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Contact CEH NORA team at  
[noraceh@ceh.ac.uk](mailto:noraceh@ceh.ac.uk)

1 LINDA MAY<sup>1</sup> (CORRESPONDING AUTHOR), BRYAN M. SPEARS<sup>1</sup>, BERNARD J. DUDLEY<sup>1</sup>  
2 AND IAIN D.M. GUNN<sup>1</sup>

3

4 <sup>1</sup>NERC - Centre for Ecology & Hydrology, Bush Estate, Penicuik, Midlothian EH26  
5 0QB, UK.

6

7 Research Paper

8 The response of the rotifer community in Loch Leven, UK, to  
9 changes associated with a 60% reduction in phosphorus inputs  
10 from the catchment

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13 Corresponding author contact details:

14 Dr. L. May,

15 NERC - Centre for Ecology & Hydrology,

16 Bush Estate,

17 Penicuik,

18 Midlothian EH26 0QB

19 UK

20 Email: [lmay@ceh.ac.uk](mailto:lmay@ceh.ac.uk);

21 Phone: +44(0) 131 445 4343

22

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24 zooplankton

25

## Abstract

26 Lakes across the world are suffering from anthropogenically induced nutrient  
27 enrichment problems and many attempts are being made to improve their water  
28 quality and ecosystem function. Most metrics that are being used to monitor recovery  
29 are based on relationships that have been established across a range of lakes. These  
30 may not respond quickly to in-lake changes in water quality when nutrient  
31 management strategies are put in place. This paper uses data routinely collected from  
32 Loch Leven, UK, to examine the immediate and longer-term responses of the rotifer  
33 community to a 60% reduction in phosphorus input from the catchment in the early  
34 1990s. We conclude that changes in rotifer abundance and relative species  
35 composition are sensitive indicators of lake-specific changes in water quality,  
36 responding more quickly than more widely used metrics, such as total phosphorus and  
37 chlorophyll *a* concentrations. However, like all indicators of change, such indices  
38 must be used with care in situations where rotifer populations are subject to multiple  
39 stressors.

40

41

## 1. Introduction

42

43 Lakes across the world are suffering from anthropogenically induced nutrient  
44 enrichment problems and many attempts have been made to improve their water  
45 quality and ecosystem function by reducing inputs from the catchment. However,  
46 only in a small number of case studies have the results of such interventions been  
47 recorded for long periods of time after nutrient management strategies have been put  
48 in place (e.g. see examples reviewed by Jeppesen et al. 2005). Even fewer studies

49 have reported the impacts of management intervention on the rotifer community of  
50 specific lakes.

51

52 Rotifers have long been recognised as good indicators of water quality across a range  
53 of lakes (e.g. Maemets 1983; Pejler 1981; Pejler 1983; Sladeček 1983; Matveeva  
54 1991; Duggan et al. 2001; Ejsmont-Karabin 2012), but it is unclear whether they can  
55 be used as indicators of temporal change within a single site in response to restoration  
56 measures. This is because most of the relationships between rotifer species  
57 composition and abundance, and lake trophic state, have been based on contemporary  
58 comparisons of rotifer communities across multiple lakes (e.g. Berzins & Pejler 1989;  
59 Jeppesen et al. 2000; Duggan et al. 2001; Tasevska et al. 2012) rather than in-lake  
60 changes over time. In general, although the rotifer community is an important  
61 component of lake food webs, the way that it responds to change is poorly  
62 understood. This is because our knowledge is limited to the results of relatively few  
63 long-term studies (e.g. Matveeva 1986; Walz et al. 1987; Balvay & Laurent 1990;  
64 Ejsmont-Karabin 1996), some of which have not linked the responses of the rotifer  
65 communities that they describe to changes in environmental pressures over time (e.g.  
66 Muirhead et al. 2006; Steinberg 2009).

67

68 Rotifer communities have been shown to respond quickly to a wide range of  
69 environmental stresses such as acidification, climate change, eutrophication and metal  
70 pollution (Walz et al. 1987; May & O'Hare 2005; Havas et al. 1995; Svensson &  
71 Stenson 2002; Vbra et al. 2003; Waervagen & Nilssen 2003; Dupius & Hann 2009).  
72 Of these, the impacts of changing eutrophication pressures have received the most  
73 attention (Matveeva 1986; Walz et al. 1987; Ejsmont-Karabin & Hillbricht-Ilkowska

74 1994). Once these pressures have been reduced, as with other freshwater biota,  
75 ‘recovery’ may not necessarily be characterised by the reversal of the patterns that  
76 were observed during degradation. For example, recent studies have suggested that  
77 zooplankton community responses in lakes can be delayed for many years, and by a  
78 number of confounding factors, when phosphorus (P) inputs from the catchment are  
79 reduced. These include changes to other chemical stressors (e.g. acidification), fish  
80 stocking practices, climate change, persistent internal P loading and (e.g. Jeppesen et  
81 al. 2005).

