



Phytoplankton community responses in a shallow lake following lanthanum-bentonite application

P. Lang^{a,*}, S. Meis^{b,1}, L. Procházková^c, L. Carvalho^b, E.B. Mackay^d, H.J. Woods^b, J. Pottie^e, I. Milne^f, C. Taylor^a, S.C. Maberly^d, B.M. Spears^b

^a Ecology Assessment Unit, Scottish Environment Protection Agency, 6 Parklands Avenue, Maxim Business Park, Eurocentral, North Lanarkshire ML1 4WQ, Scotland, UK

^b Freshwater Ecology Group, Centre for Ecology & Hydrology, Bush Estate, Penicuik, Midlothian EH26 0QB, Scotland, UK

^c Department of Ecology, Faculty of Science, Charles University in Prague, Viničná 7, CZ-128 44 Prague, Czech Republic

^d Lake Ecosystems Group, Centre for Ecology & Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster LA1 4AP, England, UK

^e Broombank, Loch Flemington, Inverness IV2 7QR, Scotland, UK

^f Ecology Partnership Development Unit, Scottish Environment Protection Agency, Graesser House, Fodderty Way, Dingwall Business Park, Dingwall IV15 9XB, Scotland, UK

ARTICLE INFO

Article history:

Received 2 May 2015

Received in revised form

19 February 2016

Accepted 7 March 2016

Available online 15 March 2016

Keywords:

Lanthanum-modified bentonite

Eutrophication

Recovery

Lake restoration

Cyanobacteria

ABSTRACT

The release of phosphorus (P) from bed sediments to the overlying water can delay the recovery of lakes for decades following reductions in catchment contributions, preventing water quality targets being met within timeframes set out by environmental legislation (e.g. EU Water Framework Directive: WFD). Therefore supplementary solutions for restoring lakes have been explored, including the capping of sediment P sources using a lanthanum (La)-modified bentonite clay to reduce internal P loading and enhance the recovery process. Here we present results from Loch Flemington where the first long-term field trial documenting responses of phytoplankton community structure and abundance, and the UK WFD phytoplankton metric to a La-bentonite application was performed. A Before-After-Control-Impact (BACI) analysis was used to distinguish natural variability from treatment effect and confirmed significant reductions in the magnitude of summer cyanobacterial blooms in Loch Flemington, relative to the control site, following La-bentonite application. However this initial cyanobacterial response was not sustained beyond two years after application, which implied that the reduction in internal P loading was short-lived; several possible explanations for this are discussed. One reason is that this ecological quality indicator is sensitive to inter-annual variability in weather patterns, particularly summer rainfall and water temperature. Over the monitoring period, the phytoplankton community structure of Loch Flemington became less dominated by cyanobacteria and more functionally diverse. This resulted in continual improvements in the phytoplankton compositional and abundance metrics, which were not observed at the control site, and may suggest an ecological response to the sustained reduction in filterable reactive phosphorus (FRP) concentration following La-bentonite application. Overall, phytoplankton classification indicated that the lake moved from poor to moderate ecological status but did not reach the proxy water quality target (i.e. WFD Good Ecological Status) within four years of the application. As for many other shallow lakes, the effective control of internal P loading in Loch Flemington will require further implementation of both in-lake and catchment-based measures. Our work emphasizes the need for appropriate experimental design and long-term monitoring programmes, to ascertain the efficacy of intervention measures in delivering environmental improvements at the field scale.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Shallow lakes are among the most abundant freshwater habitats worldwide (Downing et al., 2006; Verpoorter et al., 2014). They offer a valuable source of biodiversity (Williams et al., 2004;

* Corresponding author.

E-mail address: pauline.lang@sepa.org.uk (P. Lang).

¹ Current address: lanaplan GbR, Lobbericher Straße 5, 41334 Nettetal, Germany.

Scheffer et al., 2006), and provide important ecosystem services to humans (Postel and Carpenter, 1997; Millennium Ecosystem Assessment, 2005). Yet, with catchments often located in heavily-populated areas and surrounded by intense agriculture, shallow lakes are vulnerable to the detrimental effects of nutrient loading (Phillips, 2005; Smith and Schindler, 2009). An increase in the frequency and magnitude of potentially harmful cyanobacterial blooms is a common symptom of nutrient enrichment (Brookes and Carey, 2011; Downing et al., 2001).

Cyanobacterial blooms interfere with the ecological structure and function of lakes and can produce toxins with the potential to affect human and animal health (Codd et al., 1999, 2005). They therefore convey an important message regarding ecosystem health, and often trigger water quality managers to take action to resolve the core underlying environmental issues driving their excessive growth. The risk of occurrence of blooms of cyanobacteria generally increases at water column total phosphorus (TP) concentrations in excess of $10\text{--}20\text{ }\mu\text{g L}^{-1}$ (Carvalho et al., 2011, 2013a), and phytoplankton biomass decreases linearly with TP below about $100\text{ }\mu\text{g L}^{-1}$ in strongly phosphorus (P) limited lakes (Spears et al., 2013). Interventions to reduce cyanobacteria and phytoplankton biomass, normally attempt to reduce external P loads to lakes from their catchments. However, recent studies have highlighted a considerable temporal 'lag' in expected water quality improvements following successful catchment management (Søndergaard et al., 2003; Carvalho et al., 2012; Spears et al., 2012; Sharpley et al., 2014). The release of P from bed sediments (hereafter referred to as internal P loading) can delay the recovery of shallow lakes for decades following external P load reductions, depending on the pollution history, lake flushing rate, bed sediment surface redox conditions and its P-binding capacity (Sas, 1989; Søndergaard et al., 2001; Jeppesen et al., 2005a; Smolders et al., 2006; Spears et al., 2007). This temporal lag is also mirrored in phytoplankton community recovery, characterised by an increase in the biovolume of diatoms, cryptophytes and chrysophytes, and a decrease or no change in cyanobacteria relative to total phytoplankton community biovolume (Jeppesen et al., 1991, 2005b).

Crucially, internal P loading is often the mechanism restricting immediate improvements in shallow lakes following reductions in catchment contributions, preventing water quality targets being met within timeframes set out by environmental legislation (e.g. EU Water Framework Directive: EC, 2000). Therefore to enhance the recovery process, supplementary solutions for the control of internal loading such as sediment dredging, hypolimnetic aeration, and applying materials to 'cap' bed sediment P release have been explored (Hupfer and Hilt, 2008; Hickey and Gibbs, 2009; Lewandowski et al., 2013; Spears et al., 2013). This includes use of a lanthanum (La)-modified bentonite clay to manage eutrophication impacts by capping sediment P release (Douglas patent; Douglas et al., 2004, 2008) and its application at the field scale (Robb et al., 2003; Lürling and Faassen, 2012; Meis, 2012; Meis et al., 2013; van Oosterhout and Lürling, 2013; Douglas et al., 2016). Although some *in situ* studies have indicated that La-bentonite is effective at controlling internal P loading, in turn reducing water column TP, filterable reactive phosphorus (FRP) and chlorophyll *a* concentrations (Robb et al., 2003; Meis, 2012; Gunn et al., 2014; Douglas et al., 2016) and the occurrence of cyanobacteria (Lürling and van Oosterhout, 2013; Bishop et al., 2014), there is currently no comprehensive assessment on phytoplankton community responses following La-bentonite application from a long-term field trial.

To determine the efficacy of any restoration measure, it is vital that the results of field scale trials are analysed rigorously using appropriate statistical approaches. This is especially important for short lived organisms with turnover rates of days to weeks, as is the

case for phytoplankton, where natural seasonal and inter-annual variation can be mistaken for treatment responses when inappropriately analysed. In this context, the Before-After-Control-Impact (BACI) approach has been applied successfully within environmental impact assessments in other systems (Schroeter et al., 1993; Conquest, 2000). We employed this approach to distinguish natural variability from treatment effects in phytoplankton composition and abundance, following La-bentonite application to a shallow lake. Where the control of internal P loading is successful, one would expect a rapid (i.e. within a few years) and sustained response in phytoplankton community structure and biovolume (e.g. decline in cyanobacterial blooms).

In Europe, Annex V of the EU Water Framework Directive (WFD: 2000/60/EC) outlines three features of the phytoplankton community to be considered in the ecological status assessment for lakes: (1) phytoplankton biomass or abundance, (2) phytoplankton composition and (3) bloom frequency and intensity (EC, 2000). Here we examine the responses of a range of the most robust phytoplankton metrics developed in Europe (Carvalho et al., 2013b) and evaluate their responsiveness to restoration actions using the phytoplankton classification methods routinely employed by UK environment agencies. This provides important evidence for environmental regulators and water resource managers on the effectiveness of intervention measures and their capacity to restore 'failing' lakes to acceptable water quality standards, over relevant regulatory timescales (e.g. to have achieved Good Ecological Status for the WFD, or at least have the appropriate measures in place, by 2027) and, more generally, for the control of cyanobacterial blooms in shallow lakes.

We report on a long-term field trial (i.e. one year pre- and four years post-treatment monitoring) designed to quantify responses in the phytoplankton community following La-bentonite application to a shallow lake, placed into context of the WFD as a proxy target of ecological improvement. The specific objectives of the study were to: (i) quantify the seasonal and annual responses in phytoplankton community structure and biovolume following La-bentonite application, (ii) evaluate the responses in relevant phytoplankton community metrics in line with proxy WFD ecological quality targets, and (iii) discuss implications of the results in the context of eutrophication management and ecological recovery in lakes.

