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1 **Food web de-synchronisation in England's largest lake: an assessment based upon**  
2 **multiple phenological metrics**

3

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11

12 **Running head:** Seasonal synchrony of plankton and fish

13

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26 **Abstract**

27 Phenological changes have been observed globally for marine, freshwater and terrestrial  
28 species, and are an important element of the global biological “fingerprint” of climate  
29 change. Differences in rates of change could de-synchronise seasonal species interactions  
30 within a food web, threatening ecosystem functioning. Quantification of this risk is hampered  
31 by the rarity of long-term data for multiple interacting species from the same ecosystem and  
32 by the diversity of possible phenological metrics, which vary in their ecological relevance to  
33 food web interactions. We compare phenological change for phytoplankton (chlorophyll *a*),  
34 zooplankton (*Daphnia*) and fish (perch, *Perca fluviatilis*) in two basins of Windermere over  
35 40 years and determine whether change has differed among trophic levels, while explicitly  
36 accounting for among-metric differences in rates of change. Though rates of change differed  
37 markedly among the nine metrics used, seasonal events shifted earlier for all metrics and  
38 trophic levels: zooplankton advanced most, and fish least, rapidly. Evidence of altered  
39 synchrony was found in both lake basins, when combining information from all phenological  
40 metrics. However, comparisons based upon single metrics did not consistently detect this  
41 signal. A multi-metric approach showed that, across trophic levels, earlier phenological  
42 events have been associated with increasing water temperature. However, for phytoplankton  
43 and zooplankton, phenological change was also associated with changes in resource  
44 availability. Lower silicate, and higher phosphorus, concentrations were associated with  
45 earlier phytoplankton growth, and earlier phytoplankton growth was associated with earlier  
46 zooplankton growth. The developing trophic mismatch detected between the dominant fish  
47 species in Windermere and important zooplankton food resources may ultimately affect fish  
48 survival and portend significant impacts upon ecosystem functioning. We advocate that  
49 future studies on phenological synchrony combine data from multiple phenological metrics,  
50 to increase confidence in assessments of change and likely ecological consequences.

51 **Introduction**

52 Changes in the seasonal timing of recurring biological events are an important component of  
53 the global biological “fingerprint” of ongoing climate change (Parmesan & Yohe, 2003;  
54 IPCC, 2007). These phenological changes have been detected in long-term data from many  
55 plant and animal species occupying many habitats (Parmesan & Yohe, 2003; Root *et al.*,  
56 2003; Thackeray *et al.*, 2010). Such studies have shown that the pattern of change has been  
57 qualitatively consistent across many species; the majority of spring and summer biological  
58 events have shifted earlier in their seasonal timing.

59

60 Interactions between species at different levels within a food web rely on spatial overlap and  
61 temporal synchrony. However, it is also clear that there is a high level of variability in rates  
62 of phenological change among species and this has fuelled concern that seasonal species  
63 interactions could be de-synchronised, with potential negative ecosystem consequences  
64 (Visser & Both, 2005). Differences in rates of phenological change for life-history events of  
65 organisms within a food web may lead to temporal separation of periods of high demand by  
66 organisms in upper trophic levels, and periods of high supply of organisms at lower trophic  
67 levels (the “match-mismatch hypothesis”; Cushing, 1990). This would alter food web  
68 interactions, potentially leading to reductions in reproductive success and recruitment at  
69 upper trophic levels, but also potentially favouring lower trophic levels.

70

71 Although these arguments were first used to explain inter-annual variation in the recruitment  
72 success of marine fish, the possible impacts of phenological de-synchronisation are now  
73 being investigated across a range of species and habitats (e.g. Winder & Schindler, 2004;  
74 Hampton *et al.*, 2006; Visser *et al.*, 2006; Both *et al.*, 2009; Burthe *et al.*, 2012). The number  
75 of such studies is limited by the availability of datasets containing phenological information

76 on interacting organisms from different trophic levels at the same site. Using the few datasets  
77 that do exist, potential examples of de-synchronisation have been identified by the  
78 comparison of long-term changes in the seasonal timing of life-history or population events  
79 among interacting species. When between-species differences in the rate of phenological  
80 change are statistically significant, it has been assumed that de-synchronisation has occurred.  
81 However, there is methodological uncertainty surrounding assessments of phenological de-  
82 synchronisation because estimated rates of phenological change can vary markedly according  
83 to the metric chosen to quantify seasonal timing (Miller-Rushing *et al.*, 2008; Thackeray *et*  
84 *al.*, 2012). This uncertainty has not been explicitly accounted for in previous analyses of  
85 trophic-level de-synchronisation.

86

87 A classical “predator-prey” relationship that has been studied in the context of phenological  
88 change is the primary producer-grazer interaction in freshwater pelagic communities  
89 (Scheffer *et al.*, 1997; Winder & Schindler, 2004; de Senerpont Domis *et al.*, 2007). With  
90 clearly defined boundaries and marked seasonal cycles of phytoplankton, zooplankton and  
91 fish dynamics, lake ecosystems offer a perfect opportunity to investigate phenological de-  
92 synchronisation.

93

94 In this study we assessed the evidence for a developing phenological asynchrony using 40  
95 years of monitoring data relating to three trophic levels, with real trophic linkages, in two  
96 lake basins. The primary producers are represented by total phytoplankton biomass, as  
97 indicated by the widely used proxy of chlorophyll *a* concentration. The primary consumer  
98 trophic level is represented by the widespread cladoceran *Daphnia*. Species of the genus  
99 *Daphnia* commonly dominate spring zooplankton communities and are generalist herbivores,  
100 capable of suppressing spring phytoplankton populations (Lampert *et al.*, 1986; Vanni &

101 Temte, 1990). The secondary consumers are represented by the widespread fish, perch (*Perca*  
102 *fluviatilis* L.). Perch show marked ontogenetic shifts in diet but their larvae feed extensively  
103 on zooplankton, with *Daphnia* being an important dietary component for much or even all of  
104 their first summer (Guma'a, 1978a). The time of spawning is used here as an indicator of the  
105 time of subsequent larval emergence.

106

107 The broad aims of the current study are:

- 108 1) To determine whether phenological changes have occurred across three trophic levels,  
109 in two lake basins.
- 110 2) To examine the evidence for trophic level differences in rates of change, using  
111 multiple phenological metrics.
- 112 3) To conduct an exploratory analysis of drivers of change at each trophic level, using  
113 multiple phenological metrics.

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126 **Materials and methods**127 *Field methods*

128 Data were collected under an ongoing long-term monitoring programme, from the North and  
129 South basins of Windermere, UK (North 54°24'N, 2°57'W; South 54°19'N, 2°57'W). The  
130 North and South basins are separated by a region of shallow water and islands and cover  
131 areas of 8.1 and 6.7 km<sup>2</sup> respectively, having mean (maximum) depths of 25.1 m (64 m) and  
132 16.8 m (42 m) (Ramsbottom, 1976). The ecology of Windermere has been summarised in  
133 Reynolds & Irish (2000). Our analysis focussed on the period 1969 – 2008, during which  
134 time consistent methods were used to collect all of the data considered here at weekly to  
135 fortnightly intervals.