82

83 With a few notable exceptions (e.g. Lake Peipsi – Haberman et al. 2010), most long  
84 term studies of rotifers have focused on the impact of eutrophication, but not of re-  
85 oligotrophication, on the planktonic community. So, reports on the recovery of the  
86 rotifer community, once nutrient inputs have been reduced, are relatively rare. This  
87 paper documents the responses of the rotifer community in Loch Leven, UK, over a  
88 34 year period that is characterised by three key periods of eutrophication and nutrient  
89 management: (1) pre-management (1977-1982); (2) nutrient reduction and immediate  
90 post-management (1991-1998); and (3) longer-term post-management (2010-2011).  
91 Routine monitoring at this site since 1968 has provided a long time series of physical,  
92 chemical and biological variables with which to explore the response of the rotifer  
93 community to the nutrient reduction process.

94

95

## 2. Methods

96

97 *Study site*

98 Loch Leven (56° 10' N, 3° 30' W) is a large (13.3 km<sup>2</sup>), shallow lake in the east-  
99 central Scotland, UK, with mean and maximum depths of 3.9 m and 25.5 m,  
100 respectively (Kirby 1971). The lake has an average hydraulic retention time of about  
101 5.2 months (Smith 1974) and drains a predominantly agricultural catchment of about  
102 145 km<sup>2</sup>. Several small towns and villages within the catchment have a total  
103 population of about 11,000 people (Frost 1996).

104

105 Loch Leven is, primarily, phosphorus (P) limited and has a long and well documented  
106 history of eutrophication and recovery (May & Spears 2012). Between 1985 and  
107 1995, total phosphorus (TP) inputs from the catchment were reduced by about 60%  
108 (May et al. 2012). So, TP inputs fell from about 20 t y<sup>-1</sup> (1.5 g m<sup>-2</sup> yr<sup>-1</sup>) to about 8.5 t  
109 y<sup>-1</sup> (0.64 g m<sup>-2</sup> yr<sup>-1</sup>). This lower level of TP input was sustained until at least 2005,  
110 when a TP load of 8 t y<sup>-1</sup> (0.6 g m<sup>-2</sup> yr<sup>-1</sup>) was recorded (May et al. 2012).

111

112 Most of the reduction in TP input was due to better control of effluents from waste  
113 water treatment works and industrial sources, between 1987 and 1993 (May et al.  
114 2012). However, minor changes aimed at addressing inputs from diffuse sources  
115 (such as the installation of buffer strips) were also made (Castle et al. 1999). Key  
116 changes in the physics, chemistry and biology of Loch Leven over the pre-  
117 management, management and post-management periods have been reported  
118 elsewhere (May & Spears, 2012). In summary, these include an increase in growing  
119 depth and areal coverage of submerged macrophytes (May & Carvalho 2010; Dudley  
120 et al. 2012), a decrease in macroinvertebrate abundance and an increase in the number  
121 of taxa (Gunn et al. 2012), improvements in fish populations (Winfield et al. 2012),  
122 and an increase in the abundance of aquatic birds that depend on the lake for food and

123 habitat (Carss et al. 2012). Little information on the response of the rotifer community  
124 has been published to date.

125

126 ***Sample collection***

127 Water samples were collected at weekly intervals between 1977 and 1982, and  
128 fortnightly intervals 1991-1998 and 2010-2011. Sampling methods remained more or  
129 less consistent throughout the study period, with plankton and water chemistry  
130 samples collected from an open water site with a water depth of about 4 m (“Reed  
131 Bower”, Figure 1) using a weighted PVC tube with an internal diameter of 25 mm.  
132 Surface samples were also collected close to the outflow (“Sluices”, Figure 1). On  
133 some occasions, e.g. during bad weather, these were the only samples collected.

134

135 On return to the laboratory, water samples for chemistry and chlorophyll *a* analyses  
136 were shaken and sub-sampled. Those for TP analysis remained unfiltered; those for  
137 chlorophyll *a* analysis were filtered through a Whatman® GF/C grade filter within 6  
138 hours of collection. Filter papers were stored frozen at -18°C prior to analysis.

