Environment Agency

Evidence

Assessment of sediment phosphorus capping to control nutrient concentrations in English lakes

Project SC120064/R9

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Evidence at the Environment Agency

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Professor Doug Wilson
Director, Research, Analysis and Evaluation

Executive summary

The consequences of eutrophication in lakes are many, including the potential degradation of important ecosystem services such as the provision of food, the supply of clean water for drinking and industrial use, the support of tourism and recreation, and the maintenance of species and habitats of high conservation value.

Phosphorus (P) and nitrogen (N) are the dominant eutrophication pressures on lakes. At the centre of attempts to restore lakes suffering from eutrophication is the assumption that a reduction in P concentration will result in an improvement in lake ecology and the provision of ecosystem services. When P loading to a lake from its catchment is reduced significantly, lake recovery can be delayed for decades by the release of P that has accumulated in the lake sediments ('internal loading') over the period when catchment inputs of P were high. Effective control of internal loading can, therefore, greatly accelerate the ecological recovery process once external inputs have been reduced.

According to the 2013 classification results, which were the latest data available at the start of the project, 74% of lakes assessed in England and 34% of those in Wales required a reduction in total P concentration to meet the target of 'good ecological status' defined under the EU Water Framework Directive (WFD; Directive 2000/60/EC). However, knowledge of the best management practices available to control internal loading, and of the procedures needed to assess the efficacy of novel approaches at the site-specific scale, is incomplete. It is important to address these knowledge gaps to ensure timely ecological recovery of lakes from eutrophication to comply with the timescales imposed by the WFD (i.e. by 2021 or 2027).

This project was designed to meet this need through the development of the processes and understanding needed to underpin the use of P sorption materials that are being proposed for use as a geo-engineering tool to control internal releases of P in lakes, a technique known as 'P-capping'. In particular, the project aimed to test the efficacy of using a P-capping technique to force the recovery of two eutrophic lakes in England, ideally restoring them to 'good ecological status', as defined under the WFD. Results from these case studies and the wider literature are used to present considerations for future guidance on the use of this approach.

The overall aim of this project was to improve two eutrophic lakes in Cheshire, Hatchmere and Mere, ideally restoring them to 'good ecological status'. To control internal P loading, a P-capping treatment was applied in Year 2 of this four-year project.

In March 2013, Phoslock Europe GmbH applied 79.8 tonnes and 25.2 tonnes of the lanthanum-bentonite product known as Phoslock® to Mere Mere and Hatchmere, respectively. The Environment Agency and the Centre for Ecology & Hydrology (CEH) designed and implemented a monitoring programme to assess the chemical and ecological responses of these lakes to the Phoslock® application, primarily using WFD ecological status indicators.

This report summarises the project evidence drawing on previous unpublished project reports and peer-reviewed publications to document the chemical and ecological responses following sediment treatment. By providing insights into the future use of this approach and of its limitations, the report can be used to inform the development of general guidance on its wider use.

The main conclusion from the project is that the applications of Phoslock® used were insufficient to control sediment P release due confounding factors such as persistent catchment P loading and competition between dissolved organic carbon (DOC) and P for lanthanum. Hence, the desired responses in water quality and ecological structure were not achieved. However, for both lakes, the results showed a reduction in water column total P (TP) concentrations for up to two years after the date of application. Furthermore, reductions in bottom water orthophosphate-P concentrations and shifts in sediment P pools indicated that internal P loading had been reduced as a result of the Phoslock® applications.

In general, the project has delivered a comprehensive evidence base with which the ability of internal loading control, alone, to meet WFD objectives can be assessed. It concludes that, given the complex nature of ecological communities in lakes and of the multiple pressures acting upon them, it is unlikely that any single measure will be sufficient to achieve ecological recovery in any lake.

The need for site-specific assessments including mass balance calculations of candidate lakes is stressed. A separate report, which draws on lessons learned from this study, key messages and guidance on what factors to take into account if using such products is being considered.

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The Centre for Ecology & Hydrology (CEH) and the Environment Agency are grateful for the co-operation of the Broads Authority, the Cheshire Wildlife Trust and The Mere Golf Resort and Spa in granting permission for this study to be conducted on their lakes. We thank Natural England for its constructive advice on working with protected lakes and Defra for its continued support of this research. We also acknowledge Dr Kasper Reitzel, the University of Southern Denmark, Dr Miquel Lürling, Wageningen University, the Netherlands, Dr Grant Douglas, Commonwealth Scientific and Industrial Research Organisation Land and Water, Australia, and Dr Said Yasseri, Institute Dr Nowak, Germany, for comments on earlier versoins of this report and for openly discussing their research prior to publication to help in the interpretation of these results. We are grateful to Geoff Phillips, University of Stirling, for comments that led to the improvement of this report.

Contents

List of tables and figures

1 Introduction

1.1 Project summary

The overall aim of this project was to investigate the potential of a sediment phosphorus (P)-capping technique to control P release from sediments in order to restore eutrophic lakes in England.

This four-year project aimed to improve two eutrophic lakes in Cheshire, Hatchmere and Mere Mere, ideally restoring them to 'good ecological status', as required by the EU Water Framework Directive (WFD; Directive 2000/60/EC), through the control of internal P loading by treatment in year 2 of the project. There are two final reports that record and draw on the conclusions from this project. This report summarises the project and draws on previous unpublished project reports/records and peer-reviewed publications to review documented responses in the treated lakes and provide insights into future use of the approach and its limitations. Work is continuing on developing guidance as a result of this work and this will be provided in a separate report.

1.1.1 Project partners

The project partners were the Environment Agency (lead partner) and the Centre for Ecology & Hydrology (CEH), with Natural England (NE). These organisations were also represented on the project board.

1.1.2 Original project objectives

The original objectives and tasks of the project are listed below:

Overall objective

To restore two eutrophic lakes in England towards 'good ecological status', as defined under the WFD, through the control of internal P loading using a novel sediment treatment applied in year 2 of the four-year project.

Objective 1

Identify two lakes technically and practically suitable for treatment, in which the reduction of internal P loading is likely to deliver ecological improvements in WFD biological quality elements (BQEs), i.e., phytoplankton, fish, macrophytes, macroinvertebrates and benthic diatoms.

Objective 2

Monitor the lakes (water, biology and sediment) to determine the site-specific treatment dose-rates required and to establish pre-application baseline conditions, in terms of WFD BQE metrics.

Objective 3

Use specialist application equipment (following identification of product and contractor) to treat the target lakes during the winter (in line with site-specific risk assessments) when the first year of monitoring had been completed.

Objective 4

Assess changes improvements in water quality and WFD BQEs through comprehensive post-application monitoring (water, biology and sediment) and evaluation over a post-application period of two years. Use data from existing lake monitoring programmes to provide untreated 'control' (reference) site comparisons. Data used spanned from March 2013 to February 2015.

Objective 5

Conduct a site-specific cost-benefit analysis of the restoration programme, once all costs have been determined.

Objective 6

Use the results of the project to develop further evidence-based advice and guidance to inform future WFD restoration projects.

A list of the original main project tasks is shown in Table 1.1.

1.1.3 Project changes

During the course of the project it was necessary to change some of the originally agreed project tasks. The most significant of these are outlined below:

(1) Task 4: Following an assessment of suitable monitoring protocols it was decided that direct sampling of fish (via fyke netting) would not be undertaken due to the insensitivity of the recommended sampling method. This was replaced by the following sub-tasks:

- A literature review/desk study on the effects of lanthanum (La) and suspended solids on fish. Using information on the water quality data, there should be sufficient information to conduct a desk-based risk assessment that is specific to each lake treated.
- To address the gaps in knowledge, associated with the external P inputs to these lakes, a field sampling programme was implemented. The overall aim of the work was to create an overview of external P sources and inputs, and P losses from the outflow, and to construct a P budget that will inform our understanding of the impacts of product to these water bodies.

(2) Task 5: Based on initial results and project time constraints, the project board concluded that the cost-benefit analysis should be separated from the project. A decision could then be made, after project completion, as to whether a cost-benefit analysis would be appropriate.

Table 1.1 Summary of original main project tasks

Task no.	Task	Responsible party	Deliverables	Timeline
1	Identify potential lakes for monitoring and agree access with lake owners	EA, NE, CEH	Shortlist of lakes, agreements with lake owners	Year 1
2 (i)	Conduct pre-application monitoring (water, biology, sediment)	CEH, EA	Data on water quality, biology and sediment of lakes	Year 1
2 (ii)	Identify lakes for treatment	EA, NE, CEH	Revised shortlist	Year 1
2 (iii)	Develop legal agreements with lake owners and obtain consents from NE	EA, NE	Signed agreements	Year 1
3 (i)	Appoint contractor for lake treatment	EA	Procured contractor	Year 2
3 (ii)	Apply treatment	External contractor (managed by EA)	Product applied	Year 2
4 (i)	Conduct post-application monitoring (water, biology, sediment)*	CEH, EA	Data on water quality, biology and sediment of lakes	Years 2–4
4 (ii)	Data analysis and reporting	CEH	Report and presentation	Years 2–4
5	Cost-benefit analysis*	External contractor (managed by EA)	Report	Year 4
6	Develop decision support tools/guidance	EA	Report	Year 4

Notes: * see changes outlined below in section 1.1.3; CEH: Centre for Ecology & Hydrology; EA: Environment Agency; NE: Natural England

1.2 The need for research on sediment phosphorus release in lakes

When P loading from the catchment is reduced, lake recovery can take several decades. This is because P that accumulated in the lake sediments when catchment inputs were high is released ('internal loading') during the recovery process (Jeppesen et al. 2005, Spears et al. 2012, Søndergaard et al. 2013). For this reason, the effective control of internal loading can greatly accelerate ecological recovery once external inputs have been reduced significantly (Mehner et al. 2008). Studies assessing forced recovery are relatively new and have focused, mainly, on responses within the plankton (e.g. Van Wichelen et al. 2007, Mehner et al. 2008). In contrast, studies of the responses of relatively slower growing organisms, such as fish and aquatic macrophytes, are sparse, especially over longer time periods. Given that ecological recovery in lakes is known to lag behind chemical recovery (Verdonschot et al. 2013), it is important that research is conducted to assess recovery processes in all biological groups and to develop and test management practices that are aimed at enhancing recovery to meet specific management targets. This need is especially acute for environmental regulators who are tasked with improving ecological quality in standing waters as part of European legislation such as the Habitats Directive and the WFD. The latter calls for 'good ecological status' to be achieved by the end of the third river basin management planning cycle (2027) at the latest. Given the slow recovery time that is often observed in lakes that are subject to reductions in catchment nutrient load alone, the development of measures to 'speed up' recovery will be critical in enabling water quality managers to meet these deadlines.

At the beginning of the project the most recent assessment of the ecological status of lakes for the WFD was carried out by the Environment Agency in 2013, using data up to the end of 2012. Of 416 lakes with available data, 74% in England and 34% in Wales failed to achieve 'good status', based on total phosphorus (TP) concentration (Jo-Anne Pitt, personal communication). Duethmann et al. (2009) demonstrated that a combination of both catchment management measures and internal loading control measures will be required to improve TP concentrations in a large proportion of lakes currently failing WFD TP targets in England and Wales.

There are few methods available to control internal loading. Hard engineering solutions (e.g. sediment dredging) have been widely used, but these are rarely the best option when habitat destruction, waste disposal and cost are taken into account. In addition, they are not always successful (Pitt et al. 1997, Søndergaard et al. 2007). P-sorbing materials (e.g. modified clays, industrial by-products, flocculants and physical barriers; Leader et al. 2008, Hickey and Gibbs 2009, Spears et al. 2013c) have also been used to strip P from the water column and, after settling, reduce P release from the sediments (Hickey and Gibbs 2009, Meis et al. 2012). The use of these techniques in combination with external load reduction and other management methods (e.g. biomanipulation) have resulted in the rapid ecological recovery of some shallow lakes, at least in the short term (Mehner et al. 2008).

2 Selection of candidate sites, products and application to lakes

2.1 Initial site selection

Ideally, the selection of lakes for treatment would be based on a comprehensive understanding of the past and current nutrient sources in a lake, together with adequate in-lake monitoring data for water column nutrient concentrations and related biological elements, as a minimum. An understanding of the nature and nutrient characteristics of the lake sediment would also be beneficial. However, in practice, lake managers rarely have access to the full suite of information, and a pragmatic approach is necessary.

Site selection for this project started with a review of available Environment Agency monitoring data for all English WFD lake water bodies against a set criteria as outlined in Spears et al. (2011a).

These criteria were based on the need for evidence that TP and chlorophyll a (Chl*a*) concentrations in a candidate lake were in excess of the WFD type-specific targets, that the phytoplankton community was limited by P and that the main source of P to the water column was from the sediment and not from the catchment. If any of these criteria were not true for a candidate lake, then sediment treatment with P-capping materials would be unlikely to result in a sustained improvement in BQEs in line with the WFD targets. The criteria proposed were as follows:

i. Internal loading would be considered dominant when mean winter TP concentrations were < 50µg L^{-1} and summer maximum concentrations were ≥ 50µg L^{-1}. This condition was necessary given the lack of funds available at the outset of the project to determine catchment P loads to the lakes comprehensively.

ii. P limitation of the phytoplankton was assumed when dissolved inorganic nitrogen (DIN):soluble reactive phosphorus (SRP) ratios were < 10 by mass; where absolute dissolved inorganic N (DIN or TON in absence of DIN data) or SRP concentrations were less than 0.1mg L^{-1} and 0.01mg L^{-1}, respectively; or when Chl*a*:TP ratios were > 0.7. In addition to this, phytoplankton nutrient limitation assays have been performed in the current study to assess nitrogen (N) and P limitation (section 3.3.5).

Consideration was also given to the characteristics of each site that may limit the performance of P-capping materials (Spears et al. 2011a). These included:

iii. Lake depth where very shallow lakes (i.e. < 3m mean depth) may be sensitive to wind-induced disturbance of bed sediments affecting P-capping materials (Meis et al. 2012 and unpublished data). However, improvements in water quality may still be achieved in very shallow lakes (e.g. Loch Flemington in Scotland; average depth 0.7m).

iv. Lakes with a very low mean retention time (i.e. < 60 days) where it is possible that some product may be flushed downstream and lost during the settling process, which can take weeks to months (Hickey and Gibbs 2009).

v. Lakes with a low conductivity which may experience lower settling rates (e.g. > 2 weeks for Clatto Reservoir; < 100µS cm^{-1}; Meis et al. 2012) when compared to lakes with higher conductivity (e.g. < 2 weeks in Loch

Flemington; > 360µS cm^{-1}; Meis, unpublished data) will exhibit a higher risk for product loss through flushing.

Of the 743 lakes in England and Wales that were then identified as WFD lake water bodies, there were only 387 lakes for which there were some TP data available from Environment Agency routine monitoring. The monitoring data were processed to produce monthly, seasonal and annual average values for a range of parameters.

The screening process produced an initial list of 13 potential lakes that were reviewed by the project team in the light of other constraints, including estimated cost (which limited the lake size that could be considered for treatment within the project), lake use (Phoslock® has not been approved for application in drinking water supply lakes in England) and other known issues with individual lakes.

As a result of limited data being available on a significant number of lakes, and the other project constraints, including access issues and the willingness of lake owners to participate, the options available proved to be limited, and not necessarily ideal. However, a decision was made to consider three small lakes as candidates for treatment, and to supplement existing data with enhanced monitoring for one year prior to treatment. The candidate sites (see Figure 2.1) were Alderfen Broad (Norfolk), Hatchmere and Mere Mere (both in Cheshire), and information on them is summarised below in sections 2.1.1 to 2.1.3.

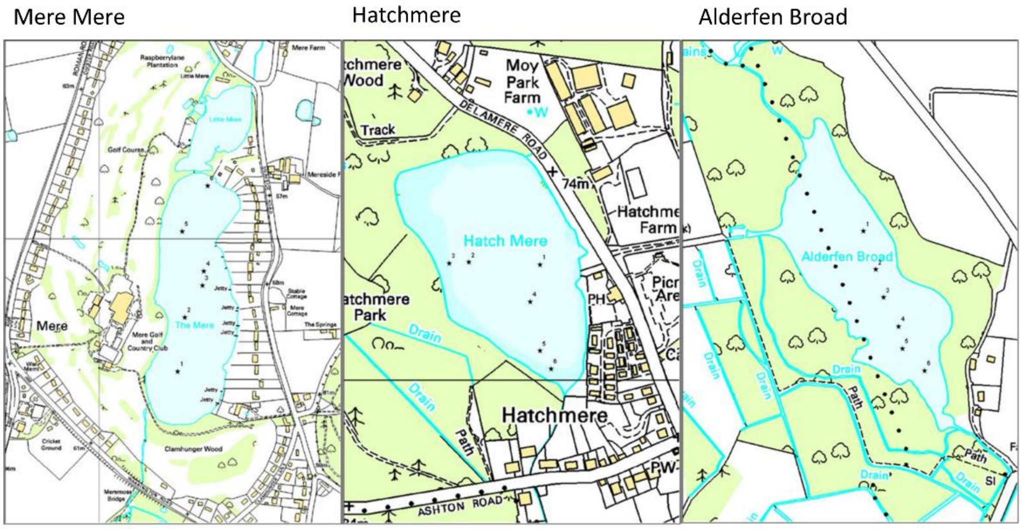

Figure 2.1 Maps showing Mere Mere, Hatchmere and Alderfen Broad and the six sampling sites used for each lake by CEH during the contemporary monitoring campaign (sample sites are further discussed in section 3) © NERC (CEH) 2009. © Crown copyright and/or database right. All rights reserved. Licence number 10001757

2.1.1 Historical evidence of water quality problems in Alderfen Broad

Alderfen Broad is a very shallow lake (maximum depth: 1.3m; mean depth: 0.8m; surface area: 4.6ha) situated in the River Ant valley. Alderfen Broad is within the globally significant Broads Ramsar site, and also forms part of the internationally designated Special Protection Area (SPA) and Special Area of Conservation (SAC). It is designated as a Site of Special Scientific Interest (SSSI) on account of its use as important supporting habitat by a wide range of water birds, vegetation and invertebrates. The broad is owned by the Norfolk Wildlife Trust. The site has a long and well-documented history of nutrient management activities (see below), including the diversion of a nutrient-rich inflow in 1979 (Perrow et al. 1994), the removal of bed sediments by the Broads Authority in 1992–1993 (Pitt et al. 1997) and biomanipulation, also by the Broads Authority (Hoare et al. 2008).

The following management practices have been undertaken on Alderfen Broad since 1979 (Andrea Kelly, personal communication):

- *Isolation – 1979:* nutrients entering from catchment reduced; macrophytes dominated 1982–83; phytoplankton dominated 1985; high nutrient concentrations due to internal release and lack of flushing; TP < 50µg L^{-1}, internal P release less important 1994–2007.
- *Sediment removal – 1992–1993*: increased water depth, removal of most of soft silt; however, rapid decomposition of exposed peat was thought to cause internal loading from dredged sediment immediately after dredging (Pitt et al. 1997).
- *Iron injection – 1995:* small areas of sediment injected with iron to lock up P in the silt; iron was lost rapidly within the mobile sediment; technique not successful at binding P within the sediment.
- *Biomanipulation, fish removal – natural fish kill 1990:* division and partial biomanipulation, including perch removal 1993; occurrence of rudd and roach indicate that it is not fully isolated.
- *Biomanipulation, perch stocking – 1999:* most introduced perch did not survive due to high water temperatures during introduction.
- *Refugia – May 2000:* cobweb brushes provided excellent habitat for invertebrates during initial stages of restoration; brushes become encrusted with sponges after two–three years; cobweb brushes and all structures removed in 2007.
- *Littoral margin scrub removal – ongoing:* 10–30m margin cleared; good for some species, but little overall regrowth of emergent plants into water over the 10 years since clearance; inflow dyke cleared in 2006 to provide connected dyke habitat and prepare for connection with the inflow stream once water quality has improved.
- *Macrophyte reintroduction – to improve species diversity*: water soldier, from dyke clearance operations, added; none survived.
- *Extend open water:* to extend open-water habitat by restoring and connecting adjacent dyke habitats.
- *Bird exclosure – 1993*: removed in 2000.
- *Inflow and outflow maintained with digger – 2010:* this will enable connection once the water quality allows.

Given the diversion of the surface water inflow in 1979, it was assumed that the main sources of nutrients entering Alderfen Broad were associated with private, on-site, sewage treatment systems and groundwater flow (Andrea Kelly, personal communication).

2.1.2 Historical evidence of water quality problems in Hatchmere

Hatchmere is a small, shallow lake (maximum depth: 3.8m; mean depth: 1.4m; surface area: 4.7ha) situated close to Delamere Forest, Norley, Cheshire. Hatchmere is designated as a SSSI (mesotrophic standing water), and forms part of the Midland Meres and Mosses Ramsar site as an example of a natural, or near-natural, wetland site that supports rare vegetation and invertebrate species (ECUS 2001a). The site is owned by the Cheshire Wildlife Trust. Although there are a small number of surface water drains entering the mere, the main source of water is thought to be groundwater. However, further work is required to map both surface and groundwater catchments (ECUS 2001a) at this site. The surrounding land is used, predominantly, for agriculture (59.87ha improved grassland; 28.27ha arable land) with coniferous plantation woodland also representing a significant contribution (55.26ha) (ECUS 2001a). Reports of at least sporadic events of high nutrient loading have been reported by various authors, with orthophosphate concentrations of around 3.5mg L^{-1} being reported (1999; ECUS 2001a) in inflowing drains. Additionally, anecdotal reports of poor agricultural practice are available. These show that silage liquor and, potentially, untreated animal waste have been allowed to enter field drains connected to Hatchmere in the past (discussed in ECUS 2001a). In addition, signs of eutrophication in surface water inputs have been reported by Wiggington (1980) and Moss et al. (1993). Open-water swimming is also a popular activity at Hatchmere and should be considered further in the context of any product application.

2.1.3 Historical evidence of water quality problems in Mere Mere

Mere Mere is a moderately sized and relatively deep lake compared with the two other study sites, although in WFD typology it would be considered to be a 'very shallow' lake (maximum depth: 8.1m; mean depth: 2.8m; surface area: 15.8ha) and is situated to the north-west of Knutsford, Cheshire. Mere Mere is designated as a SSSI and forms part of the Midland Meres and Mosses Ramsar site. It is also of high conservation value, largely due to its submerged aquatic flora (ECUS 2001b). The site is owned by The Mere Golf and Country Club, although the eastern shore consists of privately owned homes with gardens and/or jetties up to the water's edge. Mere Mere is largely surface water fed from its southern end by the Rostherne Brook, while the outflow is via a sluice gate and weir at the northern end and feeds down into Little Mere (ECUS 2001b). The surface water catchment is calculated to be 3.8km^2. Along with the private residences on the eastern shore, Mere Mere is bordered to the south and west by a golf course (including extensive plantation woodland), and to the north by the spillway separating it from Little Mere. The rest of the catchment is a mixture of arable and improved pasture incorporating some housing and farms (ECUS 2001b). Run-off from fertilised land into the Rostherne Brook is likely to be the largest contributor of nutrients and fine sediments to Mere Mere. A secondary source of pollutants has been reported to come from the main Warrington Road. A licence exists for minor water abstraction at the site in order to water the golf course during times of drought. However, this is not believed to have any negative effects on water quality or lake ecology (ECUS 2001b). There is believed to be some threat to the local ecology from invasive species, with New Zealand stonecrop (*Crassula helmsii*) being recorded in Mere Mere in 1992, and with sweet flag (*Acorus calamus*) and Japanese knotweed *(Fallopia japonica*) being found there more recently. Mere Mere is within a Catchment Sensitive Farming (CSF) priority area, and there has been a good take-up of CSF advice from farmers within the catchment, but a detailed analysis would be required to tell whether this has resulted in any water quality benefits (draft Diffuse Water Pollution plan available from G. Madgwick, Natural England).

2.2 Assessment of suitability of candidate sites

To help inform the site selection process, a monitoring programme was initiated to compile evidence with which the final sites for treatment could be selected. The results of this monitoring programme and detailed comparison of the sites was outlined in Spears et al. (2013c) and is summarised in Table 2.1. The results provided a means of assessing each site in relation to the attributes that may enhance or confound the effects of the P-capping technique. These potentially confounding factors were reviewed by Spears et al. (2011a) and are further discussed below in relation to the site attributes shown in Table 2.1.

Using the indicator values for the criteria it was apparent that all three lakes had winter mean and summer maximum TP concentrations in excess of 50µg L^{-1}, potentially indicating significant catchment P loading issues. However, summer maximum TP concentrations, relative to winter mean TP concentrations indicated dominance of internal P sources over catchment sources in all lakes. In this respect, internal loading was assumed to be most dominant in Hatchmere, followed by Alderfen Broad and then Mere Mere. As winter mean TP concentration was highest in Alderfen Broad it was possible that catchment sources were highest in this lake followed by Mere Mere then Hatchmere.

Nutrient limitation of the phytoplankton by N, or by N and P, was measured in either May or September 2012 for all lakes. In addition, the Chl*a*:TP ratio indicated that Alderfen Broad was likely to be P limited in spring but not in summer–autumn, that Hatchmere was likely to be P limited in summer, and that Mere Mere was likely to be N limited in summer–autumn prior to the application. As such, although a reduction in TP concentrations may result in achieving WFD TP targets, a similar response in Chl*a* concentration was less likely, especially in Alderfen Broad and Mere Mere. Nutrient limitation assessments reported here are in general agreement with other authors for Hatchmere and Mere Mere (Fisher et al. 2009, Maberly and Carvalho 2010).

Only a weak, if any, seasonal pattern was apparent in TP for Alderfen Broad, whereas for Hatchmere and Mere Mere a summer–autumn internal loading peak was more evident. The relatively high inter-annual variability in TP concentrations of Alderfen Broad indicated the action of other drivers important in regulating water quality in this lake, for example sporadic external loading events throughout the year and/or sporadic bed disturbance and translocation of sediment TP to the water column. The seasonal patterns of TP and Chl*a* in Mere Mere and Hatchmere were more consistent between years, although relatively few data were available with which a comprehensive comparison could be made between Hatchmere and the other candidate lakes.

The average depth of all candidate sites was below 3m, although Mere Mere had a significantly deeper maximum depth and Alderfen Broad was shallow throughout at less than about 1.3m. As such, the risk of sediment disturbance and associated translocation of P-capping material once settled on the surface of bed sediments was highest in Alderfen Broad, followed by Hatchmere and Mere Mere. Retention time and conductivity were similarly high for all lakes and no issues of product loss were expected.

Table 2.1 Summary of key site characteristics that may be used to underpin the assessment of site suitability in relation to the use of P-capping materials

Characteristic	Alderfen Broad	Hatchmere	Mere Mere
Altitude (masl)	3	76	52
Area (ha)	4.6	4.7	15.8
Catchment area (km^2)	ND	2.2	3.8
Maximum depth (m)	1.3[i]	3.8	8.1
Mean depth (m)	0.8[i]	1.4[ii]	2.8
Mean retention time (y)	ND	0.4	0.8–9.5
Main source of water[iii]	GW	GW/S	GW/S
Annual conductivity (µS cm^{-1})	594	367	374
Annual alkalinity (mequiv L^{-1})	2.28	2.12	1.55
Annual geometric mean pH	7.78	7.64	7.73
WFD Lake type[iv]	HA,VS	HA,VS	HA,VS
Type-specific WFD TP target (µg L^{-1})[v]	35^{HG}; 49^{GM}	35^{HG}; 49^{GM}	35^{HG}; 49^{GM}
Site-specific WFD TP target (µg L^{-1})	ND	35^{HG}; 48^{GM}	30^{HG}; 41^{GM}
Type-specific WFD Chl*a* target (µg L^{-1})	8.6^{HG}; 16.5^{GM}	8.6^{HG}; 16.5^{GM}	8.6^{HG};16.5^{GM}
NO_3-N targets (mg L^{-1})[vi]	1.5	1.5	1.5
Annual mean TP (µg L^{-1})[x]	89	70	63
Winter mean TP (µg L^{-1})[x]	82	68	65
Summer maximum TP (µg L^{-1})[x]	124	117	82
Annual mean Chl*a* (µg L^{-1})[x]	23	39	20
Annual mean TON (mg L^{-1})[x]	ND	3.13	0.88
Mean sediment TP (µg g^{-1} d.w.)	3,186	1,222	723
Minimum TON (mg L^{-1})	ND	1.06	0.06
Mass of P_{mobile} in top 4cm of sediment (t)[vii]	0.1	0.09	0.68
Benthivorous fish present? (Y/N)[viii]	Y	Y	N
Maximum fetch (km)[ix]	0.5	0.3	0.6
Rare/protected macrophytes present? (Y/N)	Y	N	Y
N limitation or co-limitation indicated?	Y	Y	Y

[i] = values from Cryer et al. (1986); [ii] = estimated using regression between mean and maximum depth for the Shropshire and Cheshire Meres, as outlined by Maberly and Carvalho (2010); [iii] GW = groundwater, S = surface; [iv] = HA – high alkalinity, VS – very shallow; [v] = HG – high/good WFD boundary, GM = good/moderate WFD boundary (standards from WFD-UKTAG 2008); [vi] = standards derived from James et al. (2005) and discussed for Hatchmere and Mere Mere by Maberly and Carvalho (2010); [vii] = estimates of P_{mobile} mass in the upper 4cm of sediment were calculated, as outlined in section 3.3.3; [viii] = based on both contemporary survey data and historical survey data (Moss et al. 1993, APEM 2010); [ix] = estimated from Figure 2.1; [x] = All available surface water data as supplied by the Environment Agency were processed using the Pivot Table function in Microsoft® Excel to produce a time series of average concentrations for each determinand for consecutive months between January 2000 and October 2012 and used to calculate seasonal or annual mean values across this period. ND = no data.

Based on the evidence summarised above (Table 2.1), Mere Mere and Hatchmere were selected by the project board, with reservations as noted, as the two experimental lakes that were most likely to respond to the proposed treatment.

2.3 Product selection process

A wide range of materials have been proposed for use in the control of internal loading in lakes (e.g. Hickey and Gibbs 2009). Although the P sorption properties and ecotoxicological impacts of the proposed materials have been quantified under laboratory conditions in some cases, few materials have been comprehensively assessed across a range of field conditions. The main exceptions to this are aluminium (Al) (e.g. Cooke et al. 1993, 2005, Reitzel et al. 2003) and iron (Fe)-salts (Cooke et al. 1993, Pitt et al. 1997, Perkins and Underwood 2002). In addition, no study has been published to date that assesses the ability of these products to improve the ecological quality of eutrophic lakes in the context of WFD BQEs and associated water quality metrics. Finally, few results are available with which P-capping products proposed for use in lakes can be quantitatively and comparatively assessed, making the process of objectively selecting products problematic (Leader et al. 2008, Spears et al. 2013c).

For this project the following product selection process was implemented:

- The Environment Agency released an advertisement in the *Official Journal of the European Union* describing its interest in techniques/products that can control sediment P release in lakes and inviting tenders from suppliers of such techniques/products requesting:
    - details of the technique's/product's mode of working
    - methods for site access and application
    - information on the toxicological, ecological and H&S implications of the techniques/products
    - examples of similar work carried out elsewhere
    - information on cost, sustainability and quality assurance.

Requests for full tender packs were received from potential suppliers, some of which were not known to the project group.

The tenders were evaluated against pre-determined (and advertised) selection criteria. Several minimum scores were required to progress to the evaluation, then the merits of the remaining techniques/products were assessed by an evaluation panel consisting of chemical experts, freshwater ecologists and project managers, with expert assessment from Sustainability and Health and Safety officers provided by the Environment Agency. Each panel member assessed the tenders independently according to their area of expertise. The final selection included consideration of the tender costs. The selection committee identified Phoslock® as the preferred material for use in this project.

2.4 Product application events

Phoslock Europe GmbH was commissioned by the Environment Agency to apply 25.2 tonnes of Phoslock® to Hatchmere and 79.8 tonnes to Mere Mere under Contract ID 26049B and Project Title: Trial of a Restoration Technique for Eutrophic Lakes. The dosage of Phoslock® was calculated according to the water and sediment P data provided to Phoslock Europe GmbH and other tenderers in the Tender Pack. Details of the calculations were included in their tender submission.

The application in Mere Mere commenced 4 March 2013 and finished 8 March 2013 (Figure 2.2). Weather conditions were generally favourable with only light rain falling on a number of occasions.

The application in Hatchmere commenced 11 March 2013 and finished 13 March 2013 (Figure 2.2). Weather conditions were generally favourable apart from several periods of intense snowfall during the set-up on 11 March 2013.

