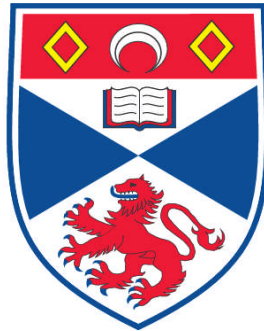# BENTHIC-PELAGIC NUTRIENT CYCLING IN SHALLOW LAKES : INVESTIGATING THE FUNCTIONAL ROLE OF BENTHIC MICROALGAE

Bryan M. Spears

A Thesis Submitted for the Degree of PhD
at the
University of St. Andrews

2007

**Full metadata for this item is available in
Research@StAndrews:FullText
at:
http://research-repository.st-andrews.ac.uk/**

**Please use this identifier to cite or link to this item:
http://hdl.handle.net/10023/371**

BENTHIC/PELAGIC NUTRIENT CYCLING IN SHALLOW

LAKES: INVESTIGATING THE FUNCTIONAL ROLE OF

BENTHIC MICROALGAE

By

Bryan Millar Spears

THESIS SUBMITTED IN PARTIAL

FULFILLMENT OF THE REQUIREMENTS FOR

THE DEGREE OF

DOCTOR OF PHILOSOPHY

School of Biology

University of St Andrews

March 2007

I, Bryan Millar Spears, hereby certify that this thesis, which is approximately 41,000 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

date   signature of candidate

I was admitted as a research student in September, 2003 and as a candidate for the degree of Doctor of Philosophy in September 2004, the higher study for which this is a record was carried out in the University of St Andrews between 2003 and 2007.

date   signature of candidate

I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the degree of Doctor of Philosophy in the University of St Andrews and that the candidate is qualified to submit this thesis in application for that degree.

date   signature of supervisor

In submitting this thesis to the University of St Andrews I understand that I am giving permission for it to be made available for use in accordance with the regulations of the University Library for the time being in force, subject to any copyright vested in the work not being affected thereby. I also understand that the title and abstract will be published, and that a copy of the work may be made and supplied to any bona fide library or research worker.

date   signature of candidate

ACKNOWLEDGEMENTS

Table of contents

ABSTRACT

Microbes, living on the boundary between the sediment and the water-column in lakes, can play a pivotal role in governing the magnitude and frequency of nutrient cycling. The purpose of this research was to focus on the role of benthic microalgae in regulating such processes and to identify spatial and temporal characteristics in their function.

Approaches included the quantification of sediment nutrient concentrations (particularly P fractionation), estimates of equilibrium phosphate concentrations (EPC0) (resuspended and undisturbed sediment estimates), and assessment of the benthic microalgal community composition, biostabilisation capacity, and its ability to regulate diffusive-nutrient flux.

This thesis highlighted the importance of biological regulation of benthic/pelagic nutrient cycling, especially the role of benthic microautotrophs. Release sensitive sediment-P fractions were observed to be highly variable (both with depth and season) and correlated well with indicators of benthic photosynthesis (e.g. DO, chlorophyll, pH). Understanding the seasonality of whole-system P partitioning can enhance future lake management programmes. EPC0 estimates were significantly higher during undisturbed as opposed to disturbed sediment conditions. Epipelon constituted < 17 % of the total sediment chlorophyll signal and was highest in the clearer winter months and at intermediate depths at which a trade off between wind-induced habitat disturbance and light limitation existed. In intact core experiments, the benthic microalgal community significantly reduced the diffusive nutrient (especially PO_4-P and SiO_2) flux. NH_4-N release was highest under light conditions at high temperatures. The mechanisms for regulation included direct uptake, photosynthetic oxygenation of the sediment surface, and regulation of nitrification/denitrification processes. Sediment

stability increased with colloidal carbohydrate concentration (extruded by benthic microbes) at 4.1 m water-depth but not at 2.1 m overlying water depth, probably indicating the role of habitat disturbance in shallow areas acting to reduce epipelic production. Additionally, in an ecosystem comparison, the nature and extent of the biotic mediation of sediment stability varied between freshwater and estuarine ecosystems.

CHAPTER 1

Introduction

Benthic algae (algae that live on or in association with substrata) are an evolutionarily diverse group of photoautotrophic organisms (prokaryotic cyanobacteria and eukaryotic algae) responsible for the performance of many important functions within aquatic ecosystems. These "ecosystem functions" ("a measurable process occurring within the system of interest"; Paterson, 2005) include primary production (Liboriussen and Jeppesen, 2003), carbon transfer through the food-web (Hillebrand, 2005), biostabilisation of sediments (Yallop *et al.,* 2000), and regulation of benthic/pelagic nutrient cycling (Woodruff *et al.,* 1999). However, much of the research that has identified such functioning has been conducted within estuarine systems with freshwater environments, shallow lakes in particular, receiving relatively little attention.

The study of biotic ecosystem functioning is important in both conceptual and practical ecology. Of growing concern are the effects of ecosystem stress and manipulation (e.g. climate change, eutrophication, catchment management) on the alteration of system ecology and related ecosystem functioning. These alterations can have drastic effects. For example, the steady state hypothesis in lakes in which the contribution of whole system primary production switches between the benthos (macrophytes) and the water-column (phytoplankton) depending on external nutrient loading from the catchment with alternative stable states (i.e. benthic algal dominated states) occurring as a result of a disturbance driven alteration in equilibrium (Scheffer, 1998). Another is the potential role of benthic autotrophs in regulating nutrient transport (ecosystem connectivity; (Heathwaite *et al.,* 2005)) through the catchment. These theories represent two of the strongest forces currently driving limnological research. The research has, however, focused more on macrophytes, ignoring other benthic

autotroph communities resulting in a relative lack of knowledge relating to benthic algae and their ecosystem functions. This is compounded by the fact that estuarine ecologists have long considered benthic microalgal communities (referred to commonly as microphytobenthos in marine studies) to perform pivotal ecosystem functions within mudflat sediment ecosystems (Paterson 1994, 1997; Paterson and Black, 1999). This introduction will outline key principles in studying the ecosystem functions of benthic algae in terms of the regulation of benthic/pelagic nutrient cycling in shallow lakes and outline the key aims of the project.

Benthic/pelagic nutrient cycling in shallow lakes

Eutrophication is defined as 'The enrichment of water by nutrients, especially compounds of nitrogen and/or phosphorus, causing an accelerated growth of algae and higher forms of plant life to produce an undesirable disturbance to the water balance of organisms present in the water and to the quality of the water concerned' (cf. Art. 2(11) of the Directive 91/271/EEC). Eutrophication is characterised by a number of undesirable water quality characteristics including potentially toxic blue-green algal blooms, decreased transparency and loss of submerged aquatic vegetation, increased settling rates of organic matter, decreased dissolved oxygen content which can lead to fish kills, retarded internal nutrient fluxes, and severely altered food webs (Boström *et al.,* 1988; Bailey-Watts and Kirika, 1993; House *et al.,* 1998; Meijer *et al.,* 1999). Early studies (e.g. Vollenweider, 1968) indicated phosphorus (P), and to a lesser extent nitrogen (N), enrichment to be the main cause of eutrophication and the majority of restoration projects have focussed on reducing the P concentration in the water-column through a number of mechanisms (Gachter and Wehrli; 1998; Perkins *et al.,* 2001, 2002; Moss *et al.,* 2002). It should be noted however that biomanipulation projects, of

the type discussed by Meijer and Hosper (1997) and Meijer *et al.* (1997) have also been conducted to minimise the symptoms of eutrophication (e.g. reduce algal blooms). Attempts to "restore" eutrophic lakes have been carried out with varying success with shallow lakes in particular showing long-term resistance to restorative measures (Bostrom *et al.,* 1988; Jenson *et al.,* 1992; Anderson and Ring, 1999; Hakanson *et al.,* 2003; Søndergaard *et al.,* 2003). The main factor delaying restoration is often the prolonged release of sediment-stored P to the overlying water-column, known as *internal loading*. The homogenous nature of the entire water-column in shallow lakes makes them extremely sensitive to internal loading where any nutrients released across the sediment-water interface may become immediately available to planktonic sequestration. A complex mosaic of biogeochemical processes, all of which may be altered by lake restoration practices, govern the movement of nutrients across the sediment-water interface. Early studies largely concerned the importance of physicochemical processes (e.g. redox conditions (Mortimer, 1971) and wind mixing (Vollenweider, 1968)). Recent studies, however, have indicated that biological mechanisms are also important in nutrient regulation. For example, bioturbation may act to increase sediment resuspension, closely linked to increased release of P (Horpilla and Kairesalo, 1990; Havens, 1991; Ogilvie and Mitchell, 1998). Benthic biofilm (autotrophic and heterotrophic communities living on/within the surface of a substrate) communities may also be of importance, having the ability not only to sequester bioavailable nutrients directly, but also to metabolically alter their chemical form and mobility (Jones, 1971; De Montigny and Prairie, 1993; Banta *et al.,* 1995; Wetzel, 2001). Additionally, the presence of a biofilm on a sediment surface may act as a stabilising agent (through intrinsic structural properties) or to increase the likelihood of disturbance through predator attraction (Patterson and Black, 1999; Yallop *et al.,* 2000).

Benthic autotrophic biofilms may also play a key role in regulating redox conditions at the sediment surface. For many reasons, therefore, benthic, particularly autotrophic, biofilms play an important role in governing internal nutrient loading in shallow lakes where conditions at the sediment surface support high biofilm production (high organic matter, flux of nutrients from sediments, and light regime to support photosynthesis; Carlton and Wetzel, 1988; Frost and Elser, 2002).

The task of identifying the role of biofilms in nutrient cycling in lakes is extremely complex. This complexity is compounded when one considers the make up of a biofilm in shallow lakes. For example, a biofilm may contain many functional groups of algae, bacteria and animals capable of altering the flux of nutrients across the sediment-water interface. An understanding of biofilm function is, therefore, clearly of great value when trying to enhance lake recovery.

Phosphorus fractions in the sediment

It is important to establish which forms of P are bioavailable and of particular importance to sediment chemistry and the conditions under which refractory forms of P are likely to become transformed to a bioavailable form. Bioavailable P in the water-column is generally considered to be soluble reactive phosphorus (SRP). However, quantifying SRP alone may not give an accurate estimate of the productivity of a system, since P may be present in a number of different forms (House *et al.,* 1998, 2003). In order to accurately assess the productivity of a water-body Vollenweider (1968) suggested using total phosphorus (TP) concentration as an index to include inorganic and organic P in both the dissolved and particulate forms. This technique is relatively robust in open water studies due to the rapid turnover of nutrients through the plankton, although a similar scenario cannot be applied to the sediment.

Wetzel (2001) suggested five possible pathways of P deposition to lake sediments:

1. The sedimentation of phosphorus minerals imported directly from the drainage basin (mineral complexes).

2. The sedimentation of phosphorus contained within allochthonous organic matter (e.g. external detritus, aquatic and terrestrial plants and animals).

3. The sedimentation of phosphorus contained within autochthonous organic matter (e.g. internal detritus, aquatic plants and animals).

4. Translocation of P in the water-column to the sediments by attached algal and microbial communities.

5. Adsorption and precipitation of phosphorus through one of three mechanisms;

    a. Coprecipitation within iron and manganese mineral complexes.

    b. Adsorption to amorphous materials (e.g. clays and oxyhydroxides).

    c. Association with carbonates.

Lake restoration attempts have generally concentrated on the reduction of allochthonous sources of P, however successful recovery is greatly dependant on the assumption that external sources have direct effects (i.e. are bioavailable on entry). Unfortunately, in many restoration projects this is not the case, with P concentrations remaining elevated for many years post-restoration (Farmer *et al.,* 1994, Reynolds, 1996, Moss *et al.,* 2002, and Søndergaard *et al.,* 2003). This phenomenon suggests that much of the allochthonous P may not be bioavailable (i.e. dissolved inorganic in form) when entering a lake but may be stored in the sediment until conditions are such that transformation of unavailable P can occur. Such mechanisms are many and may act in concert to produce a highly dynamic sediment-water interface extremely sensitive to environmental fluctuations.

It has proven increasingly difficult to quantify the flux of each depositional pathway in nature, however, laboratory studies have resulted in the development of chemical extraction techniques capable of fractionating sediment-P into a variety of operational classifications. These classifications vary in complexity, depending on the author (e.g. Perkins *et al.,* 2001; Friedrich *et al.,* 2002), though the techniques suggested by Psenner *et al.* (1988) have been the basis for many recent studies. Such techniques enable observations of spatial and temporal variations in fractionated sediment-P and have evolved out of a necessity to study nutrient mobility. In general, the common forms of sediment-P considered in most studies are derived, in some way, from the pathways discussed above and include:

1. *Labile P* (e.g. PO_4-P)

Labile/loosely bound P represents an operational fraction that may be considered immediately available (or mobile). This fraction includes easily mobilised adsorbed P and P contained in pore water. Labile P may be removed from sediment by extraction with NH_4Cl. This extraction step also removes $CaCO_3$-P resulting in the dissolution of $CaCO_3$. The addition of NH_4Cl may also dissolve some iron- and manganese-bound P.

2. *Reductant-soluble P* (e.g. $FePO_4$, $Fe_3(PO_4)_2 8H_2O$)

This represents the operational P fraction that may become bioavailable under conditions of anoxia. Reductant-soluble P represents the operational fraction bound mainly to iron hydroxides. It is extractable with buffered dithionite that also promotes the extraction of mainly iron oxides and may therefore differentiate between iron-P and P bound to other metal complexes.

3.   *P adsorbed to metal oxides* (e.g. Al OH_3 (ads.), Al PO_4)

P adsorbed to metal oxides may be extracted with NaOH and is therefore also known as the NaOH-reactive phosphorus fraction (NaOH-rP). NaOH-unreactive phosphorus (NaOH-nrp) represents the organic fraction and may be quantified by the difference between TP of the extract and SRP of the extract. P bound to AlO_3 is considered to be refractory.

4.   *Apatite-bound P* (e.g. Calcite (ads.))

This fraction represents carbonate bound P and P released through the dissolution of oxides. Extraction in HCl is generally used in isolating this fraction. Calcium-bound P is generally considered refractory.

5.   *Residual P* (refractory)

Residual P contains refractory compounds and is persulfate digestible.

Most studies concerned with the mobility and bioavailability of sediment-P have tried to quantify some of the operational fractions discussed above (See table 1.1). Such studies have highlighted the fact that mobility and bioavailability are governed not only by the chemical association between P and specific sediment types, but also by a suite of dynamic physical and biogeochemical processes. Such complexity in control has resulted in a variety of sediment-P fraction signatures specific to a given water body (Table 1.1). By examining each P "fingerprint" one can make inferences concerning the sediment nutrient status. For example, high sediment TP concentration (e.g. Lake Sodra Bergundasjon and Lake Windermere 6.5 and 4.0 mg g^{-1}dw respectively) indicates a high sediment-P retention capacity. However, for Lake Sodra Bergundasjon and Lake Windermere the main P fraction is reductant-P (4.0 and 3.0 mg g^{-1}dw respectively).

Thus, significant amounts of sediment-P may be released under conditions of anoxia in these lakes. Similar inferences can be made with regards to the sorption capacity of sediments. For example, when levels of labile-P are high (e.g. 0.163 mg g^{-1}dw in Lake Kaiavere), relative to sediment-TP, we may expect near saturation of the sorption sites in the sediments. Similarly, when levels of apatite-bound P are high, the sediments must contain soils of a calcareous nature. Although the dominant form of sediment-P is difficult to predict, the labile-P fraction is most commonly the smallest fraction.

Other nutrients in the sediment

The benthic/pelagic cycling of other plant nutrients is also important, however, few studies have considered biotic regulation of a suite of nutrients at the same time. Of particular interest are the relative effects of benthic microalgae on the release of soluble reactive silica (SiO_2) and nitrogenous compounds (e.g. nitrate and ammonium).

The cycling of N through the sediment is complex and is mainly reliant on microbial remineralisation of organic matter and subsequent nitrification/denitrification reactions. The extent to which the nitrification/denitrification balance is altered is governed mainly by the dissolved oxygen concentration where nitrification (oxidation of NH_4-N to NO_3-N) occurs under well-oxygenated conditions and denitrification (the reduction of NO_3-N to N_2 gas) under anoxic conditions (Jansson, 1980; Golterman, 2004). As both processes are governed by temperature regulated microbial processes, similar seasonal trends to that of P may be expected for N where NH_4-N and PO_4-P concentrations should be high, and NO_3-N low, during periods of high temperature (i.e. in the summer), microbial metabolism and low dissolved oxygen concentration. However, in winter when temperatures are typically low and dissolved oxygen concentrations high, PO_4-P and NH_4-N concentrations should be low and NO_3-N high.

Table 1.1. *Compilation of fractionated sediment-P chemistry as quantified using methods described by Psenner et al. (1988). * indicates combination of reductant-P and metal oxide adsorbed-P in same fraction. NA indicates fraction not analysed. Shaded boxes show dominant operational P form. L: Lake.*

Lake	Operational P fraction in sediment (mg PO$_4$-P g^{-1}dw)						Reference
	Labile	Reductant-soluble	Metal oxide-adsorbed	Apatite-bound	Residual	Total P	
Eshwaite Water	0.029	2.124	1.846	0.137	0.692	4.828	Spears, 2006. unp. data
L. Karhijarvi	0.060	0.500	0.100	0.336	0.400	1.400	Krogerus & Ekholm, 2003
L. Leven	0.037	0.564	0.408	0.497	0.363	2.288	Present study, 2004
L. Vallentunasjon	0.137	*	0.257	0.315	1.100	1.81	Boström *et al.,* 1988
L. S. Bergundasjon	0.253	*	4.017	0.707	1.519	6.49	Boström *et al.,* 1988
L. Ersken	0.015	*	0.137	0.461	0.617	1.23	Boström *et al.,* 1988
L. St. Hastevatten	0.010	*	0.060	0.019	0.861	0.95	Boström *et al.,* 1988
Alton Water	0.08	*	0.46	0.46	N/A	N/A	Perkins & Underwood, 2001
Coniston Water	0	*	1.0	0.3	1.5	2.8	Davies, 1996
Lake Winderemere	0.01	*	3.0	0.5	0.5	4.01	Davies, 1996
Bassenthwaite	0.03	*	1.1	0.25	0.75	2.13	Davies, 1996
Malham Tarn	0.05	*	0.45	0.1	0.4	1.00	Davies, 1996
White Mere	0.1	*	0.65	0.2	0.9	1.85	Davies, 1996
L. Vortsjarv	0.070-0.200	*	0.060-0.727	0.214-0.462	0.3-0.42	0.9-1.4	Noges & Kisand, 1999
L. Arreskov	0.032-0.033	0.112-0.149	0.036-0.040	0.107-0.155	0.262-0.271	0.600-0.135	Anderson & Ring, 1999
L. Prossa	0.06-0.075	0.06-0.075	0.405	0.206	NA	0.737	Kisand & Noges, 2003
L. Kaiavere	0.163	0.063-0.08	0.395	0.063-0.08	NA	0.697	Kisand & Noges, 2003

SiO_2 remineralisation is also dependent on temperature (Rippey, 1983). However, the main flux of silicon into the sediment in eutrophic lakes is contained within settling phytoplankonic diatom frustules and so sediment SiO_2 release can show great seasonality (Bailey-Watts, 1976a and b; Gibson *et al.,* 2000). Particulate sediment Si concentrations should be high in late spring/early summer following the "fall-out" of the spring diatom bloom from the water-column. This store of particulate Si will be remineralised, via microbial processes and dissolution of diatom frustules, to SiO_2 (dissolved form). These processes will again be temperature dependent with release rates increasing (during late summer) as concentration gradients across the sediment-water interface increase.

Benthic algal categorisation

Benthic algae in freshwater habitats (e.g. lakes, rivers, wetlands, and streams) are mainly dominated by cyanobacteria, green algae, diatoms and red algae. In addition, resting stages and settled cells (still capable of photosynthesis) of many planktonic algae can be found in the benthos (Sicko-Goad e*t al*., 1989; Belmonte *et al.,* 1997). Even though these groups have great evolutionary, genetic, and chemical variation, they share many of the same growth forms which are generally found for unicellular, colonial or filamentous existences (Stevenson *et al.,* 1996). The term "periphyton" (Wetzel and Westlake, 1969) has been commonly used in the literature to include all organisms (heterotrophs and autotrophs) growing in association with submerged substrata. Periphyton consists of a large number of organismal groups separable on consideration of their life traits and preferred substrata. When assessing benthic algal ecosystem functioning it is important to accurately describe the microalgal groups present at a level that allows attribution of a given function. A summary of the

definitions between each group is provided in an attempt to clarify terminology used in this thesis (Figure 1.1).

Periphyton can be categorised in to two key groups depending on type of substrata; haptobenthos (associated/attached with a solid substratum) and herpobenthos (associated/moving on a mobile particulate sediment). Haptobenthos can be further split into epiphyton (growing on other plants), epilithon (growing on inert rock), epipsammon (growing on sand grains) and epizoon (growing on animals). Endolithon lives within rocks (some of which can bore into the substratum), epilithon colonises the surface of rocks, and endozoon and endophyton live within other organisms (animals and plants, respectively). The herpobenthos (Margalef, 1960) is the community living on, or moving through, sediments and is subdivided into epipelon (living and moving on the sediment surface; Round, 1956), endopelon (living within the sediment) and endopsammon (living within sandy sediment). Since the surface sediments are always being moved by water currents, and especially by animals, these communities occupy the sediment to a variable, but generally fairly shallow, sediment depth.

Some organisms can exist within the benthos and the water-column depending on a number of factors including life histories and susceptibility to resuspension. They can be split into meroplankton, tychoplankton, and metaphyton. Meroplankton can graduate between the water-column and sediment depending on life stages. For example *Gleotrichia spp.* (Cyanobacteria) is known to perform buoyancy regulation to satisfy light (to the water-column) and nutrient (to the sediment) requirements (Head *et al.,* 1999). Tychoplankton are those organisms that are circumstantially (i.e. by forces outwith their control) distributed between the sediment and the water-column (Lincoln *et al.,* 1998). These may include all phytoplankton incapable of motility or regulating their buoyancy and, therefore, are susceptible to settling onto the sediment during calm

periods and resuspension into the water-column during turbulent periods. Metaphyton (Behre, 1956), clouds of filamentous algae (e.g. *Spirogyra, Mougeotia*, or *Zygnema* (Stevenson *et al.,* 1996)) loosely associated with the littoral substrata or with aquatic macrophytes but lacking any attachment mechanisms, are also considered tychoplanktonic although little attention has been paid to this group in the literature.
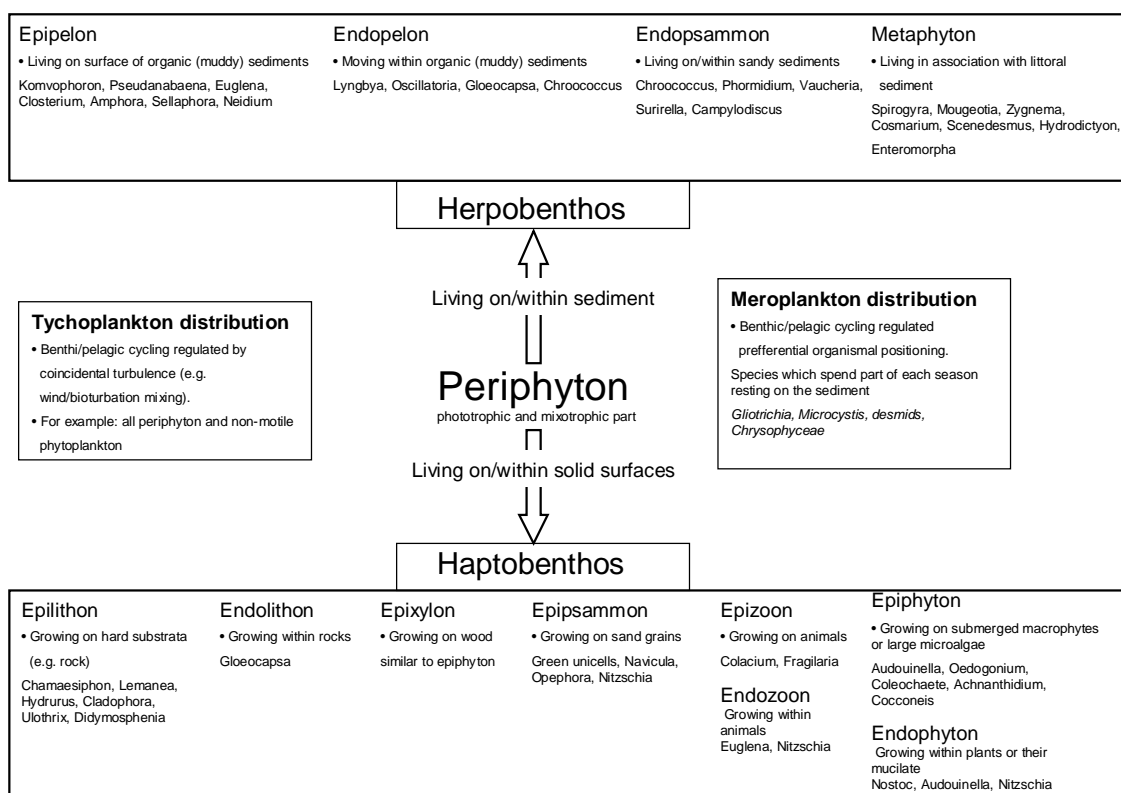
Figure 1.1. *Conceptual diagram of periphyton (all benthic microalgae) groups differentiated by association with habitat.*

Many of the above groups form resting stages (epipelic resting stages discussed later) which further complicates separation by habitat preference and adds to the complexity of the taxonomic requirements. As resting stages can remain viable for long periods of time (from years to centuries (Hariston *et al.,* 1995)) they constitute a viable "seed bank". However, the ecological role of resting stages is very rarely considered in freshwater benthic algal studies, although they may contribute to the photosynthetic

pigment signal and most definitely have an ecological role, as has been shown in marine systems (McQuoid and Hobson, 1995 & 1996).

It should be noted that benthic communities in nature are not composed exclusively of one particular group (Figure 1.1) but instead are likely to be composed of a number of groups (e.g. Figure 1.2). The level of exclusivity will depend greatly on the homogeneity of the habitat substratum and the frequency of disturbance events. It is therefore important to consider community composition when attempting to apportion cause and effect to specific periphytic algal groups. This study addresses the role of all viable autotrophs in the sediment of shallow lakes of which the epipelon are supposedly the major group.

Microalgal regulation of benthic/pelagic nutrient cycling

Epipelon have been shown to uptake nutrients (especially P) from the water-column (Havens *et al.,* 1999). Additionally, Jarvie *et al.* (2002) observed uptake of water-column P into algal biofilms in a lowland chalk river where uptake was highest under low-flow conditions. The ecological significance of water-column uptake by epipelon varies depending on the system under consideration. For example, the effects in eutrophic lakes are likely to be negligible because of the high water-column concentrations. However, in oligotrophic lakes, competition between epipelon and phytoplankton may result in a lower overall phytoplankton biomass and in streams, will act to prolong the transfer of nutrients down the system.

The epipelon may also play a major role in regulating benthic/pelagic nutrient cycling. In oligotrophic lakes and in eutrophic lakes recovering from eutrophication following a reduction in external nutrient load, nutrient cycling through the sediment may be a major source of nutrients to the water-column (Sas, 1989). Any sediment

nutrient release is subject to a number of processes regulated by the epipelon including the buffering of physicochemical nutrient release via direct sequestration of nutrients (either from water-column or sediment; Woodruff *et al.,* 1999; Jarvie *et al.,* 2002), a reduction in hypoxic nutrient release (i.e. redox sensitive P complexes and denitrification/nitrification processes; Dong *et al.,* 2000) via the maintenance of oxygenated surface sediment, and a decrease in resuspension related sediment nutrient release via increased sediment stability through the production of extracellular polymeric substances (EPS illustrated in Figure 2; Yallop *et al.,* 2000). However, it is extremely difficult to quantitatively separate these processes in nature as they occur on very small scales and are therefore extremely difficult to quantify. Some exceptions include the work of Woodruff *et al.* (1999) in which sediment silica release was observed to be limited in the presence of epipelic diatoms (most likely via direct uptake) and P was concentrated (from the sediment and water-column) via a combination of direct nutrient sequestration and oxic coprecipitation of P with Fe (III), into the surface sediment.

Sediment resuspension events may result in a relatively high nutrient release through the disruption of chemical gradients. Littoral zones in lakes are susceptible to wind-induced sediment disturbance with bioturbation also resulting in re-working of sediments throughout the lake bed. Recent work within estuarine mudflat ecosystems has highlighted the role of motile benthic diatoms in increasing sediment stability through the maintenance of an EPS (produced during motility) matrix in the upper microns of the sediment surface (Paterson, 1994; Yallop *et al.,* 2000; Deco, 2000). Few studies have assessed the role of EPS production in regulating sediment stability in freshwater ecosystems though this process may be important in littoral habitats where susceptibility to disturbance and epipelic production are high. One notable exception is

the work carried out by Cyr and Morton (2006) in which carbohydrate concentration in Canadian Shield lakes was positively correlated with sediment chlorophyll concentration. This is in agreement with much of the estuarine mudflat work (Underwood and Smith, 1998). The role of epipelic biodiversity may be critical in the biostabilisation of freshwater sediments. For example, epipelic diatoms may reduce sensitivity to erosion via biochemical processes (i.e. production of EPS) whereas mat forming cyanobacteria (e.g. *Oscillatoria sp.*) may reduce sensitivity to erosion via the maintenance of physical barriers (i.e. the mat itself; Dodds, 2003). Further investigation is required to assess the relative roles of different epipelic functional groups.
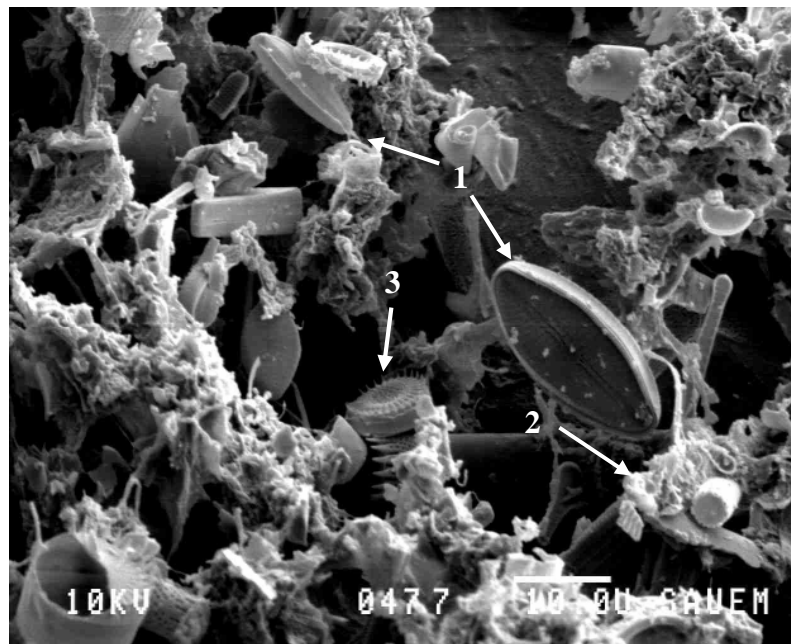
Figure 1.2. Low-temperature *scanning electron micrograph of a benthic microalgal community. Epipelic diatoms (1), extracellular polymeric substances (stringy type substance) (2) and phytoplanktonic contamination of the sediment (3) are indicated. Sediment was collected from Loch Leven (Scotland, 21 March 2006) at a depth of 2.3 m by first collecting a sediment core and then freezing the sediment surface using liquid nitrogen in the field. The image was taken using low-temperature scanning electron microscopy of the surface sediment and is courtesy of Irvine Davidson (Sediment Ecology Research Group, University of St Andrews). The scale bar (10 μm) is located in the bottom right hand legend of the image.*

It is evident that the ecosystem processes performed by the epipelon will be governed by community structure and species composition. The importance of this interaction requires more attention. For example, diatoms are the main producers of EPS and therefore may contribute most to enhancing sediment stability. However, with respect to nutrient uptake kinetics (discussed in detail by Borchardt, 1996), species-specific variations have been observed, although the extent to which these variations will regulate the cycling of different nutrients is not known.

Loch Leven: setting the scene

Loch Leven is one of the most extensively studied bodies of water in the world with detailed limnological data sets spanning almost 40 years. It is located in southeast Scotland (latitude $56^o10'$ N, longitude $3^o30'$W) and is placed within a glaciated valley banked by the Ochil and Lomond hills in the northwest, Bishop hill to the east, and Benarty and Cleish hills to the south. The surface area of the lake is some 13.3 km^2 which makes it the 13th largest in Scotland. Loch Leven is uncharacteristically shallow, in comparison to other Scottish lakes, with a mean depth of about 3.9 m. For this reason the major portion of the water body rarely stratifies with intermittent stratification occurring only in two deep basins (about 25 m deep). The importance of the lake is reflected by the massive amount of scientific attention it has received. With studies ranging from plankton identification surveys, at the turn of the century (Bachman, 1906), to pioneering eutrophication and climate change studies in the last few decades (e.g. Bailey-Watts *et al.,* 1991, 1993; Farmer *et al.,* 1994; Carvalho and Kirika, 2003), the lake has long been a site centred on scientific excellence and advancement.

Loch Leven holds great scientific importance, but its use as a recreational site and ecological reserve ensures a strong relationship between the public and their surrounding environment. The lake's ecological importance has been recognised by its designation as a Site of Special Scientific Interest (SSSI; 1956), a National Nature Reserve (NNR in 1964), a Special Protected Area (SPA in 2000) and a Ramsar wetland site of international importance (in 1976). It has also been an internationally-renowned trout fishery for over one hundred years.

The ecology of the lake is tightly coupled to its continued use as a recreational site. Early plankton studies, published at the turn of the century, indicated that Loch Leven's natural status is one of high productivity. It is this intrinsic level of high productivity that threatens the success of Loch Leven as a world-famous recreational and ecological site. The main nutrient limiting phytoplankton productivity is P as is evident from studies concerned with nutrient loading, effects of nutrient dynamics on phytoplankton community, and laboratory-controlled nutrient enrichment experiments (Bailey-Watts, 1978, 1988; Bailey-Watts and Kirika, 1993). In an attempt to reduce the eutrophication effects in Loch Leven, nutrient export studies were conducted which identified the main external sources of P being from a woollen mill and a wastewater sewage treatment plant (Bailey-Watts and Kirika, 1993). Sources of P have since been controlled and were reduced from ~13 tonnes SRP per year in 1985 to ~5 tonnes per year in 1994 (Farmer *et al.,* 1994). However, as is the case in many restoration projects (especially in shallow lakes) the lake has failed to meet recovery expectations. This was due to continued elevated internal loading following a reduction in external nutrient load, a phenomenon common in many recovering shallow lakes (Sas, 1989). Additionally, Loch Leven has a low flushing rate (about 1.88 lake volumes per year)

which restricts the removal of nutrients and has resulted in a prolonged recovery period (Farmer *et al.,* 1994).

Farmer *et al.* (1994) carried out a study to investigate the potential mobility and bioavailability of sediment-bound P in Loch Leven. In 1994 the fractionated sediment-P composition in the upper 1 cm was comprised of 31% reductant-soluble, 27.9% organic, 25.6% metal-oxide adsorbed, 9.9% apatite-bound, 5.1% residual, and 0.5% loosely bound (Farmer *et al.,* 1994).

Objectives of the thesis

The role of benthic microalgae in regulating benthic/pelagic nutrient cycling has received little attention in the literature. Whether this community acts as a consistent source, sink, or regulator of sediment nutrients is as yet untested and the level at which testing is required is at the broad community scale. Although this provides little information with respect to the effects of benthic microalgal biodiversity on regulating their ecosystem functioning, this approach represents a first step at quantifying the processes performed by benthic algae as a broad functional group at the interface between benthic and pelagic ecology. The main scope of this study was to identify the effects of the benthic algal community on benthic/pelagic nutrient cycling, in a shallow eutrophic lake, using a range of biological and chemical techniques. The study was divided into 6 key areas, the hypotheses specifically relating to which are outlined in each chapter:

1) An assessment of the historical variation in sediment phosphorus content following restorative measures in Loch Leven.

2) An investigation into the factors controlling the spatial and seasonal variations

in sediment phosphorus content, with particular focus on the role of benthic microalgae.

3) Examination of the seasonality of sediment phosphorus fluxes.

4) An assessment of the spatial and seasonal variation in benthic microalgal community structure.

5) An assessment of the role of benthic microalgae in regulating sediment diffusive nutrient fluxes (phosphorus, silica, and nitrogen) across gradients of light and temperature.

6) An ecosystem comparison designed to assess the effects of benthic microalgae in regulating sediment stability within freshwater and marine depositional sediment environments.

CHAPTER 2

Spatial and historical variation in sediment phosphorus

fractions and mobility in a large shallow lake

Spears, B.M., Carvalho, L., Perkins, R., Kirika, A., and Paterson, D.M. 2006

Water Research. 40: 383-391.
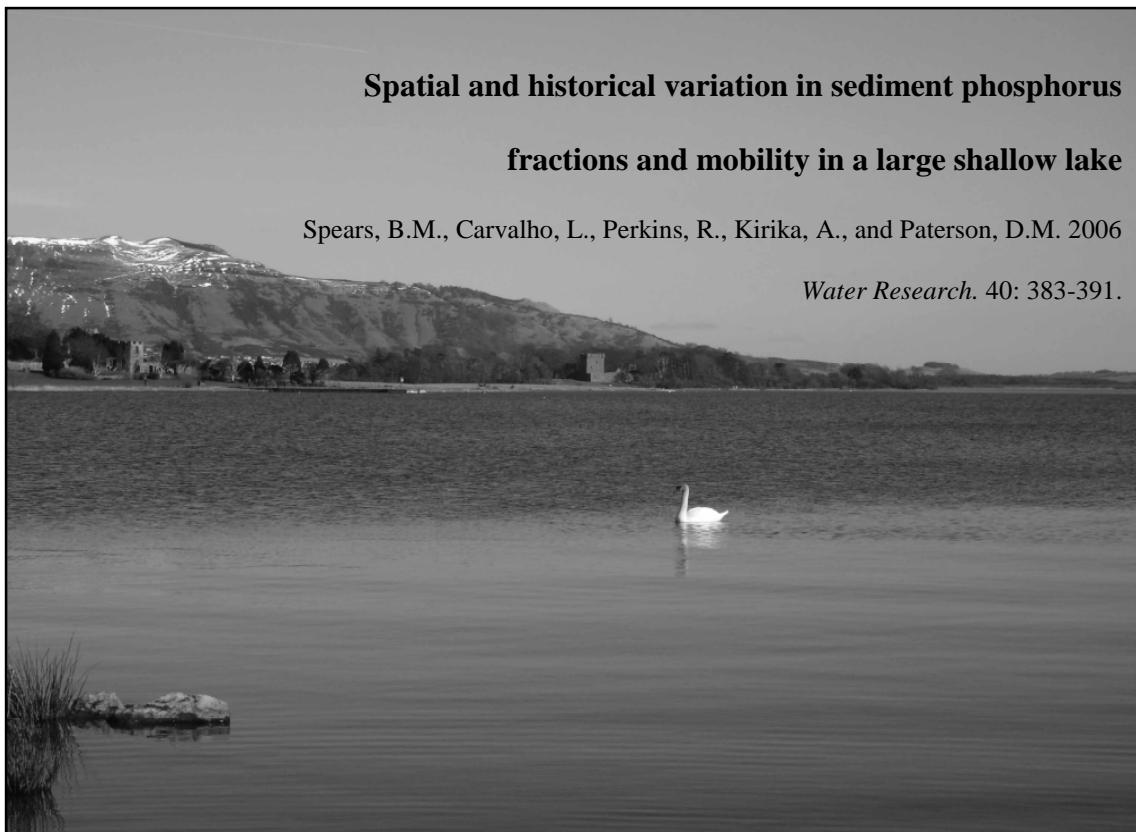
Spatial and historical variation in sediment phosphorus

fractions and mobility in a large shallow lake

ABSTRACT

Temporal and spatial variation in sediment-P composition and mobility were investigated in Loch Leven. Little change was observed in total sediment-P (surface sediment at 4 m depth), in comparison to a previous study (1990), despite significant reduction of external point sources of P to the lake. Labile P and residual P have both increased (0.007 to 0.039 mg PO_4-P and 0.121 to 0.420 mg PO_4-P per gram dry weight of sediment respectively) since 1990. An analysis of P fractions, along a depth transect, indicated elevated labile P concentrations in shallow water sediment (< 12 m overlying water depth).

The spatial variability in reductant-soluble P was significantly related to sediment chlorophyll *a* concentration as assessed using regression analysis ($R^2 = 0.733$, $p < 0.05$). This may be linked to the production of oxygen by benthic algae resulting in the maintenance of an oxygenated layer at the sediment surface. Variation in labile P was best explained by overlying water temperature and equilibrium phosphate concentration (EPC0).

INTRODUCTION

Internal loading is the mechanism through which sediment bound phosphorus (P) is released to the overlying water-column in lakes. The prolonged period required for recovery from eutrophication, characteristic in many shallow lakes, is commonly explained by physico-chemical mechanisms of P storage. However, less attention is paid to key physical (e.g. light and temperature) and biological (action of benthic algae) processes, which may be of great importance, especially in shallow lakes where the light environment favours benthic algal production (Wetzel, 2001).

A variety of physical, chemical, and biological variables have been monitored within Loch Leven at weekly to monthly frequency since January 1968 (Carvalho and Kirika, 2003). The importance of P as a limiting nutrient has been confirmed in a number of studies concerned with nutrient loading, effects of nutrient dynamics on the phytoplankton community, and laboratory-controlled nutrient enrichment experiments (Bailey-Watts, 1978; and Bailey-Watts and Kirika, 1993). For this reason, in a move to improve water quality, external total phosphorus (TP) loading was reduced from 20 t TP y^{-1} in 1985 to 8 t TP y^{-1} in 1995 (Bailey-Watts and Kirika, 1999). As is the case in many shallow lakes, however, reduction in TP and soluble reactive phosphorus (SRP) concentrations were not observed immediately following the implementation of restorative measures, significant reductions having been observed only in the last five years (Carvalho and Kirika, 2003). This phenomenon is commonly attributed to the process of internal loading whereby unfavourable water quality can be maintained in lakes, post-restoration, via P release from the sediments (Sas, 1990).

Data from the long-term monitoring of Loch Leven indicate that, in the early 1990s, SRP peaks in late summer were high. These peaks may be attributed to the mixing of hypolimnetic waters, high in sediment released P, with epilimnetic waters,

following lake de-stratification. The magnitude of the late-summer P release has declined significantly since 1997 indicating decreased internal loading (Figure 2.1). If this reduction were a result of the dynamic equilibration of internal loading then one would expect a similar decrease in sediment-P fractions sensitive to release. However, this decrease may also be due to a switch in mechanistic control of internal loading. For example, the regulatory action of benthic algae may increase as a result of decreasing phytoplankton biomass (and therefore improved sediment light environment) initiated by a decrease in external P load. In this case, little change would be expected in sediment-P fractions. The purpose of this study was to assess the impact of external P load reduction on the current status of Loch Leven sediment-P composition, with particular reference to spatial P mobility (along a depth transect), and also in comparison to historical data from 1990 (Farmer *et al.,* 1994). The results of this study will have implications for P management in shallow systems and these are discussed.
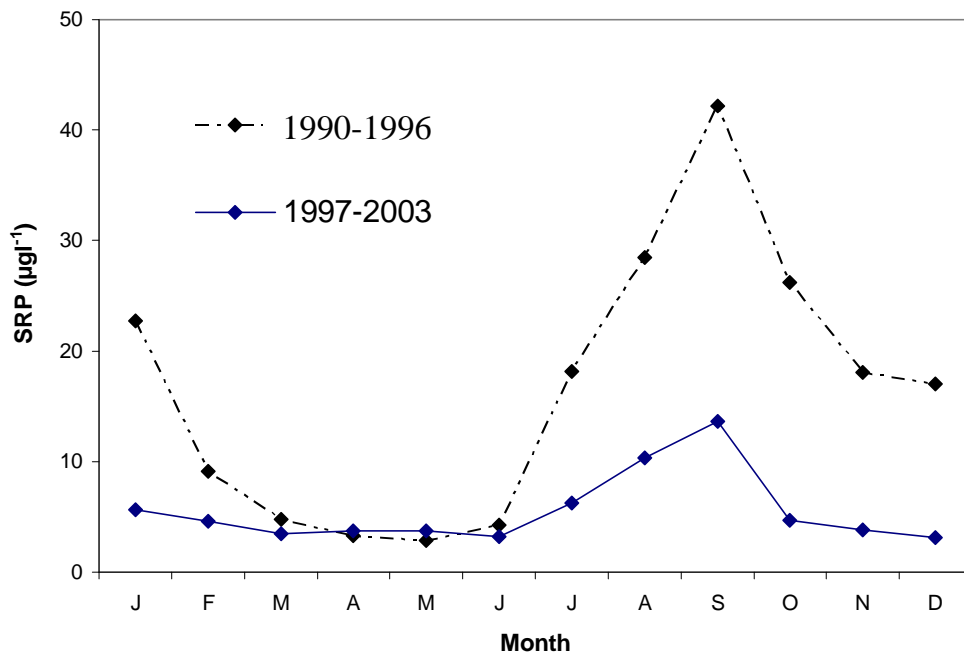

Figure 2.1 *Comparison of monthly means of SRP between 1990-1996 and 1997-2003.*

METHODS

Loch Leven is situated in the southeast of Scotland (latitude $56^o10'$ N, longitude $3^o30'$ W) and covers 13.3 km^2. It is characteristically shallow with a mean depth of 3.9 m exhibiting intermittent stratification in two small basins (~ 25 m water depth). Water chemistry has been monitored in the lake since 1968 (Carvalho and Kirika, 2003).