2. Material and methods

2.1. Description of treatment (T) site and sampling design

Loch Flemington ($57^{\circ} 32' \text{ N}$, $3^{\circ} 59' \text{ W}$) is located around 20 km east of Inverness, Scotland, UK (Fig. 1). It is a small, high alkalinity ($>50\text{ mg L}^{-1}$ as CaCO_3), shallow lake (Table 1), with no natural outflow (groundwater flows to north-east) and a water retention time of around 2 months (May et al., 2001). The international conservation importance of the site is summarised elsewhere (e.g. Gunn et al., 2014). Located in a largely agricultural lowland catchment, Loch Flemington has suffered a long-standing history of cyanobacterial blooms associated with high catchment P loading resulting in a fish kill in the 1990s (May et al., 2001). Initially, catchment management activities were undertaken to reduce external P loading: treated effluent from a nearby wastewater treatment works (WwTW) was re-directed away from its inlet in 1989, and the WwTW was upgraded during 1993 because sporadic effluent spillages continued to enter the Croy Burn (the primary feeder stream for the lake) during periods of overload (May et al., 2001). A recent assessment of TP loads to Loch Flemington indicated that the dominant external sources were now diffuse (mainly agricultural) and from septic tanks, and was estimated at

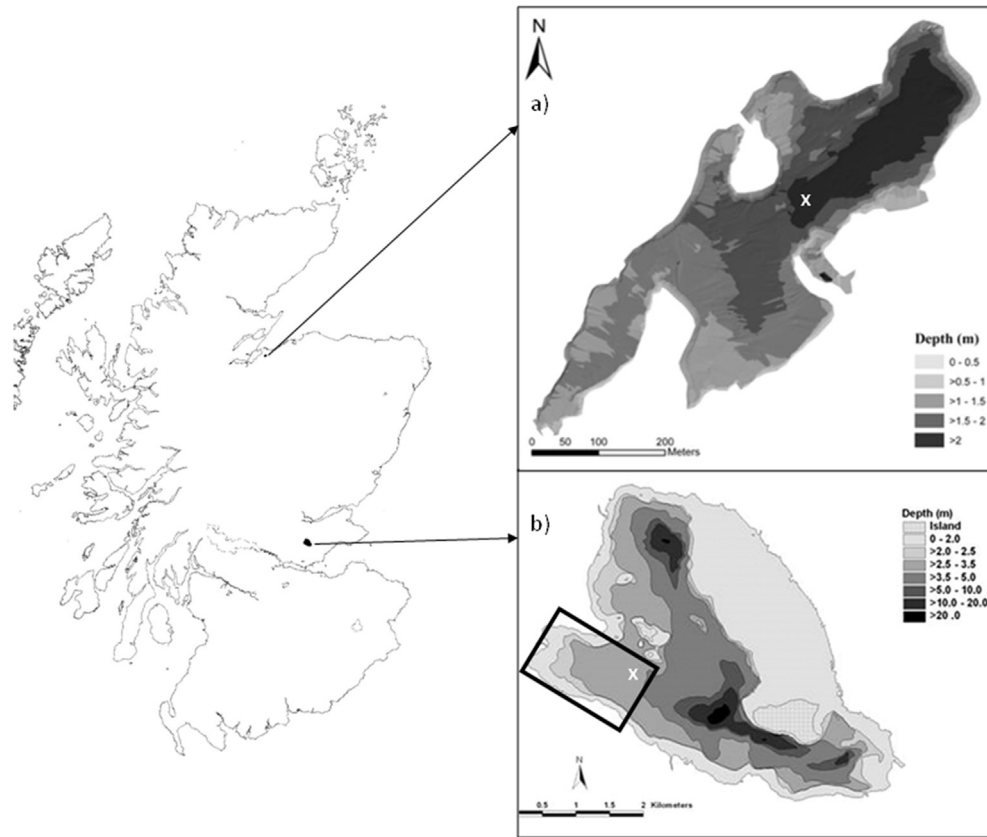


Fig. 1. Map of Scotland showing location and bathymetry of Loch Flemington (treatment site; inset 'a') and Loch Leven west basin (control site; inset 'b') with sampling sites indicated by 'x'.

$0.8 \text{ g m}^{-2} \text{ yr}^{-1}$ with internal loading providing up to $4.3 \text{ g m}^{-2} \text{ yr}^{-1}$, respectively equating to 16% and 84% of the TP load (May et al., 2001). In late March 2010, 25 tonnes of La-bentonite (170 g m^{-2} or $<0.5 \text{ mm}$ layer) was applied (over 3 days, as slurry from a pontoon) to control internal P loading, in an effort to reduce the loch's susceptibility to phosphorus-driven cyanobacterial blooms and thereby improve water quality conditions (Meis, 2012; Meis et al., 2013). The applied dose had the potential to bind 25% of potentially release-sensitive P ($P_{\text{mobile}} = \text{sum 'labile P', 'reductant-soluble P' and 'organic P' fraction}$) present in the top 4 cm or 10% of P_{mobile} present in the top 10 cm (Meis et al., 2013). Responses in TP, FRP and chlorophyll *a* concentrations following La-bentonite application have been reported elsewhere (Robb et al., 2003; Meis, 2012; Gunn et al., 2014; Douglas et al., 2016). Specifically, Gunn et al. (2014) documented a significant reduction in the annual mean TP concentration from $60 \mu\text{g L}^{-1}$ (2009) to $27 \mu\text{g L}^{-1}$ (2011) and chlorophyll *a* concentration from $51 \mu\text{g L}^{-1}$ (2009) to $12 \mu\text{g L}^{-1}$ (2011) of Loch Flemington following treatment with La-bentonite.

These responses were strongest in summer and the phytoplankton community of Loch Flemington was assessed to be strongly P limited both before and after the application (Meis, 2012).

2.2. Description of control (C) site and sampling design

The control site is situated within the shallow, west basin of Loch Leven ($56^{\circ} 10' \text{ N}$, $3^{\circ} 30' \text{ W}$), a large (13.3 km^2), high alkalinity ($>50 \text{ mg L}^{-1}$ as CaCO_3), shallow lake in eastern central Scotland, UK (Fig. 1; Table 1). The entire lake has an average hydraulic retention time of about 5.2 months (Smith, 1974), and drains a predominantly agricultural catchment of about 145 km^2 . Loch Leven lies about 160 km south from Loch Flemington and, therefore, experiences similar weather conditions (Meis, 2012). The west basin of Loch Leven was suitable for use as a control site as it shares similar morphological and physico-chemical characteristics to Loch Flemington (Table 1) and likewise, being shallow, does not thermally

Table 1

Comparison of the limnological characteristics of Loch Leven west basin (control site) with Loch Flemington (treatment site).

Site attribute	Loch Flemington treatment (T) site	Loch Leven west basin control (C) site
Location	$57^{\circ} 32' \text{ N}$, $3^{\circ} 59' \text{ W}$	$56^{\circ} 10' \text{ N}$, $3^{\circ} 30' \text{ W}$
Surface area	0.15 km^2	0.95 km^2
Mean depth	0.75 m	1.5 m
Maximum depth	2.8 m	3.0 m
Annual mean TP concentration (2009)	$60 \mu\text{g L}^{-1}$	$39 \mu\text{g L}^{-1}$
Annual mean chlorophyll <i>a</i> concentration (2009)	$51 \mu\text{g L}^{-1}$	$15 \mu\text{g L}^{-1}$
Annual mean alkalinity (2009–2013)	62.2 mg L^{-1} as CaCO_3	72.1 mg L^{-1} as CaCO_3
Estimated catchment TP load	$0.80 \text{ g m}^{-2} \text{ yr}^{-1}$ (2000)	$0.87 \text{ g m}^{-2} \text{ yr}^{-1}$ (2005)

stratify. This site also has sufficient data available and satisfies the BACI requirements for the differences between sites to be relatively constant in the before period and not subject to localised long-term random influences (e.g. large storms) during the study. The phytoplankton community in Loch Leven is, primarily, P limited and the loch has a long and well documented history of eutrophication and recovery (May and Spears, 2012). Between 1985 and 1995, TP inputs fell from about $1.9 \text{ g m}^{-2} \text{ yr}^{-1}$ to about $0.53 \text{ g m}^{-2} \text{ yr}^{-1}$ in 1995 and increased slightly to $0.87 \text{ g m}^{-2} \text{ yr}^{-1}$ in 2005 (Spears and May, 2015). To satisfy the conditions of the BACI design, we assume no significant changes in catchment P load or internal P load during the period of monitoring included in this study, although we fully recognise when experiments such as ours are conducted at the lake-scale, controlling the occurrence of 'chance' events is impossible. However, Stewart-Oaten et al. (1986) point out that short lived or small chance effects will not necessarily impinge on data analysis if samples are spaced far enough apart, as these changes would not be detected and subtle effects would be overwhelmed by the noise contributed from other errors. For example it is acknowledged that localised disturbance, in the form of dilute sewage discharges, due to engineering works (i.e. WwTW upgrade) was recorded in the west basin of Loch Leven by the Scottish Environment Protection Agency during 2011 (J. Best, pers. comm.). However, this did not result in long-term shift in TP at this site (CEH data, unpubl.), indicating that their impact on the BACI analysis was limited.

2.3. Phytoplankton sampling and enumeration

Over a five year period from 2009 to 2013, monthly sub-surface (between 0.5 and 1 m depth) water samples were taken from both Loch Flemington and the control site, Loch Leven west basin. This monitoring period represents one year before (from May 2009 to early March 2010) and four years after (from April 2010 to December 2013) La-bentonite treatment to Loch Flemington i.e. pre-application year and post-application years one to four, respectively.

The water samples from both lakes were stored in 1 L Nalgene bottles and transferred to the testing laboratory in a cool box, where they were analysed for physico-chemical parameters (e.g. TP; FRP) and phytoplankton abundance (chlorophyll *a*), and processed separately for quantitative phytoplankton analysis. The phytoplankton samples were preserved with Lugol's iodine, and sub-sampled as appropriate. Phytoplankton sub-samples were examined using an inverted microscope and analysed quantitatively, with approximately 400 phytoplankton units counted per sub-sample at a range of magnifications and biovolume determinations made according to standard procedures (CEN, 2004, 2008; Mischke et al., 2012). Identification largely followed John et al. (2011). The taxonomic richness (*S*), Shannon diversity (*H*) and Pielou's evenness (*J*) of the phytoplankton assemblage of each sample were calculated using the SDR software package (Seaby and Henderson, 2006).

2.4. Determination of ecological quality indicators

Ecological quality assessment was conducted by applying the accepted UK phytoplankton tool for WFD classification, known as PLUTO, which uses annual average chlorophyll *a* data (as a proxy for phytoplankton abundance) representing all months of the year, combined with phytoplankton community (composition and biovolume) analysis results derived from phytoplankton samples collected in summer between July and September (WFD-UKTAG, 2014). This taxonomic data corresponds to a sampling window when phytoplankton responsiveness to nutrient enrichment in the

UK is usually most discernible and blooms of cyanobacteria are most likely to occur (Carvalho et al., 2013b). The tool uses metrics which assess phytoplankton abundance (annual mean chlorophyll *a* concentration), taxonomic composition, expressed as the Phytoplankton Trophic Index (PTI: Phillips et al., 2013) (using phytoplankton biovolume data and a taxonomy based sensitivity index) and cyanobacterial bloom intensity (using cyanobacteria biovolume) (see also Table 3 caption). PLUTO combines information from the three component metrics to generate an overall Ecological Quality Ratio (EQR: deviation from minimally-disturbed reference conditions) to facilitate robust classification of phytoplankton in UK lakes according to WFD reporting requirements (Carvalho et al., 2013b; Thackeray et al., 2013). EQR values lying nearer to 1 or 0 are respectively indicative of closest to or furthest from expected reference conditions (WFD-UKTAG, 2014). In addition to the outputs of quantitative phytoplankton analysis (expressed as annual arithmetic mean values) for each season and/or year, and PLUTO for each year, we include the annual geometric mean chlorophyll *a* concentration (following the prescribed calculation methods for WFD) and annual arithmetic mean for TP concentration for each year, for both Loch Flemington (monthly data provided by SEPA) and Loch Leven west basin (monthly data provided by CEH's long-term monitoring programme).