136

137 Plankton samples were collected over the deepest point of each basin. Integrated water  
138 samples from the top 7 m were collected using a weighted plastic tube (Lund, 1949). The  
139 concentration of chlorophyll *a* in these samples was determined spectrophotometrically  
140 following extraction in boiling methanol (Talling, 1974). In order to estimate the time of year  
141 at which food availability exceeded the limitation threshold for *Daphnia*, chlorophyll *a*  
142 concentrations were approximated to carbon concentrations according to Reynolds (2006).  
143 Two sources of data were available for the crustacean zooplankton. Firstly, in the North  
144 Basin, zooplankton were collected by 40 m vertical net hauls (mesh size 250 µm, mouth  
145 diameter 0.3 m). Samples were initially fixed with a small quantity of 70% ethanol, before  
146 being preserved in 4% formaldehyde. Zooplankton were examined under a stereozoom  
147 microscope and all individuals were counted unless high population densities made this  
148 unfeasible. If this was the case, zooplankton were enumerated in sub-samples drawn from the  
149 homogenised whole sample using a Stemple pipette. Data from these net hauls were only  
150 available for a subset of years within each decade (Thackeray *et al.*, 2012) and none were

151 available from the South Basin. Therefore a second zooplankton data set was derived from  
152 counts of Cladocera retained on filter papers used in the determination of chlorophyll *a*  
153 concentration. Though at a coarser taxonomic resolution and based on a smaller volume of  
154 water than the net-haul, these data provide a continuous record over the study period for both  
155 basins of the lake and can be used to derive phenological information (Talling, 2002; George,  
156 2012).

157

158 Perch, which is the most abundant planktivore in the system (Mills & Hurley 1990; Winfield  
159 *et al.* 2008), were sampled by unbaited, wire-netting traps that produce a size-unbiased  
160 sampling of perch between 90 and 300 mm in total length. Traps were deployed weekly for 6  
161 weeks in gangs of five between late April and early June of each year, on two known  
162 spawning grounds at depths of 2 to 7 m at Green Tuft in the North Basin and Lakeside in the  
163 South Basin. Traps were set during daylight and subsequently lifted during daylight usually 7  
164 days later, the precise time of lifting being occasionally influenced by bad weather. All perch  
165 taken in the traps were pooled for each site. In the laboratory, the entire catch was identified,  
166 enumerated and the total length of each individual measured. Further sampling details are  
167 given in Paxton *et al.* (2004).

168

169 Depth-profiles of water temperature were collected over the deep point of each basin using a  
170 Mackereth oxygen electrode in the 1960s and 1970s and a Yellow Springs Instruments probe  
171 in and since the 1980s (George *et al.*, 2000). To avoid any bias in the data, introduced from  
172 uneven sampling intervals, the raw data from each basin were linearly interpolated vertically  
173 and then through time to give temperatures on a 1-m daily grid (Jones *et al.*, 2008). These  
174 data were used to calculate daily Schmidt stability (Hutchinson, 1957), and the day of the  
175 year on which a series of threshold Schmidt stability values were exceeded as a proxy for the



176 onset of thermal stratification (Thackeray *et al.*, 2008; Feuchtmayr *et al.*, 2012). Sunlight data  
177 (in the form of hours of bright sunshine per day) were provided by a Campbell Stokes  
178 sunshine recorder located adjacent to the North Basin of Windermere at Ambleside (George  
179 & Hewitt, 1998). Concentrations of soluble reactive phosphorus (SRP) and silica were  
180 determined from the integrated surface water samples, according to Mackereth *et al.* (1978).  
181 Daily data on the discharge ( $\text{m}^3 \text{s}^{-1}$ ) of the River Leven at Newby Bridge, at the southern end  
182 of Windermere (54°16'N, 2°58'W), were derived from measurements of river stage converted  
183 to discharge using a rating curve.

184

### 185 ***Phenological metrics***

186 Phenological studies of aquatic systems routinely use a variety of phenological metrics even  
187 though estimated rates of phenological change, and the strength of the relationship between  
188 phenological change and potential driving variables, vary with metric choice (see Thackeray  
189 *et al.*, 2012 and references therein). Herein, the seasonal timing of plankton population  
190 development and perch spawning were quantified using a range of phenological metrics,  
191 explicitly incorporating this aspect of methodological uncertainty into our analyses. These  
192 methods cover the breadth of approaches currently used in the literature and represent  
193 different classes of phenological events: the onset, peak or mid-point of the spring population  
194 development (Supporting Table S1; see Thackeray *et al.* 2012 for a more detailed  
195 description). For the perch spawning data, only three of the metrics could be applied because  
196 the lack of year-round sampling of this highly seasonal event and the small number of annual  
197 observations precluded determination of onset-type metrics and methods based on curve-  
198 fitting approaches.

199

200 In order to focus upon spring dynamics, January-June data and February-July data (inclusive)

201 were used for the phytoplankton and zooplankton, respectively. These periods encapsulated  
202 the period of spring population development for these groups. For phytoplankton (chlorophyll  
203 *a*) and zooplankton (filter counts and microscope counts), nine different metrics were  
204 calculated for spring population development in each year.

205

### 206 ***Detection of phenological change and trophic level comparisons***

207 The evidence for significant long-term changes in spring phenology was assessed by linear  
208 regression of each metric, for each trophic level, against year. Slope coefficients from these  
209 regressions quantified rates of phenological change ( $\text{d yr}^{-1}$ ). Residuals from each regression  
210 were checked for normality and homoscedasticity by examining quantile - quantile and  
211 residual - fit plots. Cook's distances were checked to ensure that each trend was not unduly  
212 influenced by any one observation. In each case, residuals were plotted against year to  
213 determine whether they were non-stationary with respect to time as a result of fitting linear  
214 models to potentially non-linear phenological series. This was further examined by fitting  
215 each trend with a quadratic year term. In each case the change in residual deviance associated  
216 with fitting the non-linear vs. linear trend was assessed by an F test.

217

218 The validity of the filter count *Daphnia* data was checked by comparison with the microscope  
219 count data using two approaches. Firstly, seasonal timings derived from the two datasets were  
220 correlated with each other (Pearson's correlation). Secondly, for each metric, the rates of  
221 phenological change derived from each dataset were compared by running a multiple  
222 regression of seasonal timing on year, data set and the interaction between year and data set.  
223 This allowed formal testing of the possibility that rates of changing seasonal timing with year  
224 depend upon the data set used.

