Article (refereed) - postprint

Thackeray, Stephen J.; Henrys, Peter A.; Feuchtmayr, Heidrun; Jones, Ian D.; Maberly, Stephen C.; Winfield, Ian J. 2013. **Food web de-synchronization in England's largest lake: an assessment based on multiple phenological metrics**. *Global Change Biology*, 19 (12). 3568-3580. 10.1111/gcb.12326

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

Stephen J. Thackeray*, Peter A. Henrys, Heidrun Feuchtmayr, Ian D. Jones, Stephen C. Maberly & Ian J. Winfield

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

**Corresponding author: Tel: 00 44 (0)1524 595852. Fax: 00 44 (0)1524 61536. email: sjtr@ceh.ac.uk*

Running head: Seasonal synchrony of plankton and fish

Keywords: Chlorophyll, *Daphnia*, Hierarchical models, Match-mismatch, Perch, Phytoplankton, Temperature, Uncertainty, Windermere, Zooplankton

Type of paper: Primary Research Article

Abstract

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

Introduction

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

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

Although these arguments were first used to explain inter-annual variation in the recruitment success of marine fish, the possible impacts of phenological de-synchronisation are now being investigated across a range of species and habitats (e.g. Winder & Schindler, 2004; Hampton *et al.*, 2006; Visser *et al.*, 2006; Both *et al.*, 2009; Burthe *et al.*, 2012). The number of such studies is limited by the availability of datasets containing phenological information on interacting organisms from different trophic levels at the same site. Using the few datasets that do exist, potential examples of de-synchronisation have been identified by the comparison of long-term changes in the seasonal timing of life-history or population events among interacting species. When between-species differences in the rate of phenological change are statistically significant, it has been assumed that de-synchronisation has occurred. However, there is methodological uncertainty surrounding assessments of phenological de-synchronisation because estimated rates of phenological change can vary markedly according to the metric chosen to quantify seasonal timing (Miller-Rushing *et al.*, 2008; Thackeray *et al.*, 2012). This uncertainty has not been explicitly accounted for in previous analyses of trophic-level de-synchronisation.

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

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

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

The broad aims of the current study are:

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

Materials and methods

Field methods

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

Plankton samples were collected over the deepest point of each basin. Integrated water samples from the top 7 m were collected using a weighted plastic tube (Lund, 1949). The concentration of chlorophyll *a* in these samples was determined spectrophotometrically following extraction in boiling methanol (Talling, 1974). In order to estimate the time of year at which food availability exceeded the limitation threshold for *Daphnia*, chlorophyll *a* concentrations were approximated to carbon concentrations according to Reynolds (2006). Two sources of data were available for the crustacean zooplankton. Firstly, in the North Basin, zooplankton were collected by 40 m vertical net hauls (mesh size 250 μm, mouth diameter 0.3 m). Samples were initially fixed with a small quantity of 70% ethanol, before being preserved in 4% formaldehyde. Zooplankton were examined under a stereozoom microscope and all individuals were counted unless high population densities made this unfeasible. If this was the case, zooplankton were enumerated in sub-samples drawn from the homogenised whole sample using a Stemple pipette. Data from these net hauls were only available for a subset of years within each decade (Thackeray *et al.*, 2012) and none were available from the South Basin. Therefore a second zooplankton data set was derived from counts of Cladocera retained on filter papers used in the determination of chlorophyll *a* concentration. Though at a coarser taxonomic resolution and based on a smaller volume of water than the net-haul, these data provide a continuous record over the study period for both basins of the lake and can be used to derive phenological information (Talling, 2002; George, 2012).

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

Depth-profiles of water temperature were collected over the deep point of each basin using a Mackereth oxygen electrode in the 1960s and 1970s and a Yellow Springs Instruments probe in and since the 1980s (George *et al.*, 2000). To avoid any bias in the data, introduced from uneven sampling intervals, the raw data from each basin were linearly interpolated vertically and then through time to give temperatures on a 1-m daily grid (Jones *et al.*, 2008). These data were used to calculate daily Schmidt stability (Hutchinson, 1957), and the day of the year on which a series of threshold Schmidt stability values were exceeded as a proxy for the onset of thermal stratification (Thackeray *et al.*, 2008; Feuchtmayr *et al.*, 2012). Sunlight data (in the form of hours of bright sunshine per day) were provided by a Campbell Stokes sunshine recorder located adjacent to the North Basin of Windermere at Ambleside (George & Hewitt, 1998). Concentrations of soluble reactive phosphorus (SRP) and silica were determined from the integrated surface water samples, according to Mackereth *et al.* (1978). Daily data on the discharge (m^3 s^{-1}) of the River Leven at Newby Bridge, at the southern end of Windermere (54°16'N, 2°58'W), were derived from measurements of river stage converted to discharge using a rating curve.

