressively higher accumulation over that at the ambient P concentration. At lower P concentrations however, the highest increase in biomass was observed and this probably compensates for the fact that absorption of P from the water did not result in excessive accumulation of P in the tissue. In addition, this possibly suggests that growth of *C. hispida* shoots was limited by P and not N. Furthermore, the comparatively lower increase in biomass above 150 $\mu g\ L^{-1}$ FRP enrichment was probably due to a decrease of tissue N to critical levels and does not really indicate any adverse effects of P. However, with crude cultures it is not possible to investigate carefully the tolerance of P, since negative effects of an increased concentration could be of a secondary effect caused by stimulated growth of microorganisms, since bacteria for example can rapidly absorb and convert inorganic P to organic forms, which can be difficult to assimilate by charophytes.

Therefore, the results of this experiment demonstrate high capacity of *C. hispida* to absorb P from water in a wide range of FRP concentrations. Owing to this potential *C. hispida* can probably rapidly compensate seasonal inputs of P into the pond by increasing levels of P in plant tissues as well as by higher biomass production. In addition, the fact that almost no FRP was left in the pond water after 31 days of incubation for most of the treatments, suggests that *C. hispida* growing in pond water enriched with FRP may still remain P limited. Moreover, apart from the large amount of P stored, wintergreen species like *C. hispida* can further immobilize P for long periods and therefore act as P sinks, indicating their ecological importance in freshwater ecosystems.

Finally, a substantial positive increase in length and weight of all *C. hispida* apical shoots was observed at temperature incubations (range 12 to 21 °C), with the highest

growth at 18 °C and 21 °C. Incubation at 18 °C resulted in the highest biomass production and incubation at 21 °C promoted the highest shoot elongation. In addition, acclimation of *C. hispida* shoots was better at 18 °C as was demonstrated by the highest % length and weight increase after the first fortnight of incubation. Therefore, the results obtained after about 75 days of incubation, suggest that *C. hispida* should be able to grow quite well at the highest water temperatures observed at both ponds (18 °C ≈ the highest surface water temperature observed in the ponds). However, due to the comparatively short-term incubation, definite conclusions cannot be made for the response of *C. hispida* shoots to such temperatures over a longer time-period. In addition, the decrease in the relative growth rates of height and weight over time, as well as the observed lower chl *a* contents in comparison with ambient levels and the progressive decrease in chl *a* over time, in particular for the 21 °C incubation, probably suggest that growth became limited due to lack of nutrients in the pond water.

6.9 Summary

1. Carbonate deposition on the cell walls of *C. hispida* apical tips comprised, on average between 35% and 40% d.wt and undoubtedly contributed to the fresh to dry weight quotient of the plants.
2. Rinsing with 2 % HCl acid as described in Section 2.62 probably removes most of the $CaCO_3$ deposition without influencing elemental composition of *C. hispida*.
3. Vertical gradients in the elemental composition (in particular for N and P) of *C. hispida* plants, with higher contents in the youngest apical part of shoots than the lower parts were identified.
4. Tissue N and P contents of *C. hispida* apical tips from Croft and Double Kettle showed clear seasonal patterns, with generally higher concentrations in winter and late autumn, than in summer and early spring.
5. PMEase assayed with MUP (highest affinity) showed mean values as follows: Croft Kettle, 11.8 ± 3.3; Double Kettle, 11.7 ± 2.0 μmol of MU g $d.wt^{-1}h^{-1}$. Epiphytes showed much higher PMEase activity than *C. hispida*. PDEase was much lower than PMEase throughout the year.
6. The P enrichment experiments indicated that increased pond water FRP concentrations (especially up to 150 μg L^{-1}) enhance biomass production in *C. hispida* shoots and promote P accumulation in the tissues.
7. Incubations at water temperatures of 12, 15, 18 and 21 °C indicated a substantial positive increase in length and weight of all *C. hispida* apical shoots, with the highest production of biomass at 18 °C and 21 °C.

CHAPTER 7 General discussion

7.1 Introduction

Field studies have produced interesting results concerning seasonal and temporal changes of oxygen, temperature, N and P fractions in Hell Kettle ponds as well as in the relationship between water chemistry and the nutrient content of *C. hispida*. The seasonal study on sequential P fractionation of surface sediments have provided information on a number of aspects concerning P dynamics in the sediments and their spatial and temporal changes. In addition, *in situ* studies on *C. hispida* apical shoots have shown its importance as a regulator of aqueous P concentrations as well as support the hypothesis that low aqueous P concentrations are not directly toxic on its growth.

The main aim of this study was to assess the P status in Hell Kettle ponds in particularly Croft Kettle as well as a number of other physical and chemical parameters and to determine their effect on growth of *C. hispida*. However, unlike what happened in the summer of 1996, *C. hispida* remained healthy in Croft Kettle for all the period studied and so only indirect observations and conclusions can be made.

7.2 Water chemistry

Seasonal and spatial trends were evident at Croft and Double Kettle for many of the environmental variables determined, typical of many standing waters in temperate regions (Moss *et al.*, 1996a; Heathwaite *et al.*, 1997). Croft Kettle showed very low N (mean: 144.3 $\mu g\ L^{-1}$ TN) and P (mean: 15.5 $\mu g\ L^{-1}$ TP) concentrations with winter maxima and some district peaks in the summer. Double Kettle in contrast, showed higher concentrations of inorganic, organic and particulate nutrients, with mean TP of 31.2 $\mu g\ L^{-1}$ and TN of 256.4 $\mu g\ L^{-1}$. Nonetheless, within-year and intra-annual variability at both ponds was quite high, indicating that a high frequency in sampling is required to provide representative annual mean data and this should be taken into account for future monitoring and management of the ponds. In accordance, an episodic event (as the June 2000 flood) might change completely the water chemistry and potentially toxic levels could be reached which could alter the vegetation in the ponds. Using the lake trophic classification (OECD, 1982), Croft Kettle may be classified as oligotrophic and Double Kettle as mesotrophic. The borehole water, showed much more stable physicochemical characteristics and substantially lower N

(mean: 74.6 μg L^{-1} TN) and P (mean: 5.2 μg L^{-1} TP) concentrations with limited temporal variation, indicating that the source of water was from a deep fairly unpolluted aquifer (Freeze & Cherry, 1979).

Bennion & Smith (2000) examined the variability in water chemistry of 31 shallow ponds in southeast England and found annual mean concentrations of NO_3-N varying between 0.7-5.6 mg L^{-1} and of TP varying between 25-646 μg L^{-1}. In general, most lakes and ponds in temperate regions usually have TP concentrations above 25 μg L^{-1} and TN above 1 mg L^{-1} while those contaminated by sewage effluent exceed 100 μg L^{-1} TP and 10 mg L^{-1} TN (Heathwaite *et al.*, 1997). Therefore, Hell Kettle ponds and especially Croft Kettle, apparently have very low N and P concentrations, indicative of pristine conditions. Such low N concentrations in particular are exceptions, for standing waters in temperate regions. Supportive evidence for comparatively lower N concentrations and periods of N limitation in Croft Kettle also arise from N:P ratio, which are often lower than the Redfield stoichiometry (Redfield *et al.*, 1963) as well as the presence of *Epithemia* and *Rhopalodia*(Appendix 1), the only two diatom genera with a capacity for nitrogen fixation, through a symbiotic association with a cyanobacterium (DeYoe & Lowe, 1992).

A possible explanation could be the fact that point and non-point sources of nutrients in the ponds have also very low N concentrations. Croft Kettle directly and Double Kettle indirectly (through the balancing pipe), accept inflows of groundwater but these are of modest N and P concentrations, usually below even the minima experienced in the ponds. Therefore, in the absence of other direct inputs, surface runoff and direct precipitation are probably the only other allochthonous sources in the ponds. In addition, Hell Kettle ponds have a relatively small catchment area with the presence of a wetland area adjacent to the ponds and no direct application of fertilisers for at least the previous 20 years in the adjacent agricultural land. Wetlands areas usually show high denitrification rates and overall high retention of N (Saunders & Kalff, 2001). Accordingly, the low nitrate (especially) concentrations in both ponds are probably consistent with this for the catchment since only through relatively very low and slow percolation could nitrate levels be kept well below those experienced in other agricultural catchments. Nevertheless, even though calculations of soil leaching and water inputs from the catchment cannot be easily measured, the steady pond level, especially of Croft Kettle in combination with low nitrate concentration, even in winter, indicate a low input, particularly in Croft Kettle.

Hence, the differences in nutrient concentrations between Croft and Double Kettle, most likely indicate that Double Kettle receives higher surface runoff and field drainage. This is probably due to the less dense and defined fringing vegetation around the pond, which facilitates better access to the living stock for water supply usage, suggesting higher bank erosion and subsequent inputs from the catchment. Other possible explanations could be morphology and shape of the basin as well as the presence of carp (only in Double Kettle), which through disturbance and resuspension of sediments as well as excreta, increases nutrient concentrations in surface waters (Williams *et al.*, 2002).

However, in addition to external inputs, internal cycling of nutrients was evident in Croft Kettle and was suspected for Double Kettle. Evidence for internal regeneration of nutrients in Croft Kettle arose from the fact that nutrient concentrations were higher in the hypolimnion than the epilimnion particularly during the summer. Elevated hypolimnetic concentrations of N and P, could originate from the sedimentation of epilimnetic detritus and phytoplankton and release of large amounts of P*i* and NH_4 from the sediment in association with deoxygenation of the hypolimnion (Kilinc & Moss, 2002). However, due to low planktonic growth, as was indicated by the low chlorophyll *a* concentrations in the water and the fact that increases in hypolimnetic P*i* and NH_4 did not develop immediately upon the development of hypolimnetic anoxia, owing to the time required for P transport to the overlying waters (Townsend, 1999), the best explanation is probably release from the sediment. Increases in P*i* and NH_4 concentrations are not unusual in anaerobic hypolimnia of many ponds and lakes and the mechanisms of release have been understood since Mortimer's (1941; 1942; 1971) early studies. However, Golterman (2001) suggested, that Mortimer's hypothesis that release of P from sediments is caused by the reduction of a Fe(OOH)≈P complex, is not so straight forward and may be controlled by other factors too (i.e. H_2S concentration). Hydrogen sulphide concentrations in the hypolimnion of Croft Kettle were probably quite high (intense smell) and according to Golterman's (2001) suggestions probably favoured P release from the sediment by the precipitation of FeS. Further evidence for precipitation of FeS arises from the fact that Croft Kettles sediments have a intensive black colour (Appendix 2), indication of FeS presence (Golterman, 2001).

However, even though concentrations of N and P in the hypolimnion rose after the start of stratification, the same was not observed at the epilimnion during the summer, rendering the importance of internal regeneration of nutrients. The physical and chemical data reported indicated that Croft Kettle was stratified during the whole

summer, with definite zones of severe deoxygenation, eventually complete anaerobiosis. In 2000 for example, there was a stable thermocline for at least four months from late spring through summer until about late September. This probably indicates that stratification was quite stable with relatively low eddy conductivity, hence not favouring upwards mixing of hypolimnion water and thus allowing rapid tracking by epilimnion concentrations of those in the hypolimnion. High stability of stratification may be a function of the shape of the basin, the dense and extensive beds of charophytes, which reduce water movements (Graneli & Solander, 1988; Blindow, 1992b; Scheffer, 1999) as well as the surrounding fringing vegetation, which probably reduces wind speed.

Nonetheless, despite the rather stable stratification, an increase in nutrient concentrations into the epilimnion of Croft Kettle was observed in May and June 1999. This increase was only detected by the surface standard survey and coincided with the unusual contamination of the groundwater (pers. comm. with J. Lamont-Black), suggesting that there was considerable transfer of water between hypolimnion and epilimnion. In turn, this suggests absence of stratification within these areas covered by charophytes as well as the possibility that point of groundwater input is located within the charophyte beds. Moreover, supportive evidence for absence of stratification within the dense charophyte meadows arises from the dissolved oxygen studies (Figure 4.15) as well as the morphological differences between *C. hispida* found above and below approximately 4 m in depth.

In addition, as mentioned previously, N and P concentrations in surface water showed periodical distinct peaks, mainly after autumn turnover as well as after the summer flood in June 2000. However, P concentrations in surface water after these events showed a marked decrease over time and almost an order of magnitude lower values were recorded in the subsequent months. This rapid decrease in P concentrations could probably be attributed to co-precipitation of P*i* with calcium carbonate, mostly as calcite (Penn & Auer, 1997) due to the hardwater nature of the ponds, suggesting that mineral precipitation of P*i* plays an important role in controlling levels of P*i* in Croft Kettle. Supportive evidence for this observation arose from the relatively high concentrations of $CaCO_3$ related P found in the sediments (Section 7.3). The importance of the interactions between P and $CaCO_3$ in the water column particularly during periods of intense photosynthesis (House & Donaldson, 1986), which mediate the downward flux of P from surface waters , has been shown for many hardwater lakes and rivers (Dobolyi & Herodek, 1980; Koschel *et al.*, 1983; Murphy *et al.*, 1983; Gonsiorczyk *et al.*, 1998; Neal *et al.*, 2000). Thus, high water column

concentrations of $CaCO_3$ due to groundwater inputs are probably regulating sedimentation of P into the sediments. These suggestions have important management implications for Croft Kettle, since declines in water column $CaCO_3$ concentrations due to reductions in groundwater input may result in conditions of $CaCO_3$ undersaturation and thus reductions in calcite precipitation as well as increase release of P from sediments to the water column.

7.3 Sediment

Sediment digestions for total N and P and N:P ratio and a sequential P fractionation scheme developed mainly by Psenner (Psenner *et al.*, 1984; Psenner *et al.*, 1988; Psenner & Pucsko, 1988) and tested for calcareous sediments, were used to explore vertical and temporal changes in Croft Kettle's surface sediments (0-10 cm). The uppermost sediment section (0-2 cm), which showed the highest N and P contents, yield an annual mean total-N content of 9133 ± 191.9 μg g^{-1} d.wt and total-P of 883 ± 23.1 μg g^{-1} d.wt. The values obtained for both total-N and total-P are comparable with concentrations quoted in the literature of many other freshwater lakes and rivers, and do not show either N and/or P rich sediments nor N and/or P poor sediments. For example total-N content in surface sediments; of shallow Lake Petersdorf, Germany varied between 9000 and 20000 μg g^{-1} d.wt (Kleeberg *et al.*, 1999b); of Lake Ontario had a concentration of 7500 μg g^{-1} d.wt (Kemp & Mudrochova, 1972); and of 17 rivers in southern, lowland England ranged between 1000 and 9000 μg g^{-1} d.wt (Clarke & Wharton, 2001). However, lower values have also been measured and Serruya (1971) determined that total-N of surface sediments of Lake Kinneret, Israel ranged between 800 and 2400 μg g^{-1} d.wt and Malley *et al.*, (2000) calculated that the total-N of surface sediments of four Canadian lakes varied between 600 and 3100 μg L^{-1} d.wt.

Clarke & Wharton (2001) also determined the total-P concentrations of the 17 rivers in southern, lowland England, which ranged from 154 μg g^{-1} to 2247 μg g^{-1} and Owens & Walling (2002) estimated that the total-P content of suspended sediments in Rivers Swale, Aire, Calder and some tributaries ranged between 736 to 7538 μg g^{-1} d.wt. In addition, in 66 lakes of eastern North America, P content of surface sediments ranged from 1300 to 9200 μg g^{-1} d.wt (Ostrofsky, 1987). Furthermore, according to Holtan *et al.* (1988), P content varies from 10 μg g^{-1} d.wt or less in sandy coastal sediments, up to 10000 μg g^{-1} d.wt in iron- and carbonate-rich gyttja. Finally, Blindow (1992b) showed that P content of the upper 5 cm of the surface sediments of the shallow, *Chara* lake,

Lake Takern, Sweden was 710 μg P g^{-1} d.wt and calculated that the amount of total sediment-P was much higher than the quantity stored in charophytes. However, he also mentioned that the sediment was calcium-rich and thus most of this P is not released to the water even under anaerobic conditions.