Figure 2.2 Photographs documenting the Phoslock® applications at Hatchmere and Mere Mere. From top left: Phoslock® in the hopper prior to slurry application; product being loaded from shore onto the pontoon at Mere Mere; cleaning of equipment following application as part of the biosecurity measures. From bottom left: product application track; plume of product during application; site visit by project board and stakeholders at Mere Mere during application

3 Assessing chemical and biological responses in Mere Mere and Hatchmere following Phoslock® application

3.1 Assessment approach

The project team developed a list of scientific hypotheses (Appendix 1) relating to the application of Phoslock® to the treated lakes that, collectively, would provide evidence to address Objective 4 and, in part, Objective 6 of the project (section 1.1.2). In this report, we attempt to address these hypotheses, where this was possible within constraints of the available data. Where it was not possible to test the hypotheses the reasons have been noted in Appendix 1. A working group was set up within the project to consider the most appropriate statistical methods with which to provide confidence in the conclusions drawn from these hypotheses. Following initial exploration of the suitability of 'control lake' data (i.e. Tatton Mere) it was concluded that responses indicated using a before-after-control-impact (BACI) approach compared to a simple before-after (BA) approach were, at times, contradictory. Given the BACI analysis was only possible for a small subset of physicochemical responses for which sufficient data were available across all lakes it was decided that only BA analyses would be reported here. In this way, the project team have attempted to standardise the statistical approach used to confirm responses following the Phoslock® application across both physicochemical and ecological determinands.

In addition to detecting chemical and ecological responses in line with eutrophication recovery, an important objective of the project was to assess any responses in the context of the WFD. Where possible, available metrics were used to provide this context but it is important to make clear that this analysis did not reflect the WFD reporting methodology adopted by the Environment Agency for these, and other, lakes. One important distinction is that individual annual (at times not within calendar years) or seasonal values were assessed as opposed to average values over the full reported period.

3.2 Contemporary monitoring activities

3.2.1 Monitoring activities

For the purposes of this project, pre-application monitoring activities were initiated by CEH in May 2012 and by the Environment Agency in June 2012 and extended to the sample date prior to product application, February 2013. Post-application monitoring activities commenced March 2013 and continued to March 2015. Both partners were responsible for monitoring a specific list of determinands, as outlined in Table 3.1. The monitoring work was conducted from the shore or from a boat, as necessary. In summary, the following surveys were conducted:

- monthly water chemistry, phytoplankton and zooplankton surveys (Environment Agency /CEH)
- quarterly water chemistry and macroinvertebrate surveys (CEH)
- annual macrophyte surveys (CEH)

Six sampling sites were identified at each lake in May 2012 (Figure 2.1). These were used as fixed sampling point locations throughout the project for the CEH quarterly field surveys. The sampling sites were selected to cover the surface area of each lake, and to represent a depth gradient from shallow to deeper water. A central sampling point was identified to represent average conditions of the lake and this was generally used as the Environment Agency sample point for monthly surveys. The procedures employed during the CEH quarterly surveys are described in detail by Spears et al. (2013a) and those methods used for the collection of data included in this report are briefly outlined. The following sample procedures were conducted at each of six sample sites and quarterly for both Mere Mere and Hatchmere:

- surface water pH, conductivity, water temperature, dissolved oxygen concentration (and % saturation), Secchi depth, local weather conditions and water depth recorded
- one litre of surface water collected and stored in a sample bottle
- one sediment core collected using a HTH gravity corer for collection of bottom water (i.e. 10cm above sediment surface)
- at three sites only, one sediment core (upper 10cm) collected for profundal macroinvertebrates and stored in labelled white tubs (fixed in 4% formaldehyde)

Table 3.1 Monitoring plan for the project for Mere Mere and Hatchmere

Variable	Sample location	Frequency	Responsible partner
Local weather (rainfall, wind speed and direction, etc.)	Met station (BADC)	Daily	CEH
TP, SRP, TON, SiO_2, TSi, DLa, TLa, conductivity, pH, water temperature, dissolved oxygen and chlorophyll *a*	6 open-water sites	Quarterly (May, September, December and March)	CEH
Profundal macroinvertebrates	3 open-water sites	Quarterly (May, September, December and March)	CEH
Littoral macroinvertebrates	3 shoreline sites	Quarterly (May, September, December and March)	CEH
Fish	Open-water survey	Annual (August)	CEH
Macrophytes	Open-water survey	Annual (August)	CEH
Elemental composition and P fractions in upper 4cm of sediment	6 open-water sites	Annual (May)	CEH
Sediment P dose assays	1 open-water site (6 cores)	Annual (August)	
Depth, TP, SRP, TN, TON, DOC, conductivity, pH, chlorophyll *a*, colour, ammonia, alkalinity, water temperature, dissolved oxygen and Secchi depth	1 open-water site	Monthly	EA
Phytoplankton, zooplankton, dissolved metals and total metals	1 open-water site	Monthly	EA – sample CEH – analysis
Littoral diatoms	Shoreline	Bi-annual	EA
Chironomid pupal exuviae (CPET)		Quarterly	EA
Littoral macroinvertebrates	Shoreline	Annual	EA

Notes: DLa, dissolved lanthanum; DOC, dissolved organic carbon; SiO_2, silica; SRP, soluble reactive phosphorus (ortho-phosphorus); TLa, total lanthanum; TN, total nitrogen; TON, total oxidised nitrogen; TP, total phosphorus; TSi, total silicon. BADC, British Atmospheric Data Centre; CEH, Centre for Ecology & Hydrology; EA, Environment Agency

3.3 Data collection, processing and analysis

3.3.1 Weather

Weather data were extracted from the British Atmospheric Data Centre (BADC). No single weather station provided a complete data series for wind speed, air temperature or rainfall during the contemporary monitoring period of the study (i.e. March 2012 to March 2015). However, a composite of data from sites allowed comparisons of each determinand; the use of each site being validated against available data from ‘Knutsford’. Data were available for each determinand from the ‘Knutsford’ site during the period 2003 to 2011, and monthly values of average air temperature, average wind speed and total monthly rainfall were calculated and used as a validation dataset. Using the same data processing procedures, a common dataset was compiled to produce time series (monthly frequency) from January 2013 to March 2015 for average monthly air temperature (data available for the following stations: ‘Nantwich’ and ‘Rochdale’), monthly total rainfall (data available for the following station: ‘Alderly’), and average monthly wind speed (data available for the following station: ‘Rochdale’).The ‘Nantwich’, ‘Rochdale’ and ‘Alderly’ sites are about 20 miles, 34 miles and 10 miles (32km, 55km and 16km), respectively, from the ‘Knutsford’ site. All sites, including Mere Mere and Hatchmere are situated in around about a 17 mile (27km) radius area. Correlation analysis confirmed that the air temperature data from ‘Rochdale’ was most closely matched with ‘Knutsford’ (Pearson’s correlation coefficient: 0.998; $p < 0.001$) and was used to represent both Hatchmere and Mere Mere in the analysis below. The correlation between ‘Alderly’ and ‘Knutsford’ for rainfall indicated good agreement between the two sites (Pearson’s correlation coefficient: 0.886; $p < 0.001$) and so ‘Alderly’ data were used to represent both Hatchmere and Mere Mere in the analysis below. The correlation for average monthly wind speed between ‘Rochdale’ and ‘Knutsford’ indicated good agreement between the two sites (Pearson’s correlation coefficient: 0.741; $p < 0.001$) and so ‘Rochdale’ data were used to represent both Hatchmere and Mere Mere in the analysis below.

A generalised linear model (GLM) using a Gamma distribution and log-link function, with treatment year and season as the explanatory variables, was applied to assess differences in the three weather determinands between seasons and treatment years. Spring was defined as March, April, May; summer as June, July and August; autumn as September, October and November and winter as December (of the previous year), January and February. In addition, weather conditions in all seasons in all treatment years were compared with long-term average conditions (i.e. 2003 to 2013).

3.3.2 Physicochemical responses in the water column

Contemporary physicochemical data were obtained from the Environment Agency for each lake and have been discussed in detail previously (Spears et al. 2013a). Chemical analyses for these data (roughly monthly in frequency) were conducted using standard Environment Agency analytical procedures. Data were processed to produce monthly average values for each lake with data being available between 2006 and 2015. For our planned comparisons described below, data from January 2012 to February 2013 were used to represent pre-application conditions (i.e. year 0), with data from April 2013 to March 2015 being used to represent post-application years (i.e. years 1 and 2). A subset of the available determinands are analysed in this report including orthophosphate-P[1] (abbreviated to ortho-P); total P (TP); total oxidised nitrogen (TON), total nitrogen (TN), chlorophyll *a* (Chl*a*) concentrations, and functional ratios of Chl*a*:TP, ortho-P:TP,

[1] The terminology used to describe different phosphorus determinands by different laboratories is not standardised and can lead to confusion. The Environment Agency reports soluble phosphorus as ‘orthophosphate’, whether from filtered or unfiltered water samples. All routine Environment Agency WFD lake monitoring since 2007 has involved the collection of filtered samples for soluble nutrient analysis, and the phosphorus from these samples is labelled ‘orthophosphate – filtered, as P’ (Environment Agency determinand code 9856). Therefore any reference to orthophosphate in this report should be taken as meaning soluble reactive phosphorus (SRP), and is therefore comparable with SRP results reported from other laboratories, including CEH.

TN:TP and TON:SRP (all by weight). Average quarterly concentrations of dissolved oxygen (DO) across all treatment years for bottom waters were calculated.

All statistical analyses were conducted using R (Ihaka and Gentleman 1996, R Core team 2011) using the packages mgcv, dplyr, gridExtra and multcomp (Hothorn et al. 2008). Five approaches were used to determine responses in surface water determinands. Firstly, available raw data were plotted between 2012 and 2015 and visually assessed to identify responses in determinands. Secondly, a 'before-after' (BA) analysis tested whether there were differences in the responses in water determinands in years 1 and 2 post-application following the application of La-bentonite. To assess annual differences, generalised additive mixed models (GAMMs) with a Gamma family distribution and log-link function were used. The model assigned treatment year as a fixed effect, and an AR1 autocorrelation term was used to account for temporal autocorrelation between residuals. Seasonal variation was accounted for using a smoothing spline function. Tukey's HSD multiple comparison tests were used to assess seasonal differences. Spring was defined as March, April, May; summer as June, July and August; autumn as September, October and November and winter as December (of the previous year), January and February. A GLM was applied to these data using a Gamma distribution and log-link function, with season as the explanatory variable. All statistical tests (GAMMs and GLMs) were conducted with an alpha level of 0.05. Thirdly, comparisons of pre- (average of monthly values for January 2006 to March 2012) and post-application (using all available data between April 2013 and March 2015) conditions were made for a subset of determinands using long-term monthly time series data. Fourthly, we compared the relative change (i.e. pre-application minus post-application values) per month in TP, ortho-P and Chl*a* concentration before (using available data between January 2008 and March 2012) and after the application (using all available data between April 2013 and March 2015). Finally, annual mean ortho-P, TP and Chl*a* concentrations were calculated using available values between April 2012 and March 2013 (pre-application year 0), April 2013 and March 2014 (post-application year 1) and April 2014 and March 2015 (post-application year 2). We compared these annual mean values to WFD type-specific water quality targets for TP and Chl*a* concentrations but acknowledge the methods used here do not conform to WFD methodology.

Bottom water samples were collected quarterly during the monitoring period from each of the six sample sites. TP and ortho-P analyses of these samples were conducted following the molybdenum blue spectrophotometric method after filtration through Whatman GF/F filters (nominal pore size 0.7 µm), for ortho-P. Comparisons of bottom water concentrations were made between conditions before (average concentrations for each depth between May 2012 to March 2013) and after (average concentrations for each depth between May 2013 and December 2014) the Phoslock® application. The effects of treatment were assessed using analysis of variance on log transformed ortho-P and TP data where comparisons were made between all pre-application and post-application observations (i.e. four quarterly samples per year times six sample sites per date = 24 pre-application versus 48 post-application values). The interactions between year and depth were assessed using two-way analysis of variance with Tukey's post hoc analysis.

Water samples for determination of metal concentrations were collected monthly by the Environment Agency from open-water sample stations in each lake and were shipped to CEH for analysis, usually within 24 hours of collection. Once received by CEH, filterable lanthanum (FLa; having passed through a 0.45µm filter) and total lanthanum (TLa) samples were stored frozen (-18°C) until analysis. La analysis was conducted on filtered and unfiltered water samples using inductively coupled plasma mass spectrometry (ICP-MS) following acidification (1% v/v, nitric acid) according to Lawlor and Tipping (2003) at the chemistry laboratory at CEH Lancaster. A comparison of monthly data spanning pre-application and post-application conditions is presented for both treated lakes.

3.3.3 Responses in sediment P composition following Phoslock® applications

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 difference between TSP and SRP is expected to be small within those fractions in sediments of eutrophic lakes. The forms of sediment P associated with this extraction procedure are summarised (Table 3.2).

Table 3.2 Overview of common operational sediment phosphorus (P) fractions based on sequential sediment P extraction procedures (Meis et al. 2012)

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	i, ii, iii, iv	High	1, 2, 3
‘Reductant soluble P’	P bound to Fe-hydroxides and Mn-compounds	Anoxia, redox potential	iii, iv	High	1, 2, 3, 4, 5, 6
‘Organic P’	Allochthonous/ autochthonous material; detritus	Mineralisation (temperature)	iii, iv	Medium to 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)	iii, iv	Medium to 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

Notes: ‘Seasonality of P release’ shows the seasons in which P release will most likely occur in shallow, temperate lakes; i, winter; ii, spring; iii, summer; iv, autumn; -, not likely; Al, aluminium; Fe, iron; Mn, manganese; OH, hydroxyl ion. 1 = Boström et al. (1988), 2 = Hupfer et al. (1995), 3 = Spears et al. (2007), 4 = Psenner et al. (1984), 5 = Psenner and Pucsko (1988) and Psenner et al. (1988), 6 = Lukkari et al. (2007)

Subsamples of homogenised sediments, from the upper 4cm of sediment cores collected from each site, were subjected to the following sequential extraction procedure: (i) ‘labile P’ – extraction in 1 M NH_4Cl for 30min [TSP], (ii) ‘reductant soluble P’ – extraction with 0.11 M $NaHCO_3$/0.11 M $Na_2S_2O_4$ for 1h, repeated for 5min [TSP], (iiia) extraction in 1 M NaOH for 16h, repeated for 5min [SRP], (iiib) supernatant from (iiia) analysed for TSP to determine ‘organic P’ [TSP] from ‘metal-oxide adsorbed P’, (iv) ‘apatite bound P’ – extraction with 0.5 M HCl for 16h, repeated for 5min [TSP], and (v) ‘residual P’ – digestion with 30% (v/v) H_2SO_4 and 8% $K_2S_2O_4$ at 121°C for 30min [TSP]. The sediment slurries and extraction mediums were continually shaken (60 rpm on 360° rotator; PTR-60, Grant-bio, Shepreth, UK) in the dark at 25°C for the periods outlined above. Supernatants were collected following centrifugation (4000 rpm for 10 minutes) and filtration (Whatman® GF/F filter, nominal pore size 0.7µm) at the end of each extraction step. SRP and TSP analyses were conducted on filtered water samples according to Murphy and Riley (1962) and Eisenreich et al. (1975), respectively. Total sediment P was estimated as the sum of all P fractions.

Responses in sediment P fractions were assessed in years 1 and 2 following the application relative to pre-application conditions (year 0) by combining all values across sites and quarterly sampling visits for each lake and application year. A BA ANOVA approach was used to test for the fixed effect of treatment year on the log transformed concentration of the major P pools outlined in

Table 3.2. A two-way ANOVA was used to determine interactions between treatment year and water depth across all P pools for both lakes.

Estimates of the mass of sediment P across the P pools were calculated for the upper 4cm surface sediment layers in each lake. These estimates were based on the following assumptions: (1) the mean concentrations of sediment P fractions recorded at the six sites represented whole lake bed conditions for each lake, and (2) 1g v.v. of sediment equals 1cm^3 volume of sediment in each lake.

3.3.4 Responses in sediment P flux following Phoslock® applications

Laboratory controlled sediment core incubation experiments were conducted to assess the effectiveness of the added Phoslock® to limit P release. The experiments were conducted following the application of the product to the lake and to confirm reduction in internal loading. Experiments were run in both winter and summer under anoxic and aerobic conditions at 4°C and 20°C, respectively. The effect of a simulated Phoslock® 'top-up' dose was also tested.

Twelve sediment cores (70mm (64mm ID) x 500mm clear acrylic tube) were collected from the deepest part of both Mere Mere (*c*.7.8m) and Hatchmere (*c*.3.7m) using a HTH gravity corer in May 2014, 1 year and 2 months after Phoslock® application. An additional frame was fitted to the outside of the corer to create resistance on impact with the sediment and achieve a consistent sediment depth of *c.*250mm (half core depth). Cores were then capped and secured to prevent water loss during transport to the laboratory. A further 5L of surface water was collected from each lake and filtered through 47mm GF/F glass microfibre filters, and maintained at the same temperature as the cores for the duration of the incubation experiments.

For each lake, six sediment cores were kept either at 4°C or 20°C in the dark within upright air incubators, with temperatures being chosen to represent summer and winter conditions. Each core was sampled weekly following an initial 24-hour acclimatisation period. Sampling involved the removal of about 85mL surface water from about 5cm above the sediment surface using a syringe and subsamples were stored either unfiltered or following filtration through a 47mm GF/F glass microfibre filter. Cores were bubbled with N_2 gas for 72 hours following the initial 24-hour acclimatisation period to promote the onset of anoxia. To ensure that cores remained anoxic following exposure to air during sampling, bubbling with N_2 gas was repeated following every sampling occasion. Anoxia was achieved by slowly bubbling pressurised gas through the water column using permanent tubing passed through the bung at the surface of the core. Following each sample occasion 85mL filtered lake water was added to replace the volume removed and P analyses for ortho-P and TP concentrations were conducted as described in Spears et al. (2013a). Data were processed to produce average concentrations of TP and ortho-P across the replicate cores for each temperature.

Phoslock® treatment occurred on day 23 of the incubation experiment where Phoslock® (1.625g per core for Mere Mere and 1.720g per core for Hatchmere) was distributed in 50mL of water extracted from the overlying water in each core. This dose of Phoslock® represents a 'top-up' dose, added to the cores as slurry; these doses being chosen to represent similar doses applied by Phoslock Europe GmbH to each mere, March 2013.

3.3.5 Phytoplankton community responses

Phytoplankton density was assessed primarily using the concentration of the ubiquitous green light-harvesting pigment Chl*a*. Water samples were returned to the laboratory in the cool and dark where they were filtered (Whatman GF/C filter papers), the pigments extracted in an organic solvent (acetone or methanol) and the concentration of Chl*a* calculated from absorbance measured using a spectrophotometer. Detailed method descriptions are given in Spears et al. (2013a).

Phytoplankton composition and biovolume of individual species or genera were addressed by direct observation and measurements using a microscope. Open-water samples were preserved with Lugol's iodine in the field. Phytoplankton were counted by means of 4–10mL Utermöhl sedimentation chambers at a range of magnifications (x50 to x400) depending on cell size. In general low magnification full-chamber counts, intermediate magnification transects and high magnification fields of view were used to measure approximately 400 counting units. Cell, colony and filament counts and biovolume estimates were made following the approach outlined by CEN (2004) and Brierley et al. (2007). A GLM using a Gamma distribution and log-link function, with treatment year as the explanatory variable, was applied to assess differences in growing-season phytoplankton biovolume between treatment years.

Nutrient limitation was assessed using laboratory bioassays. Water samples collected during the quarterly CEH sampling were kept cool and in the dark until returned to CEH Lancaster. Phytoplankton bioassays were undertaken as described in Maberly et al. (2002). Briefly, water was passed through a 250µm net to remove larger zooplankton and 35mL samples were placed in 50mL foam-stoppered test tubes at 20°C and about 50µmol photon m^{-2} s^{-1} continuous light (photosynthetically available radiation; spectral band 400 to 700 nm) from fluorescent tubes. Triplicate incubation tubes with the following treatments were used: Control (no addition), +P (6µM NaH_2PO_4), +N (90µM $NaNO_3$) and +P+N (concentrations as previously). After 14 to 21 days, the water in each incubation tube was filtered through a Whatman GF/C filter paper and analysed for Chl*a* after extraction in boiling methanol. Results of the nutrient limitation assays are presented to provide a comparison between the nutritional status of the phytoplankton community during the growing season of each treatment year.

3.3.6 Zooplankton community responses

In both Hatchmere and Mere Mere open-water crustacean zooplankton samples were collected monthly, pre- (i.e. May 2012–February 2013) and post-Phoslock® (i.e. March 2013–March 2015) applications, at the six water chemistry sampling locations, as described above. From each of these six locations crustacean zooplankton samples were collected by two vertical pulls of a zooplankton tube and by passing the water through a 120µm mesh net. On each sampling occasion the crustacean zooplankton samples from each of the six sample sites were aggregated into a single composite sample for each lake. All the samples were fixed in 4% formaldehyde solution. In the laboratory, each composite sample was placed in a glass vessel and made up to a final volume of 250mL with distilled water. The sample was thoroughly mixed, to distribute the animals randomly, and then subsampled with a Stempel pipette (volume 5mL). The animals present in each subsample were identified and counted under a low power binocular microscope. In all cases, a minimum of three subsamples were examined. The subsample counts were converted to numbers of individuals per litre (ind. L^{-1}) using appropriate multiplication factors. The response of the crustacean zooplankton community to the Phoslock® applications in both lakes was assessed by comparing the relative abundance of the major crustacean zooplankton genera and the total crustacean zooplankton abundance over the period of the study.

After counting the dominant genera the samples were reconstituted and archived for later size analysis and biomass measurements. Cladoceran length measurements were made from the top of the head of an adult individual animal to the point of insertion of the tail spine while copepod length measurements were made from the top of the head of an adult or copepodite individual to the tip of the distal rami. Up to a maximum of ten individuals per taxa were measured in each examined crustacean zooplankton sample using the graticule on a binocular microscope. For the biomass measurements the average measured length (mm) of all the crustacean zooplankton taxa in each collected sample, derived from this size analysis, was placed in an appropriate size class and assigned a single representative dry weight (µg) taken from published length/weight regressions for common species of copepods and cladocerans (Dumont et al. 1975). The biomass for each crustacean zooplankton taxa on each sample occasion was then calculated by multiplying the number of animals recorded by the representative dry weight figure. The total crustacean zooplankton sample biomass for each sample was calculated by the sum of all the individual taxa biomass estimates. The total crustacean biomass for both lakes were then compared, pre- and post-Phoslock® application, to see if any there was any response to the application of Phoslock® in either of the study lakes.

3.3.7 Macroinvertebrate community responses

The profundal benthos was sampled quarterly in both Hatchmere and Mere Mere, pre- (i.e. May 2012, September 2012 and January 2013) and post-Phoslock® application (i.e. March 2013, June 2013, September 2013, December 2013, March 2014, June 2014, September 2014 and December 2014). Three profundal samples were collected per lake per quarterly sampling – each sample being taken from the top 10cm of sediment collected using the HTH gravity corer described above. The profundal samples were fixed in 4% formaldehyde solution. In the laboratory each profundal sample was washed through a 500µm mesh sieve and any macroinvertebrates present in the sample were picked out and identified to at least family level. The number of macroinvertebrate taxa and mean total macroinvertebrate abundance for both lakes were compared, pre- and post-Phoslock® application.

The littoral benthos was sampled quarterly in both Hatchmere and Mere Mere, pre- (i.e. May 2012, September 2012 and January 2013) and post-Phoslock® application (i.e. March 2013, June 2013, September 2013, December 2013, March 2014, June 2014, September 2014 and December 2014). The aim was to collect three littoral macroinvertebrate samples per lake per quarterly sampling. The thick, impenetrable fringes of emergent vegetation and deep soft sediments had made access to the littoral zones of both lakes from the shore difficult. After the first sampling trip in May 2012, shore samples (collected using standard sampling methods based on Environmental Change Network (ECN) protocols (Sykes et al. 1999)) were supplemented with samples collected by sweep sampling from a boat in order to achieve the target of three littoral samples collected per lake per quarterly sampling. The littoral samples were fixed in 4% formaldehyde solution. In the laboratory each littoral sample was washed through a 500µm mesh sieve and any macroinvertebrates present in the sample were picked out and identified. Identification was generally taken to species level, where it was reasonably practical to do so in the time available, and based on the guidance on taxonomic identification levels required as a minimum for ECN (Sykes et al. 1999). For more difficult taxa, for example, Chironomidae, Oligochaeta and Hydracarina, and early instars of some insect larvae, for example, Limnephilidae cased caddis, identification was taken to genus, family or order level, as necessary. The number of macroinvertebrate taxa and the mean total macroinvertebrate abundance for both lakes were compared, pre- and post-Phoslock® application.

3.3.8 Macrophyte community responses

The annual aquatic macrophyte surveys of Hatchmere and Mere Mere were based on the Joint Nature Conservation Committee (JNCC) Common Standards Monitoring (CSM) methodologies (JNCC 2005, 2015). The CSM methodology, which was originally developed by Gunn et al. (2004), now forms the basis for both WFD monitoring (WFD-UKTAG 2014a) and site condition monitoring (SCM) of aquatic macrophyte surveys of standing waters in the UK. The CSM methods, which are based on sampling representative sectors of a lake, are designed to be practical and efficient, with the aim of producing quantitative data that are both suitable for characterising the aquatic macrophyte community and statistically robust enough to detect real changes over time (Gunn et al. 2010). This survey methodology does not aim to try and record all the aquatic macrophyte species that are present in a lake. The three elements of the aquatic macrophyte survey methodology (i.e. perimeter strandline searches, shore-wader depth transect surveys and boat-based depth transect surveys) are described in detail by Spears et al. (2013a). These survey methods were successfully used in a similar project to assess the responses of aquatic macrophytes in Loch Flemington in Scotland to the application of the P-capping agent Phoslock® (Gunn et al. 2014). The pre-application aquatic macrophyte surveys of Hatchmere and Mere Mere were carried out on 30 and 31 July 2012, respectively. The post-application aquatic macrophyte surveys of Hatchmere were carried out on 30 July 2013 and 28 July 2014. The post-application aquatic macrophyte surveys of Mere Mere were carried out on 31 July 2013 and 29 July 2014.

The results of the 2012 pre-application and 2013–14 post-application aquatic macrophyte surveys of Hatchmere and Mere Mere were examined by comparing against the data collected from earlier surveys of each lake. For both Hatchmere and Mere Mere, the 2012, 2013 and 2014 aquatic macrophyte survey data were compared primarily with CSM survey data documented in unpublished survey reports for both lakes (UCL 2007, 2008). These CSM surveys used the same survey methods and similar locations as employed in this study. In all these surveys, of both Hatchmere and Mere Mere, a maximum of three sectors were surveyed rather than the ‘normal’ target of four survey sectors. Adopting this survey approach at both lakes ensured a roughly similar survey effort per lake (i.e. about one survey sector per 300–500m length of accessible lake perimeter), thereby allowing direct data comparisons. Supplementary historical information on macrophyte species previously recorded at both sites, was also consulted (G. Madgwick, personal communication).

3.3.9 Water Framework Directive assessment using phytoplankton metrics

Phytoplankton metrics for each lake were calculated following UK biological standards for WFD using the PLUTO tool (WFD-UKTAG 2014b). In short, the metrics assess phytoplankton abundance, phytoplankton composition and bloom frequency and intensity (Carvalho et al. 2013). Phytoplankton abundance was assessed using monthly Chl*a* concentrations, although this dataset was incomplete. The composition metric required biovolume data from summer (July to September) phytoplankton samples and a taxonomic-based sensitivity index, the phytoplankton trophic index (PTI), for its assessment. Finally, the bloom metric was assessed using cyanobacterial biovolume data from the summer months.

Each metric was used to calculate an ecological quality ratio (EQR) based on the ratio of observed to predicted reference values. The reference values used were based upon lake type, which was determined using lake predictor variables: alkalinity (ideally, annual mean), mean lake depth and water colour (ideally, annual mean). Each EQR was then used to assign an ecological status class (high, good, moderate, poor or bad) to each metric, and finally an overall status class for each lake based on the combined metric result (WFD-UKTAG 2014b).

3.3.10 Water Framework Directive assessment using macrophyte metrics

In order to evaluate if there had been any responses in the aquatic macrophyte communities of Hatchmere and Mere Mere, both lake macrophyte assemblages were assessed using the current UK WFD-compliant Lake LEAFPACS2 methodology (WFD-UKTAG 2014a). Lake LEAFPACS2 was designed to primarily detect the impact of nutrient enrichment on the condition of the macrophyte and phytobenthos quality element although it may also detect the impact of other pressures or combination of pressures by combining information on the five metrics listed below:

- lake macrophyte nutrient index (LMNI)
- number of functional groups of macrophyte taxa (NFG)
- number of macrophyte taxa (NTAXA)
- mean percentage cover of hydrophytes (COV)
- relative percentage cover of filamentous algae (ALG)

The LMNI scores are based on the unweighted average of annual mean TP concentrations of lakes in which aquatic macrophyte species were reported in archived data. The number of functional groups of macrophyte taxa (NFG) is a diversity metric with individual taxa allocated to one of 18 ‘functional groups’. The number of macrophyte taxa (NTAXA) is another diversity metric indicating the number of scoring taxa recorded in the field survey. Both the mean percentage cover of hydrophytes (COV) and the relative percentage cover of filamentous algae (ALG) metrics are derived from the lake macrophyte survey data.

Each macrophyte metric was used to calculate an EQR based on the ratio of observed to predicted reference values. The reference values used were based upon lake type, which was determined using the following lake predictor variables: alkalinity (ideally, annual mean), mean lake depth, lake altitude, lake surface area and freshwater sensitivity class (i.e. the relative capacity of geology and soils to neutralise incoming acidity and hence limit acid loadings to fresh surface waters). The EQRs for the different metrics were combined to produce an overall EQR that was then used to assign an overall ecological status class (high, good, moderate, poor or bad) for each lake (WFD-UKTAG 2014a).

3.3.11 Monitoring of fish

After the first year of monitoring it was clear that the assessment of fish populations for this project presented several significant challenges. These included (a) the longevity of individual fish which 'dampens' the responses of populations to environmental changes, (b) the lack of established WFD methodologies and targets, (c) more general technical sampling problems which vary by specific water body, and (d) the acceptability or otherwise of destructive sampling such as gill netting which also varies by water body. In water bodies in some other areas of the UK and in many places elsewhere in Europe, such lake fish sampling is being carried out mainly by standardised gill netting, hydroacoustics or the combination of these two approaches (e.g. Winfield et al. 2009, Jeppesen et al. 2012, Winfield et al. 2012, Winfield et al. 2013, Bonar et al. 2017) although a recent review shows that point abundance electric fishing may also be appropriate at shallow sites (Perrow et al. 2017).

Hatchmere was considered too shallow for typical vertical hydroacoustics and in addition this issue would be further compromised, at least partially, by macrophyte or macroalgae growths for horizontal hydroacoustics. In addition, gill netting was deemed unacceptable to local stakeholders at both Mere Mere and Hatchmere. Fyke netting was selected as the only feasible sampling method and was deployed at each site in the summer of 2012. However, catches at all three sites were very low and gave only weak and vague descriptions of the fish communities present in each location. As such, available data on fish communities in both treated lakes is of very low confidence.

Options for assessing responses in the fish community for the duration of the project were reviewed and a number of recommendations (outlined below) were made:

- **Further fyke netting:** One option was to carry on with initial plans and repeat the first year of fyke netting at the two treated water bodies. However, given the very low catches experienced in 2012 this was not recommended as an effective use of resources. After considered reflection, it was concluded that robust results using this approach would require an increase of sampling effort of greater than an order of magnitude which was unlikely to be feasible due to resource and other constraints. Further fyke netting was, thus, not recommended.

- **Change of monitoring to make comparable with existing data:** Some fish data obtained by APEM Ltd and the Environment Agency by seine netting and hydroacoustics already existed for both Hatchmere and Mere Mere. However, for reasons given above these data had their limitations and their adoption for post-treatment monitoring in the present project was deemed to be of questionable scientific rigour. Adoption of these techniques was, thus, not recommended.

- **Change of monitoring to assess WFD targets:** WFD targets for lake fish populations and communities are yet to be defined and are the subject of continuing research and discussion within the Environment Agency. As a result, post-treatment monitoring could not be changed to assess WFD targets because they are not yet established.

- **Options for other work:** Given the above effectively insurmountable challenges to robust post-treatment monitoring of fish in the two study sites, other options for further fish work were considered. Firstly, a laboratory-based experimental study could have been undertaken on the effects of Phoslock® on fish. However, such work would have been resource-intensive and for practical reasons would have to been carried out on species (such as three-spined sticklebacks (*Gasterosteus aculeatus*)) in relatively small aquaria of debatable relevance to the conditions in a treatment site. Secondly, a literature review of such effects, and the related effects of inert suspended sediments and La on selected lake fish species, could be undertaken and this course of action was recommended. Thirdly, under-yearling fish sampling, using either proven point abundance electric fishing or some unproven form of fry trap, could have been undertaken. Such work would had relevance to the assessments of the current project, although pre-treatment data was unavailable, and also for the more general sampling for lake fish assessments under the WFD. However, given the naturally variable levels of under-yearling abundance, correspondingly high levels of sampling effort that would have been required and the limited project resources, such activities were difficult to justify robustly within the present project.