Phenological metrics

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

In order to focus upon spring dynamics, January-June data and February-July data (inclusive) were used for the phytoplankton and zooplankton, respectively. These periods encapsulated the period of spring population development for these groups. For phytoplankton (chlorophyll *a*) and zooplankton (filter counts and microscope counts), nine different metrics were calculated for spring population development in each year.

Detection of phenological change and trophic level comparisons

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

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

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

Drivers of change

Phenological shifts in plankton populations are indicative of environmental factors acting upon population-level processes, specifically the balance between rates of replication/reproduction and loss/mortality (Thackeray *et al.*, 2008). A range of biologically meaningful drivers of phenological change were considered at each trophic level. For phytoplankton (chlorophyll *a* concentration), the candidate drivers were spring temperature, water column stability, incident solar radiation, hydrological flushing, nutrient (phosphorus and silicate) concentrations, overwintering inoculum size and the timing of the spring population growth of zooplankton. For zooplankton (filter counts) the candidate drivers were spring temperature, incident solar radiation, hydrological flushing, overwintering inoculum size and the timing of the spring growth of phytoplankton. The inclusion of zooplankton phenological variables as predictors of phytoplankton phenology, and *vice versa*, recognises that zooplankton populations increase in response to the spring phytoplankton (food resource) peak, but may also drive some aspects of phytoplankton phenology by contributing to the decline of spring phytoplankton blooms through grazing (Lampert *et al.*, 1986). For perch, the candidate drivers were spring water temperature, incident solar radiation and the median length of the fish on the spawning grounds. See Supporting Table S2 for a justification of the selected driving variables.

Analysing drivers of change

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

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

Results

Comparison of phenological changes based upon different zooplankton data sources

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

Rates of phenological change at different trophic levels

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

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

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

Drivers of phenological change

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

Hierarchical modelling of phenological change was based upon mean values of the metrics within each species-metric class combination i.e. phytoplankton onset, phytoplankton peak/mid-growing season, zooplankton onset, zooplankton peak/mid-growing season, perch peak/mid-spawning period (Fig. 1). The seasonal timing of events at all trophic levels, in both lake basins, was earlier in years with higher water temperature apart from the chlorophyll *a* peak/mid-point in the South Basin (Tables 1-3). The time of year at which temperature change was most influential differed among trophic levels: March-April temperatures were the strongest predictors of phytoplankton phenological change, while April-May temperatures were more influential for the zooplankton and fish. The magnitude of the temperature effect, in terms of the days change in seasonal timing per °C, was greatest for *Daphnia* spp. (approximately 8 – 10 days earlier per ºC increase).

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

Discussion

Multi-metric approaches to phenological change and de-synchronisation

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

Multi-metric detection of de-synchronisation

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

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

Temperature as a driver of phenological change

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

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

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

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

Resource availability as a driver of phenological change

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

In Windermere, diatom taxa such as *Asterionella formosa* and *Aulacoseira* spp. collectively dominate this spring community (Feuchtmayr *et al.*, 2012). Diatoms require silicate to build their cell walls and, as a result, the carrying capacity of the system for these taxa is constrained by the availability of this constituent (Reynolds, 1990, 1997). The spring phytoplankton bloom occurred later in years with higher silicate concentrations. Increased silicate availability raises the maximum attainable population size for diatoms and hence prolongs the period of growth that can occur prior to silicate limitation. As a result, higher silicate concentrations would permit phytoplankton population growth to continue later into the year (Reynolds, 1990, 1997; Thackeray *et al.*, 2008; Feuchtmayr *et al.*, 2012). Although this mechanism might explain the response seen for peak populations in the North Basin, it is unlikely to be the causative factor behind the response of the onset metrics in both basins. These observations once again highlight the difficulty in making causal inferences, based upon analysis of observational data.