Sites were selected on a depth transect designed to represent key regions of the lake, samples being collected on the 13th of April 2004. Site location (GPS ±6m) and local morphometry were recorded (Table 2.1).

Single sediment cores were taken from each site using a Jenkin surface-sediment sampler (internal diameter 6.7cm), collecting 20 cm deep cores of the lake sediment along with the overlying water. The sample core tubes were sealed, chilled (4°C), and stored in darkness until analysis.

In the laboratory, water overlying the sediment surface was carefully siphoned off to minimise disturbance of the sediment-water interface. Bottom water (1 cm above the sediment surface) and surface water was sampled and filtered through a Whatman GF/C filter and stored in acid-washed plastic bottles at 4°C for SRP analysis. The upper 3 cm of sediment was removed by sectioning and thoroughly mixed before being divided and either stored for analyses or centrifuged for collection of pore water (filtered as above and stored for SRP analysis). Inorganic nutrient analysis was carried out in accordance with the procedures described by Wetzel and Likens (2000). Chlorophyll *a* analysis (bottom and surface waters and sediment sub samples) was conducted spectrophotometrically according to APHA (1992).

The phosphorus extraction scheme was modified from that of Psenner *et al.* (1988) and is described by Farmer *et al.* (1994).

The error within each step of the extraction scheme was assumed to be the same as those values reported by Farmer *et al.* (1994). These were ±40% for the labile fraction, ±10% for organic, apatite-bound and residual fractions, and ±20% for the reductant-soluble fraction. The errors in the SRP and TP analyses reported by Farmer *et al.* (1994) were both ±10%. The average concentrations of each P fraction in the upper 3 cm were compared between 1990 and the present study.

Phosphorus adsorption capacity (PAC) and equilibrium phosphate concentration (EPC0) experiments were conducted and used as measures of sediment-P release sensitivity. Controlled laboratory aerobic uptake/release experiments were conducted on surface sediment collected from each site, as described by Perkins and Underwood (2001). Aerobic conditions were deemed most representative of the overlying water on the day of sampling. Results from the PAC and EPC0 analysis were used to construct Langmuir adsorption isotherms from which EPC0, PAC, sediment-P saturation concentration, and current sediment-P saturation were estimated (Hwang *et al.*, 1976). Current % P saturation was estimated using the assumption that the sediment was in a state of equilibrium with the observed pore-water concentration at each site, which was then compared with the constructed isotherm data. These adsorption values are known to vary with a number of biogeochemical factors (e.g. DO, pH, benthic community composition) and should, therefore, only be considered indicative of sediment status under the laboratory-controlled conditions described above. Similarly, this analysis was conducted on mixed sediment and, therefore, only represents the physico-chemical relations between the sediment and the pore water and not necessarily the bottom water. Error within the PAC (±26%) and EPC0 (±17%) analysis was determined by calculating the relative standard deviation (RSD %) of six replicate samples taken from site 3 on an earlier date.

Multiple regression analysis was used to explain the variation in release-sensitive sediment-P fractions in relation to other measured variables. Statistical analysis of labile and reductant-soluble P was carried out using MINITAB version 14. Stepwise multiple regression analyses (p to enter = 0.15) were carried out to identify which of the measured variables (pH, conductivity, DO, temperature, sediment chlorophyll *a*, surface-water chlorophyll *a*, bottom-water chlorophyll *a*, EPC0, and bottom water, pore water and surface water SRP, NH_4, and SiO_2) best explained the variation in the two P pools.

RESULTS

A summary of the locations, mean water depths, % lake area of each depth class and sediment types for the six sites sampled is provided (Table 2.1). Site 3 was the sample site included in the 1990 study (Farmer *et al*., 1994). Vertical profiles of DO, temperature, conductivity, and pH observed on the day of sampling are shown (Figure 2.2).

PO_4-P, NH_4-N and SiO_2 concentration gradients between the pore and bottom waters were observed at all depths sampled (Table 2.2). Chlorophyll *a* concentration increased between 2 m and 5 m overlying water depth, decreasing in deeper water sediments (Figure 2.3).

Historical comparison of sediment-P composition

Sediment-P composition, in the upper 3 cm of sediment, was compared between 1990 and 2004 (site 3, Figure 2.4). A significant increase in both labile (0.007 mg PO_4-P g^{-1}dw to 0.039 mg PO_4-P g^{-1}dw) and residual P (0.121 mg PO_4-P g^{-1}dw to 0.420 mg

PO$_4$-P g^{-1}dw) was observed. No other significant changes were found. A direct

comparison of the results from this study and the 1990 study are given (Table 2.3).
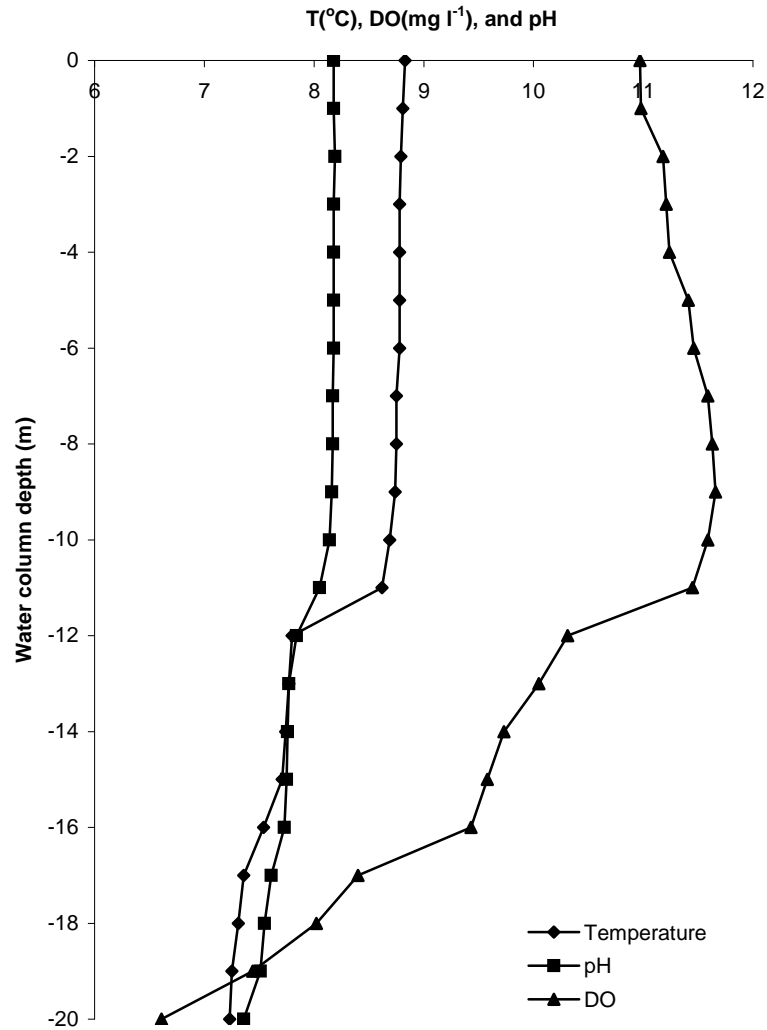

Figure 2.2. *Depth profiles of dissolved oxygen concentration, water temperature and pH. The depth transect was conducted at the 20 m site.*

Spatial variation in sediment-P composition

The spatial variation in sediment-P composition was investigated (Figure 2.5). TP, organic, reductant-soluble, and metal-oxide adsorbed P fractions all showed similar variation with water depth. A general increase in the sediment-P fractions with overlying water depth was observed up to 5 m after which concentrations levelled off. Residual and apatite-bound P showed similar trends with overlying water depth where P concentrations were similar at all depths except below 2 m where concentrations were significantly lower. Labile P in the sediment was similarly high over a depth range of 0-5 m with concentrations decreasing significantly in sites of water depth greater than 12 m. The dominant P fraction also varied (Table 2.3). The average TP concentration taken from the six sites was 2.29 mg PO_4-P g^{-1}dw. The average TP composition was 1.6% labile P, 24.6% reductant-soluble SRP, 3.2% reductant-soluble SURP, 17.9% metal-oxide adsorbed P, 21.7% organic P, 15.0% apatite-bound P, and 15.9% residual P.

Spatial variation in P-release potential

PAC ranged from 0.421 mg PO_4-P g^{-1}dw at site 4 to 0.155 mg PO_4-P g^{-1}dw at site 6 (Table 2.4). Saturation concentration increased with overlying water depth from 0.775 mg PO_4-P l^{-1} at 2 m to 1.150 mg PO_4-P l^{-1} at 20 m. Estimated current sediment-P saturation values ranged from ~16% at 2 m and 3% at 12 m overlying water depth (Table 2.4). Pore water SRP concentration was significantly higher than the EPC0 concentration for all sites (Figure 2.6). Bottom water SRP concentration was similar to EPC0 values up to 4 m, significantly less than EPC0 at 5 m, and significantly greater than EPC0 at depths greater than 12 m. Pore-water SRP exceeded bottom-water

concentrations at all water depths except 12 m where both pools were similar. Pore-water SRP concentration at 4 m was 40 µg PO_4-P l^{-1} in 1990 (Farmer *et al*., 1994).

The spatial variation in reductant-soluble SRP was significantly positively related (R^2 = 0.73) to sediment chlorophyll *a* concentration as assessed using stepwise multiple-regression analysis. However, the spatial variation in labile P was significantly related to a combination of temperature and EPC0 (R^2 = 93.4%) as assessed using stepwise multiple regression analysis (Figure 2.7).

DISCUSSION

Historical comparison of sediment-P composition

The current sediment-P composition of site 3 was shown to be similar to the P regime observed in 1990 with the exception of the labile and residual P fractions. A five-fold increase (in mass terms) was observed in labile P. This fraction is of particular interest as it is the P form that may be immediately available for sequestration by aquatic autotrophs. This increase is in excess of the analytical uncertainty of the extraction procedure (40%; Farmer *et al*., 1994) and so may be considered significant. It should be noted, however, that the labile P fraction is the most susceptible to release and so large-scale spatial fluctuations in concentration may be expected.

SRP concentrations in the water-column have been low over the last five years in Loch Leven indicating both a successful reduction in external P load and the maintenance of a significant P concentration gradient between sediment and lake water. Additionally, the total sediment-P concentration was ~ 2.5 mg PO_4-P g^{-1}dw characteristic of a scenario where restorative measures would induce net annual sediment release for a period exceeding 5 years (Sas, 1989). These observations would suggest a decrease in sediment-P. However, concentration gradients may not be the

dominant controlling mechanism of internal loading. It is widely accepted that internal loading is governed not only by chemical diffusion but also by a complex matrix of interacting 'within-lake' biogeochemical processes (Sas, 1989; Boström *et al.*, 1988; Woodruff *et al.*, 1999; Søndergaard *et al.*, 2003). For example, elevated overlying-water DO concentrations, characteristically observed in shallow lakes, might act to reduce the intensity of internal loading thereby prolonging the equilibration of SRP (Carlton and Wetzel, 1988). The maintenance of DO concentrations in excess of 2 mg l^{-1} has been shown to result in an oxygenated surface-sediment layer with a high affinity for P adsorption (Mortimer, 1971; Marsden, 1989). The field data indicated that these conditions were exceeded in the overlying water of all areas sampled, even in deeper-water sites. Thus, regardless of the onset of anoxia within the sediment, sediment

Table 2.1 *Summary of sample site location, depth, % lake area (depth range in brackets), and physical characteristics of the sediment (latter from Calvert, 1974).*

Site number	O.S. grid reference	% lake area	Depth (m)	Sediment fraction		
				Sand	Silt	Clay
1	12303, 01311	34 (0-2)	2	50-70%	30-50%	10-30%
2	12689, 01211	6 (2-2.5)	2.5	50-70%	30-50%	30-50%
3	13497, 01189	29 (2.5-4)	4	10-30%	10-30%	50-70%
4	14804, 01092	11 (4-5)	5	<10%	30-50%	50-70%
5	14195, 02640	15 (5-12)	12	10-30%	30-50%	30-50%
6	14090, 03981	5 (12-20)	20	10-30%	30-50%	30-50%

Table 2.2 *Inorganic nutrient (PO_4-P, NH_4-N, and SiO_2) and chlorophyll a concentrations in the surface, bottom, and pore waters at each depth sampled.*

Depth (m)	PO_4^{3-} (µg l^{-1})			NH_4^- (µg l-1)			SiO_2 (mg l^{-1})			Chl *a* (µg l^{-1})	
	Surface	Bottom	Pore	Surf	Bot	Pore	Surf	Bot	Pore	Surf	Bot
2	5	14	125	10	44	1733	0.80	1.60	39.56	12	42
2.5	3	12	86	45	86	1058	0.30	1.15	38.37	9	23
4	4	14	125	57	80	668	0.29	0.86	33.28	11	29
5	4	11	93	32	190	1426	0.17	1.37	43.95	8	31
12	2	51	42	32	77	1703	0.22	0.98	43.12	15	33
20	2	25	117	13	650	5101	0.25	2.57	56.68	14	25

released P will have to percolate an oxygenated boundary layer between sediment and overlying lake water. One would expect an increase in benthic algal production as a result of improved water clarity following external P reduction. Release of sediment-P across an oxygenated layer of sediment is more common at high temperatures when elevated oxygen consumption, induced by bacterial activity in deeper sediments, acts to temporarily remove the oxygenated surface layer. Laboratory studies indicate that P release becomes independent of DO concentration at temperatures exceeding $17^{o}C$ (Kamp-Nielson, 1975) to $21^{o}C$ (Holdren and Armstrong, 1980). Oxygen supply is also critical to the maintenance of surface layers. For example, reduced P release has been linked to improved sediment oxidation at elevated wind velocities (DeGroot, 1981). Sufficient Fe (III) needs to be present to support P precipitation and a number of chemical species including the hydroxyl and silicate ions have been shown to out-compete P for Fe (III) sorption sites in conditions of elevated pH (common in shallow lakes with high benthic primary production; Boström *et al*., 1988; Marsden, 1989; Koski-Vahala *et al*., 2001). Excessive wind or biologically-induced sediment resuspension may also increase sediment-P release (Paterson and Black, 1999).

The increase in residual P is less easily explained. The refractory nature of this P fraction was highlighted by Farmer *et al*. (1994) where similar values of residual P were observed throughout a 15 cm sediment core sectioned at 1 cm intervals. This lack of variation with sediment depth indicates little change in residual P concentration over many years. The mechanisms controlling the residual P fraction are expected to be the inherent physical characteristics of the allochthonous suspended solids. This observation may therefore represent a catchment-derived variation.

Spatial variation in sediment-P composition

Every fraction, with the exception of labile P, generally increased with overlying water depth. The variability observed within the sediment-P fractions can be used to infer the release potential of each site.

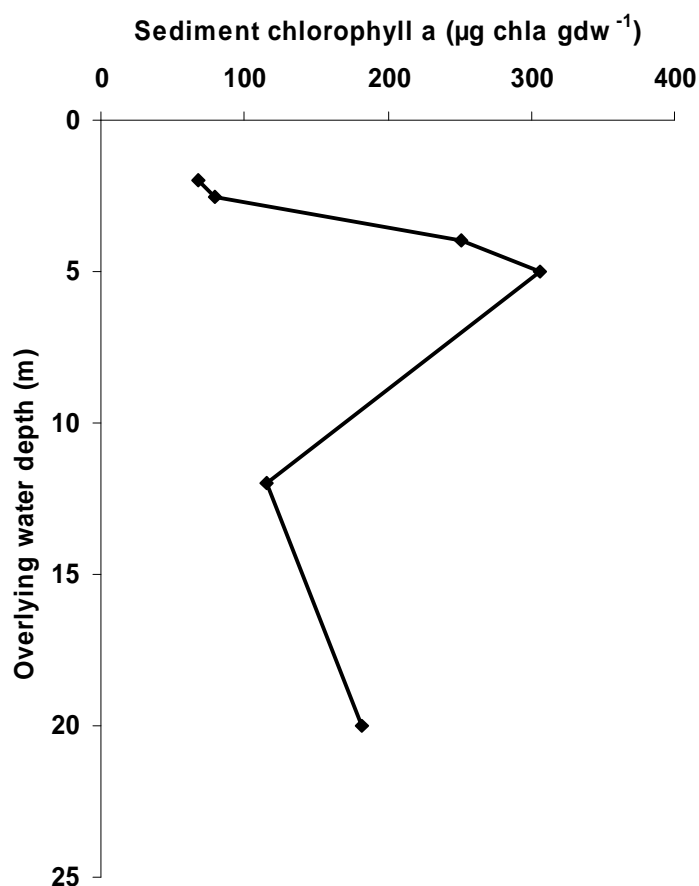**Figure 2.3** *The spatial variation in the surficial sediment chlorophyll a concentration over the six sites.*

Labile P concentration was highest at the four shallowest sites. Thermal stratification appeared to be present at depths greater than ~ 12 m and sediment-P release would be more likely in the deeper water regions (low labile P concentration) of Loch Leven where anoxia is more likely to occur in the surface-sediment. Labile P

concentration may therefore be low in the deeper-water sediments as a result of higher rates of internal release in the hypolymnetic waters. The effects of this internal release would, therefore, only be realised following lake-turnover, thus explaining the characteristic autumnal P peaks. Additionally, the recent decrease in the intensity of the autumnal peaks may be reflected in the lower concentrations of labile P in deeper-water sediment, though this is difficult to substantiate due to a lack of historical data from these sites. The release of labile P is mainly controlled by chemical diffusion and sediment resuspension, though in shallow waters oxic surface sediment may buffer such release (Boström *et al*., 1988). Multiple-regression analysis indicated that 92.9% of the variation in labile P was explained by a direct relationship with EPC0 (P release potential) and bottom-water temperature. The positive relationship between overlying-water temperature and labile P may be indicative of increased microbial decomposition and subsequent P mineralisation (e.g. Jones, 1985; Boström *et al*., 1988). The organic P pool decreased with overlying water depth, although correlation analysis with labile P proved to be insignificant. However, this relationship may still represent a temperature-regulated switch between organic and labile P pools at high temperatures. The relationship between labile P and EPC0 may be explained by considering physical adsorptive constraints. High values of EPC0 represent high P-release buffering capacities (House *et al*., 1998). Reductant-soluble SRP showed a significant relationship ($R^2 = 0.733$) with sediment chlorophyll *a* concentration. This agrees with much of the recent literature where high autotrophic photosynthesis has been coupled with high sediment-P retention. The mechanism behind this relationship is the oxidative immobilisation of P with Fe (III) when dissolved oxygen concentrations are high. Such conditions have commonly been attributed to elevated photosynthetic activity and the resultant production of O_2 in surface sediments (Hansson, 1989; Boström *et al*., 1988;

Carlton and Wetzel, 1988; House *et al.*, 1998; Brunberg and Bostrom, 1992; Woodruff *et al.*, 1999; and Søndergaard, 2001). Redox-mediated release is, therefore, more likely to occur when sediment chlorophyll *a* concentrations are low. The occurrence of the sediment chlorophyll *a* peak at 5 m overlying water depth agrees with the findings of Hansson (1990) where periphyton biomass was observed to be relatively moderate or high (compared to other depths) at depths below 4 m. Hansson (1990) also estimated a reduction of about 40% sediment-P release under conditions of high peryphyton biomass. Cyr (1998) made similar observations where chlorophyll peaks in the sediment surface seemed to be regulated by fetch and temperature as opposed to light availability.
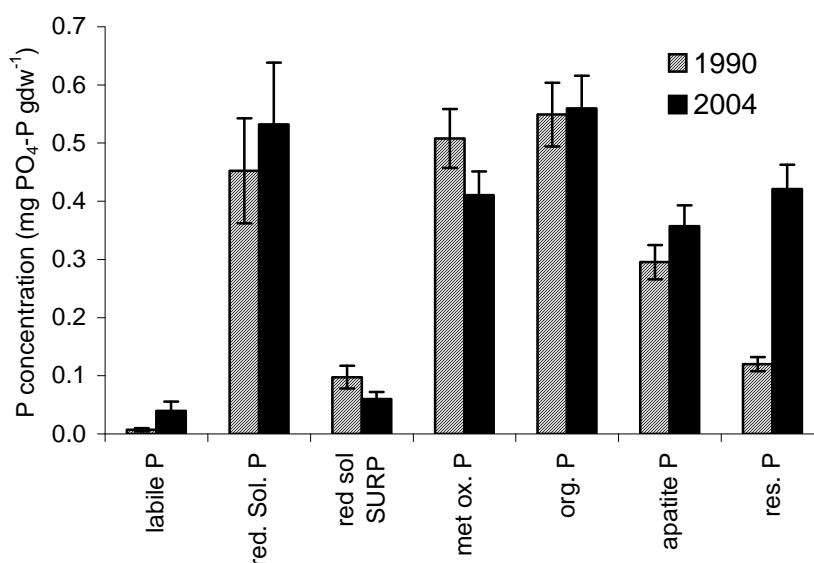
Figure 2.4. *Comparison of sediment-P fractions in the upper 3 cm of sediment in 1990 (Farmer et al., 1994) and 2004. Fractions include labile P, reductant-soluble SRP (red. sol. SRP), reductant-soluble SURP (red. sol. SURP), metal-oxide adsorbed SRP (met ox. P), Organic P (org. P), Apatite bound P (apatite P), and residual P (res. P). Sediment-P concentrations are expressed as mg PO$_4$-P g^{-1}dw. Error bars represent the error within each extraction step as reported by Farmer et al. (1994).*

Figure 2.5. *Variation in sediment-P fractions with overlying water depth. Fractions include a) labile P, b) reductant-soluble SRP (Red-sol P), c) reductant-soluble SURP (Red-sol SURP), d) metal-oxide adsorbed SRP (Met Ox P), e) Organic-P (Org. P), f) Apatite-bound P (Apat. P), g) residual P (Res. P), and h) total P (TP). Sediment-P concentrations are expressed mg PO$_4$-P g^{-1}dw. Error bars represent the error within each extraction step as reported by Farmer et al. (1994).*

Table 2.3. *Summary of % P composition of total P at each depth sampled. Each value represents the soluble reactive phosphorus extract unless otherwise indicated (i.e. SURP = soluble unreactive phosphorus). Shaded boxes represent the dominant fraction at each depth.*

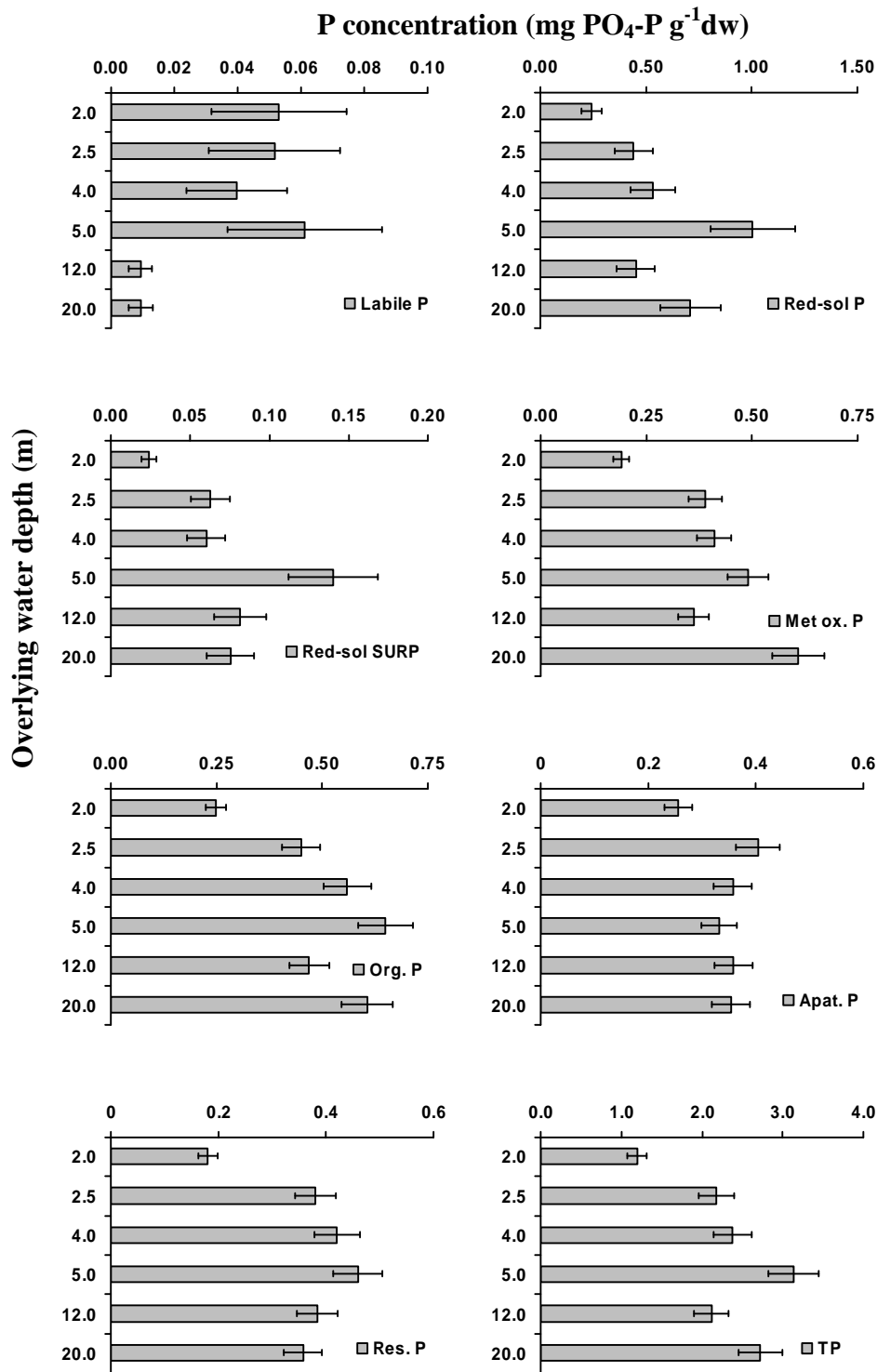| Overlying water depth (m)	Labile	Reductant Soluble	Reductant soluble SURP	Metal-Oxide adsorbed	Organic	Apatite-bound	Residual
2	2.83	20.29	2.01	15.93	20.80	21.41	15.12
2.5	1.49	20.26	2.88	17.88	20.68	18.52	17.42
4 (1990)	0.35	22.29	4.81	25.03	27.05	14.53	5.91
4 (2004)	1.03	22.35	2.53	17.24	23.51	15.01	17.68
5	1.16	32.10	4.48	15.63	20.69	10.58	14.65
12	0.27	21.33	3.85	17.14	22.15	16.94	18.15
20	0.21	26.07	2.77	22.39	22.27	13.02	13.13

Table 2.4. *Summary of results taken from phosphorus adsorption capacity (PAC) experiments. 100% P-Sat indicates the concentration in the overlying water that resulted in 100% saturation of the sediments with P. Actual P-Sat indicates the estimated % saturation of the sediments at time of sampling. All values were taken from the constructed Langmuir adsorption isotherms.*

Overlying water depth (m)	PAC (mg PO_4-P g^{-1}dw)	100% P-Sat conc. (mg PO_4-P l^{-1})	Actual % P-Sat	EPC0 (mg PO_4-P l^{-1})
2	0.14	0.78	16%	0.012
2.5	0.26	0.98	9%	0.021
4	0.13	1.07	12%	0.011
5	0.36	1.03	9%	0.030
12	0.14	1.10	3%	0.008
20	0.13	1.15	10%	0.009

Spatial variation in P-release potential

The results of the PAC analysis showed spatial variation in the P adsorption properties of the sediment. No sediment sample was found to be near PAC. The saturation concentration (concentration of the overlying water that would result in PAC) was found to increase with overlying water depth. These results are indicative of sediment-P uptake from the pore water at all sites. However, strong chemical gradients between pore and bottom water SRP, favouring release, were observed in five of the six sites with the 12 m site exhibiting near-equilibrium conditions. The low bottom-water SRP concentrations in shallow-water sediments, coupled with elevated labile P and pore

water SRP, suggest that factors other than chemical diffusion are regulating the mobility of sediment-bound P across the sediment-water interface in shallow waters. The reduced bottom-water SRP concentrations in the shallow-water sites may be indicative of a higher level of autotrophic control (e.g. direct uptake or photosynthetic DO production) resulting from elevated biomass under conditions of favourable light. Similarly, the shallow sites should be more homogenously mixed (as the field measurements suggest) and, therefore, well aerated by a mixture of photosynthetic oxygen production and wind mixing of the euphotic zone. The position of the deeper sites, below the thermocline, resulted in a less-aerated overlying water-column compared to the shallower sites. Thus, the deep sites may be considered sensitive to anoxic release, in contrast to the shallow sites, which may be governed mainly by sediment resuspension (e.g. wind mixing and bioturbation).

CONCLUSIONS

Sediment-P release in Loch Leven

The sediment fractions at the 4 m site included in both studies have not been reduced as a result of external loading reduction. Unfortunately, it is impossible to tell whether the deeper sites have seen a significant decrease in P fractions sensitive to internal release. It is, however, clear that the mechanisms of release differ with depth.

The spatial variation in sediment-P composition and loading would suggest that single-core surveys may not be sufficient to allow accurate representation of a lake's chemical condition and potential for phosphate release. Only multi-core surveys may provide adequate coverage of the variable biological and physico-chemical mechanisms driving sediment-P composition and release potential. These results offer strong evidence to support the importance of biologically mediated control of sediment-P

release, both by autotrophs and heterotrophs. The average dominant sediment-P fraction was observed to be organic P, which may be indicative of the high levels of planktonic detritus common in shallow eutrophic lakes. Temperature-controlled bacterial

SRP concentration (mg PO$_4$-P L^{-1})
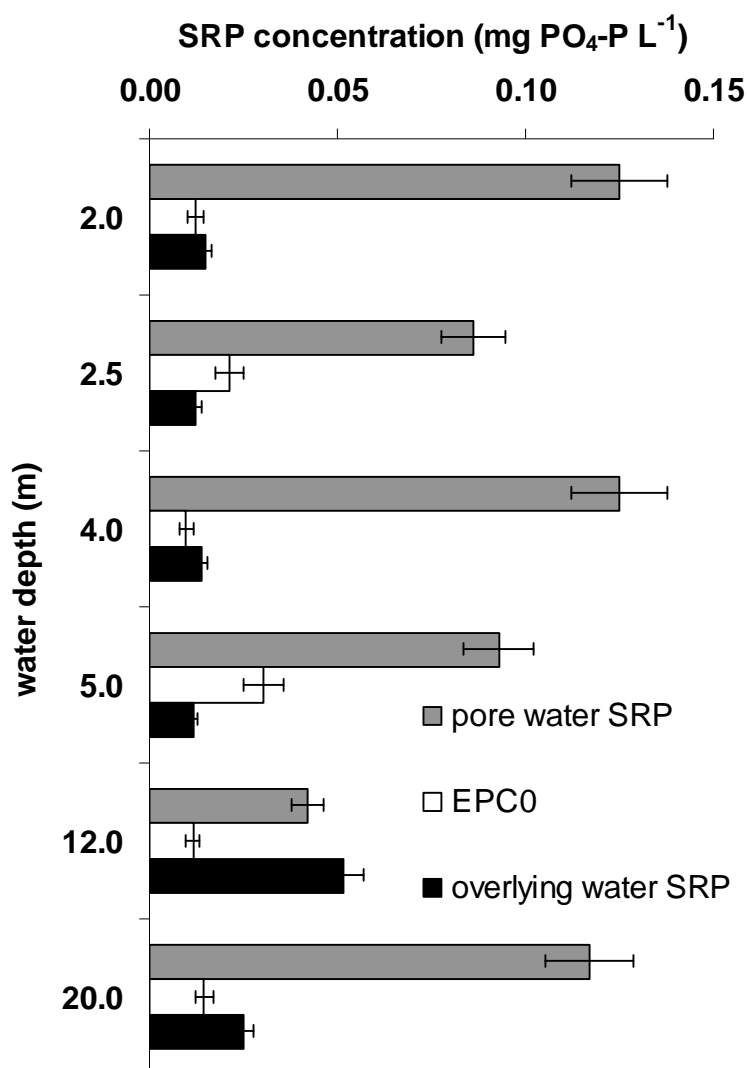
Figure 2.6. *Variation in phosphorus equilibrium concentration (EPC0), bottom water SRP concentration, and pore water SRP concentration with overlying water depth. Error bars represent the standard error taken from the analysis of six replicate cores from the 4m site, conducted in January 2004.*
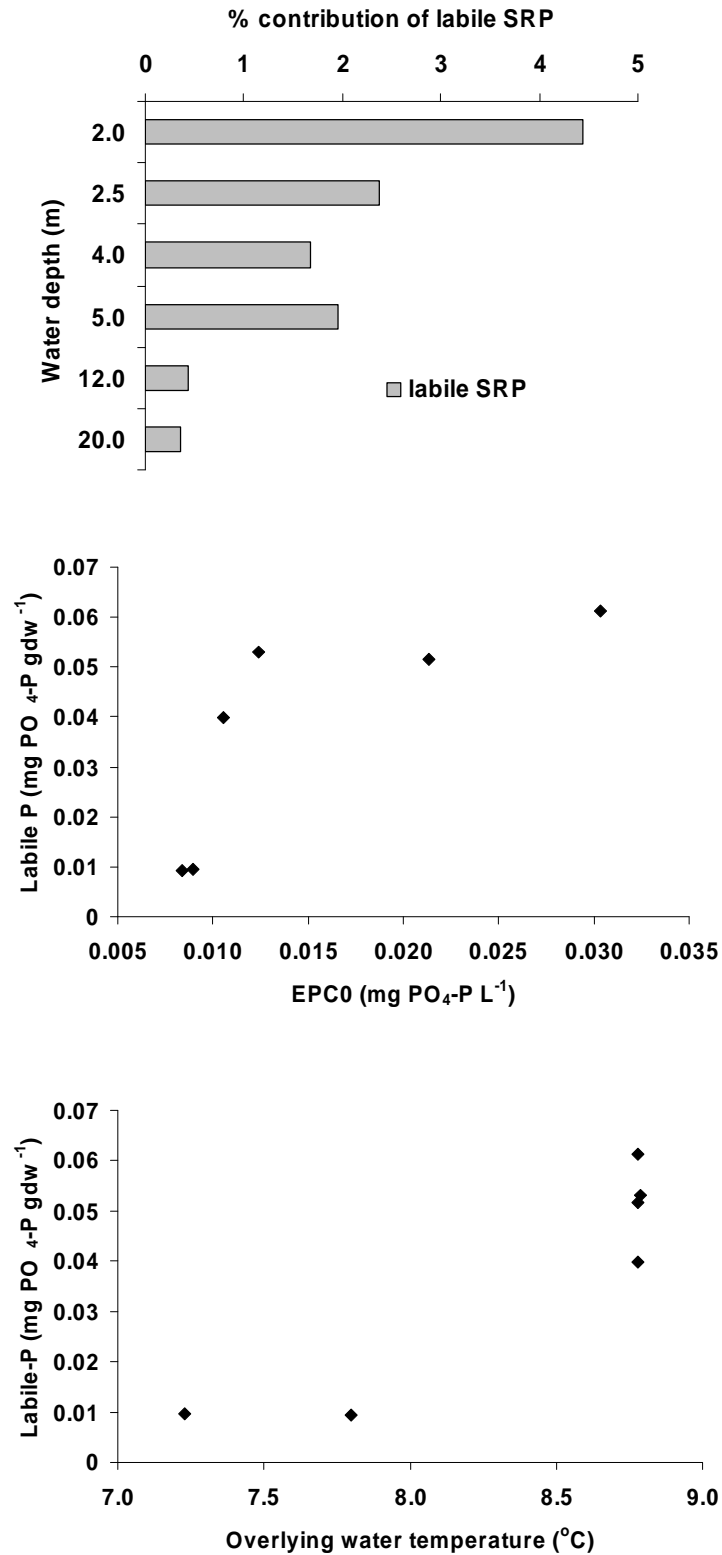
Figure 2.7. *(a) Variation in % contribution of labile P to total sediment-P with overlying water depth, and relationships between labile P,EPC0 (b) and temperature (c). ($R^2 = 0.934$, y = 0.024 Temperature + 0.9402 EPC0 - 0.1776).*

decomposition of organic matter will, therefore, be the dominant mechanism driving sediment-P release through the transfer of organic P, to a form more accessible to phytoplankton. Though it is unclear as to which mechanism is most dominant, both wind-mixing of the water-column and periphyton production should act to reduce the release of reductant-soluble P through the maintenance of an oxygenated layer in the surface sediment, though high rates of sediment resuspension, induced by wind mixing, may also increase the release of labile P.

The release of sediment-P in Loch Leven will therefore occur on a smaller but more continuous scale in shallow waters due to high rates of bacterial decomposition and a high likelihood of sediment resuspension. Deeper sites, however, may release reductant-soluble P during periods of stratification and high temperatures, conditions that correspond with the late-summer peaks in SRP concentration routinely observed within the annual SRP fingerprint for Loch Leven.

Wider implications of findings

This paper highlights the complexity of biogeochemical mechanisms controlling internal loading in shallow eutrophic water bodies. The spatial variation in a lake's sediment-P fractions, and the factors controlling them, must be fully assessed when characterising a site's potential for release/recovery from eutrophication. Autotrophic biofilms act to inhibit the release of labile P through direct uptake and the maintenance of an oxygenated microlayer, whereas heterotrophic organisms act to increase the release potential of surface sediments through P mineralisation and subsequent accumulation of labile P within the sediment. As internal loading in Loch Leven is seasonally consistent, an effort should be made to assess the temporal variation of sediment-P fractions on at least a monthly frequency. Additionally, further

investigations should be carried out to try and quantify the effects of autotrophic biofilms in inhibiting sediment-nutrient release in shallow lakes.

CHAPTER 3
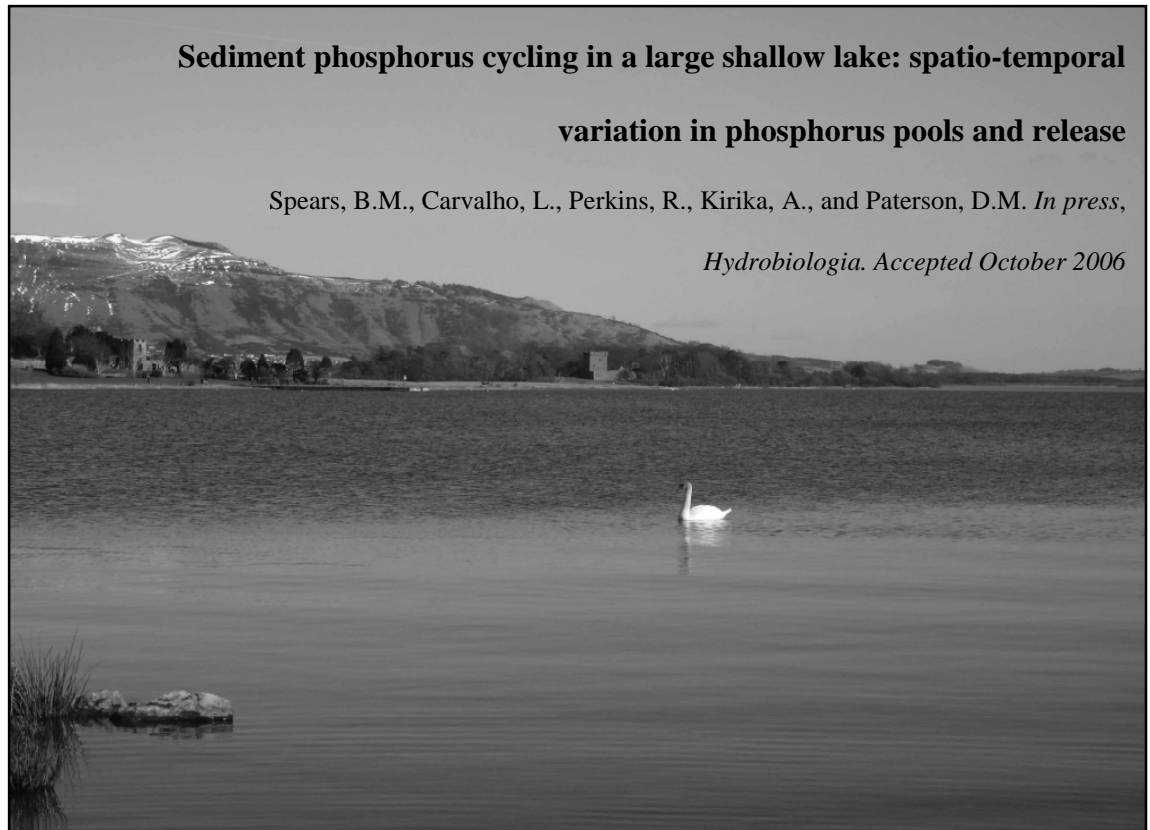
Sediment phosphorus cycling in a large shallow lake: spatio-temporal variation in phosphorus pools and release

Sediment phosphorus cycling in a large shallow lake: spatio-temporal

variation in phosphorus pools and release

ABSTRACT

Sediment and water-column phosphorus fractions were recorded monthly for one-year (April 2004 - April 2005) in a shallow lake recovering from nutrient pollution (Loch Leven, Scotland). Equilibrium phosphate concentration (EPC0) and gross sediment phosphorus (P) release rates were estimated from laboratory experiments. Pore water and organic P pools were lowest during warm water periods whereas bottom water P was lowest during cold water periods. Reductant-soluble, organic, metal oxide-adsorbed, residual and sediment total phosphorus pools all varied significantly with overlying water depth. Short-term, high magnitude, redox initiated P release events occurred in late summer and winter as a result of anoxic sediment conditions. Lower magnitude long-term release conditions were maintained for most of the year, most likely as a result of organic P cycling and maintenance of high concentration gradients between the pore and bottom water P pools. Estimates of summer P uptake/release rates, across an intact sediment-water interface, suggested that maximum gross internal release was ~ 12 mg SRP m^{-2} lake surface area d^{-1} with EPC0 values ranging between 180 and 270 µg P L^{-1}. This study highlights the biological mediation of internal loading in shallow eutrophic lakes, and in particular, the role of sediment algae in decreasing, and sediment bacteria in enhancing, sediment-P release.

INTRODUCTION

Slow recovery from eutrophication, following a reduction in external phosphorus loading, is common in shallow lakes (Sas, 1989). Attention has been focussed on the physico-chemical processes that maintain poor water quality after the reduction of external loads. Early studies highlighted the role of lake sediment as a redox mediated phosphorus (P)-buffer, where P is released under conditions of anoxia and retained under oxic conditions (Mortimer, 1941; 1942). Additionally, adsorption/desorption mechanisms play an important role in P cycling within shallow lakes (House *et al.,* 1998; Perkins *et al.,* 2001). Despite the fact that these processes were recognised as being biologically-mediated, very little attention has been paid to the biological communities acting on sediment-P release. The total phosphorus (TP) pool in sediment is composed of several operationally-defined components, each with their own release pathways. Temperature is commonly regarded as an important driver of internal loading through elevated microbial remineralisation (depletion of organic sediment-P), the resultant onset of anoxia (depletion of reductant-soluble sediment-P), and raised diffusion rates (depletion of labile and pore water P).

Benthic communities (autotrophs, heterotrophs, invertebrates etc.) may, both directly and indirectly, regulate sediment-P release (Paterson and Black, 1999). With sufficient light, benthic micro-algae proliferate and cap internal loading from sediment-P pools sensitive to anoxia (Woodruff *et al.,* 1999), inhibit resuspension through increased sediment stability (Paterson, 1988), and reduce P availability through direct uptake (Van Luijn *et al.,* 1995). However, where light is limited, heterotrophs dominate biofilms and can enhance regeneration of previously unavailable organic and refractory P, release P from redox sensitive fractions, and absorb pore water P (Gächter and Meyer, 1993). Finally, settling phytoplankton cells and plankton migrating back into the

water-column may further regulate organic P-partitioning between the sediment and the water-column.

Sediment-P uptake/release is a complex process, and affected by equilibrium conditions between the surface sediment and the overlying water. Traditional estimates of equilibrium phosphate concentrations (EPC0; SRP concentration in overlying water that results in no sediment-P uptake/release) have often used sediment suspensions and, therefore, only represent equilibrium conditions of resuspended sediment. Recently, intact sediment cores, incubated under laboratory-controlled conditions, have been used to assess sediment-P uptake/release (e.g. Søndergaard, 1989). The aims of this study were to (1) investigate the seasonality of concentration gradients between water-column and sediment-P pools in terms of internal loading mechanisms; (2) assess the effects of seasonal temperature on sediment-P composition; (3) assess the effects of overlying water depth on the spatial distribution of sediment-P composition; and (4) to use laboratory controlled intact sediment core experiments to estimate the current uptake/release conditions (including EPC0) of a shallow lake recovering from nutrient pollution (Loch Leven, Scotland).

