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Assessing the responses of aquatic macrophytes to the application of a lanthanum modified bentonite clay, at Loch Flemington, Scotland, UK

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ABSTRACT

Loch Flemington is a shallow lake of international conservation and scientific importance. In recent decades its status has declined as a result of eutrophication and the establishment of non-native invasive aquatic macrophytes. As previous research had identified the lake bed sediments as an important source of phosphorus (P), the P-capping material Phoslock® was applied to improve water quality. This paper documents the responses of the aquatic macrophyte community by comparing data collected between 1988 and 2011. Summer water-column total P concentrations decreased significantly and water clarity increased following treatment.

24   Aquatic plant colonisation depth increased and plant coverage of the lake bed extended. However, the

25   submerged vegetation remained dominated by the non-native *Elodea canadensis* Michx. Aquatic macrophyte

26   community metrics indicated no significant change in trophic status. Species richness and the number of

27   'natural' eutrophic characteristic species remained broadly similar with no records of rare species of

28   conservation interest. Loch Flemington is still classified as being in 'unfavourable no change' condition based

29   on its aquatic macrophytes despite the water quality improvements. The implications of these results are

30   discussed in relation to the future management of Loch Flemington and in the wider context of trying to

31   improve our understanding of lake restoration processes.

32   **KEYWORDS**: Eutrophication, Loch Flemington, lake restoration, P-capping material, aquatic macrophytes,

33   lake management

34

35   **INTRODUCTION**

36

37   Eutrophication is regarded as being one of the most important factors causing degradation of lakes throughout

38   Europe (Withers & Haygarth, 2007) and the rest of the world (Bennett *et al*., 2001; Schindler, 2006). In shallow

39   (< 3 m depth) freshwater lakes, elevated nutrient inputs from the catchment, resulting from agricultural, cultural

40   and industrial activity, are the primary cause of this eutrophication (Scheffer, 2004). Increased loadings of

41   phosphorus (P) commonly result in a shift from macrophyte to planktonic production, with associated

42   phytoplankton blooms (particularly cyanobacterial), leading to deterioration in water quality and associated

43   changes in the biological communities. European legislation has led in the UK to the setting of P targets for high

44   alkalinity, shallow lakes to achieve 'favourable condition' (JNCC, 2005) under the Habitats Directive (Council

45   of the European Communities, 1992) and 'good' and 'high' ecological status (UKTAG, 2008) under the Water

46   Framework Directive (WFD) (Council of the European Communities, 2000). However, many shallow lakes in

47   Europe, because of a legacy of eutrophication, already have nutrient concentrations which exceed these targets

48   (EEA, 2012). For shallow lakes, designated to be of conservation importance, this deterioration in condition is

49   also indicated by the decline or disappearance of rare and scarce vascular plants and/or assemblages of notable

50   vascular plants and charophytes (JNCC, 2005).

51 On a global scale, research on eutrophication in relation to lake management has received considerable attention

52 (Jeppesen *et al*., 2003). Successful lake restoration depends on a good understanding of the site-specific drivers

53 of eutrophication and using targeted management strategies which are based on good scientific evidence. In

54 many lakes the main factor limiting phytoplankton abundance is P availability, so it is this nutrient which is

55 usually targeted for control by most lake restoration strategies, although nitrogen (N) limitation in the summer

56 months can also be important (Fisher *et al.*, 2009; May *et al.,* 2010). Reducing the external P load from a lake

57 catchment is often the preferred remedial option where P is the limiting factor. However, its effectiveness is

58 often reduced or delayed by internal recycling of P from the lake sediments (Søndergaard *et al*., 2007). In such

59 cases, alternative approaches to lake recovery must be used, coupled with reducing the external P load, such as

60 bio-manipulation, biomass harvesting, lake flushing, sediment removal, vertical mixing, and P inactivation

61 (Cooke *et al*., 2005). The latter technique has in the past relied on adding iron, aluminium or similar salts to bind

62 and precipitate available dissolved P from the water-column to the lake sediments. This prevents internal

63 loading by maintaining, for example, a layer of iron over the sediments, but, often, after short-term success, pre-

64 treatment levels of P release return within a few years (e.g. Foy, 1985). There have also been concerns that such

65 techniques may have adverse effects on lake ecosystems (Randall *et al.*, 1999; Spears *et al.*, 2013a). A range of

66 P-capping materials have been proposed for use in the UK (Spears *et al*., 2013b). Phoslock[®] is a lanthanum (La)

67 modified bentonite clay designed to enhance the capacity of lake sediments to adsorb dissolved (i.e. bio-

68 available) P and significantly reduce its subsequent release (manufacturers reported binding capacity of 100g

69 Phoslock[®]: 1 g of P). La^{3+} ions that are strongly bound within the clay matrix react with dissolved P to form the

70 mineral rhabdophane ($LaPO_4$), a compound that is expected to be stable in the environment (i.e. beyond the pH

71 range 4 – 9; Ross *et al*., 2008).

72 The expected general responses of the aquatic macrophyte community, following eutrophication management,

73 have recently been summarised in a literature review which incorporated long-term data from oligotrophication

74 case studies (Jeppesen *et al.*, 2005; Verdonschot *et al*., 2011). Typical macrophyte community responses

75 included increases in colonisation depth, species richness (including relative characean abundance), the number

76 of nutrient intolerant species and species distribution as TP concentrations decreased. Submerged macrophyte

77 vegetation becomes increasingly restricted to shallower waters in response to decreasing light levels at the

78 sediment surface as phytoplankton population levels increase. One simple measure of macrophyte abundance in

79 lakes is maximum macrophyte growing depth (MMGD). MMGD is sensitive to eutrophication (Jupp & Spence,

80 1977; May & Carvalho, 2010; Søndergaard *et a*l., 2005; Spears *et al*., 2009) and can provide a direct measure of

81 the success of lake management measures to shift primary production in lakes from a phytoplankton-dominated

82 to a more macrophyte-dominated state (Sas, 1989).