- **Recommendations:** Following an assessment of the options above it was recommended that contemporary fish monitoring field surveys be removed from the project and replaced with a review of literature of effects of inert suspended sediments and La on selected lake fish species in the context of chemical and ecological monitoring data in Mere Mere and Hatchmere.

4 Results

4.1 Physicochemical responses in the water column

4.1.1 Before-after assessment of seasonal weather conditions during the contemporary monitoring period

The differences between seasonal values in the two post-application years were compared with seasonal values during the pre-application year and also with long-term average conditions (i.e. January 2003 to March 2013). Results of the BA analysis of weather conditions indicated that seasonal conditions of air temperature, wind speed and total monthly rainfall followed similar seasonal patterns in each year and were not significantly different between years. However, a comparison of post-application conditions against long-term data indicated that conditions in spring of 2012 (pre-application year) and 2013 (first post-application year) were significantly drier and colder than the long-term average for the respective weather station sites. The winter of 2014 (second post-application year) was significantly warmer than the long-term average. Given the results across the treatment years indicated no significant differences across seasons it is unlikely that weather conditions during the experiment were the cause of any responses detected.

4.1.2 Before-after assessment of seasonal and annual physicochemical responses following Phoslock® applications

Average quarterly bottom water DO (dissolved oxygen) concentrations are shown in Figure 4.1 and vary with bottom water depth. In Hatchmere, no significant differences were observed between treatment years and DO concentrations at 1.1m, 1.7m and 2.1m were significantly higher than the deeper three sites across the treatment years. In Mere Mere, again no significant differences in DO concentrations were observed following the application and the shallowest two sites at 1.1m and 2.5m had DO concentrations that were significantly higher than all other sites across the treatment years.

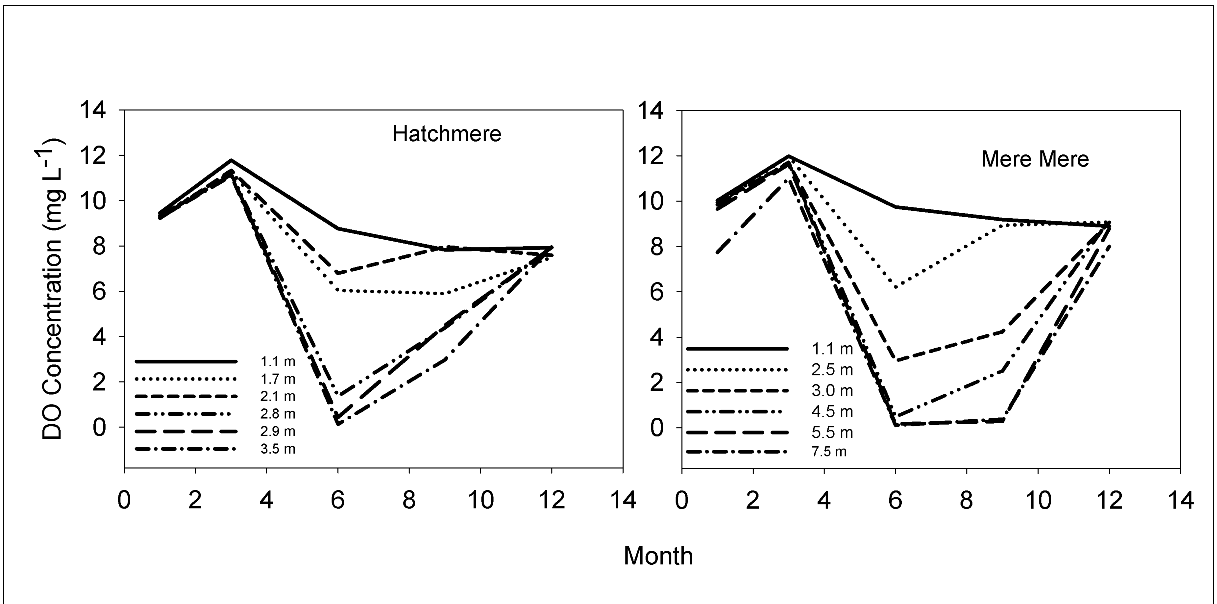

Figure 4.1 Average quarterly dissolved oxygen (DO) concentrations across all treatment years and sample depths. Average quarterly concentrations of DO across all treatment years for bottom waters are shown

Time series, at roughly monthly frequency, of surface water TP, Chl*a*, ortho-P, TON, TN, Chl*a*:TP, ortho-P:TP and TN:TP are presented for Hatchmere (Figure 4.2) and Mere Mere (Figure 4.3). A reduction in summer and autumn TP, ortho-P and Chl*a* concentration was expected in response to a reduction in internal P loading. In addition, where ortho-P was reduced we would expect to see an increase in the ortho-P:TP ratio and where P limitation of phytoplankton was enhanced through P reductions we would expect to see an increase in Chl*a*:TP and TN:TP ratios.

Although no clear responses were apparent in any of the surface water determinands following Phoslock® application in these time series, some subtle changes are evident and discussed below. This is in the context of other study sites where ortho-P, and to a lesser degree TP and Chl*a* concentrations, have reduced markedly and rapidly following treatment (Spears et al. 2016). A decrease in ortho-P concentration in winter was apparent in Hatchmere. In addition, evidence of high ortho-P concentrations in August in the pre-application year (> 25µg L^{-1}) was largely absent in both post-application years, perhaps indicating reduced release of ortho-P from bed sediments. Peak winter ortho-P concentrations were also lower (about half of pre-application values which exceeded 40µg L^{-1}) following the application in Hatchmere. Values of ortho-P:TP ratio appeared to decrease both in the summer low values and winter peak values in Hatchmere following the application. However, Chl*a* concentrations appeared to increase during spring and summer following the applications in Hatchmere. An apparent general decline in TP concentrations was observed from the Phoslock® application date in March 2013 to July 2014 in Hatchmere, although TP concentrations again increased in autumn of the same year, albeit to lower peak concentrations when compared to pre-application conditions. The responses of TP and Chl*a* concentrations resulted in an increase in the ratio of the two during summer and autumn of the years following the application. The general trend in Chl*a*:TP and in TN:TP indicate an increase in the likelihood of P limitation of phytoplankton in summer and autumn in Hatchmere, and this apparently increased following the application. An increase in winter TON and TN concentrations were observed in the years following application, perhaps indicating reduced demand for nitrogen as phosphorus becomes more limiting relief from N limitation of phytoplankton in those years resulting in increased phytoplankton yield. This was confirmed by an increase in the peak value of the TN:TP ratio during spring and summer of post-application year 2.

In Mere Mere, high ortho-P concentrations in winter were apparent both before and after the application, although sporadic increases during summer were largely absent during post-application years. With the exception of a peak value shortly following the Phoslock® application, TP concentrations remained low relative to the pre-application year (i.e. below about 60µg L^{-1}) in the years following the application. The ortho-P:TP ratio indicated a decrease in ortho-P relative to TP during summer months following the application. Chl*a* concentrations were generally low both before and after the application, with the exception of a peak value in autumn of the pre-application year. TON concentrations indicated N limitation of the phytoplankton community during summer of all years and winter maximum concentrations were lower following the application, compared to pre-application conditions. However, TN concentrations during summer appeared to increase following the application. The Chl*a*:TP ratio appeared to be higher during summer in post-application years compared to pre-application conditions and a general increase in the TN:TP ratio was also observed following application, perhaps indicating an increased likelihood of P limitation of phytoplankton.

Although no clear responses are evident in the raw data, the results of the Before-After analysis (Appendix 2) to assess seasonal and annual responses following Phoslock® application in post-application years 1 and 2 relative to pre-application year 0 indicate statistically significant differences. The magnitude of these changes relative to pre-application conditions are discussed in the following sub-sections, the Before-After analysis including transformation of the data prior to analysis. Significant decreases in ortho-P were confirmed in annual, winter, summer and autumn periods in Hatchmere in post-application years 1 and 2. Similar responses in Mere Mere were confirmed for winter, summer and autumn in year 1, but only for summer in year 2. Decreases in annual and summer TP concentrations were confirmed for both lakes in both post-application years, with the exception of summer in year 2 for Hatchmere, where no difference was reported. Increases in Chl*a* concentration were confirmed in annual and summer conditions in Hatchmere for both post-application years. However, Chl*a* concentrations decreased significantly in Mere Mere in autumn of both post-application years. TON concentrations decreased significantly in Hatchmere in summer of year 2 and a decrease in annual TON concentrations was confirmed in Mere Mere in both post-application years. A decrease in DOC concentration was confirmed across all seasons and annual concentrations for both lakes in both post-application years, with the exception of annual conditions and summer conditions in Mere Mere in year 2, and winter conditions in Hatchmere in year 1. No significant differences in Secchi disk depth were reported at an annual period (Figure 4.4).

The Chl*a*:TP ratio increased in Hatchmere during all years and seasons after application except for the autumn of year 1 and both years during winter. There was no change in the Chl*a*:TP ratio during any year or season for Mere Mere. In Hatchmere, the SRP:TP ratio increased during all seasons and annually, with the exception of autumn in both years and the year 2 winter conditions. The SRP:TP ratio did not change significantly following the application in Mere Mere. The TN:TP ratio increased in Hatchmere only during winter of post-application year 1. However, in Mere Mere TN:TP increased in both years during winter and year 2 of summer and annual responses. There was no significant change in TON:SRP ratios in either lake, with the exception of winter in post-application year 1 in Hatchmere.

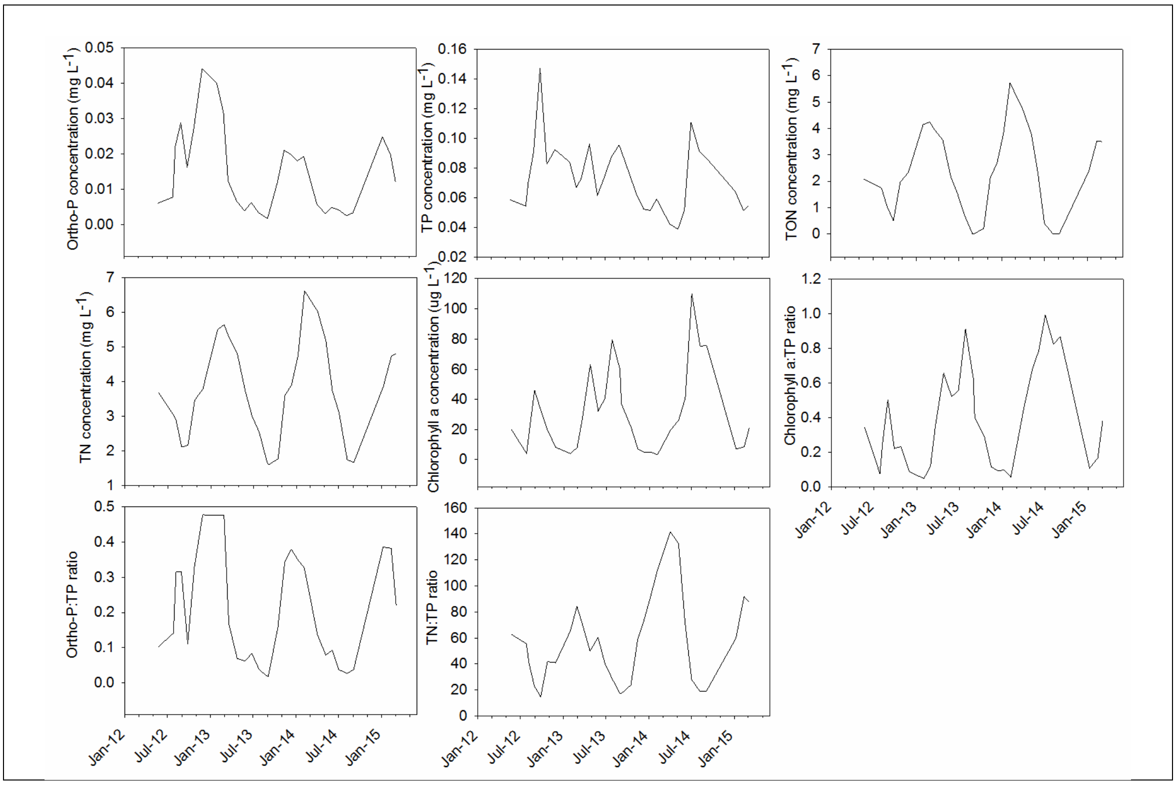

Figure 4.2 Raw data (2012–2015) for Hatchmere of orthophosphate-P (ortho-P), total phosphorus (TP), total oxidised nitrogen (TON), total nitrogen (TN) and chlorophyll *a* concentrations, and chlorophyll *a*:TP, ortho-P:TP and TP:TN ratios. The Phoslock application commenced 11 March 2013 and finished 13 March 2013.

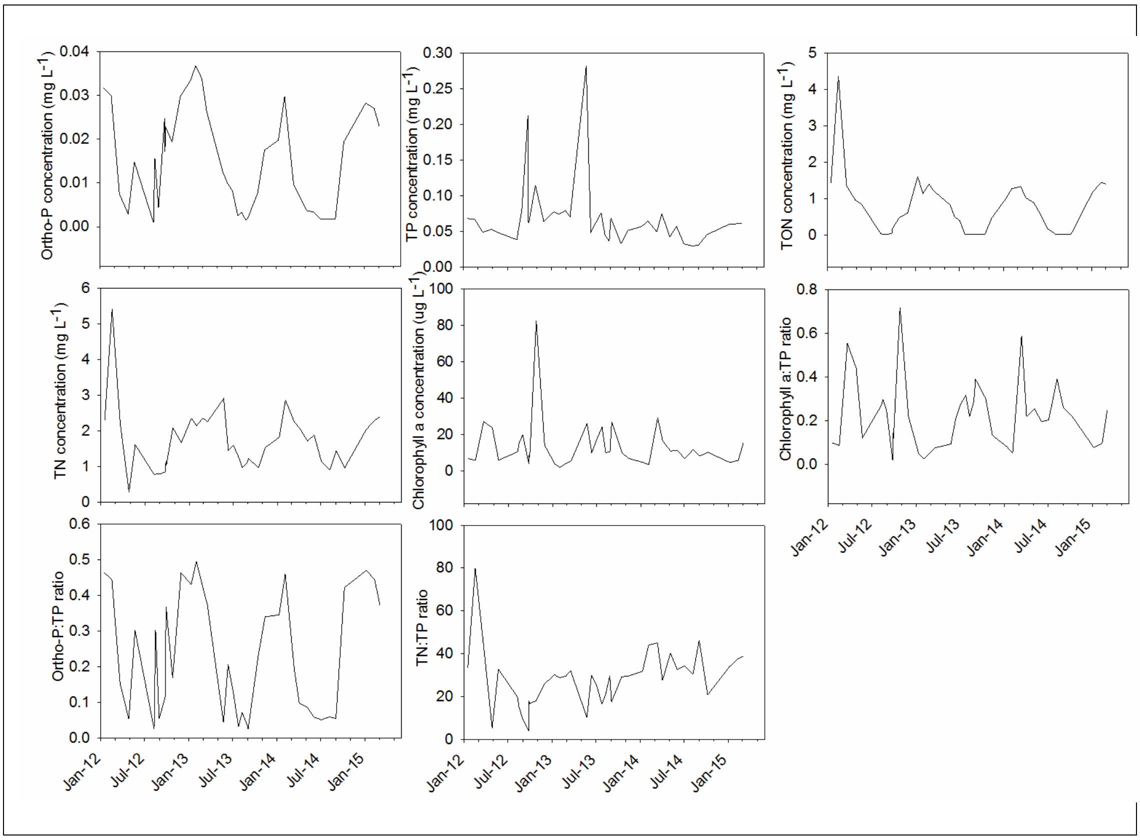

Figure 4.3 Raw data (2012–2015) for Mere Mere of orthophosphate-P (ortho-P), total phosphorus (TP), total oxidised nitrogen (TON), total nitrogen (TN) and chlorophyll *a* concentrations, and chlorophyll *a*:TP, ortho-P:TP and TP:TN ratios. The Phoslock application commenced 4 March 2013 and finished 8 March 2013.

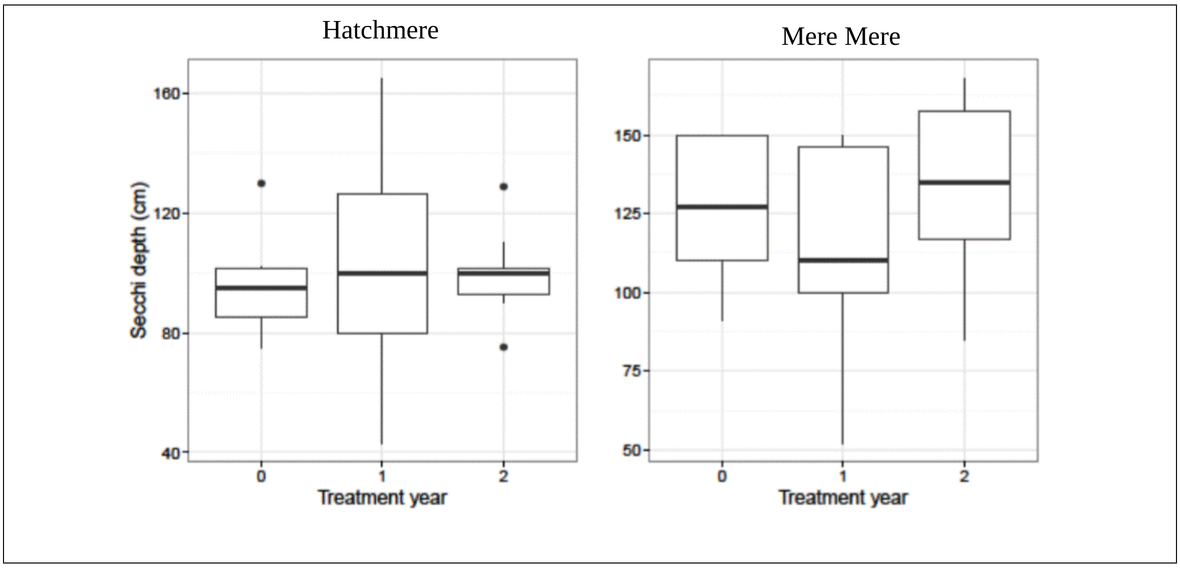

Figure 4.4 Ranges for Secchi disk depth recorded in Hatchmere and Mere Mere between June 2012 and March 2013 (i.e. pre-application year 0), April 2013 and March 2014 (post-application year 1) and April 2014 and December 2014 (post-application year 2). The 25th, 50th and 75th percentiles are indicated as the lower to upper lines on the box; the 10th and 90th percentiles as lower and upper limits of lines; outliers are shown.

4.1.3 Assessment of change in physicochemical conditions following Phoslock® application relative to pre-application conditions

Consideration of the general seasonality of Chl*a*, ortho-P, TP and TON can be useful when interpreting the drivers of water quality changes in shallow temperate lakes (Figure 4.5). For example, high concentrations of ortho-P and TP in winter may be related to high catchment loads during periods of high run-off from surrounding land related to elevated rainfall. In contrast, where summer and autumn concentrations of ortho-P and/or TP are high, internal P loading is likely to be the main driver of water quality, when run-off from the surrounding land is low. Where internal loading has been reduced but catchment loading has not, a reduction in summer ortho-P and TP are expected. In addition, where the phytoplankton primary production is predominantly P limited in summer and autumn, a corresponding reduction in Chl*a* concentration is expected. Although catchment P loading assumptions will be confirmed within the project in the form of comprehensive catchment P loading surveys, we examine the evidence drawn from in-lake data here. Consideration of relative changes in TP, Chl*a* and ortho-P concentrations throughout the year can be useful when exploring responses following Phoslock® application in the context of the hypotheses above and this analysis is also presented (Figure 4.5).

In Hatchmere, ortho-P concentrations were lower during June to October, following the application (Figure 4.5). In Mere Mere, ortho-P concentrations were also lower, following the application, between July and November. Processes acting to reduce ortho-P concentrations during this period can include uptake into macrophytes and phytoplankton, and in the case of these lakes the action of Phoslock® reducing sediment ortho-P release. Where uptake into phytoplankton is the main driver of ortho-P concentrations an increase in Chl*a* and TP concentrations would be expected to accompany the reduction in ortho-P. The lower TON concentrations following the application in Hatchmere may also indicate a period of N limitation of the phytoplankton community during the period when ortho-P concentrations were also low. This is in agreement with the nutrient limitation assays reported in section 3.4. Therefore, it is difficult to conclude that the lower post-application Chl*a* concentrations observed in autumn in Hatchmere were caused by reduced internal P loading. In contrast, TON concentrations in Mere Mere were similar before and after the application and so it is likely that the reduced Chl*a* and TP concentrations during July to October were associated with the reduction in ortho-P concentration, and, therefore, may be linked to reduced internal P loading.

In both Mere Mere and Hatchmere ortho-P concentrations followed a common general trend of high winter concentrations decreasing to lower summer/autumn concentrations, both before and after the application (Figure 4.6). This trend indicates persistent loading of ortho-P from the catchment and either relatively low internal loading in summer or autumn or efficient sequestration into the phytoplankton during this period. Given that trends in Chl*a* concentration increase towards summer and autumn the latter process is most likely to be important in both lakes. However, TP concentrations before the application in both sites were lower in winter and spring and higher in summer and autumn. This trend in TP indicates internal loading as the main driver of both TP and Chl*a* concentrations in these sites. Based on these general observations we propose that persistent catchment loading is apparent in both sites and that the Phoslock® application is likely to have reduced internal loading to elicit a response in Chl*a* and TP concentration in Mere Mere, but not Hatchmere.

The extent and timing of changes varied between lakes (Figure 4.6). Reductions in ortho-P were apparent across most months following the Phoslock® application and were most intense in January and August in Hatchmere and November in Mere Mere. Ortho-P reductions were greatest in summer (August) in Hatchmere (reaching about 10µg L^{-1}) although a significant reduction in concentration in January (reaching about 40µg L^{-1}) was also reported following the application. In Mere Mere ortho-P concentration reductions were greatest in autumn and winter (reaching 16µg L^{-1}) compared to long-term average conditions. Reductions in TP concentrations following Phoslock® application reached around 40µg L^{-1} in autumn for both lakes, although this reduction appeared more sustained from month to month in Mere Mere. When compared against long-term monthly average values, Chl*a* concentrations were higher following the application in Hatchmere between April and August (up to 25µg L^{-1} higher following application) but in May only (5µg L^{-1} higher post-application) in Mere Mere.

4.1.4 Changes in annual mean ortho-P, TP and chlorophyll *a* concentrations in the context of WFD targets

The annual mean (i.e. April of 2012 to March of 2013) ortho-P concentration in Hatchmere decreased from 24.9µg L^{-1} (average of 25.3µg L^{-1} for all pre-application years with available data; n = 4) in the pre-application year to 8.9µg L^{-1} by post-application year 2. Annual mean TP concentration in Hatchmere was 83.2µg L^{-1} (average of 76.6 for all pre-application years with available data; n = 3) before the application, declining to 64.4µg L^{-1} by post-application year 2, this concentration being higher than the WFD good/moderate lake type specific target for these lakes. Annual mean Chl*a* concentration in Hatchmere increased from 17.8µg L^{-1} (average of 38.0µg L^{-1} for all pre-application years with available data; n = 3) before the application to 40.8µg L^{-1} by post-application year 2 compared to a WFD good–moderate target of 16.5µg L^{-1} (Table 4.1).

In Mere Mere, annual mean ortho-P concentrations decreased from 18.9µg L^{-1} (average of 24.4µg L^{-1} for all pre-application years with available data; n = 6) in the year prior to the application to 11.6µg L^{-1} by post-application year 2. Annual mean TP concentrations decreased from 76.6µg L^{-1} (average of 65.9µg L^{-1} for all pre-application years with available data; n = 6) before the application to 49.8µg L^{-1} by post-application year 2. Annual mean Chl*a* concentration in Mere Mere decreased from 16.4µg L^{-1} (average of 20.6µg L^{-1} for all pre-application years with available data; n = 6) before the application to 11.8µg L^{-1} by post-application year 2 (Table 4.1).

It should be noted that the annual mean concentrations reported here are not directly comparable to the WFD targets which are designed to be compared against average values across multiple years. The values here are based only on single-year averages.

Table 4.1 Annual mean (March to March of each year) orthophosphate-P (ortho-P), total phosphorus (TP) and chlorophyll *a* (Chla) concentrations for Hatchmere and Mere Mere. Year 0 indicates pre-application conditions and years 1 and 2 are post-application years. Water Framework Directive type-specific targets for good–moderate (G/M) are shown for comparison only

	Year	Hatchmere	Mere Mere	WFD type G/M
Ortho-P	0	24.9	18.9	
(µg L^{-1})	1	10.5	11.7	
	2	8.9	11.6	
TP	0	83.2	76.6	49.0
(µg L^{-1})	1	73.3	74.9	49.0
	2	64.4	49.8	49.0
Chla	0	17.8	16.4	16.5
(µg L^{-1})	1	31.6	12.9	16.5
	2	40.8	11.8	16.5

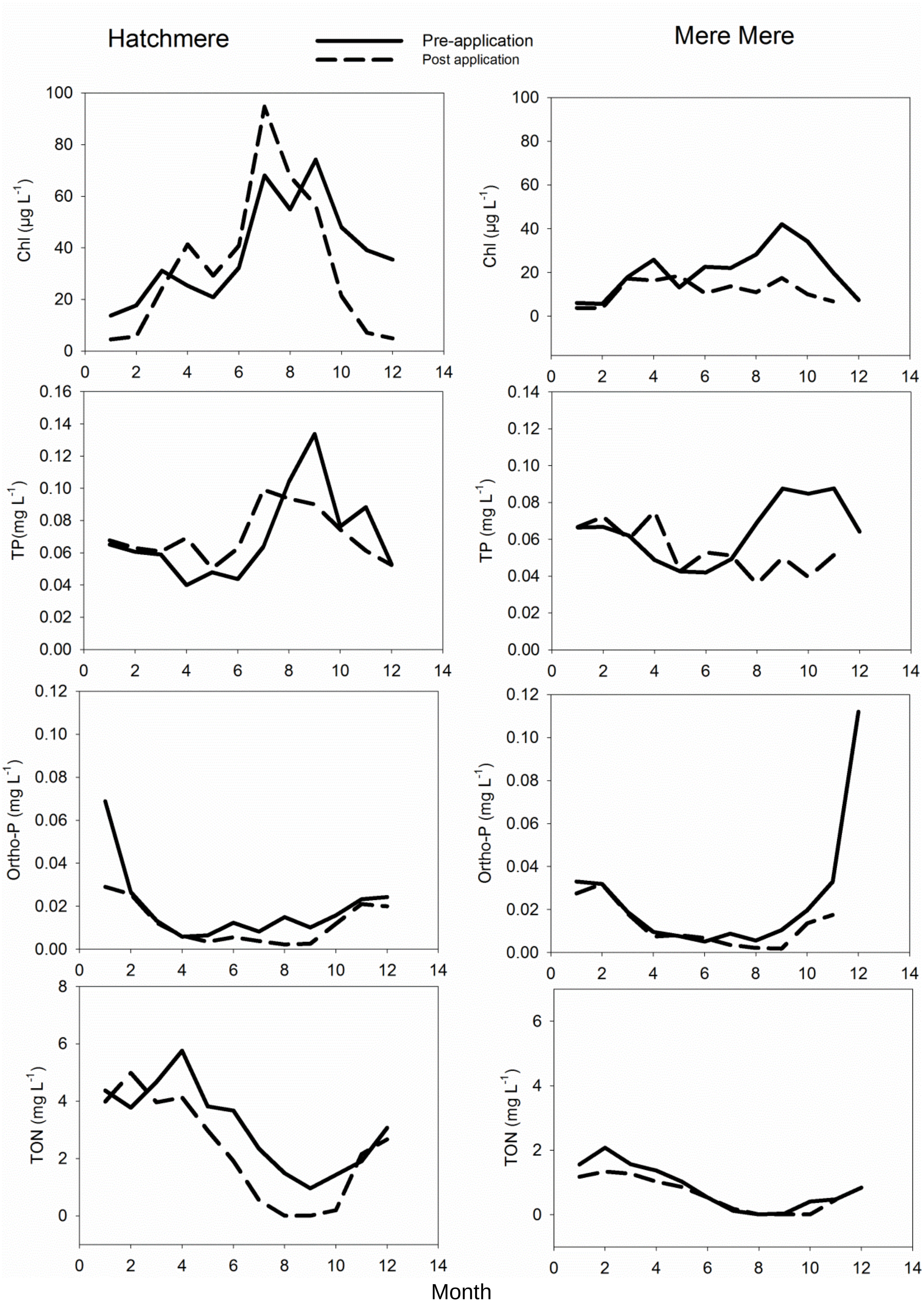

Figure 4.5 Plots of average values for each month across both before (average of monthly values for 2006–2008, 2012 for Hatchmere and 2006–2012 for Mere Mere) and after (April 2013 to March 2015) Phoslock® application for Hatchmere and Mere Mere . Determinands include chlorophyll *a* (Chl), total phosphorus (TP), orthophosphate-P (ortho-P), and total oxidised nitrogen (TON)

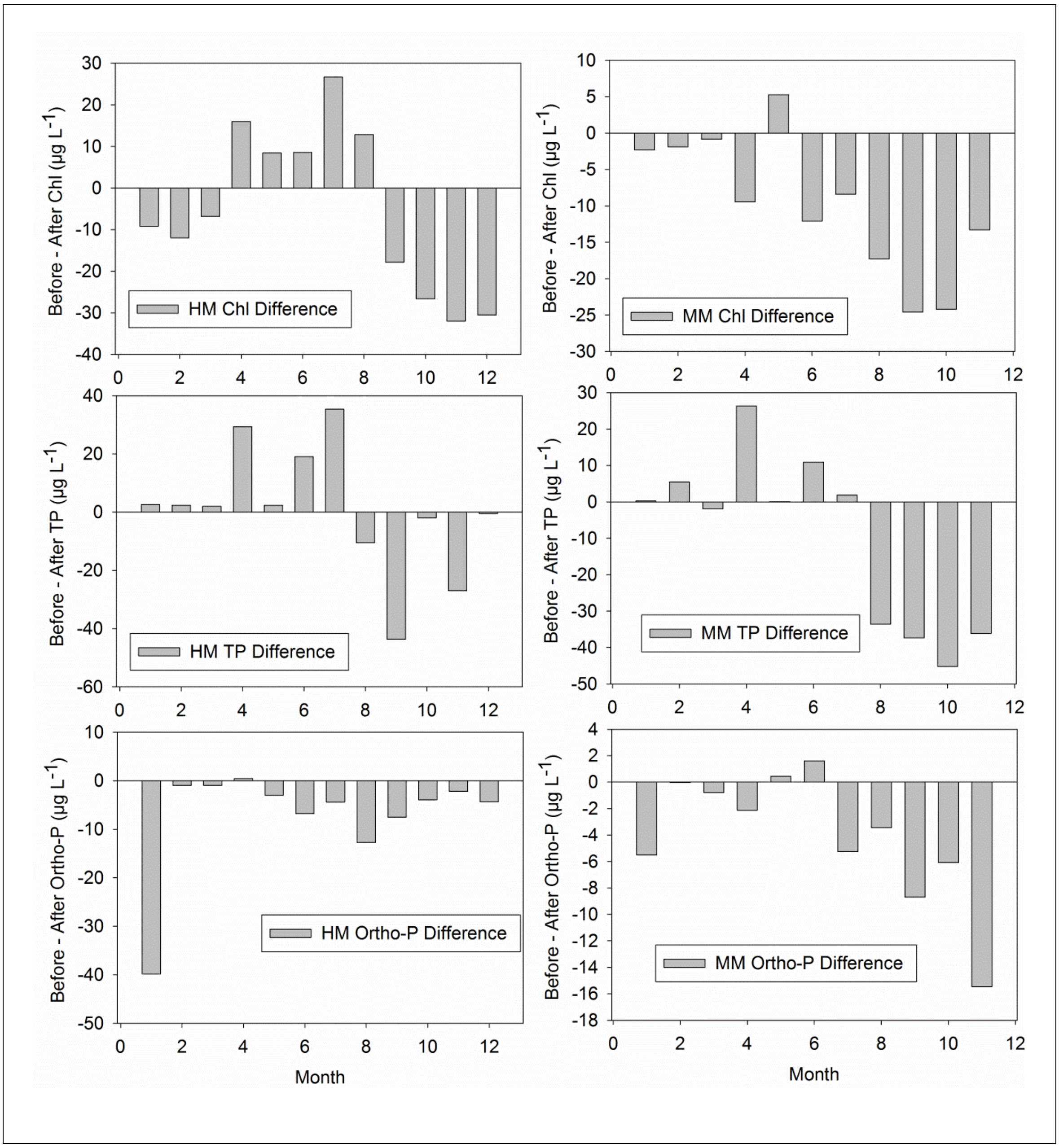

Figure 4.6 Difference between before (average of monthly values for 2006–2008, 2012 for Hatchmere and 2006–2012 for Mere Mere) and after (April 2013 to March 2015) Phoslock® application monthly values for chlorophyll *a* (Chla), total phosphorus (TP) and orthophosphate-P (ortho-P) concentrations for Hatchmere (HM) and Mere Mere (MM). Negative values indicate a post-application reduction in the determinand relative to pre-application conditions and vice versa

4.1.5 Responses of bottom water TP and ortho-P concentrations following Phoslock® applications

To examine further the evidence for reduced internal P loading in Mere Mere and Hatchmere the concentrations of TP and ortho-P recorded in the water overlying the sediment surface are plotted across depth gradients both before and after the Phoslock® application (Figure 4.7). Where internal P loading has been controlled, a reduction in TP, and especially ortho-P, would be expected. This reduction would be expected to be most intense in deeper water sediment zones where reducing conditions, and therefore high rates of ortho-P release, are most likely. In Mere Mere, TP concentrations were similar in bottom waters at lake depths of up to 3m, and at 7.5m. However, concentrations were higher at 5.5m lake depth following the application. Ortho-P concentration in the bottom waters of Mere Mere were low following the application up to about 4.5m overlying water depth and were similar to pre-application conditions at 5.5m and 7.5m overlying water depth. These results indicate that a reduction in ortho-P concentrations was, at least partly, achieved in the shallower waters of Mere Mere but that the treatment was insufficient to control ortho-P release from deeper water sediments up to 2 years following the application.