2.5. Statistical analysis

A BACI approach requires the comparison of an impacted site i.e. Loch Flemington, with a control site i.e. west basin of Loch Leven, both before and after the La-bentonite application at the impacted site. The analysis tests for a change in the difference in the mean between these sites, in the before and after periods (Stewart-Oaten et al., 1986). The null hypothesis is that there is no change in the difference in the two time periods i.e. no effect of the La-bentonite application and where a significant difference in the differences in the two time periods is detected at the 5% level, the null hypothesis is rejected and we infer there has been an effect of the La-bentonite application on the impacted site. There is a requirement within the analysis that the data from the control and impact sites are paired. Therefore, the two datasets from Loch Flemington and Loch Leven west basin were initially linearly interpolated onto a daily time step and then re-sampled at a monthly frequency centred on the middle of each month. This created a paired dataset with 53 observations for each variable in each lake, from which the differences between sites were calculated.

Statistical comparisons were carried out between the before sample data in 2009 and 2010 prior to the La-bentonite application, which occurred in late March 2010 and each of the sample data corresponding to the four year period after application i.e. the remainder of 2010 and 2011 to 2013. For annual comparisons of the differences, General Additive Mixed Models with a Gamma distribution and log-link function were used with a fixed effect of the treatment year, a smoother to account for seasonal variation and an autocorrelation term to account for temporal autocorrelation in the model residuals. Seasonal comparisons were made between summer (June, July and August), autumn (September, October and November) and winter (December, January and February), but not for spring (March, April and May) due to insufficient data. A simple General Linear Model was applied to these data using a Gamma distribution and log-link function, with treatment year as the response variable. To account for multiple testing, Tukey's multiple comparison tests were carried out. In all cases agreement with model assumptions were assessed through the examination of residual plots. All statistical analyses were conducted in R (Ihaka and Gentleman, 1996; R Core Team, 2011) using the mgcv and multcomp packages (Wood, 2006; Hothorn et al., 2008).

Only phyla considered, on average, the major contributors to total phytoplankton biovolume in Loch Flemington over the monitoring period i.e. cyanobacteria (32%), dinoflagellates (25.1%), chlorophytes (11.8%), diatoms (11.6%), cryptophytes (9.4%), euglenophytes (4.2%), and chrysophytes (4.1%) were chosen for BACI analysis. Other groups including the eustigmatophytes, haptophytes, and nanoplankton (unidentifiable cells or flagellates <20 µm diameter) were excluded from the BACI analysis as their contribution to total phytoplankton biovolume were each, on average, minimal (<1%).

3. Results

3.1. Phytoplankton community variation in Loch Flemington – BACI analysis

Annual variation in the phytoplankton community composition of Loch Flemington showed that both mean summer cyanobacteria biovolume and total phytoplankton biovolume initially decreased but then increased, over the monitoring period (Fig. 2a). The BACI analysis confirmed significant reductions relative to the control site (Fig. 2b) observed in the summer season for annual mean

Table 2

Results of the Before-After-Control-Impact (BACI) analysis showing the direction of change where a significant response was reported. Note that the arrows represent a change in the treatment site relative to the control site i.e. a downward arrow indicates the treatment site is significantly lower than the control site after the La-bentonite application and do not, necessarily, represent the same form of change in the treatment lake. 'na' denotes where the test was not performed due to insufficient data.

Mean variable	Significant direction of change relative to pre-application year			
	Post-application year 1 (2010)	Post-application year 2 (2011)	Post-application year 3 (2012)	Post-application year 4 (2013)
Total biovolume				
Annual				
Summer		↓		
Autumn				
Winter	na		na	
Cyanobacteria biovolume				
Annual				
Summer	↓	↓		
Autumn				↑
Winter	na		na	
Dinoflagellate biovolume				
Annual			↑	↑
Summer			↑	↑
Autumn				↑
Winter	na		na	
Diatom biovolume				
Annual				
Summer				
Autumn				
Winter	na		na	
Chlorophyte biovolume				
Annual			↑	↑
Summer			↑	↑
Autumn			↑	↑
Winter	na		na	↑
Cryptophyte biovolume				
Annual			↑	↑
Summer	↑	↑	↑	↑
Autumn			↑	↑
Winter	na		na	↑
Euglenophyte biovolume				
Annual			↑	↑
Summer	↓		↑	↑
Autumn		↑	↑	↑
Winter	na		na	↑
Chrysophyte biovolume				
Annual		↑	↑	↑
Summer				↑
Autumn			↑	↑
Winter	na	↑	na	↑
Taxa richness (S)				
Annual				↑
Summer				
Autumn				
Winter	na		na	
Diversity (H)				
Annual				
Summer		↑		
Autumn				
Winter	na		na	
Evenness (J)				
Annual	↑			
Summer		↑		
Autumn				
Winter	na		na	

Table 3
Qualitative inter-annual comparison of ecological quality assessment determined using the phytoplankton abundance metric (chlorophyll *a* EQR = Chl *a* EQR), phytoplankton compositional metric (Phytoplankton Trophic Index EQR = PTI EQR), cyanobacterial bloom intensity metric (Cyano EQR) and the combination of these (i.e. PLUTO EQR) together with physico-chemical conditions (total phosphorus, $\mu\text{g L}^{-1}$ = TP; filterable reactive phosphorus, $\mu\text{g L}^{-1}$ = FRP) and phytoplankton abundance (chlorophyll *a*, $\mu\text{g L}^{-1}$ = Chl *a*) for Loch Flemington (treatment site) and Loch Leven west basin (control site), corresponding to the monitoring period both before and after La-bentonite application. Note EQR values lying nearer to 1 or 0 are respectively indicative of closest to or furthest from expected reference conditions.

Annual variable	2009	2010	2011	2012	2013
Loch Flemington (T)					
Arithmetic mean TP	46.3	38.0	33.6	50.7	46.3
Arithmetic mean FRP (proportion of TP)	16.5 (36%)	13.4 (35%)	10.1 (30%)	9.2 (18%)	9.0 (19%)
Geometric mean Chl <i>a</i>	35.0	20.0	15.6	19.2	13.6
Cyano EQR	0.556	0.706	0.896	0.739	0.633
PTI EQR	0.237	0.204	0.296	0.358	0.361
Chl <i>a</i> EQR	0.336	0.353	0.400	0.404	0.481
PLUTO combined EQR	0.287	0.279	0.348	0.381	0.421
WFD ecological status (% confidence of class)	Poor (100%)	Poor (100%)	Poor (99%)	Poor (88%)	Moderate (81%)
Loch Leven west basin (C)					
Arithmetic mean TP	42.3	32.0	44.9	39.7	54.4
Arithmetic mean FRP (proportion of TP)	9.3 (22%)	6.2 (19%)	5.0 (11%)	5.5 (14%)	11.0 (20%)
Geometric mean Chl <i>a</i>	12.8	22.1	19.3	24.8	22.5
Cyano EQR	0.311	0.769	0.735	0.799	0.469
PTI EQR	0.388	0.588	0.365	0.546	0.438
Chl <i>a</i> EQR	0.531	0.376	0.410	0.386	0.324
PLUTO combined EQR	0.410	0.482	0.387	0.466	0.381
WFD Ecological Status (% confidence of class)	Moderate (81%)	Moderate (61%)	Poor (72%)	Moderate (93%)	Poor (65%)

cyanobacteria biovolume of post-application years one and two compared to the pre-application year and year two for annual mean total phytoplankton biovolume, but also that this initial cyanobacterial response was not sustained beyond post-application year two (Table 2). Annual variation in the phytoplankton community composition of Loch Flemington was also characterised by a general increase in the mean biovolume of all major phyla (except for cyanobacteria) across the seasons, over the monitoring period (Fig. 2a). The BACI analysis confirmed these general responses with significant increases relative to the control site (Fig. 2b) in the mean biovolume being reported for cryptophytes, chlorophytes, chrysophytes, dinoflagellates and euglenophytes, especially in summer during post-application years three and four (Table 2). Overall, these changes indicated a general decline in the relative contribution of cyanobacteria and increase in all other phyla to annual mean total phytoplankton biovolume after the La-bentonite application, through to post-application year four (Fig. 3).

Annual variation in the phytoplankton community composition of Loch Flemington revealed that mean taxa richness generally increased across all seasons, whilst the mean diversity and evenness indices showed some increases over the monitoring period (Fig. 2a). The BACI analysis confirmed significant increases, relative to the control site (Fig. 2b), for mean phytoplankton community evenness and diversity indices occurred in summer during post-application year two, and significant increases in annual mean phytoplankton community evenness and taxa richness also occurred in post-application years one and four, respectively (Table 2).

3.2. Responses in WFD ecological quality metrics

The PLUTO combined EQR indicated that Loch Flemington showed, with fairly high confidence, an overall improvement in WFD phytoplankton classification from *poor* to *moderate* ecological status (increasing from 0.287 to 0.421) over the monitoring period (Table 3). This status change was mostly driven by annual increments in the component phytoplankton abundance (Chl *a* EQR) and compositional (PTI EQR) metrics, which have both shown continual improvement over the monitoring period (increasing from 0.336 to 0.481 and 0.237 to 0.361, respectively: Table 3). The cyanobacterial bloom intensity metric (Cyano EQR) showed no such

consistent trend and fluctuated inter-annually, though a notable decline (corresponding to an increase in summer cyanobacteria abundance) was observed in summer 2013, for both lakes. However in contrast to Loch Flemington, the other two component phytoplankton metrics (i.e. Chl *a* EQR and PTI EQR) were less consistent for Loch Leven west basin, hence why ecological status (PLUTO combined EQR) alternated between moderate (0.410–0.482) and poor (0.381–0.387) over the monitoring period. This implies that the control site is stationed in the moderate to poor region, whereas the treatment site has shown perceivable year on year progress, at the class-boundary level, towards ecological improvement (Table 3). Despite this, Loch Flemington remained below the proxy target of WFD Good Ecological Status four years after La-bentonite application.

Annual arithmetic mean TP concentration initially decreased in Loch Flemington from its pre-application status ($46.3 \mu\text{g L}^{-1}$), to its lowest range ($33.6 \mu\text{g L}^{-1}$ and $38.0 \mu\text{g L}^{-1}$) but thereafter reverted (ranging between 46.3 and $50.7 \mu\text{g L}^{-1}$) to pre-application concentrations or above (Table 3). Annual arithmetic mean FRP concentration (and its proportional contribution to TP) showed a continual decrease from its pre-application status ($16.5 \mu\text{g L}^{-1}$ or 36%) and, on average, remained lower ($10.4 \mu\text{g L}^{-1}$ or 26%) throughout the monitoring period (Table 3). Annual geometric mean chlorophyll *a* concentrations decreased from its pre-application status ($35.0 \mu\text{g L}^{-1}$) and remained relatively low (ranging between 13.6 and $20.0 \mu\text{g L}^{-1}$) throughout the monitoring period (Table 3).