83 This paper examines how the aquatic macrophyte community in Loch Flemington responded following the

84 application of the P-capping material Phoslock® to the lake bed sediments and, on the assumption that growing

85 conditions will improve as a result of better water quality, tested the following hypotheses:

86 • There will be no negative impacts of the Phoslock® application on aquatic macrophyte community

87 • Macrophyte maximum growing depth and coverage of lake bed will increase

88 • Species diversity will increase and species of conservation interest will re-appear

89 • Aquatic macrophyte community attributes and metrics will indicate a change towards improvement in
90 Loch Flemington's condition and trophic status

91 • Mechanical control of non-native aquatic species *Elodea canadensis* Michx. may encourage the
92 recovery of native aquatic macrophyte species when used in combination with Phoslock®

93

94 **METHODS**

95 **Study site**

96 Loch Flemington (N 57^0 32.570$^{'}$, W 3^0 59.399$^{'}$) is a shallow (mean and maximum depths of 0.75 m and 2.9 m),

97 lowland (altitude 46 m), high alkalinity (1240 μeql^{-1}), eutrophic lake, of glacial origin situated near Inverness in

98 Scotland, UK (Figure 1). It has a surface area of 15 ha, a maximum fetch of 0.75 km and a perimeter of 2.7 km

99 (UKLakes Database). The lake is situated in an area consisting of glaciofluvial sand and gravel within the

100 Kildrummie Kames esker system (Gordon & Auton, 1993). Significant hydrological modifications were made to

101 the lake in the 19th century by blocking the natural surface-water outflow and significantly increasing the surface

102 area and depth of the loch (May *et al*., 2001). Today, the lake has no natural surface water outflow and the Croy

103 Burn is the only surface water inflow. Water leaves the lake by evaporation and by percolating through

104 permeable gravels along the northwest shore, leading to an estimated water retention time of around 40 days

105 (May *et al*., 2001).

106 Loch Flemington lies within the Kildrummie Kames Site of Special Scientific Interest (SSSI) which was

107 notified in 1974, in part, for Loch Flemington's eutrophic lake habitat and for supporting populations of aquatic

108 macrophyte species of conservation interest: the Nationally Rare *Potamogeton rutilus* Wolfg. and the

109 Nationally Scarce *Potamogeton filiformis* Pers. In addition, macrofossils of the Nationally Scarce and European

110 Protected Species *Najas flexilis* (Willd.) Rostk. & W. L. E. Schmidt (listed in Annexes II and IV of the Habitats

111 Directive (Council of the European Communities, 1992)) have recently been recorded in Loch Flemington

112 (Bennion *et al.,* 2008). From the 1960s onwards Loch Flemington had become increasingly eutrophic due to

113 nutrient-laden waste entering from the catchment resulting in a lake flora dominated by three submerged

114 species *E. canadensis*, *Myriophyllum spicatum* L. and *Potamogeton crispus* L. (May *et al.,* 2001).

115 Palaeolimnological records indicated that the aquatic macrophyte community had actually changed from a rich

116 macrophyte flora characteristic of mesotrophic conditions before 1850 towards a more species poor community

117 indicative of nutrient enrichment (Bennion *et al*., 2008). This resulted in a build up of P within the lake which

118 caused, by the mid 1970s, troublesome algal blooms to develop and fish kills to occur. Nuisance algal blooms,

119 particularly those dominated by cyanobacteria, continued throughout the 1980s and 1990s with increasing

120 frequency, threatening the conservation status of the lake. By 2003/04 the SSSI eutrophic lake habitat feature

121 was found to be in 'unfavourable condition'. The main reasons for this negative classification were the lake's

122 continuing poor water quality and the domination of the submerged vegetation by the non-native *E. canadensis*

123 plus the disappearance of all the aquatic macrophyte species of national conservation interest. Restoration

124 efforts at Loch Flemington up to this point had been focussed solely on improving water quality by reducing

125 catchment point sources of nutrients. An assessment of total P (TP) loads to the lake indicated that, by 2001,

126 diffuse (mainly agricultural) sources, represented ~ 80% and septic tanks ~ 18% of the external TP load,

127 respectively (May *et al*., 2001). However, the same study also found that the internal, sediment-driven load to

128 the water-column (680 kg TP yr^{-1}) was probably far greater than the external load from the catchment (120 kg

129 TP yr^{-1}).

Phoslock® application

131 Phoslock® was applied to Loch Flemington by Phoslock® Europe GmbH over a three day period, March 13th –

132 15th 2010. A pilot study had estimated that the dosage of Phoslock® required to be applied to Loch Flemington

133 was 25 tonnes in order to control a maximum of ~ 22 kg total phosphorus (TP) in the water column and ~ 210

134   kg TP in the upper 3 cm of lake sediment, on the basis that 100 g of Phoslock® binds 1 g of phosphorus (Meis *et*

135   *al*., 2012).

136   **Pre- and post-application water quality and ecological (non-aquatic macrophyte) monitoring**

137   A range of water quality and ecological (non-aquatic macrophyte) parameters were monitored at Loch

138   Flemington on a monthly basis, pre- and post-application of Phoslock®, at five open water sampling sites

139   between May 2009 and March 2011, and at three of these sites from April 2011 to November 2011, with the

140   exception of July 2011 when all five sites were sampled. Field water quality measurements included surface

141   water pH, conductivity, temperature and dissolved oxygen concentrations while water clarity was assessed using

142   a Secchi disc. Water samples were collected for later analysis. Parameters measured included soluble reactive P

143   (SRP), TP, total lanthanum (TLa), soluble lanthanum, chlorophyll *a* and phytoplankton abundance, community

144   composition and bioassessment of biovolume. Crustacean zooplankton and macroinvertebrates were also

145   collected for later analysis at the open water sample sites. For further details of sampling processing and

146   analyses see Spears *et al.* (2012). In this paper only the mean annual surface water TP and chlorophyll *a*

147   concentration results are reported on.