METHODS

Study site

Loch Leven (Figure 3.1) is a shallow eutrophic lake (mean depth 3.9 m) situated in the southeast of Scotland (latitude $56^{o}10$'N, longitude $3^{o}30$'W) with a surface area of 13.3 km^2. The majority of the lake remains well-mixed throughout the year, with intermittent stratification in two deep basins of ~ 25 m. Water chemistry has been recorded approximately every two weeks since 1968 providing a detailed history of recovery from eutrophication. External loading to Loch Leven was reduced from 4.05

mg TP m^{-2} lake surface area d^{-1} in 1985 to 1.62 mg TP m^{-2} d^{-1} in 1995 (Bailey-Watts and Kirika, 1999). However, prolonged internal loading coupled with a low flushing rate (2-3 lake volumes per year) resulted in poor water quality being maintained, with an improvement in water-column SRP concentration only being observed in recent years (Carvalho and Kirika, 2003). This improvement in water quality has not been mirrored in the sediment-P composition (Farmer *et al*., 1994; Spears *et al*., 2006a).
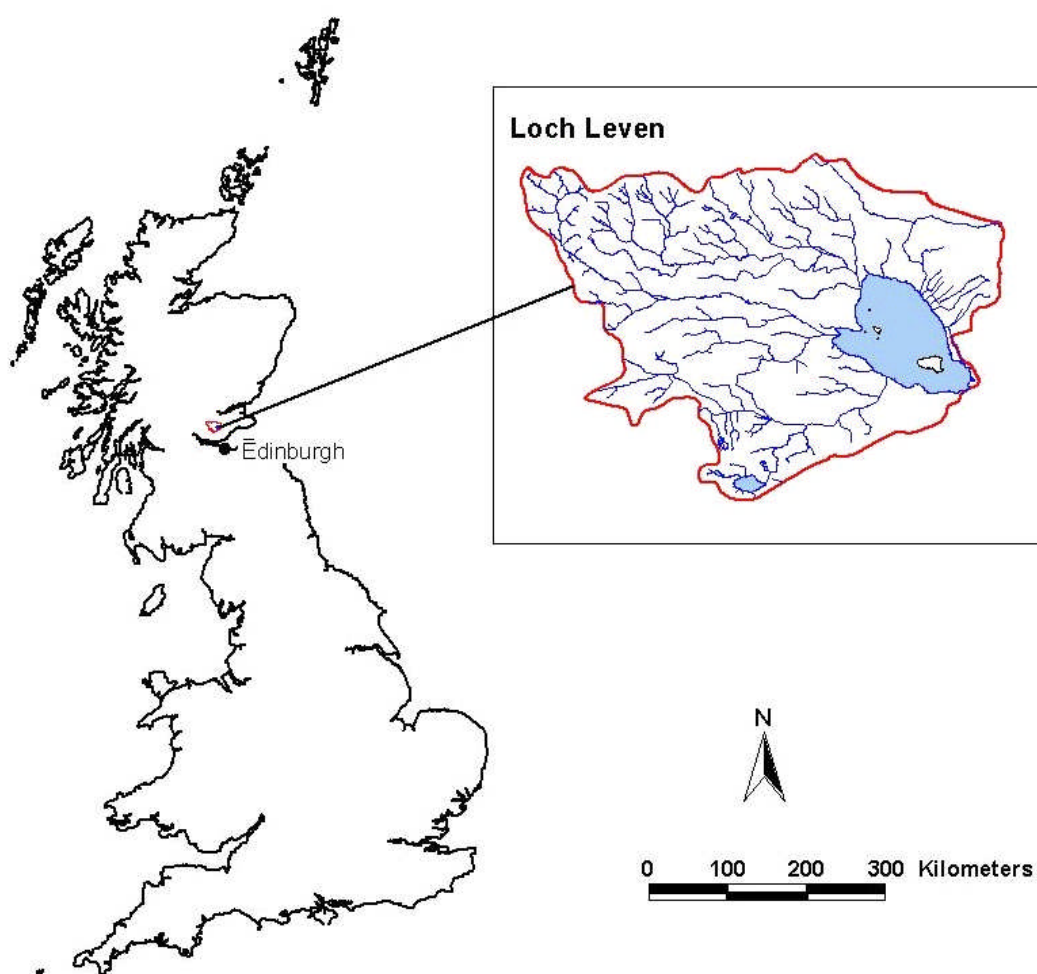

Figure 3.1. *Loch Leven and the surrounding catchment.*

Field measurements, water chemistry, and sediment-P fractionation

Monthly sampling was conducted between April 2004 and April 2005 from six sites along a depth transect (2, 2.5, 3.5, 5.5, 10, 22 m overlying water depth), to allow comparison of shallow and deep regions of the lake throughout the year. Detailed

sampling locations are described in Spears *et al.* (2006a). Surface waters were collected and stored in acid-washed Azlon bottles. Depth profiles of water temperature, dissolved oxygen % saturation (DO%), pH and conductivity were recorded on each visit (Hydrolab Water Quality Monitoring System, Hydrolab Corporation, Colorado, USA). On each date, single sediment cores were collected from each site using a Jenkin surface-sediment sampler (internal diameter 67mm) and transported to the laboratory for processing. The cores had about 30 cm overlying water and 20 cm sediment.

In the laboratory, bottom water was sampled in the cores from 1 cm above the sediment surface. Both bottom and lake surface waters were filtered (Whatman GF/C) and stored at 4^oC in acid washed Nalgene containers for chemical analysis. The filters were frozen and stored for analysis of photosynthetic pigments. The upper 3 cm of sediment from each core was removed, homogenised, then split preceding both centrifugation and filtration (as above) for pore water chemical analysis, or storage for pigment and chemical analysis. Sediment for chlorophyll *a* analysis was filtered (GF/C) before dark extraction in 90% v/v cold acetone for 24 hours at 0^oC. Chlorophyll *a* was quantified spectrophotometrically, with correction for phaeopigment concentration by acidification (APHA, 1992). Inorganic nutrient analyses were carried out using the methods of Wetzel and Likens (2000). Sediment-P fractionation was carried out using a sequential chemical extraction technique based on Psenner *et al.* (1988) and Farmer *et al.* (1994). Two sub-samples of homogenised slurry, from each site, were subject to the following extraction procedure on each sample date: (1) repeated extraction in 1 M NH_4Cl for 2 h and quantification of SRP [labile P]; (2) repeated extraction with 0.11 M $NaHCO_3$/0.11 M $Na_2S_2O_4$ for 1 h and quantification of SRP [reductant-soluble P] and total soluble phosphorus (TSP) [reductant-soluble soluble un-reactive phosphorus (SURP)]; (3) repeated (for 1 h) extraction in 1 M NaOH for 18 h followed by

quantification of SRP (metal-oxide adsorbed P), and TSP; (4) repeated (for 1 h) extraction with 0.5 M HCl for 18 h and quantification of SRP [apatite-bound P]; (5) digestion with 30% (v/v) H_2SO_4 and 8% $K_2S_2O_4$ at 121 ^{o}C for 30 min followed by SRP quantification [residual P]. Organic P was quantified by subtracting NaOH-SRP from NaOH-TSP. Sediment slurry and extraction medium were continually shaken in 50 ml centrifuge tubes in the dark at 20°C throughout each extraction. Supernatants were collected following centrifugation and filtration (GF/C filter) at the end of each extraction step.

Sediment release experiments

EPC0 experiments were conducted using intact sediment cores on the 6th August and the 8th September 2005. On each date, 12 sediment cores were collected from a site with 3.5 m of overlying water, selected to represent an average water depth. Cores were held in a water bath at 17°C with an irradiance of 63.3 µmol m^{-2} s^{-1} for 1 h. The temperature of the overlying water on collection of the cores was 15.4^{o}C on the 8th of August 2005 and 16.2^{o}C on the 6th September 2005. Following acclimatisation, three sets of triplicate cores were spiked with KH_2PO_4 to achieve overlying water-column phosphorus concentrations of 0.5, 5, and 10 mg P L^{-1}. A fourth set of triplicate cores had no additional phosphate added and represented ambient lake conditions. To maintain oxic conditions at the sediment-water interface, air was bubbled through the overlying water with care being taken to ensure minimal sediment disturbance.

Water samples (10ml) were removed from the bottom water (0-1 cm above the sediment) at the beginning and end of a 72 h incubation. A pilot study indicated that, under these conditions, phosphorus equilibrium across the interface was achieved over this time period. Uptake/release values were calculated as the difference between initial

and final overlying water SRP concentration. The range of concentrations used resulted in a linear plot of uptake/release versus overlying-water SRP concentration, enabling the EPC0 values to be estimated using linear regression. Estimates of daily sediment-P release were taken from the three cores that had had no P additions, after 24 h of incubation and are expressed as mg SRP per m^{-2} lake surface area d^{-1}.

Statistical analysis

Seasonal changes in average lake sediment-P composition were assessed by comparing the average monthly conditions of the six sites. The spatial (depth) and temporal (seasonal) variation in bottom water temperature and DO%, pore and bottom water SRP, labile P, reductant-soluble SRP, organic P, and sediment TP was assessed using two-way analysis of variance (ANOVA; total df = 59; α = 0.05). Comparisons undertaken were: 1) between warm water (11th May - 7th October 2004; n = 5) and cold water (23rd November 2004 - 14th April 2005; n = 5) conditions (seasonal variation df = 1), and 2) along a gradient of overlying water depth (spatial variation; df = 5). Interaction effects between season and depth were also considered (df = 5).

The spatial (with overlying water depth) and temporal (annual, cold water season, and warm water season means) relationships between a number of P fractions (the absolute concentrations of labile P, reductant-soluble SRP, reductant-soluble SURP, metal-oxide adsorbed P, organic P, apatite-bound P, residual P, and total sediment-P) were assessed with significant relations observed using regression analysis. Stepwise regression was first used to identify the variables that explained significant additional independent variance (p to enter = 0.15) for each of the P fractions. Explanatory variables included in the stepwise regression were: pore, surface, and bottom water SRP; surface water TP; surface water, bottom water and sediment

chlorophyll *a* concentration; bottom water (*in situ* measurements conducted at ~30cm above sediment surface), DO %, temperature, pH and conductivity. Only significant relationships ($p < 0.05$) were included in the final regression models. All statistical analyses were carried out using Minitab statistical software version 14 (Minitab Ltd., Coventry, UK).

RESULTS

Physical variables measured

Seasonal and spatial variation

The water-column was generally well-mixed throughout the year, with very weak temperature and oxygen gradients present in the warm water period. Weak stratification was only observed in May 2004, with a thermocline between 8 and 9 m water depth. The DO% saturation at 0.5 m above the deepest sediment sampled (22 m overlying water depth) was never below 62%. Average bottom water temperature (Table 3.1) was 14.0 ^{o}C in the "warm water period" (May 2004 - October 2004) and 4.9 ^{o}C in the "cold water period" (November 2004 - April 2005). DO % decreased with increasing water depth and was significantly lower in the warm water period (Table 3.1).

Water-column phosphorus and chlorophyll a concentrations

Temporal variation

Bottom water SRP concentrations (Figure 3.2a) exceeded surface water concentrations for the majority of the year and both showed peaks in winter (November 2004- February 2005) and summer (July 2004 – September 2004). Bottom water

chlorophyll *a* concentrations were higher than surface chlorophyll *a* concentrations throughout the year (Figure 3.2b) and similar seasonal variation was observed in both.

Seasonal and spatial variation

Bottom water SRP concentrations increased with overlying water depth under both warm and cold water conditions (Figure 3.4 and Table 3.1). Pore water SRP concentrations were higher in all sites under cold water conditions, showing little spatial variation throughout. Regression analysis (Table 3.3) indicated no significant relationships between pore water SRP and other variables under cold water conditions. Under warm water conditions, however, a significant positive relationship between pore water SRP and sediment chlorophyll *a* was observed. Bottom water SRP decreased with increasing DO% and sediment TP under cold water conditions but solely with increasing DO%, under warm water conditions.

Table 3.1. *Effects of season (warm v cold water) and overlying water depth on bottom water dissolved oxygen % saturation, temperature and phosphorus concentrations in bottom water and sediments.*

Variable	Season		Depth		Interaction	
	P value	F value	P value	F value	P value	F value
Bottom water						
DO%	<0.01	21.74	<0.01	5.52	<0.01	4.61
Temperature	<0.01	243.01				
SRP	0.02	2.95				
Sediment						
Pore water SRP	<0.01	26.77				
Reductant-soluble P			<0.01	3.95		
Organic P	0.04	4.35	0.01	3.28		
Apatite bound P	<0.01	14.23	0.01	3.25	0.03	2.79
Residual P			0.05	2.42		
Sediment TP			<0.01	4.40		
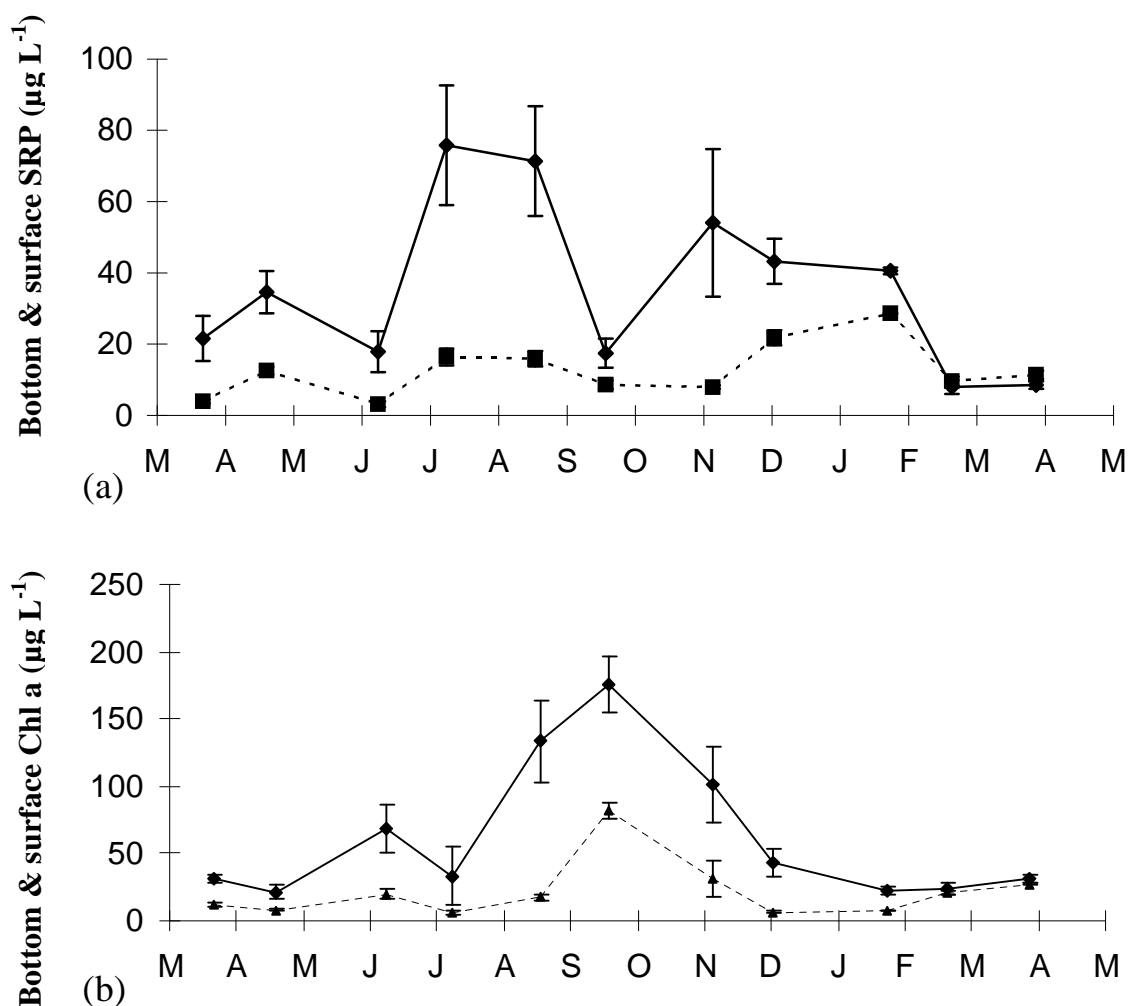
Figure 3.2. *Temporal variation in (a) bottom (full line) and surface (dashed line) water SRP concentration and (b) bottom (full line) and surface (dashed line) water chlorophyll a concentration. Each value represents the average value of the six sites sampled. Error bars represent the standard error of the mean.*

Sediment phosphorus fractions

Temporal variation

Monthly-average concentrations of labile P peaked in May and remained low for the rest of the year (Figure 3.3). Reductant-soluble SRP decreased in April, August, and December but was above 0.80 mg g^{-1} dw in all other months. The low values in August and December corresponded with the lowest concentrations of labile P and the peaks in

surface and bottom water SRP concentrations. Organic P varied little with the exception of a marked decrease in September corresponding with peaks in both labile and reductant-soluble P fractions. Total sediment-P showed little temporal variation with the exception of a peak in mid-winter.

Table 3.2. *Composition of sediment-P fractions expressed as % annual composition of total sediment-P. Values in brackets represent the standard error of the mean (n = 11). Red Sol = reductant-soluble SRP; Red Sol SURP = reductant-soluble soluble un-reactive P; Met ox = Metal oxide adsorbed P; Org = organic P; Res = residual P.*

Depth (m)	Labile	Red Sol	Red Sol SURP	Met ox	Org	Apatite	Res
2.0	2.3 (0.8)	23.2 (4.4)	2.9 (1.3)	22.9 (2.9)	15.9 (2.5)	20.9 (5.0)	12.0 (1.9)
2.5	2.1 (0.7)	28.2 (4.4)	5.5 (2.8)	18.1 (3.0)	16.0 (2.3)	15.9 (2.2)	14.1 (1.8)
3.5	2.4 (1.0)	30.6 (4.6)	4.3 (1.2)	16.0 (4.0)	19.0 (2.8)	13.0 (1.2)	14.7 (1.6)
5.5	1.5 (0.4)	39.5 (5.0)	3.3 (1.1)	16.2 (5.3)	16.8 (1.8)	9.5 (0.6)	13.2 (1.5)
10.0	1.1 (0.3)	33.4 (5.4)	4.7 (1.5)	16.2 (4.8)	16.5 (2.5)	14.4 (1.3)	13.6 (1.5)
22.0	1.4 (0.4)	34.2 (5.0)	5.1 (1.8)	17.7 (4.5)	15.3 (3.6)	12.2 (1.6)	14.0 (1.1)

Seasonal and spatial variation

The reductant-soluble SRP pool was the most abundant fraction throughout the lake (Table 3.2). The labile P pool was the smallest fraction of sediment TP. On an annual basis, the spatial variation in sediment TP was significantly related to organic P (Table 3.3).

Reductant-soluble P varied significantly with water depth with the highest values observed at intermediate depths (Figure 3.4 and Table 3.1). Organic P was consistently higher under cold water conditions and increased with overlying water depth. Apatite bound P varied significantly with season and depth. Residual P and sediment TP both varied spatially but not seasonally.

Table 3.3. *Summary of results of the multiple regression analysis used to assess the relations between the spatial variation observed in pore water P ($\mu g\ L^{-1}$), bottom (bot) water SRP ($\mu g\ L^{-1}$), organic P (org), labile P, reductant-soluble P (Red), and sediment TP (sed TP) (all as mg g^{-1} dw). Sed chl a = sediment chlorophyll a ($\mu g\ g^{-1}$ dw), DO% = dissolved oxygen % saturation, bot chl a = bottom water chlorophyll a ($\mu g\ L^{-1}$).*

Relations	P-value	Equation of line y = ….	R^2
Annual			
Sed TP	<0.01	0.98 + 4.11 org	0.96
Cool			
Pore SRP	N/A	N/A	N/A
Bot SRP	<0.01	1804 - 17.1 DO% - 16.3 sed TP	0.97
Red	0.02	-0.59 + 3.0 org	0.76
Labile	0.04	0.06 – 0.0006 bot SRP	0.69
Warm			
Pore SRP	<0.01	19.7 + 1.04 sed chl a	0.87
Bot SRP	<0.01	228.1 - 2.03 DO%	0.96
Red	0.02	1.45 - 0.05 Apat - 2.97 labile	0.92
Org	<0.01	-0.4 + 0.26 sed TP + 0.0008 bot Chl a	0.98
Labile	0.02	-0.001 + 0.17 org	0.79

Reductant-soluble P was positively related to organic P under cold water conditions but negatively related to a combination of apatite-bound and labile P fractions under warm water conditions (Table 3.3). Labile P was negatively correlated with bottom water SRP under cold water conditions but positively correlated with organic P under warm water conditions. Organic P was positively correlated with reductant-soluble P under cold water conditions and positively correlated with a combination of sediment TP and bottom water chlorophyll *a* under warm water conditions.
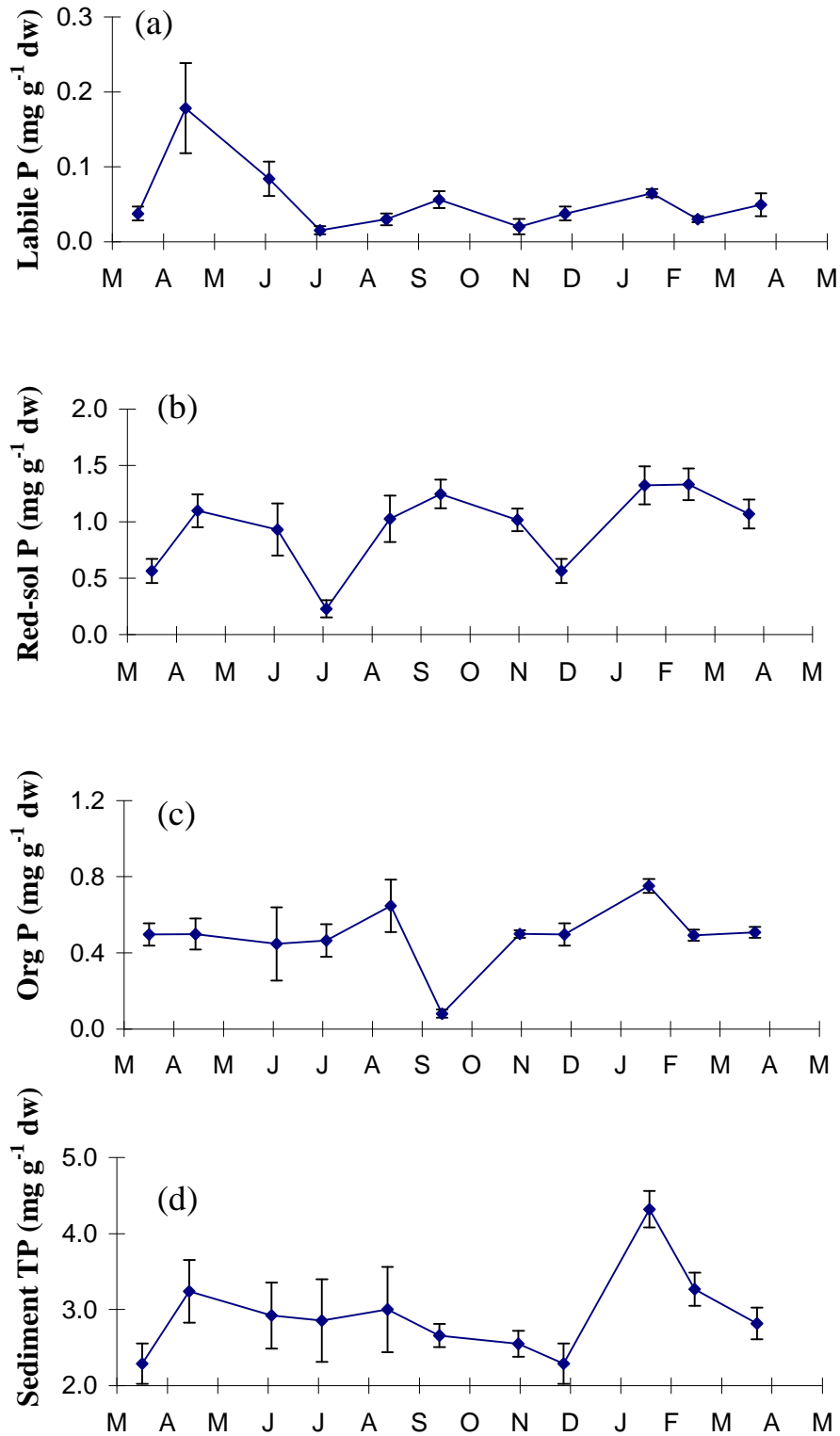
Figure 3.3. *Temporal variation in (a) labile P, (b) reductant-soluble P, (c) organic P, and (d) total sediment-P. Each value represents the average value of the six sites sampled. Error bars represent the standard error of the mean.*
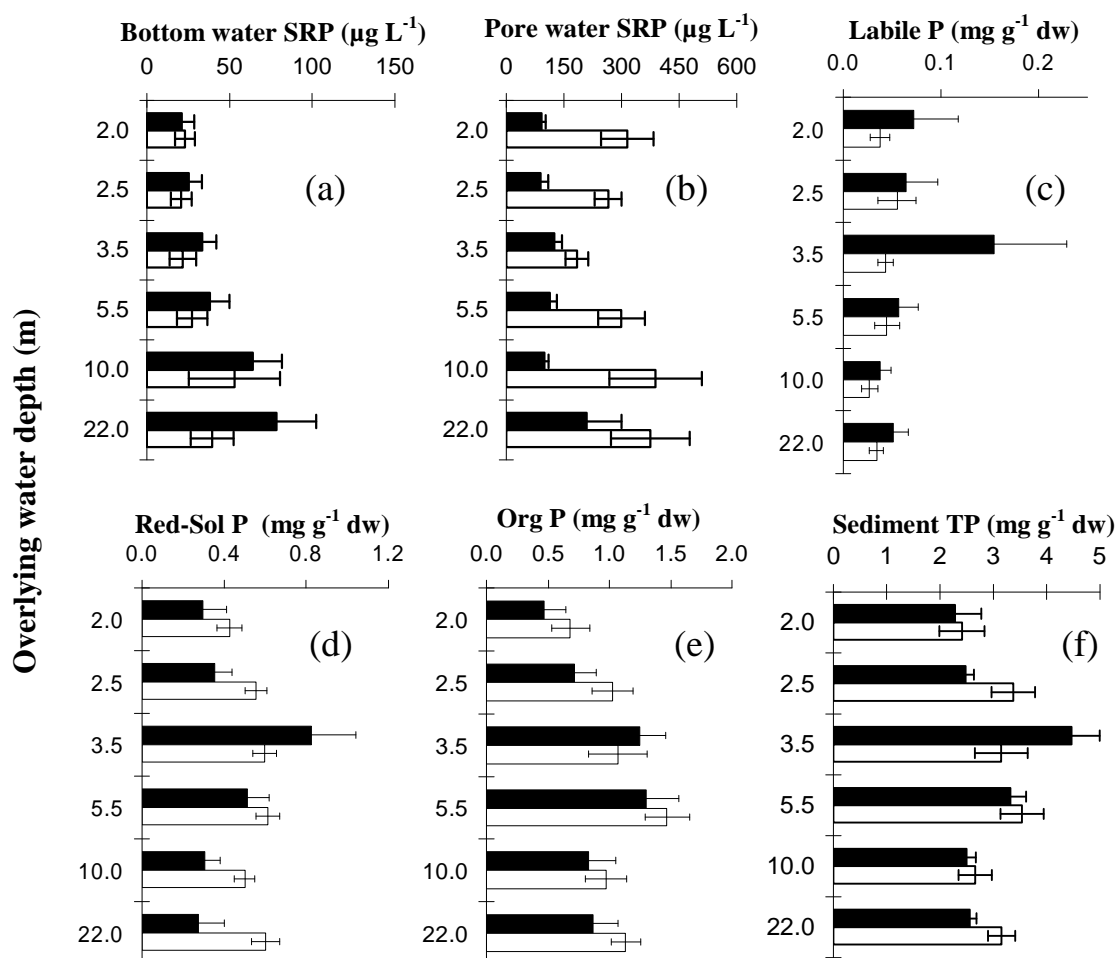
Figure 3.4. *The spatial (depth gradient) versus seasonal (warm versus cool bottom water) variation in (a) bottom water SRP concentration, (b) pore water SRP concentration, (c) labile P, (d) reductant-soluble P, (e) organic P, and (f) total sediment-P. Each bar represents either the cold (white bar) or warm (black bar) water period average value at a given depth. Error bars represent the standard error of the mean.*

Table 3.4. *Ambient conditions of bottom water on the 8th of August and the 6th of September 2005.*

Date	DO%	Conductivity (μS cm)	pH	Temperature (^{o}C)	Secchi depth (m)
8/8/05	94.4	217	8.92	15.38	0.98
6/9/05	90.4	233	8.37	16.20	0.93

Equilibrium phosphorus concentration experiments

The ambient lake conditions on both experimental dates are given (Table 3.4). The results of equilibrium phosphorus concentration experiments should only be considered as representative of the experimental conditions described in the methods section for each date. Sediment-P uptake increased linearly in both experiments with increasing overlying water SRP concentration (Figure 3.5) and EPC0 estimates were similar on both dates, with P release estimates for both experiments ~ 12 mg P m^{-2} d^{-1}.

Table 3.5. *Summarised results of the intact core sediment-P release experiments. Table includes the linear relationship between the initial overlying water-column concentration (x) and P uptake/release (y), the equilibrium phosphate concentration (EPC0 in µg L^{-1}), and the gross sediment-P release values (mg SRP m^{-2} d^{-1}). Values in brackets represent the percent error of the estimate.*

Date	Equation of line	P	R^2	EPC0	P release
8-8-2005	Y = 0.798x –215.7	0.0024	0.995	270.4	12.91 (8%)
6-9-2005	Y = 0.862x – 155.3	0.0009	0.998	180.2	12.60 (10%)

DISCUSSION

Temporal variation of concentration gradients

Bottom water SRP was consistently higher than surface water SRP and this difference was greatest during peaks of both, suggesting the main P source of SRP to the surface water was from the bed rather than from the catchment. The troughs in both the bottom and surface water SRP concentrations were associated with peaks in chlorophyll *a* concentrations in bottom and surface waters. However, using chlorophyll *a* to interpret processes occurring in the epilimnion and hypolimnion requires caution since bottom water chlorophyll *a* concentrations may represent both settling and

upwardly migrating phytoplankton (Petterson *et al.,* 1993) and resuspended benthic algae. Also, cyanobacteria capable of migrating between the sediment and the water-column characteristically dominate the phytoplankton during mid-summer in Loch Leven (Carvalho and Kirika, 2005).
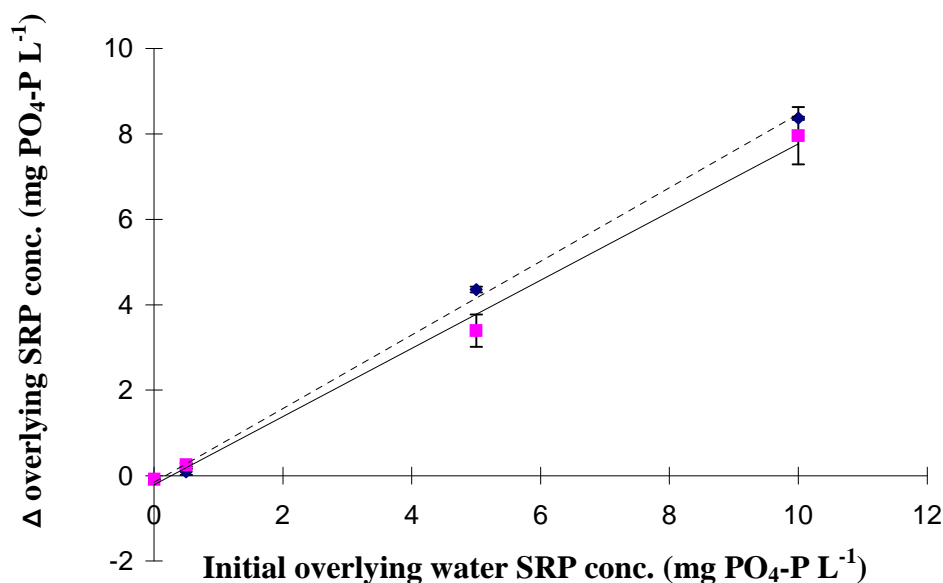
Figure 3.5. *Intact sediment core EPC0 experiments. Square tabs represent the SRP uptake/release (Δ overlying SRP concentration) versus initial overlying water SRP concentration plots for the 8th August 2005 and triangular tabs represent the results for the 6th of September 2005.*

Both water-column SRP peaks corresponded with the lowest values of reductant-soluble P and labile P indicating that the main source of P was redox-sensitive and sediment-derived. Additionally, the multiple regression analysis indicated a negative correlation between bottom water SRP and DO% saturation under both warm and cold conditions. This uptake/release pathway agrees with the classical P mobility model where SRP is released from the sediment under anoxic conditions (Mortimer

1941; 1942; Boström *et al.,* 1988). It, therefore, seems evident that the P fraction responsible for the large, short-term, P release events from the sediments of Loch Leven is the reductant-soluble P pool.

The effects of increased temperature may be important in causing the mid-summer peak where high P release rates have been observed (Kamp-Nielson, 1975). However, other factors must be driving the winter peaks in water-column SRP. Part of the winter peak in water-column SRP may be derived from increased external loads, however, the synchronised occurrence of the winter reductant-soluble P decrease and water-column SRP peaks suggests that a redox initiated release from sediments may also make an important contribution. Although the bottom water DO% levels are higher (on average) in winter than in summer, this finding agrees with studies of internal loading in deep stratifying lakes. Penn *et al.* (2000) observed two main release events, the largest of which occurred in mid-summer with an event of lesser magnitude occurring in early winter. This seasonal pattern has also been reported by Kamp-Neilson (1975a). Anoxia may occur in winter as a result of an overall shift from an autotrophically dominated system in summer (high nutrients, high intensity/duration light, high temperatures) to a heterotrophically dominated system in winter (low nutrients, low intensity/duration light, low temperatures). Thus, under summer conditions, temperature may drive DO concentrations down through a mixture of decreased solubility and increased microbial demand, with photosynthetic production of DO acting to buffer anoxia. However, it is hypothesised that in winter, the low rates of photosynthetic production may be insufficient to maintain sediment DO levels, resulting in localised anoxia at the sediment surface. It should be noted that further study is required, both within the laboratory and field, to substantiate these hypotheses.

Temperature regulated P cycling

Pore-bottom water concentration gradients

Pore water SRP concentrations generally exceeded bottom water SRP concentrations suggesting chemical gradients supportive of P release throughout the year. A comparison of the seasonal variations in pore and bottom water SRP concentrations revealed that concentration gradients were reduced during the warm water months where pore water SRP concentrations were lowest and bottom water SRP concentrations were highest. This suggests that the transfer of P from pore to bottom waters was greater under warm water conditions.

Under cold water conditions, pore water SRP concentrations were highest and bottom water SRP concentrations were lowest. This suggests less release from pore to bottom waters under cold water conditions. In this colder period, however, concentration gradients were greatest in the two deepest sites indicating that the key release mechanisms acting to facilitate the transfer of P from pore to bottom waters in winter were greatest in shallower waters and least in deeper waters. It is likely that the release from shallow water sediments is enhanced by sediment resuspension (Marsden, 1989).

The positive relationship between pore water P and sediment chlorophyll *a* under warm water conditions indicates that, even though P release from pore to bottom waters is highest in the warm water period, release is less in areas of the lake bed where sediment autotrophic biomass is highest. There are two plausible explanations for this capping of pore water P by autotrophic biofilms: firstly, this community helps maintain an oxygenated boundary layer at the sediment-water interface, and secondly, there is strong evidence, albeit from estuarine mudflat ecosystem studies, that benthic algae can

reduce resuspension-related release via biostabilisation of the sediment surface (e.g. Underwood and Paterson, 1993; Van Luijn *et al.,* 1995; Perkins *et al.,* 2004).

Sediment-P fractions

Reductant-soluble P is the dominant form of phosphorus within the sediment. Regression analysis indicated that, under cold water conditions, reductant-soluble P varied positively with organic P. This relationship may result from increased microbial remineralisation under aerobic conditions. Under warm water conditions, reductant-soluble P varied negatively with apatite-bound P and labile P. Again this suggests P transfer between sediment pools. The driving mechanism may be pH. Labile P and pH were significantly and positively correlated under cold water conditions. This agrees with Penn *et al*. (2000) where release rates of P from the labile P pool were highest at lower pH conditions. The release mechanism here is expected to be the pH-induced dissolution of $CaCO_3$ (Driscoll *et al*., 1993). Additionally, Søndergaard (1989) observed a positive relationship between labile P and pore water pH. If release from any pool occurs under oxic conditions, P is likely to be transferred into the reductant-soluble P pool.

The effects of temperature in regulating internal P release have been well documented (e.g. Søndergaard, 1989; Boström *et al*., 1988; Jensen and Anderson, 1992). It is generally agreed that, under warm conditions, the organic P pool is mineralised by increased microbial activity. Deoxygenation of the sediment-water interface through increased biological oxygen demand, as demonstrated here, is well documented (Boström *et al*., 1988). Another temperature-driven organic P related process is the translocation of P through the recruitment of cyanobacterial tychoplankton (Karlsson-Elfgren *et al*., 2004). Upward translocation rates of TP from

the sediment, mostly attributable to cyanobacterial recruitment, have been estimated at 2 - 40 mg P m^{-2} d^{-1} for a range of sites (Pettersson *et al*., 1993; Barbiero and Kann, 1994, Head *et al*., 1999). Further evidence of cyanobacterial recruitment is the sudden reduction in organic P corresponding with the peak in water-column chlorophyll *a* concentration and SRP reduction in late summer. This explanation is supported by Pettersson *et al*. (1993) who estimated that the P contribution of *Gloeotrichia echinulata* in sediment (before migration) could be 45% of sediment TP.

Regression analyses of the spatial variation in organic P also highlighted the importance of biogenic P cycling. Under warm water conditions organic P was significantly related to bottom water chlorophyll *a*. This suggested that algae may be the main component of the organic P pool under warm water conditions. The bottom chlorophyll *a* concentrations also indicated that translocation of organic P (through both settling and recruitment) was highest under warm water conditions.

Sediment-P release estimates

The EPC0 experiments indicated that Loch Leven sediment was still capable of significant P release. The release rates measured did not, however, represent the average conditions throughout the year. The temperatures on the days of the experiments were the maximum temperatures recorded throughout the sediment-P fractionation survey. However, under these experimental conditions, the sediment continually released phosphorus and so physico-chemical adsorption into the sediment was unlikely. Instead the main source of P to the sediment is likely to be particulate (e.g. planktonic detritus). This argument is supported by the high content of organic P in the sediment of this, and many other eutrophic shallow lakes (e.g. Phillips *et al.,* 1994).

The linearity of the sediment PO_4-P uptake/release plots would suggest that Loch Leven sediments are currently far below their adsorptive saturation levels (Hwang *et al.,* 1976). This finding agrees with previous work where estimates of saturation levels were <16% (Spears *et al.,* 2006a) indicating that Loch Leven has a very high P buffering capacity and is capable of storing much more P within the sediment. The estimated EPC0 values indicated that under experimental conditions, bottom water SRP concentration would have to exceed at least 180 µg L^{-1} for physico-chemical sediment uptake to occur. Surface water SRP concentrations have never exceeded this value in recorded history (Carvalho and Kirika, 2003). Estimates from traditional (i.e. shaken sediment) EPC0 measurements for Loch Leven sediment yielded EPC0 estimates of between 9-30 µg L^{-1} (Spears *et al.,* 2006a). These estimates may be considered representative of re-suspended sediment suggesting that, under conditions of re-suspension, P would be stripped from the water-column at SRP concentrations in excess of 30µg L^{-1}. The difference between EPC0 estimates taken from intact cores and continuously shaken slurry clearly highlights the importance of an undisturbed sediment-water interface in regulating internal P release.

Estimated release rates, though probably indicative of near maximum rates in warm conditions, varied little between the two experiments (~12 mg P m^{-2} d^{-1}). With an external P load of 1.62 mg P m^{-2} d^{-1} (in 1995), internal loading may play an important role in the maintenance of eutrophic conditions within the lake (Bailey-Watts and Kirika, 1999). These release rates are in agreement with other comparable studies, under warm, aerobic conditions, for example 10.3 mg P m^{-2} d^{-1} (hypereutrophic Onondaga Lake, N.Y. USA, Penn *et al.,* 2000) and 278 mg P m^{-2} d^{-1} (South Walsham Broad, UK, Phillips *et al.,* 1994).

CONCLUSIONS

This study highlighted several factors that drive the seasonality of P release. Firstly the sedimentation and mineralisation of organic P is important in replenishing the release-sensitive P fractions. Under aerobic conditions, P released as a result of microbial mineralisation is transferred to the reductant-soluble, labile and pore water P pools. This process maintains release conditions for much of the year with concentration gradients indicative of negative retention present for 10 months. In shallow waters, pore water P concentrations remain low, probably due to wind-induced sediment resuspension. Large short-term release events result from the onset of anoxia where the reductant-soluble P pool is greatly depleted in mid-summer and winter. We need to extend the measurements further to determine whether the gross release rates reported here are characteristic of annual average conditions. Evidence is provided here on the importance of heterotrophic and autotrophic microbial communities in regulating sediment-P release. The specific roles of functional groups within both communities and the environmental conditions under which they operate require further attention.

These results provide valuable lake management information where a large proportion of the release sensitive sediment-P is transferred from the sediment to the water-column in mid-summer, either via redox-regulated processes or through organic P translocation. In systems where flow regulation is achievable, increasing the flushing rates to correspond with the above transfer of P from the sediment to the water-column will help recovery from eutrophication.

CHAPTER 4
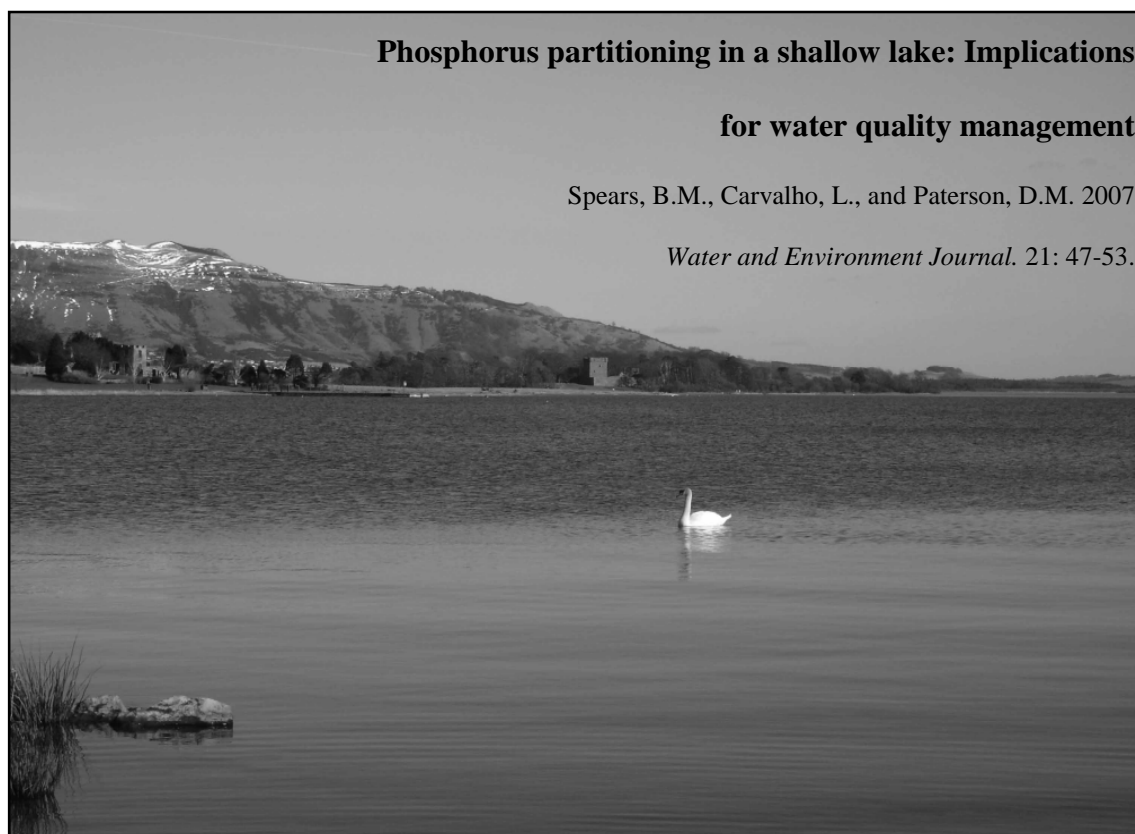
Phosphorus partitioning in a shallow lake: Implications

for water quality management

Spears, B.M., Carvalho, L., and Paterson, D.M. 2007

Water and Environment Journal. 21: 47-53.

(Winning paper in the Chartered Institute of Water and Environmental Management Young Scottish Author of the Year Award 2006 and voted the best student paper at the 2006 Freshwater Biological Association Annual Scientific Meeting). The structure of this chapter follows the format of the Water and Environment Journal in which the discussion and results are provided in one general section.