P concentrations in river and lake sediments are often a function of the capacity of sediments to bind P on particle surfaces and as minerals with calcium, iron and aluminium (Prairie *et al.*, 2001). With low P loadings and low productivity, the sediments generally contain less than 1000 μg P g^{-1} d.wt, but as the P loading increases, so does autochthonous and allochthonous sedimentation of organic matter and with it both the C, N and P contents of the surface sediment (Gibson, 1997). The increased C input creates a greater oxygen demand, which in turns promotes reducing conditions in the sediment and this, combined with the greater P content, causes a progressive tendency for more P to be released from the sediment-water interface. Croft Kettle's surface sediments total-P contents are below 1000 μg g^{-1} d.wt and are probably rich in $CaCO_3$ and Fe, suggesting high adsorption of P and limited capacity for P release.

In addition, significant differences were found between the surface profoundal and littoral sediments of Croft Kettle, even though in both sites submerged vegetation was absent. The N and P contents and N:P ratio of sediments collected from the middle of the pond were much lower (especially N) than the deep cores collected from site 6 (Figure 3.5), which was much closer to the charophyte beds. This is probably due to higher sedimentation of organic matter into the sediments closer to the charophyte beds as well as probably due to longer and more severe deoxygenation of the profoundal sediments (higher denitrification potential). Differences in nutrient composition between littoral and profoundal sediments in lakes as well as marine nearshore and offshore sediments are common in literature (e.g. Szmant & Forrester, 1996; Andersen & Ring, 1999) and could be of an order of magnitude, even in unpolluted environments. Furthermore, total-N and P and N:P ratio of the profoundal sediments showed significant differences with depth (0-35 cm), indicating historical changes in allochthonous and autochthonous sedimentation of organic matter as well as possible changes of environmental variables influencing N and P dynamics in sediments, such as water temperature and oxygen and overall water chemistry of Croft Kettle.

Differences in depth distribution, however, were not only found for the deep core from the middle of the pond, but also for total N and P contents as well as some P fractions and species for the seasonal survey of surface sediments (0-10cm). In

contrast, N:P ratio did not show such variation with depth suggesting that total N and P showed relatively similar changes with depth. Total-N and total-P concentrations in the surface sediment section were higher than that in deeper-lying sediments, independent of temporal variation. Thus, surface sediment total-P was 15 %, 25 % and 22 % higher than the other sections with decreasing order over depth respectively and total-N was 14%, 22 % and 23 % respectively. Similar results have been shown from a number of studies on freshwater environments and particularly Søndergaard *et al.* (1996), who showed that total-P concentration was higher in surface than deeper-lying sediments and more than twice as high at that at a depth of 20-30 cm, in 32 shallow Danish lakes.

NaOH-P (annual mean) was the biggest P fraction in Croft Kettle sediments, consisted 44 % of total FTP of surface sediments, of which only 12 % was FRP (reactive) and 32 % FOP (unreactive) and was the P fraction that showed the highest changes with depth particularly for FOP, which showed a mark decrease with depth. The quantitative difference between NaOH-FTP content of the surface sediment section (0-2 cm) and the deeper-lying ones in decreasing order with depth was 31%, 44 % and 50 % respectively. However, relative proportions of NaOH-FTP to total-FTP at each sediment layer did not show such high differences, with the highest being 14 %, observed between the uppermost (0-2 cm) and the deepest sediment section (7-10 cm), indicating that NaOH-P tended not to be relatively higher at high total-P. In addition, the changes in the relative proportions were mostly due to the NaOH-FOP fraction, since NaOH-FRP was more or less independent of depth averaging 20 % of total-FRP. Assuming, that NaOH-P represents humic, Fe and Al bound P, these findings indicate that there is much more humic and loosely organic material on the top sediment layer than in deeper ones and that the observed decrease with depth of total-P is probably due to this fraction. In addition, the difference between FTP and FRP extracted by NaOH indicates that most of the organic P which is not further differentiated is being extracted at this stage.

Baldwin (1996) found a significant correlation between NaOH extractable P and phospholipid concentration of sediments, suggesting some interaction between the two and indicating that even though phospholipids in the sediments could derive from a number of different sources (e.g. planktic organisms, detritus), give a good estimate of sedimentary bacterial biomass. In addition, Törnblom & Rydin (1998) indicated that the NaOH-FOP sediment P fraction consisted of 31-50 % bacterial poly-P as well as it extracts phytate (a stable cyclic P-compound associated with bacterial metabolism) present in the sediment and Goedkoop & Pettersson (2000) suggested that NaOH-FRP

is a conservative measure of bacterial poly-P. Finally, it has been shown that NaOH extracts a large proportion of the P associated with the bacteria *Pseudomonas* and that most of this P was in the non-reactive form (Waara *et al.*, 1993; Baldwin, 1996). Therefore, it is possible that a relatively high proportion of the P extracted by NaOH could have been associated with sedimentary bacteria suggesting that the differences observed could be due to bacterial biomass. Supportive evidence for this hypothesis arises from ^{31}P n.m.r. spectroscopy (DG, unpublished), where higher poly-P contents were found in the surface sediment section than the deeper lying ones.

BD-P (annual mean) consisted 13 % of total-FTP of surface sediments from Croft Kettle, of which 8 % was FRP and 5 % FOP and did not show any marked changes with depth. BD-P content in a series of Australian reservoirs ranged between 5 and 20 % of sediment total-P (Baldwin, 1996), but higher values were observed in the shallow eutrophic Lake Donghu, China, range 17-62% of total-P (Yong-ging *et al.*, 2000). Assuming that BD-P represents reductant soluble P (P bound to Fe and Mn hydroxides), its presence in the deeper sediment sections as well as the substantial presence of NaOH-P, indicate that iron-P complexes (e.g. vivionite), are still present in deeper sediment layers independent of redox potential conditions (presumably even lower), suggesting that P can be permanently immobilised in these forms. Thus, adding Fe to the sediment could be a valuable restoration measure, something which has been shown for many freshwater bodies, even hardwater lakes (Burley *et al.*, 2001). However, as was mentioned briefly above, the competitive role of sulphur for iron may be important quantitatively in such measures since it has been shown that dissolved sulphides either in the sediment surface or in anoxic hypolimnia during stratification displace iron from P and enhance release of P to the overlying water (Kleeberg, 1998; Golterman, 2001; Holmer & Storkholm, 2001).

HCl-P (annual mean) consisted 18 % of total-FTP of surface sediments from Croft Kettle, of which 15 % was FRP and only 3 % was FOP and did not show any vertical gradients. However, even though quantitatively HCl-P remained stable, its relative proportion to total-FTP increased with depth. Assuming, that HCl extracts Ca-bound P (apatite or other Ca-P complexes), these findings suggest that Ca bound P did not dissolve at deeper sediments (presumably lower pH). However, decrease (Gomez *et al.*, 1998) as well as increase (Reimers *et al.*, 1996) in pH values with depth have been shown in literature, suggesting that a possible explanation could be that sediment surface pH values ($\approx$7) of Croft Kettle did not change substantially with depth and remained to relatively neutral values. In addition, concentration of HCl-P in surface

sediments varies significantly, range between 2 % to 50 % of total-P in different types of lakes (Pettersson *et al.*, 1988), and is usually independent of Ca content of sediments (Søndergaard *et al.*, 1996; Golterman, 1998). Instead, Søndergaard *et al.* (1996) found significant correlations between HCl-P of surface sediments and phytoplankton biovolume, suggesting that sediment HCl-P is strongly related to biological processes and mostly depends on biogenic co-precipitation of P with calcite.

H_2O-P (annual mean) consisted 2 % of total-FTP of surface sediments from Croft Kettle, of which 0.4 % was FRP and 1.6 % FOP and did not show any vertical gradients. Assuming, that H_2O extracts water soluble P and interstitial P, these findings indicate that minor amounts of sediment P were found in this form, suggesting strong adsorption by the other constituents of the sediment (e.g. iron bound and Ca-bound P) and indicating very low interstitial concentrations of P as well as limited potential for release. Finally, $NaOH_{(85\ °C)}$-P (annual mean) consisted 23 % of total-FTP of surface sediments from Croft Kettle, of which 13 % was FRP and 10 % was FOP and did not show any vertical gradients too. However, a difference of about 20 % was found in the determination of total-P between digestion (showed higher total-P content) and extraction techniques, which could be regarded as refractory P too and was not extracted by any of the reagents used.

Seasonal variations in total N and P content but not for N:P ratio were identified for all sediment sections (0-10 cm) from Croft Kettle. Observations on temporal variation however, cannot be made for each P fraction separately or each month since analytical variation was higher than seasonal or monthly variability of P fractions, but some remarks will be made about P species such as total-FRP and total-FOP. The seasonal variations however, indicate that changes in temperature, oxygen and subsequently biological activity are key factors. In addition, these findings (seasonal changes for all sediment sections) indicate that this part of the sediment (0-10 cm) must be regarded as highly dynamic, comprising not only refractory P, but also of a major inorganic and organic (possible biogenic) fraction, which could eventually be decomposed and released. Changes in total P concentrations and release of P have been shown at depths up to 20-23 cm (Søndergaard *et al.*, 1996) and are in accordance with Golterman's (2001) assumption that sediment depth of 10 cm should be considered 'active'.

The general trend was higher concentrations in spring and summer than autumn and winter and there may be several possible explanations for these findings. Assuming, that overall P release from the sediment surface was not substantial, or that that the rate

of release was not much greater than the rate of deposition, as was indicated from the relatively low increases in aqueous P concentrations as well as higher summer water pH values, which probably favoured co-precipitation of Ca-PO_4 minerals, the following model could probably best explain these changes. During winter, when the sediment has a good P-sorption capacity because oxygen from the overlying water penetrates and mineralisation processes are slow, a gradual accumulation of organic matter is observed on the sediment surface. However, this does not necessarily suggests that concentrations of total-P calculated per g d.wt sediment should also increase, since either high density of sediment (Andersen & Ring, 1999) or low P content of settled organic matter could result to lower sediment P concentrations. During spring, when availability of substrates is probably high (settled organic matter during winter), oxygen content of the overlying water is still relatively high and temperature increases, bacterial biomass in the sediment surface increases and thus biological activity as well as mineralisation of organic matter. Changes in bacterial biomass could be substantial enough to explain relative higher sediment P contents (Törnblom & Rydin, 1998; Goedkoop & Pettersson, 2000).

During late spring and summer stagnation, when increased bacterial decomposition of organic matter has exhausted oxygen from the overlying water, the oxidised surface layer is diminished, bacterial biomass is reduced (Törnblom & Rydin, 1998) and redox potential decreases, suggesting potential P release either according to the classic model of Fe reduction or due to bacterial mortality (Prairie *et al.*, 2001). Therefore, lower sediment total-P concentrations should be expected. However, the data suggested that total-P concentrations in the two uppermost surface sediment sections increased during the summer. Two possible explanations could be accounted for this increase. First of all this increase coincided with the summer flood, which washed a lot of allochthonous organic and particulate matter into the pond and resulted in higher sedimentation of detritus as well as detritus-associated bacteria. Thus, these maxima in total-P could be due to this episodic event and are not a general trend of temporal variation in P contents of Croft Kettle's surface sediments. Secondly, the increase in the upper sediment sections coincided with a decrease of total-P concentration in the deeper lying sediment sections, particularly the 4-7 cm one, suggesting P release from this sediment section. Upward movement of P as well as downward seepage after decreases in redox potential, have been shown (Keizer & Sinke, 1992) and it can explain changes in total-P content of either higher or lower sediment layers if they still sustain a positive P-sorption capacity. Finally, during autumn overturn, oxygen content of overlying water increase, the sediment surface regains its oxidised microzone and good P-sorption capacity.

However, surface sediments can continue to be a net source of P to the overlying water mainly by bacterial activities. Indeed, numerous studies suggest that in aerobic environments bacterial activity is responsible for an increased rate of P release by oxidation of the organic P forms or by dissolution of the $CaCO_3$-P following a drop in pH (Prairie *et al.*, 2001).

7.4 Status of *Chara hispida*

Calcium carbonate comprised on average between 35% and 40% d.wt of *C. hispida* apical tips, suggesting that $CaCO_3$ deposition on *C. hispida* shoots undoubtedly contributes to the fresh and dry weight of the plants. These findings are in accordance with values in literature (e.g. Welch, 1952) and explain why charophytes show lower tissue chemical compositions in comparison to other submerged macrophytes (e.g. Riemer & Toth, 1969). Encrustation of charophytes depends on bicarbonate use during photosynthesis (Blindow, 1987), as well as pH (Graneli & Solander, 1988) and is mainly attributed to the presence of local ion pumps, which precipitate $CaCO_3$ directly onto the plant surface (Coletta *et al.*, 2001). Vertical gradients in $CaCO_3$ content were observed in whole *C. hispida* plants from Croft Kettle, with higher % $CaCO_3$ d.wt in the apex than the lower parts. However, these observations are probably in contrast; with the findings that tissue Ca content was slightly lower in the apex than downwards; Coletta *et al.* (2001) findings that carbonate content of *C. hispida* plants from Pandy pool, North Yorkshire remained approximately constant at 58 % d.wt; as well as Carpenter & Adams (1977) observation that the amount of marl on macrophyte shoots increases in part with the ages of the shoots. The seasonal survey indicated that $CaCO_3$ content showed considerable seasonal and monthly variation in *C. hispida* apical tips in both ponds, with overall higher contents in the summer, but no apparent seasonal trends. Moreover, the differences in $CaCO_3$ content of *C. hispida* apical tips collected from Croft and Double Kettle indicate that $CaCO_3$ deposition does not solely depend on Ca and $CaCO_3$ concentration of the water, but is a function of other environmental parameters too (e.g. light intensity).

Seasonal changes in tissue N and P content were also observed in *C. hispida* apical tips during the study period, with overall higher tissue N and P contents during winter and lower values during summer. In addition, *C. hispida* apical tips collected from Double Kettle had on average higher tissue N and P contents than those collected from Croft Kettle, probably corresponding to the higher nutrient content of Double Kettle's water. Indeed, increasing ambient inorganic N corresponded with an increase in tissue

N, but the effect on tissue P was inconsistent. Accordingly, significant positive correlations between tissue and aqueous P concentrations were only found for Double Kettle.

Riemer & Toth (1969) showed a range between 0.93 and 2.69 % d.wt and 0.10 to 0.41 for tissue N and P respectively for *Chara* sp. from a range of freshwater bodies in New Jersey, Zimba *et al.* (1993) showed a range between 1.43 and 1.71 % d.wt tissue N and 0.07 to 0.94 tissue P for *Chara* sp. from Lake Okeechobee, Florida, Kufel & Ozimek (1994) found tissue P content of 0.14 % d.wt in *C. vulgaris* in June from Lake Łuknajno, Poland and Fraser *et al.* (1984) found tissue P content of 0.06 % d.wt in *Chara* sp. from lakes in Provincial Park, Ontario. However, most of the studies mentioned above did not refer to time of sampling, species and part of shoot analysed and did not account for $CaCO_3$ deposition to the determination of dry weight. The range of tissue N and P contents of *C. hispida* shoots sampled during this study were similar but mostly to the upper limits (especially for N) to those reported above for *Chara* sp.

Nevertheless, the seasonal changes as well as the high range of tissue N and P values (about fivefold between lowest and highest N and P contents) found during this seasonal survey indicate that *C. hispida* is an opportunistic species, capable of rapid nutrient uptake and storage for growth under favourable conditions. Supportive evidence for this hypothesis also arose from the P enrichment experiment as well as the seasonal study on phosphatase activity. Incubations under a series of aqueous P concentrations, demonstrated a high capacity of *C. hispida* shoots to absorb from the pond water and accumulate P into the tissues. However, a possible drawback to these findings could be precipitation of $CaPO_4$ salts (e.g. $CaHPO_4$) on the cell wall, which is known to increase under higher aqueous P concentrations (Kiyosawa, 2001), suggesting that part of the observed increase into tissue P contents could be due to $CaPO_4$ precipitation and not due to P acquisition and storage. In addition, the seasonal survey on phosphatase activity indicated that rates of activity were not dependent on tissue P content (no inverse relationship was found), but suggested that it was a short-term response to ambient P. Accordingly, significant positive relationships were found between phosphatase activities and aqueous P concentrations. These findings are in contrast to Christmas & Whitton (1998) observations on mosses that phosphatase activity showed significant positive relationships with internal nutrient status and thus could be used as a quantitative test of the deficiency of P for aquatic organisms.

Finally, tissue N and P contents of *C. hispida* shoots after 31 days incubation into the wide range of aqueous P concentrations, ranged between 0.36 % d.wt and 1.3 for tissue N and 0.01 and 0.24 for tissue P (values not corrected for $CaCO_3$ deposition). These values were much lower than the ones observed at the seasonal survey, suggesting that both N and P concentrations in *C. hispida* are probably in excess of critical levels and that even during the growing season these nutrients were available in adequate amounts in the tissues.