148   **Aquatic macrophyte community monitoring**

149   Assessment of the aquatic macrophyte communities at Loch Flemington, pre - and post - application of

150   Phoslock®, involved three separate but related sampling monitoring programmes: Site Condition Monitoring

151   (SCM) surveys, monthly qualitative assessments of the submerged vegetation and monitoring of the maximum

152   macrophyte growing depths. Aquatic macrophyte data from a comprehensive 1988 survey of Loch Flemington,

153   carried out by the Nature Conservancy Council Scottish Loch Survey team (NCC, 1988), were used to help

154   draw historical comparisons.

155   *Site Condition Monitoring (SCM) surveys*

156   The Site Condition Monitoring (SCM) survey method followed the Common Standards Monitoring (CSM)

157   protocols produced by the UK Joint Nature Conservation Committee (JNCC) for assessing the aquatic

158   macrophyte communities of standing waters of conservation importance (JNCC, 2005; JNCC, 2009). Pre-

159   Phoslock® application SCM surveys were carried out in 2003/04 and 2009 and post-Phoslock® application

160   aquatic macrophyte surveys were carried out in 2010 and 2011 (Table 1). Although the sampling effort differed

161   slightly between these different SCM-style surveys, the resultant data are considered to be broadly comparable.

162   The SCM aquatic macrophyte survey techniques employed at Loch Flemington involved three main elements

163   and are described in detail by (Gunn *et al.*, 2010; JNCC, 2005; JNCC, 2009). These were as follows: (1)

164   perimeter strandline searches; (2) shore-wader depth transect surveys; and (3) boat-based depth transect surveys.

165   All three methods were based on representative 100 m sections or sectors selected around the perimeter of the

166   lake in areas thought to be suitable for sustaining good aquatic macrophyte populations. Both the shore-wader

167   and boat-based depth transects involved 20 sampling points per survey sector. These SCM methods were

168   designed to produce data which are statistically robust (Gunn *et al.*, 2010).

169   *Qualitative assessments of aquatic macrophyte vegetation*

170   Seven monthly pre-application and thirteen monthly post-application qualitative assessments of the aquatic

171   macrophyte community composition and abundance were carried out at Loch Flemington between May 2009

172   and July 2011. These assessments involved random searches, by boat, for a fixed period of two hours. These

173   searches were standardised by starting at a fixed point and going back and forth across the lake in straight

174   transect lines sampling the submerged vegetation en route using a double-headed rake and/or using a

175   bathyscope.

176   *Macrophyte maximum growing depth (MMGD) monitoring*

177   The MMGD of submerged macrophytes at Loch Flemington were assessed using a boat along five fixed

178   transects that were evenly spaced around the deepest, north-eastern bay of the lake. Pre-application monitoring

179   of the MMGD was carried out on a monthly basis from July 2009 to November 2009 and just before the

180   Phoslock$^®$ application on the 11th March 2010. Post-application monitoring of MMGD was also carried out on a

181   monthly basis from April 2010 through to March 2011 with an additional monitoring occasion in July 2011

182   using methods as described by Jupp *et al*. (1974). Water depth and plant occurrence were measured at about 2 m

183   horizontal intervals along each transect until a point was reached at which no plants were collected. MMGD was

184   corrected for changes in water level by measuring water level at a common datum and subtracting water level

185   height from the measured MMGD. The common datum was fixed at an average water depth of 0.38 m (range

186   0.14 to 0.69 m). The methodology followed Spears *et al*. (2009). Data sets were arranged into seasons consisting

187   of three months in line with the standard meteorological definition (Trenberth, 1983) resulting in four seasons:

188   summer (June – August), autumn (September – November), winter (December – February) and spring (March –

189   May). Statistical analyses were conducted using the software package Minitab 16 (Minitab$^®$ 16.1.1, Minitab

190   Ltd., Coventry, UK). Data sets of MMGD were not normally distributed (Anderson-Darling test, $\alpha < 0.05$) even

191   after a range of transformations (including $x' = \log(x)$, $x' = \ln(x)$, $x' = sqrt(x)$, $x' = x^2$). Therefore a non-

192   parametric Mann-Whitney U-test (MWU) was used to test for significant variation in MMGD between years for

193   a given season, for seasons consisting of at least two out of three possible months as it is assumed that at least

194   two months are required to represent a season. This procedure resulted in the following statistical comparisons:

195   summer 2009 vs. summer 2010 and autumn 2009 vs. autumn 2010.

196   **Clear-cutting experiment to mechanically control *E. canadensis***

197   To assess the response of native macrophytes species in the absence of the non-native *E. canadensis* in Loch

198   Flemington, a replicated clear-cutting experiment was carried out (summer 2010) following the application of

199   Phoslock®. A diver manually cleared five 4 m² plots of *E. canadensis* and marked a further five plots as

200   controls. The percentage volume inhabited (PVI) and the aquatic plant community composition were tracked in

201   each plot over the growing season (May till October) using visual underwater inspection. Statistical analyses

202   were conducted using Minitab 16 (Minitab® 16.1.1, Minitab Ltd., Coventry, UK). Data sets were normally

203   distributed (Anderson-Darling test, $\alpha > 0.05$) but failed of a test for equal variance (Levene's test, $\alpha < 0.05$) even

204   after a range of transformations (including $x' = \log(x)$, $x' = \ln(x)$, $x' = sqrt(x)$, $x' = x^2$). Therefore, a non-

205   parametric MWU test was used to test for significant variation in PVI between clear cut plots and controls (both

206    $n = 5$) in a given month.