139

140 ***Total phosphorus and chlorophyll a analyses***

141 Total phosphorus concentrations were determined on samples that were digested with  
142 a solution of sulphuric acid and potassium persulphate, following the method  
143 described for TP by Wetzel and Likens (2000), with an additional acidification step.  
144 This involved adding 0.1 ml of 30% H<sub>2</sub>SO<sub>4</sub> to the samples before adding persulphate.  
145 Phosphorus concentrations were determined on a spectrophotometer following the  
146 method of Murphy and Riley (1962).

147

148 For chlorophyll *a* determinations, the frozen filter papers were submerged in 90%  
149 methanol overnight in a dark refrigerator. After centrifugation, the extracted  
150 chlorophyll *a* was measured spectrophotometrically at 665 nm with a turbidity  
151 correction conducted at 750 nm. Concentrations were determined using equation 1 of  
152 APHA (1992).

153

#### 154 *Rotifer community analysis*

155 Rotifer samples were narcotised in the field by adding sufficient procaine  
156 hydrochloride ( $\text{NH}_2\cdot\text{C}_6\text{H}_4\cdot\text{COO}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{N}(\text{C}_2\text{H}_5)_2\cdot\text{HCl}$ ) to give a final concentration  
157 of  $0.2 \text{ g l}^{-1}$  (May 1985). This ensured that soft bodied species, such as *Synchaeta*,  
158 would be recognisable in the fixed samples. Samples were then preserved in 4%  
159 formaldehyde and concentrated by sedimentation. Multiple sub-samples of the  
160 concentrate with an individual volume of 3 ml (1977-1982, and 1991-1998) or 5 ml  
161 (2010-2011) were counted under  $\times 100$  magnification until at least 200 rotifers, or the  
162 whole sample, had been counted. Species identifications (following Koste 1978) were  
163 carried out on live specimens collected separately from the preserved samples,  
164 because many species cannot be fully identified, in preserved samples.

165

166

### 3. Results

167

168 The 60% reduction in P input to Loch Leven led to a slow but steady decline in  
169 annual average open water TP concentrations (Figure 2). Before restoration, these  
170 values were about  $63 \mu\text{g P l}^{-1}$ . During and shortly after the P reduction period, the  
171 average concentrations rose to about  $71 \mu\text{g P l}^{-1}$ . In the longer term, this value fell to  
172 about  $34 \mu\text{g P l}^{-1}$ .

173

174 Annual mean chlorophyll *a* (chl*a*) concentrations showed a similar pattern of  
175 reduction (Figure 3). Before restoration, these values were about 31 µg chl*a* l<sup>-1</sup>.  
176 During and immediately after management intervention, these values increased to  
177 about 47 µg chl*a* l<sup>-1</sup>. In the longer term, the values fell to about 23 µg chl*a* l<sup>-1</sup>.

178

179 Similar to P and chlorophyll *a* concentrations, total annual mean rotifer densities  
180 followed a steady decline throughout the restoration period (Figure 4). Before  
181 restoration, average annual rotifer population densities were about 1000 ind. l<sup>-1</sup>.  
182 During the P reduction phase and shortly afterwards, these values fell to about  
183 630 ind. l<sup>-1</sup>. Although the rotifer data are less complete than the TP and chlorophyll *a*  
184 data, these lower population densities appeared to be maintained in the longer term,  
185 with the equivalent value for 2010-2011 being about 604 ind. l<sup>-1</sup>. This represented an  
186 overall reduction in rotifer numbers of about 40%. However, while both TP and  
187 chlorophyll *a* concentrations initially increased before showing appreciable decreases  
188 from 2000 onwards, rotifers densities responded to the change in P load almost  
189 immediately. Their numbers had declined by about 40% by the mid 1990s.

190

191 Changes in species diversity over the study period are shown in Table 1. The 13 most  
192 common and more abundant species remained in the lake over the entire study period.  
193 Although their absolute abundances fell, in line with the overall reduction in total  
194 rotifer numbers, their relative abundances remained similar throughout (Figure 5).  
195 The most noticeable exceptions to this were the relative proportion of *Keratella tecta*  
196 in relation to the total number of rotifers, and of the ratio of this species to the  
197 abundance of *Keratella cochlearis*. Both of these values decreased over time. The

198 proportion of *Pompholyx sulcata* within the rotifer community as a whole also  
199 decreased.