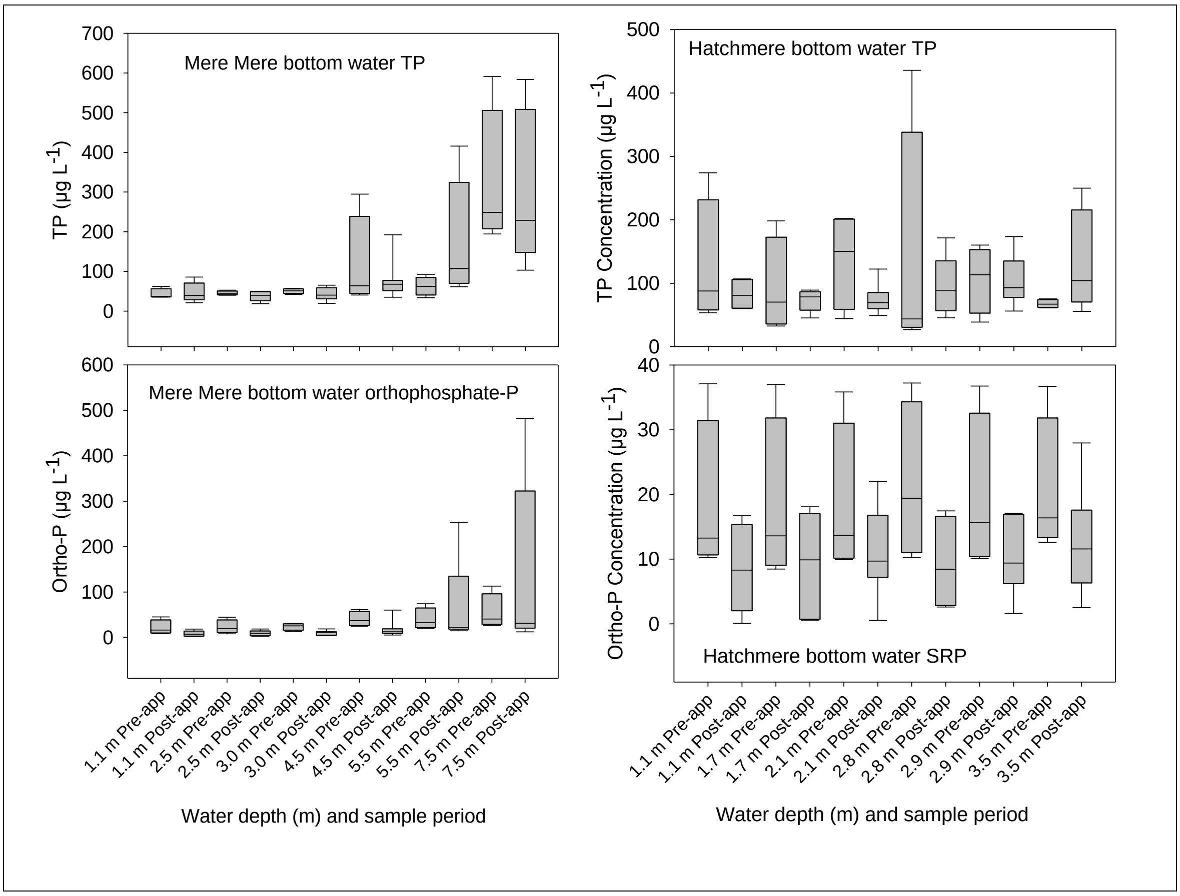

Figure 4.7 Ranges of bottom water total phosphorus (TP) and orthophosphate-P (ortho-P) concentrations with depth for Mere Mere and Hatchmere for pre-application (Pre-app: May and September 2012 and January and March 2013) and post-application (Post-app: May, September and December 2013 and March, June, September and December 2014) sample periods. The 25th, 50th and 75th percentiles are indicated as the lower to upper lines on the box; the 10th and 90th percentiles as lower and upper limits of lines.

In Hatchmere, TP concentrations were lower following the application in bottom waters up to 2.1m overlying water depth. Again, in deeper water sediment zones (i.e. 2.8m to 3.5m overlying water depth) no apparent reduction in TP concentrations was observed following the application. However, ortho-P concentrations were consistently lower following the application across the depth gradient. It should be noted that the range of bottom water ortho-P concentrations in Hatchmere was lower than the range of concentrations reported for Mere Mere, especially in deeper water sediment zones, indicating a lower internal loading rate in Hatchmere relative to Mere Mere. It is likely that the role of the sediments in driving water quality conditions should be more prominent in Mere Mere when compared to Hatchmere. This trend, coupled with the similar concentrations of ortho-P and TP across the depth gradients, also indicates that bed disturbance is unlikely to be a driver of bottom water conditions in Hatchmere.

BA ANOVA indicated that the differences between pre- and post-application ortho-P concentrations in Hatchmere and Mere Mere were significant across depths ($p < 0.001$, and $p = 0.03$, respectively), but no significant differences were indicated for TP in either lake. In addition, no significant interactions were reported for ortho-P or TP in either lake, although variation between sites was significant, regardless of treatment effect for TP ($p < 0.001$) and ortho-P ($p < 0.001$) in Mere Mere only.

4.1.6 Lanthanum responses – before-after analysis

Total lanthanum (TLa) and filterable lanthanum (FLa) concentrations in Mere Mere and Hatchmere are shown in Figure 4.8. Prior to the Phoslock® application both TLa and FLa concentrations did not exceed 0.5µg L^{-1} in Hatchmere and 0.2µg L^{-1} in Mere Mere. Following the Phoslock® application in March 2013 TLa concentrations increased rapidly to 1,250µg L^{-1} in Mere Mere and 1,760µg L^{-1} in Hatchmere before steadily declining towards June 2014. TLa concentrations fell to 18µg L^{-1} and 28µg L^{-1} in Mere Mere and Hatchmere, respectively, by March 2015. A similar result was reported for FLa, where concentrations peaked at 961µg L^{-1} and 140µg L^{-1} in Mere Mere and Hatchmere, respectively. FLa concentrations declined to 8µg L^{-1} and 7µg L^{-1} for Mere Mere and Hatchmere, respectively, by March 2015. The implications of this response in La in relation to potential ecotoxicological effects are considered further in section 6.3 and more comprehensively for the two study sites by Winfield and Lofts (2015).

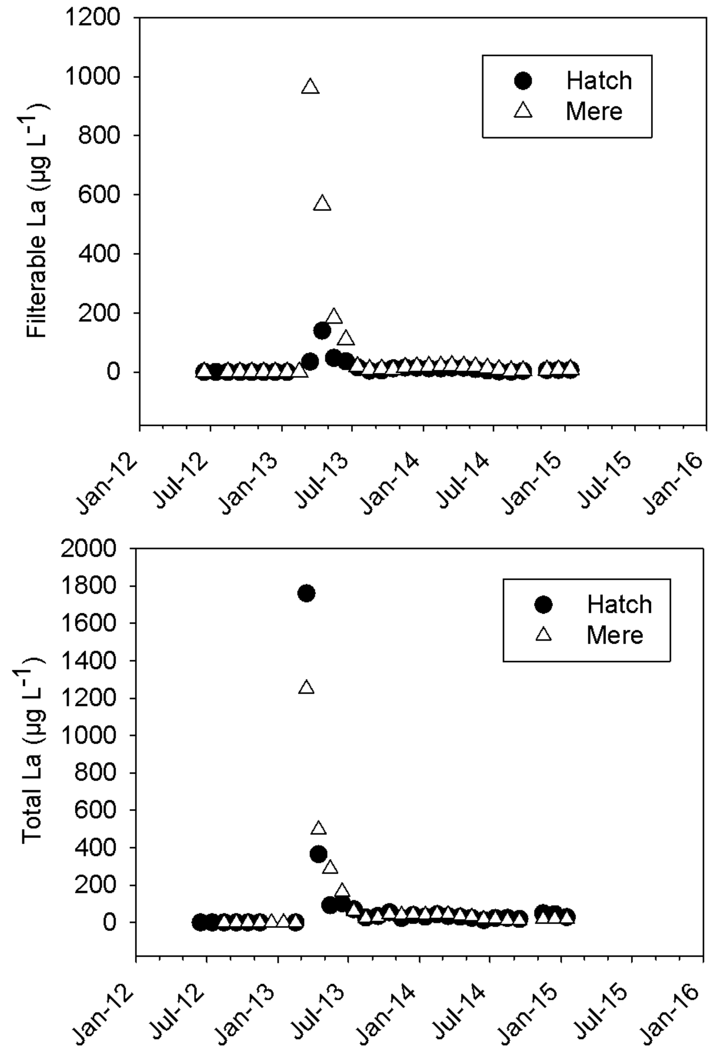

Figure 4.8 Monthly total and filterable lanthanum (La) concentrations in surface waters before and after Phoslock® application (March 2013) in Mere Mere and Hatchmere. The data run from June 2012 to March 2015

4.2 Responses in sediment P composition following Phoslock® applications

The variation in sediment P pools with depth and treatment year are shown for both lakes (Figures 4.9 and 4.10). Significant differences between years indicated using BA ANOVA are reported in Appendix 2. In general, a significant decrease in labile P (post-application year 1 for both lakes and year 2 for Hatchmere only) and an increase in metal adsorbed P (post-application year 2 for both lakes) were found following the application. An increase in apatite bound P (post-application year 2 only), organic P (post-application year 1 only) and TP (both post-application years) were reported also in Hatchmere. Significant effects of water depth were reported for all sediment P pools in Mere Mere ($p < 0.001$), regardless of treatment year, whereas this was true only for TP, labile P, reductant soluble P ($p < 0.001$) and apatite P ($p = 0.03$) pools in Hatchmere. Where variations with depth were reported this was generally reflected by an increase in the sediment P pool concentration with overlying water depth. No interaction between treatment year and depth was reported for any sediment P pool in either lake. Although no significant interaction between treatment year and depth were reported the effects of the La-bentonite are apparently more pronounced where P concentrations are highest. For example, in the deepest sample site of Mere Mere the largest effects between treatment years for TP, metal adsorbed P, apatite P and residual P pools were observed. These effects are less pronounced in Hatchmere where differences across the depths between years were less marked.

The changes in each P pool are also presented as P concentrations in Table 4.2. It is apparent that although statistically significant responses were observed across the pools, that the responses in metal adsorbed P and apatite P were dominant, in terms of mass of P. In March 2013, 79.8 tonnes and 25.2 tonnes of Phoslock® were added to Mere Mere and Hatchmere, respectively. Theoretically, assuming a P uptake capacity of 10.67mg P kg^{-1} Phoslock® based on La content, only (i.e. the general ratio of 100:1 used when calculating dose of the material), these amounts should have been sufficient to bind about 798 kg P in Mere Mere and 252 kg P in Hatchmere. Therefore, although the reduction in labile P indicates control of P release from bed sediments to water column (i.e. theoretically, P released from all other P pools has to pass through the labile P pool under undisturbed sediment conditions), the mass change indicated in Table 4.2 is negligible in terms of the mass of product added.

Table 4.2 Average concentrations of phosphorus across the operationally defined sediment pools. Average values are taken from six surface sediment (4cm) samples collected quarterly before (i.e. year 0) and after (i.e. years 1 and 2) the application of Phoslock® to Hatchmere and Mere Mere in March 2013. Numbers in brackets indicate the standard error across all sample dates and sites within a given year.

Phosphorus pool	Hatchmere (μg P g^{-1} dw)			Mere Mere (μg P g^{-1} dw)		
	Yr 0	Yr 1	Yr 2	Yr 0	Yr 1	Yr 2
Labile P	28 (5)	10 (1)	14 (2)	11 (3)	6 (2)	4 (1)
Reductant soluble P	813 (111)	791 (71)	886 (67)	850 (306)	852 (282)	1000 (358)
Metal adsorbed P	236 (46)	308 (61)	560 (59)	250 (65)	455 (180)	901 (323)
Organic P	311 (69)	680 (119)	235 (31)	398 (119)	527 (147)	381 (141)
Apatite bound P	230 (35)	334 (72)	514 (62)	171 (51)	291 (127)	643 (245)
Residual P	73 (8)	91 (10)	96 (7)	77 (15)	97 (27)	115 (32)
Total P	1,690 (195)	2,213 (218)	2,324 (198)	1,756 (492)	2,228 (710)	3,045 (1,086)

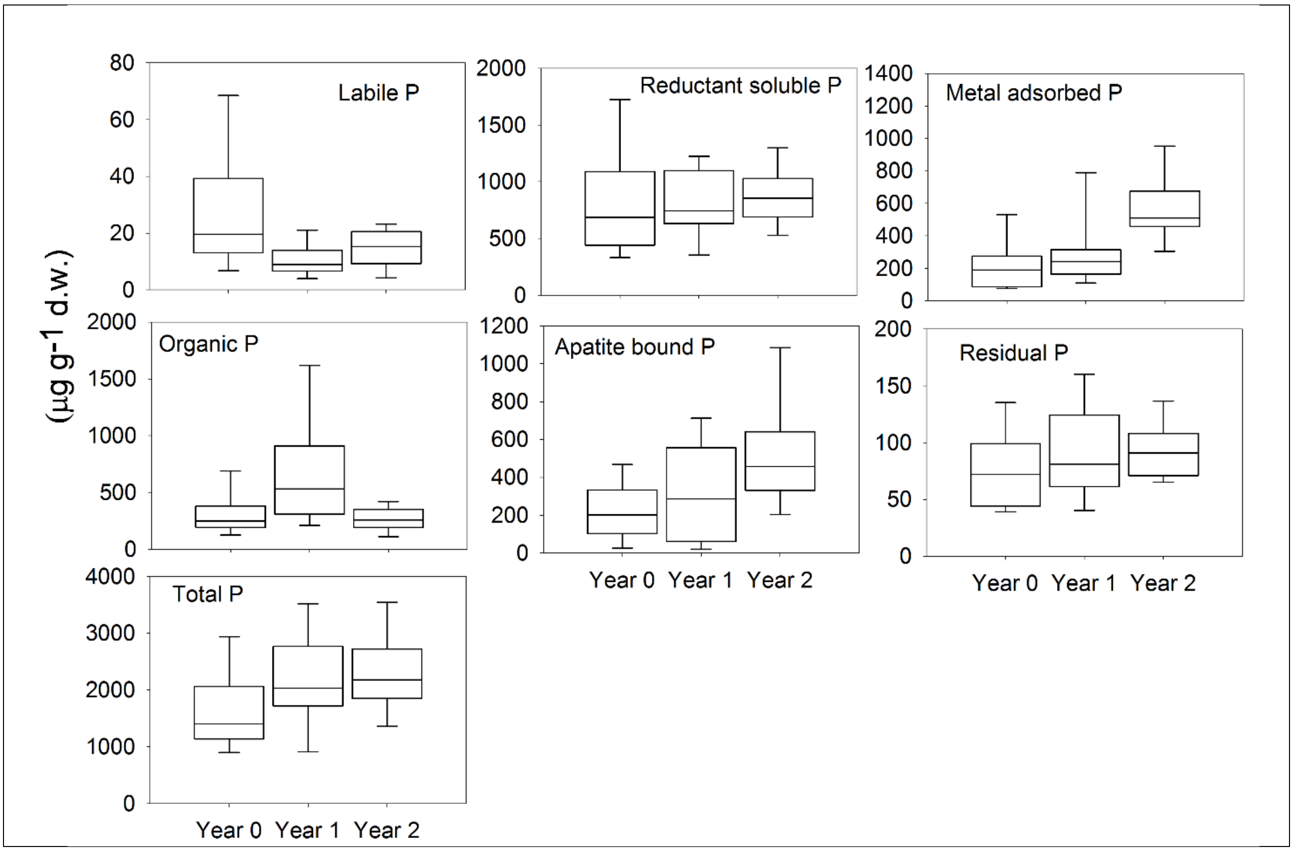

Figure 4.9 Hatchmere mean values of surface sediment phosphorus (P) fractions from quarterly sample dates across six water depths and three treatment years. Pre-treatment year 0; post-application year 1; post-application year 2. The 25th, 50th and 75th percentiles are indicated as the lower to upper lines on the box; the 10th and 90th percentiles as lower and upper limits of lines and 5th and 95th as dots are shown.

4.2.1 Responses in sediment P release following Phoslock® applications

The results of the intact sediment core experiments designed to identify persistent sediment P release following Phoslock® application are shown in Figure 4.11. Cores were collected in May 2014, following the application of Phoslock® to Hatchmere and Mere Mere in March 2013. The cores were incubated under laboratory conditions either at 4°C (n = 6 per lake) or 20°C (n = 6 per lake), in the dark, and maintained under anaerobic conditions by bubbling with N_2. A 'repeat' Phoslock® application was conducted on day 23 of the incubation as described earlier (section 3.3.4).

TP and ortho-P concentrations in the water overlying sediment within Hatchmere cores increased from day 1 of the incubation (about 20µg L^{-1}) at 20°C reaching a peak concentration of about 150µg L^{-1} and 75µg L^{-1}, respectively, by day 23. In contrast, TP and ortho-P concentrations decreased gradually throughout the incubation period in the 4°C treatment cores from Hatchmere. Following the repeat application on day 23, both TP and ortho-P concentrations in the 20°C treatment cores decreased markedly towards about 75µg L^{-1} and 25µg L^{-1}, respectively, towards the end of the incubation period. These results indicate that the original dose of Phoslock® applied to Hatchmere was insufficient to completely control sediment P release processes. Similar observations were reported for the Mere Mere cores, although the intensity of ortho-P and TP release in the 20°C treatment cores was higher, reaching concentrations of about 500µg L^{-1} and 525µg L^{-1}, respectively, by incubation day 23. As in Hatchmere, reductions in both ortho-P and TP concentration were observed following the 'repeat' dose although the final concentrations were, again, significantly higher in Mere Mere (i.e. about 200µg L^{-1} and 400µg L^{-1} for ortho-P and TP concentrations, respectively) compared to Hatchmere. TP concentrations in the 4°C treatment cores from Mere Mere gradually declined throughout the incubation although a steepening in this decline was apparent following the ‘repeat dose’. In contrast, ortho-P concentrations increased in the 4°C treatment cores from Mere Mere up to day 23 and decreased markedly following the ‘repeat’ dose on day 23, towards conditions at the start of the experiment. For Mere Mere, these results indicate that neither the original applied dose of Phoslock® or the addition of the ‘repeat’ dose were sufficient to fully control sediment P release processes, especially at higher temperatures.

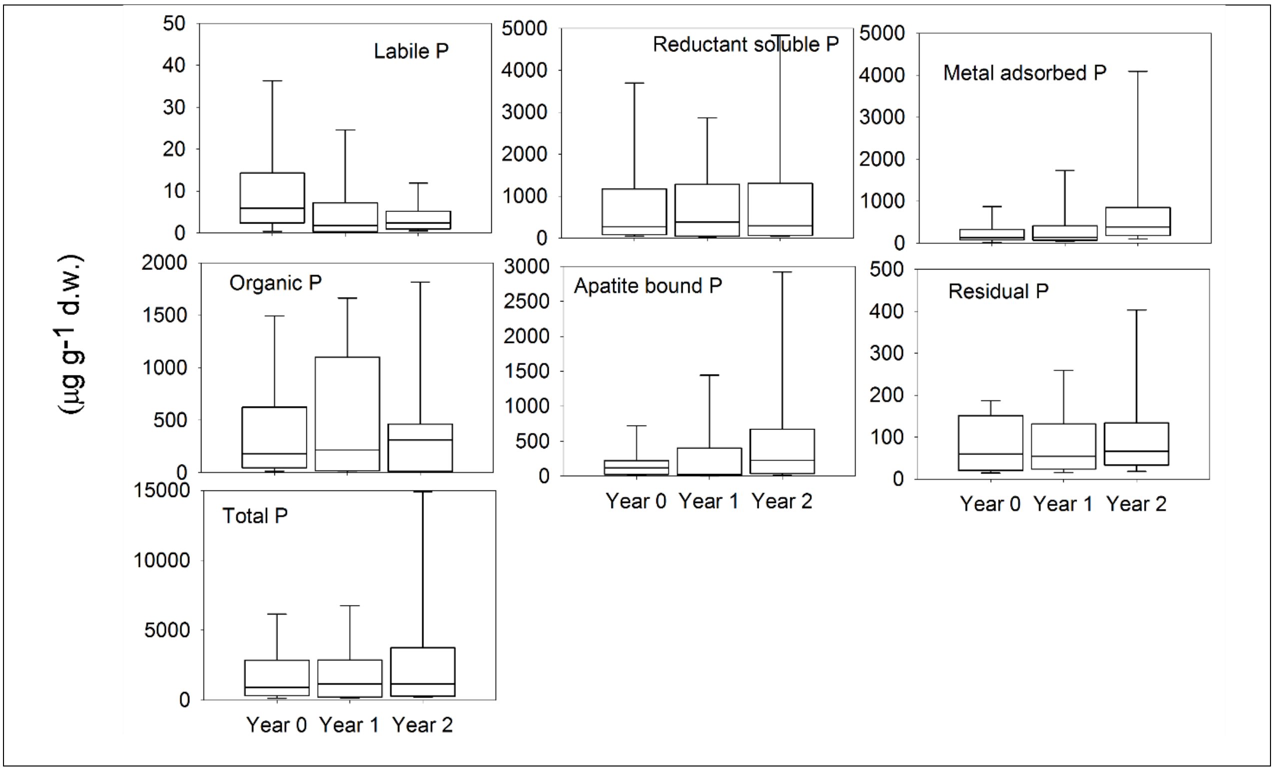

Figure 4.10 Mere Mere mean values of surface sediment phosphorus (P) fractions from quarterly sample dates across six water depths and three treatment years. Pre-treatment year 0; post-application year 1; post-application year 2. The 25th, 50th and 75th percentiles are indicated as the lower to upper lines on the box; the 10th and 90th percentiles as lower and upper limits of lines and 5th and 95th as dots are shown.

Taken collectively, these results indicate that Phoslock® is capable of reducing ortho-P and TP concentrations in both lakes but that the original dose was insufficient to control sediment P release, especially at high temperatures characteristic of summer conditions. These experimental conditions should not be considered fully representative of conditions in the lake. That said, these results are in agreement with similar intact-core experiments conducted on Loch Flemington in Scotland where persistent sediment P release was observed following Phoslock® application (Meis et al. 2013). The increased ortho-P and TP concentrations in the 20°C treatment cores relative to the 4°C treatment cores from Hatchmere and Mere Mere are likely the result of a combination of processes including enhanced microbial productivity at the higher temperature. The precise mechanisms acting here are difficult to determine without more targeted experimental manipulation of the microbial community. In addition, both the original dose added to the lake and the ‘repeat’ dose added experimentally here, should have been sufficient to strip all of the ortho-P from the water column. That this did not happen suggests some form of chemical interference limiting the capacity of the product to bind ortho-P in the water column of these lakes. Lürling et al. (2014) and Winfield and Lofts (2015) have highlighted the competitive role of DOC, and more specifically humic compounds, in isolating either La^{3+} ions in solution or in binding to Phoslock® particles in the water column and reducing the capacity of the product to remove ortho-P from solution. These processes are expected to be highest in high DOC lakes (i.e. > 10mg L^{-1}; Lürling et al. 2014), as is the case for both Mere Mere and Hatchmere (Spears et al. 2014), although this threshold value is based on laboratory controlled experimental assays.

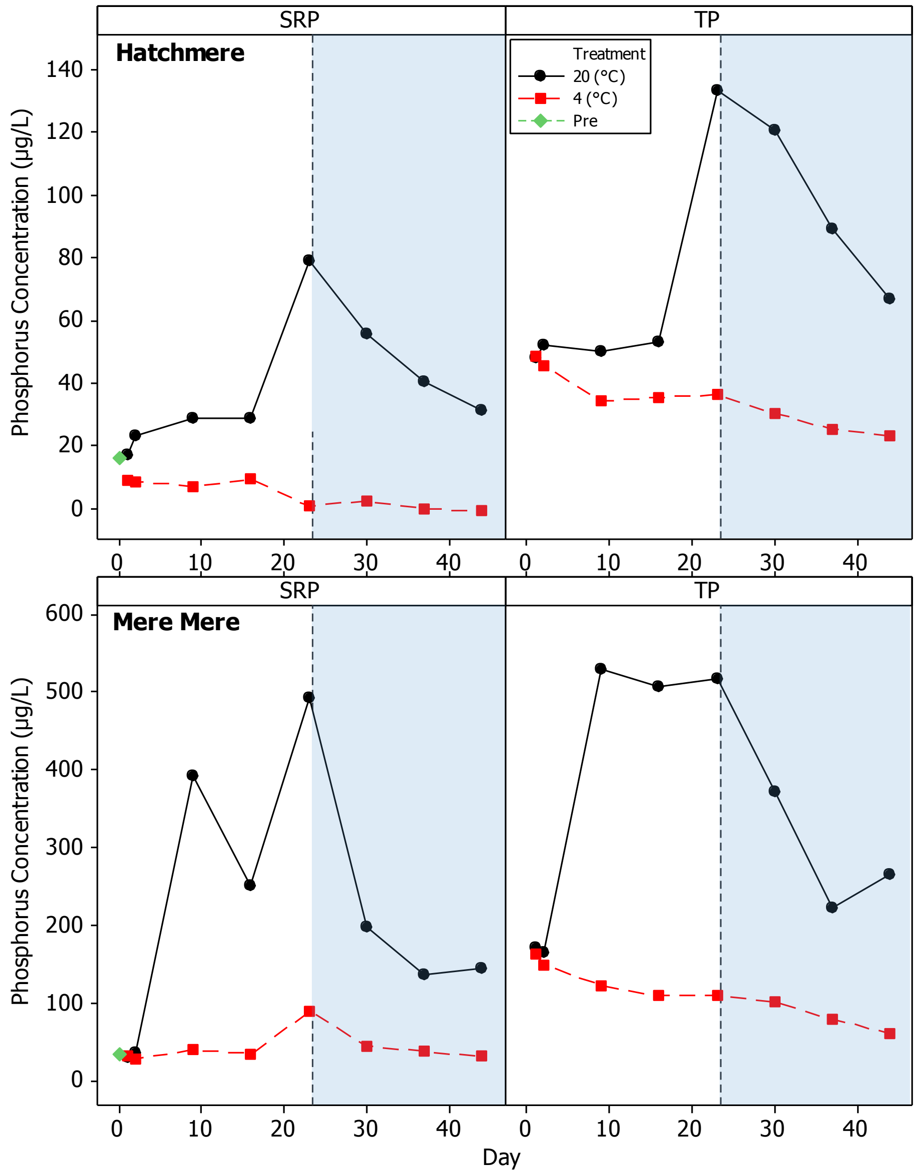

Figure 4.11 Average concentrations of total phosphorus (TP) and orthophosphate-P (ortho-P; SRP) recorded in water above the sediment in cores collected from Hatchmere and Mere Mere. A 'repeat' Phoslock® dose was added on day 23 to all cores, indicated by the blue shading

4.3 Phytoplankton community responses

The average biovolume of each phytoplankton group in each lake before and after Phoslock® application was calculated and presented as pie charts (Figure 4.12). Simple t-tests were calculated for each lake comparing biovolumes before and after Phoslock® treatment. BA GLM analysis indicated that a significant decrease in total phytoplankton biovolume was observed in post-application years 1 and 2 in Mere Mere, but not in Hatchmere. Decrease in Cryptophyta biovolume was observed in post-application year 2 in Hatchmere and post-application years 1 and

2 in Mere Mere. An increase in Chrysophyta biovolume was reported in both post-application years in Mere Mere but only in post-application year 2 in Hatchmere. Conflicting responses were observed in Bacillariophyta (increased in Hatchmere and decreased in Mere Mere) and Euglenophyta biovolume (decreased in Hatchmere and increased in Mere Mere).

Bioassays were carried out to assess which nutrients were limiting phytoplankton in the two lakes. Nutrient limitation is judged on the basis of stimulating phytoplankton biomass (Chl*a*) in response to the addition of different potentially limiting nutrients under standard conditions. If the Phoslock® treatment was effective one would expect to see an increase in the frequency of P limitation, as P availability is reduced. Table 4.3 suggests that there has been little change in the nutrients limiting the phytoplankton in the two lakes. In the case of Hatchmere, P was still limiting in all three years in winter, spring and summer. In autumn, phytoplankton were co-limited in 2012 and N limited in 2013 and 2014. There is, thus, only slight evidence for an increase in P limitation in Hatchmere. Nutrient limitation is slightly more variable, seasonally, in Mere Mere. Summers and winters were co-limited in all years. In the two years after the Phoslock® treatment phytoplankton were P limited in spring, but unfortunately there are no data from before the treatment to assess if this is caused by the Phoslock® treatment.

Table 4.3 Phytoplankton nutrient limitation in Hatchmere and Mere Mere (Phoslock® application completed 13 March 2013 in Hatchmere and 8 March 2013 in Mere Mere; bioassays prior to application indicated by box around border)

Mere	Season	2012	2013	2014
Hatch	Winter		**P**	**P**
Hatch	Spring		**P**	**P**
Hatch	Summer	**P**	**P**	**P**
Hatch	Autumn	**Co**	**N**	**N**
Mere	Winter		**Co**	**Co**
Mere	Spring		**P**	**P**
Mere	Summer	**Co**	**Co**	**Co**
Mere	Autumn	**N**	**Co**	**N**

P, phosphorus limiting; N, nitrogen limiting; Co, co-limiting

In an attempt to investigate this further, a numerical approach was taken to assess the extent of P limitation. The ratio of the mean Chl*a* concentration in the P treatment compared to that in the control was calculated. A high number, therefore, represents a large stimulatory effect of P. P had, on average, a greater stimulating effect compared to the control in Hatchmere than in Mere Mere with an average ratio of 4.2 compared to 2.4. The extent of P stimulation in Hatchmere changed strongly with season, varying between 8.5 in March 2014 and 1.0 in September 2012 when the lake was co-limited by P and N. Similarly in Mere Mere, the greatest P limitation was in September 2014, with a Chl*a* ratio of 6.4, and the lowest was close to 1 during summer N limitation. There is no clear indication of a different seasonal pattern of nutrient limitation before or after the Phoslock® treatment. Overall, while the two lakes differ in the frequency and strength of nutrient limitation, and there are clear seasonal changes, there is little evidence for a switch to greater P limitation in either lake.