207   **Condition assessment of Loch Flemington based on aquatic macrophyte monitoring, pre- and post-**

208   **Phoslock® application**

209   Loch Flemington was judged against the targets set for each of the attributes listed for natural lakes with

210   *Magnopotamion* or *Hydrocharition*-type vegetation in the UK CSM Guidance for Standing Waters (JNCC

211   2005; JNCC, 2009). To be classified as being in 'favourable condition' Loch Flemington should have an aquatic

212   macrophyte community with species characteristic of a natural eutrophic lake and comply with the specific

213   aquatic macrophyte community composition and structure targets for that type of standing water (JNCC 2005;

214   JNCC, 2009).

215   In order to evaluate if there has been any change in the trophic status in Loch Flemington, following the

216   application of Phoslock®, the whole lake macrophyte assemblages recorded in the pre-and post-application

217   surveys were assessed using two indices: the Trophic Ranking Score (TRS) (Palmer, 1992; Palmer *et al.*, 1992)

218 and the Plant Lake Ecotype Index (PLEX) (Duigan *et al.*, 2006; Duigan *et al.*, 2007). Both scoring systems are

219 based around assigning a score to species based on their affiliation to particular trophic conditions. Scores of

220 qualifying species can be summed for a site, and an average score per taxon can be calculated to give a site

221 TRS/PLEX score.

222

223 **RESULTS**

224 **Summary of key water quality findings from pre- and post-Phoslock® application monitoring**

225 The application of Phoslock® led to a significant decrease in annual mean TP concentrations to levels below the

226 WFD target for the lake (Table 2). This led, in turn, to a significant reduction in algal levels in Loch Flemington,

227 as indicated by decreases in annual mean chlorophyll *a* concentrations (to below the WFD target (Table 2)) and

228 by 77% (P <0.001) and 95% (P <0.001) reductions in summer chlorophyll *a* concentrations in 2010 and 2011,

229 respectively. There was also an increase in Secchi disc water clarity (from <0.5 m in summer 2009 to *c.* 1.4 m in

230 summer 2011) (Spears *et al.*, 2012).

231 Monthly estimates of MMGD between 2009 and 2011 showed that the aquatic macrophyte colonisation depth

232 had significantly increased following the application of Phoslock®. An increase in MMGD was observed in

233 summer (57% deeper; $W = 55$; $p < 0.001$; $n_1 = 10$, $n_2 = 15$) and autumn 2010 (15% deeper; $W = 162$; $p < 0.01$;

234 $n_1 = n_2 = 15$) when compared to 2009, indicating an overall improvement in water quality corresponding with

235 the increase in water clarity (Figure 2). With the lowering of the seasonal and annual mean chlorophyll *a*

236 concentrations (Table 2), following the Phoslock® application, it is estimated that the depth limits of the

237 dominant elodeid plant functional group (i.e. *E. canadensis*) increased (depending on the background light

238 attenuation) from *c.* 1.4 - 1.6 m in 2009 to *c.* 2.3 - 2.9 m in 2011. Linked to this increase in MMGD, the

239 coverage of aquatic macrophytes was estimated to also have increased by between 30 and 40% to c. 80% of the

240 lake bed, after the application of Phoslock®.

241 **Condition assessment of Loch Flemington based on aquatic macrophyte monitoring, pre- and post-**

242 **Phoslock® application**

243 The number and abundance of 'natural eutrophic' characteristic species recorded in the post-application 2010

244 and 2011 SCM surveys marginally increased. Although three characteristic eutrophic species were recorded in

245 these surveys, occurring in 28% and 36% of sampling points, respectively (Table 3), this was well below the

246 target of six characteristic species occurring in at least 60% of sampling points. Nevertheless, this was an

247 improvement on the situation in 1988 and 2003 (at a frequency of occurrence of 13% of sampling points), when

248 only one characteristic species, *Potamogeton obtusifolius* Mert. & W. D. J. Koch was recorded and two in 2009

249 (at a frequency of occurrence of 16% of sampling points) (Table 3). Two other characteristic species,

250 *Potamogeton perfoliatus* L. and *P. x zizii* W. D. J. Koch ex Roth were recorded on the monthly qualitative

251 assessments in 2009 and assessed as "rare" (Table 3). Overall, the aquatic macrophyte community, as indicated

252 by the 2010 and 2011 post-Phoslock® application surveys, remained broadly similar in terms of species

253 composition and richness to the 2009 pre-application surveys and improved compared with the 1988 and the

254 2003/04 surveys (Table 3). There were no obvious negative impacts of the Phoslock® application on the aquatic

255 vegetation. However, the submerged vegetation of Loch Flemington remained dominated by the non-native *E.*

256 *canadensis,* which occurred at a frequency well above the 25% target threshold in the SCM survey sampling

257 points (Table 3).

258 Analysis of the monthly qualitative assessments of the aquatic macrophyte vegetation indicated that the relative

259 abundance of *E. canadensis* and another invasive non-native *Crassula helmsii* (Kirk) Cockayn had increased

260 following the Phoslock® application, although this was not statistically significant. *C. helmsii* was first recorded

261 as having colonised areas of shallows and margins along the northern shore Loch Flemington in 2009, prior to

262 the Phoslock® application. In addition, none of the three species of national conservation value *N. flexilis, P.*

263 *filiformis* and *P. rutilus* were re-recorded during any of the post-application aquatic macrophyte surveys

264 including the extensive targeted searches carried out as part of the 2010 SCM survey. Overall, on the basis of

265 the above assessments, measured against the set targets for a naturally eutrophic lake, Loch Flemington would

266 still be classified as being in 'unfavourable no change' condition, post-Phoslock® application.