200

201 The main changes in species diversity were observed amongst the less common  
202 species, which represented only 14%-17% of the rotifer community. Of these,  
203 *Colurella adriatica* and *Polyarthra euryptera* were lost in the longer term. *Collotheca*  
204 *mutabilis*, *Conochilus hippocrepis*, *Polyarthra major* and *Synchaeta oblonga* were  
205 recorded for the first time during the period of P load reduction and remained in the  
206 lake thereafter. *Kellicottia longispina*, *Polyarthra vulgaris* and *Synchaeta tremula*  
207 were recorded during the P load reduction period, only, but not in the longer term.

208

209

#### 4. Discussion

210

211 When the P input to Loch Leven was reduced by about 60%, the rotifer community  
212 responded almost immediately with a reduction of 40% in its annual mean densities.

213 This was in contrast to TP and chlorophyll *a* concentrations, which showed a lag of  
214 several years before beginning to decline. The reduction in rotifer abundance

215 associated with the reduction in TP inputs to Loch Leven is consistent with  
216 expectations based on earlier studies of eutrophication impacts. These showed that

217 higher levels of rotifer abundance are associated with increasing levels of  
218 eutrophication across a range of different lake types (Blancher 1984, Karabin 1985,

219 Walz et al 1987, Berzins & Pejler 1989; Matveeva 1991; Jeppesen *et al.* 2000;  
220 Duggan et al. 2001; Tasevska et al. 2012) and within individual lakes (Matveeva

221 1986; Walz et al. 1987; Balvay & Laurent 1990; May & O'Hare 2005; Ejsmont-  
222 Karabin 1996; Muirhead *et al.* 1996; Ejsmont-Karabin 2003; Steinberg et al 2009).

223 The results suggest that total rotifer abundance is a good indicator of both

224 eutrophication and recovery, and that it responds more rapidly to change than other  
225 determinands that are used to measure change in water quality more routinely, such as  
226 TP and chlorophyll *a* concentrations.

227

228 At the species level, many authors have reported a strong relationship between certain  
229 rotifer ‘indicator’ species and lake trophic state. For example, based on contemporary  
230 studies across a number of lakes, it has been reported that more eutrophic  
231 environments are more likely to have species such as *Brachionus angularis*, *Filina*  
232 *longiseta*, *Keratella cochlearis*, *Keratella tecta*, *Keratella quadrata*, *Pompholyx*  
233 *sulcata* and *Trichocerca pusilla*, than less eutrophic environments (Pejler 1983:  
234 Karabin 1985; Berzins & Pejler 1989; Matveeva 1991; Ejsmont-Karabin 2003). Many  
235 authors have also reported a greater proportion of *Keratella tecta* in rotifer  
236 communities as an indicator of increasing lake trophic state (Hillbricht-Ilkowska  
237 1972; Pejler 1981; Karabin 1985; Berzins & Pejler 1989; Ejsmont-Karabin 2012).  
238 However, Ejsmont-Karabin & Hillbricht-Ilkowska (1994) suggest that it is unlikely  
239 that all of these indicators of trophic state determined across a range of lakes will also  
240 reflect in-lake changes in water quality when conditions change. The current study on  
241 Loch Leven provides an opportunity for this hypothesis to be explored. In general, the  
242 results suggest that the species composition of the rotifer community in Loch Leven,  
243 and the proportional contribution of each species to overall abundance, changed very  
244 little during the recovery period. However, there was some evidence that the  
245 proportion of *Keratella tecta* increased in relation to overall rotifer abundance as  
246 water quality improved, as did the ratio of *Keratella tecta*:*Keratella cochlearis*. So,  
247 the results of this study support the hypothesis posed by Ejsmont-Karabin &  
248 Hillbricht-Ilkowska (1994) that, although overall rotifer abundance is a good,

249 dynamic indicator of change in the level of trophy of a given lake, other indicators –  
250 such as species composition – are less useful. The results also suggest that the  
251 proportion of *Keratella tecta* in relation to total rotifer abundance, and in relation to  
252 the abundance of *Keratella cochlearis*, may be a suitable indicator for monitoring in-  
253 lake change in water quality, especially trophic state.