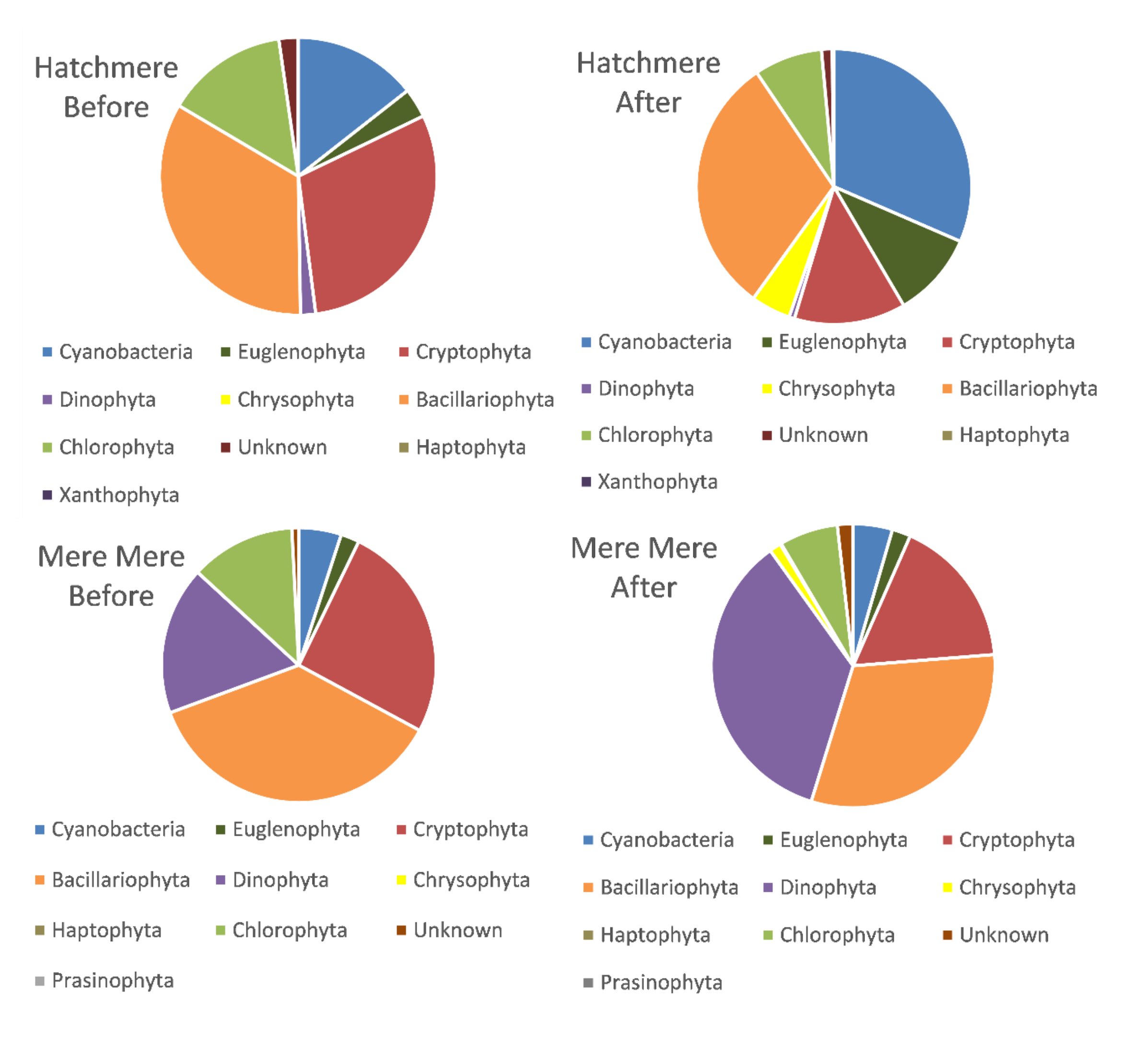

Figure 4.12 AvContribution of different phytoplankton groups to total phytoplankton biovolume before and after Phoslock® application in March 2013

4.4 Zooplankton community responses

Both Hatchmere and Mere Mere had similar crustacean zooplankton communities, composed of five main taxa, *Bosmina* sp., *Ceriodaphnia* sp., Cyclopoid copepods, *Daphnia* sp(p)., *Diaphanosoma brachyurum* and the calanoid copepod *Eudiaptomus gracilis* (Figure 4.13), with other taxa, such as Chydroridae and *Diaphanosoma brachyurum,* recorded occasionally. The taxon composition and the relative abundance of the major crustacean zooplankton taxa remained broadly the same, pre- and post-Phoslock® application, in both lakes (Figure 4.13).

A statistical analysis of the whole crustacean zooplankton dataset was conducted using ANOVA by rank to account for the data failing normality tests. The year relative to Phoslock® application was used for a one-way ANOVA treatment. *Eudiaptomus* and Cyclopoid copepods were the predominant zooplankton taxa in terms of biomass and there were no significant differences between any of the years either in terms of total biomass or in the total abundance of the crustacean zooplankton (Figures 4.14 and 4.15).

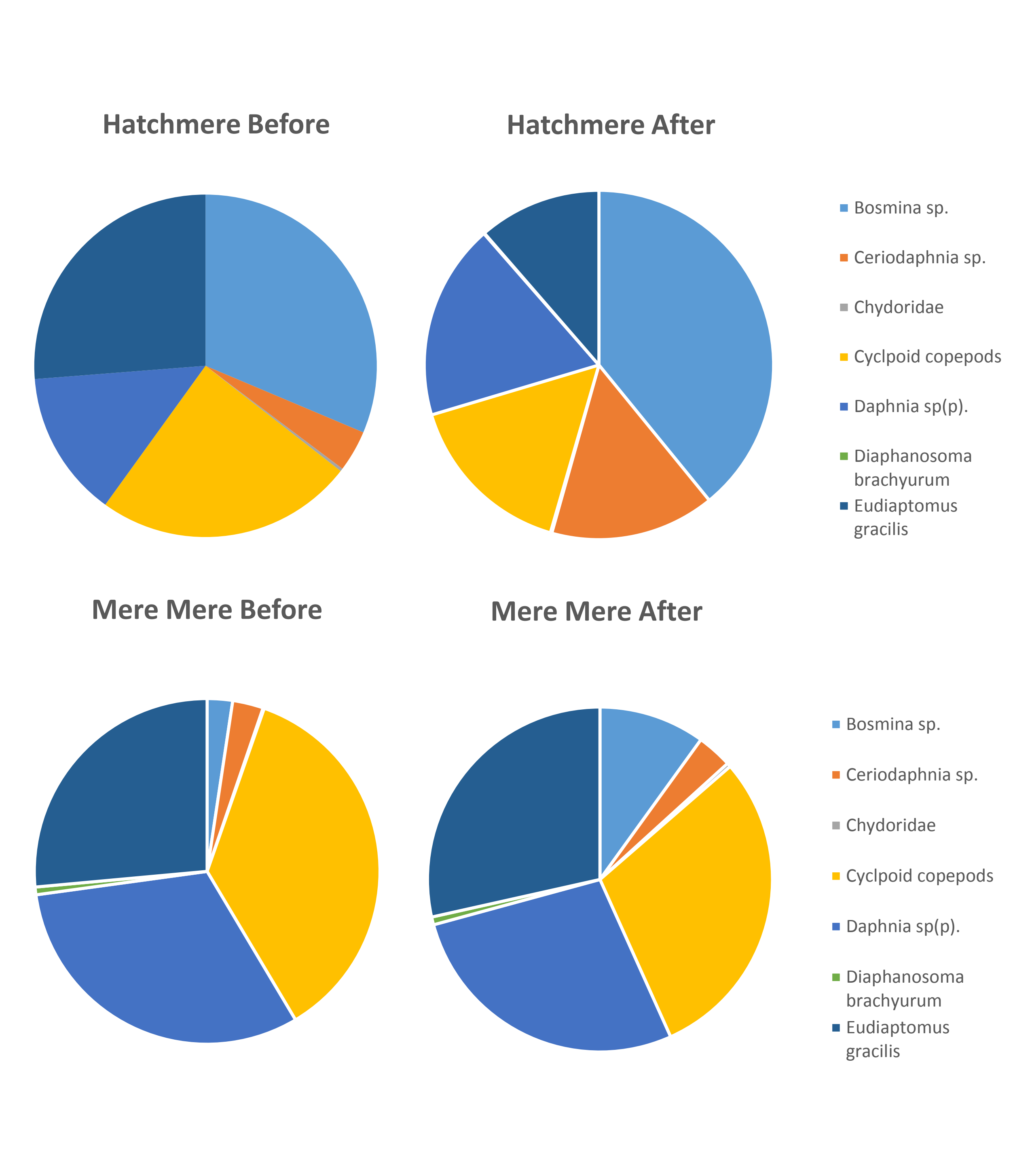

Figure 4.13 Relative abundance of crustacean zooplankton in monthly aggregated samples in Hatchmere and Mere Mere before and after Phoslock® application in March 2013

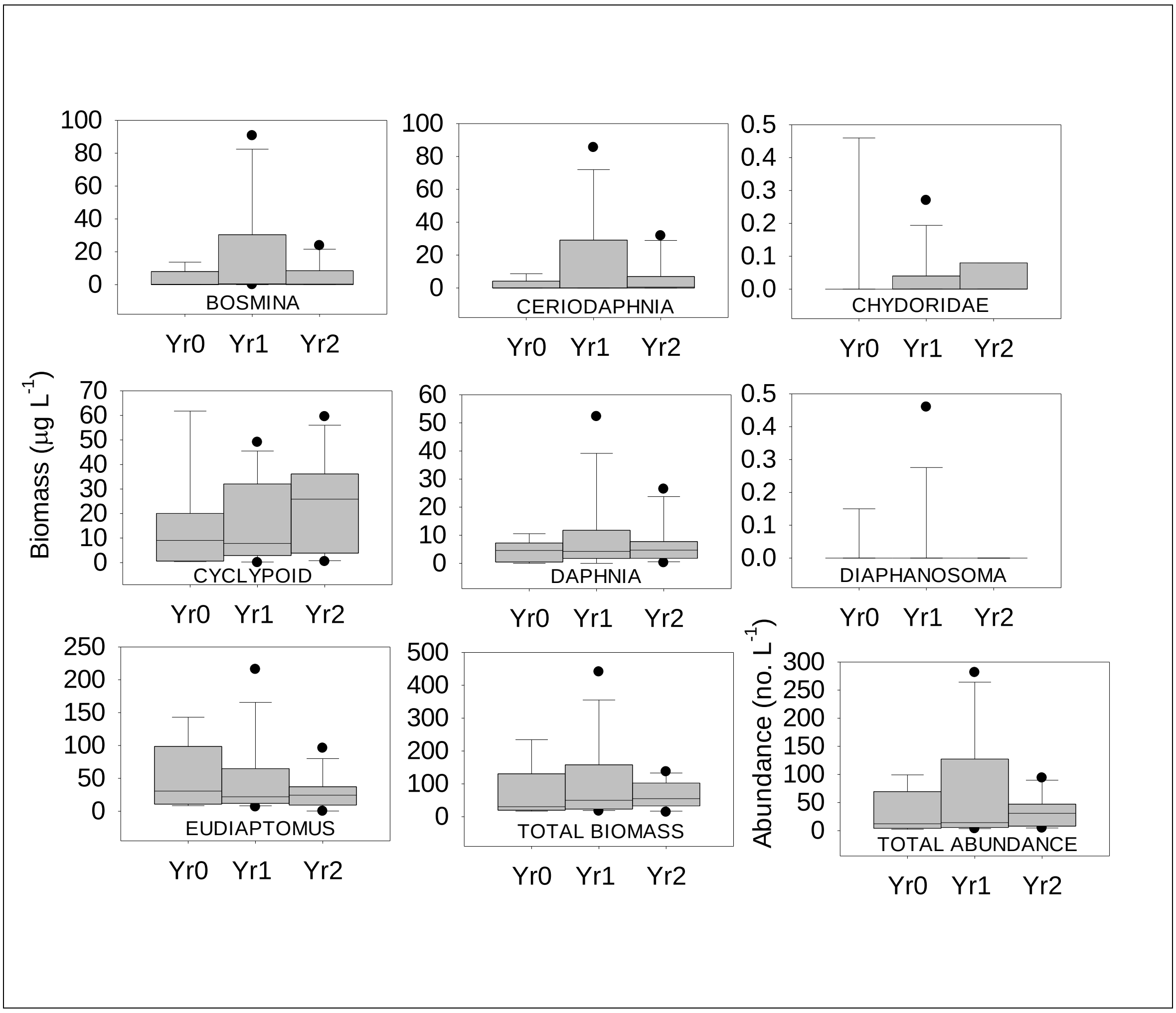

Figure 4.14 Biomass of major crustacean zooplankton groups and total crustacean zooplankton abundances, pre (Yr0) and post (Yr1, Yr2) Phoslock® application, in Hatchmere. The 25th, 50th and 75th percentiles are indicated as the lower to upper lines on the box; the 10th and 90th percentiles as lower and upper limits of lines and 5th and 95th as dots are shown.

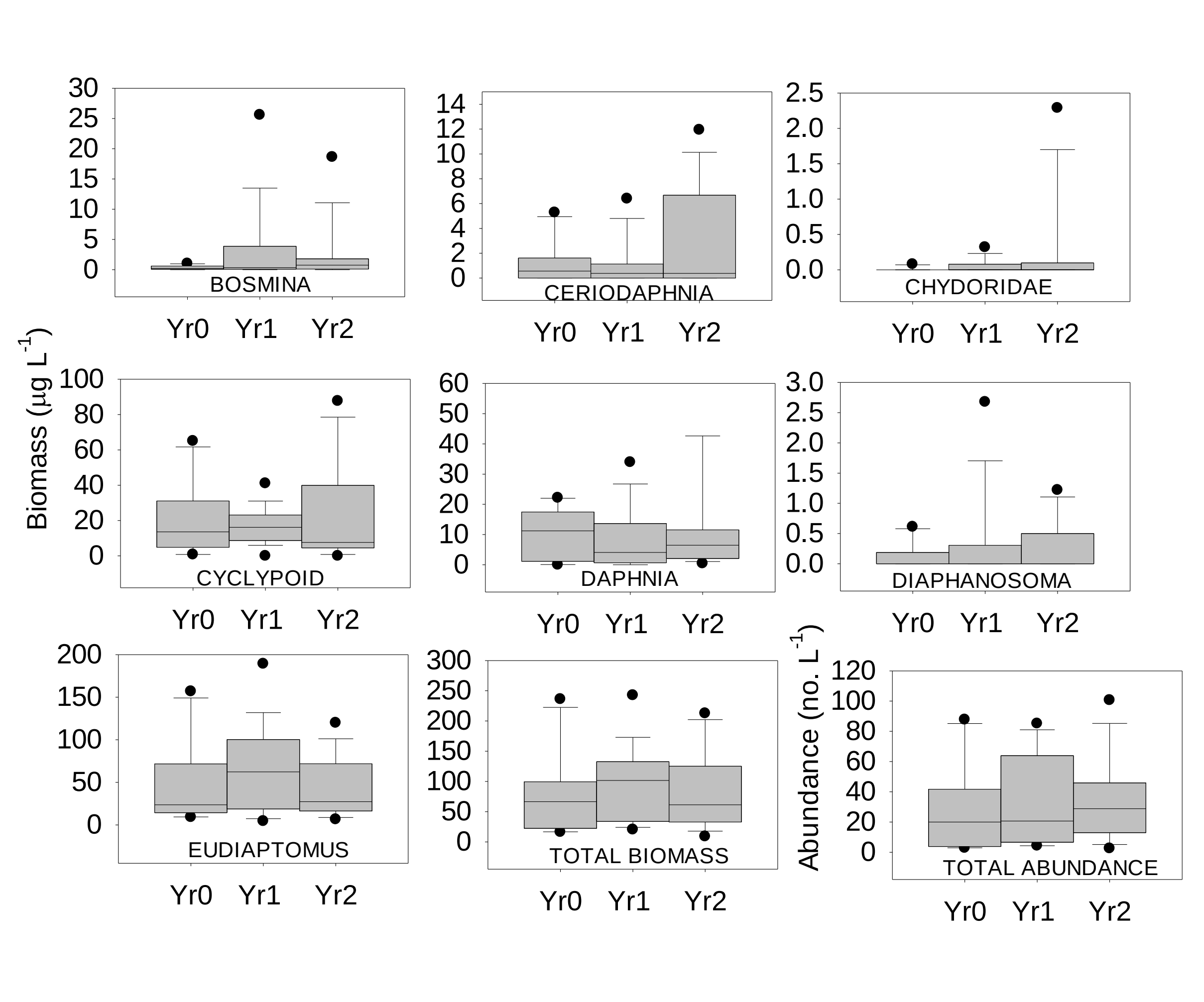

Figure 4.15 Biomass of major crustacean zooplankton groups and total crustacean zooplankton abundances, pre (Yr0) and post (Yr1, Yr2) Phoslock® application, in Mere Mere. The 25th, 50th and 75th percentiles are indicated as the lower to upper lines on the box; the 10th and 90th percentiles as lower and upper limits of lines and 5th and 95th as dots are shown.

4.5 Macroinvertebrate community responses

Spears et al. (2011b) carried out a literature review analysing the cause–effect–recovery chains for lakes recovering from eutrophication, including macroinvertebrates. Although Spears et al. (2011b) found few publications that reported comprehensively on the recovery of lake macroinvertebrate communities they did conclude that, over a 10–20-year period, the following responses might be expected in lakes recovering from eutrophication:

- increases in DO concentrations in the benthic zone leading to increases in macroinvertebrate species richness and diversity
- reductions in organic matter deposited to sediments leading to a lowering of overall macroinvertebrate abundance
- changes in fish grazing pressures leading to increases in the relative abundance of Coleoptera, Ephemeroptera, Plecoptera and Trichoptera
- expansion of macrophytes into deeper water leading to increases in the relative abundance of indicator taxa (e.g. Cladocera and Gastropoda) plus the colonisation of deeper water

The macroinvertebrate samples, collected in both the profundal and littoral benthic habitats of Hatchmere and Mere Mere, pre- and post- Phoslock® application, were analysed to see whether there was evidence of any short-term responses in the macroinvertebrate communities although, as described above, we would not necessarily expect one within this timeframe.

Overall, the profundal benthos samples, pre- and post-application, in both Hatchmere and Mere Mere, although not identified to species level, were both relatively poor in terms of taxon diversity (i.e. fewer than five different taxa per sample) and total macroinvertebrate abundance (i.e. fewer than 15 individuals per sample in Hatchmere and fewer than 40 individuals per sample in Mere Mere). The profundal benthos in both sites were mainly composed of Chaorboridae (phantom midges), with Tubificidae worms and Chironomidae (non-biting midges) larvae also commonly recorded. Chaoboridae are swimming taxa while the other two taxa live in lake bed sediments. Any variation in diversity was largely due to other taxa such as *Valvata piscinalis* (valve snail), *Pisidium* sp. (pea mussel), Ceratopogonidae (biting midges) larvae and *Erythromma najas* (red-eyed damselfly) larvae being occasionally recorded in the samples. However, in the case of Hatchmere, the non-parametric Mann-Whitney U Test (p values < 0.05) did indicate that there had been a significant drop both in mean total macroinvertebrate abundance and in the mean number of taxa recorded in the samples collected between pre- and post-Phoslock® application (Figure 4.16). The same statistical analyses were carried out on the Mere Mere profundal dataset but in this case it provided no evidence of a significant change in either the mean total macroinvertebrate abundance or in the mean number of taxa recorded in the pre- and post-Phoslock® application collected samples (Figure 4.16). If the study lakes were showing signs of recovering from eutrophication, one would expect, based on the scientific literature, an increase in the number of taxa and a decrease in total macroinvertebrate abundance. However, neither of the two study lakes followed this expected recovery pattern although the literature review, carried out by Spears et al. (2011b), suggests that such a recovery is unlikely within such a short timescale and is dependent on other elements being in place as well. The results from Hatchmere did indicate a significant decrease in total macroinvertebrate abundance but this was not matched by an increase in taxa diversity but rather the reverse, an actual decline in taxa diversity. The reasons for this pattern of change in Hatchmere are unclear but the results do, perhaps, suggest that at this site there may have been be an ecotoxicological effect on the profundal benthos from the application of the Phoslock® or, possibly, that it was an effect of physical disturbance of the lake sediment.

As might have been expected, the littoral benthos was composed of a much more diverse range of taxa than was found in the comparatively uniform habitat conditions experienced by the profundal benthos in both study lakes. One of the expected responses of lakes that are recovering from eutrophication is an increase in the relative abundance of Coleoptera, Ephemeroptera, Plecoptera and Trichoptera, in relation to changes in fish grazing pressure. In this study, only a couple of plecopteran specimens (Nemouridae) were recorded at Hatchmere and there was no clear pattern of change in the relative abundance of Coleoptera, Ephemeroptera and Trichoptera at either site (Figure 4.17). In total about 92 separate taxa were identified in Hatchmere including 16 aquatic mollusc, 10 lesser water boatman and 15 caddisfly species. Mere Mere had a similar, relatively rich macroinvertebrate diversity with a total of about 93 separate taxa identified including 21 caddisfly species. Noteworthy taxa such as *Ranatra linearis* (water stick insect) and *Erythromma najas* (red-eyed damselfly) were recorded in both lakes while the uncommon *Mesovelia furcata* (pondweed bug) was also found in Mere Mere. By applying the non-parametric Mann-Whitney U Test (p values > 0.05) to the whole littoral sample dataset we found that there was a significant drop in the mean number of macroinvertebrate taxa recorded per sample in the littoral benthos following the Phoslock® application, in both Hatchmere and Mere Mere (Figure 4.16). In terms of mean total macroinvertebrate abundance, the same statistical analysis indicated a significant drop in numbers in Mere Mere but not in the case of Hatchmere although there was an observed decline in the median values (Figure 4.16). Although overall macroinvertebrate abundance appeared to have dropped, the general pattern of decline in taxa richness and diversity in the littoral benthos, between pre- and post-Phoslock® application samples, as for the profundal benthos, is not in agreement with the expected macroinvertebrate community response pattern in lakes recovering from eutrophication (i.e. of increased taxa richness and diversity with increasing DO concentrations in the benthos). However, it should be stressed that in the relatively short time following the application of Phoslock® we would not necessarily expect to detect such a macroinvertebrate community response. The combination of declining taxa diversity and abundance in the littoral benthos, following the application of Phoslock®, may instead be indicating an adverse impact of the treatment on the benthic macroinvertebrate community.

To conclude, the above analysis indicated that the macroinvertebrate communities, in both the profundal and littoral benthic habitats, in Hatchmere and Mere Mere, did not exhibit any clear evidence of an eutrophication recovery response, following the application of Phoslock®. Given the very short timescale of the post-application assessment (under two years) reported here, it is, perhaps, unsurprising that no obvious pattern of changes has yet been detected given that a 10–20-year recovery period from eutrophication is more the norm for benthic communities (Spears et al. 2011b). We highlight potential limitations of the survey design (Hill et al., 2016) where the months May, September and January were used to charactaruise the community prior to Phoslock application compared with March, June, September and December following application.

4.1 Macrophyte community responses

At each of the three 100m survey sectors in Hatchmere, perimeter surveys were carried out in a boat by travelling parallel to shore in both 2012 (pre-Phoslock® application) and 2013–2014 (post-Phoslock® application). These were supplemented by boat-based depth transects out from the mid-point of the shore line transect to the point of deepest aquatic macrophyte growth in open water. The boat-based depth transects in Hatchmere were restricted to fewer than 20 sample points because the aquatic vegetation was limited to a relatively narrow zone around the perimeter dominated by the floating-leaved *Nuphar lutea*. Overall, despite a relatively diverse marginal and emergent plant community, dominated by *Phragmites australis*, Hatchmere had an extremely poor aquatic macrophyte flora consisting of only two floating-leaved species, *Nuphar lutea* and *Lemna minor*, with no submerged aquatic plants recorded. The various aquatic macrophyte WFD metrics derived from the results of these surveys (Table 4.4) indicated no change, thus far, in the status of aquatic macrophyte community between the pre- and post-Phoslock® application surveys. However, it should be noted that it was not possible to derive mean percentage cover of hydrophytes (COV) metric scores based on these surveys. A CSM survey of Hatchmere, carried out in September 2007 (UCL 2007), classified the lake as being in ‘unfavourable condition’. Comparisons of these survey results with historical species records indicated that in the past Hatchmere had a much more diverse aquatic flora, including *Callitriche obtusangula*, *C. platycarpa*, *Nymphaea alba*, *Potamogeton alpinus*, *P. gramineus*, *P. polygonifolius*, *P. pusillus*, *Utricularia minor* and *Zannichellia palustris,* suggesting that a major decline had occurred in the aquatic plant community of Hatchmere.

At each of the three 100m sectors in Mere Mere it was possible to carry out perimeter surveys, shore-wader and boat-based depth transects. The various aquatic macrophyte metrics derived from the results of these surveys indicated no significant change, thus far, in the status of the aquatic macrophyte community between the pre- and post-application surveys (Tables 4.4 and 4.5). A total of nine submerged and floating-leaved plants were recorded in the 2012 pre-application aquatic macrophyte survey: *Elatine hexandra*, *Eleocharis acicularis*, *Elodea canadensis*, *Lemna minor*, *Nymphaea alba*, *Nuphar lutea*, *Potamogeton crispus* and *P. obtusifolius*. All these species were subsequently recorded in the post-application aquatic macrophyte surveys carried out in 2013 and 2014. In addition, a further two aquatic macrophyte species, *Callitriche hermaphroditica* and *Potamogeton perfoliatus,* were found in the macroinvertebrate samples collected in the littoral zone, pre- and post-application, respectively. The open-water vegetation of Mere Mere is characterised by a relatively narrow band of the floating-leaved *Nuphar lutea* growing around the perimeter of the lake with clumps of *Potamogeton obtusifolius* occurring beyond this zone at a maximum recorded macrophyte growing depth varying between 2.0m and 2.3m. *Nuphar lutea* was the dominant aquatic macrophyte in the shore-wader and boat-based depth transects occurring in 67% to 80% of the sampled area with another floating-leaved species *Persicaria amphibia* occurring in 11% to 17% of the sampled area. The recorded submerged species were all relatively scarce, occurring in less than 12% of the sampled area in both the pre- and post-application aquatic macrophyte surveys. Of particular interest was finding specimens of the Nationally Scarce *Elatine hexandra* in all three aquatic macrophyte surveys in Mere Mere.

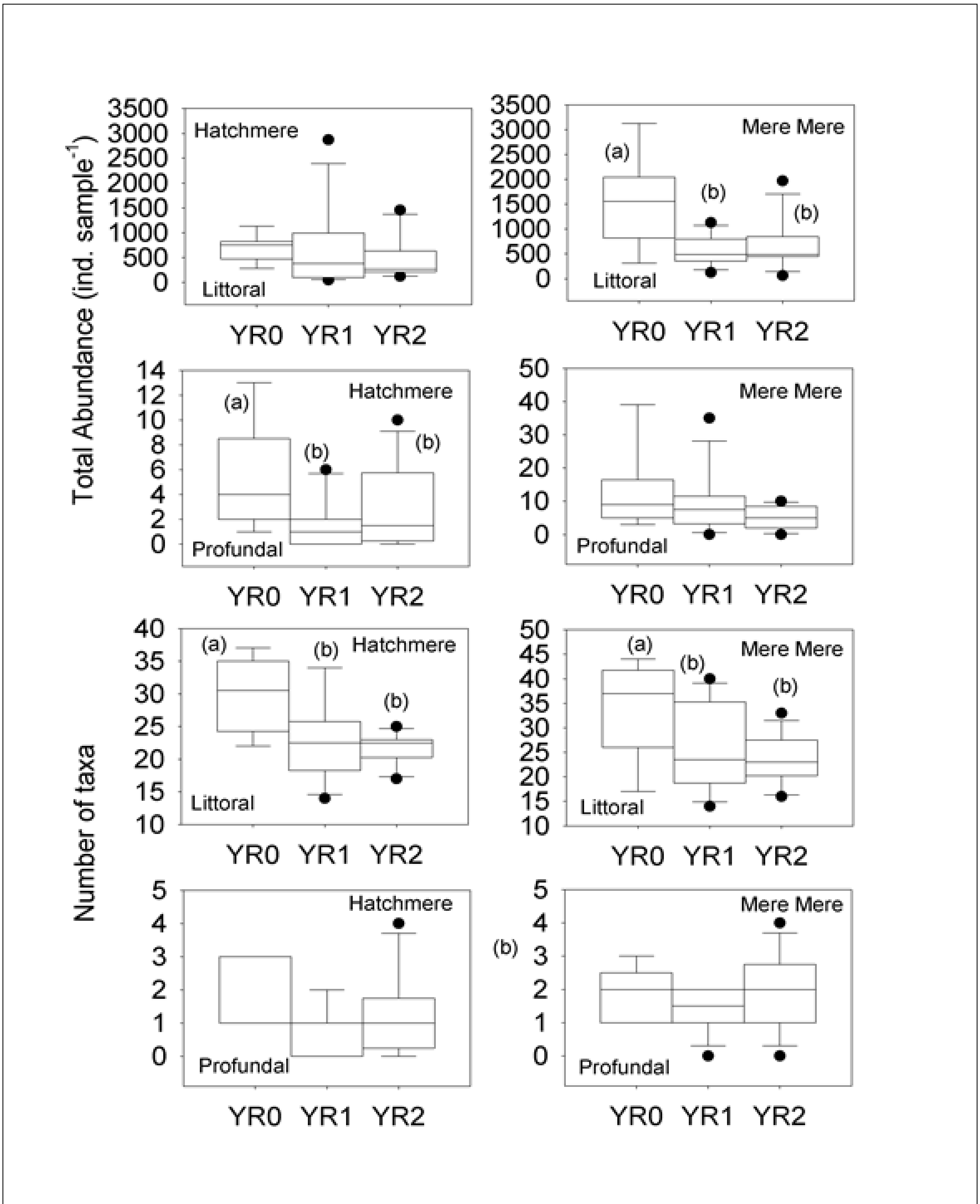

Figure 4.16 Number of different taxa and total abundance of macroinvertebrates found in littoral and profundal samples collected from Mere Mere (right panels) and Hatchmere (left panels), pre (YR0) and post (YR1, YR2) Phoslock® application. Bracketed letters denote significance groups. Groups with the same letter are not significantly different and vice versa. No lettering denotes no significant difference. The 25th, 50th and 75th percentiles are indicated as the lower to upper lines on the box; the 10th and 90th percentiles as lower and upper limits of lines and 5th and 95th as dots are shown.

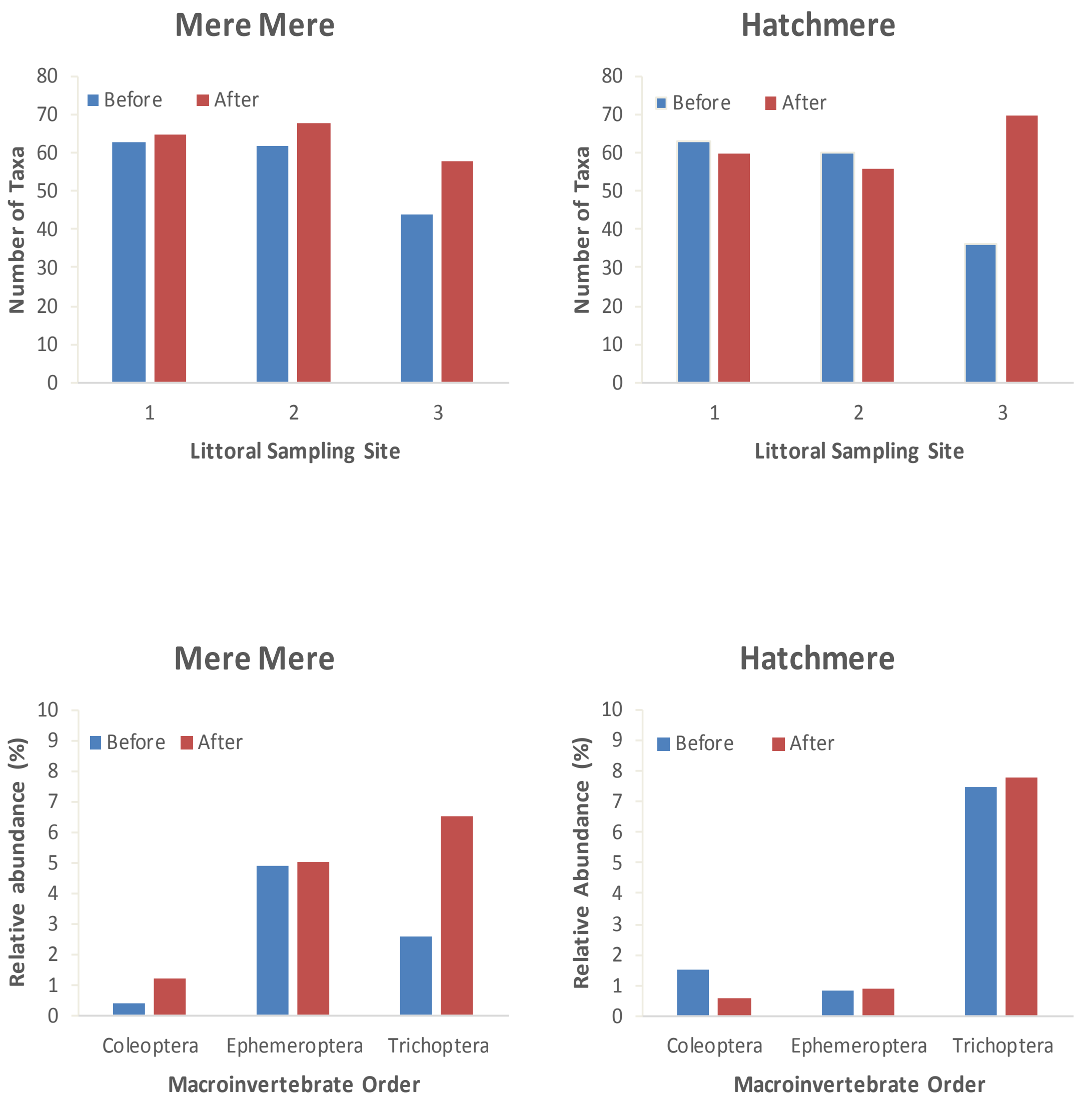

Figure 4.17 Number of taxa and relative abundance of Coleoptera, Ephemeroptera and Trichoptera taxa in littoral samples collected from Mere Mere (left panels) and Hatchmere (right panels), pre- and post-Phoslock® application

The diversity of aquatic macrophyte species recorded in 2012, 2013 and 2014 was higher compared with the CSM survey of Mere Mere carried out in July 2008 in which only six species were recorded, namely *Eleocharis acicularis*, *Lemna minor*, *Nuphar lutea*, *Nymphaea alba*, *Persicaria amphibia* and *Potamogeton crispus* (UCL 2008). However, despite this apparent improvement in water quality since 2008, the current aquatic flora, if compared with earlier records for the site, appears to be in 'unfavourable condition'. For example, in July 1992, nine submerged species and four floating-leaved species were recorded (Carvalho 1993) and the 1985 SSSI citation for Mere Mere highlights a diverse aquatic flora with 12 species of submerged macrophytes present, the highest number recorded for any of the Shropshire and Cheshire Meres. Species specifically mentioned in the citation include *Callitriche hermaphroditica* ('locally abundant'), *Elatine hexandra* ('occurs in a number of places'), *Eleocharis acicularis* (abundant), *Littorella uniflora* (abundant) and three species of *Potamogeton* (*P. berchtoldii*, *P. natans* and *P. perfoliatus*). Other aquatic species which have been recorded at Mere Mere in the last 30 years are as follows: *Callitriche obtusangula*, *C. platycarpa*, *C. stagnalis*, *Chara virgata*, *Lemna trisulca*, *Myriophyllum spicatum*, *Nitella flexilis* agg., *Potamogeton alpinus*, *P. pectinatus*, *P. pusillus*, *Ranunculus circinatus* and *Zannichellia palustris*. None of these species were recorded in Mere Mere during the pre- and post-Phoslock® aquatic macrophyte surveys (or the 2008 survey), although *Callitriche hermaphroditica* and *Potamogeton perfoliatus* were found in littoral macroinvertebrate samples collected in May 2012 and September 2013, respectively.