Phosphorus partitioning in a shallow lake: Implications

for water quality management

ABSTRACT

This paper describes the seasonal partitioning of phosphorus across the sediment-water interface in Loch Leven, Scotland, and discusses the implications for future lake management strategies with respect to recovery from eutrophication. In a 10 month survey, surface water total phosphorus concentrations were highest in late-summer and lowest in early-spring. In contrast, sediment total phosphorus concentrations were highest in mid-winter and lowest in late-summer. Water discharge at the main outflow of the loch was highest when water-column total phosphorus was low and sediment total phosphorus high and *vice versa*. Monthly sediment-P uptake/release values showed significant cycling between the water-column and the sediment with seasonal variation in four release-sensitive P-pools. Regulating water level to increase flushing during sediment release periods and decrease flushing during uptake periods has the potential to significantly enhance the recovery of shallow lakes and reservoirs following historic nutrient loading.

INTRODUCTION

The restoration of freshwater lakes, impacted by elevated external nutrient load (eutrophication), is recognised as one of the most widespread problems in water quality management. When high external nutrient (mainly nitrogen (N) and phosphorus (P)) loads are sustained for a considerable length of time, the ecological balance within a lake is tipped from a desirable macrophyte dominated clear-water system, to an undesirable turbid system of high nutrients and elevated phytoplankton biomass (Scheffer, 2001). The change in status (both ecological and socio-economic) is generally driven by an increase in total phosphorus (TP) concentration which, in turn, increases phytoplankton productivity and biomass, particularly of toxic blue-green algae. For this reason, the majority of freshwater lake restoration programmes strive towards an improvement in water quality through the reduction of within-lake TP concentrations (Sas, 1989).

The scale of the problem facing water managers was highlighted in a recent risk assessment of all 14,000 lakes in Great Britain (GB) >1 ha in size (Carvalho *et al.*, 2005). This study indicated that 56% of GB lakes are likely to require phosphorus-reduction measures in order to pass environmental objectives set by the Water Framework Directive (WFD) - to achieve 'good status' by the year 2015. In England the picture is even worse, with 64% of lakes currently being in either poor or bad status in relation to likely WFD TP standards. Significant reductions in TP concentrations will, therefore, be required in the majority of GB lakes. This poses a major challenge to lake managers since achieving significant improvements in shallow lake water quality is well known to take many years (if not decades) and is often very costly (Sas, 1989; Jeppesen *et al.*, 2005).
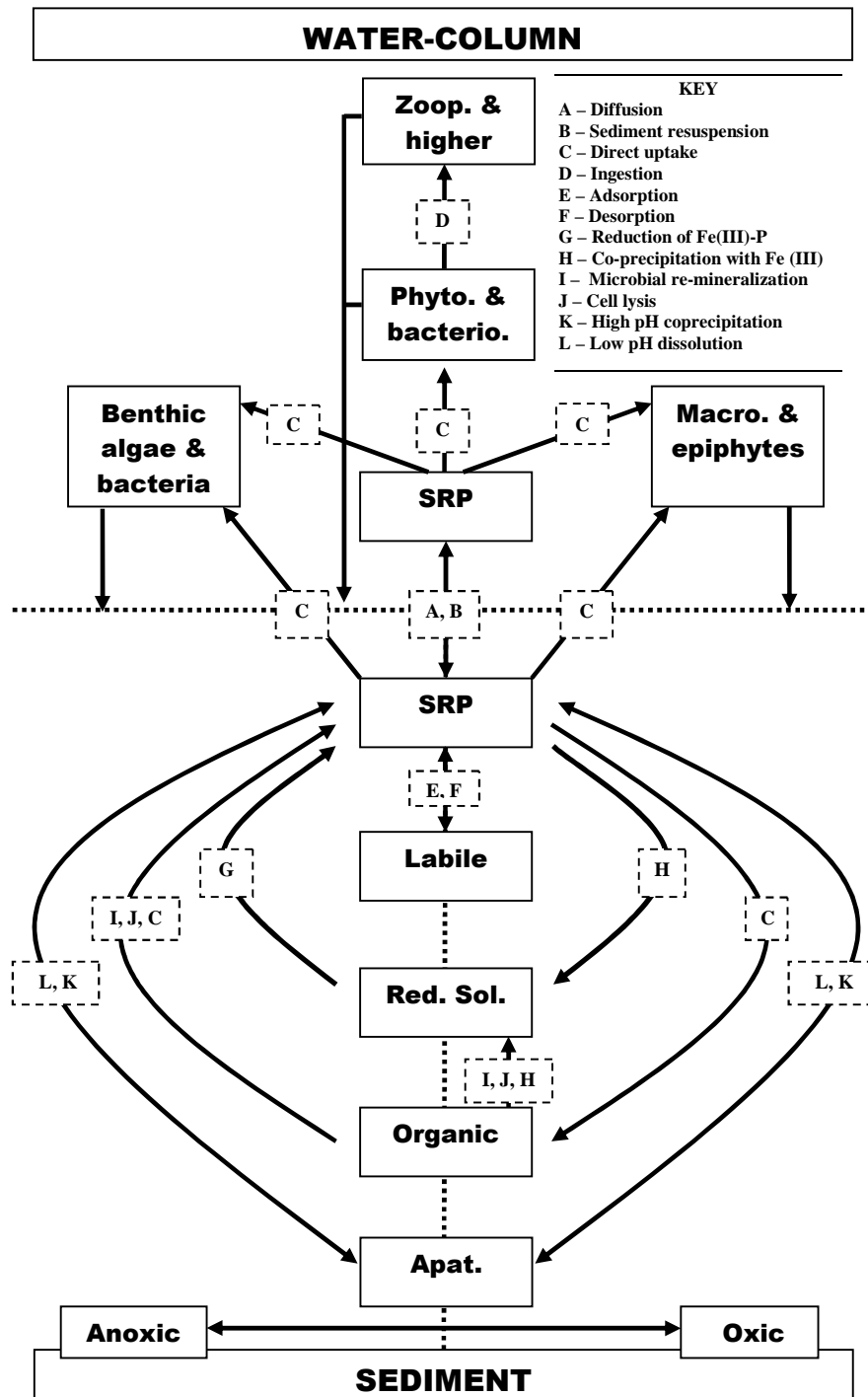
Figure 4.1. *The pathways of release-sensitive phosphorus within anoxic and oxic sediment and between the surface sediment and the water-column. The horizontal dashed line represents the interface between the sediment and the water-column (Zoop. = zooplankton; higher = organisms higher than zooplankton in the food web; phyto. = phytoplankton; bacterio. = bacterioplankton; macro. = macrophytes; SRP = soluble reactive phosphorus; Labile = Labile P; Red. Sol. = reductant-soluble phosphorus; Organic = dead and live organic P; Apat. = Apatite-bound phosphorus).*

A number of management practices are available for the remediation of eutrophic sites (discussed in detail by Moss *et al*., 1996). These practices can be split into primary and secondary treatments. Primary treatments involve the control of the pollutant source (external P load reduction) and include a number of point- and diffuse-source management practices. Secondary treatments act to reduce the effects, or symptoms, of eutrophication by either diverting (e.g. diversion/abstraction of P rich inflows), removing (e.g. sediment dredging), or containing P (e.g. by addition of binding/oxygenating agents) within a system. The removal or containment of lake P may result in short-term benefits, though these efforts have no effect on the ultimate cause of the problem, the external sources of P (Perkins and Underwood, 2001). However, a reduction in external P load alone, may not be sufficient to produce an improvement in water quality, particularly in the short-term (e.g. within 5 years: Carvalho and Kirika, 2003). For this reason, a combination of primary and secondary treatments is likely the most effective restoration strategy for successful recovery.

Prolonged recovery from eutrophication, following primary management practices, has been observed worldwide and is caused by the release of bioavailable P (soluble reactive phosphorus – SRP) from enriched lake sediments (internal loading) (Phillips *et al*., 2005). The ultimate success of primary management practices is, therefore, governed by internal loading and the flushing of sediment released P from the system. If this flushing can be achieved, a gradual reduction in whole system TP (both sediment and water pools) will occur. A lake's flushing rate, and therefore its ability to relinquish P, may greatly influence the speed of recovery (Sas, 1989). Lakes with a low flushing rate provide conditions that promote the cycling of autochthonous nutrients and therefore act to maintain internal loading for many years, post attempted restoration.

Where possible, management of flushing rates may, therefore, be a key tool in restoring freshwaters to 'good status'.

Internal loading is part of a natural cycle within a lake (Figure 4.1), a process that is by no means one-way. Even when conditions of sediment-P release are favoured, significant amounts of P may be returned to the sediment as detritus (both inorganic and organic; Boström *et al*., 1989) or as phytoplankton resting stages (Heaney *et al.,* 1983; McQuoid and Hobson, 1996).

The magnitude of sediment-P release depends upon a number of biogeochemical variables (Figure 4.1). These variables act to control the partitioning of sediment-P within a range of sediment pools. These pools include labile P (e.g. loosely bound sediment-P), reductant-soluble P (e.g. Fe- and Mn-P complexes), organic P (e.g. P contained within dead and live organisms and their breakdown products) and apatite P (e.g. Ca-P complexes). The environmental release mechanisms for each pool vary (Figure 4.1), resulting in a number of different P release pathways. Although it is important to understand the mechanisms acting to partition P within the sediment, it is also important to study the factors regulating P mobility across an intact sediment-water interface. Recent studies have suggested that benthic algae may play an important role in limiting P release (Woodruff *et al.*, 1999; Spears *et al.*, 2006 a and b). The main mechanisms of regulation include the maintenance of a well-oxygenated sediment surface through the photosynthetic production of dissolved oxygen (i.e. promoting co-precipitation of P with Fe (III)), minimising sediment re-suspension via the formation of stabilising bio-matrices, and the buffering of P release through direct uptake (Paterson 1989; Paterson *et al*., 2003; Woodruff *et al*., 1999; Underwood and Paterson, 2003). Sediment colonisation by macrophytes is thought to play a similar role, though the relative roles of each assemblage remain greatly understudied (Dudley and Walker,

2000; Dudley *et al.*, 2001). Other variables known to exert pressure on P mobility across a sediment-water interface include turbulent mixing, bioturbation, gas ebullition, and the development of concentration gradients (Boström *et al.*, 1989).

By investigating these variables, we can begin to predict the likelihood of release and, from this, the dominant pools of P in a lake (i.e. in the sediment or water-column) throughout the year. The effectiveness of removal/containment practices depends greatly on the partitioning of P at the time of their application. In an attempt to aid the planning of future management strategies, this paper uses recent work conducted within a recovering shallow lake (Loch Leven, Kinross, Scotland) to; (a) highlight the seasonality of P-partitioning between the water-column and the sediment; (b) investigate the seasonality of P uptake/release from four release-sensitive sediment-P pools; and (c) discuss the implications of P-partitioning for improving water quality *via* regulated lake flushing.

QUANTIFYING SEDIMENT PHOSPHORUS RELEASE

Loch Leven (latitude $56^o10'$ N, longitude $3^o30'$ W) is a shallow (mean depth 3.9 m) eutrophic lake of surface area 13.3 km^2 (Figure 4.2). Intermittent stratification occurs in two deep basins (constitute about 5% total lake surface area), both of about 25 m overlying water depth. Water-column and sediment TP concentrations and sediment uptake/release estimates reported in this study represent the average values taken from six sites within Loch Leven between April 2004 and April 2005. The six sites were chosen to represent a depth gradient (2 m, 2.5 m, 3.5 m, 5.5 m, 10 m, and 22 m overlying water depth). Further site information is discussed in Spears *et al.* (2006a). Sediment samples were collected using a Jenkin surface-sediment sampler which facilitated the collection of the upper 3 cm of sediment. Sediment-P pool concentrations

were quantified using a sequential chemical extraction technique (Farmer *et al*., 1994) and subsequent P analysis (Wetzel and Likens, 2000). Whole-system sediment-P concentrations were estimated using an active sediment surface area of 7 km^2 (Farmer *et al.*, 1994). Monthly sediment-P uptake/release values were estimated from the change in concentration of each pool between sampling dates. Data for discharge at the outflow were provided by the Tullis Russel Group.
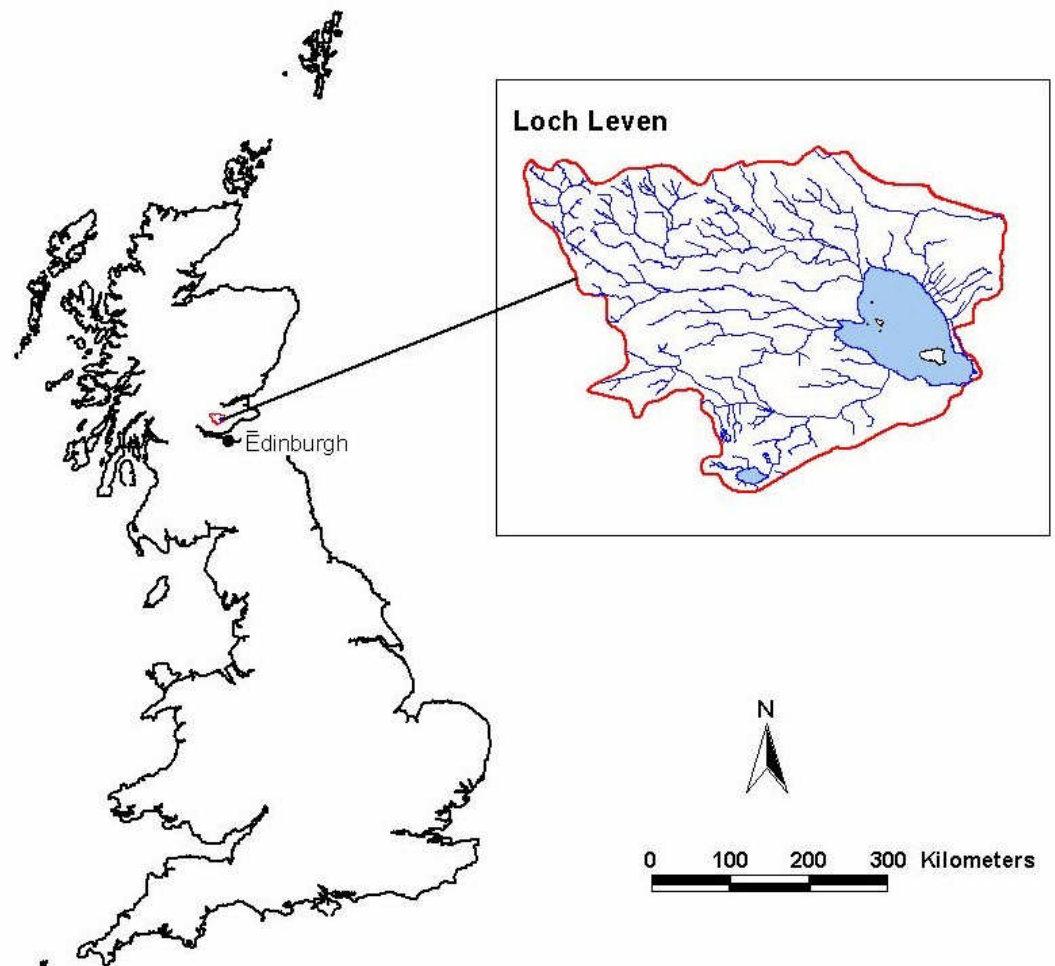

Figure 4.2. *Loch Leven and its catchment.*

SOLUBLE REACTIVE PHOSPHORUS VARIATION IN A RECOVERING LAKE

A wide range of limnological variables have been monitored at Loch Leven since 1967 providing one of few global long-term data sets with which the effects of eutrophication, and the recovery process itself, can be investigated (Carvalho *et al*., 2003; Jeppesen *et al*., 2005). The Loch was recently valued at £1.5 m to the local economy but poor water quality events (e.g. toxic algal bloom in 1992) have been estimated to cost the local economy up to £783,000 and increase the cost of downstream industry by £160,000 (LLAMAG, 1993). In an attempt to improve the water quality at the site, primary management practices were carried out (costing in excess of £4 million), successfully reducing external TP load from 20 t TP y^{-1} (1985) to 8 t TP y^{-1} (1995). However, as is the case in many shallow lakes post-restoration (Søndergaard *et al.*, 2001), it has taken many years for improvements in water quality to become evident and even after a period of 10-15 years, these improvements appear limited relative to the reduction in external load (Carvalho and Kirika, 2003; unpublished data).

The average seasonal variation in water-column SRP concentration reveals late-summer peaks (Figure 4.3). This pattern is characteristic of many recovering shallow lakes and, in the absence of significant external P sources, is shaped mainly by the cycling of P between the sediment and the water-column (Søndergaard *et al*., 2005). The depletion of SRP in the spring is caused by the spring algal-bloom which sequesters P within organic particulate matter. The late-summer peak is driven by high magnitude internal release of redox-sensitive (reductant-soluble) sediment-P. This release event is triggered mainly by a depletion of dissolved oxygen in the deeper waters, during periods of high temperatures and microbial metabolism, which results in the reduction of Fe- and Mn-P (reductant-soluble P) sediment complexes, and their subsequent release

(Spears *et al.*, 2006b). Other, lower magnitude, release events may occur throughout the year and are controlled by a number of environmental variables including pH (apatite-bound P), equilibrium conditions (labile P), microbial remineralisation (organic P), and temperature (all release-sensitive sediment forms). Once P has been released from the sediment, it may follow a number of pathways (Figure 4.1). These pathways either return the P to the sediment (e.g. by oxic co-precipitation of SRP with Fe (III) or in settling of P contained within particulate organic/inorganic matter) or result in its removal from the system by flushing. Recent studies have shown that the intensity of internal loading of P in Loch Leven has greatly diminished in recent years, indicating more sustained recovery (Carvalho and Kirika, 2003; Spears *et al.*, 2006a and b).
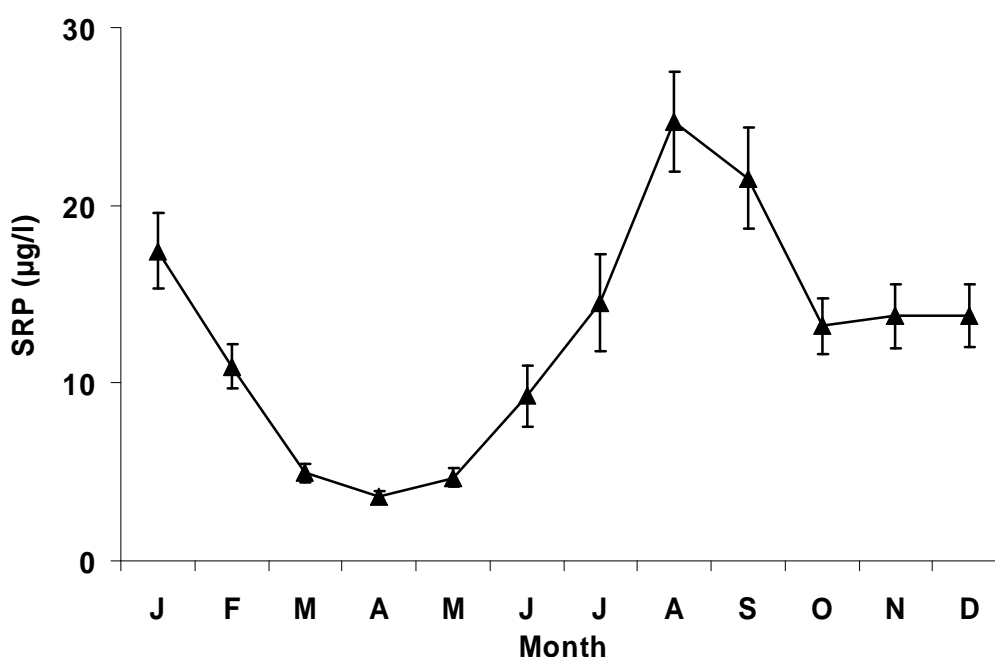

Figure 4.3. *Average (1968-2004) monthly water-column soluble reactive phosphorus (SRP) concentrations in Loch Leven (vertical bars represent the standard error of the mean).*

PHOSPHORUS PARTITIONING BETWEEN THE WATER-COLUMN AND THE SEDIMENTS

Water-column P removal/containment practices (e.g. Fe (III) addition, aeration, flow regulation) will be most effective during high magnitude sediment-P release events, of the type seen in Loch Leven (Figure 4.3). It is, therefore, important to estimate the release-potential of sediment bound P and to identify conditions that greatly affect the pattern and timing of P-partitioning. In general, the concentration of release-sensitive sediment-P will increase with external loading until the sediment is P saturated and, thereafter, any additional SRP entering a lake will persist in the water-column. The sorption capacity of sediment is site specific and varies depending on physico-chemical properties (Hwang *et al*., 1976; House *et al*., 1998). Thus, a site with a high sorption capacity will have a higher release-sensitive sediment-P pool than a site with a low sorption capacity, and where all other considerations are identical, will maintain higher magnitude internal loading for longer. In Loch Leven, a large proportion of the lake's P content is transferable between the water-column and the surface sediment (Figure 4.4). Surface water TP concentrations are highest in mid-summer coinciding with some of the lowest sediment TP values. Conversely, the highest sediment TP values are observed during periods where water-column TP is low (winter/spring). The large scale variation in sediment TP concentration would suggest that a major proportion of the sediment-P pool is sensitive to release. Additionally, the fact that the sediment TP values recovered in winter suggests that the majority of the summer-released P was returned to the sediment in the winter. The return of P to the sediment represents the rate limiting factor in lake recovery from eutrophication and is greatly dependent on lake flushing rate, a variable that is known to be very low (2-3 loch volumes/y) in Loch Leven (Bailey-Watts and Kirika, 1987).
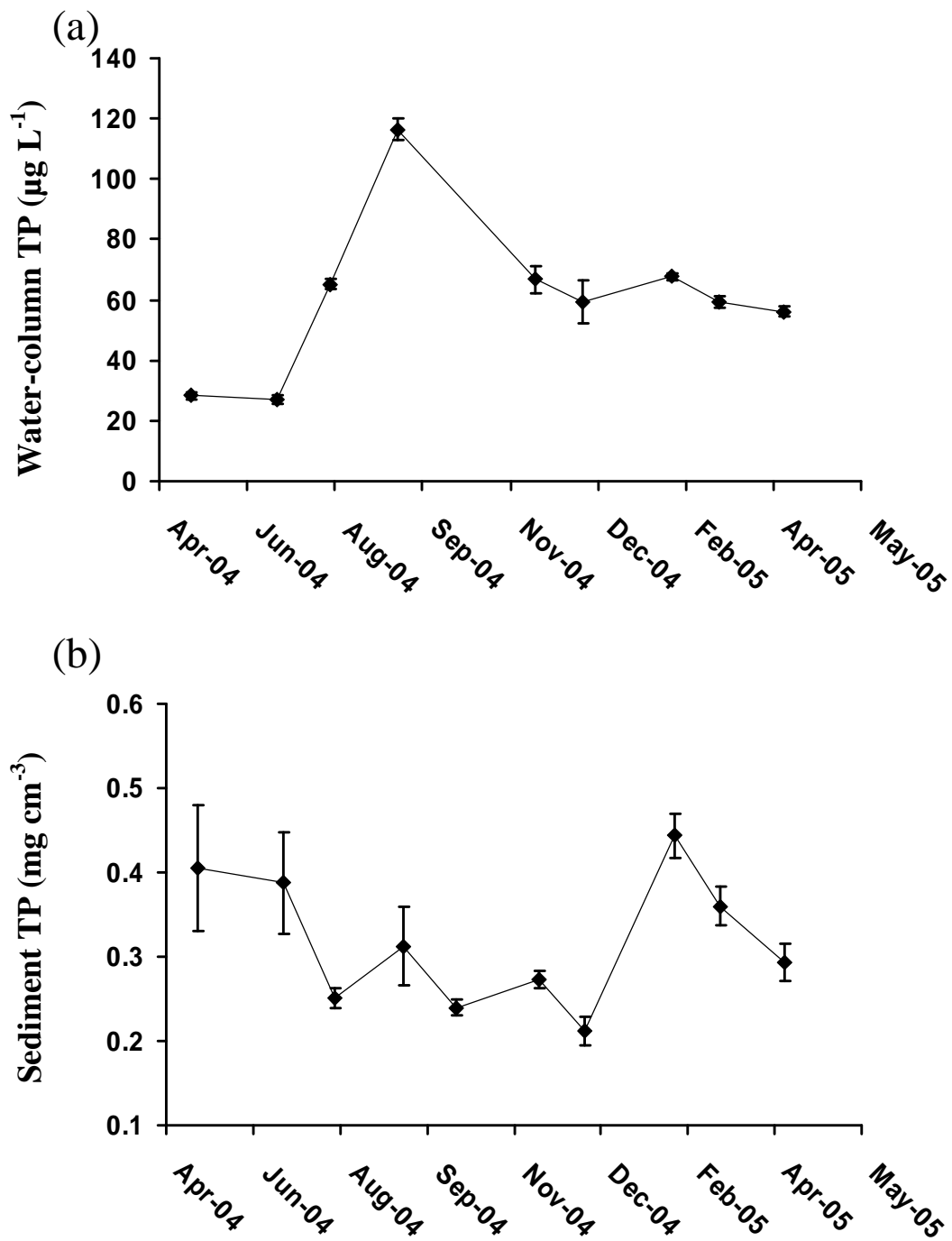
Figure 4.4 *Seasonal variation in (a) water-column and (b) surface sediment (upper 3 cm) total phosphorus (TP) concentrations (vertical bars represent the standard error of the mean of six sample sites).*

SEDIMENT PHOSPHORUS RELEASE AND RETENTION

The ability of the sediment to retain or release P was estimated using monthly uptake/release values calculated from sediment-P pool concentrations (Figure 4.5). In terms of whole-lake sediment TP retention, on average, more P was released from the sediment (92.09 t) than entered it (68.65 t), during the study period, resulting in a net loss of 23 t of TP from the sediment. This is more than double the 1995 external loading estimate of 8 t TP/y and indicates that the lake is still in a state of recovery. Whether all of this P was removed from the system or sequestered into the plankton or macrophytes is not yet known, though a loading survey, designed to estimate whole-loch P-retention, is currently under way. Previous loading surveys conducted in 1985 and 1995 recorded retention of P by the sediment (~10 and ~1 t yr^{-1} respectively) although a reduction in the level of retention was observed (Bailey-Watts and Kirika, 1999). As a large proportion of the sediment released P is returned, it is hypothesised that sediment-P pools will remain high for some time. Additionally, if the recent loss of P from the sediment is sustained, further improvements in water quality should be observed.

It is clear that large quantities of P are cycled between the sediment and the water-column with the largest observed P release (29 t) occurring between June and July 2004 and the largest P gain (49 t) occurring between December 2004 and February 2005. It is the latter that is most significant in terms of prolonging recovery as this process renders almost 50 t of P unavailable for removal from the system, via flushing, during periods of high flow.

The variation in sediment-P composition is highlighted when one considers the percent contribution of the relative pools to net sediment-P uptake/release. The change in sediment TP in May – June, appeared to show the sediment and the water-column P
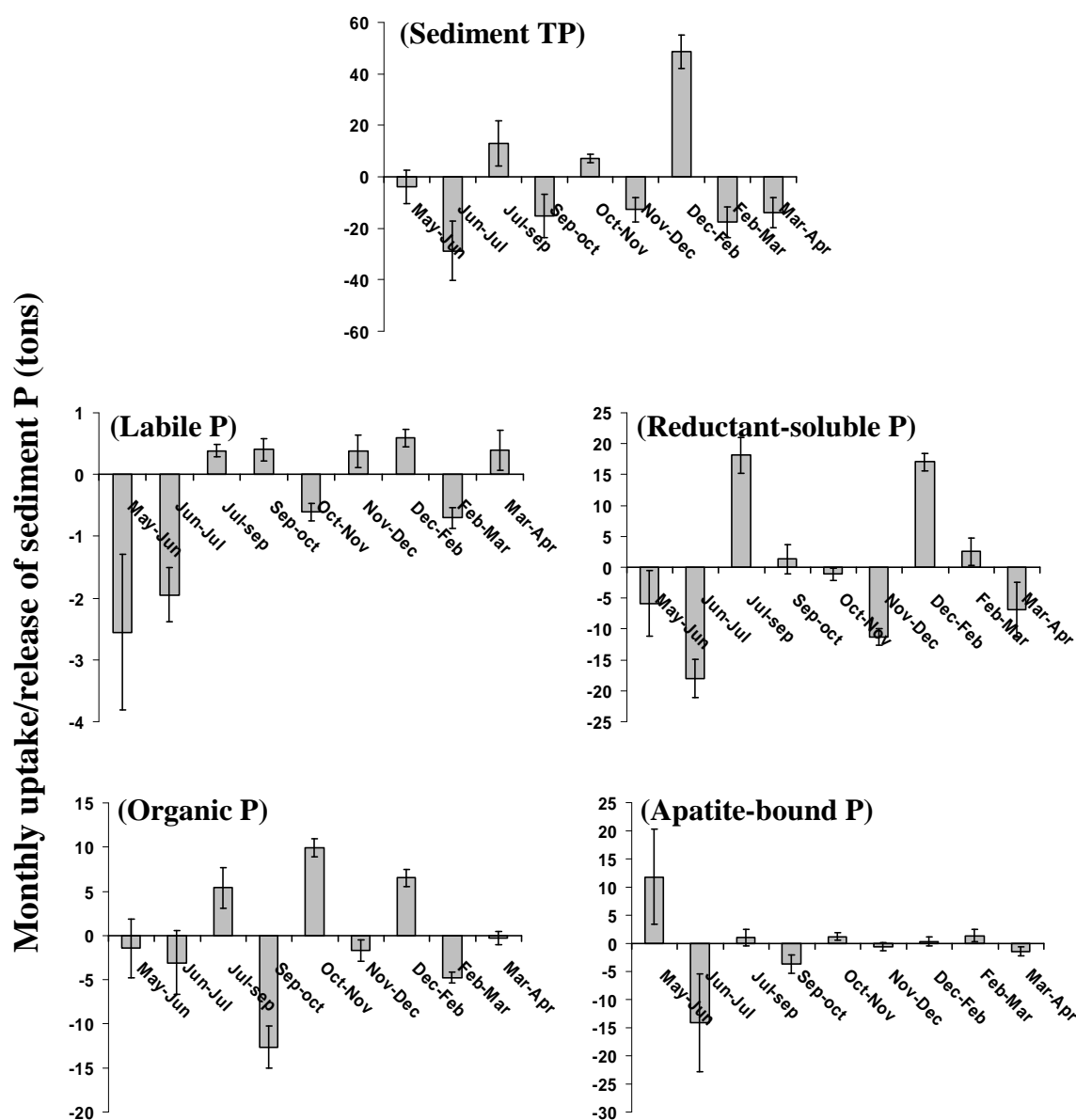
Figure 4.5. *Monthly uptake (positive values) or release (negative values) of sediment TP, labile P, reductant-soluble P, organic P, and apatite-bound P in Loch Leven between May 2004 and April 2005. Negative values represent sediment-P loss and positive values sediment-P gain (vertical bars represent the standard error of the mean of six sample sites).*

concentrations in equilibrium. However, a closer inspection of the release-sensitive fractions indicated that significant amounts of P were being released from the labile, reductant-soluble, and organic P-pools (~ 10 t). This released P was cancelled out by an uptake of P (~ 10 t) into the apatite-bound fraction. This scenario highlights the

interconnectivity between the release-sensitive P-pools and suggests a highly dynamic uptake/release regime in Loch Leven. P reduction was dominated by P release of reductant-soluble P in June and by loss of organic P in September. Sediment-P uptake was concentrated mainly within the apatite-bound and reductant-soluble fractions in July and December and in gain of organic P in October (most likely in the form of planktonic detritus and resting stages).

Understanding of this P-flux regime can be used to increase the effectiveness of many secondary management practices. For example, aeration (acts to bind P within Fe- and Mn-complexes (reductant-soluble P) within the sediment) could be applied in late-summer to reduce the release of reductant-soluble P. Similarly, sediment dredging may be best carried out in mid-winter to correspond with the highest uptake of sediment-P and also the highest sediment TP concentrations of the year. When applied correctly, information of this type can greatly improve the efficiency of lake management practices.

WHOLE-SYSTEM PHOSPHORUS REMOVAL

Where a reduction in external P load has been achieved, and no other remedial practices are conducted, the most important factor limiting a lake's recovery is the natural removal of P *via* lake flushing. Simply, lakes with a high flushing rate will recover more quickly than lakes with a low flushing rate, though this process can be greatly hampered by internal P cycling. The flushing rate in Loch Leven is known to be low (2-3 loch volumes/y) and, coupled with the rapid cycling of P between the sediment and the water-column, helps to explain the prolonged recovery (Sas, 1989). Loch Leven, however, represents an interesting case in that outflow rates have been regulated, by managed sluice gates positioned across the main outflow, since *ca*. 1830. The ability to

regulate the outflow of water provides a capability to regulate the P removal flux and thus optimise recovery. It should be noted that, at present, the main considerations for flow regulation are based on the requirements of local industries and, at the time of writing, a collaborative re-assessment of the flow regulation strategy, including current ecological and industrial requirements, was being conducted.
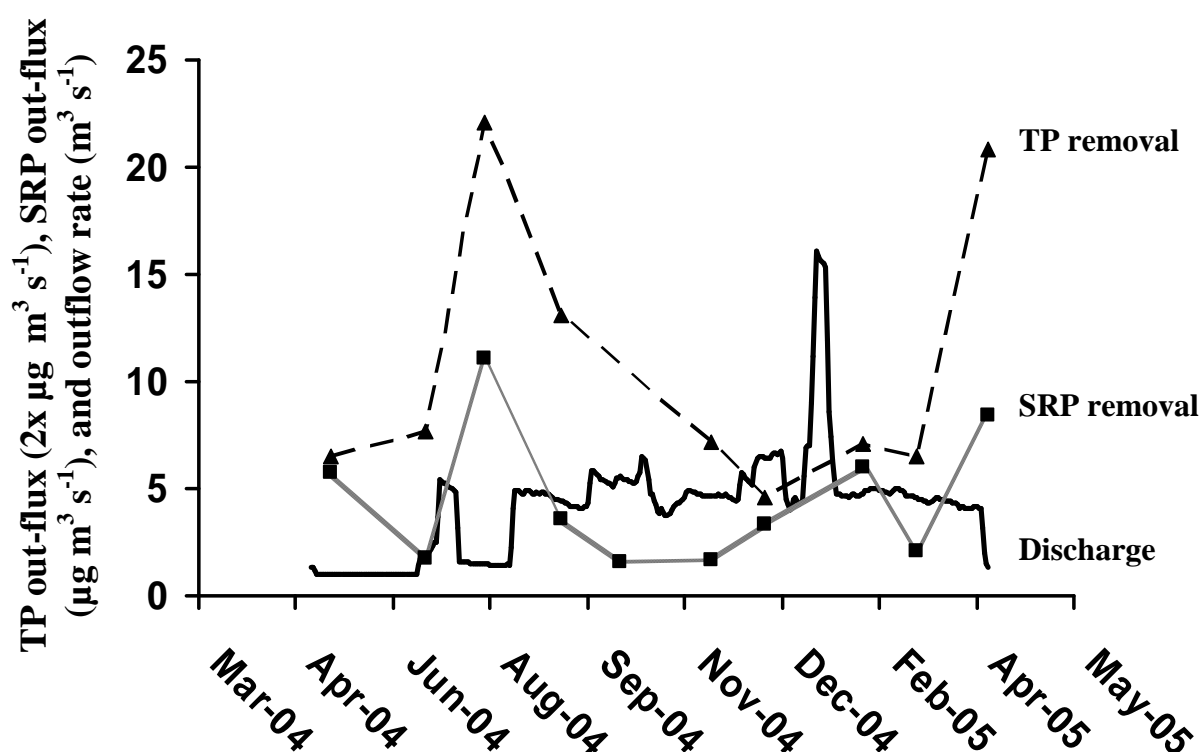

Figure 4.6. *Seasonal variation in (a) discharge at the outflow (full black line), (b) water-column soluble reactive phosphorus (SRP) removal (squares), and (c) water-column total phosphorus (TP) removal (triangles) in Loch Leven.*

The monthly SRP and TP removal fluxes between May 2004 and April 2005 were also observed to fluctuate seasonally (Figure 4.6). It is apparent that the lowest

flow rates of the year coincided with the highest monthly P out-fluxes (August 2004). Similarly, the highest flow rates corresponded with low monthly P out-fluxes (December 2004). In the interest of aiding the recovery of Loch Leven, an ideal flow regime would include high outflow rates to coincide with peak water-column TP concentrations (late-summer), therefore ensuring maximal removal of TP. Natural water levels, however, are commonly low in summer, when water-column TP concentrations are highest, and high in winter, when TP concentrations are lowest (Bailey-Watts and Kirika, 1987). Adequate planning of outflow management may act to counter this natural variation through the maintenance of high lake water levels during flooding events, thus providing a higher volume of water which may be used for flushing water-column TP during characteristic summer internal loading events. This flow regime, or at least some shift towards it, would result in enhanced P removal from the system and would therefore increase the speed and extent of recovery.

A number of other factors should also be considered when contemplating the timing and magnitude of flow regulation. These may include the effects of altered lake water levels on lake macrophyte communities, and the effects of altering outflow rates on downstream fish and invertebrates, and the productivity of local industries. It could be argued that flushing the system of P may result in downstream ecosystems receiving a pulse of P in late summer, therefore shifting the problem from lake to river/estuary. It is, however, widely accepted that standing freshwaters are more sensitive to elevated nutrient concentrations than most rivers or estuaries because of their lower flushing rates. Loch Leven is not unusual in having a regulated outflow; many impacted standing waters have managed hydrological regimes where flow regulation could become a very useful management tool for the recovery of eutrophic waters.

CONCLUSIONS

1. Loch Leven water-column total phosphorus (TP) and soluble reactive phosphorus (SRP) concentrations are highest in mid-summer and lowest in late-winter/early-spring. This pattern is common in many recovering lakes.

2. Sediment TP concentrations in Loch Leven are highest in winter and lowest in summer indicating strong P-partitioning between the sediment and the water-column. The seasonal variation in sediment TP indicates strong replenishment of sediment released P in winter.

3. The seasonal variation in P-fluxes, from four release-sensitive sediment-P pools (labile, reductant-soluble, organic and apatite-bound P-pools), was not synchronous. P-uptake/release conditions varied seasonally within the P-pools. The reductant-soluble P-pool was responsible for the majority of the late-summer internal loading event.

4. Loch Leven sediment appears to have recently shifted from an accumulation to a loss of P, suggesting that the recovery process is still having an effect and still in progress. Under this scenario, further improvement in water quality should be expected.

5. Secondary lake management practices may be enhanced where P-partitioning information is available. In Loch Leven, practices that involve the removal/containment of P from the water-column (e.g. enhanced flushing) should be carried out in late-summer when water-column TP concentrations are highest. Similarly, practices involving the containment of P within the sediment, or removal of P-rich sediment, should be carried out in mid-winter when sediment TP concentrations are highest.

CHAPTER 5

The effects of season and water-column depth on benthic microalgal composition

and abundance in Loch Leven (Scotland) sediment

Spears, B.M., Carvalho, L., Perkins, R., Kirika, A., and Paterson, D.M.
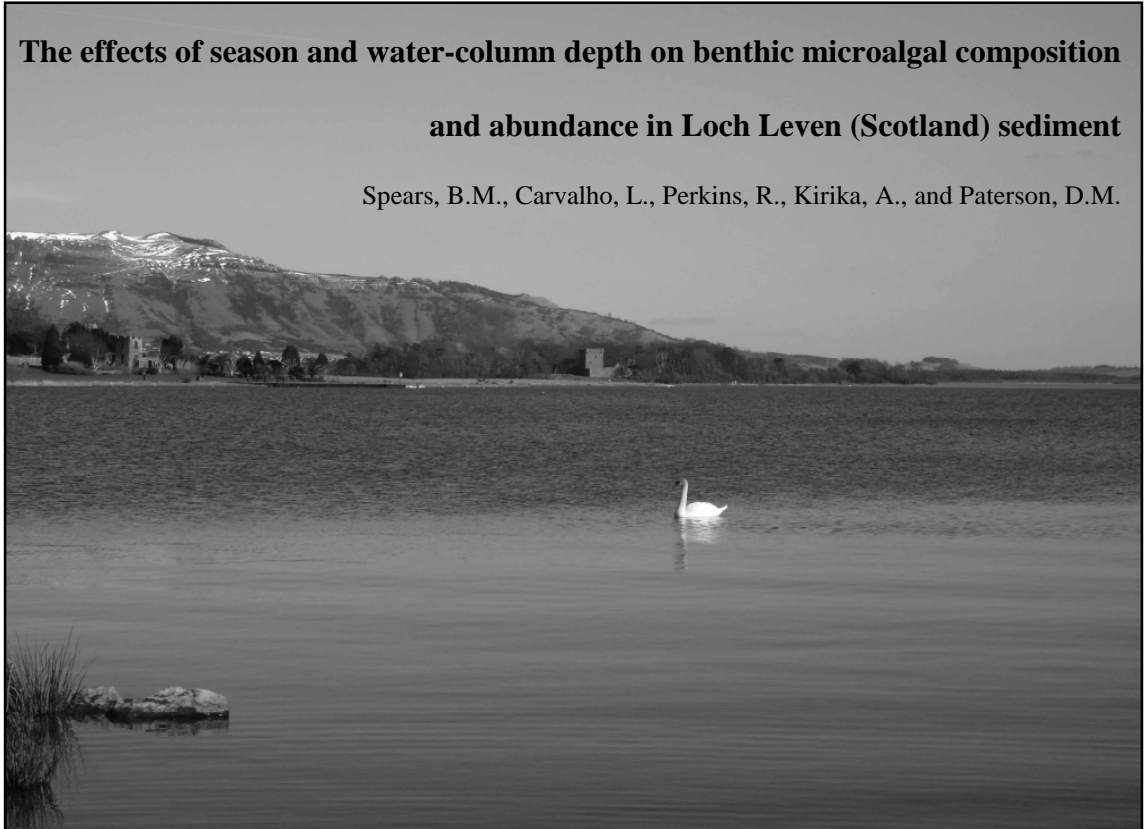
The effects of season and water-column depth on benthic microalgal composition and abundance in Loch Leven (Scotland) sediment

ABSTRACT

In an attempt to further the understanding of the factors governing benthic microalgal community composition in shallow lakes, we assessed the spatial (6 depths between 2 and 22 m overlying column) and seasonal variation in total benthic microalgal (i.e. all viable microautotrophs in the sediment; TS-chl) and epipelon biomass (Epi-chl) in a shallow eutrophic lake (Loch Leven, Scotland). We also assessed the variation in the epipelon community composition with depth using microscopic identification. TS-chl (range: 24 to 617 µg chl a g^{-1}dw) was highest in winter and at the deepest site, most likely as a result of increased deposition of phytoplankton coupled with high sporadic wind-induced sediment resuspension. High TS-chl values also corresponded with high light attenuation indicating that the main source of this biomass probably did not originate from the epipelon. Epi-chl (range: 0.16 to 2.69 µg chl a g^{-1}dw) was highest in late winter/early spring, a period when water clarity was highest and TS-chl and light attenuation were lowest. The relative contribution of Epi-chl to TS-chl never exceeded 17 % indicating a strong dominance by non-epipelic biomass at all sites across all seasons. The community composition showed high abundance of genera

known for shade tolerance (filamentous cyanobacteria) in deep-water sediments and genera known to have high light requirements (e.g. epipelic diatoms) in shallow-water sediments. Results indicate that light limitation and habitat disturbance both play key roles in governing the spatial and seasonal variation in benthic microalgal biomass and community composition and that care should be taken when apportioning cause and effect in relation to benthic microalgae in shallow lake sediments due to the influences of phytoplanktonic biomass in measurements.

INTRODUCTION

The role of benthic microalgae (all viable microautotrophs in the sediment) in regulating ecosystem functioning has received recent attention in the literature (e.g. Woodruff *et al.,* 1999; Bartoli *et al.,* 2003; Spears *et al.,* 2006a). These communities may act to minimise the release of sediment bound nutrients (e.g. phosphorus and nitrogen) *via* a number of proposed mechanisms (ecosystem functions) including stabilisation of the sediment surface through the production of extra-cellular polysaccharides (Paterson, 1989), photosynthetic oxygenation of surface sediment resulting in the reduction of anoxic nutrient release (Carlton and Wetzel, 1988), and direct sequestration of dissolved nutrients (Jarvie *et al.,* 2002). The majority of previous studies (conducted mainly in estuarine and fluvial sediments) have focussed on the role of the benthic algae in performing ecosystem functions. Significant spatial and seasonal variations in community structure have been reported in a number of studies in estuarine and fluvial sediments (Moreno and Niell, 2004; Jesus *et al.,* 2005), however, the spatiotemporal variability in the total benthic algal community biomass and composition in lakes remains relatively poorly studied. However, a few studies have reported significant spatial variation in benthic diatom communities (Oppenheim *et al*., 1989; Ollikainen *et al*., 1993).

In eutrophic shallow lake sediments (i.e. with high light attenuation and phytoplanktonic settling rates), the benthic algal community is likely to be dominated by deposited phytoplankton, with the true epipelon representing a secondary component. However, the true composition of total benthic microautotrophic biomass is made up of a number of functional groups (e.g. epipelon, epipsammon, epiphyton, metaphyton, resting stages/spores etc.) with varying associations within the sediment (Round, 1981; Stevenson, 1996). For the purpose of this paper, we consider all

microautotrophs found in the sediment and capable of photosynthesis (i.e. that contribute to the total sediment chlorophyll *a* signal) as benthic microalgae and those truly associated with the sediment-water interface and capable of repositioning themselves within it as epipelon.

