
Thackeray, S.J.; **Henrys, P.A.**; **Jones, I.D.**; **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 (2). 345-359. 10.1111/j.1365-2427.2011.02614.x

Eight decades of phenological change for a freshwater cladoceran: what are the consequences of our definition of seasonal timing?

Journal:	Freshwater Biology
Manuscript ID:	FWB-S-Sep-10-0462.R2
Manuscript Type:	Special Issue
Date Submitted by the Author:	n/a
Complete List of Authors:	Thackeray, Stephen; Lake Ecosystem Group, Centre for Ecology and Hydrology, Lancaster Environment Centre Henrys, Peter; Centre for Ecology and Hydrology Jones, Ian; CEH; Centre for Ecology and Hydrology Feuchtmayr, Heidrun; Centre for Ecology & Hydrology; Centre for Ecology and Hydrology
Keywords:	Climate change < Applied Issues, Lakes < Habitat, Population < Level of Organisation, Other species interactions < Process / Approach / Methods, Zooplankton < Taxonomic Group / Assemblage

Eight decades of phenological change for a freshwater cladoceran: what are the consequences of our definition of seasonal timing?

S. J. THACKERAY*, P. A. HENRYS*, I. D. JONES* & H. FEUCHTMAYR*

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

Corresponding author: Stephen J. Thackeray, Centre for Ecology & Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster, LA1 4AP, UK. Fax: 01524 61536.

E-mail: sjtr@ceh.ac.uk

Abbreviated title: Daphnia phenology over eight decades

Keywords: Daphnia galeata, phytoplankton, phenology, temperature, metrics

SUMMARY

1. Changes in the seasonal timing of re-occurring biological events, or phenology, are a widely reported ecological response to environmental change. Previous studies have demonstrated that plankton populations have shifted their phenology in recent decades but there is a lack of consistency with respect to the phenological metrics analysed.
2. We analysed an eight-decade data set (1934-2009) on the seasonal dynamics of Daphnia galeata in the North Basin of Windermere, UK. Rates of phenological change derived from ten different phenological metrics were compared. We evaluated the evidence for effects of spring water temperature, phytoplankton phenology and over-wintering population size on D. galeata phenology.
3. Nine of the ten phenological metrics showed statistically significant trends towards earlier seasonal timing, though rates of change varied (3.7-6.7 days per decade). Regression analyses showed a consistent effect of spring water temperature and phytoplankton phenology on the timing of D. galeata spring population development. The amount of variability explained by these drivers, the precise phytoplankton metric related most closely to D. galeata phenology and the importance of over-wintering population size differed markedly among D. galeata metrics.
4. Hierarchal models showed that the seasonal timing of the phytoplankton peak had the most consistent effect upon D. galeata phenology and that temperatures in the month previous to the average timing of population development were influential.
5. Phenological metrics differ mathematically and conceptually. They indicate different population dynamical processes and are influenced by different ecological mechanisms. Combining information from different phenological metrics will greatly improve mechanistic understanding of the factors influencing phenological change.

Introduction

Changes in the timing of seasonally re-occurring biological events, or phenology, are one of the most powerful biological responses to environmental change, particularly climate change (IPCC, 2007). There have been numerous demonstrations of a shift towards earlier spring timing for a range of life-history events, from a diversity of plant and animal taxa across marine, freshwater and terrestrial environments (Parmesan & Yohe, 2003; Root et al., 2003; Thackeray et al., 2010). Such changes raise concern as they may de-synchronise key trophic interactions within ecosystems and therefore impinge upon ecosystem functioning (Harrington, Woiwod & Sparks, 1999; Visser & Both, 2005; Thackeray et al., 2010). In freshwater environments, the potential for de-synchronisation is apparent given that variable rates of phenological change have been reported in phytoplankton (Thackeray, Jones & Maberly, 2008; Meis, Thackeray & Jones, 2009), zooplankton (Winder & Schindler, 2004; Adrian, Wilhelm & Gerten, 2006), macroinvertebrates (Doi, 2008), amphibians (Chadwick, Slater & Ormerod, 2006; Carroll et al., 2009) and fish (Winfield et al., 2004).

The potential ecosystem consequences of phenological shifts necessitate that we develop an understanding of the processes driving them. Changes in the timing of spring zooplankton populations, compared to changes at adjacent trophic levels, are key to understanding the consequences of phenological change upon food web structure. The larvae of spring-spawning fish depend upon spring zooplankton populations as a food resource and the latter, in turn, depend upon the seasonal pulse in edible phytoplankton. The phenology of zooplankton populations is likely to be affected by ambient temperature and food resource availability, which influence rates of population growth. Variation in water temperature affects age at maturation, rates of egg development and the frequency with which offspring are released at moulting (Hall, 1964; Vijverberg, 1980; Weetman & Atkinson, 2004). Food availability affects clutch size, and also the proportion of the population bearing eggs (Guisande & Gliwicz, 1992; George & Reynolds, 1997). Recent studies have suggested that long-term trends in spring temperature and food phenology have a particularly strong influence on Daphnia phenology (Hampton, Romare & Seiler, 2006; Schalau et al., 2008). The magnitude of the over-wintering population also influences the phenology of the subsequent Daphnia spring maximum (Romare et al., 2005; Hampton et al., 2006), presumably by affecting the time needed for the population to increase above a predetermined population size threshold, or to reach carrying capacity.

Despite growing interest in phenological changes in plankton communities, their drivers, and the consequences of change, there is a surprising lack of consistency in the metrics used to describe their seasonal timing of population development. A diverse array of metrics have been used which can be categorised broadly as measures of the seasonal timing of i) the onset of population growth, ii) peak abundance or iii) the centre of the growing season. Measures of the onset of population growth have included the day of the year on which a species is first detected by a sampling programme (Adrian et al., 2006), the time at which the population increases above a selected absolute or relative abundance threshold (Greve et al., 2005; Romare et al., 2005; Hampton et al., 2006; Thackeray et al., 2010) or have been based upon quantiles of the area under a curve fitted to the seasonal abundance data (Rolinski et al., 2007). The timing of peak abundance has also been determined based upon such curve-fitting approaches (Rolinski et al., 2007), as well as by identifying the sampling date on which the maximum abundance was recorded in the original data (Winder & Schindler, 2004; Adrian et al., 2006; Thackeray et al., 2008). Measures of the centre of the growing season include the centre of gravity of the seasonal population maxima (Edwards & Richardson, 2004;

Thackeray et al., 2008; Meis et al., 2009) or the 50th percentile of the cumulative seasonal abundance (Greve et al., 2005).

Aside from the obvious mathematical differences, there are also clear conceptual differences among these metrics. The corollary of this is that the mechanistic interpretation of the factors affecting observed phenological change depends strongly upon the definition of phenology being employed. Plankton phenological metrics represent changes at the population level, and are influenced by temporal changes in the balance between the rate of replication or birth, and the rate of various loss processes (Thackeray et al., 2008). The onset of population growth occurs when the rate of population growth exceeds the rate of population loss (i.e. positive net population growth), whereas the timing of peak abundance occurs when the rate of growth is balanced by the rate of loss (Thackeray et al., 2008). After the population peak, a phase of negative population growth occurs since loss processes exceed the rate of population growth.

As a result of the conceptual differences among metrics we would expect our estimates of phenological changes, and the interpretation of the drivers behind them, to be metric-dependent. As yet no study has sought to examine patterns and drivers of plankton phenological change, while considering the full range of phenological metrics that are commonly used. By collating metrics in this way, inferences regarding the evidence for change and the potential drivers of change should be more robust. The primary aim of the present study was, therefore, to use a multi-metric approach to examine the evidence for phenological shifts in a Daphnia population and to explore potential drivers of these changes. A suite of phenological metrics were used to determine long-term trends in the seasonal timing of Daphnia spring population development. Phenological changes calculated using each metric were then related to three possible determinants of spring phenology; 1) spring water temperature, 2) the seasonal timing of spring phytoplankton growth and 3) the magnitude of the over-wintering Daphnia population. We then compared results derived from each phenological metric to assess the extent to which phenological trends and the effects of driving variables depend upon the choice of phenological metric. Hierarchal modelling was also used to group driving variables into conceptual classes in order to assess consistent, higher-level patterns in the drivers of spring D. galeata phenology.

Materials and methods

Field methods

Data were collected under an ongoing long-term monitoring programme in the pelagic zone of the North Basin of Windermere, UK (54^{o}20'N, 2^{o}57'W). The basin covers an area of 8.1 km^2 and has a mean depth of 25 m (maximum depth 64 m, Ramsbottom (1976)). The ecology of Windermere was summarised by Reynolds & Irish (2000). Samples of crustacean zooplankton have been collected from the North Basin of Windermere since the mid-1930s, while other physical and chemical variables have been recorded over shorter time periods.

The present analysis focussed on two time periods. Firstly phenological trends for Daphnia were analysed over the whole period for which zooplankton data were available (1934 – 2009). Secondly, the relationships between phenological data and potential driving variables were analysed over the period 1964 – 2009, for which data on both chlorophyll a and in-lake water temperature were available. Throughout these two time periods, consistent methods were used to collect physical, chemical and biological data at one to two-weekly intervals.