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

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

Final remarks

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

While consideration of these issues will strengthen our interpretation of observed patterns of long-term change, there are limitations to what can be gleaned from even long and comprehensive data sets (Maberly & Elliott 2012). Approaches based upon analyses of long-term data would be complemented by enhanced mechanistic understanding derived from experimentation at different scales (Feuchtmayr *et al*. 2010) and the encapsulation of this information in mechanistic models (De Senerpont Domis *et al.* 2007; Schalau *et al*. 2010). When evaluating the results of studies, an appreciation of ecological scale is key: some phenological events represent behavioural changes acting at the individual level (for example perch spawning), while some represent the balance between population-level processes (for example phyto- and zooplankton population development). By developing mechanistic insight it will be possible to increase understanding of past phenological change and, ultimately, allow the development of predictive models, capable of forecasting responses to future environmental change.

Acknowledgements

We are indebted to everyone that has maintained the long-term monitoring programme on Windermere, past and present. This work was funded by the Centre for Ecology & Hydrology Environmental Change Integrating Fund Project, SPACE (Shifting Phenology: Attributing Change across Ecosystems) with additional support from NERC grants NE/H000208/1 and NE/J02080X/1. We are also grateful to the Environment Agency for providing the outflow discharge data, Mr Bernard Tebay for providing the meteorological data from Ambleside and the Freshwater Biological Association for funding additional sample processing. We thank the editor and three anonymous reviewers for helping us improve an earlier version of the manuscript.

References

Adrian R, Wilhelm S, Gerten D (2006) Life-history traits of lake plankton species may govern their phenological response to climate warming. *Global Change Biology,* **12**, 652-661.

Askeyev OV, Sparks TH, Askeyev IV, Tishin DV, Tryjanowski P (2010) East versus West: contrasts in phenological patterns. *Global Ecology and Biogeography,* **19**, 783-793.

Berger SA, Diehl S, Stibor H, Trommer G, Ruhenstroth M (2010) Water temperature and stratification depth independently shift cardinal events during plankton spring succession. *Global Change Biology,* **16**, 1954-1965.

Both C, Van Asch M, Bijlsma RG, Van Den Burg AB, Visser ME (2009) Climate change and unequal phenological changes across four trophic levels: constraints or adaptations? *Journal of Animal Ecology,* **78**, 73-83.

Burthe S, Daunt F, Butler A *et al.* (2012) Phenological trends and trophic mismatch across multiple levels of a North Sea pelagic food web. *Marine Ecology-Progress Series,* **454**, 119-133.

Craig JF (2000) *Percid Fishes: Systematics, Ecology and Exploitation,* Oxford, Blackwells, 352 pp.

Cushing DH (1990) Plankton production and year class strength in fish populations - an update of the match mismatch hypothesis. *Advances in Marine Biology,* **26**, 249-293.

De Senerpont Domis LN, Mooij WM, Hulsmann S, Van Nes EH, Scheffer M (2007) Can overwintering versus diapausing strategy in *Daphnia* determine match-mismatch events in zooplankton-algae interactions? *Oecologia,* **150**, 682-698.

Feuchtmayr H, Moss B, Harvey I, Moran R, Hatton K, Connor L, Atkinson D (2010) Differential effects of warming and nutrient loading on the timing and size of the spring zooplankton peak: an experimental approach with hypertrophic freshwater mesocosms. *Journal of Plankton Research,* **32**, 1715-1725.

Feuchtmayr H, Thackeray SJ, Jones ID, De Ville M, Fletcher J, James B, Kelly J (2012) Spring phytoplankton phenology - are patterns and drivers of change consistent among lakes in the same climatological region? *Freshwater Biology,* **57**, 331-344.

George DG (2012) The effect of nutrient enrichment and changes in the weather on the abundance of *Daphnia* in Esthwaite Water, Cumbria. *Freshwater Biology,* **57**, 360-372.

George DG, Hewitt DP (1998) The influence of year-to-year changes in position of the Atlantic Gulf Stream on the biomass of zooplankton in Windermere North Basin, UK. In: *Management of Lakes and Reservoirs during Global Climate Change* pp 223-244, Kluwer Academic Publishers.

George DG, Reynolds CS (1997) Zooplankton-phytoplankton interactions: the case for refining methods, measurements and models. *Aquatic Ecology,* **31**, 59-71.

George DG, Talling JF, Rigg E (2000) Factors influencing the temporal coherence of five lakes in the English Lake District. *Freshwater Biology,* **43**, 449-461.