7.5 Biological surveys

Some botanical changes in addition to the event of the summer of 1996 have taken place in Croft Kettle since 1970 (Section 3.9). Possible factors, which could have influenced the aquatic vegetation, are changes in water temperature, light penetration, herbivory, hydrostatic pressure, mechanical disturbance, water chemistry and sediment composition. However, there is no firm evidence that such changes have occurred, though all are plausible and the lack of detailed data on P as well as other physical and chemical variables from the 1970s makes it hard to be sure about the reasons for the disappearence or major decrease in these plants. Furthermore, as mentioned earlier, unlike what happened in the summer of 1996, *C. hispida* remained healthy in Croft Kettle for all the period studied and so only indirect observations and conclusions can be made. In addition, considering the rich development of freshwater charophytes and their importance in the nutrient cycling of standing waters (Kufel & Kufel, 2002), there are surprisingly few studies of their growth and production in relation to seasonal changes in physico-chemical conditions, so conclusions cannot also be drawn from the literature.

However, it is apparent that Croft Kettle remains a habitat with favourable conditions for the growth of *C. hispida*. Important environmental conditions are probably high calcium concentration and alkalinity and clear water with low P concentration (Wood & Imahori, 1965; Forsberg, 1965b; Hutchinson, 1975; Kufel & Kufel, 2002), parameters that still apply. Regarding P, an upper critical growth level of 20 $\mu g\ L^{-1}$ was postulated by Forsberg (1964; 1965a) from experimental work on *Chara globularis* and *Chara zeylanica* as well as field observations on Swedish charophytes (Forsberg, 1965b). However, more recent studies have not confirm Forsberg observations and have shown no inhibition of growth of a number of charophyte species, including *C. hispida*, for aqueous P concentrations up to 1000 $\mu g\ L^{-1}$ (Blindow, 1988; Kufel & Ozimek, 1994; Simons *et al.*, 1994). In addition, the in situ experiment

(aqueous P enrichment) on *C. hispida* apical shoots reported previously (Section 6.7) showed that P concentrations at least till 150 $\mu g\ L^{-1}$ were not growth inhibiting and these results are in accordance with Blindow (1988) observations on *C. hispida* and *C. tomentosa* as well as Simons & Nat (1994) on *C. connivens* and *C. major*. Nevertheless, aqueous P levels in Croft Kettle were overall quite low (<20 $\mu g\ L^{-1}$) for most of the years monitored with the exception of relatively limited periods of increased availability, indicating that aqueous P concentration should not posse a direct threat to *Chara hispida*'s growth.

Moreover, the transfer of water from Croft to Double Kettle in 1977 led to the invasion of *Chara hispida* in the latter pond (B.A. Whitton, pers. comm.). *C. hispida* grew quite well in the shallow waters of Double Kettle during the warm mid-1990 years (B.A. Whitton, pers. comm.) as well as during the period 1998-2000. Thus, as the Double Kettle reaches higher P levels than Croft Kettle, it seems unlikely that elevated aqueous P concentrations have been the key factor for the event of 1996. In turn, water temperature alone also seems unlikely to have been a key factor, because the Double Kettle shows similar surface temperatures to Croft Kettle, yet *C. hispida* remained fairly healthy there throughout the summer of 1996. However, it seems more likely that temperature has an influence on oxygen concentrations in the hypolimnion, resulting to periods of low oxygen or anoxic conditions, which could posse a direct threat to *C. hispida* or an indirect through release of hydrogen sulphide from increasingly anoxic sediments and thus cannot be ruled out as a factor. Studies on oxygen concentrations reported here were confined to daylight hours, when *C. hispida* contributes photosynthetic oxygen to the epilimnion as well as part of the hypolimnion, so further conclusions cannot be drawn out. However, dissolved oxygen is probably also not the main factor that caused the whitening of some *C. hispida* shoots in 1996 since that was observed only on surface shoots (B.A. Whittton, pers. comm.), and not on deeper growing ones, most likely to suffer from oxygen depletion.

A possible factor, which could explain the whitening of *C. hispida* shoots, but will only be discussed briefly due to lack of data, is the possibility of carbon limitation. In retrospect, it would be better to have taken measurements of inorganic carbon concentrations, but initially that was not suggested as a possible factor. This scenario arose from the observations of Chambers *et al.* (2001) and Prepas *et al.* (2001) that short-term (23-33 days) addition ($\geq$ 200 mg L^{-1}) of slaked lime ($Ca(OH)_2$) and calcite ($CaCO_3$) in lake water resulted in loss of pigmentation of aquatic macrophytes but with no consistent biomass reduction. Their explanation for this phenomenon was that a

short-term rise in pH due to the $Ca(OH)_2/CaCO_3$ addition, in relatively warm (22-23 °C) alkaline (2.28-3.94 mequiv L^{-1}) conditions, could have resulted in low concentrations of free CO_2 and bicarbonate near the leaf surface as well as limit diffusion by increased $CaCO_3$ precipitation on the macrophyte leaves, preventing efficient photosynthesis. Similarly, Van den Berg *et al.* (2002) showed that aqueous inorganic carbon limitation within dense charophyte beds could occur at periods of maximal photosynthesis even under natural conditions, when relatively high pH values are found and especially since water movement is quite restricted at maximum biomass (Sorrell *et al.*, 2001; Van den Berg *et al.*, 2002).

Finally, a possible scenario, which could explain the reduction in distribution of *C. hispida* thin as well as the dissapearence of *Potamogeton pectinatus*, is through the fact that the surrounding belt of reeds has become much more prominent since 1970s. Two possible reasons could explain this change; firstly, the construction of a fence in the winter of 1970/1971 in order to protect the pond fringe and fen from grazing and trampling by anglers and secondly, the application of sewage sludge in the nearby fields, which could have increase the nutrient content of the surrounding fields. Accordingly, the expansion of the reed beds have increased organic input in the pond as well as created much more sheltered conditions by reducing wind penetration onto the water surface, affecting parameters like wave action, water mixing, light penetration and water temperature. In turn, all these changes could promote the spatial and temporal formation as well as stability of the thermocline (extended, for longer periods and closer to water surface), resulting to reduced oxygen content and light penetration at the area of the pond in which the thermocline extends. Light attenuation with depth is now widely accepted as the main control of maximum depth limit of macrophytes (Blindow, 1992b; Sorrell *et al.*, 2001; Blindow *et al.*, 2002) and could probably explain the pattern of change of *Potamogeton pectinatus* and *C. hispida* thin (Section 3.9), since charophytes show higher shade tolerance (Kufel & Kufel, 2002) than angiosperms in clear lakes. In contrast, *P. pectinatus* outcompetes *C. hispida* (Menendez & Sanchez, 1998) as well as many other charophyte species (Coops & Doef, 1996; Van den Berg *et al.*, 1999) in shallow and turbid waters due to its ability to form a canopy of leaves near the water surface (Van den Berg *et al.*, 1998). However, light attenuation in the surface water layers is not affected from such changes, thus posing no direct threat to *C. hispida* growing at shallow areas.

SUMMARY

i. A study was carried out on aspects of water chemistry, in particular of nitrogen and phosphorus dynamics, the phosphorus status of surface sediments and the influence of aqueous phosphorus on growth of *Chara hispida*, in Hell Kettle ponds. This included seasonal studies of surface as well as depth profiles of water chemistry over a period of more than 24 months.

ii. Croft Kettle was isothermal at temperatures between 3 and 10 °C in winter and stratified in summer (approximately May to October), with surface temperatures up to 20 °C and hypolimnion temperatures just below 10 °C. Distinct layers of severe deoxygenation, eventually complete anaerobiosis were maintained in the hypolimnion in 1999 and 2000 but not in 2001. Borehole water had relatively low dissolved oxygen content and stable temperature of approximately 11 °C, suggesting that source of water was from a deep aquifer.

iii. pH of water at Croft and Double Kettle was alkaline with relatively low within year variability (typical of hardwater bodies) and summer maxima, probably indicating that submerged photosynthesis influences pH on a whole pond scale. Borehole water pH was neutral, typical of carbonate-rock aquifer systems. Hardness of water, as indicated by alkalinity and conductivity estimates, was higher in groundwater and lower in Double Kettle indicating that Croft Kettle receives overall higher amounts of groundwater than Double Kettle.

iv. Nitrogen and phosphorus fractions of surface water from Croft Kettle were very low through out the year in comparison with other standing waters of temperate regions, with the exception of episodic events and during autumnal circulation. Double Kettle had overall higher N and P concentrations than Croft, particularly of fractions of organic and particulate nature, probably due to higher surface runoff as well as an internal effect by biota. Borehole water had low N and P content indicative of a fairly unpolluted aquifer and the variability shown is believed to be due to contamination with soil particles as a result of the disturbance caused by abstraction.

v. High (>16) and low (<10) nitrogen:phosphorus ratio of water was identified at different times of the year at Croft and Double Kettle indicating periods of nitrogen and phosphorus limitation. Presence of *Epithemia* and *Rhopalodia*, the only two diatom genera capable of nitrogen fixation also provides evidence for

periods of nitrogen limitation. However, nitrogen and phosphorus concentrations were such that either is likely to be limiting, irrespective of high or low ratio.

vi. Higher values of nitrogen and phosphorus, particularly of ammonium and filterable reactive phosphorus fractions, were recorded in the hypolimnion compared with the epilimnion of Croft Kettle during the summer, suggesting internal regeneration of nutrients, during hypolimnetic anoxia.

vii. Dissolved silica showed little variation in Croft Kettle and borehole water, but considerable inter-annual changes in Double Kettle. However, dissolved silica levels were relatively higher from nitrogen and phosphorus, indicating that it is unlikely that SiO_2-Si concentrations limit primary productivity.

viii. Mean annual total-N (9133 ± 191.9 μg g^{-1} d.wt) and total-P (883 ± 23.1 μg g^{-1} d.wt) contents of Croft Kettle's surface sediments (0-2 cm) are comparable to values found in the literature for other freshwater bodies and indicate that are neither nitrogen nor phosphorus poor. Differences found between littoral and profoundal bare sediments, suggest that even in small ponds like Croft Kettle, spatial variability is significant.

ix. Depth profile studies (1-cm intervals) of total nitrogen and total phosphorus content as well as nitrogen:phosphorus ratio on a 35-cm core collected from the middle of Croft Kettle suggest historical changes in nitrogen and phosphorus dynamics of the sediments as well as nitrogen limitation.

x. On average (annual mean) 2 % (0.6 % reactive and 1.4 % non-reactive) of the surface sediment total phosphorus was H_2O extractable, 13 % (8 % reactive and 6 % non-reactive) BD extractable, 44 % (12 % reactive and 32 % non-reactive) NaOH extractable and 18 % (15 % reactive and 3 % non-reactive) HCl extractable, with the remaining 23 % being residual phosphorus ($NaOH_{(85\ °C)}$ extractable).

xi. There were only mirror changes in the relative contribution of different P fractions at increasing depth suggesting that the relationship between the various phosphorus forms is relatively stable despite probable variations in sediment composition, aging, pH and redox conditions.

xii. NaOH-P fraction showed the highest vertical gradients and seems to be mainly related to bacterial biomass and therefore directly coupled to biological

parameters. The always higher concentration of NaOH-P mostly of the non-reactive form in surface sediments than in deeper sediments shows most organically bound phosphorus is only stored temporarily as well explains the highest total phosphorus content found in the sediment surface section.

xiii. The redox potential (Eh) in Croft Kettle, at a depth of 14 mm below sediment-water interface, was always negative, irrespective of season and varied between -74 and -364 mV.

xiv. Seasonal variations in total N and P content but not for N:P ratio were identified for most sediment sections (0-10 cm) from Croft Kettle, suggesting that this part of the sediment must be regarded as highly dynamic, comprising not only of refractory P, but also of a major inorganic and organic (possible biogenic) fraction, which could eventually be decomposed and released. Observations on temporal variation of each P fraction or for each month however, could not be made since analytical variation was higher than seasonal or monthly variability.

xv. No direct evidence of undergoing diagenesis of P (solubilisation and release to the water column) during the summer stagnation period, was found. In contrast, it is suggested that Croft Kettle sediments show high adsorption for P and limited capacity for P release.

xvi. Carbonate deposition on the cell walls of *C. hispida* apical tips comprised, on average between 35% and 40% d.wt and undoubtedly contributed to the fresh and dry weight of the plants. Differences in $CaCO_3$ content of *C. hispida* apical tips collected from Croft and Double Kettle indicate that $CaCO_3$ deposition does not solely depend on Ca and $CaCO_3$ concentration of the water, but is a function of other environmental parameters too (e.g. light intensity). Rinsing with 2 % HCl acid as described in Section 2.62 probably removes most of $CaCO_3$ deposition without influencing elemental composition of *C. hispida*.

xvii. Vertical gradients in elemental composition (in particular for N and P) of *C. hispida* plants, with higher contents in the youngest apical parts than the lower (oldest) parts were identified, suggesting.that estimation of total N and P content of *C. hispida* plants cannot be based on concentrations found in the shoot.

xviii. The seasonal survey on phosphatase activity indicated that rates of activity were not dependent on tissue P content (no inverse relationship was found), but suggested that it was a short-term response to ambient P concentration.

xix. The seasonal changes as well as the high range of tissue N and P contents in C. hispida apical shoots found during this survey, indicate that *C. hispida* is capable of rapid nutrient uptake and storage. In addition, incubations under a series of aqueous P concentrations, demonstrated a high capacity of *C. hispida* shoots to absorb from the pond water and accumulate P into the tissues.

xx. Thus, regarding P, these findings suggest that current aqueous P concentrations in Croft Kettle posse no direct threat to *Chara hispida*'s growth.

xxi. Possible factors, which could have driven the vegetation changes (Section 3.9), are increase in water temperature, deoxygenation of the hypolimnion, reduced light penetration, and other changes in water chemistry and sediment composition. However, there is no firm evidence that such changes have occurred, though all are plausible and the lack of detailed data from the 1970s makes it hard to be sure about the reasons.

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APPENDIX 1

Brief report on diatom analysis of Croft Kettle core

Martyn Kelly - Bowburn Consultancy

Introduction

Palaeoecological methods provide insights into the history of sites that are often useful for conservation and management purposes (Huntley, 1991). Studies on lakes have been used to characterise "natural" (pre-impact) conditions and knowledge of the ecological preferences of species occurring in a core over time can be used to determine the factors responsible for changes from this natural condition (Flower & Battarbee, 1983). In this study, a sediment core from Hell Kettle has been analyzed in order to identify major changes in the recent past (i.e. from 100 years BP) and to consider the implications of these for present management strategies.

Methods

Core collection

- A coring trip to Hell Kettles took place on 12 March 1999. A raft was placed in the middle of Croft Kettle and was secured with cross ropes. The depth deck to sediment interface was 5.7 m. Two perspex tube cores (40 cm long) and a larger core (9.82 m long) were collected.
- 15 core levels from the first metre were examined for the quantification and identification of diatoms. The 80 cm horizon was devoid of diatoms. However, the first 60 cm of the core represent the time period of interest.

Sampling contemporary habitats

Samples from six habitats within the lake were sampled on 31 May 1999. Habitats sampled were plankton, "aufwuchs" associated with *Chara*, submerged aquatic plants (mainly *Potamogeton*) and *Cladium*, algae attached to a submerged piece of wood and the flora associated with marginal mud. Plankton was sampled in a 1 L bottle. Lugol's iodine was added on return to the laboratory and the sample was allowed to settle in a large measuring cylinder for 3 days before most of the supernatent was decanted and the remainder (about 100 mL) was transferred to a smaller measuring cylinder and the settling process repeated. The sedimented algae were then transferred to a boiling tube for the prepartion procedure described below. Aufwuchs was sampled by shaking entire plants in a plastic bag to dislodge associated algae and then allowing

the suspension of water plus algae to settle for 24 h. The supernatent was then decanted and the sedimented material processed as described below. Samples from submerged stems of *Cladium* and from submerged wood were scraped off using a pocket knife and transferred to sample bottles whilst the surface of marginal mud was sampled using a Pasteur pipette.