Vertical temperature profiles were recorded over the deep point of the lake. Measurements were taken with a Mackereth oxygen electrode in the 1960s and 1970s and a Yellow Springs Instruments probe since the 1980s (George, Talling & Rigg, 2000). To avoid any inherent bias in the data, due to among-season differences in the depth resolution of the data, the raw data were linearly interpolated vertically and then linearly interpolated through time to give temperatures on a one-metre, daily grid (Jones, Winfield & Carse, 2008). A volume-weighted 0-10 m mean water temperature was calculated for comparison with the D. galeata data since this is the part of the water column typically inhabited by this species during spring (Thackeray et al., 2005). These temperature data were then averaged by month and changes in each D. galeata phenological metric were analysed with respect to monthly mean temperatures from the month containing the mean day of year on which that phenological phase occurred (hereafter the current month) and the previous month. This allowed the possibility of time-lagged temperature effects and resulted in different metrics being analysed with respect to March – April, April – May or May – June monthly mean temperatures. Integrated surface water samples for the determination of chlorophyll a concentrations were collected using a weighted plastic tube (Lund, 1949) and analysed spectrophotometrically according to Talling (1974).

Zooplankton were collected by 40 m vertical net hauls (mesh size 120 µm, mouth diameter 0.3 m) in the pelagic zone. Samples were initially fixed with a small quantity of 70% ethanol, before being preserved in 4% formaldehyde. Zooplankton were examined under a stereo-zoom 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 Stempel pipette. The present analysis uses data on the total numbers of Daphnia galeata (Sars) collected in each sample. The long-term record of zooplankton community composition is somewhat fragmented; data from these net hauls were available only for a subset of years within each decade. However, the available data have a good temporal distribution, with at least 3 full years of data available for every decade from the 1930s to the 2000s. At present, full seasonal cycles of D. galeata abundance are available for 1934-1936, 1944-1946, 1954-1956, 1961-1974, 1976-1978, 1985-1987, 1994-1996, 2000-2006 and 2009. Mean January abundances of D. galeata were used to indicate the magnitude of the over-wintering population at the start of each year.

Phenological metrics

For both the D. galeata and chlorophyll a data, a range of different metrics (ten in all) were used to quantify the seasonal timing of spring population development. These were selected to represent the various approaches currently used in the plankton phenology literature. Three broad conceptual categories of metric were used in the analysis. We distinguished measures of the onset of spring population development, the timing of maximum abundance and the central point of the growing season.

Onset of spring population development (“Onset”)

Absolute abundance thresholds. The day of each year on which population size, or chlorophyll a concentration, first exceeded an absolute abundance threshold was determined (Romare et al., 2005; Hampton et al., 2006; Berger et al., 2010). For D. galeata data a threshold abundance of 0.2 individuals L^{-1} was set, as each year this value was exceeded during the early stages of the spring peak but not during the small abundance fluctuations that typically occurred during the preceding winter months. Given that the phenology of phytoplankton biomass was being used as a statistical predictor of *D. galeata* phenology, the threshold abundance was chosen to be one that was relevant to the grazer population. Chlorophyll *a* data were approximated to carbon concentrations according to Reynolds (2006) and a threshold concentration of 0.1 mg C L^{-1} was set since low zooplankton population growth and lower percentages of egg-bearing females for *Daphnia hyalina* and *D. galeata* have been observed below this threshold (Geller, 1985; George & Reynolds, 1997).

Relative abundance thresholds. Two different types of relative abundance thresholds were employed. The first type was the day of the year on which the abundance exceeded 25%, or 50%, of the maximum spring abundance for each year (Thackeray et al., 2010). The second type was the day of the year on which cumulative *D. galeata* abundance, or chlorophyll *a* concentration, exceeded 25% of the spring total (Greve et al., 2005). In order to focus the analysis upon spring dynamics and avoid the influence of summer/autumn population dynamics, these metrics were calculated between February and July for *D. galeata* and January and June for chlorophyll *a*. The exclusion of January data for *D. galeata* prevented fluctuations in declining overwintering pelagic populations from influencing this measure of spring dynamics.

Curve-fitting approaches. Following Rolinski et al. (2007), a six-parameter Weibull-type function was fitted to the chlorophyll *a* and the *D. galeata* abundance data for each year and was used to determine the day of the year on which the population exceeded a defined quantile of the area under the curve (set at 5% of the integrated population size).

The timing of maximum abundance ("Peak")

Smoothing the seasonal data. A generalised additive model (GAM) with a Gamma error distribution was fitted to each seasonal data set. The usefulness of GAMs to describe patterns of seasonal variation in plankton data was demonstrated by Ferguson et al. (2008). A Gamma error distribution was appropriate since the data sets varied on a continuous scale and had a positive skew. The day of the year corresponding to the maximum fitted value was determined for D. galeata and chlorophyll a.

Day of maximum abundance. A commonly used metric in the plankton phenology literature is simply the day of the year on which the maximum abundance was recorded (Winder & Schindler, 2004; Adrian et al., 2006; Thackeray et al., 2008). In the present analysis, the days of the year corresponding to the maximum observed D. galeata abundance and the maximum observed chlorophyll a concentration were determined. Since this metric is potentially sensitive to sampling frequency and sample processing errors, various authors have smoothed the data before determining the seasonal timing of peak abundance in the hope that this will produce a more robust phenological metric. In the present analysis we addressed this issue by using complementary approaches based upon curve-fitting and smoothing of the seasonal data.

Curve-fitting approaches. As for the determination of onset dates, the six-parameter Weibull-type function fitted to each set of seasonal D. galeata and chlorophyll a data was used to determine the day of maximum abundance. This corresponded to the time at which the fitted Weibull function reached its maximum value (Rolinski et al., 2007).

The central point of the growing season ("Growing Season").

Centre of gravity. For each year of D. galeata and chlorophyll a data we calculated the centre of gravity of the spring population (Edwards & Richardson, 2004; Thackeray et al., 2008; Meis et al., 2009). The centre of gravity was calculated using data collected between January and June for chlorophyll a and between February and July for D. galeata.

50th percentile of cumulative abundance. For each year we determined the first day of the year on which the D. galeata abundance, or chlorophyll a concentration, exceeded 50% of the cumulative spring abundance (Greve et al., 2005). The analysis was based upon the cumulative abundance between February and July for D. galeata and between January and June for chlorophyll a.

Statistical modelling

Long-term changes in spring phenology were assessed by linear regression of each metric against year. Residuals from each regression were checked for normality and homoscedasticity using quantile - quantile and residual - fit plots. Cook's distances were checked to ensure that each trend was not unduly influenced by any one observation. These diagnostics did not reveal any problems associated with non-normality of residuals, heteroscedasticity or influence. In each case, residuals were plotted against year to establish whether trends showed evidence of non-linearity. This was further examined by fitting each trend with a quadratic year term and by modelling the effect of year using a smooth term, fitted using a GAM. The change in residual deviance associated with fitting the non-linear vs. linear trend was assessed by an F-test. For all metrics, long-term changes in phenology were best approximated as a linear trend over time. Quadratic temporal trends and smoothers fitted using additive models did not result in a significantly better description of the long-term trend (nested model comparisons based on F tests, all $P>0.05$, results not shown). The possible influence of temporal autocorrelation was checked by plotting autocorrelation functions of residuals and comparing parameter values and significance levels in models including and excluding empirical (exponential) variograms that would capture temporal error structure. The estimated slope parameters of the statistical models and their statistical significance were little affected by the incorporation of a temporal error structure (generalised least squares regression, not shown).

To complement this assessment of phenological change, the decadal “average” seasonal pattern in D. galeata abundance was compared between the first and last decades of the dataset; the 1930s and the 2000s. While the analysed phenological metrics did not yield information on the seasonal timing of population collapse, this approach allowed direct comparison of the whole seasonal distribution of D. galeata abundance in the two decades. This method respected the nested temporal structure of the dataset; for each decade data were available for a subset of years. Within both decades, data from each year were pooled and a GAM (with Gamma error distribution and log link function) was used to fit a single smoother term to model the effect of day of the year on abundance. To compare the two decades, the estimated means, standard errors and assumed Gamma distribution for the 1930s data were used to draw 1000 random values from the distribution at each day of year. Under the null hypothesis of no difference between the two decades, the fitted smoother to the 2000s data should be comparable to the 1000 simulated series' based upon the 1930s parameters. This approach is the commonly used technique of parametric bootstrapping (Efron & Tibshirani, 1993). In this case the test statistic used to assess the significance was:

$$T_p = \frac{p}{\quad}$$

where $E\ t$ and $V\ t$ are the mean and variance of Daphnia abundance at day of year t respectively, estimated from the fitted model, and $_p$ is the estimated abundance at day of year t for the pth set of simulated data. $p=999$ sets of simulations were drawn using the 1930s parameters and the P-value was given by assessing how extreme the test statistic from the observed 2000s data, T, was compared with each of the test statistics from the simulated data $T_{p,}$ based upon the 1930s parameters. More formally this is given by $\#\{T_p \geq T\}/1000$ (Efron & Tibshirani, 1993).