254

255 The results of this study suggest that even simple measures of rotifer abundance and  
256 relative species composition could provide sensitive indicators of changing water  
257 quality within a lake in response to the control of eutrophication by management  
258 intervention. They also suggest that the rotifer community responds to change at a  
259 much faster rate than the more commonly used metrics of TP and chlorophyll *a*  
260 concentration, both of which may actually increase during the early stages of  
261 recovery. However, such rotifer-based indices must be developed and applied with  
262 care, as recent studies have also suggested that the response of zooplankton  
263 communities in recovering lakes can be subject to a number of confounding factors,  
264 such as changes in fish predation, climate change and other chemical stressors, such  
265 as acidification (e.g. Jeppesen et al. 2005), that make results difficult to interpret. This  
266 is especially true where water quality in general, and rotifer populations in particular,  
267 are influenced by multiple stressors.

268

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270

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276

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444

## 7. Tables

445 Table 1. Rotifer species found in Loch Leven over different time periods

<b>Species</b>	<b>1977-1982</b>	<b>1991-1998</b>	<b>2010-2011</b>
<i>Asplanchna priodonta</i> Gosse	X	X	X
<i>Brachionus angularis</i> Gosse	X	X	X
<i>Conochilus unicornis</i> Rousselet	X	X	X
<i>Filinia longiseta</i> (Ehrenberg)	X	X	X
<i>Keratella cochlearis</i> (Gosse)	X	X	X
<i>Keratella quadrata</i> (Müller)	X	X	X
<i>Keratella tecta</i> (Gosse)	X	X	X
<i>Notholca squamula</i> (Müller)	X	X	X
<i>Polyarthra dolichoptera</i> Idelson	X	X	X
<i>Pompholyx sulcata</i> Hudson	X	X	X
<i>Synchaeta grandis</i> Zacharias	X	X	X
<i>Synchaeta kitina</i> Rousselet	X	X	X
<i>Trichocerca pusilla</i> (Jennings)	X	X	X
<i>Colurella adriatica</i> Ehrenberg	X	X	-
<i>Polyarthra euryptera</i> Wierzejski	X	X	-
<i>Conochilus hippocrepis</i> (Schrank)	-	X	X
<i>Collotheca mutabilis</i> Hudson	-	X	X
<i>Polyarthra major</i> Burckhardt	-	X	X
<i>Synchaeta oblonga</i> Ehrenberg	-	X	X
<i>Kellicottia longispina</i> (Kellicott)	-	X	-
<i>Polyarthra vulgaris</i> Carlin	-	X	-
<i>Synchaeta tremula</i> (Müller)	-	X	-

446

447

## 8. Figure legends

448

449 Figure 1. Map of Loch Leven, showing routine sampling sites (squares), inflows and  
450 outflow (River Leven) (—), major roads (—) and urban areas (grey). Map data from  
451 Ordnance Survey Strategi dataset. Contains Ordnance Survey data © Crown copyright  
452 and database right 2012.

453

454 Figure 2. Annual mean total phosphorus (TP) concentrations in Loch Leven, 1977-  
455 2011.

456

457 Figure 3. Annual mean chlorophyll *a* concentrations in Loch Leven, 1977-2011.

458

459 Figure 4. Annual mean total rotifer densities in Loch Leven, 1977-1982, 1992-1998  
460 and 2010-2011.

461

462 Figure 5. Relative abundances of common and less common ('other') rotifer species  
463 in Loch Leven, 1977-1982, 1992-1998 and 2010-2011.