225

226 A multi-model inference approach was adopted to assess the weight of evidence in favour of  
227 the hypothesis that de-synchronisation has occurred. For each trophic pairing (phytoplankton-  
228 zooplankton, zooplankton-fish), we ran a series of models that collectively compared long-  
229 term trends in seasonal timing between trophic levels, based upon each possible pair of  
230 phenological metrics. For a given trophic level, long-term changes in the timing of each  
231 metric were compared with similar changes for all metrics at the next trophic level. Each  
232 combination of metric data was modelled against main effects of year and trophic level, and  
233 an interaction between year and trophic level. This interaction was included in each model as  
234 a specific test of the hypothesis that the rate of phenological change has differed between  
235 trophic levels. The coefficients and significance levels of the interaction terms in the different  
236 models were used to evaluate the proportion of the resulting models that showed statistically  
237 significant evidence of de-synchronisation. (i.e. contained a significant year:trophic level  
238 interaction) and the direction of this effect (i.e. which trophic level showed the most rapid  
239 phenological change). The models were categorised according to the classes of phenological  
240 metric being compared between trophic levels in order to distinguish whether de-  
241 synchronisation had occurred for certain classes of phenological event but not others. With  
242 the assumption of homoscedasticity rejected using simple linear regression, models were re-  
243 run using generalised least squares (GLS) regression in which residual variance was allowed  
244 to change as a function of trophic level. Model residuals were checked for serial dependence  
245 by plotting autocorrelation functions.

246

#### 247 ***Drivers of change***

248 Phenological shifts in plankton populations are indicative of environmental factors acting  
249 upon population-level processes, specifically the balance between rates of  
250 replication/reproduction and loss/mortality (Thackeray *et al.*, 2008). A range of biologically

251 meaningful drivers of phenological change were considered at each trophic level. For  
252 phytoplankton (chlorophyll *a* concentration), the candidate drivers were spring temperature,  
253 water column stability, incident solar radiation, hydrological flushing, nutrient (phosphorus  
254 and silicate) concentrations, overwintering inoculum size and the timing of the spring  
255 population growth of zooplankton. For zooplankton (filter counts) the candidate drivers were  
256 spring temperature, incident solar radiation, hydrological flushing, overwintering inoculum  
257 size and the timing of the spring growth of phytoplankton. The inclusion of zooplankton  
258 phenological variables as predictors of phytoplankton phenology, and *vice versa*, recognises  
259 that zooplankton populations increase in response to the spring phytoplankton (food resource)  
260 peak, but may also drive some aspects of phytoplankton phenology by contributing to the  
261 decline of spring phytoplankton blooms through grazing (Lampert *et al.*, 1986). For perch,  
262 the candidate drivers were spring water temperature, incident solar radiation and the median  
263 length of the fish on the spawning grounds. See Supporting Table S2 for a justification of the  
264 selected driving variables.

265

### 266 *Analysing drivers of change*

267 A two-stage analysis was used to explore potential drivers of phenological change at each  
268 trophic level, in each basin. The first stage brought together the information contained within  
269 the suite of phenological metrics calculated for each trophic level (9 metrics each for phyto-  
270 and zooplankton in each basin, 3 metrics for the fish in each basin, Fig. 1 and Supporting Fig.  
271 S1) by calculating the mean of *i*) the onset-type metrics and *ii*) the peak/mid-point metrics,  
272 for each trophic level in each year. This allowed the drivers of phenological change to be  
273 compared for the different trophic levels and for the different classes of event “within” each  
274 trophic level.

275

276 In the second stage, a hierarchical modelling approach was used to identify associations  
277 between phenology and potential environmental drivers. The hierarchical approach allows us  
278 to conceive a model with uncertainty attached to different levels, and hence form a model  
279 where one component with some associated error feeds into another component which itself  
280 has some error structure. This is similar to the analysis presented in Thackeray *et al.* (2012),  
281 and an excellent overview of this generic approach is presented in Grace (2006). Here, we  
282 wished to explore a broad range of potential drivers, including those that have had relatively  
283 weak associations with seasonal timing. Drivers that have, to date, had relatively weak effects  
284 may become more significant in the future if they show sustained change over the longer  
285 term. We therefore considered associations between drivers and phenological change to be  
286 significant if  $P < 0.10$ . The hierarchical approach allowed different (but related) driving  
287 variables to be modelled as components of higher level grouping variables. Changes in  
288 phenology at each trophic level were modelled as a function of a series of hypothesised  
289 relationships with abiotic and biotic drivers, summarised in Supporting Table S2. More  
290 details of the model structure for each trophic level are given in the Supporting Information.  
291 The models were implemented using Monte Carlo simulations to arrive iteratively at  
292 converged parameter estimates. The distribution of the parameter estimates from the 10 000  
293 simulations performed was used to assess the significance of each term in the model. All  
294 analyses were conducted in the base, mgcv, carditates and nlme packages of R (Wood &  
295 Augustin, 2002; Rolinski *et al.*, 2007; Pinheiro *et al.*, 2010, R Development Core Team,  
296 2011) and in WinBUGS version 1.4.3 (Lunn *et al.*, 2000).

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300

## 301 **Results**

### 302 *Comparison of phenological changes based upon different zooplankton data sources*

303 The seasonal timings of spring population development calculated from the filter count and  
304 microscope data for all different metrics were very strongly correlated (Supporting Table S3,  
305 mean  $R^2$  of 0.82). Furthermore, rates of phenological change estimated from the two types of  
306 zooplankton data were not significantly different. These analyses suggest that the filter count  
307 data for cladocerans are a reliable indicator of the phenology of *Daphnia* spring population  
308 development in Windermere. These data were therefore used as a contiguous record of  
309 changing *Daphnia* phenology in subsequent analyses.

310

### 311 *Rates of phenological change at different trophic levels*

312 Analysis of the full range of phenological metrics for each trophic level, and in each basin of  
313 Windermere, provided consistent evidence of a shift towards earlier seasonal timing for  
314 plankton population development and fish spawning over the study period (Fig. 1).  
315 Statistically significant trends were found across all metrics, with only one exception: the day  
316 of year on which estimated phytoplankton carbon exceeded  $0.1 \text{ mg L}^{-1}$  in the South Basin ( $P$   
317  $= 0.48$ ; Supporting Fig. S1). However, even in this case, the direction of change was the same  
318 as that for all of the other metrics. For the phytoplankton and zooplankton, the choice of  
319 metric resulted in considerable differences in the estimated rate of change. For chlorophyll *a*,  
320 estimated rates of advancement ranged between  $0.25 - 0.92 \text{ d yr}^{-1}$  (North Basin) and  $0.14 -$   
321  $0.69 \text{ d yr}^{-1}$  (South Basin), dependent upon the metric chosen. For *Daphnia* estimated rates  
322 ranged between  $0.52 - 1.02 \text{ d yr}^{-1}$  (North Basin) and  $0.46 - 0.91 \text{ d yr}^{-1}$  (South Basin). For the  
323 three metrics calculated for perch spawning times, among metric differences in rates of  
324 change were modest:  $0.40 - 0.50 \text{ d yr}^{-1}$  (North Basin) and  $0.25 - 0.36 \text{ d yr}^{-1}$  (South Basin).