For each of the ten phenological metrics in turn, multiple linear regression was used to assess the evidence for driving effects of spring water temperatures, phytoplankton phenology and the magnitude of the over-wintering Daphnia population (January mean D. galeata abundance). As there was no a priori reason to believe that one particular driver would be particularly influential, a series of models was run which collectively encompassed all possible combinations of temperature and food phenology variables. For each D. galeata phenological metric, candidate models included the mean temperature either of the current month or of the previous month (see earlier), and contained one of the ten possible phenological metrics for the seasonal timing of spring phytoplankton growth:

$$\mathrm{oa\ \ h\ \ hn}_{n} \ = \ �\mathrm{Thrra}_{P} \qquad \mathrm{h\,I\,oh\ \ hn} \qquad \mathrm{oa\ \ W\ n}$$

Where each of the i = 1,…,10 phenological metrics for D. galeata (DaphPhen) is a function of one of j = 1,…,2 possible temperature variables (Temp), one of k = 1,…,10 possible phenological metrics for chlorophyll a (ChlorPhen) and the overwintering D. galeata population (DaphWin). α and $\beta_1 - \beta_3$ represent the intercept and slope parameters, respectively, of the fitted models. For each D. galeata phenology metric, models were run with all possible combinations of one temperature predictor and one chlorophyll a phenology predictor. All nested models were also run (i.e. all possible models containing 2 and 1 predictors).

Model performance was assessed by ranking all models by their respective AICc values and calculating Akaike weights (Burnham & Anderson, 2002). The latter indicate the relative level of support for each model, given the data. The difference in AIC between each model and the most highly ranked model (ΔAIC) was used to assess which models received similar levels of support from the data. For each model nested within the top model, the significance of the ΔAIC was determined using the likelihood ratio test (LRT), with the extra penalty term the AIC adds to the likelihood accounted for (Conner, Seborg & Larimore, 2004). Models that yielded a non-significant result in this comparison were considered to have very similar levels of support. We therefore interpreted all models with AICc values lower than the first nested model judged less optimal than the top model. In what follows, it is these models alone that we present. To give a simple assessment of the structure of the set of top models for each D. galeata metric, we calculated the total number of fitted predictor terms within all constituent models and determined the proportion of these terms that related to effects of temperature, chlorophyll a phenology and over-wintering population size. Top models, judged by AICc, and their residuals were examined for normality, homoscedasticity, autocorrelation and influence as outlined previously. As in the case of the trend analyses, these diagnostics did not reveal any problems associated with non-normality of residuals, heteroscedasticity or influence, and model parameters were little affected by the inclusion of temporal error structures (generalised least squares regression, results not shown). Furthermore, correlations between predictor variables that co-occurred in the statistical models were examined and found to be generally weak (range of absolute values for Pearson's r = 0.07-0.57), indicating that results were unlikely to be adversely affected by colinearity among predictors. All of the above analyses were conducted using the base, mgcv, nlme and cardidates packages in R version 2.9.2 (Wood & Augustin, 2002; Rolinski et al., 2007; R Development Core Team, 2009; Pinheiro et al., 2010).

In an attempt to unify the results from the regression analyses performed upon each of the D. galeata metrics, a hierarchal modelling procedure was adopted. Specific predictor variables (two temperature variables, ten chlorophyll a phenological metrics and D. galeata over-wintering abundance) were grouped into dummy high-level grouping variables that reflected the conceptual classes to which those predictors belonged i.e. temperature (irrespective of the month being considered) and the onset, peak or central point of the growing season for phytoplankton. This made it possible to test if the higher-level variables were significant predictors across the D. galeata responses. Specifically, hierarchal models were used to examine the evidence for the overall effect of temperature upon phenology and were used to identify which broad phenological attributes of spring phytoplankton growth (onset, peak, growing season mid-point) were important and consistent predictors. The primary advantage of the approach is that it can be used to resolve the key predictors of D. galeata phenological change, while considering a range of possible descriptors for each predictor. This reduces the extent to which results depend upon the specific choice of predictor variable.

For the analysis of each D. galeata metric, changes in phenology were related to the magnitude of the over-wintering population, temperature (within which the temperature data for the current and previous month were nested) and spring chlorophyll a phenology. In the latter case, the three conceptual classes of phenological metric (Onset, Peak and GrowingSeason) were nested within the high-level chlorophyll predictor, and the specific phenological metrics were nested within each of these conceptual classes. The modelling structure was as follows:

oa h hn$_{\text{n}}$ = $_{◊}$Thrra h▮oh hn oa W n

where:

Thrra = uooM▮n hohvM▮n

h▮oh hn = on h $_{?}$hhok $_{\text{s}}$Go▮w ng ho ▮n

Once again, α and $\beta_1 – \beta_8$ represent the intercept and slope parameters, respectively, of the fitted models. The model was implemented using Monte Carlo simulations to arrive iteratively at a converged solution for the values of each of the parameters. The distribution of the parameter estimates from the 10000 simulations performed was used to assess significance of each term in the model. Analyses were run in WinBUGS version 1.4.3 (Lunn et al., 2000).

Results

Patterns of changing D. galeata phenology

During the study period, D. galeata consistently produced a single spring peak each year, and additional summer or late autumn peaks in some years (Fig. 1). Visual inspection of the data suggested that the main spring peak had advanced in its seasonal timing since the initiation of the monitoring programme. Indeed, the calculated phenological metrics suggested that this was the case, though estimated rates of change were rather variable, ranging between 0.37 and 0.67 days per year (Table 1, Fig. 2). All but one of the trends were statistically significant, the exception being that derived when using 25% of the peak spring abundance as a phenological indicator. Comparison of the average seasonal pattern in the 1930s and 2000s, using generalised additive models, showed a considerable and significant advance in the seasonal timing of both the spring population increase and population collapse over the eight decades of study (P=0.001, Fig. 3).

Drivers of changing D. galeata phenology: individual metrics

Data derived using each phenological metric were analysed to determine the evidence for an effect of spring temperature, phytoplankton phenology and over-wintering abundance on the timing of spring population development. Calculated AICc weights indicated that, for each of the D. galeata phenological metrics under consideration, no single statistical model received overwhelming support. Rather, sets of top-ranking models received similar levels of support.

Examination of the top-ranked models, judged by AICc and likelihood ratio tests, showed that the strength of the relationships between spring D. galeata phenology and the driving variables differed markedly when using different phenological metrics (Supporting tables 1-3). Two of the onset metrics were related only weakly to the selected drivers. When the timing of the spring population development was based upon the population size exceeding 25% of the peak abundance, the top-ranked model had a less optimal AICc value than the corresponding null model and explained only 3% of the variability in phenology (Supporting table 1). Similarly, when using the absolute abundance threshold as a phenological metric the top five models had AICc values that were only slightly more optimal than that of the null model, and explained between 7% and 13% of the variability in phenology. These models contained effects of previous and current temperatures and chlorophyll a phenology.

Considering the remaining indicators of the onset of spring D. galeata population development (50% of the peak abundance, 25% of the cumulative spring abundance, Weibull curve onset) some commonalities were evident. For all three of these metrics, top models consistently included an effect of temperature, specifically in April, such that the onset of population development was earlier in warm years (Fig. 4, Supporting table 1). Given that the mean seasonal timing of all three metrics fell within May, this suggested that D. galeata phenology was exhibiting a lagged response to previous temperature conditions. There was some support for an effect of May temperatures when using the accrual of 25% of the spring cumulative abundance as a phenological metric. Top models also consistently included an effect of phytoplankton phenology (Fig. 4), though no single metric appeared consistently throughout these models (Supporting table 1). Rather, models containing a range of different phytoplankton phenology metrics received similar levels of support. Nevertheless, the consistent result was that the phenology of the spring phytoplankton bloom influenced that of the spring D. galeata population. Most effects indicated that D. galeata spring population development occurred later when spring phytoplankton biomass development occurred later. A key difference in the structure of the top models occurred with respect to the relationship between the timing of spring population development and the magnitude of the over-wintering population. Only when phenology was defined using 25% of the cumulative spring abundance, did this effect appear in the top-ranked statistical models. Furthermore, these three measures of the onset of D. galeata spring population development differed considerably with respect to the amount of phenological variability explained by the selected driving variables.

Top models associated with the three measures of the timing of peak D. galeata population size (day of maximum, Weibull peak, GAM peak) consistently included an effect of temperature, particularly previous spring temperatures (Fig. 4, Supporting table 2). The mean seasonal timing of all three metrics fell within June and the majority of the top models associated with these variables included an effect of May temperatures. As was the case for the analyses of the onset of population development, peak population size occurred earlier in warm years. Many of these models also included an effect of phytoplankton phenology on D. galeata phenology, though a number of different phytoplankton phenology metrics appeared in these models with no overwhelming support for one metric in particular (Fig. 4, Supporting table 2). Adjusted R^2 values suggested that peak timings determined by GAMs were more strongly predicted by water temperature and chlorophyll a phenology than timings derived from Weibull functions and the day of the maximum abundance (Supporting table 2).