464

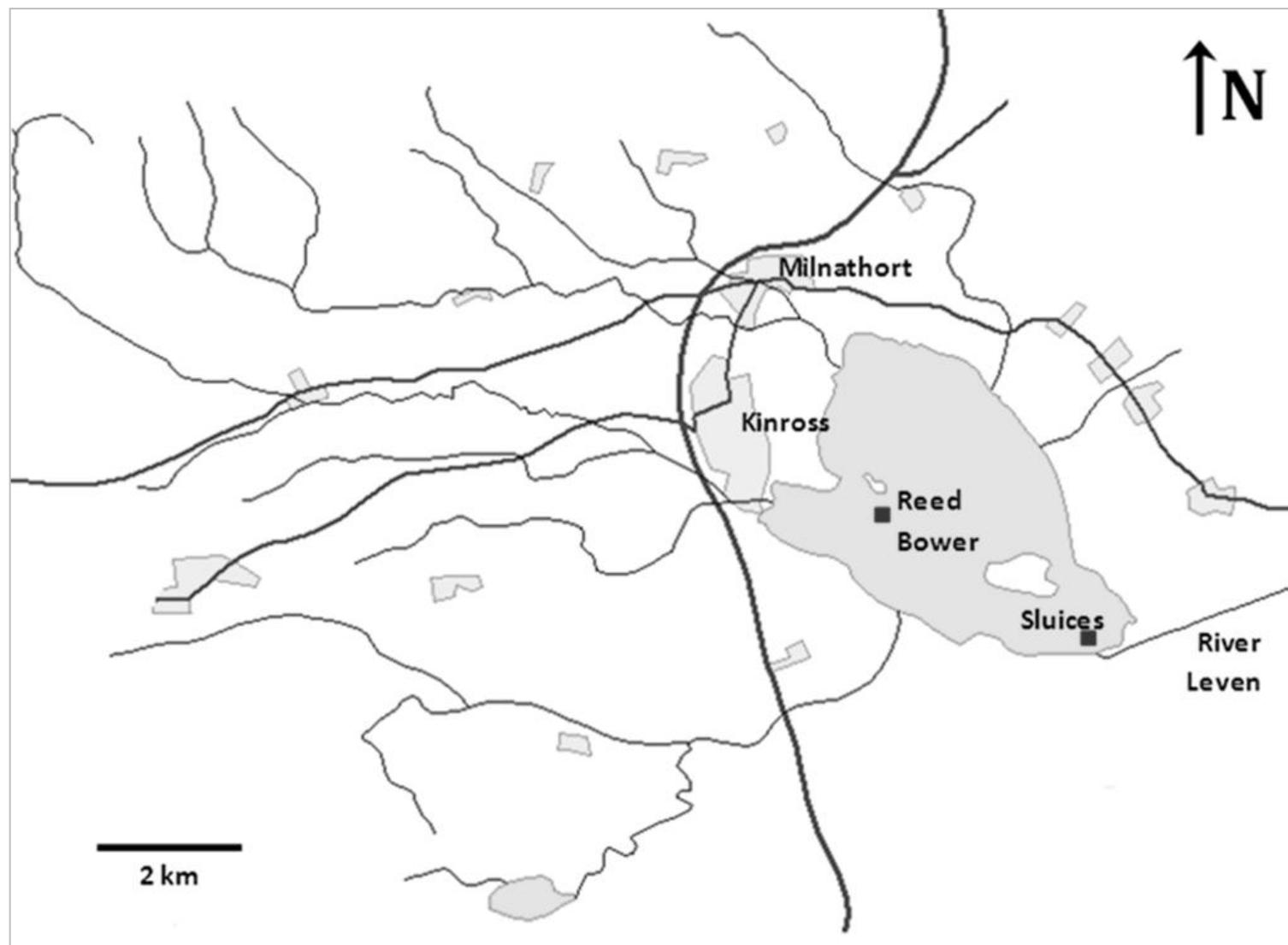


Figure 1.