325

326 Multi-model analyses showed that the statistical significance of trophic level differences in  
327 phenological change varied depending upon the metrics used to represent seasonal timing at  
328 each trophic level, and the trophic level comparison under scrutiny. There was a tendency for  
329 phenological changes to have been more rapid for *Daphnia* than for chlorophyll *a* (Fig. 2).  
330 However, the difference in rate of change was only statistically significant in a small  
331 proportion of the models evaluated. There was more evidence for zooplankton phenology  
332 becoming de-synchronised with the onset of phytoplankton growth than with the peak/mid-  
333 point of phytoplankton growth. Irrespective of the metrics used in the comparison,  
334 phenological change for *Daphnia* population development was always more rapid than that  
335 for perch spawning (Fig. 3). Furthermore, a large proportion of the models that were  
336 evaluated indicated that this difference was statistically significant.

337

338 Based on the three metrics that were common across trophic levels (the day of maximum  
339 abundance, centre of gravity and timing of 50% cumulative abundance), the more rapid  
340 advances in the seasonal timing of *Daphnia* spring population development resulted in a  
341 long-term decrease in the seasonal time difference (number of days) between this event and  
342 both phytoplankton population development and fish spawning (Supporting Fig. S2). For the  
343 phytoplankton-zooplankton comparison, this long-term decrease was statistically significant  
344 when using differences based upon the centre of gravity and 50% cumulative abundance  
345 metrics, but not when based upon the day of maximum abundance (Supporting Table S4). All  
346 the zooplankton-fish comparisons were significant apart from the North Basin comparison  
347 based upon the day of maximum abundance.

348

#### 349 ***Drivers of phenological change***

350 The potential physical, chemical and biological drivers of phenological change varied

351 markedly during the study period. Winter-spring water temperatures increased, particularly  
352 since the late 1980s (Supporting Fig. S3a,b), and the early stages of thermal stratification (as  
353 indicated by the weaker Schindt stability thresholds) also occurred earlier in later years  
354 (Supporting Fig. S3c,d). Both winter-spring mean bright sunshine hours (Supporting Fig.  
355 S3e) and River Leven discharge (Supporting Fig S3f; a proxy for flushing) showed much  
356 inter-annual variation, with the latter showing a tendency for long-term increase. In both  
357 basins of Windermere, winter mean concentrations of soluble reactive phosphate increased  
358 until the early 1990s and then either stabilised (Supporting Fig. S4a, North Basin) or declined  
359 (Supporting Fig. S4b, South Basin), following enhancements to the treatment of effluent  
360 entering the lake. Winter mean silicate concentrations showed little evidence of a long-term  
361 trend, though they declined at the time of highest SRP concentrations (Supporting Fig.  
362 S4a,b). Winter phytoplankton and zooplankton inocula demonstrated much inter-annual  
363 variation (Supporting Fig. S4c,d). In both basins, perch median length declined throughout  
364 the study period (Supporting Fig. S4e,f). Patterns of long-term change were largely dissimilar  
365 among the potential drivers, as indicated by pairwise correlations among them (Supporting  
366 Table S5). The only exceptions to this were certain combinations of monthly mean  
367 temperatures and Schmidt stability thresholds.

368

369 Hierarchical modelling of phenological change was based upon mean values of the metrics  
370 within each species-metric class combination i.e. phytoplankton onset, phytoplankton  
371 peak/mid-growing season, zooplankton onset, zooplankton peak/mid-growing season, perch  
372 peak/mid-spawning period (Fig. 1). The seasonal timing of events at all trophic levels, in both  
373 lake basins, was earlier in years with higher water temperature apart from the chlorophyll *a*  
374 peak/mid-point in the South Basin (Tables 1-3). The time of year at which temperature  
375 change was most influential differed among trophic levels: March-April temperatures were



376 the strongest predictors of phytoplankton phenological change, while April-May temperatures  
377 were more influential for the zooplankton and fish. The magnitude of the temperature effect,  
378 in terms of the days change in seasonal timing per °C, was greatest for *Daphnia* spp.  
379 (approximately 8 – 10 days earlier per °C increase).

380

381 In addition to the consistent association with temperature across trophic levels, a number of  
382 other environmental drivers were associated with seasonal timing (Tables 1-3). With the  
383 exception of the peak/mid-point in the South Basin, spring phytoplankton population growth  
384 was later in years with higher winter silicate concentrations. In the North Basin, seasonal  
385 timing of the peak/mid-point of phytoplankton growth was earlier in years with higher winter  
386 phosphorus concentrations and, in the South Basin, with earlier peaks/mid-points of spring  
387 zooplankton populations. In the South Basin, the onset of phytoplankton growth was delayed  
388 in years with earlier weak thermal stratification (Schmidt stability  $20 \text{ J m}^{-2}$ ). In the case of the  
389 zooplankton, in both basins there was some evidence that population growth occurred earlier  
390 in years where phytoplankton seasonal growth was also earlier (onset in the North Basin, and  
391 peak/mid-point in the South Basin). In the North Basin, the onset of zooplankton spring  
392 population growth occurred later in years with greater flushing, as indicated by the discharge  
393 of the outflowing River Leven. For the perch, the only additional driver of phenological  
394 change was change in the median length of the population, with later spawning occurring in  
395 the North Basin in years where the median length of the population was greater.

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402 **Discussion**403 ***Multi-metric approaches to phenological change and de-synchronisation***

404 Many metrics have been used to define seasonal timing and to quantify phenological change.  
405 Metrics vary at two levels. At a conceptual level, a seasonal cycle can be described by  
406 distinct and ecologically meaningful events such as the onset of population growth or the  
407 timing of peak abundance. At an operational level, these classes of events can each be  
408 quantified in different ways e.g. the timing of the observed peak abundance *vs* the timing of  
409 the peak based upon a curve fitted to the observed data. Metric choice can fundamentally  
410 alter the conclusion of a study. Taking the example of phytoplankton and zooplankton from  
411 the North Basin (Supporting Fig. S1), a large range of trophic-level differences in rates of  
412 change ( $-0.77$  to  $+0.40$  d yr<sup>-1</sup>) could be derived depending on metric selection. Conclusions  
413 drawn from studies based upon single metrics are therefore intrinsically uncertain unless a  
414 clearly formulated research question and strong mechanistic knowledge of a system allow  
415 unambiguous selection of an appropriate phenological metric to test a specific hypothesis.  
416 For example, if the recruitment success of a particular consumer is impaired below a given  
417 prey abundance, the time of year at which prey abundance exceeds this threshold may be the  
418 most relevant phenology metric to compare with the higher trophic level. However, there  
419 may be no *a priori* reason to select a particular metric (i.e. incomplete mechanistic  
420 understanding). Alternatively, there may be concerns regarding the sensitivity of any single  
421 metric to methodological inconsistencies in sampling and sample processing. In such  
422 situations, it may be possible to increase the strength of inference by using multiple metrics  
423 for a given class of phenological event. This is the approach adopted here.