Little is known about variations in the relative composition of microautotrophs living within the sediment surface. As such, recently reported correlations between sediment nutrient concentrations and release, and sediment chlorophyll *a* concentrations in these systems may not represent an independent ecosystem function of epipelic forms (Spears *et al.*, 2006a and b). In order to accurately apportion cause and effect, there is a need to assess the relative proportions of the benthic microalgal community, with respect to epipelic biomass, and to investigate the factors driving variation within the community.

In eutrophic sediments where nutrient concentrations are high, the main factors regulating the spatial and temporal variation in benthic microalgae are expected to be light and habitat disturbance. The epipelic community composition varies with light (and therefore overlying water depth) such that diatoms and euglenids are known to have higher light requirements than cyanobacteria (Ploug *et al.,* 1993; Kromkamp *et al.,* 1998). However, light may have little effect on the presence of phytoplankton in the sediment which is expected to be regulated by an increase in settling rates during calm periods. Furthermore, light effects may be overshadowed by habitat disturbance where the flocculent surface sediment layers (that include the benthic microaalgae) are extremely sensitive to turbulent disturbance and are commonly resuspended and transported to areas of accumulation (regulated by the bathymetry of the basin) *via* bottom-water currents, known as "sediment focussing" (Hilton *et al.,* 1986). This process may result in the homogenisation and relocation of all organisms present in the

surface sediments at the time of disturbance, throughout the lake. Disturbance events are commonly observed in shallow-water sediments where wave induced turbulence is highest (Wetzel, 2001). Therefore, although photosynthesis may only be supported in the surface sediments of the photic zone, the presence of viable autotrophs in the benthos may be more wide spread within the lake. This paper tests the hypothesis that total benthic microalgal biomass and epipelic biomass increase with decreasing overlying water depth under calm, high light, conditions (light regulation) and increase with increasing overlying water depth under turbulent conditions (habitat disturbance regulation). Data was collected monthly over a 1 year period to assess the effects of seasonality (characterised by average wind speed, light attenuation, and water temperature) and depth on the variation in benthic microalgal biomass (measured as chlorophyll *a* concentration) in the surface sediments of a shallow eutrophic lake (Loch Leven, Scotland). As well as total benthic algal biomass (total sediment chlorophyll *a* concentration) epipelic biomass was selectively sampled to provide a comparative (with total benthic algal biomass) biomass estimate. The variation in the epipelon community composition with depth (and, therefore, light) was also assessed.

MATERIALS AND METHODS

Study site

Loch Leven (latitude $56^o10'$N, longitude $3^o30'$W) is a shallow (mean depth ~3.9 m) eutrophic lake of surface area 13.3 km^2 (Figure 5.1). Intermittent stratification is observed in two deep-water basins (~25 m water depth) that together constitute ~5% of the total lake surface area. A weak thermocline usually develops between July and August at between 10 and 12 m overlying water depth with turnover occurring in September. Six sample sites were included in this survey selected to represent a gradient

of depth (2 m, 2.5 m, 3.5 m, 5.5 m, 10 m, 22 m overlying water depth) and light within

Loch Leven. Water and sediment chemistry during the study period were assessed and

are discussed in Spears *et al.* (2006a and b).
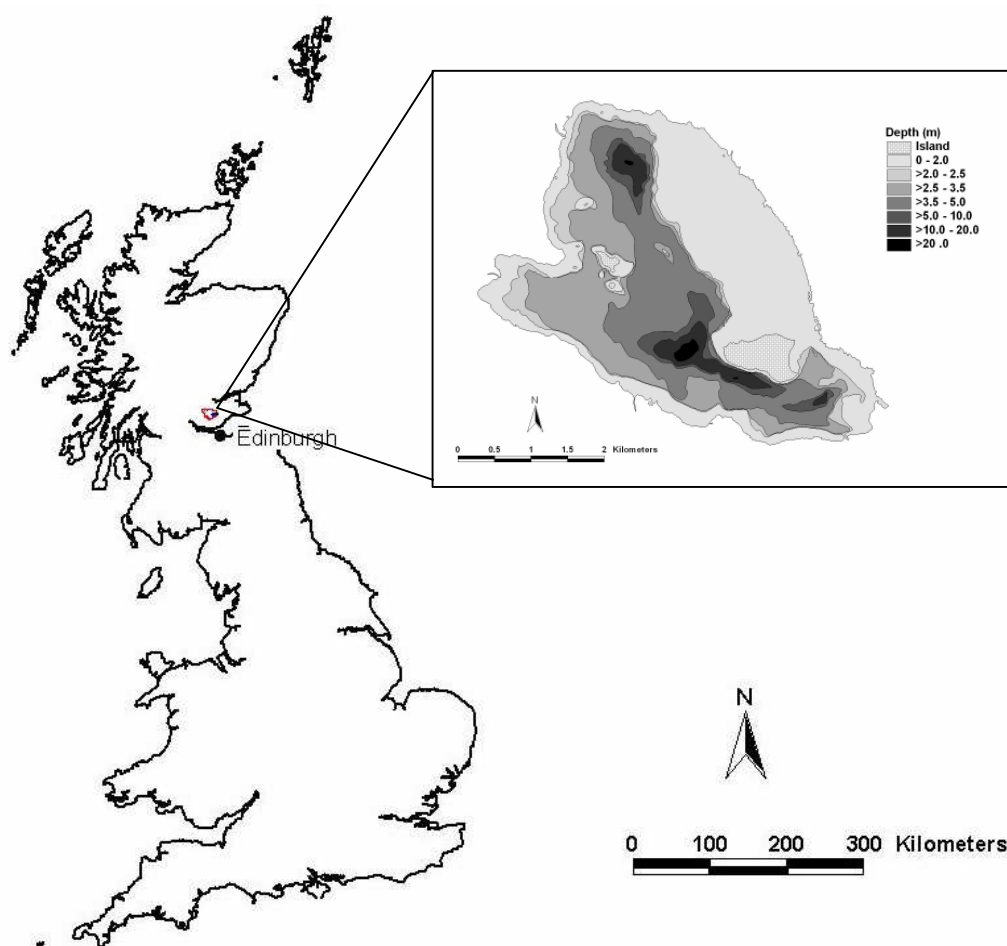
Figure 5.1. *Bathymetric map of Loch Leven.*

Field measurements

Light levels were measured using a Macam quantum radiometer/photometer

Q101 (Macam Photometrics Limited, Livingston, Scotland). Monthly light attenuation

coefficients (April 2004 – April 2005) were calculated from vertical light profiles (taken

at the 22 m site only) according to Fee *et al.* (1988). On each sampling date, bottom-

water (~30 cm above lake bed) dissolved oxygen saturation (DO %) and temperature

were quantified at each site using a Quanta probe (Hydrolab Water Quality Monitoring System, Hydrolab Corporation, Colorado, USA). Daily wind speed measurements were provided by the Loch Leven Estates and were recorded using a cup counter at the edge of the loch.

Sample collection

Sediment cores were collected from a boat using a Jenkin surface-sediment sampler. Cores (1 per site) were collected monthly from the six sites between April 2004 and April 2005. An additional 6 cores were collected (1 from each site) on the 30th June 2005 for the microscopic assessment of epipelon community composition. All cores were sealed and transported to the laboratory where water overlying the sediment surface was carefully siphoned off using a syringe to leave exposed sediment. The upper 3 cm of sediment was sampled and homogenised to facilitate sub-sampling for different analyses.

Total algal biomass in sediment

The benthic microalgal biomass was expressed as total sediment chlorophyll *a* concentration (TS-chl; µg chl a g^{-1}dw). 1 g of wet homogenised sediment was weighed into an acid washed 15 ml centrifuge tube and diluted to 10 ml with autoclaved (121°C for 30 minutes) distilled water. A 2 ml sub-sample of the solution was then filtered (Whatman GF/C filter) to concentrate the algal cells and then photosynthetic pigments were extracted in 90% cold acetone for 24 h in the dark at 0°C. Phaeophytin corrected chlorophyll *a* concentrations were quantified spectrophotometrically within 3 h of the extraction (APHA, 1992). Previous analysis indicated that repeated extraction in 90%

acetone at these sediment concentrations yielded negligible further photosynthetic pigments.

Epipelic biomass

The epipelon was harvested (i.e. separated from the sediment) using a glass Petri-dish trap. 5 g wet weight of homogenised sediment (from each site and on each date) was poured into an upturned Petri-dish top (10 cm internal diameter) within 4 hours of sediment collection. A sheet of lens tissue was laid over the sediment surface (to reduce sediment contamination) before placement of a Petri-dish bottom (9 cm internal diameter) on top of the lens-tissue. The traps were left under natural light and temperature conditions overnight and harvested at 11am the following day. The incubation time was chosen following a pilot study in which no increase in trapped epipelic biomass was observed on the glass surface between 11am and 1pm (unpublished data). The upturned Petri-dish bottom was removed and thoroughly washed through a filtration system (GF/C filter) to ensure the collection of harvested cells. The filters were then treated as above for photosynthetic pigment quantification (Epi-chl). This method of selectively removing epipelon was critically discussed by Round (1981) and is expected to result in a harvesting efficiency of greater than 90% for freshwater systems.

Epipelon community composition

The variation in epipelon community composition with overlying water depth (i.e. between the six sites) was assessed on the 30th June 2005. Epipelic algae were harvested using the Petri-dish method with glass cover slips used in place of the upturned Petri-dish bottoms to collect the epipelon. Traps were incubated under the

same conditions as above and cover slips were removed at 11am the following day and mounted onto slides. Live cells were counted using a Vickers M41 Photoplan microscope (Vickers Limited, UK) with 10x eyepiece. Counts were terminated following enumeration of 200 individual live cells or 55 fields using a 25 x objective lens. Cell biovolumes were estimated using the equations outlined in Hillebrand (1999).

Statistical analyses

The similarity in the temporal variation in mean lake (i.e. average of six sites on each sample date) TS-chl (n = 12) and Epi-chl (n = 12) values was assessed using correlation analysis ($\alpha = 0.05$). Samples were allocated to season based on the variables described in Table 5.1. Variation in the average seasonal (DF = 3) values of TS-chl and Epi-chl between the four seasons (Table 5.1) and between the 6 depth classes (DF = 5) were assessed using two-way analysis of variance (ANOVA: total DF = 71, $\alpha = 0.05$). All statistical analyses were carried out using Minitab statistical software, version 14 (Minitab Ltd., Coventry, UK).

Table 5.1. *Average conditions of lake bottom water: temperature, wind speed, and light attenuation during each season. Values in parenthesis represent the standard error of the mean (n=3).*

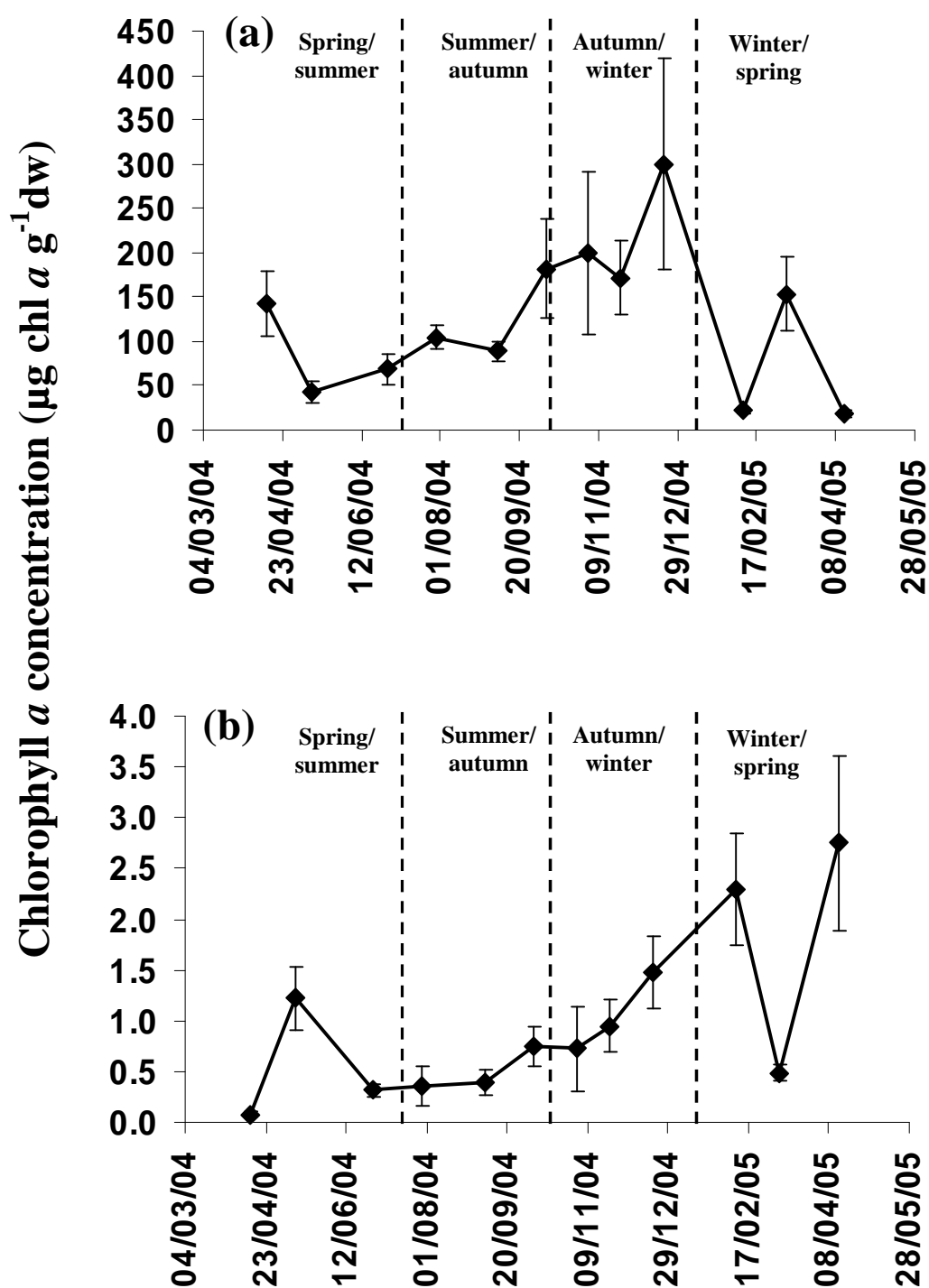| Season	Sample period	Bottom water Temperature (^{o}C)	Weekly average wind speed (km h^{-1})	Light attenuation (m)
Spring/summer	14/04/09 – 29/06/04	13.73 (0.52)	11.74 (5.44)	0.67 (0.18)
Summer/autumn	29/07/04 – 07/10/04	13.95 (1.99)	13.32 (6.11)	1.46 (0.47)
Autumn/winter	02/11/04 – 20/12/04	6.79 (1.29)	15.44 (1.67)	1.60 (0.33)
Winter/spring	09/02/05 – 14/04/05	4.78 (1.56)	8.43 (1.72)	1.07 (0.16)

Figure 5.2. *Average (n = 6) monthly variation in (a) total sediment chlorophyll a (TS-chl) and (b) epipelic chlorophyll a (Epi-chl) concentration expressed as μg chlorophyll a g^{-1}dw sediment. Error bars represent standard error of the mean of six replicate sites.*

RESULTS

Temporal variation in total and epipelic sediment chlorophyll *a* fractions

Average lake TS-chl was highest in December 2004 and lowest in February 2005 (Figure 5.2a). Epi-chl was lowest in April 2004 and highest in April 2005 (Figure 5.2b). No significant correlation was observed between TS-chl and Epi-chl monthly mean lake values (p = 0.296).

Variation in total sediment chlorophyll *a* with season and depth

TS-chl varied across the 4 seasons and with depth (Figure 5.3a). Concentrations ranged between 24 µg chl *a* g^{-1}dw (2 m site in season 4) to 617 µg chl *a* g^{-1}dw (22 m site season 3). The results of the two-way ANOVA analysis show that TS-chl was significantly affected by season (P < 0.01) and depth (P < 0.01) and by an interaction between the two (P = 0.02). TS-chl was significantly lower in winter/spring than in all other seasons and was significantly higher in autumn/winter than in all other seasons. Additionally, TS-chl was significantly higher at 22 m water-column depth than it was in all other sites. The TS-chl value observed in autumn/winter at 22 m overlying water depth was much higher than all other observed values.

Variation in epipelic chlorophyll *a* with season and depth

The seasonal and depth variations in Epi-chl reveal a different pattern in comparison to TS-chl (Figure 5.3). Concentrations ranged between 0.16 µg chl *a* g^{-1}dw (20 m site in spring/summer) and 2.69 µg chl *a* g^{-1}dw (3.5 m site in winter/spring). Two-way ANOVA analysis showed that Epi-chl varied significantly with season (P < 0.01) but not with depth. Epi-chl was significantly higher in winter/spring than in all other seasons. Epipelic biomass was clearly highest between 2-3 m overlying water

depth and declined in the 10 m and 20 m site. The percent contribution of Epi-chl to TS-chl varied between 0.14 % $\pm$ 0.13 % (20 m site in spring/summer ($\pm$ values indicate standard error; n = 3)) and 17.05 % $\pm$ 6.06 % (5.5 m site in winter/spring).

DISCUSSION

Variation in total sediment chlorophyll *a* with season and depth

Significant seasonal and spatial variation was observed in TS-chl concentration. The peak in TS-chl in autumn/winter corresponded with the season of lowest light availability (period of high light attenuation). This suggests that the peak was more likely driven by elevated levels of phytoplanktonic detritus following the characteristic summer bloom (routinely observed in August: Carvalho and Kirika, 2005) as opposed to increased epipelic production at the sediment surface. This hypothesis is in agreement with Lesen (2006) who reported a lagged correlation (2-3 months) between surface water chlorophyll *a* concentration and sediment chlorophyll *a* concentrations. Additionally, TS-chl concentration increased with overlying water depth with the highest concentration almost always corresponding with the deepest site where light levels can not support photosynthesis. Similar trends have been observed in other shallow eutrophic lakes and are generally attributed to turbulence-induced sediment focusing to deep water (e.g. Cariou-Le Gall and Blanchard, 1995; Cyr, 1998). Fine particles (e.g. fine grained sediment, benthic algae and settled phytoplankton) in shallow-water sediment are susceptible to resuspension, whilst deeper water sediments are not, thus over time sediments will naturally be transported to greater depths (Hilton *et al.*, 1986). This is especially true in Loch Leven where under-water currents can reach up to 1.15 km h^{-1} (Smith, 1974). Calvert (1974) and Spears *et al.* (2006b) have

Chlorophyll a concentration
(µg chlorophyll *a* g^{-1}dw)
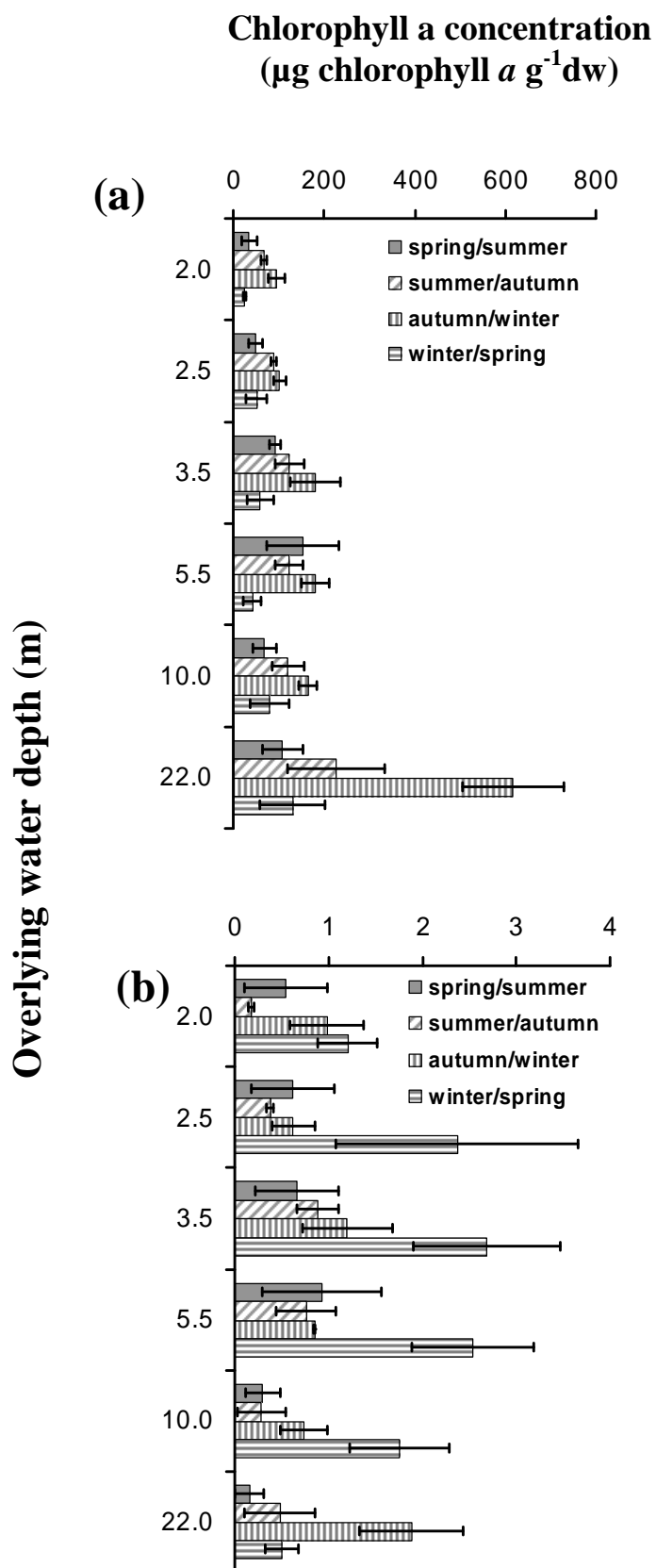
Figure 5.3. *Variation in (a) benthic microalgal and (b) epipelic biomass with season and depth. Error bars represent standard error (n = 5).*
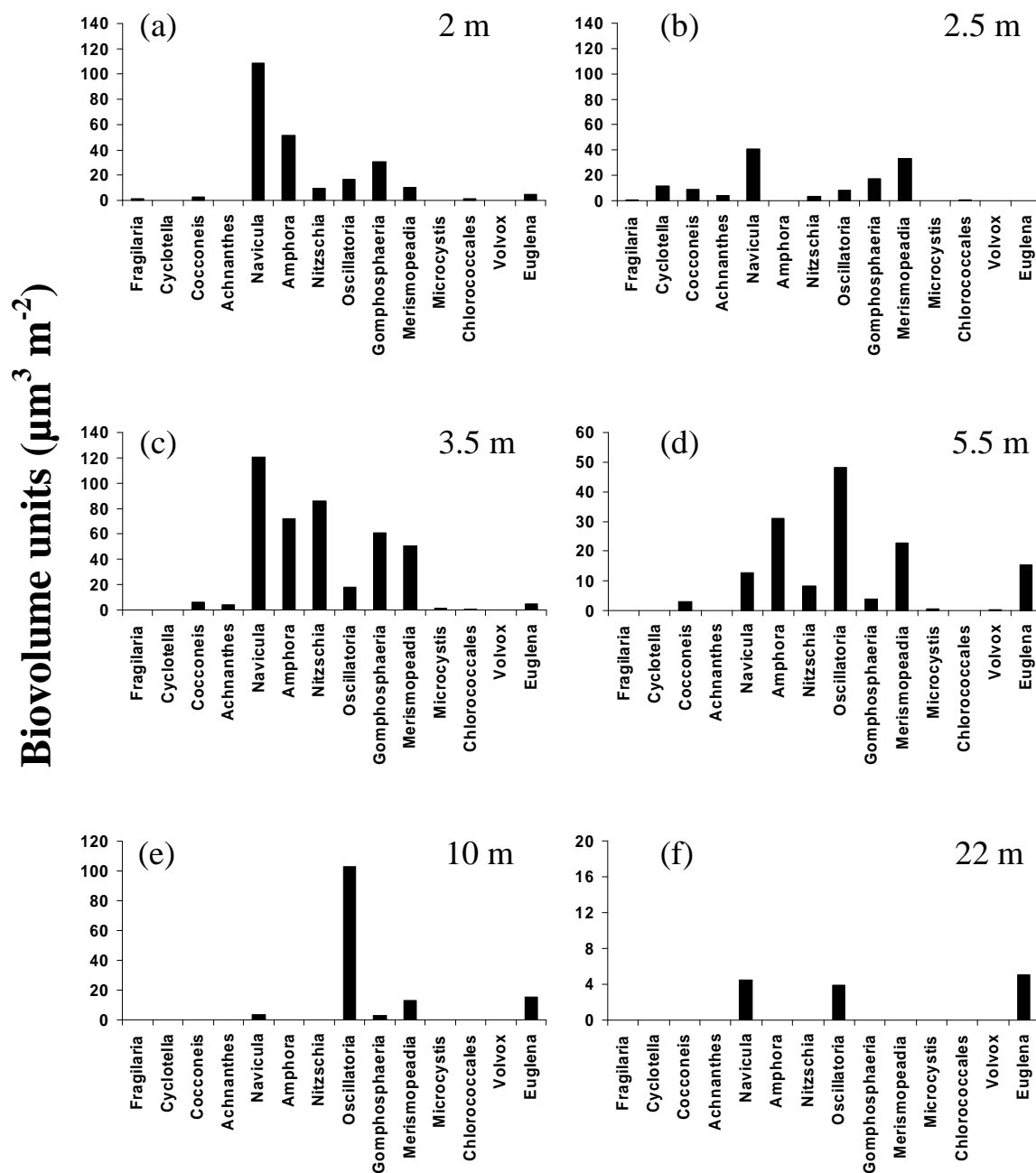
Figure 5.4. *Variation in epipelon community composition across the six sites (sampled 30th June 2005).*
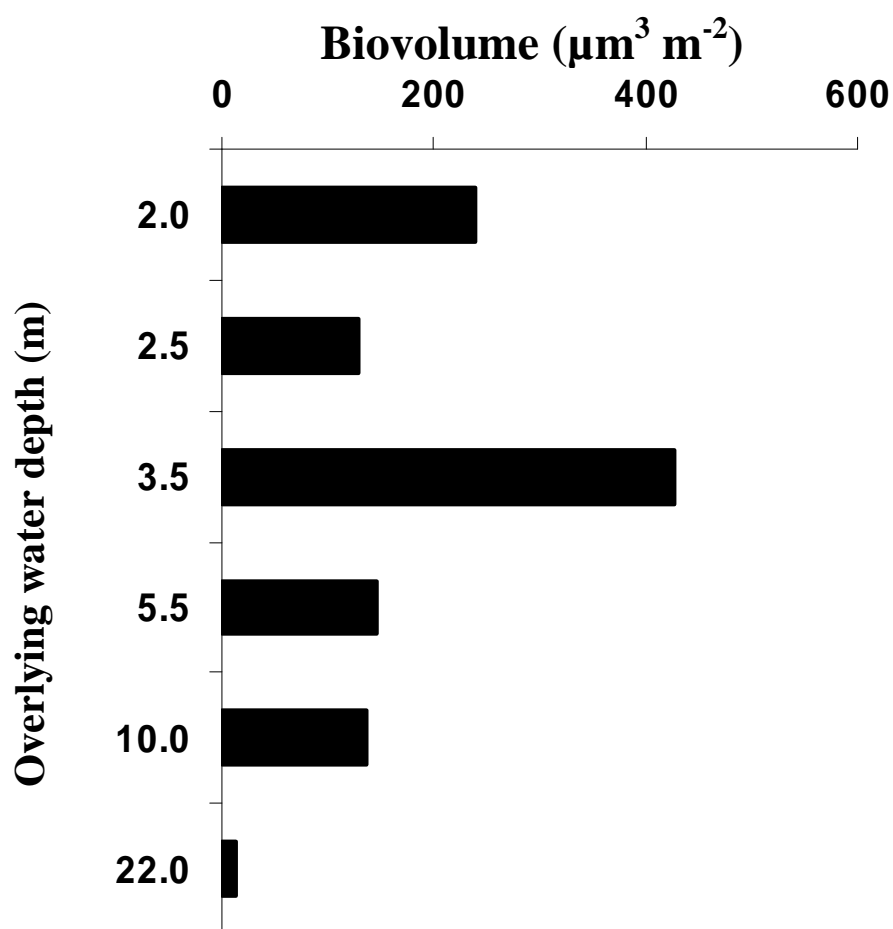
Figure 5.5. *Spatial (with depth) variation in total community biovolume across the six sites (sampled 30th June 2005).*

previously reported that the highest concentrations of organic matter in Loch Leven sediments were found in deep-water sediments further supporting the hypothesis that the main process regulating the spatial variation in TS-chl is physical habitat disturbance, regardless of season.

Variations in epipelic chlorophyll *a* with season and depth

Epi-chl concentration showed significant seasonal variation. It was high during periods of high water clarity (low light attenuation) and *vice versa*. The reduction in phytoplankton in the water-column through winter into spring resulted in improving

water clarity (decreased light attenuation), leading to light conditions at the sediment surface that would support increased epipelic production. Additionally, wind speed values were lowest in winter/spring indicating a reduced likelihood of habitat disturbance. The observed epipelic chlorophyll *a* concentration range was low compared with other studies (e.g. concentration range of 1.5-34.5 µg chl *a* g^{-1}dw and <0.1-19.2 µg chl *a* g^{-1}dw reported for the Firth of Lorne (Tett, 1982) and Loch Ewe (Steele and Baird, 1968) respectively). This is most likely explained by the poorer light climate in a eutrophic lake, such as Loch Leven, compared with these other more nutrient poor sites.

We expected spatial variation in epipelic biomass to be greatest during calm periods, with greater biomass at depths where light was not limiting and (e.g. winter/spring) where physical disturbance was low. Similarly spatial variability was expected to be less during high wind speed conditions (autumn/winter). Certainly, the results indicated that at Loch Leven, the shallowest sediments (2 m), receiving most light, were also sites of the lowest biomass. Highest epipelon biomass was instead recorded in intermediate depths (particularly 3.5 and 5.5 m depth sites). Concentrations declined in deeper sediments (10 and 22 m depth sites) where light availability clearly limits production. The statistical analysis, however, revealed no significant spatial variation within any of the 4 seasons. This may be in part because a non-monotonic response with depth was observed, but may also be in part due to spatial homogenisation of the epipelon community resulting from strong habitat disturbance (i.e. wind induced sediment resuspension) down to relatively deep waters. This may be particularly true at Loch Leven because of its large surface area, and consequent wind fetch. Smith (1974) used hydraulic models to estimate the maximum wave-mixed layers in Loch Leven at varying surface wind speeds and reported a range of ~1.5 to 4.5

m depth corresponding to surface wind speeds (from the south-west) of 16 – 68 km h^{-1}. Recent studies within Loch Leven suggest that the photic zone may only extend to between 5 and 5.5 m overlying water depth (unpublished data). Although about 80 % (area of lake shallower than 5 m) of the surface area of Loch Leven sediment, therefore, receives enough light to support photosynthesis, only 14 % of the sediment within the photic zone may be considered deep enough to escape wind induced habitat disturbance (i.e. is deeper than 4.5 m). In summary, epipelic production appears to be limited by strong habitat disturbance in shallow water sediments and light limitation in deep water sediments, with the sediment at intermediate depths representing a trade-off between the two.

Spatial variation in epipelon community composition

The epipelon community composition changed with increasing water depth as expected with high-light requirement species, such as the diatom *Navicula* spp. being more abundant in shallow-water sediment and low-light tolerant species, such as *Oscillatoria* spp. being dominant in deeper-water sediment. This further supports the hypothesis of increasing light limitation of epipelon with increasing water depth and highlights the importance of biovolume measurements in identifying community shifts where pigment quantification can not. The compositional changes observed agree with a number of studies that have observed a lower light dose acclimation in filamentous cyanobacteria when compared to epipelic diatoms (e.g. Ploug *et al.,* 1993; Kromkamp *et al.,* 1998).

Implications for future studies

During the course of this study, epipelic chlorophyll *a* concentration constituted less than 17 % of the total sediment chlorophyll *a* concentration. This stresses the fact that, in eutrophic shallow lakes, the total sediment chlorophyll *a* signal can be comprised mainly of phytoplanktonic detritus or resting stages and should not be considered representative of epipelic biomass. The proportion of epipelon may, however, be greater in oligotrophic systems where low nutrient concentrations limit phytoplankton production and, therefore, deposition to the sediment.

The phytoplankton in the sediment may still be considered photosynthetically viable and numerous studies have reported upon the importance of the sediment as a source of pelagic microautotrophs (Bernhardt and Classen, 1982; de Jonge and Beusekom, 1995; Head *et al*., 1999; Marcus and Boero, 1998). In the case of Loch Leven, this "seed bank" constitutes the entire lake bed, regardless of the light regime, with the highest non-epipelic biomass occurring in winter in deep-water sediment.

As the biomass of each sedimentary microalgal component measured in this study showed divergent seasonal and spatial variation, there is a need to examine the potentially varying ecosystem functions performed by different functional groupings of algae (e.g. true benthic diatoms versus planktonic diatoms, benthic diatoms versus benthic cyanobacteria etc) in shallow eutrophic lakes. Only with a greater understanding of the spatial and temporal variability in benthic communities can we really understand how they contribute to nutrient transfer between sediment and water-column and how they integrate into lake food-web models.

CHAPTER 6
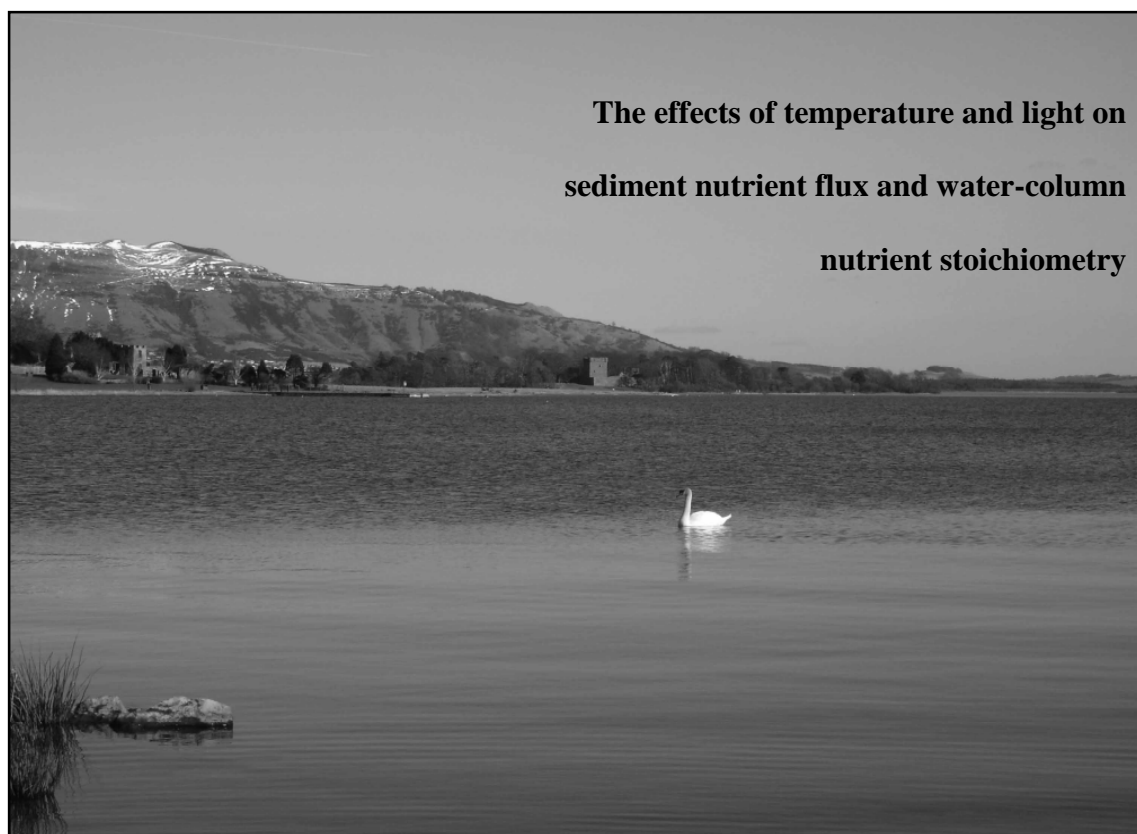
The effects of temperature and light on sediment nutrient flux and water-column nutrient stoichiometry

The effects of temperature and light on sediment nutrient flux and water-column nutrient stoichiometry

ABSTRACT

The effects of light and temperature on benthic/pelagic nutrient (silica (Si), nitrogen (N) and phosphorus (P)) cycling and water-column nutrient stoichiometry were assessed using a series of intact sediment core incubation experiments. Estimates of actual seasonal dark and light P-fluxes were assessed using 24-hour incubations. Sediment-P uptake was observed in spring (7^oC) and release in autumn (12^oC) and summer (17^oC), with the highest release rates (~17 mg PO_4-P m^{-2} sediment surface area d^{-1}) occurring in summer. In a longer (21 day) experiment in which the effects of light (light (n=6) and dark (n=6)) and temperature (five 4-day cycles to represent: 7^oC=>13^oC=>23^oC=>13^oC=>7^oC) on water-column nutrient concentrations were assessed, PO_4-P, TP, SiO_2 (soluble reactive silicate) and TSi were all significantly higher under dark conditions (ANOVA, $\alpha = 0.05$). NH_4-N (ammonium nitrogen) water-column concentrations were observed to be higher under dark conditions at low temperatures and higher under light conditions following a high temperature (23^oC) treatment. No significant light effects were observed for water-column total nitrogen (TN) concentration. Flux estimates for all nutrients measured are given. In terms of water-column nutrient stoichiometry, TN:TP ratio was significantly higher under

light conditions, TSi:TN was significantly lower under light conditions, and TSi:TP did not vary significantly between the dark and light treatments. The main processes acting to regulate diffusive nutrient release were photosynthetic elevation of bottom water pH and dissolved oxygen concentration (both significantly higher under light conditions) and direct microalgal sequestration. Improvements to the illumination of lake bed sediments should reduce the availability of both P and Si. The likelihood of water-column N-limitation will also be reduced because of the disproportionate regulation of diffusive sediment nutrient release.

INTRODUCTION

Benthic microalgae exist at the interface between the sediment and the water-column and, as such, are well placed to regulate benthic/pelagic nutrient cycling. Their ability to perform this ecosystem service may be affected by variations in light and temperature. This includes a range of processes linked to benthic microalgae including the replenishment of organic nutrient compounds to the sediment (Spears *et al.,* 2006b), direct uptake of nutrients (N-uptake: Axler and Reuter, 1996; Webster *et al.,* 2003; P-uptake: Barlow-Bush *et al.,* 2006; DeNicola *et al.,* 2006; Si-uptake: Del Amo and Brzezinski, 1999; Penna *et al.,* 2003), oxygenation of the colonisation zone and therefore reduction in the release of those nutrients that are redox-sensitive (Woodruff *et al.,* 1999), and biostabilisation of the sediment surface via either alteration of the surface structure through mat formation (Dodds, 2003) or by the enhanced cohesion of the sediment caused by the diatomaceous extrusion of extra-cellular polymers (Paterson, 1989). Although, benthic microalgae have been observed to reduce P, N and Si release from sediments (Jansson, 1980; Kelderman *et al.,* 1988; Jarvie *et al.,* 2002), it is unclear whether this regulation is constant over a diurnal cycle and over a temperature range and whether nutrient-specific regulation occurs resulting in alterations in seasonality of water-column stoichiometry. Additionally, no study has considered the effects of these processes on the benthic/pelagic cycling of a range of plant nutrients and the resultant shifts in water-column stoichiometry in accordance to the Redfield ratio (i.e. 16 silica (Si) : 16 nitrogen (N): 1 phosphorus (P); Redfield *et al.,* 1963). This may be particularly important in shaping phytoplankton communities in eutrophic shallow lakes where nutritional sources have been altered (i.e. from the catchment to the sediment) following a reduction in external nutrient load (Sas, 1989).

Recent long-term studies have highlighted the seasonality of benthic/pelagic nutrient cycling and water-column stoichiometry in recovering shallow lakes (e.g. Figure 6.1.; Søndergaard *et al.,* 2005; Jeppesen e*t al.,* 2005). The contrasting seasonality of important plant nutrients can drive phytoplankton community succession where short term Si- and N-limitation events are observed in spring and late summer, respectively, and P-limitation is common during all other periods. The occurrence of N-limitation in late-summer is thought to be driven by high magnitude sediment-P and Si release (main source to recovering lakes) coupled with denitrification of NO_3^-, (Søndergaard *et al.,* 2005). All of these processes are diffusive in nature and increase with sediment heterotrophic bacterial production and, therefore, temperature; sediment-P release occurring on the onset of anoxia resulting from a switch in sediment energy-production from autotrophy to heterotrophy (Boström *et al.,* 1988), and denitrification from an increase in denitrifying bacterial production (Risgaard-Petersen *et al.,* 1994). Si is released from the sediment in late summer following the dissolution of planktonic diatom frustules (also increases with temperature) that accumulate during spring and summer deposition (Bailey-Watts, 1976a and b; Gibson *et al.,* 2000). N is mainly released from sediment as NH_4-N which is efficiently converted to NO_3-N via nitrification under oxygenated conditions. All of these processes are expected to be altered under conditions that favour benthic microalgal photosynthesis (i.e. sufficient light conditions); P release should be reduced via the photosynthetic maintenance of dissolved oxygen concentration in the surface sediment and direct algal uptake, Si release should be reduced via direct algal uptake, and NH_4-N release should be reduced via algal uptake and increased nitrification via the photosynthetic maintenance of surface sediment dissolved oxygen concentrations. These algal processes are also expected to increase with temperature.
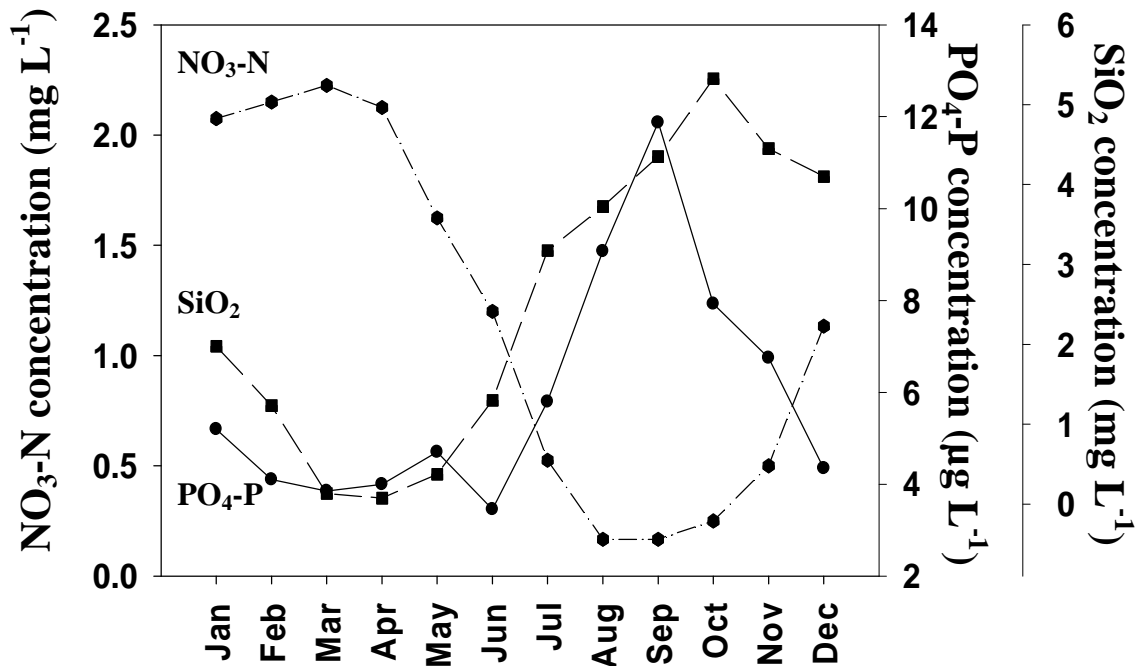
Figure 6.1. *Average monthly water-column concentrations of PO₄-P, NO₃-N, and SiO₂ during the recovery period in Loch Leven. Years included in the average values are 1997, 1998, 1999, and 2001.*

The effects of light and temperature on benthic/pelagic nutrient cycling were tested using a series of laboratory based intact sediment-core incubation experiments designed to investigate light and dark sediment nutrient fluxes across a temperature gradient in a recovering shallow lake (Loch Leven, Scotland). Two approaches were taken: (1) seasonal estimates of ambient daily light and dark P-flux rates were taken on three separate dates, using 24 hour incubations conducted at the temperature recorded at the time of sampling, and (2) estimates of daily water-column nutrient (PO₄-P, NH₄-N, and SiO₂) concentrations and stoichiometry (TSi:TN:TP) were tracked in intact sediment cores over a 21 day incubation period for both light and dark treatments in which temperatures were manipulated to mimic seasonal variations.
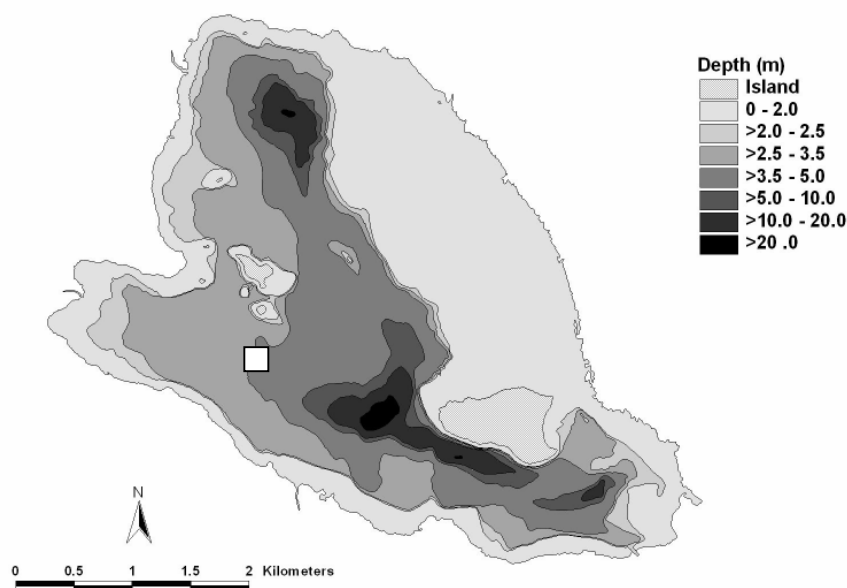
Figure 6.2. *Bathymetric map of Loch Leven showing sample site (white box).*

METHODS

Study site

Loch Leven (Figure 6.2) is a shallow lake situated in the southeast of Scotland (Latitude $56^{o}10'$ N, longitude $3^{o}30'$ W) with a surface area of 13.3 km^2 and a mean depth of 3.9 m. The lake has a well documented history of eutrophication and, in an attempt to improve water quality, external TP load was reduced from 4.05 mg TP m^{-2} lake surface area d^{-1} in 1985 to 1.62 mg TP m^{-2} lake surface area d^{-1} in 1995 (Bailey-Watts and Kirika, 1999). A recent loading survey estimated the external TP load in 2005 at 2.02 mg TP m^{-2} lake surface area d^{-1} (unpublished data). However, improvements in water quality conditions have only recently (i.e. post 2002) been observed with significant summer internal loading events still commonly observed. At the time of this study, therefore, Loch Leven represented a shallow lake under recovery from eutrophication.

