INVESTIGATING FORCED RECOVERY FROM EUTROPHICATION IN SHALLOW LAKES

Thesis submitted in partial fulfillment of the requirements

for the degree of **PhD**

SEBASTIAN MEIS

School of Earth & Ocean Sciences

Cardiff University

2012

This work has not been submitted in substance for any other degree or award at this or any other university or place of learning, nor is being submitted concurrently in candidature for any degree or other award.

Signed …… Date ……

This thesis is being submitted in partial fulfillment of the requirements for the degree of PhD

Signed …… Date ……

This thesis is the result of my own independent work/investigation, except where otherwise stated. Other sources are acknowledged by explicit references. The views expressed are my own.

Signed …… Date ……

I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loan, and for the title and summary to be made available to outside organisations.

Signed …… Date ……

I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loans after expiry of a bar on access previously approved by the Academic Standards & Quality Committee.

Signed …… Date ……

ACKNOWLEDGEMENTS

An incredible number of people have helped throughout the process of this project and I would like to say thank you to all of you. However, a few people deserve a special mention. These include my supervisors Bryan Spears, Rupert Perkins and Stephen Maberly for their continuous intellectual and personal support. Bryan deserves special thanks for his ability to fix apparently unsolvable problems and his enthusiasm for mud, which even seemed to exceed mine at times. Bryan I will nominate you for the best people manager alive award!

Michael O'Malley and Amy Anderson deserve a paragraph to themselves. Thanks to their support, including giving me a birthday cake on Loch Flemington, ensured that I enjoyed my fieldwork as much as I did.

I would also like to thank Alastair Noble and John Pottie (Loch Flemington Catchment Management Group), for their inspiring personal commitment to their local loch! In addition, thanks to all the great and helpful people at CEH Edinburgh, particularly Linda May, Iain Gunn, Bernard Dudley, Laurence Carvalho and Claire McDonald, for their support in the lab, field and during data analysis. Also, thanks to Mitzi De Ville (CEH, Lancaster), particularly for her belief that I am doing the right thing!

Furthermore, I wish to thank Iain McDonald, Ley Woolley and Anthony Oldroyd at Cardiff University for their valuable help with elemental analysis. Thanks to Nigel Traill (Phoslock® Europe GmbH, Germany), Said Yasseri (Institut Dr Novak, Germany), Patrick Van Goethem (Phoslock® Europe GmbH, Belgium) and Nicolai Nowak (Bentophos® GmbH, Germany) for their logistical support during the Phoslock® applications. Also thanks to Ian Milne (SEPA) for providing historical water quality data, Ben Leyshon (SNH) for comments on site selection and Stuart Benn (RSPB) for

providing historical fish data and for their continued support of this research. Furthermore, I wish to thank Ian Winfield (CEH, Lancaster) for his advice on sampling fish and Lenka Procházková (Charles University, Czech Republic) for her help with phytoplankton identification. Many thanks also to Michael Hupfer and colleagues (IGB, Germany) and Henning Jensen (University of Southern Denmark) for their advice on sediment analysis techniques. Thanks to Klaus van de Weyer (lanaplan, Germany) for providing office space and plenty of coffee back in the 'promised land' during the write-up phase.

Finally, to my family (Anne, Georg and Julia) and friends, thank you all so much for your great and unwavering support! I am lucky to know such great people!

TABLE OF CONTENTS

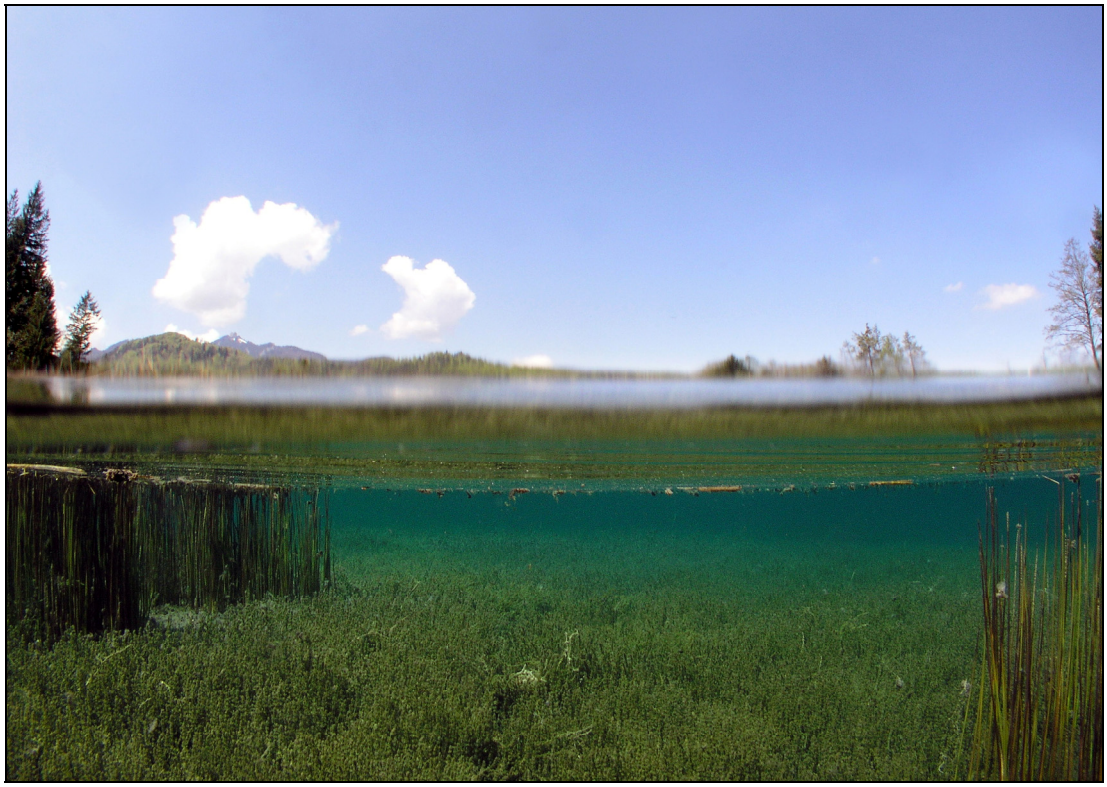
SUMMARY

Release of phosphorus (P) from bed sediments to overlying waters (internal P-loading) can detrimentally alter ecological structure and function in shallow lakes. The theory of regime shifts states that shallow lakes can exist in alternative states and that shifts between states can occur as a result of disturbance. The main hypothesis of this study was that the use of a P-capping agent (Phoslock®) in shallow lakes (i.e. a controlled disturbance) will break down the internal P-loading feedback mechanism resulting in a regime shift.

Experiments, ranging in scale from mesocosm to whole-lake, showed that Phoslock® significantly reduced internal P-loading by increasing the mass of P stored in more refractory sediment P-fractions relative to potentially release-sensitive P-fractions. Intact sediment core experiments highlighted that the application of high areal loads of Phoslock® can, at least temporarily, significantly alter the vertical distribution of sediment dissolved oxygen concentrations and cycling of nutrients other than P, suggesting that the application of high areal loads of Phoslock® to lakes should be avoided. Disruption of internal P-loading in Loch Flemington (Inverness, UK) caused a significant reduction of in-lake P concentrations, a decrease of phytoplankton biomass and an increase in water clarity. The observed changes were generally comparable to those observed in long-term multi-lake studies investigating the recovery of shallow lakes following external P-load control, but occurred over a shorter time scale (1 year compared to decades). Alterations in ecological structure and function indicated that a change in state from a 'phytoplankton dominated turbid state' to a 'macrophyte dominated clear water state' was achieved in Loch Flemington. This study confirms that it is possible to force a state change in shallow lakes by disturbing feedback mechanisms and documents further work required to improve the efficacy of the P-capping approach in lake remediation.

Chapter 1

Introduction



INTRODUCTION

Anthropogenic nutrient enrichment (cultural eutrophication) has greatly impacted the world's freshwater resources, mainly due to inputs of phosphorus (P) and nitrogen (N) (Smith *et al*., 1999). Lakes, and to a lesser extent rivers and estuaries, are extremely sensitive to enhanced P enrichment (Vollenweider, 1968; Schindler, 1977). Shallow lakes with a mean depth of less than about 3 m are the most abundant freshwater habitats of the world (Wetzel, 2001). As a habitat for a wide range of organisms they are a valuable source of local, regional and national biodiversity (Williams *et al*., 2004; Scheffer *et al*., 2006) and provide important ecosystem services (ES) to humans, including water supply for drinking and irrigation, provision of food and facilitating recreation (Postel and Carpenter, 1997). As the catchments of shallow lakes are frequently located in populated regions, intensive agricultural use often represents a significant threat to the ecological structure and function (Scheffer, 1998; Jeppesen *et al*., 2000; Vadeboncoeur *et al*., 2003; Phillips, 2005) as well as socioeconomic quality of many shallow lakes (Harper, 1992). This is a problem across many highly populated areas of the world that has been recognised through the implementation of legally binding directives to improve water quality and prevent degradation of freshwater resources, such as the Water Framework Directive (WFD; 2000/60/EC) in Europe and the Clean Water Act in the USA (CWA; 33 U.S.C. §1251 et seq., 1972). However, climate change has been predicted to increase the sensitivity of shallow lakes to eutrophication and intensify some eutrophication related problems in shallow lakes, with possible detrimental impacts on lake ecosystem structure and function in the near future (Winder and Schindler, 2004; Mooij *et al*., 2005; Kundzewicz *et al*., 2007; Meerhoff *et al*., 2007; Paerl and Huisman, 2008; Feuchtmayr *et al*., 2009; Jeppesen *et al*., 2009). As a result, research in the field of shallow lake functioning and remediation has been the focus of a wide range of recent studies (Moss *et al*., 1996; Gulati and van

Donk, 2002; Jeppesen *et al*., 2007a; Søndergaard *et al*., 2007; Hickey and Gibbs, 2009) which has led to the development of key theories, including the steady state hypothesis (Scheffer *et al*., 1993; Scheffer, 1998) and concept of ecosystem resilience (Holling, 1973; Gunderson, 2000; Scheffer *et al*., 2001; Folke *et al*., 2004). Recent studies have highlighted the fact that shallow lake responses to remediation measures is extremely complex and that sediment P-release (internal P-loading) is a key factor delaying recovery for years to decades following external load reductions (Søndergaard *et al*., 2003; Jeppesen *et al*., 2005b; Smolders *et al*., 2006; Søndergaard, 2007). Consequently, research has focussed on secondary in-lake remediation measures (for overview see e.g. Hupfer and Hilt, 2008; Hickey and Gibbs, 2009) in order to control internal P-loading and thereby 'speed up' the recovery process. This research area is, besides delivering practical information for lake mangers, likely to enhance knowledge about recovery trajectories in degraded ecosystems and provide insights into how 'controlled system disturbance' may be used to overcome feedback mechanisms that hamper recovery (e.g. internal P-loading). This introduction will outline the effects of nutrient enrichment on shallow lake functioning, with a particular focus on processes controlling internal P-loading. Furthermore, an overview of remediation and management options will be presented, and the key aims of the project will be outlined.

Eutrophication of shallow lakes

Cultural eutrophication is defined as the anthropogenic enrichment of water by nutrients, especially compounds of N and/or P (Smith *et al*., 1999), which ultimately cause the breakdown of resilience mechanisms leading to structural and functional deterioration of aquatic ecosystems (Harper, 1992; Carpenter and Lathrop, 1999). Sources of N and P include diffuse sources like surface run-off from agricultural areas and point sources like sewage effluents (Moss, 1980). In recent years the latter is of

decreasing importance in Europe due to more sophisticated waste water treatment techniques, while diffuse sources are a continuing and unresolved problem (Kristensen and Hansen, 1994). It is anticipated that the main drivers of eutrophication are population growth, industrialisation, agricultural intensification, tourism and recreation (Carpenter and Cottingham, 1997). Overall, eutrophication is accepted to be one of the most important anthropogenic causes for deteriorating ecological quality of freshwater ecosystems (Bennett *et al*., 2001; Schindler, 2006; Smith and Schindler, 2009) causing estimated financial costs of billions of dollars per year on a global scale (Smith and Schindler, 2009).

Common consequences of eutrophication are deterioration of the ecological structure and function of shallow lakes (Phillips, 2005; Smith and Schindler, 2009). Commonly observed structural changes during enrichment with P and N include an increase in primary production (Smith *et al*., 1999), an increase in magnitude and frequency of occurrence of potentially toxic cyanobacterial blooms (Brookes and Carey, 2011), a decrease in species richness (Jeppessen *et al*., 2000), a loss of functional diversity (Hulot *et al*., 2000), a loss of submerged macrophytes and fish kills (Harper, 1992). Structural changes often imply changes in function, for example a shift of primary production from benthic to pelagic habitats (Vadeboncoeur *et al*., 2003), changes in the biogeochemical cycling of nutrients (Søndergaard *et al*, 1999), changes in food web structure and trophic level interactions (Jeppesen *et al*., 2000).

Such structural and functional changes ultimately lead to a reduction in ES provided by shallow lakes (Harper, 1992; Carpenter and Cottingham, 1997; Folke *et al*., 2004; Dobson *et al*., 2006). ES comprise the benefits people obtain from ecosystems (MEA, 2005) and include in the case of shallow lakes provisioning services (e.g. delivery of fresh water for drinking, irrigation and industry, food, pharmaceuticals, energy), regulating services (e.g. climate and water regulation, carbon sequestration,

water purification and waste treatment), supporting services (e.g. nutrient cycling), and cultural services (e.g. providing inspiration, facilitating recreation, enabling scientific discovery) (Postel and Carpenter, 1997; MEA, 2005). The ES provisioning capacity of shallow lakes has been reduced by nutrient enrichment and is expected to be further impacted by climate change.

In Europe, climate change predictions for the 21st century forecast a rise in temperature, particularly in winter, and variation in precipitation patterns (Alcamo *et al*., 2007). In Northern Europe precipitation is expected to increase, with intense precipitation events particularly in winter, while a decline in precipitation is forecasted for Southern Europe (Alcamo *et al*., 2007). Furthermore, the occurrence of extreme weather events is expected to increase as a result of climate change (Alcamo *et al*., 2007). The predicted climate scenarios may affect shallow lakes, for example those in Northern Europe, by increasing nutrient transport to the lakes, and enhance sediment P-release (Jeppesen *et al*., 2009; Kosten *et al*., 2011), which may act in concert to increase nutrient supply to phytoplankton in the pelagic zone. Additionally it is expected that warming will favour plankti-benthivorous fish species which might additionally favour pelagic phytoplankton production via reduction of zooplankton grazing pressure and increased release of nutrients from the sediment as a consequence of bioturbation during benthic foraging (Jeppesen *et al*., 2007b, 2009). Phytoplankton community composition is generally expected to change towards a higher dominance of dinophytes and cyanobacteria (Jeppesen *et al*., 2009). Consequently, water clarity may decrease either due to higher phytoplankton biomass or indirectly by reduced capacity of submerged macrophytes to act as a refuge for zooplankton from a fish community dominated by small omnivorous species (Jeppesen *et al*., 2007b).

Shallow lakes appear to be particularly sensitive to predicted extreme weather events which could cause changes in ecological state triggered by high intensity rainfall

(Rip, 2007; Nöges *et al*., 2010), drought (Coops *et al*., 2003), changes in water level (Dokulil *et al*., 2011), extended periods of winter ice events leading to anoxia and winter fish kills (Greenbank, 1945; Hargeby *et al*., 2004), and summer heat waves (Schindler, 2006). Moss *et al*. (2009) reviewed the importance of climate change for the future of policy making in the EU and outlined mitigation options for freshwater ecosystems under different warming scenarios. In conclusion, climate change is likely to impact on the structure and function of shallow lakes via multiple processes, including: i) alteration of nutrient loading and cycling to and in shallow lakes (Jeppesen *et al*., 2009); ii) alteration of the community structure (Mooij *et al*., 2005; Jeppesen *et al*., 2010); and/or iii) variation in phenological events (Winder and Schindler, 2004). Thus, being able to manipulate key processes and feedback mechanisms may provide an increased resilience capacity in shallow lakes facing multiple and interacting pressures.

The majority of remediation projects have therefore focussed on reducing water-column P concentrations (Sas, 1989; Carpenter, 2008; Schindler *et al*., 2008) by various means, to control the detrimental effects of eutrophication. Many remediation efforts have aimed to control phytoplankton biomass, including potentially toxin producing cyanobacteria, since phytoplankton can play a pivotal role in shallow lake functioning (Scheffer, 1998). This remediation approach is based on the general assumption that phytoplankton production is commonly P limited in shallow lakes. This has been indicated by multi-lake studies that showed a strong relationship between phytoplankton biomass (often measured as chlorophyll *a* concentration) and total phosphorus (TP) concentration across geographical regions (Dillon and Rigler, 1974; Schindler, 1978; Vollenweider and Kerekes, 1980; Phillips *et al*., 2008). Additionally, whole-lake experiments presented evidence that the addition of P rather than N increased phytoplankton biomass (Schindler, 1977; Perkins and Underwood, 2000). However, it should be noted that there are exceptions where either N or N and P-co-limitation occurs

(Maberly *et al*., 2002; Lewis and Wurtzbaugh, 2008; Elser *et al*., 2009; May *et al*., 2010). It is also known that the relationship between chlorophyll *a* and TP concentration is known to 'break down' at TP concentrations in excess of around 100 μg TP L^{-1} in northern temperate lakes (Vollenweider and Kerekes, 1980; Phillips *et al*., 2008). This phenomenon has been generally attributed to N-limitation under P-replete conditions (Chow-Fraser *et al*., 1994). However, if considering the connectivity between freshwater and marine ecosystems (i.e. the 'freshwater-marine continuum') both N and P should be controlled to prevent aggravation of eutrophication related problems particularly downstream towards marine ecosystems (Pearl, 2009).

Shallow lake remediation

Various attempts have been made in the remediation of eutrophic shallow lakes. A wide range of case studies have highlighted a lag effect following management attempts in the catchment to reduce external nutrient inputs suggesting that recovery can take many years if not decades to occur (Sas, 1989; Marsden, 1989; Søndergaard *et al*., 2003).

Theoretical background of shallow lake remediation

Ecological functioning is influenced by some unique characteristics of shallow lakes, including: i) lack of long-term stratification leading to permanent contact of the surface sediment with a circulating water-column (Scheffer, 1998); ii) a high sediment surface to water volume ratio (Sas, 1989); and iii) a euphotic zone extending to the majority of the sediment bed (Scheffer, 1998). These characteristics play an important role in the response of shallow lakes to nutrient enrichment. Shallow lakes can exist in alternative states along a P gradient (Scheffer, 1998). Although alternative states have been described for terrestrial ecosystems (e.g. woodlands; Scheffer *et al*., 2001) and marine ecosystems (e.g. coral reefs; Scheffer *et al*., 2001) they have been most intensively

investigated and described in shallow lakes subjected to eutrophication (Scheffer *et al*., 1993; Scheffer, 1998; Scheffer *et al*., 2001; Blindow *et al*., 2006). At low nutrient concentrations shallow lakes are predicted to exist in a 'macrophyte dominated clear water state' while a 'phytoplankton dominated turbid state' persists at high nutrient concentrations (Scheffer *et al*., 1993). Various feedback mechanisms exist that buffer the respective states against change following disturbance (Scheffer *et al*., 1993; Scheffer, 1998). The macrophyte dominated clear water state may be stabilized against a state change following an increase in P-loading for example by the following feedback mechanisms: i) macrophyte beds serving as refugia for zooplankton and enhancing piscivorous fish species that limit top-down control of plankti-benthivorous fish species (Jeppesen *et al*., 1998a); ii) control of phytoplankton biomass by enhanced zooplankton grazing (Carpenter *et al*., 1992, 1996); and/or iii) reduction of phytoplankton production via the reduction of wind-induced sediment re-suspension, often associated with sediment P-release, by macrophytes (Dieter, 1990; Madsen *et al*., 2001). In contrast, the phytoplankton dominated turbid state may be stabilized against state change following a decrease in P-loading by the following feedback mechanisms: i) phytoplankton or non-algal shading (e.g. by wind or fish induced sediment re-suspension (Ibelings *et al*., 2007)) hampering the establishment of submerged macrophytes (Sand-Jensen and Borum, 1991); ii) truncated food-webs dominated by planktivorous fish species causing high grazing pressure on zooplankton (Moss, 1990); and/or iii) release of P from the sediment fuelling phytoplankton growth until a new P-equilibrium is reached (Søndergaard *et al*., 2003).

The role of internal P-loading

Many studies have highlighted that cycling of P between lake sediments and the water-column (internal P-loading) is a key feedback mechanism buffering the phytoplankton

dominated turbid state against state change following reduction of external nutrient load, thereby delaying the recovery of shallow lakes (Sas, 1989; Marsden, 1989; Søndergaard *et al*., 1999, 2003; Mehner *et al*., 2008). Sediments play a major role in the 'P metabolism' of shallow lakes, acting both as a sink and a source of P depending on nutrient loading history, sediment P-binding capacity and a range of other biogeochemical factors (Boström *et al*., 1988; Sas, 1989; Søndergaard, 2007). Sediment P-binding capacity may be dependent on sediment elemental composition, particularly the presence of elements that are involved in P-binding and P-cycling (e.g. aluminium, calcium, manganese, iron; Boström *et al*., 1988; Søndergaard *et al*, 1996). In the sediment, P is present in dissolved, loosely bound and various organically and inorganically bound forms (Søndergaard, 2007), with dissolved and loosely bound sediment P (i.e. '*labile P*' as defined by common sequential extraction procedures discussed later), commonly representing a very small fraction of total sediment P (generally <1%; Boström *et al*., 1988; Søndergaard, 1990). However '*labile P*' plays an important role since it is involved in the exchange of P between bound fractions in the sediment, and the exchange of P across the sediment-water interface (Søndergaard, 2007). Cycling of P between sediments and the water-column (hereafter termed P-cycling) is controlled by a complex mosaic of variables, including physicochemical variables that can vary seasonally and spatially, including temperature (Jensen and Andersen, 1992; Spears *et al*., 2012), pH (Boström *et al*., 1988; Seitzinger, 1991), redox-conditions (Einsele, 1936; Mortimer, 1971), concentration gradients (Boström *et al*., 1988), P-sorption properties (Søndergaard, 2007), water clarity (Spears *et al*., 2012), wind induced sediment re-suspension (Vollenweider, 1968; Boström *et al*., 1988; Spears *et al*., 2012) and/or biological factors including bioturbation by benthic macroinvertebrates and fish (Phillips *et al*., 1994), microbial activity (Hupfer and Lewandowski, 2008), macrophyte cover (Stephen *et al*., 1997; Boros *et al*., 2011), or

benthic algae (Boström *et al*., 1982, 1988; Spears *et al*., 2008a). In order to characterise potentially release-sensitive sediment P-fractions, various chemical extraction techniques capable of fractionating sediment P into a variety of operational classifications have been developed. The fractions are defined by the solubility in, or reactivity with the used chemical extraction solutions (Lukkari *et al*., 2007), however such techniques also allow estimation of general sediment P-sorption and release processes. Techniques suggested by Psenner *et al*. (1988), with modifications (e.g. by Hupfer *et al*., 1995), have been the basis for many recent studies. These techniques enable investigation into spatial and temporal variations in sediment P-fractions and have evolved out of a necessity to study and quantify potentially release-sensitive P-fractions in lake sediments (Lukkari *et al*., 2007). Although total sediment P is unlikely to change quickly for several years due to the large amount retained in the lake sediment, the proportion of different fractions may vary seasonally (Søndergaard, 2007; Spears *et al*., 2007b). The operationally defined P-fractions within sediments and their release conditions are summarised in Table 1.1. Commonly the sum of the '*labile P*', '*reductant-soluble P*' and '*organic P*' fraction (hereafter termed P_{mobile}) may be used as a proxy for the mass of P-cycling between the sediment and the water-column. Although P_{mobile} can be relatively well estimated using standard fractionation techniques, the depth from which P_{mobile} cycles between the sediment and the water-column is likely to be site specific due to the various processes acting on sediment P-cycling. Common estimates of the 'active' sediment depth vary between the top 4 cm (Cooke *et al*., 2005) and the top 10 cm of the sediment (Boström *et al*., 1982) but P-release has been reported from sediment depths of up to 25 cm (Søndergaard *et al*., 1999).

Table 1.1 Overview of common operational sediment phosphorus (P) fractions based on sequential sediment P extraction procedures.

Operational fraction	Expected P species in fraction	Driver of P-release	Seasonality of P-release	Likelihood of release under natural conditions	Ref.
'*Labile P*'	Directly available P; pore-water P; loosely bound or adsorbed P	Desorption; diffusion; steep concentration gradients	1,2, 3,4	High	1, 2, 3
'*Reductant-soluble P*'	P bound to Fe-hydroxides and Mn-compounds	Anoxia, redox potential	3, 4	High	1, 2, 3, 4, 5, 6
'*Organic P*'	Allochthonous/autochthonous material; detritus	Mineralisation (temperature)	3, 4	Medium - High	1, 2, 3, 4, 5, 6
'*Metal-oxide adsorbed P*'	P adsorbed to metal oxides (mainly Al, Fe); P exchangeable against OH^-	High pH (e.g. photosynthetic activity)	3, 4	Medium - High	1, 2, 4, 5, 6
'*Apatite bound P*'	P bound to carbonates and apatite P	Low pH	-	Medium	1, 2, 3, 4, 5
'Residual P'	Refractory compounds		-	Low	1, 2, 4, 5

'Seasonality of P-release' highlights the most likely seasons in shallow, temperate lakes in which P-release will occur (-, not likely; 1, likely in winter; 2, likely in spring; 3, likely in summer; 4, likely in autumn); Al, aluminium; Fe, iron; Mn, manganese; OH^-, hydroxyl ion; Ref., reference; **1**, Boström *et al*, 1988; **2**, Hupfer *et al*. ,1995; **3**, Spears *et al*. 2007a; **4**, Psenner *et al*., 1984; **5**, Psenner *et al*., 1988; **6**, Lukkari *et al*., 2007

Alteration of internal P-loading: the role of biological processes

Recent studies have highlighted the importance of biological processes in altering P-cycling causing a complex interrelation between physicochemical and biological processes that control internal P-loading. For example it has long been accepted that bacteria inhabiting sediments are predominantly affecting P-cycling by altering redox state e.g. by utilising oxygen, nitrate or iron compounds, for example, as electron acceptors (Gächter and Meyer, 1993; Dodds and Whiles, 2010), that may enhance the release of P from the '*reductant-soluble P*' fraction, and by alteration of sediment pH conditions that may affect the release of P from the '*metal-oxide adsorbed P*' fraction. However the role of bacteria in P-cycling appears to be more complex since bacteria may reduce sediment P-release by acting as a temporary P sink (Gächter and Meyer, 1993) or by producing refractory P compounds that add to the permanent storage of P in sediments (Gächter and Meyer, 1993). Benthic algae, often living associated with bacteria, in biofilms at the sediment-water interface can have opposing influences on sediment P-cycling. Benthic algae may directly reduce sediment P-release by nutrient sequestration (van Luijn *et al*., 1995; Woodruff *et al*., 1999; Spears *et al*., 2008a) or indirectly via stabilisation of the sediment by mat formation (Dodds, 2003) or excretion of extracellular polymeric substances (EPS) (Yallop *et al*., 2000; Spears *et al*., 2007c, 2008b), both of which may reduce sediment re-suspension. Additionally, benthic algae may increase sediment and bottom water oxygen concentrations (Carlton and Wetzel, 1988; Christensen *et al*., 1990; Spears *et al*., 2008a) which may reduce P-release from the '*reductant-soluble P*' fraction. On the contrary, an increase in bottom water pH during photosynthesis (Spears *et al*., 2008a) might alter the release of P from the '*metal-oxide adsorbed P*' fraction (Drake and Heaney, 1987). Macrophytes, which may colonize large areas of the sediment bed in shallow lakes, may also significantly alter sediment P-cycling. For example, macrophytes may alter sediment P-cycling by

alteration of wind-induced sediment re-suspension events (Dieter, 1990; Madsen *et al*., 2001), amendment of sedimentation rates (Horppila and Nurminen, 2003), modification of sediment oxygen concentrations (Sand-Jensen *et al*., 1982; Hupfer and Dollan, 2003), alteration of sediment redox-conditions (Boros *et al*., 2011), modification of pH (Barko and James, 1997; Hupfer and Dollan, 2003), direct nutrient uptake (Carignan and Kalff, 1980; Chambers *et al*., 1989) and P-release following decay and senescence (Barko and Smart, 1980). However, it appears that the effects of macrophytes on P-cycling varies considerably with species, life cycle traits and the area covered by submerged plants (Granéli and Solander, 1988; Stephen *et al*., 1997; Boros *et al*., 2011). For example, high macrophyte cover (i.e. in excess of 80%) may reduce sediment redox conditions (Boros *et al*., 2011) which in turn may enhance release of P from the '*reductant-soluble P*' fraction (Frodge *et al*., 1991). In addition, macrophyte cover may be strongly linked to other important drivers of sediment P-cycling including bioturbation by fish. Benthic foraging fish may alter sediment P-cycling by increasing sediment re-suspension (Horpilla and Kairesalo, 1990; Meijer *et al*., 1990). Following re-suspension of sediment particles, P-equilibrium conditions in the water-column and properties of the re-suspended sediment (e.g. elemental composition) can either lead to an increase (Tarvainen *et al*., 2005), a decrease (Lougheed *et al*., 1998) or no change in water-column soluble reactive phosphorus (SRP) concentrations (Breukelaar *et al*., 1994), whereas water-column TP concentrations increase (Breukelaar *et al*., 1994). Other impacts of fish on P-cycling in lakes include their role as a P sink (Griffiths, 2006) or as a vector for the translocation of P between benthic and pelagic zones associated with feeding and excretion respectively (Brabrand *et al*., 1990; Horpilla and Kairesalo, 1990). Similar to fish, bioturbation by benthic macroinvertebrates (often dominated by species belonging to Chironomids and Tubifex in shallow eutrophic lakes; Köhler *et al*., 2005; Dodds and Whiles, 2010) may increase the exchange between sediment pore-

water and the overlaying water-column thereby enhancing translocation of '*labile P*' upwards across the sediment-water interface (Boström *et al*., 1988). Furthermore, benthic macroinvertebrates may amend sediment P-cycling by alteration of their physicochemical environment, including alteration of sediment redox-conditions (generally causing an increase) that may affect release of P from the '*reductant-soluble P*' fraction (Matisoff *et al*., 1985; Lewandowski and Hupfer, 2005; Lewandowski *et al*., 2007). On the contrary, benthic macroinvertebrates may enhance sediment P-release directly via excretion (Gallepp, 1979; Vanni, 2002) or indirectly by augmenting microbial mineralisation rates or capacity (Hansen *et al*., 1997; Kristensen, 2000). Again, the effect of macroinvertebrates on P-cycling depends primarily on species composition, their life stage and abundance (Fukuhara and Sakamoto, 1987; Biles *et al*., 2002; Lewandowski and Hupfer, 2005). Finally, phytoplankton may impact on sediment P-cycling, for example via elevated pH during high photosynthetic activity that may enhance release of P from the '*metal-oxide adsorbed P*' fraction from re-suspended sediment particles (Koski-Vähälä and Hartikainen, 2001). Similarly, in shallow lakes with an often homogenous water-column, high water-column pH may directly affect sediment pH which in turn may impact on sediment P-cycling (Søndergaard, 1988).

The increasing awareness about the complex interplay between biological and physicochemical processes in controlling P-cycling is further confounded by the fact that such processes are highly variable on both a spatial and temporal scale (Spears *et al*., 2007b), leading to 'hot spots' of sediment P-release/uptake (Lewandowski and Hupfer, 2005). It is therefore a complex task to investigate the effect of any remediation measure on the various ecosystem components regulating sediment P-cycling, however remediation studies assessing multiple physicochemical and biological variables at the same time may add to the understanding of processes controlling sediment P-cycling.

Resilience and disturbance

Internal P-loading is one of the major feedback mechanisms increasing the resilience of the phytoplankton dominated turbid state to changes following external load reductions (Søndergaard *et al*., 2003). 'Resilience' is defined as the '*capacity of a system to absorb disturbance and reorganize while undergoing change so as to retain essentially the same function, structure, identity and feedbacks*' (Folke *et al*., 2004; Walker and Meyers, 2004). Resilience of shallow lakes is highest under very low nutrient concentrations (i.e. macrophyte dominated clear water state) or at very high nutrient concentrations (i.e. phytoplankton dominated turbid state) with each state being stabilized by a range of feedback mechanisms (Moss, 1980; Jeppesen, 1998b; Scheffer, 1998), as referred to above. However, resilience is weakened at intermediate nutrient concentrations (i.e. during recovery from eutrophication e.g. following reductions of external load) where a lake can switch between states (Scheffer, 1998; Scheffer *et al*., 2001). At intermediate nutrient concentrations it is hypothesised that disturbances will result in a forced alteration of state. A range of natural disturbances can affect this change including storms, floods, droughts, disease and toxic pollution events, and extreme wind conditions (Coops *et al*., 2003; Rip, 2007; Nöges *et al*., 2010). All of these natural disturbance mechanisms may result in the selective alteration of a lakes ecological structure and function.

This study hypothesises that by harnessing such disturbance mechanisms it may be possible to control a state change in a recovering lake. Generally, a state change can only be achieved if the disturbance is strong enough to move a system far enough from its origin so that function, structure and feedback mechanisms change sufficiently to stabilize another state (Walker and Meyers, 2004). Recent studies have indicated that systems susceptible to a state change are characterised by slow recovery from disturbances (Scheffer *et al*., 2009; Veraart *et al*., 2011). Various methods have been

trialled to initiate a state change in shallow lakes towards the macrophyte dominated clear water state including methods of resource control ('bottom up' approach; McQueen *et al*., 1986) and food web manipulations ('top down' approach; Carpenter *et al*., 1985). Such trials have resulted in various success in terms of water quality targets met, permanency and costs (Gulati and van Donk, 2002; Cooke *et al*., 2005; Søndergaard *et al*., 2007). It appears that resource ('bottom up') and predator ('top down') control may often interact during recovery (Perkins and Underwood, 2002; Jeppesen *et al*., 2007a; Lund *et al*., 2010) and that lake specific characteristics including nutrient concentration, macrophyte cover, lake size and depth may alter the effectiveness of 'top down' or 'bottom up' measures (Jeppesen *et al*., 1997; Genkai-Kato and Carpenter, 2005; Jeppesen *et al*., 2007a).

Studies investigating structural and functional recovery trajectories in shallow lakes recovering from eutrophication are comparatively new compared to studies assessing structural and functional degradation during the process of nutrient enrichment (Carpenter and Lathrop, 1999; Schindler, 2006; Smith *et al*., 2006; Spears *et al*., 2011). Hence the knowledge about recovery trajectories in ecological structure and function is still limited and therefore an area of ongoing research. Shallow lake ecosystems are expected to show recovery trajectories that fail to return to pre-impact status upon nutrient reduction (Hosper and Jagtman, 1990; Perkins and Underwood, 2002) and reasons for this might include loss of structural and functional diversity (Hulot *et al*., 2000; Jeppesen *et al*., 2000), altered nutrient cycling (Søndergaard *et al*., 1999) and/or amended energy flows (Vadeboncoeur *et al*., 2003). A range of studies have shown that reducing internal P-loading alone can partly improve the degraded structure and function of shallow lakes (Reitzel *et al*., 2005; van Oosterhout and Lürling, 2011), while results of other studies suggest that simultaneous management of multiple pressures, e.g. in-lake nutrient concentration and food web structure, might be

required to facilitate more rapid, complete (relative to reference state) and resilient structural and functional recovery (Benndorf, 1987; Hosper and Jagtman, 1990; Worm *et al*., 2002; van Wichelen *et al*., 2007; Mehner *et al*., 2002, 2008).

Managing internal P-loading as a 'controlled disturbance'

Northern, temperate shallow lakes in which internal P-loading plays a major role commonly show a seasonal pattern of high water-column P concentrations in summer and autumn (negative sediment P-retention) and low concentrations in winter and spring (positive sediment P-retention) (Spears *et al*., 2007a; Søndergaard *et al*, 2012). Extensive investments have been made to reduce external nutrient inputs and although controlling external nutrient loading is a pre-requisite for lasting remediation efforts (Cooke *et al*., 2005), expected improvements in water quality commonly only occur after a considerable time lag of years to decades, following external load reductions (Søndergaard *et al*., 2003). Sediment P content is dependent upon the pollution history (i.e. intensity and period; Søndergaard *et al*., 2001), sediment P-binding capacity (Sas, 1989) and lake flushing rate (Spears *et al*., 2007a). The expected length of the transient state following external load reductions, at which net sediment P-release occurs, is influenced by the magnitude of external load reduction and sediment P content (Sas, 1989). A low flushing rate, high inflow P concentrations and high sediment P content will result in an extended transient period, and vice versa. Eventually, a new P-equilibrium state will be reached at which net release of P from the sediment stops, assuming no alteration in external nutrient loading during the transient period (Sas, 1989). A range of predictive models have been developed to assess the length of the transient state (hereafter termed recovery time) in lakes. These models generally estimate the period taken for re-equilibration of P between sediment and overlying water-column given a known reduction in external P-load to the lake, sediment TP

content and flushing rate (e.g. Jensen *et al*, 2006). The estimated response times to reach a new nutrient equilibrium state for northern temperate lakes after the reduction of external loading and without additional remediation measures are 10 to 15 years for P and 5 to 10 years for N (Søndergaard *et al*., 1999; Jeppesen *et al*., 2005b, 2007a).

'Controlled disturbance'

To 'speed up' the recovery process increasing attention has been recently paid to additional in-lake remediation techniques (Cooke *et al*., 2005; Hupfer and Hilt, 2008; Hickey and Gibbs, 2009). Such techniques include, for example, sediment removal (Does *et al*., 1992; Hupfer and Hilt, 2008), hydraulic flushing (Hosper and Meyer, 1986), oxidation of the sediment surface using nitrate (Hupfer and Hilt, 2008) and the application of active P-stripping/sediment P-capping agents (hereafter termed P-capping agents; Cooke *et al*., 2005; Hupfer and Hilt, 2008; Hickey and Gibbs, 2009; Gibbs *et al*., 2011). In general, P-capping agents are applied to lakes in a similar manner (Fig. 1.1); they are first added to the water-column as slurry, powder or granules before settling to the lake bed. This results in a two-phase 'controlled disturbance'. Firstly, soluble P is stripped from the water-column (*phase I*) and secondly the particles settle on to the lake bed forming a layer at the sediment-water interface that selectively enhances the physicochemical composition of sediments to favour P-retention (*phase II*). P-capping agents are expected to reduce internal P-loading through the interception of release-sensitive P (P_{mobile}; sum of '*labile P*', '*reductant-soluble P*' and '*organic P*' fraction) following release events (e.g. release of P from ferric iron Fe(III)-P complexes under reducing conditions in sediments) at the sediment-water interface. It is generally hypothesised that controlling internal P-loading (feedback mechanism) will lead to changes in ecological structure, function and feedback mechanisms. These may include a reduction of phytoplankton growth in the pelagic zone through an initial reduction in

water-column soluble P concentrations and reduced replenishment of water-column soluble P from the sediment. Consequently, shading, a feedback mechanisms commonly associated with high phytoplankton biomass, will be reduced allowing the establishment and growth of benthic algae and submerged macrophytes on the sediment bed. Given the disturbance by the applied P-capping agent was sufficient to allow macrophytes to grow and cover large areas of the sediment bed, feedback mechanisms associated with the macrophyte dominated clear water state may naturally control internal P-release. It has been indicated, that reducing internal P-loading by increasing sediment P-retention capacity, particularly in summer, might be an important measure in addition to external load control (Søndergaard *et al*, 2012) and that the disruption of internal P-loading may be the decisive remediation measure in eutrophic shallow lakes (Mehner *et al*., 2008).

Sediment P-capping agents

Various P-capping agents have been used in lake remediation projects. These vary in chemical structure (e.g. P-binding elements like Al, Fe, Ca-based agents) and function (uptake/release pathways of P regulated by e.g. redox conditions, pH, temperature). To date, iron- (Fe; e.g. $FeSO_4$, $FeCl_3$; Perkins and Underwood, 2001), aluminium- (Al; e.g. $Al_2(SO_4)_3$, $AlCl_3$, Al modified zeolite (Aqual P™); Welch and Cooke, 1999; Reitzel *et al*., 2005; Özkundakci *et al*., 2010; Gibbs *et al*., 2011), calcium- (Ca; e.g. $CaCO_3$; Hupfer and Hilt, 2008) and La-based (e.g. Phoslock®; Gibbs *et al*., 2011) products have been used as P-capping agents.

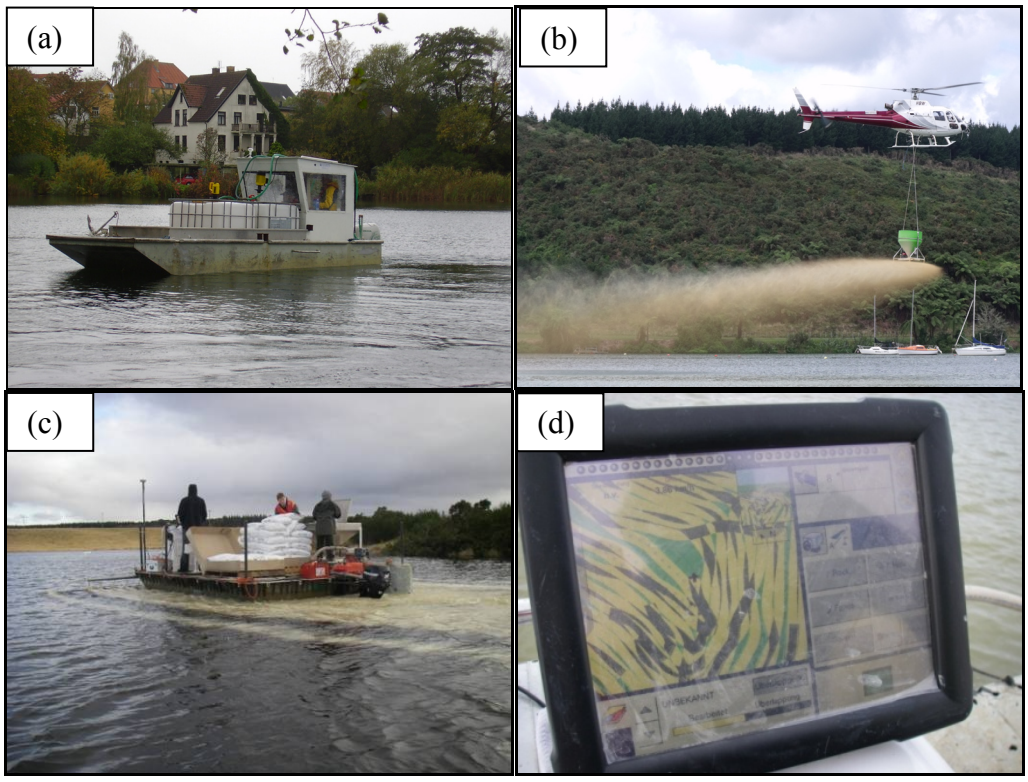


Figure 1.1 Photographs showing (a) application of aluminium hydroxide (Lake Nordborg, DK; courtesy of Inmaculada de Vicente), (b) helicopter application of an aluminium modified zeolite (Aqual P™, Blue Pacific Minerals, Okawa Bay, NZ; courtesy of Bernard Novak), (c) application of Phoslock® from a barge (Loch Flemington, UK) and (d) GPS log of transects followed during application of Phoslock® to facilitate even coverage of the sediment bed (Loch Flemington, UK).

However, natural seasonal variation in sediment redox state or pH conditions in eutrophic lakes can cause the release of P bound to Al-, Fe-, and Ca-based products (Stumm and Morgan, 1996; Hupfer and Hilt, 2008). Studies have shown that P-binding to Fe (and Mn) complexes is enhanced under high sediment redox conditions (i.e. around >200 mV) typically present in aerobic sediment layers, while P can be released from these complexes under low redox conditions (i.e. below 200 mV) often occurring in anaerobic sediment layers (Boström *et al*., 1988). Generally sediment redox conditions can be strongly coupled with temperature and/or nitrate concentrations

(Jensen and Anderson, 1992). Furthermore, an increase in pH can reduce the availability of binding sites on Fe- and Al-oxides due to competition between hydroxyl ions (OH-) and the bound P ions for binding sites (Andersen, 1975; Drake and Heaney, 1987). In contrast, low pH can enhance the release of P from Ca complexes (Boström *et al*., 1988). Overall, these processes are complicated by biological mechanisms (e.g. maintenance of oxygen, pH and sediment stability conditions in the sediment surface by the benthos; Perkins and Underwood, 2001; Spears *et al*., 2008a) that can cause spatial and seasonal variation in physicochemical drivers controlling sediment P uptake/release.

Theory of Phoslock® in controlling internal P-loading

In the 1990s, Phoslock® was developed by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) of Australia (Douglas, 2002). Phoslock® is a lanthanum (La)-modified bentonite clay, produced by exchanging ions (mainly Ca and Na) of the bentonite clay (dominated by montmorillonite) with La in a cation exchange process (Yuan and Wu, 2007; Hagsheresht *et al*., 2009). La is one of the P-binding elements in Phoslock® which has a nominal La content of 50,000 mg La kg^{-1} Phoslock® (Afsar and Groves, 2009). La bound within the clay matrix can react with dissolved P anions to form the insoluble mineral Rhabdophane ($LaPO_4 \cdot nH_2O$) (Douglas *et al*., 1999; Douglas 2002), with a solubility product of Ksp =$10^{-24.7}$ to $10^{-25.7}$ at 25 °C (Johannesson and Lyons, 1994; Liu and Byrne, 1997). It should be noted that La is a common inorganic constituent of lake sediments, with natural La concentrations in sediments of ten European lakes being found to range from 10 mg La kg^{-1} dry weight (DW) to 45 mg La kg^{-1} DW (Spears; unpublished data). Elemental analysis of Phoslock® using inductively coupled plasma mass spectrometry (ICP-MS) indicated that Phoslock® contained in addition to La (45,380 mg La kg^{-1} DW) other elements commonly involved

in P-binding and cycling including Al (11,701 mg Al kg^{-1} DW), Fe (4,498 mg Fe kg^{-1} DW), Ca (1,233 mg Ca kg^{-1} DW) and Mn (29 mg Mn kg^{-1} DW) (Gibbs *et al*, 2011).

In recent years Phoslock® has been increasingly used as a P-capping agent for the remediation of eutrophic water bodies (Robb *et al*., 2003; Lürling and Faassen, 2011; Lürling and van Oosterhout, 2012). It is reported that P bound by Phoslock® is retained under anaerobic conditions (Ross *et al*. 2008; Gibbs *et al*., 2011) and that Phoslock® is effective over pH 5 to 9 (Ross *et al*. 2008). Based on a nominal La content of 50,000 mg La kg^{-1} Phoslock® (Afsar and Groves, 2009) and a molar binding ratio of La:P of 1:1 (Haghseresht *et al*., 2009) 1 kg of Phoslock® is estimated to bind 11,147 mg P. However, reported P-adsorption capacities of Phoslock® (i.e. 11,100 to 15,100 mg P kg^{-1} Phoslock®; Yuan and Wu, 2007; Haghseresht *et al*., 2009; Gibbs *et al*., 2011) are pH dependent (tested range pH 5-9), although the effects of pH on Phoslock® P-binding properties are contentious. Gibbs *et al*. (2011) measured an increase in P-adsorption capacity with increasing pH, while a decrease in P-adsorption capacity was observed with increasing pH by Ross *et al*. (2008) and Haghseresht *et al*. (2009). Furthermore, studies have highlighted that humic substances are likely to reduce the P-adsorption capacity of Phoslock® (Ross *et al*., 2008; Lürling and Faasen, 2011) by reducing the availability of La for P-binding (Sonke and Salters, 2006; Tang and Johannesson, 2003, 2010). It appears therefore that *in situ* conditions (e.g. pH, humic substances, presence of algae) may alter the P-binding capacity of Phoslock® (Ross *et al*., 2008; Lürling and Faasen, 2011).

Effects of Phoslock® on sediment properties, nutrient cycling and biological communities

Laboratory studies have highlighted a range of effects of Phoslock® on sediment processes, including increased sediment consolidation and stability thresholds (Egemose

et al., 2010), temporary suppression of nitrification and denitrification processes (Gibbs *et al*., 2011) and alteration of the depth of the oxygenated aerobic sediment layer beneath the capping layer (Vopel *et al*., 2008). Alterations in sediment dissolved oxygen (DO) concentrations were attributed to both the thickness and the gas diffusivity of the applied capping layer (Vopel *et al*., 2008). In the field, Phoslock® has been applied at areal loads ranging from 170 – 590 g Phoslock® m^{-2} (Phoslock, 2012). *In situ* applications of Phoslock® have been made in impounded river systems (Robb *et al*., 2003) and in shallow lakes (Lürling and van Oosterhout, 2012) focussing primarily on the effects of Phoslock® on water-column P concentrations and phytoplankton biomass (particularly cyanobacteria) with partially opposing results. Robb *et al*. (2003) reported a reduction in water-column SRP concentrations and attributed this to the application which was assumed to reduce sediment P-release. In contrast, Lürling and van Oosterhout (2012) did not detect a significant reduction in water-column TP and SRP concentrations in a recently impounded section of the Gouden Ham which was treated with Phoslock®. Although cyanobacteria biomass decreased significantly, Lürling and van Oosterhout (2012) raised the question whether the impoundment or the Phoslock® treatment was the driver of the observed trend in water-column P concentrations and cyanobacteria abundance.

A wide range of ecotoxicological studies have been conducted to quantify the effects of Phoslock®, and its P-binding ingredient La, on various components of the food web, although few of these studies follow common standard methods and few have been published in the peer reviewed literature (Stauber, 2000; Clearwater, 2004; Martin and Hickey, 2004). Notable exceptions include Lürling and Tolman (2010) who showed that 0.001% of La can be released from Phoslock®. La had no toxic effect on *Daphnia magna* (Straus) up to concentrations of 1 mg La L^{-1} in P-free medium but life history traits of *Daphnia magna* can be affected at this concentration in medium with P through

rhabdophane formation ($LaPO_4 \cdot n\ H_2O$) and consequent precipitation of the food source, algae (Lürling and Tolman, 2010). It should be noted that The Netherlands are the only European country that has a maximum permissible concentration for La of 0.01 mg La L^{-1} (Sneller *et al*., 2000).

Scope of study

Internal P-loading plays, as a major feedback mechanism of the phytoplankton dominated turbid state, an important role in recovering shallow lakes by influencing their structure and function. Controlling internal P-loading is a challenging scientific task since it is regulated by complex often interrelated physicochemical and biological variables that vary on a spatiotemporal scale. According to the stable states theory, shallow lakes may be susceptible to a state change from a phytoplankton dominated turbid state towards a macrophyte dominated clear water state at intermediate nutrient concentrations. The main scope of this study was, therefore, to investigate the effects of reducing internal P-loading by a controlled disturbance using a sediment P-capping agent (Phoslock®) in recovering shallow lakes and to assess if this approach may be used to initiate a state change towards the macrophyte dominated clear water state. Over the course of this study, Phoslock® was applied to two shallow eutrophic lakes. Clatto Reservoir (Dundee, UK) represented a contained and artificial pilot site while Loch Flemington (Inverness, UK) represented a eutrophic shallow lake, more typical of a natural shallow lake system.

This study was divided into 6 key areas, the hypotheses specifically relating to which are outlined in each chapter:

i) An experimental assessment of P-retention/release from Phoslock® to forecast the effect of Phoslock® on P-partitioning between potentially release-sensitive and more refractory P-fractions.

ii) Examination of the effect of Phoslock® on sediment DO concentrations, nutrient cycling and sediment particle entrainment under different dose scenarios.

iii) An evaluation of the conceptual framework in which P-capping agents form a distinct layer on the sediment surface by assessing positioning of Phoslock® in the sediment and investigating effects of Phoslock® burial on its efficiency to control water-column P concentrations.

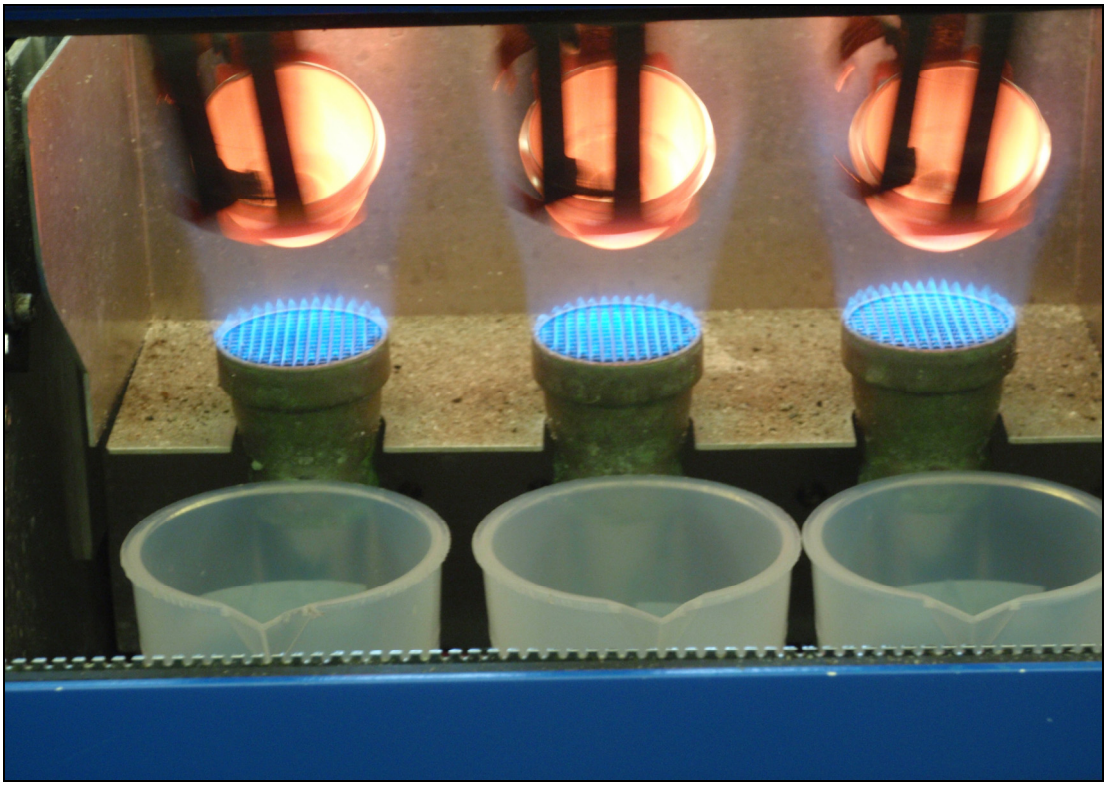
iv) An assessment of short-term physicochemical responses in the water-column including potential La leaching following a whole-lake Phoslock® application.

v) Effects of Phoslock® amendment on sediment elemental composition and sediment P-partitioning, particularly the timing of P-partitioning processes.

vi) An investigation of the effects of a Phoslock® application on whole-lake ecology by assessing seasonal variation in a range of physicochemical and biological responses pre- and post-application in the sediment and the water-column.

Chapter 2

Material and methods



MATERIAL AND METHODS

This chapter outlines the common methods used in this study. The 'Material and methods' section in each individual chapter will refer to numbered sections of this chapter. It should be noted that information on sample frequency, the level of replication and/or specific details of the experimental design used are presented in each individual chapter.

2.1 Study sites

2.1.1 Clatto Reservoir

Clatto Reservoir is a shallow reservoir, constructed in 1874, situated in the southeast of Scotland (N 56° 29.946', W 3° 01.734') at an altitude of 162 m above sea level. It has a surface area of 9 ha, a mean depth of 2.75 m, a maximum fetch of 0.35 km and a rectangular perimeter of 1.2 km. In the past the reservoir was charged via pipes connected to a series of upstream reservoirs, but it has been rain water fed since 2007. Its elevated position in relation to its catchment limits run-off and associated external nutrient loads. Groundwater inputs and agricultural inputs are likely to be negligible at this site because the majority of the surrounding land is lower than the reservoir sill and is residential or maintained parkland. It is therefore likely that the main historical sources of phosphorus (P) to Clatto Reservoir have been the upstream reservoirs, atmospheric P-deposition and the resident bird population, with the current inputs reduced to the latter two forms. The site has no natural outflow although the water level can be managed via a draw-down tower towards the south western shore. Use of the site as a drinking water reservoir ceased in 1972 (Dundee City Council, pers. comm.) and recently it has been used solely for recreation, including boating and fishing. From 2007

onwards cyanobacterial abundance was assessed against World Health Organisation (WHO) guidelines of 20,000 cells mL^{-1} (equates to lowest risk level defined as 'relatively low probability of adverse health effects'; WHO, 2003) and the site was commonly closed in summer months of 2007 and 2008 due to exceeding this target (Dundee City Council, pers. comm.).

2.1.2 Loch Flemington

Loch Flemington is a high alkalinity, eutrophic lake of glacial origin situated in the northeast of Scotland (N 57° 32.570', W 3° 59.399'). It has a surface area of 15 ha, a maximum fetch of 0.75 km, a perimeter of 2.7 km and a mean and maximum depth of 0.75 m and 2.9 m, respectively (Gunn *et al*., 2010). The lake is situated in an area consisting of glaciofluvial sand and gravel (Gordon and Auton, 1993) within the Kildrummie Kames Site of Special Scientific Interest (SSSI). The site is designated as a Special Protection Area (SPA) due to its recent use as a nesting site for the rare Slavonian Grebe (*Podiceps auritus* L.). Furthermore the site has been known to host the rare European macrophyte *Najas flexilis* (Willd.) Rostk. & Schmidt in the past (Bennion *et al*., 2008), which is protected by national and international legislation (e.g. Natura 2000, EC Habitats Directive, UK Biodiversity Action Plan). Significant hydrological modifications were made to the lake in the 19th century by blocking the natural surface-water outflow causing a significant increase in the surface area and depth of the lake (May *et al*., 2001). Consequently, the lake has no natural surface water outlet and has one surface water inlet, the Croy Burn. Water leaves the lake by evaporation and by draining through the permeable gravels along the northwest shore, leading to an estimated water retention time of around 40 days (May *et al*., 2001). The site has a long and relatively well documented history of cultural eutrophication problems. Paleolimnological studies indicated that the macrophyte community changed from a

diverse community characteristic of mesotrophic conditions (before 1850) towards a meso-eutrophic community (around 1850) and finally towards a species poor community (after 1850) indicative of eutrophic conditions (Bennion *et al*., 2008). Surveys of the macrophyte community conducted on behalf of Scottish Natural Heritage (SNH) in 2004 (SNH, 2004) and 2010 (SNH, 2010) indicated that the community was dominated by *Elodea canadensis* Michx. in both years. In 2004, a total of seven different floating and submerged species was recorded, while in 2010 eleven different species were recorded (Appendix: Table A2.1). Furthermore, regular summer blooms of potentially toxin producing cyanobacteria have been reported from the mid 1970s to 2009 (Barrett, 2000; May *et al*., 2001; Bennion *et al*., 2008). Reductions in external nutrient loads are summarised by May *et al*. (2001). The most important changes included the re-directing of treated effluent from a nearby waste-water treatment works (WwTW) away from the catchment in 1989 and the upgrading of the WwTW in 1993 to reduce sporadic effluent spillages into the Croy Burn. The most recent nutrient budget indicated that the external load to Loch Flemington equals 120 kg TP yr^{-1}, while internal sources were estimated to contribute 680 kg TP yr^{-1} (May *et al*., 2001).

2.2 Sampling

2.2.1 Water samples

Surface water samples (1 L) were collected at each sampling site by holding a bottle 5 cm below the water surface. Bottom water samples (0.1 L) were taken from sediment cores (Section 2.2.2) 5 cm above the sediment surface using a syringe. All samples were collected in acid washed plastic bottles and stored unfrozen at <4 ºC in the dark before being processed upon return to the laboratory (Clatto Reservoir) or on site (Loch Flemington).

2.2.2 Sediment samples

Unless otherwise stated, sediment cores were taken using an HTH gravity corer (core internal diameter 65 mm, length 500 mm; Pylonex, Umeå, Sweden). Cores were extruded and sectioned (2 cm slices) to a sediment depth of 10 cm on site and stored unfrozen at <4 ºC in the dark on transport to the laboratory before being frozen (-18 ºC) prior to processing for chemical analyses.

2.3 Measurements

2.3.1 Surface water

Measurements of surface water pH, conductivity, temperature and dissolved oxygen (DO) concentration were made 5 cm below the water surface using a HACH multi-parameter meter (HQ30d, HACH Lange GmbH, Düsseldorf, Germany) calibrated against standard pH and conductivity buffer solutions (HACH Lange GmbH, Düsseldorf, Germany). Water clarity was assessed using a Secchi disk (25 cm diameter quadrat). Secchi depth measurements were excluded if the disk was visible on the sediment surface at the deepest point of the lake or when the disk disappeared between dense vegetation. Euphotic depth (Z_{eu}) was estimated based on Secchi depth measurements (Z_S) according to equation 1 following Reynolds (1984):

$$Z_{eu} = 2.7 * Z_S \qquad \text{(eq. 1)}$$

Vertical underwater light profiles were measured using a LI-COR® light meter (LI-250A, LI-COR® Environmental UK Ltd, Cambridge, UK).

2.3.2 Sediment dissolved oxygen concentration

Sediment dissolved oxygen (DO) concentrations were measured using an oxygen microptode (PSt1 oxygen sensor, tip diameter <50 µm, Precision Sensing GmbH, Regensburg, Germany) connected to a temperature compensated oxygen meter (Microx TX3, Precision Sensing GmbH, Regensburg, Germany). The software used was OxyView (OxyView TX3-V5.31, Precision Sensing GmbH, Regensburg, Germany). Vertical positioning of the oxygen microptode was controlled using a manual micromanipulator (MM33, 10 µm resolution, pyroscience, Aachen, Germany) mounted on a heavy stand HS1 (pyrosciene, Aachen, Germany). The limit of detection (LOD) was set to 0.05 mg L^{-1}.

2.3.3 Sediment particle entrainment

Sediment particle entrainment (SPE) was measured by magnetic particle induction (MagPI). This method quantifies the adhesive capacity of the sediment surface, which in this case was taken as a proxy for bed stability (Anderson *et al*., 2011). A defined volume of ferrous particles (150 – 250 µm, Partrac, UK) were distributed over an area of around 1 cm^2 of the sediment surface using a syringe. The particles were then retrieved by an electromagnet positioned in the water-column at a distance of 5 mm above the sediment surface. A variable DC power supply (Skytronic, 650.676, Schorndorf, Germany) was used to gradually increase the voltage/current supplied to the electromagnet in small increments (0.2 V/~0.1 A) and thus precisely control the increasing inductive force acting on the particles. The response of the particles to each increment of electromagnetic force was carefully visually monitored. The voltage was recorded when total clearance of particles from the area below the electromagnet was achieved. The attractive magnet force acting on the particles at the point of total clearance was calculated from a standard curve. To calibrate, the electromagnet was

submerged in distilled water and placed 5 mm above a sensor connected to a Gauss meter (Shanghai Heng Tong Magnetoelectricity Co., Ltd., HT201, Shanghai, China). The voltage and current were increased incrementally (2 V/ ~0.05 A per increment) while all other factors remained constant. The magnetic flux density (MFD) for each voltage increase was measured by the Gauss meter in mTesla and recorded. The calibration was carried out in triplicate for each voltage/current increase and averaged. Straight lines were fitted to the lower range (0.0 to 5.0 V) and upper range (5.2 to 7.6 V) of values, the equations of which were used to calculate MFD at the point of total particle clearance.

2.4 Chemical analysis

2.4.1 Carbon

Water samples taken for the analysis of total dissolved carbon (TDC) and dissolved organic carbon (DOC) were stored frozen (-18 °C) until analysis. TDC analysis was conducted on unfiltered samples, while analysis of DOC was conducted on filtered (Whatman GF/C filter, nominal pore size 1.2 μm; Whatman Ltd., Kent, UK) samples. Analysis was carried out using a PPM LABTOC Analyser (Pollution & Process Monitoring Ltd, Kent, UK) with a detection range of 0.1 – 4000 mg L^{-1}. TDC was measured by mixing the sample with a sodium persulfate containing reagent and exposing the mixture to ultra-violet light in the reaction vessel, causing oxidation of all carbon present to carbon dioxide (CO_2; 'ultra-violet light promoted persulfate oxidation'). Carbon dioxide and the carrier gas (N_2) were separated from the liquid and dried. The amount of carbon dioxide was measured with an infra-red detector and compared against a standard curve constructed from a potassium hydrogen phthalate ($C_8H_5KO_4$) standard. DOC was measured after removal of inorganic carbon through

acidification and sparging with carrier gas (N_2). The pre-treated sample was then fed to the reaction vessel and CO_2 was produced and measured through ultra-violet light promoted persulfate oxidation as described above. Dissolved inorganic carbon (DIC) concentration was calculated using equation 2.

$$DIC\ (\mu g\ L^{-1}) = TDC\ (\mu g\ L^{-1}) - DOC\ (\mu g\ L^{-1}) \qquad \text{(eq. 2)}$$

2.4.2 Lanthanum

Water samples for the analysis of total lanthanum (TLa) and dissolved lanthanum (La) were stored frozen (-18 °C) until analysis. TLa analysis was conducted on unfiltered water samples by inductively coupled plasma optical emission spectrometry (ICP-OES) according to standard ISO methods (ISO 11885-E22:1997-11) following nitric acid digestion (ISO 15587-2-A23:2002-03) at the Institute Dr Nowak (Ottersberg, Germany). The LOD for TLa concentration was 2 µg L^{-1} and concentrations below the LOD were set to 1 µg L^{-1}. La analysis was conducted on filtered water samples (Whatman GF/F filter, nominal pore size 0.7 µm; Whatman Ltd., Kent, UK) by inductively coupled plasma mass spectrometry (ICP-MS) following acidification (1% v/v, nitric acid ≥ 68%, Primar Plus, Fisher) according to Lawlor and Tipping (2003) at the chemistry laboratory at the Centre of Ecology and Hydrology (Lancaster, UK).

2.4.3 Nitrogen fractions

Water samples for the analysis of nitrate-nitrogen (NO_3-N) and ammonium-nitrogen (NH_4-N) were filtered (Whatman GF/C filter, nominal pore size 1.2 µm) and stored frozen (-18 °C) until analysis. NO_3-N and NH_4-N analysis were conducted using ion chromatography. Anions (NO_3-N) were separated on a Metrosep A Supp 5 column (150 mm x 4.0 mm; Metrohm Ltd., Herisau, Switzerland) using an eluant of 3.2 mM Na_2CO_3 and 1.0 mM $NaHCO_3$, while cations (NH_4-N) were separated on a Metrosep C1 column

(125 mm x 4.6 mm; Metrohm Ltd., Herisau, Switzerland) using an eluant of 5 mM tartaric acid, 1 mM dipicolinic acid and 24 mM boric acid. Concentrations were determined by comparing against known standards. Dissolved inorganic nitrogen (DIN) concentration was calculated using equation 3.

$$DIN\ (\mu g\ L^{-1}) = NO_3\text{-}N\ (\mu g\ L^{-1}) + NH_4\text{-}N\ (\mu g\ L^{-1}) \quad \text{(eq.3)}$$

2.4.4 Phosphorus

Water samples for the analysis of total phosphorus (TP) and soluble reactive phosphorus (SRP) were stored frozen (-18 °C) until analysis. Acid washed glassware was used for all analysis steps. SRP analysis was conducted on filtered water samples (Whatman GF/C filter, nominal pore size 1.2 μm) according to Murphy and Riley (1962). In this procedure, orthophosphate reacts in an acidified medium with ammonium molybdate and potassium antimonyl tartrate to phosphomolybdic acid. This is reduced using ascorbic acid to form molybdenum blue with the adsorbance at 882 nm used to quantify the concentration using a standard curve constructed from potassium di-hydrogen orthophosphate (KH_2PO_4). TP analysis was conducted on unfiltered samples according to Eisenreich *et al*. (1975). All forms of P were oxidised to SRP using potassium persulfate digestion (30 mins at 120 °C in autoclave) in an acidified (H_2SO_4) medium and SRP concentration was determined as described before. The LOD for TP and SRP analyses was set to 3 μg L^{-1}.

2.4.5 Silicon

Water samples for the analysis of total diatom silica ($TSiO_2$) and dissolved silica (SiO_2) were stored at 4 °C in the dark prior to analysis. SiO_2 analysis was conducted on filtered water samples (Whatman GF/C filter, nominal pore size 1.2 μm) according to Golterman *et al*. (1978). In this procedure, silicon in solution (silicate, SiO_3^{2-}; silicic

acid, H_4SiO_4) reacts with acidic ammonium molybdate, forming a yellow silicomolybdate complex. This complex is reduced with ascorbic acid to form the molybdate blue colour with the adsorbance at 810 nm used to quantify the concentration using a standard curve constructed from sodium silicofluoride (Na_2SiF_6). Oxalic acid is used to eliminate interferences from any phospho-molybdate complex formation. $TSiO_2$ analysis was conducted according to Golterman *et al*. (1978). During a heated (30 mins at 120 °C in autoclave) sodium carbonate (Na_2CO_3) digestion, opaline silica of diatom frustules is converted to SiO_2 which is measured as described before. The digestion is not considered strong enough to include any suspended sand particles. All apparatus used during $TSiO_2$ and SiO_2 analysis which were in contact with samples and/or reagents were made of plastic.

2.4.6 Sediment elemental composition

Sediment samples were analysed for major elements (Al, Ba, Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Sc, Si, Sr, Ti, V, Y, Zn and Zr) with an ICP-OES, while trace elements (Ce, Cs, Dy, Er, Eu, Ga, Gd, Hf, Ho, La, Lu, Mo, Nb, Nd, Pb, Pr, Rb, Sm, Sn, Ta, Tb, Th, Tm, U and Yb) were analysed using an ICP-MS following the methodology described in McDonald and Viljoen (2006). Prior to analyses, sediment samples of each depth interval were homogenized and freeze dried. Approximately 1,500 mg of freeze dried sample was weighed and heated in a muffle furnace at 900 ºC for 90 minutes to release water (H_2O), CO_2 and other volatiles. The mass of ignited residue was determined and loss on ignition (LOI) calculated. A mass of 100 mg of ignited residue was weighed and mixed with 600 mg lithium (Li) borate flux mixture (50% metaborate and 50% tetraborate) in a crucible before approximately 0.5 mL Li iodide solution (25% w/v) was added as a non-wetting agent. The mixture was fused over a propane burner on a automated fusion system. The melt was poured into a 250 mL beaker containing 50

mL of 4% HNO_3 and the solution was stirred on a heated magnetic stirrer/hotplate until all glass fragments had dissolved. The solution was spiked with 1 mL of a 100 ppm rhodium (Rh) spike solution and made up to 100 mL with deionised water.

An ICP-OES system was used to analyse the solution for the elements named above. During ICP-OES analysis, the aqueous sample is converted to an aerosol via a nebulizer and transported into a plasma. The plasma, a zone of high temperatures, is created by the interaction of ionized argon (Ar) gas and an electromagnetic field. In the plasma, the sample is completely atomized, creating excited atoms/ions that exist in an unstable energy state. Upon return to the ground state, atoms/ions emit light with wavelengths specific to the elements present in the sample. Emissions are separated based on wavelengths and intensity, which is proportional to the concentration of the element in the aqueous sample, is measured and compared to known standards. Blanks were prepared in the same way as describe above, but omitting the sample material. Calibration was performed using a reagent blank and standard solutions, prepared as above, and using the 1 ppm Rh spike as internal standard to correct for instrumental drift during the run. Analysis of a standard solution was repeated every 10 samples as an external check on instrumental drift.

ICP-MS analysis for the elements mentioned above was carried out using the same solutions as prepared for ICP-OES, except that the initial sample solution was diluted by 10 times with 2% HNO_3 and spiked with 5 ppb of indium (In) and thallium (Tl) internal standards to correct for instrumental drift. In this analytical technique, instead of separating light according to its wavelengths (as in ICP-OES), single charged ions generated in the inductively coupled plasma (ICP) are separated by a mass spectrometer (MS) according to their mass-to-charge ratio which is measured by a detector. Sample preparation and analysis followed the methodology described in McDonald and Viljoen (2006).

2.4.7 Sediment phosphorus fractionation

The P extraction scheme followed Hupfer *et al*. (1995), based on Psenner *et al*. (1988), except that only total soluble phosphorus (TSP) was determined for the '*labile P*', '*reductant-soluble P'* and '*apatite bound P*' fractions. This modification has been used elsewhere (Lewandowski *et al*., 2003; Hupfer *et al*., 2009) and was deemed appropriate as the differentiation between TSP and SRP is small in those fractions in sediments of eutrophic lakes (Hupfer pers. comm.). Furthermore, the methods of Hupfer *et al*. (1995), Lewandowski *et al*. (2003) and Hupfer *et al*. (2009) produce comparable results (Hupfer pers. comm.).

Subsamples of homogenised sediments, from each depth interval, were subjected to the following sequential extraction procedure: (i) extraction in 1 M NH_4Cl for 30 min to determine loosely adsorbed and pore-water P ('*labile P*'); (ii) extraction with 0.11 M $NaHCO_3$ / 0.11 M $Na_2S_2O_4$ for 1 h terminated by removal of supernatant, repeated for 5 min with fresh extractant, to determine P mainly bound to iron (Fe)-hydroxides (OH^-) or manganese (Mn) compounds ('*reductant-soluble P*'); (iiia) extraction in 1 M NaOH for 16 h terminated by removal of supernatant, repeated for 5 min with fresh extractant, to mobilize P which is mainly exchangeable against OH^- ions determined as SRP ('*metal-oxide adsorbed P'*), and (iiib) organic bound P in the same fraction quantified by subtracting NaOH-SRP from NaOH-TSP ('*organic P*'); (iv) extraction with 0.5 M HCl for 16 h terminated by removal of supernatant, repeated for 5 min with fresh extractant, to determine P bound to carbonates and apatite P ('*apatite bound P*'); (v) digestion with 30% (v/v) H_2SO_4 and 8% $K_2S_2O_4$ at 121 ºC for 30 min followed by TSP quantification to determine refractory P ('*residual P*'). The sediment slurry and extraction medium was continually shaken (60 rpm on 360º rotator; PTR-60, Grant-bio, Shepreth, UK) in the dark at 25 ºC for the periods outlined above. Supernatant was collected following centrifugation and filtration (Whatman GF/C filter,

nominal pore size 1.2 µm) at the end of each extraction step. TSP was analysed according to the methods of Eisenreich *et al*. (1975) as described above for TP (Section 2.4.4), while SRP was analysed as described above (Section 2.4.4). The sum of '*labile P*', '*reductant-soluble P'* and '*organic P*' fraction was termed P_{mobile} and considered to represent potential release-sensitive P (Boström *et al*., 1982; Søndergaard *et al*., 2003).

2.4.8 Total sediment phosphorus content

A subsample of around 1 g wet sediment, taken from a homogenized sediment sample, was weighed into a centrifuge tube. After addition of 50 mL distilled water the tube was vortexed for 1 min to create a homogenous mixture. A subsample (1 mL) was taken and mixed with 4 mL distilled water. TP analysis was conducted as described above (Section 2.4.4). Additionally, three subsamples (around 1 g) of wet sediment were taken from the same homogenized sediment sample and weighed before and after drying (105 °C for 48h). Average dry weight (DW) per wet weight (WW) (g g^{-1}) was calculated by dividing average DW (g) by average WW (g). Consequently, the DW of the sample used to determine TP concentration was calculated by multiplying WW (g) used with the average DW per WW (g g^{-1}). Following this, TP concentration (µg L^{-1}) was multiplied by the volume of sample (L), yielding the mass of P in the sample (µg). Consequently the amount of P per g DW sediment (µg P g^{-1} DW sediment) was calculated by multiplying the mass of P in the sample (µg) with the reciprocal of the average DW per WW (g g^{-1}).