424

425 ***Multi-metric detection of de-synchronisation***

426 Previous studies, based on single metrics, have drawn varying conclusions on the existence of  
427 de-synchronisation between phytoplankton and zooplankton (e.g. Winder & Schindler 2004;  
428 Adrian *et al.* 2006). Using a multi-metric approach, evidence of a long-term convergence of  
429 phenological events was found for phytoplankton and zooplankton in Windermere. Although  
430 the signal of phenological convergence was highly consistent among metric comparisons,  
431 relatively few were statistically significant. The convergence occurred because the timing of  
432 vernal *Daphnia* population development advanced more rapidly than that of their  
433 phytoplankton food resource. Results suggested that the convergence was more pronounced  
434 when comparing *Daphnia* seasonal events with the onset, rather than the peak/mid-point, of  
435 phytoplankton growth. This is perhaps indicative of tight coupling between peak events at the  
436 two trophic levels (Scheffer *et al.* 1997), and a greater relative influence of exogenous (non-  
437 grazer) factors on the onset of phytoplankton growth.

438

439 The difference in seasonal timing between *Daphnia* population development and perch  
440 spawning has also diminished over time. The convergence was highly consistent among  
441 metric comparisons, but unlike the phytoplankton-*Daphnia* system, most comparisons were  
442 statistically significant. In the present analyses, perch spawning behaviour was used as an  
443 indicator of the timing of subsequent larval emergence, and it is these larvae that feed  
444 extensively on zooplankton (Guma'a 1978a). The convergence of *Daphnia* and perch  
445 spawning phenology (Supporting Fig. S2) could result in subsequent larval emergence  
446 occurring after *Daphnia* populations have declined. This, in turn, could influence the survival  
447 of perch during their vulnerable juvenile stages (Paxton *et al.* 2004) and have negative  
448 consequences for the status of the adult population. Since perch are the most numerous fish  
449 species in Windermere (Mills & Hurley 1990; Winfield *et al.* 2008), such changes have the  
450 potential to impact upon whole ecosystem functioning. The possible impacts of altered

451 synchrony for larval perch diet, growth and survival, and the extent to which temperature-  
452 driven changes in incubation time (Guma'a 1978b) might mediate the synchrony between  
453 larval emergence and zooplankton dynamics following spawning, are therefore priorities for  
454 future research.

455

#### 456 *Temperature as a driver of phenological change*

457 Long-term changes in water temperature were associated with the seasonal timing of events  
458 at all three trophic levels. The seasonal timing of biological events occurred significantly  
459 earlier with increases in water temperature, with the exception of the phytoplankton  
460 peak/mid-point in the South Basin. This temperature sensitivity is consistent with  
461 observations made on many taxa across a wide range of ecosystems (Roy & Sparks, 2000;  
462 Menzel *et al.*, 2006; Askeyev *et al.*, 2010), as well as for freshwater communities in  
463 particular (Gerten & Adrian, 2000; Berger *et al.*, 2010; Feuchtmayr *et al.*, 2010; Thackeray *et*  
464 *al.*, 2012). Increases in water temperature would be expected to promote earlier  
465 phytoplankton and zooplankton population development, by increasing rates of  
466 replication/reproduction early in the spring (Hall, 1964; Munro & White, 1975; Vijverberg,  
467 1980; Reynolds, 1989; Weetman & Atkinson, 2004). In the case of the perch, seasonal  
468 changes in water temperature are thought to be an important environmental cue for the  
469 initiation of spawning behaviour (Craig, 2000) rather than having a direct physiological  
470 effect.

471

472 It has been hypothesised that seasonal variation in rates of long-term temperature change may  
473 lead to trophic-level de-synchronisation when interacting species are sensitive to temperature  
474 at different times of year (Visser & Both, 2005). In Windermere, phenological changes for  
475 *Daphnia* and perch were responsive to temperatures later in the year than were phytoplankton

476 (April-May for *Daphnia* and perch, March-April for phytoplankton). This is a necessary  
477 condition for the above hypothesis and is consistent with the fact that, on average, the spring  
478 phytoplankton peak was the earliest of the three considered events in each basin. However,  
479 over the study period, rates of temperature change were similar throughout the spring period  
480 at approximately  $+0.04$  to  $0.05^{\circ}\text{C yr}^{-1}$  reducing the likelihood of this as a mechanism behind  
481 the observed changes. Furthermore, the above hypothesis implicitly assumes that the  
482 temperature sensitivity of seasonal timing (in days per  $^{\circ}\text{C}$ ) is similar among trophic levels. In  
483 Windermere, the sensitivity of *Daphnia* population development to temperature change (rate  
484 of change per unit temperature increase) was greater than that of phytoplankton or perch.  
485 This is consistent with previous studies showing that the sensitivity of seasonal timing to  
486 temperature varies among taxa (Roy & Sparks, 2000; Winder & Schindler, 2004; Adrian *et al.*,  
487 2006; Menzel *et al.*, 2006; Askeyev *et al.*, 2010). Therefore, the observed de-  
488 synchronisation in Windermere is likely to have been driven by differential temperature  
489 sensitivity in conjunction with additional drivers of phenological change.

490

491 In addition to the observed associations between seasonal timing and water temperature,  
492 phytoplankton onset was also correlated with the onset of thermal stratification in the south  
493 basin of Windermere. However, inter-annual variations in some monthly mean water  
494 temperatures and stratification thresholds were strongly correlated (Supporting Table S5),  
495 making it difficult to disentangle the effects of these two drivers. In the South Basin, the  
496 onset of phytoplankton growth typically occurred before the onset of thermal stratification.  
497 The mean onset for phytoplankton is on day of the year 84 (range: 58 to 104), while the mean  
498 day of year for the attainment of a Schmidt stability of  $20 \text{ J m}^{-2}$  is 107 (range: 83 to 128).  
499 This would suggest that the observed “effect” of the timing of this Schmidt stability threshold  
500 may be correlative, rather than causative. Further process-modelling work would be needed

501 to resolve the unique contributions of water temperature and stratification to inter-annual  
502 variations in phytoplankton onset phenology.

503

504 ***Resource availability as a driver of phenological change***

505 For phytoplankton and zooplankton, resource availability is a key factor that controls rates of  
506 population growth (Reynolds, 2006). Inter-annual variations in the concentration of chemical  
507 resources were associated with the seasonal timing of the phytoplankton spring bloom, in  
508 concert with changes in temperature. There was some evidence from the North Basin of  
509 Windermere that the seasonal timing of phytoplankton spring growth advanced with an  
510 increase in soluble reactive phosphorus (SRP) concentration. This is consistent with the  
511 hypothesis that higher SRP concentrations would permit a longer period of more rapid light-  
512 limited growth, thus allowing earlier attainment of the maximum population size (Reynolds,  
513 1990, 1997). Empirical support for this mechanism exists in studies of the spring growth of  
514 individual phytoplankton taxa in Windermere (Thackeray *et al.*, 2008; Feuchtmayr *et al.*,  
515 2012).