4. Discussion

4.1. Annual and seasonal responses in the phytoplankton community of Loch Flemington indicated by the BACI analysis

Discernible structural changes occurred in the phytoplankton community of Loch Flemington during the four years following La-bentonite application. This constituted a significant reduction in the abundance of cyanobacteria during summer, and an ecological shift from cyanobacteria dominance, towards a more functionally diverse and evenly-distributed algal community. This initial cyanobacterial response was in line with a reduction of internal P loading from the bed sediments in Loch Flemington, following La-

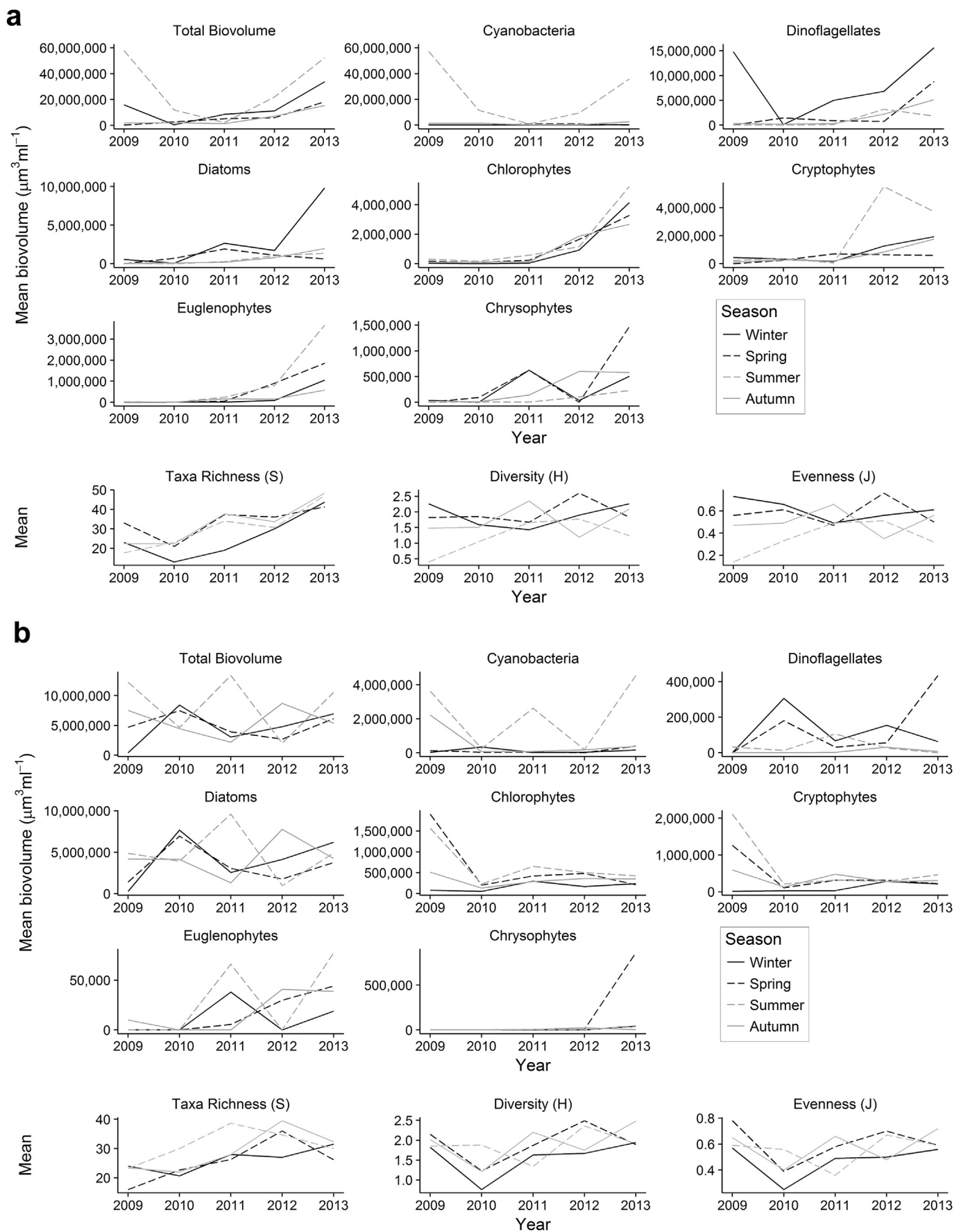


Fig. 2. Seasonal and annual variation in total phytoplankton biovolume, biovolume of major contributing phyla and community diversity indices for **(a)** Loch Flemington and **(b)** Loch Leven west basin, corresponding to the monitoring period both before and after La-bentonite application.

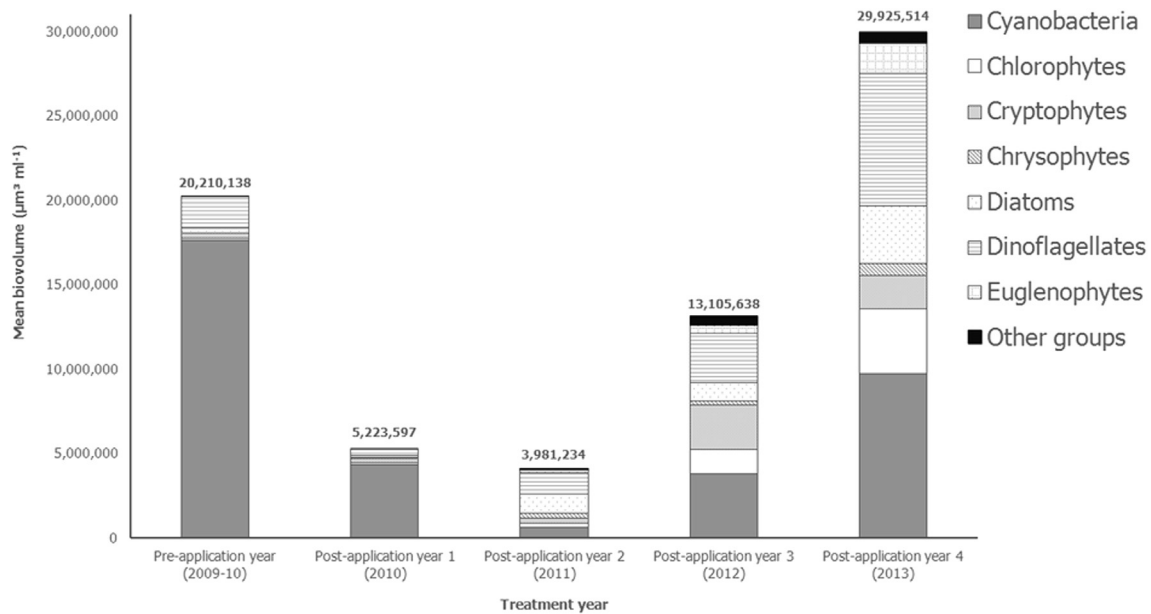


Fig. 3. Annual variation in the proportion of major contributing phyla to total phytoplankton community biovolume of Loch Flemington, corresponding to the monitoring period both before and after La-bentonite application.

bentonite application, reported elsewhere (Meis, 2012; Meis et al., 2013; Gunn et al., 2014).

The decrease of mean total phytoplankton biovolume in summer, during the first two years following La-bentonite application, was explained by the relative decline in cyanobacteria biovolume, and suggests an initial period of P limitation. Similar responses have been observed in multi-lake studies assessing the recovery of shallow lakes from eutrophication following reductions of external P loads alone, albeit over longer time scales (Jeppesen et al., 2005b; Carvalho et al., 2012), and *in situ* control of sediment P release after P-capping (Lürling and van Oosterhout, 2013). In this study, summer cyanobacteria and total phytoplankton biovolume increased between post-application years three and four. However annual mean cyanobacteria biovolume remained proportionally, and in absolute terms, lower, while aggregate biomass contributions from other algal groups (e.g. cryptophytes, chlorophytes, chrysophytes, dinoflagellates and euglenophytes) increased and explained the relative increase in total phytoplankton community biovolume. Further to this, new UK algal records (e.g. *Pseudostaurastrum limneticum*; *Dinobryon stokesii* var. *neustonicum*) appeared in the phytoplankton community of Loch Flemington following treatment (Lang et al., 2014, 2016).

These results are in general agreement with the ecological shifts reported from shallow lakes elsewhere: that reductions in phytoplankton biomass following nutrient reduction are often accompanied by changes in phytoplankton community structure, including a decrease in the relative abundance of cyanobacteria (Downing et al., 2001) and relative increase in the proportion of other planktonic algae (Jeppesen et al., 2005a; Bellinger and Sigee, 2010). In Loch Flemington, the responses in phytoplankton community composition following the La-bentonite application also included an increase, relative to the control site, in the annual mean biovolume of cryptophytes, chlorophytes, chrysophytes, dinoflagellates and euglenophytes, during post-application years three and four. The results from Loch Flemington follow a similar pattern to reported increases in the biovolume of diatoms, cryptophytes and chrysophytes, and a decrease or no change in cyanobacteria relative to total phytoplankton community biovolume in

lakes to which external P loading had been reduced (Jeppesen et al., 1991, 2005b). In particular, reduced P concentrations in spring and summer can reduce the competitive advantage of cyanobacteria, leading to decreases in bloom formation (Phillips et al., 2005). Robb et al. (2003) reported significant reductions in the concentrations of FRP, chlorophyll *a*, and cyanobacteria, compared to control areas, after La-bentonite was applied to an impounded river system in Western Australia. Bishop et al. (2014) also documented reduced cyanobacteria densities and a corresponding increase in the prevalence of chlorophytes, following La-bentonite treatment to a shallow reservoir in California.

4.2. Responses in phytoplankton community metrics in line with proxy WFD ecological quality targets

There was a significant reduction in the magnitude of summer cyanobacterial blooms in the two years following La-bentonite application to Loch Flemington, which was not observed at the control site, and generally agrees with the results of other *in situ* studies (Robb et al., 2003; Lürling and van Oosterhout, 2013; Bishop et al., 2014). However, this initial cyanobacterial response was short lived and not sustained beyond post-application year two. This suggests that the initial reduction in annual mean TP concentration, and consequent reduction of summer cyanobacterial abundance during the two years following La-bentonite application, was associated with a short lived reduction in internal P loading as a result of capping sediment release. The reversal trend in cyanobacterial abundance in Loch Flemington may have been a response to the observed increase in annual mean TP concentration, associated with resumption in the internal P load. Furthermore, this increase in annual mean TP concentration was closely mirrored at the control site, suggesting evidence that a large-scale driver i.e. weather, was influencing changes at both sites. Hence the increase in annual mean TP concentration may have been due to enhanced internal P loading, associated with the drier and warmer than average summer of 2013, when compared across the five year monitoring period (Table 4). It was also apparent that the cyanobacterial bloom intensity metric (Cyano EQR) varied over the

Table 4

Qualitative inter-annual comparison of mean summer rainfall and water temperature, representing a change relative to the 5 year average, for Loch Flemington (treatment site) and Loch Leven west basin (control site), corresponding to the monitoring period both before and after La-bentonite application.