267 Table 3 summarises all the species and their abundances as recorded in the various different aquatic macrophyte

268 surveys in Loch Flemington over the period of 1988-2011 plus their associated TRS and PLEX scores. Overall,

269 there had been a decline in both the TRS and PLEX average score per taxon values between 1988 and 2011,

270 reflected in the re-appearance of *Apium inundatum* (L.) Rchb, f., *Chara virgata* Kütz. and *Myriophyllum*

271 *alterniflorum* DC.*,* species characteristic of more mesotrophic conditions (Preston & Croft, 1997; Stewart, 2004)

272 although *M. alterniflorum* was recorded in the lake before the Phoslock® application. Pre- and post application

273 the mesotrophic characteristic species *Potamogeton gramineus* L. has also regularly been recorded. Recordings

274   of these more mesotrophic species are consistent with the palaeoecological results which indicated the lake

275   supported mesotrophic species in the past (Bennion *et al*., 2008). However, Loch Flemington on the basis of its

276   current aquatic plant community would still be classified as a Type 10 eutrophic standing water body (Palmer,

277   1992; Palmer *et al*., 1992) and as a species-poor example of a Group G Central and Eastern, above neutral,

278   lowland lake with *Lemna minor*, *Elodea. canadensis*, *Potamogeton natans* and *Persicaria amphibia* (Duigan *et*

279   *al.,* 2006; Duigan *et al*., 2007).

280   **Clear-cutting experiment to mechanically control *E. canadensis***

281   The result of the replicated clear-cutting experiment on *E. canadensis* indicated that although the PVI was

282   reduced significantly ($W = 5$; $p < 0.05$; $n_1 = n_2 = 5$) in the cleared plots the presence of *E. canadensis* was not

283   reduced and no desirable macrophyte species began to colonise the cleared patches (Figure 3).

284

285   **DISCUSSION**

286   The application of Phoslock[®] to Loch Flemington led to a reduction in water column P concentrations and

287   summer algal blooms, resulting in increased water clarity which potentially improved the light climate for

288   aquatic macrophytes. As expected, the treatment appeared to have had no negative effect on the aquatic

289   macrophyte community. However, although the light climate had significantly improved the only evidence of

290   changes in the diversity of the aquatic macrophyte community were several new records of mesotrophic species

291   (reflected in a slight decline in the average TRS/PLEX metric scores). Analysis of plant macrofossils taken

292   from a sediment core, collected from the littoral zone of Loch Flemington in 2006, indicated a greater diversity

293   of species characteristic of meso-eutrophic conditions in the years post-1850 than in more recent times (Bennion

294   *et al.,* 2008). These findings suggest that a recovering Loch Flemington should exhibit greater species diversity

295   with more species present which are indicative of meso-eutrophic lakes, including *Chara* species, *Isoetes*

296   *lacustris* L. *N. flexilis*, *Nitella* species and *Potamogeton praelongus* Wulfen. Overall, Loch Flemington, post-

297   Phoslock[®] application, remains classified as a eutrophic lake in 'unfavourable' condition on the basis of its

298   failure to achieve a number of its set conservation targets. These included the continued absence of species of

299   conservation interest, *N. flexilis*, *P. filiformis* and *P. rutilus* and the continued dominance of the aquatic

300   vegetation by the non-native *E. canadensis* and the recent colonisation by the invasive *C. helmsii*.

301 Although in the short-term the Loch Flemington aquatic macrophyte community has yet to show the response to

302 the Phoslock® treatment that are consistent with community shifts reported in other re-oligotrophication studies

303 (e.g. Jeppesen *et al*., 2005), this is perhaps not surprising. Verdonschot *et al*. (2011), in their literature review on

304 the ecological recovery of macrophyte communities from eutrophication, indicated that full recovery of species

305 composition was rarely recorded, often as a result of physical barriers to distribution and/or the loss of nutrient

306 intolerant seed banks in cases where eutrophic conditions had been prevalent for many years. At a structural

307 level, macrophyte colonisation responses are generally observed relatively quickly (i.e. less than five years) after

308 reductions in TP load, as was the case in this study. However, at a community composition level, the recovery

309 timescales for macrophytes to shift from eutrophic to more mesotrophic conditions were generally reported to

310 take place on a longer time-scale from 2 to 40+ years than reported here for Loch Flemington. Verdonschot *et*

311 *al*. (2011) highlighted a number of factors which could explain such delayed responses in shallow lakes

312 including the following: the suppression of native aquatic macrophyte species by the invasion of more

313 competitive species; constant disturbance of lake sediments, e.g. by wind, linked to changes in climatic

314 conditions (Spears & Jones, 2010), leading to a turbid state predominating (e.g. Lake Apopka, USA; Havens *et*

315 *al*., 2001); recovery of macrophyte community being, in part, dictated by the presence of individual species seed

316 banks, growth traits, distribution networks and pathways with species characterised by being slower growing,

317 having higher root: shoot ratios, and being longer lived becoming increasingly prevalent as nutrient

318 concentrations reduce (Riis & Biggs, 2011).