It should be noted that the aquatic macrophyte survey methods employed in 2008, 2012, 2013 and 2014 were very different (being focused on only three representative 100m sectors), compared with the earlier surveys, which examined the whole lake shoreline. The SSSI citation also lists aquatic macrophyte species that had been recorded in the past but which may have been absent from Mere Mere for many years. The SSSI may have been 'unfavourable' at the time of notification.

Table 4.4 Summary of aquatic macrophyte metrics recorded at Hatchmere, pre- and post-Phoslock® application. LMNI – lake macrophyte nutrient index score; Fun. gr. – number of functional groups

Note: X = recorded

			Pre-Phoslock® species			Post-Phoslock® species	
Macrophyte taxon	**LMNI**	**Fun. gr.**	**< 2007**	**2007**	**2012**	**2013**	**2014**
Callitriche obtusangula	9.34	6	X				
Callitriche platycarpa	9.50	6	X				
Callitriche stagnalis	6.38	6	X				
Crassula helmsii	5.57	5	X				
Eleocharis acicularis	8.68	4	X				
Eleogiton fluitans	2.03	15	X				
Elodea canadensis	7.45	5	X				
Lemna minor	8.52	1	X	X	X	X	X
Littorella uniflora	3.73	4	X				
Myriophyllum alterniflorum	2.66	7	X				
Nuphar lutea	7.47	12	X	X	X	X	X
Nuphar x *spenneriana*	3.65	12	X				
Nymphaea alba	6.84	12	X				
Potamogeton alpinus	4.48	16	X				
Potamogeton gramineus	2.85	16	X				
Potamogeton polygonifolius	2.39	16	X				
Potamogeton pusillus	7.54	14	X				
Sparganium natans	2.79	13	X				
Utricularia minor	2.36	9	X				
Zannichellia palustris	8.69	15	X				
Lake macrophyte nutrient index (LMNI)				8.00	8.00	8.00	8.00
Observed number of functional groups) (NFG)				2	2	2	2
Number of taxa (NTAXA)				2	2	2	2
Mean % cover of hydrophytes (COV)				-	-	-	-
Relative % cover of filamentous algae (ALG)				0	0	0	0
Macrophyte maximum growing depth (m)				1.60	1.70	1.50	1.50
Secchi depth (m)				0.60	0.70	0.45	0.45

Table 4.5 Summary of aquatic macrophyte metrics recorded at Mere Mere, pre- and post-Phoslock® application. LMNI – lake macrophyte nutrient index score; Fun. gr. – number of functional groups

			Pre-Phoslock® species			Post-Phoslock® species	
Macrophyte taxa	**LMNI**	**Fun. gr.**	**< 2007**	**2008**	**2012**	**2013**	**2014**
Callitriche hermaphroditica	8.08	5	X		+		
Callitriche obtusangula	9.34	6	X				
Callitriche platycarpa	9.50	6	X				
Callitriche stagnalis	6.38	6	X				
Chara virgata	4.29	2	X				
Elatine hexandra	3.81	11	X		X	X	X
Eleocharis acicularis	8.68	4	X	X	X	X	X
Elodea canadensis	7.45	5	X		X	X	X
Lemna minor	8.52	1	X	X	X	X	
Lemna trisulca	7.96	1	X				
Littorella uniflora	3.73	4	X				
Myriophyllum spicatum	6.23	7	X				
Nitella flexilis agg.	5.19	2	X				
Nuphar lutea	7.47	12	X	X	X	X	X
Nymphaea alba	6.84	12	X	X	X		X
Persicaria amphibia	8.25	10	X	X	X	X	X
Pilularia globulifera	3.59	4	X				
Potamogeton alpinus	4.48	16	X				
Potamogeton berchtoldii	6.58	14	X				
Potamogeton crispus	7.50	17	X	X	X	X	X
Potamogeton natans	4.71	16	X				
Potamogeton obtusifolius	6.97	14	X		X	X	X
Potamogeton pectinatus	7.19	15	X				
Potamogeton perfoliatus	4.42	17	X			+	
Potamogeton pusillus	7.54	14	X				
Ranunculus circinatus	8.70	5	X				
Zannichellia palustris	8.69	15	X				
Lake macrophyte nutrient index (LMNI)				7.88	7.28	7.33	7.12
Observed number of functional groups) (NFG)				5	8	8	7
Number of taxa (NTAXA)				6	9	8	8
Mean % cover of hydrophytes (COV)				-	4.46	5.23	4.24
Relative % cover of filamentous algae (ALG)				0	0	0	0
Macrophyte maximum growing depth (m)				1.90	2.00	2.30	1.70
Secchi depth (m)				1.40	1.10	1.02	1.20

Note: Note: X = recorded; + = species recorded in littoral macroinvertebrate samples but not included in LEAFPACS metric scores

4.2 Water Framework Directive assessment using phytoplankton metrics

Using the PLUTO tool (WFD-UKTAG 2014b), Hatchmere achieved ‘good’ status for the phytoplankton abundance metric (i.e. chlorophyll *a*) in 2012, and ‘moderate’ status for phytoplankton composition and bloom intensity (Table 4.6). During September 2012 the metrics based on phytoplankton composition and bloom abundance indicated that the lake was of ‘poor’ status. However, the combined metric score, which included the phytoplankton abundance (i.e. chlorophyll *a*) metric, for the lake was ‘moderate’ overall (Table 4.6). For 2013 the lake showed a decline in condition according to the three metrics which gave it a combined status of ‘poor’. All three metrics showed a decline, especially in July and August, and the bloom metric showed the greatest decline in status from ‘good’ to ‘bad’. In 2014 the lake status returned to ‘moderate’ overall, with composition and bloom abundance metric scores showing the greatest improvement in August (‘poor’ to ‘good’ and ‘bad’ to ‘moderate’, respectively). It should be noted that the classification method used by the Environment Agency for site assessment for the WFD requires averages to be calculated from three full summers in three years. The summer of 2012, pre-treatment, was a particularly wet summer, not conducive to phytoplankton development, whereas the summer of 2013 was a dry, hot summer with widespread algal bloom problems reported across the UK.

Table 4.6 Summary of WFD phytoplankton metrics for July, August and September in Hatchmere and Mere Mere in 2012 (pre-application), and 2013 and 2014 (post-application). Metric scores are indicated in parenthesis.

		Hatchmere			Mere Mere		
		July	August	September	July	August	September
Chlorophyll (EQRNorm)	2012		Good (0.66)			Good (0.64)	
	2013		Moderate (0.57)			Good (0.63)	
	2014		Moderate (0.51)			Good (0.74)	
PTI Class (PTI EQRNorm)	2012	Good (0.661)	Moderate (0.43)	Poor (0.34)	Moderate (0.42)	Moderate (0.47)	Moderate (0.46)
	2013	Moderate (0.51)	Poor (0.35)	Moderate (0.42)	Moderate (0.47)	Poor (0.33)	Moderate (0.47)
	2014	Poor (0.40)	Good (0.71)	Moderate (0.48)	Moderate (0.49)	Good (0.69)	Moderate (0.50)
Cyanobacteria class (Cyan EQRNorm)	2012	Good (0.72)	Good (0.60)	Poor (0.36)	Good (0.62)	Good (0.73)	High (0.85)
	2013	Bad (0.03)	Bad (0.00)	Poor (0.21)	Good (0.75)	High (0.85)	High (0.83)
	2014	Good (0.72)	Moderate (0.54)	Poor (0.38)	Good (0.61)	Good (0.76)	Good (0.73)

In Mere Mere the phytoplankton abundance metric showed a slight improvement and was classified as ‘good’ in all years (Table 4.6). The phytoplankton composition metric score also improved slightly between 2012 and 2014. This is mostly due to an improvement in the August score from ‘moderate’ to ‘good’, but overall the 2014 score remains in the ‘moderate’ class. The bloom intensity metric improved from ‘good’ to ‘high’ between 2012 and 2013, before returning to ‘good’ status in 2014. The overall status of Mere Mere was ‘moderate’ in both 2012 and 2013 but improved to ‘good’ in 2014.

These results should be treated with caution due to the uncertainty associated with the limited data available. There are recommended minimum sampling frequencies for the metrics, with between three and six years of data required (Carvalho et al. 2013). In this study there were limited pre-application data available but, with post-application data collection continuing, the metric results should become more reliable with time. There is possibly also an issue with lake ‘type’, as there is some concern that the lake P standards for humic lakes may not be appropriate (J-A. Pitt, personal communication). For this analysis, to calculate reference conditions, it was assumed that both sites were ‘clear water’ lakes. In projects such as this, it is important that the impacts of humic materials (i.e. as DOC concentration or colour) are considered fully in the context of WFD targets and operational performance of the product in treated lakes.

4.3 Water Framework Directive assessment using macrophyte metrics

Both Hatchmere and Mere Mere were consistently at ‘poor’ ecological status based on their aquatic macrophyte communities (using the LEAFPACS2 tool), pre- and post-Phoslock® application (Table 4.7). Hatchmere showed no change while Mere Mere showed a slight improvement in its overall EQR although it still remains within the ‘poor’ ecological status classification. A review of the scientific literature suggests that as nutrient concentrations decrease, increases in colonisation depth, in species richness (including relative characean abundance), in the number of nutrient-intolerant species and in species distribution can be expected (Spears et al. 2011b). At a structural level, macrophyte colonisation responses are likely to occur relatively quickly (i.e. < five years) while, at a community composition level, the recovery timescales for macrophytes (i.e. two to 40+ years) are likely to take generally longer, as nutrient concentrations decrease.

Table 4.7 Summary of WFD macrophyte metrics in Hatchmere and Mere Mere in 2007, 2008 and 2012 (pre-application), and 2013 and 2014 (post-application)

	Hatchmere				Mere Mere			
	2007	**2012**	**2013**	**2014**	**2008**	**2012**	**2013**	**2014**
EQR_{LMNI}	0.419	0.419	0.419	0.419	0.368	0.472	0.462	0.504
EQR_{NFG}	0.335	0.335	0.335	0.335	0.809	1.293	1.293	1.132
EQR_{NTAXA}	0.217	0.217	0.217	0.217	0.596	0.896	0.795	0.795
EQR_{COV}	-	-	-	-	-	0.737	0.799	0.719
EQR_{ALGAE}	1	1	1	1	1	1	1	1
$EQR_{LEAFPACS}$	**0.352**	**0.352**	**0.352**	**0.352**	**0.273**	**0.353**	**0.340**	**0.393**
Ecological status	Poor	Poor	Poor	Poor	Poor	Poor	Poor	Poor

5 Summary of responses in sediment, water column and ecological indicators following Phoslock® applications

5.1 Evidence of responses in sediment P composition and release indicators

A driving hypothesis in the study was that Phoslock® would alter the bed sediment P composition to favour more refractory sediment P forms. In previous field and laboratory based studies, this has been evident with reductions in labile and refractory P pools and an increase in metal adsorbed P and apatite P (Meis et al. 2012, 2013, Dithmer et al. 2016b). A significant reduction in labile P, the sediment pool from which P is most readily released, was observed in both Mere Mere and Hatchmere following the application, although only in post-application year 1 for Mere Mere. An increase in metal adsorbed P was also apparent in both lakes, but not in both post-application years.

Meis et al. (2012) demonstrated that P was taken up from solution into all of the operationally defined sediment P pools determined via the sequential extraction scheme used here. The order of mass of P recovered from P-saturated Phoslock® reported by Meis et al. (2012) from each pool was apatite bound P (60.7%) > metal adsorbed P (16.9%) > reductant soluble P (14.4%) > labile P (6.6%) > residual P (1.2%) > organic P (0.2%), indicating multiple P uptake pathways into Phoslock®, as would be logical given the chemical composition of calcium-based bentonite. The responses listed above for Mere Mere and Hatchmere appear to be in general agreement with Meis et al. (2012). Finally, the greatest change in sediment P in our treatment lakes expressed as the difference between years 2 and 0 sediment P concentrations for Hatchmere was in the metal adsorbed pool (14%) followed by the apatite bound (12%), reductant soluble (3%), residual (1%), labile (-1%), and organic (-4%) pools. In Mere Mere the greatest increase was also in the metal adsorbed pool (22%) followed by apatite (16%), reductant soluble (5%), residual (1%), labile (-1%) and organic (-1%) pools. These series are in general agreement with those reported from laboratory trials by Meis et al. (2013). In addition, variation with depth in La and P fractions and translocation of material following application have been reported elsewhere (Meis et al. 2013, Dithmer et al. 2016b, Yasseri and Epe 2016).

Intact sediment core incubations were conducted in post-application year 2 (section 3.4.4) by CEH and, independently, in year 2 by Phoslock ®Europe (Yasseri 2016). These incubations were designed to assess the effectiveness of the treatment to block the release of SRP from bed sediments to the water column. The CEH study included high (20°C) versus low (4°C) temperature treatments under reducing redox conditions to force conditions that favour sediment P release in most eutrophic lakes. For both treated lakes, SRP concentrations increased under the 20°C treatment up to incubation day 23, after which SRP concentrations declined gradually following a repeat Phoslock® dose towards the end of the experimental period (i.e. > 20 days following experimental Phoslock ®treatment; 505g m^{-2} Phoslock® and 534g m^{-2} Phoslock® for Hatchmere and Mere Mere, respectively). This rate of P decrease was much lower in both lakes when compared to Loch Flemington, a shallow lake in Scotland where the control of SRP release under similar experimental conditions (i.e. 18°C; reducing conditions; dark; following initial in situ Phoslock® application; core experimental dose of 170g m^{-2} Phoslock®) occurred in under five days. Similar results were reported by Yasseri (2016), although repeated dosing treatments indicated that an addition of a further 5.5 tonnes Phoslock® to Hatchmere and 18.5 tonnes Phoslock® to Mere Mere would result in effective sediment capping. Using their estimates of P content of the upper 10cm bed sediments in both lakes they alternatively estimate the 'top-up' dose to be 6.7 tonnes for Hatchmere and 20.7 tonnes for Mere Mere. The conclusion here, from all available evidence, is that the Phoslock® doses applied to both Hatchmere and Mere Mere were insufficient to totally reduce sediment P release.

Measures of bottom water TP and ortho-P were assessed quarterly in years 0, 1 and 2 in both lakes and were used to provide evidence of a reduction in internal loading indicated by a decrease in bottom water concentrations following the Phoslock® application. The results of these analyses are in general agreement with the core incubations. Specifically, a significant decrease in bottom water ortho-P was reported in both lakes following the application. Whereas in Hatchmere this reduction was consistent across the six sample sites, elevated bottom water ortho-P concentrations were persistent in the deeper water layers of Mere Mere even though the deeper bottom waters of both sites experienced DO depletion during summer (Figure 4.1). Given reducing conditions are known to occur in both lakes at the deepest points it is apparent that the application of Phoslock® did successfully reduce internal loading in Hatchmere and, to a lesser extent, in Mere Mere.

It is apparent, from the available evidence, that the Phoslock® application decreased the potential for P release from the bed sediments (i.e. decrease in labile P) of both lakes and that the increase in metal adsorbed P indicated that all available La should have been saturated with P within 2 years of application in Hatchmere. In Mere Mere, given the La:P uptake ratio of 1:1, it is apparent that P recovered was sufficient to bind with about 80% of the La. This latter statement is only true if we assume that La only interacts with P in lake bed sediments. Dithmer et al. (2016a) conducted a sediment survey of both lakes and indicated that DOC concentrations in Mere Mere and Hatchmere may be sufficiently high to interact with La, as did the chemical modelling work conducted by Winfield and Lofts (2015).

5.2 Evidence of responses in water quality indicators

Where La-bentonite has been successful in controlling internal loading these responses should occur relatively quickly, at least within the recovery timescales known to occur following catchment nutrient load reduction alone (i.e. < five years; Jeppesen et al. 2005, Sharpley et al. 2013). We based our hypotheses of water quality responses on long-term catchment nutrient load reduction studies that indicated that a range of responses occur over the recovery time period in lakes. The recovery process for P in temperate lakes following catchment management is characterised by a rapid decline in winter concentrations followed by a gradual decline in summer P concentrations as the intensity of internal P loading diminishes with time (Phillips et al. 2005, Søndergaard et al. 2013). Whereas winter P concentrations are generally driven more by catchment inputs, sediment P release is more prominent in the warmer summer months when redox conditions of bed sediments can become reducing (i.e. liberating ortho-P from Fe–P sediment complexes) and high temperatures increase sediment-water ortho-P concentration gradients and diffusive fluxes from the sediment to the water column (Spears et al. 2007). The period over which these responses occur is lake specific and regulated by various factors including hydraulic residence time, sediment P concentrations and depth (Sas 1989). In addition to changes in the magnitude of summer TP concentrations, Phoslock® should cause a decrease in ortho-P in bottom and surface waters. With the exception of changes in P composition and concentrations in the water column, and in line with other field-scale studies, we did not expect changes in the general physicochemical conditions in the treated lakes following Phoslock® application. In this project we have focused to date on the WFD targets for Hatchmere and Mere Mere. However, to provide wider context we also draw on work by Jeppesen et al. (2000) who defined five ecological classes across 71 Danish lakes according to surface water annual mean TP concentrations and indicated that significant decreases in Chl*a* concentrations, and increases in water clarity, aquatic macrophyte community species numbers and maximum growing depths, would occur at annual mean TP concentrations < 50µg L^{-1}.

Ortho-P concentrations in surface waters were reduced significantly in both lakes, the strongest responses occurring in summer and autumn. The latter observation indicates a reduction in the magnitude of internal loading in both lakes, following Phoslock® application. However, microbial organisms may also sequester ortho-P and may have been partly responsible for the decrease, at least in Hatchmere. Annual mean ortho-P concentrations declined from 24.9µg L^{-1} to 8.9µg L^{-1} in Hatchmere and from 18.9µg L^{-1} to 11.6µg L^{-1} in Mere Mere. However, evidence to support a strengthening of P limitation of phytoplankton following Phoslock® application was only observed in summer and autumn in Hatchmere, as an increase in the Chl*a*:TP ratio and in winter of both lakes as an increase in the TN:TP ratio. Neither of these changes can be attributed solely to the Phoslock® application. For example, phytoplankton biomass increased in Hatchmere following the application and will have reduced available ortho-P in the water column. With a reduction in ortho-P we would also expect a reduction in TP, and where internal loading had been effectively controlled this should be greatest in summer and autumn. This was again confirmed in both lakes, the summer peak being observed to decline from about 140µg L^{-1} towards 80µg L^{-1} in Hatchmere and from about 90µg L^{-1} to 50µg L^{-1} in Mere Mere. When considered in combination with the evidence from the sediments it is apparent that the Phoslock® treatment was more effective in Mere Mere than Hatchmere. Reductions in annual mean TP concentrations were also observed in both lakes throughout the sampling period, from 83.2µg L^{-1} to 64.4µg L^{-1} in Hatchmere and 76.6µg L^{-1} to 49.8µg L^{-1} in Mere Mere. The reduction in TP concentrations reported here would not reflect a strong shift between lake classes as outlined by Jeppesen et al. (2000) (i.e. class 2 annual mean TP range of 50µg L^{-1} to 100µg L^{-1}; class 1 < 50µg L^{-1}). These changes are discussed in the context of WFD targets below. In summary, the reductions in TP concentrations in both lakes were insufficient to results in a shift in WFD ecological status from moderate to good status. However, should improvements in TP concentrations continue, then we would expect good status to be reached for Mere Mere (i.e. further reduction required of > 0.8µg L^{-1} in the annual mean). Conditions at Hatchmere would have to improve markedly (i.e. further reduction required of > 15.4µg L^{-1} in the annual mean) to reach good status, in accordance with WFD targets.

Other physicochemical responses were observed including a reduction in TON concentrations in both lakes (annual response for Mere Mere, summer response for Hatchmere) and DOC concentrations which decreased across most seasons in both lakes. This latter response was conspicuous given the known interactions of DOC and La discussed elsewhere in this report and likely reflects a combination of interactions with Phoslock® and changes in catchment loading of humic materials, neither of which were directly measured in this study.

Spears et al. (2016) conducted a meta-analysis of chemical and ecological responses across 16 lakes (including Hatchmere and Mere Mere) that had been treated with Phoslock® and provide a useful comparison of responses with which to place those observed in Hatchmere and Mere Mere into context. Spears et al. (2016) reported significant decreases in TP concentrations across the treated lakes over annual (median across all lakes of 0.06mg L^{-1} in the 24 months pre-application to 0.03mg L^{-1} in the 24 months post-application), autumn (0.07mg L^{-1} to 0.03mg L^{-1}) and winter (0.07mg L^{-1} to 0.02mg L^{-1}) periods. Similar reductions were observed for ortho-P concentrations at annual (0.019mg L^{-1} to 0.005g L^{-1}), summer (largest relative response; 0.018mg L^{-1} to 0.004mg L^{-1}), autumn (0.019mg L^{-1} to 0.005mg L^{-1}) and winter (0.033mg L^{-1} to 0.005mg L^{-1}) periods. The responses in TP and ortho-P for Hatchmere and Mere Mere reported here are weak in comparison to most other lakes considered by Spears et al. (2016) over the same time period. In fact, responses in TP and ortho-P were site-specific across the 16 treated lakes, the intensity of the response appearing to decrease with increasing DOC concentration. The mechanisms behind the DOC–TP reduction correlation were not directly assessed by Spears et al. (2016) although DOC concentrations and TP concentrations are correlated across many lakes as a result of catchment processes (Nürnberg and Shaw 1998) and DOC and La are known to interact to slow P uptake by Phoslock® (Dithmer et al. 2016b). The water quality responses both in the current report and those in Spears et al. (2016) were only considered over the first 24-month post-application period. The results of the laboratory incubation trial by Dithmer et al. (2016b) indicated that P uptake in batch reactions can take longer than 400 days to complete, especially when DOC concentrations are high, as in Hatchmere and Mere Mere. Taken collectively with the apparent decreasing trend in annual mean ortho-P and TP concentrations in both lakes, this evidence suggests that chemical responses in both Hatchmere and Mere Mere may not yet be complete.

5.3 Evidence of responses in ecological indicators

Where the phytoplankton community is primarily P limited, reductions in annual average TP concentrations should cause a reduction in Chl*a* concentrations, an increase in water clarity, and eventually an increase in the extent and diversity of aquatic macrophytes, with changes in these properties occurring below the 50µg L^{-1} threshold, at least in Danish lakes (Jeppesen et al. 2000). In addition to these Danish examples, Phillips et al. (2015) conducted a review of chemical and ecological responses to various restoration approaches in the Norfolk Broads and proposed that similar ecological transitions occurred below about 55µg L^{-1} annual mean TP and 30µg L^{-1} annual mean Chl*a* concentrations. These general hypotheses on ecological recovery were supplemented with a range of others (Appendix 1) that included indicators of ecological state and function across macroinvertebrates, zooplankton, macrophytes and phytoplankton communities, as discussed by Spears et al. (2011b). Spears et al. (2016), in a detailed meta-analysis, examined the water quality and macrophyte community responses across 18 lakes (including Hatchmere and Mere Mere) treated with Phoslock®, and confirmed the above expected general improvements in water quality leading to an improvement in the aquatic macrophyte community within two years, although macrophyte data were only available for a subset of six lakes. In their study, Spears et al. (2016) reported that Chl*a* concentrations across 15 lakes decreased slightly, and in summer, from mean peak values of 119µg L^{-1} to 74µg L^{-1}, whereas Secchi disk depth increased in summer from mean peak values of 398cm to 506cm. There was also a significant increase in the median number of macrophyte species from 5.5 to 7.0 and in the maximum macrophyte colonisation depth from 1.8m to 2.5m, although macrophyte responses varied significantly among the studied lakes. Hence, because these responses were highly site specific, Spears et al. (2016) stressed the need for comprehensive pre- and post-application assessments of processes driving ecological structure and function in lakes where Phoslock® or similar P-capping products are considered for future use.

Although annual mean TP concentrations declined following Phoslock® application in both Hatchmere and Mere Mere, the concentrations did not decline to levels at which significant ecological responses would be expected. In the context of both Jeppesen et al. (2000) and Phillips et al. (2015) the lakes were close to the threshold TP concentrations. However, annual mean Chl*a* concentrations in Hatch Mere increased following the application from 17.8µg L^{-1} to 40.8µg L^{-1} whereas in Mere Mere Chla concentrations decreased slightly from 16.4µg L^{-1} to 11.8µg L^{-1}. For Hatchmere this takes the lake above the threshold indicated by Phillips et al. (2015), having been below this level in the 1 year prior to the application. In Mere Mere, the annual mean Chl*a* concentration was already below the Phillips et al. (2015) threshold. The reduction in Mere Mere brought the Chl*a* concentrations to below the WFD type-specific good/moderate boundary (16.5µg L^{-1}) in post-application years 1 and 2. It is apparent that factors other than sediment derived P are driving phytoplankton biomass in Hatchmere, and to a lesser degree Mere Mere. One obvious confounding factor here may be persistent loading of P from the catchment and this is discussed further in section 6.2.

The lack of evidence to support P limitation of the phytoplankton using the indicators of Chl a:TP, ortho-P concentrations, TN:TP ratios and nutrient limitation assays makes it difficult to conclude that P limitation of the phytoplankton in either lake has been strengthened. However, when shifts in within-year correlations between ortho-P, TON and Chl*a* are considered, following Phoslock® application, the situation is complex. (Table 5.1). A negative correlation between TON and Chl*a* was observed in both lakes only prior to the Phoslock® application. In addition, a strong negative correlation in ortho-P concentrations emerges only after the Phoslock® application in Hatchmere, while a weak negative correlation is present both before and after the application in Mere Mere. Where dissolved nutrients are available in excess one would not expect to see a correlation between ortho-P, TON and Chl*a*. Where phytoplankton biomass is increasing as dissolved nutrients are decreasing then the rate of removal of the dissolved nutrient exceeds its supply, indicating increased likelihood of limitation. We acknowledge that the mechanisms behind such correlations are unclear and present these data as weak evidence to support the hypothesised shift towards stronger P limitation, at least in Hatchmere, in the years following the application of Phoslock®, but do not attempt to substantiate the mechanism of the change. In other studies, where changes in nutrient limitation following Phoslock® application have been examined, the potential for P limitation of the phytoplankton has increased markedly (Douglas et al. 2016).

The series of responses required to support a deeper growing and more diverse macrophyte community includes a reduction in Chl*a* concentrations (i.e. to < 30µg L^{-1}) and a corresponding increase in water clarity. In Hatchmere, where Chl*a* concentrations increased significantly following Phoslock® application, the macrophyte community should, in theory, have responded negatively although there was no evidence of any change, as it was already very sparse prior to treatment. In Mere Mere, Chl*a* concentrations indicated favourable conditions for macrophytes both before and after the application, and these conditions improved only slightly, but significantly. Correspondingly, no significant changes to indicate recovery in ecological communities were observed in the macrophyte community of either lake. Given the hypothesised zooplankton responses are reliant upon increased cover of macrophytes it is unsurprising that no evidence of zooplankton responses were reported either although such responses can also be mediated by fish, which are not considered here. Given their reported long-term responses to recovery from eutrophication (Spears et al. 2011b) it is perhaps to be expected that there was no obvious pattern of changes in the benthic macroinvertebrate communities. The observed mixed pattern of response of the benthos in the two study sites, instead, may indicate that the application of Phoslock® had an adverse short-term impact on the benthic macroinvertebrate communities of the two study sites or may, perhaps, be a reflection of natural high inter-annual variability. Given the potential for ecotoxicological effects associated with materials being added to lakes we address this in detail in section 6.3.

Table 5.1 Correlation of total phosphorus (TP), orthophosphate-P (ortho-P; SRP) and total oxidised nitrogen (TON) versus chlorophyll *a* concentrations for average monthly values before (i.e. January 2006 to March 2013; where available) and after (i.e. April 2013 to December 2014) the application. Correlation analysis was conducted following log transformation using Minitab version 16 using Pearson Correlation analysis. *** p < 0.001; ** p > 0.001 < 0.01; * p > 0.01 < 0.05

	Hatchmere		Mere Mere	
	Before	After	Before	After
TP	0.6*	0.6*		
SRP		-0.9***	-0.6*	-0.6*
TON	-0.8**		-0.6*	

Although chemical responses associated with Phoslock® application have been relatively well documented, no study has, to date, reported on an objective assessment of ecological responses across trophic levels in multiple lakes. In the present study, only one ecological response can be considered with relatively high confidence to be the result of the Phoslock® application: the reduction in phytoplankton biomass in Mere Mere. When we extend this community level analysis by considering responses in WFD metrics for phytoplankton, we see little obvious improvement in ecological status to suggest that the overall objective of the project had been met. That is, neither lake moved across the good/moderate WFD boundary for any ecological metric used. Although there was an improvement in the phytoplankton in Mere Mere, the phytoplankton community metric indicated Mere Mere was in good ecological status in the year prior to the application anyway. The macrophyte community metric was stable and indicative of poor status throughout the project. We should again stress that the WFD-based analyses conducted in this report are not directly comparable with EA reporting on these lakes. In addition, Hatchmere is not part of the EA WFD monitoring programme, as it is below the 5ha size threshold. Only one other study has considered ecological responses in line with WFD targets using standard UK assessment metrics. Lang et al. (2016) reported a general reduction in phytoplankton biovolume for two years following a Phoslock® application to Loch Flemington, Scotland, a sustained (i.e. to post-application year 4) increase in phytoplankton community diversity; a sustained decrease in annual geometric mean Chl*a* concentration from 35µg L^{-1} to 13µg L^{-1} and a shift in overall ecological status, based on scores of combined phytoplankton community metrics from 'poor' to 'moderate' in post-application year 4. If we assume that the responses reported by Lang et al. (2016) are a result of the Phoslock® application then the post-application monitoring period of the present study would be insufficient to capture similar responses in Hatchmere and Mere Mere.

6 Assessing potentially confounding factors

6.1 Consideration of product application procedures and chemical interactions

In Table 4.2 we saw that when changes in both apatite and metal adsorbed P pools are combined they represent an increase in the P content of the upper 4cm of bed sediments of 1,123kg P in Mere Mere and 608kg P in Hatchmere, the major changes post-application. For metal adsorbed P alone, the change in concentrations represents an increase of 651kg P in Mere Mere (i.e. 81% of the theoretical La–P yield) and 324kg P in Hatchmere (i.e. 129% of the theoretical La–P yield). It should be noted that mass balance estimates of this kind carry significant uncertainty. For example, in these calculations we assume that the six sample sites represent average conditions of the lake bed. This is unlikely to be the case across most sites and will vary with physical processes. In addition, we give equal weighting to each site even though P concentrations were observed to be higher in deeper water sediments, especially in Mere Mere. These results are in agreement with other studies that indicate significant uncertainty associated with estimating effective product dose based on the simple sediment and water column P mass balance approach commonly used. To address this uncertainty, Meis et al. (2013) propose the application of multiple smaller doses until the desired chemical effect is achieved. This is a fundamental shift from the single ‘bulk dose’ approach employed in this study and may be undesirable given the time and cost implications of achieving restoration targets in the context of the WFD. A balance is required between effective application of measures, technical feasibility, cost and timescales of effectiveness.