Gerten D, Adrian R (2000) Climate-driven changes in spring plankton dynamics and the sensitivity of shallow polymictic lakes to the North Atlantic Oscillation. *Limnology and Oceanography,* **45**, 1058-1066.

Grace JB (2006) Structural Equation Modelling and Natural Systems. Cambridge University Press, UK. 365 pp.

Guisande C, Gliwicz ZM (1992) Egg size and clutch size in two *Daphnia* species growth at different food levels. *Journal of Plankton Research,* **14**, 997-1007.

Guma'a SA (1978a) The food and feeding habits of young perch, *Perca fluviatilis*, in Windermere. *Freshwater Biology,* **8**, 177-187.

Guma'a SA (1978b). The effects of temperature on the development and mortality of eggs of perch, *Perca fluviatilis. Freshwater Biology,* **8**, 221-227.

Hall DJ (1964) An experimental approach to the dynamics of a natural population of *Daphnia galeata mendotae*. *Ecology,* **45**, 94-112.

Hampton SE, Romare P, Seiler DE (2006) Environmentally controlled *Daphnia* spring increase with implications for sockeye salmon fry in Lake Washington, USA. *Journal of Plankton Research,* **28**, 399-406.

Hutchinson GE (1957) *A treatise on limnology. Volume 1: Geography, physics and chemistry,* London, Chapman and Hall Ltd, 1015 pp.

IPCC (2007) Summary for policymakers. In: *Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of working group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.* (eds Parry ML, Canziani OF, Palutikof JP, Van Der Linden PJ, Hanson CE). Cambridge, Cambridge University Press.

Jones ID, Winfield IJ, Carse F (2008) Assessment of long-term changes in habitat availability for Arctic charr (*Salvelinus alpinus*) in a temperate lake using oxygen profiles and hydroacoustic surveys. *Freshwater Biology,* **53**, 393-402.

Lampert W (1978) A field study on the dependence of the fecndity of *Daphnia* spec. on food concentration. *Oecologia,* **36**, 363-369.

Lampert W, Fleckner W, Rai H, Taylor BE (1986) Phytoplankton control by grazing zooplankton: A study on the spring clear-water phase. *Limnology and Oceanography,* **31**, 478-490.

Lund JWG (1949) Studies on *Asterionella* I. The origin and nature of the cells producing seasonal maxima. *Journal of Ecology,* **37**, 389-419.

Lunn DJ, Thomas A, Best N, Spiegelhalter D (2000) WinBUGS - a Bayesian modelling framework: concepts, structure and extensibility. *Statistics and Computing,* **10**, 325-337.

Maberly SC, Elliott JA (2012) Insights from long-term studies in the Windermere catchment: external stressors, internal interactions and the structure and function of lake ecosystems. *Freshwater Biology*, **57**, 233-243.

Mackereth FGH, Heron J, Talling JF (1978) *Water analysis: some revised methods for limnologists,* Ambleside, Freshwater Biological Association, 120 pp.

Menzel A, Sparks TH, Estrella N *et al.* (2006) European phenological response to climate change matches the warming pattern. *Global Change Biology,* **12**, 1969-1976.

Miller-Rushing AJ, Inouye DW, Primack RB (2008) How well do first flowering dates measure plant responses to climate change? The effects of population size and sampling frequency. *Journal of Ecology,* **96**, 1289-1296.

Mills CA, Hurley MA (1990) Long-term studies on the Windermere populations of perch (*Perca fluviatilis*), pike (*Esox lucius*) and Arctic charr (*Salvelinus alpinus*). *Freshwater Biology*, **23**, 119-136.

Munro IG, White RWG (1975) Comparison of the Influence of Temperature on the Egg Development and Growth of *Daphnia longispina* O.F. Müller (Crustacea:Cladocera) from Two Habitats in Southern England. *Oecologia,* **20**, 157-165.

Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature,* **421**, 37-42.

Paxton CGM, Winfield IJ, Fletcher JM, George DG, Hewitt DP (2004) Biotic and abiotic influences on the recruitment of perch (*Perca fluviatilis*) in Windermere, U.K. *Journal of Fish Biology,* **65**, 1622-1642.

Pinheiro J, Bates D, Debroy S, Sarkar D, R Development Core Team (2010) nlme: Linear and nonlinear mixed effects models. R package version 3.1-97.

R Development Core Team (2011) R: A language and environment for statistical computing. pp Page, Vienna, Austria, R Foundation for Statistical Computing, ISBN 3-900051-07-0, url = http://www.R-project.org/.