Annual mean variable	Summer 5 year average (2009–13)	Change relative to 5 year average				
		Summer 2009	Summer 2010	Summer 2011	Summer 2012	Summer 2013
Loch Flemington (T)						
Rainfall (mm)*	63.7	+8.8	+3.5	+14.1	–2.5	–23.8
Water temperature (°C)	17.5	+0.4	–0.5	–0.1	–0.6	+0.9
Loch Leven west basin (C)						
Rainfall (mm)*	98.1	+7.4	–6.0	+9.5	+29.6	–40.6
Water temperature (°C)	17.4	+0.6	–0.1	–1.2	–0.2	+0.9

* Met Office (2006) data.

monitoring period and dipped notably (reflecting a prevalence of cyanobacteria) during summer 2013, both in the treatment and control lakes, which reinforces the importance of weather factors in promoting cyanobacterial growth. This particular ecological quality indicator is sensitive to inter-annual variability (Carvalho et al., 2013b) and again, may reflect the importance of climate-related drivers of cyanobacterial blooms, particularly their responsiveness to temperature (Rigosi et al., 2014), flushing (Carvalho et al., 2011) and water column stability (Dokulil and Teubner, 2000). Otherwise, annual patterns of rainfall (Fig. 4a and b) and water temperature (Fig. 5a and b) were relatively consistent for both lakes throughout the monitoring period, corresponding to pre- and post-application years, further supporting interpretation of the BACI analysis i.e. evidence of a treatment impact. This also suggests that a subset of unusual seasonal weather conditions (e.g. summer 2013) can exert pronounced effects on cyanobacterial blooms, of which, a better grasp of the underlying mechanistic factors controlling their severity is essential and could potentially be discerned using long-term monitoring data.

The phytoplankton compositional metric (PTI EQR) showed a sustained improvement during the monitoring period, reflecting an increase in diversity, as the phytoplankton community became progressively characterised by taxa indicative of better water quality (Phillips et al., 2013). The other main change was a continued reduction in phytoplankton abundance (Chl *a* EQR). These sustained phytoplankton responses, which did not occur in the control lake, suggest continual improvement of ecological quality in the treatment lake, and correspond to the monitoring period after La-bentonite application. It is interesting that both the phytoplankton compositional (PTI EQR) and abundance (Chl *a* EQR) metrics for Loch Flemington continued to improve over the monitoring period, despite increases in annual mean TP concentrations. This may suggest an ecological response to the sustained reduction in annual mean FRP concentration following La-bentonite application, or infers that changes in the phytoplankton community structure indicate shifting environmental conditions (e.g. nutrient limitation) or grazer behaviours (e.g. selectivity) which are not captured by phytoplankton metrics used to detect TP impacts.

The output from PLUTO indicated an increase in the ecological quality of Loch Flemington following the La-bentonite application, from a continued change in the phytoplankton community structure relative to the control site. This general improvement resulted in a shift in phytoplankton classification from *poor* to *moderate* ecological status over the monitoring period, highlighting a response which was not observed at the control site. However, the results of BACI analysis (discussed in Section 4.1) indicate that at least some of the encouraging responses (i.e. reduced magnitude of cyanobacterial blooms in summer) have gradually begun to reverse within four years of the La-bentonite application to Loch Flemington. We hypothesise that this improvement may not be sustained and lead to a longer term deterioration of ecological status, if

the TP concentration continues to increase.

4.3. Implications of the results for future eutrophication management in lakes

We have demonstrated the use of a BACI approach for detecting responses in phytoplankton community composition at seasonal and annual timescales. This method enables an assessment of the impact of the treatment, whilst taking natural inter-annual variability, such as seasonal patterns of change, into account. By examining the change between the two sites before and after treatment and assuming the control site is representative of only natural inter-annual variability, changes seen in Loch Flemington, relative to the west basin of Loch Leven, following the La-bentonite application could be assessed as evidence of a treatment effect with greater confidence. These results demonstrate the need for appropriate experimental design when assessing responses to restoration measures at the lake-scale, and we encourage the use of BACI analysis more widely in this field. In particular, there is a real need to collect adequate monitoring data for several years prior to an intervention being made, in order to properly assess and detect the effectiveness of the lake management carried out. However, these experiments are resource intensive and there may be scope for citizen science to sustain long-term monitoring programmes (e.g. Loch Flemington).

Our results indicate that the La-bentonite application to Loch Flemington was sufficient to induce a rapid (i.e. within 2 years) response in the phytoplankton community. However, this response was short lived for some important measures of water quality (e.g. TP, cyanobacterial blooms) and did not result in a shift from poor to good ecological status, in line with the proxy WFD target. Furthermore, conspicuous blooms continue to form (albeit smaller in magnitude compared with the pre-application year) during summer in Loch Flemington, signifying a failure to improve water quality conditions through a sustained reduction in cyanobacteria abundance within relevant regulatory timescales. The resumption in TP concentrations suggest that the La-bentonite application was insufficient to control internal P loading longer than two years. It is clearly important, therefore, to ask why? Five possible explanations can be offered. The first is that insufficient La-bentonite was added to control internal P loading because the layer of La-bentonite applied to Loch Flemington was lower than the recommended (~2 mm) thickness to adequately cap sediment P release (Douglas et al., 2008). This is in agreement with Meis et al. (2013), who estimated that the dose was sufficient only to bind about 25% of the release-sensitive sediment P pool in the top 4 cm of the sediment. The second explanation is that external P loading has increased in recent years, leading to an enhanced supply to the lake itself, and hindered ecological recovery. Although there is no direct evidence to support this scenario at Loch Flemington, insufficient control of catchment loading has been cited as a reason for failure of P-

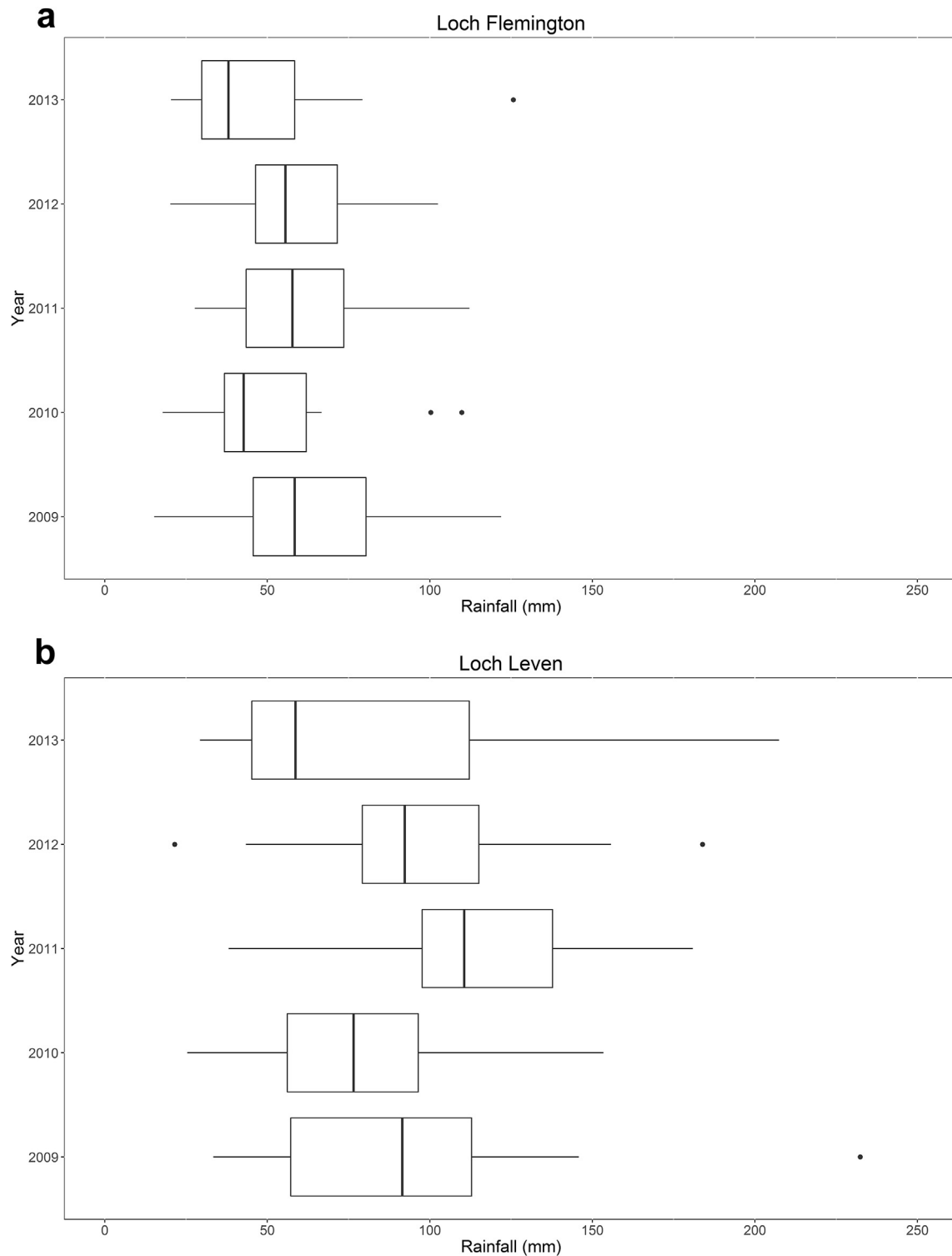


Fig. 4. Boxplots representing annual variation in monthly rainfall* of (a) Loch Flemington and (b) Loch Leven west basin, corresponding to the monitoring period both before and after La-bentonite application. *Met Office (2006) data.

capping agents elsewhere in Europe (Egemoose et al., 2011; Lürling and van Oosterhout, 2013). Thirdly, the fish community in Loch Flemington was altered following intense cyanobacterial blooms of the 1990s, which resulted in the disappearance of piscivorous brown trout and led to dominance by planktivorous three-spined stickleback. Hence it is likely that ‘top down’ control on zooplankton abundance has reduced grazing pressure on the

phytoplankton community in Loch Flemington (Meis, 2012). Under these conditions, ecological resilience to subtle changes in TP concentrations is low and phytoplankton responses can be relatively pronounced (Jeppesen et al., 2007). A fourth scenario to consider is sediment remobilisation or the redistribution of La-bentonite material within the lake itself, leading to patchy coverage and areas of unaltered P release (Robb et al., 2003). Lastly,