516

517 In Windermere, diatom taxa such as *Asterionella formosa* and *Aulacoseira* spp. collectively  
518 dominate this spring community (Feuchtmayr *et al.*, 2012). Diatoms require silicate to build  
519 their cell walls and, as a result, the carrying capacity of the system for these taxa is  
520 constrained by the availability of this constituent (Reynolds, 1990, 1997). The spring  
521 phytoplankton bloom occurred later in years with higher silicate concentrations. Increased  
522 silicate availability raises the maximum attainable population size for diatoms and hence  
523 prolongs the period of growth that can occur prior to silicate limitation. As a result, higher  
524 silicate concentrations would permit phytoplankton population growth to continue later into  
525 the year (Reynolds, 1990, 1997; Thackeray *et al.*, 2008; Feuchtmayr *et al.*, 2012). Although

526 this mechanism might explain the response seen for peak populations in the North Basin, it is  
527 unlikely to be the causative factor behind the response of the onset metrics in both basins.  
528 These observations once again highlight the difficulty in making causal inferences, based  
529 upon analysis of observational data.

530

531 Changes in the seasonal timing of *Daphnia* spring population development were linked to  
532 inter-annual variations in the seasonal timing of phytoplankton growth in both basins of  
533 Windermere. This interdependence is to be expected since rates of *Daphnia* population  
534 growth would be affected by birth rates that are dependent upon the proportion of egg bearing  
535 females in the population, and their corresponding clutch sizes; factors that are strongly  
536 responsive to food availability (Lampert, 1978; Guisande & Gliwicz, 1992; George &  
537 Reynolds, 1997). The dependence of *Daphnia* population phenology upon that of their  
538 phytoplankton resources has been noted from analyses of an independent *Daphnia* data set  
539 from the North Basin (Thackeray *et al.*, 2012), and is in agreement with the findings of  
540 process-based modelling approaches (Schalau *et al.*, 2008). Put simply, spring *Daphnia*  
541 populations cannot increase until phytoplankton food resources themselves increase above  
542 limiting levels. Though a statistically significant relationship between phytoplankton and  
543 *Daphnia* phenology was found in the present study, it was also apparent that rates of  
544 phenological change were more rapid for *Daphnia*. The seasonal “window” of *Daphnia*  
545 population development is necessarily restricted to the period with sufficient resource supply,  
546 though it is likely that the temperature dependence of *Daphnia* grazing and development  
547 render the rate of the whole consumer-resource interaction temperature dependent (Schalau *et*  
548 *al.*, 2008). Thus, the time taken for the whole consumer-resource cycle to complete may be  
549 shorter under warmer conditions, causing the observed seasonal convergence of  
550 phytoplankton and *Daphnia* phenology.

551

552 For perch, and indeed most probably for all lake fish species, the resources used during  
553 spawning will have been acquired over the weeks and months preceding spawning (Craig,  
554 2000). Consequently, vernal resource availability does not drive the precise timing of  
555 spawning.

556

557 ***Final remarks***

558 Phenological change has now been reported for a great diversity of species. It is therefore  
559 crucial to determine the potential for de-synchronisation of seasonal species interactions and  
560 the drivers behind any loss of synchrony. The wider application of multi-metric approaches  
561 would make analyses more robust to the methodological uncertainties associated with  
562 phenology metric choice, thereby increasing confidence in our assessments of the ecological  
563 significance of phenological change. Furthermore, analysis of long-term data sets that are  
564 spatially replicated within a system will improve the precision of estimates of phenological  
565 events; however such datasets in lakes are extremely rare.

566

567 While consideration of these issues will strengthen our interpretation of observed patterns of  
568 long-term change, there are limitations to what can be gleaned from even long and  
569 comprehensive data sets (Maberly & Elliott 2012). Approaches based upon analyses of long-  
570 term data would be complemented by enhanced mechanistic understanding derived from  
571 experimentation at different scales (Feuchtmayr *et al.* 2010) and the encapsulation of this  
572 information in mechanistic models (De Senerpont Domis *et al.* 2007; Schalau *et al.* 2010).  
573 When evaluating the results of studies, an appreciation of ecological scale is key: some  
574 phenological events represent behavioural changes acting at the individual level (for example  
575 perch spawning), while some represent the balance between population-level processes (for



576 example phyto- and zooplankton population development). By developing mechanistic  
577 insight it will be possible to increase understanding of past phenological change and,  
578 ultimately, allow the development of predictive models, capable of forecasting responses to  
579 future environmental change.

580

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591

592

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- 758



759 **Supporting information legends**

760

761 **Supporting Figure S1.** Rates of phenological change (1969-2008) across three trophic levels

762 in a) the North Basin and b) the South Basin of Windermere, UK.

763

764 **Supporting Figure S2.** Examples of long-term change in the seasonal time difference

765 between events at different trophic levels in the North and South basins of Windermere.

766

767 **Supporting Figure S3.** Long-term variations in potential physical drivers of phenological

768 change.

769

770 **Supporting Figure S4.** Long-term variations in potential chemical and biological drivers of

771 phenological change.

772

773 **Supporting Table S1.** The phenological metrics used to quantify the seasonal timing of

774 plankton development and perch spawning.

775

776 **Supporting Table S2.** Driving variables used in the analyses of phenological change, with

777 justification for their inclusion and supporting references.

778

779 **Supporting Table S3:** Comparison of phenological metrics derived using filter count and

780 microscope data for *Daphnia*.

781

782 **Supporting Table S4:** Summaries of exemplar linear models of long-term changes in the

783 seasonal time difference between phenological events at adjacent trophic levels.

784

785 **Supporting Table S5:** Correlation matrix for predictors included in the hierarchical

786 modelling of phenological change at three trophic levels.

787

788 **Supporting Information:** hierarchical model structure for analysis of drivers

789

790 **Table 1.** Summary of hierarchical model analyses of drivers of phenological change, for phytoplankton. In each cell a simple “change in  
 791 driver=>change in response” statement indicates the direction of the relationship and the symbol “=>” should be read as “leads to”. For  
 792 temperature and stratification the specific (nested) variable related to phenology is indicated. For temperature these are individual monthly  
 793 means, for stratification these are the seasonal timings of Schmidt stability thresholds of 20, 50, 150 and 300 J m<sup>-2</sup>. Statistically significant  
 794 (P<0.10) terms are shown, and non significant terms are denoted “ns”. For each significant term the parameter estimate (days of change per unit  
 795 of the driver) is given, with the 90% Bayesian credible interval shown in brackets. See text for details on modelling approach.