Diatom preparation and analysis

Methods for preparing and enumerating diatom samples followed Kelly (1996). This uses a mixture of sulphuric and oxalic acids plus potassium permanaganate to oxidise organic material in the samples, following which permanent slides are prepared using Naphrax (Northern Biological Supplies, Ipswich). At least 300 valves were counted from each sample, using a Leica microscope with 1000 × oil-immersion objective and phase contrast condenser. All valves were identified to the lowest possible taxonomic level using Krammer and Lange-Bertalot (1986-1991) and Hartley *et al.* (1996) as the primary floras. Nomenclature follows that of Whitton *et al.* (1998). As this was conceived as a preliminary study, no attempt to estimate absolute numbers of diatoms was made and all results are presented as the percentage of the total count represented by a particular species or group of species.

Data analysis

Environmental preferences of taxa found within the pond were obtained from the literature. Due to the unusual flora found in the pond, it was not possible to use the most sophisticated indices and models available to interpret trophic changes. However, all taxa were allocated to one of three categories (plankton, epiphyton, epipelon) depending upon their preferred habitats and changes in the relative representation of these are used to follow possible changes within the pond. Bennion (1995) identifies several problems associated with reconstructing trophic changes in shallow ponds due to the high proportions of non-planktonic taxa in assemblages. However, changing abundances of epiphytic and planktonic taxa were important features of the eutrophication of the Norfolk Broads (e.g. Moss *et al*, 1986) and can also be used to indicate changes in water levels within lakes (Wolin & Duthie, 1999). In Croft Kettle, where transparency is high and total surface area low, then changing abundances of different life forms (particulary the epipelon) may also indicate the density of the macrophyte "canopy" in the pond.

Results

Taxonomic composition

104 diatom taxa were found in Croft Kettle, of which 60 were represented in contemporary samples. Of the remaining taxa, only *Aulacoseira crenulata* was found in large numbers and 24 were only found on one occasion. Other taxa present at >10% on at least one occasion were: *Achnanthidium minutissimum, Amphora libyca, Gomphonema angustum, Navicula oblonga, N. radiosa* and *Staurosirella lapponica.* Several of these were absent from a study that included underwater springs in the Malham area (Round, 1960) which serves to highlight the interest of the Croft Kettle flora, and the problems for data interpretation.

The status of *Aulacoseira crenulata* has been debated. Hartley (1986) considers it to be a form of *A. italica* whilst Krammer and Lange-Bertalot (1991) prefers to recognise it as a distinct species. They comment that it is found in more electrolyte-rich habitats than *A. italica*, but makes no specific comment on nutrient preferences. (*A. italica* is normally considered to prefer eutrophic conditions).

Distribution of diatoms in contemporary habitats

Distributions of dominant diatoms in contemporary samples are shown in Fig. 1. The "phytoplankton" sample was dominated by taxa typical of benthic habitats and it is assumed that these represent opportunist "tychoplankter" or, simply, benthic taxa that had been dislodged. There were relatively few differences between different epiphytic habitats, although *Cymbella cymbiformis* and *Gomphonema angustum* were both more abundant on *Cladium* leaves whilst *Achnanthdium minutissimum, Epithemia* and *Rhopalodia* were more abundant in the loose aufwuchs around *Chara* and other submerged plants. The sample of marginal mud had a markedly different flora, dominated by *Staurosirella lapponica.*

Analysis of the core

14 samples from the top 100 cm were examined. Of these, the sample from -80 cm was devoid of diatoms, and subsequent analyses concentrated on the upper 60 cm. Based on assumed dates from a pollen diagram from Croft Kettle, this represents, at most, the last 350 years. In the lower part of the core (> -20 cm depth), *Aulacoseira crenulata* was relatively abundant (Fig. 2). This declined towards the surface of the core and the associated rise in *Achnanthidium minutissimum* suggests an increase in

macrophytes. This pulse in turn declined and *Staurosirella lapponica*, a species typical of marginal mud, increased.

A further point worthy of note is that the period following the peak in phytoplankton is also characterised by relatively high numbers of *Epithemia* and *Rhopalodia* - the only diatom genera which are capable of nitrogen fixation (via endosymbiotic cyanobacteria). The reasons for this will be discussed in more detail below.

Fig. 2 and 4 illustrate the changes in dominant life-forms throughout the top 60 cm of the core and illustrate the change from plankton dominance, to epiphyton to epipelon. The surface of the core has about 50% epipelon, despite the rich macrophyte flora, indicating that changes must be interpreted as differences in either the patch size or architecture of the macrophyte canopy. There is a negative relationship between the percentage of epipelon and that of epiphyton (Fig. 4), which also suggests that the changes in life form may represent changes in the structure of the macrophyte flora in and around the margins of the pond.

Discussion

General comments

The paucity of studies on the palaeoecology of calcareous ponds means that any interpretation here must be regarded as tentative. Further sampling of calcareous ponds will do much to understand the habitat preferences of the dominant diatoms found here and to assess the value of studies of this kind for the management of these habitats.

Interpretation of changes in dominant life forms

The problems of interpreting changes in nutrient status in the core have already been raised; however, recent changes do not suggest a significant increase in nutrients. Although a rise in epiphyton is sometimes reported as a sign of the early stages of eutrophication, the nature of the species present in Croft Kettle suggests that this is not the case here, as *Achnanthidium minutissimum* is relatively intolerant of high nutrients and *Epithemia* and *Rhopalodia* suggest a low N:P (see below). Moreover, the subsequent rise in epipelon would not have been expected if the lake were getting more eutrophic, as the amount of light penetrating to the bottom of the lake would be expected to decrease.

A more plausible explanation is that the changes observed in the upper 20 cm of the core indicate changes in the relative proportions of macrophyte growth forms, possibly related to changing water levels. The greater the area of shallow land around the pond that is flooded, the greater the potential for colonisation by *Cladium* and the associated marshy areas in which *Staurosirella lapponica* thrives. A lower water level, by contrast, would lead to less development of fringing *Cladium* and a greater representation within the sediment diatom assemblage of the aufwuchs associated with *Chara* and other submerged plants. If this hypothesis were correct, then the implication is that the water level in the pond is now higher than it was 100 years ago. Some support is lent by the pollen diagram of S.L. Dodsworth, which indicates an increased level of Cyperaceae pollen in the topmost layers of the core (although the resolution of this diagram is too low for a detailed comparison). This, however, does not easily explain the high representation of phytoplankton found at -20 cm and deeper.

Significance of Epithemia and Rhopalodia

The peak in *Epithemia* and *Rhopalodia* at about -20 cm is unusual in palaeolimnological analyses. The ecological preferences of these genera are vague, and generally concentrate on a preference for relatively electrolyte-rich conditions. Drum and Pankratz (1965) were the first to recognise that these genera contained endosymbiotic Cyanobacteria and subsequent workers (e.g. DeYoe *et al.*, 1992) have demonstrated a capacity for nitrogen fixation. On this basis, Stevenson and Pan (1999) suggested that the proportion of *Rhopalodia* and *Epithemia* could be used as indicators of low N conditions. An alternative interpretation, based on observations made on streams in Northumberland, is that these genera indicate low to moderate P concentrations coupled with low N (Kelly, unpublished data). Their relatively robust frustules suggest that high Si might also be a prerequisite. Their presence in Croft Kettle alongside N-fixing Cyanobacteria (some of which are also capable of utilising organic P sources) would tend to support this.

In Croft Kettle, *Epithemia* and *Rhopalodia* were associated particularly with the epiphyton and, in view of the interpretation of changes in diatoms in terms of lake levels, the primary explanation for changing abundance of these genera must be the relative availability of habitats within the lake. However, a further important issue that cannot be thoroughly explored here is interspecific competition within particular assemblages and it is possible that the period when *Epithemia* and *Rhopalodia* were most abundant coincided with a period of relatively low N:P. Does the decline, perhaps, coincide with the use of artificial fertilizers on the land?

Future work

This work provides some insights into reasons for recent changes in Croft Kettle. However, in order to put these into context, some other important questions need to be addressed:

1. What time period is represented by the section of the core analyzed here? A series of Pb^{210} dates from the core which would enable much of the above account to be put into a more precise context.
2. Are the conclusions about fluctuating lake levels supported by the result of a high-resolution pollen and macrofossil analysis in the upper 60 cm of the core? Cyperaceae pollen is usually "lumped" during pollen analyses, but it would be useful to see if *Cladium* pollen had any distinguishing features.
3. What is the magnitude of natural variation in the diatom flora over a longer (i.e. >1000 years) time period?

A second survey of contemporary habitats in autumn would also be helpful, as some of the species common in the upper part of the core (e.g. *Amphora libyca*) were not found during the early summer survey.

A "training set" of calcareous ponds, including surface sediment samples, chemistry, geomorphological characteristics and samples from major habitats within the ponds would enable the environmental factors determining the diatom flora to be identified. This would be a medium-sized project, but would provide some insights of value to the future management of calcareous ponds.

Acknowledgement

Thanks to Keith Clarke (UEA) for confirming the identification of *Aulacoseira crenulata*.

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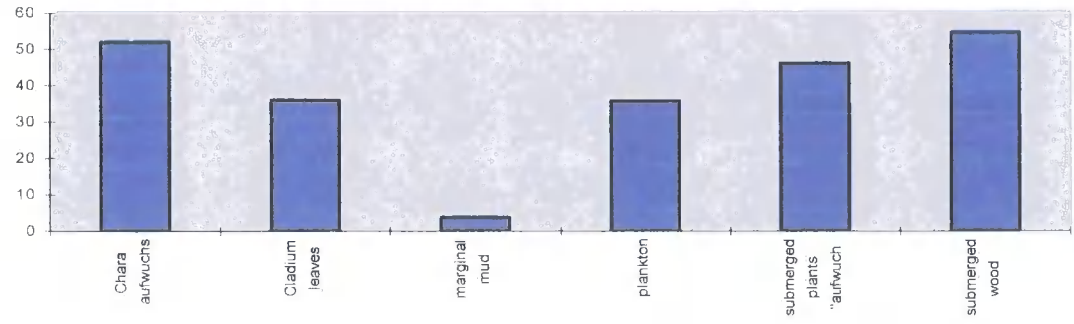
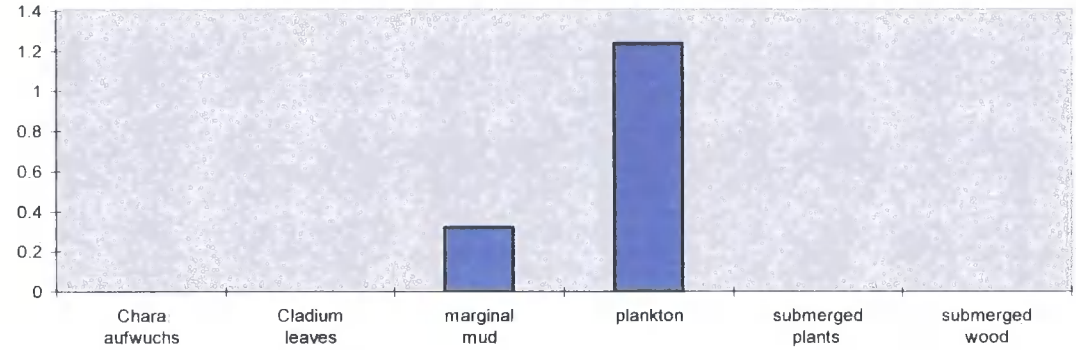
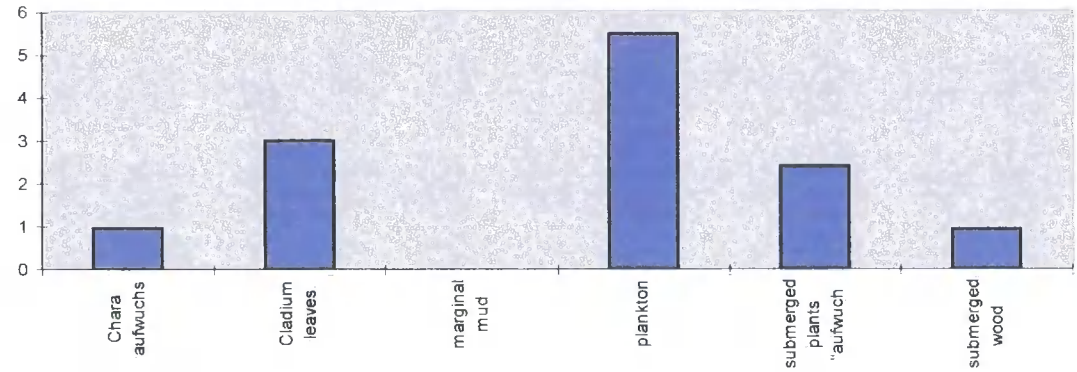
Achnanthidium minutissimum



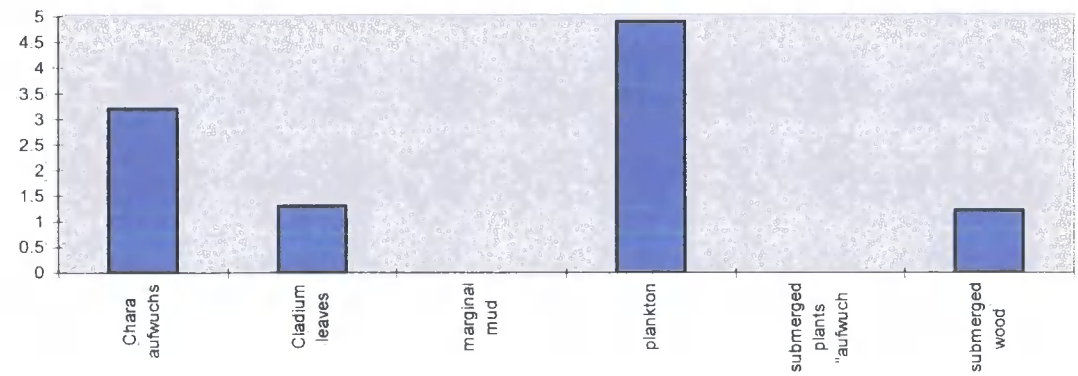
Amphora libyca



Cymbella cymbiformis



Epithemia sp.



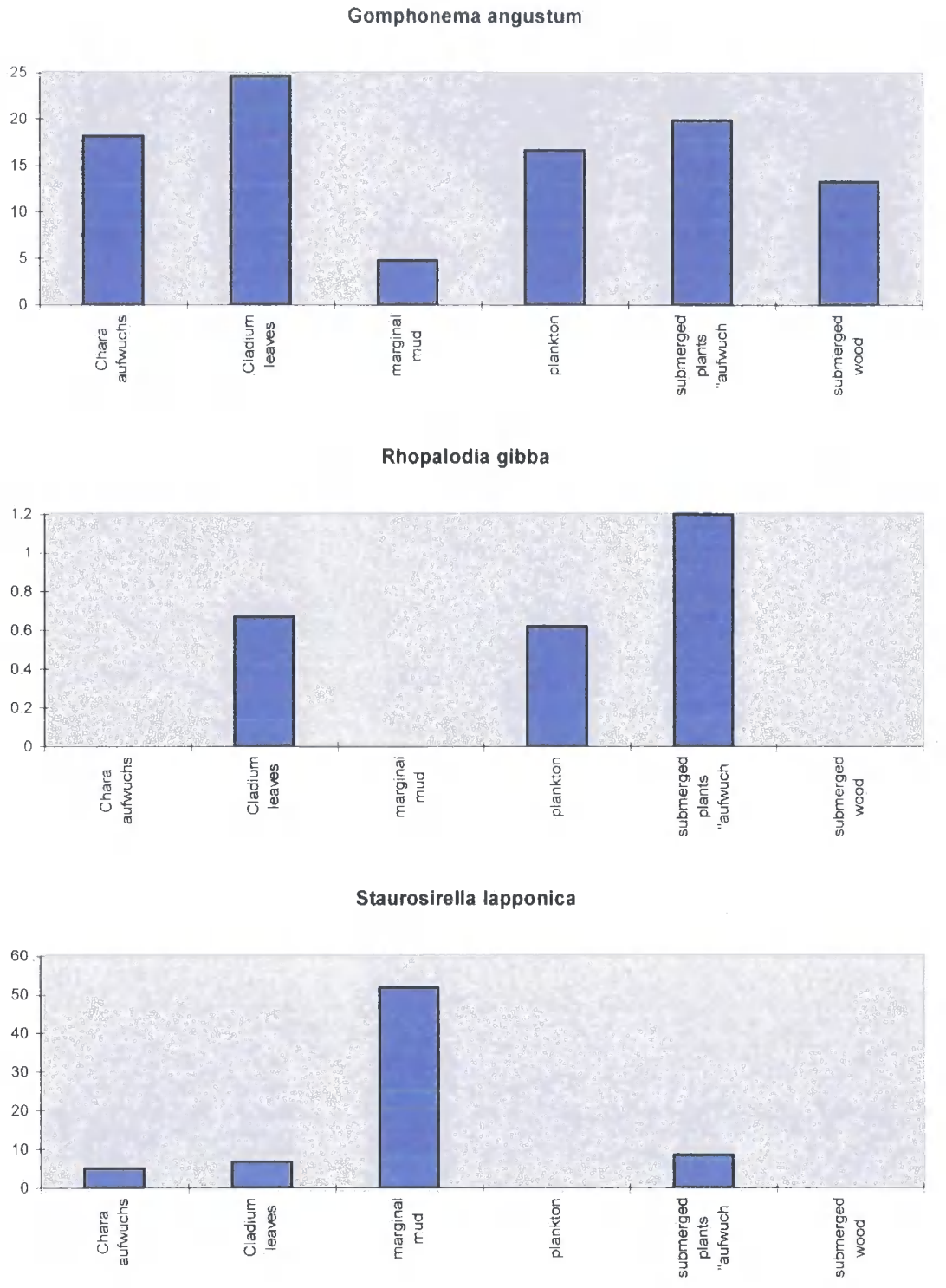


Fig. 1. Representation of common diatom species in microhabitats in Croft Kettle, May 1999.