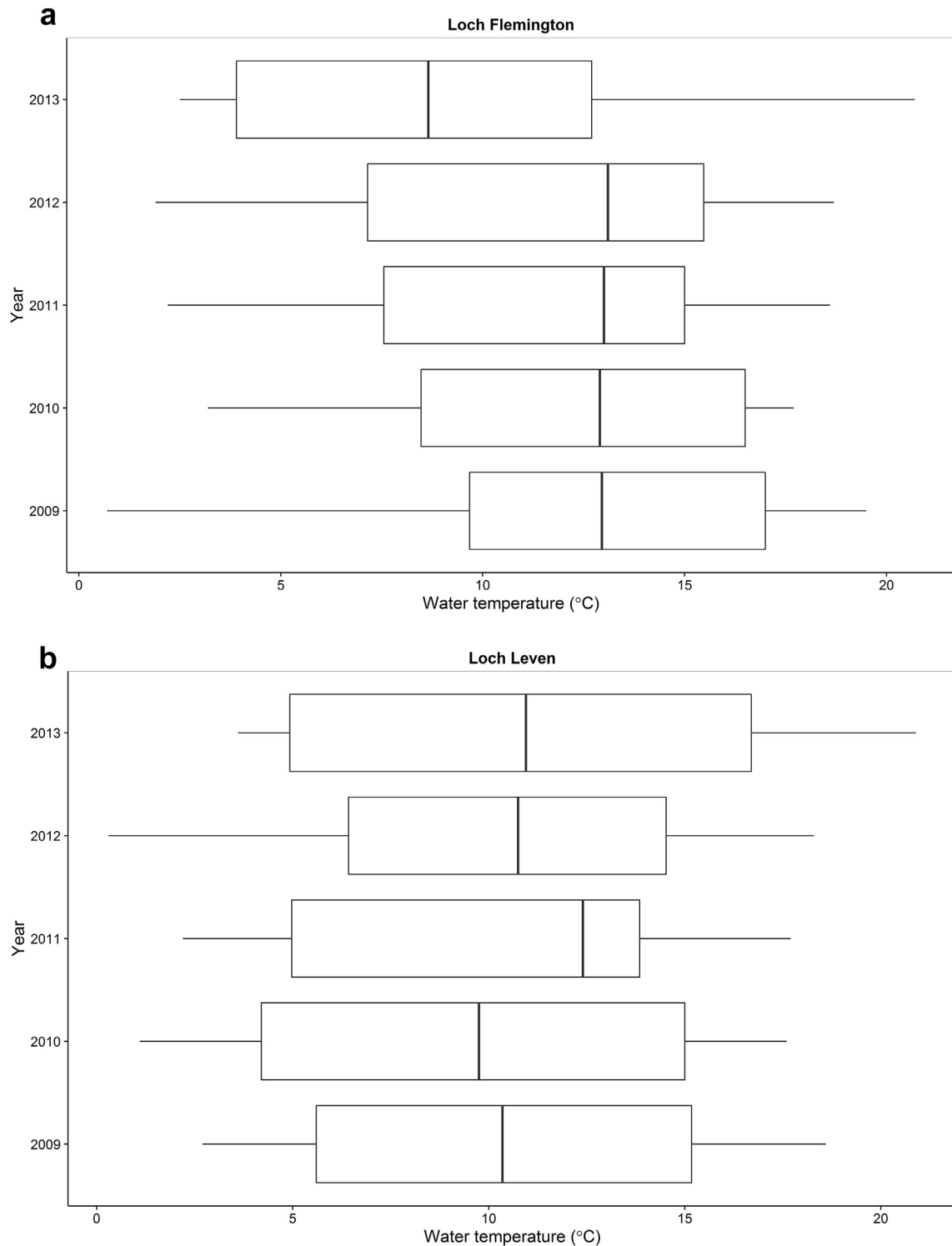


Fig. 5. Boxplots representing annual variation in monthly water temperature of (a) Loch Flemington and (b) Loch Leven west basin, corresponding to the monitoring period both before and after La-bentonite application.

inter-annual variation in weather was probably responsible for driving variation in the intensity of cyanobacterial blooms in both the treatment and control lakes. This large-scale effect was most apparent during the summer of 2013 when prevailing weather consisting of lower than average rainfall combined with warmer lake surface conditions, favoured cyanobacterial growth, perhaps due to enhanced internal P loading (Søndergaard et al., 2001),

reduced flushing rate (Carvalho et al., 2011), increased water column stability (Dokulil and Teubner, 2000), sensitivity to temperature (Rigosi et al., 2014) or indeed, an interaction of these factors. Therefore the 'envelope' of possible responses needs to be recognised.

It is highly likely that in Loch Flemington, as in many other shallow lakes, the effective control of internal P loading will require

the implementation of both in-lake and catchment-based measures. This places increasing weight on simultaneously managing integrated approaches (e.g. control of external N and P sources and internal P loading; bio-manipulation of fish community; management of submerged macrophytes) to facilitate more stable structural and functional recovery in impacted, shallow lake ecosystems (Hosper and Jagtman, 1990; Søndergaard et al., 2000; van Wichelen et al., 2007; Mehner et al., 2002, 2008; Duras and Dziaman, 2010). Controlling catchment-derived nutrient loading is a prerequisite for lasting lake restoration efforts, otherwise internal stocks of nutrients will be replenished and recovery periods protracted through their sediment release (Cooke et al., 2005).

We have presented the first comprehensive assessment, using a BACI approach, which documents phytoplankton community structure and biovolume responses at the field scale. Our work emphasizes the need for appropriate experimental design and long-term monitoring programmes, to ascertain the efficacy of intervention measures for restoring lakes and driving environmental improvements towards statutory targets.

4.4. Conclusions

- The BACI analysis confirmed significant reductions in the magnitude of summer cyanobacterial blooms in Loch Flemington, relative to the control site, following La-bentonite application.
- This initial cyanobacterial response was not sustained beyond post-application year two, implying a short lived reduction in internal P loading as a result of capping sediment release.
- The resumption in annual mean TP concentration, suggests that the La-bentonite application was insufficient to control internal P loading for longer than two years.
- Continual improvements in the phytoplankton compositional and abundance metrics, not observed at the control site, may reflect a response to the sustained reduction in annual mean FRP concentration following La-bentonite application.
- The cyanobacterial bloom intensity metric is sensitive to inter-annual variability and reflects the importance of climate-related drivers of cyanobacterial blooms.
- Overall, phytoplankton classification indicated that the lake moved from *poor* to *moderate* ecological status but did not reach the proxy water quality target (i.e. WFD Good Ecological Status) within four years following La-bentonite application.
- Cyanobacteria continue to form conspicuous blooms during summer, signifying a failure to improve water quality conditions over relevant regulatory time scales.
- The effective control of internal P loading in Loch Flemington will require further implementation of both in-lake and catchment-based measures.
- Our work emphasizes the need for appropriate experimental design and long-term monitoring programmes, to ascertain the efficacy of intervention measures in delivering environmental improvements at the field-scale.

Acknowledgements

The research in this study was funded jointly by the Centre for Ecology & Hydrology (CEH), SNH and Phoslock® Europe GmbH. Phoslock® Europe GmbH was not involved in the monitoring design, sample analysis or interpretation of the data. Sebastian Meis was jointly funded by CEH and by a DAAD Scholarship (agreement number D/08/42393), with in-kind support for analysis provided by the Scottish Environment Protection Agency (SEPA) and the Charles University in Prague. Citizen Science has sustained the routine monitoring programme of Loch Flemington and

through communicating this message we hope to motivate others to become similarly engaged with their local water environment, its experts and responsible watchdogs in the future. This manuscript contains SEPA data © Scottish Environment Protection Agency and database right [2014]. All rights reserved. We are especially grateful to SEPA for providing the data utilised in this paper, also to CEH for provisioning data used for the supporting analyses, and the Met Office for licensing access and permission to use rainfall records derived from their weather stations adjacent to our study sites. The views and opinions expressed in this paper are strictly those of the authors and are not necessarily those of SEPA. Whilst every effort has been made to ensure the accuracy of the information provided SEPA cannot be held responsible for any inaccuracies or omissions, whether caused by negligence or otherwise. We thank the following: Dr Rupert Perkins (Cardiff University); Dr Stephen Thackeray, Dr Linda May and Iain Gunn (CEH); Dr Jennifer Best and Dr Jan Krokowski (SEPA); Ben Leyshon (SNH); Nigel Traill (Phoslock® Europe GmbH, Germany); Said Yasseri (Institut Dr Novak, Germany); Patrick van Goethem (Phoslock® Europe GmbH, Belgium); Nicolai Novak (Bentophos® GmbH, Germany); Dr Kevin J. Murphy (University of Glasgow); Michael O'Malley and Amy Anderson for help with fieldwork; the Loch Flemington Catchment Management Group for ongoing cooperation. We are grateful to Dr Gertrud Nürnberg and our anonymous reviewers for their comments. Finally, we declare that no conflicts of interest exist for any author of the study.