The same lagged effect of May temperatures was supported by models of the centre of gravity of the spring population and the timing of the 50th percentile of cumulative abundance (Fig. 4, Supporting table 3). Indeed, in both cases, models containing only May temperatures were judged most optimal. In addition to May temperatures, phytoplankton phenology affected spring D. galeata phenology when using either response metric. However, there was evidence for an effect of zooplankton over-wintering populations only when analysing the centre of gravity of the spring D. galeata population development.

Drivers of changing D. galeata phenology: hierarchic modelling

Separate analyses of each D. galeata phenological metric revealed consistent effects of temperature, though the choice of metric affected whether previous or current temperatures were well supported as predictors. While there was consistent evidence for an effect of chlorophyll a phenology on the timing of D. galeata population development, there was little consistency among models regarding the most influential chlorophyll a metric. Furthermore, for some chlorophyll a metrics, the direction of the relationship with D. galeata spring population development was inconsistent when different metrics were used to define D. galeata phenology. For example, the sign of the relationship between D. galeata phenology and the chlorophyll Weibull onset metric differed when D. galeata phenology was quantified using the Weibull onset method and the GAM peak method (Supporting tables 1 and 2). Given these inconsistencies, hierarchal models were used to assess whether consistent higher level relationships could be observed between particular conceptual classes of chlorophyll a phenological metrics and D. galeata phenology.

Table 2 shows the significant results obtained using this approach. Unlike in Supporting tables 1 to 3, all models were run with all terms included due to the Bayesian method of parameter estimation. A significant effect of temperature upon phenology was found for nine of the ten D. galeata metrics. Temperatures from the current and previous months were important predictors for almost all of the onset-type metrics, while for peak and centre of growing season type metrics previous temperatures were consistently important. Significant effects of chlorophyll a phenology were found when using six of the ten metrics to quantify the seasonal timing of D. galeata population development (Table 2). In every case, the timing of the spring chlorophyll peak was a significant component of this overall effect. As was the case in the multi-model analyses, the magnitude of the over-wintering population was found to be a significant predictor only when analysing the accrual of 25% of the cumulative spring abundance or the centre of gravity of the spring population.

Discussion

The principal aim of the present study was to investigate the seasonal dynamics of D. galeata over eight decades for evidence of phenological change, and to explore potential drivers of change. To our knowledge this is the longest existing phenological data set for a freshwater plankton population. Crucially we analysed patterns and drivers of change by simultaneously using multiple phenological metrics, all of which have been employed in the existing literature. Metrics differ mathematically and conceptually and thus reveal different aspects and drivers of phenological change. While the value of multi-metric approaches has been recognised in long-term studies of some plankton populations (Maberly et al., 1994) and in ecologically based water quality assessment (Coates et al., 2007) this approach has rarely been used specifically to examine phenological changes in lake communities (but see Rolinski et al., 2007), with disparate studies essentially employing different and singular definitions of phenology.

The present multi-metric analysis provided robust evidence for a phenological advance in spring D. galeata populations over eight decades. All ten of the metrics showed a tendency towards earlier spring development of D. galaeta populations, and additive modelling showed that this was accompanied by earlier seasonal population declines. When using 25% of the peak abundance as a phenological metric, the seasonal timing of population development appeared to occur extremely early in some years. In these years small and short-lived increases in winter populations, prior to the main period of spring increase, surpassed 25% of the peak spring abundance for the year. Though these seasonal “false starts” resulted in the overall trend being non-significant, the direction of change was in qualitative agreement with that based upon the remaining metrics. These results are in broad agreement with other studies that have documented phenological advances in zooplankton communities (Adrian et al., 2006). However, depending upon which metric was considered, estimated rates of phenological change varied between 3.7 and 6.7 days per decade. This degree of variability is comparable with that found among species, in comparative studies (Parmesan &

Yohe, 2003; Root et al., 2003; Root et al., 2005; Parmesan, 2007). Visser and Both (2005), suggested that phenological changes in focal organisms be compared to the "yardstick" of similar changes in the organisms with which they interact. Differences in metric choice among studies have the potential to influence the outcome of such comparisons. The conceptual class of phenological metric has rarely been included as an explicit determinant of change in interspecific comparisons, since many phenological data sets contain information on only one metric (but see Thackeray et al., 2010).

We explored the influence of spring water temperature, phytoplankton phenology and over-wintering population size upon spring D. galeata phenology. Many of the analyses provided evidence for an effect of spring temperature upon the timing of spring population development, as has been noted in other long-term studies (Gerten & Adrian, 2000; Hampton et al., 2006) and large-scale experiments (Feuchtmayr et al., 2010). Indeed, population models have suggested that long-term changes in temperature have a more significant influence on Daphnia phenology that the seasonal timing of resource availability (Schalau et al., 2008). Warming increases rates of population growth via effects upon rates of maturation, neonate release and egg development (Hall, 1964; Vijverberg, 1980; Weetman & Atkinson, 2004). Furthermore, warming enhances the population grazing rate via increases in individual grazing rates (McMahon, 1965; Burns, 1969) and the increase in the number of grazers. We would therefore expect warming to bring about an earlier onset of population growth, assuming sufficient food resources, and an earlier over-exploitation of phytoplankton food resources leading to an advance in the seasonal collapse of the D. galeata population. Both of these changes were evident when using additive models to compare average seasonal dynamics during the 1930s and 2000s. However, it must be noted that changes in temperature could have indirect effects upon zooplankton population development, by influencing phytoplankton communities via temperature effects on growth and due to correlated changes in the physical structuring of the water column (Feuchtmayr et al., 2011).

The results of this study support the assertion that the phenological effects of warming depend upon the seasonal timing of the warming trend (Wagner & Benndorf, 2007; Huber, Adrian & Gerten, 2010). Specifically, both the multi-model analyses for each metric and the hierarchal modelling suggest that the phenology of spring population development depends upon temperatures at a specific time of year, prior to the main period of population growth. This agrees with the findings of Madgwick et al. (2006); the structure of a plankton community at a particular point in time is a biological response to previous environmental conditions. For eight of the ten metrics, the majority of top regression models included an effect of previous temperatures. For onset-type metrics this equated with April temperatures, while for the later occurring peak and mid-growing season-type metrics May temperatures were more important. Hierarchal modelling confirmed that a significant effect of previous temperatures upon phenology was evident for seven of the ten D. galeata metrics. Aside from this dependence of the precise temperature effect upon the conceptual D. galeata metric class, for two of the onset variables none of the fitted models represented a substantial improvement on the null model. Therefore, the ability to detect a relationship between water temperature and D. galeata phenology, and the precise nature of this relationship, depends upon the chosen phenological metric.

Less consistency was found among regression models with respect to the effects of phytoplankton phenology. The most well supported models for many of the D. galeata metrics in the multi-model analyses included an effect of phytoplankton phenology. In the majority of cases these terms indicated that D. galeata population development occurred later when spring phytoplankton growth occurred later. This is in keeping with the idea that the seasonal timing of food availability should be a decisive factor for grazer phenology, since clutch sizes, and proportions of egg-bearing females will increase in response to seasonal food increase (Guisande & Gliwicz, 1992; George & Reynolds, 1997) However, the top statistical models in these analyses contained a range of chlorophyll a phenological metrics as predictors and did not provide overwhelming support for the importance of one phytoplankton metric in particular. Furthermore, the effects of some phytoplankton metrics were not consistent when different metrics were used to define D. galeata phenology. Hierarchal modelling was therefore used to search for more consistent, higher-level, relationships between D. galeata phenology and phytoplankton phenology. These analyses suggested that, when chlorophyll a phenology was a significant predictor of D. galeata phenology, it was typically the timing of the chlorophyll peak that was most important. So, although a consistent effect of a specific aspect of phytoplankton phenology could not be observed at the metric level, it could be at a higher conceptual level.

The direction of causality in the relationship between phytoplankton and D. galeata phenology may be equivocal. While the seasonal increase in phytoplankton concentrations will permit D. galeata population growth, grazing by the latter will contribute to the decline in spring phytoplankton peak. However, grazing is not the sole determinant of the collapse of spring phytoplankton blooms. For example, spring phytoplankton populations may decline in response to nutrient limitation (Reynolds, 2006; Thackeray et al., 2008) and sedimentation (Huisman & Sommeijer, 2002). Therefore, it is conceivable that factors external to, and independent of, the grazer-phytoplankton interaction influence the phenology of the latter, and in turn influence the phenology of the grazer. We therefore feel that our approach of

logy as a predictor is justified. Changes in the phenology of zooplankton and their phytoplankton resources must be considered within the context of their joint dynamics, but also with respect to external driving forces. The corollary of this dynamic interplay between grazers and resources is that there is the potential for changes in lake trophic state to influence the phenology of zooplankton populations, via effects of nutrient availability on the phenology of phytoplankton (Thackeray et al., 2008; Feuchtmayr et al. 2010). Long-term changes in nutrient availability influence the balance between phytoplankton replication rates and grazing losses, potentially affecting the timing of the onset of food limitation in the grazers. While it is recognised that changing trophic state can influence phytoplankton phenology, there is a need to develop a mechanistic understanding of how this effect might propagate to higher trophic levels.