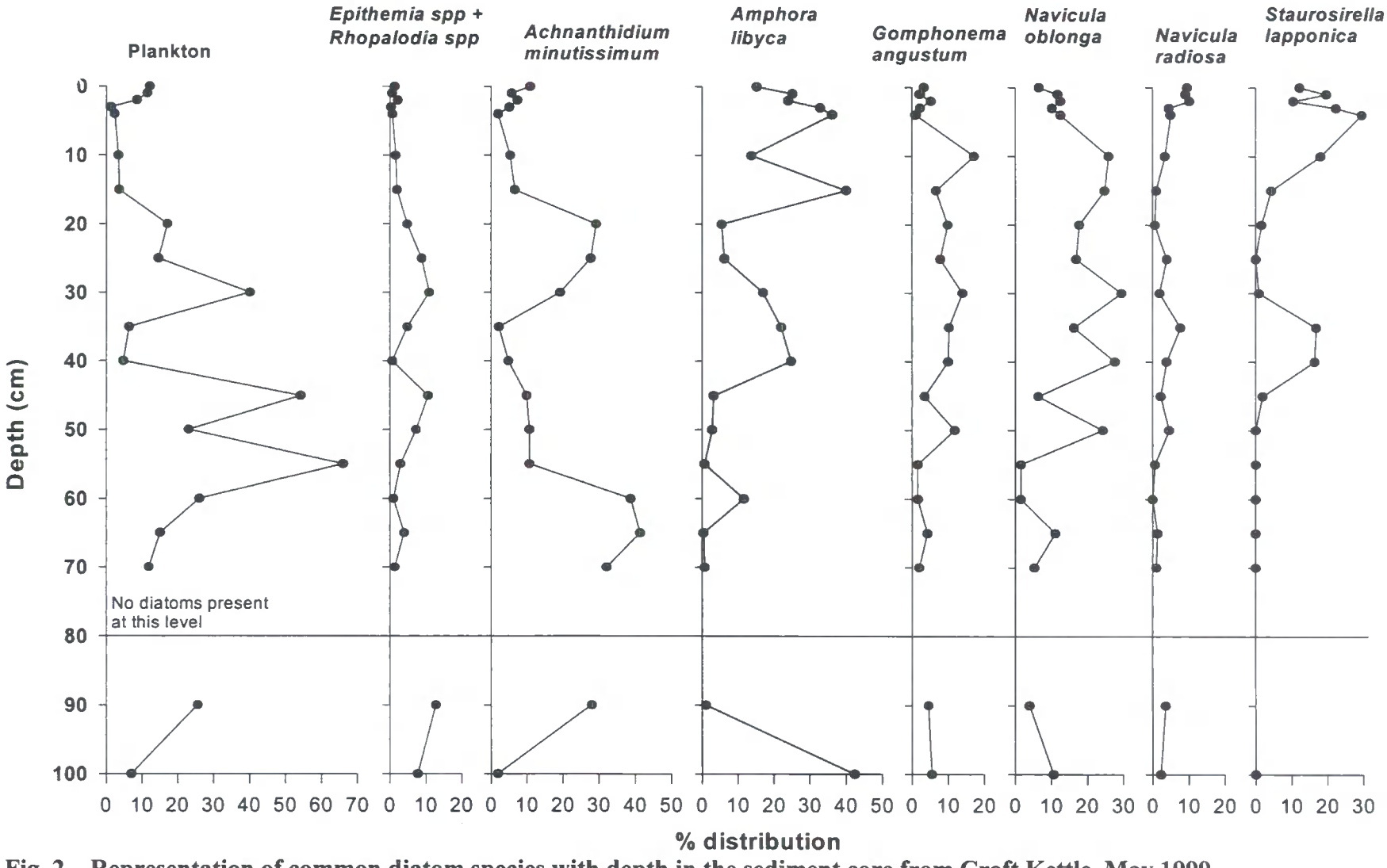


Fig. 2. Representation of common diatom species with depth in the sediment core from Croft Kettle, May 1999.

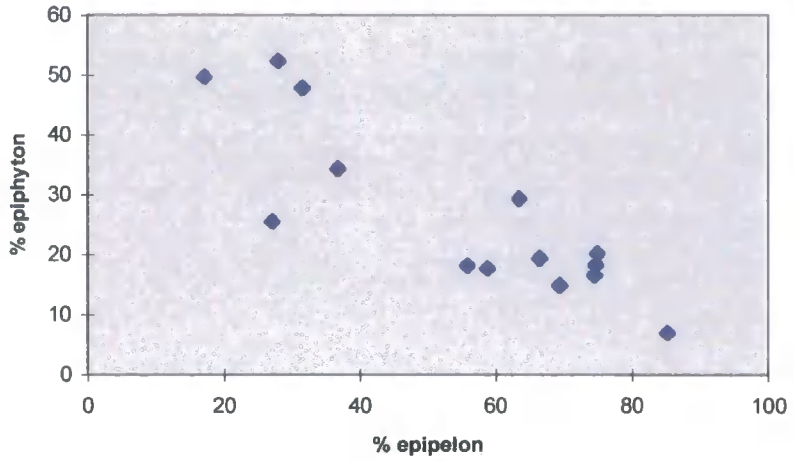


Fig. 3. Relationship between epiphytic and epipelic diatom taxa in samples from Croft Kettle core.

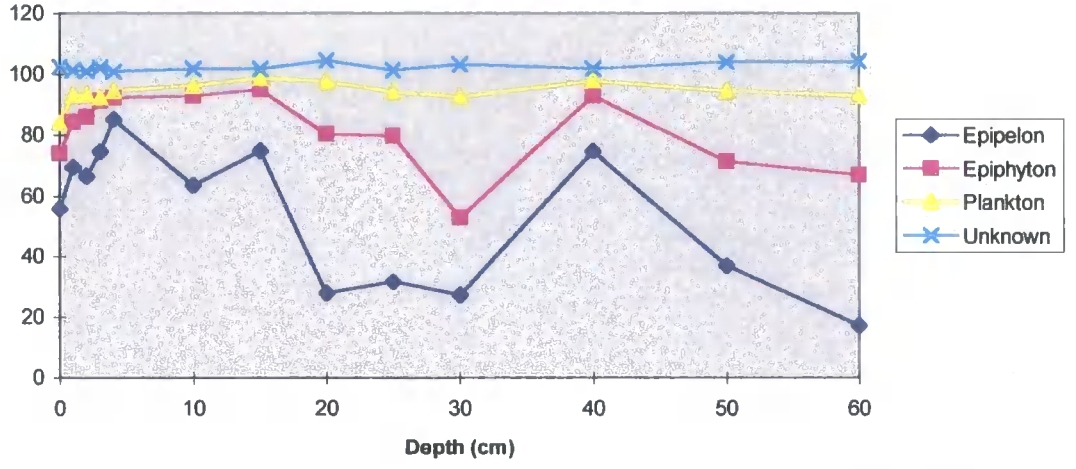


Fig. 4. Relative proportions of main life-forms in top 60 cm of core from Croft Kettle.

Table 1. Diatom taxa found in Croft Kettle. 104 taxa were found in total, of which 60 (in bold) were found in contemporary samples.

Taxon	frequency	maximum (%)
Achnanthes sp. Bory 1822 (sensu lato)	**6**	**0.7**
Achnanthes conspicua A.Mayer 1919	**3**	**0.3**
Achnanthidium minutissimum (Kütz.) Czarnecki 1994	**21**	**54.5**
Amphipleura pellucida Kütz. 1844	**20**	**7.9**
Amphora libyca Ehrenb. 1840	**17**	**42.4**
Amphora normanii Rabenh. 1864	1	0.3
Amphora ovalis (Kütz.) Kütz. 1844	2	3.6
Amphora pediculus (Kütz.) Grunow in Schmid et al. 1875	**12**	**3.2**
Amphora sp. Ehrenb. ex Kütz. 1840	**5**	**1.6**
Amphora veneta Kütz. 1844	1	0.3
Aneumastus stroesei (Østrup) D.G.Mann 1990	**1**	**0.3**

Asterionella formosa Hassall 1855	1	0.3
Aulacoseira crenulata (Ehrenb.) Thwaites 1848	14	20.0
Brachysira serians (Bréb. ex Kütz.) Round et D.G.Mann 1981	8	0.9
Brachysira vitrea (Grunow) R.Ross in Hartley 1986	**9**	**3.7**
Caloneis bacillum (Grunow) Cleve 1894	**1**	**1.2**
Caloneis latiuscula (Kütz.) Cleve 1938	**1**	**2**
Cocconeis pediculus Ehrenb. 1838	1	0.6
Cocconeis placentula Ehrenb. 1838 var. euglypta (Ehrenb.) Grunow 1884	**11**	**2**
Cocconeis placentula Ehrenb. 1838	**15**	**3.9**
Cosmioneis pusilla (W.Sm.) D.G.Mann et A.J.Stickle in Round et al. 1990	**1**	**0.3**
Craticula sp. Grunow 1868	**1**	**0.3**
Cyclotella sp. Kütz. ex.Bréb. 1838	**8**	**1.7**
Cyclotella meneghiniana Kütz. 1844	**5**	**3.7**
Cyclotella radiosa (Grunow) Lemmerm. 1900	2	1.8
Cymatopleura librile (Ehrenb.) Pant. 1902	1	0.3
Cymbella sp. C.Agardh 1830	**1**	**0.7**
Cymbella affinis Kütz. 1844	**3**	**3.9**
Cymbella cesatii (Rabh.) Grunow in A. Schmidt 1881	**1**	**1.2**
Cymbella cistula (Ehrenb.) Kirchner 1878	1	0.6
Cymbella cymbiformis Agardh 1830	**16**	**5.8**
Cymbella microcephala Grunow 1880	**9**	**3.7**
Denticula tenuis Kütz. 1844	1	0.3
Diatoma tenue Agardh 1812	3	0.6
Diploneis elliptica (Kütz.) Cleve 1891	**1**	**0.3**
Diploneis oblongella (Nägeli) A.Cleve 1922	1	0.3
Diploneis oculata (Bréb.) Cleve 1894	**1**	**0.3**
Diploneis ovalis (Hilse) Cleve 1891	**4**	**0.3**
Diploneis sp. Ehrenb. ex P.T.Cleve 1894	**1**	**0.3**
Encyonema minutum (Hilse in Rabenh.) D.G.Mann in Round et al. 1990	**2**	**0.3**
Epithemia turgida (Ehrenb.) Kütz. 1844	**17**	**5.5**
Epithemia sorex Kütz. 1844	2	1.2
Fragilaria sp. H.C.Lyngb. 1819	**3**	**1.5**
Fragilaria bidens Heib. 1863	1	0.3
Fragilaria capucina Desm. 1825	**10**	**1.6**
Fragilaria capucina Desm. 1825 var. mesolepta (Rabenh.) Rabenh. 1864	1	0.3
Fragilaria vaucheriae (Kütz.) Petersen 1938	1	0
Fragilariforma virescens (Ralfs) D.M.Williams et Round 1988	1	0.3
Frustulia sp. Rabenh. 1853	1	0.3
Frustulia vulgaris (Thwaites) De Toni 1891	**1**	**0.6**
Gomphonema angustum Agardh 1831	**21**	**24.7**
Gomphonema insigne Greg. 1856	2	0.3
Gomphonema olivaceum (Hornem.) Bréb. 1838	**3**	**0.7**
Gomphonema parvulum (Kütz.) Kütz. 1849	1	0.3

Gomphonema sp. Ehrenb. 1832	6	1.0
Gyrosigma acuminatum (Kütz.) Rabenh. 1853	2	0.3
Hantzschia amphioxys (Ehrenb.) Grunow 1877	1	0.3
Mastogloia smithii Thwaites 1856	**12**	**3.1**
Melosira varians Agardh 1827	2	0.7
Navicula capitata Ehrenb. 1838 var. hungarica (Grunov) R.Ross 1947	**1**	**0.3**
Navicula capitata Ehrenb. 1838	2	0.3
Navicula cryptotenella Lange-Bert. 1985	**14**	**6.7**
Navicula gregaria Donkin 1861	**2**	**0.3**
Navicula lanceolata (Agardh) Ehrenb. 1838	**2**	**0.6**
Navicula oblonga Kütz. 1844	**15**	**27.7**
Navicula radiosa Kütz. 1844	**20**	**10.2**
Navicula reichardtiana Lange-Bert. 1989	1	0.6
Navicula sp. Bory 1822	**2**	**0.3**
Navicula unidentified small species Bory 1822	**5**	**4.3**
Navicula veneta Kütz.	**2**	**0.6**
Neidium binodis (Ehrenb.) Hust. 1945	**1**	**0.3**
Neidium sp. E.Pfitzer 1871	1	0.3
Nitzschia amphibia Grunow 1862	**4**	**0.6**
Nitzschia dissipata (Kütz.) Grunow 1862	1	0.7
Nitzschia gracilis Hantzsch 1860	9	2.3
Nitzschia lacuum Lange-Bert. 1980	**3**	**4.3**
Nitzschia linearis (Agardh) W.Sm. 1853	**8**	**2.3**
Nitzschia recta Hantzsch ex. Rabenh. 1861	1	0.3
Nitzschia sigmoidea (Nitzsch.) W.Sm. 1853	1	0.3
Nitzschia sp. Hassall 1845	**7**	**1.2**
Pinnularia appendiculata (Agardh) Cleve 1895	**2**	**0.3**
Planothidium lanceolatum (Bréb.) Round et L.Bukhtiyarova 1996	4	0.7
Pseudostaurosira brevistriata (Grunov in Van Heurck) Williams et Round 1987	**9**	**9.2**
Reimeria sinuata (Greg.) Kociolek et Stoermer 1987	2	0.6
Rhoicosphenia abbreviata (C.Agardh) Lange-Bert. 1980	**5**	**1.5**
Rhopalodia gibba (Ehrenb.) O.Müll. 1895	**19**	**5.9**
Sellaphora pupula (Kütz.) Mereschk. 1902	3	0.7
Stauroneis smithii Grunow 1860	**2**	**0.3**
Staurosira construens Ehrenb. 1843	**5**	**8.9**
Staurosira elliptica (Schumann) D.M.Williams et Round 1987	**1**	**0.3**
Staurosirella lapponica (Grunov in Van Heurck) D.M.Williams et Round 1987	**16**	**51.8**
Staurosirella pinnata (Ehrenb.) D.M.Williams et Round 1987	**8**	**7.6**
Stephanodiscus parvus Stoermer et Håk. 1984	2	6.1
Stephanodiscus sp. Ehrenb. 1845	4	4.2
Synedra acus Kütz. 1844	3	1.2

Synedra capitata Ehrenb. 1836	6	7.1
Synedra minuscula Grunov in Van Heurck 1881	4	6.8
Synedra parasitica (W.Sm.) Hust. 1930	**3**	**0.3**
Synedra tenera W.Sm. 1856	**8**	**1.3**
Synedra ulna (Nitzsch) Ehrenb. 1836	2	0.6
Tabularia fasiculata (Agardh) D.M.Williams et Round 1986	4	1.0
Thalassiosira weissfloggii (Grunov) Fryxell et Hasle 1977	1	0.3
Tryblionella acuminata W.Sm. 1853	1	0.3

APPENDIX 2

Summary report for pollen analysis and radiocarbon dating of Croft Kettle

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1 Introduction

1.1 Aim

The aim of this report is to investigate the vegetation history and floristic changes that have occurred within and around Hell Kettles since their formation. Any changes in the vegetation over the last 100 years are of particular interest, following a decrease in the floristic diversity of the ponds and their surrounding vegetation.