References

- Bellinger, E.G., Sigeo, D.D., 2010. *Freshwater Algae: Identification and Use as Bio-indicators*. John Wiley & Sons, UK.
- Bishop, W.M., McNabb, T., Cormican, I., Willis, B.E., Hyde, S., 2014. Operational evaluation of Phoslock® phosphorus locking technology in Laguna Niguel Lake, California. *Water Air Soil Pollut.* 225 (7), 2018. <http://dx.doi.org/10.1007/s11270-014-2018-6>.
- Brookes, J.D., Carey, C.C., 2011. Resilience to blooms. *Science* 334 (6052), 46–47. <http://dx.doi.org/10.1126/science.1207349>.
- Carvalho, L., Miller, C.A., Scott, E.M., Codd, G.A., Davies, P.S., Tyler, A., 2011. Cyanobacterial blooms: statistical models describing risk factors for national-scale lake assessment and lake management. *Sci. Total Environ.* 409 (24), 5353–5358. <http://dx.doi.org/10.1016/j.scitotenv.2011.09.030>.
- Carvalho, L., Ferguson, C.A., Gunn, I.D.M., Bennion, H., Spears, B., May, L., 2012. Water quality of Loch Leven: responses to enrichment, restoration and climate change. *Hydrobiologia* 681 (1), 35–47. <http://dx.doi.org/10.1007/s10750-011-0923-x>.
- Carvalho, L., McDonald, C., de Hoyos, C., Mischke, U., Phillips, G., Borics, G., Poikane, S., Skjelbred, B., Lyche Solheim, A., van Wichelen, J., Cardoso, A.C., 2013a. Sustaining recreational quality of European lakes: minimising the health risks from algal blooms through phosphorus control. *J. Appl. Ecol.* 50 (2), 315–323. <http://dx.doi.org/10.1111/1365-2666.12059>.
- Carvalho, L., Poikane, S., Lyche Solheim, A., Phillips, G., Borics, G., Catalan, J., De Hoyos, C., Drakare, S., Dudley, B.J., Jarvinen, M., Laplace-Treytore, C., Maileht, K., McDonald, C., Mischke, U., Moe, J., Morabito, G., Noges, P., Noges, T., Ott, I., Pasztaleniec, A., Skjelbred, B., Thackeray, S.J., 2013b. Strength and uncertainty of phytoplankton metrics for assessing eutrophication impacts in lakes. *Hydrobiologia* 704 (1), 127–140. <http://dx.doi.org/10.1007/s10750-012-1344-1>.
- CEN, 2004. *Water Quality – Guidance Standard for the Routine Analysis of Phytoplankton Abundance and Composition Using Inverted Microscopy (Utermohli Technique)*. CEN/TC230/WG2/TG3.
- CEN, 2008. *Water Quality – Phytoplankton Biovolume Determination by Microscopic Measurement of Cell Dimensions*. CEN/TC230/WG2/TG3.
- Codd, G.A., Bell, S.G., Kaya, K., Ward, C.J., Beattie, K.A., Metcalf, J.S., 1999. Cyanobacterial toxins, exposure routes and human health. *Eur. J. Phycol.* 34 (4), 405–415. <http://dx.doi.org/10.1080/09670269910001736462>.
- Codd, G.A., Morrison, L.F., Metcalf, J.S., 2005. Cyanobacterial toxins: risk management for health protection. *Toxicol. Appl. Pharmacol.* 203 (3), 264–272. <http://dx.doi.org/10.1016/j.taap.2004.02.016>.
- Cooke, G.D., Welch, E.B., Peterson, S.A., Nichols, S.A., 2005. *Restoration and Management of Lakes and Reservoirs*. Taylor & Francis, London.
- Conquest, L.L., 2000. Analysis and interpretation of ecological field data using BACI designs: discussion. *J. Agric. Biol. Environ. Stat.* 5 (3), 293–296.
- Dokulil, M.T., Teubner, K., 2000. Cyanobacterial dominance in lakes. *Hydrobiologia* 438 (1–3), 1–12. <http://dx.doi.org/10.1023/A:1004155810302>.
- Douglas, G.B., Robb, M.S., Coad, D.N., Ford, P.W., 2004. A review of solid phase adsorbents for the removal of phosphorus from natural and wastewaters. In: Valsami-Jones, E. (Ed.), *Phosphorous in Environmental Technology: Principles*

- and Applications. IWA Publishing, pp. 291–320.
- Douglas, G.B., Robb, M.S., Ford, P.W., 2008. Reassessment of the performance of mineral-based sediment capping materials to bind phosphorus: a comment on Akhurst et al. 2004. *Mar. Freshw. Res.* 59 (9), 836–837. <http://dx.doi.org/10.1071/MF07183>.
- Douglas, G., Lüring, M., Spears, B., 2016. Assessment of changes in potential nutrient limitation in an impounded river after application of lanthanum-modified bentonite. *Water Res.* 97, 47–54. <http://dx.doi.org/10.1016/j.watres.2016.02.005>.
- Downing, J.A., Watson, S.B., McCauley, E., 2001. Predicting cyanobacteria dominance in lakes. *Can. J. Fish. Aquat. Sci.* 58 (10), 1905–1908. <http://dx.doi.org/10.1139/f01-143>.
- Downing, J.A., Prairie, Y.T., Cole, J.J., Duarte, C.M., Tranvik, L.J., Striegl, R.G., McDowell, W.H., Kortelainen, P., Caraco, N.F., Melack, J.M., Middelburg, J.J., 2006. The global abundance and size distribution of lakes, ponds, and impoundments. *Limnol. Oceanogr.* 51 (5), 2388–2397. <http://dx.doi.org/10.4319/lo.2006.51.5.2388>.
- Duras, J., Dziaman, R., 2010. Recovery of shallow recreational Bolevecký pond, Plzeň, Czech Republic. In: Nedzarek, A. (Ed.), *Anthropogenic and Natural Transformations of Lakes*. Polish Limnological Society, Turon, pp. 43–50.
- EC, 2000. Directive 2000/60/EC of the European parliament and of the council of 23 October 2000 establishing a framework for community action in the field of water policy. *Off. J. Eur. Commun. L* 327, 1–72.
- Egemoose, S., de Vicente, I., Reitzel, K., Flindt, M.R., Andersen, F.O., Lauridsen, T.L., Søndergaard, M., Jeppesen, E., Jensen, H.S., 2011. Changed cycling of P, N, Si, and DOC in Danish Lake Nordborg after aluminum treatment. *Can. J. Fish. Aquat. Sci.* 68 (5), 842–856. <http://dx.doi.org/10.1139/f2011-016>.
- Gunn, I.D.M., Meis, S., Maberly, S.C., Spears, B.M., 2014. Assessing the responses of aquatic macrophytes to the application of a lanthanum modified bentonite clay, at Loch Flemington, Scotland, UK. *Hydrobiologia* 737 (1), 309–320. <http://dx.doi.org/10.1007/s10750-013-1765-5>.
- Hickey, C.W., Gibbs, M.M., 2009. Lake sediment phosphorus release management – decision support and risk assessment framework. *N. Z. J. Mar. Freshw. Res.* 43 (3), 819–856. <http://dx.doi.org/10.1080/00288330909510043>.
- Hosper, S.H., Jagtman, E., 1990. Biomanipulation additional to nutrient control for restoration of shallow lakes in The Netherlands. *Hydrobiologia* 200–201 (1), 523–534. <http://dx.doi.org/10.1007/BF02530369>.
- Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. *Biom. J.* 50 (3), 346–363. <http://dx.doi.org/10.1002/bimj.200810425>.
- Hupfer, M., Hilt, S., 2008. In: Jørgensen, S.E., Fath, B. (Eds.), *Ecological Engineering, Encyclopedia of Ecology*, vol. 3. Elsevier, Oxford, pp. 2080–2093.
- Ihaka, R., Gentleman, R., 1996. R: a language for data analysis and graphics. *J. Comput. Graph. Stat.* 5 (3), 299–314. <http://dx.doi.org/10.1080/10618600.1996.10474713>.
- Jeppesen, E., Kristensen, P., Jensen, J.P., Søndergaard, M., Mortensen, E., Lauridsen, T.L., 1991. Recovery resilience following a reduction in external phosphorus loading of shallow eutrophic Danish lakes: duration, regulating factors and methods for overcoming resilience. *Mem. Dell'Istituto Ital. Idrobiol.* 48, 127–148.
- Jeppesen, E., Jensen, J.P., Søndergaard, M., Lauridsen, T.L., 2005a. Response of fish and plankton to nutrient loading reduction in eight shallow Danish lakes with special emphasis on seasonal dynamics. *Freshw. Biol.* 50 (10), 1616–1627. <http://dx.doi.org/10.1111/j.1365-2427.2005.01413.x>.
- Jeppesen, E., Søndergaard, M., Jensen, J.P., Havens, K.E., Anneville, O., Carvalho, L., Coveney, M.F., Deneke, R., Dokulil, M.T., Foy, B., Gerdeaux, D., Hampt, S.E., Hilt, S., Kangur, K., Köhler, J., Lammens, E.H.H.R., Lauridsen, T.L., Manca, M., Miracle, M.R., Moss, B., Nöges, P., Persson, G., Phillips, G., Portielje, R., Romo, S., Schelske, C.L., Straila, D., Tatray, I., Willén, E., Winder, M., 2005b. Lake responses to reduced nutrient loading – an analysis of contemporary long-term data from 35 case studies. *Freshw. Biol.* 50 (10), 1747–1771. <http://dx.doi.org/10.1111/j.1365-2427.2005.01415.x>.
- Jeppesen, E., Meerhoff, M., Jacobsen, B.A., Hansen, R.S., Søndergaard, M., Jensen, J.P., Lauridsen, T.L., Mazzeo, N., Branco, C.W.C., 2007. Restoration of shallow lakes by nutrient control and biomanipulation – the successful strategy varies with lake size and climate. *Hydrobiologia* 581 (1), 269–285. <http://dx.doi.org/10.1007/s10750-006-0507-3>.
- John, D.M., Whittom, B.A., Brook, A.J., 2011. *The Freshwater Algal Flora of the British Isles*, second ed. Cambridge University Press, Cambridge.
- Lang, P., Procházková, L., Krokowski, J., Meis, S., Spears, B.M., Milne, I., Pottier, J., 2014. The bizarre Eustigmaticean alga, *Pseudostaurastrum limneticum* (Borge) Chodat, in a shallow, nutrient-enriched Scottish loch: new to British Isles. *Glasg. Nat.* 26 (1), 107–109.
- Lang, P., Meis, S., Spears, B.M., Krokowski, J., Milne, I., Pottier, J., 2016. *Dinobryon stokesii* var. *neustonicum* in Loch Flemington, Scotland: a rarely observed variety of golden alga new to UK freshwaters. *Glasg. Nat.* 26 (2), 99–100.
- Lewandowski, J., Hoehn, E., Kasprzak, P., Kleeborg, A., Kurzreuther, H., Lücke, N., Mathes, J., Meis, S., Röncke, H., Rothe, M., Sandrock, S., Wauer, G., Hupfer, M., 2013. Gewässerinterne Ökotechnologien zur Verminderung der Trophie von Seen und Talsperren. *Korresp. Wasserrwirtsch.* 6 (12), 718–728. <http://dx.doi.org/10.3243/kwe2013.12.004>.
- Lüring, M., Faassen, E.J., 2012. Controlling toxic cyanobacteria: effects of dredging and phosphorus-binding clay on cyanobacteria and microcrusts. *Water Res.* 46 (5), 1447–1459. <http://dx.doi.org/10.1016/j.watres.2011.11.008>.
- Lüring, M., van Oosterhout, F., 2013. Case study on the efficacy of a lanthanum-enriched clay (Phoslock®) in controlling eutrophication in Lake Het Groene Eiland (The Netherlands). *Hydrobiologia* 710 (1), 253–263. <http://dx.doi.org/10.1007/s10750-012-1141-x>.
- May, L., Gunn, I., Kirika, A., 2001. Phosphorus Study at Loch Flemington. Report to Scottish Natural Heritage. Centre for Ecology & Hydrology, Edinburgh.
- May, L., Spears, B.M., 2012. Managing ecosystem services at Loch Leven, Scotland, UK: actions, impacts and unintended consequences. *Hydrobiologia* 681 (1), 117–130. <http://dx.doi.org/10.1007/s10750-011-0931-x>.
- Mehner, T., Benndorf, J., Kasprzak, P., Koschel, R., 2002. Biomanipulation of lake ecosystems: successful applications and expanding complexity in the underlying science. *Freshw. Biol.* 47 (12), 2453–2465. <http://dx.doi.org/10.1046/j.1365-2427.2002.01003.x>.
- Mehner, T., Diekmann, M., Gonsiorczyk, T., Kasprzak, P., Koschel, R., Krienitz, L., Rumpf, M., Schulz, M., Wauer, G., 2008. Rapid recovery from eutrophication of a stratified lake by disruption of internal nutrient load. *Ecosystems* 11 (7), 1142–1156. <http://dx.doi.org/10.1007/s10021-008-9185-5>.
- Meis, S., 2012. Investigating Forced Recovery from Eutrophication in Shallow Lakes. PhD thesis. Cardiff University.
- Meis, S., Spears, B.M., Maberly, S.C., Perkins, R.G., 2013. Assessing the mode of action of Phoslock® in the control of phosphorus release from the bed sediments in a shallow lake (Loch Flemington, UK). *Water Res.* 47 (13), 4460–4473. <http://dx.doi.org/10.1016/j.watres.2013.05.017>.
- Met Office, 2006. UK Daily Rainfall Data, Part of the Met Office Integrated Data Archive System (MIDAS). NCAS British Atmospheric Data Centre, 8th February 2016. <http://catalogue.ceda.ac.uk/uuid/c732716511d3442f05cdccbe99b8f90>.
- Millennium Ecosystem Assessment, 2005. *Ecosystems and Human Well-being: Synthesis*. Island Press, Washington DC.
- Mischke, U., Thackeray, S., Dunbar, M., McDonald, C., Carvalho, L., de Hoyos, C., Järvinen, M., Laplace-Treytore, C., Morabito, G., Skjelbred, B., Lyche Solheim, A., Brierley, B., Dudley, B., 2012. WISER Deliverable D3.1-4: Guidance Document on Sampling, Analysis and Counting Standards for Phytoplankton in Lakes, pp. 15–31.
- Phillips, G.L., 2005. In: O'Sullivan, P.E., Reynolds, C.S. (Eds.), *The Lakes Handbook - Lake Restoration and Rehabilitation*. Blackwell Publishing, Oxford.
- Phillips, G.L., Kelly, A., Pitt, J.-A., Sanderson, R., Taylor, E., 2005. The recovery of a very shallow eutrophic lake, 20 years after the control of effluent derived phosphorus. *Freshw. Biol.* 50 (10), 1628–1638. <http://dx.doi.org/10.1111/j.1365-2427.2005.01434.x>.
- Phillips, G., Lyche-Solheim, A., Skjelbred, B., Mitschke, U., Drakare, S., Free, G., Järvinen, M., de Hoyos, C., Morabito, G., Poikane, S., Carvalho, L., 2013. A phytoplankton trophic index to assess the status of lakes for the water framework directive. *Hydrobiologia* 704 (1), 75–95. <http://dx.doi.org/10.1007/s10750-012-1390-8>.
- Postel, S., Carpenter, S.R., 1997. In: Daily, G. (Ed.), *Freshwater Ecosystem Services*. Island Press, Washington, D.C. pp. 195–214.
- R Core Team, 2011. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>.
- Rigosi, A., Carey, C.C., Ibelings, B.W., Brooks, J.D., 2014. The interaction between climate warming and eutrophication to promote cyanobacteria is dependent on trophic state and varies among taxa. *Limnol. Oceanogr.* 59 (1), 99–114. <http://dx.doi.org/10.4319/lo.2014.59.01.0099>.
- Robb, M., Greenop, B., Goss, Z., Douglas, G., Adeney, J., 2003. Application of Phoslock™, an innovative phosphorus binding clay, to two Western Australian waterways: preliminary findings. *Hydrobiologia* 494 (1), 237–243. <http://dx.doi.org/10.1023/A:1025478618611>.
- Sas, H., 1989. Lake restoration by Reduction of Nutrient Loading: Expectations, Experiences, Extrapolations. Academia Verlag Richarz GmbH, St. Augustin.
- Scheffer, M., Van Geest, G.J., Zimmer, K., Jeppesen, E., Søndergaard, M., Butler, M.G., Hanson, M.A., Declerck, S., De Meester, L., 2006. Small habitat size and isolation can promote species richness: second-order effects on biodiversity in shallow lakes and ponds. *Oikos* 112 (1), 227–231. <http://dx.doi.org/10.1111/j.0030-1299.2006.14145.x>.
- Schroeter, S.C., Dixon, J.D., Kastendiek, J., Smith, R.O., Bence, J.R., 1993. Detecting the ecological effects of environmental impacts: a case study of kelp forest invertebrates. *Ecol. Appl.* 3 (2), 331–350. <http://dx.doi.org/10.2307/1941836>.
- Seaby, R.M.H., Henderson, P.A., 2006. *Species Diversity and Richness (SDR) Software Version 4.1.2*. Pisces Conservation Ltd., Hampshire, UK.
- Sharpley, A., Jarvie, H.P., Buda, A., May, L., Spears, B.M., Kleinman, P., 2014. Phosphorus legacy: overcoming the effects of past management practices to mitigate future water quality impairment. *J. Environ. Qual.* 42 (5), 1308–1326. <http://dx.doi.org/10.2134/jeq2013.03.0098>.
- Smith, I.R., 1974. The structure and physical environment of Loch Leven, Scotland. *Proc. R. Soc. Edinb. Sect. B. Biol.* 74, 81–100. <http://dx.doi.org/10.1017/S0080455X00012339>.
- Smith, V.H., Schindler, D.W., 2009. Eutrophication science: where do we go from here? *Trends Ecol. Evol.* 24 (4), 201–207. <http://dx.doi.org/10.1016/j.tree.2008.11.009>.
- Smolders, A.J.P., Lamers, L.P.M., Lucassen, E.C.H.E.T., Van Der Velde, G., Roelofs, J.G.M., 2006. Internal eutrophication: how it works and what to do about it – a review. *Chem. Ecol.* 22 (2), 93–111. <http://dx.doi.org/10.1080/02757540600579730>.
- Søndergaard, M., Jeppesen, E., Jensen, J.P., Lauridsen, T., 2000. Lake restoration in Denmark. *Lake Reserv. Res. Manag.* 5 (3), 151–159. <http://dx.doi.org/10.1046/j.1440-1770.2000.00110.x>.