2.5 Biological analysis

2.5.1 Chlorophyll a analysis

Samples for chlorophyll *a* analysis were prepared by filtering (Whatman GF/C filter, nominal pore size 1.2 µm) between 200 and 400 mL of surface lake water (Section

2.2.1). The filter was stored frozen (-18 °C) in 15 mL centrifuge tubes in the dark until analysis. Pigments were extracted by submersing the frozen filter papers overnight in 90% (v/v) cold acetone. Following extraction, tubes were centrifuged for 5 min at 3,600 rpm. Analysis were carried out under dim light and samples were stored cold (< 4 °C) until analysis. Chlorophyll *a* analysis was conducted spectrophotometrically at 664, 665 and 750 nm wavelength using a PU 8670 VIS/NIR spectrophotometer (Philips), with correction for phaeopigments by acidification, according to APHA (1992), using pure acetone as blank. Samples were corrected for turbidity by subtracting the 750 nm optical density values from the 664 nm and 665 nm optical density values before (664 nm) and after (665 nm) acidification. Chlorophyll *a* concentration was calculated using equation 4.

$$\text{Chlorophyll } a \ (\mu g\ L^{-1}) = [26.7 * (664_b - 665_a) * V_1] / [V_2 * L] \qquad \text{(eq. 4)}$$

where:

664_b, 665_a = turbidity corrected optical density of 90% acetone before (b) and after (a) acidification
V_1 = volume of the extract in L
V_2 = volume of water filtered in m^3
L = light path length of cuvette in cm

2.5.2 Sediment chlorophyll a content

The methodology followed HIMON (2005). This method differed from the method used for the measurement of water column chlorophyll *a* concentrations as the mass of chlorophyll *a* was expressed against mass of sediment ($\mu g\ g^{-1}$) as opposed to volume of water ($\mu g\ L^{-1}$). The trichoromatic spectrophotometric equation used is considered the most accurate for mixed phytoplankton populations (Jeffrey and Humphrey, 1975) characteristic of the study site. Subsamples of homogenised sediments from each depth interval were frozen (-18 °C) and freeze dried prior to analysis. Pigments were extracted from 50 mg freeze dried sediment in 12 mL cold acetone (90% v/v) for 24 h in the

freezer (-18 °C). Following this period samples were vortexed and extraction was continued for another 24 h in the freezer (-18 °C). Samples were centrifuged (5 min at 3000 rpm) prior to spectrophotometric pigment analysis according to Jeffrey and Humphrey (1975). Extractants were decanted in a 1 cm cuvette and absorbances were read at wavelengths of 630, 647, 664 and 750 nm using a PU 8670 VIS/NIR spectrophotometer (Philips). Analysis were carried out under dim light and samples were stored cold (< 4 °C) until analysis. Sediment chlorophyll *a* content (µg g^{-1}) was calculated using equation 5.

Chlorophyll *a* (µg g^{-1}) = (eq. 5)

$$[(11.85(E_{664}-E_{750}) - 1.54(E_{647}-E_{750}) - 0.08(E_{630}-E_{750})) * V] / W$$

where:

$E_{630, 647, 664, 750}$ = absorbance at 630, 647, 664 and 750 nm
V = volume of the extract in mL
W = weight of sample in g

2.5.3 Phytoplankton counts and assessment of biovolume

At each sample site an algal sample (0.05 L) was taken from the water surface, fixed using Lugol's Iodine solution and stored in a cool and dark place. Samples were shipped to Charles University for microscopic enumartion by Lenka Procházková (Charles University, Prague, CZ). Prior to analysis, samples were carefully homogenised by manual mixing using a combination of alternating horizontal rolling and vertical tumbling for 2 minutes to achieve a random distribution of phytoplankton cells. Subsamples were filled in calibrated Ütermohl sedimentation chambers (5 – 10 mL) and phytoplankton was counted following settling over night using a Zeiss Axiovert inverted microscope according to the CEN methodology (CEN TC 230/WG 2/TG 3/N73, 2004) and the WISER guidance on the quantitative analysis of phytoplankton (WISER, 2012). The procedure involved counting of approximately 400 taxonomic

units per sample using a combination of: i) a whole chamber count at low magnification (40x) to pick up large taxa; ii) transect counts at intermediate magnification (100x) to enumerate intermediate sized taxa; and iii) high magnification counts (400x to 630x) using fields of view to pick up small taxa. Identification was conducted to the highest possible taxonomic level following John *et al*. (2005). Variation in phytoplankton abundance was assessed on the taxonomic class level according to the database of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/ taxonomy). Biovolume (μm^{-3} mL^{-1}) was calculated using the '*WISER Phytoplankton Counter Spreadsheet*' based on Carvalho *et al*. (2007).

2.5.4 Fluoroprobe measurements

Phytoplankton biomass was measured using a bbe fluoroprobe (bbe Moldaenke GmbH, Kiel-Kronshagen, Germany). Gregor and Maršálek (2004) have shown that phytoplankton biomass measurements using this instrument provide results comparable to measurements following ISO methodology (ISO 10260, 1992). Measurements were taken every second for one minute and the average phytoplankton biomass was expressed as $\mu g\ L^{-1}$.

2.5.5 Zooplankton

Samples for the analysis of the zooplankton community composition were taken in Loch Flemington by filtering 12.5 L of surface water at each sample site except site 3 through a net (mesh size 120 µm). The combined samples were preserved in formaldehyde (final concentration 4% v/v) and stored in a cool and dark place. Zooplankton samples were counted using a low power binocular microscope. If zooplankton abundance was high, three subsamples were counted using the following procedure: each sample was placed in a glass vessel and made up to a final volume of

250 mL with distilled water. The sample was thoroughly mixed to achieve a random distribution of animals present, and then sub-sampled with a Stempel pipette (volume 5 mL) as described by May *et al*. (1993). Animals present in each subsample were counted and average abundance of subsamples calculated. Taxonomic identification was conducted to the genus level (Scourfield and Harding, 1966; Harding and Smith, 1974; Dussart and Defaye, 1995; Einsle, 1996) except for larval stages of copepods that were grouped under 'copepod nauplii'. Abundance of animals in each sample/subsample was calculated as individuals per litre (Indi. L^{-1}).

2.5.6 Macroinvertebrates

The macroinvertebrate community was analysed from cores collected using an HTH gravity corer as described above (Section 2.2.2). Following measurement of the height of the sediment in the retrieved cores, samples were sieved through a 250 µm mesh and preserved using formaldehyde (final concentration 10% v/v). Macroinvertebrate samples were analysed using a low power binocular microscope and identified to various taxonomic levels following Murray-Bligh and Ferguson (1999), including family level (Chaoboridae, Chironomidae, Sphaeriidae, Hydrobiidae, Bithyniidae), order level (Trichoptera), subclass level (Oligochaeta) and phylum level (Nematoda). Abundances were calculated as individuals per cubic meter (Indi. m^{-3}).

2.5.7 Macrophytes

Maximum colonisation depth (MCD) of submerged macrophytes was assessed along five fixed transects that were evenly spaced around the deepest, north-eastern bay of Loch Flemington. Transects were running astral from the shore towards the central deep trough of the bay. A double-headed rake, consisting of two opposing garden rake heads (38 cm long, 7 cm wide, 16 'teeth'), covered with coarse wire mesh as described by

Jupp *et al*. (1974) were fastened together back-to-back and connected to a rope. The boat was stopped about every 2 m along the transect, water depth was recorded and the rake was trawled across the sediment surface for approximately 1-2 s. Plant specimens were returned to the boat for identification and the colonisation depth was recorded until MCD was reached. MCD was corrected for changes in water level by measuring water level at a common datum and subtracting water level height from the measured MCD. The common datum was fixed at an average water depth of 0.38 m (range 0.14 to 0.69 m water depth).

2.5.8 Fish

Trap sampling

Six traps (Minnow Trap; height 230 mm; length 450 mm; double entrance openings of 12.5 mm radius; mesh size 6 mm) were randomly distributed along the north shore of Loch Flemington (N 57° 32.719', W 3° 59.146'; N 57° 32.704', W 3° 59.201'; N 57° 32.648', W 3° 59.422'; N 57° 32.612', W 3° 59.475'; N 57° 32.543', W 3° 59.552'; N 57° 32.439', W 3° 59.689'). Traps were positioned at the transitional zone between emergent macrophytes (mainly *Eleocharis palustris* (L.) Roem. & Schult., *Phalaris arundinacea* L. and *Equisetum fluviatile* L.) and the open water. Traps were left in the lake for 24 h (12:00 to 12:00 on successive days), following which fish in each trap were identified, counted and released.

Gill net sampling

Fish community composition was assessed using three benthic Multimesh Norden Gillnets (Pokorný - Sítě s.r.o., Czech Republic; EN 14 757:2005). The nets were 1.5 m in height and 36 m long, with 12 panels of equal length having bar-mesh sizes of 5, 6.25, 8, 10, 12.5, 15.5, 19.5, 24, 29, 35, 43 and 55 mm, respectively. The nets were

placed perpendicular to the south shore of Loch Flemington (net 1, open water endpoint N57° 32.625', W3° 59.182' at 1.5 m depth; net 2, open water endpoint N57° 32.570', W3° 59.349' at 1.2 m depth; net 3, open water endpoint N57° 32.517', W3° 59.393' at 0.7 m depth). Nets were left in the lake overnight (15 h). Fish were identified and counted on recovering of the nets.

Chapter 3

A Phoslock® pilot study in an artificial water-body (Clatto Reservoir, UK): physicochemical responses and effects on cyanobacteria

A Phoslock® pilot study in an artificial water-body (Clatto Reservoir, UK): physicochemical responses and effects on cyanobacteria

ABSTRACT

This pilot study investigated short-term physicochemical responses (one month pre- versus one month post-application) and longer-term effects on cyanobacteria abundance (two years pre- and three years post-application) following the application of 270 g Phoslock® m^{-2} to Clatto Reservoir, a 9 ha reservoir with a history of cyanobacterial blooms. Mean surface water soluble lanthanum (La) and total lanthanum (TLa) concentrations increased significantly from below detectable limits to 0.02 mg La L^{-1} and 0.20 mg TLa L^{-1} respectively, when comparing pre- versus post-application periods. Total phosphorus (TP) concentration and dissolved inorganic nitrogen (DIN) concentration decreased significantly from 32.1 to 26.0 µg TP L^{-1} and from 1309.9 to 1066.1 µg DIN L^{-1} respectively, while soluble reactive phosphorus (SRP) concentrations did not differ significantly when comparing pre- (4.3 µg SRP L^{-1}) and post-application (3.8 µg SRP L^{-1}). The application of Phoslock® had no effect on surface water pH while a significant increase in conductivity (from 84.6 µS cm^{-1} to 90.0 µS cm^{-1}) and a significant decrease in dissolved oxygen (DO) concentration (from 102% to 99%) occurred when comparing pre- versus post-application periods. Water clarity (measured as Secchi depth) decreased significantly by 0.95 m post-application, causing a decrease in the estimated euphotic depth from 5.7 m to 3.2 m in the first month post-application. Of all investigated physicochemical parameters only water clarity was significantly negatively correlated to TLa and La concentrations indicating that the application of Phoslock® was the primary cause of the reduction in water clarity. However, a comparison of the measured short-term physicochemical responses in Clatto Reservoir with the range of variation observed on a seasonal time scale for an

untreated site (Loch Leven, Kinross, UK), indicated that the magnitude of change in all physicochemical parameters, including water clarity, following the application of Phoslock® did not exceed seasonal variation at Loch Leven. In Clatto Reservoir, cyanobacterial abundance was significantly lower in the first year (2009) post-application, however, no significant differences in cyanobacterial abundance were apparent between pre-application years and the second and the third year post-application. Although water quality targets of 20,000 cells mL^{-1} were less frequently exceeded in all post-application years, the role of Phoslock® in controlling cyanobacterial abundance remains unclear. The results of this pilot study suggest that Phoslock® had no detrimental short-term (one month) impact on water quality parameters including pH, conductivity and DO concentration. However the decrease in water clarity post-application may have short-term negative impacts on phototrophic organisms through a reduction in light availability. The application of Phoslock® to lakes should therefore be conducted in multiple smaller doses or outside the main growing season (i.e. winter) of submerged macrophytes.

INTRODUCTION

Anthropogenic nutrient enrichment (eutrophication) has become the main cause for deteriorating ecological quality of freshwater ecosystems (Smith and Schindler, 2009) and the control of phosphorus (P) has become a focal point of remediation efforts (Carpenter, 2008). Nutrient enrichment often promotes cyanobacterial blooms that pose a risk to human health (Brookes and Carey, 2011), a fact that has long been recognised through the setting of guideline public health targets by the World Health Organisation (WHO) and the European Bathing Water Directive for the safe use of recreational water environments (WHO, 2003; European Union, 2006).

In-lake remediation

Controlling external nutrient inputs is a pre-requisite for lasting remediation efforts (Cooke *et al*., 2005), however increasing attention has recently been paid to additional in-lake remediation techniques to 'speed up' the recovery process (Hupfer and Hilt, 2008; Hickey and Gibbs, 2009). Common remediation methods for the control of internal P-loading in shallow lakes include oxidation of the sediment to favour P-binding using nitrate (Hupfer and Hilt, 2008), sediment dredging (Does *et al*., 1992) or the application of active P-stripping/sediment P-capping agents (hereafter termed P-capping agents; Cooke *et al*., 2005; Hupfer and Hilt, 2008; Hickey and Gibbs, 2009). Iron- (Fe; e.g.$FeSO_4$, $FeCl_3$; Perkins and Underwood, 2000), aluminium- (Al; e.g. $Al_2(SO_4)_3$, $AlCl_3$, Al modified zeolite; Cooke *et al*., 2005; Reitzel *et al*., 2005; Özkundakci *et al*., 2010), and calcium-based (Ca; e.g. $CaCO_3$; Hupfer and Hilt, 2008) products have been used as P-capping agents in remediation projects. Potential risks associated with the application of those products, when not applied correctly, are acidification of the water body when using aluminium and iron products (Cooke *et al*., 2005; Hupfer and Hilt, 2008; Hickey and Gibbs, 2009), or an increase in pH when using

calcium products (Hupfer and Hilt, 2008). Additionally, the formation of toxic ions (Al_3^+) can take place if the pH of the water body decreases below pH 6 during or after application (Hupfer and Hilt, 2008) which can have detrimental effects on biota (Havens and Heath, 1990; Spry and Wiener, 1991; Jančula *et al*., 2011). Finally, water clarity may be reduced during application, especially when products are applied as a slurry to the water surface and when the settling velocity of the product is slow, which may affect primary producers (Hickey and Gibbs, 2009).

In recent years lanthanum (La) modified bentonite clay (Phoslock®) has been increasingly tested as a P management tool for the remediation of eutrophic water bodies (e.g. Robb *et al*., 2003; Lürling and Faassen, 2011; Lürling and van Oosterhout, 2012). The product is designed to strip dissolved P (i.e. orthophosphate, hydrogen and dihydrogen phosphate ions; Haghseresht *et al*., 2009) from the water-column and reduce the translocation of dissolved P across the sediment-water interface once it has settled onto the bed. Laboratory studies using lake water and artificial solutions (reverse osmosis water and WC algal growth medium) suggest that applying Phoslock® up to concentrations of 3.2 g Phoslock® L^{-1} is unlikely to alter water-column pH (Ross *et al*., 2008; van Oosterhout and Lürling, 2012), while a linear increase in conductivity with increasing Phoslock® dose was measured (van Oosterhout and Lürling, 2012). Furthermore a short-term (< 1 day) decrease in water clarity was observed (van Oosterhout and Lürling, 2012), while settling velocity appeared to be higher at increased pH (Ross *et al*., 2008). However, peer-reviewed studies of whole-lake Phoslock® applications to assess changes in pH, conductivity, turbidity or toxicity following application are scarce and were absent at the time of this study. In Lake Rauwbraken (Netherlands), a short-term decrease in pH (pH 7.7 to pH 7.0; comparison 10 days pre-application versus 14 days post-application) was observed following the application of 450 g Phoslock® m^{-2} (van Oosterhout and Lürling, 2011). However

measured changes in pH cannot be ascribed with certainty to Phoslock®, as a combination of polyaluminiumchloride and Phoslock® was applied (van Oosterhout and Lürling, 2011). In contrast the application of 220 g Phoslock® m^{-2} in Lake Het Groene Eiland (Netherlands) had no significant impact on pH over a period of 29 months in comparison to a control site (Lürling and van Oosterhout, 2012). Furthermore, rapid changes in pH may also occur naturally (Maberly, 1996). The Phoslock® application to Het Groene Eiland did not significantly alter turbidity, while conductivity was significantly lower in the treated lake (376.0 µS cm^{-1} versus 445.0 µS cm^{-1}) compared to the control (Lürling and van Oosterhout, 2012). Water-column TLa and La concentrations increased significantly in Lake Rauwbraken and Het Groene Eiland, reaching mean post-application concentrations of 0.03 to 0.25 mg TLa L^{-1} and 0.00 to 0.03 mg La L^{-1} (van Oosterhout and Lürling, 2011; Lürling and van Oosterhout, 2012).

A wide range of ecotoxicological studies have been conducted to quantify the effects of Phoslock® on various components of the food web, although few of these studies follow common standard methods or have been published in peer reviewed literature (Stauber, 2000; Clearwater, 2004; Martin and Hickey, 2004). Notable exceptions include Lürling and Tolman (2010) who showed that 0.001% of La can be released from Phoslock®. La had no toxic effect on *Daphnia magna* (Straus) up to concentrations of 1 mg La L^{-1} in P-free medium, but life history traits of *Daphnia magna* can be affected at this concentration in medium with P (330 µg P L^{-1}) through rhabdophane formation (La PO_4 – n H_2O) and consequent precipitation of the food source algae (Lürling and Tolman, 2010). Furthermore, van Oosterhout and Lürling (2012) estimated the EC_{50} of Phoslock® on population growth of rotifers (*Brachionus calyciflorus* Pallas) to be 0.15 g Phoslock® L^{-1} in WC algal growth medium. It should be noted that The Netherlands are the only European country that has a maximum permissible concentration for La of 0.01 mg L^{-1} (Sneller *et al*., 2000). Generally, an

assessment of Phoslock® concentrations against published ecotoxicological thresholds is hampered by: i) availability of peer reviewed studies following common methods for a range of different species; ii) reliability of ecotoxicological tests (Jančula *et al*., 2011) and applicability of laboratory results to whole-lake ecosystems (Mayer-Pinto *et al*., 2010); and iii) species level data covering inter-annual variability at a study site.

Various examples of effects of different P-capping agents on cyanobacteria biomass in mesocosm and/or whole-lake experiments exist in the literature, however variation in magnitude and frequency of doses, site properties and length of the pre- and especially post-application period hamper comparisons between them and assessments of the various products. For example Lelková *et al*. (2008) report an initial success (first year) in decreasing cyanobacteria biomass after multiple doses of an aluminium based agent. Zhang *et al*. (2001) concluded that calcium based products were only successful in reducing cyanobacteria biomass when multiple doses were applied. Lürling and Faassen (2011) did not measure a significant reduction in cyanobacteria biomass following application of 360 g Phoslock® m^{-2} in an enclosure experiment lasting eight weeks, while Lürling and van Oosterhout (2012) observed a significant decrease in cyanobacteria chlorophyll *a* in Lake Het Groene Eiland compared to a control site following the application of 220 g Phoslock® m^{-2}.

Study outline and hypotheses

To date, reports from field-based studies investigating physicochemical responses following the application of Phoslock® and effects on cyanobacterial abundance are still scarce. However such information is crucial for lake managers to decide whether to use Phoslock® to reduce P concentrations and potentially cyanobacteria biomass. The main aims of this study were: (i) to investigate impacts and recovery of TLa and La concentrations following a Phoslock® application to gather information about potential

La leaching from Phoslock®, peak concentrations during application and the time period required to reach pre-application concentrations in the water-column; (ii) to assess the short-term responses of physicochemical parameters including pH, conductivity, DO concentration and water clarity following a Phoslock® application; and (iii) to investigate whether cyanobacteria abundance was reduced in the first 3 years after application of Phoslock® in relation to water quality targets (<20,000 cells mL^{-1}; WHO, 2003). The specific hypotheses tested were: (i) the application of Phoslock® will significantly increase surface water TLa and La concentrations; (ii) the application of Phoslock® will not alter water quality parameters including pH, conductivity, DO concentration and water clarity (measured as Secchi depth); and (iii) the application of Phoslock® will cause a decrease in the abundance of cyanobacteria.

MATERIAL AND METHODS

Study site and rationale of pilot study

A description (Chapter 2, Section 2.1.1) and maps showing the location and surrounding area of the study site (Fig. 3.1) are presented. Dundee City Council, the local governing authority responsible for managing the risk to public health from cyanobacteria at Clatto Reservoir, decided to use Phoslock® to reduce P concentrations (suspected to result from internal P-loading) and thereby reduce phytoplankton biomass (specifically cyanobacterial biomass). Permission by the legislative authority (Scottish Environment Protection Agency, SEPA) to conduct the application was granted one month prior to the proposed application date, which resulted in a short pre-application monitoring period. The short pre-application data set posed limitations on data analysis and interpretation (e.g. no assessment of seasonal variability in a given parameter). However, the opportunity to assess short-term physicochemical responses following the application of Phoslock® in Clatto Reservoir was an important and necessary precursor to a more comprehensive assessment of chemical and ecological responses in Loch Flemington.

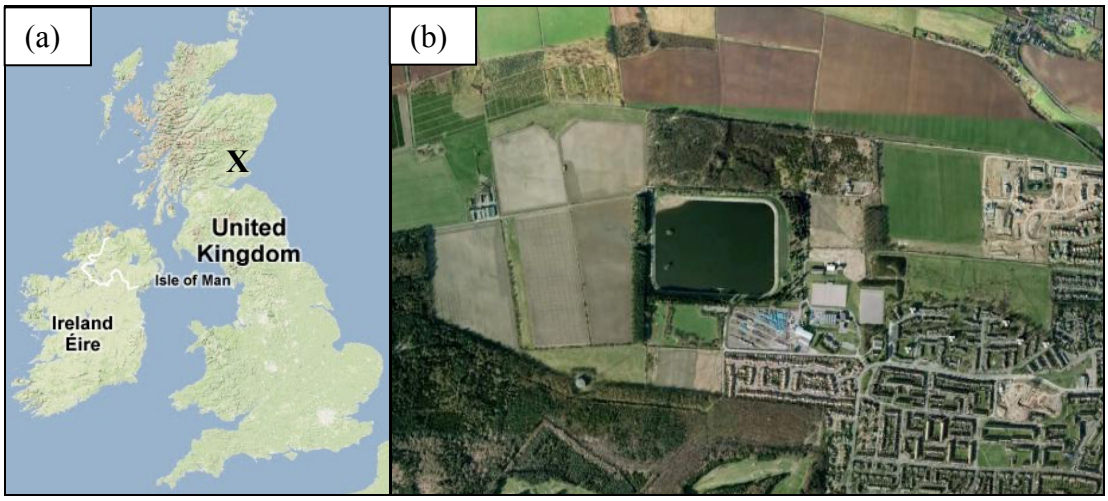


Figure 3.1 Maps showing (a) location of Clatto Reservoir (cross; Google Maps, 2013) and (b) surrounding area of Clatto Reservoir (Google Earth, 2013a).

Dosing

Phoslock® Europe GmbH calculated a required dosage of 24,000 kg of Phoslock® (270 g Phoslock® m^{-2}) to bind an estimated amount of 19 kg TP in the water-column and 244 kg of potentially release-sensitive P in the upper 4 cm of sediment. This estimate was based on mass balance estimates made using surface water TP concentrations (n = 3; October 2008) and the surface sediment TP content (n = 3; October 2008). Phoslock® was applied as a slurry from a pontoon over a period of 3 days in March 2009. On the pontoon, lake water was pumped into a mixing chamber to which Phoslock® pellets were added to produce a slurry. The slurry was pumped to a spray manifold mounted to the rear of the pontoon and applied to the water surface. Photographs of the application procedure are shown (Appendix: Fig. A3.1).

Sample collection

Pre-application sampling started one month (n = 4; day -28, -14, -3 and -1 for TP, SRP, DIN, chlorophyll *a*, TLa, La) and two weeks (n = 3; day -14, -3 and -1 for pH, conductivity, DO, water clarity) prior to the Phoslock® application. Post-application samples were taken over a period of one month (n = 4; day 7, 14, 21 and 29 for TP, SRP, DIN, chlorophyll *a*, pH, conductivity, DO, Secchi depth) and for a period of nine months (n = 14 for TLa, La; day 7, 14, 21, 29, 43, 53, 81, 109, 136, 164, 195, 228, 256, 286). On each site visit surface water samples were collected from a boat (Chapter 2, Section 2.2.1) at four permanent sample sites (n = 4), spread over a depth gradient covering deep (Site 1; 5.8 ± 0.2 m), intermediate (Site 3; 3.6 ± 0.2 m) and shallow areas of the reservoir (Site 2 and 4; 2.3 ± 0.3 m). This is with the exception of samples taken for the analysis of TLa and La concentrations that were only collected from Site 1.

Physical and chemical parameters

On each visit at each sample site, surface measurements of pH, conductivity and DO concentrations were made and water clarity was assessed using a Secchi disk (Chapter 2, Section 2.3.1). Surface water samples were analysed for TLa (Chapter 2, Section 2.4.2), La (Chapter 2, Section 2.4.2), DIN (Chapter 2, Section 2.4.3), TP (Chapter 2, Section 2.4.4) and SRP (Chapter 2, Section 2.4.4).

Phytoplankton

Surface water samples were analysed for chlorophyll *a* concentration (Chapter 2, Section 2.5.1), which was used as a proxy for phytoplankton standing stock. Cyanobacterial abundance and community composition were assessed using data supplied by SEPA. The data used in this study consisted of shore samples and covered the period April 2007 - October 2011 (2007: April-November; 2008: June-November; 2009: June-October; 2010: April – October; 2011: April - October). On each occasion, water samples were collected for the microscopic enumeration of cyanobacterial cells against WHO guidelines of 20,000 cells mL^{-1} (WHO, 2003). All counts were expressed as cells mL^{-1} apart from in June 2007 when SEPA data contained colony counts for *Gloeotrichia* spp.. Subsequently, colony counts were converted to cell counts based on personal observations of *Gloeotrichia* spp. cell numbers per colony in Clatto Reservoir from 2009. On one occasion in October 2007, abundance was only recorded as "scum" at the east side of the reservoir and this data point was excluded as no conversion was possible. To allow a comparison between the years 2007, 2008, 2009, 2010 and 2011, cyanobacterial abundance was processed to produce monthly averages using all sample occasions and sample sites (range of number of samples per month n = 1 - 3, average = 1.8).

Comparison with untreated site

Seasonal variability for a range of physicochemical and biological parameters was assessed in an untreated site (Loch Leven; N 56° 11' 42.98", W 3° 22' 42.53"). A dataset (1988 – 2007) containing physicochemical (pH, conductivity, DO concentration, Secchi depth, TP, SRP, DIN) and biological (chlorophyll *a*) data was used to calculate monthly averages of each parameter. In order to assess seasonal variation in each parameter the difference between minimum and maximum value of a parameter was calculated and expressed as percentage change. Similarly, variation in each of the above parameters was calculated between the pre- (one month) and post-application (one month) period in Clatto Reservoir. The magnitude of change (percentage change) in each parameter was compared between Clatto Reservoir and Loch Leven.

Statistical analyses

Minitab 16 (Minitab® 16.1.1, Minitab Ltd., Coventry, UK) was used for all statistical analyses. Data sets of conductivity, TP, SRP and La were normally distributed (Anderson-Darling test, $\alpha > 0.05$) and of equal variance (Levene's test, $\alpha > 0.05$) following a box-cox power transformation, while the data set for water clarity did not require any transformation to pass these criteria. A 2-Sample T-test was used to test for significant differences between pre- and post-application data sets.

Data sets of pH, DO, DIN and TLa concentration were not normally distributed (Anderson-Darling test, $\alpha < 0.05$) even after a range of transformations (including $x' = \log(x)$, $x' = \ln(x)$, $x' = \text{sqrt}(x)$, $x' = x^2$ or a box-cox transformation). The chlorophyll *a* data set was normally distributed (Anderson-Darling test, $\alpha > 0.05$) but failed of a test for equal variance (Levene's test, $\alpha < 0.05$) even after a range of transformations. A non-parametric Mann-Whitney U-test (MWU) was used to test for significant differences between pre- and post-application data sets.

Monthly average cyanobacterial abundance (cells mL^{-1}) was calculated for June to October for 2007, 2008, 2009, 2010 and 2011 in order to produce comparable populations for those years. The data were log transformed to pass a normality criterion (Anderson-Darling test, $\alpha > 0.05$) but were not of equal variance (Levene's test, $\alpha < 0.05$) even after a range of transformations (including $x' = \ln(x)$, $x' = \text{sqrt}(x)$, $x' = x^2$ or a box-cox transformation). A non-parametric Kruskal-Wallis test (KW) was used on un-transformed data to assess variations in mean June-October cyanobacterial abundance across years. Where significant variation was evident (KW test, $\alpha < 0.05$), a non-parametric MWU test was used to determine between which years significant differences in cyanobacterial abundance occurred.

Correlation analysis (Pearson correlation) was carried out between surface water TLa concentration, La concentration, TP concentration, SRP concentration, DIN concentration, chlorophyll *a* concentration, DO concentration, pH, conductivity and water clarity. Correlation analysis was carried out on a data set covering one month pre- (n = 4, day -28, -14, -3 and -1 for TLa, La, TP, SRP, DIN, chlorophyll *a*; n = 3, day -14, -3 and -1 for pH, conductivity, DO, water clarity) and one month post-application data (n = 4; day 7, 14, 21 and 29 all variables). Data sets were normally distributed (Anderson-Darling test, $\alpha > 0.05$) following a log transformation ($x' = \log(x+1)$). Bonferroni corrections were not required because single hypothesis testing instead of multiple hypotheses testing was performed.

RESULTS

Changes in lanthanum concentration

Surface water TLa concentration (Fig. 3.2a; Table 3.1) increased significantly ($W = 10.0$; $p < 0.05$; $n_1 = n_2 = 4$) from below detectable limits pre-application to 0.20 mg TLa L^{-1} in the first month post-application, with peak concentrations of 0.52 mg TLa L^{-1} during application of the Phoslock® slurry. TLa concentrations similar to pre-application concentrations were reached four months post-application (below detectable limits in the period June – December). Surface water La concentrations (Fig. 3.2b; Table 3.1) increased significantly ($T = 13.95$; $p < 0.001$; $n_1 = n_2 = 4$) from below detectable limits pre-application to 0.02 mg La L^{-1} in the first month post-application. However, La concentrations did not recover to pre-application concentrations within nine months of the application.

Short-term physicochemical and biological responses (one month pre- versus one month post-application)

Conductivity increased significantly ($T = -15.16$; $p < 0.001$; $n_1 = 12$, $n_2 = 16$) from 84.6 µS cm^{-1} pre-application to 90.0 µS cm^{-1} in the first month post-application, while surface water pH did not vary significantly (Table 3.1). DO concentrations were significantly ($W = 218.0$; $p < 0.05$: $n_1 = 12$; $n_2 = 16$) lower in the post-application period (99%) compared to the pre-application period (102%). Water clarity, measured as Secchi depth, decreased significantly ($T = -6.32$; $p < 0.001$; $n_1 = 8$, $n_2 = 16$) from 2.1 m to 1.2 m, equivalent to a decrease in euphotic depth from 5.7 m (pre-application) to 3.2 m (post-application). Water clarity reached pre-application levels after approximately 47 days post-application (data not shown). TP concentrations decreased significantly (T

= 4.16; $p < 0.001$; $n_1 = 13$, $n_2 = 16$) from 32.1 µg TP L^{-1} pre-application to 26.0 µg TP L^{-1} post-application, while SRP concentrations did not vary significantly.

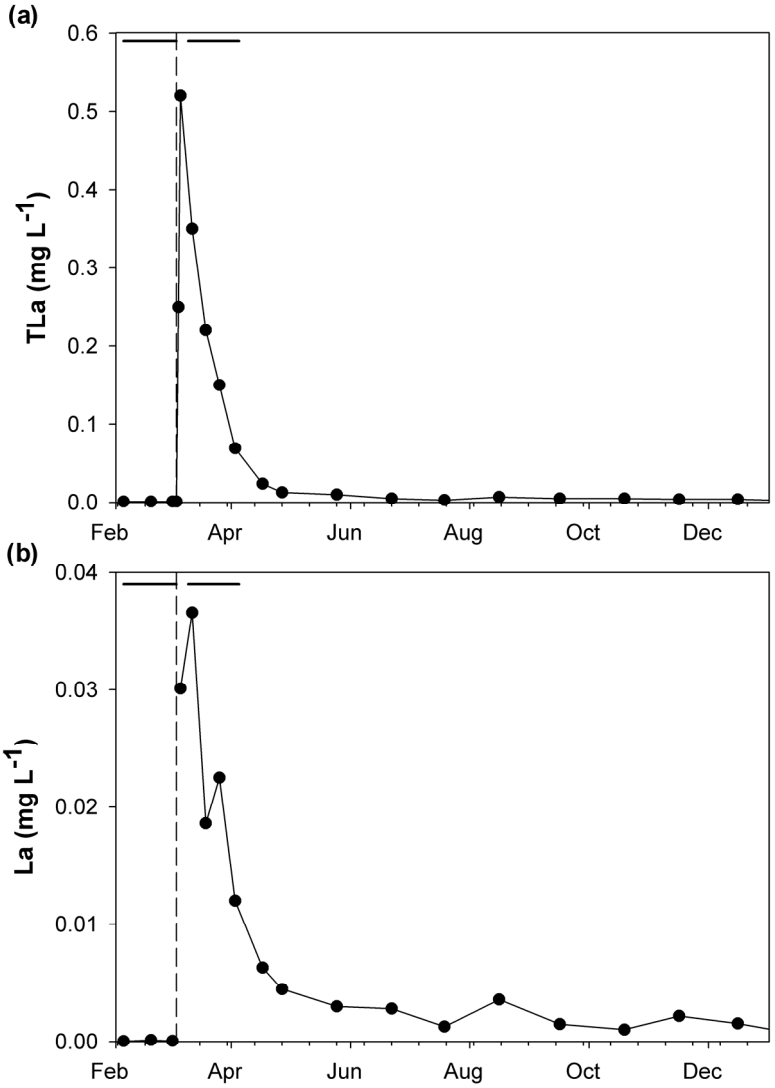


Figure 3.2 Seasonal variation in (a) surface water total lanthanum concentration (TLa) and (b) soluble lanthanum concentration (La) in Clatto Reservoir in 2009. Dashed vertical line represents timing of the Phoslock® application (March 2009) and bold horizontal lines indicate period of pre- and post-application monitoring for physicochemical parameters.

DIN concentrations were significantly ($W = 273$; $p < 0.05$; $n_1 = 14$; $n_2 = 16$) lower post-application (1066.1 μg L^{-1}) compared to pre-application concentrations (1309.8 μg L^{-1}). Chlorophyll *a* concentration decreased significantly ($W = 290.0$; $p < 0.01$; $n_1 = 14$; $n_2 = 16$) from 10.3 μg L^{-1} to 6.2 μg L^{-1} following application.

The percent change (i.e. pre- versus post-application) for each of the variables measured in Clatto Reservoir was compared to the calculated seasonal variation in the untreated lake (Loch Leven). This comparison indicated that the responses observed in Clatto Reservoir across all parameters did not exceed seasonal variation at the untreated site (Table 3.1).

Correlation analysis between physicochemical and biological parameters

Surface water La concentrations were significantly ($p < 0.001$) positively correlated with surface water TLa concentrations (Table 3.2), indicating that La was released during the application of Phoslock®. Of all parameters assessed only water clarity (measured as Secchi depth) was significantly negatively correlated with TLa ($p < 0.01$) and La ($p < 0.01$) concentrations (Table 3.2), indicating that the application of Phoslock® was the primary cause of the significant ($T = -6.32$; $p < 0.001$; $n_1 = 8$, $n_2 = 16$) decrease in water clarity post-application.

Table 3.1 Physicochemical and biological short-term responses following the Phoslock® application in Clatto Reservoir and seasonal variation in an untreated site (Loch Leven). Presented are mean values with standard error in parenthesis. Differences in pre- and post-application values (Clatto Reservoir) and minimum and maximum monthly mean values (Loch Leven) are expressed as percentage change (% change).

Variable	Clatto Reservoir								Loch Leven				
	Pre-application	n =	During application	n =	Post-application	n =	p-value	% change	minimum	n =	maximum	n =	% change
pH	7.7 (0.08)	12	7.8 (0.03)	8	7.7 (0.07)	16	n.s.[a]	**+0**	7.9 (0.08)	15	8.9 (0.08)	14	**± 12.1**
cond.	84.6 (0.17)	12	86.3 (0.22)	8	90.0 (0.34)	16	*** [b]	**+6**	230.1 (10.61)	10	250.3 (3.37)	13	**± 8.8**
DO	102 (0.79)	12	103 (0.22)	8	99 (0.73)	16	* [a]	**-3**	89 (3.77)	12	102 (3.83)	9	**± 13.8**
clarity	2.13 (0.14)	8	0.94 (0.08)	8	1.18 (0.06)	16	*** [b]	**-45**	1.10 (0.06)	18	2.12 (0.22)	17	**± 92.3**
TLa	0.00 (0.00)	4	0.39 (0.14)	2	0.20 (0.06)	4	* [a]	**+19900**	n/a	n/a	n/a	n/a	**n/a**
La	0.00 (0.00)	4	0.03 (0.00)	2	0.02 (0.01)	4	*** [b]	**+1900**	n/a	n/a	n/a	n/a	**n/a**
TP	32.1 (1.50)	13	28.2 (0.97)	8	26.0 (0.42)	16	*** [b]	**-19**	38.5 (2.65)	17	92.4 (7.04)	17	**± 140**
SRP	4.3 (0.28)	14	4.1 (0.22)	8	3.8 (0.26)	16	n.s.[b]	**-12**	4.5 (0.54)	17	26.1 (5.17)	18	**± 476**
DIN	1309.8 (57.29)	14	1285.7 (15.23)	8	1066.1 (59.18)	16	* [a]	**-19**	75.6 (16.78)	16	2211.8 (187.30)	11	**± 2825**
chl *a*	10.3 (1.08)	14	7.9 (0.69)	8	6.2 (0.30)	16	** [a]	**-40**	23.9 (5.07)	18	52.4 (19)	19	**± 119**

cond., conductivity (µS cm^{-1}); DO, dissolved oxygen (%); clarity, water clarity (measured as Secchi depth; m); TLa, total lanthanum (mg L^{-1}); La, dissolved lanthanum (mg L^{-1}); TP, total phosphorus (µg L^{-1}); SRP, soluble reactive phosphorus (µg L^{-1}); DIN, dissolved inorganic nitrogen (µg L^{-1}); chl *a*, chlorophyll *a* (µg L^{-1}); n/a, not applicable as no or insufficient data available; [a], non-parametric Mann-Whitney U-test; [b], 2-Sample T-test; n.s., $p \geq 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

Table 3.2 Summary of correlation analysis including surface water total lanthanum (TLa; mg L^{-1}), soluble lanthanum (La; mg L^{-1}), total phosphorus (TP; µg L^{-1}), soluble reactive phosphorus (SRP; µg L^{-1}), pH, conductivity (cond.; µS cm^{-1}), water clarity (clarity; m), dissolved inorganic nitrogen (DIN; µg L^{-1}), chlorophyll *a* (chl *a*; µg L^{-1}) and dissolved oxygen (DO; %) concentration. Significant correlations are presented in bold with p-values in superscript (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

Variable	TLa	La	TP	SRP	pH	cond.	clarity	DIN	chl *a*
La	**+0.969*** **								
TP	-0.493	-0.440							
SRP	-0.404	-0.413	-0.084						
pH	-0.219	+0.228	-0.086	+0.297					
cond.	+0.550	+0.586	**-0.837***	-0.077	+0.305				
clarity	**-0.915****	**-0.928****	+0.702	-0.003	-0.070	-0.740			
DIN	-0.541	-0.071	+0.084	+0.456	-0.652	-0.650	+0.645		
chl *a*	-0.463	-0.371	**+0.919****	-0.140	+0.099	-0.728	+0.745	-0.127	
DO	-0.593	-0.454	+0.571	+0.487	+0.559	-0.232	+0.359	-0.261	+0.729

Changes in cyanobacterial abundance

Data sets of cyanobacterial abundance (Fig. 3.3) were used to assess changes over longer time periods in phytoplankton standing stock. In pre-application years, monthly average cyanobacterial abundance (Fig. 3.3) exceeded WHO guidelines of 20,000 cells mL^{-1} in summer (2007 and 2008: June) and in autumn (2007: September, October and November; 2008: October). In 2009, the first year post-application, cyanobacterial abundance did not exceed WHO guidelines in any month. In 2010, guidelines of 20,000 cells mL^{-1} were exceeded in only one month (August). However, during this month cyanobacterial abundance reached a new maximum of around 1.7 million cells mL^{-1}. In 2011, mean cyanobacterial abundance did not exceed WHO guidelines of 20,000 cells mL^{-1}.

Monthly average cyanobacterial abundance (June – October; Fig. 3.3) differed significantly between years ($H = 11.71$; $p < 0.05$; $DF = 4$; $n = 5$). A pair wise comparison between years showed that cyanobacterial abundances in 2009, the first year following application, were significantly lower compared to pre-application years (2007 vs. 2009: $W = 40.0$; $p < 0.05$; $n_1 = n_2 = 5$; 2008 vs. 2009: $W = 29$; $p < 0.05$; $n_1 = n_2 = 5$). However, no significant differences in cyanobacterial abundance were measurable when comparing 2010, the second year following application, with pre-application years or when pre-application years were compared with 2011. Comparing cyanobacterial abundance of post-application years with each other revealed that cyanobacterial abundance in 2010 was not significantly different to 2009, while cyanobacterial abundance in 2011 was significantly higher compared to 2009 ($W = 15$; $p < 0.05$; $n_1 = n_2 = 5$).

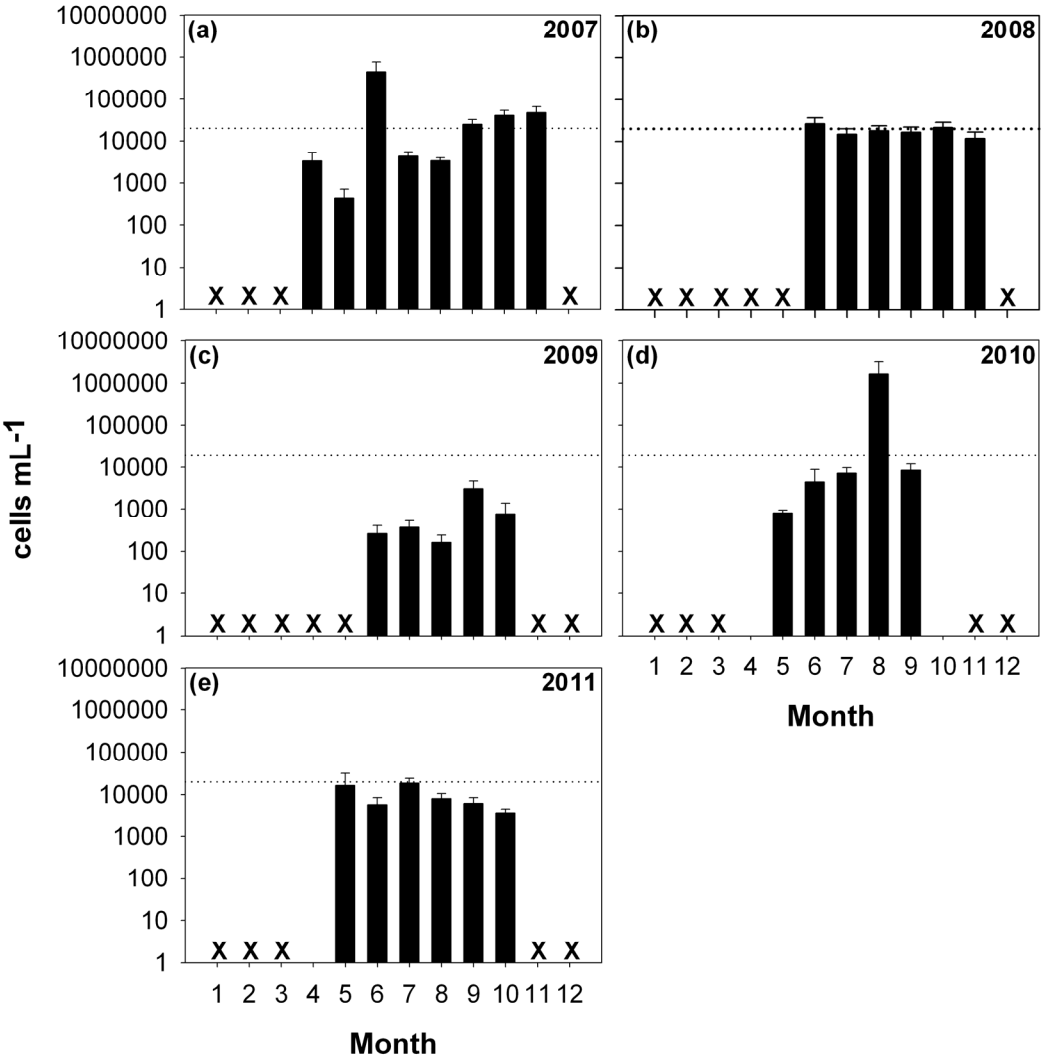


Figure 3.3 Inter-annual changes in cyanobacteria abundance (cell counts) in Clatto Reservoir in: (a) 2007, (b) 2008, (c) 2009, (d) 2010, and (e) 2011. Phoslock® was applied to Clatto Reservoir in March 2009. Error bars represent standard error of the mean (n = 1 – 3 sample occasions in a given month at variable sites n = 1 - 4) and dotted horizontal line represents abundance of 20,000 cells mL^{-1}. Months for which Scottish Environment Protection Agency (SEPA) data is absent are marked with X. In March and October 2010 and March 2011 cyanobacteria cell counts were zero. Note logarithmic scale on y-axis.

DISCUSSION

The application of Phoslock® to Clatto Reservoir resulted in a significant elevation in surface water TLa and La concentrations in the first month post-application. Of all investigated physicochemical and biological parameters only water clarity was significantly negatively correlated to TLa and La concentrations. This indicates that the application of Phoslock® was the primary cause for the reduction in water clarity post-application. Furthermore, cyanobacteria abundance was significantly lower in the first year following the application of Phoslock® and water quality targets of 20,000 cells mL^{-1} were less frequently exceeded in all post-application years. However, the role of Phoslock® in controlling cyanobacteria abundance remains unclear.

Variation in lanthanum concentration

Surface water TLa and La concentrations were significantly higher in the first month post-application compared to pre-application conditions, with La concentrations exceeding the maximum permissible concentration standard (0.01 mg La L^{-1}) used in The Netherlands (Sneller *et al*., 2000). Concentrations were comparable with measurements in Lake Rauwbraken of 0.25 mg TLa L^{-1} and 0.03 mg La L^{-1} respectively in the first two weeks post-application, although around 1.7 times more Phoslock® were applied per area in Lake Rauwbraken (van Oosterhout and Lürling, 2011). In a sediment incubation experiment Gibbs *et al*. (2011) measured La release following the application of 300 g Phoslock® m^{-2} of around 2.00 mg La m^{-2} d^{-1} over a period of at least 14 days. Correlation analysis indicated that La was either released from Phoslock® or La not bound in the clay matrix was dissolved into the water. For Clatto Reservoir, which received a similar areal load to that used by Gibbs *et al*. (2011) in an experiment, a release rate of 3.64 mg La m^{-2} d^{-1} was estimated over the first 14 days following application based on reservoir area, volume and changes in surface water La

concentration. The estimated rate of La release in Clatto Reservoir is comparable to the finding of Gibbs *et al*. (2011), especially when taking potential confounding factors like spatial variability of this whole-lake experiment into account. However, an assessment of potential toxicity of the observed TLa and La concentrations is hampered by: i) availability of peer reviewed studies following common methods for a range of different species; ii) absence of species data for Clatto Reservoir; and iii) reliability of ecotoxicological tests. Regarding the latter point, Jančula *et al*. (2011) point out in a test with polyaluminium chloride, that a prediction of the effects of a whole-lake application based on standard ISO methods is insufficient as EC_{50} values can differ markedly based on the composition of water used for the assay. Jančula *et al*. (2011) suggest ecotoxicological tests should be conducted with water from the site which should be restored (i.e. 'site specific ecotoxicological assessment'). However, even the suggested approach from Jančula *et al*. (2011) is unlikely to incorporate spatio-temporal variation and interactions between biotic and abiotic components of the ecosystem.

Short-term effects on water quality parameters

Although mitigation of eutrophication and cyanobacterial blooms is important to reduce human health risks (Brookes and Carey, 2011), any remediation method should not have any detrimental impacts on non-target biota. However, the application of commonly used P-capping agents (Al-, Fe- and Ca-based products) may impact on non-target species when not applied correctly (Cooke *et al*., 1993; Hickey and Gibbs, 2009). For example, changes in pH resulting from incorrect application procedures (e.g. insufficient buffering) may have direct detrimental impacts on biota with low tolerance to pH variation, through toxicity of the applied P-capping agent under particular pH conditions (Dietrich and Schlatter, 1989) and/or on primary producers via knock-on effects on available carbon dioxide (CO_2) and bicarbonate (HCO_3^-) for photosynthesis

(Chambers *et al*., 2001). It was expected that any effects on water quality parameters (e.g. pH, conductivity, turbidity) are likely to be strongest in the first month post-application since TLa and La concentrations were significantly elevated over this period. However in this pilot study no significant short-term variation in pH was detected. No additional buffering was required during application, which reduces the risk of human-induced application errors. This finding is in agreement with van Oosterhout and Lürling (2012) who suggested that Phoslock® up to concentrations of 3.2 g Phoslock® L^{-1} (compared to around 0.1 g Phoslock® L^{-1} in Clatto Reservoir) were unlikely to alter pH under experimental conditions. However the applicability of this finding to other types of water bodies (different ionic composition, conductivity, alkalinity) requires further testing.

The short-term increase in conductivity observed in Clatto Reservoir following the application of Phoslock® was both significant and of small magnitude. While experiments by Ross *et al*. (2008) in lake water and reverse osmosis water suggested that Phoslock® had no effect on conductivity, van Oosterhout and Lürling (2012) report a linear increase in conductivity with increasing concentration of Phoslock® However, correlation analysis indicated that variation in conductivity was unlikely to be caused by the application of Phoslock®. Additionally the magnitude of change in conductivity observed in Clatto Reservoir between the pre- versus post-application period was smaller than seasonal variability in conductivity at an untreated site, indicating that any detrimental impact on biota was unlikely. Similar to the change in conductivity was the significant but small magnitude change in DO concentration reported in Clatto Reservoir. Laboratory experiments indicated that Phoslock® has no significant effect on DO concentrations (van Oosterhout and Lürling, 2012) which is supported by the observation that DO concentrations were not significantly correlated with TLa or La concentrations in this study. Variation in DO concentrations may have been influenced

by reduced photosynthetic activity due to a reduction in water clarity and/or variation in oxygen solubility depending on temperature. However the observed magnitude of change did not exceed the seasonal variability at an untreated site. In contrast, water clarity which was significantly negatively correlated to TLa and La concentrations decreased significantly post-application, a period in which phytoplankton biomass (chlorophyll *a* concentration) was low. The results of the correlation analysis indicated that Phoslock® caused the decrease in water clarity post-application, with water clarity recovering as TLa and La concentrations declined. This could potentially affect benthic microalgae and macrophyte growth by light limitation over a period of 47 days (data not shown). However it should be noted that the decrease in euphotic depth, from 5.7 m to 3.2 m, is still above the mean depth of 2.75 m of the reservoir, so that more than half the sediment bed remained in the euphotic zone at this shallow site. In addition, seasonal variation in water clarity in the untreated site was higher than the pre- and post-application response reported for Clatto Reservoir.

Short-term changes in nutrient concentrations

Both, TP and DIN concentrations decreased significantly post-application but the observed magnitude of change was small compared to the seasonal variability in those parameters at an untreated site. In an enclosure experiment, Lürling and Faassen (2011) did not find significant differences in TP and DIN concentrations when comparing controls with a Phoslock® treatment of similar dosage (360 g Phoslock® m^{-2}). It is therefore likely that the measured changes in Clatto Reservoir may be attributed to events coinciding with the remediation efforts (e.g. seasonal patterns in nutrient cycling) rather than the application itself, which is supported by the observation that neither TP nor DIN concentrations were significantly correlated to TLa or La concentrations. In laboratory incubation experiments 300 g Phoslock® m^{-2} was reported to cause a

temporary suppression of nitrification/denitrification under aerobic conditions, leading to significantly elevated water-column ammonium-nitrogen (NH_4-N) and reduced nitrate-nitrogen (NO_3-N) concentrations relative to the control (Gibbs *et al*., 2011). However the overall effect on nitrogen cycling was not estimated by Gibbs *et al*. (2011) and up-scaling of laboratory-based studies to whole-lake manipulation experiments is questionable (Schindler, 1998).

Short and long-term changes in phytoplankton

Post-application, cholorophyll *a* concentration decreased significantly in Clatto Reservoir. Short-term (<7 days) laboratory studies have shown that Phoslock® can reduce growth of various phytoplankton species including *Scenedesmus obliquus* (Turpin) Kuetzing and *Microcystis aeruginosa* (Kutzing) via a combination of light limitation, flocculation of cells with bentonite and reduction of available SRP (van Oosterhout and Lürling, 2012). Post-application underwater light availability was reduced which may reduce phytoplankton growth, while SRP concentrations did not vary significantly. Although Verspagen *et al*. (2006) found that bentonite concentrations as low as 0.00015 g L^{-1} were able to induce increased flocculation and sedimentation of *Microcystis* cells, Han and Kim (2001) point out that flocculation also depends on the ionic strength of the water. Lürling and Faasen (2011), did not observe a significant decrease in chlorophyll *a* concentrations, concluded that increased flocculation would be unlikely to have been observed in their study system (mesocosm tubes in Lake De Ploeg) in which conductivity was 312 µS cm^{-1} and Phoslock® concentration was 0.277 g Phoslock® L^{-1}. Therefore, a significant reduction in phytoplankton biomass (and therefore chlorophyll *a* concentration) via flocculation appears unlikely in Clatto Reservoir, since conductivity (< 100 µS cm^{-1}) and Phoslock® concentration (around 0.1 g Phoslock® L^{-1}) were lower than those considered by Lürling and Faasen (2011).

Furthermore, the chlorophyll *a* concentration was not significantly correlated to TLa or La concentration so that a causal link between the application of Phoslock® and the significant decrease in chlorophyll *a* concentration can not be concluded.

Cyanobacterial abundance decreased significantly in the first year post-application but no significant difference could be detected between pre-application years and the second and third year post-application. Therefore, the results indicate that the Phoslock® application had an initial limited effect on cyanobacterial abundance that lasted for one year but failed to control cyanobacterial abundance in the longer term. In an enclosure experiment running for eight weeks, Lürling and Faassen (2011) used 360 g Phoslock® m^{-2} to treat similar sediment P contents (2850 mg P m^{-2} compared to 2710 mg P m^{-2} in Clatto; Chapter 4) but varying water-column TP concentration (680.0 µg L^{-1} compared to around 32.0 µg L^{-1} in Clatto Reservoir). However, no significant impact of the Phoslock® application on cyanobacteria biomass was detected (Lürling and Faassen, 2011). It is likely that an underdose of Phoslock® is the main reason behind the failure to control cyanobacterial abundance in the enclosure experiment conducted by Lürling and Faassen (2011) and in Clatto Reservoir. Abundances and dominance of cyanobacteria are known to be driven by a range of factors including: i) nutrient concentrations; ii) underwater light climate; iii) water temperature; iv) lake morphometry, mixing conditions and retention time; as well as v) food web structure (Hyenstrand *et al*., 1998; Dokulil and Teubner, 2000). In the absence of such information it is impossible to investigate the drivers of the observed pattern in Clatto Reservoir. However, this opportunistic pilot study clearly highlighted the need to investigate these complex physicochemical and biological parameters in order to draw firm conclusions about the effects of a Phoslock® application on cyanobacteria (or more generally phytoplankton) abundance; a fact that influenced the design of the study at the main site (Loch Flemington). Furthermore, the observed pattern in cyanobacterial

abundance showed that the evaluation of remediation success greatly depends on the post-application monitoring period. While monitoring Clatto Reservoir for one year post-application would suggest that the application was successful, longer term post-application monitoring showed that the system appeared to return to pre-application conditions in terms of cyanobacteria abundances within two years. This raises a more general question about assessing remediation success in a range of published remediation projects as costs and time limitations often restrict the length of the post-application period. Here the data suggests that at least two to three years of post-application monitoring were required to capture the cyanobacterial response to the Phoslock® application.

CONCLUSIONS

This pilot study offered the opportunity to test short-term effects of Phoslock® on a range of physicochemical and biological parameters in a contained and artificial system. The results showed that the application of Phoslock® caused a significant increase in surface water TLa and La concentrations in the first month post-application while confidence was gained that Phoslock® had no detrimental impacts on water quality parameters (pH, conductivity, DO concentration). However, significantly lower water clarity post-application which was attributed to the application of Phoslock® may have negative impacts on phototrophic organisms through light limitation in the short-term. Phoslock® should, therefore, be applied outside the main growing season of submerged macrophytes. Cyanobacterial abundance was significantly lower in the first year post-application but returned to pre-application conditions in the second and third year post-application. The role of Phoslock® in controlling cyanobacterial abundance remained unclear. This study clearly demonstrates the requirement to assess a range of physical (e.g. light availability, pH, DO), chemical (e.g. TP, SRP, DIN) and biological (e.g. food

web structure, community composition, species data) parameters across multiple pre- and post-application seasons and years in order to draw conclusions about complex changes in lake ecology following the application of P-capping agents.

Chapter 4

Assessing physicochemical changes in the bed sediments of a shallow reservoir following the application of Phoslock® I: Clatto Reservoir, UK

[*Meis et al. 2012. Sediment amendment with Phoslock® in Clatto Reservoir (Dundee,UK): Investigating changes in sediment elemental composition and phosphorus fractionation. Journal of Environmental Management* ***93****:185-193; Role of authors: (i) Meis, S.: study and experimental design, collection and analysis of samples, data analysis, preparation of manuscript; (ii) Spears, B. M.: assistance during study and experimental design, comments on manuscript; (iii) Maberly, S. C.: comments on manuscript; (iv) O'Malley, M. B.: fieldwork assistant; and (v) Perkins, R. G.: comments on manuscript*]

Assessing physicochemical changes in the bed sediments of a shallow reservoir following the application of Phoslock® I: Clatto Reservoir, UK

ABSTRACT

Phoslock® is a lake remediation tool designed to strip dissolved phosphorus (P) from the water-column and increase the sediment P-sorption capacity of bed sediments. This study investigated short-term (1 month) alterations in sediment elemental composition and sediment P-fractions and longer term (7 months) variation in sediment lanthanum (La) content based on sediment cores taken 2 days before and 1, 4 and 7 months following the application of 270 g Phoslock® m^{-2} to a 9 ha, man-made reservoir (Clatto Reservoir, Dundee, Scotland) in March 2009. Laboratory experiments were used to assess the effects of Phoslock® burial in bed sediments on its efficiency to control water-column P concentrations (Phoslock® burial experiment) and to investigate partitioning of P between release-sensitive sediment P-fractions and Phoslock® (P-adsorption/extraction experiment). Sediment La content was significantly higher in the top 8 cm of the sediment 1 month after the application of Phoslock® while the sediment content of other elements commonly involved in P-binding and P-cycling including aluminium (Al), calcium (Ca), iron (Fe) and manganese (Mn) was not significantly different. Assessing longer term (7 month) variation in vertical sediment La distribution showed a significant increase in sediment La content in the 6 – 8 cm sediment depth layer between post-application month 4 and 7, indicating that La (and Phoslock®) was subjected to vertical transport processes. On the whole-lake scale, the applied mass of La had the capability of binding 250 kg P. Consequently mass balance calculations were used to estimate the theoretical binding of release-sensitive P (P_{mobile}; the sum of '*labile P'*, '*reductant-soluble P*' and '*organic P'* fraction) by La across the top 4 cm and 10 cm depths of sediment. The amended mass of La in the sediment had the potential to bind

42% of P_{mobile} present in the top 4 cm or 17% of P_{mobile} in the top 10 cm. However, with the exception of a significant increase in the mass of P present in the '*residual P'* fraction, sediment P-fractions, including P_{mobile}, did not differ significantly 1 month following the Phoslock® application compared to pre-application conditions. Field results indicated that no short-term (1 month) alterations in the mass of P present in the P_{mobile} fraction are to be expected following the application of Phoslock®. Results of the Phoslock® burial experiment indicated that water-column total phosphorus (TP) concentrations were significantly lower following a 21 day incubation period when Phoslock® formed a layer on the sediment surface (no vertical mixing) compared to a situation when Phoslock® was manually mixed through the top 3 cm of surface sediment (vertical mixing). The results of the burial experiment indicate that the ability of Phoslock® to control water-column TP concentrations decreases when Phoslock® is subjected to vertical transport processes in the sediment. Results of the P-adsorption/extraction experiment indicated a P-saturation value of 21,670 mg P kg^{-1} Phoslock®. Furthermore, sequential extraction of P from saturated Phoslock® under laboratory conditions indicated that 21% of P bound by Phoslock® was release-sensitive, while 79% of bound P was unlikely to be released over a range of pH 5 to 9 or under reducing conditions in shallow lakes. The study therefore indicates that applying Phoslock® is likely to increase the mass of P permanently bound in lake sediments, even under reducing conditions, but that P-partitioning between P_{mobile} and more refractory P-fractions does not occur in the short-term (1 month) following application.

INTRODUCTION

Internal phosphorus (P) loading can prolong the recovery of shallow lakes from cultural eutrophication following the reduction of external P-sources (Sas, 1989; Søndergaard *et al*., 2003; Jeppesen *et al*., 2005b). Many studies have assessed methods to control internal P-loading in shallow lakes (Hickey and Gibbs, 2009), including hypolimnetic withdrawal (Cooke *et al*., 2005), hydraulic flushing (Hosper and Meyer, 1986) and the application of P-stripping/sediment capping agents (hereafter termed P-capping agents; Gibbs *et al*., 2011). P-capping agents commonly used in remediation projects include iron- (Fe; e.g. $FeSO_4$, $FeCl_3$; Perkins and Underwood, 2001, 2002), aluminium- (Al; e.g. $Al_2(SO_4)_3$, $AlCl_3$, Al modified zeolite; Welch and Cooke, 1999; Reitzel *et al*., 2005; Özkundakci *et al*., 2010) and calcium-based (Ca; e.g. $CaCO_3$; Hupfer and Hilt, 2008) products. P-capping agents are expected to reduce internal P-loading through the interception of release-sensitive P (P_{mobile}; the sum of '*labile P'*, '*reductant-soluble P*' and '*organic P'* fraction) following release events (e.g. release of P from Fe(III)-P complexes under reducing conditions in sediments) at the sediment-water interface. However variation in redox and pH conditions in eutrophic lakes can cause the release of P bound to Fe-based/Al-based products (Stumm and Morgan, 1996; Hupfer and Hilt, 2008). Recently a lanthanum (La)-modified bentonite clay (Phoslock®) has been developed. It is reported that P bound by Phoslock® is retained under reducing conditions (i.e. redox sensitive P complexes will be reduced below 200 mV; Boström *et al*., 1988; Stumm and Morgan, 1996) and that Phoslock® is effective over a range of pH 5 to 9 (Ross *et al*. 2008). However reported P-adsorption capacities of Phoslock® (i.e. 11,100 to 15,100 mg P kg^{-1} Phoslock®; Haghseresht *et al*., 2009; Gibbs *et al*., 2011) are pH dependent, although the effects of pH on Phoslock® P-binding properties are contentious. Gibbs *et al*. (2011) measured an increase in P-adsorption capacity with

increasing pH, while a decrease in P-adsorption capacity was observed with increasing pH by Ross *et al*. (2008) and Haghseresht *et al*. (2009).

Laboratory studies have highlighted a range of effects of Phoslock® on sediment processes including the positioning of the aerobic-anaerobic boundary in surface sediments (Vopel *et al*., 2008), increased sediment consolidation and stability thresholds (Egemose *et al*., 2010), and temporary suppression of nitrification and denitrification processes (Gibbs *et al*., 2011). Field scale applications of Phoslock® have reported reductions in water-column P concentrations and changes in cyanobacterial community composition (Robb *et al*., 2003). However, little is known about the amendment effects on sediment chemical properties and alterations in operationally defined sediment P-fractions following a whole-lake Phoslock® application. The operationally defined P-fractions within sediments and their release conditions are summarised (Chapter 1, Table 1.1). For Phoslock®, it is hypothesised that P from the '*labile P'*, '*reductant-soluble P*' and '*organic P*' fractions will be transferred to the '*apatite bound P*' fraction, and that the binding capacity of the latter fraction will increase as a result of elevated sediment La content. Little is known about the timing of this partitioning process or the relative increase in the binding capacity of the specific sediment P-fractions following amendment with Phoslock®. Additionally, little is known about alterations in the sediment content of other elements commonly involved in P-binding and cycling following a Phoslock® application. Gibbs *et al*. (2011) reported that Phoslock® contained significant amounts of Al, Fe, Ca and manganese (Mn), in addition to La. However, Phoslock® may contain other elements not considered in previous studies that may alter sediment elemental composition following application.

For other P-capping agents it has been shown that burial (i.e. vertical positioning) in the sediment may affect their efficiency to control sediment P-release. In an experimental study Lewandowski *et al*. (2003) showed that the ability of a buried

alum layer to control P-release from a newly formed sediment layer above the alum layer was strongly reduced. For Phoslock®, it is hypothesised that burial or mixing of Phoslock® to greater sediment depths will reduce its ability to intercept upwards diffusing P before it enters the water-column.

Study outline and hypotheses

To investigate the above physical and chemical processes and their effects on the binding capacity of Phoslock®, this study combined field surveys and experimental manipulations to assess: (i) the short-term effects of Phoslock® amendment on the elemental composition and P-binding properties of sediments; (ii) the vertical translocation of La within sediments; (iii) the effects of Phoslock® burial on its efficiency to control water-column P concentrations; and (iv) the partitioning of P between release-sensitive P-fractions and Phoslock®. The specific hypotheses tested were: (i) sediment La content will increase in the uppermost (0 – 2 cm) sediment layer following the application of Phoslock®; (ii) the application of Phoslock® will cause an increase in the mass of P present in the '*apatite bound P*' fraction; and (iii) vertical mixing of Phoslock® into the sediment will decrease its ability to reduce water-column P concentrations.

MATERIAL AND METHODS

Study site and dosing

A description of the study site is presented (Chapter 2, Section 2.1.1). Following a preliminary survey in 2008, Phoslock® Europe GmbH calculated a required dosage of 24,000 kg of Phoslock® (270 g Phoslock® m^{-2}) to bind an estimated amount of 19 kg total phosphorus (TP) in the water-column and around 244 kg of potentially release-sensitive P in the upper 4 cm of sediment. This dose of Phoslock® was applied as a slurry from a pontoon over a period of 3 days in March 2009.

Sample collection

Single sediment cores were collected (Chapter 2, Section 2.2.2) from four permanent sample sites 2 days pre- and 1, 4 and 7 months post-application of Phoslock®. Sample sites included deep (site 1; 5.8 ± 0.2 m), intermediate (site 3; 3.6 ± 0.2 m) and shallow areas of the reservoir (site 2 and 4; 2.3 ± 0.3 m). Cores were extruded and sectioned (2 cm slices) to a sediment depth of 10 cm on site.

Sediment analysis

Analysis of sediment elemental composition (Chapter 2, Section 2.4.6) was conducted on cores taken on each sampling date on all sediment sections (2 cm slices) over the top 10 cm of the core. Sediment samples of cores taken 2 days before (n = 4) and 1 month after the application of Phoslock® (n = 4) were analysed for all elements possible to investigate short-term changes in sediment elemental composition. Sediment of cores taken 4 and 7 months after the application of Phoslock® (each n = 4) were analysed for sediment La content (Chapter 2, Section 2.4.6) to assess longer term variation in sediment La content. Sediment P-fractionation (Chapter 2, Section 2.4.7) was conducted

on sediment cores taken 2 days before (n = 4) and 1 month (n = 4) after the Phoslock® application on all sediment sections (2 cm slices) over the top 10 cm of the core.

Mass balance calculations

Mass balance estimates were based on lake area, sediment water content, sediment La and sediment P_{mobile} content as defined by sequential P-extraction techniques. Mass of P_{mobile} on the whole-lake scale was calculated for each sample site and sediment depth interval (1 cm intervals) based on the assumption that 1 g wet weight (WW) equals 1 cm^3 by:

$$M_P = (A * M_{DW/WW}) * M_{Fraction}$$

where M_P equals mass of P on the whole-lake scale (kg), A equals lake area (cm^2), $M_{DW/WW}$ equals mass dry weight (DW) sediment per mass WW (g DW g^{-1} WW) and $M_{Fraction}$ equals mass of P_{mobile} per DW sediment (kg P g^{-1} DW). Consequently the sum of M_P was calculated for the top 4 cm and top 10 cm for each sample point individually before calculating the mean (n = 4) for each depth. Mass balance estimates were made for the top 4 cm and top 10 cm of the sediment as these depths are common estimates of the 'active' sediment depth from which P_{mobile} cycles between the sediment and the water-column (Boström *et al*., 1982; Cooke *et al*., 2005). Comparisons of sediment P_{mobile} content estimates using wet density (as outlined by Jensen *et al*., 1995 based on DW and loss on ignition) versus the assumption that 1 g WW equals 1 cm^3 indicated differences in P_{mobile} content estimates of less than 3% underestimation (data not shown). Mass of La on the whole-lake scale was calculated as described for P_{mobile} above by exchanging mass of P_{mobile} per DW sediment (kg P g^{-1} DW) with mass of La per DW (kg La g^{-1} DW).

P-adsorption/extraction from Phoslock® experiment

Phoslock® was saturated with P, by adding 5,000 ± 10 mg Phoslock® to 0.0475 L of a 2,500 mg P L^{-1} solution (KH_2PO_4, AnalaR; pH 4.5) in 0.05 L centrifuge tubes (n = 5). The final calculated amount of P per tube equalled 23,750 mg P kg^{-1} Phoslock® and exceeded reported P-binding capacities of Phoslock® by 1.6 to 2.2 times (Haghseresht *et al*., 2009; Gibbs *et al*., 2011). Tubes were shaken (60 rpm on 360º rotator) in the dark at 25 ºC for 36 h. The pH of the Phoslock®:P solution mixture was not adjusted and changed from pH 4.7 at the beginning to 5.1 at the end of the shaking period. Residual dissolved P (not bound by Phoslock®) was removed prior to the sequential P-extraction (Chapter 2, Section 2.4.7) from 1 g WW Phoslock® by the following procedure: (i) centrifugation (5 min at 3,800 rpm) of the Phoslock®:P solution mixture and decanting of supernatant; (ii) repeated washing of the Phoslock® pellet (3 times) consisting of (iia) addition of 0.05 L distilled water to pellet, (iib) mixing of pellet with distilled water, (iic) shaking slurry (5 min at 60 rpm on 360º rotator), and (iid) centrifugation (5 min at 3,800 rpm) and removal of supernatant. This washing procedure mimics the procedure used in the production of Phoslock® to remove excess La not bound in the bentonite clay structure (Douglas, 2002).

Phoslock® burial experiment

Homogenized sediment collected prior to the application of Phoslock® in March 2009 from multiple locations (n = 25) covering deep, intermediate and shallow areas of Clatto Reservoir was used to provide a consistent substrata across burial treatments. Sediment was collected using an Ekman grab, combined and stored in a large plastic container in the dark for 20 months. Prior to using the collected sediment for the experiment all surplus water was siphoned off and the sediment was rigorously stirred for 10 minutes to produce a homogenous mixture. The experiment was conducted in sediment cores (n

= 20; height 250 mm; internal diameter 68 mm) which were sealed at the bottom with rubber bungs. Control cores (n = 5) were prepared by adding 400 g of wet sediment to the cores, resulting in a sediment height of 10 cm. In addition to the control cores three treatments (n = 5 per treatment) were prepared to represent burial of Phoslock® across 0 cm, 1 cm and 3 cm of surface sediment. The Phoslock® dose was set at 0.97 g Phoslock®, equivalent to an aerial load of 270 g Phoslock® m^{-2} originally applied to Clatto Reservoir. The '0 cm mixing' treatment was prepared by adding 400 g of wet sediment into the core tubes and adding Phoslock® as a slurry on the sediment surface using a syringe. The '1 cm mixing' treatment was prepared by filling 360 g of wet sediment into the core tube and overlaying this with 40 g of wet sediment (equivalent to a sediment depth of 1 cm in the core tubes used) into which Phoslock® had been mixed. Finally, the '3 cm mixing' treatment was prepared by filling 280 g of wet sediment into the core tube and overlaying this with 120 g of wet sediment into which Phoslock® had been mixed. Distilled water (360 mL) was added to all cores to create a water-column using a plastic sheet to cover the sediment surface during the filling process to avoid sediment disturbance. Distilled water was used to create a steep concentration gradient between the sediment and the overlying water-column that would favour sediment P-release. All cores consisted of 10 cm sediment depth and 10 cm overlying water-column depth.

All cores were incubated in a temperature control room at 15 °C in the dark for 21 days. Cores were initially bubbled for 24 h with oxygen free N_2 gas to create low water-column oxygen concentrations that favour sediment P-release, with care being taken not to disturb the sediment surface. On all remaining days cores were bubbled daily for 5 h with N_2 gas and cores were sealed with Parafilm and kept in an N_2 filled plastic bag when N_2 bubbling was stopped. Measurements of water-column dissolved oxygen (DO) concentrations (day 0, 1, 3, 7 and 21), pH and conductivity (day 0, 7 and

21) were made 5 cm above the sediment surface (Chapter 2, Section 2.3.1). Samples (50 mL) for the analysis of water-column soluble reactive phosphorus (SRP) and TP concentrations (Chapter 2, Section 2.4.4) were taken 5 cm above the sediment on day 3, 7 and 21 using a syringe. Water was replaced with distilled water following sample removal from the water-column.

The period required to establish constant P-equilibrium conditions in experiments using the experimental sediment core set up is likely to vary depending on the sediment used and parameters/processes under investigation. Estimates of the period required to establish constant P-equilibrium conditions range from 5 to 20 days (Lewandowski *et al*., 2003; Gibbs *et al*., 2011). It was therefore assumed that day 21 represented conditions of constant P-equilibrium.

Statistical analyses

Minitab 16 (Minitab® 16.1.1, Minitab Ltd., Coventry, UK) was used for all statistical analyses unless otherwise stated.

Short-term changes in sediment elemental composition and P-fractions

Sediment elemental composition and sediment P-fractions were similar across all sites indicating that water depth was not a factor influencing their distribution with sediment depth. Consequently data from all four sites were pooled to allow a statistical assessment of variation in elemental composition and P-fractions with sediment depth between pre- and post-application periods.

Data sets of i) sediment elemental composition and ii) sediment P-fractions were not normally distributed (Anderson-Darling test, $\alpha < 0.05$) even after transformation (including $x' = \log(x)$, $x' = \ln(x)$, $x' = \text{sqrt}(x)$, $x' = x^2$). Therefore, a non-parametric Mann-Whitney U-test (MWU) was used: i) to test for significant variation, for each

depth interval, in sediment elemental composition between sediment samples taken 2 days before and 1 month following the application; and ii) to test for significant variation, for each depth interval, in sediment P-fractions between sediment samples taken 2 days before and 1 month post-application of Phoslock®.

Longer term changes in sediment La content

Data sets of sediment La content were not normally distributed (Anderson-Darling test, $\alpha < 0.05$) even after transformation (including $x' = \log(x)$, $x' = \ln(x)$, $x' = \sqrt{x}$, $x' = x^2$). Therefore a non-parametric Kruskal-Wallis (KW) test was used to test for significant variation, for each depth interval, in sediment La content between sample dates (2 days before and 1, 4 and 7 months after the Phoslock® application). Due to loss of sample, this analysis was not possible for the 8 – 10 cm layer. Where significant variation was evident (KW test, $\alpha < 0.05$), a non-parametric MWU test was used to determine, for each depth interval, between which consecutive sampling dates (2 days before vs. 1 month after; 1 month after vs. 4 month after; 4 month after vs. 7 month after) significant differences in sediment La content occurred.