319 In the case of Loch Flemington the recovery of the aquatic macrophyte community may have been hampered by

320 the presence of the invasive species *E. canadensis* and *C. helmsii*. *E. canadensis* holds a competitive advantage

321 over many native species because it can maintain some above-sediment plant growth in the winter (Simpson,

322 1984) enabling it to outcompete other non-evergreen species that have to regenerate totally from turions or

323 seeds. *E. canadensis* can also utilise bicarbonate rather than carbon dioxide for photosynthesis (Bowmer *et al*.,

324 1995; Maberly, 1983), which may give it a competitive advantage over species such as *N. flexilis,* which is

325 reliant on carbon dioxide (Wingfield, *et al*., 2005). *N. flexilis* is an annual plant which spreads by underwater

326 pollination and cannot reproduce vegetatively, relying instead on seed production to survive (Wingfield *et al*.,

327 2004). Whilst it is possible that some seeds may survive in the seed bank for more than one year, their longevity

328 is not well understood (Wingfield *et al.,* 2004). However, although no survey of contemporary aquatic

329 macrophyte communities have ever recorded *N. flexilis* in Loch Flemington, a recent palaeoecological study

330 found macrofossils of the species in the lake sediments indicating that it had been present for a minimum of 100

331 years (Bennion *et al.,* 2008). This suggests that there would be a readily available seed bank for *N. flexilis,*

332 depending on seed longevity, to extend its coverage in the lake should suitable areas of the lake bed be opened

333 up from *E. canadensis* dominance. Like *N. flexilis, P. rutilus* is typically found in unpolluted mesotrophic lakes

334 in northern Scotland (Preston, 1995) and as such it might be expected to re-establish itself naturally in Loch

335 Flemington if there were any local sources from which the plant could re-colonise. Partly because it is rare and

336 partly because it grows often in deeper water where it is difficult to locate (Preston & Croft, 1997), little is

337 known about the long-term survival of *P. rutilus* turions in the sediment. However, it is apparent from the clear-

338 cutting experiment carried out at Loch Flemington, following the Phoslock® application, that local scale

339 mechanical control methods did not help the process of re-establishing the native aquatic macrophyte

340 community, at least in the short term.

341 While *E. canadensis* is well established at the site and is the dominant submerged macrophyte species, *C.*

342 *helmsii* appears to be in the early stages of colonisation having first been recorded in Loch Flemington in 2009,

343 prior to the Phoslock® application. Therefore, the options for management of these two species are very

344 different. In the case of *E. canadensis,* although P concentrations in Loch Flemington have significantly dropped

345 following the Phoslock® application, they are still too high to cause a decline in the population, as the species is

346 known to exist in a wide range of nutrient concentrations in the UK (Preston & Croft, 1997). Although

347 mechanical control methods such as clear-cutting may be used to contain the spread of *E. canadensis* it requires

348 implementation on a regular basis, is often labour intensive and even then success in achieving long-term

349 control is unlikely (Howard-Williams *et al.,* 1996). Wade (1990) showed that the recovery of pre-existing

350 submerged vegetation is often rapid after being cut, particularly by species such as *E. canadensis,* which can re-

351 colonise and spread quickly from undifferentiated plant fragments left behind and which is even less susceptible

352 to control by cutting compared to other competitive disturbance-tolerant species such as *Myriophyllum spicatum*

353 (Abernethy *et al.,* 1996). As well as clear cutting a number of other measures have been proposed for the control

354 of dense stands of *E. canadensis*, including the use of jute mats (Caffrey *et al.,* 2010), and in extreme cases,

355 draw-down followed by the application of a herbicide (Cooke *et al*., 2005) but as far as the authors are aware,

356 there have been no examples of the successful complete eradication of *E. canadensis* from lakes similar to Loch

357 Flemington. In contrast to *E. canadensis*, an opportunity to eradicate *C. helmsii* from the lake exists as it is not

358 abundant or widespread. In 2011, colonies of *C. helmsii* were restricted to shoreline areas and had not yet

359 reached the open water areas of the lake. Control measures of *C. helmsii*, so far, have focused on the use of

360 shading. This has involved identification and mapping existing colonies of *C. helmsii* prior to laying plastic

361   sheeting in an attempt to shade out some of the colonies but this work is extremely time consuming and labour

362   intensive but important, particularly as it may aid the potential re-colonisation by the Nationally Scarce *P.*

363   *filiformis*, which typically grows in shallow open water areas such as where the *C. helmsii* populations are

364   currently concentrated. Future efforts to manage the *C. helmsii* population, during these early stages of

365   colonisation, are likely to focus on the continued use of plastic sheeting supplemented by the possible use of

366   selective herbicides.

367   The sediment treatment work in Loch Flemington has, so far, been shown to be successful in reducing P

368   concentrations and summer algal blooms, and improving macrophytes growing conditions without any

369   noticeable negative impacts on the ecology of the aquatic macrophyte community. However, it is also clear that

370   the biological community, as exemplified by the aquatic macrophytes, will take longer than the water chemistry

371   to recover. This study shows that reducing P loading is not sufficient in itself to rapidly restore the aquatic

372   macrophyte species diversity of a lake, including the desirable re-colonisation of species of conservation value,

373   if there is a pre-existing problem with invasive, non-native species or if there is a lack of plant propagules. In the

374   case of Loch Flemington, while *C. helmsii* can be hopefully eradicated before becoming fully established*,*

375   management *of E. canadensis* will need to focus on control and containment, with the aim of facilitating the

376   recovery of some of the former aquatic macrophyte species diversity, for which the site was renowned. It is also

377   important to emphasise that without reducing nutrient loads from the catchment, the water quality improvements

378   so far brought about by the application of a P-capping agent such as Phoslock$^{®}$, may not persist. Therefore, it is

379   crucial that catchment nutrient sources continue to be monitored and managed where necessary.