Ramsbottom AE (1976) *Depth charts of the Cumbrian lakes.*, Freshwater Biological Association, 39 pp.

Reynolds CS (1989) Physical determinants of phytoplankton succession. In: *Plankton Ecology.* (ed Sommer U) pp 9-56. Madison, Brock-Springer.

Reynolds CS (1990) Temporal scales of variability in pelagic environments and the response of phytoplankton. *Freshwater Biology,* **23**, 25-53.

Reynolds CS (1997) Successional development, energetics and diversity in planktonic communities. In: *Biodiversity: An ecological perspective.* (eds Abe T, Levin SR, Higashi M) pp 167-202. New york, Springer.

Reynolds CS (2006) *Ecology of phytoplankton,* Cambridge, Cambridge University Press, 535 pp.

Reynolds CS, Irish AE (2000) *The phytoplankton of Windermere (English Lake District).* Ambleside, Freshwater Biological Association, 73 pp.

Rolinski S, Horn H, Petzoldt T, Paul L (2007) Identifying cardinal dates in phytoplankton time series to enable the analysis of long-term trends. *Oecologia,* **153**, 997-1008.

Root TL, Price JT, Hall KR, Schneider SH, Rosenzweig C, Pounds JA (2003) Fingerprints of global warming on wild animals and plants. *Nature,* **421**, 57-60.

Roy DB, Sparks TH (2000) Phenology of British butterflies and climate change. *Global Change Biology,* **6**, 407-416.

Schalau K, Rinke K, Straile D, Peeters F (2008) Temperature is the key factor explaining interannual variability of *Daphnia* development in spring: a modelling study. *Oecologia,* **157**, 531-543.

Scheffer M, Rinaldi S, Kuznetsov YA, Van Nes EH (1997) Seasonal dynamics of *Daphnia* and algae explained as a periodically forced predator-prey system. *Oikos,* **80**, 519-532.

Talling JF (1974) Photosynthetic pigments. General outline of spectrophotometric methods; specific procedures. In: *A Manual on Methods for Measuring Primary Productivity in Aquatic Environments.* . (ed Vollenweider RA) pp 22-26. Oxford, Blackwell Scientific Publications.

Talling JF (2002) Phytoplankton-zooplankton seasonal timing and the "clear-water" phase in some English lakes. *Freshwater Biology,* **48**, 39-52.

Thackeray SJ, Henrys PA, Jones ID, Feuchtmayr H (2012) Eight decades of phenological change for a freshwater cladoceran: what are the consequences of our definition of seasonal timing? *Freshwater Biology,* **57**, 345-359.

Thackeray SJ, Jones ID, Maberly SC (2008) Long-term change in the phenology of spring phytoplankton: species-specific responses to nutrient enrichment and climatic change. *Journal of Ecology,* **96**, 523-535.

Thackeray SJ, Sparks TH, Frederiksen M *et al.* (2010) Trophic level asynchrony in rates of phenological change for marine, freshwater and terrestrial environments. *Global Change Biology,* **16**, 3304-3313.

Vanni MJ, Temte J (1990) Seasonal patterns of grazing and nutrient limitation of phytoplankton in a eutrophic lake. *Limnology and Oceanography,* **35**, 697-709.

Vijverberg J (1980) Effect of temperature in laboratory studies on development and growth of Cladocera and Copepoda from Tjeukemeer, The Netherlands. *Freshwater Biology,* **10**, 317-340.

Visser ME, Both C (2005) Shifts in phenology due to global climate change: the need for a yardstick. *Proceedings of the Royal Society of London Series B-Biological Sciences,* **272**, 2561-2569.

Visser ME, Holleman LJM, Gienapp P (2006) Shifts in caterpillar biomass phenology due to climate change and its impact on the breeding biology of an insectivorous bird. *Oecologia,* **147**, 164-172.

Weetman D, Atkinson D (2004) Evaluation of alternative hypotheses to explain temperature-induced life history shifts in *Daphnia*. *Journal of Plankton Research,* **26**, 107-116.

Winder M, Schindler DE (2004) Climate change uncouples trophic interactions in an aquatic ecosystem. *Ecology,* **85**, 2100-2106.

Winfield IJ, Fletcher JM, James JB (2008) The Arctic charr (*Salvelinus alpinu*s) populations of Windermere, U.K.: population trends associated with eutrophication, climate change and increased abundance of roach (*Rutilus rutilus*). *Environmental Biology of Fishes*, **83**, 25-35.