Phoslock® burial experiment

Variation in water-column TP, SRP, DO, pH and conductivity was assessed between treatments for each day of the experiment. A Grubb's test was performed using the software QuickCalcs (QuickCalcs Online Calculator, GraphPad Software Inc., La Jolla, USA) to identify significant ($p < 0.01$) outliers, leading to the exclusion of one data point from the TP data set (0 cm mixing treatment on day 21). Data sets of water-column TP and DO concentration were normally distributed (Anderson-Darling test, $\alpha > 0.05$) and of equal variance (Levene's test, $\alpha > 0.05$) following transformation (TP: $x' = \log(x)$; DO: Box-Cox power transformation, $x' = x^{-0.37}$) and deletion of an outlier in the

TP data set. One-way analysis of variance (ANOVA) with Tukey's *post hoc* test was used to test for significant variation in a given parameter between treatments for each day of the experiment.

Data sets of water-column SRP concentration, pH and conductivity were not normally distributed (Anderson-Darling test, $\alpha < 0.05$) and not of equal variance (Levene's test, $\alpha < 0.05$) even after a range of transformations (including: $x' = \log(x)$, $x' = \ln(x)$, $x' = \text{sqrt}(x)$, $x' = x^2$). Therefore a non-parametric KW test was used to test for significant variation in a given parameter between treatments for each day of the experiment. Where significant variation was evident (Kruskal-Wallis test, $\alpha < 0.05$) a non-parametric MWU test was used to determine between which treatments significant differences in a given parameter occurred.

RESULTS

Short-term (1 month) changes in sediment elemental composition

This section refers to individual sediment depth layers in which significant variation in elemental composition between pre- and post-application was detected. Variation in sediment La content (Fig. 4.1) showed the same vertical pattern for all sample sites. Mean pre-application sediment La content (Table 4.1; Fig. 4.2) ranged from 27.8 to 34.8 mg La kg^{-1} DW sediment depending on sediment depth. Mean sediment La content was significantly higher in the 0 – 2 cm, 2 – 4 cm, 4 – 6 cm and 6 – 8 cm sediment depth layer ($W = 10$; $p < 0.05$; $n_1 = n_2 = 4$), 1 month post-application ranging from 204 to 8,803 mg La kg^{-1} DW sediment.

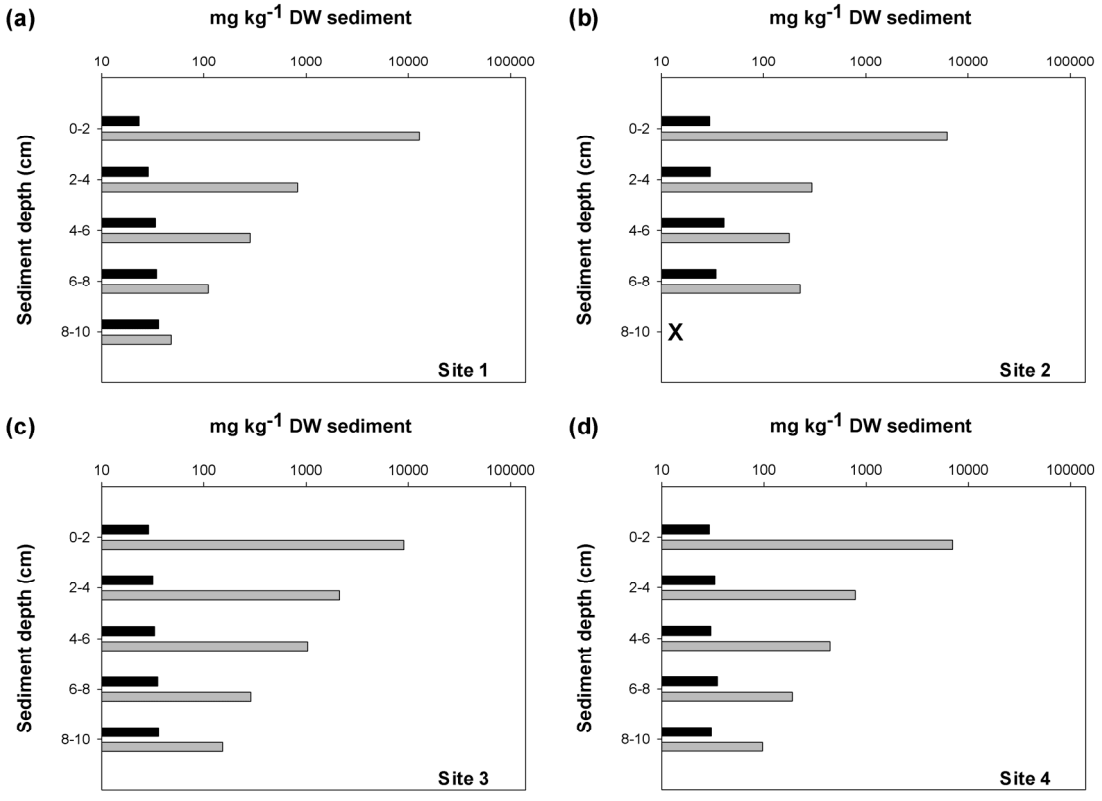


Figure 4.1 Sediment lanthanum content at individual sample sites in Clatto Reservoir 2 days before (black bars) and 1 month after (grey bars) the application of Phoslock®. Depth for which no sediment was sampled is marked with X. Note log scale on the x-axis.

Mean surface sediment (0 – 2 cm) contents of barium (Ba), cerium (Ce), neodymium (Nd) and praseodymium (Pr) were significantly higher ($W = 10$; $p < 0.05$; $n_1 = n_2 = 4$) 1 month following the application compared to pre-application conditions (Table 4.1). No significant differences were detected in other analysed elements, including elements involved in P-binding and cycling, for example, Al, Ca, Fe and Mn.

Longer-term changes in sediment La content (up to 7 months post-application)

Mean sediment La content differed significantly across all months in the 0 – 2 cm ($H = 9.26$; $p < 0.05$; $DF = 3$; $n = 4$), 2 – 4 cm ($H = 8.49$; $p < 0.05$; $DF = 3$; $n = 4$), 4 – 6 cm ($H = 9.33$; $p < 0.05$; $DF = 3$; $n = 4$) and 6 – 8 cm ($H = 12.26$; $p < 0.01$; $DF = 3$; $n = 4$) sediment layers. Mean sediment La content (Fig. 4.2) was significantly higher in the 0 – 2 cm, 2 – 4 cm, 4 – 6 cm and 6 – 8 cm sediment depth layer ($W = 10$; $p < 0.05$; $n_1 = n_2 = 4$) during all post-application months when compared to pre-application conditions. A pair-wise comparison of post-application months indicated a significant increase ($W = 10$; $p < 0.05$; $n_1 = n_2 = 4$) from 128.89 to 293.25 mg La kg^{-1} DW sediment between months 4 and 7 post-application in the 6 – 8 cm sediment layer (Fig. 4.2).

Table 4.1 Changes in sediment elemental composition at Clatto Reservoir before (2 days) and after (1 month) the application of Phoslock®. Sediment content of any given element is expressed as mg kg^{-1} dry weight sediment. Elements commonly involved in natural sediment phosporus-binding and cycling processes (Al, Ca, Fe, Mn) and elements that varied significantly pre- and post-application of Phoslock® are shown.

Depth(cm)	n =	Element	Al	Ba	Ca	Ce	Fe	La	Mn	Nd	Pr
0-2	4	before	49,717	507	14,016	57.1	57,497	27.8	2,845	25.5	7.0
		after	53,657	604	13,663	66.4	49,139	8,803	2,630	28.9	8.3
		% change	8	19	-3	16	-15	31,619	-8	13	19
		MWU		*		*		*		*	*
2-4	4	before	54,862	514	13,756	64.8	53,059	30.9	1,455	28.7	7.9
		after	55,446	2,449	14,827	62.3	46,400	1,006	1,244	27.9	7.8
		% change	1	376	8	-4	-13	3,154	-14	-3	-2
		MWU						*			
4-6	4	before	58,254	558	13,178	70.7	51,107	34.5	1,163	31.2	8.6
		after	58,087	538	13,561	72.5	49,548	486	1,137	32.2	8.9
		% change	0	-4	3	3	-3	1,309	-2	3	4
		MWU						*			
6-8	4	before	60,042	556	12,506	75.3	48,634	34.8	973	32.9	9.0
		after	64,050	569	13,247	66.5	48,337	204	1,005	29.7	8.2
		% change	7	2	6	-12	-1	487	3	-10	-9
		MWU						*			
8-10	3	before	61,262	591	11,298	72.5	46,039	34.3	699	32.4	8.9
		after	68,236	579	11,344	78.4	52,925	99.4	988	34.5	9.5
		% change	11	-2	0	8	15	190	41	7	7
		MWU									

MWU: Mann-Whitney U-test; *: $p < 0.05$.

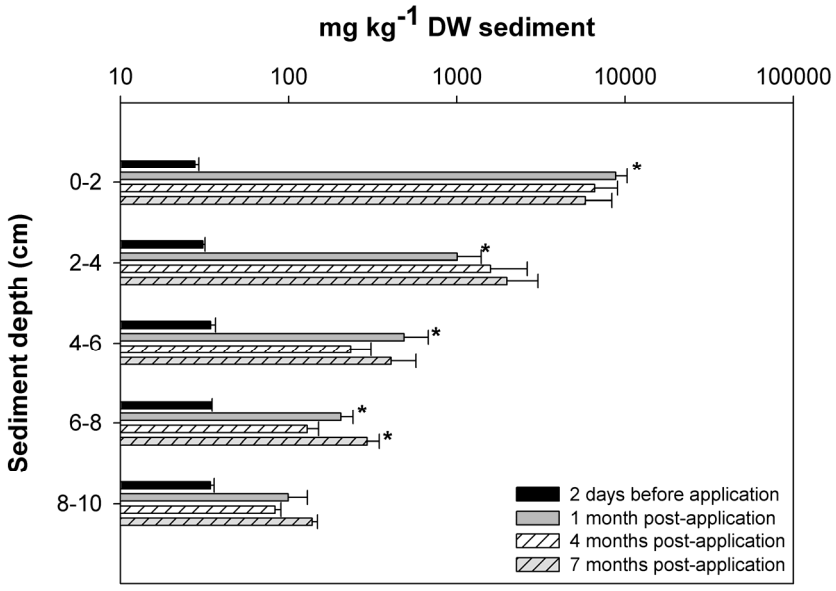


Figure 4.2 Sediment lanthanum content as the mean of all sample sites in Clatto Reservoir 2 days before (black bars), 1 month after (grey bars), 4 months after (white hatched bars) and 7 months (grey hatched bars) after the application of Phoslock®. Error bars represent the standard error of the mean (n = 4 for 0 – 8 cm; n = 3 for 8 – 10 cm for 2 days before, 1 and 4 months after; n = 2 for 8 – 10 cm for 7 months after) and significant differences between consecutive months are marked with asterisks (* $p < 0.05$). Note log scale on the x-axis.

Mass balance estimates

Mass balance estimates (Table 4.2) extrapolating to the whole-lake scale indicated that 594 kg P_{mobile} were present in the top 4 cm of the sediment, while the top 10 cm contained 1,502 kg P_{mobile} pre-application. Mass balance estimates also indicated that 1,119 kg La were added to the sediment. Based on a molar binding ratio of La:P of 1:1 (Haghseresht *et al*., 2009) and assuming all measured La is available for P-binding, the added amount of La can bind 250 kg P. It was, therefore, estimated that the applied mass of La would be sufficient to bind around 42% of P_{mobile} in the top 4 cm or around 17% of P_{mobile} in the top 10 cm of the sediment (Table 4.2). Based on a nominal La

content of Phoslock® of 50,000 mg La kg^{-1} Phoslock® (Afsar and Groves, 2009) the measured mass of La equalled the addition of 22,380 kg of Phoslock® compared to the applied 24,000 kg Phoslock®.

Table 4.2 Calculated sediment mobile phosphorus content (P_{mobile}) and sediment lanthanum (La) content in Clatto Reservoir. Based on the molar La:P binding ratio of 1:1, the added amount of 1,119 kg La can bind 250 kg P.

Sediment depth	Pre-application P_{mobile} cumulative	Pre-application P_{mobile} cumulative	Post-application La content cumulative	Potential control of pre-application P_{mobile} by total cumulative La content
(cm)	(kg)	(%)	(kg)	(%)
top 2	276	18	787	91
top 4	**594**	**40**	**934**	**42**
top 6	888	59	1,039	28
top 8	1,136	76	1,087	22
top 10	**1,502**	**100**	**1,119**	**17**

Short-term (1 month) changes in P-partitioning across sediment fractions

This section will refer to individual sediment depth layers in which significant variation in sediment P-fractions between pre- and post-application was detected. The amount of P in the '*residual P*' fraction (Fig. 4.3f) increased significantly ($W = 10$; $p < 0.05$; $n_1 = n_2 = 4$) following the application in the 0 – 2 cm sediment depth layer from 71 mg P kg^{-1} DW sediment to 113 mg P kg^{-1} DW sediment, while differences in other P-fractions were not significant (Fig. 4.3). The largest amount of sediment P, both before and after the application, was present in the '*reductant-soluble P*' fraction (Fig. 4.3b).

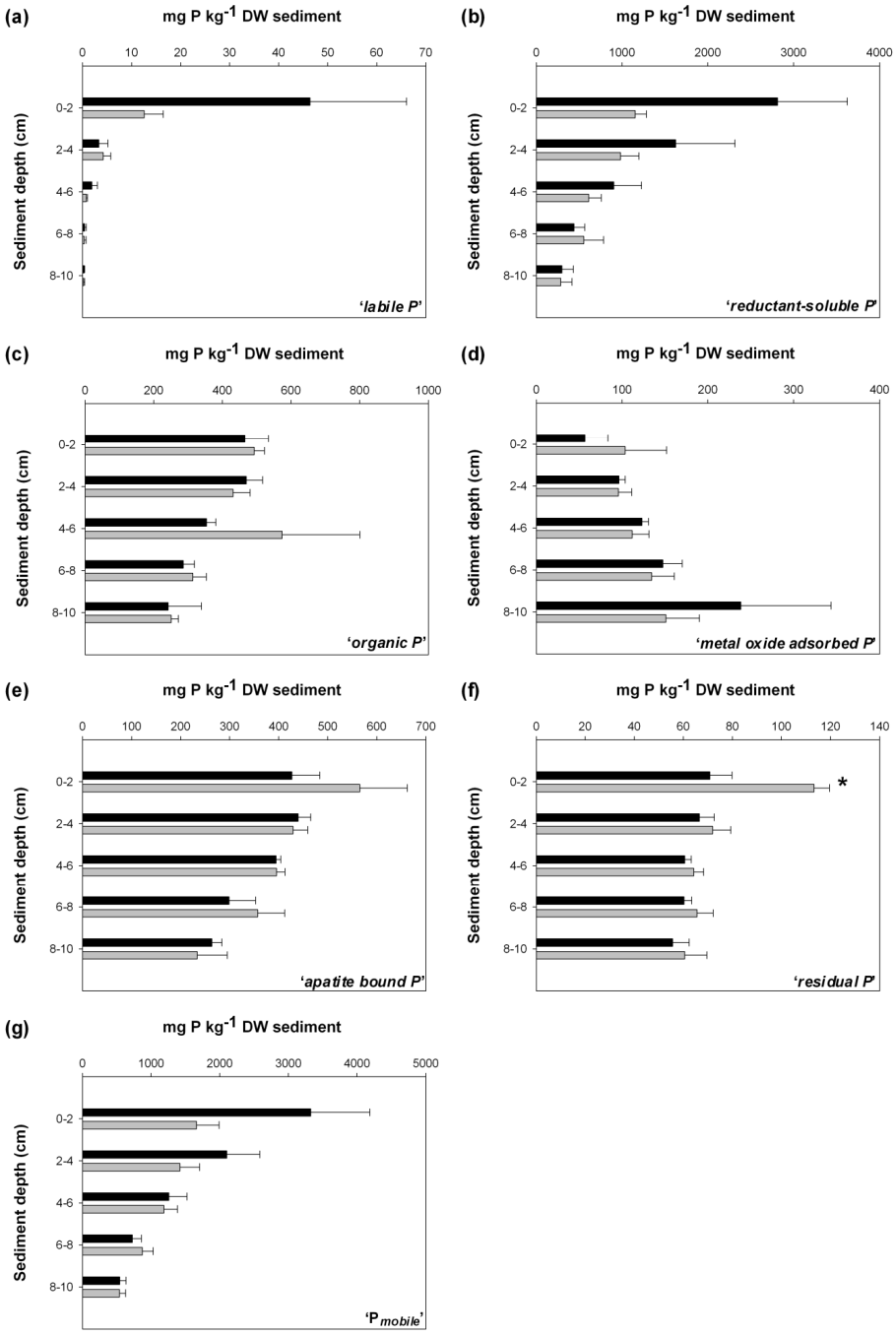


Figure 4.3 Sediment phosphorus (P) fractions in Clatto Reservoir 2 days before (black bars) and 1 month after (grey bars) the application of Phoslock® including a) '*labile P*', b) '*reductant-soluble P*', c) '*organic P*', d) '*metal-oxide adsorbed P*', e) '*apatite bound P*', f) '*residual P*', and g) P_{mobile} (sum of '*labile P*', '*reductant-soluble P'* and '*organic P*' fraction). Error bars represent standard error of the mean (n = 4 for 0 – 8 cm; n = 3 for 8 – 10 cm) and significant differences are marked with asterisks (* $p < 0.05$).

P extraction from Phoslock®

The mass of P extracted from P-saturated Phoslock® was 21,670 mg P kg^{-1} Phoslock® (Fig. 4.4). The largest mass of P of 13,150 mg P kg^{-1} Phoslock® was extracted from the '*apatite bound P*' fraction. Based on a nominal La content of 50,000 mg La kg^{-1} Phoslock® (Afsar and Groves, 2009) and a molar binding ratio of La:P of 1:1 (Haghseresht *et al*., 2009) the P-binding capacity of La contained in 1 kg of Phoslock® is estimated to equal 11,147 mg P. The results indicate that the P-binding capacity of Phoslock® is nearly two times greater than the expected P-binding capacity of the La content alone.

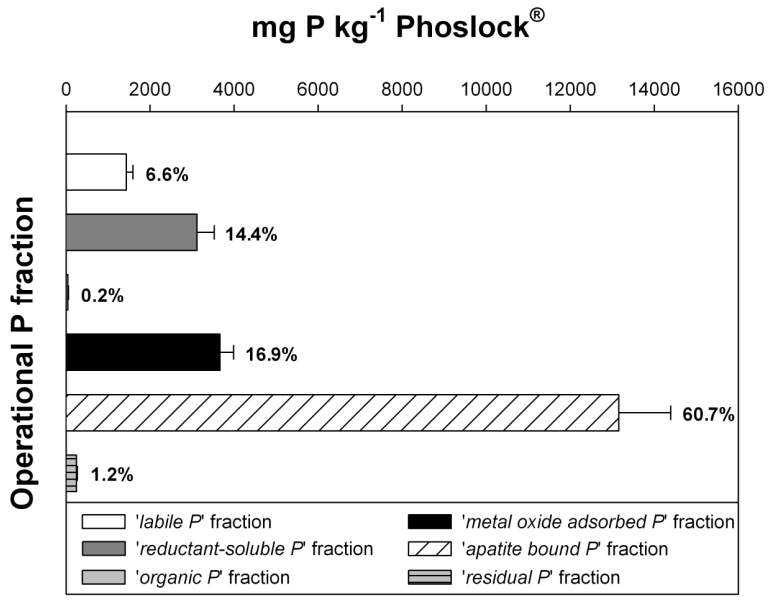


Figure 4.4 Fractions from which phosphorus (P) was extracted from saturated Phoslock® following a 36 h contact period and mass of P extracted. Error bars represent standard error of the mean (n = 5). The contribution of each fraction is expressed relative to the total mass of extracted P (21,670 mg P).

Phoslock® burial experiment

Variation in water-column TP (Fig. 4.5a) and SRP (Fig. 4.5c) concentration between treatments is presented. Water-column TP concentrations in the control were significantly higher compared to all treatments on day 3 ($F = 83.82$; $p < 0.001$; $DF = 3$; $n = 5$), day 7 ($F = 86.03$; $p < 0.001$; $DF = 3$; $n = 5$) and day 21 ($F = 153.53$; $p < 0.001$; $DF = 3$; $n = 5$, except $n = 4$ for 0 cm mixing treatment). On day 21, which was assumed to represent constant P-equilibrium conditions, water-column TP concentrations were significantly lower in the 0 cm mixing treatment compared to the 3 cm mixing treatment, indicating that the ability of Phoslock® to control water-column TP concentrations is higher when Phoslock® forms a layer on the sediment surface. Comparing the reduction in water-column TP concentration on day 21 to the control revealed that the 0 cm mixing treatment caused a 97.0% reduction, while the 1 cm mixing and 3 cm mixing treatment caused a 96.0% and 94.7% reduction in TP concentration, respectively (Fig. 4.5b).

Similarly, water-column SRP concentrations were significantly higher in the control compared to all treatments on day 3 ($H = 12.95$; $p < 0.01$; $DF = 3$; $n = 5$), day 7 ($H = 11.78$; $p < 0.01$; $DF = 3$; $n = 5$) and day 21 ($H = 13.03$; $p < 0.01$; $DF = 3$; $n = 5$), indicating that all treatments significantly reduced the release of SRP across the sediment-water interface. Water-column SRP concentrations did not vary significantly between the 0 cm mixing, 1 cm mixing and 3 cm mixing treatment on any day of the experiment. On day 21 the reduction in SRP concentration relative to the control ranged from 98.6 to 99.6% (Fig. 4.5d).

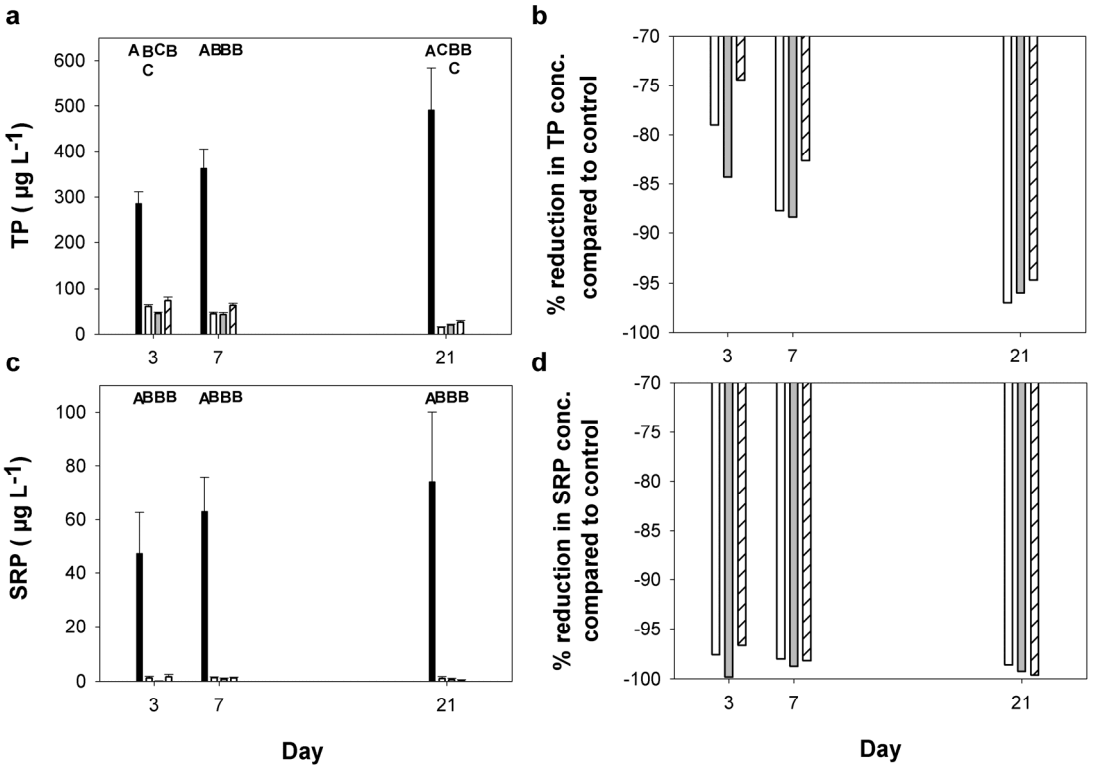


Figure 4.5 Variation in water-column (a) total phosphorus (TP) and (c) soluble reactive phosphorus (SRP) concentrations over the course of the mixing experiment, including control (black bars), 0 cm mixing treatment (white bars), 1 cm mixing treatment (grey bars) and 3 cm mixing treatment (white hatched bars). Percent reduction in (b) TP and (d) SRP concentration of treatments (0 cm mixing, 1 cm mixing, 3 cm mixing) compared to the control. Error bars (a, c) represent the standard error of the mean (n = 5; n = 4 for 0 cm mixing treatment on day 21).

Variation in water-column DO concentration, pH and conductivity between treatments is presented (Table 4.3). Water-column DO concentrations varied significantly between treatments on day 0 ($F = 6.84$; $p < 0.01$; $DF = 3$; $n = 5$), day 3 ($F = 3.76$; $p < 0.05$; $DF = 3$; $n = 5$) and day 21 ($F = 4.27$; $p < 0.05$; $DF = 3$; $n = 5$), while no significant differences were observed on day 1 and day 7. DO concentrations ranged from 7.2 to 8.4 mg L^{-1} on day 0 and decreased after the onset of N_2 bubbling to 0.3 to 0.6 mg L^{-1} on day 1. On the

remaining days of the experiment, DO concentrations varied between 1.2 to 3.1 mg L^{-1} (Table 4.3). A pair wise comparison (Tukey's *post hoc* test) between treatments on day 21 indicated that water-column DO concentrations did not vary significantly between the 0 cm mixing, 1 cm mixing and 3 cm mixing treatment, however DO concentrations of the 1 cm mixing treatment were significantly higher compared to the control (Table 4.3). Water-column pH (Table 4.3) varied significantly between treatments on day 0 ($H = 13.95$; $p < 0.01$; $DF = 3$; $n = 5$), while no significant differences were detected between treatments on day 7 and day 21. Water-column conductivity (Table 4.3) varied significantly between treatments on day 0 ($H = 12.95$; $p < 0.01$; $DF = 3$; $n = 5$), day 7 ($H = 10.91$; $p < 0.05$; $DF = 3$; $n = 5$) and day 21 ($H = 10.13$; $p < 0.05$; $DF = 3$; $n = 5$). On day 21 conductivity did not vary significantly between the 0 cm mixing, 1 cm mixing and 3 cm mixing treatment, while conductivity in the control was significantly lower ($W = 10$; $p < 0.05$; $n_1 = n_2 = 5$) compared to the 0 cm mixing, 1 cm mixing and 3 cm mixing treatment.

Table 4.3 Mean water-column dissolved oxygen (DO; mg L^{-1}) concentrations, pH and conductivity (cond.; µS cm^{-1}) with standard error of the mean (n = 5) in brackets of the control, 0 cm mixing treatment (0 cm), 1 cm mixing treatment (1 cm) and 3 cm mixing treatment (3 cm). Table includes results of the pair wise comparison (Tukey's *post hoc* test (Tukey) or Mann-Whitney U-test (MWU)). Groups that do not share the same letter are significantly different.

Parameter	Treatment	Day 0	Day 1	Day 3	Day 7	Day 21
DO (Tukey)	control	8.4 (0.13)^{B}	0.3 (0.04)^{A}	2.4 (0.18)^{AB}	1.4 (0.32)^{A}	1.5 (0.21)^{A}
	0 cm	8.0 (0.21)^{B}	0.6 (0.37)^{A}	2.2 (0.26)^{A}	1.2 (0.15)^{A}	2.4 (0.35)^{AB}
	1 cm	7.2 (0.12)^{A}	0.3 (0.08)^{A}	3.0 (0.13)^{B}	1.3 (0.08)^{A}	3.1 (0.44)^{B}
	3 cm	7.6 (0.28)^{AB}	0.3 (0.12)^{A}	2.7 (0.14)^{AB}	1.6 (0.25)^{A}	2.7 (0.19)^{AB}
pH (MWU)	control	6.3 (0.02)^{B}	-	-	7.0 (0.04)^{A}	6.7 (0.02)^{A}
	0 cm	7.0 (0.06)^{A}	-	-	7.5 (0.61)^{A}	6.8 (0.04)^{A}
	1 cm	6.3 (0.04)^{BC}	-	-	7.0 (0.01)^{A}	6.7 (0.12)^{A}
	3 cm	6.2 (0.01)^{C}	-	-	7.0 (0.04)^{A}	6.7 (0.15)^{A}
Cond. (MWU)	control	10.9 (1.46)^{B}	-	-	55.6 (3.18)^{A}	78.8 (5.08)^{A}
	0 cm	77.3 (2.73)^{A}	-	-	95.6 (8.39)^{B}	114.3 (3.19)^{B}
	1 cm	16.3 (2.10)^{B}	-	-	101.2 (3.81)^{B}	116.9 (4.71)^{B}
	3 cm	11.3 (1.14)^{B}	-	-	93.6 (4.14)^{B}	122.0 (4.69)^{B}

DISCUSSION

Field observations made 1 month following the application of Phoslock® to Clatto Reservoir indicated that a significant increase in sediment La content was not confined to the surface sediment layer (0 – 2 cm). Experimental results indicated that the ability of Phoslock® to control water-column TP concentrations decreased when Phoslock® was mixed vertically into the sediment, as observed in Clatto Reservoir. P-extraction from P-saturated Phoslock® under experimental conditions indicated that the application of Phoslock® will cause an increase in the mass of P present in the '*apatite bound P*' fraction. However, field observations showed that alterations in sediment P-partitioning in particular an increase the mass of P present in the '*apatite bound P*' fraction did not occur in the short-term (1 month) post-application.

Short-term effects on sediment elemental composition and sediment P-binding properties

Following the application of Phoslock®, sediment La content increased significantly, whereas the sediment content of other elements, commonly involved in sediment P-binding and cycling including Al, Ca, Fe and Mn (Boström *et al*., 1988; Søndergaard *et al*., 1996), did not differ significantly. Sediment P-binding capacity was, therefore, theoretically higher post-application due to an increase in La-P binding sites. Mass balance estimates extrapolating to the whole-lake scale indicated that 1,119 kg La (equivalent to the addition of 22,380 kg Phoslock®) were added to the sediment. The nominal La content of Phoslock® is 50,000 mg La kg^{-1} Phoslock® (Afsar and Groves, 2009), which would equal a mass of 1,200 kg La applied to Clatto Reservoir. The difference in La measured and added, although small, may be the result of: i) a proportion of the applied Phoslock® persisting in the water-column during the sample period (Chapter 3); ii) variation in the nominal La content, for example Gibbs *et al*.

(2011) reported 45,000 mg La kg^{-1} Phoslock®; or iii) low spatial resolution La measures in this study resulting in error in the mass balance calculations. However, based on a molar binding ratio of La:P of 1:1 (Haghseresht *et al*., 2009) and assuming all measured La is available for P-binding, the added amount of La should be sufficient to bind up to 250 kg P.

The dose estimates calculated by Phoslock® Europe GmbH were based on an estimated amount of P_{mobile} of 244 kg in the upper 4 cm. However this study estimated that the mass of P_{mobile} in the upper 4 cm exceeded estimates by Phoslock® Europe GmbH by around 2.4 times. Variation in estimated sediment P_{mobile} content may result from seasonal variation in sediment P-fractions (Søndergaard, 2007; Spears *et al*., 2007b), spatial variation in sediment P_{mobile} content (James *et al*., 2000; Spears *et al*., 2006) or error in P-fractionation (Farmer *et al*., 1994). In addition Phoslock® Europe GmbH considered only the upper 4 cm of the sediment to calculate dose. However the 'active' sediment depth (sediment depth from which P_{mobile} cycles between sediment and water-column) and associated P_{mobile} content is likely to be site specific (Søndergaard *et al*., 1996). For example bioturbation, macrophyte density, microbial activity (Boström *et al*., 1988; Phillips *et al*., 1994; Stephen *et al*., 1997), pH and oxygen concentration profiles (Boström, 1984) have all been shown to regulate P-release from the 'active' layer. Common estimates of the 'active' sediment depth vary between the top 4 cm (Cooke *et al*., 2005) and the top 10 cm (Boström *et al*., 1982). However, P-release from sediment depths of up to 20 cm to 25 cm has been reported (Søndergaard *et al*., 1999). Based on the results of the current study detailing depth profiles of operational P-fractions in Clatto Reservoir it is apparent that significant quantities of P_{mobile} are present up to at least 10 cm depth. These results indicate that 594 kg P were present in the top 4 cm, while the top 10 cm contained 1,502 kg P.

Consequently, the added La would be sufficient to control 42% of P_{mobile} in the top 4 cm and 17% of P_{mobile} in the top 10 cm.

Phoslock® (modified bentonite clay) contains, in addition to La, a range of other elements commonly involved in P-binding (Nagy and Kónya, 2010), so the total P-binding capacity of Phoslock® is likely to be larger than the P-binding capacity of La alone. This study showed that 1 kg of Phoslock® was able to bind 21,670 mg P. This exceeded the theoretical P-binding capacity by La of 11,147 mg P, assuming a molar binding ratio of La:P of 1:1 (Haghseresht *et al*., 2009), by 1.9 times. Furthermore the measured P-binding capacity in this study exceeded reported P-binding capacities of Phoslock® of other studies by 1.4 to 2.0 times (Haghseresht *et al*., 2009; Gibbs *et al*., 2011). Potential reasons for this include: i) a higher nominal La content in the Phoslock® used in the present study; ii) unmodified elements in bentonite clay may play a larger role in P-binding than formerly estimated, Haghseresht *et al*. (2009), for example, reported that the P-binding capacity of unmodified bentonite was <500 mg P kg^{-1}; iii) the elemental composition, and therefore the P-binding capacity of bentonite may vary; or iv) experimental conditions (e.g. continuous shaking, low pH, high temperature) may have increased Phoslock® P-binding capacity.

Experimental results indicated that 21% of TP bound by Phoslock® was release-sensitive (i.e. P_{mobile}), while 79% was unlikely to be released under typical lake pH ranges (pH 5 – 9) and reducing conditions. The release-sensitive P was present as '*labile P*' (6.6%), '*reductant-soluble P*' (14.4%) and '*organic P'* (0.2%). The release of P from Phoslock® (i.e. from elements other than La) would therefore be predominantly driven by desorption and diffusion of P from the '*labile P*' fraction across steep concentration gradients and reducing conditions would trigger the release of P contained in the '*reductant-soluble P*' fraction. Assuming Phoslock® performs similarly in the field as it did in the laboratory (i.e. P-binding capacity of 21,670 mg P kg^{-1} Phoslock), it

is estimated that the 24,000 kg of Phoslock® applied to Clatto Reservoir are sufficient to control 69% of P_{mobile} in the top 4 cm or 27% of P_{mobile} in the top 10 cm of sediment. These findings indicate that Phoslock® acts by increasing the binding capacity of sediments by selectively enhancing the more refractory sediment P-fractions relative to P_{mobile}. This is important as internal P-loading can only be reduced in the longer term by increasing the capacity of sediments to retain P under reducing conditions (Lijklema, 1994; Hupfer and Lewandowski, 2008).

The P release experiment did not directly assess the release of P from La-P complexes. Under the assumption that the La-P bond is broken during a single extraction step, it is hypothesised that La bound-P is most likely to be recovered from the '*apatite bound P*' fraction. This hypothesis is based on the observation that the amount of P extracted from this fraction (13,150 mg P kg^{-1} Phoslock®) was similar to that expected to be bound (11,147 mg P) by the mass of La in 1 kg of Phoslock®. This hypothesis may be tested by analysing extraction supernatants for La in future experiments.

Short-term changes in P composition following Phoslock® application

No significant differences in the P_{mobile} fraction were observed up to 1 month following the application, despite significantly higher post-application sediment La content. Significantly more P was stored in the '*residual P*' fraction in the top 2 cm of sediment post-application, although this increase was small on the whole reservoir scale (additional 4 kg P bound in '*residual P*' fraction post-application). These findings indicated that the application of Phoslock® did not trigger the release of P from other sediment P-fractions or an immediate change in sediment P-partitioning between P_{mobile} and more refractory P-fractions in the first month post-application. Long-term studies of change in sediment P-fractions (Chapter 5) could give insights into the timing and

mechanisms of P-release from P_{mobile} followed by potential interception of P by Phoslock® into the more refractory '*apatite bound P*' fraction.

Vertical translocation of La and effects of Phoslock® burial on its ability to control sediment P-release

A significant increase in sediment La content was not confined to the sediment surface (0 - 2 cm), but was observed just one month after application over the upper 8 cm of sediment. Although not significant, it appeared that surface sediment La content (0 – 2 cm) decreased between post-application month 1 and 7 while subsurface sediment La content (2 – 4 cm) appeared to increase over this period. However, assessing longer term changes in sediment La content indicated a significant increase in sediment La content in the 6 – 8 cm sediment layer between post-application months 4 and 7. This indicates that La (and Phoslock®) was subjected to vertical sediment transport processes which may include bioturbation (Fischer *et al*., 1980; Meysman *et al*., 2006) and wind induced sediment re-suspension (Hilton *et al*., 1986; Douglas and Rippey, 2000) and that this process became more pronounced with time. Consequently, the conceptual framework in which sediment P-capping agents form a distinct surface layer which intercepts upwardly diffusing P following release events may have to be adapted for shallow lakes. The vertical translocation of La observed in the present study implies that La may be transported below the 'active' sediment depth from which cycling of P_{mobile} between sediment and water-column is expected (here Phoslock® Europe GmbH considered the top 4 cm). This raises the question, is the P-capping efficiency of Phoslock® reduced following vertical translocation? Experiments suggest that the burial of alum strongly reduces its ability to control sediment P-release (Lewandowski *et al*., 2003). All treatments in the Phoslock® burial experiment significantly reduced the release of SRP across the sediment-water interface relative to the control, but there were

no significant differences between mixing treatments. These results suggest that mixing of Phoslock® across surface sediment layers, at the dose used in this experiment, will be unlikely to reduce the efficiency of Phoslock® to reduce SRP release. However water-column TP concentrations were significantly lower at the end of the experimental period when Phoslock® formed a surface layer (0 cm mixing treatment) compared to when it was mixed through the top 3 cm of the sediment. The reason for this observation is not clear. However, the sediment surface layer may have been disturbed during bubbling with N_2 resulting in an increase in water-column TP but not SRP. Alternatively a surface layer of Phoslock® may act as a physical barrier to the migration of organisms for example cyanobacteria (Barbiero and Welch, 1992; Head *et al*., 1999) or protozoa (Finlay, 1981; Beaver and Crisman, 1989) and/or phytoplankton resting stages (Pettersson, 1998) that may have been viable in the sediments used (Hašler *et al*., 2004; Poulíčková *et al*., 2008). Vertical migration of such organisms from the sediment into the water-column may increase water-column TP but not SRP concentration. To clarify the reasons behind these results, future mixing experiments should either incorporate measurements of phytoplankton and protozoan biomass and/or assess La concentrations in the water-column as a tracer for sediment and Phoslock® disturbance.

CONCLUSIONS

The application of Phoslock® did not significantly alter the amount of P_{mobile} over a 1 month post-application sample period in the bed sediments of Clatto Reservoir, indicating that Phoslock® is unlikely to be a suitable remediation measure to reduce the mass of P present in the P_{mobile} fraction in the short-term. The pre-application results indicated that significant amounts of P_{mobile} were present at sediment depths of up to at least 10 cm. It is therefore evident that the original dose estimates, based on P_{mobile} in the upper 4 cm, were underestimates, assuming that P_{mobile} across all sediment depths are

subject to release processes. Consequently the applied dose of Phoslock® was not sufficient to control the mass of P_{mobile} present in the top 4 cm (or top 10 cm) in the bed sediments of Clatto Reservoir. More intensive pre- and post-application monitoring in conjunction with sediment P release experiments are recommended to improve future dosing calculations. Natural sediment disturbance processes (e.g. bioturbation, wind induced sediment re-suspension) most likely caused the translocation of La down to sediment depths of 8 cm and these processes acted over a period of 7 months. Experimental studies indicated that vertical Phoslock® (and La) mixing reduced the efficiency of Phoslock® in controlling water-column TP concentrations. Under laboratory conditions, the Phoslock® P-binding capacity was estimated at 21,670 mg P kg^{-1} Phoslock®, with P being bound in release-sensitive (21%) and refractory fractions (79%). Applying Phoslock® is, therefore, likely to increase the amount of P permanently bound in sediments, even under reducing conditions. However, whole-lake experiments investigating sediment P-partitioning over longer time scales (> 1 month) are required (Chapter 5) to validate the experimental results.

Chapter 5

Assessing physicochemical changes in the bed sediments of a shallow lake following the application of Phoslock® II: Loch Flemington, UK

Assessing physicochemical changes in the bed sediments of a shallow lake following the application of Phoslock® II: Loch Flemington, UK

ABSTRACT

Phoslock®, a lanthanum (La) modified bentonite clay, is a lake remediation tool designed to reduce sediment phosphorus (P) release. This study investigated changes in sediment elemental composition and sediment P-partitioning based on sediment cores taken 8 and 5 months pre-application and 4, 7 and 12 months post-application of 170 g Phoslock® m^{-2} to a eutrophic lake (Loch Flemington, Inverness, UK) in March 2010. Additionally, a laboratory dosing experiment using intact sediment cores collected in month 16 post-application was used to assess the effectiveness of the applied dose on the control of sediment P-release under aerobic and anaerobic conditions. At the whole-lake scale sediment La content was significantly higher in the top 10 cm of the sediment 4 months after treatment while the sediment content of other elements commonly involved in P-binding and cycling including aluminium (Al), calcium (Ca), iron (Fe) and manganese (Mn) were not significantly different. Mass balance calculations indicated that the applied mass of La had the capability of binding 25% of potentially release-sensitive P (P_{mobile}; the sum of '*labile P*', '*reductant-soluble P*' and '*organic P*' fraction) present in the top 4 cm or 10% of P_{mobile} present in the top 10 cm of the sediment. Field results indicated that the applied dose equalled a 'low dose' compared to dose estimates aiming at controlling total sediment P_{mobile} content. Assessing variation in sediment P-partitioning indicated that the application caused a significant increase in the mass of P present in the more refractory '*apatite bound P*' fraction compared to P_{mobile}, with the largest mass of P measured in the '*apatite bound P*' fraction 12 months post-application. The laboratory experiment 16 months after treatment indicated that the original Phoslock® dose was sufficient to control sediment P-release under aerobic

conditions but that significant P-release may still occur should prolonged anaerobic conditions persist in Loch Flemington. However Phoslock® may be a viable option to control sediment P-release under anaerobic conditions which would require an estimated additional application of up to 75,000 kg of Phoslock®. A conceptual model is proposed for the use of P-capping agents in lake remediation projects, which is likely to increase cost-effectiveness and reduce non-target effects by applying multiple smaller doses compared to a single high dose.

INTRODUCTION

Cycling of phosphorus (P) between lake sediments and the water-column (internal P-loading) can delay the recovery of shallow lakes following reductions in external P-load (Marsden, 1989; Sas, 1989; Søndergaard *et al*., 2003; Jeppesen *et al*., 2005b). Various methods have been trialled to 'speed up' the recovery process by reducing internal P-loading (Cooke *et al*., 2005; Hupfer and Hilt, 2008; Hickey and Gibbs, 2009), including sediment removal (Hupfer and Hilt, 2008), hydraulic flushing (Hosper and Meyer, 1986), oxidation of the sediment surface (Hupfer and Hilt, 2008) and the application of active P-stripping/sediment P-capping agents (hereafter termed P-capping agents; Gibbs *et al*., 2011). P-capping agents are designed to increase the P-binding capacity of sediments in order to increase P-retention in bed sediments. To date, iron- (Fe; e.g. $FeSO_4$, $FeCl_3$; Perkins and Underwood, 2001), aluminium- (Al; e.g. $Al_2(SO_4)_3$, $AlCl_3$, Al modified zeolite; Welch and Cooke, 1999; Reitzel *et al*., 2005; Özkundakci *et al*., 2010), calcium (Ca; e.g. $CaCO_3$; Hupfer and Hilt, 2008) and lanthanum (La)-based products (e.g. Phoslock®; Gibbs *et al*., 2011; Meis *et al*., 2012; Chapter 4) have been used as P-capping agents. However, large uncertainty is involved in calculating the 'effective dose' (i.e. dose required to achieve site specific water quality targets) when using P-capping agents. It has been suggested that dosing relative to the amount of P_{mobile} (sum '*labile P*', '*reductant-soluble P*' and '*organic P*' fraction) in the sediment is the most appropriate approach (Reitzel *et al*., 2005; Vicente *et al*., 2008). Although P_{mobile} can be relatively accurately estimated using standard sequential extraction techniques (e.g. Psenner *et al*., 1988; Hupfer *et al*., 1995; Paludan and Jensen, 1995), the depth from which P_{mobile} cycles between the sediment and the water-column (hereafter termed 'active' sediment depth) is likely to be a site specific variable, since sediment P-release is determined by physicochemical conditions such as pH, redox, temperature, P-equilibrium concentrations, concentration gradients and wind induced

sediment re-suspension (Boström *et al*., 1982, 1988; Lukkari *et al*., 2007; Spears *et al*., 2012) and/or biological factors such as bioturbation, microbial activity, macrophyte cover and deposition of organic material to the sediment (Boström *et al*., 1982, 1988; Phillips *et al*., 1994; Stephen *et al*., 1997) that can vary seasonally and spatially (Spears *et al*., 2006). Common estimates of the 'active' sediment depth vary between the top 4 cm (Cooke *et al*., 2005) to the top 10 cm (Boström *et al*., 1982) but P-release has been reported from sediment depths of up to 25 cm (Søndergaard *et al*., 1999). Additionally, studies have shown that P stored in the '*reductant-soluble P*' fraction (which generally contributes to P_{mobile}) can in certain cases contribute to the permanent storage of P in the sediment (Kozerski and Kleeberg 1998) suggesting that estimates of the sediment P_{mobile} content are only a proxy for estimating required dose. Furthermore, other confounding factors exist that potentially influence the P-binding capacity of P-capping agents. In the case of Phoslock® these include a dependency of the P-binding capacity on pH (Ross *et al*., 2008; Haghseresht *et al*., 2009; Gibbs *et al*., 2011) and/or interference of humic substances (Ross *et al*., 2008) that might reduce availability of La, the active P-binding element in Phoslock®, for P-binding (Tang and Johannesson, 2003; Sonke and Salters, 2006; Tang and Johannesson, 2010). Finally the role of biological factors (e.g. macrophytes, benthic algae) on sediment P-release is not sufficiently understood with respect to their potential impact on P-cycling at the whole-lake scale (Stephen *et al*., 1997).

Using Phoslock® as an example, variation in dose estimates and/or remediation targets have lead to a variety of areal loads applied in lake remediation projects (range 170 – 590 g Phoslock® m^{-2}; Phoslock, 2012). Laboratory studies have indicated that areal loads above 200 g Phoslock® m^{-2} can cause a decrease in the depth of the aerobic sediment layer beneath the capping layer (Vopel *et al*., 2008) and temporary suppression of nitrification and denitrification processes (Gibbs *et al*., 2011). Studies

investigating effectiveness of Al-based P-capping agents highlighted that burial of the capping layer can reduce its ability to control sediment P-release (Lewandowski *et al*., 2003) and that ageing in the absence of P can decrease maximum adsorption capacity (Vicente *et al*., 2008). Both studies suggest that repeated doses are likely to increase effectiveness. Given uncertainties in calculating effective dose, potential non-target effects at high dose and potential advantages in adding multiple smaller doses, it is proposed that an initial 'low dose' followed by monitoring and, if required, calculation of a 'top-up' dose is likely to be the best approach using P-capping agents in terms of cost-effectiveness and avoidance of detrimental effects.

Study outline and hypotheses

This study investigated the effects of a 'low dose' Phoslock® application using sediment surveys conducted in Loch Flemington starting 8 months pre-application and continuing 12 months post-application to assess: (i) the effects of Phoslock® on sediment elemental composition and sediment P-binding properties; and (ii) variation in sediment P-partitioning. Subsequently, dosing experiments on intact sediment cores in the laboratory under aerobic and anaerobic conditions were used to assess: (iii) the effectiveness of the applied Phoslock® dose on the control of sediment P-release; and (iv) the effectiveness of additional application of Phoslock® on P-cycling. Based on the results, a conceptual model demonstrating the procedures for using P-capping agents in lake remediation projects was produced. The specific hypotheses tested were: (i) sediment La content will increase following a low dose Phoslock® application; (ii) the mass of P present in the '*apatite bound P*' fraction will increase following a low dose Phoslock® application; and (iii) Phoslock® reduces sediment P-release under aerobic and anaerobic conditions.

MATERIAL AND METHODS

Study site and dosing

A description of the study site is presented (Chapter 2, Section 2.1.2). At sediment P concentrations in excess of 2,500 mg P kg^{-1} dry weight (DW) sediment in Loch Flemington, as inferred from May *et al*. (2001), net annual sediment P-release is expected to occur for more than 5 years (Sas, 1989). Such estimates are based on the assumption that P can be washed out via an outflow (Sas, 1989). However Loch Flemington has no surface outflow, therefore impeding P flushing. As such, the estimated recovery time at Loch Flemington with no further management and assuming no increase in catchment P-loading is greater than 5 years. In order to reduce internal P-loading 25,000 kg of Phoslock® were applied to Loch Flemington as a slurry from a pontoon over a period of 3 days in March 2010. On the pontoon, lake water was pumped into a mixing chamber to which Phoslock® pellets were added to produce a slurry. The slurry was pumped to a spray manifold mounted to the front of the pontoon and applied to the water surface. The pontoon was a equipped with a GPS to facilitate an even coverage of the sediment bed with Phoslock®. Photographs of the application procedure are shown (Appendix: Fig. A5.1, A5.2). Based on a nominal La content of 50,000 mg La kg^{-1} Phoslock® (Afsar and Groves, 2009) and a molar binding ratio of La:P of 1:1 (Haghseresht *et al*., 2009) the applied amount of Phoslock® was designed to control 279 kg P.

Sample collection

Sediment cores (n = 5) were collected (Chapter 2, Section 2.2.2) at 5 permanent sample sites (Fig. 5.1c) 8 and 5 months pre-application (July 2009 and October 2009) and 4, 7 and 12 months post-application of Phoslock® (July 2010, October 2010 and March 2011).

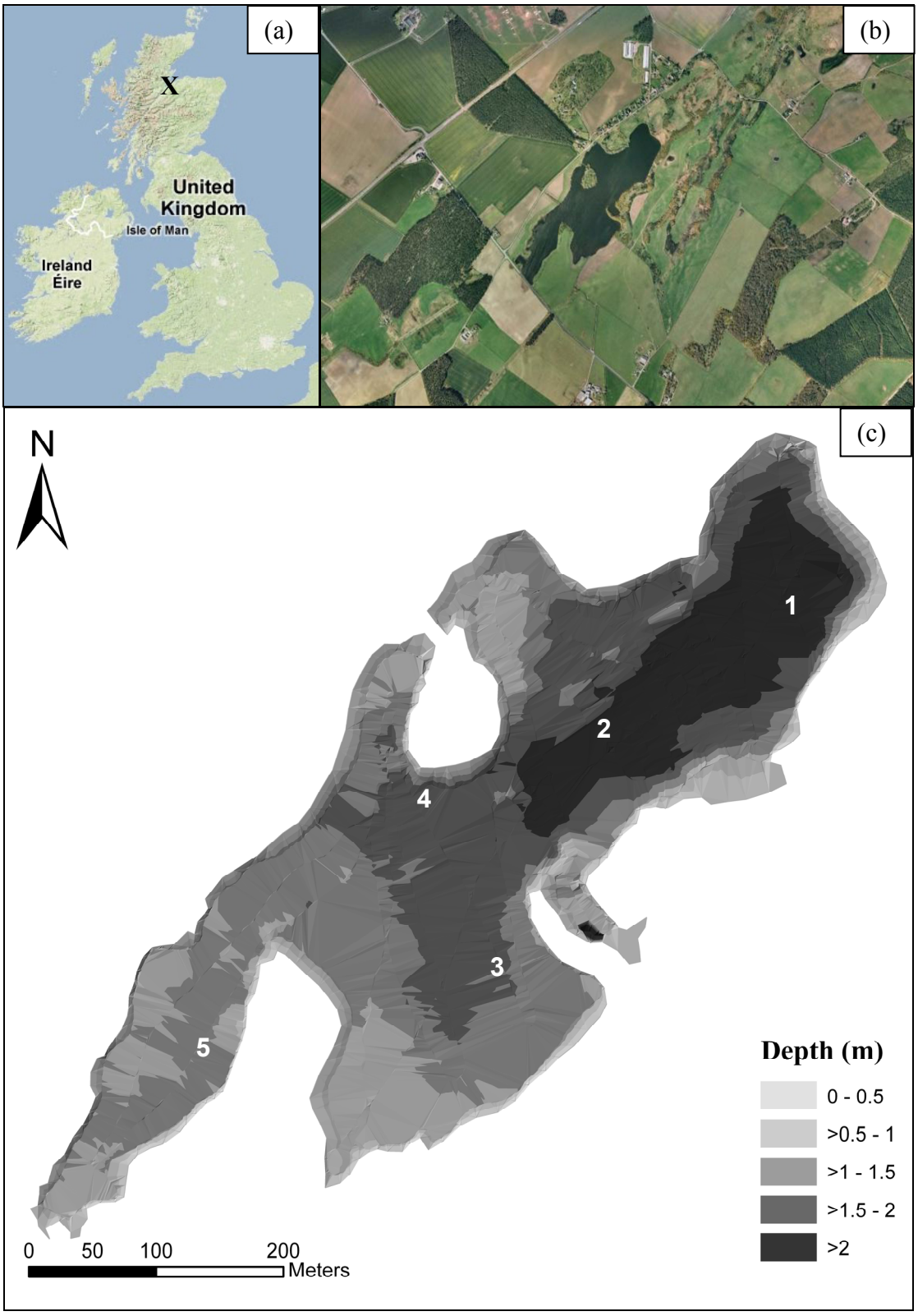


Figure 5.1 Maps showing (a) location of Loch Flemington (cross; Google Maps, 2013), (b) the surrounding area of Loch Flemington (Google Earth, 2013b), and (c) bathymetric map of Loch Flemington including the location of sample sites (numbers).

Sediment analysis

Analysis of sediment elemental composition (Chapter 2, Section 2.4.6) was conducted on sediment samples from all cores, except cores taken 12 months post-application, for each 2 cm depth interval over the top 10 cm of the core. Variation in sediment content of elements commonly involved in P-binding and cycling including Al, Ca, Fe, Mn (Boström *et al*., 1988; Søndergaard *et al*, 1996) and La are reported. Sediment P-fractionation (Chapter 2, Section 2.4.7) was conducted on sediment samples from all dates for each 2 cm depth interval over the top 10 cm of the core. The sum of the '*labile P*', '*reductant-soluble P*' and '*organic P*' fractions is termed P_{mobile} and is considered to represent the release-sensitive sediment P-pool (Boström *et al*., 1982; Søndergaard *et al*., 2003).

Mass balance calculations

Sediment P-partitioning

Mass balance estimates were based on lake area, sediment water content and sediment P content as defined by sequential P-extraction techniques. Mass of P on the whole-lake scale was calculated for each sample site and sediment depth interval (1 cm intervals) based on the assumption that 1 g wet weight (WW) equals 1 cm^3 by:

$$M_P = (A * M_{DW/WW}) * M_{Fraction}$$

where M_P equals mass of P on the whole-lake scale (kg), A equals lake area (cm^2), $M_{DW/WW}$ equals mass DW sediment per mass WW (g DW g^{-1} WW) and $M_{Fraction}$ equals mass of P in a given fraction per DW sediment (kg P g^{-1} DW). Consequently the sum of M_P was calculated for the top 4 cm and top 10 cm for each sample point individually before calculating the mean for each depth (n = 5). Mass balance estimates were made for the top 4 cm and top 10 cm of the sediment as these depths are common estimates of the 'active' sediment depth from which P_{mobile} cycles between the sediment and the

water-column (Boström *et al*., 1982; Cooke *et al*., 2005). Comparisons of sediment P content estimates using wet density (as outlined by Jensen *et al*., 1995 based on DW and loss on ignition) versus the assumption that 1 g WW equals 1 cm^3 indicated differences in P content estimates of less than 3% underestimation (data not shown). In the absence of loss on ignition values for cores taken 12 months post-application, all mass balance estimates were calculated based on the assumption that 1 g WW equals 1 cm^3.

Sediment P-binding capacity

Mass balance estimates were based on lake area, sediment water content, sediment La and sediment P_{mobile} content. Calculations were based on the assumption that 1 g WW of sediment equals 1 cm^3. Mass of La on the whole-lake scale was calculated as described for P above by exchanging mass of P per DW sediment (kg P g^{-1} DW) with mass of La per DW (kg La g^{-1} DW). Average P_{mobile} content was calculated for the pre-application period based on mean sediment P_{mobile} content of cores taken 8 and 5 months pre-application. Average sediment La content was calculated for the post-application period based on mean sediment La content of cores taken 4 and 7 months post-application.

Phoslock® dosing experiment

Sample collection and experimental set-up

Sediment cores (n = 20) were taken 16 months post-application (July 2011) from a depth of 2.7 m from Site 1 (Fig. 5.1c). Cores were collected from a boat using a Jenkin corer (core internal diameter 66 mm, core length 500 mm) that facilitated the collection of an undisturbed sediment-water interface, with each core consisting of approximately 21 cm sediment core depth with 23 cm overlying water-column depth. Assuming a relative uniform Phoslock® coverage during application in Loch Flemington in March 2010, each core had been previously subjected to an areal load of 170 g Phoslock® m^{-2}.

Cores were transported to the laboratory in the dark within 4 h of collection. In the laboratory cores were left overnight at ambient lake temperature (18 ºC) in the dark. The next morning cores were randomly assigned to four treatments (each n = 5): i) bubbled with air (termed 'aerobic control'); ii) bubbled with air and treated with Phoslock® ('aerobic dosed'); iii) bubbled with oxygen free N_2 ('anaerobic control'); and iv) bubbled with oxygen free N_2 and treated with Phoslock® ('anaerobic dosed'). Core incubations were conducted over a period of 29 days at 18 ºC in the dark. Measurements of water-column dissolved oxygen (DO) concentration, pH and conductivity were conducted weekly, 15 cm above the sediment surface using a HACH multi-parameter meter (HQ30d, HACH Lange GmbH, Düsseldorf, Germany). The first measurement (day 0) was taken after leaving the cores under ambient temperatures overnight and before the onset of air and N_2-bubbling. Water samples (15 mL) for the analysis of soluble reactive phosphorus (SRP; Chapter 2, Section 2.4.4) and total phosphorus (TP; Chapter 2, Section 2.4.4) were taken 5 cm above the sediment surface on a bi-weekly basis using a syringe. Water removed during sampling was replaced with filtered (Whatman GF/C; nominal pore size 1.2 µm) surface water collected at the same location as the cores and stored at 18 ºC in the dark. The average total soluble phosphorus (TSP; Chapter 2, Section 2.4.4) concentration of water used for replacement was 16 µg L^{-1}.

Dosing

Dosing was conducted weekly, following measurements (pH, conductivity and DO concentration) and sampling (TP and SRP), by applying 0.58 g Phoslock® (equivalent to 170 g Phoslock® m^{-2}) as a slurry to the treatments aerobic dosed and anaerobic dosed. Air and N_2-bubbling was omitted for 24 h after dosing in all cores to allow Phoslock® to

settle. Sediment cores of the treatments anaerobic control and anaerobic dosed were sealed with rubber bungs during this period to minimise aeration.

Assessment of Phoslock® layer

On days 22 and 29 a visual assessment of the area of the Phoslock® layer covered with sediment was made in cores of the aerobic dosed and anaerobic dosed treatments and expressed as percentage cover.

Statistical analyses

Minitab 16 (Minitab® 16.1.1, Minitab Ltd., Coventry, UK) was used for all statistical analyses. Data sets of: i) sediment elemental composition; ii) mass balance estimates (mass of P in sediment P-fractions over the top 4 cm and top 10 cm of the sediment); and iii) water-column pH, conductivity, DO, TP and SRP concentration (dosing experiment) were not normally distributed (Anderson-Darling test, $\alpha < 0.05$) even after transformation (including $x' = \log(x)$, $x' = \ln(x)$, $x' = \text{sqrt}(x)$, $x' = x^2$). Therefore a non-parametric Kruskal-Wallis (KW) test was used to: i) test for significant variation in sediment elemental composition between months (July 2009, October 2009, July 2010, October 2010) for each depth interval; ii) test for significant variation in the mass of P in a sediment P-fraction between months (July 2009, October 2009, July 2010, October 2010, March 2011); and iii) test for significant variation in a given parameter between treatments for each day of the experiment. Where significant variation was evident (KW test, $\alpha < 0.05$), a non-parametric Mann-Whitney U-test (MWU) was used: i) to determine, for each depth interval, between which consecutive months (July 2009 vs. October 2009; October 2009 vs. July 2010; July 2010 vs. October 2010) significant differences in sediment elemental composition occurred; ii) to determine between which months significant differences in the mass of P in a sediment P-fraction occurred; and

iii) to determine between which treatments significant differences in a given parameter occurred.

Additionally a non-parametric MWU test was used: i) to compare TP and SRP concentrations between day 0 and other days of the dosing experiment individually and for each treatment separately; and ii) to test for significant differences in percentage cover of the Phoslock® layer with sediment between cores of the aerobic dosed and anaerobic dosed treatment on day 22 and day 29 in the dosing experiment.

Results of statistical analyses are presented in the text except in cases in which this would result in a disproportional long statistical result sections. In such cases only sample size (n) and p-values are reported followed by a cross reference to the relevant appendix section where full results of statistical analyses are shown.

RESULTS

Mass balance estimates

Mass balance estimates (Table 5.1) extrapolating to the whole-lake scale indicated that 829 kg P_{mobile} was contained in the top 4 cm of the sediment pre-application and 2,115 kg P_{mobile} was contained in the top 10 cm. Mass balance estimates also indicated that 914 kg La were added to the sediment. Based on a molar binding ratio of La:P of 1:1 (Haghseresht *et al*., 2009) and assuming all measured La is available for P-binding, the added amount of La can bind 204 kg P. It was, therefore, estimated that the applied mass of La would be sufficient to bind around 25% of P_{mobile} in the top 4 cm or around 10% of P_{mobile} in the top 10 cm of the sediment. Based on a nominal La content of Phoslock® of 50,000 mg La kg^{-1} Phoslock® (Afsar and Groves, 2009) it was calculated that the added mass of La equalled the addition of a total mass of 18,280 kg Phoslock® compared to the applied 25,000 kg Phoslock®.

Table 5.1 Calculated sediment mobile phosphorus content (P_{mobile}) and sediment lanthanum (La) content in Loch Flemington. Based on a molar La:P-binding ratio of 1:1, the added amount of 914 kg La can bind 204 kg P. Pre-application P_{mobile} content was based on the average P_{mobile} content in month 8 (July 2009) and 5 (October 2009) pre-application, while the post-application La content was based on the average sediment La content in month 4 (July 2010) and 7 (October 2010) post-application.

Sediment depth (cm)	Pre-application P_{mobile} accumulative (kg)	Pre-application P_{mobile} accumulative (%)	Post-application La content accumulative (kg)	Potential control of pre-application P_{mobile} by total La content (%)
top 2	340	16	436	60
top 4	**829**	**39**	**650**	**25**
top 6	1,276	60	785	16
top 8	1,722	81	863	12
top 10	**2,115**	**100**	**914**	**10**

Changes in sediment elemental composition

This section only refers to sediment depths layers in which significant variation in elemental composition of an element was detected. Pre-application sediment La content (Table 5.2; Fig. 5.2) ranged from 17.4 to 20.9 mg La kg^{-1} DW sediment. Sediment La content was significantly higher in the top 10 cm of the sediment four months post-application ($W = 15$; $p < 0.05$; $n_1 = n_2 = 5$) compared to pre-application conditions, ranging from 197 to 3,469 mg La kg^{-1} DW sediment (Table 5.2; Appendix: Table A5.1). Sediment La content decreased with increasing sediment depth (Table 5.2; Fig. 5.2). In comparison there was no significant difference in the other P-binding and cycling elements including Al, Ca, Fe and Mn between pre-application month 5 and post-application month 4. Sediment Ca content (Table 5.2; Appendix: Table A5.1) decreased significantly in the 4 – 6 cm ($W = 39$; $p < 0.05$; $n_1 = n_2 = 5$) and 6 – 8 cm ($W = 40$; $p < 0.05$; $n_1 = n_2 = 5$) sediment depth layer between post-application month 4 and 7.

Table 5.2 Mean sediment content (n = 5) of calcium (Ca) and lanthanum (La) expressed as mg kg^{-1} dry weight sediment in Loch Flemington pre- (July 2009, October 2009) and post-application (July 2010, October 2010) of Phoslock®. Comparisons were made pre-application (July 2009 vs. October 2009), pre- vs. post-application (October 2009 vs. July 2010) and post-application (July 2010 vs. October 2010) using a non-parametric Mann-Whitney U-test (MWU). Variation is expressed as percentage change (% change). Significant differences are marked (*, $p < 0.05$; **, $p < 0.01$).

Element	Depth	Sediment content				MWU and % change		
		Jul 09	Oct 09	Jul 10	Oct 10	Jul. 09 vs. Oct. 09	Oct. 09 vs. Jul. 10	Jul. 10 vs. Oct. 10
Ca	0-2	18,125	15,836	18,548	15,910	-13	17	-14
	2-4	14,630	15,994	15,986	14,141	9	0	-12
	4-6	14,200	15,420	15,920	13,888	9	3	-13*
	6-8	14,304	15,975	15,563	13,447	12	-3	-14*
	8-10	15,463	15,399	14,970	14,115	0	-3	-6
La	0-2	19.9	17.4	3,469	2,387	-13	19,787*	-31
	2-4	20.9	18.3	1,335	1,010	-13*	7,200*	-24
	4-6	19.2	18.7	669	594	-3	3,470*	-11
	6-8	19.9	19.2	329	368	-4	1,615*	12
	8-10	20.9	19.5	197	213	-7	909*	9

Figure 5.2 Sediment lanthanum content as means (n = 5) of all sample sites 8 (July 2009; black bars) and 5 (October 2009; white bars) months pre- and 4 (July 2010; grey bars) and 7 (October 2010; white hatched bars) months post-application in Loch Flemington. Error bars represent standard error of the mean (n = 5) and significant differences between consecutive months are marked with asterisks (non-parametric Mann-Whitney U-test; *, $p < 0.05$). Note log scale on the x-axis.

Changes in sediment P-partitioning

Variation in sediment P-fractions (Fig. 5.3; Appendix: Table A5.2, A5.3) showed similar trends in the top 4 cm and top 10 cm depth layers. This is with the exception of variation in the '*metal oxide adsorbed P*' fraction (Fig. 5.3g, h) in which the mass of P present varied significantly ($H = 12.30$; $p < 0.05$; $DF = 4$; $n = 5$) between months over the top 10 cm sediment but not when considering the top 4 cm. Pre- and post-application, the largest amount of P was present in the '*reductant-soluble P*' and the '*organic P*' fractions (Fig. 5.3c-f).

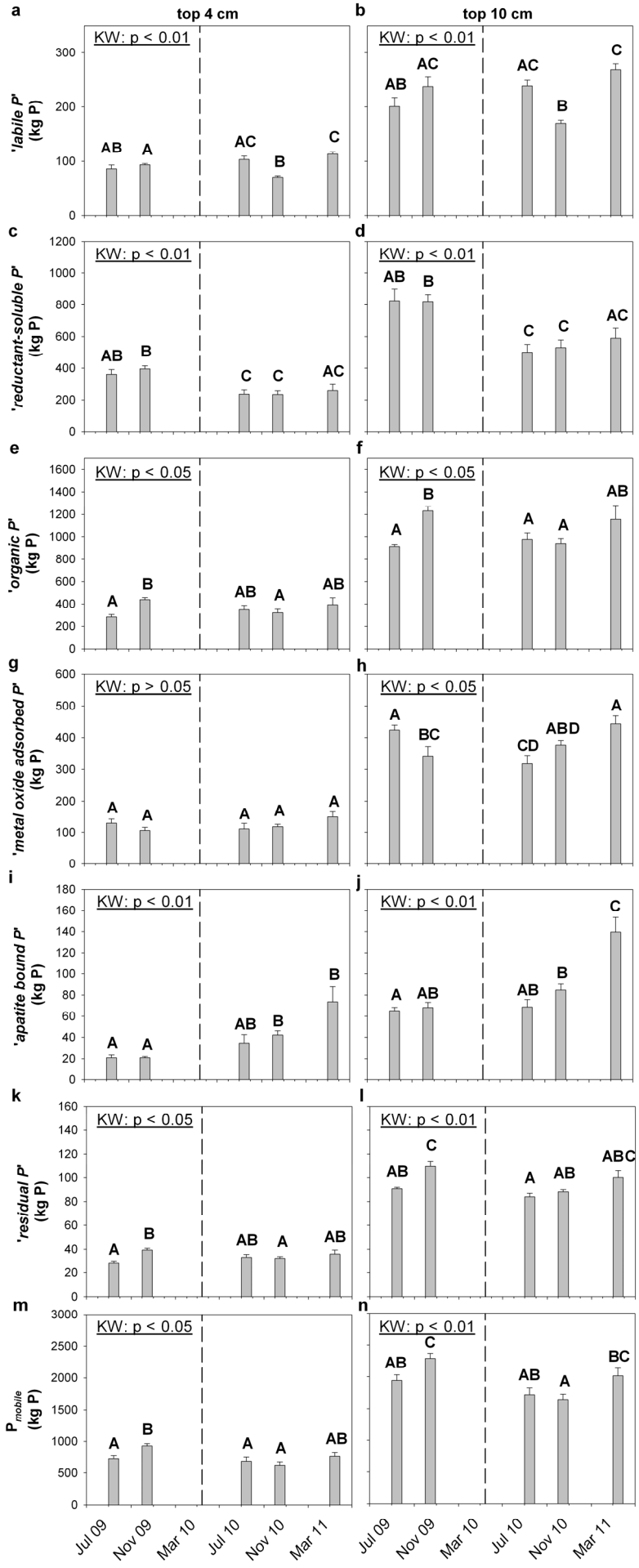

a
top 4 cm
b
top 10 cm
KW: p < 0.01
'labile P' (kg P)
c
d
KW: p < 0.01
'reductant-soluble P' (kg P)
e
f
KW: p < 0.05
'organic P' (kg P)
g
h
KW: p > 0.05
KW: p < 0.05
'metal oxide adsorbed P' (kg P)
i
j
KW: p < 0.01
'apatite bound P' (kg P)
k
l
KW: p < 0.05
KW: p < 0.01
'residual P' (kg P)
m
n
KW: p < 0.05
KW: p < 0.01
P mobile (kg P)
Jul 09
Nov 09
Mar 10
Jul 10
Nov 10
Mar 11

Figure 5.3 (p. 126) Variation in estimated mass of sediment phosphorus (P) in the top 4 and top 10 cm of the sediment across sediment P-fractions in Loch Flemington, including (a, b) '*labile P*', (c, d) '*reductant-soluble P*', (e, f) '*organic P*', (g, h) '*metal-oxide adsorbed P*', (i, j) '*apatite bound P*', (k, l) '*residual P*', and (m, n) P_{mobile}. Error bars represent standard error of the mean (n = 5). The dashed vertical line indicates the timing of the Phoslock® application (March 2010). Results of non-parametric Kruskal Wallis (KW) test (p-values) are underlined. Groups that do not share the same symbol (A, B, C) are significantly different (non-parametric Mann-Whitney U-test; $p < 0.05$).

The mass of P present in the '*apatite bound P*' fraction (Fig. 5.3i, j) increased over time post-application and was significantly higher 7 months post-application ($p < 0.05$; $n_1 = n_2 = 5$; Appendix: Table A5.3) compared to pre-application conditions. The mass of P present in the '*apatite bound P*' fraction in the top 10 cm of the sediment increased significantly ($W = 15$; $p < 0.05$; $n_1 = n_2 = 5$) from 67 kg P pre-application to 139 kg P in post-application month 12. The mass of P_{mobile} (Fig. 5.3m) increased significantly ($W = 15$; $p < 0.05$; $n_1 = n_2 = 5$) between pre-application months 8 and 5, while no significant variation was detected in the mass of P_{mobile} in the top 4 cm between post-application months.

Phoslock® dosing experiment

After day 5, water-column DO concentrations (Table 5.3; Fig. 5.4) were significantly lower ($W = 40$; $p < 0.05$; $n_1 = n_2 = 5$) in the anaerobic control and anaerobic dosed treatments compared to the aerobic control and aerobic dosed treatments. In contrast, water-column pH (Table 5.3; Fig. 5.4) was significantly higher ($W = 15$; $p < 0.05$; $n_1 = n_2 = 5$) in the anaerobic control and anaerobic dosed treatments compared to the aerobic control and aerobic dosed treatment following day 5. The pH did not vary significantly between aerobic control and aerobic dosed treatments (except on day 22; $W = 15$; $p < 0.05$; $n_1 = n_2 = 5$) or between anaerobic control and anaerobic dosed treatments. Conductivity (Table 5.3; Fig. 5.4) did not vary significantly between anaerobic control and anaerobic dosed treatments, whereas conductivity in the aerobic dosed treatment was significantly higher ($W = 15$; $p < 0.05$; $n_1 = n_2 = 5$) than the aerobic control following day 22. Additionally conductivity in the aerobic dosed treatment was significantly higher ($p < 0.05$; $n_1 = n_2 = 5$; Appendix: Table A5.4) compared to anaerobic control and anaerobic dosed treatments after day 5.

Temporal variation in water-column TP and SRP concentrations (Table 5.4; Fig. 5.5) showed a significant continuous increase ($W = 15$; $p < 0.05$; $n_1 = n_2 = 5$) in both TP and SRP concentrations in the anaerobic control treatment between day 0 and day 29, whereas no such pattern was observed in the other treatments. TP concentrations (Table 5.5) did not vary significantly between aerobic control and aerobic dosed treatments, except on day 29 when TP concentration was significantly higher ($W = 40$; $p < 0.05$; $n_1 = n_2 = 5$) in the aerobic control. In contrast, TP concentrations in the anaerobic control treatment significantly exceeded ($p < 0.05$; $n_1 = n_2 = 5$; Appendix: Table A5.6) TP concentrations of all other treatments from day 5 onwards.

Table 5.3 Results of non-parametric Mann-Whitney U-test (MWU) investigating variation in water-column dissolved oxygen (DO) concentrations, pH and conductivity (cond.) between treatments. Treatments included aerobic control (A), aerobic dosed (B), anaerobic control (C) and anaerobic dosed (D). A non-parametric MWU test was conducted where significant variation between treatments was evident (Kruskal Wallis (KW) test, $\alpha < 0.05$). In cases where KW test $\alpha > 0.05$ this was marked with 'n/a' (not applicable). Significant differences are marked (*, $p < 0.05$; **, $p < 0.01$), while n.s. denotes $p \geq 0.05$.

Parameter	Day	MWU					
		A vs. B	A vs. C	A vs. D	B vs. C	B vs. D	C vs. D
DO	0	n/a	n/a	n/a	n/a	n/a	n/a
	5	n.s.	*	*	*	*	n.s.
	8	n.s.	*	*	*	*	n.s.
	15	n.s.	*	*	*	*	n.s.
	22	*	*	*	*	*	n.s.
	29	n.s.	*	*	*	*	n.s.
pH	0	n.s.	*	*	n.s.	*	n.s.
	5	n.s.	*	*	*	*	n.s.
	8	n.s.	*	*	*	*	n.s.
	15	n.s.	*	*	*	*	n.s.
	22	*	*	*	*	*	n.s.
	29	n.s.	*	*	*	*	n.s.
cond.	0	n/a	n/a	n/a	n/a	n/a	n/a
	5	n.s.	n.s.	n.s.	*	*	n.s.
	8	n.s.	n.s.	n.s.	*	*	n.s.
	15	n.s.	n.s.	n.s.	*	*	n.s.
	22	*	n.s.	n.s.	*	*	n.s.
	29	*	n.s.	n.s.	*	*	n.s.