Some previous studies have suggested that the magnitude of over-wintering populations can have a significant effect upon spring D. galeata phenology (Romare et al., 2005; Hampton et al., 2006). There was only weak evidence for this in the North Basin of Windermere. Such an effect was consistently observed only when two metrics were used to indicate D. galeata phenology. This particular result highlights the importance that the choice of phenological metric can have on the ecological interpretation of change.

In the regression analyses of individual D. galeata phenological metrics, maximal adjusted R^2 values indicated that approximately half of the variability in the seasonal timing of spring population development could be explained by the environmental drivers that were investigated. While some of this unexplained variability may have arisen due to the effects of sampling and sub-sampling errors, other important drivers of phenological change are

585 and in turn influence the phenology of the grazer. We therefore feel that our approach of

probably present. In the analysis chlorophyll concentrations were used to quantify the timing of the seasonal phytoplankton peak, making the implicit assumption that all taxa in the spring phytoplankton community were ingestible to Daphnia. Cryptomonas spp., Chlorella spp. and Asterionella formosa (Hassall) that are typically present and abundant throughout the spring bloom in Windermere (Reynolds & Irish, 2000) can be consumed by Daphnia (Schindler, 1971; Nadin-Hurley & Duncan, 1976; Reynolds, 2006). There is also evidence that Daphnia are capable of consuming filamentous phytoplankton (Nadin-Hurley & Duncan, 1976; Fulton, 1988; Epp, 1996) such that Aulacoseira, which also makes a substantial contribution to the Windermere spring phytoplankton bloom, could also be consumed. However, at present, detailed phytoplankton species data are not available for all of the study years, reducing the feasibility of a more detailed assessment of the food spectrum. The role of seasonal changes in food quality in determining D. galeata phenology would perhaps be better studied by examining sestonic carbon, phosphorus and fatty acid content; attributes that are known to affect Daphnia reproductive parameters (Sterner & Schulz, 1998). Since the nutritive content of phytoplankton cells is a variable property (Sterner et al., 1998), detailed and direct study would be needed to resolve this issue rather than making simple assumptions based upon the available phytoplankton species data. It is also plausible that long-term changes in the phenology and abundance of potential competitors and predators (Wagner & Benndorf, 2007), could affect D. galeata phenology by affecting population growth and loss rates, respectively.

In the case of the phenological metric based upon an absolute abundance threshold, it must also be noted that changes in phenology could be confounded with changes in population size. Miller-Rushing, Inouye & Primack (2008) noted that changes in the population size of flowering plants may alter the time of year at which first flowering dates are detected even if the timing of peak flowering remains unchanged, while Stine, Huybers & Fung (2009) noted that analyses of long-term changes in the phase of the annual temperature cycle will be confounded with changes in annual mean temperature if analyses are based upon the seasonal timing of absolute temperature thresholds. Therefore, it is conceivable that changes in mean D. galeata abundance among years might affect the seasonal timing of any given population size being achieved, even if the timing of population increase has not changed. There is a need to examine the influence of changing population size upon different phenological metrics, as this has not been rigorously analysed in the plankton literature.

The effect of metric choice on observed patterns of change has been discussed in studies of terrestrial plants and birds (Miller-Rushing et al., 2008; Lehikoinen & Sparks, 2010), but had not yet received in-depth consideration for plankton communities. The former studies have prompted the general recommendation that, when possible, phenological studies should use metrics that capture the whole seasonal distribution of activity for the focal organisms (Miller-Rushing et al., 2008). While this might not be possible for many existing phenological data sets, the temporal organisation of plankton monitoring programmes allows such an approach. There is probably no single, “best”, metric to use in such studies since individual metrics or classes of metrics will be more or less suited to the ecological questions being addressed. The exact choice of metrics used in any one analysis should be informed by conceptual considerations of the ecological processes under study, and by the underlying hypotheses being tested. If a range of candidate metrics are relevant to those processes, testing all of them may strengthen inferences about ecological processes. It is clear that there is a need for an underlying mechanistic theory of the drivers of plankton phenological change and for experimental and modelling approaches that can disentangle the drivers of phenology for different broad classes of phenological event. Future studies should explore combining multiple metrics to create a basket of phenological indicators that might then be used to explore the drivers and consequences of phenological 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). In addition, some of the required sample processing was undertaken by the Freshwater Biological Association.

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.

Berger S.A., 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.

Burnham K.P. & Anderson D.R. (2002) Model Selection and Multimodel Inferences: A Practical Information-theoretic Approach, Springer-Verlag, New York.

Burns C.W. (1969) Relation between filtering rate, temperature, and body size in four species of Daphnia. Limnology and Oceanography, 14, 693-700.

Carroll E.A., Sparks T.H., Collinson N. & Beebee T.J.C. (2009) Influence of temperature on the spatial distribution of first spawning dates of the common frog (Rana temporaria) in the UK. Global Change Biology, 15, 467-473.

Chadwick E.A., Slater F.M. & Ormerod S.J. (2006) Inter- and intraspecific differences in climatically mediated phenological change in coexisting Triturus species. Global Change Biology, 12, 1069-1078.

Coates S., Waugh A., Anwar A. & Robson M. (2007) Efficacy of a multi-metric fish index as an analysis tool for the transitional fish component of the Water Framework Directive. Marine Pollution Bulletin, 55, 225-240.

Conner J.S., Seborg, D. E. & Larimore W.E. (2004) Analysis of the ΔAIC statistic for optimal detection of small changes in dynamic systems. Proceedings of the 2004 American Control Conference, 5, 4408-4413.

Doi H. (2008) Delayed phenological timing of dragonfly emergence in Japan over five decades. Biology Letters, 4, 388-391.

Edwards M. & Richardson A.J. (2004) Impact of climate change on marine pelagic phenology and trophic mismatch. Nature, 430, 881-884.

Efron B. & Tibshirani R.J. (1993) An Introduction to the Bootstrap, Chapman & Hall, New York.

Epp G.T. (1996) Grazing on filamentous cyanobacteria by Daphnia pulicaria. Limnology and Oceanography, 41, 560-567.

Ferguson C.A., Carvalho L., Scott E.M., Bowman A.W. & Kirika A. (2008) Assessing ecological responses to environmental change using statistical models. Journal of Applied Ecology, 45, 193-203.

Feuchtmayr H., Thackeray S.J., Jones I.D., De Ville M., Fletcher J., James B. & Kelly J. (this issue) Spring phytoplankton phenology: are patterns and drivers of change consistent among lakes in the same climatological region? Freshwater Biology, xx, xxx-xxx.

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, 1715-1726.

Fulton R.S. (1988) Grazing on filamentous algae by herbivorous zooplankton. Freshwater Biology, 20, 263-271.

Geller W. (1985) Production, food utilisation and losses of two coexisting, ecological different Daphnia species. Archiv für Hydrobiologie Ergebnisse der Limnologie, 21, 67-79.

George D.G. & Reynolds C.S. (1997) Zooplankton-phytoplankton interactions: the case for refining methods, measurements and models. Aquatic Ecology, 31, 59-71.

George D.G., Talling J.F. & 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.

Greve W., Prinage S., Zidowitz H., Nast J. & Reiners F. (2005) On the phenology of North Sea ichthyoplankton. ICES Journal of Marine Science, 62, 1216-1223.

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

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

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

Harrington R., Woiwod I. & Sparks T. (1999) Climate change and trophic interactions. Trends in Ecology and Evolution, 14, 146-150.

Huber V., Adrian R. & Gerten D. (2010) A matter of timing: heat wave impact on crustacean zooplankton. Freshwater Biology, 55, 1769-1779.

Huisman J. & Sommeijer B. (2002) Maximal sustainable sinking velocity of phytoplankton. Marine Ecology-Progress Series, 244, 39-48.

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 M.L. Parry & O.F. Canziani & J.P. Palutikof & P.J. Van Der Linden & C.E. Hanson), pp. 7-22. Cambridge University Press, Cambridge.

Jones I.D., Winfield I.J. & 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.

Lehikoinen E. & Sparks T.H. (2010) Changes in migration. In: Effects of Climate Change on Birds. (Eds A.P. Møller & W. Fiedler & P. Berthold), pp. 89-112. Oxford University Press, Oxford.

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

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

Maberly S.C., Hurley M.A., Butterwick C., Corry J.E., Heaney S.I., Irish A.E., Jaworski G.H.M., Lund J.W.G., Reynolds C.S. & Roscoe J.V. (1994) The rise and fall of Asterionella formosa in the south basin of Windermere: analysis of a 45-year series of data. Freshwater Biology, 31, 19-34.

Madgwick G., Jones I.D., Thackeray S.J., Elliott J.A. & Miller H.J. (2006) Phytoplankton communities and antecedent conditions: high resolution sampling in Esthwaite Water. Freshwater Biology, 51, 1798-1810.

Mcmahon J.W. (1965) Some physical factors influencing the feeding behaviour of Daphnia magna Straus. Canadian Journal of Zoology, 43, 603-611.

Meis S., Thackeray S.J. & Jones I.D. (2009) Effects of recent climate change on phytoplankton phenology in a temperate lake. Freshwater Biology, 54, 1888-1898.

Miller-Rushing A.J., Inouye D.W. & Primack R.B. (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.