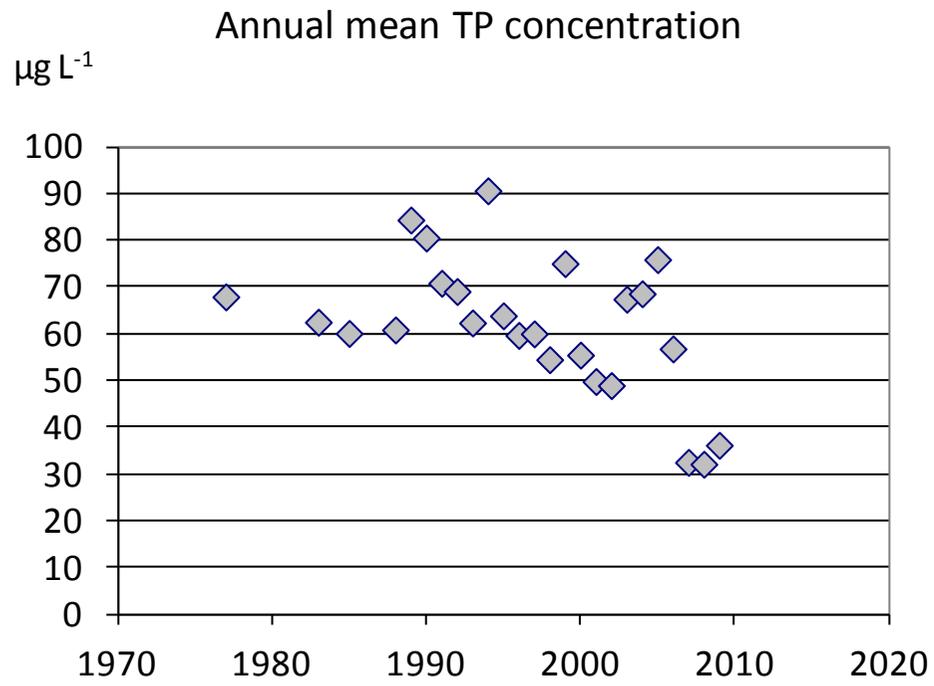


Figure 2

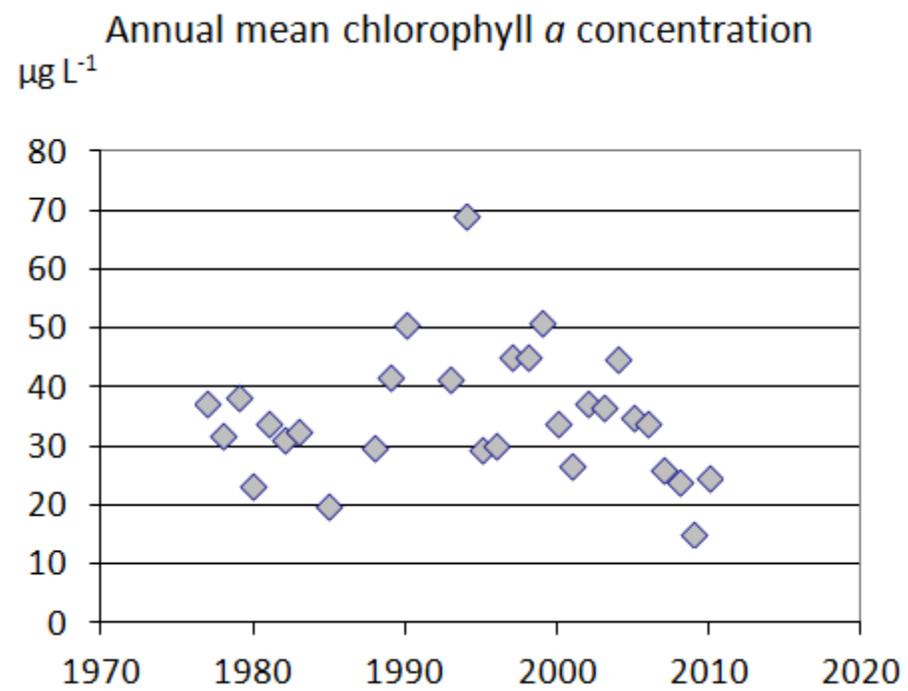


Figure 3