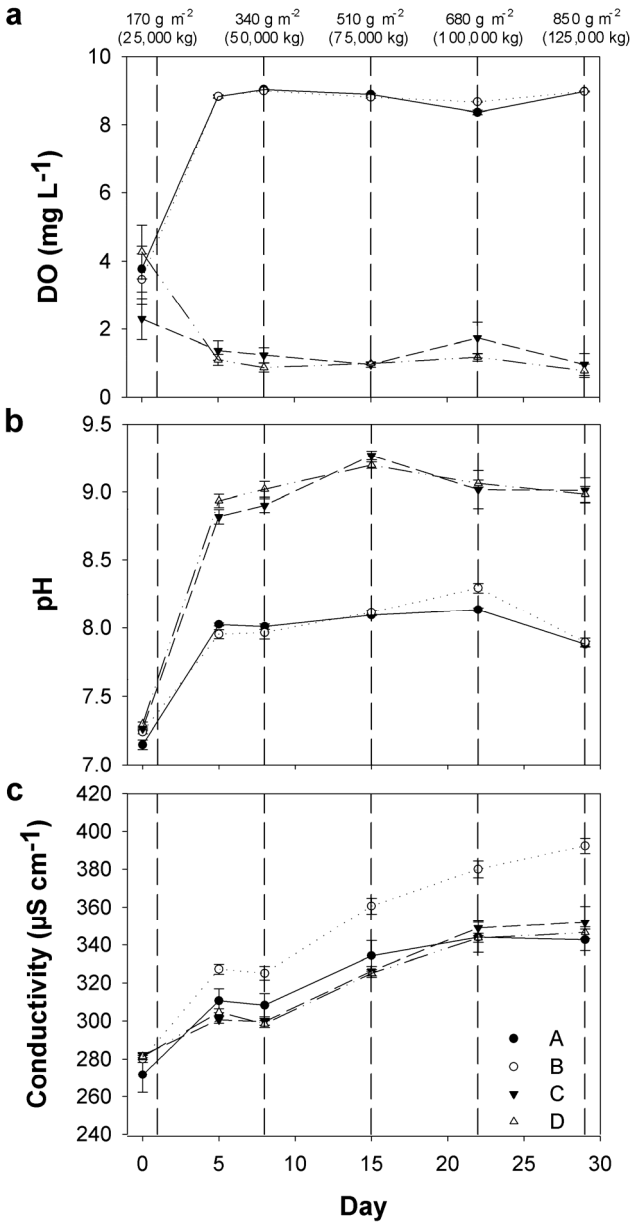


Figure 5.4 Variation in water-column dissolved oxygen (DO) concentration (a), pH (b) and conductivity (c) in sediment core experiment, including aerobic control (A; black circles), aerobic dosed (B; open circles), anaerobic control (C; black triangles) and anaerobic dosed (D; open triangles). Error bars represent standard error of the mean (n = 5) and vertical dashed lines indicate timing of Phoslock® dosing. Note all cores were taken 16 months post application of Phoslock® at Loch Flemington, therefore representing an initial areal load of 170 g Phoslock® m^{-2}. Superimposed are the equivalent additional areal load (g m^{-2}) and the equivalent additional Phoslock® dose for Loch Flemington (kg).

When comparing day 0 and day 29, TP concentrations (Table 5.4) did not vary significantly in aerobic control, while TP concentrations decreased significantly in aerobic dosed ($W = 40$; $p < 0.05$; $n_1 = n_2 = 5$) and anaerobic dosed treatments ($W = 39$; $p < 0.05$; $n_1 = n_2 = 5$), whereas a significant increase ($W = 15$; $p < 0.05$; $n_1 = n_2 = 5$) was detected in anaerobic control. In cores of the anaerobic control treatment, SRP represented 15% of TP at the start and 82% of TP at the end of the experiment. SRP concentrations (Table 5.5) did not vary significantly between aerobic control and aerobic dosed treatments except on days 15 ($W = 40$; $p < 0.05$; $n_1 = n_2 = 5$) and 22 ($W = 38$; $p < 0.05$; $n_1 = n_2 = 5$) when SRP concentrations were significantly higher in the aerobic control treatment. In contrast, SRP concentrations in the anaerobic control significantly exceeded ($p < 0.05$; $n_1 = n_2 = 5$; Appendix: Table 5.6) SRP concentrations of all other treatments from day 5 onwards. SRP concentrations (Table 5.4) increased significantly ($W = 15$; $p < 0.05$; $n_1 = n_2 = 5$) in all treatments when comparing day 0 and day 29.

Visual inspections of the Phoslock® layer in both the aerobic dosed and the anaerobic dosed cores indicated partial coverage with organic sediments over the course of the experiment (Fig. 5.6). The percentage cover of the Phoslock® layer by sediment was significantly higher in the aerobic dosed compared to the anaerobic dosed treatment (Fig. 5.6) on day 22 (59% vs. 13%; $W = 40$; $p < 0.05$; $n_1 = n_2 = 5$) and day 29 (61% vs. 13%; $W = 38.5$; $p < 0.05$; $n_1 = n_2 = 5$).

Table 5.4 Variation in water-column total phosphorus (TP) and soluble reactive phosphorus (SRP) concentrations between day 0 and all other days (each n = 5) of the experimental period as indicated by non-parametric Mann-Whitney U-test. Treatments included aerobic control (A), aerobic dosed (B), anaerobic control (C) and anaerobic dosed (D). Significant differences are marked (*, $p < 0.05$), while n.s. denotes $p \geq 0.05$.

Variable	Treatment	Day	5	8	12	15	19	22	26	29
TP	A	0	n.s.	*	n.s.	n.s.	n.s.	n.s.	*	n.s.
		% change	-22	-34	-29	-28	-13	-26	-33	-13
	B	0	n.s.	n.s.	n.s.	n.s.	n.s.	*	*	*
		% change	-12	-20	-19	-18	2	-35	-34	-42
	C	0	*	*	*	*	*	*	*	*
		% change	60	140	172	256	317	280	339	379
	D	0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	*
		% change	-2	-9	-31	-25	-7	-28	-33	-36
SRP	A	0	n.s.	n.s.	n.s.	*	*	*	*	*
		% change	56	116	130	171	263	402	314	296
	B	0	n.s.	n.s.	n.s.	*	*	*	*	*
		% change	50	120	93	83	308	419	429	407
	C	0	*	*	*	*	*	*	*	*
		% change	221	608	968	1367	1824	1741	2163	2604
	D	0	n.s.	n.s.	n.s.	n.s.	n.s.	*	*	*
		% change	-18	32	50	2	26	324	392	345

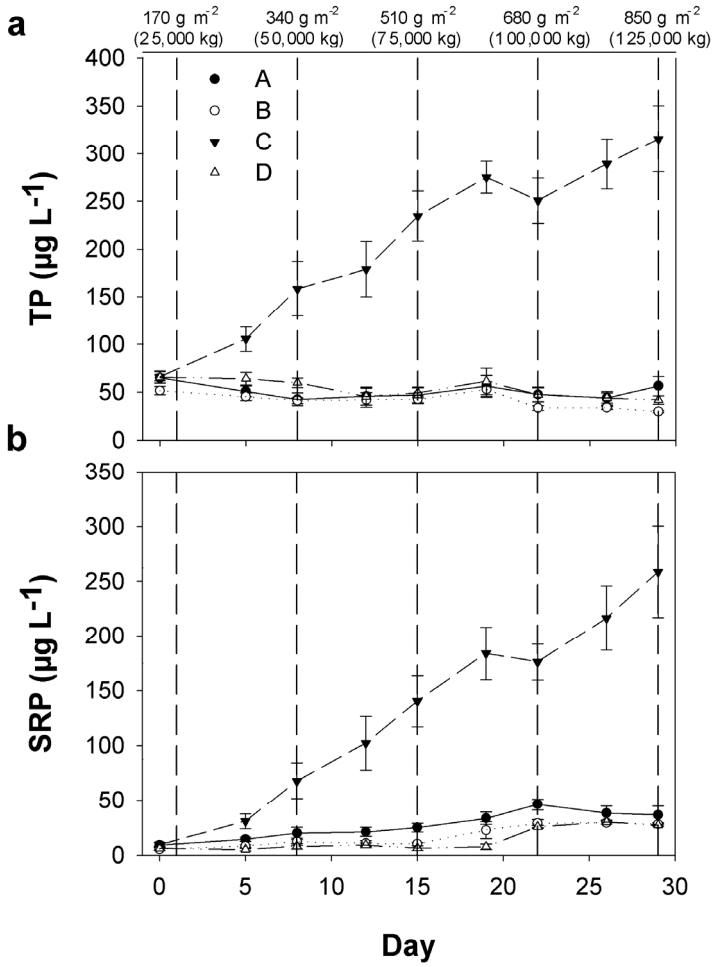


Figure 5.5 Variation in water-column total phosphorus (TP) concentration (a) and soluble reactive phosphorus (SRP) concentration (b) in sediment core experiment, including aerobic control (A; black circles), aerobic dosed (B; open circles), anaerobic control (C; black triangles) and anaerobic dosed (D; open triangles). Error bars represent standard error of the mean (n = 5) and vertical dashed lines indicate timing of Phoslock® dosing. Note all cores were taken 16 months post application of Phoslock® at Loch Flemington, therefore representing an initial areal load of 170 g Phoslock® m^{-2}. Superimposed are the equivalent additional areal load (g m^{-2}) and the equivalent additional Phoslock® dose for Loch Flemington (kg).

DISCUSSION

This study highlighted that a low dose Phoslock® application was sufficient to cause significant alterations in sediment elemental composition and sediment P-binding capacity. In particular sediment La content increased significantly while the sediment content of other elements commonly involved in P-binding and P-cycling was not significantly different between pre- and post-application months. Field observations showed that the mass of P present in the more refractory '*apatite bound P*' fraction increased significantly over time post-application whereas laboratory experiments indicated that Phoslock® can significantly reduce sediment P-release under aerobic and anaerobic conditions. Observed alterations in sediment P-partitioning *in situ* and laboratory experiments indicated that Phoslock® can be a useful measure to reduce internal P-loading by increasing the mass of P permanently bound in the sediment even under anaerobic conditions.

Effects on sediment elemental composition and sediment P-binding properties: classification of dose

The application of Phoslock® increased the sediment P-binding capacity due to an increase in La-P-binding sites post-application. Similar changes have been observed in Clatto Reservoir (Dundee, UK), where sediment La content was significantly higher in the top 8 cm of the sediment one month post-application of 270 g Phoslock® m^{-2} (Meis *et al*., 2012; Chapter 4). The measured mass of La in the sediment in Loch Flemington equalled the addition of 18,280 kg of Phoslock®. The difference of 6,720 kg to the applied 25,000 kg of Phoslock® may result from: i) a proportion of the applied Phoslock® persisting in the water-column during the sampling period (range 4 to 68 µg La L^{-1}; average 16 µg La L^{-1} measured during monthly post-application sampling; Chapter 6); ii) variation in the nominal La content; and/or iii) low spatial resolution La

measurements in a complex lake system resulting in error in the mass balance estimates. However, the added amount of La should be sufficient to bind up to 204 kg P. Consequently, the measured amount of La in the sediment post-application would be sufficient to control 25% of P_{mobile} in the top 4 cm and 10% of P_{mobile} in the top 10 cm. Reitzel *et al*. (2005; Lake Sønderby, Denmark) successfully reduced internal P-loading for at least two years using an Al-based P-capping agent with dose estimates based on P_{mobile} in the upper 10 cm of sediment. In comparison to the P-binding potential of the masses of product applied in other studies, the mass of Phoslock® applied to Loch Flemington can be considered a low dose.

Vertical sediment La distribution indicated that La (and Phoslock®) was subjected to vertical sediment transport processes which may include wind induced sediment re-suspension (Hilton *et al*., 1986; Douglas and Rippey, 2000) and/or bioturbation (Fischer *et al*., 1980; Meysman *et al*., 2006). Similar distribution patterns have been observed in Clatto Reservoir (Meis *et al*., 2012; Chapter 4). Therefore, the conceptual framework in which P-capping agents form a distinct surface layer which intercepts upwards diffusing P following release events may have to be adapted for shallow lakes. The vertical translocation of La (and Phoslock®) implies that La might be transported below the 'active' sediment depth from which cycling of P_{mobile} between sediment and the water-column is expected. Studies suggest that burial of alum reduces its ability to control sediment P-release (Lewandowski *et al*., 2003), which has also been observed for burial of Phoslock® (Chapter 4). The impact of vertical La translocation on the efficiency of controlling sediment P-release and water-column P concentrations therefore requires further investigation to improve calculations of effective dose in the future.

Table 5.5 Variation in water-column total phosphorus (TP) and soluble reactive phosphorus (SRP) concentrations between treatments, including aerobic control (A), aerobic dosed (B), anaerobic control (C) and anaerobic dosed (D). A non-parametric MWU test was conducted where significant variation between treatments was evident (Kruskal Wallis (KW) test, $\alpha < 0.05$). In cases where KW test $\alpha > 0.05$ this was marked with 'n/a' (not applicable). Significant differences are marked (*, $p < 0.05$; **, $p < 0.01$), while n.s. denotes $p \geq 0.05$.

Parameter	Day	MWU					
		A vs. B	A vs. C	A vs. D	B vs. C	B vs. D	C vs. D
TP	0	n/a	n/a	n/a	n/a	n/a	n/a
	5	n.s.	*	n.s.	*	n.s.	*
	8	n.s.	*	n.s.	*	*	*
	12	n.s.	*	n.s.	*	n.s.	*
	15	n.s.	*	n.s.	*	n.s.	*
	19	n.s.	*	n.s.	*	n.s.	*
	22	n.s.	*	n.s.	*	n.s.	*
	26	n.s.	*	n.s.	*	n.s.	*
	29	*	*	n.s.	*	*	*
SRP	0	n/a	n/a	n/a	n/a	n/a	n/a
	5	n.s.	*	*	*	n.s.	*
	8	n.s.	*	n.s.	*	n.s.	*
	12	n.s.	*	n.s.	*	n.s.	*
	15	*	*	*	*	n.s.	*
	19	n.s.	*	*	*	n.s.	*
	22	*	*	*	*	n.s.	*
	26	n.s.	*	n.s.	*	n.s.	*
	29	n.s.	*	n.s.	*	n.s.	*

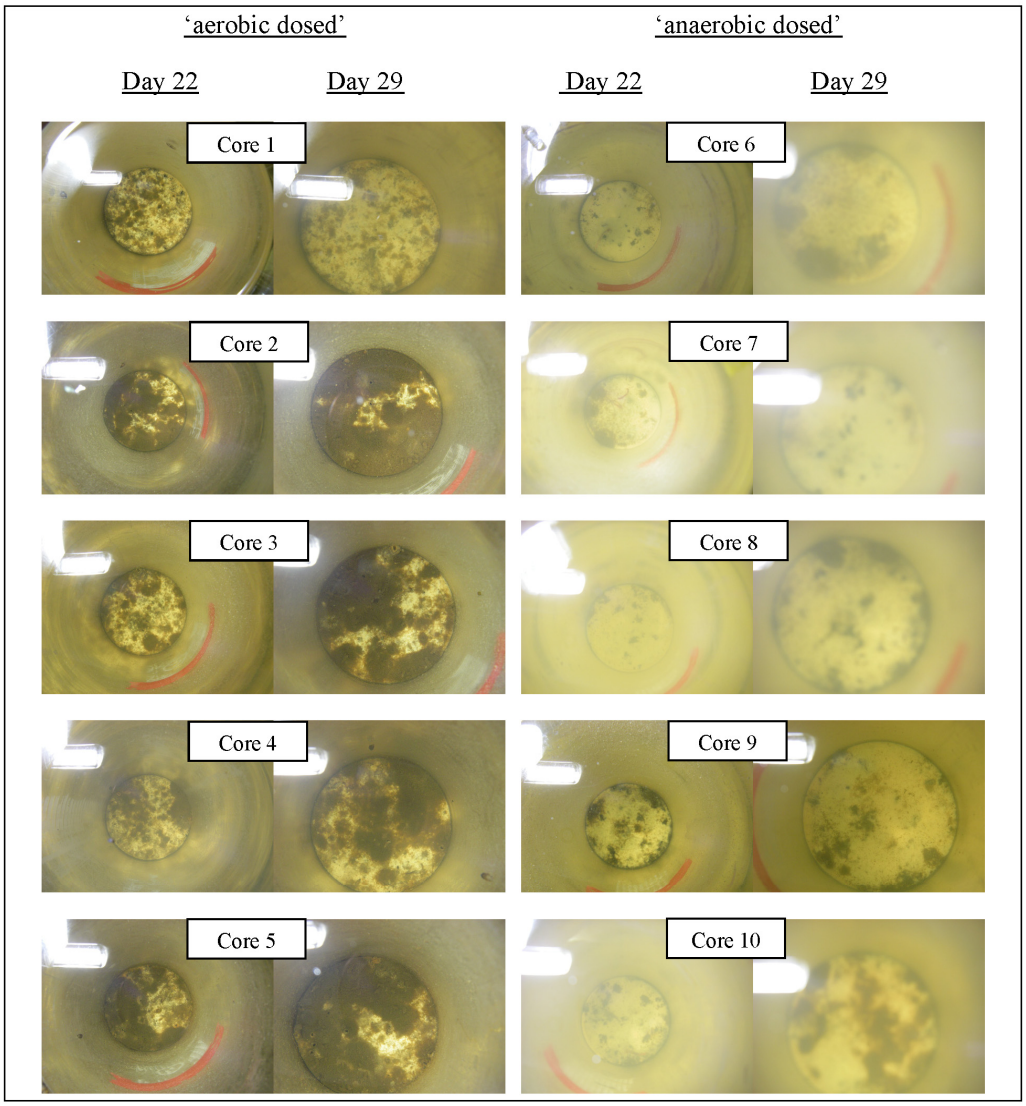


Figure 5.6 Phoslock® layer covered by sediment on days 22 and 29 in aerobic dosed and anaerobic dosed treatments.

Changes in sediment P-partitioning across operational fractions following Phoslock® application

The significant increase in sediment P_{mobile} content between pre-application month 8 (July) and 5 (October) was driven by a significant increase in the amount of P present in the '*organic P*' fraction, since no significant variation was detected in the '*reductant-soluble P*' and '*labile P*' fractions (which also contribute to P_{mobile}) over this period. The '*organic P*' fraction represents P in allochthonous/autochthonous material and detritus (Boström *et al*., 1988) and it is hypothesised that settling phytoplankton and/or material

from decomposing macrophytes caused the significant increase in the mass of P stored in this fraction in the autumn sample. This is supported by observations that phytoplankton biomass (measured as chlorophyll *a* concentration) peaked between pre-application month 8 and 7, and had sharply decreased by a factor of 10 by pre-application month 6 (Chapter 6). Post-application, no significant variation was found in the amount of P present in the P_{mobile} fraction in the top 4 cm of the sediment, indicating that no major P-release/settling events occurred. The observed pattern raises the question: which sediment P-partitioning processes prevented significant variation in sediment P_{mobile} content in the top 4 cm of the sediment post-application? Of all sediment P-fractions investigated only the amount of P present in the '*apatite bound P*' fraction showed a significant increase over time. Experimental work has shown that around 60% of P bound by Phoslock® was extracted from the '*apatite bound P*' fraction (Meis *et al*., 2012; Chapter 4), the binding capacity of which is expected to increase following the application of Phoslock®. However, no significant increase in the '*apatite bound P*' fraction was observed in Clatto Reservoir one month post-application (Meis *et al*., 2012; Chapter 4), indicating that no short-term P-partitioning processes occurred to partition P between release-sensitive P-fractions (P_{mobile}) and the '*apatite bound P*' fraction. Similarly, the significant increase in the amount of P stored in the '*apatite bound P*' fraction did not occur immediately following the application in Loch Flemington but was observed 7 months post-application. The mass of P present in the '*apatite bound P*' fraction increased gradually over time to approximately 2 to 4 times higher than the pre-application level for the top 10 cm or 4 cm of the sediment respectively. These findings suggest that P released from release-sensitive P-fractions (P_{mobile}) is transferred into the more refractory '*apatite bound P*' fraction. However, the timing of this sediment P-partitioning process appears to follow a time lag post-application which suggests that the application of Phoslock® is unlikely to reduce the

mass of P_{mobile} immediately. Considering that the 914 kg of La added to the sediment can bind up to 204 kg P, it is hypothesised that the mass of P present in the '*apatite bound P*' fraction will increase further with time, since this fraction contained approximately 139 kg P 12 months post-application. However, since P-binding by La following release events from release-sensitive sediment P-fractions (P_{mobile}) appears to occur long after Phoslock® application (i.e. between 4 and 7 months) it is important to assess if ageing of Phoslock® under *in situ* conditions has any effects on its P-binding capacity. For Al-based P-capping agents it has been shown that ageing can decrease the maximum adsorption capacity by up to 75% (Vicente *et al*., 2008). However such reductions in maximum adsorption capacity of Al-based P-capping agents have been observed in the absence of P in the surrounding medium (Vicente *et al*., 2008) which is unlikely to occur at the sediment-water interface of eutrophic shallow lakes.

Dosing experiment: Investigating effectiveness of applied Phoslock® dose

The aerobic control and anaerobic control treatments to which no further Phoslock® was added during the experiment were used as proxies to investigate whether the original dose to Loch Flemington (170 g Phoslock® m^{-2}) was sufficient to control sediment P-release under aerobic and anaerobic conditions. Variation in water-column TP concentrations over the experimental period indicated that no sediment P-release occurred under aerobic conditions, while significant amounts of P were released from the sediment under anaerobic conditions. At the end of the experimental period, water-column TP concentrations were approximately six times higher under anaerobic conditions compared to aerobic conditions. The significant increase in TP concentration in the anaerobic control was most likely caused by P released from the '*reductant-soluble P*' fraction, which represents P bound to Fe- and Mn-compounds. Under anaerobic conditions Fe(III) and Mn(III/IV) are reduced to Fe(II) and Mn(II), causing

the release of P (Psenner *et al*., 1984; Boström *et al*., 1988; Stumm and Morgan, 1996; Perkins and Underwood, 2001). The results, therefore, indicated that the applied dose of 170 g Phoslock® m^{-2} to Loch Flemington in March 2010 was not sufficient to prevent sediment P-release under anaerobic conditions, suggesting significant sediment P-release will occur in Loch Flemington should prolonged periods of anoxia persist.

Alteration of sediment P-release under aerobic and anaerobic conditions using Phoslock®

Variation in physicochemical properties of a water body including pH (Ross *et al*., 2008; Hagsheresht *et al*., 2009; Gibbs *et al*., 2011) and/or presence of humic substances (Tang and Johanesson, 2003; Sonke and Salter, 2006; Tang and Johanesson, 2010) can alter the P-binding capacity of Phoslock® *in situ*. It is therefore important to assess if the additional application of Phoslock® under semi 'natural' conditions (e.g. sediment core incubations) can alter sediment P-release. Water-column TP concentrations did not differ significantly between aerobic control and aerobic dosed treatments except on day 29, suggesting that the additional application of Phoslock® did not alter sediment P-release under aerobic conditions over the experimental period. However, experiments running for longer periods are required to assess whether significant differences in TP observed at the end of the experiment were the onset of a longer-term trend. The reasons for this result are not known, however, sediment P-release (e.g. mineralisation and subsequent release of P present in the '*organic P*' fraction) under aerobic conditions in the aerobic control treatment and/or disturbance of the sediment surface in the aerobic control treatment caused by bubbling in the experiment may have caused this. In contrast, water-column TP concentrations were significantly lower in the anaerobic dosed compared to the anaerobic control treatment indicating that Phoslock® was able to significantly reduce sediment P-release under anaerobic conditions. It is therefore

concluded that the application of additional Phoslock® to Loch Flemington could be a viable option if anaerobic conditions are the principal cause of sediment P-release in the lake.

Estimated top-up dose to prevent sediment P-release under anaerobic conditions

Water-column TP concentrations in the anaerobic dosed treatment did not vary significantly between day 0 and any day up to day 22 (equivalent to 1 - 3 additional doses of Phoslock®), indicating that between 25,000 to 75,000 kg of Phoslock® should be sufficient to control sediment P-release under anaerobic conditions in Loch Flemington. However, it has been suggested that sediment P-release under laboratory conditions may be initially higher following artificial creation of anaerobic conditions compared to long-term P-release under anaerobic conditions (Hupfer and Lewandowski, 2008) which may lead to overestimation of required dose. It was assumed that predominantly P released from the '*reductant-soluble P*' fraction caused the significant increase in water-column SRP and TP concentrations in the anaerobic control. It was estimated that, on the whole-lake scale, around 244 kg P were present in the '*reductant-soluble P*' fraction pre-application in the top 4 cm of the sediment, while the top 10 cm contained around 540 kg P. Based on a nominal La content of 50,000 mg La kg^{-1} Phoslock® (Afsar and Groves, 2009) and a molar binding ratio of La:P of 1:1 (Haghseresht *et al*., 2009) between 21,890 and 48,440 kg Phoslock® would be required to treat the mass of '*reductant-soluble P*' in Loch Flemington in the top 4 cm or 10 cm of the sediment respectively. Consequently, laboratory experiments and dose estimates based on sediment P-fractions indicated 'top-up' doses of similar magnitude required to control sediment P-release under anaerobic conditions. Therefore the estimated maximum amount of Phoslock® required to control sediment P-release under anaerobic conditions is 75,000 kg. Given the time lag between application and sediment P-

partitioning towards more refractory P-fractions (e.g. '*apatite bound P*'), it is recommended that an application be conducted early in the year to prevent release of P from the '*reductant-soluble P*' fraction which occurs predominantly in later summer to early autumn in northern temperate shallow lakes (Spears *et al*., 2007a; Søndergaard *et al*., 2012).

Effects of multiple Phoslock® doses on pH and conductivity

The effect of multiple Phoslock® doses on water-column pH and conductivity was investigated by comparing response of control and treated cores under aerobic or anaerobic conditions since physicochemical properties of water bodies can set limits to the amount of P-capping agents that can be applied. For example, alkalinity can limit the maximum dose of Al-based P-capping agents; commonly, Al-based P-capping agents can be applied until pH decreases to a value of 6 (Kennedy and Cooke, 1982; Cooke *et al*., 2005). In the dosing experiment, a total of 3.5 g Phoslock® L^{-1} were added to the cores (equivalent to an additional areal load of 850 g Phoslock® m^{-2}). The results suggest that up to an additional areal load of 850 g Phoslock® m^{-2} variation in pH due to Phoslock® is unlikely in Loch Flemington. This is supported by a laboratory experiment highlighting that Phoslock® had no effect on pH over an investigated dose range of 0.05 to 3.2 g Phoslock® L^{-1} (van Oosterhout and Lürling, 2012). The significant higher pH in the 'anaerobic' group was likely to be an artefact caused by the N_2-bubbling which removes dissolved CO_2 and causes the pH to rise (Stumm and Morgan, 1996). In contrast, water-column conductivity was significantly higher in the aerobic dosed treatment compared to all other treatments towards the end of the experimental period. A laboratory study by van Oosterhout and Lürling (2012) observed an increase in conductivity with increasing concentrations of Phoslock® in the water-column. It would therefore be expected that conductivity of both the aerobic dosed and anaerobic dosed

treatment should be significantly elevated. However, it is hypothesised that bioturbation activity by benthic macroinvertebrates was higher under aerobic conditions, causing an increase in conductivity either due to increased mixing of Phoslock® and/or sediment particles into the water-column or by causing enhanced diffusion of ions across the sediment-water interface. This hypothesis was supported by the observation that, under aerobic conditions, a significant larger area of the Phoslock® layer was covered with fresh sediment compared to anaerobic conditions.

Implications for lake management

Calculating effective dose to control sediment P-release, particularly in the long-term, is one of the most challenging processes in lake remediation projects (Schauser *et al*., 2003; Vicente *et al*., 2008; Hickey and Gibbs, 2009). Given uncertainties involved in calculating effective dose (Schauser *et al*., 2003; Vicente *et al*., 2008; Hickey and Gibbs, 2009), potential non-target effects at high dose (Vopel *et al*., 2008; Gibbs *et al*., 2011) and potential advantages in adding multiple smaller doses (Lewandowski *et al*., 2003; Vicente *et al*., 2008), the following conceptual model for the use of P-capping agents is proposed and discussed, using Loch Flemington as an example (Fig. 5.7). The model suggests that, to avoid overdosing a lake with a P-capping agent, an initial low dose should be applied, followed by continuous monitoring and top-up doses where required, until the required improvements in water quality are met. This should be conducted simultaneously with continuous catchment management and monitoring of catchment nutrient loading to the lake. The proposed conceptual model differs predominantly from other conceptual models by the fact that it assumes that calculating effective dose is impossible, so that a continuous cycle of dosing, monitoring and assessment is required.

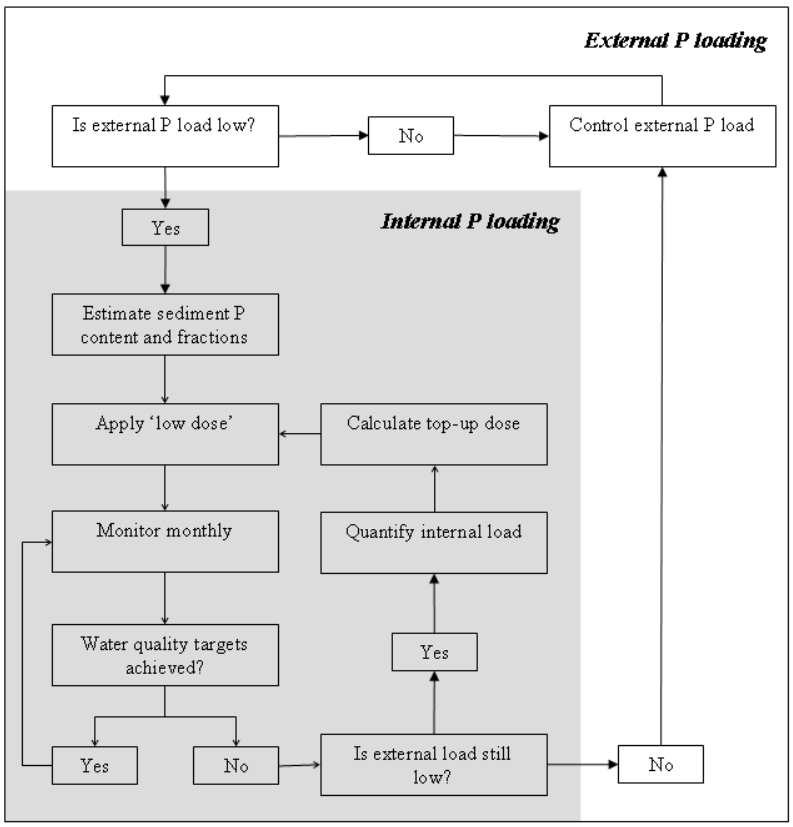


Figure 5.7 Conceptual model for the application of P-capping agents in lake remediation projects.

At Loch Flemington, an initial low dose was applied deliberately, and water quality was monitored pre- and post-application. Laboratory experiments were used to estimate a required 'top-up' dose should the initial low dose fail to control sediment P-release. If, initially, the sediment P_{mobile} content of the top 4 cm or top 10 cm of the sediment had been targeted, between 75,000 to 190,000 kg of Phoslock® would have been applied to Loch Flemington. Despite the applied low dose no major sediment P-release events occurred up to 12 months post-application and experiments indicated that the dose was sufficient to control sediment P-release under aerobic conditions. Should monitoring indicate deterioration of water quality, for example due to sediment P-release under anaerobic conditions as indicated by the experiment, it was estimated that a 'top-up' dose should range between 25,000 to 75,000 kg of Phoslock®. Based on potential

advantages of adding multiple doses (Lewandowski *et al*., 2003; Vicente *et al*., 2008) it is proposed that an initial 'top-up' dose of 25,000 kg should be applied as early as possible in the year and effects on sediment P-release should be monitored. In the case of Loch Flemington, this approach would require less Phoslock® up to at least two additional 'top-up' doses compared to dosing relative to the sediment P_{mobile} content, thereby potentially increasing cost-effectiveness and reducing the risk of non-target effects.

CONCLUSIONS

The application of Phoslock® caused a significant increase in the mass of P present in the more refractory '*apatite bound P*' fraction in Loch Flemington. However, this sediment P-partitioning process occurred gradually over time and not immediately following application. Laboratory experiments indicated that the applied amount of Phoslock® was likely to be sufficient to control sediment P-release under aerobic conditions, even though the application equalled a low dose compared to dose estimates relative to the total sediment P_{mobile} content. Laboratory experiments indicated that significant sediment P-release is to be expected should extended periods (> 1 week) of anaerobic conditions occur in Loch Flemington. The potential for this release may be reduced by the additional application of between 25,000 to 75,000 kg Phoslock®. The proposed conceptual model underpins the use of P-capping agents in lakes. This model prescribes a smaller multiple dosing strategy that will reduce the likelihood of undesirable effects associated with the application of a high single dose. These undesirable effects include changes in sediment DO concentrations and nitrogen transformation processes in the organic sediment layer. In addition, the proposed dosing strategy will allow more accurate and cost-effective determination of required dose at the whole-lake scale.

Chapter 6

Seasonal responses of physicochemical and biological variables to reduced sediment phosphorus release following the application of Phoslock®

Seasonal responses of physicochemical and biological variables to reduced sediment phosphorus release following the application of Phoslock®

ABSTRACT

The release of phosphorus (P) from bed sediments (internal P-loading) is a key factor delaying the recovery of shallow lakes for years to decades following reduction in external P-loading. This study investigated the seasonal response of physical, chemical and biological variables following disruption of the internal P-loading feedback mechanism in a shallow, eutrophic lake (Loch Flemington, Inverness, UK) by the application of 170 g Phoslock® m^{-2}. Monitoring was conducted over a period of 30 months, covering 10 month pre- and up to 20 months post-application. Following application, in-lake surface and bottom water soluble reactive phosphorus (SRP) and total phosphorus (TP) concentrations decreased significantly, particularly in summer, indicating a reduction in sediment P-release. Consequently, phytoplankton biomass (chlorophyll *a* and total phytoplankton biovolume) which was significantly positively correlated to surface water TP concentration, was significantly reduced. In turn, water clarity (measured as Secchi depth) was significantly negatively correlated to phytoplankton biovolume, chlorophyll *a* concentrations and surface water TP concentration and increased significantly in the first and second summer post-application. A significant increase in maximum colonisation depth (MCD) of aquatic macrophytes was observed post-application, probably as a result of the higher water clarity. Assessing variation in abundance of organism groups of higher trophic levels indicated a significant decrease in dominant benthic macroinvertebrate groups (Chironomidae, Oligochaeta and Sphaeriidae) in summer and autumn in the first year post-application, whereas abundance of zooplankton groups did not change significantly in the first year following the application of Phoslock®. Similarly, abundance of fish

(three-spined stickleback, *Gasterosteus aculeatus* L.) did not change significantly post-application. This study showed that the disruption of the internal P-loading feedback mechanism caused significant changes in the community structure of Loch Flemington. The observed changes, particularly the reduction of in-lake P concentrations, the decrease of phytoplankton biomass and the increase in water clarity, were generally comparable to those observed in long-term multi-lake studies investigating the recovery of shallow lakes from eutrophication. However in the current study the observed changes occurred over a shorter time scale (1 year), probably due to the rapid decrease in sediment P-release. Alterations in ecological structure indicated a change in state from a 'phytoplankton dominated turbid state' to a 'macrophyte dominated clear water state'. However further remediation measures, particularly biomanipulation of the fish community which appeared to consists solely of a single planktivorous species, may be required to increase resilience of the macrophyte dominated clear water state against environmental pressures.

INTRODUCTION

Common consequences of excessive nutrient loading to shallow lakes include severe changes in the structure and function of these habitats (Phillips, 2005; Smith and Schindler, 2009). Commonly observed structural changes during the enrichment of shallow lakes with phosphorus (P) and nitrogen (N) compounds include an increase in primary production, a decrease in species richness and a loss of functional diversity (Harper, 1992; Smith *et al*., 1999; Jeppessen *et al*., 2000; Hulot *et al*., 2000). Structural changes often imply changes in function, for example a shift of primary production from benthic to pelagic habitats, changes in the biogeochemical cycling of nutrients, changes in food web structure and trophic level interactions (Søndergaard *et al*, 1999; Jeppesen *et al*., 2000; Vadeboncoeur *et al*., 2003). Regime shifts and alternative states have been intensively investigated and described in shallow lakes subjected to eutrophication (Scheffer *et al*., 1993; Scheffer, 1998; Blindow *et al*., 2006). Various feedback mechanisms exist that buffer the respective states against change following perturbation (Scheffer *et al*., 1993; Scheffer 1998). However, a change in state occurs if a disturbance is strong enough to overcome resilience so that structure, function and feedback mechanism of a system change sufficiently to stabilize an alternative state (Walker and Meyers, 2004). Changes in ecological structure and function of shallow lakes following nutrient enrichment are often linked to a loss of system resilience (Scheffer and Carpenter, 2003; Folke *et al*., 2004), thereby rendering the system more prone to a regime shift following disturbance. Additionally, eutrophication related alterations in structure and function are often associated with the loss of ecosystem services (ES) provided by shallow lakes (Folke *et al*., 2004).

Many studies have highlighted the problem of internal P-loading delaying the recovery of shallow lakes once external loads have been reduced (Marsden, 1989; Søndergaard *et al*., 2003). Internal P-loading is likely to be one of the major feedback

mechanisms buffering the phytoplankton dominated turbid state against change following the reduction of external nutrient loading (Søndergaard *et al*., 1999; Mehner *et al*., 2008). In order to control internal P-loading and to 'speed up' the recovery process different P-stripping/sediment P-capping agents (hereafter termed P-capping agents; Cooke *et al*., 2005; Hickey and Gibbs, 2009; Gibbs *et al*., 2011) have been developed and tested. A range of studies have shown that reducing internal P-loading alone can partly improve the degraded structure and function of shallow lakes (Reitzel *et al*., 2005; van Oosterhout and Lürling, 2011), while results of other studies suggest that management of multiple pressures (e.g. control of internal P-loading and biomanipulation of fish community) simultaneously might be required to facilitate more complete structural and functional recovery (Hosper and Jagtman, 1990; van Wichelen *et al*., 2007; Mehner *et al*., 2002, 2008).

Structural and functional changes in shallow lakes recovering from eutrophication

Studies investigating structural and functional changes in shallow lakes recovering from eutrophication are comparatively new compared to studies assessing structural and functional degradation during the process of nutrient enrichment (Carpenter and Lathrop, 1999; Schindler, 2006; Smith *et al*., 2006; Spears *et al*., 2011). Hence, knowledge about recovery trajectories is still limited (Dokulil *et al*., 2011). However, available studies have identified the following common structural and functional responses in shallow lakes recovering from eutrophication.

In-lake total phosphorus (TP) concentrations often decrease in winter, spring and autumn, while summer TP concentrations are often maintained close to or exceeding pre-management concentrations due to internal P-loading (Søndergaard *et al*., 2005). It has been estimated that internal P-loading can delay recovery for up to about 10 - 15 years (Jeppesen *et al*., 2005b), depending on retention time, pollution history, sediment

P content and lake depth (Sas, 1989). Over time, the magnitude of sediment P-release in summer decreases, leading to a decrease in summer in-lake P concentrations (Søndergaard *et al*., 2005). In most lakes, chlorophyll *a* concentrations are strongly related to TP concentrations (Dillon and Rigler, 1974; Schindler, 1977, 1978; Phillips *et al*., 2008), at least up to TP concentrations of about 100 μg TP L^{-1} (Phillips *et al*., 2008) although this is in part a covariate relationship as the planktonic biomass contributes to the TP pool. Consequently, phytoplankton biomass also decreases during recovery when TP concentrations are reduced below 100 μg TP L^{-1} (Jeppesen *et al*., 2005b; Jeppesen *et al*., 2007a) due to both reduced P supply and this covariate relationship. In shallow lakes, changes in phytoplankton biomass following nutrient reduction are often accompanied by changes in phytoplankton community structure, including a relative increase in the biomass of diatoms, cryptophytes and chrysophytes (Jeppesen *et al*., 2005b), a decrease in the relative biomass of cyanobacteria (Downing *et al*., 2001) and an increase in species richness (Bellinger and Sigee, 2010). Such structural changes can lead to functional changes including a reduction of pelagic primary production (Liboriussen and Jeppesen, 2003; Vadeboncoeur *et al*., 2003), a decrease in organic matter deposition to the sediment (Lang, 1998, 1999), a decrease in quantity and an increase in quality of food supply for zooplankton (Ahlgren *et al*., 1990; Gliwicz and Lampert, 1990; Jeppesen *et al*., 2002). Higher water clarity following a reduction in phytoplankton biomass in particular can have profound impacts on macrophyte colonisation depth and therefore the area of sediment covered with submerged plants (Jupp and Spence, 1977; Canfield *et al*., 1985; Perkins and Underwood, 2002; Jeppesen *et al*., 2005b). Additionally, species richness and the number of nutrient intolerant macrophyte species have been observed to increase at lower nutrient concentrations (Jeppesen *et al*., 2000; James *et al*., 2005). Since macrophytes can play a pivotal role in shallow lakes, changes in structure can have profound functional changes including

alteration of sediment P-release depending on macrophyte abundance (Stephen *et al*., 1997; Boros *et al*., 2011), changes in P-partitioning between phytoplankton and macrophytes (van Donk *et al*., 1993) and a general increase in benthic primary production (Vadeboncoeur *et al*., 2003). In turn, changes in phytoplankton and macrophyte community composition and abundance can influence zooplankton community composition and overall abundance (Lauridsen *et al*., 1996; Jeppesen *et al*., 1998a). Common responses of zooplankton in shallow lakes recovering from eutrophication include an increase in the relative abundance of *Daphnia* spp. compared to total cladoceran biomass (Jeppesen *et al*., 2005a, b; Jeppesen *et al*., 2007a), an increase in the biomass ratio of zooplankton to phytoplankton (Jeppesen *et al*., 2005b), a decrease in zooplankton biomass (Jeppesen *et al*., 2000) and an increase in zooplankton species diversity (Jeppesen *et al*., 2000). Associated structural and functional changes include, for example, an increase in the relative grazing pressure (Jeppesen *et al*., 2005b), an extended clear water phase in spring (Lampert *et al*., 1986) and decreased organic matter deposition to the sediment (Lang, 1998, 1999). Commonly observed responses of the benthic macroinvertebrate community during the recovery from eutrophication are a reduction in abundance (Köhler *et al*., 2005; Gunn *et al*., 2012), an increase in diversity (Gunn *et al*., 2012), an increase in the chironomid to oligochaete ratio (Wetzel, 2001) and an increase in abundance of nutrient intolerant species (Wiederholm, 1980; Gunn *et al*., 2012). Associated functional responses have received little attention in the literature most likely as small scale heterogeneity in macroinvertebrate abundances hampers the assessment with respect to their potential impact on processes at the whole-lake scale (Lewandowski *et al*., 2005). However, associated functional changes include a decrease in filtration rates (Hornbach *et al*., 1984; Way, 1989), reduced solute exchange between sediment and water-column (Matisoff *et al*., 1985; Lewandowski and Hupfer, 2005) and lower decomposition rates

of organic material in the sediment (Wallace and Webster, 1996). Finally, common responses of the fish community during recovery from eutrophication include a decrease in fish biomass (Jeppesen *et al*., 2005a, b; Jeppsen *et al*., 2007a) and a general increase in the abundance of piscivorous fish species (Jeppesen *et al*., 2000; Jeppesen *et al*., 2005a, b). Such structural changes in fish community composition and abundance are likely to cause changes in function including increased predation pressure on zooplanktivorous fish (Jeppesen *et al*., 2000; Jeppesen *et al*., 2005b), a decrease in chlorophyll *a* to P ratio as a result of the trophic cascade (Carpenter and Kitchell, 1993; Jeppesen *et al*., 2005b) and transferral and storage of P in higher trophic levels (Carpenter *et al*., 1992, 1996; Schindler *et al*., 1996). Structural and functional responses of different trophic groups observed in shallow lakes recovering from eutrophication have been summarised (Table 6.1).

Table 6.1 Summary of structural and functional responses of different trophic groups in shallow lakes recovering from eutrophication.

Trophic group	Structural response	Functional response	Ref.
phytoplankton	-decrease in biomass -change in community structure -increase in species richness	-decrease in pelagic primary production -decrease in organic matter deposition to the sediment -decrease in shading -decrease in quantity and increase in quality of food for zooplankton	1-11
macrophytes	-increase in colonisation depth -increase in species richness -increase in nutrient intolerant species	-increase in benthic primary production -alteration of sediment P-release -change in P-partitioning between phytoplankton and macrophytes	8, 9, 12-18
zooplankton	-decrease in biomass -increase in relative abundance of *Daphnia* to total cladoceran biomass -increase in biomass ratio of zooplankton to phytoplankton -increase in species richness	-increase in relative grazing pressure -decrease in organic matter deposition to the sediment -storage of P in higher trophic levels -reduced phytoplankton shading	1, 4, 9, 10, 16, 19, 20
benthic macro-invertebrates	-decrease in biomass -increase in Chironomid to Oligochaeta ratio -increase in nutrient intolerant species -increase in species richness	-decrease in decomposition rate -reduced filtration rate -reduced solute exchange between sediment and water-column	21-29
fish	-decrease in biomass -increase in abundance of piscivorous species	-increase in predation pressure on zooplanktivorous fish -decrease in chlorophyll *a* to P ratio -storage of P in higher trophic levels	9, 10, 16, 20, 30-34

Ref., reference; P, phosphorus; **1**, Lang, 1998; **2**, Ahlgren *et al*., 1990; **3**, Gliwicz and Lampert, 1990; **4**, Lang,1999; **5**, Downing *et al*., 2001; **6**, Jeppesen *et al*., 2002; **7**, Liboriussen and Jeppesen, 2003; **8**, Vadeboncoeur *et al*., 2003; **9**, Jeppesen *et al*., 2005b; **10**, Jeppesen *et al*., 2007a; **11**, Bellinger and Sigee, 2010; **12**, Jupp and Spence, 1977; **13**, Canfield *et al*., 1985; **14**, van Donk *et al*., 1993; **15**, Stephen *et al*., 1997; **16**, Jeppesen *et al*., 2000; **17**, James *et al*., 2005; **18**, Boros *et al*., 2011; **19**; Lampert *et al*., 1986; **20**, Jeppesen *et al*., 2005a; **21**, Wiederholm, 1980; **22**, Hornbach *et al*., 1984; **23**, Matisoff *et al*., 1985; **24**, Way, 1989; **25**, Wallace and Webster, 1996; **26**, Köhler *et al*., 2005; **27**, Lewandowski and Hupfer, 2005; **28**, Lewandowski *et al*., 2005; **29**, Gunn *et al*., 2012; **30**, Carpenter *et al*., 1992; **31**, Carpenter and Kitchell, 1993; **32**, Carpenter *et al*., 1996; **34**, Schindler *et al*., 1996

Study outline and hypotheses

This study investigated the seasonal response of physical, chemical and biological variables following disruption of the internal P-loading feedback mechanism by a 'controlled disturbance' using Phoslock® in a shallow, eutrophic lake. Furthermore, this study investigated whether observed responses are comparable to findings of long-term studies. Based on studies investigating structural and functional changes in shallow lakes recovering from eutrophication (e.g. Jeppesen *et al*., 2005b; Søondergaard *et al*. 2005) and studies investigating the use of P-capping agents (e.g. Reitzel *et al*., 2005; van Oosterhout and Lürling, 2011) it was hypothesized that the disruption of internal P-loading will result in: (i) A decrease in surface and bottom water P concentrations particularly in summer; (ii) a decrease in phytoplankton biomass (chlorophyll *a* concentration and total biovolume) particularly in summer; (iii) an increase in water clarity (Secchi depth) accompanied by an increase in maximum colonisation depth (MCD) of macrophytes; and (iv) a decrease in zooplankton and benthic macroinvertebrate abundance. Overall it was hypothesised that a reduction of the internal P-loading feedback mechanism following the controlled disturbance using Phoslock® would force a change in state from a phytoplankton dominated turbid state to a macrophyte dominated clear water state.

MATERIAL AND METHODS

Study site and dosing

A description of the study site is presented (Chapter 2, Section 2.1.2). Past surface water data for TP in Loch Flemington were available from the Scottish Environmental Protection Agency (SEPA) for the years 2000 until 2003. Mean seasonal surface water TP concentrations for this period were 44.43 µg L^{-1} (± 4.75 standard error (s.e.)) for spring, 141.31 µg L^{-1} (± 43.41 s.e.) for summer, 72.00 µg L^{-1} (± 27.79 s.e.) for autumn and 36.75 µg L^{-1} (± 2.50 s.e.) for winter. Furthermore, at sediment P concentrations in excess of 2,500 mg P kg^{-1} dry weight (DW) sediment in Loch Flemington, as inferred from May *et al*. (2001), net annual sediment P-release is expected to occur for more than 5 years (Sas, 1989). Such estimates are based on the assumption that P can be washed out via an outflow (Sas, 1989). However Loch Flemington has no outflow, thus impeding P flushing. Therefore, the estimated recovery time at Loch Flemington with no further management and assuming no increase in catchment P-loading is greater than 5 years. In order to reduce internal P-loading, 25,000 kg of Phoslock® (equivalent to an areal load of 170 g Phoslock® m^{-2}) was applied to Loch Flemington as a slurry from a pontoon over a period of 3 days in March 2010. Based on a nominal La content of 50,000 mg La kg^{-1} Phoslock® (Afsar and Groves, 2009) and a molar binding ratio of La:P of 1:1 (Haghseresht *et al*., 2009), the applied amount of Phoslock® was sufficient to bind 279 kg P. Mass balance calculations (Chapter 5) indicated that the applied mass of lanthanum (La) was sufficient to bind approximately 25% of P_{mobile} (sum of '*labile P'*, '*reductant-soluble P*' and '*organic P'* fraction) in the top 4 cm or around 10% of P_{mobile} in the top 10 cm of the sediment. It was anticipated that the applied mass of 25,000 kg of Phoslock® was likely to be an under-dose since it was not sufficient to bind all P_{mobile} in the top 4 or top 10 cm, with these depths representing common estimates of the 'active' sediment depth from which P_{mobile} cycles between the sediment

and the water-column (Boström *et al*., 1982; Cooke *et al*., 2005). However, the purpose of this study was to reduce the internal P-loading feedback mechanism which promotes the phytoplankton dominated turbid state in order to investigate lake recovery.

Field measurements and sample collection

Samples and measurements were taken on a monthly basis at five permanent open water sampling sites (n = 5) between May 2009 and March 2011 (Fig. 6.1). Following this period, measurements and samples were taken on a monthly basis at three of the five permanent sampling sites (n = 3; Site 1, 2 and 5) by a local person up to November 2011. This is with the exception of July 2011 in which five sites were sampled (n = 5). Ice cover prevented sampling and measurements of most parameters in February 2010 and December 2010. In December 2009 only one sample site (n = 1; Site 1) was accessible, while in January 2010 only three sample sites (n = 3; Site 1, 2 and 5) were accessible due to ice cover. The number of observations and measurements made for different variables are shown (Appendix: Table A6.1). Exceptions from the general sampling and measurement scheme for individual variables are outlined in the text.

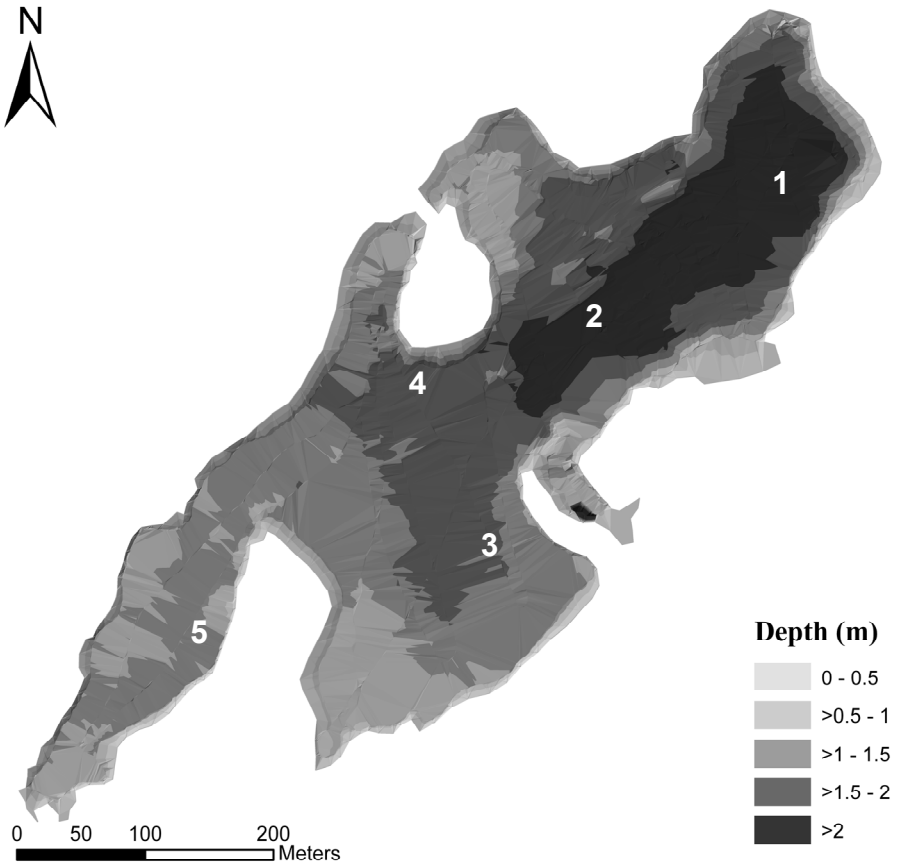


Figure 6.1 Bathymetric map of Loch Flemington including the location of sample sites (numbers).

Field measurements and sample collection

On each visit at each sample site, surface and bottom water samples were taken (Chapter 2, Section 2.2.1) and surface measurements of pH, conductivity, temperature and dissolved oxygen (DO) concentrations were made and water clarity was estimated using a Secchi disk (Chapter 2, Section 2.3.1). Additionally, sediment cores were taken (Chapter 2, Section 2.2.2) and maximum colonisation depth (MCD) of submerged macrophytes was assessed (Chapter 2, Section 2.5.7). The number of observations and measurements made for different variables is shown (Appendix: Table A6.1).

Chemical analysis

Surface water samples (Chapter 2, Section 2.2.1) were analysed for total dissolved carbon (TDC; Chapter 2, Section 2.4.1), dissolved organic carbon (DOC; Chapter 2, Section 2.4.1), total lanthanum (TLa; Chapter 2, Section 2.4.2), soluble lanthanum (La; Chapter 2, Section 2.4.2), dissolved inorganic nitrogen (DIN; Chapter 2, Section 2.4.3), TP (Chapter 2, Section 2.4.4), soluble reactive phosphorus (SRP; Chapter 2, Section 2.4.4), total diatom silica ($TSiO_2$; Chapter 2, Section 2.4.5) and soluble silica (SiO_2; Chapter 2, Section 2.4.5). Bottom water samples (Chapter 2, Section 2.2.1) were analysed for TLa (Chapter 2, Section 2.4.2), La (Chapter 2, Section 2.4.2), TP (Chapter 2, Section 2.4.4.) and SRP (Chapter 2, Section 2.4.4).

Sampling of surface and bottom water TLa and La concentrations deviated from the general sampling scheme outlined above. Samples for the analysis of surface water TLa and La concentration were taken monthly from three sample sites (n = 3; Site 1, 2 and 5). Samples for the analysis of bottom water TLa and La concentration were taken monthly from one sample site (n = 1; Site 1). Every third month, starting from July 2009 till January 2011 samples for the analysis of bottom water TLa and La concentrations were taken from three sample sites (n = 3; Site 1, 2 and 5). The number of observations and measurements made for different variables is shown (Appendix: Table A6.1).

Biological analysis

Surface water samples were taken for the analysis of chlorophyll *a* concentration (Chapter 2, Section 2.5.1), for the assessment of phytoplankton community composition and biovolume (Chapter 2, Section 2.5.3) and for the analysis of zooplankton abundance and community composition (Chapter 2, Section 2.5.5). Sediment cores were taken at each site for the analysis of benthic macroinvertebrate abundance (Chapter 2, Section

2.5.6). Fish community composition was assessed using traps (August 2010; Chapter 2, Section 2.5.8) and gill nets (July 2011; Chapter 2, Section 2.5.8). Fish traps were supplied by the Royal Society for the Protection of Birds (RSPB, Inverness, contact person Stuart Benn). Trapping was conducted by the RSPB between 2003 and 2006 using the same traps at the transitional zone between emergent macrophytes and the open water along the northern shore. Fish counts for the years 2003 until 2006 were kindly provided by the RSPB. The number of observations and measurements made for different variables is shown (Appendix: Table A6.1).

Weather data

Air temperature (hourly measurements) and precipitation (daily measurements) data were provided by the British Atmospheric Data Centre (BADC). The data were based on land surface observations from the Meteorological (Met) Office station network, which is accessible via the Met Office MIDAS database (http://badc.nerc.ac.uk). Data from a Met station situated 12 km northeast of Loch Flemington were used. Data on air temperature and precipitation was expressed as monthly means for three periods: i) 1999-2008, which represents a 10 year pre-monitoring average; ii) 2009, pre-application year; iii) 2010, year of application and first post-application seasons; and iv) 2011 the second year post-application. The number of observations and measurements made for different variables is shown (Appendix: Table A6.1).

Statistical analyses

All data sets were arranged into seasons consisting of three months. Average air temperature between 1998 till 2008 was highest in July, consequently July was defined as the middle of summer in line with the standard meteorological definition of seasons (Trenberth, 1983) and the four seasons were: summer (June – August), autumn

(September – November), winter (December – February) and spring (March – May). Samples collected in March 2010 were taken two weeks after the application of Phoslock® hence the period March till May 2010 represented the first post-application season (i.e. spring 2010).

All statistical analyses were conducted using Minitab 16 (Minitab® 16.1.1, Minitab Ltd., Coventry, UK). Data sets were assessed for normal distribution (Anderson-Darling test) and equal variance (Levene's test). A one-way analysis of variance (ANOVA) with Tukey's *post hoc* test was used if data from at least three years (pre- and both post-application years) were available in cases where data sets were normally distributed (Anderson-Darling test, $\alpha > 0.05$) and of equal variance (Levene's test, $\alpha > 0.05$) before or after transformation (including $x' = \log(x)$, $x' = \ln(x)$, $x' = \text{sqrt}(x)$, $x' = x^2$). A 2-Sample t-test was also used in cases where normality (Anderson-Darling test, $\alpha > 0.05$) and homogeneity (Levene's test, $\alpha > 0.05$) assumptions were fulfilled and data from only two years were available. A non-parametric Kruskal-Wallis (KW) test was used if data from at least three years (pre- and both post-application years) were available in cases i) where data sets were not normally distributed (Anderson-Darling test, $\alpha < 0.05$) even after a range of transformations (including $x' = \log(x)$, $x' = \ln(x)$, $x' = \text{sqrt}(x)$, $x' = x^2$) or ii) where data sets were normally distributed (Anderson-Darling test, $\alpha > 0.05$) but not of equal variance (Levene's test, $\alpha < 0.05$) even after a range of transformations (including $x' = \log(x+4)$, $x' = \ln(x+4)$, $x' = \text{sqrt}(x)$, $x' = x^2$). A KW test was used to test for significant variation in a given variable between years for a given season. Where significant variation was evident (KW test, $\alpha < 0.05$), a non-parametric Mann-Whitney U-test (MWU) was used to determine between which years significant differences in a given variable for a given season occurred. A non-parametric MWU test was also used in cases where normality (Anderson-Darling

test, $\alpha < 0.05$) and/or homogeneity (Levene's test, $\alpha < 0.05$) assumptions were not fulfilled and data from only two years were available.

All statistical analyses followed the following presumptions: (i) Only seasons consisting of at least two out of three possible months were compared against each other, as it is assumed that at least two months are required to represent a season reasonably well; (ii) if sample sizes (number of observations or measurements) differed between seasons a non-parametric test was used. If differences in sample size between seasons exceeded $n \geq 6$ no comparison was made (McDonald CEH pers. comm.). This was indicated in tables showing statistical results by '▪', (iii) if the sample size of a season was smaller than $n = 3$ no comparison was made, indicated in tables showing statistical results by '▪' (presumption (iii) required the fulfilment of presumption (i) and (ii)). An overview of data distribution, variance and statistical tests (Appendix: Table A6.2) and a full description of statistical analysis for each parameter is given (Appendix: 6.3 Statistical analysis).

Correlation analysis (Spearman's rank correlation) was carried out between surface water TP concentration, surface water SRP concentration, chlorophyll *a* concentration, total phytoplankton biovolume, water clarity, MCD, total zooplankton and total benthic macroinvertebrate abundance. Monthly means were calculated for each parameter to produce comparable populations. Data sets did not follow a normal distribution even after a range of transformations (including $x' = \log(x)$, $x' = \ln(x)$, $x' = \text{sqrt}(x)$, $x' = x^2$). Months for which data were not available for all variables were deleted before ranking the data as an even number of data points is required by the Spearman's rank correlation (resulted in deletion of: May and June 2009; January, March and December 2010; April till November 2011). Correlation analyses (Spearman's rank correlation) were carried out on the remaining data set (July-December 2009; April-

November 2010; January – March 2011), covering a total of 17 months (6 months pre-application and 11 months post-application).

RESULTS

Air temperature and precipitation

Mean seasonal air temperature and precipitation (Fig. 6.2) did not differ significantly between a ten year period prior to the first year of sampling (1999-2008), the first year of pre-application sampling (2009) and the first (2010) and second year (2011) of post-application sampling.

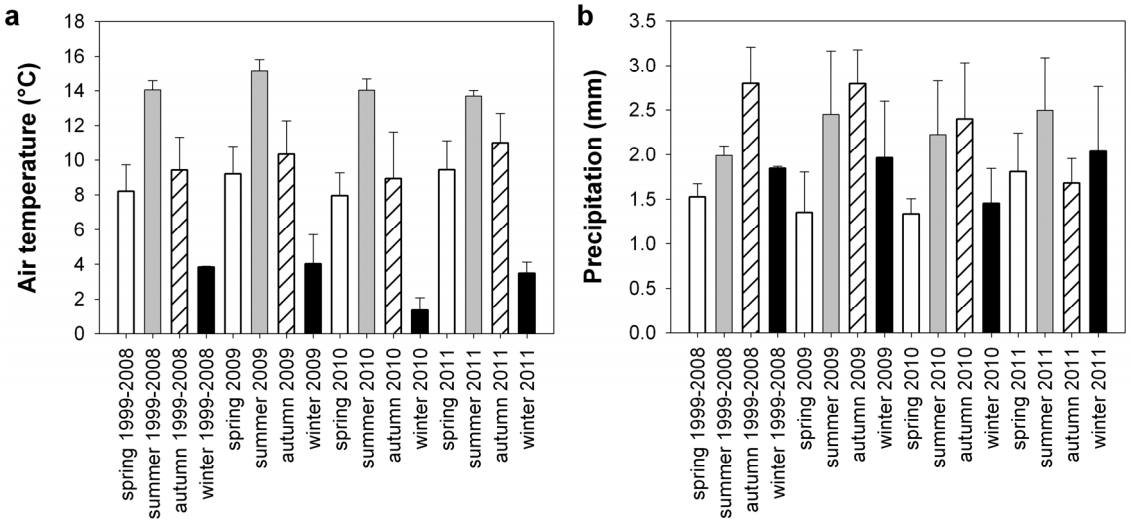


Figure 6.2 Variation in mean seasonal air temperature (a) and precipitation (b), including an average of a ten year period prior to the first year of sampling (1999-2008), the first year of pre-application sampling (2009), and the first (2010) and second year of post-application sampling (2011). Error bars represent the standard error of the mean (n = 3). Seasons are spring (white bars), summer (grey bars), autumn (white hatched bars) and winter (black bars).

Physical parameters

Seasonal variation in surface water pH, conductivity, temperature, DO concentrations and water clarity between pre- (2009) and post-application (2010, 2011) years are presented (Fig. 6.3; Table 6.2). pH (Fig. 6.3a) was highest in summer in pre- and post-application years. pH in summer did not differ significantly between 2009 and 2010, while pH in summer 2011 was significantly lower compared to pH in summer 2009 ($W = 268$; $p < 0.001$; $n_1 = 15$, $n_2 = 11$) and summer 2010 ($W = 263$; $p < 0.01$; $n_1 = 15$, $n_2 = 11$). Water temperature (Fig. 6.3c) in summer 2009 was significantly higher compared to the first ($W = 309.5$; $p < 0.01$; $n_1 = n_2 = 15$) and second year post-application ($W = 242.5$; $p < 0.05$; $n_1 = 15$, $n_2 = 11$) although air temperature (Fig. 6.2) did not differ significantly between years. Surface water DO concentrations (Fig. 6.3d) were highest during summer in all years, with a maximum observed in summer 2009. Post-application, summer DO concentrations were significantly ($W = 297$; $p < 0.01$; $n_1 = n_2 = 15$) lower in 2010 compared to 2009. Lowest water clarity (Fig. 6.3e) was measured in summer 2009 and water clarity increased significantly in the first ($W = 135$; $p < 0.001$; $n_1 = 15$, $n_2 = 11$) and second summer ($W = 120$; $p < 0.001$; $n_1 = 15$, $n_2 = 10$) post-application compared to pre-application conditions.

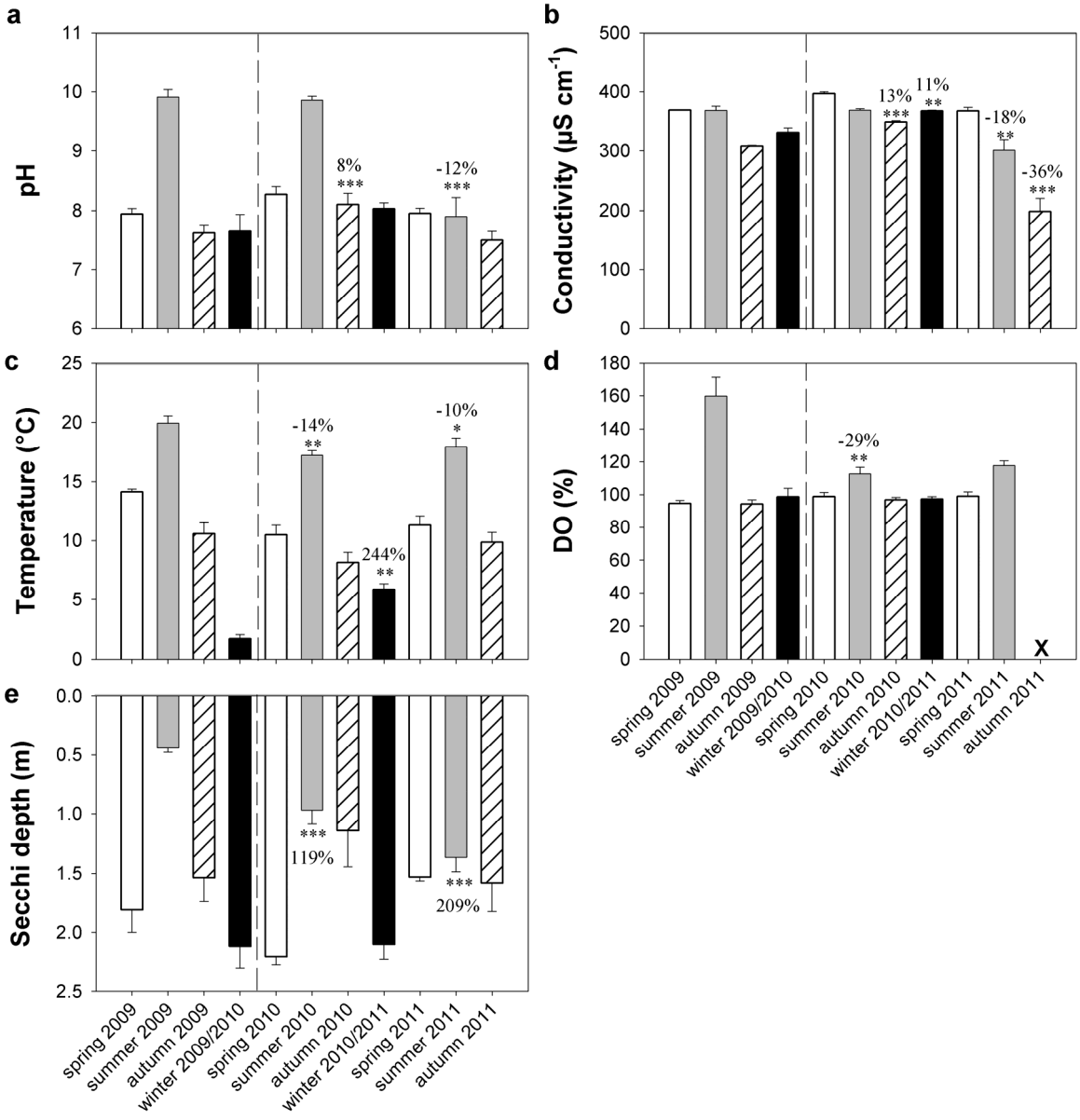


Figure 6.3 Seasonal variation in surface water (a) pH, (b) conductivity, (c) temperature, (d) dissolved oxygen (DO) concentration, and (e) water clarity (measured as Secchi depth). Dashed vertical line indicates the timing of the Phoslock® application and seasons in which no sampling occurred are marked with 'X'. Error bars represent standard error of the mean (for sample size see Appendix: Table A6.1). Significant differences (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$) from seasonal comparisons between pre- and post-application years (i.e. 2009 vs. 2010; 2009 vs. 2011) and percent change are indicated. Seasons are spring (white bars), summer (grey bars), autumn (white hatched bars) and winter (black bars).

Table 6.2 Results of non-parametric Kruskal-Wallis test (KW; degrees of freedom = 2) and Mann-Whitney U-test (MWU) investigating variation in surface water pH, conductivity (cond.), temperature (temp.), dissolved oxygen concentration (DO) and water clarity (measured as Secchi depth; clarity) in a given season between years.

Parameter	Season	n_{09}=	n_{10}=	n_{11}=	KW	MWU		
						09/10	09/11	10/11
pH	spring	5 [I]	15	11	-	▪	▪	*
	summer	15	15	11	***	n.s.	***	**
	autumn	15	15	9	***	***	n.s.	**
	winter	4	10	0	-	n.s.	▪	▪
cond.	spring	5 [I]	15	11	-	▪	▪	**
	summer	15	15	11	*	n.s.	**	*
	autumn	15	15	9	***	***	***	***
	winter	4	10	0	-	**	▪	▪
temp.	spring	5 [I]	15	11	-	▪	▪	n.s.
	summer	15	15	11	**	**	*	n.s.
	autumn	15	15	9	n.s.	n/a	n/a	n/a
	winter	4	10	0	-	**	▪	▪
DO	spring	5 [I]	15	5 [I]	-	▪	▪	▪
	summer	15	15	5 [I]	-	**	▪	▪
	autumn	15	15	0	-	n.s.	▪	▪
	winter	4	10	0	-	n.s.	▪	▪
clarity	spring	2 [I]	5	10	-	▪	▪	**
	summer	15	11	10	***	***	***	n.s.
	autumn	6	7	5	n.s.	n.s.	n.s.	n.s.
	winter	2	3	0	-	▪	▪	▪

09, 2009; 10, 2010; 11, 2011; /, versus; [I], season consists of less than two out of three possible months; -, not applicable as data from at least three years required; ▪, no test possible as season consists of less than two months, difference in number of observations between seasons ≥ 6 or sample size of season smaller than n = 3; n/a, MWU test not applicable as no significant variation indicated by KW test ($\alpha > 0.05$); n.s., $p \geq 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

Chemical parameters

Peak concentrations of surface and bottom water La and TLa concentrations (Fig. 6.4; Table 6.3) were measured in spring 2010. Surface and bottom water TLa and La concentrations (Table 6.3) were significantly higher in summer (surface TLa and La: $W = 45$; $p < 0.001$; $n_1 = n_2 = 9$; bottom TLa and La: $W = 15$; $p < 0.05$; $n_1 = n_2 = 5$), autumn (surface TLa and La: $W = 45$; $p < 0.001$; $n_1 = n_2 = 9$; bottom TLa and La: $W = 15$; $p < 0.05$; $n_1 = n_2 = 5$) and winter (surface TLa and La: $W = 10$; $p < 0.05$; $n_1 = 4$, $n_2 = 6$; bottom TLa and La: $W = 10$; $p < 0.05$; $n_1 = n_2 = 4$) in the first year post-application

compared to pre-application conditions. Bottom water TLa concentrations decreased at a comparatively slow rate in the post-application monitoring period when compared to surface TLa, bottom La and surface La concentrations. The latter (surface TLa, bottom La and surface La concentrations) appeared to show a step change between spring and summer 2010 after which no major changes occurred.

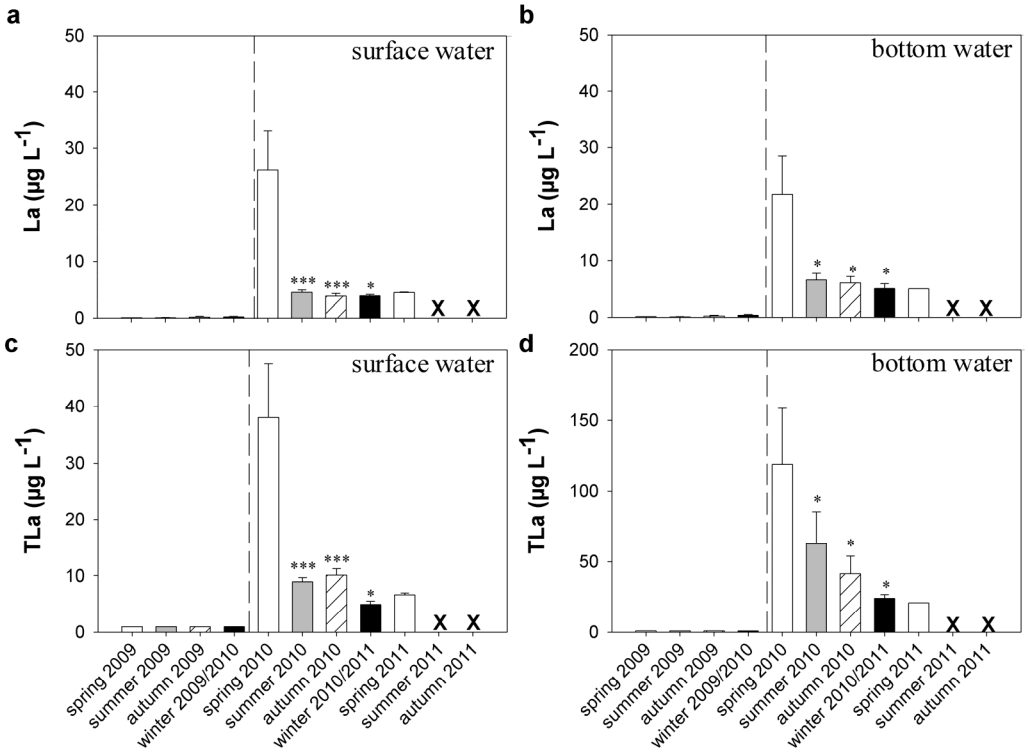


Figure 6.4 Seasonal variation in water lanthanum concentrations, including (a) surface water soluble lanthanum (La) concentration, (b) bottom water soluble La concentration, (c) surface water total lanthanum (TLa) concentration, and (d) bottom water TLa concentration (note different scale). Dashed vertical line indicates the timing of the Phoslock® application and seasons in which no sampling occurred are marked with 'X'. Error bars represent standard error of the mean (for sample size see Appendix: Table A6.1). Significant differences (*, $p < 0.05$; ***, $p < 0.001$) from seasonal comparisons between pre- and post-application years (i.e. 2009 vs. 2010; 2009 vs. 2011) are indicated. Seasons are spring (white bars), summer (grey bars), autumn (white hatched bars) and winter (black bars).

Table 6.3 Results of non-parametric Mann-Whitney U-test (MWU) investigating variation in surface (surf.) and bottom (bot.) water soluble lanthanum (La) and total lanthanum (TLa) concentrations in a given season between years.