Nadin-Hurley C.M. & Duncan A. (1976) A comparison of daphnid gut particles with the sestonic particles present in two Thames Valley reservoirs throughout 1970 and 1971. Freshwater Biology, 6, 109-123.

Parmesan C. (2007) Influences of species, latitudes and methodologies on estimates of phenological response to global warming. Global Change Biology, 13, 1860-1872.

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

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 (2009) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ramsbottom A.E. (1976) Depth Charts of the Cumbrian Lakes., Freshwater Biological Association.

Reynolds C.S. (2006) Ecology of Phytoplankton, Cambridge University Press, Cambridge.

Reynolds C.S. & Irish A.E. (2000) The Phytoplankton of Windermere (English Lake District). Freshwater Biological Association, Ambleside.

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.

Romare P., Schindler D.E., Scheuerell M.D., Scheuerell J.M., Litt A.H. & Shepherd J.H. (2005) Variation in spatial and temporal gradients in zooplankton spring development: the effect of climatic factors. Freshwater Biology, 50, 1007-1021.

Root T.L., Macmynowski D.P., Mastrandrea M.D. & Schneider S.H. (2005) Human-modified temperatures induce species changes: Joint attribution. Proceedings of the National Academy of Sciences of the United States of America, 102, 7465-7469.

Root T.L., Price J.T., Hall K.R., Schneider S.H., Rosenzweig C. & Pounds J.A. (2003) Fingerprints of global warming on wild animals and plants. Nature, 421, 57-60.

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.

Schindler J.E. (1971) Food quality and zooplankton nutrition. Journal of Animal Ecology, 40, 589-595.

Sterner R.W., Clasen J., Lampert W. & Weisse T. (1998) Carbon:phosphorus stoichiometry and food chain production. Ecology Letters, 1, 146-150.

Sterner R.W. & Schulz K.L. (1998) Zooplankton nutrition: recent progress and a reality check. Aquatic Ecology, 32, 261-279.

Stine, A. R., Huybers, P. & Fung, I. Y. (2009) Changes in the phase of the annual cycle of surface temperature. Nature, 457, 435-441.

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

Thackeray S.J., George D.G., Jones R.I. & Winfield I.J. (2005) Vertical heterogeneity in zooplankton community structure: a variance partitioning approach. Archiv fur Hydrobiologie, 164, 257-275.

Thackeray S.J., Jones I.D. & Maberly S.C. (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 S.J., Sparks T.H., Frederiksen M., Burthe S., Bacon P.J., Bell J.R., Botham M.S., Brereton T.M., Bright P.W., Carvalho L., Clutton-Brock T., Dawson A., Edwards M., Elliott J.M., Harrington R., Johns D., Jones I.D., Jones J.T., Leech D.I., Roy D.B., Scott W.A., Smith M., Smithers R.J., Winfield I.J. & Wanless S. (2010) Trophic level asynchrony in rates of phenological change for marine, freshwater and terrestrial environments. Global Change Biology, 16, 3304-3313.

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 M.E. & 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.

Wagner A. & Benndorf J. (2007) Climate-driven warming during spring destabilises a Daphnia population: a mechanistic food web approach. Oecologia, 151, 351-364.

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 D.E. (2004) Climate change uncouples trophic interactions in an aquatic ecosystem. Ecology, 85, 2100-2106.

Winfield I.J., Fletcher J.M., Hewitt D.P. & James J.B. (2004) Long-term trends in the timing of the spawning season of Eurasian perch (Perca fluviatilis) in the North Basin of Windermere, U.K. In: Proceedings of Percis III: The Third International Percid Fish Symposium (Eds T.P. Barry & J.A. Malison), pp. 95-96.

Wood S.N. & Augustin N.H. (2002) GAMs with integrated model selection using penalized regression splines and applications to environmental modelling. Ecological Modelling, 157, 157-177.

Table 1 Linear models of trends in the seasonal timing of Daphina galeata spring population development. Shown are the slope parameter of each model (in d yr^{-1}) and its respective standard error [B(s.e.)], the F statistic and its associated P value (F, P) and the adjusted R-squared (Adj. R^2). F statistic degrees of freedom are 1,36 for Weibull curve onset/peak models and 1,38 for all other models.

Phenological metric	Metric type	B (s.e.)	F	P	Adj. R^2
First day abundance exceeds 0.2 L^{-1}	Onset	-0.67 (0.12)	31.68	<0.001	0.44
Exceed 25% peak abundance	Onset	-0.40 (0.21)	3.65	0.064	0.06
Exceed 50% peak abundance	Onset	-0.41 (0.11)	14.19	<0.001	0.25
Accrual of 25% of cumulative abundance	Onset	-0.40 (0.11)	13.60	<0.001	0.24
Weibull curve onset	Onset	-0.38 (0.11)	12.06	0.001	0.23
GAM peak	Peak	-0.55 (0.09)	35.69	<0.001	0.47
Day of maximum abundance	Peak	-0.53 (0.11)	21.74	<0.001	0.35
Weibull curve peak	Peak	-0.50 (0.10)	23.87	<0.001	0.38
Centre of gravity	Growing season	-0.37 (0.09)	15.36	<0.001	0.27
Accrual of 50% of cumulative abundance	Growing season	-0.47 (0.09)	26.47	<0.001	0.40

Table 2 Hierarchal models for Daphnia galeata phenological change, as indicated by each calculated metric. All models were run with all terms present and only those terms significant at the 10% level are shown here. Estimated slope parameters together with 95% confidence intervals are given for each high-level predictor [B(95% C.I.)], as well as the corresponding P value (P). Units for slope parameters are d °C^{-1} for temperature effects, d d^{-1} for chlorophyll phenology effects and d Daphnia L^{-1} for effects of the overwintering population. For each high-level predictor, the associated lower-level predictor variables which have a significant effect on D. galeata phenology are indicated. Temperature effects are grouped by whether the key variable is the mean value from the Previous or Current month. The effects of Chlorophyll a phenology are subdivided according to whether onset, peak or centre of growing season type metrics are important predictors.

High-level predictors	B (95% C.I.)	P	Lower-level predictors
First day abundance exceeds 0.2 L^{-1}			
Temperature	-7.41 (-13.19,-1.63)	0.017	Current
Exceed 25% peak abundance			
No significant terms			
Exceed 50% peak abundance			
Temperature	-6.29 (-11.17,-1.41)	0.017	Previous
Chlorophyll a phenology	0.30 (-0.03,0.63)	0.079	Peak
Accrual of 25% of cumulative abundance			
Temperature	-7.44 (-12.66,-2.23)	0.003	Previous Current
Chlorophyll a phenology	0.55 (0.22,0.88)	0.003	Peak
Over-wintering population	-130.20 (-237.7,-22.78)	0.024	
Weibull curve onset			
Temperature	-5.73 (-10.11,-1.35)	0.015	Current

Chlorophyll a phenology	0.31 (-0.02,0.64)	0.073	Onset Peak
GAM peak			
Temperature	-9.26 (-13.25,-5.29)	0.000	Previous
Chlorophyll a phenology	0.32 (-0.04,0.67)	0.085	Peak
Day of maximum abundance			
Temperature	-8.72 (-15.12,-2.32)	0.012	Previous
Chlorophyll a phenology	0.42 (0.09,0.76)	0.018	Peak
Weibull curve peak			
Temperature	-5.89 (-10.87,-0.91)	0.027	Previous
Centre of gravity			
Temperature	-5.89 (-10.05,-1.73)	0.010	Previous
Chlorophyll a phenology	0.34 (0.03,0.65)	0.038	Peak
Over-wintering population	-73.92 (-154.9,7.1)	0.008	
Accrual of 50% of cumulative abundance			
Temperature	-6.81 (-10.89,-2.73)	0.003	Previous

Figure legends

Fig. 1 Proportional symbol plot of seasonal and inter-annual variations in Daphnia galeata abundance in the North Basin of Windermere. Scale bar shows the relationship between symbol size and population size.

Fig. 2 Inter-annual variation in the seasonal timing of spring Daphnia galeata population development, according to the ten phenological metrics described in the text. On each plot the fitted regression line for the long-term trend (see Table 1) has been superimposed. All data are plotted as annual anomalies from the mean of the respective series.

Fig. 3 Smoothers of seasonal variation in Daphnia galeata abundance in the 1930s (dashed line) and 2000s (solid black line). Grey lines are a series of realisations of abundance based upon sampling from a Gamma distribution centred on the 1930s, baseline, period. The 1930s smoother is fitted to pooled 1934-1936 data and the 2000s smoother is fitted to pooled 2004-2006 data.

Fig. 4 For each Daphnia galeata metric, the percentage of predictor terms in top models that indicate effects of temperature (black), chlorophyll a phenology (grey) and the magnitude of the over-wintering population (white). D.galeata metrics are grouped according to conceptual class (onset, peak and growing season), with classes separated by vertical dashed lines. See online supporting information for more details of fitted models.