Sample collection

All sediment cores were collected from the same sampling point (Figure 6.2) corresponding to the mean depth (3.9 m) of the lake. Cores were collected from a boat using a Jenkin surface-sediment sampler which facilitated the collection of an undisturbed sediment-water interface (core internal diameter 6.7 cm and height 50 cm) consisting of about 20 cm sediment and 30 cm overlying water-column. Cores were transported to the laboratory within 3 hours of collection. All incubations were conducted within an environmental chamber in which light and temperature were regulated.

Table 6.1. *Summary of field observations taken on each sampling date. Inc. temp. = incubation temperature, Temp. = water-column temperature at time of sampling, Secchi = secchi depth, Cond. = water-column conductivity, DO = water-column dissolved oxygen concentration.*

Date	Inc. temp. (^{o}C)	Temp. (^{o}C)	Secchi (m)	Cond. (μS cm)	pH	DO (mg L^{-1})
23 August 2005	17.0	17.0	1.0	224	8.5	9.9
2 November 2005	12.0	10.2	1.0	220	8.5	10.5
18 April 2006	7.0	7.6	1.1	232	8.3	11.1

Ambient light/dark P- flux incubations

These incubations were designed to estimate the daily dark/light P-flux rates under ambient lake conditions. 12 cores were collected on three dates (23 August 2005, 2 November 2005, and 18 April 2006). Water-column temperature, dissolved oxygen concentration, conductivity, and pH were measured on each date using an underwater sensor (Hydrolab Water Quality Monitoring System, Hydrolab Corporation, Colorado, USA) and are summarised in Table 6.1. Cores were returned to the laboratory and allowed to acclimatise for 1 hour in an incubator at temperatures similar to those

observed in the lake at time of sampling (7°C on 18 April 2004, 12°C on 2 November 2005, and 17°C on 23 August 2005). On each date, 6 cores were left in the light (15 µmol s^{-1} m^{-2} between 8 am and 4 pm) and 6 were darkened by covering with aluminium foil and black polyethylene bags. Cores were continually bubbled (commenced at the beginning of the incubation period) with air using a pump (Tetratec Whisper AP 200, TetraWerke, Melle, Germany) producing approximately 60 bubbles per minute with a tubing height of 23 cm above the sediment surface. Visual inspection of the cores indicated no disturbance of the sediment surface at this bubbling rate and chemical analysis of the water-column in a pilot study showed efficient mixing. Water overlying the sediment surface (sampled from 1 cm above the sediment surface) was collected at 3:30 pm on the day of sampling using a syringe immediately after the acclimatisation period and at the end of a 24-hour incubation. The differences in the concentrations were corrected for sediment surface area and dark and light treatment fluxes expressed as mg PO_4-P m^{-2} sediment surface area d^{-1}. Only soluble reactive phosphorus (PO_4-P) flux was considered in the ambient P-flux experiments and P analysis was conducted according to Wetzel and Likens (2000).

Light manipulation experiment

The cores collected on 18 April 2006 were also used for this experiment. Again 6 cores were left uncovered and 6 were incubated in the dark. The light levels and cycle period and the bubbling rate were identical to the conditions described for the ambient P-flux experiments. However, temperatures were manipulated at 4 pm on day 1 (initial 7°C), day 5 (initial 13°C), day 9 (23°C), day 13 (final 13°C), and day 17 (final 7°C) in this experiment. The temperatures were maintained for 4 days following each change. Total phosphorus and phosphate (PO_4-P) analyses were conducted, using persulfate

digestion for the former, follow the acid-molybdenum-blue colorimetric method (Wetzel and Likens, 2000). Ammonium nitrogen (NH_4-N) was analysed using the phenol-hypochlorite method (Wetzel and Likens, 2000). Total nitrogen was analysed using ion chromatography, following a persulfate digestion. Total silica (TS; following a Na_2CO_3 digestion) and silicate (SiO_2) concentrations were analysed using the acid-ammonium-molybdate method described by Golterman *et al.* (1978). Nutrient flux estimates were made using the difference in water-column concentrations over each 24 hour period and were standardised to sediment surface area. pH was measured at the end of the incubation experiment (using an HI 99121 pH meter equipped with HI 1292D soil electrode; Hanna Instruments Limited, Leighton Buzzard, Bedfordshire, UK) at 1 cm above the sediment surface and 0.5 cm, 1.5 cm, and 8 cm below the sediment surface. Dissolved oxygen concentration was measured at about 0.5 cm above the sediment surface at the end of the incubation experiment using a Hach Luminescent Dissolved Oxygen probe (Model HQ10; Hach + Lang Europe, Dusseldorf, Germany).

Statistical analyses

The overall effects of temperature and light in the seasonal ambient P-flux incubations were assessed using two-way analysis of variance (ANOVA; total degrees of freedom (TDF) = 35, $\alpha = 0.05$).

In the light manipulation experiment, repeated measures one-way ANOVAs (TDF = 251, $\alpha = 0.05$) were used to assess the overall effects of light on the water-column concentrations of NH_4-N, PO_4-P, SiO_2, TP, TSi, and TN as well as ratios of TN:TP, TSi:TP, and TSi:TN. It was impossible to statistically differentiate between the effects of temperature and incubation time in the 21 day incubation experiments. As a

result, visual inspection of the data was used to identify gradual 'incubation effects'
from more distinct 'threshold' temperature effects following temperature shifts.

The effects of light on variations in pH and water-column DO concentrations at
the end of the incubation experiment were assessed using one-way ANOVA.

RESULTS

Ambient light/dark P flux incubations

PO_4-P flux increased significantly with temperature ($p < 0.01$) but not with light
(Figure 6.3). Sediment uptake of PO_4-P was observed at 7^oC with release occurring at
12^oC and 17^oC.

Light manipulation experiment: water-column nutrient concentrations

The results of the repeated measures one way ANOVA analysis used to
determine significance of the light and dark treatments are summarised (Table 6.2). The
ranges of nutrient flux estimates over the 21 day incubation period are reported (Table
6.3). Light and dark water-column PO_4-P (Figure 6.4a) and TP (Figure 6.4b)
concentrations showed similar trends throughout the incubation period remaining low
until day 11 following which a sharp increase was observed which coincided with an
increase from 13^oC to 23^oC to suggest more rapid temperature effects. The trends
differed from day 17, with a decrease in TP concentration in contrast to the continued
increase in PO_4-P concentration, during the same period. Concentrations of both were
significantly higher under dark conditions than light (Table 6.2). NH_4-N concentrations
also differed significantly between the light treatments being higher under dark
conditions prior to day 10. Concentrations under light conditions gradually increased
from day 7 with a sharper increase on day 12 and 13. However, NH_4-N concentrations

decreased from day 12 until day 16, and appeared to be triggered by the temperature change. TN decreased between day 1 and 8 and did not vary significantly between light and dark treatments. No sharp temperature effects were observed. SiO_2 and TSi concentrations increased gradually throughout the incubation. Concentrations were significantly higher under dark as opposed to light conditions over the 21 days.

Table 6.2. *Results of the one-way repeated measures ANOVA (α = 0.05) analysis used to assess the significance between water-column nutrient concentrations (NH_4-N (µg L^{-1}), TN (mg L^{-1}), PO_4-P (µg L^{-1}), TP (µg L^{-1}), TSi (mg L^{-1}), SiO_2 (mg L^{-1})) and stoichiometry (TN:TP, TSi:TP, and TSi:TN) under dark and light treatments. Mean values over the incubation period (n = 21) and standard deviation of the mean are given. Df = degrees of freedom, SS = sum of squares, MS = mean square.*

		Mean light	Mean dark	df	SS	MS	F	p-value
PO_4-P	Light	24 (24)	41 (40)	1	17583	17583	14.41	0.000
	Error			250	305009	1220		
	Total			251	322593			
TP	Light	47 (25)	67 (42)	1	29589	29589	14.98	0.000
	Error			250	493772	1975		
	Total			251	523361			
NH_4-N	Light	92 (63)	62 (36)	1	57172	57172	5.40	0.021
	Error			250	2644672	10579		
	Total			251	2701844			
TN	Light	0.60 (0.17)	0.57 (0.18)	1	0.0650	0.0650	1.45	0.230
	Error			250	11.2432	0.0450		
	Total			251	11.3082			
SiO_2	Light	5.6 (3.8)	7.9 (5.1)	1	323.2	323.2	15.73	0.000
	Error			250	5135.5	20.5		
	Total			251	5458.7			
TSi	Light	6.2 (3.3)	8.4 (4.5)	1	313.7	313.7	19.35	0.000
	Error			250	4052.3	16.2		
	Total			251	4365.9			
TN:TP	Light	42 (25)	30 (19)	1	9962	9962	13.34	0.000
	Error			250	186734	747		
	Total			251	196697			
TSi:TP	Light	78 (32)	81 (43)	1	576	576	0.26	0.613
	Error			250	561087	2244		
	Total			251	561663			
TSi:TN	Light	3.0 (1.8)	4.2 (2.7)	1	99.37	99.37	15.21	0.000
	Error			250	1633.70	6.53		
	Total			251	1733.07			

Table 6.3. *The range of sediment flux estimates measured during the 21 day light manipulation incubation experiment for total phosphorous (TP), PO$_4$-P, total nitrogen (TN) ammonium nitrogen (NH$_4$-N), total silica (TSi), and silicate (SiO$_2$). Negative values indicate sediment uptake of nutrients from the water-column and positive sediment release to the water-column. All flux estimates are expressed as mg per m^2 sediment surface area per day.*

	TP (mg m^2 d^{-1})	PO$_4$-P (mg m^2 d^{-1})	TN (mg m^2 d^{-1})	NH$_4$-N (mg m^2 d^{-1})	TSi (mg m^2 d^{-1})	SiO$_2$ (mg m^2 d^{-1})
Light	-17 to +19	-2 to +11	-59 to +42	-8 to +7	-189 to +781	-120 to +1126
Dark	-8 to +8	-1 to +4	-34 to +52	-25 to +22	-135 to +674	-101 to +777
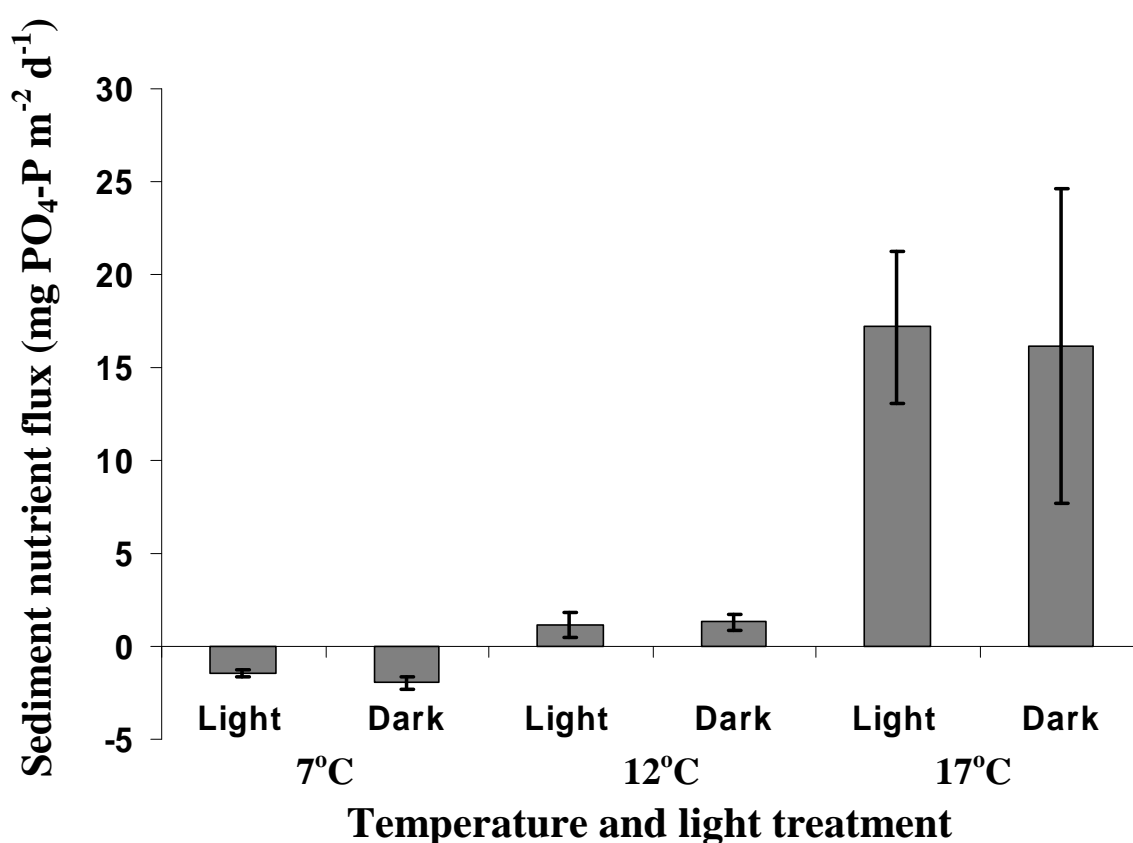
Figure 6.3. *Light/dark release under ambient lake conditions. Error bars represent standard error of the mean (n = 6).*

Light manipulation experiment: water-column nutrient stoichiometry

TN:TP ratio was significantly higher under light conditions (Table 6.2). TSi:TP did not vary significantly under the different light treatments. TSi:TN was significantly higher under dark conditions.

Light manipulation experiment: pH and dissolved oxygen profiles

The DO concentration was significantly higher under light conditions at the end of the incubation period (dark DO = 9.25 mg L^{-1}; light DO = 9.85 mg L^{-1}; ANOVA, α = 0.05, n = 6). pH conditions (Figure 6.5) were significantly higher under light conditions at 1 cm above and 0.5 cm below the sediment surface but no difference was observed at 1.5 cm and 8 cm below the sediment surface (ANOVA; α = 0.05).

DISCUSSION

Ambient P-flux estimates

Estimates of P-flux rates under field conditions showed uptake of P by the sediment at $7^{o}C$ (i.e. in spring; 18 April 2006) and release at $12^{o}C$ (i.e. autumn; 2 November 2005) increasing further at $17^{o}C$ (i.e. summer; 23 August 2005). This is in agreement with a number of recent studies in which P release has been observed in the (warmer) summer, and sediment-P uptake observed in the (cooler) winter months (e.g. Søndergaard *et al.*, 2005; Spears *et al.*, 2006c). Other laboratory studies have found that P release across an oxygenated sediment-water interface becomes independent of DO concentrations at temperatures between $17^{o}C$ (Kamp-Nielson, 1975) and $21^{o}C$ (Holdren and Armstrong, 1980). This would suggest that sediment oxygen demand exceeds sediment oxygen production at these temperatures, creating zones of anoxia under
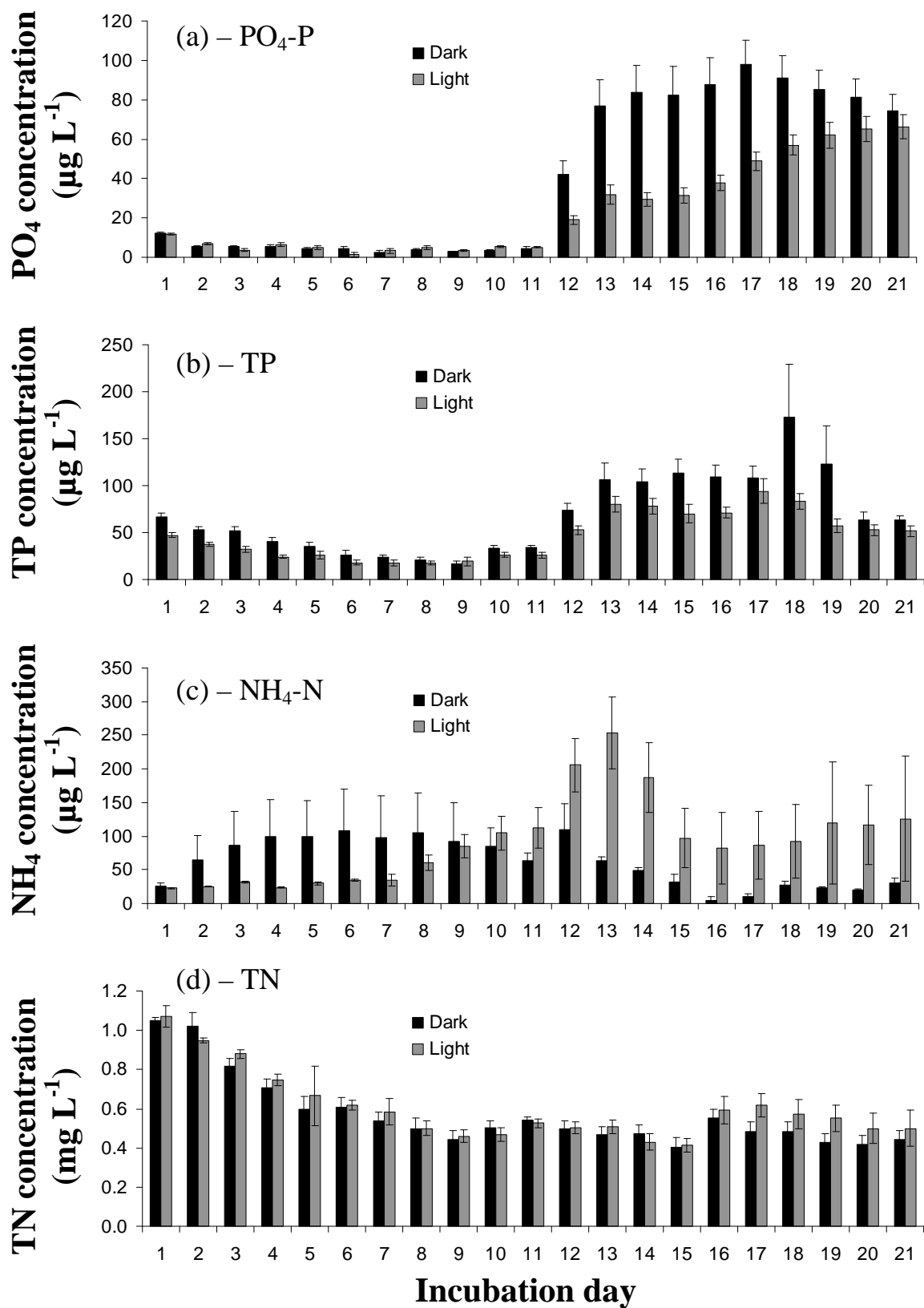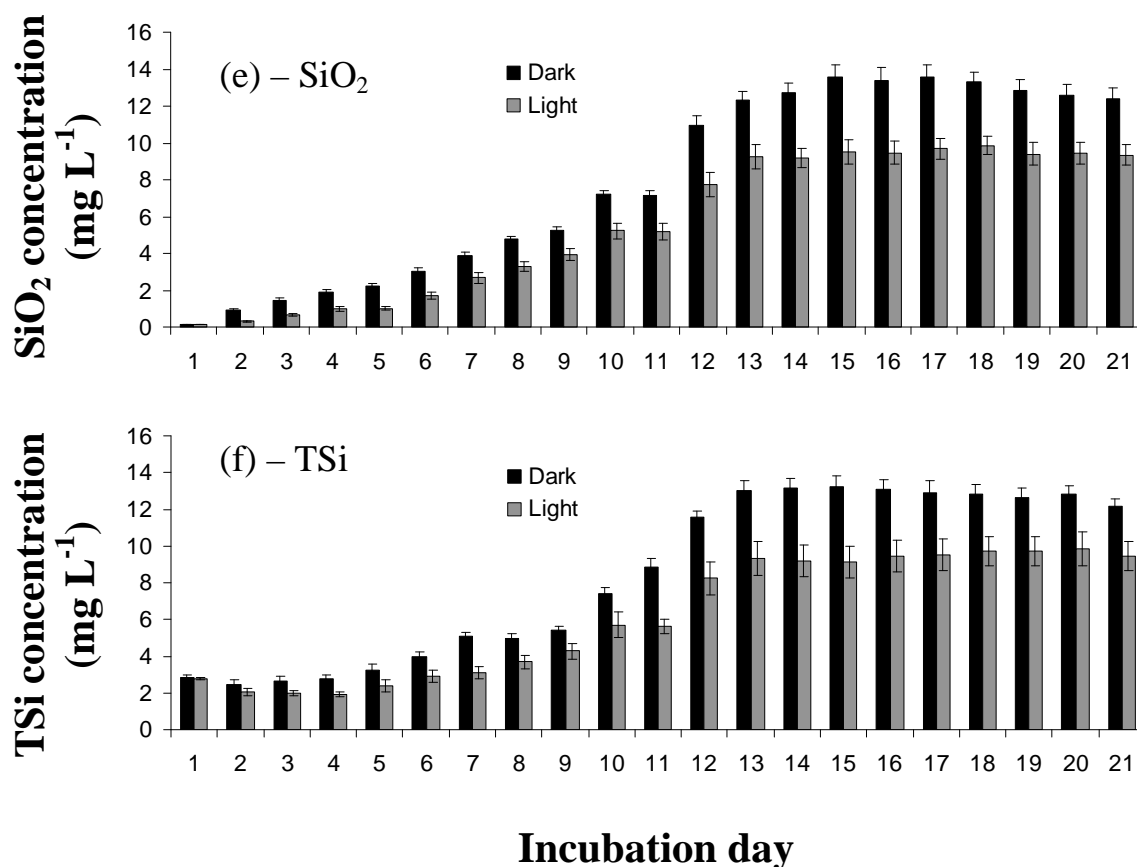
Figure 6.4. *Variation in water-column nutrient concentrations (SRP (a), TP (b), NH$_4$ (c), TN (d), SiO$_2$ (e), TSi (f)) during the 21 day incubation experiment. Temperature treatments: day 1 - 5: 7°C, day 5 – 9: 13°C, day 9 – 13: 23°C, day 13 – 17: 13°C, day 17 - 21: 7°C.*

which redox-sensitive P complexes can be reduced and PO$_4$-P released from the sediment. However, no significant difference was observed between light and dark ambient P-fluxes. This can be explained by 1) low initial benthic algal biomass, or 2) 24 hours is insufficient time for DO shifts to occur. The low secchi depth (~1 m on each date) observed on each of the three sampling dates indicated low light conditions at the sediment surface (~3.5 m below the water surface) and, therefore, it is likely there was a low initial biomass of benthic microalgae (not measured in this study). In a previous study, the highest epipelic biomass was observed to occur in December/January (i.e. during clearer water periods) in Loch Leven (chapter 5).
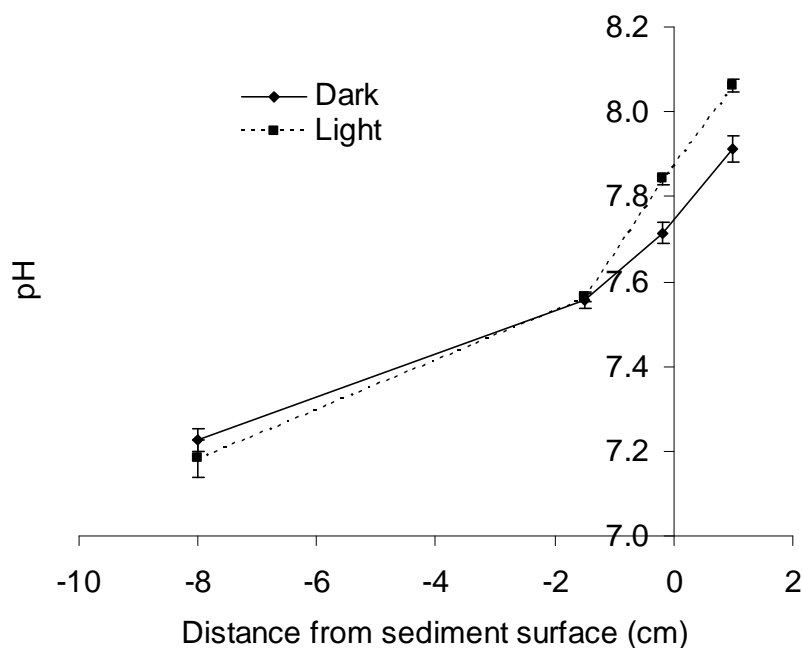
Figure 6.5 *Variation in pH across the sediment/water interface following the long-term incubation experiment.*

The measured release rates (Table 6.3) are comparatively low for eutrophic sediments with similar estimates of ambient P release ranging from $10 - 278$ mg P m^{-2} sediment surface area d^{-1} (Penn *et al.,* 2000 and Phillips *et al.,* 1994 respectively) compared with the light P-flux at 17°C in Loch Leven, using the same incubation techniques, estimated at 24 mg P m^{-2} sediment surface area d^{-1} (Spears *et al.,* 2006b).

Light manipulation experiment: effects of light on benthic/pelagic nutrient cycling

Light and dark NH$_4$-N water-column concentrations showed conflicting trends throughout the incubation experiment where concentrations under light conditions were lowest (relative to dark conditions) between days 1-8 and concentrations under dark conditions were lowest following day 12. The former observation is in agreement with our original hypothesis where increased nitrification (i.e. lower NH$_4$-N concentrations) was expected under light conditions. The higher concentrations under dark conditions

are in agreement with a number of other studies in which NH_4-N release has been reduced by benthic microalgal production either by direct uptake or increased nitrification through the photosynthetic maintenance of DO levels (Jansson, 1980; Risgaard-Petersen *et al.,* 1994; Axler and Reuter, 1996). The shift in apparent impacts of light on NH_4-N fluxes occurs from day 9 when the temperature was increased from $13^{o}C$ to $23^{o}C$, suggesting that at this point NH_4-N release increased to the extent of masking any "dampening" effects of benthic algae. Apart from the sharp change in NH_4-N concentrations under light conditions during days 12 to 14, all other concentration changes were gradual and therefore it is impossible to separate temperature effects from incubation effects. TN and NH_4-N variation was not synchronous throughout the incubation period presumably as the result of other nitrification/denitrification processes involving NO_3-N and N_2 gas. The observed flux rates are in agreement with other studies (e.g. $52 - 71$ mg NH_4-N m^2 d^{-1} in Lake Vechten, Netherlands (Sweerts *et al.,* 1991)).

SiO_2 concentrations increased steadily up to day 9. This rate of release increased markedly during day 10 to 13, particularly under dark conditions coinciding with a temperature change from $13^{o}C$ to $23^{o}C$. The lack of change following day 13 may represent the onset of equilibrium between the sediment and the water-column in combination with limited sequestration rates of Si by benthic algae. TSi and SiO_2 variation was very similar, indicating strong dependence of TSi on sediment derived SiO_2. Flux estimates were in agreement with estimates reported in other studies (e.g. $0.1 - 0.8$ g SiO_2 m^{-2} d^{-1} in Loch Leven (Bailey-Watts, 1976b) and 0.1 g SiO_2 m^{-2} d^{-1} in Lake Grevelingen, Netherlands (Kelderman *et al.,* 1988)). Water-column concentrations were consistently higher under dark conditions indicating strong regulation of Si release by benthic microalgae. This is likely due to direct uptake. The regulation of sediment Si

release by benthic microalgae has also been observed by Kelderman *et al.* (1988) and, although not observed in this study, sediment Si release has been reported to increase under high temperatures (i.e. highest release in summer; Bailey-Watts, 1976a and b; Rippey, 1983; Gibson *et al.,* 2000).

The pH and DO profiles measured at the end of the 21 day incubation experiment substantiated the hypothesis that actively photosynthesising benthic algae alter the biogeochemical dynamics of sediment N and P cycling through the maintenance of elevated DO and/or pH conditions. Few studies have directly assessed this phenomeonon in relation to nutrient fluxes, although the profiles observed in this study are in agreement with those observed in others. Carlton and Wetzel (1988) observed elevated pH conditions in the sediment surface under light conditions with significant decreases in pH (about 1 pH unit) observed down through the sediment. The removal of P from the water-column in Ca-P complexes is enhanced under elevated pH conditions (i.e. above pH 6.5 – 7.0: Wetzel, 2001; Otsuki and Wetzel, 1972). Therefore, under the conditions observed at the end of the incubation experiments, one may expect release of P from Ca-P complexes in the deeper sediment layers and precipitation of Ca-P in the water-column and surface sediments. However, the observed difference of 0.1 pH unit in this study, between dark and light conditions, is lower than the 1 pH unit difference observed in other studies (Dodds, 2003). DO concentrations were also higher under light (9.85 mg L) when compared to dark (9.25 mg L) conditions. Again, this difference is low in comparison to other studies, for example supersaturated DO concentrations (>150% for *Phormidium/Cymbella* and *Ulothrix* dominated assemblages) have been observed in the surface layers (upper 2 mm) of dense algal mats (Dodds, 2003). The elevated DO concentrations under light conditions may

decrease the release of P from sediment Fe-P and Mn-P complexes to the overlying water-column.

Although these experiments were aerated, the significantly higher DO concentrations observed under light conditions reflect the elevated DO consumption of the sediment in the absence of benthic primary production. These observations strengthen the hypothesis that benthic autotrophic production can limit P and N release through the maintenance of elevated DO and pH conditions. Under such conditions the likelihood of Ca-P and Fe-P release is reduced (Boström *et al.,* 1988) and the reduction of NH_4-N by microbial nitrification is enhanced (Risgaard-Petersen *et al.,* 1994). However, direct uptake of nutrients by benthic microalgae must also be considered and is likely to be a key process affecting SiO_2 release from the sediment. Additionally, *in situ* processes may not always be so homogenous, and so the actual "in-lake" effects may be greater than those observed in these experiments.

Light manipulation experiment: effects of light on water-column nutrient stoichiometry

The TN:TP ratio was highest, and TSi:TN ratio lowest, under light conditions. Thus, dependent upon absolute concentrations, water-column N-limitation may become less likely under light conditions. The main factors regulating this variation are likely to be driven by benthic photosynthesis and include elevated DO concentrations and pH conditions at the sediment surface under light conditions regulating P release more than N release, as discussed above.

Few other studies have assessed the effects of sediment nutrient cycling on water-column nutrient stoichiometry with the notable exception of Levine and Schindler (1992) in which TN:TP ratio was observed to decrease (i.e. alleviation of N-limitation)

as a result of sediment processes. This discrepancy may indicate a difference in the loading histories of the study lakes where Loch Leven has been impacted by elevated external nutrient loading for several centuries (estimated using paleolimnological techniques; unpublished data) and, as such, experiences large sediment-P release events in comparison to the lakes considered by Levine and Schindler (1992).

WIDER IMPLICATIONS OF STUDY

The sediment nutrient release scenario presented in this paper is representative of a shallow lake recovering from eutrophication. Under other circumstances (i.e. different nutrient loading history) the effects of temperature and light may be altered, as indicated by the comparison of this study with the ELA example (Levine and Schindler, 1992). Eutrophication is a very widespread problem and the need for understanding the processes that could enhance or speed up restoration has never been greater, particularly given European legislation (the EC WFD) which requires waters to achieve "good" ecological management by 2015. This study provides valuable information on the role of the sediment community in influencing the recovery period. Improved water clarity (and resultant enhancement of sediment light regime) will be a positive feedback on recovery and, in general, N limitation should be reduced as water quality improves.

CHAPTER 7

On the boundaries: sediment stability measurements across aquatic ecosystems

Spears, B.M., Funnell, J., Saunders, J., & Paterson, D.M. *In press.*

Proceedings of the International Symposium of Sediment Dynamics

and Pollutant Mobility in River Basins (SEDYMO)
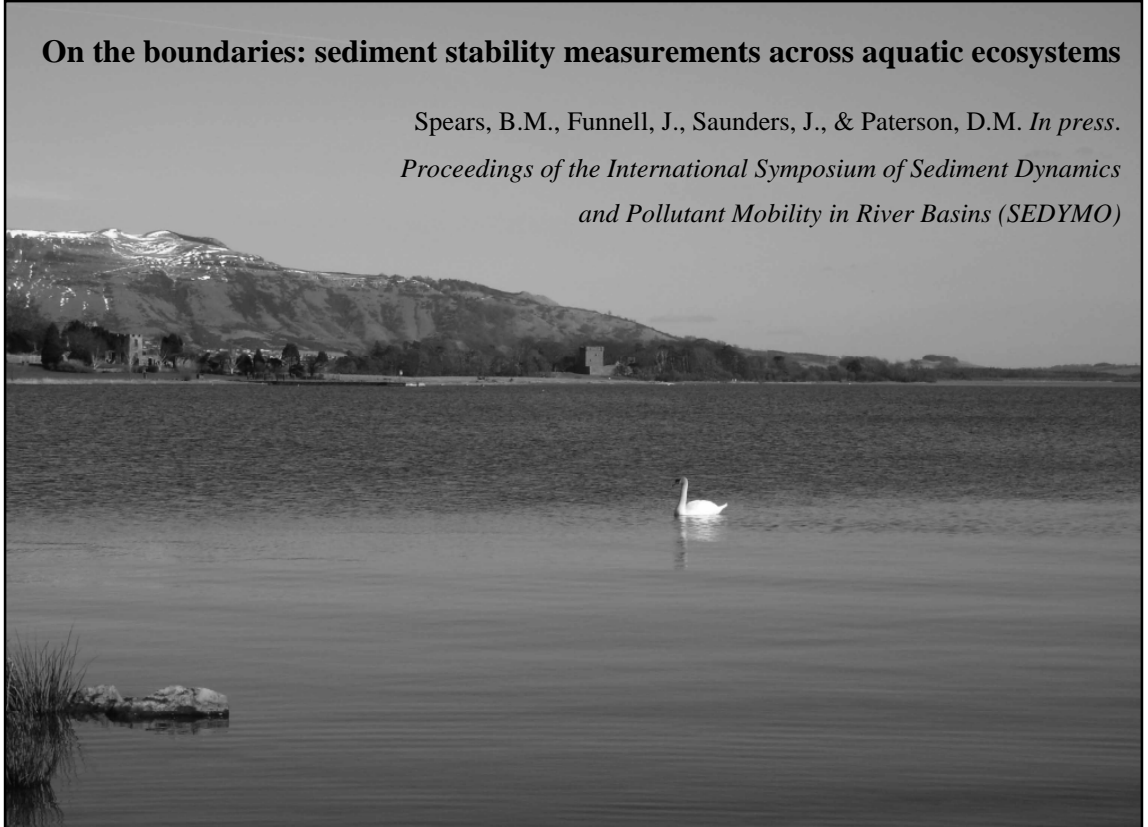
On the boundaries: sediment stability measurements across aquatic ecosystems

ABSTRACT

Over the last decades, fluid dynamics has undergone a revolution in the understanding of the importance of flow to the ecology of aquatic ecosystems. This advance has been driven in two ways: firstly as a by-product of improved system analysis that allows the determination of flow patterns at ever decreasing scales of space and time; secondly, many new methods have been specifically developed to examine questions in ecology related to flow dynamics and organism and sediment behaviour. Early work was fairly predictable, employing standard fluid dynamics approaches to test biological theory under laboratory conditions (e.g "flume and flora" experiments), then a period began in which many laboratories produced devices to measure the stability of sediment under field conditions. Examples range from linear flumes, carousel or circular chamber systems, to suction or jet devices. They produce useful data but rarely on a comparative scale across ecosystems. This paper introduces some initial comparative measurements and discussion relating to the measurement of the erosive dynamic in varied and often extreme (intertidal, salt marsh) systems.

INTRODUCTION

Few studies in the literature compare the sediment stability of depositional habitats across marine, freshwater and brackish ecosystems. This is partly because there is conceptual difficulty in comparing different erosional devices but also because scientists often focus on specific habitats. In addition, many field devices generate shear stresses over the 0-1 Nm^{-2} range, with few capable of generating erosive forces beyond this level (Tolhurst *et al*., 2000). However, habitats such as intertidal deposits and salt marshes are often quite resistant to hydrodynamic forcing and are considered to provide an "ecosystem service" of coastal protection. Most existing measurements have been made within a "measurement comfort zone" (Figure 7.1), usually where a bed shear stress between approximately 0.1 and 1 Nm^{-2} surpasses the critical threshold. However, the study of a wider range of habitats is fundamental to the understanding of ecosystem dynamics in aquatic environments.
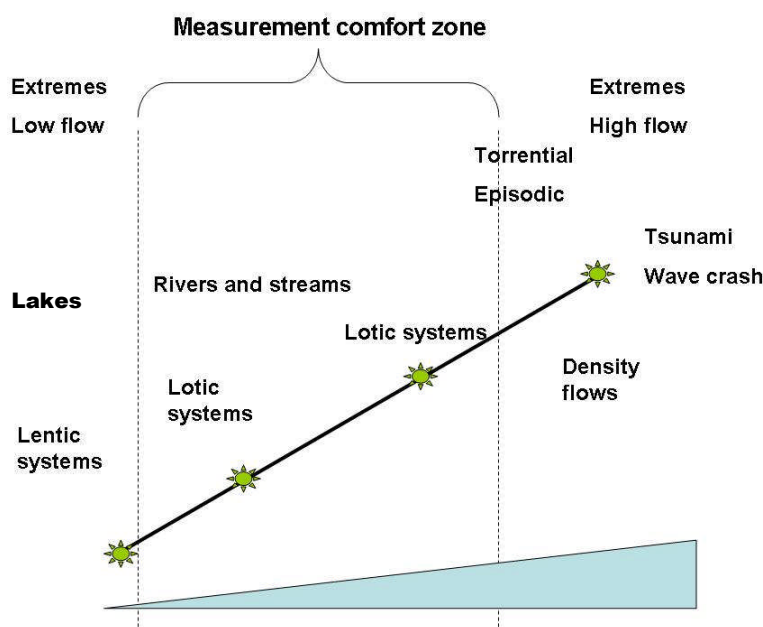
Figure 7.1. *Schematic diagram of the measurement comfort zone for many erosion devices.*

Resistance to sediment disturbance can be enhanced via physicochemical (particle-particle attraction/resistance; Lerman 1979; Lick and Huang, 1993), bio-physical (e.g. increased structural integrity within root beds; Kenworthy *et al*., 1982; Benoy and Kalff, 1999) and bio-chemical processes (the production of extracellular polymeric substances: EPS) (Paterson, 1994; Yallop *et al*., 2000; Deco, 2000). While the majority of field based sediment stability studies have been conducted within estuarine mudflat ecosystems, the relevance of these findings across different sediment ecosystems remains largely unknown. Variation should be expected since significant variations in ecosystem processes, known to affect sediment stability, occur within, and between, different ecosystems (e.g. lakes, rivers, and estuaries). These include exposure/submersion cycles, the deposition/removal of fine particulate matter (FPM), the community structure of benthic fauna and flora, and the shear stress exerted across the sediment surface (O'Sullivan and Reynolds, 2004; Nedwell and Raffaelli, 1999).

In estuarine systems, the presence of EPS, secreted mainly by benthic diatoms (Goto *et al*., 1999; Yallop *et al*., 2000), has been shown to enhance sediment stability (Paterson 1997; Tolhurst, 1999; Decho, 2000). Recent studies have also highlighted the role of vascular plant root-systems in enhancing sediment stability and accumulation by increasing the structural integrity and, therefore, the bed shear strength of colonised riverine and estuarine sediments (Grady, 1981). Similar ecosystem engineering may occur in shallow freshwater lakes in which light regimes favour the colonisation of macrophytes and microphytobenthos. However, a number of differences occur between ecosystems. For example, tidal cycles drive diurnal fluctuations in estuarine sediment exposure/submersion and compaction. In contrast, lacustrine sediments are continually submerged, with fewer disturbances and a higher depositional flux of fine particulate matter. Another key difference between freshwater and estuarine systems is the strength

of the electrostatic attractions (cohesion) between sediment particles as influenced by salinity (Packman and Jerolmack, 2004).

A relatively simple comparative assessment of sediment stability across estuarine and freshwater habitats, using the same measurement system, was undertaken. Additionally, spatial variation within each ecosystem was assessed (lake depth, intertidal position). The specific objectives of the study were to (1) assess the spatial variation (i.e. inter- and intra-site) of sediment stability and related sediment characteristics; (2) identify the key variables regulating stability in each site; and (3) identify trends in stability regulation across ecosystem types.

Figure 7.2. *Loch Leven and the Eden Estuary.*
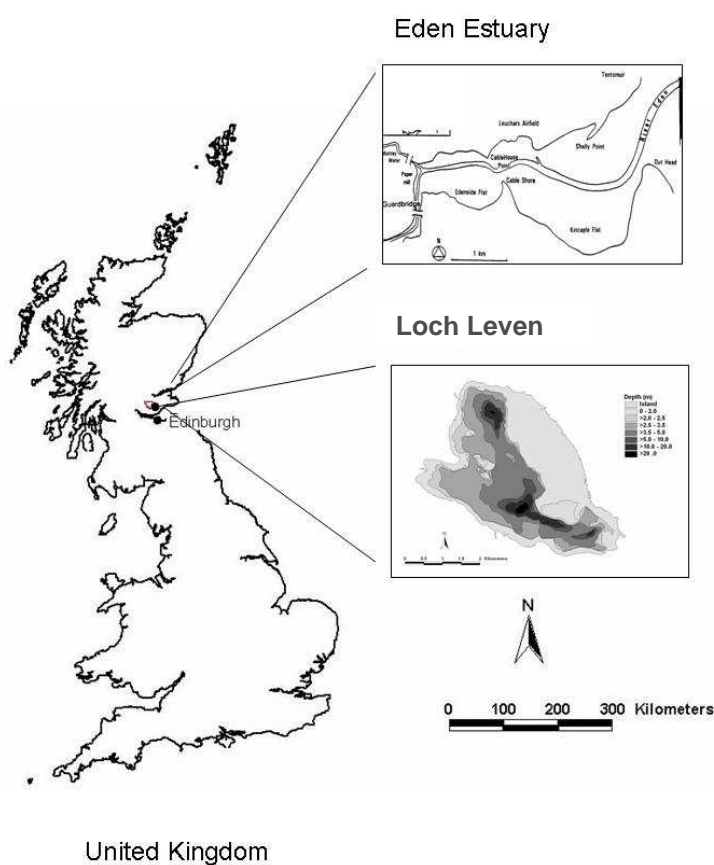
METHODS

Study sites

Loch Leven is a shallow eutrophic lake situated in the southeast of Scotland (56° 10' N, 3° 30' W), with a mean depth of 3.9 m, surface area of 13.3 km^2 and is generally dominated by *Potamogeton sp.* Two sample sites were chosen at 2.1 m (shallow Loch Leven site) and 4.3 m (deep Loch Leven site) depth (Figure 7.2) to represent macrophyte and microphytobenthos dominated sediment, respectively.

The Eden estuary is situated in southeast Scotland (56° 22' N, 2° 50' W) and is composed of both mudflat (8 km^2 surface area) and saltmarsh (0.11 km^2). The mudflat is generally dominated by microphytobenthos (mainly diatoms) and the saltmarsh mainly by *Puccinellia maritima*. Three sample sites were chosen on the southern shore near the mouth of the estuary to represent low shore and mid shore mudflat, and saltmarsh sediment areas (Figure 7.2). Sediment is predominantly sandy mud.