1.2 Site Description and Location

The area known as Hell Kettles, refers to two small ponds called Croft Kettle and Double Kettle, which are located near to Oxneyfield, County Durham, just a couple of miles south of Darlington, near to the junction of the River Tees and River Skerne. The two Kettles are situated upon an outcrop of Magnesium Limestone overlain by boulder clay and are fed by an underground spring located at the bottom of Croft Kettle. The formation of the Kettles is recorded in local folklore and is thought to have occurred in 1179 AD, due to the subsidence of the local Magnesium Limestone. The subsidence of the local limestone has previously been attributed to coal, lime, allum, marl or limestone workings.

2 Methods

2.1 Sampling

On 12th March 1999, a 4.1m sediment core (HK-2), was retrieved from the middle of Croft Kettle using a Livingstone Corer. The cores were wrapped and sealed in clingfilm and foil to prevent moisture loss and contamination. They were then labelled and placed in split drainage pipes, before being transported to the University of Durham where they were stored at 4°C to until analysis was possible.

2.2 Stratigraphic Description

The sediment core consisted of 4m of post-glacial sediment. The detailed stratigraphy is outlined below.

Depth in cm (below lake bottom)	Sediment Description
30-45	Dark olive-brown organic lake mud with fine organic plant remains
45-74	Olive green slightly silty organic mud with occasional visible plant remains
74-85	Light brown slightly silty organic mud
85-124	Olive- brown slightly silty organic mud.
124-133	Red-Brown organic mud with organic plant remains.
133-134	Rust coloured (Red-orange) organic mud
134-137	Olive-brown organic mud.
137-174	Red-brown, very fine, banded clay (contains lighter coloured bands)
174-180	Olive-brown, very fine, slightly organic, clay with black speckles of coal or manganese
180-186	Dark brown organic mud with 50% irregular gravels (approx. 2mm in diameter) also black pieces of coal or manganese.
186-190	Orange-cream, banded organic marl.
190-200	Very Dark brown, banded organic marl with plant remains.
200-215	Olive brown slightly organic clay.
215-220	Olive, fairly saturated clay.
220-241	Grey-olive, fairly compact clay.
241-245	Olive-brown, clayey organic mud with small molluscan remains
245-250	Dark brown, organic mud with mica (sand) and very fine organic remains
250-261	Orange, sandy, Moss/Sphagnum layer, interrupted by sandy OM layer 257-259cm
261-330	Chocolate brown, slightly sandy (mica) organic mud with molluscs and fine organic plant remains.
330-385	Dark brown very compacted /unsaturated, sandy organic mud with some molluscan remains.
395-400	Olive brown organic clay.

2.3 Pollen Analysis

Pollen analysis of HK-2 should produce a reliable record of local vegetation change because of the small surface area of the pond (approximately 40m in diameter). Jacobson and Bradshaw's (1981) model of pollen transport and deposition predicts that the vast majority of the pollen deposited within the kettle will be derived from the local or extra local vegetation (i.e. vegetation within a few hundred meters of the pond).

The surface of the core was carefully scraped away to remove any contaminated sediment, and then sub-samples were taken for pollen analysis every 20 cm down the profile, using a known sediment volume (usually between 0.5-1cm^3). Following

analysis of the initial results, subsequent pollen samples were taken at 2.5cm, 5cm or 10cm intervals at selected horizons throughout the core, in order to investigate periods of significant interest.

Pollen samples were prepared using standard chemical procedures with minerogenic content being removed by the use of Hydrofloric Acid. Finally, the samples were mounted on microscope slides using silicon oil. Microscope analysis was undertaken using a Nikon microscope at magnifications of x400 and x600. Pollen and spores were identified using the aid of various pollen keys (e.g., Moore et al 1991, Faegri and Iversen 1989 and Andrew 1984). A total of more than 400 pollen grains (excluding aquatics and spores) were identified for each level, with the exception of a couple of samples which contained very low pollen concentrations. The addition of a known concentration of exotic *Lycopodium* spores enabled calculation of pollen and spore concentrations.

Pollen counts are expressed as a percentage of Total Pollen (excluding obligate aquatics and spores). Obligate aquatics, spores and fungi are also expressed as a percentage of the total pollen count. In Figs 2-5, pollen percentages with values less than or equal to 1% are represented by a small + symbol. Absolute pollen diagrams (concentration of grains per unit time), would provide a more direct representation of actual vegetation change and therefore permit a more penetrating analysis of vegetation change. However this requires extensive dating, and the results of the Pb^{210} analysis are not yet available.

3.0 Interpretation

The pollen diagram has been split up into three local pollen assemblage zones (LPAZ's) to aid the interpretation of the data. The pollen diagram has been split into four separate diagrams and arranged so that species which occupy broadly similar habitats are presented together.

Pollen grains have been assigned to the types or groups assigned by Moore et al (1991). Table 1.0 lists the species of plants recorded from Hell Kettles between 1777-1996. The corresponding pollen groups or pollen types have been appended alongside. In addition, the following species have been used as indicator species to aid the interpretation of the pollen zones:

Land Use	Indicator Species Used
Cultivation plants and associated weeds	*Cerealia*, *Cannabis* type, Brassicaceae, *Anthemis* type, *Sinapsis* type, Lactuceae.
Pastoral plants	Poaceae (Wild grasses, *Glyceria* type), *Plantago lanceolata, Rumex* ssp, Ranunculaceae.
Ruderals	*Artemisia* type, Chenopodiaceae, *Centaurea nigra.*

4 Dating of the Profile

4.1 Radiocarbon Dating HK-2.

No radiocarbon dates have been obtained from HK-2 due to the lack of terrestrial plant remains in the sediment examined to date. Analysis of the levels of the radioactive isotopes Pb^{210} and Cs^{137} have been undertaken, but the results are not yet available. Nevertheless, the sediments can still be dated by correlating HK-2 with another core, HK-1, which was taken in 1991 (Dodsworth 1991). A single radiocarbon date has been obtained from HK-1, at a depth of 310-315cm and thus the approximate age of the sediments in HK-2 can be estimated by correlating between the two cores.

4.2 Correlation of HK-1 and HK-2

The radiocarbon date from HK-1 was obtained from remains of *Equisetum* found between 310-315 cm. The radiocarbon date for the seed has been calculated as 1240 +/- 40 BP. This calibrates to between 680-890 Cal AD (1270-1060 Cal BP). The intercept of the radiocarbon calibration curve occurs at 780 Cal AD (1170 Cal BP).

Unfortunately the stratigraphy of the two cores is significantly different and this causes problems when correlating between the two cores. Nonetheless, correlation between the cores has been attempted using key similarities in the pollen diagrams. Correlation between HK-1 (Fig 6) and HK-2 (Figs 2-5) has been based on the curves for *Calluna, Sphagnum*, Lactuceae, *Corylus, Alnus* and *Betula*. Consequently, a depth of 310-315 cm on HK-1 is thought to be approximately equivalent to between 300-320 cm on core HK-2. Thus, a depth of 310 cm on core HK-2 is proposed to be equivalent to c.780 Cal AD.

If one assumes a constant rate of sediment accumulation through to the top of the profile, then the sediment in HK-2 will have accumulated at a rate of approximately 0.25 cm yr^{-1} or 3.94 yrs cm^{-1}. It must be noted that sediment accumulation rates are

unlikely to be linear through time, especially if a sudden catastrophic subsidence event had indeed occurred. However, in the absence of any other radiocarbon dates, the rate of sediment accumulation is tentatively assumed to be correct. Naturally and any conclusions reached in this study will require further testing when further radiocarbon dates or the results of Pb^{210} dating become available.

Using a sedimentation rate of 3.94 yrs cm^{-1}, the bottom of the diagram then dates to c. 425 Cal AD, which is just after the Roman withdrawal from England. The subsidence of the Magnesium Limestone would then have occurred at a depth of 208 cm.

4.3 Other Pollen work from the Northeast of England.

Further age estimates can be obtained by correlating HK-2 with other radiocarbon dated pollen profiles from within northeast England. Pollen analysis of the historical past has been done at several other sites e.g., Bartley, Chambers and Hart-Jones (1976), Roberts, Turner and Ward (1973) and Turner (1979). Some of these sites may provide useful comparisons with the work at Hells Kettles. The nearest lowland sites are: Neasham Fen, Hutton Henry and Thorpe Bulmer (Bartley et al 1976).

5 Results

5.1 Vegetation and Environmental History

LPAZ HK2-A 4m-1.36m Wet Meadow and Arable Landscape

This zone has been split up into three sub-zones.

LPA Z HK2-A1 4m-3.5m

The bottom of the pollen diagram is virtually devoid of trees. The low percentages (10-15%) indicates that trees and shrubs were restricted to a few scattered occurrences e.g. *Alnus* and *Betula* around the site and *Quercus, Corylus* and *Fraxinus* along the edges of pasture fields or in their midst. The lack of woodland also implies that extensive deforestation of the surrounding area occurred at an earlier date. Turner (1979) concluded that within Northeast England, deforestation took place on an extensive scale between approximately 100 BC-200 AD. The two nearest sites to Hell Kettles are the sites of Thorpe Bulmer and Hutton Henry. At these two sites deforestation occurred at 190-240 AD and 70 –110 AD respectively (Bartley et al 1976).

The rest of the surrounding land was comprised mainly of wet meadows and pasture, due to the low concentrations of tree pollen and the high levels for *Plantago lanceolata* and Poaceae pollen which belong mostly to Wild Grasses (see Table 1.0). Single grains of *Primula veris* type pollen and *Sanguisorba minor* pollen demonstrate the calcareous nature of the grassland. Some arable farming must also have taken place due to the sporadic occurrences of pollen from Cereals, *Cannabis* type and *Anthemis* type and the higher levels of the pollen from *Sinapsis* type, Brassicaceae and Lactuceae. A mixed farming regime must therefore have been in use.

The actual site now occupied by Croft Kettle was an area of marsh land or wet meadow. *Sphagnum* moss, Umbelliferae, *Cirsium* (maybe Marsh Thistle), Sedges, including *Cladium* and wild grasses (some of which appear to belong to *Phragmites, Festuca* and *Glyceria*), were growing at the site. Scattered tall herbs e.g. *Equisetum, Filipendula* and *Thalictrum* and tall aquatic herbs such as *Sparganium*, and *Typha latifolia* also grew nearby.

Areas of shallow, standing water occurred, due to the presence of *Hottonia palustris* (Water violet) which grows in shallow water, ponds and ditches. *Triglochin* (c.f. *palustris*, Marsh Arrowgrass) and *Thelypteris palustris* (Marsh fern) may also have been present. The former is based on a peak in pollen grains that are very similar to *Potamogeton* pollen, but have a smaller surface network. The presence of Marsh fern is based on a small peak in spores belonging to Filicales undifferentiated.

Pre-Quaternary Spores reach levels of between 10-20%, which indicates that soil was in-washing into the marsh area on a regular basis. These soil influxes may either have been caused by soil in-wash from agriculture fields or due to periods of flooding. (Pre-Quaternary Spores are ancient plant spores that have been eroded out of the surrounding strata e.g. carboniferous coal seams or the Magnesium Limestone and then deposited within the soils or boulder clay).

LPAZ HK2-A2 3.5-2.7m

During this sub-zone there is a slight increase in pollen from trees and shrubs. This is due to a rise in *Betula* pollen, followed by an increase in pollen from *Alnus, Salix* and *Corylus*. Despite these increases, trees and shrubs did not form a significant part of the landscape. The trees and shrubs were probably growing locally as there is a definite decline in the inputs of Pre-Quaternary Spores, suggesting stabilisation of the surrounding soils. This zone also shows an increase in the importance of arable farming

within the catchment, shown by a rise in *Cerealia* and *Cannabis* pollen and pollen from associated arable weeds e.g. *Centaurea cyanus, Anthem*is, Brassicaceae and *Sinapsis* type. Arable cultivation must have occurred close to the site in order for there to be a noticeable peak in the pollen, because pollen from domestic crops is poorly dispersed (Vuorela 1973). Despite the increased importance of arable around the site, pasture is still an important feature in the landscape shown by the continual deposition of pollen from wild grasses and *Plantago lanceolata.*

Standing water still occurred at the site due to the presence of the shallow water species *Potamogeton*; presumably *P. natans,* which usually occurs in water less than 1m deep. Standing water is also indicated by the occurrence of a single grain of *Myriophyllum verticillatum* pollen. Huntley and Birks (1983) state that low percentages of *Myriophyllum* pollen are often found even when the plant is abundant. However, the reduction in pollen and spores from *Triglochin*, Apiaceae, *Filipendula*, *Equisetum, Sphagnum* and grasses e.g., *Phragmites* type and Wild Grass (e.g. *Festuca*) types, suggests that the water was gradually shallowing and that the soils on the periphery of the marsh were slowly drying out. A slight increase in the abundance of sedges, *Glyceria* type pollen and Ranunculaceae also supports this. This would explain why shrubs such as *Betula* and *Alnus* were able to encroach into the wetland area.

The occurrence of occasional grains of *Cannabis* type pollen demonstrates that hemp cultivation for rope, drugs or fruits was occurring somewhere in the catchment. Although, the low pollen percentages suggest that hemp cultivation did not occur within the nearby fields. The presence of *Cannabis* type pollen could imply Anglo Saxon occupation, which is in consistent with the proposed chronology (above).

LPAZ HK2-A3 2.7m - 1.36m

The bottom of this sub-zone is very similar to sub-zone HK2-A2 above, because it contains substantial evidence for local arable cultivation as well as the presence of meadow and pastureland. However, the sub-zone has been differentiated because of the higher levels of pollen from *Plantago* ssp, *Rumex* ssp and ferns (Filicales), which suggest well-drained soils and pastureland. A phase of arable cultivation, continued from the preceding zone, continues on until a depth of c.1.60 m. Bartley et al (1976) noticed an apparent phase of extended arable cultivation in the North-east of England throughout many of their pollen diagrams. At Neasham Fen, Mordon Carr, Thorpe Bulmer and Hutton Henry the extended agriculture phase is shown to end at approx.

1100 AD (Bartley et al 1976). Although the rate of sedimentation calculated in section 4.2 (above) would date the end of this agriculture phase to 1371 Cal AD.

Mid-way through sub-zone HK2-A3, and starting just below the 2m level, there is a significant rise and peak in Pre-Quaternary Spores. Spore reach levels equivalent to c.50% of the Total pollen count, before declining rapidly. Towards the top of the sub-zone, as the influx in Pre-Quaternary spores reaches a peak, there is a gradual rise in pollen from heaths species e.g. *Calluna* and a definite resurgence in pollen from grass and wetland species such as Apiaceae, *Filipendula, Sphagnum* and wild grasses. There is also a slight resurgence in tree pollen e.g. from *Quercus* and *Fraxinus*.

The stratigraphy suggests that the water level in the marsh became gradually shallower throughout HK2-A1 and HK2-A2, culminating in the development of a moss layer when the water level became level with the ground. However, this moss layer was subsequently overlain by lake muds, clays and then marls, which suggests a sudden and substantial rise in the water level at the site.

The gradual but substantial influx of Pre-Quaternary Spores could therefore represent the sudden subsidence of the limestone, which is recorded in Brompton's Chronicle of 1179. The gradual but high influx of Pre-Quaternary Spores would be generated by the sudden opening of the kettle holes and the subsequent influx of materiai being (gradually?) washed back into the ponds. Alternatively, the spores may be the result of substantial and prolonged periods of flooding within the catchment.

It is possible that the creation of the Hell Kettles could have been caused by an increase in water pressure, following dissolution of Gypsum within the limestone. A gradual increase in water pressure at the weakest point in the strata would eventually push up the soil surface (as chronicled) and eventually lead to the creation of the Kettles. Very similar occurrences have also been observed in areas containing old mine workings, when pumping of the ground water has ceased. The increase in water pressure at Hell Kettles might have been exacerbated by a gradual sea level rise (and thus an increase in ground water), in the North-east of England between 650-1350 AD (Tooley 1974).