One other source of uncertainty when assessing the amount of P removed per unit Phoslock® after an application is the assumption that the uptake capacity is driven solely by La–P interactions and conforms to the 100:1 La:P ratio typically employed to calculate dose. The measured uptake capacity of the product under laboratory conditions and saturated SRP has been reported up to 21,670mg P kg^{-1} Phoslock® (Meis et al. 2013) across all pools and the kinetics of P uptake are expected to vary with chemical composition of the receiving waters (Dithmer et al. 2016a, 2016b, Lürling et al 2016, Spears et al. 2016). If we assume the upper reported uptake capacity then the theoretical yields are 1,669kg P for Mere Mere and 529kg P for Hatchmere based on uptake into all operational pools compared with 795kg P for Mere Mere and 252kg P for Hatchmere, assuming uptake by La into the metal adsorbed P pool. It is apparent that increases in sediment TP mass following the applications are in general agreement with the estimates based on maximum uptake capacity (i.e. increases of 1,289kg P and 634kg P for Mere Mere and Hatchmere, respectively).

The reason behind the apparent slow responses in ortho-P in the incubation experiments following Phoslock® addition for both lakes is not clear. Yasseri (2016) proposed that Fe–P cycling is behind the timing of sediment P control in both lakes, where elevated Fe concentrations in bed sediments may provide a significant pathway for P from bed sediment to water column that bypasses the applied Phoslock® layers. Given that Fe–P cycling is expected to be a major pathway of P across the sediment–water interface in most eutrophic lakes it is difficult to reconcile this hypothesis with the operational performance of Phoslock®. It is common that La can be distributed vertically with sediment depth up to and exceeding 10cm below the bed in treated lakes (Meis et al. 2012, 2013, Dithmer et al. 2016b). The processes of translocation are likely to vary between lakes but may include bioturbation and wind-induced wave disturbance. As La is mixed throughout the upper few centimetres of sediments, a discrete physical ‘cap’ persisting at the sediment–water interface is unlikely, and the likelihood of P released from Fe–P complexes under reducing conditions reaching the water column should increase. What this means is that complete control of internal loading may be unlikely, due to the absence of a surface cap in shallow lakes, especially where bed sediment disturbance is likely.

An alternative hypothesis is that other chemical constituents of the receiving water limit the rate of P uptake by La, but not the nature of the chemical interactions. In P uptake incubation studies in Danish lakes, Dithmer et al. (2016b) demonstrate that P removal from the water column by La was slower under high DOC concentrations (51mg L^{-1}), apparently following a first-order decay trend, and that decreases in P concentrations were not complete up to 400 days incubation. In addition, Dithmer et al. (2016b) report that the initial effect of DOC on La–P binding diminished with time so that the P uptake capacity at the end of the 400-day incubation was similar both in the presence and absence of DOC. It is likely that P adsorption following Phoslock® application in Mere Mere and Hatchmere was slower than observed in other lakes as a result of elevated concentrations of humic compounds, indicated by elevated DOC concentrations. Lürling et al. (2014) indicated that humic compounds may act as a ligand donor in the complexation of bentonite, forming particles of several micrometres in diameter (Bilanovic et al. 2007) and propose the use of a flocculent (polyaluminium chloride; PAC) to reduce the humic content of the receiving waters where DOC is high, preceding a Phoslock® application. Although Lürling et al. (2014) reported an increase in FLa concentration in the presence of 10mg L^{-1} DOC, the results of Winfield and Lofts (2015) indicate that the majority of this pool will be rapidly sequestered through complexation with humic substances. During the monitoring period of the current study (i.e. May 2012 to September 2013) ranges of DOC concentrations reported by the EA for open-water samples were 16mg L^{-1} to 25mg L^{-1} and 13mg L^{-1} to 20mg L^{-1} in Hatchmere and Mere Mere, respectively (Spears et al. 2013a). Nevertheless, saturation of the applied Phoslock® is also apparent from the sediment P analysis reported above and it is probable that a combination of the two hypotheses outlined above is an important confounding factor in Hatchmere and Mere Mere. Other forms of chemical competition for La with P are outlined by Copetti et al. (2016) and include interactions with pH and salinity.

We recommend that these potentially confounding factors be explored on a case-by-case basis for future candidate lakes using a combination of targeted chemical speciation modelling, analysis of field data on variations in chemical composition of receiving waters, and repeat application on intact sediment core (ideally extending to mesocosm scale) incubation trials to estimate effective dose and likely response timescales and endpoints.

6.2 Evidence of persistent catchment nutrient loading

In-lake restoration and management activities, such as sediment removal or the addition of P binding products, can be used to address the problems associated with in-lake recycling of P. In general, these techniques are applied in an attempt to improve water quality and significantly reduce recovery times. However, both short- and/or long-term improvements in water quality may be difficult to achieve or sustain if the original source of the problem has not been addressed. This issue was raised by the project team during the site selection process (Spears et al. 2011a, 2013a), citing results published by Søndergaard et al. (2007) and Lürling and Van Oosterhout (2013) that illustrate this problem. In addition, the high open-water TP values observed following the Phoslock® application (Spears et al. 2015), especially in winter, strongly suggest that there is a persistently high catchment nutrient loading to both Mere Mere and Hatchmere.

Historical information on the P load to Mere Mere from its catchment between 1990 and 1992 are available from Carvalho (1993). Over that period, the average rate of inflow to the lake from the main feeder stream, the Rostherne Brook, was estimated to be about 27.8 L s^{-1}. The corresponding average ortho-P, TSP and TP concentrations recorded were 81µg P L^{-1}, 119µg P L^{-1} and 191µg P L^{-1}, respectively. More details of the values collected by Carvalho (1993) are given in Table 6.1. The overall ortho-P, TSP and TP loads to the lake from this source were estimated to be 43, 67 and 112 kg yr^{-1}, respectively, or 0.28, 0.44 and 0.74g P m^{-2} yr^{-1}, respectively.

Table 6.1 Summary average, maximum and minimum concentrations of orthophosphate-P (ortho-P), total soluble phosphorus (TSP) and total phosphorus (TP) in Mere Mere, 1990–1992 (after Carvalho 1993)

Summary statistic	Ortho-P (µg P L^{-1})	TSP (µg P L^{-1})	TP (µg P L^{-1})	Flow (L s^{-1})
Average	81	119	193	27.8
Maximum	260	563	439	242
Minimum	20	35	42	0

There was no existing historical P loading data for Hatchmere, but some sporadic events of high P inputs have been reported by various authors, with ortho-P concentrations of around 3.5mg L^{-1} being reported in an inflowing drain in 1999 (ECUS 2001a). However, there are historical anecdotal reports of poor agricultural practice within the catchment. These suggest that, in the past at least, silage liquor and, potentially, untreated animal waste have been allowed to enter field drains that connect to Hatchmere (ECUS 2001a). In addition, signs of eutrophication in surface water inputs to this lake were reported by Wiggington (1980) and Moss et al. (1993).

May et al. (2016) report on nutrient loading surveys of the surface water catchments of Hatchmere and Mere Mere conducted between 27 January 2015 and 13 July 2015 for Mere Mere and between 21 October 2014 and 16 October 2015 for Hatchmere (i.e. following the application of Phoslock®). These surveys included the collection of water quantity and P concentrations allowing instantaneous estimates of P load to be extrapolated to annual loads. Given the infrequency of sample collection for water chemistry, it is advised that these extrapolations be treated with caution. However, it is likely that infrequent sampling as utilised here (i.e. monthly frequency or less) often underestimates annual loads, as outlined by Defew et al. (2013). Nonetheless, the results of the survey of Hatchmere and Mere Mere are outlined below and compared with estimates of the ‘critical TP load’ estimated using the generally accepted relationship between TP input to a lake, its flushing rate and its in-lake P concentration (OECD 1982, May et al. 2016).

In 2014–2015, the TP load to Mere Mere from its main inflow was estimated to be about 167kg yr^{-1} (1.09g P m^{-2} yr^{-1}), which is about 3.8 times the maximum annual TP load that would be appropriate for achieving moderate water quality, and 5.5 times the maximum TP load required to achieve good water quality at the site, in relation to the WFD site-specific target (Table 6.2). A similar situation was found at Hatchmere, where the TP load from the surface water catchment via the main inflow was estimated to be about 136kg yr^{-1} (3.46g P m^{-2} yr^{-1}), that is 10 or 15 times the maximum load that would be recommended to ensure sustainable recovery to moderate or good WFD status, respectively. In addition to these inputs from surface water inflows, it is possible that influxes of groundwater may also increase the external TP load. In addition, direct run-off from the ungauged parts of both surface water catchments (31% of the catchment area for Mere Mere, 21% of the catchment area for Hatchmere) will contribute additional P too. The main source of the high TP load to Mere Mere that is being delivered by the Rostherne Brook appears to be coming from an area that is upstream of the culvert sampling point.

In relation to Hatchmere, the data strongly suggest that a ditch that was discharging very high TP concentrations (i.e. 3.5mg P L^{-1}) into the lake when surveyed by ECUS (2001a) is still doing so; in this study we recorded values of up to 7mg P L^{-1} in this small inflow. However, because the flow in this drain is very low, it may represent only a very small proportion (about 1.5%) of the overall P load to the lake from its catchment. The most likely source is an on-site sewage treatment system (such as a septic tank) serving a single household. It is recommended that this is investigated.

Given the hypothetical P uptake capacity of Phoslock® added to Mere Mere and Hatchmere were between 529 and 1,669kg and 252 and 795kg, respectively, the mass of P estimated to be entering both lakes annually is significant and likely to have limited the water column responses to Phoslock® application.

Table 6.2 Measured total phosphorus (TP) load to Mere Mere and Hatchmere from their main inflows in comparison to the calculated 'critical' or maximum load of TP that would be expected to take the P concentration in the lakes above the target WFD water quality boundary values indicated

WFD boundary for lake water quality	Site-specific target TP conc. (µg L^{-1})	Hydraulic load from main inflow (m^3 yr^{-1})	Estimated direct rainfall to lake (m^3 yr^{-1})	Water retention time (yr)	'Critical' TP load (kg yr^{-1}/ mg P m^{-2} yr^{-1})	Measured TP load (kg yr^{-1}/ mg P m^{-2} yr^{-1})
Mere Mere						
High/good	30	432,043	71,100	0.9	30/0.20	167/1.10
Good/moderate	41	432,043	71,100	0.9	44/0.29	167/1.10
Hatchmere						
High/good	35	129,298	21,150	0.4	9/0.23	136/3.46
Good/moderate	48	129,298	21,150	0.4	14/0.36	136/3.46

To place this into context, we can compare our calculated values of P load with those of Janse et al. (2008) who used the model PCLake to generate ranges of critical P loads that should support clear water, macrophyte-dominated conditions across a wide range of lake types. For a lake with a fetch of between 300m and 1,000m, a water inflow rate of between 20 and 40mm day^{-1}, and a mean depth in excess of 1.5m, Janse et al. (2008) report that critical P loads to support clear water conditions should be less than about 0.73g P m^{-2} yr^{-1}. Rast and Lee (1978) demonstrated a range of critical P loads in the North American portion of the original OECD analysis across a gradient of mean depth/hydraulic residence time reporting 'excessive loading' thresholds for lakes with mean depth/hydraulic residence time values of about 3 (i.e. both Hatchmere and Mere Mere) of about 0.3mg P m^{-2} yr^{-1}. Janse et al. (2010) report the range of catchment P loads across 43 Dutch shallow lakes as between 0.2g m^{-2} yr^{-1} and 492g m^{-2} yr^{-1}. In comparison, Jeppesen et al. (1991) reported a P load range of between 0.06 and 217g m^{-2} yr^{-1} for 131 Danish lakes prior to nutrient reduction measures. Based on Hughes et al. (2004) and Carvalho et al. (2005), and using export coefficient modelling, the distribution of P loads for UK lakes constrained to below 5g P m^{-2} yr^{-1} is shown (Figure 7.1; data provided by G. Phillips, 10 March 2016). It should be noted that modelled P load estimates of this type carry with them significant uncertainty when applied at the site-specific scale and are not directly comparable with measured data. However, these estimates provide some context with respect to the distribution of lakes around the range of critical P values outlined above. From Table 6.2, the critical areal P loads for the good/moderate boundary for Hatchmere and Mere Mere are about 0.36 and 0.29g P m^{-2} yr^{-1}, respectively.

6.3 Potential ecotoxicological effects associated with lanthanum

The assessment of fish populations presented significant difficulties in this project due mainly to (a) the lack of established WFD methodologies and targets, (b) more general technical sampling problems which vary by specific water body, and (c) the acceptability or otherwise of destructive sampling such as gill netting which also varies by water body. It was therefore recommended that contemporary fish monitoring field surveys be removed from the project and be replaced with a review of literature of effects of inert suspended sediments and La on selected lake fish species in the context of chemical and ecological monitoring data in Mere Mere and Hatchmere.

Concern has been raised regarding the potential for release of FLa following Phoslock® application and the potential unintended ecological implications of this release (Stauber and Binet 2000, Hickey and Gibbs 2009, Lürling and Tolman 2010, Spears et al. 2013b). The speciation of FLa ions is also important when considering ecotoxicological impact, and of the FLa species (e.g. La^{3+}, $La(OH)^{2+}$) the La^{3+} ion carries the greatest risk of biological effects (Das et al. 1988). In particular, it is important to assess the concentration of FLa in receiving lakes and to relate this concentration to FLa speciation.

As part of this project, Winfield and Lofts (2015) reviewed the published and grey literature on the effects of inert suspended solids and La on selected lake biota, with a focus on fish, and used this evidence to determine the likely corresponding effects of the Phoslock® applications at Hatchmere and Mere Mere. In addition, chemical speciation modelling using data available from the treated lakes was conducted to examine the likelihood of persistent La^{3+} concentrations following Phoslock® application. The key results of these studies are discussed below and the potential pathways of La through the food web considered are outlined (Figure 6.1).

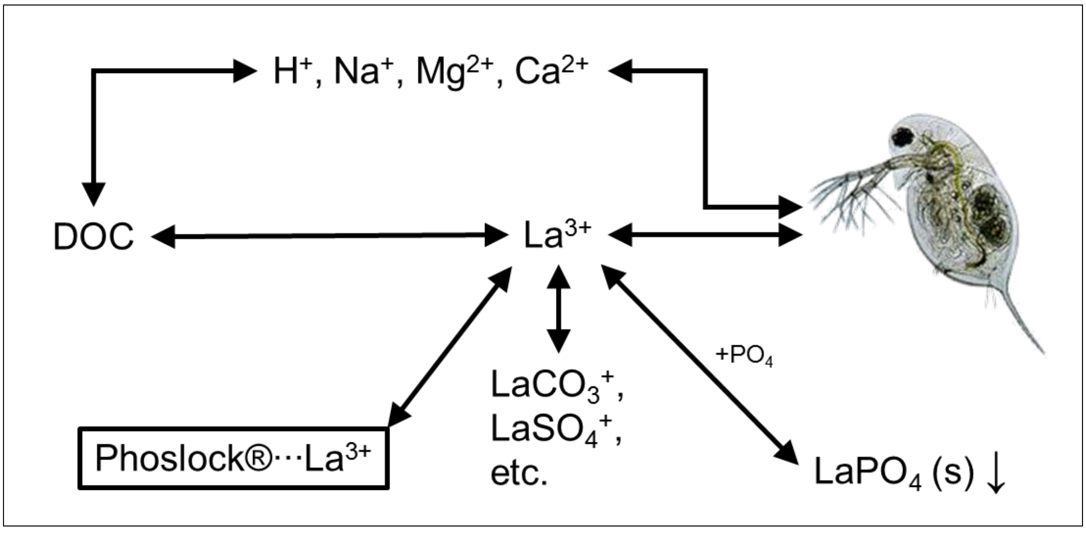

Figure 6.1 Conceptual diagram of the chemical speciation of lanthanum and of the potential interactions between other chemical components of lake water and with organisms following Phoslock® addition to a lake

6.3.1 Review of studies reporting ecotoxicological assessments

Searches of the published literature were conducted between 5 May 2014 and 9 January 2015, supplemented by the use of articles of grey literature already assembled within the project and by approaches to personal contacts between 20 September 2013 and 9 January 2015. Approximately 70 relevant articles (of which approximately 50% contained significant amounts of information on fish) were identified. However, many of these studies were focused on lotic rather than lentic water bodies or species and very few of them addressed macrophytes or algae in sufficient detail for the present purposes. The application of this body of knowledge to the interpretation of likely effects of elevated concentrations of suspended solids at Hatchmere and Mere Mere was also compromised by the lack of recent field data from either site. The measurement of suspended solids should be considered in future applications.

Conclusions regarding the biological effects of elevated concentrations of suspended solids and La varied considerably with taxa. Increases in suspended solids and La were considered unlikely to have had any effects on the fish or macroinvertebrates, as were increases of suspended solids for zooplankton, in Hatchmere and Mere Mere. In contrast, increases in La had the potential to have had a short-term effect on zooplankton in relation to laboratory based ecotoxicology studies, although effects from suspended solids were considered to be unlikely (Winfield and Lofts 2015). Conclusions regarding macrophytes were based on a limited evidence base, but suspended solids were thought unlikely to have had any effect while it was considered impossible to reach any robust conclusions regarding La. Reviewed studies on algae also provided only a very limited evidence base and it was considered impossible to draw any robust conclusions about the effects of suspended solids, although the observed increases in La were considered to have a higher likelihood of a negative effect (Winfield and Lofts 2015).

Significant bioaccumulation of La was unlikely in the two longest-lived and largest-sized groups of biota (i.e. fish and macrophytes) considered in the present review (Winfield and Lofts 2015). This is an important conclusion because it indicates that even if, in contrast to current common practice, fish were to be consumed from either lake there would be no consequences for human health related to La.

Table 6.3 Summary of the ecotoxicological thresholds for lanthanum-bentonite (LMB) and filterable lanthanum (FLa). EC_{50} = 50% effect concentration (mg L^{-1}); NOEC = no effect concentration (mg L^{-1}); LOEC = lowest observed effect concentration (mg L^{-1}), after Copetti et al. (2016)

Test organism	Test conditions	Stressor	Endpoint	EC_{50}	NOEC	Reference
Zooplankton						
Daphnia carinata	$LaCl_3$, solution, soft water, 48 hours	FLa	Mortality	0.04		Barry and Meehan (2000)
Daphnia carinata	$LaCl_3$, solution, hard water, 48 hours	FLa	Mortality	1.18		Barry and Meehan (2000)
Daphnia carinata	$LaCl_3$, solution, hard water, 6 days	FLa	Survival, growth		< 0.06	Barry and Meehan (2000)
Daphnia magna	Not specified, solution, 48 hours	FLa	Reproduction	24		Sneller et al. (2000)
Daphnia magna	$La(NO_3)_3{\bullet}6H_2O$, food suspension, P-containing medium, 14 days	FLa	Growth (length)		LOEC = 0.1	Lürling and Tolman (2010)
Daphnia magna	$LaCl_3$, solution, hard water, 21 days	FLa	Reproduction		0.1	Sneller et al. (2000)
Daphnia magna	LMB, suspension, 5 days	LMB	Juvenile growth (weight)	871	100	Lürling and Tolman (2010)
Daphnia magna	LMB, suspension, 5 days	LMB	Juvenile growth (length)	1,557	500	Lürling and Tolman (2010)
Daphnia magna	LMB, suspension, 48 hours	LMB	Immobilisation	> 50,000		Martin and Hickey (2004)
Daphnia magna	LMB, suspension, 48 hours	LMB	Mortality	4,900		Watson-Leung (2008)
Ceriodaphnia dubia	$LaCl_3$, solution, 48 hours	FLa	Immobilisation	5	2.6	Stauber and Binet (2000)
Ceriodaphnia dubia	$LaCl_3$, solution, 7 days	FLa	Reproduction	0.43	0.05	Stauber and Binet (2000)
Ceriodaphnia dubia	LMB, leachate, 48 hours	FLa	Mortality	0.08		Stauber (2000)
Ceriodaphnia dubia	LMB, leachate, 7 days	FLa	Mortality	0.82		Stauber (2000)
Ceriodaphnia dubia	LMB, leachate, 7 days	FLa	Reproduction	0.28		Stauber (2000)
Ceriodaphnia dubia	LMB, suspension, 48 hours	LMB	Immobilisation	> 50		ECOTOX (2008)
Ceriodaphnia dubia	LMB, suspension, 7 days	LMB	Immobilisation and reproduction	> 1		ECOTOX (2008)
Brachionus calyciflorus	LMB, suspension, 48 hours	LMB	Population growth rate	154	100	Lürling and Van Oosterhout (2013)
Fish						
Melanotaenia duboulayi	$LaCl_3$, solution, 96 hours	FLa	Immobilisation	< 0.6	< 0.6	Stauber and Binet (2000)
Oncorhynchus mykiss	LMB, suspension, 48 hours	LMB	Mortality	> 13,600		Watson-Leung (2008)
Macroinvertebrates						
Hyalella azteca	$LaCl_3$, solution, soft water, 7 days	FLa	Mortality	0.02		Borgmann et al. (2005)

Hyalella azteca	$LaCl_3$, solution, hard water, 7 days	FLa	Mortality	1.67 (nominal)		Borgmann et al. (2005)
Hyalella azteca	LMB, suspension, 14 days	LMB	Survival and growth	> 3,400		Watson-Leung (2008)
Hexagenia sp.	LMB, suspension, 21 days	LMB	Survival and growth	> 450		Watson-Leung (2008)
Chironomus dilutus	LMB, suspension, 38 days	LMB	Survival and growth	> 450		Watson-Leung (2008)
Chironomus zealandicus	LMB, suspension, 38 days	LMB	Survival, emergence, sex ratio	> 400	400	Clearwater (2004)
Nematodes						
Caenorhabditis elegans	$LaCl_3$, solution, 72 hours	FLa	Growth, reproduction		1.39	Zhang et al. (2010)
Macrophytes						
Hydrocharis dubia	$La(NO_3)_3$, solution, 7 days	FLa	Chlorophyll content	2.78		Xu et al. (2012)
Hydrilla verticillata	$La(NO_3)_3$, solution, 10 days	FLa	Chlorophyll content, oxidative stress		1.39	Wang et al. (2007)

6.3.2 Chemical speciation analysis

Chemical speciation modelling of La in Hatchmere and Mere Mere during the time period before, during and after Phoslock® addition predicted that La^{3+} concentrations were well below any of the threshold values (lowest threshold value of 20µg L^{-1}) reported in Table 6.3, even though total filterable La (Fla) concentrations may indicate otherwise. Chemical equilibrium modelling indicated that any La^{3+} released from the bentonite would be effectively scavenged from the water column within $LaPO_4$ (s) and $La_2(CO_3)_3$ and through the formation of complexes with DOC (Figures 6.2 and 6.3). When simulated with a lower DOC concentration than that observed in the treated lakes, predicted La^{3+} concentrations were higher, and at the highest FLa concentrations reported for both sites carbonate was an important binding ligand for La^{3+}. A simple assessment based on La toxicity to a single species (*Daphnia carinata*) suggested that this species would not be at acute risk under the chemical conditions prevailing in Hatchmere and Mere Mere; however, a potential risk at the highest FLa concentration was indicated in a lake of the chemistry of Mere Mere but with a much lower DOC concentration (i.e. 2.5mg L^{-1}). Not accounting for differences in La^{3+} bioavailability between the laboratory and field may overestimate risk by not taking DOC complexation of La^{3+} into account.

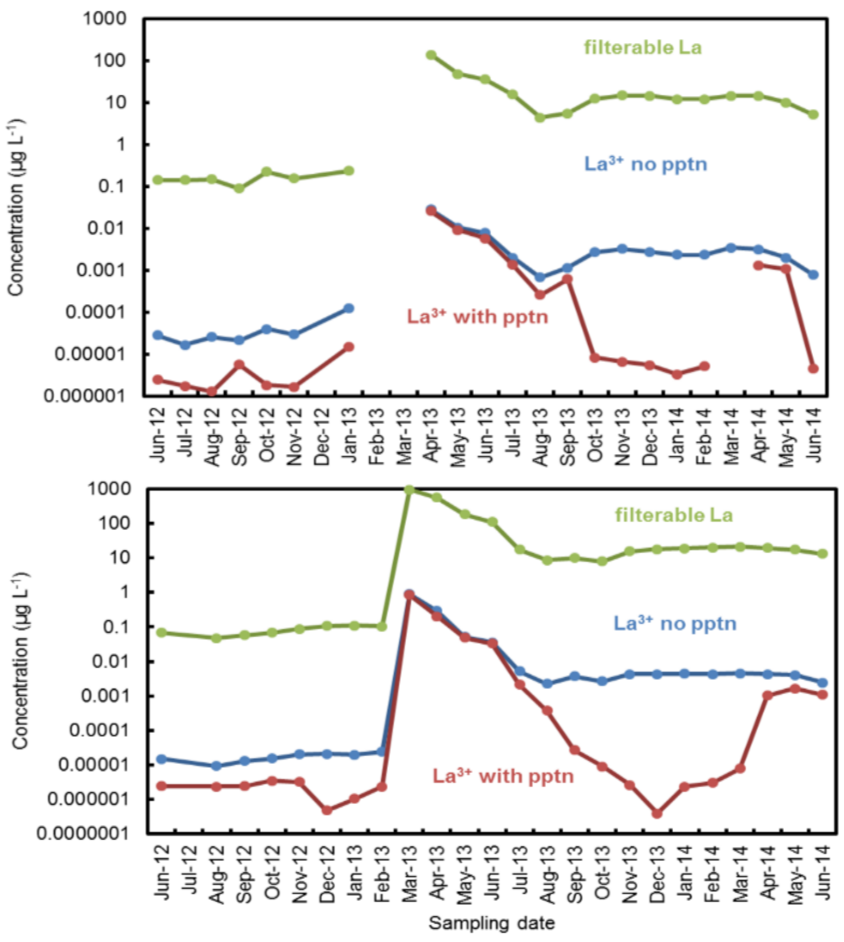

Figure 6.2 Basic lanthanum speciation for Hatchmere and Mere Mere. Temporal trends in filterable La and predicted free La^{3+} with precipitation (pptn) of $LaPO_4$ (s) and $La_2(CO_3)_3$ (s) allowed

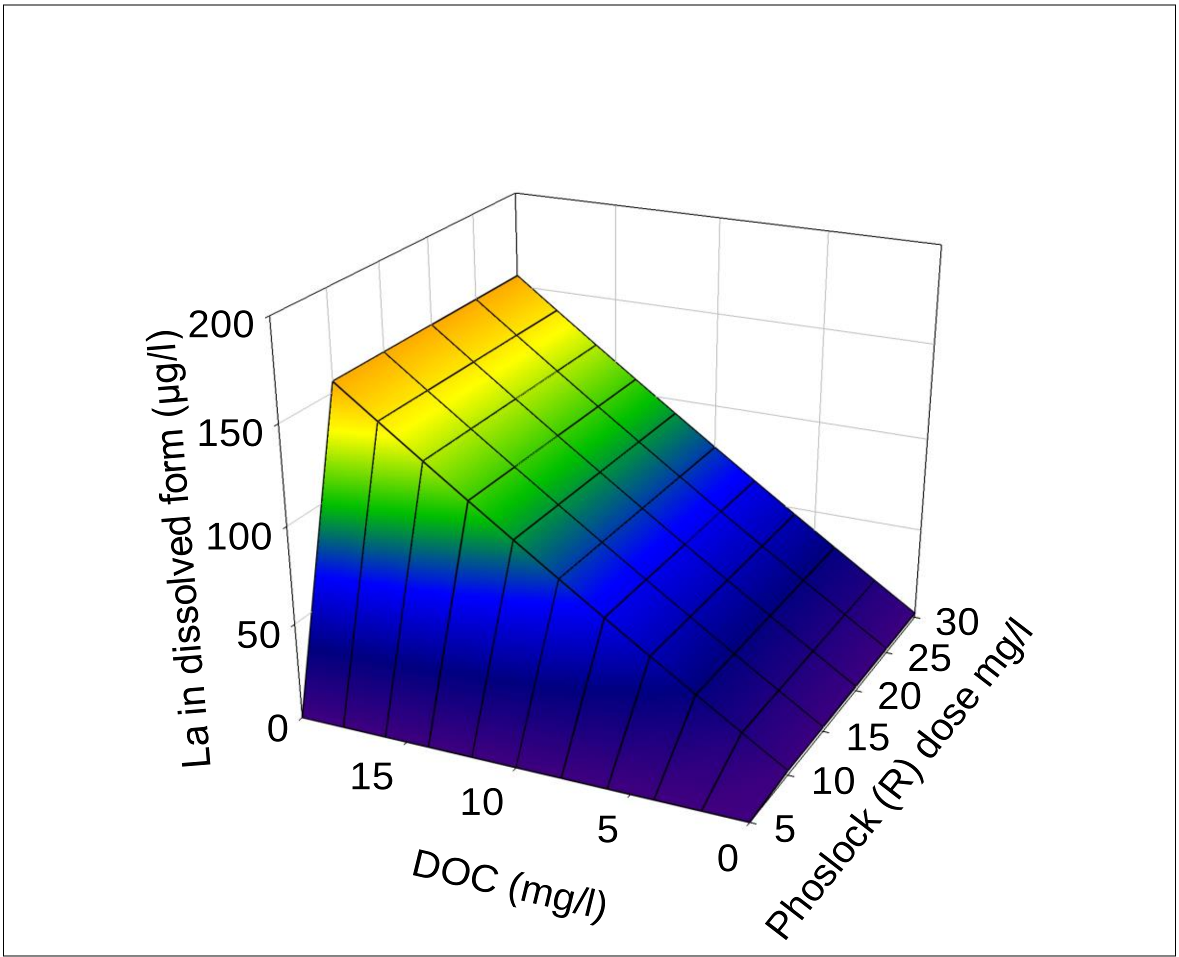

Figure 6.3 Predicted dissolved lanthanum (La) in Hatchmere water of April 2012 as a function of Phoslock® (and La) dose and water column DOC concentration. Predictions assume that $LaPO_4$ (s) and $La_2(CO_3)_3$ (s) may precipitate

The speciation modelling predicted a strong influence of the DOC concentration on the concentration of La in dissolved form at chemical equilibrium (Figure 6.3). With the exception of the lowest Phoslock® dose where, as noted, all La forms La-rhabdophane, the predicted concentration of La in dissolved form increases with increasing DOC concentration, but does not increase with Phoslock® dose. However, the predicted La^{3+} activity was constant at just over 0.0002µM (0.028µg L^{-1}) at all the Phoslock® doses except the lowest, where it is approximately four orders of magnitude lower. The proportion of La remaining bound to the bentonite clay is consistently predicted to be negligible (< 0.001%) in all the circumstances modelled.

Effective and useful interpretation of speciation modelling requires an appreciation that the system under investigation may not be at chemical equilibrium. For example, slow kinetics of solid formation may confound predictions. For this reason, we have made predictions of La speciation in the meres both allowing for and not allowing for precipitation of solids, in order to provide information on the potential importance of this process while accounting for the uncertainty regarding its actual occurrence in the field. Speciation calculations should be seen as providing information on the likely trends in La chemistry in relation to water chemistry. In the field, rates of physical processes such as particle settling, water mixing and flushing are likely to further influence speciation over time.

Speciation modelling consistently predicted that filterable La in the waters of Hatchmere and Mere Mere was largely present either as complexes with DOC or as a La-rhabdophane solid. The predicted presence of La-rhabdophane able to pass the filters used does not imply an error, since it is well known that naturally occurring solids, such as iron oxyhydroxides, may pass conventional filters with pore sizes down to 0.1µm (Fox 1988). Allowing this precipitation to be modelled produces much lower prediction of the free La^{3+} activity than if it is not modelled, demonstrating the importance of precipitation as a control on the free ion activity. Complexation of La by other inorganic ligands (e.g. carbonate) is generally less important, although it may become more important in systems where the DOC concentration is lower than in Hatchmere and Mere Mere.

The theoretical calculations also showed the importance of precipitation as a control on the free La^{3+} activity. Of particular note is the predicted importance of $La_2(CO_3)_{3\,(s)}$ formation as a sink for La released from Phoslock®. Our calculations used a fixed alkalinity, which provides an effectively infinite supply of CO_3^{2-} ions for $La_2(CO_3)_{3\,(s)}$ formation. In real-world situations this corresponds to a situation where loss of carbonate ions from the water due to $La_2(CO_3)_{3\,(s)}$ formation is completely buffered by dissolution of gaseous CO_2 from the atmosphere, to maintain a constant activity of CO_3^{2-}. In practice this buffering may not be complete, due to slow dissolution of gaseous CO_2, and may also be influenced by inorganic carbon uptake and loss due to photosynthesis and respiration, and so $La_2(CO_3)_{3\,(s)}$ formation may not be as important as implied by the theoretical calculations. Nonetheless, given that $La_2(CO_3)_{3\,(s)}$ formation would reduce Phoslock® efficiency, there is a need to better establish the importance of $La_2(CO_3)_{3\,(s)}$ formation in real-world situations.