Sample collection

20 cores were collected from each site (i.e. 100 cores in total). At each site, 10 cores were used for stability analysis and 10 cores for the quantification of other sediment characteristics. In the latter, the upper 2 mm of sediment was cryogenically preserved using the contact coring technique (HIMOM, 2005). Cores (8 cm internal diameter and 5 cm deep) were collected manually (7-15th February, 2006) from within a 2 m^2 area of exposed sediment at both mudflat sites and the saltmarsh site. To maintain the natural structure of the sediment, macrophytes present in the saltmarsh cores were not removed. Freshwater sediment cores (6.7 cm internal diameter) were collected (21st March 2006 by boat) at each site, within a 10 m^2 area of submerged sediment using a Jenkin surface-sediment sampler. The cores consisted of 20 cm sediment overlain by 30

cm water. The overlying water was either left (for the analysis of sediment stability) or carefully siphoned off to leave an exposed sediment surface as required for contact coring.

Sediment stability

Sediment stability was measured using a Mark IV Cohesive Strength Meter (CSM) (Paterson *et al*., 1989). The CSM operates by firing a sequence of water jets with incremental increases in pressure onto the sediment surface inside a small (2 cm diameter) erosion chamber filled with filtered seawater. Eroded sediment is detected by an infrared transmission across the inside of the erosion chamber. The critical erosion threshold is deemed to have passed when suspended sediment levels result in a 10% drop in the original transmission value encountered at the beginning of the test (Tolhurst *et al*., 2000). The test programme "Fine 1" was selected due to its high resolution at low critical erosion thresholds which were expected after initial trials upon the sediments. The CSM was calibrated and results expressed as the stagnation pressure upon the sediment surface expressed as Nm^{-2}.

Cores from the saltmarsh and mudflat sites were analysed in the laboratory. The erosion chamber was set flush with the exposed sediment surface and manually filled with filtered saline water by syringe. Stability in freshwater cores was analysed on a boat, immediately after collection of the cores, with the overlying water-column intact. Filtered saline water was used as the erosive medium in saltmarsh and mudflat cores and distilled water was used in the freshwater cores.

Other sediment characteristics

At each site, 10 contact cores were collected for the analysis of wet bulk density, water content, and organic content (HIMOM protocols, 2005). Bound and colloidal carbohydrates were separated following the addition of 5 ml distilled water to 50 mg freeze dried sediment and subsequent centrifugation (1500 rpm for 15 minutes). The resultant pellet was analysed for bound (attached), and the supernatant colloidal, carbohydrates (Underwood *et al.*, 1995). Both colloidal and bound carbohydrate concentrations were determined using Dubois assays as described within HIMOM protocols (2005) and are expressed against a glucose standard curve as glucose equivalents ($\mu g\ g^{-1}$ dry weight glucose equivalents). Chlorophyll *a* was extracted from freeze dried sediment in 90% methanol. The extraction was conducted in an ultrasound bath for 90 min. Following this, samples were stored in the dark at $-80^{o}C$ for 24 hours before being vortexed and stored for a further 24 h in the same conditions. Samples were centrifuged (2000 rpm) for 3 min prior to spectrophotometric pigment analysis according to HIMOM (2005).

Statistical analysis

Variation was assessed using one-way analysis of variance (ANOVA) followed by Fisher's least significant difference post-hoc analysis (critical value = 2.014, α = 0.05), on observation of a significant ANOVA result. An analysis of the relationships between variables within each site (intra-site relationships) was conducted using correlation analysis (n = 10). An analysis of the relationships between variables across the five sites (inter-site relationships) was also conducted using correlation analysis (n = 5). All error bars shown represent the standard error of the mean (n = 10) taken from each variable.

RESULTS AND DISCUSSION

Variation in sediment stability properties between ecosystem types

Significant intra-site variation (Table 7.1 and Figure 7.3a; ANOVA analyses; p < 0.05) was observed in sediment stability, colloidal carbohydrate concentration, bound carbohydrate concentration, chlorophyll *a* concentration, organic matter content, water content, and bulk density. Sediment stability was lowest in the freshwater sites, intermediate in the mudflat sites and highest in the saltmarsh site. Stability was observed to increase with depth in the freshwater sites and with increasing distance up the shore in the estuary. The saltmarsh site was more stable than the intertidal low shore site, and both freshwater sites. Sediment stability was significantly higher in the mid shore intertidal site than it was in the shallow freshwater site (Figure 7.3a). Colloidal carbohydrate and bound carbohydrate concentrations were significantly higher in the deep freshwater site than at all other sites (Figure 7.3b). The bound carbohydrate concentration in the shallow freshwater site was higher than for mid and low shore mudflat sites but similar to the values observed in the saltmarsh site (Figure 7.3c). Chlorophyll *a* concentration was significantly higher in the deep freshwater site than any other site (Figure 7.3d). The saltmarsh and the shallow freshwater site had similar chlorophyll *a* concentrations, both being higher than the mudflat sites. Sediment organic composition was similar in the saltmarsh and the deep freshwater sites, values for both of which were significantly higher than all other sites (Figure 7.3e). The water content of the mudflat sites were both low and did not differ from each other. Intermediate water content was observed in the saltmarsh site followed by the shallow freshwater site with the highest water content found in the deep freshwater site (Figure 7.3f).

Table 7.1. *Summary of results taken from Fishers post hoc analysis. Direct comparisons of primary variables versus all other variables included where '+' represents instances where primary variables are higher than other variables and '-' represents instances where the primary variable is less than other variables. 2.1m: 2.1 m freshwater lake site; 4.3 m: 4.3 m freshwater lake site; Low: low shore mudflat site; Mid: mid shore mudflat site; salt: saltmarsh site.*

Primary variable	2.1 m	4.3 m	Low	Mid
Sediment stability				
2.1 m				-
4.3 m				-
Low				
Mid			+	
Salt	+	+	+	+
Colloidal carbohydrates				
2.1 m		+		
4.3 m				
Low		+		
Mid		+		
Salt		+		
Bound carbohydrates				
2.1 m			+	+
4.3 m	+		+	+
Low				
Mid				
Salt	-	-	+	+
Chlorophyll a				
2.1 m			+	+
4.3 m	+		+	+
Low				
Mid				
Salt		-	+	+
Organic matter content				
2.1 m				
4.3 m	+		+	+
Low				
Mid				
Salt	+		+	+
Water content				
2.1 m			+	+
4.3 m	+		+	+
Low				
Mid			+	
Salt	-	-	+	+
Bulk density				
2.1 m			-	-
4.3 m			-	-
Low				
Mid				
Salt	+	+	-	-

The bulk density of the intertidal sites was greatest, followed by the salt marsh site and then the freshwater sites. Bulk density in the mid shore site was significantly greater than all other sites with the exception of the low shore tidal site (Figure 7.3g).

Intra-site relationships between sediment stability properties

A positive correlation between sediment stability and colloidal carbohydrate concentration was found for the deep freshwater site (Table 7.2). Negative correlations were observed between sediment stability and chlorophyll *a*, and sediment stability and organic matter composition in the mid shore tidal site and between sediment stability and bulk density in the salt marsh site. Sediment stability was not significantly correlated with any of the measured variables in the shallow freshwater site or the low shore site.

Inter-site relationships between sediment stability properties

No significant relationships were observed between sediment stability and any of the other measured variables across the sites. Colloidal carbohydrate, bound carbohydrate and chlorophyll *a* concentrations were significantly positively inter-correlated. The measured sediment characteristics varied significantly both across and within the five sites. The majority of these differences can be accounted for by considering the key ecosystem processes acting within each. Within the estuarine environment, wet bulk density was lowest in the saltmarsh site and similar in both low and mid shore sediment. This is probably the result of a number of factors including bioturbation, the effect of vegetative cover on the saltmarsh and the consequent retention of fine sediments. In comparison, the bulk density values of both freshwater sites were significantly lower than all the estuarine sites. This is due to the depositional

Table 7.2. *Summary of correlation analysis (n = 10; p < 0.05) for each site. Only significant correlations reported. Coll. Carb: colloidal carbohydrates; Bound carb.: bound carbohydrates; Chl a: chlorophyll a; Org.: Organic content; Bulk. Den.: bulk density. Values in bold indicate relationships including sediment stability.*

Relations	Variable X	Variable Y	P-value	R^2
Loch Leven 2.1 m	Coll. carb.	Chl. a	0.043	0.65
	Coll. carb.	Bulk den.	0.021	-0.71
	Bulk den.	Water	0.025	-0.70
	Chl. a	Org.	0.043	0.64
Loch Leven 4.3 m	**Stability**	**Coll. carb.**	**0.032**	**0.675**
	Coll. carb.	Water	0.002	0.85
	Bound carb.	Chl. a	0.009	-0.77
	Chl. a	Water	0.015	0.74
Eden low shore	Chl a	Org.	0.007	0.79
	Water	Chl. a	0.014	0.74
	Water	Org.	0.004	0.81
Eden mid shore	**Stability**	**Chl. a**	**0.044**	**-0.64**
	Stability	**Org.**	**0.016**	**-0.73**
	Coll carb.	Bound carb.	0.016	0.73
	Chl. a	Org.	0.011	0.76
	Chl. a	Water	0.034	0.67
	Org.	Water	0.008	0.78
	Bulk den.	Water	0.013	-0.75
Eden saltmarsh	**Stability**	**Bulk den.**	**0.033**	**-0.67**
	Coll. carb.	Chl a.	0.028	0.69
	Coll. carb.	Org.	0.013	0.75
	Coll. carb.	Water	0.025	0.70
	Chl a.	Org.	0.000	0.90

environment in productive freshwater lakes where high fluxes of fine particle matter and flocs combined with low sediment flushing rates occur (Hilton *et al*., 1986; Weyenmeyer *et al.,* 1995). These processes result in the accumulation of fine, unconsolidated organic matter (Nedwell *et al*., 1999).

Water content was expected to decrease up the shore line as a result of increased exposure and evaporation. However, the opposite trend was observed, most likely

resulting from biologically-mediated ecosystem engineering where biofilms and rooted plant beds can trap moisture and prevent desiccation (Paterson and Black, 1999; Yallop *et al.*, 2000).

Table 7.3. *Correlation analysis of sediment characteristics across five sites (Loch Leven 2.2 m, Loch Leven 4.3 m, Eden low shore, Eden mid shore, and Eden saltmarsh). Coll. Carb: colloidal carbohydrates; Bound carb.: bound carbohydrates; Chl a: chlorophyll a; Org.: Organic content; Bulk. Den.: bulk density. Significant correlations in bold with p values in parenthesis.*

Variable	Stability	Coll. carb.	Bound carb.	Chl. a	Org.	Water
Coll. carb	-0.309					
	(0.613)					
Bound. carb.	-0.371	**0.984**				
	(0.538)	**(0.002)**				
Chl. a	-0.223	**0.966**	**0.983**			
	(0.718)	**(0.008)**	**(0.003)**			
Org.	0.360	0.662	0.673	0.797		
	(0.552)	(0.223)	(0.213)	(0.106)		
Water	-0.525	0.779	0.878	0.856	0.551	
	(0.363)	(0.120)	(0.050)	(0.064)	(0.336)	
Bulk den.	0.436	-0.690	-0.815	-0.815	-0.631	**-0.936**
	(0.462)	(0.197)	(0.093)	(0.093)	(0.254)	**(0.019)**

Chlorophyll *a*, organic matter, and carbohydrate concentrations

Similar trends in chlorophyll *a* concentration, organic matter content, and bound and colloidal carbohydrate concentrations were observed across the sites. The correlations between chlorophyll *a* concentration and bound and colloidal carbohydrate concentrations within freshwater and saltmarsh sediments suggest that the main source

of carbohydrates in these systems are derived from autotrophic processes. The absence of a significant correlation between chlorophyll *a* and carbohydrate concentrations in the mudflat sites agrees with the observations of Perkins *et al*. (2003) in which carbohydrate concentration was shown to be more sensitive to a simulated tidal cycle than chlorophyll *a* concentration as a result of the greater solubility of carbohydrate in water. Thus, an uncoupling of the two variables can be expected in sediment ecosystems exposed to severe tidal processes. Both bound and colloidal carbohydrate concentrations were higher in freshwater than they were in estuarine sediments. Coupled with the chlorophyll *a* concentrations, this suggests that the highest autotrophic production of carbohydrates occurred in freshwater sediment which may be due to the relatively high autotrophic (i.e. combination of phytoplankton, microphytobenthos, epiphytes etc) production in these high nutrient systems.

The higher chlorophyll *a* values in the freshwater sites compared to the mudflat sites are likely due to a higher accumulation of phytoplanktonic detritus. This process is especially important in eutrophic lakes where movement of phytoplankton between the sediment and the water-column is regulated by environmental variables and can account for major portions of total phosphorus and nitrogen partitioning (Head *et al*., 1999). The high organic content and chlorophyll *a* concentrations observed in the saltmarsh, in comparison to the mudflat sites, is likely due to an increase in *P. maritime* in combination with microphytobenthos biomass as a result of increased exposure time (Austin *et al*., 1999).

Sediment stability regulation: an ecosystem comparison

Significant spatial variation was observed in sediment stability between freshwater, estuarine mudflat, and estuarine saltmarsh sites. Stability was highest in the
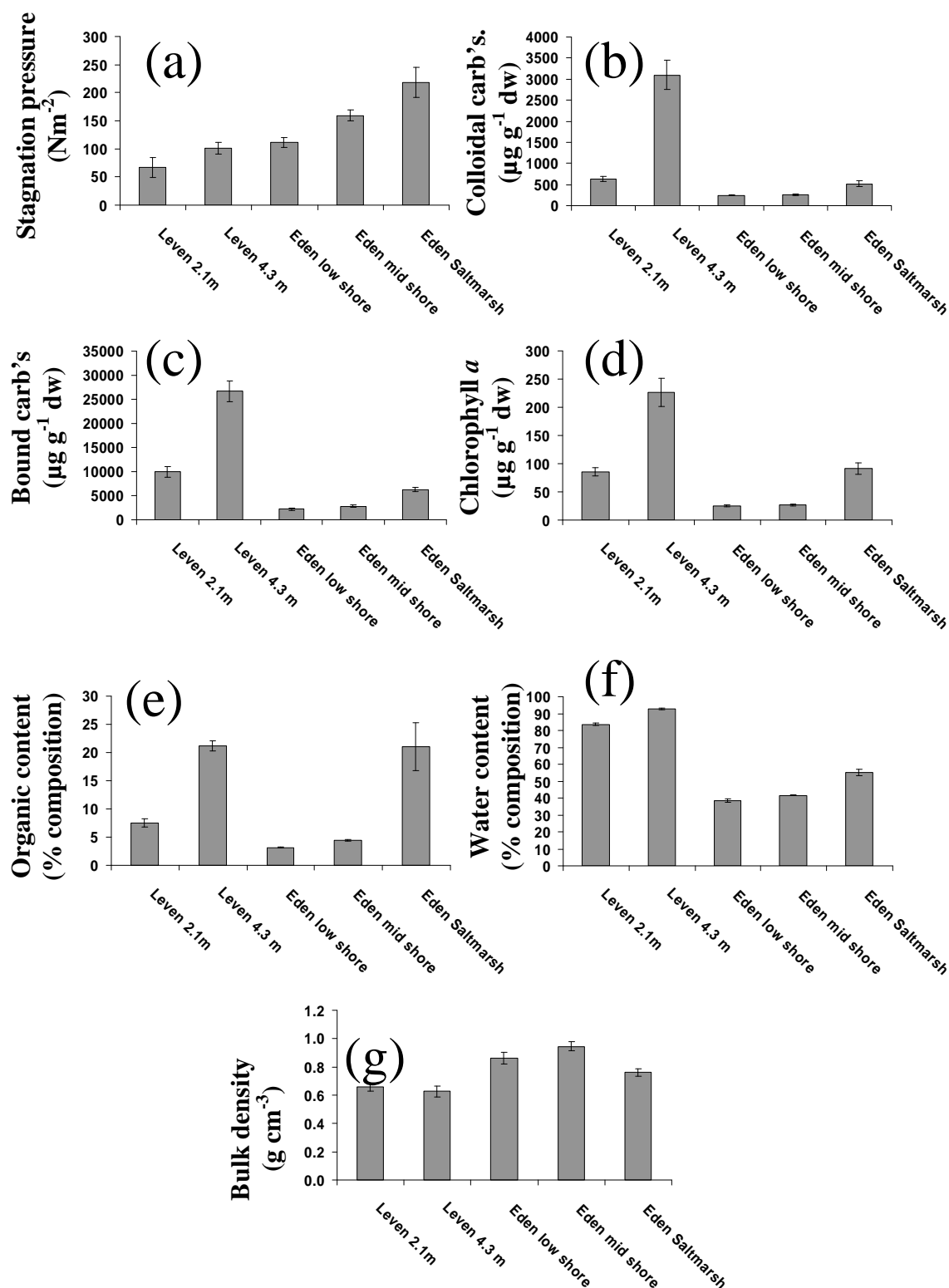
Figure 7.3. *Average cross site trends in (a) stagnation pressure that corresponds to the critical erosion threshold, (b) colloidal carbohydrate concentration, (c) bound carbohydrate concentration, (d) chlorophyll a concentration, (e) organic content, (f) water content, and (g) wet bulk density. Error bars represent the standard error of the mean (n = 10).*

saltmarsh site and lowest in the shallow freshwater site. Sediment stability was observed to increase with depth in the freshwater sites and with distance up the shore in the estuarine sites. In general, sediment stability was higher in the estuary than it was in the lake. No significant correlations were observed between sediment stability and any of the measured variables across the five sites indicating differing mechanisms of stability regulation between sites or the existence of controlling mechanisms not considered in this study. One example of the former is the influence of electrostatic and physico-chemical particle-particle attractions increasing with sodium chloride concentration (i.e. salinity; Lerman, 1979; Packman and Jerolmack, 2004). Thus, cohesive aggregation increases across the salinity gradient such that estuarine sediments are more 'cohesive' than freshwater sediments. However, this would represent a sedimentological view and it is almost certain that extracellular polymers are also influenced by the ionic nature of their surrounding and contribute more to sediment cohesion under saline conditions. This hypothesis suggests that physico-chemical processes are the main drivers of sediment stability across ecosystem types with bio-physical and bio-chemical processes also being affected by the physicochemical changes. This explains why, although levels of extracellular polymers are high in the deep freshwater sites, the enhanced stability is less in comparison to the intertidal sites. This possibility requires further research.

Stability was significantly correlated with a number of variables within sites. The positive correlation between sediment stability and colloidal carbohydrate concentration in the deep freshwater site was in agreement with conventional diatom biostabilisation theory (e.g. Madsen *et al*., 1993; Paterson, 1994). The absence of this relation in the shallow freshwater site may be the result of excessive wind mixing reducing microphytobenthos biomass, and hence the production of carbohydrates.

CONCLUSIONS

The variation in sediment stability was not explained by a single variable across ecosystems. Instead, stability was found to be system specific and, therefore, care must be taken when defining general rules of stability across ecosystem types. The stability of the bed was higher in the estuarine sites than it was in the freshwater system, probably as a result of elevated electrostatic effects on particle-particle attraction and polymer related adhesion in the former. Stability was higher at 4.3 m overlying water depth compared to 2.1 m overlying water depth in the freshwater site most likely as a result of higher autotrophic production of carbohydrates. Colloidal carbohydrate concentrations were higher in the freshwater ecosystem than in the estuarine ecosystem but less effective at stabilisation.

CHAPTER 8

Understanding the biogeochemical processes acting to transform nutrients within the sediment and regulate transfer between the sediment and the overlying water-column is a complex scientific discipline. Spatially, variations in such processes can be observed from the geographic- (governed by land-use and variations in seasonal extremes; Lehner and Döll, 2004) to the micro-scale (Downing and Rath, 1988). Similarly, temporal variations can occur over thousands of years (e.g. natural aging of a lake; Engstrom *et al*., 2000) or in the space of a few minutes (Froelich, 1988). Gaining a true comprehension of such variation is crucial to our ability to understand the effects of anthropogenic pressures on the aquatic environment. However, our ability to realise this goal is limited by our capacity to observe the spatial and temporal dynamics of the system, and by our lack of knowledge concerning key processes acting to regulate it. This discussion aims to highlight the advances provided by this body of work against the background of current knowledge of the spatial and temporal variations in sediment nutrient processes and of the importance of benthic/pelagic biologically mediated nutrient cycling in regulating such processes. However, the advancements to the methodologies available for quantifying sediment phosphorus content and benthic microalgae in the context of the current state of the art, are first discussed.

ADVANCEMENT TO METHODOLOGIES

Sediment-P fractionation

Sequential chemical extraction has been used as a means of operationally quantifying a range of sediment-P compounds for many years (i.e. table 1.1). These methods are commonly used to infer processes leading to alterations in internal loading

in lakes. A criticism of these methods is that, in many cases, insufficient sample replication is employed to allow accurate quantification of the natural spatial and temporal variation in site-specific sediment nutrient content and so wider scale generalised conclusions, although common, may be misleading. The significance of seasonal and spatial variation in sediment-P composition was shown in the present study where variations in concentrations in a number of sediment-P fractions were observed to occur within Loch Leven. Additionally, these P fractions varied both dependently and independently of each other adding further complexity to the approach. A number of environmental drivers are responsible for these variations including chemical, biological, and physical mechanisms (discussed in detail in chapters 2-4). These mechanisms must be taken into account when comparing differences between systems. Although intra-site spatial variations were observed in this study, similar variations may not occur in other lake types due to differences in the spatial variability of environmental factors. Few studies have highlighted micro-scale spatial variation in sediment nutrient content (Downing and Rath, 1988) that might be expected when considering a range of relevant factors including variable rates of deposition, sensitivity to turbulent mixing, and variations in microalgal (i.e. epipelon (Jesus *et al*., 2005), bacterial, and zoobenthos communities (Bergtold and Traunspurger, 2004). Temporal variation is also important and attempts should be made to assess the repetitiveness of the observed seasonal trends in this study and to ensure that these variations are relevant over a range of lake types. Assuming these trends are representative of the annual patterns, samples would have to be collected from each season to provide an accurate representation of whole-system sediment conditions.

Sediment-P fractionation, when assessed using a thorough spatial and temporal design, can provide an assessment of whole-system P dynamics (chapter 4), although,

care must be taken in interpreting such results. A key assumption with this approach is that a decrease in the sediment TP concentration represents a transfer of P from the sediment to the water-column and not simply to sediment layers below those sampled. Although this is likely to be true, and is backed up by observations in the literature dealing with the vertical distribution of P in the sediment (Iqbal *et al*., 2006; Turner *et al*., 2006), further study may be necessary to assess the extent to which this process varies spatially and seasonally. This vertical distribution of P in the sediment is common and is caused by the accumulation of upwardly migrating P, originating from deeper anoxic sediment layers, in the oxygenated surface layers (Anderson *et al*., 1993; Golterman, 2004). In the scenario presented in chapter 4, the decrease in sediment-P was mirrored by an increase in water-column P and this, combined with the detailed assessment of seasonal and spatial variations in the various P fractions (chapter 2), substantiates the assumption. Improving the understanding of these mechanisms provides information, with regards to the seasonality of the sediment-P partitioning that can then facilitate more informed water-quality management decisions (as discussed in chapter 4).

Sediment-water equilibrium assessments

Equilibrium experiments are commonly employed to assess the nutrient concentrations in the overlying water-column that would illicit either uptake or release of nutrients from the sediment. However, such studies traditionally employ resuspension, conditions that may not accurately represent natural environmental situations in lakes (Sundareshwar and Morrris, 1999; Perkins and Underwood, 2001; Spears *et al*., 2006a). By disturbing the sediment-water interface these studies disrupt the processes that may be key in regulating actual sediment-water nutrient interactions

across an intact interface. The results outlined in chapters 2 and 3 highlight the fact that equilibrium conditions across an intact sediment-water interface can be greatly misrepresented using suspension techniques. Under undisturbed conditions the EPC0 of sediment collected from 3 m overlying water depth was between 180-270 μg L^{-1} in comparison to 11 μg L^{-1} under conditions of resuspension. It is, therefore, imperative that natural conditions be considered in such studies. Additionally, spatial variation is likely to occur as a result of variation in the benthic community (chapter 5). However, further work is required to assess the effects of benthic community variation on the uptake and release potential of the sediment. Our observations indicate that Loch Leven sediment will uptake PO$_4$-P under disturbed conditions when water-column PO$_4$-P concentrations are > 11 μg L^{-1} - 30 μg L^{-1} and release PO$_4$-P under undisturbed conditions.

Benthic microalgal community structure

The benthic microalgal community was found to be heterogeneous in composition with both spatial and seasonal variations observed. Although significant correlations were observed between bulk sediment chlorophyll *a* and sediment-P fractions it was difficult to determine whether a specific component of the community had a greater affect on sediment-P concentrations in relation to others. For example, strong correlations were observed between bottom water and sediment chlorophyll *a* and a number of sediment-P fractions, however, this chlorophyll signal was composed of a number of operationally defined microautotrophic groups. It was, therefore, difficult to identify the component of the microautotrophic community responsible for the correlation. Which leads the question: what groups of algae need to be distinguished to accurately apportion community functions? The fact that the major component of the

sediment microalgal biomass was made up of "non-epipelic" (phytoplankton) algae suggests that these correlations may not be indicative of actual algal regulation but instead may indicate the importance of the flux of organic nutrients from the water-column to the sediment, or *vice versa*, in driving internal nutrient cycling. This is especially conspicuous when one considers the fact that the relationships between sediment chlorophyll and sediment-P appear to be strongest in the deeper aphotic sediment habitats where active photosynthesis is not possible. However, the series of experiments designed to assess benthic microalgal control of nutrient cycling between the sediment and the water-column (chapter 6) indicated that, regardless of the community composition, benthic photosynthesis had significant regulatory effects on sediment nutrient release. This highlights a number of issues with the study of such abiotic/biotic sediment nutrient interactions. Assessment of bulk biomass (i.e. using photosynthetic pigments or Dive PAM fluorescence) is unlikely to accurately determine the active component of the community, especially in shallow eutrophic lakes where the planktonic flux to the sediment is high. As such, when considering the effects of community composition on benthic/pelagic nutrient cycling, there is a requirement for selective sampling strategies that allow the identification of key functional groups within the community. The methods reported (chapter 5) were a first attempt at separating this community and assessing the environmental variables responsible for the group-specific variations. Large-scale spatial and seasonal variations were observed in microalgal community composition further supporting the need for such selective techniques. The relatively crude nature of these techniques is recognised and it is clear that greater effort is needed to create a robust method, based on the work presented in chapter 5, capable of accurately and repeatedly sampling such functional groups. These

should include methods of assessing the vertical migration of specific groups of epipelon.

Microscopic analysis of the epipelic community indicated that spatial variation was regulated mainly by a combination of increasing light limitation and decreasing habitat disturbance with depth, where the highest biomass was observed at intermediate (i.e. a trade off between light and disturbance limitation) water depth. However, there will undoubtedly be other factors important in driving such variation. These will include depositional rate, grazing pressures, sediment nutrient stoichiometry, and bioturbation. The relative effects of these processes on community structure needs to be further understood before the functional role of these organisms, and the level to which community shifts affect changes in community function, can be fully assessed.

BENTHIC MICROALGAL ECOSYSTEM FUNCTIONING IN LAKES

Sediment-P replenishment

The observations made during the course of this work have raised more questions than have been answered with respect to the role of benthic microalgae in regulating benthic/pelagic nutrient cycling. That said, a number of key processes have been identified. Phytoplanktonic "fall out" to the sediment acts as the main feedback loop of nutrients from the water-column to the sediment in Loch Leven. This feedback mechanism is responsible for driving the process of internal loading and prolonging the recovery from eutrophication in shallow lakes where concentration gradients between the sediment and the overlying water-column nearly always favour release from the sediment. This depositional nutrient flux is seasonal with the largest inputs to the sediment occurring after the summer phytoplankton bloom. It is likely that the major functional group responsible for this transfer of nutrients is the cyanobacteria

(especially *Microcystis*). This theory stems from the commonly observed *Microcystis* blooms that follow the summer internal release events in Loch Leven and, therefore, represents the major component of the phytoplankton biomass during the period of greatest P transfer. The transfer of nutrients between the organic and inorganic phase within the sediment is driven by microbial decomposition, a fact that has been long acknowledged in the literature but little studied. The role of meroplankton (including *Microcystis* and the diatom *Aulacoseira*) migration between the sediment and the water-column is also expected to play a significant seasonal role, and although supporting evidence is presented (chapter 3), further work is required to substantiate this hypothesis.

True benthic microalgae can also play an important role in governing nutrient release from the sediment. This was assessed experimentally in this study (chapter 6) where benthic autotrophic production was observed to have a regulatory effect on the transfer of a suite of nutrients (P, Si, and NH_4-N) across the sediment-water interface. The effects of this regulation were not uniform across the suite of nutrients considered resulting in strong alteration of the nutrient stoichiometry in the overlying water-column. The effects of temperature were also indicated with the most significant effects of regulation by benthic photosynthesis observed under high temperatures. This is most probably due to the dynamics of dissolved oxygen concentrations where high temperatures increase the benthic microbial DO demand and therefore decrease the sediment DO concentration in aphotic environments. A number of observations in this study support this hypothesis suggesting that the autotrophic production of DO plays a key role in regulating the release of reductant-soluble P (e.g. iron bound P). Such observations include the inverse correlations between bottom water SRP concentration and DO (chapter 3). More succinct evidence was gained from the 21-day incubation

experiments (chapter 6) in which DO and pH concentrations were higher at the sediment surface (0.2 cm above sediment surface) under light as opposed to dark conditions at the end of the incubation period. Although these experiments were aerated, this observation reflects the elevated photosynthetic maintenance of DO (Figure 8.1) and pH conditions (chapter 6) in illuminated sediment and indicates a reduced likelihood of Ca-P and Fe-P release from an illuminated sediment surface.
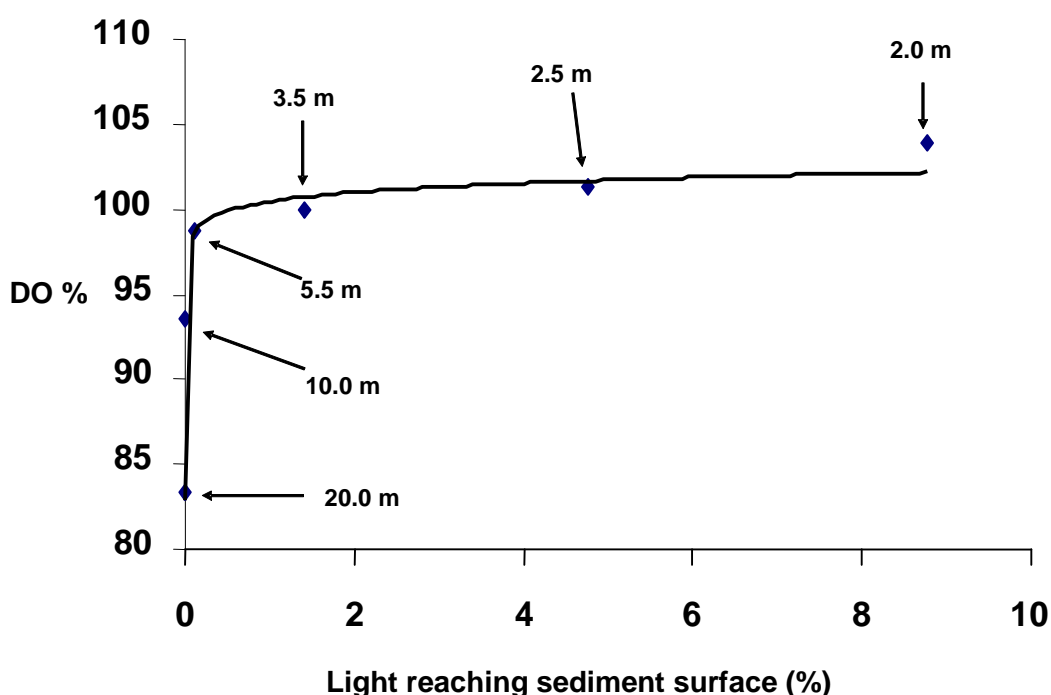
Figure 8.1. *Variation in bottom water dissolved oxygen percent saturation, percent of incident light reaching sediment surface for six points of varying overlying water depth. Values were measured between March 2004 and March 2005 in Loch Leven.*

Other mechanisms may also be important. These include biostabilisaton of the sediment via the production of extracellular polymeric substances or by the construction of physical barriers (i.e. dense cell matrices) on the sediment-water interface. Initial analyses of such mechanisms (chapter 7) suggested that stability increased with EPS concentration and sediment chlorophyll *a* concentration which is in agreement with

much of the classical research in this field (Paterson, 1989; Yallop *et al*., 2000). However, much research is still required to allow identification of the main EPS producers (i.e. cyanobacteria or diatoms; tychoplankton, meroplankton, or epipelon) and the extent to which EPS increases the erosion threshold of ("non-cohesive") freshwater sediment. Additionally, the effects of this biostabilisation on nutrient release remain untested. The direct uptake of nutrients by benthic microalgae was also not quantified specifically, and will undoubtedly (Thornton *et al.,* 2002) have had an effect in the experiments reported in chapters 3 and 6. A hierarchical experimental approach should be employed to address this knowledge gap including the assessment of nutrient uptake kinetics in pure cultures and mixed communities under laboratory controlled conditions. However, these experiments must be carried out with artificial sediment in an attempt to restrict nutrient uptake/release by natural sediment.

IMPLICATIONS FOR WHOLE-SYSTEM FUNCTIONING

Sediment-dwelling autotrophs can perform key functions with respect to benthic/pelagic nutrient cycling in shallow lakes. These functions are expected to vary with growth form (i.e. macrophytes, epipelon, tychoplankton, meroplankton (defined in chapter 1) etc) as well as community composition (e.g. diatoms versus cyanobactreia for microalgae) and, as such, can vary both spatially and temporally. What follows is the outline of a conceptual model (using data provided from this study in combination with results from a recent study in which macrophyte cover in Loch Leven was assessed (summer 2006; Spears *et al.,* in prep)) designed to partition benthic autotroph colonisation and ecosystem function. Two key areas (photic and aphotic zones) of the lake bed were identified according to observed variations in light and dissolved oxygen concentrations with depth (modified from data presented in chapter 3). These two areas

are further sub-categorised into 4 zones of distinct benthic autotroph dominance (Figure

8.2 and Table 8.1) and hypotheses are proposed in relation to the variation in nutrient

regulation mechanisms performed by the autotrophic community present within each

zone.

Table 8.1. *Summary of autotroph colonisation zones in Loch Leven detailing the dominant autotroph type (dominant autotroph), overlying water depth (depth), % incident light reaching the sediment surface taken from annual (march 2004 – march 2005) average observations (average % light), lake surface area (L.S.A. %), nutrient cycling mechanisms, and the estimated (from Smith, 1972) wind speed range that would illicit sediment resuspension (resusp. wind velocity). Eplil. = epilithon; Endo.= endopelon; Epip= epipsammon; Macro.=macrophyte; Tycho = tychoplankton; Epi.= epipelon; Endopel.= endopelon.*

	Dominant autotroph	Depth (m)	Average % light	L.S.A. (%)	Nutrient cycling environment	Resusp. wind velocity
Zone 1	Epil., Endo., Epip.	< 1.0	100–16.1	21	Oxic resuspension	< 4 m s^{-1}
Zone 2	Macro., Tycho.	1.0 – 3.5	16.0-1.4	37	Oxic diffuse, macrophyte regulated resuspension	4 – 10 m s^{-1}
Zone 3	Epi., Endopel., Tycho.	3.5 – 5.0	1.4–0.1	23	Oxic diffuse & benthic microalgal regulated resuspension	10 – 18 m S^{-1}
Zone 4	All	> 5.0	0.1–0.0	19	Anoxic diffuse	> 18 m s^{-1}

Zone 1

Sediment-associated autotrophs are expected to be sparse in the very shallow

littoral habitats of Loch Leven as a result of two key factors. Firstly, this zone is

characterised by high wind-induced mixing which will limit autotrophic colonisation

via regular habitat disturbance. Secondly, shallow habitats within the loch are

characterised by rocky/sandy substratum (Calvert, 1974) and, therefore, represent poor nutrient quality for sediment associated plants and algae. However, other benthic autotrophs will still exist at this depth and will most likely be dominated by growth forms adept at sequestering nutrients from the water-column (e.g. epipsammon, epilithon, charophytes etc) as opposed to the sediment. As such, the autotrophic community within the shallow waters of Loch Leven may compete for nutrients with phytoplankton, and ultimately reduce inputs into the sediment by reducing the phytoplanktonic sedimentation of the system. The autotrophs in this zone will probably play a negligible role in controlling the transfer of nutrients from the sediment to the water-column.

Zone 2

The community composition of this zone shows great seasonality, being dominated by macrophytes between spring and summer and by epipelon and deposited phytoplankton in winter (chapter 5 and unpublished data). This seasonality will result in general shifts in nutrient turnover depending on the system state. Nutrients will be bound within the macrophytes, and therefore, removed from both sediment and water-column during the spring and summer but will be returned to the sediment in organic form during macrophyte "die-back" in autumn. During the spring and summer, epipelic biomass was lowest and is likely to be capped by light limitation via macrophytic/phytoplantkonic shading, however, the epipelon biomass increased in winter, presumably as a response to reduced shading. Although this zone is within the wind-mixed upper layers of the lake, the sensitivity to resuspension of the sediments may be dampened via high macrophyte biomass in summer and high epipelic biomass in winter. Although further work is required to substantiate this theory it is likely that

nutrient release within this zone is driven by a combination of wind-induced resuspension and diffusive release, with benthic autotrophs (during peak growing seasons in the case of macrophytes) acting to dampen release via biostabilisation and the photosynthetic oxygenation of the sediment surface.
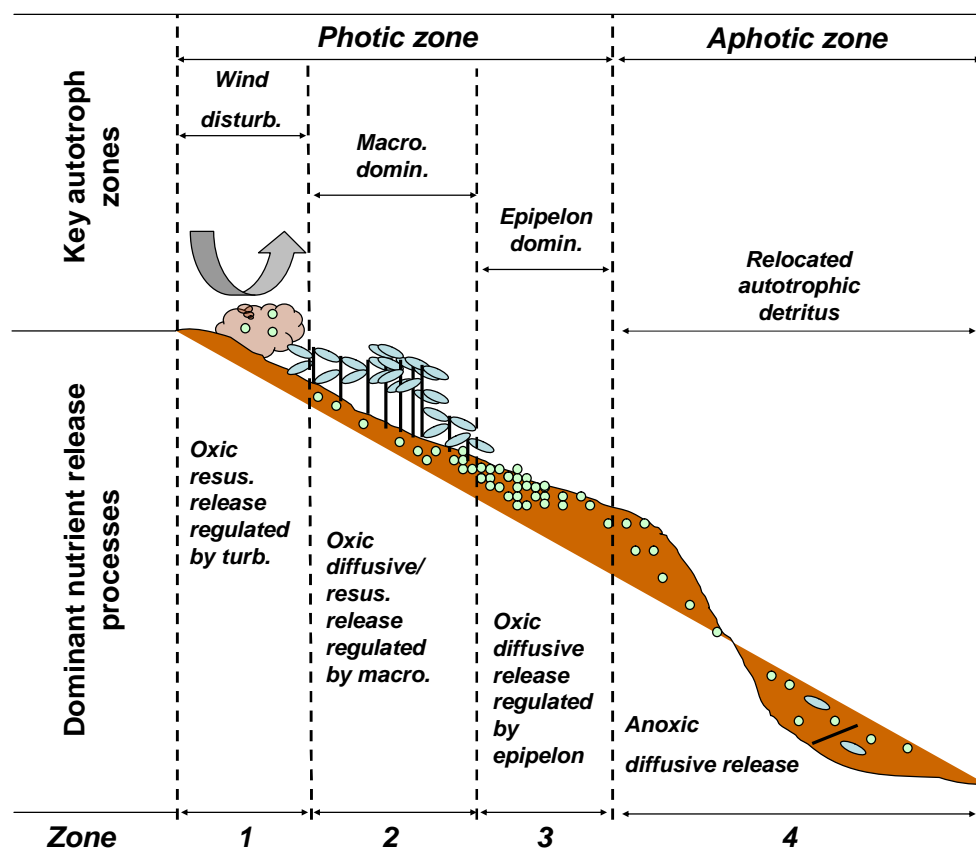
Figure 8.2. *Conceptual model of autotrophic colonisation and the associated regulation of nutrient cycling.*

Zone 3

Zone three represents an area of the lake in which autotrophic growth is light-limited for most of the season. The results suggest that macrophytes have higher photosynthetic light requirements than benthic microalgae where epipelon were observed to colonise this zone in summer, albeit at low levels, when macrophytes were

generally observed to be in low abundance or absent (chapter 5 and unpublished data). Epipelic biomass increased in winter when light penetration to the sediment increased, presumably as a result of phytoplankton and macrophyte die-back. This period of high epipelic biomass coincided with high sediment TP content and so represented a period during which the functionality of the epipelon was most important. As wind-induced resuspension is limited to extreme weather conditions at this depth, the main form of sediment nutrient release is likely to be diffusive. As such, summer periods will experience high-temperature diffusive-flux conditions and, in the absence of benthic autotrophic production, are sensitive to periods of anoxia, particularly at night. In contrast, winter periods are characterised by high epipelic biomass therefore capping sediment nutrient release by photosynthetic oxygenation under low-temperature diffusive flux conditions. This is in agreement with the P fractionation results from chapter 3 in which pore-water P concentrations were lowest under high temperature conditions suggesting more efficient release of sediment-P under warm conditions where concentration gradients are high and epipelic biomass low.

Zone 4

Light levels at the sediment surface within zone 4 were insufficient to support benthic photosynthesis. That said, the highest bulk sediment chlorophyll *a* concentrations were observed within this zone. This is probably due to the transport and focussing of organisms from zones 1 - 3 and, although observed as viable photosynthetic material, these organisms can not photosynthesise at this depth. Meroplankton may be present at this depth, though the specific effects of these organisms are largely unknown in Loch Leven. The main function performed by autotrophs present in this zone will be as a vector of nutrient transfer from areas of high

primary production (both water-column and sediment) to one of high heterotrophic production and associated remineralization rates of organic matter. This zone is sensitive to conditions of temperature mediated anoxia during summer and so represents the area of the lake that likely supports the summer water-column P maxima.

WIDER IMPLICATIONS OF STUDY

Understanding the variable positioning of each of the key zones described above is crucial to the understanding of lake functioning and water quality management practices in Loch Leven, and other shallow lakes. Zone 1 is unlikely to be altered by improvements in water quality as the main force driving colonisation in this zone is wind-induced mixing. However, recent improvements in water quality have undoubtedly altered the boundaries of zones 2 and 3 in recent years with improvements in water clarity (unpublished data) resulting in the deepening of zone 2 (measured as macrophyte maximum growing depth; unpublished data) to depths observed previously under un-impacted conditions (i.e. 5 m maximum growing depth observed in 1910; Jupp and Spence, 1977). It is also likely that the lower boundary of zone 3 has been deepened, although the extent to which the relative aerial cover of zones 2 and 3, and therefore the relative importance of benthic microalgae versus macrophytes in regulating benthic/pelagic nutrient cycling, have been affected is not known. It is, however, possible to infer an overall increase in the area of the lake in which sediment nutrient release is regulated by benthic primary production. The main consequence of this increase in the overall photic zone will be a decrease in the area of the lake sensitive to high magnitude anoxic P release (Zone 4). Further improvements in water clarity should therefore compound this phenomenon and lead to a continuation of the recent improvements in water quality, and the desired regime shift.

Summary

Reduction of external nutrient load, the typical approach to the management of eutrophic lakes, may often result in nutritional dependence switching from external to internal supply. Internal nutrient recycling may delay or prevent state change, from turbid (phytoplankton dominated) to clear water (macrophytes and benthic microalgal dominated), for years to decades (Sas, 1989). The role of sediment phosphorus (P) release in maintaining high productivity in this context has been well-documented (Jeppesen *et al*., 2005; Phillips *et al*., 2005; Søndergaard *et al*., 2005), however, benthic/pelagic cycling of other nutrients (e.g. nitrogen and silica) important in sustaining planktonic productivity have received less attention. Differences in internal P loading potential are most pronounced in shallow lakes where sediment resuspension favours nutrient release but where favourable benthic light regimes, if present, can also support high benthic autotrophic production and well oxygenated surface sediment, both of which are linked to a reduction in sediment nutrient release (Hilton *et al*., 1985; Woodruff *et al*., 1999; Hakanson, 2005). It is in shallow lake systems that alternative stable states are most widely recognised and where the role of macrophytes in these regime shifts has been developed (Scheffer *et al*., 1993; Scheffer, 2001). The functional roles/ecosystem services provided by microphytobenthos are also expected to be important in shallow lakes although few examples of such performance exist within the literature (exceptions include: Bartoli *et al*., 2003; Risgaard-Peterson *et al*., 2005; Qu *et al*., 2005).

The main purpose of this thesis was to investigate the linkages between benthic microalgal ecology and benthic/pelagic nutrient cycling in a shallow eutrophic lake (Loch Leven, Scotland). The work was designed to (1) identify the mechanisms by which nutrients (mainly P, nitrogen (N), and silica (Si)) are transferred between the

water column and the sediment, (2) investigate the composition of the benthic microalgal community and assess the extent of seasonal and spatial variation, and (3) investigate specific ecosystem functions performed by benthic microalgae including the regulation of diffusive sediment nutrient release and the reduction of resuspension related sediment nutrient release via biostabilisation.