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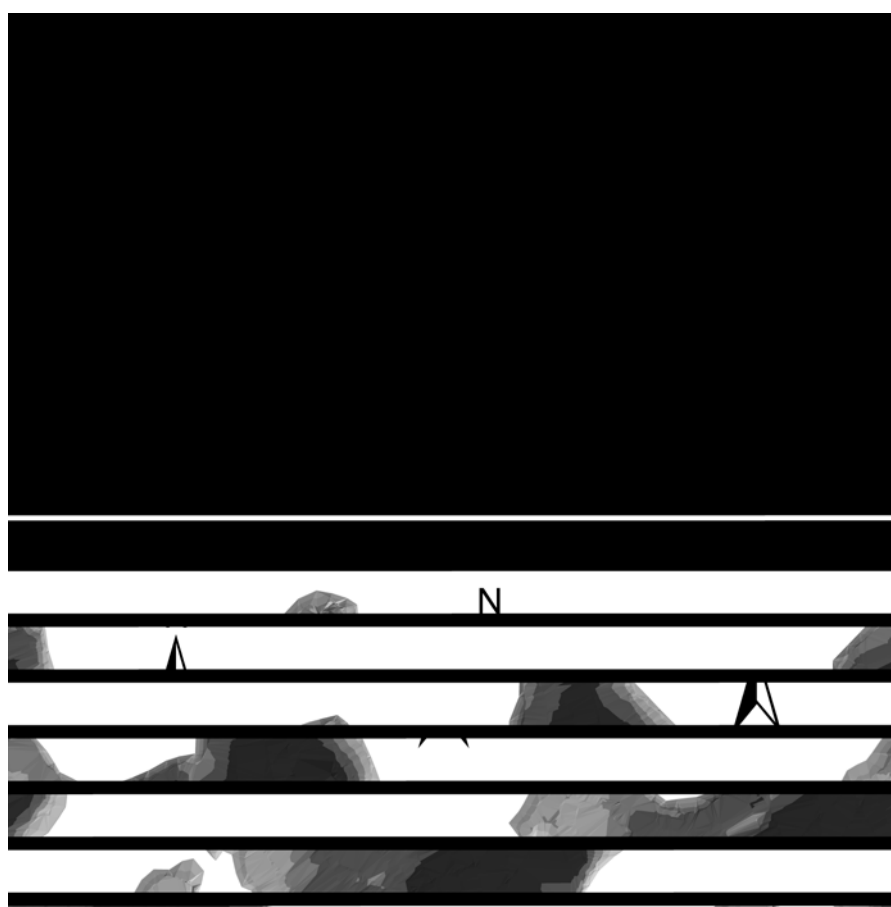
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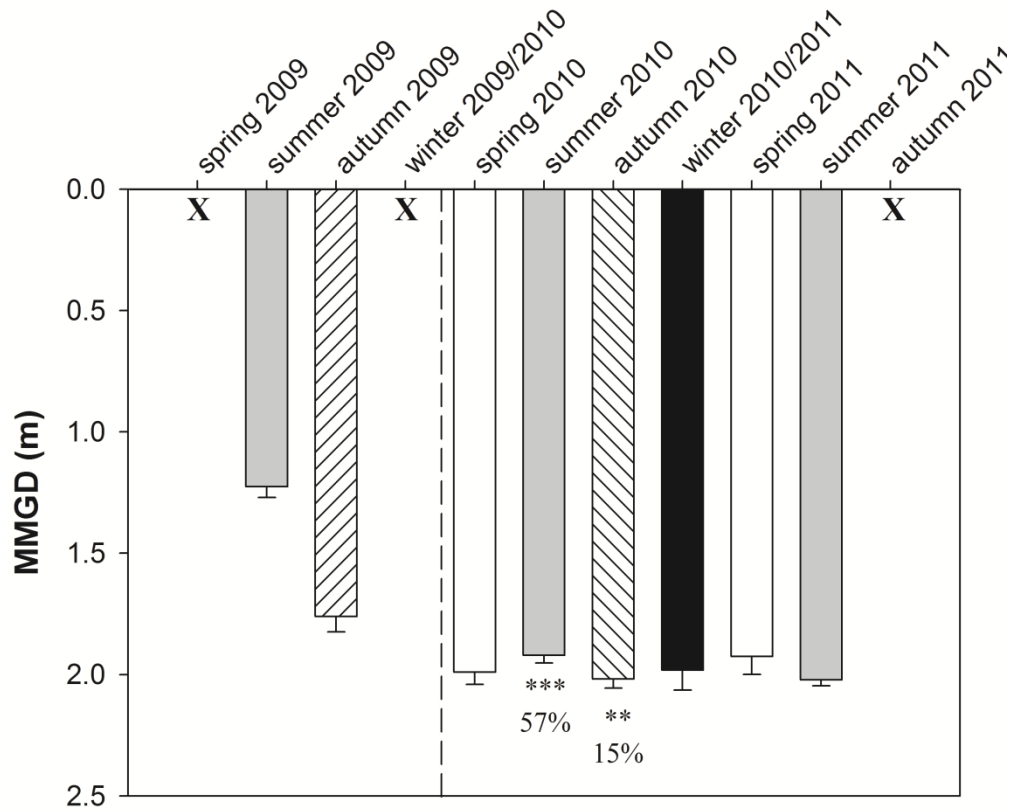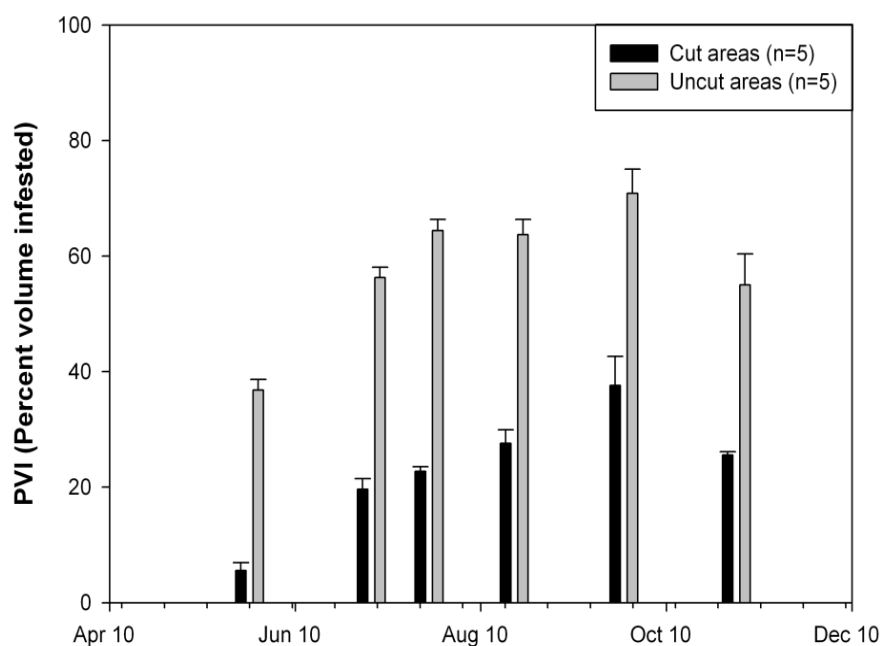**Loch Flemington bathymetric map**