A further aspect of note in the theoretical calculations is the poor predicted retention of La by the Phoslock® itself – competition by solution complexation and precipitate formation was predicted to remove essentially all the La from the Phoslock® which is unrealistic. For example, Dithmer et al. (2015) have previously reported formation of $LaPO_4$ close to the clay matrix indicating that a major proportion of the La is retained within the clay following application. Given the importance of La retention in settling Phoslock® particles for longer-term sequestration of P released from the bottom sediments, further work to establish the actual retention of La by Phoslock® in field situations is needed.

Formation of $La_2(CO_3)_{3\,(s)}$ and solution complexes (particularly with DOC) does not imply that the La is no longer available for P sequestration in the longer term, in response to further P additions to the water column. In particular, La complexed to DOC is likely to remain available for La-rhabdophane formation, unless and until it is physically flushed from the system. Formation of $La_2(CO_3)_{3\,(s)}$ may be followed by settling of the precipitate to the bottom sediments (assuming that the settling time is less than the lake flushing time). It is possible that subsequent dissolution of this $La_2(CO_3)_{3\,(s)}$ may provide La for P sequestration in response to further P inputs to the water column; however, the degree to which this actually occurs is likely to depend on the dissolution kinetics of $La_2(CO_3)_{3\,(s)}$ in the presence of PO_4 at the sediment–water interface:

$$La_2(CO_3)_{3\,(s)} + 2PO_4 \rightarrow 2LaPO_{4\,(s)} + 3CO_3^{2-}$$

Thus, more information on dissolution of $La_2(CO_3)_{3\,(s)}$ in the presence of La-rhabdophane may be useful to better understand how $La_2(CO_3)_{3\,(s)}$ formation ultimately influences the efficacy of Phoslock® addition for P sequestration in the longer term.

The simple assessment done here demonstrates the importance of accounting for bioavailability when assessing the risks of La in the water column to aquatic organisms. Risks to *Daphnia carinata* estimated solely by comparing the observed filterable La with endpoint concentrations expressed as total dissolved La are much higher than when risks are assessed by comparing free La^{3+} activities in the toxicity tests to field waters, after accounting for the effects of water chemistry. The control of La^{3+} activity by solid formation and DOC complexation is indicated to be important in determining the level of risk. More comprehensive bioavailability modelling, as has been developed for other metals such as copper (European Copper Institute 2007) and nickel (European Commission 2008), would enable more comprehensive and robust assessment of the risks.

7 Summary and recommendations

This section presents a review of the evidence and lessons learned from this study in relation to wider use of the P-capping approach in lakes to meet WFD water quality targets. We also draw on evidence from the literature, produced mainly during this project, to substantiate, where possible, the evidence from Hatchmere and Mere Mere.

During the course of this project, evidence and approaches have been developed that are being used to support the development of guidance on what factors to take into account if using such products. This will be presented in a separate report when completed.

The main conclusion drawn from the project is that the applications of Phoslock® used were (as a result of confounding factors including persistent catchment P loading competition between DOC and P for La) insufficient to control sediment P release and hence the desired responses in water quality and ecological structure. However, for both lakes the results indicated reductions in water column TP concentrations up to two years following the application. Furthermore, reductions in bottom water ortho-P concentrations and shifts in sediment P pools indicated a reduction in internal loading, especially in Mere Mere, as a result of the Phoslock® applications.

In general, the project has delivered a comprehensive evidence base with which the use of internal loading control alone to meet WFD objectives can be assessed. Given the complex nature of ecological communities in lakes and of the multiple pressures acting upon them, it is unlikely that any single measure will be sufficient to achieve ecological recovery in any lake. While the use of materials like Phoslock® to reduce P concentrations in lakes can be effective, they do not necessarily offer a high likelihood of achieving ecological recovery. Ecological recovery can lag behind chemical recovery as a result of a number of site-specific factors including biological connectivity and physical habitat structures (as is the issue in the Broads) (Phillips et al. 2005, Verdonschot et al. 2013). Active management of ecological communities may therefore be necessary to achieve specific targets, for example of community composition. In the case of Hatchmere and Mere Mere, although reductions in water column TP concentrations were apparent, they were not substantial, and did not result in significant improvements in water clarity and resulted in no major improvements in phytoplankton, macrophytes, macroinvertebrates or zooplankton. Although fish populations were not monitored for reasons given above, given their relatively high positions in the lakes' food chains, their individual longevities and the conclusions of the ecotoxicological review, it is highly unlikely that the fish of either lake were significantly influenced by the applications of Phoslock®.

Given the need to address the general issues raised above, we stress the need for site-specific assessments including mass balance calculations of candidate lakes.

7.1 Factors affecting phosphorus control

One of the most common reasons for failure to reach targets in the management of eutrophication in lakes is insufficient reduction of catchment P loading (Søndergaard 2007). This is also consistent with studies in which materials have been used to control internal P loading including Phoslock® (Lürling and van Oosterhout 2013). Estimates of longevity for improved water quality (measured as TP response) in lakes treated with aluminium salts range from 0 to 41 years (average of 11 years; n = 114 lakes; Huser et al. 2016b). Given the relatively short period of time over which field trials using Phoslock® have been conducted it is difficult to draw comparisons with aluminium-treated lakes. However, examples of limited or no responses in water quality following Phoslock® application as a result of insufficient catchment load reduction have been reported (Spears et al. 2016).

In the current study, it was assumed from the outset (based on expert knowledge and on the general assessment of seasonal trends) that both Hatchmere and Mere Mere would respond well following the control of internal P loading alone. Following a lack of response in water quality in both lakes in year 1 post-application the project team examined potentially confounding factors and reshaped the project to reduce monitoring of fish responses[2] in place of monitoring of catchment P load.

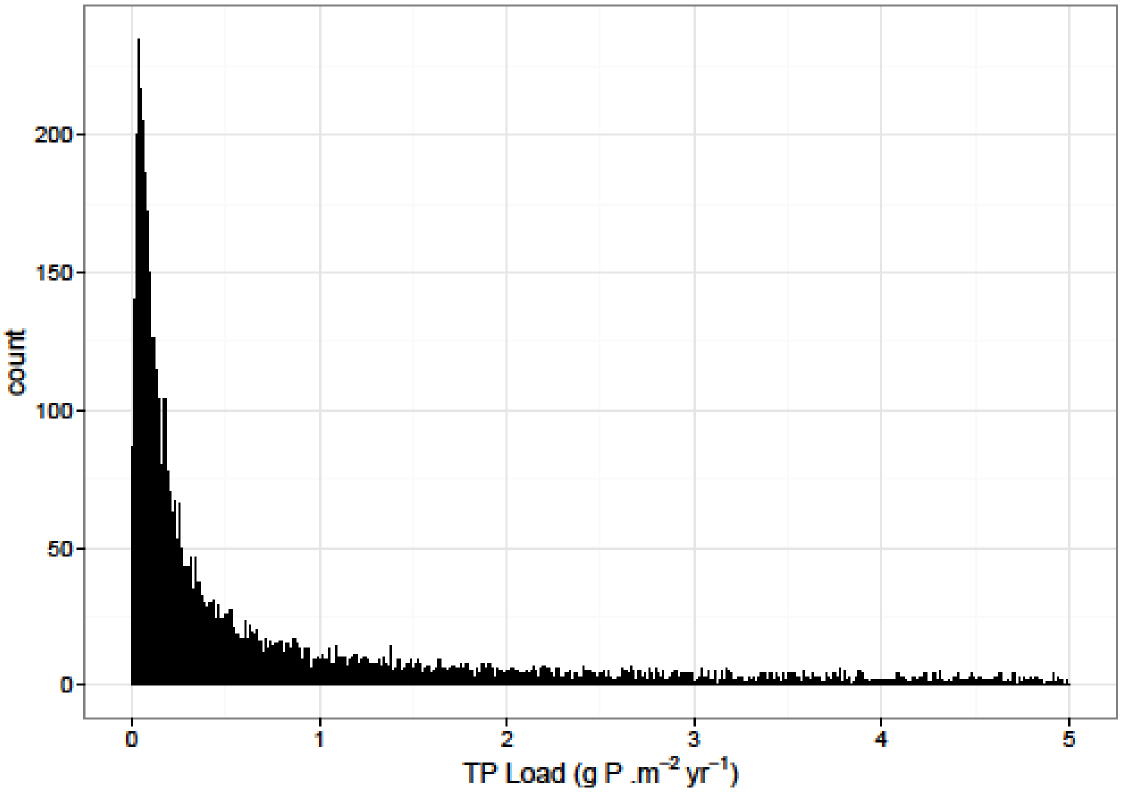

Figure 7.1 Distribution of areal phosphorus (P) loads to UK lakes constrained to include only those lakes with a P load of less than 5g P m^{-2} yr^{-1}. Methods for the determination of P loads described by Hughes et al. (2004) and Carvalho et al. (2005)

Estimates of annual P load from the catchments of both lakes indicated levels that were likely to limit the effects of internal loading control. Assuming the distribution of areal P loads in Figure 7.1 is broadly representative of UK lakes it is apparent that a large proportion of lakes in the UK have P loads that are likely to confound the chemical and ecological responses associated with an application of Phoslock®, in the absence of catchment management to reduce P loads. A useful approach to identify

[2] It should be noted that monitoring of the fish communities of both lakes was constrained to qualitative sampling methodologies (Spears et al. 2014). The absence of comprehensive and quantitative fish community data means that we can draw no conclusions on (1) the direct ecotoxicological effects of Phoslock use on fish, (2) indirect effects of Phoslock use on fish community composition, or (3) the effects of fish as regulators of ecological responses to reduced nutrient loading.

these lakes is the comparison of measured catchment P loads with critical P loads to meet WFD good/moderate ecological status as reported for Hatchmere and Mere Mere in Table 6.1.

Evidence on P loading is essential to inform site-specific management measures. Seasonal trends in TP and Chl*a* can indicate importance of internal loading relative to catchment loading (Phillips et al. 2005, Søndergaard 2007, Sharpley et al. 2013) as demonstrated here. However, site-specific P mass balance assessments should be used to calculate the loads of P from both catchment and bed sediments to be controlled to meet critical P loads (Lürling et al. 2016). Ideally, whole system P mass balances should be constructed to allow assessment of the effects of P reduction scenarios, including cost-benefit assessments (Hupfer et al. 2016, Huser et al. 2016b). These mass balance models should include estimates of P stocks in bed sediments and an assessment of the proportion of these stocks responsible for water quality deterioration during periods of intense internal loading (Phillips et al. 2015). For example, Hupfer et al. (2016) used a simple 'one-box' P mass balance model to demonstrate shortened recovery time in lakes with long water residence times only when catchment P load reduction is combined with internal load control. Using the constructed P mass balances, the longevity of any application of material to control sediment P release should be estimated in line with cumulative catchment P load.

Huser et al. (2016b) used similar mass balance approaches to predict the longevity of alum treatments in Minneapolis lakes. The Minneapolis lakes study indicated that different dosing approaches would lead to improvements in water quality for between four and 20 years. However, in some circumstances (i.e. in urban lakes with elevated P loads), continuous sediment treatment was proposed as the only option for water quality improvements. Had this approach been adopted in the present study it would have been apparent that for Hatchmere and Mere Mere the applied dose of Phoslock® would be saturated based on catchment load alone, within three and ten years for Mere Mere and two and six years for Hatchmere, regardless of internal load. It is essential that the cost-effectiveness of monitoring, catchment management and in-lake measures be assessed when planning lake management programmes.

In addition to the need to control catchment and internal sediment P sources, other management measures may be necessary to increase the likelihood of successful eutrophication management. Huser et al. (2016a) demonstrate the importance of benthivorous fish (e.g. grass carp, *Ctenopharyngodon idella*) in disturbing bed sediments and redistributing sediment P latterly across the lake bed. Under these conditions it is important to consider an appropriate sediment depth when estimating effective dose. It may also be necessary to conduct biomanipulation of the fish community to favour increased abundance of large cladocerans and reduced phytoplankton biomass. Phillips et al. (2015) present a model for achieving ecological recovery in the Norfolk Broads, indicating that combined control of catchment and internal P loads with biomanipulation to strengthen the response of the phytoplankton community to P reductions should be an effective approach, especially in isolated water bodies. This approach was demonstrated in field experiments by Waajen et al. (2016), where Chl*a* concentrations were about 6µg L^{-1} when Phoslock® was combined with fish biomanipulation, were less than 44.5µg L^{-1} when biomanipulation was conducted alone and were between 52.4 and 269µg L^{-1} with no treatment. Waajen et al. (2016) also demonstrated the successful introduction of macrophytes in the field experiments establishing ecological recovery within one year following Phoslock® plus biomanipulation interventions, although the long-term effectiveness of this approach has yet to be confirmed.

An authoritative review of biomanipulation is provided by Hansson et al. (1998). Such studies have relevance to the present project because biomanipulation in Europe frequently involves the removal, or at least great reduction, of roach populations due to this species frequently being a major predator of zooplankton populations, especially in eutrophic waters. Such biomanipulated fish populations have been removed or reduced by manual removal through netting or electric fishing, by the addition of predatory fish, or by the use of chemicals such as rotenone. The UK experience with biomanipulation, where fish removal/reduction methods are tightly restricted, is reviewed by Moss et al. (1996), with Perrow et al. (1997) providing a more fish-focused account and Phillips et al. (1999, 2015) concentrating on its practical aspects. In the UK, such work has been focused almost entirely on the Norfolk Broads of south-east England, with the manual removal of roach and, to some extent, other species, by repeated electric fishing, some netting and some egg removal being reviewed by Perrow et al. (1997) and Phillips et al. (1999).

The potential for ecotoxicological effects associated with the Phoslock® applications to Mere Mere and Hatchmere were assessed. This assessment considered evidence drawn from a comprehensive review of available literature coupled with chemical speciation modelling. The results of these studies indicated that long-term ecotoxicological effects on the food web of both sites were unlikely. However, evidence from presence–absence observations of macroinvertebrates indicated a general reduction in biomass and abundance following Phoslock® applications. It was also reported that the comparisons between laboratory based ecotoxicological assessments and field conditions are invalid as a result of methodological issues (Copetti et al. 2016). This is due to the importance of relatively complex chemical composition of receiving waters in comparison to the simple, and unrealistic, chemical compositions used in most laboratory based assessments.

The chemical speciation modelling approach proved useful in addressing these methodological shortfalls and provided evidence that laboratory based studies can overestimate the potential for ecotoxicological effects in the field. This approach was useful, and should be developed to produce a comprehensive assessment of wider ecotoxicological risk associated with Phoslock® and other materials proposed for the control of internal loading by combining data from multiple laboratory based studies across many test species to examine the relationships between environmental risk related to the chemical and biological composition of receiving waters. To confirm the results of this modelling approach at the site-specific scale, lake enclosure studies could be used to ensure no undesirable ecotoxicological effects of Phoslock® application prior to applying products at the field scale.

Interactions between humic substances and Phoslock® were identified as a key mechanism maintaining low La^{3+} concentrations in the water column of the treated lakes. The importance of these processes in retarding the ortho-P uptake capacity and kinetics of Phoslock® were also reviewed. This review confirmed that the operational performance of Phoslock® can vary depending on the chemical composition of the receiving waters and these interactions are reviewed in detail by Copetti et al. (2016), drawing on multiple laboratory and field based trials. It is recommended that site-specific studies are conducted prior to any future application to examine the operational performance of the product and to set expectations for timescales and endpoints of P reductions. In this report, intact-core incubations were useful in determining likely kinetics of P removal from the water column and the control of P release from bed sediments. These simple experiments demonstrated that insufficient product had been added to both lakes to effectively control sediment P release under reducing conditions.

Repeated dose incubation trials have been used previously to estimate the dose at which sediment P release is effectively controlled (Meis et al. 2013). In this study, chemical equilibrium modelling confirmed that the apparent slow response was likely due to interactions between humic compounds and La in both lakes. The simple single dose estimate procedure employed in this study and many others based on estimates of sediment P stocks appears to be insufficient to ensure effective control of internal P loading. This conclusion is consistent with evidence from other studies and suggests the need to change expectations of the timescales of the approach to reach management targets (Meis et al. 2013, Dithmer et al. 2016b). Serial dosing may be considered in future where a larger dose in year 1 is followed by smaller doses in following years to ensure internal loading is effectively controlled. We acknowledge that this approach will extend the treatment period and increase application costs from single years to multiple years and that this treatment period will depend on a range of physicochemical processes that vary at the site-specific level (Dithmer et al. 2016b).

An approach not used in this study was the assessment of product dose-response within lake enclosures prior to field scale application. Lürling et al. (2016) recommend that such studies be conducted during pre-application monitoring to provide more realistic evidence of product behaviour in candidate lakes, and to confirm estimates of effective dose, environmental risk and short-term ecological responses following product application. Clearly a trade-off must be reached between gathering sufficient evidence with which management measures can be selected and implemented with sufficient chances of success, without conducting a full research programme on each candidate lake.

7.2 Conclusions and recommendations

In general, the standard WFD monitoring programme employed during this project was sufficient to detect physicochemical and ecological responses following the Phoslock® applications. These observations provided some insights into both structural and functional responses, and responses in WFD metrics relative to targets across ‘biological quality elements’. However, some supplementary data were also necessary to determine the responses, or their lack thereof, following the Phoslock® application. These included quarterly sediment P composition and content, monthly metal concentrations in the water column and sediments, and annual sediment core dose-response incubations to confirm whether or not internal P loading had been controlled. In terms of ecological responses, annual surveys of aquatic macrophytes, quarterly surveys of macroinvertebrates (both littoral and profundal) and monthly surveys of zooplankton communities served to document short-term ecological responses following the Phoslock® application. The assessment of fish responses were limited due to a lack of acceptable survey approaches, as discussed in detail by Spears et al. (2014). However, bespoke survey approaches to determine the likely impact of fish on other elements could be considered. It should be stressed that monitoring to inform remediation activities (i.e. designed to detect early onset of subtle responses in ecological structure and function) will require more comprehensive activities when compared to monitoring for assessment of ecological structure alone.

One limitation of this study was the short length of pre-application water quality monitoring data available for Hatchmere, which limited the statistical power associated with the assessment of responses. In particular, our analysis approach was limited in its ability to detect inter-annual variability with which the responses measured could be confirmed. To address this issue the project team attempted to identify data from a suitable control lake with which a Before-After-Control-Impact (BACI) analysis could be performed. This proved difficult due to a lack of available long-term data across nearby lakes and due to a lack of monitoring across all necessary determinands. It is suggested that finding an ideal control site that is not experiencing some form of environmental change may be challenging. As such, our analysis is limited to a BA approach with validation using long-term variations in weather and physicochemical data prior to the application. We acknowledge that this approach may not account for conditions that may confound the detection of responses (e.g. extreme weather or catchment loading events). That said, available data indicated that the former, at least, had not occurred during the course of the experiments.

To increase the confidence in detection of responses following an application we recommend that suitable control lakes be identified, and that the use of populations of control lakes be assessed statistically using the guidelines outlined by Schroeter et al. (1993) and Lang et al. (2016) and include estimates of confidence ratings for detection. Ideally, suitable monitoring programmes should be in place across a range of control lakes to provide national-scale baseline data against which future restoration programmes can be assessed with sufficient confidence. In practice, we acknowledge that this is unlikely. We also acknowledge that the implementation of long-term monitoring programmes is costly, but the value of such activities across strategic sites is that a high degree of confidence in the detection of responses can be assured against the effects of uncontrollable experimental conditions in the field.

The general approach considered in this project included the development of improved water quality conditions only, with no additional measures to support responses in ecological communities. This approach provides a 'window of opportunity' for ecological recovery but does not ensure responses in ecological communities. Given the improvements in water quality achieved here were limited, the lack of responses across ecological communities was expected. However, in other studies where Phoslock® has been used to control internal loading and where water quality improvements have been more dramatic (i.e. for Phoslock® applications see Spears et al. 2016), short-term (i.e. < 2–5 years) responses in phytoplankton (Lang et al. 2016) and macrophytes (Spears et al. 2016) have been apparent, although not necessarily instantaneous. Given the potential for time-lags between chemical and ecological recovery, and due to the wide range of estimated periods of effective internal loading control (e.g. 0 to 41 years; Huser et al. 2016a), annual assessments of responses will be useful and this is not consistent with the WFD assessment approaches that consider multi-year averages.

It is important that management responses remain reactive during the initial phases of treatment to ensure that the effectiveness of internal loading control is not limited. Rapid and effective management of unforeseen confounding factors can ensure that investment in management measures are not lost. The post-application phase is the most crucial period throughout which monitoring and management responses should be prioritised and the scope of work revised according to regular reporting programmes, as was the case in the current project.

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Appendix 1 Scientific hypotheses relating to the application of Phoslock® to the treated lakes

	Hypothesis	Example response variables	Frequency	Hypothesis confirmed?
Assessing lake experiment controls				
1	Weather conditions during the monitoring period will not be significantly different from the 10-year mean	Water temperature, wind conditions, rainfall	Daily	Y
Assessing expected responses to the Phoslock® application				
2	Summer and autumn phosphorus concentrations will be significantly lower in surface and bottom waters	TP, SRP, TSP	M	Y
3	Apatite/metal bound P pools will increase in the sediment and depth effects will be insignificant	Sediment surveys (six sites)	A	N
4	Total and filterable lanthanum concentrations will be significantly higher	TLa, FLa, FLa species from modelling	M	Y
5	No significant changes will be observed in other water chemistry parameters after the Phoslock® application	TON, SiO_2, pH, conductivity, DO, DOC, colour	M	N
6	Summer and autumn chlorophyll *a* concentration, water clarity and cyanobacteria abundance will be significantly lower and phytoplankton community more diverse	Chlorophyll *a* concentration, Secchi depth, total suspended solids concentration, phytoplankton community abundance/biovolume	M	N
7	Summer and autumn large-bodied cladoceran zooplankton abundance and biomass will increase as will zooplankton species richness	Zooplankton biomass and abundance data	M	N

	Hypothesis	Example response variables	Frequency	Hypothesis confirmed?
8	Macrophyte maximum growing depth will be significantly deeper and the community more diverse	Macrophyte survey data	A	N
9	An increase in species richness and a decrease in abundance will occur in the macroinvertebrate community	Macroinvertebrate survey	Q	N
10	Responses in WFD metrics will be significant and will result in an improvement in ecological quality	Phytoplankton and macrophyte metrics	M, A	N
Quantifying lake-specific recovery trajectories following the Phoslock® application				
11	Recovery times for water chemistry (and clarity) determinands will be significantly different and lake specific	TLa, FLa, La species, TP, SRP, TSP, chlorophyll *a*, cyanobacteria abundance, Secchi depth, Total suspended solids (TSS), DOC, colour, TON, SiO_2	M	Insufficient data frequency to run GAMM
12	Recovery times for zooplankton, phytoplankton and macroinvertebrate communities will vary across functional groups and will be lake specific	Zooplankton, phytoplankton, macroinvertebrate community composition	M, Q	Insufficient data frequency to run GAMM
13	Recovery times of WFD metrics will vary across metrics and lakes	Metrics for phytoplankton and macrophytes	M	Insufficient data frequency to run GAMM
Quantifying ecotoxicological risk of the approach				
14	La^{3+} concentration will remain below ecotoxicological thresholds published in the literature for all organisms for the duration of the monitoring period	La^{3+} data from modelling + threshold values from literature	M	N
15	TSS concentration will not exceed threshold values for all organisms for the duration of the post-application monitoring period	TSS + threshold data from literature	M	Y

	Hypothesis	Example response variables	Frequency	Hypothesis confirmed?
Identifying process responses following application				
16	The dose applied to both lakes will be sufficient to control sediment P release in both lakes at 20°C and under reducing redox state	Intact-core experiments	W	N
17	Phytoplankton nutrient limitation will be predominantly P limited following the application in both lakes	Nutrient limitation assays	Q	N
18	Chl*a*:TP ratio will increase following the Phoslock® application indicating stronger P limitation	Chl*a*:TP ratio	M	Y
19	The ratio of large-bodied Cladocera biomass to total zooplankton biomass will increase following the application of Phoslock®	Zooplankton biomass data	M	N
20	The relative abundance of Coleoptera, Ephemeroptera, Plecoptera and Trichoptera will increase as an indicator of increased fish predation and Cladocera and Gastropoda abundance will increase as an indicator of improved benthic habitat	Macroinvertebrate surveys	Q	N
Assessing common responses across multiple lakes				
21	Recovery in TLa and FLa will occur within 2 years of application across all lakes and will never exceed ecotoxicological thresholds reported in the literature	TLa, FLa from 16 treated lakes	M–Q	Y
22	In general, a decrease in TP, SRP, chlorophyll *a* and an increase in water clarity will be observed across treated lakes and these responses will reach endpoint within 2 years of application	TP, Chl*a*, SRP, Secchi depth from *c.*10 treated lakes	M–Q	Y

Notes: A = annual; M = monthly; Q = quarterly

Appendix 2 Data analysis

Response indicator	Treatment year and direction of change				Analysis type
	Hatchmere		Mere Mere		
	1	2	1	2	
WEATHER					
Total monthly rainfall					
Winter	-	-	-	-	B-A GLM
Spring	-	-	-	-	B-A GLM
Summer	-	-	-	-	B-A GLM
Autumn	-	-	-	-	B-A GLM
Average monthly air temperature					
Winter	-	-	-	-	B-A GLM
Spring	-	-	-	-	B-A GLM
Summer	-	-	-	-	B-A GLM
Autumn	-	-	-	-	B-A GLM
Average monthly wind speed					
Winter	-	-	-	-	B-A GLM
Spring	-	-	-	-	B-A GLM
Summer	-	-	-	-	B-A GLM
Autumn	-	-	-	-	B-A GLM
WATER CHEMISTRY					
Ortho-P					
Annual	↓	↓	-	-	B-A GAMM
Winter	↓	↓	↓	-	B-A GLM
Spring	N/A	N/A	N/A	N/A	B-A GLM
Summer	↓	↓	↓	↓	B-A GLM
Autumn	↓	↓	↓	-	B-A GLM
TP					
Annual	-	-	-	-	B-A GAMM
Winter	↓	↓	↓	↓	B-A GLM
Spring	N/A	N/A	N/A	N/A	B-A GLM
Summer	-	-	-	-	B-A GLM
Autumn	↓	-	↓	↓	B-A GLM
Chlorophyll *a*					
Annual	↑	↑	-	-	B-A GAMM
Winter	-	-	-	-	B-A GLM
Spring	N/A	N/A	N/A	N/A	B-A GLM
Summer	↑	↑	-	-	B-A GLM
Autumn	-	-	↓	↓	B-A GLM
Secchi depth					
Annual	-	-	-	-	
Winter	↓	-	-	-	B-A GLM
Spring	N/A	N/A	N/A	N/A	B-A GLM
Summer	-	-	-	-	B-A GLM
Autumn	-	-	-	-	B-A GLM
TON					
Annual	-	-	↓	↓	B-A GAMM
Winter	-	-	-	-	B-A GLM
Spring	N/A	N/A	N/A	N/A	B-A GLM
Summer	-	↓	-	-	B-A GLM
Autumn	-	-	-	-	B-A GLM

Response indicator	Treatment year and direction of change				Analysis type
	Hatchmere		**Mere Mere**		
	1	**2**	**1**	**2**	
DOC					
Annual	↓	↓	↓	-	B-A GAMM
Winter	-	↓	↓	↓	B-A GLM
Spring	N/A	N/A	N/A	N/A	B-A GLM
Summer	↓	↓	↓	-	B-A GLM
Autumn	↓	↓	↓	↓	B-A GLM
SEDIMENT CHEMISTRY					
Labile P					
Annual	↓	↓	↓	-	B-A ANOVA
Reductant soluble P					
Annual	-	-	-	-	B-A ANOVA
Metal adsorbed P					
Annual	-	↑	-	↑	B-A ANOVA
Organic P					
Annual	↑	-	-	-	B-A ANOVA
Apatite bound P					
Annual	-	↑	-	-	B-A ANOVA
Residual P					
Annual	-	↑	-	-	B-A ANOVA
Sediment TP					
Annual	↑	↑	-	-	B-A ANOVA
PHYTOPLANKTON					
Total biovolume					
Growing season	-	-	↓	↓	B-A GLM
Cyanophytota biov.					
Growing season	-	-	-	-	B-A GLM
Cryptophyta biov.					
Growing season	-	↓	↓	↓	B-A GLM
Bacillariophyceae biov.					
Growing season	-	↑	↓	-	B-A GLM
Chlorophyta biov.					
Growing season	-	-	-	-	B-A GLM
Chrysophyta biov.					
Growing season	-	↑	↑	↑	B-A GLM
Dinophyta biov.					
Growing season	↓	↓	-	-	B-A GLM
Euglenophyta biov.					
Growing season	↓	↓	↑	-	B-A GLM
ZOOPLANKTON					
Bosmina					
Annual	-	-	-	-	B-A ANOVA
Ceriodaphnia					
Annual	-	-	-	-	B-A ANOVA
Chydoridae					
Annual	-	-	-	-	B-A ANOVA
Cyclopoid					
Annual	-	-	-	-	B-A ANOVA
Daphnia					
Annual	-	-	-	-	B-A ANOVA
Diaphanosoma					
Annual	-	-	-	-	B-A ANOVA
Eudiaptomus					
Annual	-	↓	-	-	B-A ANOVA

Response indicator	Treatment year and direction of change				Analysis type
	Hatchmere		**Mere Mere**		
	1	**2**	**1**	**2**	
Total biomass					
Annual	-	-	-	-	B-A ANOVA
Total abundance					
Annual	-	-	-	-	B-A ANOVA
MACROINVERTEBRATES					
Littoral total abundance					
Annual	-	-	↓	↓	B-A ANOVA
Profundal total abundance					
Annual	↓	↓	-	-	B-A ANOVA
Littoral taxa number					
Annual	↓	↓	↓	↓	B-A ANOVA
Profundal taxa number					
Annual	-	-	-	-	B-A ANOVA
MACROPHYTES					
Species number (wader)					
Summer	-	-	-	-	B-A ANOVA
Species number (boat)					
Summer	-	-	-	-	B-A ANOVA
Species number (perimeter)					
Summer	-	-	-	-	B-A ANOVA
FUNCTIONAL INDICATORS					
Daphnia:total biovolume					B-A ANOVA
Annual	-	-	-	-	
Chl*a*:TP					
Annual	↑	↑	-	-	B-A GAMM
Winter	-	-	-	-	B-A GLM
Spring	N/A	N/A	N/A	N/A	B-A GLM
Summer	↑	↑	-	-	B-A GLM
Autumn	-	↑	-	-	B-A GLM
Ortho-P:TP					
Annual	↑	↑	-	-	B-A GAMM
Winter	↑	-	-	-	B-A GLM
Spring	N/A	N/A	N/A	N/A	B-A GLM
Summer	↑	↑	-	-	B-A GLM
Autumn	-	-	-	-	B-A GLM
TN:TP					
Annual	-	-	-	↑	B-A GAMM
Winter	↑	-	↑	↑	B-A GLM
Spring	N/A	N/A	N/A	N/A	B-A GLM
Summer	-	-	-	↑	B-A GLM
Autumn	-	-	-	-	B-A GLM
TON:ortho-P					
Annual	-	-	-	-	B-A GAMM
Winter	↑	-	-	-	B-A GLM
Spring	N/A	N/A	N/A	N/A	B-A GLM
Summer	-	-	-	-	B-A GLM
Autumn	-	-	-	-	B-A GLM

List of abbreviations

BA or B-A	before-after
BACI	before-after-control-impact
BQE	biological quality elements
CEH	Centre for Ecology & Hydrology
Chl*a*	chlorophyll *a*
CPET	Chironomid pupal exuviae
CSF	Catchment Sensitive Farming
CSM	Common Standards Monitoring
DIN	dissolved inorganic nitrogen
DLa	dissolved lanthanum
DO	dissolved oxygen
DOC	dissolved organic carbon
ECN	Environmental Change Network
EQR	ecological quality ratio
FLa	filterable lanthanum
GAMM	generalised additive mixed model
GLM	generalised linear model
JNCC	Joint Nature Conservation Committee
La	lanthanum
LMB	lanthanum-bentonite
LMNI	lake macrophyte nutrient index
N	nitrogen
ortho-P	orthophosphate
P	phosphorus
PTI	phytoplankton trophic index
SAC	special area of conservation
SCM	site condition monitoring
SiO_2	silica
SPA	special protection area
SRP	soluble reactive phosphorus
SSSI	Site of Special Scientific Interest
TLa	total lanthanum
TN	total nitrogen
TON	total oxidised nitrogen
TP	total phosphorus
TSi	total silicon
TSP	total soluble phosphorus
TSS	total suspended solids
WFD	Water Framework Directive

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