Parameter	Season	n_{09}=	n_{10}=	n_{11}=	MWU		
					09/10	09/11	10/11
surf. La	spring	3 [I]	9	3 [I]	▪	▪	▪
	summer	9	9	0	***	▪	▪
	autumn	9	9	0	***	▪	▪
	winter	4	6	0	*	▪	▪
bot. La	spring	1 [I]	5	1 [I]	▪	▪	▪
	summer	5	5	0	*	▪	▪
	autumn	5	5	0	*	▪	▪
	winter	4	4	0	*	▪	▪
surf.TLa	spring	3 [I]	9	3 [I]	▪	▪	▪
	summer	9	9	0	***	▪	▪
	autumn	9	9	0	***	▪	▪
	winter	4	6	0	*	▪	▪
bot. TLa	spring	1 [I]	5	1 [I]	▪	▪	▪
	summer	5	5	0	*	▪	▪
	autumn	5	5	0	*	▪	▪
	winter	4	4	0	*	▪	▪

09, 2009; 10, 2010; 11, 2011; /, versus; [I], season consists of less than two out of three possible months; ▪, no test possible as season consists of less than two months, difference in number of observations between seasons ≥ 6 or sample size of season smaller than n = 3; n.s., $p \geq 0.05$; *, $p < 0.05$; ***, $p < 0.001$

Seasonal variation in surface and bottom water TP and SRP concentrations are presented (Fig. 6.5; Table 6.4). Surface and bottom water TP and SRP concentrations (Fig. 6.5) were highest in summer 2009. Following the application of Phoslock® surface water SRP concentrations (Fig. 6.5a) were significantly lower in summer 2010 ($W = 294$; $p < 0.05$; $n_1 = n_2 = 15$) and summer 2011 ($W = 265$; $p < 0.01$; $n_1 = 15$, $n_2 = 11$). In contrast, surface water SRP concentrations were significantly higher ($W = 144$; $p < 0.05$; $n_1 = 15$, $n_2 = 9$) in autumn compared to pre-application concentrations in the second year post-application (2011). Summer bottom water SRP concentrations (Fig. 6.5b) decreased significantly ($W = 332$; $p < 0.001$; $n_1 = n_2 = 15$) between 2009 and 2010. Post-application, summer surface water TP concentrations (Fig. 6.5c) decreased significantly in summer 2010 ($W = 328$; $p < 0.001$; $n_1 = n_2 = 15$) and in summer 2011

(W = 284; $p < 0.001$; $n_1 = 15$, $n_2 = 11$) compared to pre-application concentrations. A further significant decrease (W = 250; $p < 0.05$; $n_1 = 15$, $n_2 = 11$) in summer surface water TP concentrations was observed between 2010 and 2011. Following application, bottom water TP concentrations (Fig. 6.5d) decreased significantly in summer (W= 309; $p < 0.01$; $n_1 = n_2 = 15$) and in autumn (W = 313; $p < 0.001$; $n_1 = n_2 = 15$) in 2010.

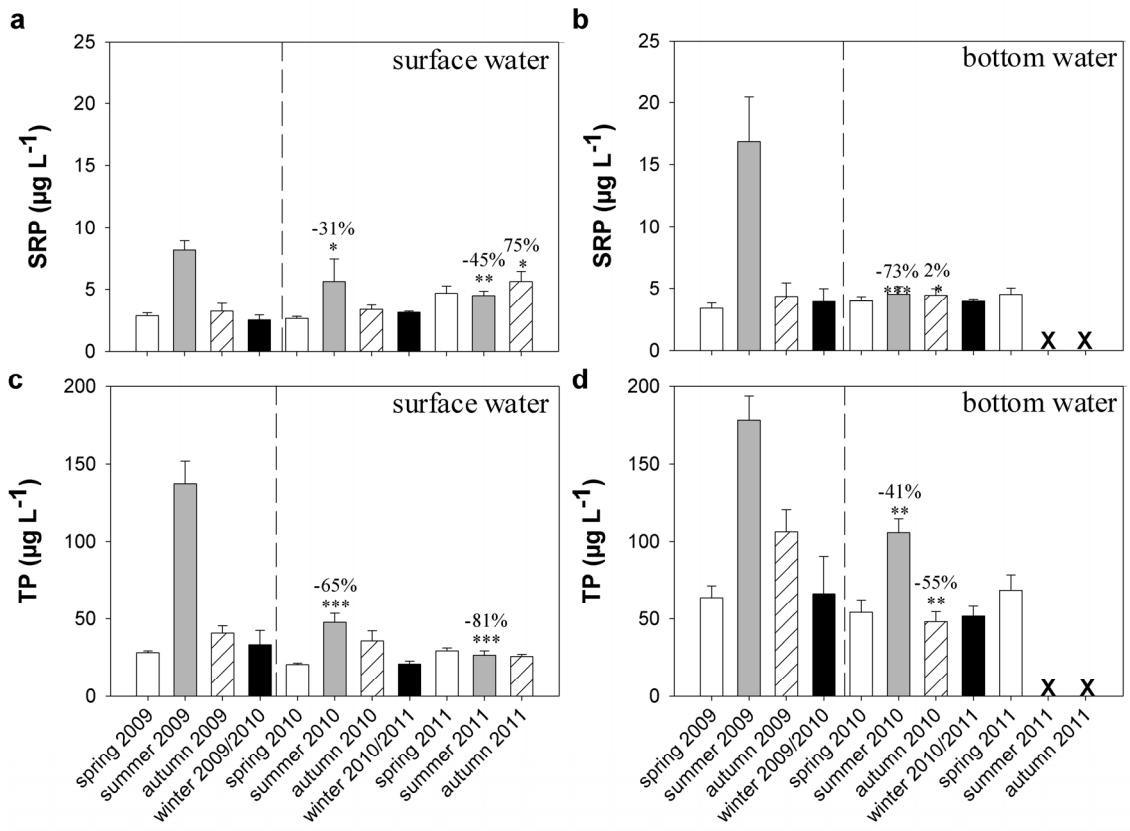


Figure 6.5 Seasonal variation in phosphorus concentration, including (a) surface water soluble reactive phosphorus (SRP) concentration, (b) bottom water SRP concentration, (c) surface water total phosphorus (TP) concentration, and (d) bottom water TP concentration. Dashed vertical line indicates the timing of the Phoslock® application and seasons in which no sampling occurred are marked with 'X'. Error bars represent standard error of the mean (for sample size see Appendix: Table A6.1). Significant differences (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$) from seasonal comparisons between pre- and post-application years (i.e. 2009 vs. 2010; 2009 vs. 2011) and percent change are indicated. Seasons are spring (white bars), summer (grey bars), autumn (white hatched bars) and winter (black bars).

Table 6.4 Results of non-parametric Kruskal-Wallis test (KW; degrees of freedom = 2) and Mann-Whitney U-test (MWU) investigating variation in surface (surf.) and bottom (bot.) water total phosphorus (TP) and soluble reactive phosphorus (SRP) concentrations in a given season between years.

Parameter	Season	n_{09}=	n_{10}=	n_{11}=	KW	MWU		
						09/10	09/11	10/11
surf. SRP	spring	5[I]	15	11	-	▪	▪	***
	summer	15	15	11	**	*	**	n.s.
	autumn	15	15	9	**	n.s.	*	**
	winter	4	12	0	-	▪	▪	▪
bot. SRP	spring	5[I]	15	5[I]	-	▪	▪	▪
	summer	15	15	0	-	***	▪	▪
	autumn	15	15	0	-	*	▪	▪
	winter	4	10	0	-	n.s.	▪	▪
surf.TP	spring	5[I]	15	11	-	▪	▪	**
	summer	15	15	11	***	***	***	*
	autumn	15	15	9	n.s.	n/a	n/a	n/a
	winter	4	12	0	-	▪	▪	▪
bot. TP	spring	5[I]	15	5[I]	-	▪	▪	▪
	summer	15	15	0	-	**	▪	▪
	autumn	15	15	0	-	***	▪	▪
	winter	4	10	0	-	n.s.	▪	▪

09, 2009; 10, 2010; 11, 2011; /, versus; [I], season consists of less than two out of three possible months; -, not applicable as data from at least three years required; ▪, no test possible as season consists of less than two months, difference in number of observations between seasons ≥ 6 or sample size of season smaller than n = 3; n/a, MWU test not applicable as no significant variation indicated by KW test ($\alpha > 0.05$); n.s., $p \geq 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

Seasonal variation in surface water DIN, TDC, dissolved inorganic carbon (DIC), DOC, $TSiO_2$ and SiO_2 concentrations are presented (Fig. 6.6; Table 6.5). Surface water DIN, $TSiO_2$ and SiO_2 concentrations did not vary significantly in a given season between pre- and post-application years (Table 6.5). However, maximum concentrations of $TSiO_2$ and SiO_2 were measured in summer pre-application, while maximum concentrations were measured in winter post-application. In contrast, surface water TDC ($W = 327$; $p < 0.001$; $n_1 = n_2 = 15$), DIC ($W = 342$; $p < 0.001$; $n_1 = n_2 = 15$) and DOC ($W = 291.5$; $p < 0.05$; $n_1 = n_2 = 15$) concentrations were significantly lower in summer 2010 compared to pre-application concentrations (Fig. 6.6; Table 6.5), whereas autumn DOC

concentrations increased significantly ($W = 179.5$; $p < 0.05$; $n_1 = n_2 = 15$) in the first year post-application.

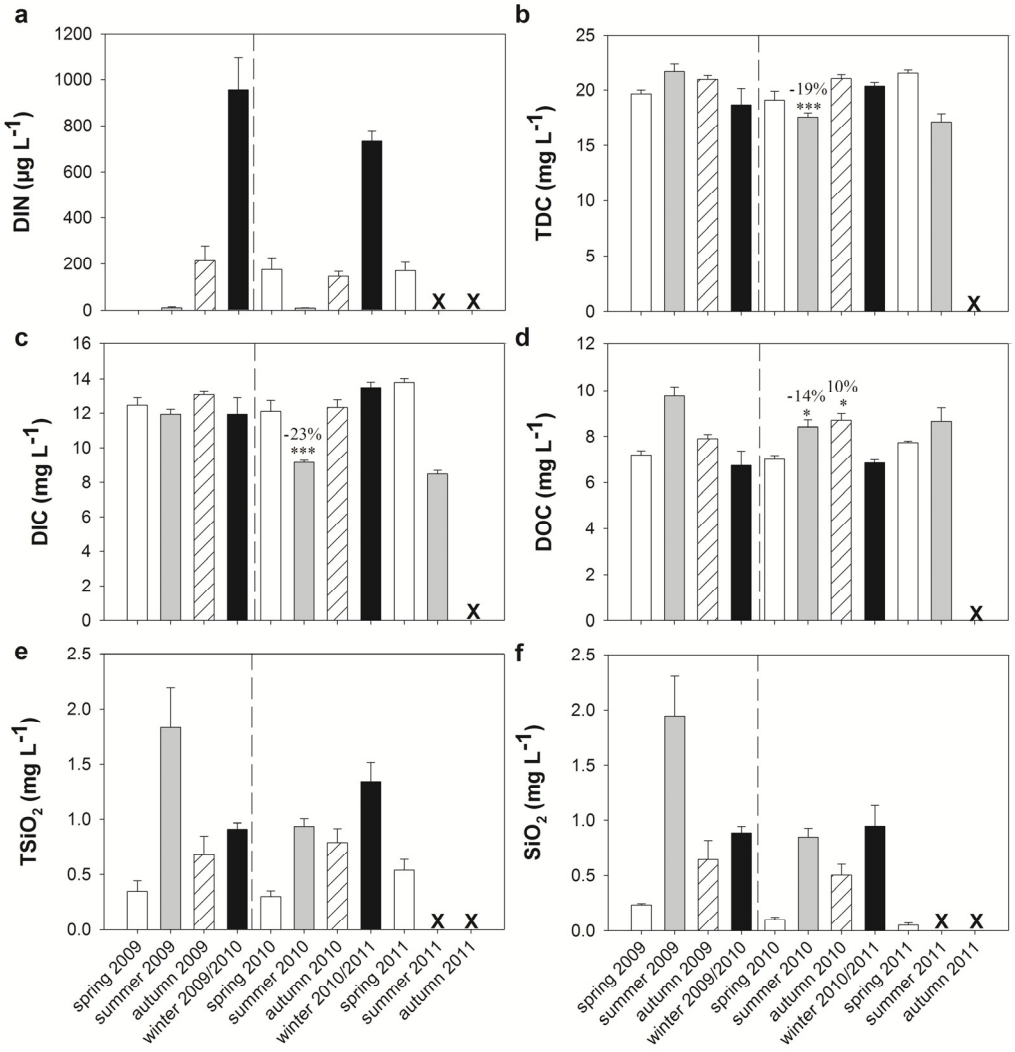


Figure 6.6 Seasonal variation in surface water carbon, nitrogen and silica concentration, including (a) dissolved inorganic nitrogen (DIN), (b) total dissolved carbon (TDC), (c) dissolved inorganic carbon (DIC), (d) dissolved organic carbon (DOC), (e) total diatom silica ($TSiO_2$), and (f) dissolved silica (SiO_2). Dashed vertical line indicates the timing of the Phoslock® application and seasons in which no sampling occurred are marked with 'X'. Error bars represent standard error of the mean (for sample size see Appendix: Table A6.1). Significant differences (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$) from seasonal comparisons between pre- and post-application years (i.e. 2009 vs. 2010; 2009 vs. 2011) and percent change are indicated. Seasons are spring (white bars), summer (grey bars), autumn (white hatched bars) and winter (black bars).

Table 6.5 Results of non-parametric Mann-Whitney U-test (MWU) investigating variation in surface water dissolved inorganic nitrogen (DIN), total dissolved carbon (TDC), dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), total diatom silica ($TSiO_2$) and soluble silica (SiO_2) concentration in a given season between years.

Parameter	Season	n_{09}=	n_{10}=	n_{11}=	MWU		
					09/10	09/11	10/11
DIN	spring	5 [I]	15	5 [I]	▪	▪	▪
	summer	13	15	0	n.s.	▪	▪
	autumn	15	15	0	n.s.	▪	▪
	winter	4	10	0	n.s.	▪	▪
TDC	spring	5 [I]	15	5 [I]	▪	▪	▪
	summer	15	15	5 [I]	***	▪	▪
	autumn	15	15	0	n.s.	▪	▪
	winter	4	10	0	n.s.	▪	▪
DIC	spring	5 [I]	15	5 [I]	▪	▪	▪
	summer	15	15	5 [I]	***	▪	▪
	autumn	15	15	0	n.s.	▪	▪
	winter	4	10	0	n.s.	▪	▪
DOC	spring	5 [I]	15	5 [I]	▪	▪	▪
	summer	15	15	5 [I]	*	▪	▪
	autumn	15	15	0	*	▪	▪
	winter	4	10	0	n.s.	▪	▪
$TSiO_2$	spring	5 [I]	15	5 [I]	▪	▪	▪
	summer	15	15	0	n.s.	▪	▪
	autumn	15	15	0	n.s.	▪	▪
	winter	4	10	0	n.s.	▪	▪
SiO_2	spring	5 [I]	15	5 [I]	▪	▪	▪
	summer	15	15	0	n.s.	▪	▪
	autumn	15	15	0	n.s.	▪	▪
	winter	4	10	0	n.s.	▪	▪

09, 2009; 10, 2010; 11, 2011; /, versus; [I], season consists of less than two out of three possible months; ▪, no test possible as season consists of less than two months, difference in number of observations between seasons ≥ 6 or sample size of season smaller than n = 3; n.s., $p \geq 0.05$; *, $p < 0.05$; ***, $p < 0.001$

Biological parameters

Phytoplankton

Mean summer biovolume of Cyanophyceae (Fig. 6.7a) did not vary significantly between 2009 and 2010 but decreased significantly ($F = 28.84$; $p < 0.01$; $DF = 2$; $n = 3$) between 2009 and 2011. In contrast, mean summer biovolume of Euglenophyceae (Fig. 6.7h) increased significantly ($F = 8.29$; $p < 0.05$; $DF = 2$; $n = 3$) between 2010 and 2011. Mean summer total phytoplankton biovolume (Fig. 6.7i) was significantly lower ($F = 21.25$; $p < 0.01$; $DF = 2$; $n = 3$) in 2010 and 2011 compared to pre-application estimates. It should be noted that no assessment could be made against spring 2009 for all phytoplankton classes, because the spring season 2009 consisted of only one month (May).

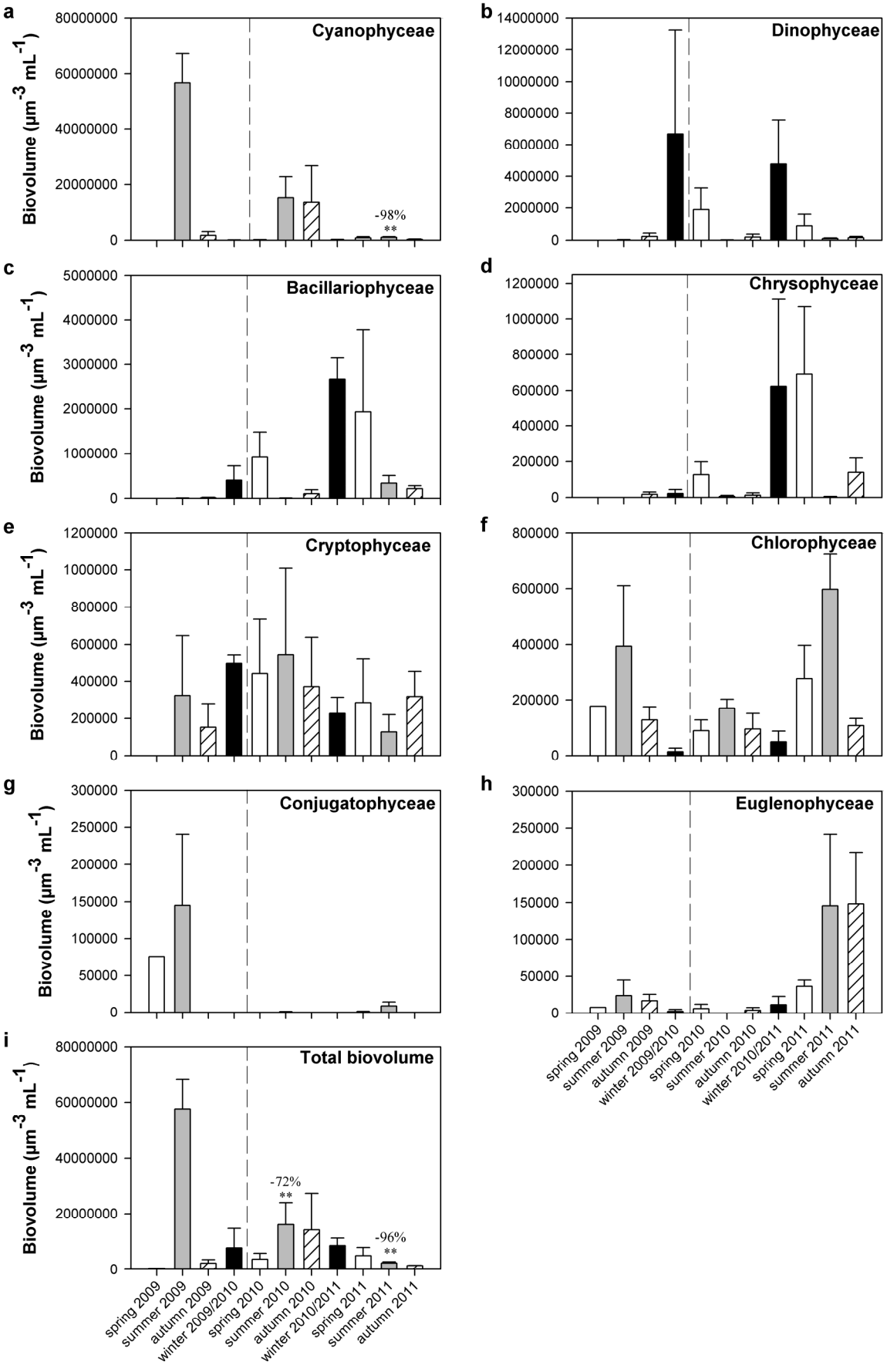

a
Cyanophyceae
Biovolume (µm-3 mL-1)
80000000
60000000
40000000
20000000
0
-98%
**
b
Dinophyceae
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
c
Bacillariophyceae
5000000
4000000
3000000
2000000
1000000
0
d
Chrysophyceae
1200000
1000000
800000
600000
400000
200000
0
e
Cryptophyceae
1200000
1000000
800000
600000
400000
200000
0
f
Chlorophyceae
800000
600000
400000
200000
0
g
Conjugatophyceae
300000
250000
200000
150000
100000
50000
0
h
Euglenophyceae
300000
250000
200000
150000
100000
50000
0
i
Total biovolume
80000000
60000000
40000000
20000000
0
-72%
**
-96%
**
spring 2009
summer 2009
autumn 2009
winter 2009/2010
spring 2010
summer 2010
autumn 2010
winter 2010/2011
spring 2011
summer 2011
autumn 2011

Figure 6.7 (p. 176) Seasonal variation in biovolume of individual phytoplankton classes in Loch Flemington, including (a) Cyanophyceae, (b) Dinophyceae, (c) Bacillariophyceae, (d) Chrysophyceae, (e) Cryptophyceae, (f) Chlorophyceae, (g) Conjugatophyceae, (h) Euglenophyceae and (i) total phytoplankton biovolume. Phytoplankton classes are presented in order of peak biovolume values. Dashed vertical line indicates the timing of the Phoslock® application. Error bars represent standard error of the mean (for sample size see Appendix: Table A6.1). Significant differences (**, $p < 0.01$) from seasonal comparisons between pre- and post-application years (i.e. 2009 vs. 2010; 2009 vs. 2011) and percent change are indicated. Seasons are spring (white bars), summer (grey bars), autumn (white hatched bars) and winter (black bars).

Seasonal variation in the contribution of individual phytoplankton classes to total phytoplankton biovolume is presented (Fig. 6.8). The percentage contribution of species belonging to the class Cyanophyceae (Fig. 6.8a) to total phytoplankton biovolume was significantly lower ($F = 19.58$; $p < 0.01$; $DF = 2$; $n = 3$) in summer 2011 compared to summer 2009 and summer 2010. In contrast, the contribution of species of the phytoplankton class Dinophyceae (Fig. 6.8b) was significantly higher ($F = 7.56$; $p < 0.05$; $DF = 2$; $n = 3$) in summer 2011 compared to summer 2009 and summer 2010. Similarly, the contribution of species of the phytoplankton class Chlorophyceae (Fig. 6.8f) was significantly higher ($F = 13.75$; $p < 0.01$; $DF = 2$; $n = 3$) in summer 2011 compared to summer 2009 and summer 2010. The contribution of species of the class Cryptophyceae (Fig. 6.8e) to total phytoplankton biovolume decreased significantly ($T = 3.43$; $p < 0.05$; $n_1 = n_2 = 3$) between spring 2010 and spring 2011. It should be noted that no assessment could be made against spring 2009 for all phytoplankton classes, because the spring season 2009 consisted of only one month (May).

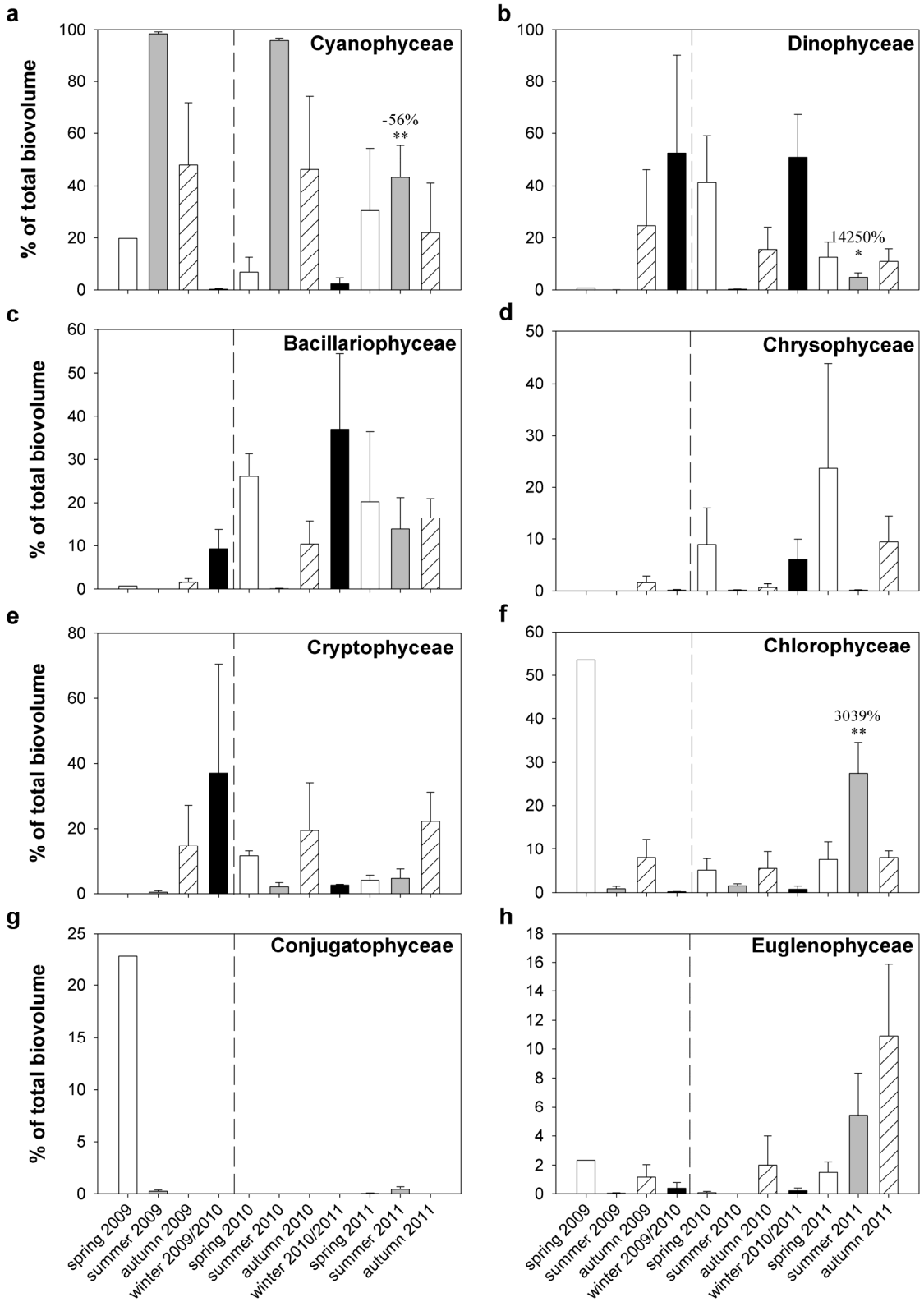

a
Cyanophyceae
-56%
**
b
Dinophyceae
14250%
*
c
Bacillariophyceae
d
Chrysophyceae
e
Cryptophyceae
f
Chlorophyceae
3039%
**
g
Conjugatophyceae
h
Euglenophyceae
% of total biovolume
spring 2009
summer 2009
autumn 2009
winter 2009/2010
spring 2010
summer 2010
autumn 2010
winter 2010/2011
spring 2011
summer 2011
autumn 2011

Figure 6.8 (p. 178) Seasonal variation in the percentage contribution of individual phytoplankton classes to total phytoplankton biovolume in Loch Flemington, including (a) Cyanophyceae, (b) Dinophyceae, (c) Bacillariophyceae, (d) Chrysophyceae, (e) Cryptophyceae, (f) Chlorophyceae, (g) Conjugatophyceae and (h) Euglenophyceae. Dashed vertical line indicates the timing of the Phoslock® application. Error bars represent standard error of the mean (for sample size see Appendix: Table A6.1). Significant differences (*, $p < 0.05$; **, $p < 0.01$) from seasonal comparisons between pre- and post-application years (i.e. 2009 vs. 2010; 2009 vs. 2011) and percent change are indicated. Seasons are spring (white bars), summer (grey bars), autumn (white hatched bars) and winter (black bars).

Seasonal variation in chlorophyll *a* concentration between years is presented (Fig. 6.9; Table 6.6). Highest chlorophyll *a* concentrations were measured in summer 2009. Summer chlorophyll *a* concentrations were significantly lower in 2010 ($W = 337$; $p < 0.001$; $n_1 = n_2 = 15$) and 2011 ($W = 285$; $p < 0.001$; $n_1 = 15$, $n_2 = 11$) compared to pre-application concentrations.

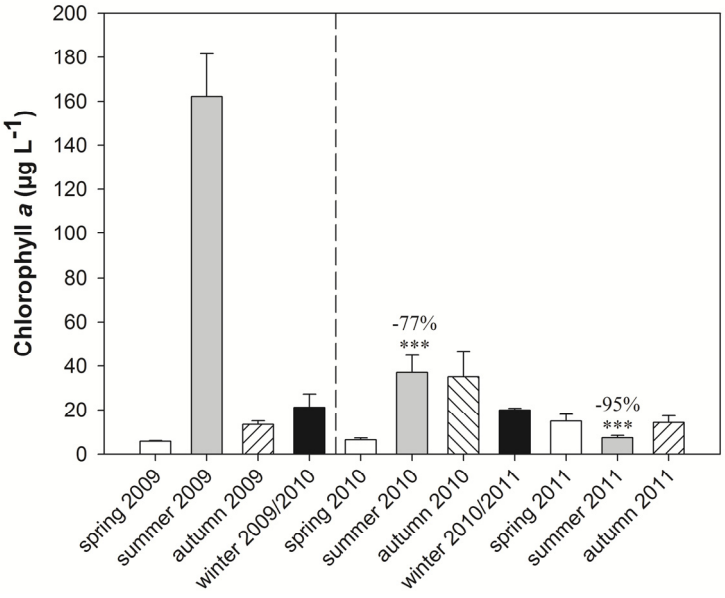


Figure 6.9 Seasonal variation in chlorophyll *a* concentrations in Loch Flemington. Dashed vertical line indicates timing of Phoslock® application and error bars represent standard error of the mean (for sample size see Appendix: Table A6.1). Significant differences (***, $p < 0.001$) from seasonal comparisons between pre- and post-application years (i.e. 2009 vs. 2010; 2009 vs. 2011) and percent change are indicated. Seasons are spring (white bars), summer (grey bars), autumn (white hatched bars) and winter (black bars).

Table 6.6 Results of non-parametric Kruskal-Wallis test (KW; degrees of freedom = 2) and Mann-Whitney U-test (MWU) investigating variation in chlorophyll *a* concentrations in a given season between years.

Season	n_{09}=	n_{10}=	n_{11}=	KW	MWU		
					09/10	09/11	10/11
spring	5[1]	15	11	-	▪	▪	*
summer	15	15	11	***	***	***	***
autumn	15	15	9	n.s.	n.s.	n.s.	n.s.
winter	4	9	0	-	n.s.	▪	▪

09, 2009; 10, 2010; 11, 2011; /, versus; [1], season consists of less than two out of three possible months; -, not applicable as data from at least three years required; ▪, no test possible as season consists of less than two months, difference in number of observations between seasons ≥ 6 or sample size of season smaller than n = 3; n.s., $p \geq 0.05$; *, $p < 0.05$; ***, $p < 0.001$

Macrophyte maximum colonisation depth

MCD (Fig. 6.10; Table 6.7) increased significantly in summer ($W = 55$; $p < 0.001$; $n_1 = 10$, $n_2 = 15$) and autumn ($W = 162$; $p < 0.01$; $n_1 = n_2 = 15$) between 2009 and 2010.

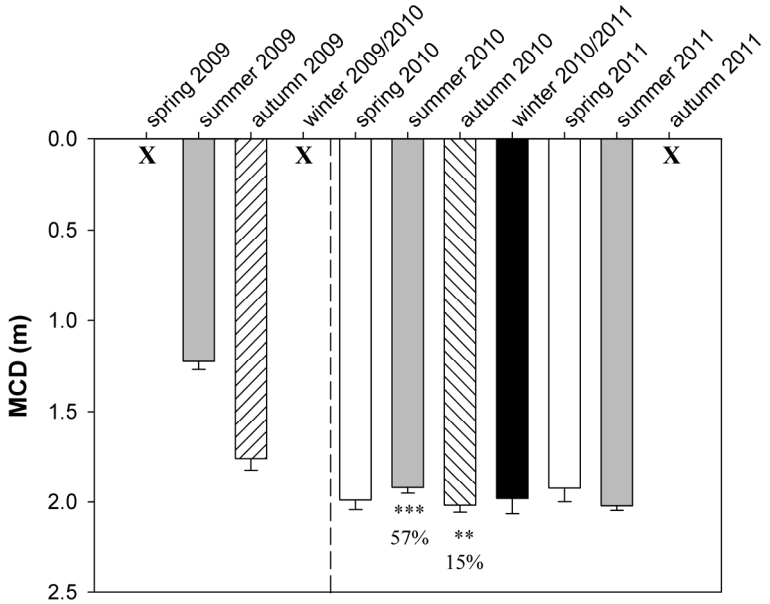


Figure 6.10 Seasonal variation in maximum colonisation depth (MCD) of submerged macrophytes in Loch Flemington. Dashed vertical line indicates timing of the Phoslock® application, error bars represent standard error of the mean (for sample size see Appendix: Table A6.1) and seasons in which no sampling occurred are marked with 'X' Significant differences (**, $p < 0.01$; ***, $p < 0.001$) from seasonal comparisons between pre- and post-application years (i.e. 2009 vs. 2010; 2009 vs. 2011) and percent change are indicated. Seasons are spring (white bars), summer (grey bars), autumn (white hatched bars) and winter (black bars).

Table 6.7 Results of non-parametric Mann-Whitney U-test (MWU) investigating variation in maximum colonisation depth (MCD) of macrophytes in a given season between years.

Season	n_{09}=	n_{10}=	n_{11}=	MWU		
				09/10	09/11	10/11
spring	0	15	5 [I]	▪	▪	▪
summer	10	15	5 [I]	***	▪	▪
autumn	15	15	0	**	▪	▪
winter	0	10	0	▪	▪	▪

09, 2009; 10, 2010; 11, 2011; /, versus; [I], season consists of less than two out of three possible months; ▪, no test possible as season consists of less than two months, difference in number of observations between seasons ≥ 6 or sample size of season smaller than n = 3;**, $p < 0.01$; ***, $p < 0.001$

Zooplankton

Seasonal variation in different zooplankton groups is presented (Fig. 6.11). No significant changes in seasonal abundance of zooplankton were detected.

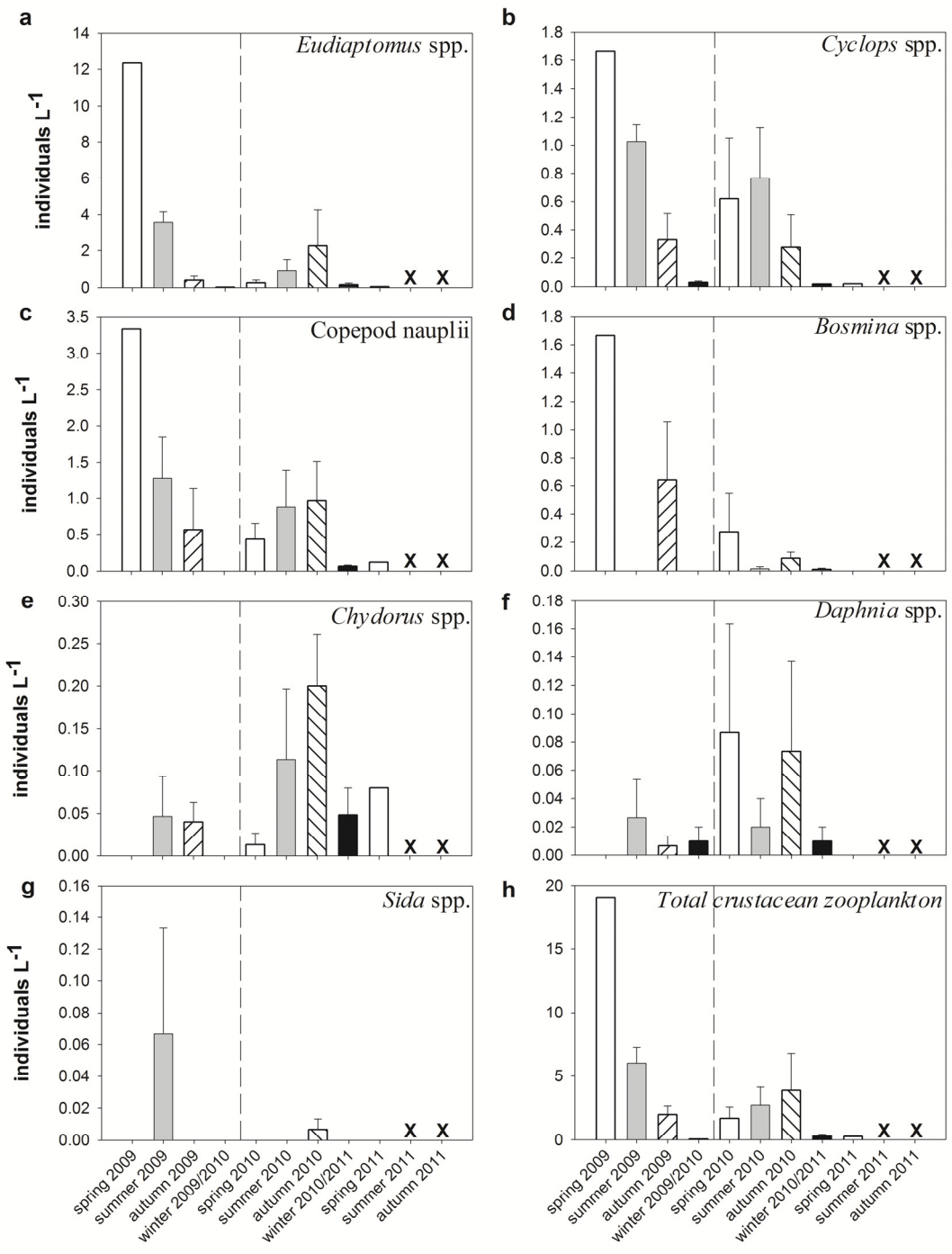


Figure 6.11 Seasonal variation in zooplankton groups in Loch Flemington, including (a) *Eudiaptomus* spp., (b) *Cyclops* spp., (c) copepod nauplii, (d) *Bosmina* spp., (e) *Chydorus* spp., (f) *Daphnia* spp., (g) *Sida* spp., and (h) total crustacean zooplankton. Dashed vertical line indicates the timing of the Phoslock® application, error bars represent standard error of the mean (for sample size see Appendix: Table A6.1) and seasons in which no sampling occurred are marked with 'X'. Seasons are spring (white bars), summer (grey bars), autumn (white hatched bars) and winter (black bars).

Benthic macroinvertebrates

Seasonal variation in benthic macroinvertebrate abundances are presented (Table 6.8; Fig. 6.12). Abundances of species belonging to the groups Chironomidae, Oligochaeta and Sphaeridae (Fig. 6.12b, c, d) significantly decreased (for details see Appendix: Table A6.4) in summer and autumn 2010 compared to the same seasons in 2009. In contrast, abundances of species of the group Trichoptera increased significantly ($W = 178.5$; $p < 0.05$; $n_1 = n_2 = 15$) in autumn 2010 compared to autumn 2009 (Fig. 6.12g).

Table 6.8 Results of non-parametric Mann-Whitney U-test (MWU) investigating variation in benthic macroinvertebrate abundance in a given season between years.

Group	Season	n_{09}=	n_{10}=	n_{11}=	MWU		
					09/10	09/11	10/11
Chaoboridae	spring	5 [I]	15	5 [I]	▪	▪	▪
	summer	14	15	0	n.s.	▪	▪
	autumn	15	15	0	n.s.	▪	▪
	winter	4	10	0	n.s.	▪	▪
Chironomidae	spring	5 [I]	15	5 [I]	·	▪	▪
	summer	14	15	0	***	▪	▪
	autumn	15	15	0	***	▪	▪
	winter	4	10	0	n.s.	▪	▪
Oligochaeta	spring	5 [I]	15	5 [I]	▪	▪	▪
	summer	14	15	0	**	▪	▪
	autumn	15	15	0	*	▪	▪
	winter	4	10	0	n.s.	▪	▪
Sphaeriidae	spring	5 [I]	15	5 [I]	▪	▪	▪
	summer	14	15	0	*	▪	▪
	autumn	15	15	0	**	▪	▪
	winter	4	10	0	n.s.	▪	▪
Hydrobiidae	spring	5 [I]	15	5 [I]	▪	▪	▪
	summer	14	15	0	n.s.	▪	▪
	autumn	15	15	0	n.s.	▪	▪
	winter	4	10	0	n.s.	▪	▪
Bithyniidae	spring	5 [I]	15	5 [I]	▪	▪	▪
	summer	14	15	0	n.s.	▪	▪
	autumn	15	15	0	n.s.	▪	▪
	winter	4	10	0	n.s.	▪	▪
Trichoptera	spring	5 [I]	15	5 [I]	▪	▪	▪
	summer	14	15	0	n.s.	▪	▪
	autumn	15	15	0	*	▪	▪
	winter	4	10	0	n.s.	▪	▪
Nematoda	spring	5 [I]	15	5 [I]	▪	▪	▪
	summer	14	15	0	▪	▪	▪
	autumn	15	15	0	▪	▪	▪
	winter	4	10	0	▪	▪	▪

09, 2009; 10, 2010; 11, 2011; /, versus; [I], season consists of less than two out of three possible months; ▪, no test possible as season consists of less than two months, difference in number of observations between seasons ≥ 6 or sample size of season smaller than n = 3; n.s., $p \geq 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

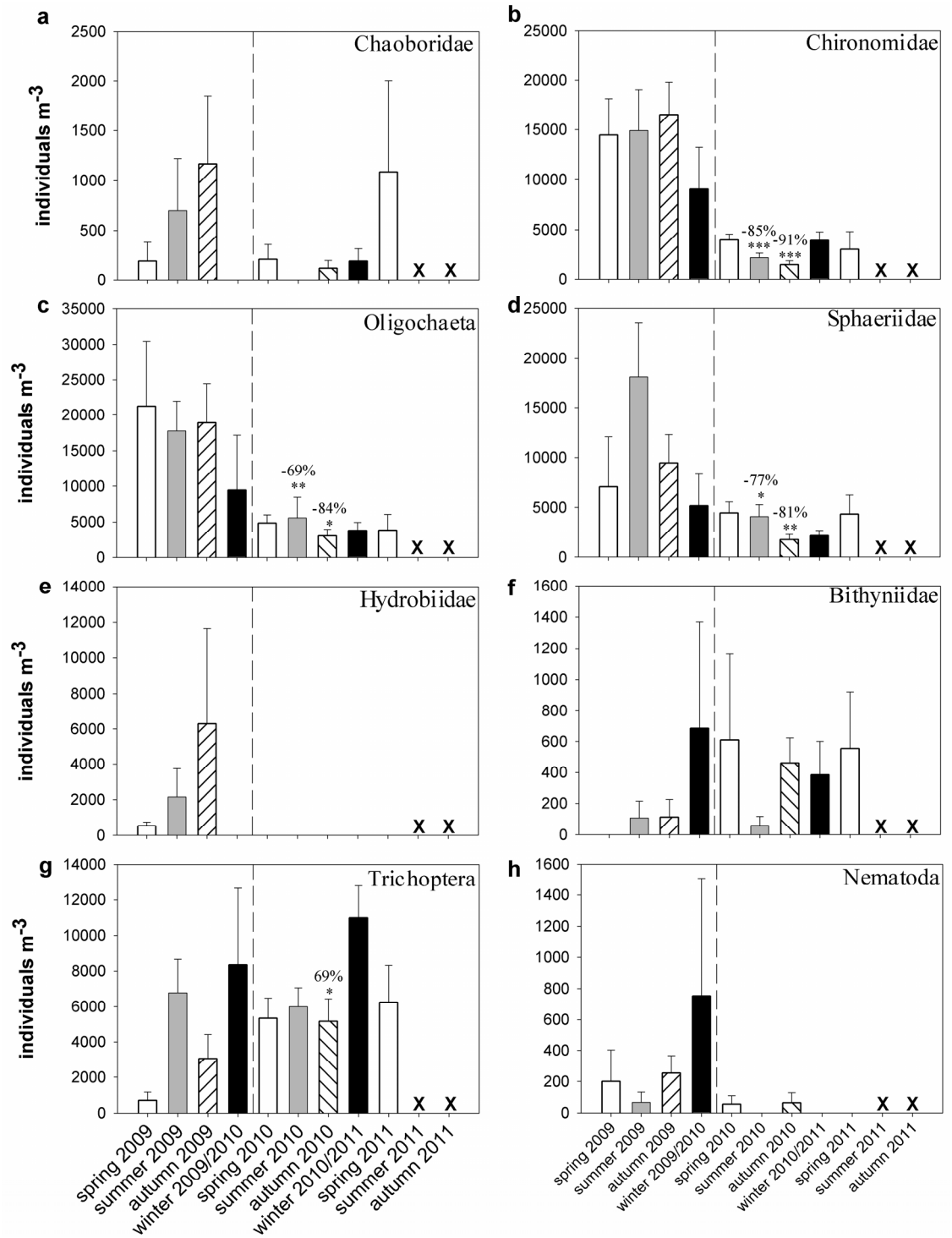

a
Chaoboridae
individuals m-3
b
Chironomidae
-85%
***
-91%
***
c
Oligochaeta
-69%
**
-84%
*
d
Sphaeriidae
-77%
*
-81%
**
e
Hydrobiidae
f
Bithyniidae
g
Trichoptera
69%
*
h
Nematoda
spring 2009
summer 2009
autumn 2009
winter 2009/2010
spring 2010
summer 2010
autumn 2010
winter 2010/2011
spring 2011
summer 2011
autumn 2011

Figure 6.12 (p. 186) Seasonal variation in abundance of benthic macroinvertebrate groups in Loch Flemington, including (a) Chaoboridae, (b) Chironomidae, (c) Oligochaeta, (d) Sphaeriidae, (e) Hydrobiidae, (f) Bithyniidae, (g) Trichoptera, and (h) Nematoda. Dashed vertical line indicates the timing of the Phoslock® application and seasons in which no sampling occurred are marked with 'X'. Error bars represent standard error of the mean (for sample size see Appendix: Table A6.1). Significant differences (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$) from seasonal comparisons between pre- and post-application years (i.e. 2009 vs. 2010; 2009 vs. 2011) and percent change are indicated. Seasons are spring (white bars), summer (grey bars), autumn (white hatched bars) and winter (black bars).

Fish community abundance and composition

Variation in the number of fish caught using traps between 2003-2006 and 2010 is presented (Fig. 6.13). Three-spined sticklebacks (*Gasterosteus aculeatus*) were the only species caught in all years. No significant variation was detected in mean fish numbers between years 2003-2006 and 2010. The gill net survey (n = 3) in July 2011 resulted in the catch of 1021 three-spined sticklebacks (catch per unit effort 330 fish net^{-1} $night^{-1}$). No other fish species were caught during the gill net survey.

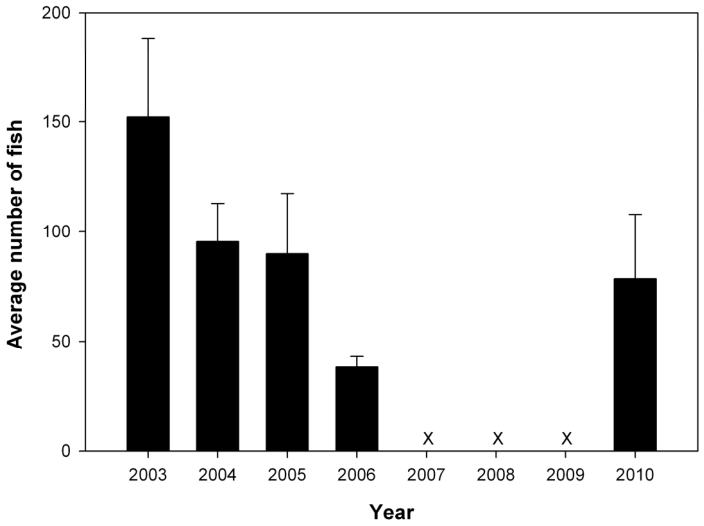


Figure 6.13 Three-spined stickleback (*Gasterosteus aculeatus*) caught in Loch Flemington using Gee Minnow Traps between 2003 and 2010. Error bars represent standard error of the mean (n = 6 for 2003 – 2006; n = 5 in 2010), years were no sampling was conducted are marked with 'X'.

Correlation analysis between physicochemical and biological parameters

The results of the correlation analysis are presented (Table 6.9). Phytoplankton biomass (measured as chlorophyll *a* and total phytoplankton biovolume) was significantly positively correlated to surface water TP ($p < 0.001$) and SRP ($p < 0.05$) concentration. Similarly total zooplankton abundance was significantly positively correlated to surface water TP ($p < 0.05$) and SRP concentration ($p < 0.05$) but not to phytoplankton biomass. In contrast, water clarity was significantly negatively correlated to surface water TP concentration ($p < 0.001$), SRP concentration ($p < 0.05$), chlorophyll *a* concentration ($p < 0.001$) and total phytoplankton biovolume ($p < 0.01$). MCD did not show a significant correlation to water clarity, phytoplankton biomass (chlorophyll *a* and total phytoplankton biovolume) or surface water P concentrations (SRP or TP). Total macroinvertebrate abundance was significantly negatively ($p < 0.001$) correlated to MCD.

Table 6.9 Summary of correlation analysis (n = 17 months; 6 months pre-application and 11 months post-application), including surface water total phosphorus concentrations (TP; µg L^{-1}), surface water soluble reactive phosphorus concentrations (SRP; µg L^{-1}), chlorophyll *a* concentrations (chl *a*; µg L^{-1}), total phytoplankton biovolume (biovol.; μm^{-3} mL^{-1}), water clarity (clarity; m), maximum colonisation depth of macrophytes (MCD; m), total zooplankton abundance (tot. zoo.; individuals L^{-1}) and total macroinvertebrate abundance (tot. invert.; individuals m^{-3}). Significant correlations are presented in bold with p-values in superscript (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

Variable	TP	SRP	chl *a*	biovol.	clarity	MCD	tot. zoo
SRP	**+0.625****						
chl a	**+0.877*****	**+0.588***					
biovol.	**+0.794*****	**+0.485***	**+0.951*****				
clarity	**-0.893*****	**-0.605***	**-0.763*****	**-0.723****			
MCD	-0.324	-0.338	-0.211	-0.071	+0.264		
tot. zoo.	**+0.493***	**+0.485***	+0.311	+0.292	**-0.578***	-0.346	
tot. invert.	+0.086	+0.203	-0.042	-0.127	-0.133	**-0.787*****	+0.230

DISCUSSION

The application of Phoslock® caused significant changes in ecological structure and nutrient cycling processes that were consistent with a reduction of internal P-loading. These included a decrease in summer in-lake P concentrations, a decline in phytoplankton biomass and an increase in water clarity in particular. The response of physical, chemical and biological variables to the reduction of internal P-loading was most pronounced in the summer season in both post-application years. Similar responses, albeit over longer time scales, have been observed in multi-lake studies assessing the recovery of shallow lakes from eutrophication following reductions of external P-loads (Jeppesen *et al*., 2005b). Alterations in ecological structure indicated a change in state from a phytoplankton dominated turbid state to a macrophyte dominated clear water state in Loch Flemington following application.

Pre-application conditions

A comparison of mean seasonal air temperature and precipitation between the period 1999-2008 and 2009 indicated similar weather conditions with peak temperatures and precipitation patterns of comparable magnitude in all seasons. In addition, a comparison of surface water TP concentrations between the period 2000-2003 and 2009 showed a similar seasonal pattern with peak concentrations of comparable magnitude particularly in summer. This indicated that 2009 can be considered as a suitable baseline year, representing common peak TP concentrations and seasonal patterns of TP concentrations as well as common weather conditions prior to the application of Phoslock®.

Structural changes following the reduction of internal P-loading

A comparison of surface water TP concentrations between the period 2000-2003 and 2009 showed a similar seasonal pattern with peak concentrations of comparable magnitude particularly in summer. This indicated that P-cycling between bed sediments and the water-column did not diminish notably over nearly a decade in Loch Flemington, although external P-loads were already reduced between 1989 and 1993 (May *et al*., 2001). This is in line with assumptions that sediment P-release can delay the recovery of shallow lakes for up to 10-15 years following reduction of external P-loading (Søndergaard *et al*., 1999, 2005; Jeppesen *et al*., 2005b). However, these estimates are commonly derived from lakes in which P may be flushed from the water body via an outflow. In contrast, Loch Flemington has no surface water outflow and hence retains all particulate P (May *et al*., 2001) and thus it is likely that this lack of an outflow, and associated retention of P within the lake, facilitated internal P-loading between 2000-2003 and 2009.

In 2009, the year prior to the application of Phoslock®, peak surface and bottom water SRP and TP concentrations were measured in summer, as observed in other shallow lakes in which internal P-loading drives the seasonal P cycle (Søndergaard *et al*., 1999, 2001, 2005, 2012; Spears *et al*., 2012). Post-application, summer surface and bottom water SRP and TP concentrations decreased significantly, most probably as a consequence of an increase in sediment P-binding capacity (Chapter 5; Meis *et al*., 2012). The magnitude of change in TP concentrations were in agreement with findings of other whole-lake studies using P-capping agents (Reitzel *et al*., 2005), whereas the decrease in bottom water SRP concentrations is indicative of reduced sediment P-release in shallow lakes (Boström *et al*. 1988; Jensen pers. comm.). The significant reduction in in-lake P concentrations indicated that the application of Phoslock® caused

a disruption of internal P-loading, which is a major feedback mechanism stabilizing the phytoplankton dominated turbid state in many shallow lakes.

Response of phytoplankton

Controlling internal P-loading has been shown to cause rapid changes in ecological structure, including alterations in phytoplankton abundance and community composition (Cooke *et al*., 2005; Mehner *et al*., 2008). Similarly, the reduction of internal P-loading in Loch Flemington caused a significant reduction in summer phytoplankton biomass (measured as chlorophyll *a* concentration and phytoplankton biovolume) in the first and second year post-application of similar magnitude as observed by Mehner *et al*. (2008). A range of studies have shown that phytoplankton biomass can be effectively controlled in many cases by reducing P concentrations (Dillon and Rigler, 1974; Schindler, 1977, 1978; Vollenweider and Kerekes, 1980; Phillips *et al*., 2008). The data from Loch Flemington showed that phytoplankton biomass (measured as chlorophyll a concentration and phytoplankton biovolume) was significantly positively correlated with surface water P concentrations (particularly TP). This significant relationship was also reflected by a comparable magnitude of change (decrease) in phytoplankton biomass (first year: -72 to -77%; second year: -95 to -96%) and summer surface water TP concentration (first year: -65%; second year: -81%) post-application. Although phytoplankton biomass decreased significantly in summer in the first year post-application (2010), the contribution of individual phytoplankton classes to total phytoplankton biomass did not change significantly. However, in 2011, the second year post-application, the percentage contribution of Cyanophyceae to the total phytoplankton biovolume in summer decreased significantly as observed by other studies (Downing *et al*., 2001; Jeppesen *et al*., 2005b), while the percentage contribution of Dinophyceae and Chlorophyceae increased significantly in summer

2011. Such alterations in phytoplankton community structure are considered to represent an increase in food quality for zooplankton (Ahlgren *et al*., 1990; Ferrão-Filho *et al*., 2003; Elliot, 2012). It appeared that the phytoplankton community composition prior to the application of Phoslock® influenced phytoplankton community composition in the first year post-application, although phytoplankton biomass was significantly reduced. However, changes in phytoplankton community composition were observed in the second year post-application. The decrease in phytoplankton biomass and therefore pelagic primary production was also reflected by a significant decrease in surface water pH in 2011.

Response of macrophytes

Reductions in phytoplankton biomass (i.e. chlorophyll *a* concentrations and total phytoplankton biovolume) most probably caused the significant increase in water clarity as reported in similar studies (e.g. Mazumder *et al*., 1990). This was also indicated through the significant negative correlation reported between water clarity and phytoplankton biomass in Loch Flemington. Water clarity is an important controlling factor in the inverse relationship between phytoplankton and macrophytes, both important ecological structural components in shallow lakes (Scheffer, 1998; Scheffer *et al*., 1993; Scheffer and Jeppesen, 1998). Consequently, the observed increase in MCD of macrophytes in summer and autumn in the first year post-application was probably driven by increased water clarity as reported in other studies (Jupp and Spence, 1977; Canfield *et al*., 1985), although water clarity and MCD did not significantly correlate in Loch Flemington. Commonly, changes in MCD are reported to occur gradually over longer, up to decadal time scales (Perkins and Underwood, 2002; May and Carvalho, 2010). However, the macrophyte community of Loch Flemington was dominated by a single species, *Elodea canadensis*. This species is reported to maintain a large standing

crop over the winter period (Haag, 1979), spread primarily vegetatively from stem fragments and grow rapidly from dormant apices in spring (Nichols and Shaw, 1986). These life-cycle traits, and the dominance and presence of *E. canadensis* prior to the application, are probably the best explanation for the rapid increase in MCD post-application. Similarly, the extensive growth of *Elodea* spp. following a fish kill in Alton Water Reservoir has been considered as a major ecological structural change during the recovery of this water body from eutrophication (Perkins and Underwood, 2002). This response was considered to facilitate a change in state, enhancing feedback mechanisms associated with the macrophyte dominated clear water state (Perkins and Underwood, 2002). The functional role of macrophytes in shallow lakes is likely to be greater when a larger area and volume of the water-column is occupied and when the growing season is long (Schutten *et al*., 1994; Scheffer, 1998). It is hypothesised that the increase in MCD, leading to a potential increase in the area of the sediment covered, and a potential increase in the percent volume inhabited, is likely to strengthen the functional role of macrophytes in Loch Flemington, which has also been concluded for Alton Water Reservoir (Perkins and Underwood, 2002).

Response of zooplankton

In Loch Flemington, total zooplankton abundance was significantly positively correlated to surface water P concentrations. However, abundance and community composition of zooplankton did not differ significantly between 2009 and 2010, despite a reduction in the in-lake P concentrations, particularly in summer. Multi-lake studies report a reduction in zooplankton biomass over longer time scales following reduction of external P-load as a consequence of food limitation (Jeppesen *et al*., 2000, 2005b). In Loch Flemington, phytoplankton biomass decreased significantly post-application while phytoplankton community composition did not differ significantly between 2009 and

2010. In both years, the phytoplankton community was dominated in summer and autumn by species of the class Cyanophyceae. It was expected that a decrease in food quantity (i.e. phytoplankton biomass measured as chlorophyll *a* concentration and total biovolume) without improvements in food quality (e.g. dominance of Cyanophyceae; Ahlgren *et al*., 1990; Jeppesen *et al*., 2002) in 2010 would cause a decrease in zooplankton abundance. However, no significant relationship was detected between zooplankton and phytoplankton abundance. The sampling approach of using a combined sample, which lowers sample size, might have impacted on the statistical power to detect change between 2009 and 2010. Additionally, higher taxonomic resolution of the phytoplankton community may be required to analyse variation in food quality. Zooplankton sampling was halted after March 2011. It cannot be excluded that changes in zooplankton community composition occurred following March 2011, especially as changes in phytoplankton community composition (particularly a decrease in contribution of Cyanophyceae and increase in contribution of Chlorophyceae) were observed.

Response of benthic macroinvertebrates

Pre-application the benthic macroinvertebrate community was dominated by species of the groups Chironomidae, Oligochaeta and Sphaeriidae, all of which declined significantly in summer and autumn in the first year post-application. The magnitude of decrease, particularly that of Chironomidae, was similar to that observed in Lake Müggelsee following reduction of external P-loading (Köhler *et al*., 2005). In Lake Müggelsee, the decline in abundance was primarily attributed to a reduction in organic matter deposition to the sediment, although a coinciding increase in grazing pressure by cyprinid fish could not be excluded (Köhler *et al*., 2005). However, the decline in abundance of Oligochaeta and Sphaeriidae were clearly attributed to reductions in

external P-loads in Lake Michigan (Nalepa *et al*., 1998). In contrast, total macroinvertebrate abundance in Loch Flemington was not significantly correlated to TP concentrations or chlorophyll *a* concentrations which can be used as a proxy for organic matter deposition to the sediment, so that a causal link cannot be stated. However, total macroinvertebrate abundance was significantly negatively correlated to MCD which may be an indirect measure of organic matter deposition since MCD is likely to increase at high water clarity and associated low concentrations of particles in the water-column. Furthermore, abundance of sticklebacks did not increase significantly in 2010 compared to pre-application years. It appears therefore unlikely that fish grazing pressure increased post-application, thereby causing a decrease in macroinvertebrate abundance. Of all benthic macroinvertebrate groups only species belonging to the group Trichoptera showed a significant increase in abundance in autumn 2010. An analysis of a different response in macroinvertebrate feeding groups (e.g. filter feeders, shredders, collectors, predators) is hampered by the low taxonomic resolution of the data. It may be argued that the increase in sediment La content had a negative effect on benthic macroinvertebrate abundance. However, studies assessing the response of macroinvertebrate abundance after external load reductions find similar declines in abundance (Köhler *et al*., 2005; Gunn *et al*., 2012), so that any potential toxic effect cannot be easily separated from effects occurring during the reduction of nutrient concentrations. However, the application of Phoslock® may be comparable to loading scenarios of fine inorganic sediment which can detrimentally affect benthic macroinvertebrate abundance (Wagenhoff *et al*., 2012). Future studies may discern the potential negative effect of fine inorganic sediment loading from potential La toxicity on macroinvertebrate abundance by assessing variation in macroinvertebrate abundance following addition of bentonite (i.e. Phoslock® without La) and Phoslock® compared to untreated controls.

Response of fish

The gill net survey in 2011 indicated that the fish community appeared to consist solely of sticklebacks. Furthermore, stickleback abundance did not change significantly when comparing 2000-2006 and 2010. Mesocosm experiments have shown that this species can reduce the number of large-bodied cladocearans, thereby leading to high chlorophyll *a* concentrations at abundances greater than 4 – 6 sticklebacks m^{-2} (Jakobsen *et al*., 2003). Additionally, it is reported that sticklebacks are known to associate with plant beds so that any potential increase in macrophyte MCD and therefore percentage volume inhabited (PVI) is unlikely to have any refugia effect for zooplankton at high stickleback abundance (Stephen *et al*., 1998). It is therefore hypothesised that sticklebacks may have an important 'top down' control on zooplankton in Loch Flemington. However, a survey of defined areas would be required to determine stickleback numbers per area and relate these against findings from other studies (e.g. Jakobsen *et al*., 2003). It has been observed that a decrease in fish biomass and an increase in abundance of piscivorous fish species occurs naturally following reduction of external P-loading but this may take up to 10 - 15 years (Jeppesen *et al*., 2005b). It may therefore be assumed that the abundance of sticklebacks in Loch Flemington might naturally decrease over longer timescales, if in-lake P concentrations remain low. However, a change in fish community composition particularly an increase in piscivorous fish species may be hampered in Loch Flemington because the only tributary to the lake has a maximum depth of approximately 0.1 m in periods of low flow and is culverted over a length of approximately 1.5 km, which may obstruct colonisation pathways for other fish species.

Alteration in nutrient concentrations

The seasonal response of nutrients, in addition to P, revealed that the application of Phoslock® did not cause significant changes in DIN, $TSiO_2$ and SiO_2 concentrations when comparing 2009 and 2010. However, alterations in the timing of peak concentrations of $TSiO_2$ and SiO_2 may be linked to higher abundance (although not significant) of Bacillariophycea in winter post-application. In a study using aluminium hydroxide, silica regeneration from the sediment was significantly reduced, likely due to adsorption and precipitation of silica by aluminium hydroxide or due to reduced mineralization of organic matter (Egemose *et al*., 2011). It appears that Phoslock® had no impact on silica cycling which is important as a decline in the availability of silica might hamper an increase in phytoplankton species of the class Bacillariophyceae (Reynolds, 2006).

Alterations in structure and function

A summary of interactions between selected biotic and abiotic variables in Loch Flemington pre- and post-application is presented in a conceptual diagram (Fig. 6.14a, b) and observed structural and inferred functional changes are summarised (Table 6.10).

Managing resilience

The controlled disturbance using Phoslock® caused a significant decrease in internal P-loading in Loch Flemington. The observed alterations in ecological structure following application indicated a change in state from a phytoplankton dominated turbid state to a macrophyte dominated clear water state. However, resilience, i.e. the ability of a system to maintain a given state following disturbance (Holling, 1973), is influenced by structural and functional components of ecosystems (Jones and Lawton, 1995; Power *et al*., 1996; Carpenter and Cottingham, 1997; Ludwig *et al*., 1997). In order to increase

resilience of the macrophyte dominated clear water state in Loch Flemington the following measures need consideration: i) further reduction of external P-loading as far as it is technically and financially feasible; ii) follow-up applications of P-capping agents (if financially more feasible than managing external P-loads); and iii) biomanipulation of the degraded food web structure. Estimates of external P-loads to Loch Flemington were around 120 kg TP yr^{-1} (May *et al*., 2001). Therefore, external P-loading is likely to increase in-lake P concentrations overtime causing an increase in phytoplankton biomass at the cost of submerged macrophytes. Unless external P-loading can be reduced to zero, which is impossible in populated areas, it appears inevitable that the resilience of the macrophyte dominated clear water state will decrease overtime, rendering the system prone to a reverse switch to the phytoplankton dominated turbid state.

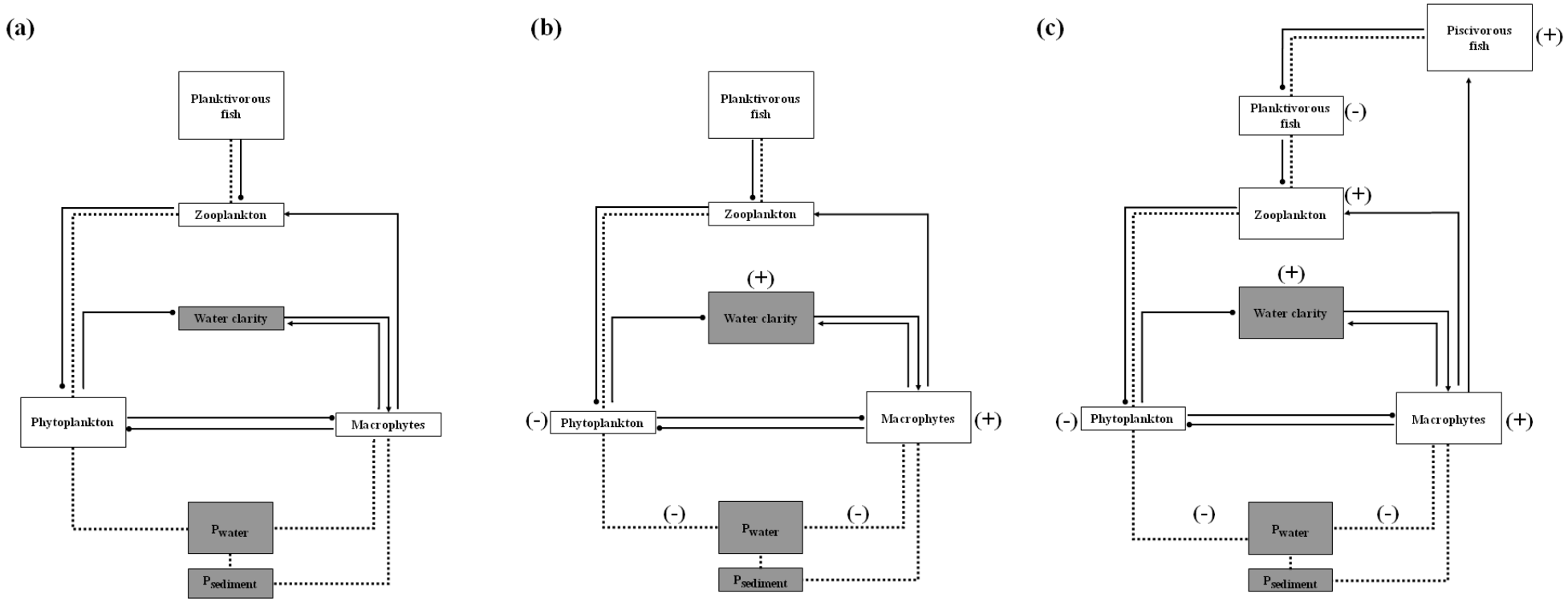


Figure 6.14 Conceptual diagram summarising selected changes in ecological structure and interactions between biotic and abiotic variables in Loch Flemington (a) before the controlled disturbance, (b) after the controlled disturbance, and (c) hypothesised changes after the controlled disturbance and following successful remediation of the fish community structure. Dotted lines indicate phosphorus (P) pathways, lines ending in a circle indicate negative interactions and lines ending in arrows indicate positive interactions. Minus sign (-) indicates a decrease while a plus sign (+) indicates an increase in the importance of a pathway or a structural component.

Table 6.10 Structural responses of various organism groups to reductions in P concentrations following the application of Phoslock® in Loch Flemington and inferred functional response from the literature.

Organism/group	Structural response	Functional response	Ref.
Phytoplankton (chlorophyll *a* and total biovolume)	Reduction in phytoplankton biomass in summer 2010 and 2011	Decrease in organic matter deposition to sediment; decrease in food quantity for zooplankton; decrease in pelagic primary production	1-7
Cyanophyceae	Reduction in biovolume and percentage contribution to total phytoplankton biomass in summer 2011	Increase in food quality for zooplankton	1, 6-8
Dinophyceae	Increase in percentage contribution to total phytoplankton biomass in summer 2011	Decrease in food quality for zooplankton	9
Chlorophyceae	Increase in percentage contribution to total phytoplankton biomass in summer 2011	Increase in food quality for zooplankton	7-9
Macrophytes	Increase in maximum colonisation depth	Increase in benthic primary production	5, 10
Zooplankton	No change in abundance of individual groups	-	-
Macroinvertebrates Chironomidae	Reduction in abundance in summer and autumn 2010	Reduction in sediment redox state promoting increased sediment P-release; reduction of microbial mediate sediment P-release; reduced solute exchange between sediment and water-column; decrease in decomposition rate	11- 14
Oligochaeta	Reduction in abundance in summer and autumn 2010	Reduction in sediment redox state promoting increased sediment P-release; reduced solute exchange between sediment and water-column; decrease in decomposition rate	11, 12, 14
Sphaeriidae	Reduction in abundance in summer and autumn 2010	Decrease in filtration rate; decrease in sediment oxygenation; reduced sediment P-release	15-17
Trichoptera	Increase in abundance in autumn 2010	Increase in filtration rate; decrease in decomposition rate	12
Fish	No change	-	-

Ref., reference; **1**, Gliwicz and Lampert, 1990; **2**, Lang, 1998; **3**, Lang, 1999; **4**, Jeppesen *et al*., 2002; **5**, Vadeboncoeur *et al*., 2003; **6**, Gulati *et al*., 2008; **7**, Elliott, 2012; **8**, Ahlgren *et al*., 1990; **9**, Ferrão-Filho *et al*., 2003; **10**, Scheffer, 1998; **11**, Matisoff *et al*., 1985; **12**, Wallace and Webster, 1996; **13**, Lewandowski *et al*., 2005; **14**, Lewandowski and Hupfer, 2005: **15**, Hornbach *et al*., 1984; **16**, Way, 1989; **17**, Vaughn and Hakenkamp, 2008

Due to the dominance of a single planktivorous fish species (sticklebacks), the potential impaired colonisation pathways for other fish species and the time lag of 10 - 15 years for changes in fish community structure to occur naturally (e.g. Jeppesen *et al*., 2005b), it appears that biomanipulation of the fish community might be a sensible remediation option in order to increase the resilience of the macrophyte dominated clear water state against a reverse switch. Sticklebacks can have cascading effects on phytoplankton biomass and community composition via 'top down' control on zooplankton abundance, community composition and size distribution (Jakobsen *et al*., 2003, 2004). High stickleback abundance may promote a shift from large bodied zooplankton grazers towards small zooplankton species which are less efficient grazers. Consequently, this would favour high phytoplankton abundance and a shift in size distribution towards larger phytoplankton taxa (Brooks and Dodson, 1965; Carpenter *et al*., 1985; Mazumder *et al*. 1990; Jakobsen *et al*., 2003, 2004). It was expected that zooplankton biomass, and therefore zooplankton grazing pressure in Loch Flemington following the application of Phoslock®, were controlled by a combination of 'bottom up' process (i.e. decrease in food quantity) and 'top down' processes (i.e. grazing pressure by sticklebacks). It is hypothesised that a reduction of planktivorous fish is likely to release zooplankton from predation pressure which in turn is likely to: i) increase the 'top down' control of zooplankton on phytoplankton (Jeppesen *et al*., 2005b); ii) cause a shift towards larger-bodied zooplankton species which can reduce the sensitivity of phytoplankton to small pulses of nutrients (Carpenter *et al*., 1992; Cottingham and Schindler, 2000); and iii) assimilate more P at the top of the food web in higher trophic levels (Carpenter *et al*. 1992; Carpenter and Kitchell 1993). Overall, this is likely to sustain lower phytoplankton biomass, higher water clarity which in turn favours growth of submerged macrophytes and feedback mechanisms associated with the macrophyte dominated clear water state. Inferred changes in the interactions between biotic and abiotic variables

following successful reduction of planktivorous fish biomass and increase in piscivorous fish biomass are presented (Fig. 6.14c).

In practice, it appears that removal of planktivorous fish to low numbers followed by stocking of piscivorous fish species is the most successful method (Benndorf, 1990; Perrow *et al*., 1997; Hansson *et al*., 1998; Søndergaard *et al*., 2000). However, repeated removal of planktivorous fish species might be required to achieve long-term effects (Søndergaard *et al*., 2007). Stocking of piscivorous fish alone without removal of planktivorous fish might not lead to low planktivorous fish abundance, and associated improvements in water clarity, as indicated by Skov and Nilsson (2007). A range of studies suggest that a combination of P reduction and biomanipulation methods may be most successful in increasing water quality (Benndorf, 1987; Hosper and Jagtman, 1990; Lathrop *et al*., 1996; Perkins and Underwood, 2002).

CONCLUSIONS

This study showed that the disruption of the internal P-loading feedback mechanism caused significant alterations in P-cycling processes and the ecological structure of Loch Flemington. In particular, in-lake P concentrations and phytoplankton biomass decreased, whereas water clarity and MCD increased. Overall, changes were most pronounced in summer, the season in which internal P-loading has the largest impact on ecological structure in shallow lakes. The observed changes are comparable with findings of long-term multi-lake studies assessing the recovery of shallow lakes from eutrophication although changes observed in this study occurred over a shorter time-scale. The longevity of reduced in-lake P concentrations is likely to be dependent on a combination of external load, stability of the La-P binding and the degree of structural and functional recovery acting to stabilize the macrophyte dominated clear water state. It is hypothesised that additional measures, including biomanipulation of the fish

community for example, are required to facilitate more complete structural and functional recovery which will increase the resilience of the system against a reverse switch towards a phytoplankton dominated turbid state.