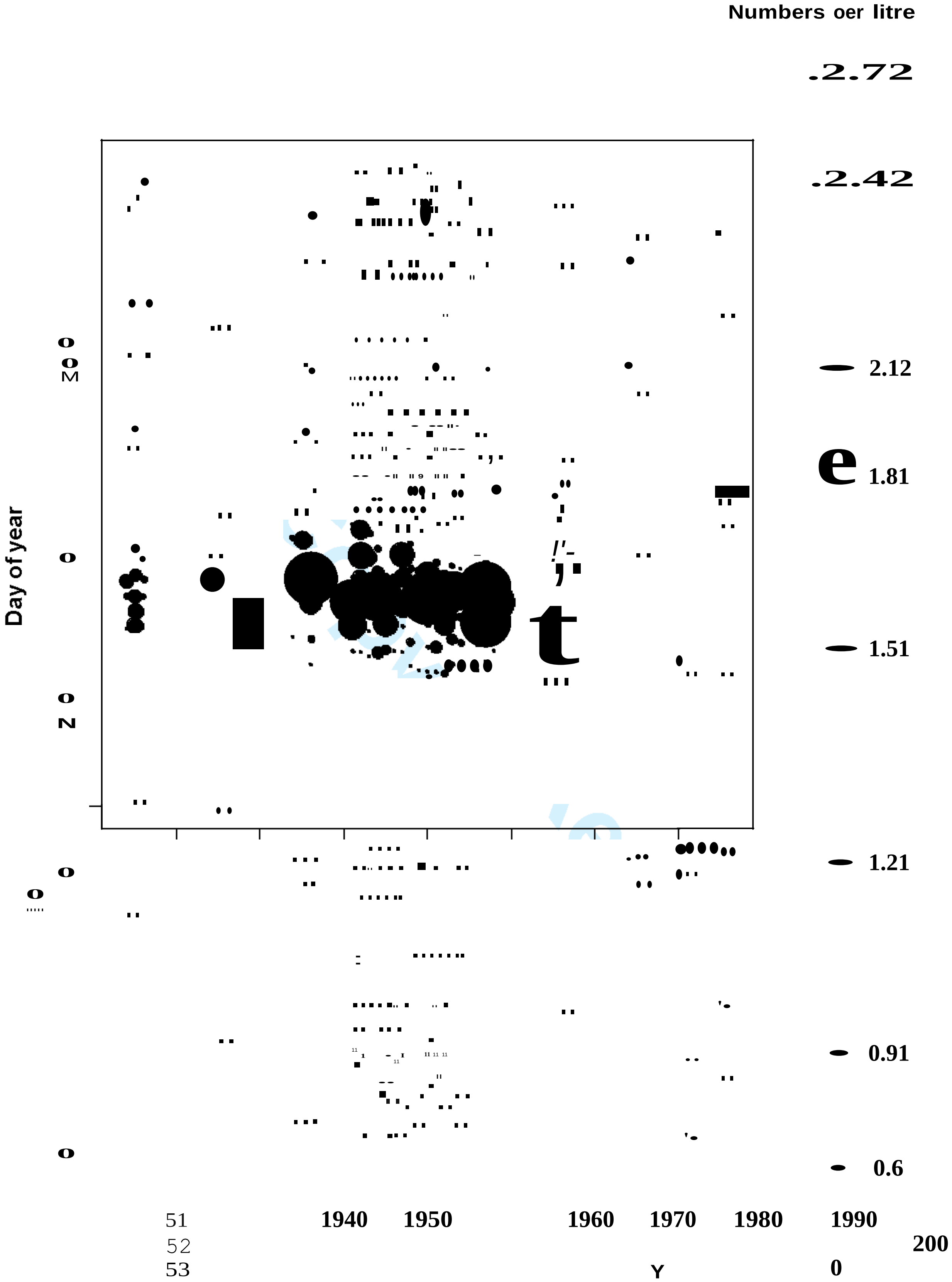
Numbers oer litre
2.72
2.42
2.12
1.81
1.51
1.21
0.91
0.6
Day of year
1940
1950
1960
1970
1980
1990
2000
Year

Fig. 1

- **0.3**

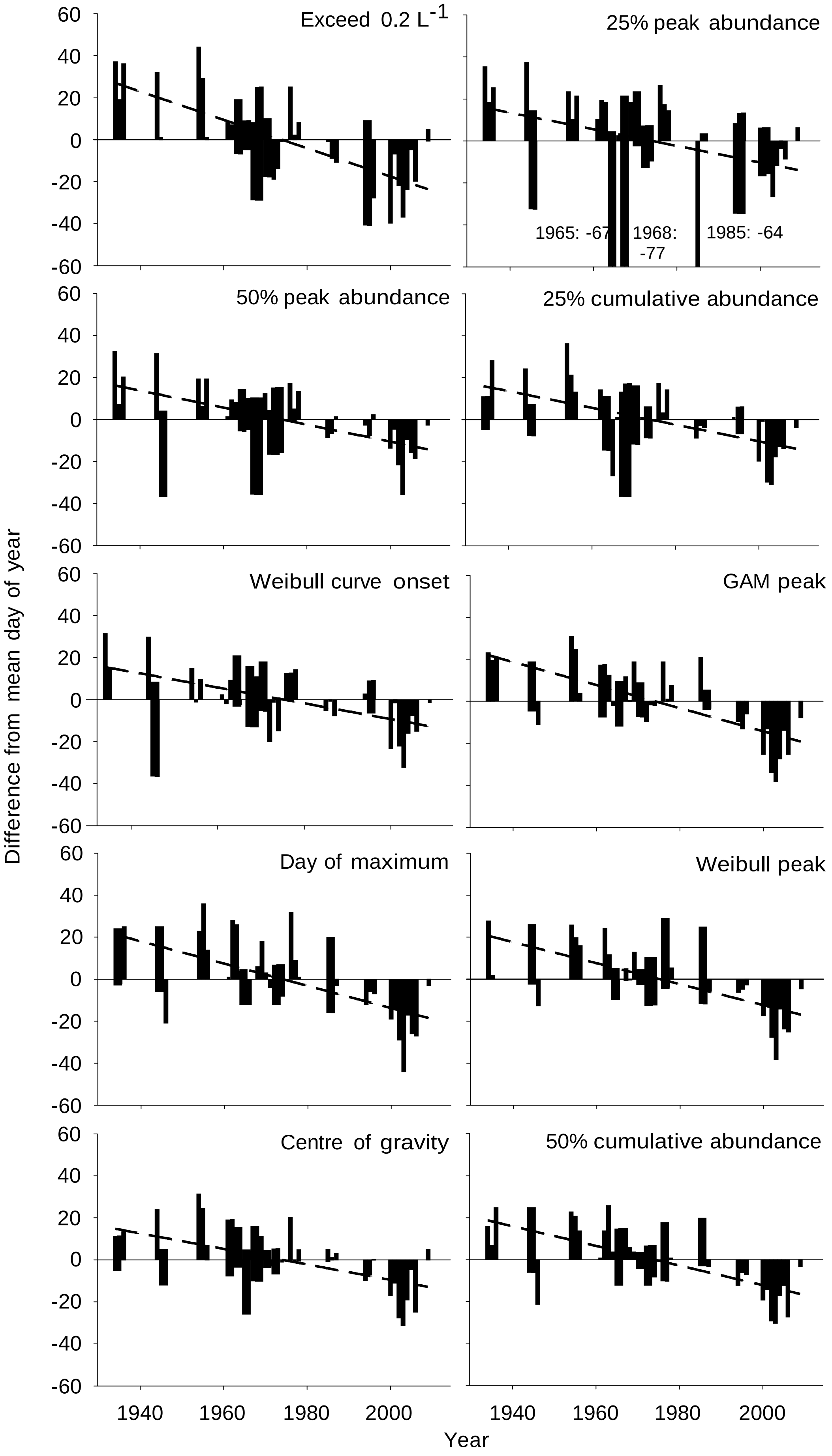
Exceed 0.2 L^{-1}
25% peak abundance
1965: -67
1968: -77
1985: -64
50% peak abundance
25% cumulative abundance
Weibull curve onset
GAM peak
Day of maximum
Weibull peak
Centre of gravity
50% cumulative abundance
Difference from mean day of year
60
40
20
0
-20
-40
-60
1940
1960
1980
2000
Year