Driver	Phytoplankton onset		Phytoplankton peak/mid-growing season	
	North Basin	South Basin	North Basin	South Basin
<b>Water temperature (°C)</b>	Warmer=>Earlier -6.69 [-9.95, -2.78] (March/April)	Warmer=>Earlier -4.03 [-7.99, -0.17] (April)	Warmer=>Earlier -5.90 [-10.27, -1.75] (April)	ns
<b>Stratification (day of year)</b>	ns	Later=>Earlier -0.41 [-0.76, -0.07] (20 J m <sup>-2</sup> )	ns	ns
<b>Leven discharge (m<sup>3</sup> s<sup>-1</sup>)</b>	ns	ns	ns	ns

<b>Winter silicate (mg m<sup>-3</sup>)</b>	Higher=>Later 0.02 [0.01, 0.03]	Higher=>Later 0.02 [0.01, 0.03]	Higher=>Later 0.03 [0.01, 0.04]	ns
<b>Winter phosphorus (mg m<sup>-3</sup>)</b>	ns	ns	Higher=>Earlier -2.45 [-4.08, -0.95]	ns
<b>Sunlight (hour d<sup>-1</sup>)</b>	ns	ns	ns	ns
<b>Inoculum (mg Chl <i>a</i> m<sup>-3</sup>)</b>	ns	ns	ns	ns
<b>Zooplankton phenology (day of year)</b>	ns	Earlier=>Earlier 0.22 [0.04, 0.42] (Peak/Mid-point)	ns	Earlier=>Earlier 0.29 [0.05, 0.54] (Peak/Mid-point)

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802 **Table 2.** Summary of hierarchical model analyses of drivers of phenological change, for zooplankton. In each cell a simple “change in  
 803 driver=>change in response” statement indicates the direction of the relationship and the symbol “=>” should be read as “leads to”. For  
 804 temperature the specific (nested) variable related to phenology is indicated i.e. individual monthly means. Statistically significant ( $P < 0.10$ ) terms  
 805 are shown, and non significant terms are denoted “ns”. For each significant term the parameter estimate (days of change per unit of the driver) is  
 806 given, with the 90% Bayesian credible interval shown in brackets. See text for details on modelling approach.

Driver	Zooplankton onset		Zooplankton peak/mid-growing season	
	North Basin	South Basin	North Basin	South Basin
<b>Water temperature (°C)</b>	Warmer=>Earlier -7.90 [-10.58, -5.20] (May)	Warmer=>Earlier -10.05 [-13.92, -6.07] (April/May)	Warmer=>Earlier -8.28 [-11.44, -4.89] (May)	Warmer=>Earlier -8.66 [-12.33, -4.78] (May)
<b>Leven discharge (m<sup>3</sup> s<sup>-1</sup>)</b>	Higher=>Later 0.97 [0.06, 1.89]	ns	ns	ns
<b>Sunlight (hour d<sup>-1</sup>)</b>	ns	ns	ns	ns
<b>Inoculum (individuals per L<sup>-1</sup>)</b>	ns	ns	ns	ns

<b>Phytoplankton phenology (day of year)</b>	Earlier=>Earlier 0.36 [0.15, 0.58] (Onset)	ns	ns	Earlier=>Earlier 0.25 [0.003, 0.49] (Peak/Mid-point)
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817 **Table 3.** Summary of hierarchical model analyses of drivers of phenological change, for perch. In each cell a simple “change in driver=>change  
 818 in response” statement indicates the direction of the relationship and the symbol “=>” should be read as “leads to”. For temperature the specific  
 819 (nested) variable related to phenology is indicated i.e. individual monthly means. Statistically significant ( $P < 0.10$ ) terms are shown, and non  
 820 significant terms are denoted “ns”. For each significant term the parameter estimate (days of change per unit of the driver) is given, with the 90%  
 821 Bayesian credible interval shown in brackets. See text for details on modelling approach.

<b>Perch peak/mid-spawning period</b>		
<b>Driver</b>	<b>North Basin</b>	<b>South Basin</b>
<b>Water temperature (°C)</b>	Warmer=>Earlier -3.80 [-5.00, -2.59] (May)	Warmer=>Earlier -4.35 [-5.76, -3.03] (April)
<b>Sunlight (hour d<sup>-1</sup>)</b>	ns	ns
<b>Median perch length (cm)</b>	Greater=>Later 0.54 [0.21, 0.88]	ns

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## Figure legends

Figure 1. Long-term changes in the seasonal timing of phytoplankton (a, b) and zooplankton (c, d) spring population growth, and perch spawning (e, f). Data are shown for the North (a, c, e) and South (b, d, f) basins of Windermere. Points show the original phenological metric data and lines show average seasonal timings for distinct metric classes. In plots a-d, solid lines show the mean seasonal timing of onset-type metrics (circles), and dashed lines show the mean seasonal timing of peak/middle-type metrics (square symbols). For the perch data, only peak/middle type metrics were calculated.

Figure 2. Comparison of rates of phenological change between phytoplankton and zooplankton in the North (a, b) and South (c, d) basins of Windermere, grouped according to whether metrics indicate the onset or peak/middle of spring population growth. In (a) and (c), the onset of zooplankton population growth is compared to changes in the onset and peak/middle of phytoplankton spring growth. In (b) and (d), the peak/middle of spring zooplankton population growth is compared to changes in the onset and peak/middle of phytoplankton spring growth. Within each group of comparisons, models based on specific combinations of metrics are classified according to the trophic level that changed most rapidly and whether or not the rates of change were significantly different ( $P < 0.05$ ).

Figure 3. Comparison of rates of phenological change between zooplankton and perch in the North (a) and South (b) basins of Windermere grouped according to whether metrics indicate the onset or peak/middle of spring zooplankton growth and perch spawning. In both panels, the peak/middle of perch spawning is compared to changes in the onset and peak/middle of zooplankton spring population growth. Within each group of comparisons, models based on

specific combinations of metrics are classified according to the trophic level that changed most rapidly and whether or not the rates of change were significantly different ( $P < 0.05$ ).