- Søndergaard, M., Jensen, J.P., Jeppesen, E., 2001. Retention and internal loading of phosphorus in shallow, eutrophic lakes. *Sci. World J.* 1, 427–442. <http://dx.doi.org/10.1100/tsw.2001.72>.
- Søndergaard, M., Jensen, J.P., Jeppesen, E., 2003. Role of sediment and internal loading of phosphorus in shallow lakes. *Hydrobiologia* 506–509 (1–3), 133–145. <http://dx.doi.org/10.1023/B:HYDR.0000008611.12704.dd>.
- Spears, B.M., Carvalho, L., Perkins, R.G., Kirika, A., Paterson, D.M., 2007. Sediment phosphorus cycling in a large shallow lake: spatio-temporal variation in phosphorus pools and release. *Hydrobiologia* 584 (1), 37–48. <http://dx.doi.org/10.1007/s10750-007-0610-0>.
- Spears, B.M., Carvalho, L., Perkins, R., Kirika, A., Paterson, D.M., 2012. Long-term variation and regulation of internal phosphorus loading in Loch Leven. *Hydrobiologia* 681 (1), 23–33. <http://dx.doi.org/10.1007/s10750-011-0921-z>.
- Spears, B.M., Meis, S., Anderson, A., Kellou, M., 2013. Comparison of phosphorus (P) removal properties of materials proposed for the control of sediment P release in UK lakes. *Sci. Total Environ.* 442 (1), 103–110. <http://dx.doi.org/10.1016/j.scitotenv.2012.09.066>.
- Spears, B.M., May, L., 2015. Long-term homeostasis of filterable un-reactive phosphorus in a shallow eutrophic lake following a significant reduction in catchment load. *Geoderma* 257–258, 78–85. <http://dx.doi.org/10.1016/j.geoderma.2015.01.005> (Special Issue on developments in soil organic phosphorus cycling in natural and agricultural ecosystems).
- Stewart-Oaten, A., Murdoch, W.W., Parker, K.R., 1986. Environmental impact assessment: “Pseudoreplication” in time? *Ecology* 67, 929–940. <http://dx.doi.org/10.2307/1939815>.
- Thackeray, S.J., Nöges, P., Dunbar, M.J., Dudley, B.J., Skjelbred, B., Morabito, G., Carvalho, L., Phillips, G., Mischke, U., Catalan, J., de Hoyos, C., Laplace, C., Austoni, M., Padeddal, B.M., Maileht, K., Pasztaleniec, A., Järvinen, M., Lyche Solheim, A., 2013. Quantifying uncertainties in biologically-based water quality assessment: a pan-European analysis of lake phytoplankton community metrics. *Ecol. Indic.* 29, 34–47. <http://dx.doi.org/10.1016/j.ecolind.2012.12.010>.
- van Oosterhout, F., Lüring, M., 2013. The effect of phosphorus binding clay (Phos-lock®) in mitigating cyanobacterial nuisance: a laboratory study on the effects on water quality variables and plankton. *Hydrobiologia* 710 (1), 265–277. <http://dx.doi.org/10.1007/s10750-012-1206-x>.
- van Wichelen, J., Declercq, S., Muylaert, K., Hoste, I., Geenens, V., Vandekerckhove, J., Michels, E., Pauw, N., Hoffmann, M., Meester, L., Vyverman, W., 2007. The importance of drawdown and sediment removal for the restoration of the eutrophied shallow Lake Kraenpoel (Belgium). *Hydrobiologia* 584 (1), 291–301. <http://dx.doi.org/10.1007/s10750-007-0611-z>.
- Verpoorter, C., Kutser, T., Seekell, D.A., Tranvik, L.J., 2014. A global inventory of lakes based on high-resolution satellite imagery. *Geophys. Res. Lett.* 41 (18), 6396–6402. <http://dx.doi.org/10.1002/2014GL060641>.
- Water Framework Directive – United Kingdom Technical Advisory Group, 2014. UKTAG Lake Assessment Method Phytoplankton: Phytoplankton Lake Assessment Tool with Uncertainty Module (PLUTO). WFD-UKTAG c/o SEPA, Stirling, UK.
- Williams, P., Whitfield, M., Biggs, J., Bray, S., Fox, G., Nicolet, P., Sear, D., 2004. Comparative biodiversity of rivers, streams, ditches and ponds in an agricultural landscape in Southern England. *Biol. Conserv.* 115 (2), 329–341. [http://dx.doi.org/10.1016/S0006-3207\(03\)00153-8](http://dx.doi.org/10.1016/S0006-3207(03)00153-8).
- Wood, S.N., 2006. Generalised Additive Models: an Introduction with R. Chapman & Hall/CRC Press, Florida, USA.