Chapter 7

Effects of Phoslock® dose on sediment oxygen concentration and nutrient cycling across the sediment-water interface: results from intact sediment core experiments

Effects of Phoslock® dose on sediment oxygen concentration and nutrient cycling across the sediment-water interface: results from intact sediment core experiments

ABSTRACT

Phoslock® is a lake remediation tool designed to strip dissolved phosphorus (P) from the water-column and reduce sediment P-release. This study investigated the effects of a 'low dose' (6.4 g Phoslock® m^{-2}) and a 'high dose' (4,300 g Phoslock® m^{-2}) Phoslock® treatment on sediment properties (dissolved oxygen (DO) concentrations, pore-water nutrient concentrations, sediment particle entrainment (SPE), sediment chlorophyll *a* content) and physicochemical parameters of the water-column (DO concentrations, pH, conductivity, nutrient concentrations) over an experimental period of 8 days under aerobic conditions using intact sediment cores collected from a shallow lake (Loch Leven, Kinross, UK). Results indicated that both the low dose and the high dose treatments were sufficient to inhibit sediment P-release, as water-column total phosphorus (TP) and soluble reactive phosphorus (SRP) concentrations did not significantly increase over the experimental period. In contrast, control cores with no added Phoslock® showed significant increases in SRP and TP concentrations over the experimental period. No visible Phoslock® layer was observed in the low dose treatment, whereas a 10 mm thick Phoslock® layer was present on the organic sediment surface in the high dose treatment. Sediment DO concentrations, SPE and sediment chlorophyll *a* content, water-column nutrient concentrations and pore-water nutrient concentrations did not vary significantly between the low dose treatment and the control cores at the end of the experimental period (day 8). This is with the exception of significantly lower water-column soluble silica (SiO_2) and pore-water nitrate (NO_3-N) concentrations in the low dose treatment. In contrast, sediment DO concentrations decreased through the Phoslock® layer in the high dose treatment and were significantly

lower (-94%) at the organic sediment surface (0 mm) beneath the inorganic capping layer compared to the organic sediment surface (0 mm) of the control cores. In addition, SPE and sediment chlorophyll *a* content were significantly lower in the high dose treatment relative to the control cores. Water-column TP, SRP, total dissolved carbon (TDC), dissolved inorganic carbon (DIC) and sediment pore-water NO_3-N concentrations were all significantly lower in the high dose treatment compared to the control cores at the end of the experimental period (day 8). In contrast, water-column ammonium (NH_4-N), dissolved inorganic nitrogen (DIN), total diatom silica ($TSiO_2$), SiO_2 and pore-water NH_4-N and DIN concentrations all significantly increased in the high dose treatment compared to the control cores. The results of this study indicated that the application of high areal loads of Phoslock® can, at least temporarily, significantly alter sediment DO concentrations and cycling of nutrients other than P. Alterations in the vertical distribution of DO in the sediment appeared to cause suppression of nitrification processes while denitrification processes appeared to be enhanced. The application of high areal loads of Phoslock® to lakes should therefore be avoided. However, the applicability of these laboratory results to the whole-lake scale requires further validation as experimental conditions are unable to mimic all biogeochemical processes affecting nutrient cycling in lakes *in situ*.

INTRODUCTION

The release of phosphorus (P) from bed sediments (internal P-loading) can prolong the recovery of shallow lakes following external load reductions (Sas, 1989; Marsden, 1989; Søndergaard *et al*., 2003; Jeppesen *et al*., 2005b). In order to meet legally binding water quality targets (e.g. Water Framework Directive) various remediation methods have been trialled to 'speed up' the recovery process (Hupfer and Hilt, 2008; Gibbs *et al*., 2011), including the application of Phoslock®, a lanthanum (La) modified bentonite clay (e.g. Robb *et al*., 2003; Lürling and Faasen, 2011; Meis *et al*., 2012). Studies investigating the use of Phoslock® as a remediation tool to reduce internal P-loading have primarily focused on alterations in sediment elemental composition and sediment P-fractionation (Meis *et al*., 2012; Chapter 4 and 5). These studies showed that the application of Phoslock® causes an increase in sediment La content, which in turn alters sediment P-partitioning by increasing the mass of P present in more refractory sediment P-fractions compared to potentially release-sensitive P-fractions (P_{mobile}) (Meis *et al*., 2012; Chapter 4 and 5). In addition, studies investigated the effects of Phoslock® on organism groups of different trophic levels (Lürling and Tolman, 2010; van Oosterhout and Lürling, 2011; Chapter 6) with a particular focus on the efficiency of Phoslock® in reducing phytoplankton biomass (Lürling and Faassen, 2011; van Oosterhout and Lürling, 2012; Chapter 3 and 6). Results of these studies are contentious. For example, cyanobacteria biomass returned to pre-application conditions in the second year post-application in Clatto Reservoir at which the applied mass of La was estimated to be sufficient to control 17% of P_{mobile} in the top 10 cm of the sediment (Chapter 3 and 4). In contrast, phytoplankton biomass (including cyanobacteria biomass) remained significantly lower in the first two years post-application in Loch Flemington although the relative proportion of P_{mobile} potentially controlled by La was lower (Chapter 3 and 6).

Laboratory studies indicated that variation in applied areal loads of Phoslock® can significantly alter sediment properties including sediment dissolved oxygen (DO) concentration (Vopel *et al*., 2008) and cycling of nutrients between the sediment and the water-column (Gibbs *et al*., 2011). In particular, results indicated a decrease in the thickness of the oxygenated sediment layer below the capping layer at areal loads in excess of 200 g Phoslock® m^{-2} and a shift of the aerobic-anaerobic interface out of the organic sediment into the capping layer at areal loads above 600 g Phoslock® m^{-2} (Vopel *et al*., 2008). Alterations in sediment DO concentration were attributed to both the thickness and the gas diffusivity of the applied capping layer (Vopel *et al*., 2008). Sediment DO distribution can influence sediment redox (reduction-oxidation) state which in turn controls various biological and chemical nutrient transformation processes (Stumm and Morgan, 1996; Wetzel, 2001; Dodds and Whiles, 2010). For example, the release of carbon compounds from sediments commonly occurs following breakdown of organic matter during aerobic and anaerobic respiration, leading to release of inorganic and organic carbon fractions (Heyer and Kalff, 1998). Since aerobic mineralisation rates of organic matter can be up to 10 times faster then anaerobic mineralisation rates, variation in sediment DO concentration may cause a decrease in the breakdown rate of organic compounds (Kristensen *et al*., 1995). The application of Phoslock® might also directly affect C cycling since humic substances might be bound by La (Sonke and Salters, 2006; Ross *et al*., 2008; Tang and Johannesson, 2003, 2010), causing a decrease in water-column dissolved organic carbon (DOC) concentrations. Nitrogen chemistry has also been reported to be impacted by Phoslock®. Ammonium (NH_4-N) produced during mineralisation of organic material is converted to nitrate (NO_3-N) via nitrification in oxygenated sediment layers (Jensen *et al*., 1993; Wetzel, 2001). Gibbs *et al*. (2011) observed an increase in the flux of NH_4-N from the sediment to the overlaying water-column at areal loads of 300 g Phoslock® m^{-2} under aerobic

conditions in the water-column and hypothesised that nitrification was temporarily suppressed due to a reduction of oxygen diffusion to nitrifying bacteria in the sediment. Phoslock® might, therefore, directly (e.g. by binding and precipitation of chemical compounds or reducing the rate of exchange of chemical compounds between the sediment and water) or indirectly (e.g. by alteration of sediment DO concentration) affect nutrient cycling between the sediment and the overlaying water-column, and the magnitude of change may be dependent on the areal load of Phoslock® applied.

Study outline and hypotheses

Variation in water-column and sediment P contents have led to the requirement for a range of areal loads of Phoslock® being applied to lakes (i.e. ranging from 170 – 590 g Phoslock® m^{-2}; Phoslock, 2012). To date studies investigating the effects of variation in areal loads of Phoslock® applied to sediments on DO concentrations (Vopel *et al*., 2008) and nutrient cycling processes (Gibbs *et al*., 2011) have been conducted in isolation. However, sediment DO distribution and biogeochemical nutrient transformation and cycling processes are probably inherently linked. This study, therefore, investigated the effects of a low dose (6.4 g Phoslock® m^{-2}; equivalent to the dose required to bind P released under short-term anaerobic conditions) and a high dose (4,300 g Phoslock® m^{-2}; equivalent to the dose required to bind all P in the sediment) Phoslock® treatment on sediment properties and physicochemical parameters of the water-column using intact sediment cores taken from a shallow lake. These dose levels were determined using a small pilot study using sediment cores from the sample site (see below, section 'pilot study'). The aims were to assess: (i) variation in sediment DO concentrations; (ii) variation in water-column and sediment pore-water nutrient concentrations; (iii) variation in water-column DO concentrations, pH and conductivity; and (iv) the effect of layer thickness on sediment particle entrainment (SPE). The specific hypotheses

tested were: (i) high areal loads of Phoslock® cause lower DO concentrations at the organic sediment surface beneath the inorganic capping layer; (ii) high areal loads of Phoslock® cause a suppression of nitrification processes in the sediment; and (iii) the application of Phoslock® will prevent the release of P from the sediment to the overlaying water-column.

MATERIAL AND METHODS

Study site

Loch Leven is a shallow lake situated in the southeast of Scotland (N 56° 11′, W 3° 22′) with a surface area of 1320 ha and a mean depth of 3.9 m (Spears *et al*., 2012). The lake has a well documented history of eutrophication, and in an attempt to improve water quality external total phosphorus (TP) loads were 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 (May and Spears, 2012). However, sediment P-release is still common particularly in summer in recent years (Spears *et al*., 2006; 2007a, b; 2012). At the time of this study, Loch Leven represented a shallow lake under recovery from eutrophication, and in which internal P-loading was the dominant P source (Spears *et al*., 2012). Loch Leven is therefore a useful comparison to Loch Flemington, the sediments of which could not be used for this experiment given the recent Phoslock® application at the site.

Sample collection

Sediment cores (n = 28) were collected from Loch Leven at a location where the water-column depth was 3.5 m (Fig. 7.1) in September 2010 (22nd of September, n = 8; 28th of September, n = 20). Cores were collected from a boat using a Jenkin surface sediment sampler (core internal diameter 66 mm, core length 500 mm) that facilitated the collection of an undisturbed sediment-water interface consisting of about 16 cm sediment and 29 cm overlying water-column. Cores were transported to the laboratory in the dark within 3 hours of collection.

On each visit *in situ* measurements of surface water temperature, pH, conductivity and DO concentrations were made and underwater light availability close to the sediment surface was assessed (Chapter 2, Section 2.3.1).

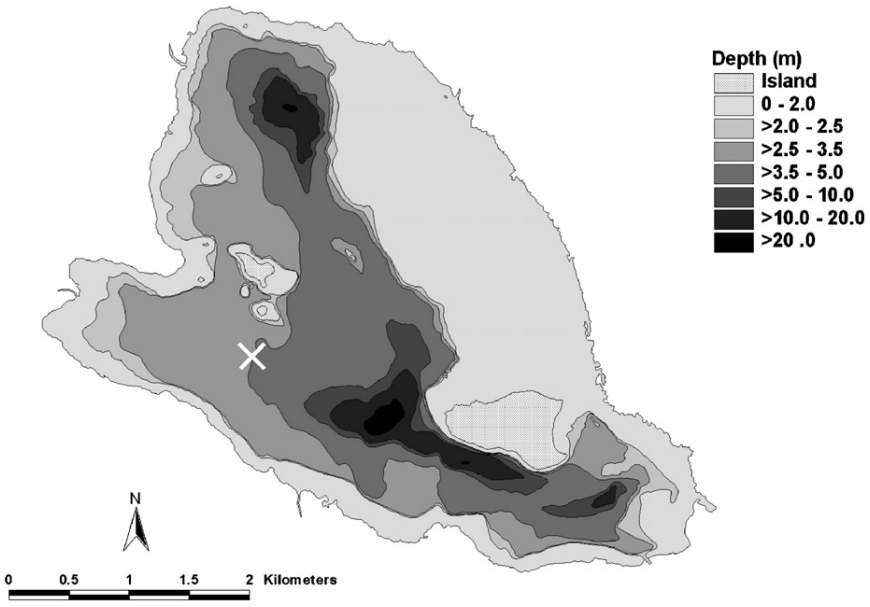


Figure 7.1 Bathymetric map of Loch Leven including the location of the sample point (white cross). Map from Spears and Jones (2010).

Pilot study

Sediment cores (n = 8; 22nd of September 2010) were used for a pilot study to determine the Phoslock® dose required to: i) bind the mass of P potentially released under anaerobic conditions (n = 5); and ii) bind all P in the sediment (n = 3). Dose estimates from the pilot study were used in the 'dosing experiment' described in the following section.

Cores (n = 5) taken for the determination of P potentially released under anaerobic conditions were incubated at ambient lake temperature (13 °C) and ambient low light conditions as measured in Loch Leven (0.5 μmol m^{-2} s^{-1} photosynthetically available radiation (PAR)) for a period of 5 days. Sediment cores were bubbled with oxygen free nitrogen gas (N_2) with care being taken to minimise disturbance of the sediment surface. Water samples (50 mL) for the analysis of soluble reactive phosphorus (SRP: Chapter 2, Section 2.4.4) and TP (Chapter 2, Section 2.4.4) were taken 10 cm above the sediment surface at the beginning and end of the experimental

period using a syringe. SRP concentrations increased from 1.8 µg L^{-1} (± 0.19 standard error (s.e.)) at the beginning to 100.2 µg L^{-1} (± 14.27 s.e.) at the end of the experimental period, while TP concentrations increased from 50.3 µg L^{-1} (± 3.48 s.e.) at the beginning to 231.0 µg L^{-1} (± 11.19 s.e.) at the end of the experimental period. Based on a volume of 1.1 L per core and water-column TP concentrations measured on day 5, it was calculated that the mass of P in the water-column, as a consequence of sediment P-release, equalled 0.24 mg P. Based on a nominal La content of 50,000 mg La kg^{-1} Phoslock® (Afsar and Groves, 2009) and a molar binding ratio of La:P of 1:1 (Haghseresht *et al*., 2009) it was estimated that around 0.022 g Phoslock® was required to bind all P present in the water-column following a 5 day experimental period. The dose equalled an areal load of 6.4 g Phoslock® m^{-2} and was termed 'low dose' treatment in the 'dosing experiment'.

Sediment from each core (n = 3) taken for the determination of total sediment P content was homogenized. Sediment TP content was determined (Chapter 2, Section 2.4.8) on three subsamples of 1 g wet weight (WW) sediment taken from each core. Cores contained approximately 595 cm^3 sediment with an average P content of 271 µg P g^{-1} WW sediment. Based on the assumption that 1 g WW equalled a volume of 1 cm^3 it was estimated that the total sediment P content was approximately 161 mg P $core^{-1}$. Based on a nominal La content of 50,000 mg La kg^{-1} Phoslock® (Afsar and Groves, 2009) and a molar binding ratio of La:P of 1:1 (Haghseresht *et al*., 2009) it was estimated that 14.7 g Phoslock® are required to bind all the P in the sediment in each core. This was equivalent to an areal load of 4,300 g Phoslock® m^{-2} and was termed 'high dose' treatment in the 'dosing experiment'.

Dosing experiment

Five sediment cores collected on the 28th of September 2010 were used to assess ambient water-column phytoplankton biomass, sediment chlorophyll *a* content and sediment pore-water nutrient concentrations (hereafter termed 'ambient cores'). On site, the overlying water was carefully siphoned from the cores and stored unfrozen at <4 °C in the dark for measurement of phytoplankton biomass (Chapter 2, Section 2.5.4). Subsequently, sediment cores were extruded and sectioned (2 cm slices) to a depth of 6 cm and stored unfrozen at <4 °C in the dark on transport to the laboratory. Each sediment slice was rigorously stirred for 3 minutes to produce a homogenous mixture. A subsample (5 g WW) was taken from the top 2 cm slice of each core for the analysis of sediment chlorophyll *a* content (Chapter 2, Section 2.5.2). Sediment slices were transferred to centrifuge tubes and centrifuged (5 min at 4,500 rpm) to separate the sediment from pore-water. Between 5 to 10 mL pore-water were collected depending on sediment depth sampled. Pore-water was divided into filtered (Whatman GF/C; nominal pore size 1.2 µm) and unfiltered subsamples and stored frozen (-18 °C) prior to analysis. Pore-water was analysed for NO_3-N (Chapter 2, Section 2.4.3), NH_4-N (Chapter 2, Section 2.4.3), TP (Chapter 2, Section 2.4.4) and SRP (Chapter 2, Section 2.4.4).

Core incubations

The remaining cores (n = 15) were incubated at ambient lake temperature (13 °C) and bubbled gently with air in an 8 h light (2.7 µmol m^{-2} s^{-1} PAR) and 16 h dark (0.05 µmol m^{-2} s^{-1} PAR) cycle for 8 days to mimic ambient conditions in Loch Leven. Cores were randomly assigned to three treatment groups: control, low dose and high dose (each n = 5). On day 1 Phoslock® was added as a slurry to the low dose (addition of 0.022 g Phoslock® per core, equivalent to an areal load of 6.4 g Phoslock® m^{-2}) and high dose

treatment cores (addition of 14.7 g Phoslock® per core, equivalent to an areal load of 4,300 g Phoslock® m^{-2}).

Measurements of water-column DO concentrations, pH and conductivity were made daily at 10 cm above the sediment surface (Chapter 2, Section 2.3.1). Water samples (50 mL) for the analysis of TP (Chapter 2, Section 2.4.4), SRP (Chapter 2, Section 2.4.4), NH_4-N (Chapter 2, Section 2.4.3), NO_3-N (Chapter 2, Section 2.4.3), $TSiO_2$ (Chapter 2, Section 2.4.5), SiO_2 (Chapter 2, Section 2.4.5), TDC (Chapter 2, Section 2.4.1) and DOC (Chapter 2, Section 2.4.1) were collected daily 10 cm above the sediment surface using a syringe and stored frozen (-18 °C) until analysis. This is with the exception of subsamples taken for the analysis of $TSiO_2$ and SiO_2 that were stored at 4 °C in the dark. The volume of water removed during sampling (50 mL) was replaced by distilled water, which was used intentionally to maintain a steep P concentration gradient between the sediment and the overlaying water-column favouring sediment P-release. Analysis of water-column TP and SRP concentration was conducted on samples taken on day 0, 2, 4, 6, and 8. Analysis of NH_4-N and NO_3-N was conducted on samples taken on day 0, 4, 6 and 8, while analysis of $TSiO_2$, SiO_2, TDC and DOC was conducted on samples taken on day 0, 4 and 8. The number of measurements (i.e. n = 5) and variation from number of measurements for all variables is presented (Appendix: Table A7.1).

Sediment DO concentration profiles were measured (Chapter 2, Section 2.3.2) on day 1 - 3 and 6 - 8 at 10, 5, 0, -2.5, -5, -7.5, -10 and -20 mm core depth relative to the organic sediment surface (control and low dose) and the inorganic Phoslock® surface (high dose). Subsequently, sediment DO measurements were normalised relative to the organic sediment surface (0 mm) in all treatments, resulting in four comparable depths (i.e. 10, 5, 0 and -10 mm). Mean sediment DO concentrations were calculated for these depths for each treatment using measurements from all days (n = 30; hereafter termed

mean sediment DO concentration). Mean sediment DO concentrations of less than 0.05 mg L^{-1} were set as the limit of detection.

At the end of the experiment (day 8) water was carefully siphoned from the cores (leaving a 1 cm water layer on top of the sediment or Phoslock® surface respectively) and phytoplankton biomass (µg L^{-1}) was measured by fluorescence using a fluoroprobe (Chapter 2, Section 2.5.4). SPE was measured in each core (Chapter 2, Section 2.3.3). Following measurement of SPE, cores were extruded and sectioned (2 cm slices) to a depth of 6 cm and each slice was homogenized. Sediment chlorophyll *a* content was measured from a subsample (5 g WW) taken from the top 2 cm slice (Chapter 2, Section 2.5.2). Sediment pore-water (5 - 10 mL) was analysed for NH_4-N (Chapter 2, Section 2.4.3), NO_3-N (Chapter 2, Section 2.4.3), TP (Chapter 2, Section 2.4.4) and SRP (Chapter 2, Section 2.4.4). Samples were stored frozen (-18 °C) prior to analysis. The number of measurements (n = 5) and variation from number of measurements for all variables is presented (Appendix: Table A7.2).

Statistical analyses

Minitab 16 (Minitab® 16.1.1, Minitab Ltd., Coventry, UK) was used for all statistical analyses. Data sets of: i) water-column nutrient concentrations (TP, NH_4-N, $TSiO_2$, DOC); ii) sediment pore-water nutrient concentrations (TP, SRP, NH_4-N, DIN); iii) sediment chlorophyll *a* content; and iv) SPE were normally distributed (Anderson-Darling test, $\alpha > 0.05$) and of equal variance (Levene's test, $\alpha > 0.05$) before or after applied transformation where required (either $x' = \log(x)$, $x' = \ln(x)$, $x' = \text{sqrt}(x)$, or $x' = x^2$). Therefore a one-way analysis of variance (ANOVA) with Tukey's *post hoc* test was used: i) to determine, for each day of the experiment, between which treatments (control, low dose, high dose) significant differences in a given parameter occurred; ii) to determine between which treatments including control, low dose and high dose

(measured on day 8) and ambient cores (measured on day 0) significant differences in a given parameter occurred; iii) to determine between which treatments including control, low dose and high dose (measured on day 8) and ambient cores (measured on day 0) significant differences in sediment chlorophyll *a* content occurred; and iv) to determine between which treatments including control, low dose, high dose (measured on day 8) significant differences in SPE occurred.

Data sets of: i) water-column nutrient concentrations (SRP, NO_3-N, DIN, SiO_2, TDC); ii) water-column physicochemical properties (pH, conductivity, DO concentration); iii) sediment pore-water NO_3-N concentration; and iv) sediment DO concentration were not normally distributed (Anderson-Darling test, $\alpha < 0.05$) or were normally distributed (Anderson-Darling test, $\alpha > 0.05$) but not of equal variance (Levene's test, $\alpha < 0.05$) even after transformation (either $x' = \log(x)$, $x' = \ln(x)$, $x' = \text{sqrt}(x)$, or $x' = x^2$). Therefore a non-parametric Kruskal-Wallis (KW) test was used for i) and ii) to test for significant variation in a given parameter between treatments (control, low dose, high dose) for each day of the experiment, iii) to test for significant variation in sediment pore-water NO_3-N concentration between treatments including control, low dose and high dose (measured on day 8) and ambient cores (measured on day 0), and iv) to test for significant variation in mean sediment DO concentration between treatments (control, low dose, high dose) for each depth interval (i.e. 10, 5, 0 and -10 mm) individually. Where significant variation was evident (KW test, $\alpha < 0.05$), a non-parametric Mann-Whitney U-test (MWU) was used for i) and ii) to determine, for each day of the experiment, between which treatments (control, low dose, high dose) significant differences in a given parameter occurred, iii) to determine between which treatments (ambient cores, control, low dose, high dose) significant differences in sediment pore-water NO_3-N concentrations occurred, and iv) to determine, for each

depth interval, between which treatments (control, low dose, high dose) significant differences in mean sediment DO concentrations occurred.

Additionally, a paired t-test was used to test for significant differences in water-column nutrient concentrations (TP, NH_4-N, $TSiO_2$, DOC; all normally distributed data sets) between day 0 and day 8 for each treatment individually. A non-parametric MWU test was used to test for significant differences in water-column physicochemical properties (pH, DO, conductivity; non normal and/or unequal variance data sets) and water-column nutrient concentrations (SRP, NO_3-N, DIN, SiO_2, TDC, DIC; non normal and/or unequal variance data sets) between day 0 and day 8 for each treatment individually.

RESULTS

Field measurements

Surface water pH, conductivity and DO concentrations were similar on both sampling dates (Table 7.1), whereas under-water light availability differed between sampling dates, although light levels were low on both dates.

Table 7.1 Summary of field observations including temperature, pH, conductivity, dissolved oxygen (DO) concentrations (measured at the water surface) and underwater light availability (measured at 3 m water depth).

Date	Depth (m)	Temperature (°C)	pH	Conductivity ($\mu S\ cm^{-1}$)	DO ($mg\ L^{-1}$)	Light ($\mu mol\ m^{-2}\ s^{-1}$)
22/09/2010	3.5	13.3	8.3	212	10.1	0.4
28/09/2010	3.4	11.8	7.9	224	10.2	2.8

Water-column dissolved oxygen concentration, pH and conductivity

Variation in water-column DO concentrations, pH and conductivity between treatments (Fig. 7.2) and between day 0 and day 8 for each treatment individually are presented (Table 7.2). Water-column DO concentrations (Fig. 7.2a) did not differ significantly between treatments except on day 3 ($H = 9.50$; $p < 0.01$; $DF = 2$; $n = 5$) and 5 ($H = 7.61$; $p < 0.05$; $DF = 2$; $n = 5$). No significant variation in DO concentrations was detected in any treatment between day 0 and day 8 (Table 7.2).

pH (Fig. 7.2b) ranged from 6.7 to 7.7 and differed significantly (for details see Appendix: Table A7.3) between treatments from day 2 onwards. On day 8, pH of the low dose treatment did not differ significantly from the control cores, while pH in the high dose treatment was significantly lower ($W = 39$; $p < 0.05$; $n_1 = n_2 = 5$) compared to the control cores. However, no significant variation was detected in pH of any treatment when comparing day 0 and day 8 (Table 7.2).

Conductivity (Fig. 7.2c) differed significantly between treatments on all days except on day 1 (for details see Appendix: Table A7.3). Conductivity in the high dose treatment was significantly higher ($W = 15$; $p < 0.05$; $n_1 = n_2 = 5$) compared to the control cores and the low dose treatment from day 2 onwards. When comparing day 0 and day 8 conductivity of the control cores and the low dose treatment decreased significantly ($W = 40$; $p < 0.05$; $n_1 = n_2 = 5$) by -17% and -24%, respectively (Table 7.2). In contrast, conductivity increased significantly ($W = 15$; $p < 0.05$; $n_1 = n_2 = 5$) by 52% in the high dose treatment over the experimental period with a peak following the application of Phoslock® on day 2. At the end of the experiment (day 8), conductivity did not differ significantly between the low dose treatment and the control cores, while conductivity in the high dose treatment was significantly higher ($W = 15$; $p < 0.05$; $n_1 = n_2 = 5$) compared to the control cores (Fig. 7.2c). The significant increase in conductivity in the high dose treatment was attributed to the addition of Phoslock® which has been shown to increase conductivity with increasing dose (van Oosterhout and Lürling, 2012).

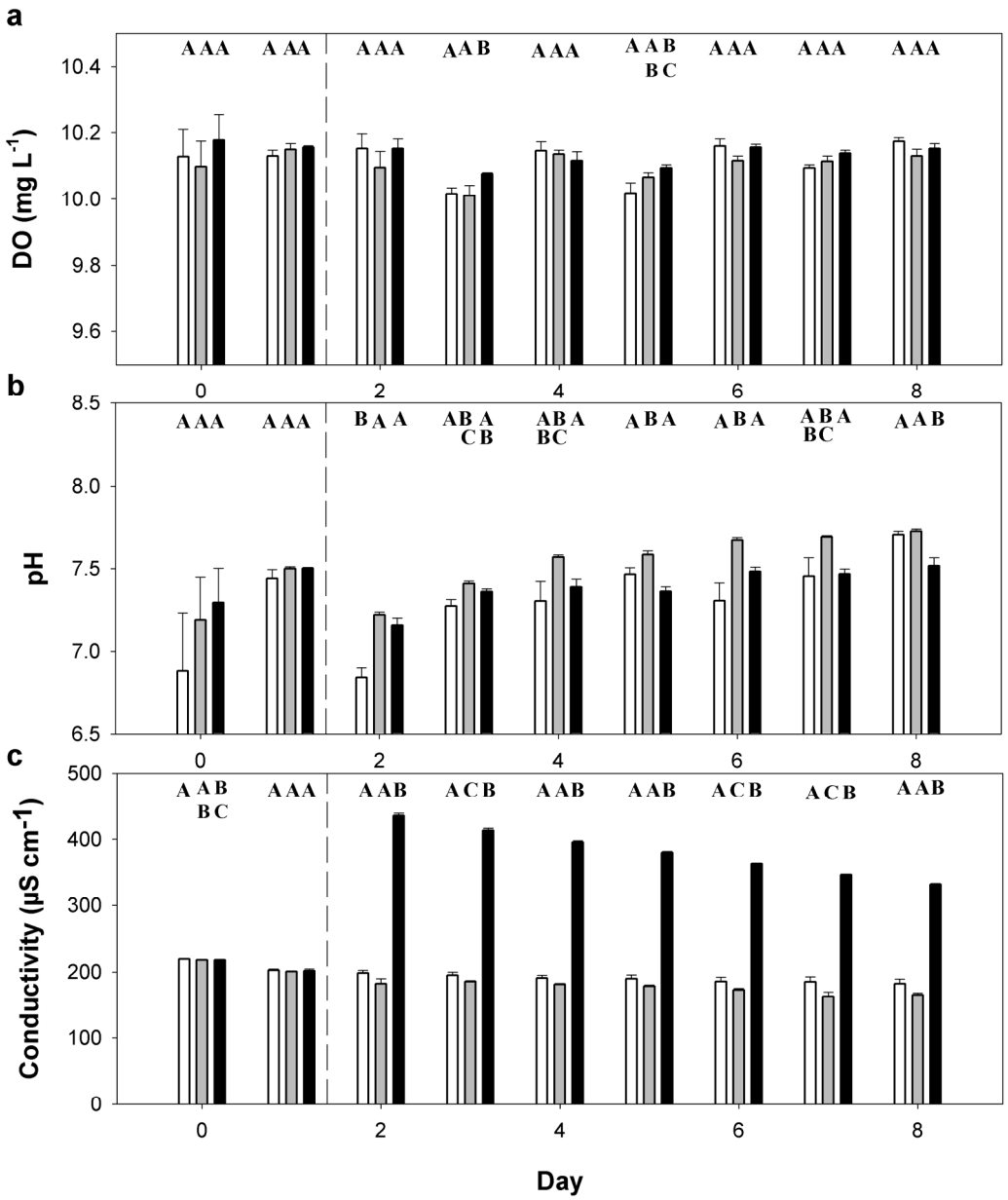


Figure 7.2 Variation in water-column (a) dissolved oxygen (DO) concentration, (b) pH and (c) conductivity between treatments for each day of the experiment, including control (white bars), low dose (grey bars) and high dose (black bars). Dashed vertical line indicates timing of the Phoslock® addition and error bars represent standard error of the mean (n = 5; for exceptions see Appendix: Table A7.1). Results of non-parametric Kruskal-Wallis and Mann-Whitney U-test are indicated by letters (A, B, C); treatments that do not share the same letter are significantly different on a given day.

Table 7.2 Variation in physicochemical parameters of the water-column and results of non-parametric Mann-Whitney U-test and paired t-test investigating variation physicochemical parameters between day 0 and day 8 for each treatment individually. Shown is mean concentration (n = 5; except n = 4 for DO of low dose treatment on day 8) with standard error in parenthesis and the percent change between day 0 and day 8.

Variable	Treatment	Day 0	Day 8	% change	p-value	W/T-value
DO	Control	10.1 (0.08)	10.2 (0.01)	+0.4	n.s.	30^{W}
	Low dose	10.1 (0.08)	10.1 (0.02)	+0.3	n.s.	24^{W}
	High dose	10.2 (0.08)	10.2 (0.02)	-0.3	n.s.	27.5^{W}
pH	Control	6.9 (0.35)	7.7 (0.02)	+2	n.s.	32^{W}
	Low dose	7.2 (0.26)	7.7 (0.01)	+2	n.s.	33.5^{W}
	High dose	7.3 (0.21)	7.5 (0.05)	+1	n.s.	26^{W}
cond.	Control	219.3 (0.26)	182.4 (7.10)	-17	*	40^{W}
	Low dose	218.3 (0.20)	165.3 (2.57)	-24	*	40^{W}
	High dose	218.1 (0.24)	332.4 (1.36)	+52	*	15^{W}
TP	Control	47.9 (2.99)	85.0 (6.51)	+78	**	-5.02^{T}
	Low dose	49.0 (1.13)	67.1 (11.19)	+37	n.s.	-1.58^{T}
	High dose	49.2 (0.87)	19.6 (3.43)	-60	**	8.02^{T}
SRP	Control	5.2 (0.37)	17.8 (2.47)	+243	*	15^{W}
	Low dose	4.9 (0.26)	8.4 (4.45)	+73	n.s.	33^{W}
	High dose	4.8 (0.31)	4.1 (0.40)	-14	n.s.	33.5^{W}
NH_4-N	Control	111.7 (16.63)	353.0 (24.63)	+216	**	-6.43^{T}
	Low dose	85.0 (23.27)	303.9 (10.90)	+258	**	-6.73^{T}
	High dose	114.9 (16.33)	2085.4 (79.64)	+1716	***	-21.26^{T}
NO_3-N	Control	277.5 (11.27)	1474.0 (218.45)	+431	*	15^{W}
	Low dose	267.0 (10.73)	1422.5 (67.89)	+433	*	15^{W}
	High dose	288.4 (14.38)	1172.0 (289.89)	+306	*	15^{W}
DIN	Control	389.1 (18.82)	1827.0 (216.65)	+370	*	15^{W}
	Low dose	352.0 (24.12)	1726.4 (77.13)	+391	*	15^{W}
	High dose	403.3 (27.52)	3257.4 (234.31)	+708	*	15^{W}
$TSiO_2$	Control	5.4 (0.39)	16.3 (2.27)	+204	***	-13.42^{T}
	Low dose	5.1 (0.40)	11.2 (0.80)	+120	***	-15.37^{T}
	High dose	4.8 (0.31	50.5 (15.82)	+947	**	-7.16^{T}
SiO_2	Control	2.8 (0.09)	10.5 (0.98)	+274	*	15^{W}
	Low dose	2.8 (0.07)	7.0 (0.62)	+149	*	15^{W}
	High dose	2.7 (0.05)	15.8 (0.93)	+482	*	15^{W}
TDC	Control	20.3 (0.32)	13.1 (0.58)	-36	*	40^{W}
	Low dose	19.7 (0.16)	12.6 (0.47)	-36	*	40^{W}
	High dose	19.4 (0.35)	7.7 (0.51)	-60	*	40^{W}

Table 7.2 continued

Variable	Treatment	Day 0	Day 8	% change	p-value	W/T-value
DIC	Control	15.7 (0.25)	8.8 (0.51)	-44	*	40^{W}
	Low dose	15.3 (0.14)	8.8 (0.15)	-43	*	40^{W}
	High dose	14.9 (0.36)	3.7 (0.13)	-75	*	40^{W}
DOC	Control	4.7 (0.09)	4.3 (0.41)	-8	n.s.	1.09^{T}
	Low dose	4.5 (0.07)	3.9 (0.40)	-14	n.s.	1.47^{T}
	High dose	4.5 (0.06)	3.9 (0.48)	-12	n.s.	1.12^{T}

DO, dissolved oxygen concentration (mg L^{-1}); cond., conductivity (µS cm^{-1}); TP, total phosphorus (µg L^{-1}); SRP, soluble reactive phosphorus (µg L^{-1}); NH_4-N, ammonium (µg L^{-1}); NO_3-N, nitrate (µg L^{-1}); DIN, dissolved inorganic nitrogen (µg L^{-1}); $TSiO_2$, total diatom silica (mg L^{-1}); SiO_2, soluble silica (mg L^{-1}); DOC, dissolved organic carbon (µg L^{-1}); DIC, dissolved inorganic carbon (µg L^{-1}), TDC, total dissolved carbon (µg L^{-1}); n.s., $p \geq 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; W, W-value of Mann-Whitney U-test; T, T-value of paired t-test

Sediment dissolved oxygen concentration

No visible Phoslock® layer was observed in cores of the low dose treatment (Fig. 7.3), while a 10 mm thick Phoslock® layer was observed above the organic sediment surface (0 mm) in cores of the high dose treatment.

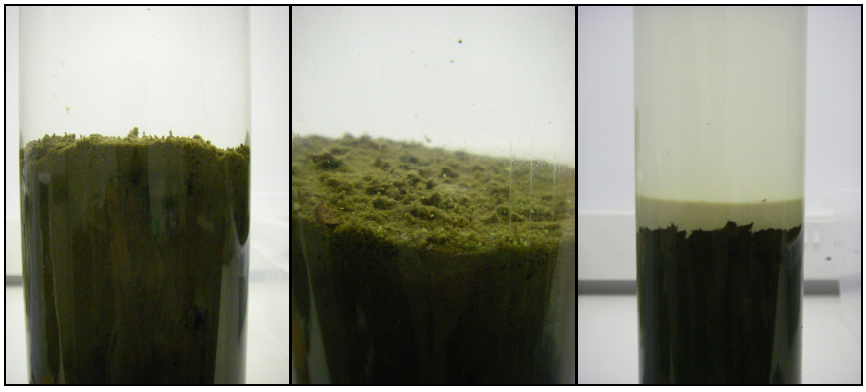


Figure 7.3 Photographs of control (left), low dose (middle) and high dose (right). Note, no visible Phoslock® layer was observed on the organic sediment surface in the low dose treatment (middle), whereas a 10 mm thick Phoslock® layer was present on the organic sediment surface in the high dose treatment (right).

It should be noted that the depth of all measurements of DO concentration were normalised relative to the organic sediment surface. Mean DO concentrations at the Phoslock®-water interface (10 mm) in the high dose treatment were significantly higher compared to DO concentrations at the sediment-water interface (0 mm) of the control cores ($W = 475$; $p < 0.001$; $n_1 = n_2 = 30$) and the low dose treatment ($W = 480$; $p < 0.001$; $n_1 = n_2 = 30$). However, when comparing mean sediment DO concentrations at the organic sediment surface (0 mm in all treatments) no significant difference was detected between the control cores and the low dose treatment (Fig. 7.4, Table 7.3), whereas DO concentrations at the organic sediment surface (0 mm) in the high dose treatment (0.24 mg L^{-1}) were significantly lower ($W = 1362$; $p < 0.001$; $n_1 = n_2 = 30$) compared to the control cores (4.20 mg L^{-1}). In both the control cores and the low dose treatment cores, mean sediment DO concentrations exceeded 0.05 mg L^{-1} at a sediment depth of -2.5 mm, whereas DO concentrations were below 0.05 mg L^{-1} at a sediment depth of -5 mm (Fig. 7.4). In the high dose treatment, mean sediment DO concentrations decreased throughout the Phoslock® layer from 9.01 mg L^{-1} (10 mm) to 0.24 mg L^{-1} (0 mm). Based on the significant lower DO concentration at the organic sediment surface (0 mm) in the high dose treatment it was inferred that the aerobic organic sediment layer, below the inorganic Phoslock® layer, was thinner compared to the control cores and the low dose treatment.

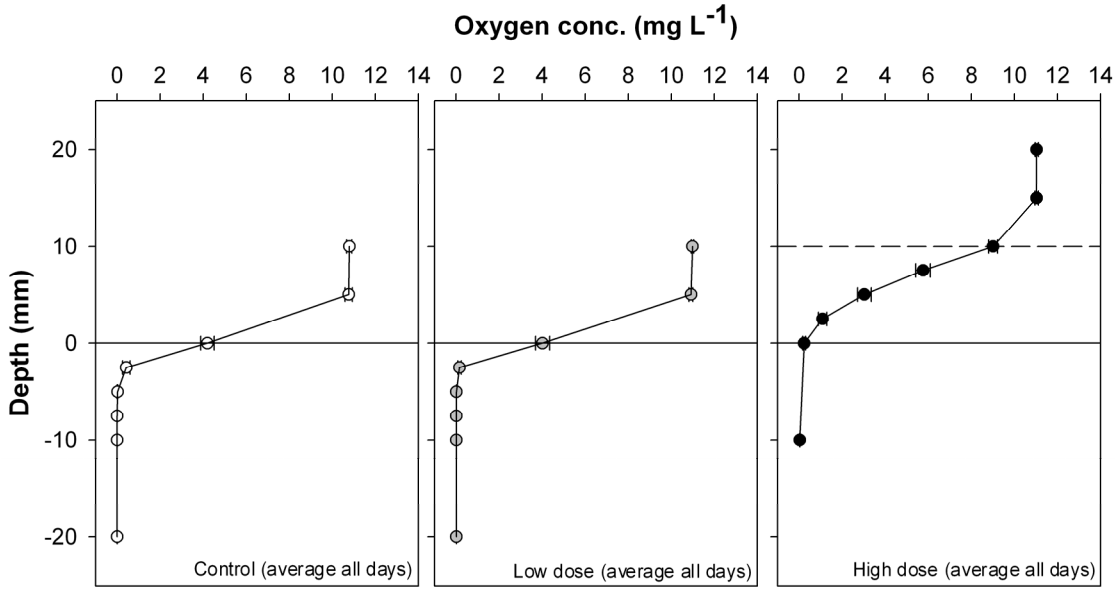


Figure 7.4 Mean sediment dissolved oxygen (DO) concentration in control (left), low dose (middle) and high dose (right). Horizontal line indicates organic sediment surface. Dashed horizontal line indicates surface of Phoslock® layer. Error bars represent standard error of the mean (n = 30).

Table 7.3 Mean (n = 30) sediment dissolved oxygen (DO; mg L^{-1}) concentrations with standard error in parenthesis and results of non-parametric Kruskal-Wallis test (KW; degrees of freedom = 2) and Mann-Whitney U-test (MWU) investigating variation in sediment DO concentration between treatments, including control (C), low dose (L) and high dose (H). Significant differences are marked (*, $p < 0.05$; ***, $p < 0.001$), while n.s. denotes $p \geq 0.05$. All depth measurements are relative to the organic sediment surface (defined as 0 mm).

Depth (mm)	C	L	H	KW	MWU		
					C vs. L	C vs. H	L vs. H
10	10.79 (0.12)	10.99 (0.06)	9.01 (0.21)	***	n.s.	***	***
5	10.76 (0.17)	10.91 (0.09)	3.03 (0.32)	***	n.s.	***	***
0	4.20 (0.31)	4.02 (0.33)	0.24 (0.07)	***	n.s.	***	***
-10	0.01 (0.00)	0.02 (0.00)	0.03 (0.01)	*	n.s.	*	n.s.

Sediment pore-water nutrient concentrations

Sediment pore-water TP and SRP concentrations (Fig. 7.5) did not vary significantly at any sediment depths between ambient cores and the control cores, indicating that experimental conditions had no effect on sediment pore-water P concentrations. Furthermore, no significant difference in sediment pore-water TP and SRP concentrations (Fig. 7.5) was detected between treatments at any sediment depth, indicating that the application of Phoslock® did not alter pore-water P concentrations.

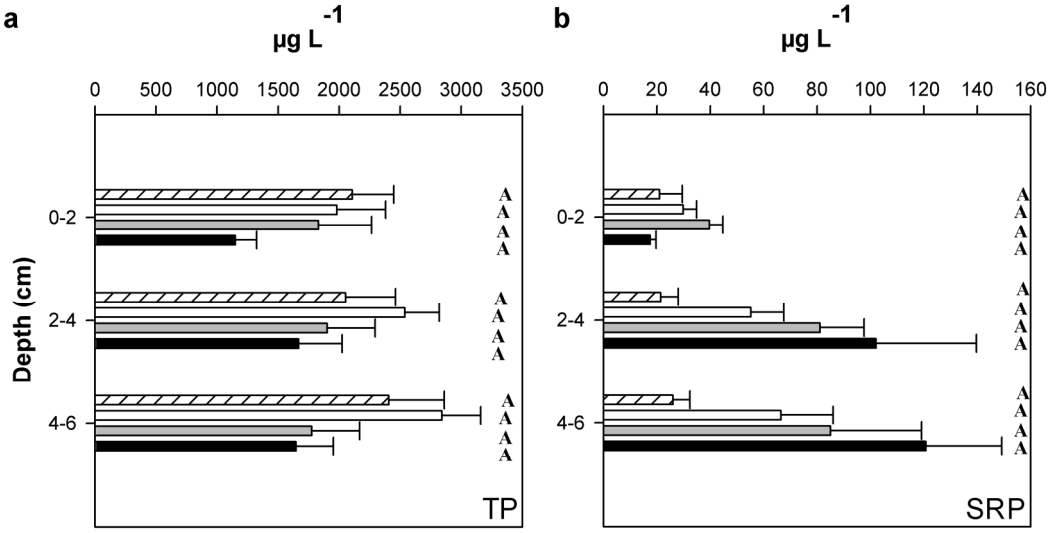


Figure 7.5 Variation in pore-water (a) total phosphorus (TP) and (b) soluble reactive phosphorus (SRP) concentration between treatments, including ambient cores (white hatched bars; day 0), control (white bars; day 8), low dose (grey bars; day 8) and high dose (black bars; day 8). Error bars represent standard error of the mean (n = 5). Results of one-way ANOVA are indicated by letters (A, B, C); treatments that do not share the same letter are significant different for a given sediment depth. Depth was measured starting from the sediment-water interface (ambient cores, control, low dose) and the Phoslock®-water interface (high dose).

Similarly, sediment pore-water NH_4-N, NO_3-N and DIN concentrations (Fig. 7.6) did not differ significantly between ambient cores and the control cores; this is with the exception of significantly lower NO_3-N concentrations ($W = 40$; $p < 0.05$; $n_1 = n_2 = 5$) between 4-6 cm sediment depths in the control cores compared to ambient cores. Overall, the results suggest that experimental conditions had little effect on sediment pore-water N concentrations. Sediment pore-water NH_4-N concentrations (Fig. 7.6a) did not vary significantly between control cores and the low dose treatment, whereas NH_4-N concentrations were significantly higher in the high dose treatment compared to the control cores between 0 – 2 cm ($F = 26.72$; $p < 0.001$; $DF = 3$; $n = 5$) and 4 – 6 cm ($F = 6.25$; $p < 0.01$; $DF = 3$; $n = 5$) sediment depth as indicated by Tukey's *post hoc* test. Sediment pore-water NO_3-N concentrations (Fig. 7.6b) were significantly lower in the low dose treatment compared to the control cores between 2 – 4 cm ($W = 39$; $p < 0.05$; $n_1 = n_2 = 5$) and 4 – 6 cm ($W = 40$; $p < 0.05$; $n_1 = n_2 = 5$) sediment depth, while NO_3-N concentrations were significantly lower (for details see Appendix: Table A7.4) in the high dose treatment compared to the control cores over all sediment depths. No significant difference in sediment pore-water DIN (Fig. 7.6c) concentrations was detected between the control cores and the low dose treatment. In contrast, DIN concentrations were significantly elevated ($F = 10.97$; $p < 0.001$; $DF = 3$; $n = 5$) in the high dose treatment compared to the control cores between 0 – 2 cm sediment depth. Variation in pore-water N concentrations between treatments indicated that the application of Phoslock® caused a decrease in pore-water NO_3-N concentrations at both low and high areal loads, while pore-water NH_4-N and DIN concentrations increased following a high dose Phoslock® application.

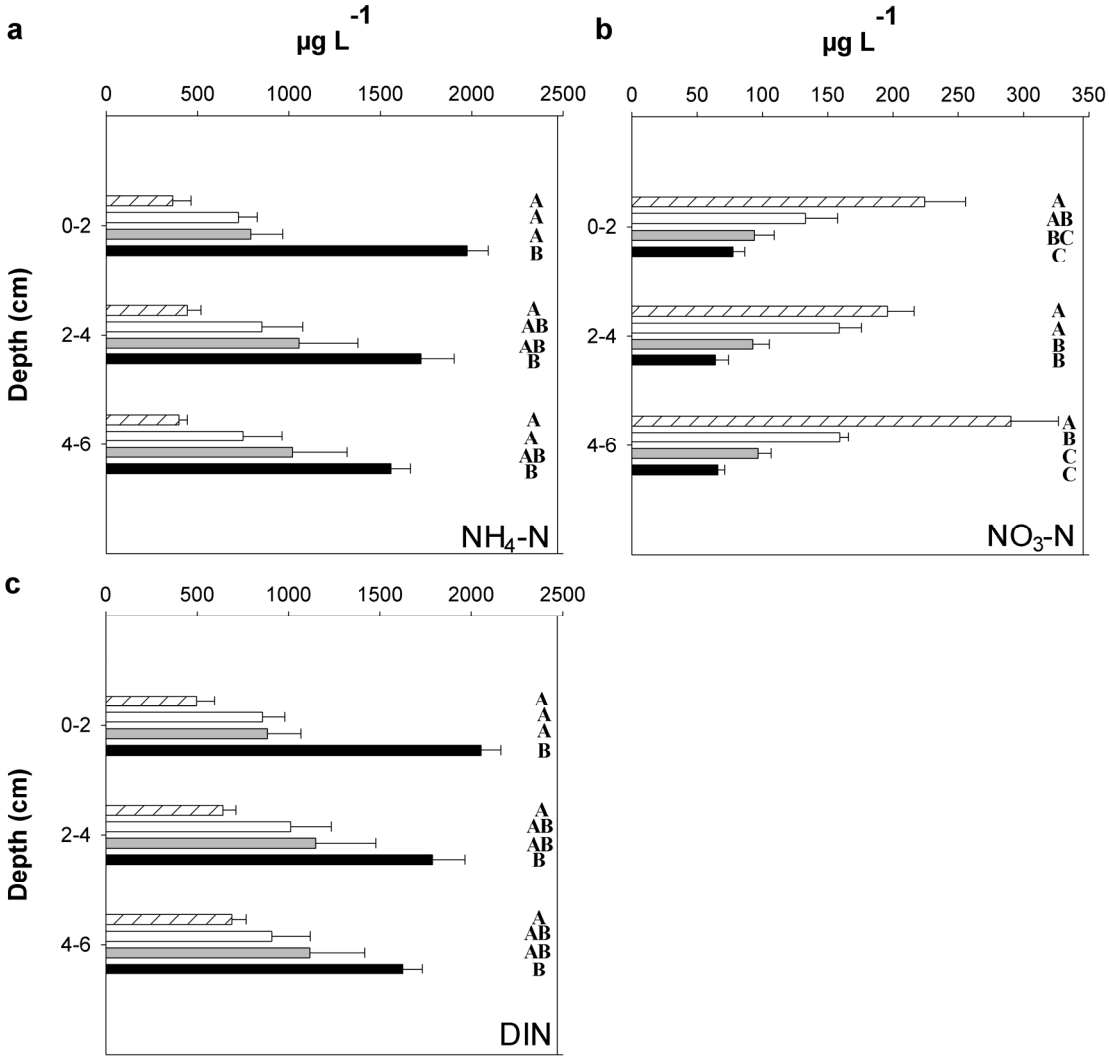


Figure 7.6 Variation in pore-water (a) ammonium (NH_4-N), (b) nitrate (NO_3-N) and (c) dissolved inorganic nitrogen (DIN) concentration between treatments, including ambient cores (white hatched bars; day 0), control (white bars; day 8), low dose (grey bars; day 8) and high dose (black bars; day 8). Error bars represent standard error of the mean (n = 5; for exceptions see Appendix: Table A7.2). Results of one-way ANOVA (NH_4-N, DIN) and non-parametric Kruskal-Wallis and Mann-Whitney U-test (NO_3-N) are indicated by letters (A, B, C); treatments that do not share the same letter are significant different for a given depth. Depth was measured starting from the sediment-water interface (ambient cores, control, low dose) and the Phoslock®-water interface (high dose).

Water-column nutrient concentrations

Water-column TP ($T = -5.02$; $p < 0.01$; $n_1 = n_2 = 5$) and SRP ($W = 15$; $p < 0.05$; $n_1 = n_2 = 5$) concentrations increased significantly between day 0 and day 8 in the control cores, indicating sediment P-release (Table 7.2; Fig. 7.7). In contrast, no significant variation in TP and SRP concentrations was observed over this period in the low dose treatment, indicating that the applied dose was sufficient to prevent sediment P-release. In the high dose treatment TP concentrations decreased significantly ($T = 8.02$; $p < 0.01$; $n_1 = n_2 = 5$) between day 0 and day 8, indicating that the dose was sufficient to prevent sediment P-release and additionally remove P from the water-column. At the end of the 8 day experimental period (Fig. 7.7), water-column TP and SRP concentrations did not differ significantly between control cores and low dose, whereas TP ($F = 19.09$; $p < 0.001$; $DF = 2$; $n = 5$) and SRP ($W = 40$; $p < 0.05$; $n_1 = n_2 = 5$) concentrations were significantly lower in the high dose treatment compared to the control cores.

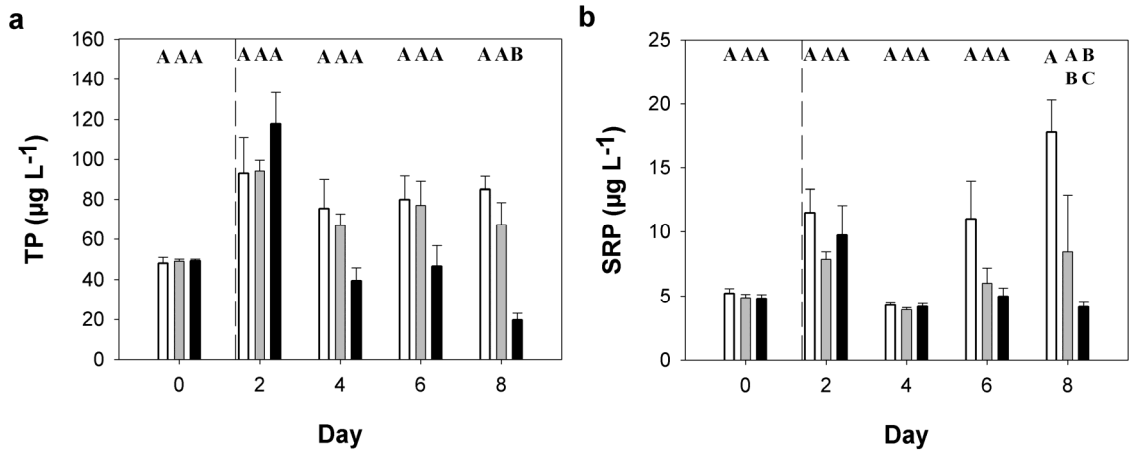


Figure 7.7 Variation in water-column (a) total phosphorus (TP) and (b) soluble reactive phosphorus (SRP) concentrations between treatments on a given day, including control (white bars), low dose (grey bars) and high dose (black bars). Dashed vertical line indicates timing of the Phoslock® addition and error bars represent standard error of the mean (n = 5; for exceptions see Appendix: Table A7.1). Results of one-way ANOVA (TP) and results of non-parametric Kruskal-Wallis and Mann-Whitney U-test (SRP) are indicated by letters (A, B, C); treatments that do not share the same letter are significantly different on a given day.

Water-column NH_4-N (for details see Table 7.2), NO_3-N ($W = 15$; $p < 0.05$; $n_1 = n_2 = 5$) and DIN ($W = 15$; $p < 0.05$; $n_1 = n_2 = 5$) concentrations increased significantly in all treatments between day 0 and day 8 (Table 7.2). The largest increase in NH_4-N and DIN concentrations (Fig. 7.8a, c) was measured in the high dose treatment, in which the lowest increase in NO_3-N concentration (Fig. 7.8b) was also observed. Following an 8 day experimental period, water-column NH_4-N, NO_3-N and DIN concentrations did not differ significantly between control cores and the low dose treatment. In contrast, NH_4-N ($F = 586.68$; $p < 0.001$; $DF = 2$; $n = 5$) and DIN ($W = 15$; $p < 0.05$; $n_1 = n_2 = 5$) concentrations were significantly higher, by 491% and 78% respectively, in the high dose treatment compared to the control cores, whereas NO_3-N concentrations did not differ significantly between the control cores and the high dose treatment. Overall, the

results suggest that high areal loads of Phoslock® enhanced the release of NH_4-N and DIN from the sediment to the overlaying water-column, which is in line with observations of significantly higher sediment pore-water N concentrations at high areal loads (Fig. 7.6).

Water-column $TSiO_2$ (for details see Table 7.2) and SiO_2 ($W = 15$; $p < 0.05$; $n_1 = n_2 = 5$) concentrations increased significantly in all treatments between day 0 and day 8 (Table 7.2). On day 8, water-column $TSiO_2$ concentrations (Fig. 7.8d) did not differ significantly between control cores and low dose, whereas SiO_2 concentrations (Fig. 7.8e) were significantly lower ($W = 38$; $p < 0.05$; $n_1 = n_2 = 5$) in the low dose treatment compared to the control cores. In contrast, $TSiO_2$ ($F = 10.96$; $p < 0.01$; $DF = 2$; $n = 5$) and SiO_2 ($W = 16$; $p < 0.05$; $n_1 = n_2 = 5$) concentrations were significantly higher, by 210% and 51% respectively, in the high dose treatment compared to the control cores. The results indicate that the high areal load of Phoslock® caused an increase in water-column $TSiO_2$ and SiO_2 concentrations.

Water-column TDC and DIC concentrations decreased significantly ($W = 40$; $p < 0.05$; $n_1 = n_2 = 5$) between day 0 and day 8 in all treatments, whereas DOC concentrations did not vary significantly over this period (Table 7.2). On day 8, water-column DOC, DIC and TDC concentrations (Fig. 7.8f, g, h) did not differ significantly between the control cores and the low dose treatment. In contrast, TDC and DIC concentrations were significantly lower ($W = 40$; $p < 0.05$; $n_1 = n_2 = 5$) in the high dose treatment, by -41% and -57% respectively, compared to the control cores. The results suggest that the high areal load of Phoslock® caused a decrease in water-column TDC and DIC concentrations.

Sediment particle entrainment

On day 8 SPE (Fig. 7.9) did not differ significantly between the control cores and the low dose treatment as indicated by Tukey's *post hoc* test. In contrast, SPE was significantly lower ($F = 8.26$; $p < 0.01$; $DF = 2$; $n = 5$) in the high dose treatment compared to the control cores and the low dose treatment as indicated by Tukey's *post hoc* test.

Sediment chlorophyll a content and water-column phytoplankton biomass

Sediment chlorophyll *a* content did not differ significantly in the top 2 cm of the sediment between ambient cores (287.5 µg g^{-1} ± 23.9 s.e.; day 0) and control cores (275.4 µg g^{-1} ± 12.2 s.e.; day 8), indicating that experimental conditions had no effect on benthic algal biomass. In contrast, water-column total phytoplankton biomass was significantly lower ($F = 98.36$; $p < 0.001$; $DF = 3$; $n = 5$) in control cores (18.4 µg L^{-1} ± 0.7 s.e.; day 8) compared to ambient cores (31.5 µg L^{-1} ± 0.4 s.e.; day 0) as indicated by Tukey's *post hoc* test, suggesting that phytoplankton settling and/or growth rate may have been altered by experimental conditions. Both, sediment chlorophyll *a* content and water-column total phytoplankton biomass did not differ significantly between control cores (275.4 µg g^{-1} ± 12.2 s.e.; 18.4 µg L^{-1} ± 0.7 s.e.; day 8) and the low dose treatment (242.7 µg g^{-1} ± 4.9 s.e.; 18.3 µg L^{-1} ± 0.4 s.e.; day 8). In contrast, sediment chlorophyll *a* content ($F = 46.05$; $p < 0.001$; $DF = 3$; $n = 5$) and water-column total phytoplankton biomass ($F = 98.36$; $p < 0.001$; $DF = 3$; $n = 5$) were significantly lower in the high dose treatment (80.9 µg g^{-1} ± 7.3 s.e.; 10.4 µg L^{-1} ± 0.1 s.e.; day 8) compared to control cores (275.4 µg g^{-1} ± 12.2 s.e.; 18.4 µg L^{-1} ± 0.7 s.e.; day 8) as indicated by Tukey's *post hoc* test.

DISCUSSION

This study shows that Phoslock®, at both low and high dose, can effectively prevent sediment P-release. However, high areal loads of Phoslock® caused significant changes in water-column nutrient concentrations, pore-water nutrient concentrations and sediment properties. In contrast no significant variation was detected in the majority of biological, physical and chemical parameters when comparing the low dose treatment and control cores. The results showed that the application of high areal loads of Phoslock® can, at least temporarily, significantly alter sediment DO concentrations and cycling of nutrients other than P, indicating that the application of single high doses of Phoslock® to lakes should be avoided.

Effect of Phoslock® dose on sediment oxygen concentration and nutrient cycling

Measurements of sediment DO concentrations were normalized relative to the organic sediment surface in this study to produce data that is comparable with other similar studies (Vopel *et al*., 2008). The decision to normalise to the organic sediment surface is also qualified by the fact that biogeochemical processes are expected to occur, at least over the short experimental period, predominantly in the organic sediment layer. The results showed that the thickness of the Phoslock® layer can alter sediment DO concentration at the organic sediment surface which has been highlighted for a range of P-capping agents (Vopel *et al*., 2008). While the low dose treatment had no effect on sediment DO concentrations, sediment DO concentrations decreased throughout the Phoslock® layer in the high dose treatment and were significantly lower at the organic sediment surface below the inorganic Phoslock® layer as observed by Vopel *et al*. (2008). It was inferred that the thickness of the oxygenated organic sediment layer beneath the inorganic capping layer was thinner in the high dose treatment which is expected to occur at areal loads in excess of 200 g Phoslock® m^{-2} (Vopel *et al*., 2008).

It should be noted that dilution of the water-column by distilled water added to replace volumes of water removed during sampling may have altered the nutrient flux values and values of water quality parameters (including conductivity, pH and DO concentrations). However, any effect of distilled water addition may be expected to be consistent across treatments. Variation in water-column P concentrations indicated release of P from the sediment in control cores despite high water-column oxygen concentrations (> 10 mg L^{-1}) and an estimated oxygenated sediment layer of between 2.5 to 5 mm depth. In contrast, both the low dose and the high dose treatments were sufficient to control sediment P-release under aerobic conditions, indicating that Phoslock® intercepts upwards diffusing P at the sediment-water or Phoslock®-water interface respectively. However, neither the low dose nor the high dose treatment had an effect on sediment pore-water P concentrations over the experimental period of 8 days, indicating that Phoslock® is unlikely to reduce pore-water SRP concentrations immediately post-application.

Laboratory studies highlighted that the application of Phoslock® may alter cycling of nutrients other then P (Gibbs *et al*., 2011). The low dose treatment had no effect on NH_4-N concentrations indicating that nitrification processes were not altered. Similar observations have been made by Gibbs *et al*. (2011) in which Phoslock®, up to an areal load of 75 g Phoslock® m^{-2}, had no effect on nitrification processes. In contrast, pore-water NO_3-N concentrations were significantly lower in the low dose treatment compared to control cores below 2 cm sediment depths. However, the reason for this observation remains unclear as sediment DO concentrations, water-column NO_3-N concentrations, sediment chlorophyll *a* content and water-column phytoplankton biomass did not differ significantly between control cores and the low dose treatment.

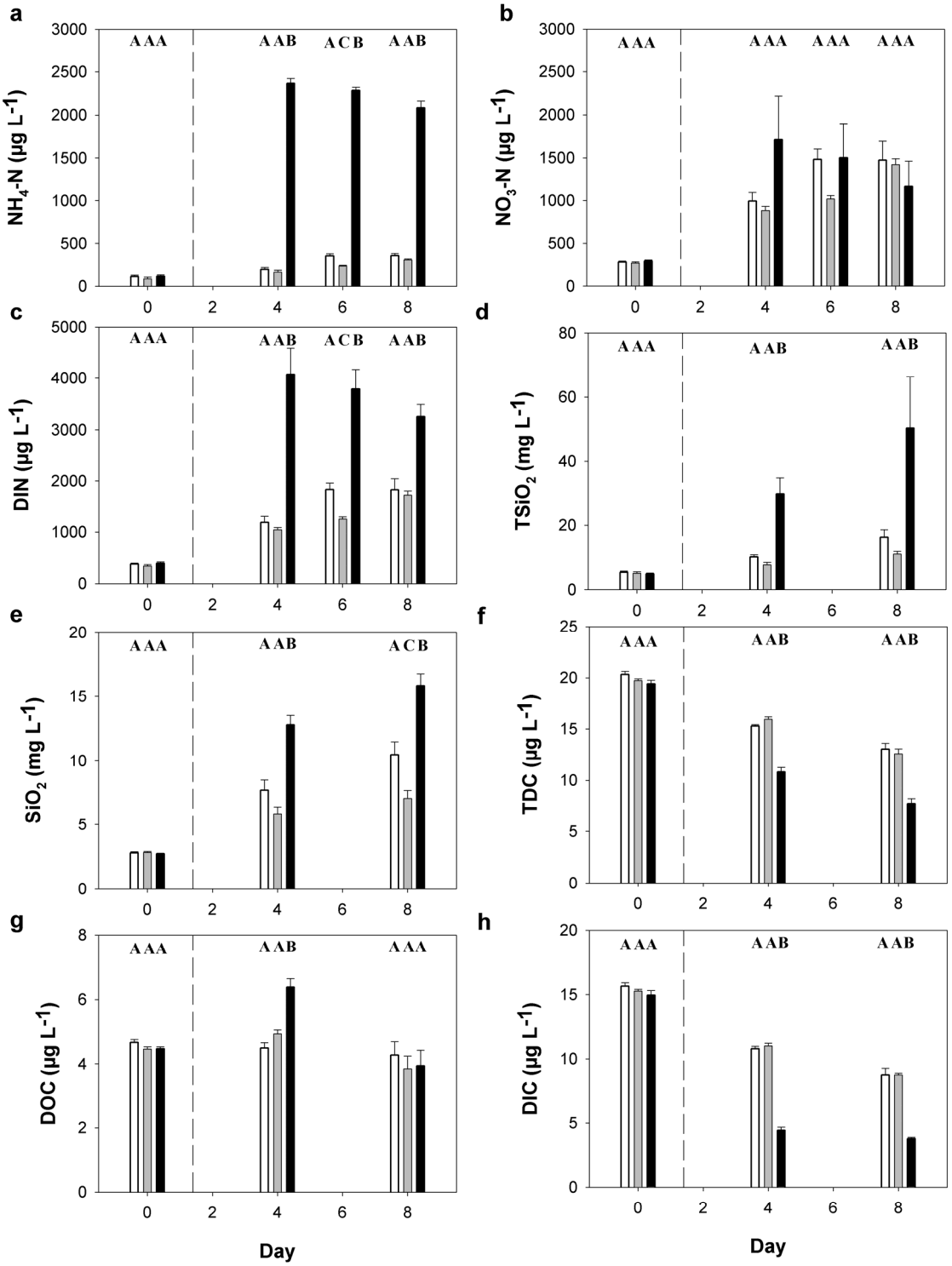

a
b
c
d
e
f
g
h
AAA
AAB
ACB
AAB
AAA
AAA
AAA
AAB
ACB
AAB
AAA
AAB
AAB
AAA
AAB
ACB
AAA
AAB
AAB
AAA
AAB
AAB
NH_4-N (µg L^{-1})
NO_3-N (µg L^{-1})
DIN (µg L^{-1})
$TSiO_2$ (mg L^{-1})
SiO_2 (mg L^{-1})
TDC (µg L^{-1})
DOC (µg L^{-1})
DIC (µg L^{-1})
Day
Day

Figure 7.8 (p. 238) Variation in water-column (a) ammonium (NH_4-N), (b) nitrate (NO_3-N), (c) dissolved inorganic nitrogen (DIN), (d) total diatom silica ($TSiO_2$), (e) soluble silica (SiO_2), (f) total dissolved carbon (TDC), (g) dissolved organic carbon (DOC), and (h) dissolved inorganic carbon (DIC) concentrations between treatments on a given day, including control (white bars), low dose (grey bars) and high dose (black bars). Dashed vertical line indicates timing of the Phoslock® addition and error bars represent standard error of the mean (n = 5; for exceptions see Appendix: Table A7.1). Results of one-way ANOVA (NH_4-N, $TSiO_2$, DOC) and results of non-parametric Kruskal-Wallis and Mann-Whitney U-test (NO_3-N, DIN, SiO_2, TDC, DIC) are indicated by letters (A, B, C); treatments that do not share the same letter are significant different on a given day.

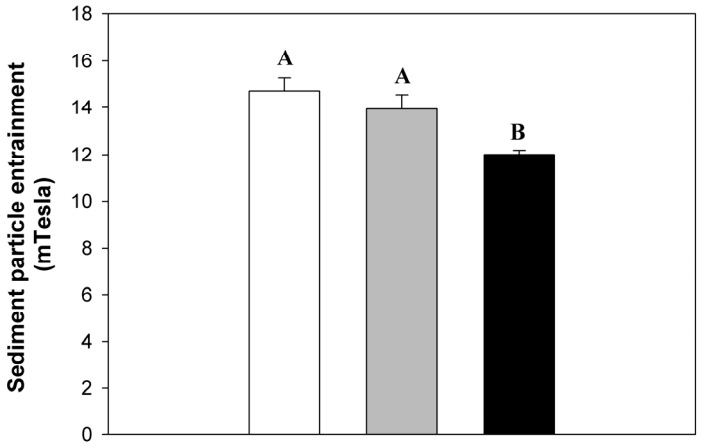


Figure 7.9 Variation in sediment particle entrainment between treatments on day 8, including control (white bar), low dose (grey bar) and high dose (black bar). Error bars represent standard error of the mean (n = 5). Groups that do not share a same letter (A, B) are significantly different as indicated by one-way ANOVA.

Although Gibbs *et al*. (2011) did not investigate pore-water NO_3-N concentrations directly they observed a significantly lower NO_3-N flux under aerobic conditions and concluded that temporary suppression of denitrification may have resulted from carbon (C) limitation of the microbial community in the inorganic capping layer. However, no significant variation in water-column C concentrations was observed between control cores and the low dose treatment. Furthermore, no visible Phoslock® layer was observed at the organic sediment surface in the low dose treatment, making C limitation of the microbial community, due to the applied (not visible) layer of inorganic material (Phoslock®) in the sediment of the low dose treatment, unlikely.

Pore-water and water-column NH_4-N and DIN concentrations were significantly elevated in the high dose treatment, indicating that high areal loads of Phoslock® alter cycling of N between the sediment and the overlaying water-column. Commonly, NH_4-N released during mineralization of organic material in anaerobic sediment layers is converted to NO_3-N by nitrifying bacteria (*Nitrosomonas* sp. and *Nitrobacter* sp.) in oxygenated sediment layers near the sediment-water interface (Boström 1981; Henriksen *et al*., 1981; Jensen *et al*., 1993). The increase in sediment pore-water NH_4-N and DIN concentrations and the subsequent diffusion of NH_4-N into the overlying water-column indicated that the high dose treatment significantly altered nitrification processes in the sediment. Measurements of sediment oxygen concentrations showed that oxygen concentrations at the organic sediment surface were 94% lower in the high dose treatment compared to control cores. It is, therefore, likely that diffusion of oxygen to nitrifying bacteria in the organic sediment was reduced by the Phoslock® layer, causing a decrease in nitrification. Gibbs *et al*. (2011) observed a significant increase in NH_4-N flux from the sediment into the overlaying water-column at an areal load of 300 g Phoslock® m^{-2} and also attributed this to a reduction in oxygen diffusion to nitrifying bacteria in the sediment. The significant decrease in pore-water NO_3-N concentrations

in the sediment of the high dose treatment indicated that denitrification was probably enhanced due to a decrease in the oxygenated organic sediment layer beneath the inorganic capping layer. The results of this study suggest that a high areal load of Phoslock® might, at least temporary, affect phytoplankton community composition due to elevated water-column NH_4-N concentrations and/or alterations of the water-column N:P ratio. This may release phytoplankton from N-limitation and/or cause alterations in phytoplankton community composition (Suttle and Harrison, 1988; Fong *et al*., 1993; Bulgakov and Levich, 1999). However, whole-lake experiments are required to investigate if alterations in N cycling are evident under *in situ* conditions at high areal loads of Phoslock® (Kimball and Levin, 1985; Schindler, 1998).

In addition to altering cycling of N, water-column $TSiO_2$ and SiO_2 concentrations were significantly elevated in the high dose treatment. In lakes, Si is released from diatom frustules during settling and dissolution of diatom frustules in the sediment by microbial mediated mineralization processes (Bailey-Watts, 1976 a,b; Bidle and Azam, 1999; Gibson *et al*., 2000; Lent and Lyons, 2001). Commonly, temperature is considered the main controlling factor of the chemical depolymerisation process (Lewin, 1961; Kamatani and Riley, 1979) with recent studies highlighting that temperature affects both the chemical depolymerisation and the microbial mediated regeneration of Si (Bidle and Azam, 1999). However, given that temperature did not vary between treatments it is concluded that the addition of large amounts of Si present in bentonite clay (Nagy and Kónya, 2010) caused elevated water-column $TSiO_2$ and SiO_2 concentrations in the high dose treatment. Application of high areal loads of Phoslock® may, therefore, enhance diatom growth at least temporarily *in situ* by removal of Si limitation if other nutrients required for growth are available.

Finally, the significant decrease in water-column TDC concentration in the high dose treatment was driven by a significant decrease in DIC concentrations. It has been

shown that humic substances (i.e. DOC) may be bound by La (Ross *et al*., 2008; Lürling and Faasen, 2011), while photosynthetic activity can lead to a decrease in DIC by converting CO_2 (inorganic C) into organic matter. However, photosynthetic activity was likely reduced in the high dose treatment since phytoplankton biomass was significantly lower in the sediment and the water-column. Based on the results of this study it is therefore hypothesised that La or other components of Phoslock® caused binding and precipitation of inorganic carbon (i.e. DIC) compounds, thereby leading to the higher observed decrease in TDC concentrations in the high dose treatment compared to control cores .

Effects of Phoslock® dose on water-column phytoplankton biomass and sediment chlorophyll* a *content

Phoslock® is designed as a tool for lake managers to control eutrophication related problems including phytoplankton blooms. The significant decrease in water-column phytoplankton biomass in the high dose treatment relative to control cores was probably caused by sedimentation of algal cells during the addition of Phoslock®. Studies have shown that bentonite concentrations as low as 15 mg L^{-1} can increase sedimentation of *Microcystis* cells (Verspagen *et al*., 2006), while Phoslock® concentrations exceeded 13,000 mg Phoslock® L^{-1} in the high dose treatment during application. Other laboratory studies attributed a decrease in phytoplankton growth to the combined effects of light limitation during application, flocculation and binding of SRP to Phoslock® (van Oosterhout and Lürling, 2012). It may be argued that significantly lower water-column SRP concentrations following application of Phoslock® in the high dose treatment (being around 4 µg L^{-1}) may have limited phytoplankton growth. However, many algal species are known to utilise luxury P uptake that can sustain growth and cell divisions (Reynolds, 2006), so that P limitation of algal growth over the short

experimental period is unlikely to be the primary cause of reduced phytoplankton biomass in the high dose treatment.

Sediment chlorophyll *a* content was significantly lower in the high dose treatment relative to the control cores at the end of the experimental period. It has been observed that viable benthic algae can be present within several centimetres of aphotic sediment layers in marine (MacIntyre and Cullen, 1995; MacIntyre *et al*., 1996) and freshwater sediments (Cyr, 1998; Spears *et al*., 2010). This has been attributed to the effects of sediment re-working and re-suspension processes in both ecosystems (i.e. marine (Consalvey *et al*., 2004) and freshwater systems (Poulícková *et al*., 2008)). Vertical migration of benthic algae has been intensively studied in biofilms of intertidal mudflat systems (e.g. Serôdio *et al*., 1997; Consalvey *et al*., 2004; Perkins *et al*., 2010a). In these marine systems, benthic algae (e.g. diatoms) have been observed to migrate vertically, with bulk cellular movements being tidal driven and modulated by light (Consalvey *et al*., 2004), while cell microcycling occurs as the primary form of down regulation of photosynthetic activity in response to changes in light dose (Perkins *et al*., 2010b). However, information on benthic algal cell positioning in fine surface layers of freshwater sediment and knowledge about vertical migration of benthic algal cells *in situ* in freshwater sediments is lacking. Furthermore, microalgal communities of shallow eutrophic lake sediments may be dominated by settled phytoplankton and cells from other habitats (e.g. epipsammon, epiphyton, metaphyton and resting stages/sprores) rather than by 'true' benthic algae (Stevenson, 1996), and Spears *et al*. (2010) have shown that this is also true for the microalgal community in sediments of Loch Leven. Therefore, even if an analogous behaviour (i.e. vertical migration) of benthic algae species would exist in freshwater systems their relative contribution to the whole community signal is likely to be masked by planktonic detritus (Spears *et al*., 2010). In conclusion, the variation in sediment chlorophyll *a* content reported here

indicates that no measurable colonisation of the inorganic Phoslock® layer by algae occurred during the experiment in the high dose treatment.