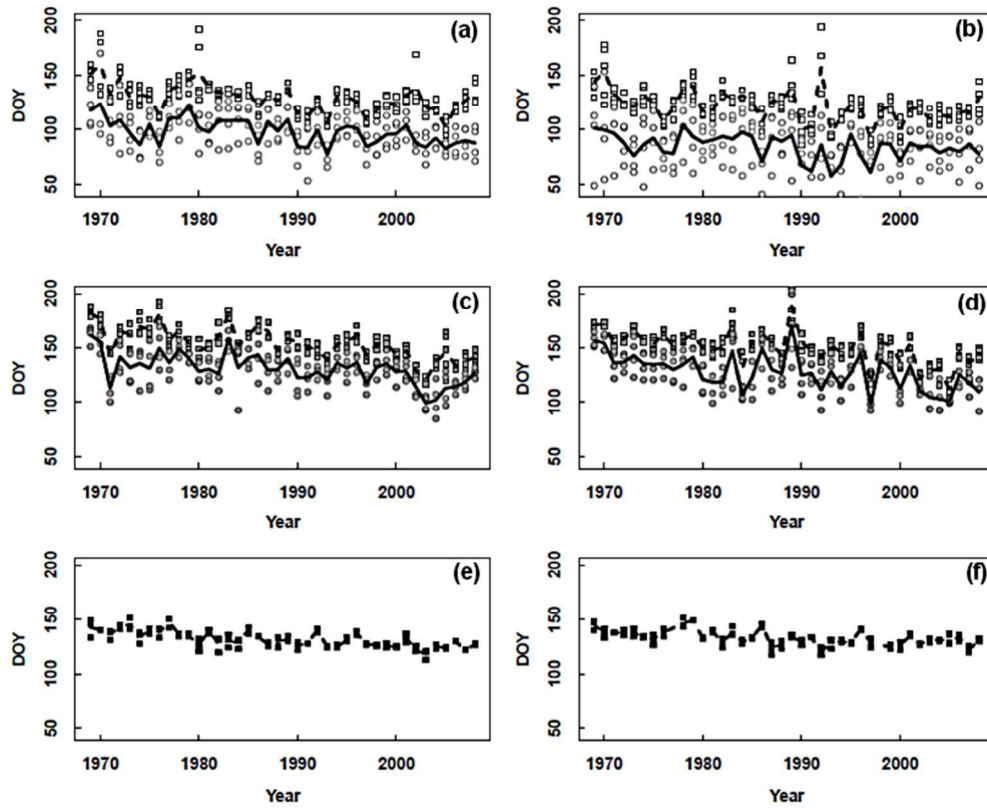


Figure 1

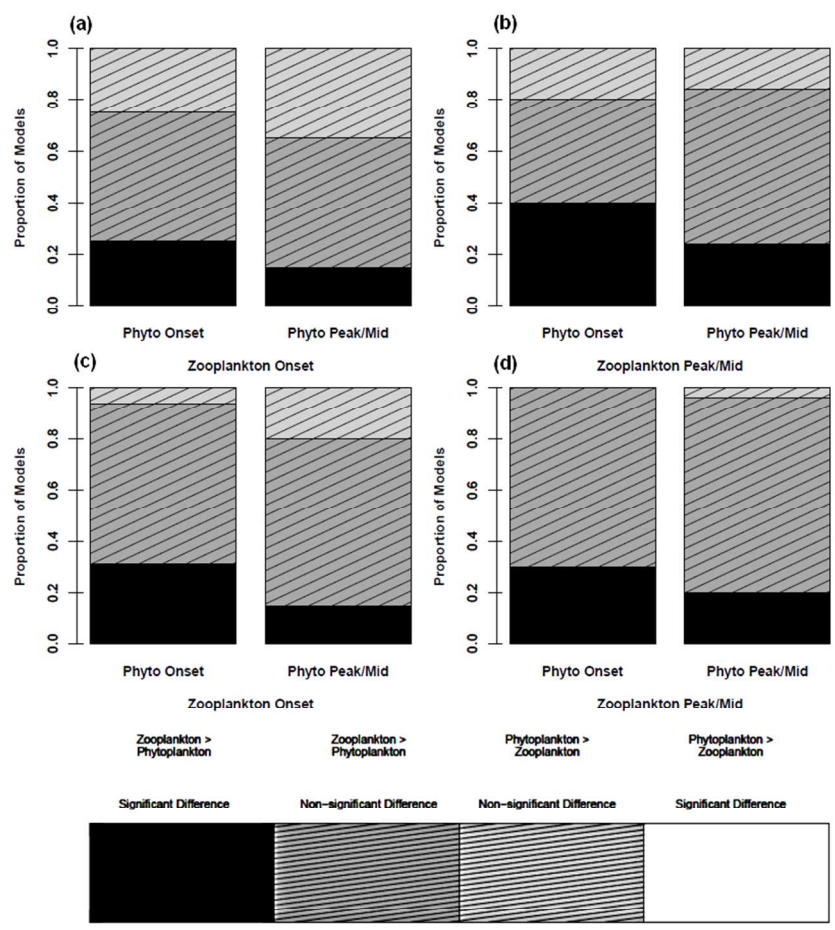


Figure 2

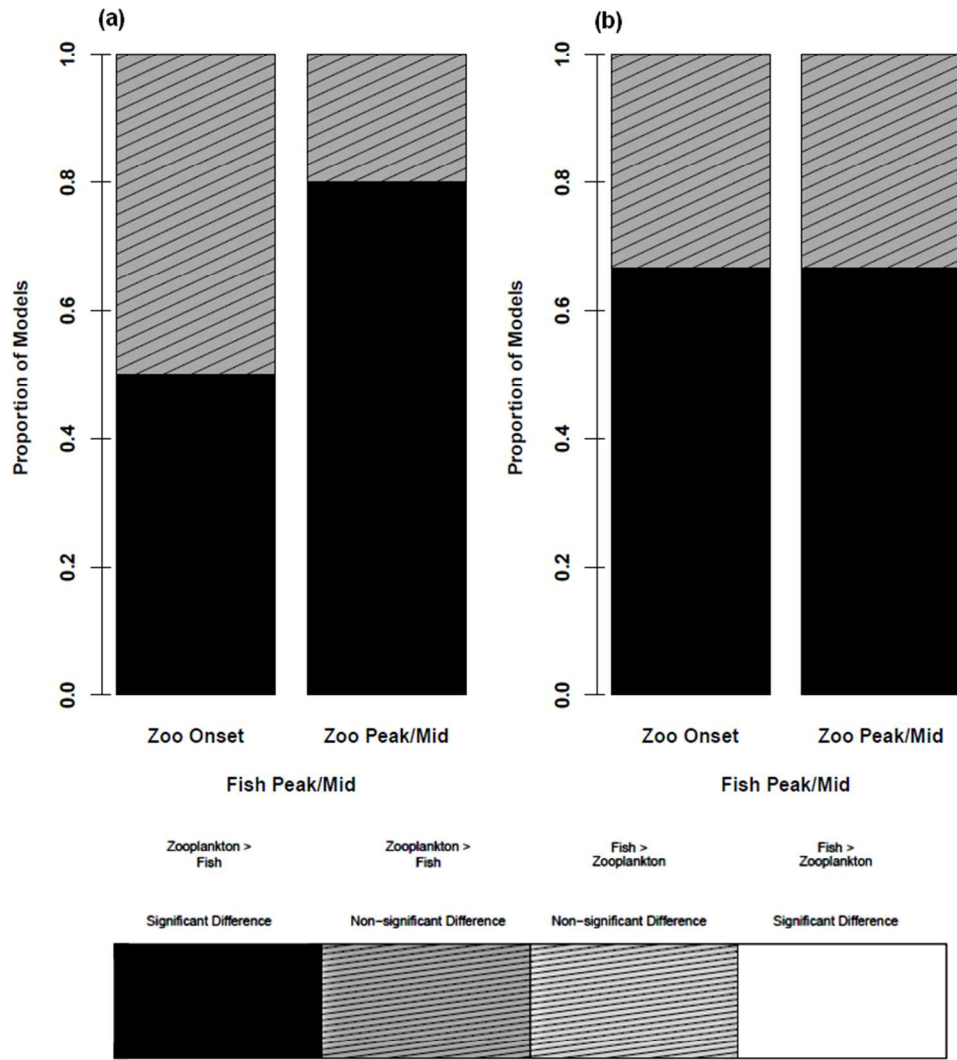


Figure 3