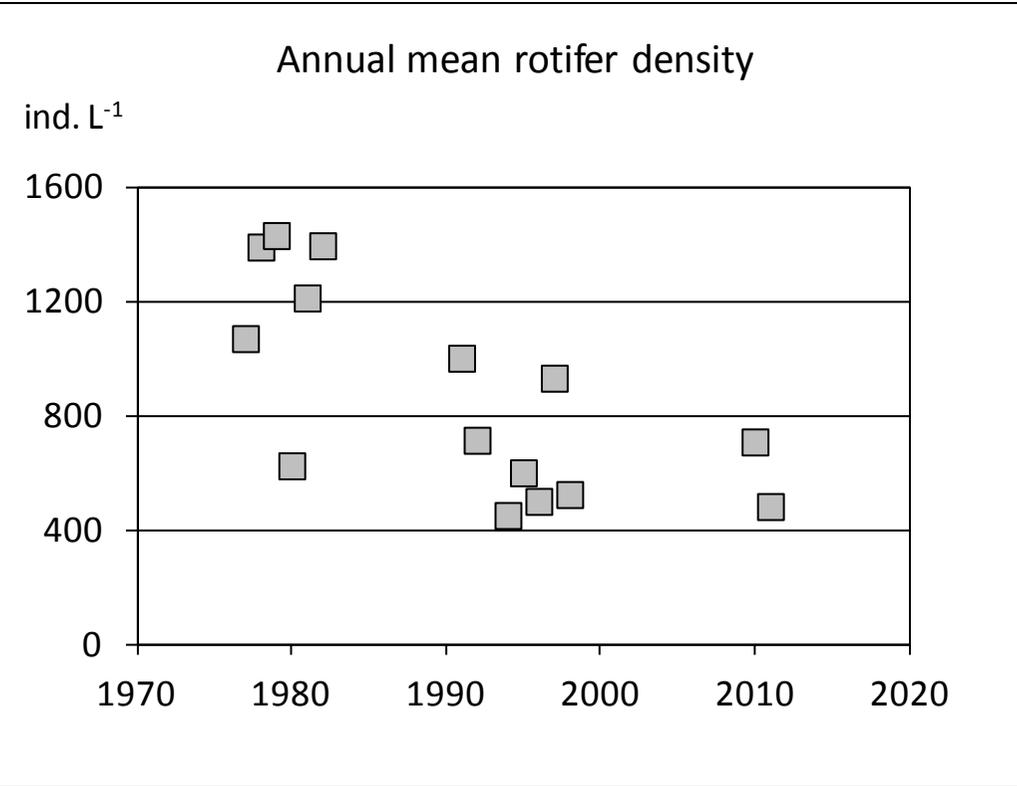


Figure 4

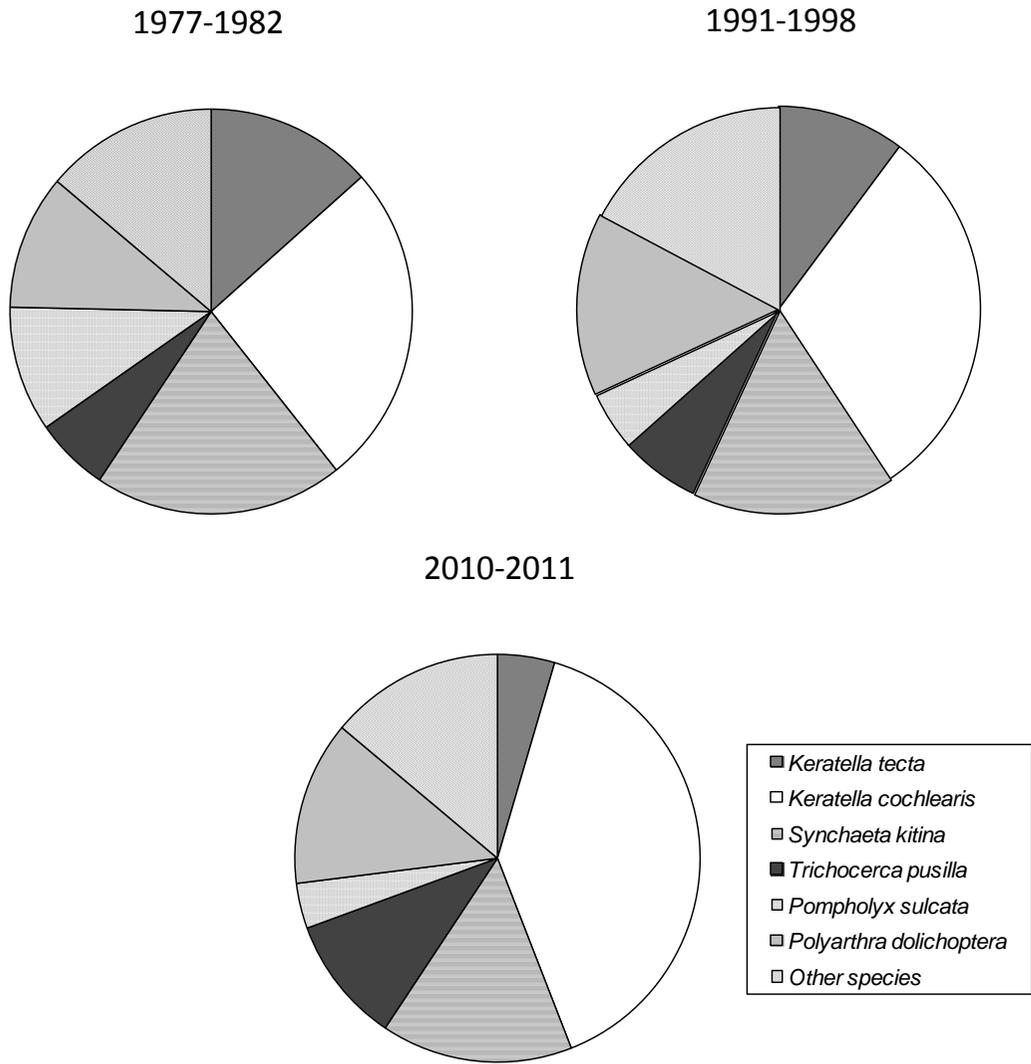


Figure 5