Historical variation in sediment phosphorus pools and mobility

In a move to improve water quality, external total P (TP) loading to Loch Leven was reduced from 20 t TP y^{-1} in 1985 to 8 t TP y^{-1} in 1995. As is the case in many shallow lakes, internal loading of P maintained poor water quality conditions for a considerable length of time (~15 years) post restoration, with reductions in internal loading only recently being observed. However, in a quantitative comparison of sediment TP composition, no significant decrease was observed in any P fraction over a period where internal loading had significantly decreased (between 1990 and 2004). Significant spatial variation was observed in sediment-P composition and mobility characteristics (e.g. sorption capacity, equilibrium phosphorus concentration (EPC0: concentration of soluble reactive phosphorus (SRP) in the overlying water column above which sediment uptake, and below which sediment release, occurs)) indicating the presence of processes acting to replenish sediment nutrients. On this occasion, redox sensitive sediment-P was positively correlated with sediment chlorophyll *a* concentration (benthic microalgal biomass) and labile sediment-P (loosely adsorbed onto sediment) was governed mainly by a multivariate relationship with temperature and EPC0. It is essential to consider spatio-temporal variations in sediment nutrient concentrations and the effects of biological and physico-chemical regulators of nutrients

in sediments when using sediment-P composition for assessing the recovery of shallow lakes from eutrophication.

Current seasonal and spatial variation in sediment phosphorus pools and mobility

The seasonality and spatial aspects of internal loading are governed by a range of physical, chemical and biological interactions. It is extremely important to understand these processes in order to better predict/enhance the likelihood of recovery of lakes from eutrophication. Temperature is widely considered the key driver of internal loading where release rates are greatly enhanced by an elevation in microbial oxygen demand (anoxic release of reductant-soluble P) and remineralisation of organic matter, both traits of high temperature conditions. The composition of the benthic micro-community may also affect sediment-P cycling where autotrophic communities dominate (reducing redox sensitive P release via production of O_2) in shallow well lit sediments and heterotrophic communities dominate (increasing P release via O_2 consumption) in deep poorly lit sediments. In shallow lakes, however, high magnitude release events (12 mg SRP m^{-2} d^{-1}) may only occur when temperatures rise sufficiently to tip the balance of the benthic community from one dominated by autotrophic, to one dominated by heterotrophic production. This event usually occurs in mid-late summer in Loch Leven and many other shallow lakes. Once released, phosphorus can be quickly taken up into the pelagic foodweb and may be returned to the sediment as organic detritus (composed mainly of phytoplanktonic "fallout" in Loch Leven).

The difference between EPC0 estimates taken from intact cores (180-270 μg SRP L^{-1}) and continuously shaken slurry (9-30 μg SRP L^{-1}) indicated that physicochemical sediment-P uptake was enhanced during resuspension events and physicochemical release conditions were maintained during calm conditions. This also

has strong implications on the spatial aspects of P release where resuspension is more likely in shallow water sediments.

Whole lake sediment phosphorus partitioning: implications for water quality management

Developing new methods that help to improve the remediation of impacted water bodies is essential to the successful implementation of the Water Framework Directive (WFD). A recent risk-assessment of the condition of the UK's standing waters indicated that about 56% (over 7000 lakes) of all UK lakes (>1 ha in surface area) currently require remedial action under the guidelines set out by the WFD. The data set collected during the course of this study can provide excellent information with regards to the partitioning of P between the water column and the sediment and can be used to better plan management programmes.

Anoxic P release may be reduced by aeration in hotter summer months. Additionally, sediment dredging activities would be better applied in winter when sediment TP concentrations are high. Lake flushing (using existing sluice gates) is likely the most effective secondary remedial option where water levels may be maintained leading up to the characteristic summer anoxic release event after which the system may be "flushed", thus enhancing P removal.

The effects of season and water-column depth on benthic microalgal composition and abundance in Loch Leven (Scotland) sediment

A number of studies have reported correlations between sediment chlorophyll *a* concentration and sediment nutrient concentration, or some other index of chemical mobility, in shallow lakes. Although these pigment concentrations are generally

attributed to epipelon biomass in estuarine and riverine sediment environments, the composition of sediment chlorophyll *a* concentration may be more complex in eutrophic shallow lake sediments where the benthic microalgal community can be dominated by settled phytoplankton with epipelon representing a secondary composite (between 0.14 and 17% in Loch Leven).

In Loch Leven, the relative (to total benthic microalgal biomass) composition of epipelon was highest during periods where phytoplankton biomass, and therefore light attenuation, was lowest (i.e. in winter) and *vice versa*. The spatial variation in the benthic microalgal community biomass was likely governed by a trade-off between light limitation (deep water sediment) and habitat disturbance (shallow water sediment: i.e. wind induced sediment disturbance). However, in large lakes where wind induced sediment resuspension is common, habitat disturbance can result in the homogenisation of sediment pigment concentrations in the surface sediment or elevated concentrations in deeper waters as a result of sediment resuspension/focussing processes. Light limitation also affected the epipelon community composition with genera possessing low photophysiological requirements (cyanobacteria) observed in deep water sediment and *vice versa* (diatoms in shallow water sediment).

The effects of benthic microalgae on diffusive sediment nutrient release

This work enhances the understanding of the effects of temperature and light on benthic microalgal regulation of diffusive nutrient fluxes across an intact sediment-water interface. Intact sediment core experiments were employed that allowed the manipulation of temperature and light levels and nutrient uptake/release rates were estimated daily over a 21 day period. Estimates of P flux, designed to mimic ambient release rates, were also conducted. P, N, and Si release all increased under high

temperature (23°C) conditions. N uptake was observed under colder temperatures (less than 13°C). Additionally, the stoichiometry of TSi:TN:TP varied with temperature and light. Nutrient release was significantly lower under light (versus dark) conditions indicating regulation of nutrient cycling by benthic microalgae.

These results stress the importance of benthic microalgal production and temperature on regulating the water column nutrient regime, especially in lakes that are nutritionally dependent on sediment sources.

On the boundaries: sediment stability measurements across aquatic ecosystems

The production of extracellular polymeric substances (EPS) by epipelon has been observed to increase the structural integrity of estuarine sediments and may, therefore, reduce the likelihood of sediment resuspension (and related nutrient release) in other sediment ecosystems (i.e. lakes). The continuity of this theory was tested by tracking sediment stability, and the factors regulating it, within and between freshwater lake (shallow and deep Loch Leven sediment) and estuarine (low shore, mid shore and saltmarsh sediment from the Eden Estuary) sediment ecosystems. Freshwater sediments were least stable (despite highest EPS concentrations) and saltmarsh sediments most stable indicating factors other than biostabilisation were important in regulating variation between ecosystems. Correlation analysis also highlighted ecosystem specific stability regulation through positive relationships with colloidal carbohydrates (sediment in 4 m overlying lake water), and negative correlations with sediment chlorophyll *a* and organic matter (mid shore estuarine sediment), and wet bulk density (salt marsh sediment). Sediment stability varied significantly within and between freshwater and estuarine sediment ecosystems and this variation was driven by differences in key ecosystem processes and structure, most likely salinity, benthic

autotrophic community structure, and deposition/removal of fine particulate organic matter.

REFERENCES

Anderson, F.O., & P. Ring. 1999. Comparison of phosphorus release from littoral and profundal sediment in a shallow, eutrophic lake. Hydrobiologia. 408/409:175-183.

Anderson, N.J., B. Rippey, & C.E. Gibson. 1993. A comparison of sedimentary and diatom-inferred phosphorus profiles: implications for defining pre-disturbance reference conditions. Hydrobiologia. 253: 357-366.

APHA. 1992. Standard methods for the examination of water and wastewater. Eds: Greenberg A. E., L. S. Clesceri, & A.D. Eaton. American Public Health Association, Washington, DC.

Austin, I., T.J. Andersen, & K. Edelvang. 1999. The influence of benthic diatoms and invertebrates on the erodibility of an intertidal mudflat, the Danish Wadden Sea. Estuarine and Coastal Shelf Science. 49: 99–111.

Axler, R.P., & J.E. Reuter. 1996. Nitrate uptake by phytoplankton and peryphyton: whole lake enrichments and mesocosm-15N experiments in an oligotrophic lake. Limnology and Oceanography. 41: 659-671.

Bachman, H. 1906. Le plankton des lacs Ecossaia. Archives of Physical and Natural Sciences. 20**:** 359-380.

Bailey-Watts, A.E. 1976a. Planktonic diatoms and some diatom-silica relations in a shallow eutrophic Scottish loch. Freshwater Biology**.** 6: 69-80.

Bailey-Watts, A.E. 1976b. Planktonic diatoms and silica in Loch Leven, Kinross, Scotland: a one month silica budget. Freshwater Biology. 6:203-213.

Bailey-Watts, A.E. 1978. A nine-year study of the phytoplankton of the eutrophic and non-stratifying Loch Leven (Kinross, Scotland). Journal of Ecology. 6:741-771.

Bailey-Watts, A.E. 1988. Studies on the control of the early spring diatom maximum in Loch Leven 1981. In Round F.E. (Ed), Algae and the Aquatic Environment. Biopress, Bristol. 53-87.

Bailey-Watts, A.E., & J. Komarek. 1991. Towards a formal description of a new species of synechococcus (cyanobacteria, Microcystaceae) from the freshwater picoplankton. Algological Studies. 61: 5-19.

Bailey-Watts, A.E., & A. Kirika. 1987. A re-assessment of phosphorus inputs to Loch Leven (Kinross, Scotland): rationale and an overview of results on instantaneous loadings with special reference to run-off. Transcripts of the Royal Society of Edininburgh: Earth Sciences. 78: 351-367.

Bailey-Watts, A.E., & A. Kirika. 1993. Loch Leven NNR: Water quality 1992-1994 with special reference to nutrientsand phytoplankton, and an assessment of phosphorus levels in the loch sediments. Interim Report to Scottish Natural Heritage, Institute of Freshwater Ecology, Edinburgh.

Bailey-Watts, A.E., & A. Kirika. 1999. Poor water quality in Loch Leven (Scotland) in 1995, in spite of reduced phosphorus loading since 1985: the influences of catchment management and inter-annual weather variation. Hydrobiologia. 403: 135-151.

Banta, G.T, A.E. Giblin, J.E. Hobbie, & J. Tucker. 1995. Benthic respiration and nitrogen release in Buzzards Bay, Massachusetts. Journal of Marine Research. 53:107-135.

Barbiero, R.P., & J. Kann. 1994. The importance of benthic recruitment to the population development of Aphanizomenon flos-aquae and internal loading in a shallow lake. Journal of Plankton Research. 16: 1581-1588.

Barlow-Bush, L., H.M. Baulch, & W.D. Taylor. 2006. Phosphate uptake by seston in the Grand River, southern Ontario. Aquatic Sciences. 68: 1615-1621.

Bartoli, M., D. Nizzoli, & P. Viaroli. 2003. Microphytobenthos activity and fluxes at the sediment-water interface: interactions and spatial variability. Aquatic Ecology. 37:341-349.

Bergtold, M., & W. Traunspurger. 2004. The benthic community in the profundal of lake Brunnsee: seasonal and spatial patterns. Archiv fur Hydrobiologie. 160:527-544.

Behre, K. 1956. Die Algenbesiedlung Seen um Bremen und Bremerhaven. Veröff. Inst. Meersforsch. Bremerhaven. 4: 221-383.

Belmonte, G. A., M.F. Rubino, & F. Boero. 1997. Morphological convergence of resting stages of planktonic organisms: a review. Hydrobiologia. 355: 159–165.

Benoy, G.A., & J. Kalff. 1999. Sediment acuumulation and Pb burdens in submerged macrophyte beds. Limnology and Oceanography. 44: 1081-1090.

Bernhardt, H., & J. Clasen. 1982. Limnological effects of the elimination of phosphorus from the Wahnbach Reservoir. Water Science and Technology. 14: 397 - 406.

Borchardt, M.A. 1996. Nutrients. In: Stevenson R.J., Bothwell M.L. and Lowe R.L. 1996. Algal Ecology: Freshwater Benthic Ecosystems. Academic Press Limited, London.

Boström, B., J.M. Anderson, S. Fleischer, & M. Jansson. 1988. Exchange of phosphorus across the sediment-water interface. Hydrobiologia. 170: 229-244.

Boström, B., A. Petterson, & I. Ahlgren. 1989. Seasonal dynamics of a cyanobacterial-dominated microbial community in surface sediment of a shallow, eutrophic lake. Aquatic Science. 51: 153-177.

Brunberg, A., & B. Boström. 1992. Coupling between benthic biomass of microcystis and phosphorus release from the sediments of a highly eutrophic lake. Hydrobiologia. 235/236: 375-385.

Calvert, S.E. 1974. The distribution of bottom sediments in Loch Leven, Kinross. Proceedings of the Royal Society of Edinburgh. B74: 69-80.

Cariou-Le Gall, V., & G.F. Blanchard. 1995. Monthly HPLC measurements of pigment concentration from an intertidal muddy sediment of Marennes-Oleraon Bay, France. Marine Ecolology - Progress Series. 121: 171-179.

Carlton, R.G., & R.G. Wetzel. 1988. Phosphorus flux from lake sediments: Effects of epipelic algal oxygen production. Limnology and Oceanography. 33:562-570.

Carvalho, L., & A. Kirika. 2003. Changes in shallow lake functioning: response to climate change and nutrient reduction. Hydrobiologia. 506-509: 789-796.

Carvalho, L., & A. Kirika. 2005. Loch Leven 2003: physical, chemical, and algal aspects of water quality. Report to Scottish Natural Heritage. Centre for Ecology and Hydrology, Edinburgh.

Carvalho, L., S. Maberly, L. May, C. Reynolds, M. Hughes, R. Brazier, L. Heathwaite, S. Lui, J. Hilton, D. Hornby, H. Benion, A. Elliott, N. Wilby, R. Dils, G. Phillips, L. Pope, & I. Fozzard. 2005. Risk assessment methodology for determining nutrient impacts in surface freshwater bodies. Report to the Environment Agency. Science Report SCO20029/SR.

Cyr, H. 1998. How does vertical distribution of chlorophyll vary in littoral sediments of small lakes? Freshwater Biology. 39: 25-40.

Cyr, H., & K.E. Morton. 2006. Distribution of biofilm exopolymeric substances in littoral sediments of Canadian Shield lakes: effects of light and substrate. Canadian Journal of Fisheries and Aquatic Sciences. 63: 1763-1776.

Davies, P.S. 1996. Phosphorus fluxes in sediments in relation to phytoplankton ecology. Ph.D. Thesis. CEH Windermere.

De Groot, W.T. 1981. Phosphate and wind in a shallow lake. Archiv fur Hydrobiologie. 91: 475-489.

De Montigny, C., & Y.T. Prairie. 1993. The relative importance of biological and chemical processes in the release of phosphorus from a highly organic sediment. Hydrobiologia. 253:141-150.

Decho, W. 2000. Microbial biofilms in intertidal systems: an overview. Continental Shelf Research. 20: 1257-1273.

Del Amo, Y., & M.A. Brzezinskio. 1999. The chemical form of dissolved Si taken up by marine diatoms. Journal of Phycology. 35: 1162-1170.

DeNicola, D.M., E. de Eyto, A. Wemaere, & K. Irvine. Periphyton response to nutrient addition in 3 lakes of different benthic productivity. Journal of the North American Benthological Society. 25: 616-631.

Dodds, W.K. 2003. The role of periphyton in phosphorus retention in shallow freshwater aquatic systems. Journal of Phycology. 39:840-849.

Dong, L.F., D.C.O Thornton, D.B. Nedwell, & G.J.C Underwood. 2000. Denitrification in sediments of the River Colne Estuary, England. Marine Ecology Progress Series. 203: 109-122.

Downing, J.A., & L.C. Rath. 1988. Spatial patchiness in the lacustrine sedimentary environment. Limnology and Oceanography. 33: 447-458.

Driscoll, C.T., S.D. Effler, M.T. Auer, S.M. Doer, & M.R. Penn. 1993. Supply of phosphorus to the water column of a productive hard water lake: controlling mechanisms and management considerations. Hydrobiologia. 253: 61-72.

Dudley, B.J., & D.I. Walker. 2000. Plant/water nutrient dynamics in a south western Australian estuary. Biologia Marina Mediterranea. 7: 43-46.

Dudley, B.J., A.M.E. Gahnstrom, & D.I. Walker. 2001. The role of benthic vegetation as a sink for elevated ammonium and nitrate in a mesotrophic estuary. Marine Ecology Progress Series. 219: 99-107.

Engstrom, D.R., S.C. Fritz, J.E. Almendinger, & S. Juggins. 2000. Chemical and biological trends during lake evolution in recently deglaciated terrain. Nature. 408: 161-166.

Farmer, J.G., A.E. Bailey-Watts, A. Kirika, & C. Scott. 1994. Phosphorus fractionation and mobility in Loch Leven sediments. Aquatic Conservation: Marine and Freshwater Ecosystems. 4: 45-56.

Fee, E.J., R.E. Hecky, S.J. Guildford, C. Anema, D. Mathew, & K. Hallard. 1998. Phytoplankton primary production and related limnological data for lakes and channels in the Mackenzie Delta and lakes of the Tuktoyaktuk peninsula, NWT. Canadian Technical Report of Fisheries and Aquatic Science. 1614.

Friedrich, J., C. Dinkel, G. Friedl, N. Pimenov, J. Wijsman, M.T. Gomoiu, A. Cociasu, L. Popa, & B. Wehrli. 2002. Benthic nutrient cycling and diagenetic pathways in the north-western black sea. Estuarine, Coastal and Shelf Science. 54: 369-383.

Froelich, P. N. 1988. Kinetic control of dissolved phosphate in natural rivers and estuaries: A primer on the phosphate buffer mechanism. Limnology and Oceanography. 33: 649-668.

Frost P.C., & J. Elser. 2002. Effects of light and nutrients on the net accumulation and elemental composition of epilithon in boreal lakes. Freshwater Biology. 47: 173-183.

Gächter, R., & J.S. Meyer. 1993. The role of microorganisms in mobilization and fixation of phosphorus in sediments. Hydrobiologia. 253: 103-122.

Gächter, R., & B. Wehrli. 1998. Ten years of artificial mixing and oxygenation: no effect on the internal phosphorus loading to two eutrophic lakes. Environmental Science and Technology. 32: 3659-3665.

Gibson, C.E., G. Wang, & R.H. Foy. 2000. Silica and diatom growth in Lough Neagh: the importance of internal recycling. Freshwater Biology. 45: 285-293.

Golterman, H.S., R.S. Clymo, & M.A.M. Olmstad. 1978. Methods for physical and chemical analysis of freshwaters. Blackwell, Oxford, UK.

Golterman, H.L. 2004. The chemistry of phosphate and nitrogen compounds in sediments. Kluwer Academic Publishers, London.

Goto, N., T. Kawamura, O. Mitamura, & H. Terai. 1999. Importance of extracellular organic carbon production in the total primary production by tidal-flat diatoms in comparison to phytoplankton. Marine Ecology - Progress Series. 190: 289-295.

Grady, J.R. 1981. Properties of seagrass and sand flat sediments from the intertidal zone of St Andrews Bay, Florida. Estuaries. 4: 335-344.

Hakanson, L., A.P. Ostapenia, & V. Boulion. 2003. A mass-balance model for phosphorus in lakes accounting for biouptake and retention in biota. Freshwater Biology. 48: 928-950.

Hansson, L. 1989. The influence of a periphytic biolayer on phosphorus exchange between substrate and water. Archiv fur Hydrobiologie. 115: 21-26.

Hansson, L. 1990. Quantifying the impact of periphytic algae on nutrient availability from phytoplankton. Freshwater Biology. 24: 265-273.

Hariston, N.G., R.A. van Brunt, C.M. Kearns, & D.R. Engstrom. 1995. Age and survivorship of diapausing eggs in a sediment egg bank. Ecology. 76: 1706-1711.

Havens, K.E. 1991. Fish-induced sediment resuspension: effects on phytoplankton biomass and community structure in a shallow hypereutrophic lake. Journal of Plankton Research. 13: 1163-1176.

Havens, K.E., T.L. East, S.J. Hwang, A.J. Rodusky, B. Sharfstein, & A.B. Steinman 1999. Algal responses to experimental nutrient addition in the littoral community of a subtropical lake. Freshwater Biology. 42: 329-344.

Head, R.M., R.I. Jones, & A.E. Bailey-Watts. 1999. Vertical movements by planktonic cyanobacteria and the translocation of phosphorus: implications for lake restoration. Aquatic Conservation: Marine and Freshwater Ecosystems. 9: 111-120.

Heaney, S., D. Chapman, & H. Morison. 1983. The role of the cyst stage of the dinoflagellate Ceratium hirundinella within a small productive lake. British Phycological Journal. 18: 47-49.

Heathwaite, A.L., P.F. Quinn, & C.J.M. Hewett. 2005. Modelling and managing critical source areas of diffuse pollution from agricultural land by simulating hillslope flow connectivity. Journal of Hydrology. 304: 446-461.

Hillebrand, H. 2005. Light regime and consumer control of autotrophic biomass. Journal of Ecology. 93: 758-769.

Hillebrand, H., C.D. Dűrselen, D. Kirschtel, U. Pollingher, & T. Zohary. 1999. Biovolume calculation for pelagic and benthic microalgae. *Journal of Phycology.* 35: 403-424.

Hilton, J., J.P. Lishman, & P.V. Allen. 1986. The dominant processes of sediment distribution and focussing in a small, eutrophic, monomictic lake. Limnology and Oceanography. 31: 125-133.

HIMOM. 2005. Heirarchical Monitoring Methods. European commission fifth framework programme. Contract: EVK3-CT-2001-00052.

Holdren, G. C., & D.E. Armstrong. 1980. Factors affecting phosphorus release from intact lake sediment cores. Environmental Science and Technology. 14: 79-87.

Horpilla, J., & T. Kairesalo. 1990. A fading recovery: the role of roach (Rutilus rutilus) in maintaining high phytoplankton productivity and biomass in Lake Vesjarvi, Southern Finland. Hydrobiologia. 200/201: 153-165.

House, W.A., T.D. Jickells, A.C. Edwards, K.E. Praska, & F.H. Denison. 1998. Reactions of phosphorus with sediments in fresh and marine waters. Soil Use and Management. 14: 139-146.

House, W.A. 2003. Geochemical cycling of phosphorus in rivers. Applied Geochemistry. 18: 739-748.

Hwang, C.P., T.H. Lackie, & P.M. Huang. 1976. Adsorption of inorganic phosphorus by lake sediments. Journal of the Water Pollution Control Federation. 48: 2754-2760.

Hwang, S.J., K.E. Havens, & A.D. Steinman. 1998. Phosphorus kinetics of planktonic and benthic assemblages in a shallow subtropical lake. Freshwater Biology. 40: 729-745.

Iqbal, M.Z., E.J. Brown, & M.E. Clayton. 2006. Distribution of phosphorus in a biologically restricted lake in Iowa, USA. Journal of Hydrology. 326: 349-366.

Jansson, M. 1980. Role of benthic algae in transport of nitrogen from sediment to lake water in a shallow Clearwater lake. Archive fűr Hydrobiologie. 89: 101-109.

Jarvie, H.P., C. Neal, A. Warwick, J. White, M. Neal, H.D. Wickham, L.K. Hill, & M.C. Andrews. 2002. Phosphorus uptake into algal biofilms in a lowland chalk river. Science of the Total Environment. 282: 353-373.

Jenson, H.S., & F.Ø. Anderson. 1992. Importance of temperature, nitrate, and pH for phosphate release from aerobic sediments of four shallow, eutrophic lakes. Limnology and Oceanography. 37: 577-589.

Jensen, H.S., P. Kristensen, E. Jeppesen, & A. Skytthe. 1992. Iron:phosphorus ratio in surface sediment as an indicator of phosphate release from aerobic sediments in shallow lakes. Hydrobiologia. 235/236: 731-743.

Jeppesen, E., M. Søndergaard, J.P. Jensen, K. Havens, O. Anneville, L. Carvalho, M.F. Coveney, R. Deneke, M.T. Dokulil, B. Foy, D. Gerdeaux, S.E. Hampton, S. Hilt, K. Kangur, J. Kohler, E.H.H.R. Lammens, T.L. Lauridsen, M. Manca, M.R. Miracle, B. Moss, P. Noges, G. Persson, G. Phillips, R. Portielje, S. Romo, C.L. Schelske, D. Straile, I. Tatrai, E. Willen, & M. Winder. 2005. Lake responses to reduced nutrient loading – an analysis of contemporary long-term data from 35 case studies. Freshwater Biology. 50: 1747-1771.

Jesus, B., M. Marani, V. Brotas, & Paterson, D.M. 2005. Spatial dynamics of microphytobenthos determined by PAM fluorescence. Estuarine Coastal and Shelf Science. 65: 30-42

Jones J.G. 1971. Studies on freshwater bacteria: Factors which influence the population and its activity. Journal of Ecology. 59: 593-613.

Jones G. 1985. Decomposition in lake sediments: bacterial action and interaction. 53rd Annual Report of the Freshwater Biological Association. Pp. 31-44. Ambleside, Cumbria.

Jupp, B.P., & D.H.N. Spence. 1977. Limitations on macrophytes in a eutrophic lake, Loch Leven. The Journal of Ecology. 65: 175-186.

de Jonge, V.N., & J.E.E. van Beusekom. 1995. Wind- and tide-influenced resuspension of sediment and microphytobenthos from tidal flats in the Ems estuary. Limnolology and Oceanography. 40: 766-778.

Kamp-Nielson, L. 1975. A kinetic approach to the aerobic sediment-water exchange of phosphorus in Lake Esrom. Ecological Modelling. 1: 153-160.

Karlson-Elfgren, I., K. Rengefors, & S. Gustafsson. 2004. Factors regulating recruitment from the sediment to the water column in the bloom-forming cyanobacterium Gloeotrichia echinulata. Freshwater Biology. 49: 265-273.

Kelderman, P., H.J. Lindeboom, & J. Klien. 1988. Light dependent sediment-water exchange of dissolved reactive phosphorus and silicon in a producing microflora mat. Hydrobiologia. 159: 137-147.

Kenworthy, W.J., J.C. Ziemen, & G.W. Thayer. 1982. Evidence for the influence of seagrasses on the benthic nitrogen cycle in a coastal plain estuary near Beaufort, North Carolina (USA). Oecologia. 54: 152-158.

Kisand A., & P. Noges. 2003. Sediment phosphorus release in phytoplankton dominated versus macrophyte dominated shallow lakes: importance of oxygen conditions. Hydrobiologia. 506-509: 129-123.

Koski-Vahala J., H. Hartikainen, & P. Tallberg. 2001. Phosphorus mobilization from various sediment pools in response to increased pH and silicate concentration. Journal of Environmental Quality. 30: 546-552.

Krogerus K., & P. Ekholm. 2003. Phosphorus in settling matter and bottom sediments in lakes loaded by agriculture. Hydrobiologia. 429:15-28.

Kromkamp, J., C. Barranguet, & J. Peene. 1998. Determination of microphytobenthos PSII quantum efficiency and photosynthetic activity by means of variable chlorophyll fluorescence. Marine Ecology - Progress Series. 162: 45-55.

Lehner, B., & P. Döll. 2004. Development and validation of a global database of lakes, reservoirs and wetlands. Journal of Hydrology. 296: 1-22.

Lerman, A. 1979. Geochemical processes: water and sediment environments. John Wiley and Sons Publishers, New York.

Lesen, A.E. 2006. Sediment organic matter composition and dynamics in San Fransisco Bay, California, USA: Seasonal variation and interactions between water-column chlorophyll and the benthos. Estuarine and Coastal Shelf Science. 66: 501-512.

Levine, S.N. & D.W. Schindler. 1992. Modification of the N:P ratio in lakes by in situ processes. Limnology and Oceanography. 37: 917-935.

Liboriussen, L. & E. Jeppesen. 2003. Temporal dynamics in epipelic, pelagic and epiphytic algal production in a clear and turbid shallow lake. Freshwater Biology. 48: 418-431.

Lick, W., & H. Huang. 1993. Flocculation and the physical properties of flocs. In: Nearshore and estuarine cohesive sediment transport (Mehta, A.J. Ed). AGU, Washington, DC, pp 21-39.

Lincoln, R., G. Boxshall, & P. Clark. 1998. A dictionary of Ecology, Evolution and Systematics. Cambridge University Press: 2nd edition.

LLAMAG. (1993) Loch Leven Area Management Advisory Group Report.

Madsen, K.N., P. Nilsson, & K. Sundbäck. 1993. The influence of benthic micro-algae on the stability of a subtidal sediment. Journal of Experimental Marine Biology and Ecology. 170: 159-177.

Marcus, N.H., & Boero, F. 1998. Minireview: the importance of benthic-pelagic coupling and the forgotten role of life cycles in coastal aquatic systems. Limnology and Oceanography. 43: 763-768.

Marsden, M.W. 1989. Lake restoration by reducing external phosphorus loading: the influence of sediment phosphorus release. Freshwater Biology. 21: 139-162.

Margalef, R. 1960. Ideas for a synthetic approach to the ecology of running waters. International Review Gesamt Hydrobiologie. 45: 133-153.

McQuoid, M.R., & L.A. Hobson. 1995. Importance of resting stages in diatom seasonal succession. Journal of Phycology. 31: 44–50.

McQuoid, M.R., & L.A. Hobson. 1996. Diatom resting stages. Journal of Phycology. 32: 889–902.

Meijer, M., & H. Hosper. 1997. Effects of biomanipulation in the large and shallow Lake Wolderwijd, The Netherlands. 1997. Hydrobiologia. 342/343: 335-349.

Meijer, M., I. de Boois, M. Scheffer, R. Portielje, & H. Hosper. 1999. Biomanipulation in shallow lakes in the Netherlans: an evaluation of 18 case studies. Hydrobiologia. 408/409: 13-30.

Moreno, S. & F.X. Niell. 2004. Scales of variability in the sediment chlorophyll content of the shallow Palmones River Estuary, Spain. Estuarine and Coastal Shelf Science. 60: 49-57.

Mortimer, C.H. 1941. The exchange of dissolved substances between mud and water in lakes 1. Journal of Ecology 29: 280-329.

Mortimer, C.H. 1942. The exchange of dissolved substances between mud and water in lakes 2. Journal of Ecology 30: 147-201.

Mortimer, C.H. 1971. Chemical exchanges between sediments and water in the Great Lakes – speculations on probable regulatory mechanisms. Limnology and Oceanography. 16: 387-404.

Moss, B., J. Madgwick, & G. Phillips. 1996. A guide to the restoration of nutrient-enriched shallow lakes. W. W. Hawkes, UK.

Moss B., L. Carvalho, & J. Plewes. 2002. The lake at Llandrindod Wells – a restoration comedy? Aquatic Conservation. 12: 229-245.

Nedwell D.B., & D.G. Raffaelli (Eds.). 1999. Advances in Ecological Research Estuaries. 29:. Academic Press, San Diego, CA.

Nedwell, D.B., T.D. Jickells, M. Trimmer, & R. Sanders. 1999. Nutrients in estuaries. In: Advances in Ecological Research: Estuaries. (Nedwell D.B., and D.G. Raffaelli., Eds). 29. Academic Press, San Diego, CA.

Noges, P., & A. Kisand. 1999. Horizontal distribution of sediment phosphorus in shallow eutrophic Lake Vortsjarv (Estonia). Hydrobiologia. 408/409: 167-174.

Ollikainen, M., H. Simola, & R. Niinioja. 1993. Changes in diatom assemblages in the profundal sediments of two large oligohumic lakes in east Finland. Hydrobiologia. 269-270: 415-413.

Ogilvie, B., & S.F. Mitchell. 1998. Does sediment resuspension have persistant effects on phytoplankton? Experimental studies in three shallow lakes. Freshwater Biology. 40: 52-63.

Oppenheim, D.R., & C. Ellis-Evans. 1989. Depth-related changes in benthic diatom assemblages of a maritime Antarctic lake. Polar Biology. 9: 525-532.

Otsuki, A., & R.G. Wetzel. 1972. Coprecipitation of phosphate with carbonates in a marl lake. Limnology and Oceanography. 17:763-766.

Packman, A.I., & D. Jerolmack. 2004. The role of physicochemical processes in controlling sediment transport and deposition in turbidity currents. Marine Geology. 204: 1-9.

Paterson, D.M. 1989. Short-term changes in the erodibility of intertidal cohesive sediments related to the migratory behaviour of epipelic diatoms. Limnolology and Oceanography. 34: 223-234.

Paterson, D.M. 1994. Microbiological mediation of sediment structure and behaviour. In: Microbial Mats. (Stal, L.J., Caumette, P., Eds.), NATO ASI Series Vol. G35. Springer, Berlin Heidelberg.

Paterson, D.M. 1997. Biological mediation of sediment erodibility, ecology and physical dynamics. In: Cohesive Sediments (Burt, N., R. Parker, & I. Watts. Eds), pp. 215-229. Wiley Interscience. Chichester, UK.

Paterson, D.M. 2005. Biodiversity and Functionality of Aquatic Ecosystems In: Biodiversity : Structure and Function, from Encyclopedia of Life Support Systems.

Paterson, D.M., & K.S. Black. 1999. Water flow, sediment dynamics, and benthic biology. In Advances in Ecological Research (Raffaelii, D. & D. Nedwell. Eds.), 155- 193. Oxford University Press, Oxford.

Paterson, D.M., R. Perkins, M. Consalvey, & G.J.C. Underwood. 2003. Ecosystem function, cell micro-cycling and the structure of transient biofilms. In: Fossil and Recent Biofilms A Natural History of Life on Earth (Krumbein W.E., D.M. Paterson, & G.A. Zavarzin. Eds.), Kluwer, London, 47-63.

Penna, A., M. Magnani, I. Fenoglio, B. Fubini, C. Cerrano, M. Giovine, & G. Bavestrello. 2003. Marine diatom growth on different forms of particulate silica: evidence of cell/particle interaction. Aquatic Microbial Ecology. 32: 299-306.

Penn, M.R., M.T. Auer, S.M. Doerr, C.T. Driscoll, C.M. Brookes & S.W. Effler. 2000. Seasonality in phosphorus release rates from the sediments of a hypereutrophic lake under a matrix of pH and redox conditions. Canadian Journal of Fisheries and Aquatic Sciences 57: 1033-1041.

Perkins, R.G., C. Honeywill, M. Consalvey, H.A. Austin, T.J. Tolhurst, & D.M. Paterson. 2003. Changes in microphytobenthic chlorophyll a and EPS resulting from

sediment compaction due to de-watering: opposing patterns in concentration and content. Continental Shelf Research. 23: 575–586

Perkins, R.G., & G.J.C. Underwood. 2001. The potential for phosphorus release across the sediment-water interface in an eutrophic reservoir dosed with ferric sulphate. Water Research. 35: 1399-1406.

Perkins, R.G., & G.J.C. Underwood. 2002. Partial recovery of a eutrophic reservoir through managed phosphorus limitation and unmanaged macrophyte growth. Hydrobiologia. 418: 75-85.

Perkins, R.G., D.M. Paterson, H. Sun, J. Watson, & M.A. Player. 2004. Extracellular polymeric substances: quantification and use in erosion experiments. Continental Shelf Research. 24: 1623-1635.

Pettersson, K., E. Herlitz, & V. Istvanovics. 1993. The role of Gloeotrichia echinulata in the transfer of phosphorus from sediment to water in Lake Erken. Hydrobiologia. 253: 123-129.

Phillips, G., R. Jackson, C. Bennett, & A. Chilvers. 1994. The importance of sediment phosphorus release in the restoration of very shallow lakes (The Norfolk Broads, England) and the implications for biomanipulation. Hydrobiologia. 275/276: 445-456.

Phillips, G., A. Kelly, J.A. Pitt, R. Sanderson, & E. Taylor. 2005. The recovery of a very shallow eutrophic lake, 20 years after the control of effluent derived phosphorus. Freshwater Biology. 50: 1628-1638.

Ploug, H., C. Lassen, & B.B. Jørgensen. 1993. Action spectra of microalgal photosynthesis and depth distribution of spectral scalar irradiance in a coastal marine sediment of Limfjorden, Denmark. FEMS Microbial Ecology. 12: 69-78.

Psenner, R., B. Boström, M. Dinka, K. Petterson, R. Pucsko, & M. Sager. 1988. Fractionation of phosphorus in suspended matter and sediment. Archive fűr Hydrobiologie: Ergebnisse der Limnologie. 30: 98-103.

Qu, W.C., R.J. Morrison, R.J. West, & C.W. Su. 2005. Diagenetic stoichiometry and benthic nutrient fluxes at the sediment-water interface of Lake Illawara, Australia. Hydrobiologia. 537: 249-264.

Redfield, A.C., B.H. Ketchum, & F.A. Richards. 1963. The influence of organisms on the composition of seawater. In: Hill M.N. ed. *The Sea 2: The composition of seawater*. Wiley, New York. pp26-77.

Reynolds, C.S. 1996. Phosphorus recycling in lakes: evidence from large limnetic enclosures for the importance of shallow sediments. Freshwater Biology. 35: 623-645.

Rippey, B. 1983. A laboratory study of the silicon release process from a lake sediment (Lough Neagh, Northern Ireland). Archive fűr Hydrobiologie. 96: 417-433.

Risgaard-Petersen, N., S. Rysgaard, L. P. Nielsen, & N.P. Revsbech. 1994. Diurnal variation of denitrification and nitrification in sediments colonized by benthic macrophytes. Limnology and Oceanography. 39: 573-579.

Risgaard-Peterson, N., R.L. Meyer, & N.P. Revsbech. 2005. Denitrification and anaerobic ammonium oxidation in sediments: effects of microphytobenthos and NO_3^-. Aquatic Microbial Ecology. 40: 67-76.

Round, F. E. 1956. A note on some communities of the littoral of lakes. Archive fűr Hydrobiologie. 52: 398-405.

Round, F.E. 1981. The ecology of algae. Cambridge University Press, Cambridge.

Sas, H. 1989. Lake Restoration by Reduction of Nutrient Loading. Academic Verlag Richarz.

Scheffer, M., S.H. Hosper, M.L. Meijer, B. Moss, & E. Jeppesen. 1993. Alternative equilibria in shallow lakes. Trends in Ecology and Evolution. 8:275-279.

Scheffer, M. 1998. Ecology of shallow lakes. Kluwer Academic Press, London, UK.

Scheffer, M. 2001. Ecology of shallow lakes. Kluwer Academic Press. London, UK.

Sicko-Goad, L., E.F. Stoermer, & J.P. Kociolek. 1989. Diatom resting cell rejuvenation and formation: Time course, species records, and distribution. Journal of Plankton Research. 11: 375-389.

Smith, I.R. (1974). The structure and physical environment of Loch Leven, Scotland. Proceedings of the Royal Society of Edinburgh. B 74: 81-100.

Søndergaard, M. 1989. Phosphorus release from a hypertrophic lake sediment: Experiments with intact sediment cores in a continuous flow system. Archive fùr Hydrobiologie. 116: 45-59.

Søndergaard, M., J.P. Jensen, & E. Jeppesen. 2001. Retention and internal loading of phosphorus in shallow, eutrophic lakes. Scientific World. 1: 427-442.

Søndergaard, M., J.P. Jensen, & E. Jeppesen. 2003. Role of sediment and internal loading of phosphorus in shallow lakes. Hydrobiologia. 506-509: 135-145.

Søndergaard, M., J.P. Jensen, & E. Jeppesen. 2005. Seasonal response of nutrients to reduced phosphorus loading in 12 Danish lakes. Freshwater Biology. 50: 1605-1615.

Spears, B.M., L. Carvalho, R. Perkins, A. Kirika, & D.M. Paterson. 2006a. Spatial and historical variation in sediment phosphorus fractions and mobility in a large shallow lake. Water Research. 40: 383-391.

Spears, B.M., L. Carvalho, R. Perkins, A. Kirika, & D.M. Paterson. 2006b. Sediment P cycling in a large shallow lake: spatio-temporal variation in phosphorus pools and release. Hydrobiologia. In press.

Spears, B.M., L. Carvalho, & D.M. Paterson. 2006c. Phosphorus partitioning in a shallow lake: implications for water quality management. Water and Environment Journal. 21: 47-53.

Spears, B.M., I. Gunn, I. Winfield, B. Dudley, L. Carvalho, E. Vourexaki, & L. May. *In prep.* An assessment of methods for determining macrophyte maximum growing depth in lakes.

Steele, J.H., & I.E. Baird, I.E. 1968. Production ecology of a sandy beach. Limnology and Oceanography. 13:14-25.

Stevenson, R.J., M.L. Bothwell, & R.L. Lowe. 1996. Algal Ecology: Freshwater Benthic Ecosystems. Academic Press Limited, London.

Sundareshwar, P.V., & J.T. Morris. 1999. Phosphorus sorption characteristics of intertidal marsh sediments along an estuarine salinity gradient. Limnology and Oceanography. 44: 1693-1701.

Sweerts, J-P.R.A., M-J. Bär-Gilissen, A.A. Cornelese, & T.E. Cappenberg. 1991. Oxygen-consumption processes at the profundal and littoral sediment-water interface of a small meso-eutrophic lake (Lake Vechten, The Netherlands). Limnology and Oceanography. 36: 1124-1133.

Tett, P. 1982. The Loch Eil Project: planktonic pigments in sediments from Loch Eil and the Firth of Lorne. Journal of Experimental Marine Biology and Ecology. 56:101-114.

Thornton, D.C.O., L.F. Dong, G.J.C. Underwood, & D.B. Nedwell. 2002. Factors affecting microphytobenthos biomass, species composition and production in the Colne Estuary (UK). Aquatic Microbial Ecology. 27: 285:300.

Tolhurst, T.J. 1999. Microbial mediation of intertidal sediment erosion. PhD thesis. University of St Andrews.

Tolhurst, T.J., K.S. Black, D.M. Paterson, H. Mitchener, R. Termaat, & S.A. Shayler. 2000. A comparison and measurement standardisation of four in situ devices for determining the erosion sheer stress of intertidal sediments. Continental Shelf Research. 20: 1397-1418.

Turner, R.E., N.N. Rabalais, B. Fry, N. Attila, C.S. Milan, J.M. Lee, C. Normandeau, T.A Oswald, E.M. Swenson, & D.A. Tomasko. 2006. Paleo-indicators and water quality change in the Charlotte Harbor estuary (Florida). Limnology and Oceanography. 51: 518-533.

Underwood, G.J.C., & D.M. Paterson. 1993. Seasonal-changes in diatom biomass, sediment stability and biogenic stabilization in the Severn estuary. Journal of the Marine Biological Association of the United Kingdom. 73: 871-887.

Underwood, G.J.C., & D.M. Paterson. 2003. The importance of extracellular carbohydrate production by marine epipelic diatoms. Advances in Botanical Research. 40: 183-240.

Underwood, G.J.C., D.M. Paterson, & R.J. Parkes. 1995. The measurement of microbial carbohydrate exopolymers from intertidal sediments. Limnology and Oceanography. 40: 1243-1253.

Underwood, G.J.C., & D.J. Smith. 1998. Predicting epipelic diatom exoploymer concentration in intertidal sediments from sediment chlorophyll a. Microbial Ecology. 35: 116-125.

Van Luijn, F., D.T. Van der Molen, W.J. Luttmer, & P.C.M. Boers. 1995. Influence of benthic diatoms on the nutrient release from sediments of shallow lakes recovering from eutrophication. Water Science and Technology. 32: 89-97.

Vollenweider, R.A. 1968. Scientific fundamentals of the eutrophication of lakes and flowing waters, with particular reference to nitrogen and phosphorus as factors of

eutrophication. Tech. Report. DAS/CSI/68.27, Organisation for Economic Cooperation and development, Paris 250p.

Webster, J.R., P.J. Mulholland, J.L. Tank, H.M. Valett, W.K. Dodds, B.J. Peterson, W.B. Bowden, C.N. Dahm, S. Findlay, S.V. Gregory, N.B. Grimm, S.K. Hamilton, S.L. Johnson, E. Martí, W.H. Mcdowell, J.L. Meyer, D.D. Morrall, S.A. Thomas, W.M. Wollheim. 2003. Factors affecting ammonium uptake in streams - an inter-biome perspective. Freshwater Biology. 48: 1329–1352.

Wetzel, R.G., & D.F. Westlake. 1969. Periphyton. In IPB Handbook. 12: 33-40. Blackwell, Oxford.

Wetzel, R.G. 2001. Limnology: Lake and river ecosystems. 3rd Ed. Academic Press. pp1006.

Wetzel, R.G., & G.E. Likens. 2000. Limnological Analyses 3rd edition. Springer-Verlag, New York.

Weyenmeyer, G.A., M. Meili, & D.C. Pierson. 1995. A simple method to quantify sources of settling particles in lakes: resuspension versus new sedimentation of material from planktonic production. Marine and Freshwater Research. 46: 223-231.

Woodruff, S.L., W.A. House, M.E. Callow & B.S.C. Leadbeater. 1999. The effects of biofilms on chemical processes in surficial sediments. Freshwater Biology. 41: 73-89.

Yallop, M.L., D.M. Paterson, & P. Wellsbury. 2000. Interrelationships between rates of microbial production, exopolymer production, microbial biomass and sediment stability in biofilms of intertidal sediments. Microbial Ecology. 39: 116-127.