Wood SN, Augustin NH (2002) GAMs with integrated model selection using penalized regression splines and applications to environmental modelling. *Ecological Modelling,* **157**, 157-177.

Supporting information legends

Supporting Figure S1. Rates of phenological change (1969-2008) across three trophic levels in a) the North Basin and b) the South Basin of Windermere, UK.

Supporting Figure S2. Examples of long-term change in the seasonal time difference between events at different trophic levels in the North and South basins of Windermere.

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

Supporting Figure S4. Long-term variations in potential chemical and biological drivers of phenological change.

Supporting Table S1. The phenological metrics used to quantify the seasonal timing of plankton development and perch spawning.

Supporting Table S2. Driving variables used in the analyses of phenological change, with justification for their inclusion and supporting references.

Supporting Table S3: Comparison of phenological metrics derived using filter count and microscope data for *Daphnia*.

Supporting Table S4: Summaries of exemplar linear models of long-term changes in the seasonal time difference between phenological events at adjacent trophic levels.

Supporting Table S5: Correlation matrix for predictors included in the hierarchical modelling of phenological change at three trophic levels.

Supporting Information: hierarchical model structure for analysis of drivers

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

	Phytoplankton onset		**Phytoplankton peak/mid-growing season**	
Driver	**North Basin**	**South Basin**	**North Basin**	**South Basin**
Water temperature (°C)	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
Stratification (day of year)	ns	Later=>Earlier -0.41 [-0.76, -0.07] (20 J m^{-2})	ns	ns
Leven discharge (m^3 s^{-1})	ns	ns	ns	ns

Winter silicate (mg m^{-3})	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
Winter phosphorus (mg m^{-3})	ns	ns	Higher=>Earlier -2.45 [-4.08, -0.95]	ns
Sunlight (hour d^{-1})	ns	ns	ns	ns
Inoculum (mg Chl *a* m^{-3})	ns	ns	ns	ns
Zooplankton phenology (day of year)	ns	Earlier=>Earlier 0.22 [0.04, 0.42] (Peak/Mid-point)	ns	Earlier=>Earlier 0.29 [0.05, 0.54] (Peak/Mid-point)

Table 2. Summary of hierarchical model analyses of drivers of phenological change, for zooplankton. In each cell a simple "change in driver=>change in response" statement indicates the direction of the relationship and the symbol "=>" should be read as "leads to". For temperature the specific (nested) variable related to phenology is indicated i.e. individual monthly means. Statistically significant (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 of the driver) is given, with the 90% Bayesian credible interval shown in brackets. See text for details on modelling approach.

	Zooplankton onset		**Zooplankton peak/mid-growing season**	
Driver	**North Basin**	**South Basin**	**North Basin**	**South Basin**
Water temperature (°C)	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)
Leven discharge (m^3 s^{-1})	Higher=>Later 0.97 [0.06, 1.89]	ns	ns	ns
Sunlight (hour d^{-1})	ns	ns	ns	ns
Inoculum (individuals per L^{-1})	ns	ns	ns	ns

Phytoplankton phenology (day of year)	Earlier=>Earlier 0.36 [0.15, 0.58] (Onset)	ns	ns	Earlier=>Earlier 0.25 [0.003, 0.49] (Peak/Mid-point)

Table 3. Summary of hierarchical model analyses of drivers of phenological change, for perch. In each cell a simple "change in driver=>change in response" statement indicates the direction of the relationship and the symbol "=>" should be read as "leads to". For temperature the specific (nested) variable related to phenology is indicated i.e. individual monthly means. Statistically significant ($P<0.10$) terms are shown, and non significant terms are denoted "ns". For each significant term the parameter estimate (days of change per unit of the driver) is given, with the 90% Bayesian credible interval shown in brackets. See text for details on modelling approach.

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

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 ($P<0.05$).