LPAZ HK2-B 1.36m –0.7m Carr and Calluna Heath

The start of this zone is marked by an increase in trees, shrubs and heaths and an overall decline in Pre-Quaternary Spores, as well as a marked reduction in the

importance of arable cultivation. As the soil in the surrounding area started to stabilise, seen by the reduction in Pre-Quaternary spores, *Calluna* and *Sphagnum* heath became locally important. The area of heathland was fringed by *Alnus* and *Betula* carr, with *Corylus* confined to the drier areas. *Corylus* may have been managed and coppiced, although there is no direct evidence to support this. The development of heath around Croft Kettle is bimodal, and interrupted by a brief transition to alder carr. During this period there is also a brief re-surgence in arable cultivation. Otherwise, the surrounding landscape seems to be predominantly used as grassland and pasture. The calcareous nature of the pond water is suggested by the occurrence of a single grain of *Myriophyllum spicatum* at 1.32m. Tall and emergent aquatics such as *Equisetum* and *Typha angustifolia* are also present in small numbers.

This period of heath development could potentially be attributed to the climatic deterioration of the Little Ice Age c. 1500-1700 AD (Lamb 1977). The repeated occurrence of *Succisa pratense* during this phase, may be taken as an indicator of the declining status of the surrounding soil. A single occurrence of *Cannabis* type pollen at 1.2m may also indicate a Medieval (Tudor) date for the start of the zone, in keeping with the suggested chronology, as in 1533 AD the cultivation of hemp was ordered by law, in order to produce rigging for the naval fleet. If one uses a linear sedimentation rate of 3.94 yrs cm^{-1}, then the Little Ice Age would have started at around 127 cm and ended at approximately 76 cm. These levels are very similar to the proposed dates for the beginning and end of zone HK2-B.

LPAZ HK2-C 0.7-0m Pasture and Arable with Scattered Woodland

This zone shows the development of stable pasture with park woodland. Arable production, primarily *Hordeum* type is still evident in the surrounding area but the land is predominantly composed of damp calcareous meadows and pastureland. The landscape and environment is relatively stable, with only minor variations in the abundance and variety of plant species. Anthropogenic impact on the landscape is evident by the high proportion of ruderal plants and plants of disturbed or waste ground e.g., *Rumex obtusifolius, Urtica dioica* and Chenopodiaceae. The climate is much warmer than in the preceding zone. Lamb (1977) states that the climate warmed after 1700 AD, thus this zone is though to represent the last 2-300 years of sediment deposition.

Tree and shrub pollen accounts for 20-40% of the total pollen but this is reflective of the fluctuating amounts of scrub and patches of woodland that occurred within the local

and regional environment. The Kettles were surrounded by mixed scrub e.g. probably *Cratageus* and *Populus* with some *Alnus, Betula* and *Salix*. Whilst the rest of the tree pollen e.g. *Quercus, Acer, Fraxinus* and *Ulmus* was derived from patches of deciduous type woodland and trees that occur within and around the field edges.

Sedges including *Carex* ssp. and *Cladium*, *Phragmites* type grasses and tall herbs, all fringed the margins of the ponds. The extent and dominance of the sedges e.g. *Cladium* and grasses e.g. *Phragmites*, fluctuates throughout the entire zone. This is clearly illustrated in Fig 7, which shows the varying pollen concentrations of selected taxa, per unit volume of sediment. At some point during the zone the pond has supported plants belonging to *Sparganium* type, *Potamogeton* type, *Typha angustifolia* type and *Hottonia palustris* (Water Violet).

5.2 Recent Vegetation Changes and Loss of Species Diversity.

It is difficult to say anything conclusive about the variety or extent of aquatic vegetation growing within the pond, due to the low levels of aquatic pollen that have been counted during this study. Pollen grains from very few aquatic species were actually observed, and the total number of aquatic pollen grains was exceedingly low (see Figure 1). A significantly higher pollen count would be needed in order to resolve this problem.

The limited pollen data and the lack of a reliable dating chronology make it impossible to ascertain whether there has been a reduction in the diversity of species over the last 100 years. It is only possible to say that the pond and surrounding areas have supported a variety of different species through time. The pollen of many species is recorded sporadically and only observed in very low quantities. It would therefore be unwise to say that a species (or pollen type), was absent from the area, based solely on the absence of its pollen. For example, pollen of *Hottonia palustris* was last recorded at 60cm, but the plant could still have been growing in the pond until much more recently.

Lastly, pollen analysis has been unable to provide information about the loss of specific plant species from in and around the ponds, because pollen grains cannot often be reliably identified to a single species (see Table 1.). Most pollen grains can only be classified to a family or group of plants e.g. Lactuceae. Differences in the pollen production, pollen source area and pollen grain taphonomy of different plants also means that the areal distribution of the plants could not be determined in this study.

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Table 1. List of Species found at Hell Kettles between 1777 –1996 and their equivalent pollen grains.

Species	Pollen type
CHAROPHYTA	Not Recorded
LICHENS	Not Recorded
LIVERWORTS	Not Recorded
MOSSES	Sphagnum is the only type recorded
PTERIDO-PHYTES	PTERIDO-PHYTES
Equisetum sp	Equisetum type
FLOWERING PLANTS	FLOWERING PLANTS

Achillea ptarmica L.	Anthemis type
Achillea millefolium L.	
Matricaria recutita L.	
Agrostis capillaris L.	Poaceae -
Agrostis stolonifera L.	Wild Grass type
Anthoxanthum odoratum L.	
Bromus hordeaceus L	
Festuca ssp	
Festulolium loliaceum Hudson	
Lolium perenne L.	
Phleum pratense L.	
Alisma plantago-aquatica L.	Pollen not observed
Alnus glutinosa (L.) Gaertn.	Alnus type
Alopecurus geniculatus L.	Wild Grass type
Angelika sylvestris L.	Apiaceae
Apium nodiflorum L.	
Berula erecta (Huds.) Coville.	
Conopodium majus Gouan	
Oenanthe fistulosa L.	
Bellis perennis L.	Aster type
Eupatorium cannabinum L.	
Senecio ssp	
Blysmus compressus (L.) Link.	Cyperaceae-Carex type
Carex ssp.	
Eleocharis ssp	
Schoenoplectus ssp	
Scirpus lacustris L.	
Briza media L.	Poaceae---Phragmites/ Wild Grass
Cynosurus cristatus L.	type
Dactylis glomerata L.	
Deschampsia cespitosa (L.)	
Beauv.	
Holcus lanatus L	
Poa ssp	
Caltha palustris L. sp. palustris	Caltha type
Capsella bursa-pastoris (L.)	Brassicaceae
Medic.	
Cardamine pratensis L.	Sinapsis type.
Rorippa ssp	
Centaurea nigra L.	Centaurea nigra type
Cerastium fontanum Baumg	Pollen not observed
Cirsium arvense (L.) Scop.	Cirsium type
Cirsium palustre (L.) Scop.	
Chenopodium rubrum L.	Chenopodiaceae
Cladium mariscus (L.)	Cyperaceae- Cladium type
Crataegus monogyna Jacq.	Crataegus type
Dactylorhiza fuchsii (Druce)	Pollen not observed

Elodea canadensis Michaux	Pollen not observed
Epilobium ssp	Pollen not observed
Equisetum palustre L.	Equisetum type
Galium ssp.	Rubiaceae
Glechoma hederacea L.	Pollen not observed
Glyceria ssp	Poaceae—Glyceria type
Hedera helix L.	Pollen not observed
Hippuris vulgaris L.	Pollen not observed
Hypericum tetrapterum Fr.	Hypericum perforatum type
Hypochaeris radicata L.	Lactuceae
Leontodon ssp	
Taraxacum ssp	
Iris pseudacorus L.	Pollen not observed
Juncus ssp	Fossil pollen not preserved
Lathyrus pratensis L.	Pollen not observed
Lemna ssp	Pollen not observed
Lotus ssp	Lotus type
Lynchis flos-cuculi L.	Lynchis type
Lythrum salicaria L.	Pollen not observed
Mentha ssp	Mentha type
Myostis ssp	Pollen not observed
Nympheae alba L.	Pollen not observed
Parnassia palustris L.	Pollen not observed
Pedicularis palustris L.	Pollen not observed
Persicaria ssp	Polygonum type
Phragmites australis (Cav.)	Poaceae---Phragmites type
Plantago lanceolata L.	Plantago lanceolata type
Plantago media L.	Plantago media
Polygonum aviculare L.	Polygonum aviculare L.
Populus canescens (Ait.) Sm.	Populus type
Potamogeton ssp	Potamogeton type
Potentilla anserins L.	Potentilla type
Sanguisorba minor	Sanguisorba minor ssp minor
Prunella vulgaris L.	Pollen not observed
Ranunculus acris L.	Ranunculus acris type
Other Ranunculus ssp	Ranunculaceae
Rubus fructicosus L. agg.	Pollen not observed
Rumex acetosa L.	Rumex acetosa type
Other Rumex ssp	Pollen not observed
Scrophularia auriculata L.	Pollen not observed
Scutellaria galericulata L.	Pollen not observed
Solanum dulcamara L.	Pollen not observed
Sparganium erectum L.	Sparganium type
Staychs officinalis	Pollen not observed
Stellaria ssp	Pollen not observed
Succisa pratensis Moench.	Succisa pratensis
Thalictrum flavum L.	Thalictrum type

Trifolium pratense L.	Trifolium pratense type
Other Trifolium ssp	Trifolium type
Triglochin palustre L.	Triglochin type
Trollius europaeus L.	Pollen not observed
Tussilago farfara L.	Pollen not observed
Urtica dioica L.	Urtica dioica type
Urticularia vulgaris L.	Pollen not observed
Valeriana officinalis L.	Pollen not observed
Veronica ssp	Veronica type
Viburnum lantana L.	Pollen not observed
Vicia cracca L.	Vicis cracca type

Appendix 3

Results of Cs-137 and Pb-210 dating

The sediment samples for Cs-137 and Pb-210 dating were sent to the Environmental Radiation Research Laboratory at the Department of Experimental Physics of University College of Dublin. The person responsible for the analysis was Dr E. McGee and the paragraph below is a direct quotation of his sayings:
"If it is from Britain or northern Europe then I would have expected to see a strong Cs-137 signal, but all we see are traces. It is possible that the traces we see at the top of the core are from the start of input from weapons testing in the early 1960. However, the Pb-210 excess values suggest that we are even further back in time with no excess signal detected in the 0-3cm section. The other possibility is that there has been significant inwash of re-worked material - is this a possibility? It is interesting that here are high levels of supported Pb-210 in the system – evidence from Bi-214 and Pb-214 (have you stumbled on a uranium mine?), and there seems to be an especially enriched band of material in the 14-30 cm zone. These data do give us some useful information concerning the sequence, but unfortunately cannot be used to give you a rate of sedimentation which I am sure is what you had hoped."

Sample Depth	Cs-137	Cs-137 error	Total Pb-210	Pb-210 error	Bi-214	Bi-214 error	Pb-214	Pb-214 error	$Pb\text{-}210_{excess}$
0-3cm	7.2	0.5	580	16	539	27	865	45	-122
8-10cm	5.7	0.5	675	18	478	24	762	38	55
14-16cm	9.7	1	1477	42	955	48	1614	82	192.5
24-26cm	8	0.3	1365	31	907	46	1455	73	184
29-30cm	2.6	0.5	1545	42	1053	51	1635	82	201
34-35cm	1.6	1.2	413	10	287	15	449	25	45
49-50cm	0	0	65	3	42	3	76	4	6
54-55cm	0	0	65	3	59	3	86	5	-7.5
64-65cm	0	0	149	4	98	5	156	8	-7

APPENDIX 4

Synoptic background review of Hell Kettles[3]

1179	The ponds dramatically appeared at Christmas.
1328	Brompton's Chronicle (Abbot of Jervaulx) referring to the origin of the ponds.
1545	Leland reported that a marked duck was put in Croft Kettle and then was found at Croft Bridge upon Tees.
1725	Daniel Defoe, who was making a tour through the North of England visited the ponds (Manson, 1898), and subsequently wrote: "As to the Hell Kettles, so much talked up for a wonder, which are to be seen as we ride from the Tees to Darlington, I had already seen so little of wonder in such country tales that I was not hastily deluded again. Its evident they are nothing but old coal pits filled with water by the river Tees.
1774 (Oct)	Mr Grose and Mr Allan visited Hell Kettles on 18 October 1774, and found the diameter of the three large pools to be each about 34.7m, and that of the smaller 25.6m. Their respective depths were 5.9, 5.2, 4.3, and 1.7 metres. All of them are nearly round: the water stands to the brim, is quite cold, and impregnated with sulphur, curdling with milk, and refusing to mix with soap. Surface channels join the three largest ponds; and the water, after supplying the neighbouring farms by a small streamlet runs into the Skerne (Fordyce, 1857).
1777	Stephen Robson's first plant list for the sites includes *Cladium mariscus* and *Chara hispida* (the oldest record of a freshwater alga in Britain).
1894	The Kettles were plumbed by the late Mr John Clervaux Chayton, of Croft. The depth of Double Kettle was 6.4m in one place and 4.5m in another. That of Croft Kettle was 7.3m in one and 3.4m in another place; the third one near the road was 1.4m. The ponds have since been sounded from a boat by W. Messrs, C. Abbey and A.E. Garbutt. They found that the greatest depth of the double pond in the half nearest Croft road was 5.8m a little west of the centre and 5.2m in the centre. In the half furthest from the road, the greatest depth was 4.6m, while the greatest depth of the large one near Croft is 6.4m at the north end and in the middle 5.9m. The surface of the water at the ponds was found to be 3.2m above the level of the Tees at Stapleton Wood.
1898	A small water shell, called *Paludina* was recorded by Manson.
1910's	*Gyrinus bicolor* was recorded which is a rare whirligig[4] (the specimens are in the Hancock).
1925	Nicholson surveys ponds
1929	Nicholson's paper on the natural history of the kettles; an excellent species baseline. Already by 1925, more than 20 sp previously recorded were missing,

[3] This list is an intergration of S. Hedley's background review with historical records for Hell Kettles.