Effects of Phoslock® dose on sediment particle entrainment

Sediment transport processes, including sediment focussing, are important processes affecting positioning and cycling of particulate material and nutrients (Blais and Kalff, 1995; Bloesch, 1995) which may detrimentally affect habitat quality (Wood and Armitage, 1997). SPE varied significantly with dose and was significantly lower in the Phoslock® surface of the high dose treatment. Lower SPE implies that particles are more easily entrained into the overlying water and moved over the sediment bed, rendering a freshly applied Phoslock® layer more prone to horizontal transport processes (e.g. wind-induced sediment re-suspension) along prevailing wind directions compared to particles present in the organic sediment surface. This might result in focusing of Phoslock® towards the downwind shore, in shallow lakes. Benthic microbial assemblages (Decho 2000; Spears *et al*., 2007c; Gerbersdorf *et al*., 2008) and benthic algae (Dodds, 2003; Paterson, 1989, Spears *et al* 2007c) can increase sediment stability and SPE by secreting extracellular polymeric substances (EPS) which enhances cohesion and adhesion between individual particles (Decho 1994; Perkins *et al*., 2004; Spears *et al* 2007c; Spears *et al*., 2008b). This study did not assess benthic microbial assemblages but it was found that sediment chlorophyll *a* content (used as a proxy for benthic algal biomass) was significantly lower in the high dose treatment compared to the control. It is therefore hypothesised that lower SPE in the high dose treatment may be associated with lower benthic algal biomass and some alteration of functional capacity to control SPE. In contrast, flume experiments indicated that Phoslock®, applied at an areal load of 2,500 g Phoslock® m^{-2}, caused an increase in sediment erosion thresholds (bed failure) compared to homogenized sediment of three meso-

/eutrophic lakes following a 1 to 10 day experimental period (Egemose *et al*., 2010). SPE is capable of measuring much smaller forces acting on the sediment bed and can be viewed as a primary stage of bed failure. However, variation in SPE between treated and untreated sediment is always dependent on the reference sediment used. Spears *et al*. (2008b) have shown that microalgal biomass, sediment EPS concentrations and salinity in particular are of major importance in affecting sediment stability of the reference sediment.

CONCLUSIONS

The results of this study indicate that a relatively low Phoslock® dose can be sufficient to control sediment P-release from Loch Leven sediment under aerobic conditions without affecting cycling of nutrients other than P. In contrast, the application of high areal loads of Phoslock® can significantly alter sediment DO concentrations and nutrient cycling between the sediment and the overlying water-column. Alterations in the distribution of DO in the sediment appeared to have 'knock-on' effects on nitrification and denitrification processes in the sediment, leading to significant elevation of water-column nitrogen concentrations. The application of high areal loads of Phoslock® to lakes should therefore be avoided, particularly in cases where the phytoplankton community is N-limited. SPE was significantly lower in a 10 mm thick Phoslock® layer, potentially rendering freshly applied Phoslock® prone to horizontal transport processes. It needs to be validated whether SPE of a Phoslock® layer increases over time once the layer has been colonised by benthic microbial and benthic algal assemblages. However, no significant colonisation of the Phoslock® layer was observed in the short-term, implying that Phoslock® should be applied during calm windless conditions to avoid an uneven coverage of the sediment bed due to horizontal transport processes during, or shortly after, application.

Chapter 8

General discussion

GENERAL DISCUSSION

Shallow lakes provide a wide range of valuable ecosystem services (ES) to humans, yet many shallow lakes are detrimentally affected by anthropogenic pressures. Investigating methods to manipulate key processes and feedback mechanisms that facilitate preservation of, or changes towards, a desired ecological state are important to safeguard the role of these ubiquitous habitats for the provision of ES. However, remediation of shallow lake ecosystems is a complex task since structural and functional responses to changes in pressures can be confounded by feedback mechanisms driven by interrelated biological and physicochemical processes (Maberly and Elliott, 2012). Nevertheless, remediation measures may be required to increase the ecological resilience of lakes to undesirable state changes in the context of multiple pressures, adding a level of insurance to the loss of ES provision. Furthermore, shallow lake remediation studies provide a model system with which complex ecological theories like the stable states theory can be tested (Scheffer *et al*., 1993) and insights gained will be helpful to guide policy making towards a sustainable use of these ecosystems and the ES that they provide (Scheffer *et al*., 2000).

The purpose of this study was to conduct a series of experiments, from mesocosm to ecosystem in scale, to develop our understanding of lake ecosystem functioning and our ability to control these functions to manipulate, and ultimately enhance the provision of ES. The main hypothesis of this study was that a 'controlled disturbance', i.e. the application of Phoslock®, a lanthanum (La) modified bentonite clay designed to strip dissolved phosphorus (P) from the water-column and increase the sediment P-sorption capacity, would result in a 'breakdown' of the internal P-loading feedback mechanism in a recovering shallow lake (Loch Flemington), and that this would force a change in state from a 'phytoplankton dominated turbid state' to a 'macrophyte dominated clear water state'. Whole-lake manipulation experiments were

conducted to assess this hypothesis in combination with a series of laboratory controlled experiments designed to quantify processes associated with the feedback mechanism.

This is the first study investigating alterations in sediment P-partitioning and timescales of P-partitioning processes following the application of Phoslock®. Vertical positioning of Phoslock® in the sediment was assessed for the first time *in situ* and probable reasons and consequences of the observed pattern were discussed. This study showed, at the ecosystem scale (Loch Flemington), that internal P-loading, a key feedback mechanism in shallow lakes, can be successfully manipulated by a controlled disturbance using Phoslock®. Alterations in ecological structure and function during the rapid recovery processes indicated the possibility to force a change in state from a phytoplankton dominated turbid state to a macrophyte dominated clear water state in a recovering shallow lake. In addition, this study combined experimental assessments formerly done in isolation, indicating that alterations in nutrient cycling processes and sediment oxygen distribution at high areal loads of Phoslock® are probably linked, adding to evidence that the application of high areal loads of Phoslock® should be avoided. Finally, a new conceptual model for the use of P-capping agents in lake remediation projects was proposed, suggesting that the application of multiple smaller doses is beneficial to single high doses in terms of cost-effectiveness and avoidance of non-target effects.

Shallow lake remediation: linking theory and practice

Manipulating feedback mechanisms in shallow lakes

According to the stable states theory, the resilience of shallow lakes to a change of state is highest under very low or very high nutrient concentrations at which each state is stabilized by multiple feedback mechanisms (Moss, 1980, 1990; Jeppensen, 1998b;

Scheffer *et al*., 1993; Scheffer, 1998). However, at 'intermediate' nutrient concentrations resilience to a change of state is weakened and shallow lakes may exist in either a phytoplankton dominated turbid state or a macrophyte dominated clear water state (Moss, 1990; Scheffer *et al*., 1993).

Biomanipulation for example represents a controlled disturbance where the food web is selectively altered to initiate a state change towards a macrophyte dominated clear water state by disrupting feedback mechanisms. In a multi-lake study considering shallow temperate lakes, biomanipulation was considered most effective when summer mean total phosphorus (TP) concentrations were between 80 to 150 µg TP L^{-1} (Jeppesen *et al*., 1990). Subsequently, Jeppesen *et al*. (1997) suggested that this range should be revised to 50 to 100 µg TP L^{-1}. However, site specific characteristics like lake area, mean depth, retention time and weather conditions may alter the threshold P concentrations at which lakes are sensitive to a change in state following disturbance (Jeppesen *et al*., 1990; Scheffer and van Nes, 2007; Janse *et al*., 2008). The range in which phytoplankton biomass is positively correlated with water-column TP concentrations (i.e. up to around 100 µg TP L^{-1}; Vollenweider and Kerekes, 1980; Phillips *et al*., 2008) corresponds markedly well with the range of estimated 'intermediate P concentrations' (50 to 150 µg TP L^{-1}; Jeppesen *et al*., 1990; 1997). It is therefore apparent that the availability of P exerts a strong control over phytoplankton biomass and associated feedback mechanisms at intermediate P concentrations.

In Loch Flemington, mean summer TP concentrations (2009: 137 µg TP L^{-1}) were within the range of intermediate TP concentrations reported above, prior to the controlled disturbance (Chapter 6). It was therefore hypothesised that the lake would be sensitive to state change following a disturbance.

Evidence of disruption of the internal P-loading feedback mechanism

Monitoring data indicated that internal P-loading significantly affected ecological structure and function in Loch Flemington prior to the controlled disturbance, thereby maintaining an undesirable ecological state (Chapter 5, 6). Characteristics included a marked seasonality with high water-column P concentrations, high phytoplankton biomass and low water clarity over the summer months which has been observed in other lakes suffering from internal P-loading (Søndergaard *et al*., 1999; Spears *et al*., 2007b; Søndergaard *et al*., 2012). Phytoplankton community composition in such lakes may be dominated by cyanobacteria potentially causing nuisance surface blooms (Phillips *et al*., 2005; Burger *et al*., 2008; Hickey and Gibbs, 2009), which was also apparent at Loch Flemington prior to the controlled disturbance.

Various alterations in ecological structure and function indicated that the controlled disturbance significantly reduced the internal P-loading feedback mechanism in Loch Flemington in the first two years (Chapter 6). Most pronounced structural changes associated with reduced water-column P concentrations (summer 2010: 48 µg TP L^{-1}; summer 2011: 27 µg TP L^{-1}) were evident in the summer season and included a reduction in phytoplankton biomass and an increase in water clarity as observed in other lakes following reduction of internal P-loading (Jeppesen *et al*., 2005b; Søndergaard *et al*., 2005; Mehner *et al*., 2008). Apparent functional changes included: i) alteration of P-cycling between the sediment and water-column, leading to significantly lower water-column P concentrations, particularly in summer, as observed elsewhere following reduction of internal P-loading (Mehner *et al*., 2008); and ii) a shift of primary production from predominantly pelagic (phytoplankton) to benthic (macrophytes) habitats as indicated by a significant reduction in phytoplankton biomass and an increase in the maximum colonisation depth (MCD) of macrophytes. Studies assessing variation in primary production between pelagic and benthic habitats indicate that

opposite trends are to be expected during nutrient enrichment (Vadeboncoeur *et al*, 2003).

Evidence of a state change

According to the definition of Walker and Meyers (2004) a state change occurs if a disturbance is strong enough to overcome resilience so that structure, function and feedback mechanism of a system change sufficiently to stabilize another state. This study hypothesised that by harnessing such disturbance mechanisms it may be possible to control a state change in shallow lakes at intermediate nutrient concentrations. Alterations in ecological structure, function and the apparent disruption of the internal P-loading feedback mechanism following the controlled disturbance indicated that a change in state to a macrophyte dominated clear water state occurred in Loch Flemington (Chapter 5, 6). However, it remains to be seen how long this alternative state persists and so the stability of the system is not well understood. The whole-lake experiment in Clatto Reservoir indicated that successful reduction of cyanobacteria abundance was limited to the first year post-application (Chapter 3).

Enhancement of recovery by feedback mechanisms associated with the macrophyte dominated clear water state

The apparently 'immediate' effect of the controlled disturbance on ecological structure and function in Loch Flemington may be explained by the inverse relationship between phytoplankton and macrophytes that are pivotal structural components in shallow lakes, with water clarity acting as the controlling factor (Scheffer, 1998; Scheffer *et al*., 1993; Scheffer and Jeppesen, 1998). Perrow *et al*., 1997 demonstrated that by creating clear water conditions in spring ('window of opportunity'), it is possible to enhance the establishment and growth of submerged macrophytes. Following the controlled

disturbance in spring in Loch Flemington, phytoplankton biomass decreased relative to previous years, especially in summer, thereby leading to increased water clarity and an increase in the macrophyte MCD (Chapter 6). An increase in MCD resulted in a larger area of the sediment bed being colonised by macrophytes. It was therefore hypothesised that the functional capacity of the macrophyte community to control internal P-loading increased as a result of the controlled disturbance. Functional processes controlling internal P-loading may include direct P sequestration by macrophytes at the cost of phytoplankton (Carignan and Kalff, 1980), and reduction of wind induced sediment re-suspension promoting and sustaining high water clarity (Madsen *et al*., 2001; Horppila and Nurminen, 2003).

The benefit of enhancing resilience in shallow lakes

Eutrophication, climate change, acidification, fishery management or the ingress of invasive species are all pressures which may act in concert on the ecological structure, function and feedback mechanisms of shallow lakes (Vitousek *et al*., 1997; Winfield *et al*., 2008; Spears *et al*. 2011). Climate change, alone, is predicted to increase nutrient transport to temperate lakes and enhance internal P-loading (Jeppesen *et al*., 2009; Kosten *et al*., 2011). Therefore, climate change is likely to promote phytoplankton growth even if all catchment properties (e.g. population size, land use) are unchanged. Consequently, feedback mechanisms associated with a phytoplankton dominated turbid state are likely to be strengthened at the cost of submerged macrophytes. Furthermore climate warming is expected to alter the food web structure of shallow lakes by favouring planktivorous and benthivorous fish species (Jeppesen *et al*., 2007b; 2009). P from external sources may, therefore, be converted more efficiently into phytoplankton biomass since zooplankton grazing pressure is suppressed by planktivorous fish, while

sediment re-suspension by benthic feeding fish hampers growth of macrophytes by non-algal shading in a warmer world. Over time, such climate associated changes are likely to erode feedback mechanisms associated with a macrophyte dominated clear water state thereby lowering system resilience to undesirable state changes.

In line with the stable states theory, it has been observed that a state change back towards a macrophyte dominated clear water state occurs at a lower nutrient concentration than the initial change towards a phytoplankton dominated turbid state (e.g. Ibelings *et al*. 2007; Dokulil *et al*., 2011). It may therefore be inferred that remediation efforts leading to an increase in resilience of shallow lakes present in a macrophyte dominated clear water state, either naturally or following remediation, may be more cost-effective and technically feasible than initiating a state change back towards a macrophyte dominated clear water state which would require nutrient concentrations to be reduced below concentrations at which the state change towards the undesired state occurred. In addition, some studies suggest that recovery to pre-impact conditions may be hampered following loss of key structural components (Ibelings *et al*. 2007) or alterations of physical habitat structure by invasive species (Crooks, 2002). Increasing resilience of a macrophyte dominated clear water state may, therefore, be required to sustain ES provided by shallow lakes facing multiple pressures.

Combining remediation measures to increase resilience

Observed structural and functional changes in Loch Flemington following the controlled disturbance have been summarised in a conceptual diagram (Chapter 6, Fig. 6.14a, b). The reduction in summer TP concentrations following the controlled disturbance to 48 µg TP L^{-1} in the first and 27 µg TP L^{-1} in the second year are estimated to sustain a macrophyte dominated clear water state (Hosper and Jagtman, 1990; Jeppesen *et al*., 1990, 1997). However, like many other shallow lakes, Loch

Flemington is located in an agricultural lowland area and despite reductions of external P-loading in the past (May *et al*., 2001) external sources may overtime increase in-lake P concentrations, thereby enhancing phytoplankton growth and associated feedback mechanisms. With time this will weaken the resilience of the system to a point when a disturbance may cause a state change back to a phytoplankton dominated turbid state. Taking this inevitable process into account highlights the need for continuous management of shallow lakes. Further remediation measures may therefore be required in Loch Flemington to increase ecological resilience, thereby ensuring that the desired state is sustained. Particularly variation and magnitude in external P-loading and variation in physicochemical conditions influencing internal P-loading (e.g. climate change) are potential detrimentally impacting upon ecological resilience. The latter being of importance since the applied dose was not deemed sufficient to control sediment P-release under extended anaerobic periods as inferred from laboratory experiments which may be a consequence of climate warming (Chapter 5).

One focal component of further remediation efforts may be the fish community structure in Loch Flemington as results suggest that only planktivorous sticklebacks were present in the lake, while top predators (i.e. piscivorous fish species) were absent (Chapter 6). It has been reported that sticklebacks can exert strong grazing pressure on large-bodied cladocearan (Jakobsen *et al*., 2003) and that this small fish species lives among plant beds thereby annulling any positive refugia effects for zooplankton at high stickleback abundance (Stephen *et al*., 1998). Whole-lake experiments in lakes dominated by either planktivorous fish (shorter food chain) or piscivorous fish (longer food chain) indicated that external P-loading pulses stimulated an increase in phytoplankton biomass more, when the fish community was dominated by planktivorous fish species (Carpenter *et al*., 1992, 1996; Schindler *et al*., 1996). Alternatively, in lakes dominated by piscivores, nutrient pulses were more effectively

transferred to higher trophic levels (Carpenter *et al*., 1992, 1996; Schindler *et al*., 1996). This has been attributed to the structuring influence of large piscivores on the food web which may enhance the role of large-bodied zooplankton species in controlling phytoplankton biomass (Kitchell and Carpenter 1993). In lakes with a longer food chain the P return rate is slower because large fish act as longer term P sinks (Carpenter and Cottingham, 1997). This study hypothesises that manipulating the fish community in Loch Flemington will increase resilience of the macrophyte dominated clear water state to pressures like external P-loading. This may be achieved by lowering planktivorous fish numbers followed by stocking with piscivorous fish species in order to facilitate a sustainable increase in the ratio of piscivorous to planktivorous fish as suggested by Jeppesen *et al*. (1990). Perrow *et al*. (1997) indicated that this combined approach of removal and stocking is likely to achieve the best results in terms of cost-effectiveness and durability. Alterations in fish community structure in Loch Flemington are expected to release zooplankton from grazing pressure, thereby increasing the control of phytoplankton by zooplankton. Consequently, any P entering the lake may be more efficiently transferred to higher trophic levels, preventing an increase in phytoplankton biomass and deterioration of the underwater light climate. Alteration of the fish community is, therefore, expected to increase resilience of the macrophyte dominated clear water state to undesirable state changes which is summarized in a conceptual graph (Fig. 8.1), while anticipated alterations in ecological structure and interactions between biotic and abiotic variables have been illustrated in a conceptual diagram (Chapter 6, Fig. 6.14c).

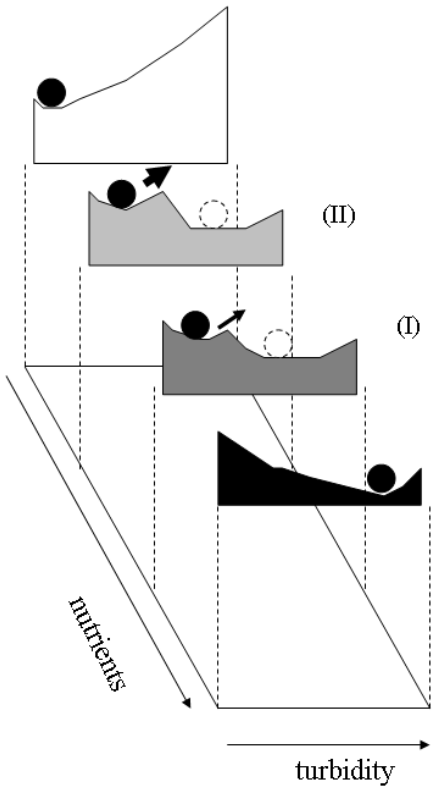


Figure 8.1 Conceptual graph summarising expected variation in ecological resilience to a change in state towards the phytoplankton dominated turbid state in Loch Flemington (I) after the controlled disturbance and (II) after the controlled disturbance and following successful remediation of the fish community structure. 'Strength' of resilience is indicted by size of arrows. Diagram following Scheffer *et al*. (2000) with modifications.

Recommended methodology for the application of Phoslock® for lake remediation

Controlling internal P-loading by increasing sediment P-binding capacity

Sequential extraction of P from P saturated Phoslock® under laboratory conditions indicated that around 79% of P bound was unlikely to be released under anaerobic conditions or over a pH range of 5 to 9 (Chapter 4). The majority of P was recovered from the '*apatite bound P*' fraction (60.7%), the fraction which is expected to increase

following a whole-lake application of Phoslock®. This has been confirmed by an assessment of *in situ* sediment P-partitioning in Loch Flemington in which the mass of P present in the '*apatite bound P*' fraction increased significantly with time (Chapter 5). Therefore, both laboratory and whole-lake experiments indicated that applying Phoslock® is likely to permanently prevent P from cycling between the sediment and the water-column, as required for the control of internal P-loading (Boström *et al*., 1988; Hupfer and Lewandowski, 2008; Hickey and Gibbs, 2009). Lake managers may therefore apply Phoslock® to reduce internal P-loading and associated problems in cases where monitoring data clearly indicates that internal P-loading opposed to external P-loading is the major factor delaying recovery of shallow lake ecosystems. Future studies should focus on the permanency of the La-P bond under *in situ* conditions particularly if algal or microbial enzymes may have the ability to obtain P from the La-P complex.

Timing of application: Prior to internal P-loading peak

Laboratory and whole-lake experiments showed that the application of Phoslock® will increase the mass of P present in the '*apatite bound P*' fraction. In Clatto Reservoir, no significant increase in the mass of P present in this fraction was found 1 month after the application of Phoslock® in spring (Chapter 4). The observed significant increase in the mass of P in the '*apatite bound P*' fraction in Loch Flemington 7 months post-application suggested that sediment P-partitioning between P_{mobile} (the sum of the '*labile P*', '*reductant-soluble P*' and '*organic P*' fraction) and the '*apatite bound P*' fraction occurred between summer and autumn (i.e. between post-application month 4 (July) and 7 (October); Chapter 5). These observations suggest that applying Phoslock® will not immediately cause alterations in sediment P-partitioning between P_{mobile} and more refractory sediment fractions. However, the results indicate that P will be bound by La following sediment P-release events which typically occur between summer and autumn

in northern temperate shallow lakes (Søndergaard *et al*., 1999; Spears *et al*., 2007b). Phoslock® should, therefore, be applied prior to the period in which sediment P-release is expected to be highest. It may be argued that Phoslock® should be applied during the period of high sediment P-release to bind P in the water-column immediately. However, P released from the sediment is quickly converted into phytoplankton biomass in shallow lakes. Although Phoslock® may initiate physical precipitation of algal cells (Verspagen *et al*., 2006), P-binding by La would not be expected to occur until mineralisation of such algal cells. In addition, it has been suggested that the application of Phoslock®, at the dose used at Clatto Reservoir (Chapter 3), may have short-term negative impacts on phototrophic organisms by reducing water clarity. This finding suggested that the application of Phoslock® to lakes should be conducted outside the main growing season (i.e. winter) of submerged macrophytes.

Conceptual model for the use of P-capping agents

Sediment elemental analysis showed that La (and Phoslock®) was subjected to vertical translocation processes in the sediment (Chapter 4, 5), which has been observed for other P-capping agents (Egemose *et al*., 2012). Laboratory core experiments indicated that the ability of Phoslock® to control water-column TP concentrations decreased with increasing vertical mixing (Chapter 4), which is in line with studies assessing the burial of alum (Lewandowski *et al*., 2003). The conceptual framework in which P-capping agents form a distinct surface layer which intercepts upwardly diffusing P following release events, therefore, appears too simplistic as bioturbation and/or wind induced sediment re-suspension are likely influencing the vertical positioning of P-capping agents *in situ* (Fischer *et al*., 1980; Hilton *et al*., 1986, Douglas and Rippey, 2000; Meysman *et al*., 2006; Egemose *et al*., 2012). The application of multiple smaller doses may therefore be beneficial compared to a single high dose as it would facilitate the

positioning of La (and Phoslock®) closer towards the sediment surface after each application.

Intact sediment core experiments indicated that a 'high dose' (equivalent to bind all sediment P) may at least temporarily alter cycling of nutrients other than P by altering the vertical distribution of sediment dissolved oxygen (DO) concentrations (Chapter 7). The observed significant increase in water-column dissolved inorganic nitrogen (DIN) concentrations may release phytoplankton from N-limitation and/or cause changes in phytoplankton community composition (Suttle and Harrison, 1988; Fong *et al*., 1993; Bulgakov and Levich, 1999). Additionally, sediment DO concentrations decreased temporarily below the P-capping layer at 'high dose' which may have detrimental impacts on benthic macroinvertebrate abundance and community composition (Pathiratne and Weerasundara, 2004; Della Bella *et al*., 2005). Again, this evidence suggests that multiple smaller doses may be beneficial as non-target effects may result from the application of high areal loads of Phoslock®.

Furthermore, it appeared that changes in ecological structure preceded measureable alterations in sediment P-partitioning (i.e. partitioning between P_{mobile} and more refractory P-fractions, particularly '*apatite bound P*') in Loch Flemington. This mismatch raises the question of how much P in the sediment and the water-column needs to be controlled to cause desired and measurable structural and functional responses? It has been shown that dosing P-capping agents relative to the P_{mobile} fraction in the top 10 cm of the sediment can successfully control internal P-loading (Reitzel *et al*., 2005). However, the results of this study have demonstrated that significant and rapid ecological responses can be achieved following even a 'low dose' application of Phoslock® (Chapter 6) with the applied mass of La sufficient to control only 10% of P_{mobile} present in the top 10 cm of the sediment (Chapter 5). Mass balance estimates based on lake area, mean depth and minimum (spring) and maximum (summer) surface

water TP concentrations pre-application indicate that only around 12 kg P were released over this period in Loch Flemington (assuming external sources were negligible), but were sufficient to sustain high phytoplankton biomass and low water clarity. This mass of P represents less than 1% of the mass of P present in the P_{mobile} fraction in any month sampled. It is therefore hypothesised that the observed changes in ecological structure and function in Loch Flemington may have been the combined result of: i) application in spring hampered the development of high phytoplankton biomass through a combination of sedimentation of phytoplankton cells during application (Verspagen *et al*., 2006) and P-limitation of phytoplankton standing stock in the water-column after application (Sas, 1989); ii) presence of submerged macrophyte species that were able to respond quickly to the higher water clarity ('window of opportunity') created by the application early in the year, thereby strengthening feedback mechanisms associated with the macrophyte dominated clear water state; and iii) reduction of sediment P-release between summer and autumn by increased sediment P-binding capacity causing an extended growing season for submerged macrophytes. Overall, this may suggest that an initial 'low dose' may be suitable in certain lakes to cause significant variation in ecological structure and function.

Given potential non-target effects at 'high dose' (Chapter 7; Gibbs *et al*., 2011; Vopel *et al*., 2008), potential impact of vertical positioning of P-capping agents on P-binding capacity (Chapter 4; Lewandowski *et al*., 2003), potential role of feedback mechanisms associated with the macrophyte dominated clear water state (Chapter 6) and the complex relationships between biological and physicochemical properties during remediation, this study proposed a conceptual model for the use of P-capping agents based on the assumption that calculating 'effective dose' is impossible (Chapter 5, Fig. 5.7). Instead the model suggests that to avoid overdosing a lake with a P-capping agent an initial 'low dose' should be applied, similar to the practice in human medicine

(Chin and Lee, 2008). This should be followed by a continuous cycle of monitoring, assessment and application of top-up doses where required, until the required improvements in water quality are met. This should be conducted simultaneously with continuous catchment management and monitoring of catchment nutrient loading to the lake. The usefulness of the proposed conceptual model needs to be rigorously tested in long-term studies (> 5 – 10 years) at multiple sites.

Phoslock® knowledge gaps

Future studies assessing the use of Phoslock® as a P-capping agent for the control of internal P-loading in lakes should focus on the following areas. Firstly, more information regarding the potential for indirect and direct ecotoxicological effects is required to underpin use of all P-capping products, especially in ecosystems of high conservation value. Indirect effects associated with the application of Phoslock® may be related to increased loading of fine suspended inorganic sediment, which may be assessed against water quality guidelines for short-term exposure of surface waters to increased suspended solid concentrations (e.g. European Union: Freshwater Fisheries Directive, 2004/44/EC). Furthermore studies investigating ecotoxicological threshold levels for a range of species across trophic levels according to standard methods and studies assessing the potential role of La bioaccumulation through the food web are required. Such information would be crucial for lake managers to decide, depending on presence of potentially sensitive organisms, in which ecosystems Phoslock® can be a useful measure for the abatement of internal P-loading. Secondly, further attention regarding the long-term efficacy of Phoslock® (i.e. La-P complexes) under *in situ* conditions is required to assess if P is permanently removed from cycling between the sediment and the water-column. Finally, future studies are required to improve dose calculations in order to achieve water quality targets with minimum dose applied. In

particular, a better understanding of the mechanisms of regime shifts between the phytoplankton dominated turbid state and the macrophyte dominated clear water state in a range of shallow lake ecosystem may be required to improve the likelihood of successful remediation associated with the use of P-capping agents. Adjusting dose calculations to different external P-loading scenarios requires further investigation as does the question of whether ageing of Phoslock® under *in situ* conditions affects its P-binding capacity? Despite advancements made by various recent studies assessing the use of Phoslock® (and other P-capping agents) there is still large uncertainty involved in the use of P-capping agents in remediation projects which results partially from a lack of knowledge concerning coupled biogeochemical processes and feedback mechanisms operating at the ecosystem scale.

CONCLUSIONS

This study showed that a controlled disturbance caused the disruption of the internal P-loading feedback mechanism in a recovering shallow lake (Loch Flemington), thereby forcing a change in state from a phytoplankton dominated turbid state to a macrophyte dominated clear water state. Significant alterations in ecological structure and function, observed following disruption of the internal P-loading feedback mechanism, are potentially associated with an increase in ES provided, especially the provision of clean and safe water. Additional remediation measures are likely to increase ecological resilience thereby reducing the likelihood of a reverse change in state and therefore securing ES provisioning capacity.

Practical implications of this study for lake managers include the finding that Phoslock® increases the mass of P present in more refractory sediment P-fractions relative to P_{mobile}. This will reduce P-cycling between the sediment and the water-column and increase the permanent sediment P-binding capacity under reducing

conditions in lake bed sediments. Phoslock® should be applied prior to the expected internal P-loading peak. However, the application of Phoslock® early in spring to Loch Flemington in this study appeared to be beneficial as natural feedback mechanisms associated with the macrophyte dominated clear water state appeared to enhance structural and functional recovery. Evidence from mesocosm and ecosystem scale experiments suggested that multiple smaller doses of Phoslock® are likely to increase cost-effectiveness and reduce the risk of non-target effects.

References

Afsar, A. and S. Groves. 2009. Eco-toxicity assessment of Phoslock®. Phoslock Water Solutions Limited. [online: http://www.phoslock.com.au/docs2/25.%20Lanthanum%20and%20Human%20Health-June%202009.pdf; accessed 6th of December 2010]

Ahlgren, G., L. Lundstedt, M. Brett, and C. Forsberg. 1990. Lipid composition and food quality of some freshwater phytoplankton for cladoceran zooplankters. Journal of Plankton Research 12:809-818.

Alcamo, J., J. M. Moreno, B. Nováky, M. Bindi, R. Corobov, R. J. N. Devoy, C. Giannakopoulos, E. Martin, J. E. Olesen, and A. Shivdenko. 2007. Europe. Pages 541-580 *in* M. L. Parry, O. F. Canziani, J. P. Palutikof, P. J. van der Linden, and C. E. Hanson, editors. Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.

Andersen, J. M. 1975. Influence of pH on release of phosphorus from lake sediments. Archiv für Hydrobiologie 76:411-419.

Anderson, A. M., B. M. Spears, H. V. Lubarsky, I. Davidson, S. U. Gerbersdorf, and D. M. Paterson. 2011. Magnetic particle induction and its importance in biofilm research. Pages 189-216 *in* R. Fazel, editor. Biomedical engineering - Frontiers and challenges. InTech.

APHA. 1992. Standard methods for the examination of water and wastewater. American Public Health Association, Washington, DC.

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

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

Barbiero, R. P. and E. B. Welch. 1992. Contribution of benthic blue-green algal recruitment to lake populations and phosphorus translocation. Freshwater Biology 27:249-260.

Barko, J. W. and W. F. James. 1997. Effects of submerged aquatic macrophytes on nutrient dynamics, sedimentation, and resuspension. Pages 197-214 *in* E. Jeppesen, M. Søndergaard, M. Søndergaard, and K. Christoffersen, editors. The structuring role of submerged macrophytes in lakes. Springer, New York.

Barko, J. W. and R. M. Smart. 1980. Mobilization of sediment phosphorus by submersed freshwater macrophytes. Freshwater Biology 10:229-238.

Barrett, P. 2000. The restoration of Loch Flemington. Progress report to the Loch Flemington Management Group (unpublished report). Inverness.

Beaver, J. R. and T. L. Crisman. 1989. The role of ciliated protozoa in pelagic freshwater ecosystems. Microbial Ecology 17:111-136.

Bellinger, E. G. and D. D. Sigee. 2010. Freshwater algae: Identification and use as bioindicators. John Wiley & Sons, Chichester.

Benndorf, J. 1987. Food web manipulation without nutrient control: A useful strategy in lake restoration? Aquatic Sciences 49:237-248.

Benndorf, J. 1990. Conditions for effective biomanipulation; conclusions derived from whole-lake experiments in Europe. Hydrobiologia 200/201:187-203.

Bennett, E. M., S. R. Carpenter, and N. F. Caraco. 2001. Human impact on erodable phosphorus and eutrophication: A global perspective. BioScience 51:227-234.

Bennion, H., G. Clarke, T. Davidson, D. Morley, N. Rose, S. Turner, and H. Yang. 2008. Palaeoecological study of seven mesotrophic lochs (Final Report to SEPA and SNH; ISSN: 1366-7300). Environmental Change Research Centre, London.

Bidle, K. D. and F. Azam. 1999. Accelerated dissolution of diatom silica by marine bacterial assemblages. Nature 397:508-512.

Biles, C. L., D. M. Paterson, R. B. Ford, M. Solan, and D. G. Raffaelli. 2002. Bioturbation, ecosystem functioning and community structure. Hydrology and Earth System Sciences 6:999-1005.

Blais, J. M. and J. Kalff. 1995. The influence of lake morphometry on sediment focusing. Limnology & Oceanography 40:582-588.

Blindow, I., G. Andersson, A. Hargeby, and S. Johansson. 2006. Long-term pattern of alternative stable states in two shallow eutrophic lakes. Freshwater Biology 30:159-167.

Bloesch, J. 1995. Mechanisms, measurement and importance of sediment resuspension in lakes. Marine and Freshwater Research 46:295-304.

Boros, G., M. Søndergaard, T. P., A. Vári, and I. Tátrai. 2011. Influence of submerged macrophytes, temperature, and nutrient loading on the development of redox potential around the sediment–water interface in lakes. Hydrobiologia 665:117-127.

Boström, B. 1981. Factors controlling the seasonal variation of nitrate in Lake Erken. International Review of Hydrobiology 66:821-836.

Boström, B. 1984. Potential mobility of phosphorus in different types of lake sediment. Internationale Revue der gesamten Hydrobiologie und Hydrographie 69:1522-2632.

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

Boström, B., M. Jansson, and C. Forsberg. 1982. Phosphorus release from lake sediments. Archiv für Hydrobiologie-Beiheft Ergebnisse der Limnologie 18:5-59.

Brabrand, A., B. A. Faafeng, and J. P. Moritz Nilssen. 1990. Relative importance of phosphorus supply to phytoplankton production: Fish excretion versus external loading. Canadian Journal of Fisheries and Aquatic Sciences 47:364-372.

Breukelaar, A. W., E. H. R. R. Lammens, J. G. P. Klein Breteler, and I. Tátrai. 1994. Effects of benthivorous bream (*Abramis brama*) and carp (*Cyprinus carpio*) on sediment resuspension and concentrations of nutrients and chlorophyll *a*. Freshwater Biology 32:113-121.

Brookes, J. D. and C. C. Carey. 2011. Resilience to blooms. Science 334:46-47.

Brooks, J. L. and S. I. Dodson. 1965. Predation, body size, and composition of plankton. Science 150:28-35.

Bulgakov, N. G. and A. P. Levich. 1999. The nitrogen : phosphorus ratio as a factor regulating phytoplankton community structure. Archiv für Hydrobiologie 146:3-22.

Burger, D. F., D. P. Hamilton, and C. A. Pilditch. 2008. Modelling the relative importance of internal and external nutrient loads on water column nutrient concentrations and phytoplankton biomass in a shallow polymictic lake. Ecological Modelling 211:411-423.

Canfield, D. E., K. A. Langeland, S. B. Linda, and W. T. Haller. 1985. Relations between water transparency and maximum depth of macrophyte colonization in lakes. Journal of Aquatic Plant Management 23: 25-28.

Carignan, R. and J. Kalff. 1980. Phosphorus sources for aquatic weeds: Water or sediments? Science 207:987-989.

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

Carpenter, S. R. 2008. Phosphorus control is critical to mitigating eutrophication. Proceedings of the National Academy of Sciences of the United States of America 105:11039–11040.

Carpenter, S. R. and K. L. Cottingham. 1997. Resilience and restoration of lakes. Conservation Ecology. [online: http://www.consecol.org/vol1/iss1/art2/; accessed 1st of July 2012]

Carpenter, S. R. and J. F. Kitchell. 1993. The trophic cascade in lakes. Cambridge University Press, Cambridge.

Carpenter, S. R., J. F. Kitchell, K. L. Cottingham, D. E. Schindler, D. L. Christense, D. M. Post, and N. Voichick. 1996. Chlorophyll variability, nutrient input, and grazing: Evidence from whole-lake experiments. Ecology 77:725-735.

Carpenter, S. R., J. F. Kitchell, and J. R. Hodgson. 1985. Cascading trophic interactions and lake productivity. BioScience 35:634-369.

Carpenter, S. R., C. E. Kraft, R. Wright, X. He, P. A. Soranno, and J. R. Hodgson. 1992. Resilience and resistance of a lake phosphorus cycle before and after food web manipulation. The American Naturalist 140:781-798.

Carpenter, S. R. and R. C. Lathrop. 1999. Lake restoration: capabilities and needs. Hydrobiologia 395/396:19-28.

Carvalho, L., B. Dudley, I. Dodkins, R. Clarke, I. Jones, S. Thackeray, and S. Maberly. 2007. Phytoplankton Classification Tool (Phase 2). Project WFD80. SNIFFER, Edinburgh.

CEN TC 230/WG 2/TG 3/N73. 2004. Water quality – Guidance standard for the routine analysis of phytoplankton abundance and composition using inverted microscopy (Utermöhl technique). Technical Committee CEN/TC 230.

Chambers, P. A., E. E. Prepas, M. L. Bothwell, and H. R. Hamilton. 1989. Roots versus shoots in nutrient uptake by aquatic macrophytes in flowing waters. Canadian Journal of Fisheries and Aquatic Sciences 46:435-439.

Chambers, P. A., E. E. Prepas, M. E. Ferguson, M. Serediak, M. Guy, and M. Holst. 2001. The effects of lime addition on aquatic macrophytes in hard water: *in situ* and microcosm experiments. Freshwater Biology 45:1121-1138.

Chin, R. and B. Y. Lee. 2008. Dosing and intervention. Pages 181-212 *in* R. Chin and B. Y. Lee, editors. Principles and practice of clinical trial medicine. Elsevier, London.

Chow-Fraser, P., D. O. Trew, D. Findlay, and M. Stainton. 1994. A test of hypotheses to explain the sigmoidal relationship between total phosphorus and chlorophyll *a* concentrations in Canadian Lakes. Canadian Journal of Fisheries and Aquatic Sciences 51:2052-2064.

Christensen, P. B., L. P. Nielsen, J. Sorensen, and N. P. Revsbech. 1990. Denitrification in nitrate-rich streams: Diurnal and seasonal variation related to benthic oxygen metabolism. Limnology & Oceanography 35:640-651.

Clearwater, S. J. 2004. Chronic exposure of midge larvae to Phoslock. NIWA Client Report. Report Number: NAU04927. National Institute of Water & Atmospheric Research, New Zealand.

Consalvey, M., D. M. Paterson, and G. J. C. Underwood. 2004. The ups and downs of life in a benthic biofilm: Migration of benthic diatoms. Diatom Research 19:181-202.

Cooke, G. D., E. B. Welch, A. B. Martin, D. G. Fulmer, J. B. Hyde, and G. D. Schrieve. 1993. Effectiveness of Al, Ca, and Fe salts for control of internal phosphorus loading in shallow and deep lakes. Hydrobiologia 253:323-335.

Cooke, G. D., E. B. Welch, S. A. Peterson, and S. A. Nichols. 2005. Restoration and management of lakes and reservoirs. 3rd edition edition. Taylor & Francis, London.

Coops, H., M. Beklioglu, and T. L. Crisman. 2003. The role of water-level fluctuations in shallow lake ecosystems – workshop conclusions. Hydrobiologia 506/509:23-27.

Cottingham, K. L. and D. E. Schindler. 2000. Effects of grazer community structure on phytoplankton response to nutrient pulses. Ecology 81:183-200.

Crooks, J. A. 2002. Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. Oikos 97:153-166.

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

Decho, A. W. 1994. Molecular scale events influencing the macroscale cohesiveness of exopolymers. Pages 135-148 *in* W. E. Krumbein, D. M. Paterson, and L. J. Stal, editors. Biostabilisation of Sediment. BIS Verlag, Oldenburg.

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

Della Bella, V., M. Bazzanti, and F. Chiarotti. 2005. Macroinvertebrate diversity and conservation status of Mediterranean ponds in Italy: water permanence and mesohabitat influence. Aquatic Conservation: Marine and Freshwater Ecosystems 15:583-600.

Dieter, C. D. 1990. The importance of emergent vegetation in reducing sediment resuspension in wetlands. Journal of Freshwater Ecology 5:467-473.

Dietrich, D. and C. Schlatter. 1989. Aluminium toxicity to rainbow trout at low pH. Aquatic Toxicology 15:197-212.

Dillon, P. J. and F. H. Rigler. 1974. The phosphorus-chlorophyll relationship in lakes. Limnology & Oceanography 19:767-773.

Dobson, A., D. Lodge, J. Alder, G. S. Cumming, J. Keymer, J. McGlade, H. Mooney, J. A. Rusak, O. Sala, V. Wolters, D. Wall, R. Winfree, and M. A. Xenopoulos. 2006. Habitat loss, trophic collapse, and the decline of ecosystem services. Ecology 87:1915-1924.

Dodds, W. and M. Whiles. 2010. Freshwater Ecology - Concepts & environmental applicaitons of limnology. Academic Press, London.

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

Does, v. d. J., P. Verstraelen, P. Boers, v. J. Roesteel, R. Roijackers, and G. Moser. 1992. Lake restoration with and without dredging of phosphorus-enriched upper sediment layers. Hydrobiologia 233:197-210.

Dokulil, M. T., K. Donabaum, and K. Pall. 2011. Successful restoration of a shallow lake: A case study based on bistable theory. Pages 285-294 *in* A. A. Ansari, S. Singh Gill, G. R. Lanza, and W. Rast, editors. Eutrophication: Causes, consequences and control. Springer, London.

Dokulil, M. T. and K. Teubner. 2000. Cyanobacterial dominance in lakes. Hydrobiologia 438:1-12.

Douglas, G. B. 2002. Remediation material and remediation process for sediments. US Patent 6350383.

Douglas, G. B., J. A. Adeney, and M. S. Robb. 1999. A novel technique for reducing bioavailable phosphorus in water and sediments. Pages 517–523 *in* Proceedings of the International Association Water Quality Conference on Diffuse Pollution.

Douglas, R. W. and B. Rippey. 2000. The random redistribution of sediment by wind in a lake. Limnology and Oceanography 45:686-694.

Downing, J. A., S. B. Watson, and E. McCauley. 2001. Predicting cyanobacteria dominance in lakes. Canadian Journal of Fisheries and Aquatic Sciences 58:1905-1908.

Drake, J. C. and S. I. Heaney. 1987. Occurrence of phosphorus and its potential remobilization in the littoral sediments of a productive English lake. Freshwater Biology 17:513-523.

Dussart, B. H. and D. Defaye. 1995. Copepoda: Introduction to the copepoda. SPB Publishing, Amsterdam.

Egemose, S., K. Reitzel, F. Ø. Andersen, and M. R. Flindt. 2010. Chemical lake restoration products: Sediment stability and phosphorus dynamics. Environmental Science & Technology 44:985-991.

Egemose, S., K. Reitzel, F. Ø. Andersen, and H. S. Jensen. 2012. Resuspension-mediated aluminium and phosphorus distribution in lake sediments after aluminium treatment. Hydrobiologia: Online First.

Egemose, S., D. I. Vicente, K. Reitzel, M. R. Flindt, F. Ø. Andersen, T. L. Lauridsen, M. Søndergaard, E. Jeppesen, and H. S. Jensen. 2011. Changed cycling of P, N, Si, and DOC in Danish Lake Nordborg after aluminum treatment. Canadian Journal of Fisheries and Aquatic Sciences 68:842-856.

Einsele, W. 1936. Über die Beziehungen der Eisenkreislaufes zum Phosphorkreislauf im eutrophen See. Archiv für Hydrobiologie 29:664-686.

Einsle, U. 1996. Copepoda: Cyclopoida: Genera cyclops, megacyclops, acanthocyclops. SFB Publishing, Amsterdam.

Eisenreich, S. J., R. T. Bannermann, and D. E. Armstrong. 1975. A simplified phosphorus analytical technique. Environmental Letters 9:45-53.

Elliott, J. A. 2012. Is the future blue-green? A review of the current model predictions of how climate change could affect pelagic freshwater cyanobacteria. Water Research 46:1364-1371.

Elser, J. J., T. Andersen, J. S. Baron, A.-K. Bergström, M. Jansson, M. Kyle, K. R. Nydick, L. Steger, and D. O. Hessen. 2009. Shifts in lake N:P stoichiometry and nutrient limitation driven by atmospheric nitrogen deposition. Science 326:835-837.

European Union. 2006. Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC. Pages 37-51. Official Journal of the European Union.

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

Ferrão-Filho, A. S., M. S. Arcifa, and C. Fileto. 2003. Resource limitation and food quality for cladocerans in a tropical Brazilian lake. Hydrobiologia 491:201-210.

Feuchtmayr, H., R. Moran, K. Hatton, L. Connor, T. Heyes, B. Moss, I. Harvey, and D. Atkinson. 2009. Global warming and eutrophication: effects on water chemistry and autotrophic communities in experimental hypertrophic shallow lake mesocosms. Journal of Applied Ecology 46:713-723.

Finlay, B. J. 1981. Oxygen availability and seasonal migrations of ciliated protozoa in a freshwater lake. Microbiology 123:173-178.

Fischer, J. B., W. J. Lick, P. L. McCall, and J. A. Robbins. 1980. Vertical mixing of lake sediments by tubificid oligochaetes. Journal of Geophysical Research 85:3997-4006.

Folke, C., S. R. Carpenter, B. Walker, M. Scheffer, T. Elmqvist, L. Gunderson, and C. S. Holling. 2004. Regime shifts, resilience, and biodiversity in ecosystem management. Annual Review of Ecology, Evolution, and Systematics 35:557-581.

Fong, P., J. B. Zedler, and R. M. Donohoe. 1993. Nitrogen vs. phosphorus limitation of algal biomass in shallow coastal lagoons. Limnology and Oceanography 38:906-923.

Frodge, J. D., G. L. Thomas, and G. B. Pauley. 1991. Sediment phosphorus loading beneath dense canopies of aquatic macrophytes. Lake and Reservoir Management 7:61-71.

Fukuhara, H. and M. Sakamoto. 1987. Enhancement of inorganic nitrogen and phosphate release from lake sediment by tubificid worms and chironomid larvae. Oikos 48:312-320.

Gächter, R. and J. S. Meyer. 1993. The role of microorganisms in mobilization and fixation of phosphorus in sediments. Hydrobiologia 253:103-121.

Gallepp, G. W. 1979. Chironomid influence on phosphorus release in sediment-water microcosms. Ecology 60:547-556.

Genkai-Kato, M. and S. R. Carpenter. 2005. Eutrophication due to phosphorus recycling in relation to lake morphometry, temperature, and macrophytes. Ecology 86:210-219.

Gerbersdorf, S. U., W. Manz, and D. M. Paterson. 2008. The engineering potential of natural benthic bacterial assemblages in terms of the erosion resistance of sediments. FEMS Microbiology Ecology 66:282-294.

Gibbs, M., C. Hickey, and D. Özkundakci. 2011. Sustainability assessment and comparison of efficacy of four P-inactivation agents for managing internal phosphorus loads in lakes: sediment incubations. Hydrobiologia 658:253-275.

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

Gliwicz, Z. M. and W. Lampert. 1990. Food thresholds in *Daphnia* species in the absence and presence of blue-green filaments. Ecology 71:691-702.

Golterman, H. L., R. S. Clymo, and M. A. M. Ohnstad. 1978. Methods for physical and chemical analysis of freshwaters. 2nd edition. Blackwell, Oxford.

Google Earth. 2013a. Clatto Reservoir and surrounding area. [online; accessed 4th of January 2013].

Google Earth. 2013b. Loch Flemington and surrounding area. [online; accessed 4th of January 2013].

Google Map. 2013. Map of the United Kingdom. [online: http://maps.google.de/; accessed 4th of January 2013].

Gordon, J. E. and C. A. Auton. 1993. The Kildrummie Kames. Pages 176-181 *in* J. E. Gordon and D. G. Sutherland, editors. Quaternary of Scotland. Chapman and Hall, London.

Granéli, W. and D. Solander. 1988. Influence of aquatic macrophytes on phosphorus cycling in lakes. Hydrobiologia 170:245-266.

Greenbank, J. 1945. Limnological conditions in ice-covered lakes, especially as related to winter-kill of fish. Ecological Monographs 15:343-392.

Gregor, J. and B. Maršálek. 2004. Freshwater phytoplankton quantification by chlorophyll *a*: a comparative study of in vitro, in vivo and in situ methods. Water Research 38:517-522.

Griffiths, D. 2006. The direct contribution of fish to lake phosphorus cycles. Ecology of Freshwater Fish 15:86-95.

Gulati, R. D., L. M. D. Pires, and E. Van Donk. 2008. Lake restoration studies: Failures, bottlenecks and prospects of new ecotechnological measures. Limnologica 38:233-247.

Gulati, R. D. and E. van Donk. 2002. Lakes in the Netherlands, their origin, eutrophication and restoration: state-of-the-art review. Hydrobiologia 478:73-106.

Gunderson, L. H. 2000. Ecological resilience - in theory and application. Annual Review of Ecology and Systematics 31:425-439.

Gunn, I. D. M., M. O'Hare, L. Carvalho, D. B. Roy, P. Rothery, and A. M. Darwell. 2010. Assessing the condition of lake habitats: a test of methods for surveying aquatic macrophyte communities. Hydrobiologia 656:87-97.

Gunn, I. D. M., M. T. O'Hare, P. S. Maitland, and L. May. 2012. Long-term trends in Loch Leven invertebrate communities. Hydrobiologia 681:59-72.

Haag, R. W. 1979. The ecological significance of dormancy in some rooted aquatic plants. Journal of Ecology 67:727-738.

Haghseresht, F., S. Wang, and D. D. Do. 2009. A novel lanthanum-modified bentonite, Phoslock, for phosphate removal from wastewaters. Applied Clay Science 46:369-375.

Han, M. Y. and K. W. 2001. A theoretical consideration of algae removal with clays. Microchemical Journal 68:157-161.

Hansen, K., S. Mouridsen, and E. Kristensen. 1997. The impact of *Chironomus plumosus* larvae on organic matter decay and nutrient (N, P) exchange in a shallow eutrophic lake sediment following a phytoplankton sedimentation. Hydrobiologia 364:65-74.

Hansson, L.-A., H. Annadotter, E. Bergman, S. F. Hamrin, E. Jeppesen, T. Kairesalo, E. Luokkanen, P. A. Nilsson, M. Søndergaard, and J. Strand. 1998. Biomanipulation as an application of food-chain theory: Constraints, synthesis, and recommendations for temperate lakes. Ecosystems 1:558-574.

Harding, J. P. and W. A. Smith. 1974. A key to the British freswater cyclopoid and calanoid copepods. Freshwater Biological Association, Ambleside.

Hargeby, A., I. Blindow, and L.-A. Hansson. 2004. Shifts between clear and turbid states in a shallow lake: multi-causal stress from climate, nutrients and biotic interactions. Archiv für Hydrobiologie 161:433-454.

Harper, D. M. 1992. Eutrophication of freshwaters - Principles, problems and restoration. Chapman & Hall, London.

Hašler, P., A. Poulíčková, and M. Lysáková. 2004. The survival and vitality of cyanobacteria and algae in fishpond bottom sediments. Czech Phycology 4:133-145.

Havens, K. E. and R. T. Heath. 1990. Phytoplankton succession during acidification with and without increasing aluminum levels. Environmental Pollution 68:129-145.

Head, R. M., R. I. Jones, and 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.

Henriksen, K., J. I. Hansen, and T. H. Blackburn. 1981. Rates of nitrification, distribution of nitrifying bacteria, and nitrate fluxes in different types of sediment from Danish Waters. Marine Biology 61:299-304.

Heyer, d. C. and J. Kalff. 1998. Organic matter mineralization rates in sediments: A within- and among-lake study. Limnology & Oceanography 43:695-705.

Hickey, C. W. and M. M. Gibbs. 2009. Lake sediment phosphorus release management—Decision support and risk assessment framework. New Zealand Journal of Marine and Freshwater Research 43:819-856.

Hilton, J., J. P. Lishman, and 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. 'Hierarchical Monitoring Methods. European Commission Fifth Framework Programme.' Contract: EVK3-CT-2001-00052. Brokman Consult, Geesthacht.

Holling, C. S. 1973. Resilience and stability of ecological systems. Annual Review of Ecology and Systematics 4:1-23.

Hornbach, D. J., C. M. Way, T. E. Wissing, and A. J. Burky. 1984. Effects of particle concentration and season on the filtration rates of the freshwater clam, *Sphaerium striatinum* Lamarck (Bivalvia: Pisidiidae). Hydrobiologia 108:83-96.

Horppila, J. and T. Kairesalo. 1990. A fading recovery: the role of roach (*Rutilus rutilus* L.) in maintaining high phytoplankton productivity and biomass in Lake Vesijärvi, southern Finland. Hydrobiologia 200/201:153-165.

Horppila, J. and L. Nurminen. 2003. Effects of submerged macrophytes on sediment resuspension and internal phosphorus loading in Lake Hiidenvesi (southern Finland). Water Research 37:4468-4474.

Hosper, H. and M.-L. Meyer. 1986. Control of phosphorus loading and flushing as restoration methods for Lake Veluwe, the Netherlands. Hydrobiological Bulletin 20:183-194.

Hosper, S. H. and E. Jagtman. 1990. Biomanipulation additional to nutrient control for restoration of shallow lakes in The Netherlands. Hydrobiologia 200/201:523-534.

Hulot, F. D., G. Lacroix, F. Lescher-Moutoué, and M. Loreau. 2000. Functional diversity governs ecosystem response to nutrient enrichment. Nature 405:340-344.

Hupfer, M. and A. Dollan. 2003. Immobilisation of phosphorus by iron-coated roots of submerged macrophytes. Hydrobiologia 506/509:635-640.

Hupfer, M., R. Gächter, and R. Giovanoli. 1995. Transformation of phosphorus species in settling seston and during early sediment diagenesis. Aquatic Sciences 57:305-324.

Hupfer, M. and S. Hilt. 2008. Lake restoration. Pages 2080-2093 *in* S. E. Jørgensen and B. Fath, editors. Ecological Engineering. Vol. 3 of Encyclopedia of Ecology. Elsevier, Oxford.

Hupfer, M. and J. Lewandowski. 2008. Oxygen controls the phosphorus release from lake sediments - a long-lasting paradigm in limnology. International Review of Hydrobiology 93:415-432.

Hupfer, M., D. Zak, R. Roßberg, C. Herzog, and R. Pöthig. 2009. Evaluation of a well-established sequential phosphorus extraction technique for use in calcite-rich lake sediments: identification and prevention of artifacts due to apatite formation. Limnology & Oceanography: Methods 7:399-410.

Hyenstrand, P., P. Blomqvist, and A. Petterson. 1998. Factors determining cyanobacterial success in aquatic systems - a literature review. Archiv für Hydrobiologie - Special Issues Advanced Limnology 51:41-62.

Ibelings, B. W., R. Portielje, E. H. R. R. Lammens, R. Noordhuis, M. S. van den Berg, W. Joosse, and M.-L. Meijer. 2007. Resilience of alternative stable states during the recovery of shallow lakes from eutrophication: Lake Veluwe as a case study. Ecosystems 10:4-16.

ISO 10260. 1992. Water quality - Measurement of biochemical parameters - Spectrometric determination of the chlorophyll-*a* concentration. International Organization for Standardization, Geneva, Switzerland.

Jakobsen, T. S., P. B. Hansen, E. Jeppesen, P. Grønkjær, and M. Søndergaard. 2003. Impact of three-spined stickleback *Gasterosteus aculeatus* on zooplankton and chl a in shallow, eutrophic, brackish lakes. Marine Ecology Progress Series 262:277-284.

Jakobsen, T. S., P. B. Hansen, E. Jeppesen, and M. Søndergaard. 2004. Cascading effect of three-spined stickleback *Gasterosteus aculeatus* on community composition, size, biomass and diversity of phytoplankton in shallow, eutrophic brackish lagoons. Marine Ecology Progress Series 279:305–309.

James, C., J. Fisher, V. Russell, S. Collings, and B. Moss. 2005. Nitrate availability and hydrophyte species richness in shallow lakes. Freshwater Biology 50:1049-1063.

James, W. F., J. W. Barko, H. L. Eakin, and D. R. Helsel. 2000. Distribution of sediment phosphorus pools and fluxes in relation to alum treatment. Journal of the American Water Resources Association 36:647-656.

Jančula, D., P. Mikula, and B. Maršálek. 2011. Effects of polyaluminium chloride on the freshwater invertebrate *Daphnia magna*. Chemistry and Ecology 27:351-357.

Janse, J. H., L. N. De Senerpont Domis, M. Scheffer, L. Lijklema, L. Van Liere, M. Klinge, and W. M. Mooij. 2008. Critical phosphorus loading of different types of shallow lakes and the consequences for management estimated with the ecosystem model PCLake. Limnologica 38:203-219.

Jeffrey, S. W. and G. F. Humphrey. 1975. New spectrophotometric equations for determining chlorophylls *a*, *b*, c_1 and *c2* in higher plants, algae and natural phytoplankton. Biochemie und Physiologie der Pflanzen 167:191-194.

Jensen, H. S. and F. Ø. Andersen. 1992. Importance of temperature, nitrate, and pH for phosphate release from aerobic sediments of 4 shallow, eutrophic lakes. Limnology & Oceanography 37:577-589.

Jensen, H. S., P. B. Mortensen, F. Ø. Andersen, E. Rasmussen, and A. Jensen. 1995. Phosphorus cycling in a coastal marine sediment, Aarhus Bay, Denmark. Limnology and Oceanography 40:908-917.

Jensen, J. P., A. R. Pedersen, E. Jeppesen, and M. Søndergaard. 2006. An empirical model describing the seasonal dynamics of phosphorus in 16 shallow eutrophic lakes after external loading reduction. Limnology & Oceanography 51:791-800.

Jensen, K., N. P. Revsbech, and L. P. Nielsen. 1993. Microscale distribution of nitrification activity in sediment determined with a shielded microsensor for nitrate. Applied and Environmental Microbiology 59:3287-3296.

Jeppesen, E. 1998. The ecology of shallow lakes - trophic interactions in the pelagial. University of Copenhagen, Silkeborg.

Jeppesen, E., J. P. Jensen, P. Kristensen, M. Søndergaard, E. Mortensen, O. Sortkjær, and K. Olrik. 1990. Fish manipulation as a lake restoration tool in shallow, eutrophic, temperate lakes 2: threshold levels, long-term stability and conclusions. Hydrobiologia 200/201:219-227.

Jeppesen, E., J. P. Jensen, and M. Søndergaard. 2002. Response of phytoplankton, zooplankton, and fish to re-oligotrophication: An 11 year study of 23 Danish lakes. Aquatic Ecosystem Health & Management 5:31-43.

Jeppesen, E., J. P. Jensen, M. Søndergaard, T. Lauridsen, and F. Landkildehus. 2000. Trophic structure, species richness and biodiversity in Danish lakes: changes along a phosphorus gradient. Freshwater Biology 45:201-218.

Jeppesen, E., J. P. Jensen, M. Søndergaard, T. Lauridsen, L. J. Pedersen, and L. Jensen. 1997. Top-down control in freshwater lakes: the role of nutrient state, submerged macrophytes and water depth. Hydrobiologia 342/343:151-164.

Jeppesen, E., J. P. Jensen, M. Søndergaard, and T. L. Lauridsen. 2005a. Response of fish and plankton to nutrient loading reduction in eight shallow Danish lakes with special emphasis on seasonal dynamics. Freshwater Biology 50:1616-1627.

Jeppesen, E., B. Kronvang, M. Meerhoff, M. Søndergaard, K. M. Hansen, H. E. Andersen, T. Lauridsen, L. Liboriussen, M. Beklioglu, A. Özen, and J. E. Olesen. 2009. Climate change effects on runoff, catchment phosphorus loading and lake ecological state, and potential adaptations. Journal of Environmental Quality 38:1930-1941.

Jeppesen, E., T. Lauridsen, T. Kairesalo, and M. R. Perrow. 1998a. Impact of submerged macrophytes on fish-zooplankton interactions in lakes. Pages 91-114 *in* E. Jeppesen, M. Søndergaard, M. Søndergaard, and K. Christoffersen, editors. The structuring role of submerged macropyhtes in lakes. Springer, London.

Jeppesen, E., M. Meerhoff, K. Holmgren, I. González-Bergonzoni, F. T. De Mello, S. A. J. Declerck, L. De Meester, M. Søndergaard, T. L. Lauridsen, R. Bjerring, J. M. Conde-Porcuna, N. Mazzeo, C. Iglesias, M. Reizenstein, H. J. Malmquist, Z. Liu, D. Balayla, and X. Lazzaro. 2010. Impacts of climate warming on lake fish community structure and potential effects on ecosystem function. Hydrobiologia 646:73-90.

Jeppesen, E., M. Meerhoff, B. A. Jacobsen, R. S. Hansen, M. Søndergaard, J. P. Jensen, T. L. Lauridsen, N. Mazzeo, and C. W. C. Branco. 2007a. Restoration of shallow lakes by nutrient control and biomanipulation—the successful strategy varies with lake size and climate. Hydrobiologia 581:269-285.

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

Jeppesen, E., M. Søndergaard, M. Meerhoff, T. L. Lauridsen, and J. P. Jensen. 2007b. Shallow lake restoration by nutrient loading reduction—some recent findings and challenges ahead. Pages 239-252 *in* R. D. Gulati, E. Lammens, N. Pauw, and E. Donk, editors. Shallow Lakes in a Changing World, Springer, Amsterdam.

Jeppesen, E., M. Søndergaard, M. Søndergaard, and K. Kristoffersen. 1998b. Structuring role of submerged macrophytes in lakes. Springer, New York.

Johannesson, K. H. and W. B. Lyons. 1994. The rare earth element geochemistry of Mono Lake water and the importance of carbonate complexing. Limnology & Oceanography 39:1141-1154.

John, D. M., B. A. Whitton, and A. J. Brook. 2005. The freshwater algal flora of the British Isles - An identification guide to freshwater and terrestrial algae. Cambridge University Press, Cambridge.

Jones, C. and J. Lawton. 1995. Linking species and ecosystems. Chapman and Hall, London.

Jupp, B. P. and D. H. N. Spence. 1977. Limitations on macrophytes in a eutrophic lake, Loch Leven. 1. Effects of phytoplankton. Journal of Ecology 65:175-186.

Jupp, B. P., D. H. N. Spence, and R. H. Britton. 1974. The distribution and production of submerged macrophytes in Loch Leven, Kinross. Proceedings of The Royal Society of Edinburgh Section B (Biology) 74:195-208.

Kamatani, A. and J. P. Riley. 1979. Rate of dissolution of diatom silica walls in seawater. Marine Biology 55:29-35.

Kennedy, R. H. and G. D. Cooke. 1982. Control of lake phosphorus with aluminum sulfate: Dose determination and application techniques. Journal of the American Water Resources Association 18:389-395.

Kimball, K. D. and S. A. Levin. 1985. Limitations of laboratory bioassays: The need for ecosystem-level testing. BioScience 35:165-171.

Kitchell, J. F. and S. R. Carpenter. 1993. Variability in lake ecosystems: complex responses by the apical predator. Pages 111-124 *in* M. McDonnell and S. Pickett, editors. Humans as components of ecosystems. Springer, New York.

Köhler, J., S. Hilt, R. Adrian, A. Nicklisch, H. P. Kozerski, and N. Walz. 2005. Long-term response of a shallow, moderately flushed lake to reduced external phosphorus and nitrogen loading. Freshwater Biology 50:1639-1650.

Koski-Vähälä, J. and H. Hartikainen. 2001. Assessment of the risk of phosphorus loading due to resuspended sediment. Journal of Environmental Quality 30:960-966.

Kosten, S., E. Jeppesen, V. L. M. Huszar, N. Mazzeo, E. H. Van Nes, E. T. H. M. Peeters, and M. Scheffer. 2011. Ambiguous climate impacts on competition between submerged macrophytes and phytoplankton in shallow lakes. Freshwater Biology 56:1540-1553.

Kozerski, H.-P. and A. Kleeberg. 1998. The sediments and benthic-pelagic exchange in the shallow Lake Müggelsee (Berlin, Germany). Internationale Revue der Gesamten Hydrobiologie 83:77-112.

Kristensen, E. 2000. Organic matter diagenesis at the oxic/anoxic interface in coastal marine sediments, with emphasis on the role of burrowing animals. Hydrobiologia 426:1-24.

Kristensen, E., S. I. Ahmed, and A. H. Devol. 1995. Aerobic and anaerobic decomposition of organic matter in marine sediment: Which is fastest? Limnology & Oceanography 40:1430-1437.

Kristensen, P. and H. Hansen. 1994. European rivers and lakes. European Environment Agency, Copenhagen.

Kundzewicz, Z. W., L. J. Mata, N. W. Arnell, P. Döll, B. Kabat, B. Jiménez, K. A. Miller, T. Oki, Z. Sen, and I. A. Shiklomanov. 2007. Freshwater resources and their management. Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Intergovernmental Panel on Climate Change, Cambridge, UK.

Lampert, W., W. Fleckner, H. Rai, and B. E. Tayloer. 1986. Phytoplankton control by grazing zooplankton: A study on the spring clear-water phase. Limnology & Oceanography 31:478-490.

Lang, C. 1998. Using oligochaetes to monitor the decrease of eutrophication: the 1982-1996 trend in Lake Geneva. Archiv für Hydrobiologie 141:447-458.

Lang, C. 1999. Contrasting responses of oligochaetes (Annelida) and chironomids (Diptera) to the abatement of eutrophication in Lake Neuchâtel. Aquatic Sciences - Research Across Boundaries 61:206-214.

Lathrop, R. C., S. R. Carpenter, and L. G. Rudstam. 1996. Water clarity in Lake Mendota since 1900: responses to differing levels of nutrients and herbivory. Canadian Journal of Fisheries and Aquatic Sciences 53:2250-2261.

Lauridsen, T., L. J. Pedersen, E. Jeppesen, and M. Søndergaard. 1996. The importance of macrophyte bed size for cladoceran composition and horizontal migration in a shallow lake. Journal of Plankton Research 18:2283-2294.

Lawlor, A. J. and E. Tipping. 2003. Metals in bulk deposition and surface waters at two upland locations in northern England. Environmental Pollution 121:153-167.

Lelková, E., M. Rulík, P. Hekera, P. Dobiáš, P. Dolejš, M. Borovičková, and A. Poulíčková. 2008. The influence of the coagulant PAX–18 on *Planktothrix agardhii* bloom in a shallow eutrophic fishpond. Fottea 8:147-154.

Lent, R. M. and B. Lyons. 2001. Biogeochemistry of silica in Devils Lake: Implications for diatom preservation. Journal of Paleolimnology 26:53-66.

Lewandowski, J. and M. Hupfer. 2005. Effect of macrozoobenthos on two-dimensional small-scale heterogeneity of pore water phosphorus concentrations in lake sediments: A laboratory study. Limnology & Oceanography 50:1106-1118.

Lewandowski, J., C. Laskov, and M. Hupfer. 2007. The relationship between *Chironomus plumosus* burrows and the spatial distribution of pore-water phosphate, iron and ammonium in lake sediments. Freshwater Biology 52:331-343.

Lewandowski, J., M. Schadach, and M. Hupfer. 2005. Impact of macrozoobenthos on two-dimensional small-scale heterogeneity of pore water phosphorus concentrations: in-situ study in Lake Arendsee (Germany). Hydrobiologia 549:43-55.

Lewandowski, J., I. Schauser, and M. Hupfer. 2003. Long term effects of phosphorus precipitations with alum in hypereutrophic Lake Süsser See (Germany). Water Research 37:3194-3204.

Lewin, J. C. 1961. The dissolution of silica from diatom walls. Geochimica et Cosmochimica Acta 21:182-198.

Lewis, W. M. and W. A. Wurtsbaugh. 2008. Control of lacustrine phytoplankton by nutrients: Erosion of the phosphorus paradigm. International Review of Hydrobiology 93:446-465.

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

Lijklema, L. 1994. Nutrient dynamics in shallow lakes: Effects of changes in loading and role of sediment-water interactions. Hydrobiologia 275/276:335-348.

Liu, X. and R. H. Byrne. 1997. Rare earth and yttrium phosphate solubilities in aqueous solution. Geochimica et Cosmochimica Acta 61:1625-1633.

Lougheed, V. L., B. Crosbie, and P. Chow-Fraser. 1998. Predictions on the effect of common carp (*Cyprinus carpio*) exclusion on water quality, zooplankton, and submergent macrophytes in a Great Lakes wetland. Canadian Journal of Fisheries and Aquatic Sciences 55:1189-1197.

Ludwig, D., B. Walker, and C. S. Holling. 1997. Sustainability, stability, and resilience. Conservation Ecology. [online; http://www.ecologyandsociety.org/vol1/iss1/art7/; accessed 2nd of June 2012]

Lukkari, K., H. Hartikainen, and M. Leivuori. 2007. Fractionation of sediment phosphorus revisited. I: Fractionation steps and their biogeochemical basis. Limnology and Oceanography: Methods 5:433-444.

Lund, S. S., F. Landkildehhus, M. Søndergaard, T. Lauridsen, S. Egemose, H. S. Jensen, F. Ø. Andersen, L. S. Johansson, M. Ventura, and E. Jeppesen. 2010. Rapid changes in fish community structure and habitat distribution following the precipitation of lake phosphorus with aluminium. Freshwater Biology 55:1036-1049.

Lürling, M. and E. J. Faassen. 2011. Controlling toxic cyanobacteria: effects of dredging and phosphorus-binding clay on cyanobacteria and microcystins. Water Research 46:1447-1459.

Lürling, M. and Y. Tolman. 2010. Effects of lanthanum and lanthanum-modified clay on growth, survival and reproduction of *Daphnia magna*. Water Research 44:309-319.

Lürling, M. and F. van Oosterhout. 2012. Case study on the efficacy of a lanthanum-enriched clay (Phoslock®) in controlling eutrophication in Lake Het Groene Eiland (The Netherlands). Hydrobiologia: Online First.

Maberly, S. C. 1996. Diel, episodic and seasonal changes in pH and concentrations of inorganic carbon in a productive lake. Freshwater Biology 35:579-598.

Maberly, S. C. and J. A. Elliott. 2012. Insights from long-term studies in the Windermere catchment: external stressors, internal interactions and the structure and function of lake ecosystems. Freshwater Biology 57:233-243.

Maberly, S. C., L. King, M. M. Dent, R. I. Jones, and C. E. Gibson. 2002. Nutrient limitation of phytoplankton and periphyton growth in upland lakes. Freshwater Biology 47:2136-2152.

MacIntyre, H. L. and J. J. Cullen. 1995. Fine-scale vertical resolution of chlorophyll and photosynthetic parameters in shallow-water benthos. Marine Ecology Progress Series 122:227-237.

MacIntyre, H. L., R. J. Geider, and D. C. Miller. 1996. Microphytobenthos: the ecological role of the "secret garden" of unvegetated, shallow-water marine habitats. I. Distribution, abundance and primary production. Estuaries 19:186-201.

Madsen, J. D., P. A. Chambers, W. F. James, E. W. Koch, and D. F. Westlake. 2001. The interaction between water movement, sediment dynamics and submersed macrophytes. Hydrobiologia 444:71-84.

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

Martin, M. and C. W. Hickey. 2004. Determination of HSNO ecotoxic thresholds for granular Phoslock (Eureka Formulation) Phase 1: acute toxicity. NIWA Client Report. Report Number: HAM2004-137. National Institute of Water & Atmospheric Research, Hamilton.

Matisoff, G., J. Berton Fisher, and S. Matis. 1985. Effects of benthic macroinvertebrates on the exchange of solutes between sediments and freshwater. Hydrobiologia 122:19-33.

May, L., A. E. Bailey-Watts, and A. Kirika. 1993. The ecology of *Synchaeta kitina* Rousselet in Loch Leven, Scotland. Hydrobiologia 255/256:305-315.

May, L. and L. Carvalho. 2010. Maximum growing depth of macrophytes in Loch Leven, Scotland, United Kingdom, in relation to historical changes in estimated phosphorus loading. Hydrobiologia 646:123-131.

May, L., I. Gunn, and A. Kirika. 2001. Phosphorus study at Loch Flemington (Report to Scottish Natural Heritage; Contract no.: HT/LE08/00/01/35) Centre for Ecology and Hydrology, Edinburgh.

May, L. and B. M. Spears. 2012. Managing ecosystem services at Loch Leven, Scotland, UK: actions, impacts and unintended consequences. Hydrobiologia 681:117-130.

May, L., B. M. Spears, B. Dudley, and T. W. Hatton-Ellis. 2010. The importance of nitrogen limitation in the restoration of Llangorse Lake, Wales, UK. Journal of Environmental Monitoring 12:338-346.

Mayer-Pinto, M., A. J. Underwood, T. Tolhurst, and R. A. Coleman. 2010. Effects of metals on aquatic assemblages: What do we really know? Journal of Experimental Marine Biology and Ecology 391:1-9.

Mazumder, A., W. D. Taylor, D. J. McQueen, and D. R. S. Lean. 1990. Effects of fish and plankton on lake temperature and mixing depth. Science 247:312-315.

McDonald, I. and K. S. Viljoen. 2006. Platinum-group element geochemistry of mantle eclogites: a reconnaissance study of xenoliths from the Orapa kimberlite, Botswana. Applied Earth Science : IMM Transactions section B 115:81-93.

McQueen, D. J., J. R. Post, and E. L. Mills. 1986. Trophic relationships in pelagic freshwater ecosystems. Canadian Journal of Fisheries and Aquatic Sciences 43:1571-1581.

MEA. 2005. Ecosystems and human well-being: Wetlands and water. Synthesis. Millennium Ecosystem Assessment. World Resources Institute. Washington, DC.

Meerhoff, M., J. M. Clemente, F. T. De Mello, C. Iglesias, A. R. Pedersen, and E. Jeppesen. 2007. Can warm climate-related structure of littoral predator assemblies weaken the clear water state in shallow lakes? Global Change Biology 13:1888-1897.

Mehner, T., J. Benndorf, P. Kasprzak, and R. Koschel. 2002. Biomanipulation of lake ecosystems: successful applications and expanding complexity in the underlying science. Freshwater Biology 47:2453-2465.

Mehner, T., M. Diekmann, T. Gonsiorczyk, P. Kasprzak, R. Koschel, L. Krienitz, M. Rumpf, M. Schulz, and G. Wauer. 2008. Rapid recovery from eutrophication of a stratified lake by disruption of internal nutrient load. Ecosystems 11:1142-1156.

Meijer, M.-L., M. W. de Haan, A. W. Breukelaar, and H. Buiteveld. 1990. Is reduction of the benthivorous fish an important cause of high transparency following biomanipulation in shallow lakes? Hydrobiologia 200/201:303-315.

Meis, S., B. M. Spears, S. C. Maberly, M. B. O'Malley, and R. G. Perkins. 2012. Sediment amendment with Phoslock® in Clatto Reservoir (Dundee, UK): Investigating changes in sediment elemental composition and phosphorus fractionation. Journal of Environmental Management 93:185-193.

Meysman, F. J. R., J. J. Middelburg, and C. H. R. Heip. 2006. Bioturbation: a fresh look at Darwin's last idea. Trends in Ecology & Evolution 21:688-695.

Mooij, W. M., S. Hülsmann, L. N. De Serpont Domis, B. A. Nolet, P. L. E. Bodelier, P. C. M. Boers, L. M. D. Pires, H. J. Gons, B. W. Ibelings, R. Noordhuis, R. Portielje, K. Wolfstein, and E. H. R. R. Lammens. 2005. The impact of climate change on lakes in the Netherlands: a review. Aquatic Ecology 39:381-400.

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

Moss, B. 1980. Ecology of freshwaters. Blackwell Scientific Publications, Oxford.

Moss, B. 1990. Engineering and biological approaches to the restoration from eutrophication of shallow lakes in which aquatic plant communities are important components. Hydrobiologia 200/201:367-377.