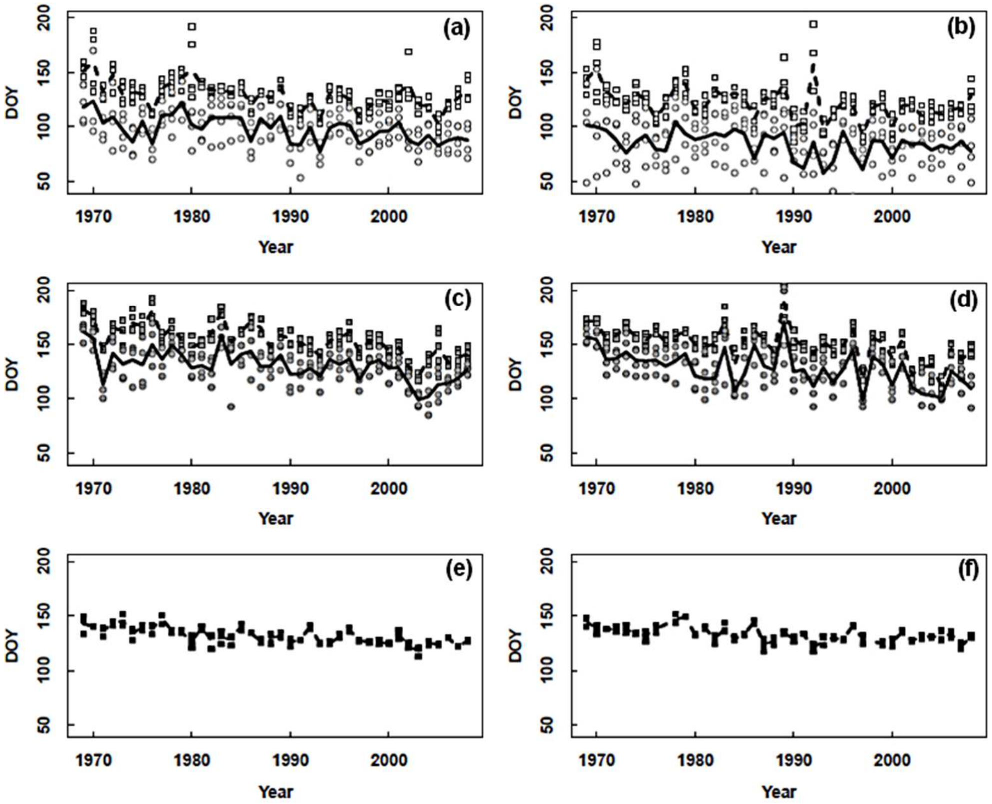

Figure 1

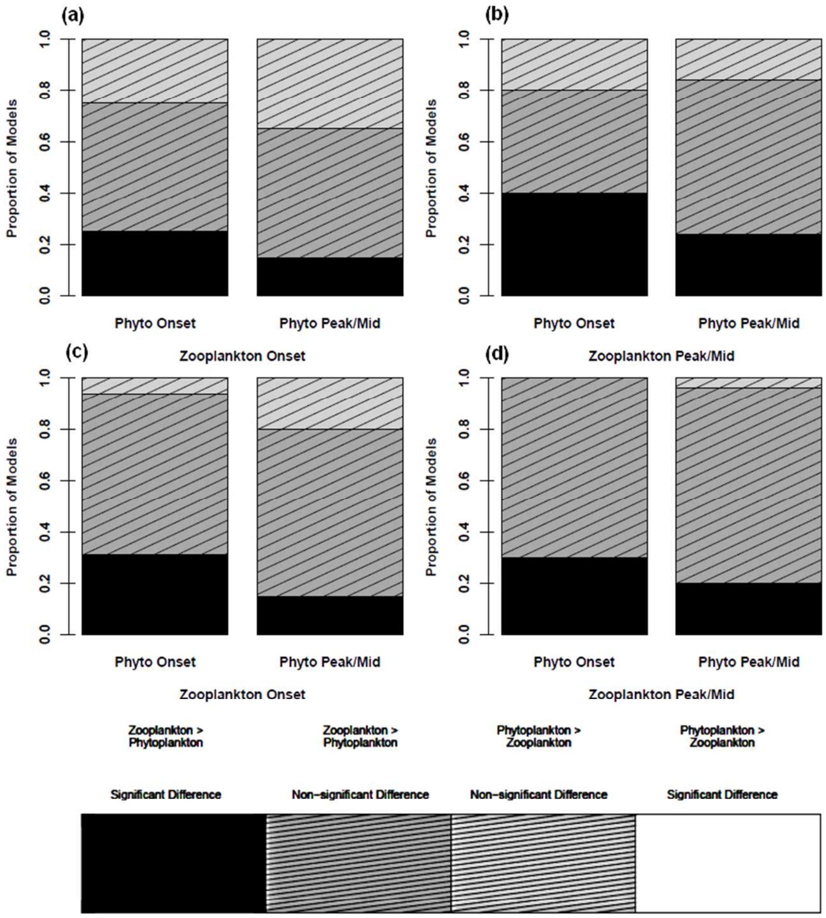
(a)
(b)
(c)
(d)
Proportion of Models
0.0
0.2
0.4
0.6
0.8
1.0
Phyto Onset
Phyto Peak/Mid
Zooplankton Onset
Zooplankton Peak/Mid
Zooplankton > Phytoplankton
Phytoplankton > Zooplankton
Significant Difference
Non–significant Difference