[4] Whirligig: any freshwater beetle of the family Gyrinidae that circles about on the surface.

	including *Carex elata*, *Parnassia palustris*, *Lythrum salicaria*, *Ranunculus lingua* and *Trollius europaeus*. It was also noted that a small surface channel permits water to flow from Croft to Double Kettle.
1960's	The owners, the Fells, install a 15.2cm supply pipe, replacing an earlier supply from an open ditch. They use a large quantity of water for their domestic and farm needs
1966	B.A. Whitton & D.S. Gibbons publish paper in "Ponds and reservoirs in Co Durham" (Journal of Durham University Natural History Society, XII).
1967 (Autumn)	Tees Valley and Cleveland Water Board begin pumping at Broken Scar works in Darlington, 4 miles to northwest, at OS 255141.
1968	J. R. Fell receives letter from Darlington & Teesdale Naturalists Field Club stating that the ponds were drier than ever before.
1968 (Nov)	The Northumbrian River Authority (NRA) installed an automatic water level recorder on Croft Kettle.
1969 (Jan)	The broken Scar borehole is opening at 10^4 m^3 d^{-1}. NRA carries out water analyses for both ponds, considering that local drainage and rainfall rather than abstraction is to blame for low levels in Double Kettle.
1969 (Apr)	Letter from NRA to J.R. Fell pointing out that the Authority had monitored water levels on all boreholes around the Broken Scar abstraction, indicating that the initial fall in the local water levels was rapid and then was gradually stabilised at significantly lower levels. Croft Kettle's water levels had altered by less than one inch in this period and the slight variations in the level can be related to local rainfall. NRA, however, gives the assurance that weekly measurements will continue at Croft Kettle.
1970	The water level in Double Kettle has fallen over a foot, permitting severe grazing of marginal vegetation. A plastic balancing pipe is being installed between the ponds. B.A. Whitton surveys the vegetation of the ponds.
1971	B.D. Wheeler & B.A. Whitton's papers "Ecology of Hell Kettles; 1. Terrestrial and sub-aquatic vegetation 2. The ponds" were published. The following species were reported to be missing since Nicholson's survey: *Ricciocarpus natans*, *Equisetum fluviatile*, *Briza media* (refound in 1996), *Eupatorium cannabinum*, *Galium uliginosum* (refound in 1996), *Pedicularis palustris*, *Potamogeton coloratus*, *Potamogeton pusillus*, *Ranunculus aquatilus* (refound in 1981), *Stellaria graminea*, *Stellaria palustris*, *Thalictrum flavum*, *Utricularia vulgaris* and *Veronica scutellata*. In addition, the surface channel connecting the two ponds, is replaced by a balancing pipe, which elevates water levels in Double Kettle. Crude flow measurements from the pipe, coupled with observation on the rate of fill of the Double Kettle after installation of the new pipe, suggest that throughflow in Croft Kettle is sufficient to re-fill it 3-4 times per year. Part of the fen vegetation is also fenced to prevent grazing and trampling by anglers.
1973	Letter from J.R. Fell to NCC informing that outflow from the pipe, was the lowest ever recorded (4.55 L per 33 s, while it was 4.55 L per 11 s).
1975	J.R. Fell replaces the balancing pipe between the ponds. Water levels were very low corresponding to the famous drought of this year.
1975	Darlington Naturalist Field Club that visited Hell Kettles mentions that at the southern end of the Double Kettle, *Cladium mariscus* was recovering slightly

	from the drastic cutting four years ago.
1976	Hell Kettles becomes a SSSI.
1980	File note by C. Shaw stating that by cattle poaching, the marginal vegetation zones are very narrow.
1981 (Jun)	Site visit to Hell Kettles by J. Hopkins, reporting that all the species present in 1971 were still present and that *Ranunculus aquatilus* was refound.
1981 (Winter)	J.R. Fell reports that Double Kettle has frozen and Croft Kettle has only a small amount of ice.
1983 (Nov)	NWA begins to abstract 18.2×10^3 m^3d^{-1} in order to clear the Tees of pollution caused by accidental release of oil from an upper Teesdale quarry.
1984 (May)	Pumping at Broken Scar stops for three months in order to assess the impact of water abstraction upon the aquifer. Site meeting between NCC and NWA to decide the abstraction license arrangements for the future of Hell Kettles.
1984 (Jul)	R.A. Fell reports algal growth in Croft Kettle (some manual clearance of which regrew in August) and also a fall in water level of Double Kettle.
1984 (Aug)	J. Hopkins reports 10% pond surface covered with algae.
1985 (May)	R.A. Fell signs first agreement with NCC – a S15 with three-year term, stipulating no fertiliser input to field surrounding the ponds.
1986 (Aug)	A new balancing pipe of 150 mm diameter replaces the previous one due to cattle damage to it. The new pipe has a doglet with weir and inspection chamber at bend.
1987	A flow gauge is installed to the farm pipe in order to measure the outfall rate.
1987 (Nov)	NWA confirms to NCC that they will be applying to DoE for a license to abstract 9.1×10^3, rising to 18.2×10^3 m^3 d^{-1} for maximum of 2 weeks if treatment plant fails or there is pollution in the river. G. Kershaw of NRA confirms that there are 6m of artesian head at the kettles, and that pumping at 9.1×10^3 will only reduce this about 5m, reducing the upflow rate by about a sixth. This is not considered serious, but a condition in the license states that the total hardness of the water should always be above 1300 mg L^{-1} $CaCO_3$, otherwise the abstraction will cease until the total hardness of the water exceeds 1400 mg L^{-1}. Water samples should be obtained from just below the surface of the water close to the overflow from the southern pond every 6 months. Results of this analysis should be forwarded to the NCC.
1987	Report of Invertebrate sites register for Durham by S.G. Ball referring to a record of shining ram's-horn snail (*Segmentina nitida*) from Hell Kettles that dates to 1970. However, this snail is not mentioned in Ralph Lowe's (1989) "Atlas of the land and freshwater Mollusca of Northumberland and Durham" and Dr M. Kerney from the Natural History Museum states that this is a southern species and that it has never been recorded north of Yorkshire. Therefore, its actual presence can be doubted.
1987 (Dec)	NCC accepts conditions of abstraction license.
1988 (May)	R.A. Fell renews the agreement with NCC- a S15 for 15 years.
1989 (Mar)	J. Barrett raises concern at perceived falling levels in Croft Kettle.

1989 (Mar)	G.M. Kershaw measures the overflow from the farm's pipe to be 52 m^3d^{-1}, also ensuring that water levels in both ponds are high.
1989 (Jul)	NWA (Kershaw) measures flow in farm supply pipe at 345.5 m^3 d^{-1}.
1989 (Aug)	Key correspondence between J. Barret and R Wright, suggesting that the combined effect of the prolonged drought and water abstraction has caused the unexpected low levels at the ponds.
1990 (Feb)	A borehole is being drilled to the farm in order to ensure a reliable water supply.
1990 (Oct)	Both ponds maintain their water levels, despite the dry summer. However, the farm's borehole water level decreased by three feet over a period of a few days, following a doubling of abstraction rate in Broken Scar for 8 days, and was not recovered after a week of the cessation of abstraction of the second pump.
1991 (Feb)	Consent sought by University College London for collection of sediment cores to assess the diatom record.
1991 (Dec)	A student project report, on the "Analytical Investigation of pollen from Hell Kettles" by S.L. Dodsworth.
1996	Fringing vegetation mapped to NVC standards. The balancing overflow water, has diversified the grassland between the ponds and given rise to an interesting fen types, notably MG8 and *Eleocharis uniglumis* was found.
1997	R.A. Fell and Dr B.A. Whitton raise concern over moribund *Chara*

APPENDIX 5

Hell Kettles Flora survey

Table I shows the plant species list for the Hell Kettles ecosystem from 1777 to 2000 and is an integration of Wheeler & Whitton (1971) table with the latest surveys. The geographical limits of some of the early records are vague, so pre-1925 records are restricted to those recognised by Nicholson. Names in parentheses are synonyms used by Nicholson or previous to 1996 authors. Species in bold were found in 1996 for the first time. Species represented by blue font were found in 2000 for the first time.

A. Species gone before 1925

B. Species gone between 1925 and 1970

C. Species found in 1970, but not in Nicholson's list

D. New records found in fen area in 1970 survey

E. New records found in association with trees in 1970 survey

F. New records found on bare mud in 1970 survey

G. New records frequently found in marshes in 1970 survey

H. Species recorded in 1996

I. Species recorded in 2000 (June 2000)

A number of people helped with identification of specific species of the June 2000 floristic survey and these include; P. Bond, P. Gates, K. Gray and D.I. Griss.

Table I Plant species list for the Hell Kettles region from 1777 to 2000.

Species	A	B	C	D	E	F	G	H	I
CHAROPHYTA									
Chara hispida								+	+
Chara vulgaris									
Nitella sp.								+	
LICHENS									
Lecanora conizaeoides Nyl. Ex. Cromb			+		+				
Lepraria incana (L.) Ach.			+		+				
Physcia adscendens (Th.Fr.) Oliv. Em. Bitt.			+		+				
LIVERWORTS									
Lophocolea bidentata (L.) Dum.									
Marchantia polymorpha L.									
Pellia endiviifolia Licks. (=P. fabbroniana)									
Ricciocarpus natans (L.) Corda		+							
MOSSES									

Species	A	B	C	D	E	F	G	H	I
Amblystegium serpens (Hedw.) B., S & G			+				+		
Amblystegium juratzkanun Schp.			+				+		
Barbula tophacea (Brid.) Mitt.			+			+			
Brachythecium rivulare B., S & G			+	+				+	
Brachythecium rutabulum (Hedw.) B., S & G			+				+		
Calliergon cuspidatum Sull								+	
Climacium dendroides Hedw.								+	
Cratoneuron filicinum (Hedw.) Spruce			+				+	+	
Drepanocladus aduncus (Hedw.) Warnst			+				+		
Eurhynchium confertum (Dicks) Milde			+		+				
Eurhynchium praelongum (Hedw.) Holk			+	+	+				
Hypnum cupressiforme (Hedw.)			+		+				
Leptodictyum riparium (Hedw.) Warnst			+				+		
Mnium affine Bland. Var. elatum		+							
Mnium longirostrum Brid.			+				+		
Mnium punctatum Hedw.			+	+					
Physcomitrium pyriforme (Hedw.) Brid.			+			+			
Plagiomnium sp Kop.								+	
Pohlia nutans (Hedw.) Lindb.			+		+				
Rhizomnium punctatum (Hedw.)								+	
Rhytidiadelphus squarrosus (Hedw.)								+	
Tortula laevipila (Brid.) Schwaegr.			+		+				
PTERIDOPHYTES									
Equisetum arvence L.								+	
Equisetum fuviatile L. (=E. limosum L.)		+							
Equisetum palustre L.								+	
FLOWERING PLANTS									
Achillea ptarmica L.			+		+				+
Achillea millefolium L.								+	+
Agrostis capillaris L.								+	
Agrostis stolonifera L.			+	+			+	+	+
Alisma plantago-aquatica L.									
Alnus glutinosa (L.) Gaertn. (=A. rotundfolia Stokes)									
Alopecurus geniculatus L.								+	+
Anacamptis pyramidalis L.									+
Angelika sylvestris L.			+						+
Anthoxanthum odoratum L.								+	+
Apium nodiflorum L.								+	
Arabidopsis thaliana L.									+
Bellis perennis L.			+	+				+	+
Berula erecta (Huds.) Coville (=Sium erectum Huds.)								+	
Blysmus compressus (L.) Link.								+	
Briza media L.		+						+	

Species	A	B	C	D	E	F	G	H	I
Briza minor L.									+
Bromus hordeaceus L								+	+
Caltha palustris L. sp. palustris (=C. palustris L.)								+	+
Capsella bursa-pastoris (L.) Medic.		+			+				
Cardamine pratensis L.								+	+
Carex acutiformis Ehrh. (=C. paludosa Good.)								+	
Carex caryophyllea Latour								+	+
Carex disticha Huds.			+	+				+	+
Carex elata All. (=C. stricta Good)	+								
Carex hirta L.								+	
Carex muricata L.		?							
Carex nigra (L.) Reichard			+	+				+	+
Carex otrubae Podp (=C. vulpina L.)								+	
Carex ovalis Gooden			+	+					
Carex panicea L.								+	
Centaurea nigra L.								+	+
Cerastium fontanum Baumg (C. holosteoides Fries)			+	+				+	+
Cirsium arvense (L.) Scop.			+	+	+			+	+
Cirsium palustre (L.) Scop.								+	+
Chenopodium rubrum L.			+			+			
Cladium mariscus (L.) Pohl.								+	+
Conopodium majus Gouan									+
Crataegus monogyna Jacq.			+		+				
Cynosurus cristatus L.			+	+				+	+
Dactylis glomerata L.								+	+
Dactylorhiza fuchsii (Druce) Dactylorchis fuchsii (Druce) Vermeulen)		?							
Deschampsia cespitosa (L.) Beauv. (=Aira caespitose L.)								+	+
Eleocharis palustris (L.) Roem. & Schultes									
Eleocharis uniglumis (Link) Schultes								+	
Elodea canadensis Michaux.			+					+	
Epilobium hirsutum L.								+	+
Epilobium palustre L.									
Epilobium parviflorum Schreber								+	+
Eupatorium cannabinum L.		+							
Festuca arundinacea Schreb.			+	+					
Festuca pratensis Hudson								+	
Festuca rubra L.			+				+	+	+
Festulolium loliaceum Hudson								+	
Filipendula ulmaria L.									+
Galium aparine L.								+	+
Galium elongatum C. Presl								+	
Galium palustre L.								+	+
Galium uliginosum L.		+							

Species	A	B	C	D	E	F	G	H	I
Gallium verum L.								+	
Glechoma hederacea L.			+	+					
Glyceria notata Chevall. (G. plicata Fr.)			+				+		
Glyceria fluitans (L.) R. Br.								+	
Hedera helix L.			+		+				
Hippuris vulgaris L.								+	+
Holcus lanatus L.			+	+			+	+	+
Hypericum elodes L.									+
Hypericum tetrapterum Fr.									
Hypochaeris radicata L.								+	+
Iris pseudacorus L.		+							
Juncus articulatus L. (=J. lamprocarpus Ehrh.)								+	+
Juncus bufonius L.									
Juncus effusus L.								+	+
Juncus inflexus L. (=J. glaucus Bibth.)								+	+
Juncus subnodulosus Schrank. (=J. obtusiflorus Hoffm.)								+	
Lathyrus pratensis L.								+	
Lemna minor L.									
Lemna trisulca L.								+	
Leontodon autumnalis L.								+	+
Leontodon hispidus L.								+	
Lolium perenne L.			+	+				+	+
Lotus corniculatus L.								+	
Lotus pedunculatus Cav. (=L. uliginosus Schkuhr)								+	+
Lychnis flos-cuculi L.								+	+
Lythrum salicaria L.	+								
Luzula sylvatica									+
Matricaria recutita L.			+			+			+
Mentha aquatica L.								+	+
Mentha arvensis L.									+
Mentha x verticillata L.									
Myosotis laxa Lehm. (M. caespitosa K. F. Schultz)									
Myosotis scorpioides L.								+	+
Nymphaea alba L.								+	
Oenanthe fistulosa L.								+	
Parnassia palustris L.		+							
Pedicularis palustris L.			+						
Persicaria amphibia L. (Polygonum amphibium L.)								+	+
Persicaria lapathifolia L. (Polygonum lapathifolium L.)			+			+			
Persicaria maculosa Gray (Polygonum persicaria L.)			+			+			
Phleum pratense L.			+	+					+
Phragmites australis (Cav.) Trin. Ex Steudel (P. communis Trin.)								+	+

Species	A	B	C	D	E	F	G	H	I
Plantago lanceolata L.		+			+			+	+
Plantago media L.			+	+	+				+
Poa annua L.			+	+					
Poa pratensis L.			+	+				+	
Poa trivialis L.			+	+				+	
Polygonum aviculare L.			+			+			+
Populus canescens (Ait.) Sm.			+		+				
Potamogeton coloratus Hornem		+							
Potamogeton pectinatus L.									+
Potamogeton pusillus L.		+							
Potentilla anserina L.			+	+				+	+
Potentilla reptans L.									+
Prunella vulgaris L.			+	+				+	+
Ranunculus acris L.			+	+				+	+
Ranunculus aquatilis L.		+							
Ranunculus bulbosus L.			+	+				+	+
Ranunculus lingua L.	+								
Ranunculus repens L.			+	+				+	+
Ranunculus sceleratus L.								+	+
Rorippa islandica (Oeder) Borbas			+			+			
Rorippa microphylla (Boenn) Hyland. (=Radicula nastrurtium L.)									
Rorippa nasturtium-aquaticum L. Hayek (Nasturtium officinale R. Br.)								+	
Rubus fruticosus L. agg.			+		+				
Rumex acetosa L.								+	+
Rumex conglomeratus Murr.			+				+	+	+
Rumex crispus L.			+	+					+
Rumex obtusifolius L.									+
Sanguisorba minor Scop.(Poterium sanguisorba L.)			+	+					
Schoenoplectus lacustris (L.) Palla (=Scirpus lacustris L)									+
Schoenoplectus tabernaemontani (C.C. Gmell) Calla (=Scirpus tabernaemontani C.C. Gmell)									
Scirpus lacustris L.								+	
Scrophularia auriculata L. (S. aquatica L.)									
Scrophularia nodosa L.									+
Scutellaria galericulata L.									
Senecio jacobaea L			+			+		+	+
Senecio vulgaris L.			+			+			
Solanum dulcamara L.		+							
Sparganium erectum L. (=S. ramosum Huds.)									+
Stachys officinalis								+	
Stellaria uliginosa Murray (S. alsine Grimm.)								+	
Stellaria graminea L.		+							
Stellaria media (L.) Vill.			+	+		+			+

Species	A	B	C	D	E	F	G	H	I
Stellaria palustris Retz.		+							
Succisa pratensis Moench.(=Scabiosa succisa L.)								+	
Taraxacum sect. Ruderalia Kirschner (T. officinale Wigg. Group)			+	+				+	+
Thalictrum flavum L.		+							
Trifolium pratense L.			+	+				+	+
Trifolium repens L.			+	+				+	+
Triglochin palustre L.								+	+
Trollius europaeus L.	+								
Tussilago farfara L.			+			+			
Urtica dioica L.			+			+		+	
Utricularia vulgaris L.		+							+
Valeriana officinalis L.									
Veronica beccabunga L.								+	+
Veronica scutellata L.		+							+
Viburnum lantana L.	+								
Vicia cracca L.			+		+				+