Moss, B., D. Hering, A. J. Green, A. Aidoud, E. Becares, M. Beklioglu, H. Bennion, D. Boix, S. Brucet, L. Carvalho, B. Clement, T. Davidson, S. Declerck, M. Dobson, E. van Donk, B. Dudley, H. Feuchtmayr, N. Friberg, G. Grenouillet, H. Hillebrand, A. Hobaek, K. Irvine, E. Jeppesen, R. Johnson, I. Jones, M. Kernan, T. L. Lauridsen, M. Manca, M. Meerhoff, J. Olafsson, S. Ormerod, E. Papastergiadou, W. E. Penning, R. Ptacnik, X. Quintana, L. Sandin, M. Seferlis, G. Simpson, C. Triga, P. Verdonschot, A. M. Verschoor, and G. A. Weyhenmeyer. 2009. Climate change and the future of freshwater biodiversity in Europe: A primer for policy-makers. Freshwater Reviews 2:103-130.

Moss, B., J. Madgwick, and G. Phillips. 1996. A guide to the restoration of nutrient-enriched shallow lakes. Broads Authority, Norwich.

Murphy, J. and J. P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica Chemica Acta 27:31-36.

Murray-Bligh, J. and A. Ferguson. 1999. Procedures for collecting and analysing macroinvertebrate samples. Environment Agency Report, 2nd Edition BT001. Environment Agency, UK.

Nagy, N. M. and J. Kónya. 2010. Interfacial chemistry of rocks and soils. CRC Press - Taylor & Francis Group, Boca Raton.

Nalepa, T., D. J. Hartson, D. L. Fanslow, G. A. Lang, and S. J. Lozano. 1998. Declines in benthic macroinvertebrate populations in southern Lake Michigan, 1980–1993. Canadian Journal of Fisheries and Aquatic Sciences 55:2402-2413.

Nichols, S. A. and B. H. Shaw. 1986. Ecological life histories of the three aquatic nuisance plants, *Myriophyllum spicatum*, *Potamogeton crispus* and *Elodea canadensis*. Hydrobiologia 131:3-21.

Nõges, P., U. Mischke, R. Laugaste, and A. G. Solimini. 2010. Analysis of changes over 44 years in the phytoplankton of Lake Võrtsjärv (Estonia): the effect of nutrients, climate and the investigator on phytoplankton-based water quality indices. Hydrobiologia 646:33-48.

Özkundakci, D., D. P. Hamilton, and P. Scholes. 2010. Effect of intensive catchment and in-lake restoration procedures on phosphorus concentrations in a eutrophic lake. Ecological Engineering 36:396-405.

Paerl, H. W. 2009. Controlling eutrophication along the freshwater–marine continuum: Dual nutrient (N and P) reductions are essential. Estuaries and Coasts 32:593-601.

Paerl, H. W. and J. Huisman. 2008. Blooms like it hot. Science 320:57-58.

Paluden, C. and H. S. Jensen. 1995. Sequential extraction of P in freshwater wetland and lake sediment: Significance of humic acids. Wetlands 15:365-373.

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

Pathiratne, A. and A. Weerasundara. 2004. Bioassessment of selected inland water bodies in Sri Lanka using benthic oligochaetes with consideration of temporal variations. International Review of Hydrobiology 89:305-316.

Perkins, R. G., J. C. Kromkamp, J. Serôdio, J. Lavaud, B. Jesus, J. L. Mouget, S. Lefebvre, and R. M. Forster. 2010a. The application of variable chlorophyll fluorescence to microphytobenthic biofilms. Pages 237-276 *in* D. J. Suggett, M. A. Borowitzka, and O. Prášil, editors. Chlorophyll *a* fluorescence in aquatic sciences: Methods and applications. Springer, London.

Perkins, R. G., J. Lavaud, J. Serôdio, J. L. Mouget, P. Cartaxana, P. Rosa, L. Barille, V. Brotas, and B. M. Jesus. 2010b. Vertical cell movement is a primary response of intertidal benthic biofilms to increasing light dose. Marine Ecology Progress Series 416:93-103.

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

Perkins, R. G. and G. J. C. Underwood. 2000. Gradients of chlorophyll *a* and water chemistry along an eutrophic reservoir with determination of the limiting nutrient by *in situ* nutrient addtition. Water Research 34:713-724.

Perkins, R. G. and 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. and G. J. C. Underwood. 2002. Partial recovery of a eutrophic reservoir through managed phosphorus limitation and unmanaged macrophyte growth. Hydrobiologia 481:75-87.

Perrow, M. P., M.-L. Meijer, P. Dawidowicz, and H. Coops. 1997. Biomanipulation in shallow lakes: state of the art. Hydrobiologia 342/343:355-363.

Pettersson, K. 1998. Mechanisms for internal loading of phosphorus in lakes. Hydrobiologia 373/374:21-25.

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

Phillips, G., A. Kelly, J. A. Pitt, R. Sanderson, and 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.

Phillips, G., O.-P. Pietiläinen, L. Carvalho, A. Solimini, A. Lyche Solheim, and A. C. Cardoso. 2008. Chlorophyll–nutrient relationships of different lake types using a large European dataset. Aquatic Ecology 42:213-226.

Phillips, G. L. 2005. Eutrophication of shallow temperate lakes. Pages 261-278 *in* P. E. O'Sullivan and C. S. Reynolds, editors. The Lakes Handbook - Lake Restoration and Rehabilitation. Blackwell Publishing, Oxford.

Phoslock. 2012. Case studies on De Kuil (Netherlands) and Loch Flemington (UK). Phoslock Water Solutions Limited. [online: http://www.phoslock.com.au/case-studies.php; accessed 13th of May 2012]

Postel, S. and S. R. Carpenter. 1997. Freshwater ecosystem services. Pages 195-214 *in* G. Daily, editor. Nature's services. Island Press, Washington, D.C.

Poulíčková, A., P. Hašler, M. Lysáková, and B. M. Spears. 2008. The ecology of freshwater epipelic algae: an update. Phycologia 47:437-450.

Power, M. E., D. Tilman, J. A. Estes, B. A. Menge, W. J. Bond, S. Mills, G. Daily, J. C. Castilla, J. Lubchenco, and R. T. Paine. 1996. Challenges in the quest for keystones. BioScience 46:609-620.

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

Psenner, R., R. Pucsko, and M. Sager. 1984. Die Fraktionierung organischer und anorganischer Phosphorverbindungen von Sedimenten - Versuch einer Definition ökologisch wichtiger Fraktionen. Archiv für Hydrobiologie 1:111-155.

Reitzel, K., J. Hansen, F. Ø. Andersen, K. S. Hansen, and H. S. Jensen. 2005. Lake restoration by dosing aluminum relative to mobile phosphorus in the sediment. Environmental Science & Technology 39:4134-4140.

Reynolds, C. S. 1984. Photosynthetic activity of phytoplankton. Pages 123-156 *in* C. S. Reynolds, editor. The ecology of freshwater phytoplankton. Cambridge University Press, Cambridge.

Reynolds, C. S. 2006. Ecology of phytoplankton. Cambridge University Press, Cambridge.

Rip, W. J. 2007. Cyclic state shifts in a restored shallow lake. Wageningen University.

Robb, M. S., B. Greenop, Z. Goss, G. Douglas, and J. Adeney. 2003. Application of Phoslock^{TM}, an innovative phosphorus binding clay, to two Western Australian waterways: preliminary findings. Hydrobiologia 494:237-243.

Ross, G., F. Haghseresht, and T. E. Cloete. 2008. The effect of pH and anoxia on the performance of Phoslock$^{(R)}$, a phosphorus binding clay. Harmful Algae 7:545-550.

Sand-Jensen, K. and J. Borum. 1991. Interactions among phytoplankton, periphyton, and macrophytes in temperate freshwaters and estuaries. Aquatic Botany 41:137-175.

Sand-Jensen, K., C. Prahl, and H. Stokholm. 1982. Oxygen release from roots of submerged aquatic macrophytes. Oikos 38:349-354.

Sas, H. 1989. Lake restoration by reduction of nutrient loading: Expectations, experiences, extrapolations. Academia Verlag Richarz GmbH, St. Augustin.

Schauser, I., J. Lewandowski, and M. Hupfer. 2003. Decision support for the selection of an appropriate in-lake measure to influence the phosphorus retention in sediments. Water Research 37:801-812.

Scheffer, M. 1998. Ecology of shallow lakes. Chapman & Hall, London.

Scheffer, M., J. Bascompte, W. A. Brock, V. Brovkin, S. R. Carpenter, V. Dakos, H. Held, E. H. Van Nes, M. Rietkerk, and G. Sugihara. 2009. Early-warning signals for critical transitions. Nature 461:53-59.

Scheffer, M., W. Brock, and F. Westley. 2000. Socioeconomic mechanisms preventing optimum use of ecosystem services: An interdisciplinary theoretical analysis. Ecosystems 3:451-471.

Scheffer, M., S. Carpenter, J. A. Foley, C. Folke, and B. Walker. 2001. Catastrophic shifts in ecosystems. Nature 413:591-596.

Scheffer, M. and S. R. Carpenter. 2003. Catastrophic regime shifts in ecosystems: linking theory to observation. Trends in Ecology & Evolution 18:648-656.

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

Scheffer, M. and E. Jeppesen. 1998. Alternative stable states. Pages 397–406 *in* E. Jeppesen, M. Søndergaard, M. Søndergaard, and K. Christoffersen, editors. The structuring role of submerged macrophytes in lakes. Springer, New York.

Scheffer, M., G. J. Van Geest, K. Zimmer, E. Jeppesen, M. Søndergaard, M. G. Butler, M. A. Hanson, S. Declerck, and L. De Meester. 2006. Small habitat size and isolation can promote species richness: second-order effects on biodiversity in shallow lakes and ponds. Oikos 112:227–231.

Scheffer, M. and E. H. Van Nes. 2007. Shallow lakes theory revisited: various alternative regimes driven by climate, nutrients, depth and lake size. Hydrobiologia 584:455-466.

Schindler, D. E., S. R. Carpenter, K. L. Cottingham, X. He, J. R. Hodgson, J. F. Kitchell, and P. A. Soranno. 1996. Food web structure and littoral coupling to pelagic trophic cascades. Pages 96-108 *in* G. A. Polis and K. O. Winemiller, editors. Food webs: integration of pattern and dynamics. Chapman & Hall, New York.

Schindler, D. W. 1977. Evolution of phosphorus limitation in lakes. Science 195:260-262.

Schindler, D. W. 1978. Factors regulating phytoplankton production and standing crop in the world's freshwaters. Limnology & Oceanography 23:478-486.

Schindler, D. W. 1998. Whole-ecosystem experiments: Replication versus realism: The need for ecosystem-scale experiments. Ecosystems 1:323-334.

Schindler, D. W. 2006. Recent advances in the understanding and management of eutrophication. Limnology & Oceanography 51:356-363.

Schindler, D. W., R. E. Hecky, D. L. Findlay, M. P. Stainton, B. R. Parker, M. J. Paterson, K. G. Beaty, M. Lyng, and S. E. M. Kasian. 2008. Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of a 37-year whole-ecosystem experiment. Proceedings of the National Academy of Sciences of the United States of America 105:11254-11258.

Schutten, J., J. A. Velden, and H. Smith. 1994. Submerged macrophytes in the recently freshened lake system Volkerak-Zoom (The Netherlands), 1987–1991. Hydrobiologia 275/276:207-218.

Scourfield, D. J. and J. P. Harding. 1966. British freshwater cladocera. Freshwater Biological Association, Ambleside.

Seitzinger, S. P. 1991. The effect of pH on the release of phosphorus from Potomac estuary sediments: Implications for blue-green algal blooms. Estuarine, Coastal and Shelf Science 33:409-418.

Serôdio, J., M. Da Silva, and F. Catarino. 1997. Nondestructive tracing of migratory rhythms of intertidal benthic microalgae using *in vivo* chlorophyll *a* fluorescence. Journal of Phycology 33:542-553.

Skov, C. and P. A. Nilsson. 2007. Evaluating stocking of YOY pike *Esox lucius* as a tool in the restoration of shallow lakes. Freshwater Biology 52:1834-1845.

Smith, V. H., S. B. Joye, and R. W. Howarth. 2006. Eutrophication of freshwater and marine ecosystems. Limnology & Oceanography 51:351-355.

Smith, V. H. and D. W. Schindler. 2009. Eutrophication science: where do we go from here? Trends in Ecology & Evolution 24:201-207.

Smith, V. H., G. D. Tilman, and J. C. Nekola. 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environmental Pollution 100:179-196.

Smolders, A. J. P., L. P. M. Lamers, E. C. H. E. T. Lucassen, G. Van Der Velde, and J. G. M. Roelofs. 2006. Internal eutrophication: How it works and what to do about it – a review. Chemistry and Ecology 22:93-111.

Sneller, F. E. C., D. F. Kalf, L. Weltje, and v. A. P. Wezel. 2000. Maximum permissible concentrations and negligible concentrations for rare earth elements (REEs). RIVM (National Institute of Public Health and the Environment), Netherlands.

SNH. 2004. Site Condition Monitoring report for Loch Flemington. Scottish Natural Heritage, Inverness.

SNH. 2010. Site Condition Monitoring report for Loch Flemington. Scottish Natural Heritage, Inverness.

Søndergaard, M. 1988. Seasonal variations in the loosely sorbed phosphorus fraction of the sediment of a shallow and hypereutrophic lake. Environmental Geology 11:115-121.

Søndergaard, M. 1990. Porewater dynamics in the sediment of a shallow and hypertrophic lake. Hydrobiologia 192:247-258.

Søndergaard, M. 2007. Nutrient dynamics in lakes – with emphasis on phosphorus, sediment and lake restorations. University of Aarhus, National Environmental Research Institute.

Søndergaard, M., R. Bjerring, and E. Jeppesen. 2012. Persistent internal phosphorus loading during summer in shallow eutrophic lakes. Hydrobiologia 408/409:145-152.

Søndergaard, M., J. P. Jensen, and E. Jeppesen. 1999. Internal phosphorus loading in shallow Danish lakes. Hydrobiologia 408/409:145-152.

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

Søndergaard, M., J. P. Jensen, and E. Jeppesen. 2003. Role of sediment and internal loading of phosphorus in shallow lakes. Hydrobiologia 506/509:133-145.

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

Søndergaard, M., E. Jeppesen, J. P. Jensen, and T. Lauridsen. 2000. Lake restoration in Denmark. Lake & Reservoirs: Research and Management 5:151-159.

Søndergaard, M., E. Jeppesen, T. Lauridsen, C. Skov, E. H. Van Nes, R. Roijackers, E. Lammens, and R. Portielje. 2007. Lake restoration: successes, failures and long-term effects. Journal of Applied Ecology 44:1095-1105.

Søndergaard, M., J. Windolf, and E. Jeppesen. 1996. Phosphorus fractions and profiles in the sediment of shallow Danish lakes as related to phosphorus load, sediment composition and lake chemistry. Water Research 30:992-1102.

Sonke, J. E. and V. J. M. Salters. 2006. Lanthanide–humic substances complexation. I. Experimental evidence for a lanthanide contraction effect. Geochimica et Cosmochimica Acta 70:1495-1506.

Spears, B. M., L. Carvalho, and D. M. Paterson. 2007a. Phosphorus partitioning in a shallow lake: implications for water quality management. Water and Environment Journal 21:47-53.

Spears, B. M., L. Carvalho, R. Perkins, A. Kirika, and D. M. Paterson. 2012. Long-term variation and regulation of internal phosphorus loading in Loch Leven. Hydrobiologia 681:23-33.

Spears, B. M., L. Carvalho, R. Perkins, M. B. O'Malley, and D. M. Paterson. 2010. The contribution of epipelon to total sediment microalgae in a shallow temperate eutrophic loch (Loch Leven, Scotland). Hydrobiologia 646:281-293.

Spears, B. M., L. Carvalho, R. Perkins, and D. M. Paterson. 2008a. Effects of light on sediment nutrient flux and water column nutrient stoichiometry in a shallow lake. Water Research 42:977-986.

Spears, B. M., L. Carvalho, R. G. Perkins, A. Kirika, and D. M. Paterson. 2006. 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. G. Perkins, A. Kirika, and D. M. Paterson. 2007b. Sediment phosphorus cycling in a large shallow lake: spatio-temporal variation in phosphorus pools and release. Hydrobiologia 584:37-48.

Spears, B. M., J. Funnell, J. Saunders, and D. M. Paterson. 2007c. On the boundaries: sediment stability measurements across aquatic ecosystems. Pages 68-79 *in* B. Westrich and U. Föstner, editors. Sediment Dynamics and Pollutant Mobility in Rivers: An Interdisciplinary Approach. Springer, Berlin.

Spears, B. M., I. D. M. Gunn, S. Meis, and L. May. 2011a. Deliverable D6.4-2: Analysis of cause-effect-recovery chains for lakes recovering from eutrophication. European Commission within the Seventh Framework Programme. Project code - WISER 226273, Edinburgh.

Spears, B. M. and I. D. Jones. 2010. The long-term (1979–2005) effects of the North Atlantic Oscillation on wind-induced wave mixing in Loch Leven (Scotland). Hydrobiologia 646:49-59.

Spears, B. M., J. E. Saunders, I. Davidson, and D. M. Paterson. 2008b. Microalgal sediment biostabilisation along a salinity gradient in the Eden Estuary, Scotland: unravelling a paradox. Marine and Freshwater Research 59:313-321.

Spry, D. J. and J. G. Wiener. 1991. Metal bioavailability and toxicity to fish in low-alkalinity lakes: A critical review. Environmental Pollution 71:243-304.

Stauber, J. L. 2000. Toxicity testing of modified clay leachates using freshwater organisms. Report No: ET/IR267R. CSIRO, Australia.

Stephen, D., B. Moss, and G. Phillips. 1997. Do rooted macrophytes increase sediment phosphorus release? Hydrobiologia 342/343:27-34.

Stephen, D., B. Moss, and G. Phillips. 1998. The relative importance of top-down and bottom-up control of phytoplankton in a shallow macrophyte dominated lake. Freshwater Biology 39:699-713.

Stevenson, R. J. 1996. An introduction to algal ecology in freshwater benthic habitats. Pages 3-30 *in* R. J. Stevenson, M. L. Bothwell, and R. L. Lowe, editors. Algal ecology: Freshwater and benthic ecosystems. Academic Press, London.

Stumm, W. and J. J. Morgan. 1996. Aquatic Chemistry - Chemical equilibria and rates in natural waters. 3rd edition. Wiley-Interscience, New York.

Suttle, C. A. and P. J. Harrison. 1988. Ammonium and phosphate uptake rates, N:P supply ratios, and evidence for N and P limitation in some oligotrophic lakes. Limnology and Oceanography 33:186-202.

Tang, J. and K. H. Johannesson. 2003. Speciation of rare earth elements in natural terrestrial waters: assessing the role of dissolved organic matter from the modeling approach. Geochimica et Cosmochimica Acta 67:2321-2339.

Tang, J. and K. H. Johannesson. 2010. Ligand extraction of rare earth elements from aquifer sediments: Implications for rare earth element complexation with organic matter in natural waters. Geochimica et Cosmochimica Acta 74:6690-6705.

Tarvainen, M., A.-M. Ventelä, H. Helminen, and J. Sarvala. 2005. Nutrient release and resuspension generated by ruffe (*Gymnocephalus cernuus*) and chironomids. Freshwater Biology 50:447-458.

Trenberth, K. W. 1983. What are the Seasons? Bulletin of the American Meteorological Society 64:1276-1277.

Vadeboncoeur, Y., E. Jeppesen, M. J. V. Zanden, H.-H. Schierup, K. Christoffersen, and D. M. Lodge. 2003. From Greenland to green lakes: Cultural eutrophication and the loss of benthic pathways in lakes. Limnology & Oceanography 48:1408-1418.

van Donk, E., R. D. Gulati, A. Iedema, and J. T. Meulemans. 1993. Macrophyte-related shifts in the nitrogen and phosphorus contents of the different trophic levels in a biomanipulated shallow lake. Hydrobiologia 251:19-26.

van Luijn, F., D. T. Van der Molen, W. J. Luttmer, and 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.

van Oosterhout, F. and M. Lürling. 2011. Effects of the novel '*Flock & Lock*' lake restoration technique on *Daphnia* in Lake Rauwbraken (The Netherlands). Journal of Plankton Research 33:255-263.

van Oosterhout, F. and M. Lürling. 2012. The effect of phosphorus binding clay (Phoslock®) in mitigating cyanobacterial nuisance: a laboratory study on the effects on water quality variables and plankton. Hydrobiologia: Online First.

van Wichelen, J., S. Declerck, K. Muylaert, I. Hoste, V. Geenens, J. Vandekerkhove, E. Michels, N. Pauw, M. Hoffmann, L. Meester, and W. Vyverman. 2007. The importance of drawdown and sediment removal for the restoration of the eutrophied shallow lake Kraenepoel (Belgium). Hydrobiologia 584:291-303.

Vanni, M. J. 2002. Nutrient cycling by animals in freshwater ecosystems. Annual Review of Ecology and Systematics 33:341-370.

Vaughn, C. C. and C. C. Hakenkamp. 2008. The functional role of burrowing bivalves in freshwater ecosystems. Freshwater Biology 46:1431-1446.

Veraart, A. J., E. J. Faassen, V. Dakos, E. H. Van Nes, M. Lürling, and M. Scheffer. 2011. Recovery rates reflect distance to a tipping point in a living system. Nature 481:357-359.

Verspagen, J. M. H., P. M. Visser, and J. Huisman. 2006. Aggregation with clay causes sedimentation of the buoyant cyanobacteria *Microcystis* spp.. Aquatic Microbial Ecology 44:165-174.

Vicente, D. I., P. Huang, F. Ø. Andersen, and H. S. Jensen. 2008. Phosphate adsorption by fresh and aged aluminum hydroxide. Consequences for lake restoration. Environmental Science & Technology 42:6650-6655.

Vitousek, P. M., H. A. Mooney, J. Lubchenco, and J. M. Melillo. 1997. Human domination of earth's ecosystems. Science 277:494-499.

Vollenweider, R. A. 1968. Scientific fundamental 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.

Vollenweider, R. A. and J. Kerekes. 1980. The loading concept as basis for controlling eutrophication philosophy and preliminary results of the OECD program on eutrophication. Progress in Water Technology 12:5-38.

Vopel, K., M. Gibbs, C. W. Hickey, and J. Quinn. 2008. Modification of sediment-water solute exchange by sediment-capping materials: effects on O_2 and pH. Marine and Freshwater Research 59:1101-1110.

Wagenhoff, A., C. R. Townsend, and C. D. Matthaei. 2012. Macroinvertebrate responses along broad stressor gradients of deposited fine sediment and dissolved nutrients: a stream mesocosm experiment. Journal of Applied Ecology 49:892-902.

Walker, B. and J. A. Meyers. 2004. Thresholds in ecological and social-ecological systems: a developing database. Ecology and Society 9:3-19.

Wallace, J. B. and J. R. Webster. 1996. The role of macroinvertebrates in stream ecosystem function. Annual Review of Entomology 41:115-139.

Way, C. M. 1989. Dynamics of filter-feeding in *Musculium transversum* (Bivalvia:Sphaeriidae). Journal of the North American Benthological Society 8:243-249.

Welch, E. B. and G. D. Cooke. 1999. Effectiveness and longevity of phosphorus inactivation with alum. Lake and Reservoir Management 15:5-27.

Wetzel, R. G. 2001. Limnology. Lake and river ecosystems. 3rd edition. Academic Press, San Diego.

WHO. 2003. Guidelines for safe recreational water environments. World Health Organization, Geneva.

Wiederholm, T. 1980. Use of benthos in lake monitoring. Water Pollution Control Federation 52:537-547.

Williams, P., M. Whitfield, J. Biggs, S. Bray, G. Fox, P. Nicolet, and D. Sear. 2004. Comparative biodiversity of rivers, streams, ditches and ponds in an agricultural landscape in Southern England. Biological Conservation 115:329-341.

Winder, M. and D. E. Schindler. 2004. Climate change uncouples trophic interactions in an aquatic ecosystem. Ecology 85:2100-2106.

Winfield, I. J., J. M. Fletcher, and J. B. James. 2008. The Arctic charr (*Salvelinus alpinus*) populations of Windermere, UK: population trends associated with eutrophication, climate change and increased abundance of roach (*Rutilus rutilus*). Environmental Biology of Fishes 83:25-35.

WISER. 2012. Annex 1: WISER Guidance on phytoplankton counting. Pages 15-31 Deliverable D3.1-4: Guidance document on sampling, analysis and counting standards for phytoplankton in lakes. European Commission within the Seventh Framework Programme.

Wood, P. J. and P. D. Armitage. 1997. Biological effects of fine sediment in the lotic environment. Environmental Management 21:203-217.

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

Worm, B., H. K. Lotze, H. Hillebrand, and U. Sommer. 2002. Consumer versus resource control of species diversity and ecosystem functioning. Nature 417:848-859.

Yallop, M. L., D. M. Paterson, and 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.

Yuan, G. and L. Wu. 2007. Allophane nanoclay for the removal of phosphorus in water and wastewater. Science and Technology of Advanced Materials 8:60-62.

Zhang, Y., A. Ghadouani, E. E. Prepas, B. Pinel-Alloul, S. Reedyk, P. A. Chambers, R. D. Robarts, G. Méthot, A. Raik, and M. Holst. 2001. Response of plankton communities to whole-lake $Ca(OH)_2$ and $CaCO_3$ additions in eutrophic hardwater lakes. Freshwater Biology 46:1105-1119.

Appendix

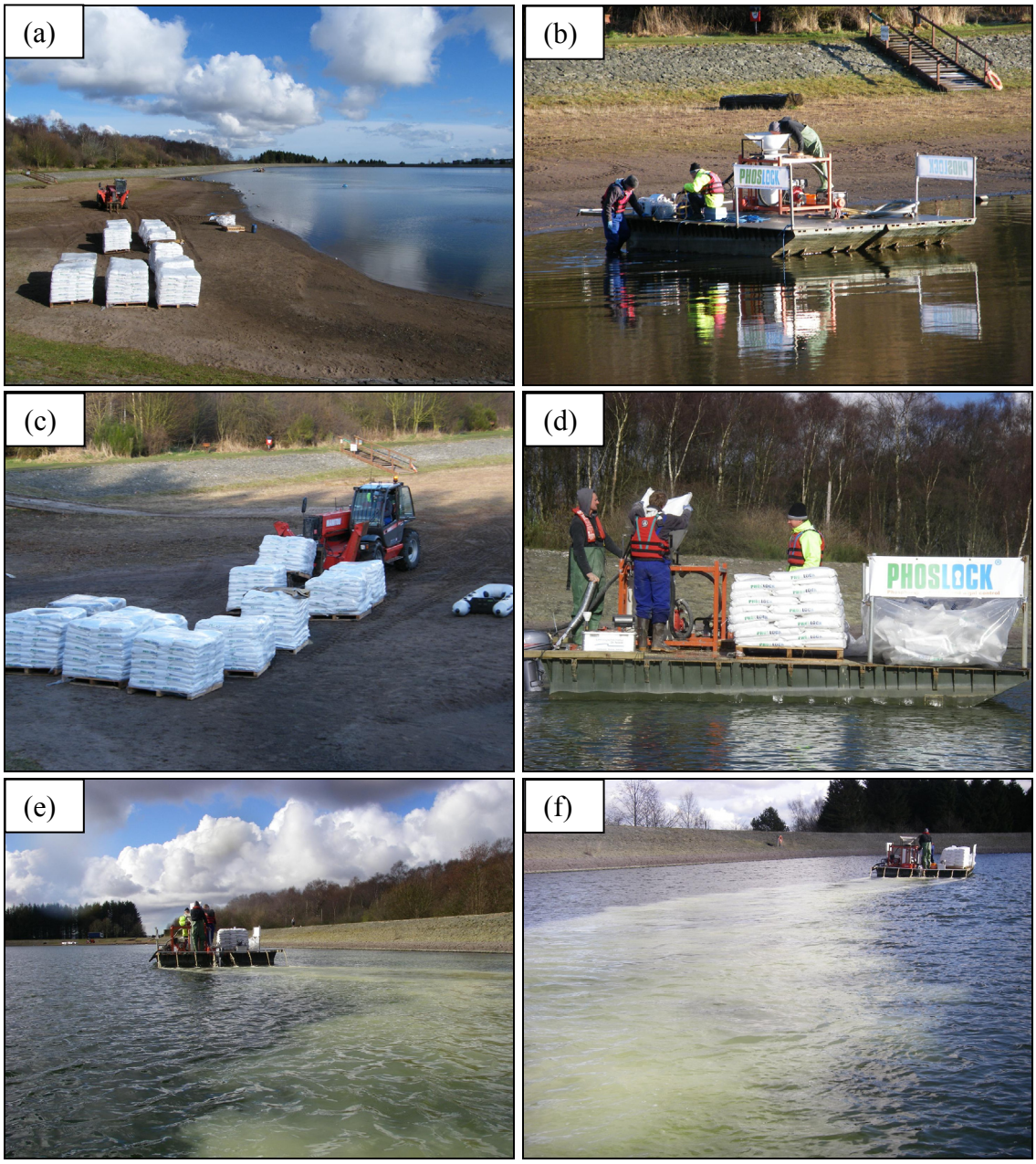
Chapter 2 – Material and methods

Appendix: Table A2.1 Species list of macrophytes recorded in Loch Flemington during the Site Condition Monitoring conducted on behalf of Scottish Natural Heritage (SNH, 2004, 2010).

Habitat	Species	2004	2010
Open water (floating/submerged)	*Apium inundatum*		X
	Chara virgata		
	Crassula helmsii		X
	Elodea canadensis	X	X
	Hydrocotyle vulgaris		
	Lemna minor		X
	Littorella uniflora	X	X
	Menyanthes trifoliata	X	X
	Myriophyllum alterniflorum		X
	Myriophyllum spicatum	X	
	Persicaria amphibia	X	X
	Potamogeton gramineus		X
	Potamogeton natans	X	X
	Potamogeton obtusifolius	X	X
Riparian zone (emergent)	*Caltha palustris*		X
	Carex rostrata		X
	Carex sp.	X	X
	Eleocharis palustris	X	X
	Epilobium sp.		X
	Equisetum fluviatile	X	X
	Juncus articulatus		X
	Juncus conglomeratus		X
	Juncus sp.	X	
	Mentha aquatica		X
	Myosotis sp.	X	X
	Phalaris arundinacea	X	X
	Potentilla anserina		X
	Potentilla palustris		X
	Ranunculus flammula		X
	Sparganium erectum	X	X

Appendix

Chapter 3 – A Phoslock® pilot study in an artificial water-body (Clatto Reservoir, UK): physicochemical responses and effects on cyanobacteria



Appendix: Figure A3.1 Photographs showing (a) telescopic loader and pallets of Phoslock® at the shore of Clatto Reservoir, (b) assembly of pontoon used for the application of Phoslock®, (c) telescopic loader transporting Phoslock® pallet from the shore onto the pontoon, (d) pontoon used for the application of Phoslock®, (e) rear view of pontoon with spray manifold used to apply Phoslock®/water slurry to the water surface, and (f) Phoslock® slurry shortly after application.

Appendix

Chapter 5 - Assessing physicochemical changes in the bed sediments of a shallow lake following the application of Phoslock® II: Loch Flemington, UK

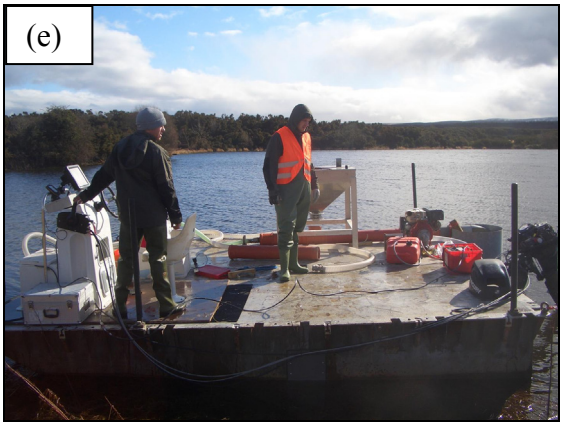
(a)

(b)

(c)

(d)

(e)

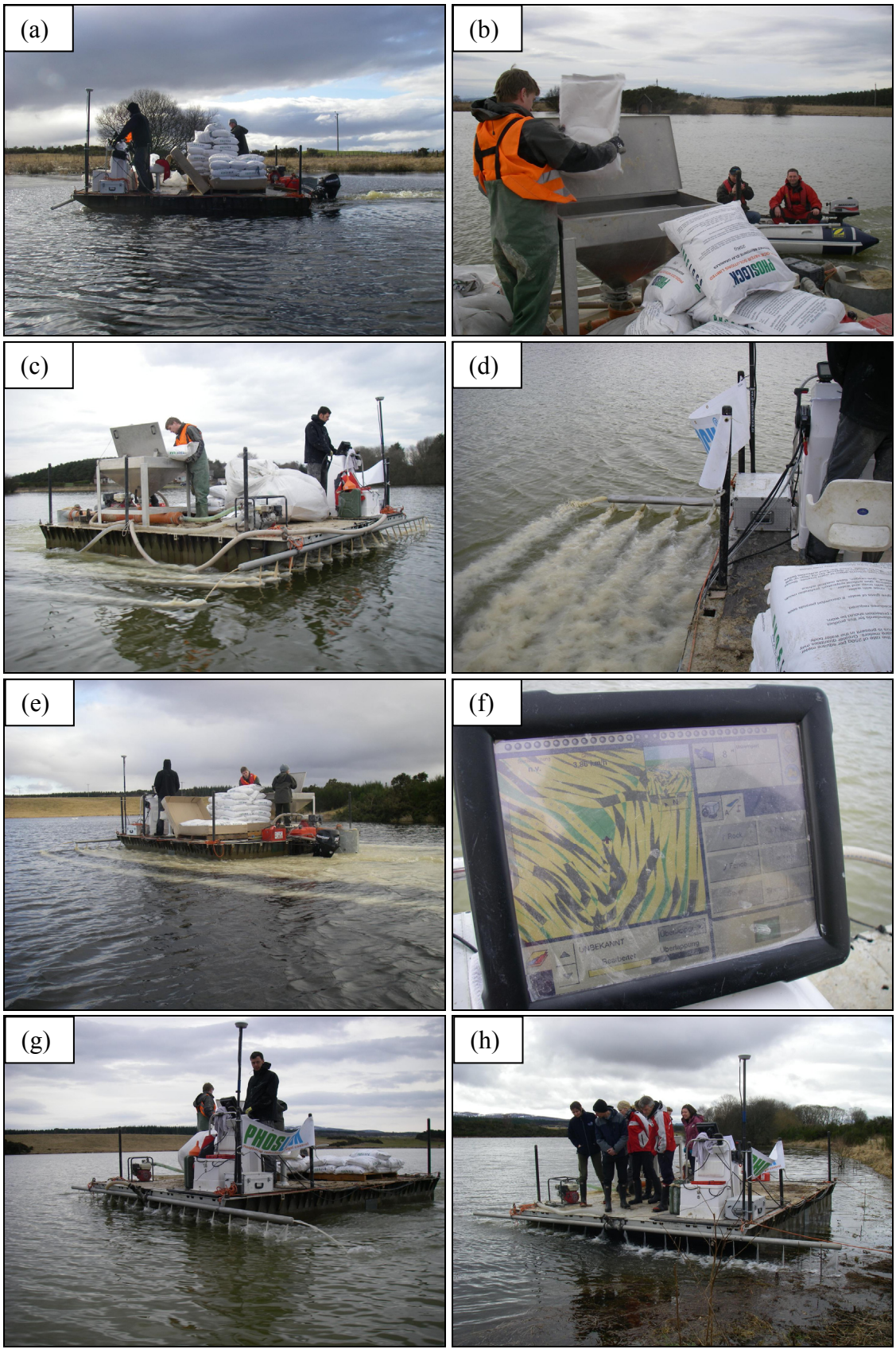


(f)

(g)

(h)

Appendix: Figure A5.1 (p. 322) Photographs showing delivery and assembly of pontoon used for the application of Phoslock® (a-e), delivery and unloading of Phoslock® pallets from lorry using a telescopic loader (f, g) and pallets of Phoslock® at the shore of Loch Flemington (h).


(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)

Appendix: Figure A5.2 (p. 324) Photographs showing (a) pontoon with Phoslock® pallets, (b, c) mixing chamber for production of Phoslock®/water slurry, (d, e) spray manifold mounted at the front of the pontoon for application of the Phoslock®/water slurry to the water surface, (f) GPS log of transects followed during application of Phoslock® to facilitate even coverage of the sediment bed, and (g, h) presentation of Phoslock® application procedure and pontoon to members of the Scottish Environment Protection Agency (SEPA).

Appendix: Table A5.1 Assessment of variation in mean (n = 5) sediment calcium (Ca) and lanthanum (La) content between months (July 2009, October 2009, July 2010, October 2010) by a non-parametric Kruskal-Wallis (KW) test and a non-parametric Mann-Whitney U-test (MWU). A non-parametric MWU test was conducted where significant variation between months was evident (KW test, $\alpha < 0.05$). In cases where KW test $\alpha \geq 0.05$ this was marked with 'n/a' (not applicable). Significant differences are marked (*, $p < 0.05$; **, $p < 0.01$), while n.s. denotes $p \geq 0.05$. DF = degrees of freedom.

Element	Depth	KW				MWU					
		H-value	p-value	DF =	n =	Jul. 09 vs. Oct. 09		Oct. 09 vs. Jul. 10		Jul. 10 vs. Oct. 10	
	(cm)					W-value	p-value	W-value	p-value	W-value	p-value
Ca	0-2	4.94	n.s.	3	5	n/a	n/a	n/a	n/a	n/a	n/a
	2-4	6.01	n.s.	3	5	n/a	n/a	n/a	n/a	n/a	n/a
	4-6	9.97	*	3	5	19	n.s.	25	n.s.	39	*
	6-8	11.89	**	3	5	18	n.s.	29	n.s.	40	*
	8-10	5.17	n.s.	3	5	n/a	n/a	n/a	n/a	n/a	n/a
La	0-2	15.80	**	3	5	34	n.s.	15	*	37	n.s.
	2-4	15.89	**	3	5	38	*	15	*	33	n.s.
	4-6	14.50	**	3	5	31	n.s.	15	*	30	n.s.
	6-8	14.50	**	3	5	31	n.s.	15	*	25	n.s.
	8-10	14.66	**	3	5	33	n.s.	15	*	26	n.s.

Appendix: Table A5.2 Results of non-parametric Kruskal-Wallis test assessing variation in the mass of phosphorus (P) in different sediment P-fractions between months (July 2009, October 2009, July 2010, October 2010, March 2011). Mass of P in a given sediment P-fraction is based on mass balance estimates over the top 4 cm or the top 10 cm of the sediment. Significant differences are marked (*, $p < 0.05$; **, $p < 0.01$), while n.s. denotes $p \geq 0.05$. DF = degrees of freedom.

Sediment	Fraction	H-value	p-value	DF =	n =
Top 4 cm	'*Labile P*'	16.67	**	4	5
	'*Reductant-soluble P*'	14.44	**	4	5
	'*Organic P*'	9.54	*	4	5
	'*Metal oxide adorbed P*'	4.68	n.s.	4	5
	'*Apatite bound P*'	15.74	**	4	5
	'*Residual P*'	9.86	*	4	5
	'P_{mobile}'	11.35	*	4	5
Top 10 cm	'*Labile P*'	15.07	**	4	5
	'*Reductant-soluble P*'	14.87	**	4	5
	'*Organic P*'	9.98	*	4	5
	'*Metal oxide adorbed P*'	12.30	*	4	5
	'*Apatite bound P*'	15.15	**	4	5
	'*Residual P*'	14.63	**	4	5
	'P_{mobile}'	15.21	**	4	5

Appendix: Table A5.3 Results of non-parametric Mann-Whitney U-test (MWU) investigating variation in the mass of phosphorus (P) in different sediment P-fractions between months (July 2009, October 2009, July 2010, October 2010, March 2011). Mass of P in a given sediment P-fraction is based on mass balance estimates over the top 4 cm or the top 10 cm of the sediment. Fractions include: (1) '*Labile P*'; (2) '*Reductant-soluble P*'; (3) '*Organic P*'; (4) '*Metal oxide adorbed P*'; (5) '*Apatite bound P*'; (6) '*Residual P*'; and (7) 'P_{mobile}'. A non-parametric MWU test was conducted where significant variation between treatments was evident (KW test, $\alpha < 0.05$). In cases where KW test $\alpha > 0.05$ this was marked with 'n/a' (not applicable).

Depth (cm)	Fraction	Jul. 09 vs. Oct. 09		Jul. 09 vs. Jul. 10		Jul. 09 vs. Oct. 10		Jul. 09 vs. Mar. 11		Oct. 09 vs. Jul. 10		Oct. 09 vs. Oct. 10		Oct. 09 vs. Mar. 11		Jul. 10 vs. Oct. 10		Jul. 10 vs. Mar. 11		Oct. 10 vs. Mar. 11	
		W	p	W	p	W	p	W	p	W	p	W	p	W	p	W	p	W	p	W	p
Top 4	1	20	n.s.	20	n.s.	37	n.s.	17	*	22	n.s.	40	*	16	*	40	*	21	n.s.	15	*
	2	23	n.s.	38	*	38	*	36	n.s.	40	*	40	*	39	*	28	n.s.	24	n.s.	23	n.s.
	3	15	*	22	n.s.	23	n.s.	20	n.s.	35	n.s.	39	*	30	n.s.	30	n.s.	23	n.s.	21	n.s.
	4	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	5	26	n.s.	19	n.s.	16	*	15	*	21	n.s.	15	*	15	*	22	n.s.	19	n.s.	19	n.s.
	6	15	*	21	n.s.	21	n.s.	20	n.s.	36	n.s.	38	*	30	n.s.	29	n.s.	23	n.s.	21	n.s.
	7	17	*	30	n.s.	34	n.s.	25	n.s.	39	*	40	*	37	n.s.	30	n.s.	23	n.s.	20	n.s.
Top 10	1	21	n.s.	19	n.s.	35	n.s.	16	*	26	n.s.	40	*	22	n.s.	40	*	21	n.s.	15	*
	2	30	n.s.	38	*	38	*	37	n.s.	40	*	40	*	40	*	26	n.s.	23	n.s.	22	n.s.
	3	15	*	24	n.s.	25	n.s.	21	n.s.	39	*	40	*	30	n.s.	30	n.s.	23	n.s.	23	n.s.
	4	38	*	38	*	37	n.s.	26	n.s.	29	n.s.	25	n.s.	17	*	20	n.s.	17	*	18	n.s.
	5	24	n.s.	24	n.s.	17	*	15	*	28	n.s.	19	n.s.	15	*	20	n.s.	15	*	16	*
	6	15	*	36	n.s.	34	n.s.	21	n.s.	40	*	40	*	35	n.s.	22	n.s.	18	n.s.	21	n.s.
	7	16	*	36	n.s.	36	n.s.	27	n.s.	40	*	40	*	37	n.s.	33	n.s.	20	n.s.	17	*

n.s., $p \geq 0.05$; *, $p < 0.05$; W, W-value of MWU test; p, p-value of MWU test

Appendix: Table A5.4 Results of non-parametric Kruskal-Wallis test (KW) and Mann-Whitney U-test (MWU) investigating variation in water-column dissolved oxygen (DO) concentrations, pH and conductivity between treatments including aerobic control (A), aerobic dosed (B), anaerobic control (C) and anaerobic dosed (D). A non-parametric MWU test was conducted where significant variation between treatments was evident (KW test, $\alpha < 0.05$). In cases where KW test $\alpha > 0.05$ this was marked with 'n/a' (not applicable). Significant differences are marked (*, $p < 0.05$; **, $p < 0.01$), while n.s. denotes $p \geq 0.05$. DF, degrees of freedom; W, W-value of MWU test; p, p-value of MWU test.

Parameter	Day	KW				MWU											
		H-value	p-value	DF =	n =	A vs. B		A vs. C		A vs. D		B vs. C		B vs. D		C vs. D	
						W	p	W	p	W	p	W	p	W	p	W	p
DO	0	4.90	n.s.	3	5	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	5	14.43	**	3	5	27	n.s.	40	*	40	*	40	*	40	*	31	n.s.
	8	15.33	**	3	5	34.5	n.s.	40	*	40	*	40	*	40	*	34	n.s.
	15	15.14	**	3	5	36	n.s.	40	*	40	*	40	*	40	*	26	n.s.
	22	16.30	**	3	5	15	*	40	*	40	*	40	*	40	*	32	n.s.
	29	14.38	**	3	5	29	n.s.	40	*	40	*	40	*	40	*	30	n.s.
pH	0	13.83	**	3	5	18.5	n.s.	15.5	*	15	*	19.5	n.s.	16	*	19	n.s.
	5	15.73	**	3	5	37	n.s.	15	*	15	*	15	*	15	*	21.5	n.s.
	8	14.81	**	3	5	29.5	n.s.	15	*	15	*	15	*	15	*	21	n.s.
	15	15.50	**	3	5	21	n.s.	15	*	15	*	15	*	15	*	35.5	n.s.
	22	16.36	**	3	5	15	*	15	*	15	*	15	*	15	*	32.5	n.s.
	29	14.47	**	3	5	23.5	n.s.	15	*	15	*	15	*	15	*	27	n.s.
Conductivity	0	1.10	n.s.	3	5	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	5	11.22	*	3	5	19	n.s.	35	n.s.	32	n.s.	40	*	40	*	20.5	n.s.
	8	10.98	*	3	5	19	n.s.	34	n.s.	35	n.s.	40	*	40	*	29	n.s.
	15	9.40	*	3	5	19	n.s.	31	n.s.	31	n.s.	40	*	40	*	29.5	n.s.
	22	11.43	*	3	5	15	*	23.5	n.s.	25.5	n.s.	40	*	40	*	32.5	n.s.
	29	10.76	*	3	5	15	*	23.5	n.s.	25	n.s.	39	*	40	*	30.5	n.s.

Appendix: Table A5.5 Variation in mean (n = 5) water-column total phosphorus (TP) and soluble reactive phosphorus (SRP) concentrations between day 0 and all other days of the experimental period as indicated by non-parametric Mann-Whitney U-test. Treatments included aerobic control (A), aerobic dosed (B), anaerobic control (C) and anaerobic dosed (D). Significant differences are marked (*, $p < 0.05$), while n.s. denotes $p \geq 0.05$.

Variable	Treatment	Day		5	8	12	15	19	22	26	29
TP	A	0	p-value	n.s.	*	n.s.	n.s.	n.s.	n.s.	*	n.s.
			W-value	37	38	35	36	29	37	38	29
	B	0	p-value	n.s.	n.s.	n.s.	n.s.	n.s.	*	*	*
			W-value	32	35	34	35	28	40	40	40
	C	0	p-value	*	*	*	*	*	*	*	*
			W-value	17	15	15	15	15	15	15	15
	D	0	p-value	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	*
			W-value	28	32	36	37	30	35	38	39
SRP	A	0	p-value	n.s.	n.s.	n.s.	*	*	*	*	*
			W-value	18	19	18	17	16	15	15	15
	B	0	p-value	n.s.	n.s.	n.s.	*	*	*	*	*
			W-value	19	19	18	17	16	15	15	15
	C	0	p-value	*	*	*	*	*	*	*	*
			W-value	15	15	15	15	15	15	15	15
	D	0	p-value	n.s.	n.s.	n.s.	n.s.	n.s.	*	*	*
			W-value	32	21	22	29	24.5	15	15	15

Appendix: Table A5.6 Results of non-parametric Kruskal-Wallis test (KW) and Mann-Whitney U-test (MWU) investigating variation in mean (n = 5) water-column total phosphorus (TP) and soluble reactive phosphorus (SRP) concentrations between treatments including aerobic control (A), aerobic dosed (B), anaerobic control (C) and anaerobic dosed (D). A non-parametric MWU test was conducted where significant variation between treatments was evident (KW test, $\alpha < 0.05$). In cases where KW test $\alpha > 0.05$ this was marked with 'n/a' (not applicable). Significant differences are marked (*, $p < 0.05$; **, $p < 0.01$), while n.s. denotes $p \geq 0.05$. DF, degrees of freedom; W, W-value of MWU test; p, p-value of MWU test.

Parameter	Day	KW				MWU											
		H-value	p-value	DF =	n =	A vs. B		A vs. C		A vs. D		B vs. C		B vs. D		C vs. D	
						W	p	W	p	W	p	W	p	W	p	W	p
TP	0	5.42	n.s.	3	5	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	5	12.12	**	3	5	31	n.s.	15	*	21	n.s.	15	*	19	n.s.	38	*
	8	14.15	**	3	5	27	n.s.	15	*	19	n.s.	15	*	16	*	40	*
	12	10.73	*	3	5	28	n.s.	15	*	27	n.s.	15	*	27	n.s.	40	*
	15	10.95	*	3	5	29	n.s.	15	*	27	n.s.	15	*	24	n.s.	40	*
	19	10.86	*	3	5	29	n.s.	15	*	25	n.s.	15	*	26	n.s.	40	*
	22	12.44	**	3	5	34	n.s.	15	*	26	n.s.	15	*	20	n.s.	40	*
	26	12.30	**	3	5	32	n.s.	15	*	26	n.s.	15	*	19	n.s.	40	*
	29	16.13	**	3	5	40	*	15	*	33.5	n.s.	15	*	16	*	40	*
SRP	0	5.15	n.s.	3	5	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	5	15.17	**	3	5	28	n.s.	11	*	30	*	15	*	37	n.s.	40	*
	8	13.86	**	3	5	35	n.s.	16	*	37	n.s.	15	*	36	n.s.	40	*
	12	13.88	**	3	5	37	n.s.	15	*	37	n.s.	15	*	30	n.s.	40	*
	15	16.06	**	3	5	40	*	15	*	39	*	15	*	34	n.s.	40	*
	19	15.42	**	3	5	33	n.s.	15	*	40	*	15	*	37	n.s.	40	*
	22	16.07	**	3	5	38	*	15	*	40	*	15	*	35	n.s.	40	*
	26	11.96	**	3	5	33	n.s.	15	*	34	n.s.	15	*	26	n.s.	40	*
	29	11.27	*	3	5	31	n.s.	15	*	31	n.s.	15	*	31	n.s.	40	*

Appendix

Chapter 6 - Seasonal responses of physicochemical and biological variables to reduced sediment phosphorus release following the application of Phoslock®

Appendix: Table A6.1 Number of samples of different variables per season in Loch Flemington between 2009 and 2011.

Parameter	Unit	2009				2010				2011			
		Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
air temperature	°C	3	3	3	3	3	3	3	3	3	3	3	3
precipitation	mm	3	3	3	3	3	3	3	3	3	3	3	3
pH[s]		5[I]	15	15	4	15	15	15	10	11	11	9	0
conductivity[s]	µS cm^{-1}	5[I]	15	15	4	15	15	15	10	11	11	9	0
temperature[s]	°C	5[I]	15	15	4	15	15	15	10	11	11	9	0
DO[s]	%	5[I]	15	15	4	15	15	15	10	5[I]	5[I]	0	0
water clarity	m	2[I]	15	6	2	5	11	7	3	10	10	5	0
TLa[s]	µg L^{-1}	3[I]	9	9	4	9	9	9	6	3[I]	0	0	0
La[s]	µg L^{-1}	3[I]	9	9	4	9	9	9	6	3[I]	0	0	0
TLa[b]	µg L^{-1}	1[I]	5	5	4	5	5	5	4	1[I]	0	0	0
La[b]	µg L^{-1}	1[I]	5	5	4	5	5	5	4	1[I]	0	0	0
TP[s]	µg L^{-1}	5[I]	15	15	4	15	15	15	12	11	11	9	0
SRP[s]	µg L^{-1}	5[I]	15	15	4	15	15	15	12	11	11	9	0
TP[b]	µg L^{-1}	5[I]	15	15	4	15	15	15	10	5[I]	0	0	0
SRP[b]	µg L^{-1}	5[I]	15	15	4	15	15	15	10	5[I]	0	0	0
DIN[s]	µg L^{-1}	5[I]	13	15	4	15	15	15	10	5[I]	0	0	0
TDC[s]	mg L^{-1}	5[I]	15	15	4	15	15	15	10	5[I]	5[I]	0	0
DIC[s]	mg L^{-1}	5[I]	15	15	4	15	15	15	10	5[I]	5[I]	0	0
DOC[s]	mg L^{-1}	5[I]	15	15	4	15	15	15	10	5[I]	5[I]	0	0
$TSiO_2$[s]	mg L^{-1}	5[I]	15	15	4	15	15	15	10	5[I]	0	0	0
SiO_2[s]	mg L^{-1}	5[I]	15	15	4	15	15	15	10	5[I]	0	0	0
chlorophyll *a*[s]	µg L^{-1}	5[I]	15	15	4	15	15	15	9	11	11	9	0
phytoplankton[s]	μm^{-3} mL^{-1}	1[I]	3	3	2	3	3	3	2	3	3	3	0
zooplankton[s]	ind. L^{-1}	1[I]	3	3	2	3	3	3	2	1[I]	0	0	0
benthic invert.	ind. m^{-3}	5[I]	14	15	4	15	15	15	10	5[I]	0	0	0
MCD	m	0	10	15	0	15	15	15	10	5[I]	5[I]	0	0

[s], surface water sample; [b], bottom water sample; [I], data of less than two months of a season available; DO, dissolved oxygen; TLa, total lanthanum; La, soluble lanthanum; TP, total phosphorus; SRP; soluble reactive phosphorus; DIN, dissolved inorganic nitrogen; TDC, total dissolved carbon; DOC, dissolved organic carbon; $TSiO_2$, total diatom silica; SiO_2, soluble silica; benthic invert., benthic macroinvertebrates; MCD, maximum colonisation depth of macrophytes; ind., individuals

Appendix: Table A6.2 Overview of data distribution (Anderson-Darling test), equal variance (Levene's test) and statistical test used for individual data sets, including non-parametric Kruskal-Wallis test (KW), non-parametric Mann-Whitney U-test (MWU), one-way analysis of variance (ANOVA) and 2-Sample t-test (t-test).

Variable	Normal distribution ($\alpha > 0.05$)	Equal variance ($\alpha > 0.05$)	KW	MWU	ANOVA	t-test
air temperature	yes	yes	-	-	yes	-
precipitation	yes	yes	-	-	yes	-
pH[s]	no	n/a	yes	yes	-	-
conductivity[s]	no	n/a	yes	yes	-	-
temperature[s]	no	n/a	yes	yes	-	-
DO[s]	no	n/a	-	yes	-	-
water clarity	no	n/a	yes	yes	-	-
TLa[s]	no	n/a	-	yes	-	-
La[s]	no	n/a	-	yes	-	-
TLa[b]	no	n/a	-	yes	-	-
La[b]	no	n/a	-	yes	-	-
TP[s]	no	n/a	yes	yes	-	-
SRP[s]	no	n/a	yes	yes	-	-
TP[b]	no	n/a	-	yes	-	-
SRP[b]	no	n/a	-	yes	-	-
DIN[s]	no	n/a	-	yes	-	-
TDC[s]	no	n/a	-	yes	-	-
DIC[s]	no	n/a	-	yes	-	-
DOC[s]	no	n/a	-	yes	-	-
$TSiO_2$[s]	no	n/a	-	yes	-	-
SiO_2[s]	no	n/a	-	yes	-	-
chlorophyll *a*[s]	no	n/a	yes	yes	-	-
phytoplankton[s]	yes	yes	-	-	yes	yes
zooplankton[s]	no	n/a	-	yes	-	-
benthic invert.	no	n/a	-	yes	-	-
MCD	no	n/a	-	yes	-	-
fish	no	n/a	yes	yes	-	-

[s], surface water sample; [b], bottom water sample; n/a, not applicable as not normally distributed even after transformation; DO, dissolved oxygen; TLa, total lanthanum; La, soluble lanthanum; TP, total phosphorus; SRP, soluble reactive phosphorus; DIN, dissolved inorganic nitrogen; TDC, total dissolved carbon; DOC, dissolved organic carbon; $TSiO_2$, total diatom silica; SiO_2, soluble silica; benthic invert., benthic macroinvertebrates; MCD, maximum colonisation depth of macrophytes

Appendix: A6.3 Statistical analysis

Weather data

Monthly means of air temperature and precipitation were calculated for four periods (i.e. 1999-2008, 2009, 2010 and 2011) in order to produce comparable populations. This resulted in a sample size of n = 3 per season. Data sets were normally distributed (Anderson-Darling test, $\alpha > 0.05$) and of equal variance (Levene's test, $\alpha > 0.05$), so a one-way analysis of variance (ANOVA) with Tukey's *post hoc* test was used to test for significant variation in a given parameter for each season between the four periods.

Physical parameter

Variation in surface water pH, conductivity, dissolved oxygen (DO) concentration, temperature and water clarity was assessed for each season between years (2009, 2010, 2011). This resulted in the following comparisons for all parameters except DO concentrations: i) spring (2010 vs. 2011); ii) summer (2009 vs. 2010 vs. 2011); iii) autumn (2009 vs. 2010 vs. 2011); and iv) winter (2009 vs. 2010). Variation in DO concentrations for each season between years was assessed by the following comparisons: i) summer (2009 vs. 2010); ii) autumn (2009 vs. 2010); iii) and winter (2009 vs. 2010). Sample sizes for each season are given (Appendix: Table A6.1). Data sets were not normally distributed (Anderson-Darling test, $\alpha < 0.05$) even after a range of transformations (including $x' = \log(x)$, $x' = \ln(x)$, $x' = \text{sqrt}(x)$, $x' = x^2$). Therefore, a non-parametric Kruskal Wallis test (KW) was used to test for significant variation in a given variable (including pH, conductivity, temperature and water clarity) between years for summer and autumn. Where significant variation was evident (KW test, $\alpha < 0.05$), a non-parametric Mann-Whitney U-test (MWU) test was used to determine between which years significant differences in a given variable for a given season

occurred. A non-parametric MWU test was also used to determine between which years significant differences in a given variable (pH, conductivity temperature and water clarity) for a given season (spring, winter) occurred.

A non-parametric MWU test was used to determine between which years significant differences in DO concentrations in summer, autumn and winter occurred, in cases where data was available and presumptions were fulfilled.

Chemical parameter

Variation in surface water total lanthanum (TLa), soluble lanthanum (La), dissolved inorganic nitrogen (DIN), total dissolved carbon (TDC), dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), total diatom silica ($TSiO_2$), soluble silica (SiO_2) and bottom water TLa, La, total phosphorus (TP) and soluble reactive phosphorus (SRP) concentration was assessed for each season between years (2009, 2010, 2011). This resulted in the following comparisons: i) summer (2009 vs. 2010); ii) autumn (2009 vs. 2010); and iii) winter (2009 vs. 2010). Sample sizes for each variable in each season are presented (Appendix: Table A6.1). Data sets were not normally distributed (Anderson-Darling test, $\alpha < 0.05$) even after a range of transformations (including $x' = \log(x)$, $x' = \ln(x)$, $x' = \text{sqrt}(x)$, $x' = x^2$), therefore a non-parametric MWU test was used to determine between which years significant differences in a given variable for a given season occurred, in cases where data was available and presumptions were fulfilled. Variation in surface water TP and SRP concentrations was assessed for each season between years (2009, 2010, 2011). This resulted in the following comparisons: i) spring (2010 vs. 2011); ii) summer (2009 vs. 2010 vs. 2011); iii) autumn (2009 vs. 2010 vs. 2011); and iv) winter (2009 vs. 2010). Sample sizes for each season are presented (Appendix: Table A6.1). Data sets were not normally distributed (Anderson-Darling test, $\alpha < 0.05$) even after a range of transformations

(including $x' = \log(x)$, $x' = \ln(x)$, $x' = \text{sqrt}(x)$, $x' = x^2$). A non-parametric KW test was used to test for significant variation in surface water TP and SRP for summer and autumn between years (2009, 2010, 2011). Where significant variation was evident (KW test, $\alpha < 0.05$), a non-parametric MWU test was used to determine between which years significant differences in a given variable for a given season (summer, autumn) occurred.

A non-parametric MWU was also used to test for significant variation in surface water TP and SRP concentrations in spring and winter between years (winter: 2009, 2010; spring: 2010, 2011).

Biological parameters

Phytoplankton

Variation in phytoplankton biovolume was assessed for each season between years (2009, 2010, 2011). This resulted in the following comparisons: i) spring (2010 vs. 2011); ii) summer (2009 vs. 2010 vs. 2011); and iii) autumn (2009 vs. 2010 vs. 2011). Sample sizes for each season are presented (Appendix: Table A6.1). Data sets were normally distributed (Anderson-Darling test, $\alpha > 0.05$) and of equal variance (Levene's test, $\alpha > 0.05$) following transformation ($x' = \ln(x+1)$). A one way ANOVA with Tukey's *post hoc* test was used to test for significant variation in phytoplankton biovolume for summer and autumn between years (2009, 2010, 2011).

A 2-Sample t-test was used to test for significant variation phytoplankton biovolume in spring between years (2010, 2011), while presumptions were not fulfilled to test variation in phytoplankton biovolume in winter between years.

Chlorophyll a concentration

Variation in chlorophyll *a* concentrations was assessed for each season between years (2009, 2010, 2011). This resulted in the following comparisons: i) spring (2010 vs. 2011); ii) summer (2009 vs. 2010 vs. 2011); iii) autumn (2009 vs. 2010 vs. 2011); and iv) winter (2009 vs. 2010). Sample sizes for each season are presented (Appendix: Table A6.1). Data sets were not normally distributed (Anderson-Darling test, $\alpha < 0.05$) even after a range of transformations (including $x' = \log(x)$, $x' = \ln(x)$, $x' = \text{sqrt}(x)$, $x' = x^2$). A non-parametric KW test was used to test for significant variation in chlorophyll *a* concentrations for summer and autumn between years (2009, 2010, 2011). Where significant variation was evident (KW test, $\alpha < 0.05$), a non-parametric MWU test was used to determine between which years significant differences in chlorophyll *a* concentrations for a given season (summer, autumn) occurred.

A non-parametric MWU test was also used to test for significant variation in surface water chlorophyll *a* concentrations in spring and winter between years.

Maximum colonisation depth

Variation in maximum colonisation depth (MCD) of macrophytes was assessed for each season between years (2009, 2010). This resulted in the following comparisons: i) summer (2009 vs. 2010); and ii) autumn (2009 vs. 2010). Sample sizes for each season are presented (Appendix: Table A6.1). Data sets were not normally distributed (Anderson-Darling test, $\alpha < 0.05$) even after a range of transformations (including $x' = \log(x)$, $x' = \ln(x)$, $x' = \text{sqrt}(x)$, $x' = x^2$). A non-parametric MWU test was used to test for significant variation in MCD between years for summer and autumn, where data was available and presumptions were fulfilled.

Appendix

Zooplankton

Variation in zooplankton abundance was assessed for each season between years (2009, 2010). This resulted in the following comparisons: i) summer (2009 vs. 2010); and ii) autumn (2009 vs. 2010). Sample sizes for each season are presented (Appendix: Table A6.1). Data sets were not normally distributed (Anderson-Darling test, $\alpha < 0.05$) even after a range of transformations (including $x' = \log(x)$, $x' = \ln(x)$, $x' = \text{sqrt}(x)$, $x' = x^2$). A non-parametric MWU test was used to test for significant variation in zooplankton abundance between years for summer and autumn, where data was available and presumptions were fulfilled.

Benthic macroinvertebrates

Variation in benthic macroinvertebrate abundance was assessed for each season between years (2009, 2010). This resulted in the following comparisons: i) summer (2009 vs. 2010); ii) autumn (2009 vs. 2010); and iii) winter (2009 vs. 2010). Sample sizes for each season are presented (Appendix: Table A6.1). Data sets were not normally distributed (Anderson-Darling test, $\alpha < 0.05$) even after a range of transformations (including $x' = \log(x)$, $x' = \ln(x)$, $x' = \text{sqrt}(x)$, $x' = x^2$). A non-parametric MWU test was used to test for significant variation in benthic macroinvertebrate abundance between years for summer, autumn and winter.

Fish

Variation in fish abundance (sampled with traps) was assessed between years (2003, 2004, 2005, 2006, 2010). Sample size equalled n = 6 for 2003-2006 and n = 5 for 2010. Data sets were not normally distributed (Anderson-Darling test, $\alpha < 0.05$) even after a range of transformations (including $x' = \log(x)$, $x' = \ln(x)$, $x' = \text{sqrt}(x)$, $x' = x^2$). A non-parametric KW test was used to test for significant variation in fish abundance

between years (2003, 2004, 2005, 2006, 2010). Where significant variation was evident (KW test, $\alpha < 0.05$), a non-parametric MWU test was used to determine between which years significant differences in fish abundance occurred.

Appendix: Table A6.4 Results of non-parametric Mann-Whitney U-test (MWU) investigating variation in benthic macroinvertebrate abundance in a given season between years.

Group	Season	n_{09}=	n_{10}=	n_{11}=	MWU					
					2009 vs. 2010		2009 vs. 2011		2010 vs. 2011	
					W	p	W	p	W	p
Chaoboridae	spring	5[I]	15	5[I]	▪	▪	▪	▪	▪	▪
	summer	14	15	0	232.5	n.s.	▪	▪	▪	▪
	autumn	15	15	0	259	n.s.	▪	▪	▪	▪
	winter	4	10	0	26	n.s.	▪	▪	▪	▪
Chironomidae	spring	5[I]	15	5[I]	▪	▪	▪	▪	▪	▪
	summer	14	15	0	287.5	***	▪	▪	▪	▪
	autumn	15	15	0	330.5	***	▪	▪	▪	▪
	winter	4	10	0	38	n.s.	▪	▪	▪	▪
Oligochaeta	spring	5[I]	15	5[I]	▪	▪	▪	▪	▪	▪
	summer	14	15	0	273.5	**	▪	▪	▪	▪
	autumn	15	15	0	292	*	▪	▪	▪	▪
	winter	4	10	0	29	n.s.	▪	▪	▪	▪
Sphaeriidae	spring	5[I]	15	5[I]	▪	▪	▪	▪	▪	▪
	summer	14	15	0	260.5	*	▪	▪	▪	▪
	autumn	15	15	0	303.5	**	▪	▪	▪	▪
	winter	4	10	0	31	n.s.	▪	▪	▪	▪
Hydrobiidae	spring	5[I]	15	5[I]	▪	▪	▪	▪	▪	▪
	summer	14	15	0	247.5	n.s.	▪	▪	▪	▪
	autumn	15	15	0	255	n.s.	▪	▪	▪	▪
	winter	4	10	0	33	n.s.	▪	▪	▪	▪

Appendix: Table A6.4 continued

Group	Season	n_{09}=	n_{10}=	n_{11}=	MWU					
					2009 vs. 2010		2009 vs. 2011		2010 vs. 2011	
					W	p	W	p	W	p
Bithyniidae	spring	5 [I]	15	5 [I]	▪	▪	▪	▪	▪	▪
	summer	14	15	0	211	n.s.	▪	▪	▪	▪
	autumn	15	15	0	197	n.s.	▪	▪	▪	▪
	winter	4	10	0	30.5	n.s.	▪	▪	▪	▪
Trichoptera	spring	5 [I]	15	5 [I]	▪	▪	▪	▪	▪	▪
	summer	14	15	0	204	n.s.	▪	▪	▪	▪
	autumn	15	15	0	178.5	*	▪	▪	▪	▪
	winter	4	10	0	25	n.s.	▪	▪	▪	▪
Nematoda	spring	5 [I]	15	5 [I]	▪	▪	▪	▪	▪	▪
	summer	14	15	0	217.5	▪	▪	▪	▪	▪
	autumn	15	15	0	255	▪	▪	▪	▪	▪
	winter	4	10	0	35	▪	▪	▪	▪	▪

n_{09}, sample size in 2009; n_{10}, sample size in 2010; n_{11}, sample size in 2011; W, W-value of MWU test; p, p-value of MWU test; [I], season consists of less than two out of three possible months; ▪, no test possible as season consists of less than two months, difference in number of observations between seasons ≥ 6 or sample size of season smaller than n = 3; n.s., $p \geq 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

Appendix

Chapter 7 - Effects of Phoslock® dose on sediment oxygen concentration and nutrient cycling across the sediment-water interface: results from intact sediment core experiments

Appendix: Table A7.1 Number of measurements and samples taken over the experimental period for control (C), low dose (L) and high dose (H).

Variable	Unit	Day 0			Day 1			Day 2			Day 3			Day 4			Day 5			Day 6			Day 7			Day 8		
		C	L	H	C	L	H	C	L	H	C	L	H	C	L	H	C	L	H	C	L	H	C	L	H	C	L	H
DO	mg L^{-1}	5	5	5	5	5	5	5	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	4	5
pH		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
cond.	µS cm^{-1}	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
TP	µg L^{-1}	5	5	5	0	0	0	5	5	5	0	0	0	5	5	5	0	0	0	5	5	5	0	0	0	5	5	5
SRP	µg L^{-1}	5	5	5	0	0	0	5	5	5	0	0	0	4	5	5	0	0	0	4	4	4	0	0	0	5	5	5
NH_4-N	µg L^{-1}	5	5	5	0	0	0	0	0	0	0	0	0	5	5	5	0	0	0	5	5	5	0	0	0	5	5	5
NO_3-N	µg L^{-1}	5	5	5	0	0	0	0	0	0	0	0	0	5	5	5	0	0	0	5	5	5	0	0	0	5	5	5
DIN	µg L^{-1}	5	5	5	0	0	0	0	0	0	0	0	0	5	5	5	0	0	0	5	5	5	0	0	0	5	5	5
$TSiO_2$	mg L^{-1}	5	5	5	0	0	0	0	0	0	0	0	0	5	5	5	0	0	0	0	0	0	0	0	0	5	5	5
SiO_2	mg L^{-1}	5	5	5	0	0	0	0	0	0	0	0	0	5	5	5	0	0	0	0	0	0	0	0	0	5	5	5
TDC	µg L^{-1}	5	5	5	0	0	0	0	0	0	0	0	0	4	5	5	0	0	0	0	0	0	0	0	0	5	5	5
DIC	µg L^{-1}	5	5	5	0	0	0	0	0	0	0	0	0	4	5	5	0	0	0	0	0	0	0	0	0	5	5	5
DOC	µg L^{-1}	5	5	5	0	0	0	0	0	0	0	0	0	4	5	5	0	0	0	0	0	0	0	0	0	5	5	5

DO, dissolved oxygen; cond., conductivity; TP, total phosphorus; SRP, soluble reactive phosphorus; NH_4-N, ammonium; NO_3-N, nitrate; DIN, dissolved inorganic nitrogen; $TSiO_2$, total diatom silica; SiO_2, soluble silica; TDC, total dissolved carbon; DIC, dissolved inorganic carbon; DOC, dissolved organic carbon

Appendix: Table A7.2 Number of samples taken for analysis of sediment properties and pore-water nutrient concentrations for ambient cores (A), control (C), low dose (L) and high dose (H). Depth was measured starting from the sediment-water interface (ambient cores, control, low dose) and the Phoslock®-water interface (high dose).

Variable	Unit	Depth	Day 0	Day 8		
		(cm)	A	C	L	H
SPE	mTesla	0	-	5	5	5
chl *a*	µg g^{-1}	0-2	5	5	5	5
TP	µg L^{-1}	0-2	5	5	5	5
		2-4	5	5	5	5
		4-6	5	5	5	5
SRP	µg L^{-1}	0-2	5	5	5	5
		2-4	5	5	5	5
		4-6	5	5	5	5
NH_4-N	µg L^{-1}	0-2	3	5	5	5
		2-4	5	5	5	5
		4-6	5	5	5	5
NO_3-N	µg L^{-1}	0-2	4	5	5	5
		2-4	5	5	5	5
		4-6	5	5	5	5
DIN	µg L^{-1}	0-2	3	5	5	5
		2-4	5	5	5	5
		4-6	5	5	5	5

SPE, sediment particle entrainment; chl *a*, sediment chlorophyll *a* content; TP, total phosphorus; SRP, soluble reactive phosphorus; NH_4-N, ammonium; NO_3-N, nitrate; DIN, dissolved inorganic nitrogen

Appendix: Table A7.3 Results of non-parametric Kruskal-Wallis (KW) and Mann-Whitney U-test (MWU) investigating variation in water-column dissolved oxygen (DO; mg L^{-1}), pH and conductivity (cond.; µS cm^{-1}) between treatments including control (C), low dose (L) and high dose (H) for each day of the experiment.

Variable	Day	n_C=	n_L=	n_H=	KW			MWU					
					H-value	p-value	DF =	C vs. L		C vs. H		L vs. H	
								W-value	p-value	W-value	p-value	W-value	p-value
DO	0	5	5	5	0.72	n.s.	2	n/a	n/a	n/a	n/a	n/a	n/a
	1	5	5	5	1.69	n.s.	2	n/a	n/a	n/a	n/a	n/a	n/a
	2	5	4	5	2.28	n.s.	2	n/a	n/a	n/a	n/a	n/a	n/a
	3	5	5	5	9.50	**	2	25	n.s.	15	*	15	*
	4	5	5	5	0.07	n.s.	2	n/a	n/a	n/a	n/a	n/a	n/a
	5	5	5	5	7.61	*	2	19.5	n.s.	16	*	19.5	n.s.
	6	5	5	5	4.33	n.s.	2	n/a	n/a	n/a	n/a	n/a	n/a
	7	5	5	5	4.82	n.s.	2	n/a	n/a	n/a	n/a	n/a	n/a
	8	5	4	5	5.39	n.s.	2	n/a	n/a	n/a	n/a	n/a	n/a
pH	0	5	5	5	0.50	n.s.	2	n/a	n/a	n/a	n/a	n/a	n/a
	1	5	5	5	0.03	n.s.	2	n/a	n/a	n/a	n/a	n/a	n/a
	2	5	5	5	9.67	**	2	15	*	15.5	*	33	n.s.
	3	5	5	5	9.07	*	2	15	*	18	n.s.	35.5	n.s.
	4	5	5	5	7.33	*	2	19	n.s.	31.5	n.s.	40	*
	5	5	5	5	9.81	**	2	16.5	*	35.5	n.s.	40	*
	6	5	5	5	9.50	**	2	15	*	25	n.s.	40	*
	7	5	5	5	8.54	*	2	18	n.s.	34	n.s.	40	*
	8	5	5	5	8.96	*	2	24	n.s.	39	*	40	*

Appendix: Table A7.3 continued

Variable	Day	n_C=	n_L=	n_H=	KW			MWU					
					H-value	p-value	DF =	C vs. L		C vs. H		L vs. H	
								W-value	p-value	W-value	p-value	W-value	p-value
cond.	0	5	5	5	7.67	*	2	37	n.s.	40	*	30.5	n.s.
	1	5	5	5	0.76	n.s.	2	n/a	n/a	n/a	n/a	n/a	n/a
	2	5	5	5	11.18	**	2	37	n.s.	15	*	15	*
	3	5	5	5	11.38	**	2	37.5	*	15	*	15	*
	4	5	5	5	11.18	**	2	37	n.s.	15	*	15	*
	5	5	5	5	11.18	**	2	37	n.s.	15	*	15	*
	6	5	5	5	11.58	**	2	38	*	15	*	15	*
	7	5	5	5	12.02	**	2	39	*	15	*	15	*
	8	5	5	5	11.18	**	2	37	n.s.	15	*	15	*

DF, degrees of freedom; n.s., $p \geq 0.05$; *, $p < 0.05$; **, $p < 0.01$; n/a, MWU test not applicable as no significant variation indicated by KW test ($\alpha > 0.05$)

Appendix: Table A7.4 Results of non-parametric Kruskal-Wallis (KW) and Mann-Whitney U-test (MWU) investigating variation in pore-water nitrate (NO_3-N; µg L^{-1}) concentrations between treatments including ambient cores (A), control (C), low dose (L) and high dose (H). Depth was measured starting from the sediment-water interface (ambient cores, control, low dose) and the Phoslock®-water interface (high dose).

Depth (cm)	n_A=	n_C=	n_L=	n_H=	KW			MWU											
					H-value	p-value	DF =	A vs. C		A vs. L		A vs. H		C vs. L		C vs.H		L vs. H	
								W	p	W	p	W	p	W	p	W	p	W	p
0 - 2	4	5	5	5	11.09	*	3	27	n.s.	30	*	30	*	34	n.s.	38	*	31	n.s.
2 – 4	5	5	5	5	15.21	**	3	34	n.s.	40	*	40	*	39	*	40	*	36	n.s.
4 – 6	5	5	5	5	17.10	**	3	40	*	40	*	40	*	40	*	40	*	37	n.s.

DF, degrees of freedom; W, W-value of MWU test; p, p-value of MWU test; n.s., $p \geq 0.05$; *, $p < 0.05$; **, $p < 0.01$