Figure 2

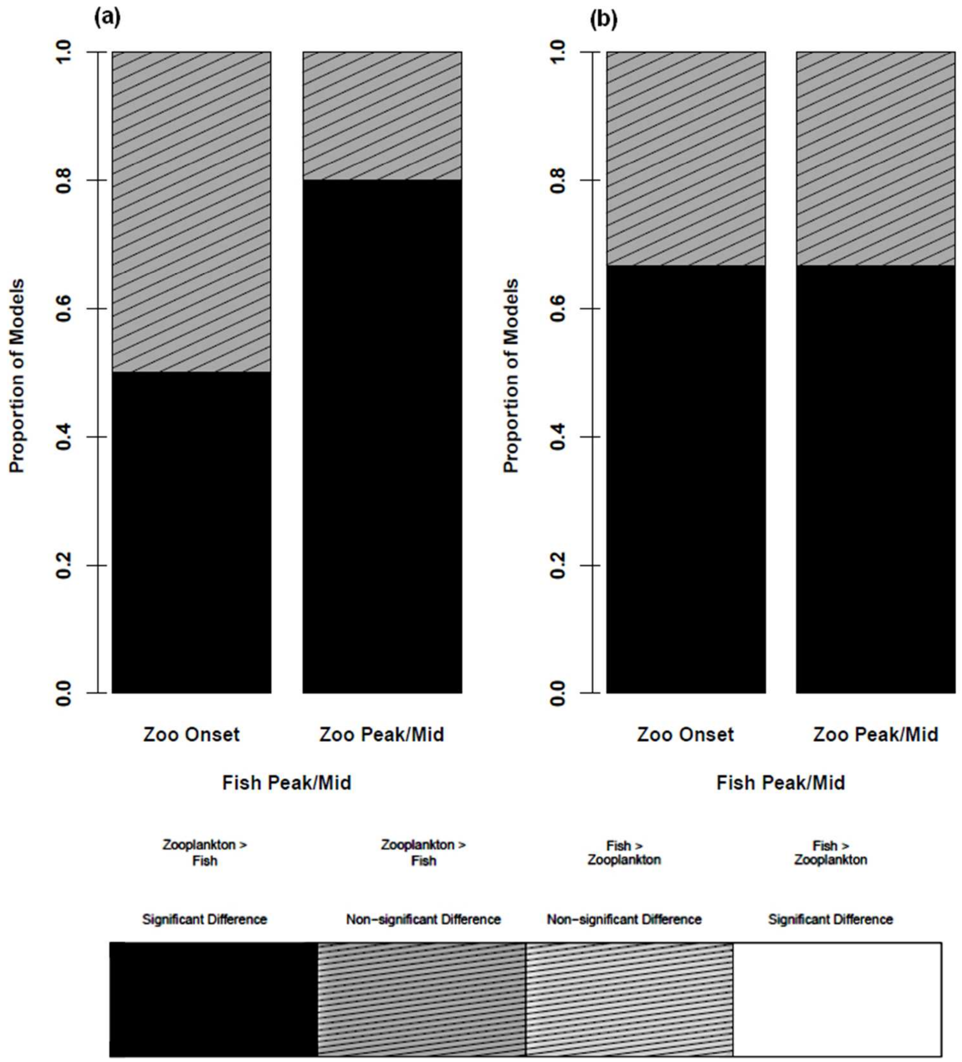
(a)
(b)
Proportion of Models
0.0
0.2
0.4
0.6
0.8
1.0
Zoo Onset
Zoo Peak/Mid
Fish Peak/Mid
Zooplankton > Fish
Significant Difference
Zooplankton > Fish
Non−significant Difference
Fish > Zooplankton
Non−significant Difference
Fish > Zooplankton
Significant Difference

Figure 3