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586   **Fig. 1.** Bathymetric Map of Loch Flemington

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Fig. 2. Seasonal variation in macrophyte maximum growing depth (MMGD) in Loch Flemington. Dashed vertical line indicates timing of the Phoslock® application, error bars represent standard error of the mean (n = variable) and seasons in which no sampling occurred are marked with 'X'. Significant differences (**, $p < 0.01$; ***, $p < 0.001$) in a season between pre- and post-application years and percent change are indicated

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594

595 **Fig. 3.** Seasonal variation in percentage volume inhabited (PVI) of *Elodea canadensis* following clear-cutting in
596 five 4 m^2 plots compared with five uncut 4 m^2 plots in Loch Flemington in 2010. Error bars represent the
597 standard error of the mean.

598

599 **Table 1.** Summary of SCM aquatic macrophyte surveys carried out at Loch Flemington, pre- and post-
600 Phoslock® application.

	SCM surveys pre-Phoslock® application		SCM surveys post-Phoslock® application	
	3 July 2003 + 5 August 2004	12 August 2009	26/27 July + 2/3 August 2010	18/19th July 2011
Number of perimeter strandline searches	4	4	5	4
Number of shore-wader depth transects	4	4	3	3
Number of boat-based depth transects	-	1	2	2
Other	Targeted search for rare species in open-water	-	Targeted search for rare species in open water	-

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602   **Table 2**. Summary table of annual mean surface water TP and chlorophyll *a* concentrations, pre - and post-
603   Phoslock[®] application

Year	Mean TP concentrations ($\mu g\ P\ l^{-1}$) (n = variable)	Mean chlorophyll *a* concentrations ($\mu g\ l^{-1}$) (n = variable)
WFD target	32	16
2009 (Pre-application)	60	51
2010 (Post-application)	31	25
2011 (post-application)	27	12

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Species	TRS	PLEX	Pre - Phoslock® application			Post – Phoslock® application	
			1988[a]	2003/04	2009[c]	2010[c]	2011[c]
Apium inundatum	7.00	7,50	-	-	-	1%	present
Chara virgata[d]	7.30	7.69	-	-	-	present	-
Crassula helmsii	X	X	-	-	present	present	present
Elodea canadensis	8.50	7.95	LD	56%	62%	52%	51%
Lemna minor[d]	9.00	8.85	-	-	3%	5%	28%
Littorella uniflora	6.70	4.23	O	6%	3%	3%	present
Myriophyllum alterniflorum	5.50	4.23	-	-	14%	9%	7%
Myriophyllum spicatum	10.00	8.85	-	1%	1%	present	-
Persicaria amphibia	9.00	7.95	LD	28%	33%	16%	22%
Potamogeton gramineus	7.30	7.31	-	present	3%	present	1%
Potamogeton natans	6.70	4.23	-	7%	17%	8%	6%
Potamogeton obtusifolius[d]	7.30	6.54	O-F	13%	13%	27%	9%
Potamogeton perfoliatus[d]	7.30	7.69	-	-	present	-	present
Potamogeton x *zizii*[d]	X[b]	7.69	-	-	present	-	-
Ranunculus aquatilis	8.50	7.95	R	-	-	-	-
Total number of species			5	7	12	12	11
Mean number of species per SCM survey transect (wader & boat)			N/A	3.75	4.00	4.17	4.20
Total number of characteristic[d] species			1	1	4	3	3
% frequency of sampling points with characteristic[d] species			N/A	13	16	28	36
Total TRS			40	55.50	77.3	84.3	74.3
Total PLEX			34.62	47.07	75.52	75.33	66.48
Mean Score Per Taxon - TRS			8.00	7.93	7.73	7.66	7.43
Mean Score Per Taxon - PLEX			6.92	6.72	6.87	6.85	6.65

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630 [a] in 1988 DAFOR (Dominance, Abundant, Frequent, Occasional, Rare) abundance scale used; LD = Locally
631 Dominant

632 [b] X indicates no scores allocated to species

633 [c] species list includes data from monthly qualitative assessments in addition to SCM survey data – only SCM
634 survey data used for abundances

635 [d] characteristic species of natural eutrophic lakes with *Magnopotamion* or *Hydrocharition*-type vegetation
636 (JNCC 2005; JNCC, 2009)

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