Fig. 2

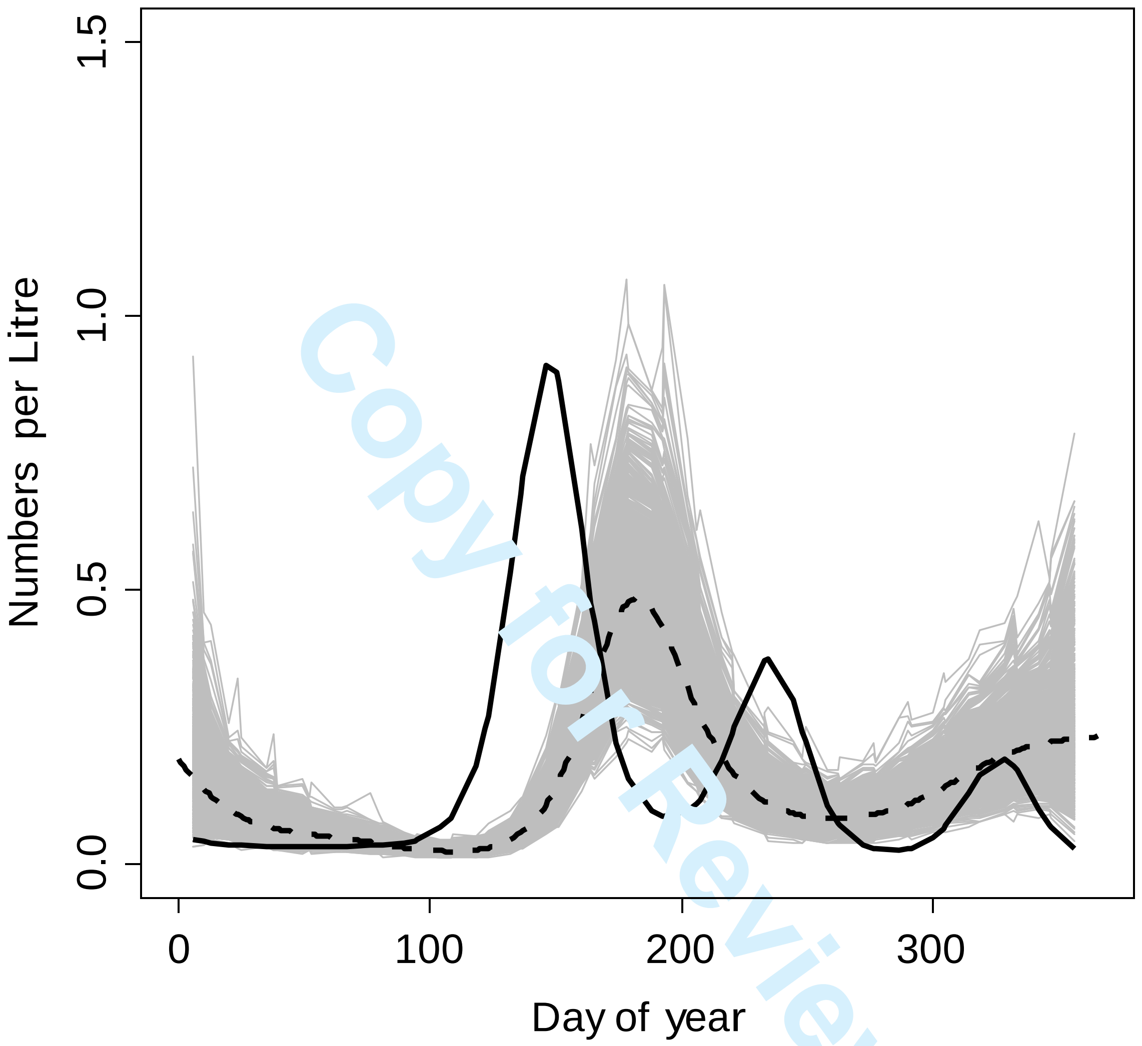

Fig. 3

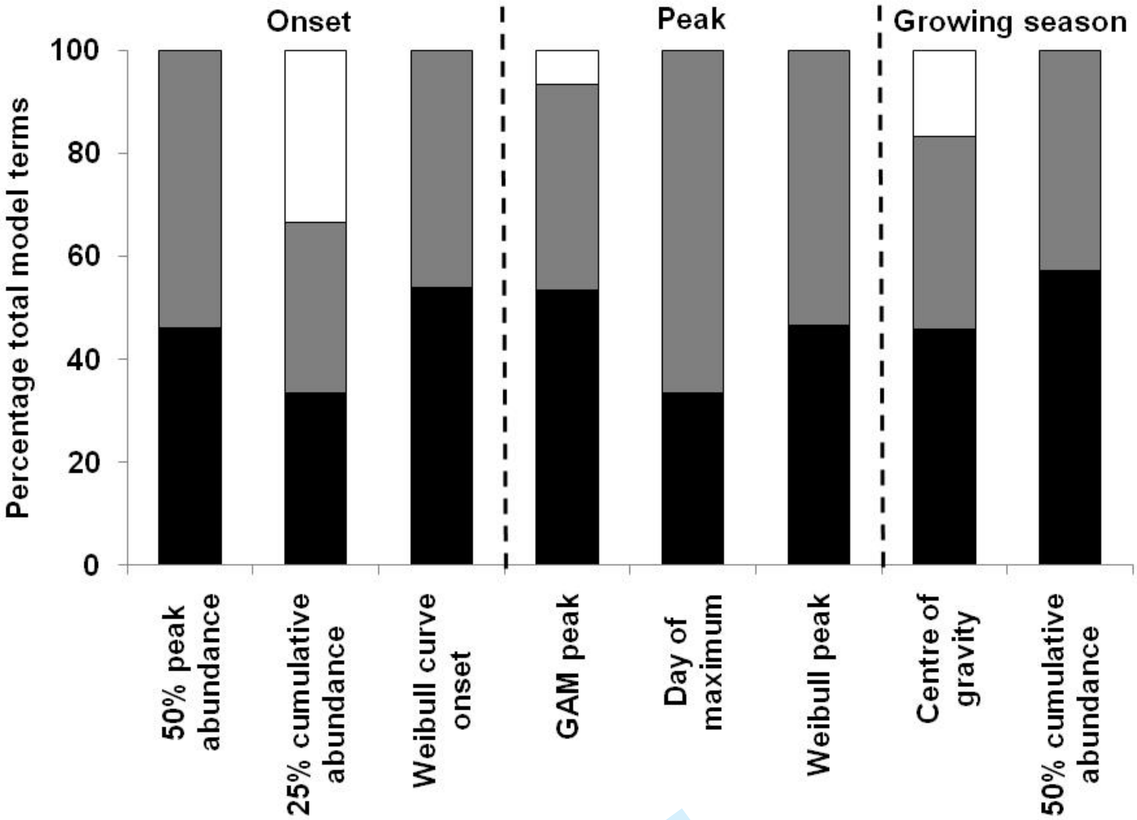
Onset
Peak
Growing season
Percentage total model terms
100
80
60
40
20
0
50% peak abundance
25% cumulative abundance
Weibull curve onset
GAM peak
Day of maximum
Weibull peak
Centre of gravity
50% cumulative abundance

Fig. 4

Supporting table 1 Models for Daphnia phenological change, using onset-type metrics. Model selection was based upon differences in Akaike's Information Criterion adjusted for small sample size (ΔAIC_c) and the Akaike weight (w_i). Also shown are the adjusted R-squared values (Adj. R^2) and the number of parameters in each model, inclusive of the error variance (k). The most parsimonious model is displayed in bold. Where models judged less optimal than the most parsimonious model, by AIC_c, are nested within the most parsimonious model a likelihood ratio test was performed on the ΔAIC value. This was done to assess the significance of the difference in support for the two models. The P value associated with this test is given only for models nested within the top model (LRT P). DOM = day of maximum, CofG = centre of gravity. + or – is used to indicate the sign of the slope parameter estimated for each relationship.

Predictors	k	Adj. R^2	ΔAIC_c	w_i	LRT P
First day abundance exceeds 0.2 L^{-1}					
Null model	2				
May temp (-)	3	0.13	0.00	0.08	
April temp (-)	3	0.12	0.38	0.06	
50% cumulative Chlorophyll (+)	3	0.07	1.58	0.03	
Chlorophyll DOM (+)	3	0.07	1.59	0.03	
May temp (-), Chlorophyll DOM (+)*	4	0.13	1.78	0.03	0.089
Exceed 25% peak abundance					
Null model	2				
Daph. Jan (-) **	3	0.03	0.00	0.07	
Exceed 50% peak abundance					
Null model	2				
April temp (-)	3	0.20	0.00	0.12	
April temp (-), Chlorophyll DOM (+)	4	0.23	0.95	0.07	0.152

April temp (-), Chlorophyll CofG (+)	4	0.21	1.49	0.06	0.108
Chlorophyll DOM (+)	3	0.13	2.17	0.04	
April temp (-), 25% cumulative Chlorophyll (+)	4	0.19	2.22	0.04	0.069
Chlorophyll CofG (+)	3	0.12	2.30	0.04	
April temp (-), Chlorophyll GAM peak (+)	4	0.19	2.34	0.04	0.064
April temp (-), 50% cumulative Chlorophyll (+)	4	0.18	2.37	0.04	0.063
Accrual of 25% of cumulative abundance					
Null model	2				
April temp (-), Chlorophyll DOM (+), Daph. Jan (-)	5	0.50	0.00	0.40	
May temp (-), Chlorophyll DOM (+), Daph. Jan (-)	5	0.45	2.46	0.12	
April temp (-), Chlorophyll GAM peak (+), Daph. Jan (-)	5	0.43	3.44	0.07	
April temp (-),50% cumulative Chlorophyll (+), Daph. Jan (-)	5	0.40	4.57	0.04	
Weibull curve onset					
Null model	2				
April temp (-)	3	0.42	0.00	0.19	
April temp (-), Chlorophyll >0.1 mgC L^{-1} (+)	4	0.42	1.61	0.09	0.104
April temp (-), Chlorophyll DOM (+)	4	0.41	2.09	0.07	0.077
April temp (-), 25% peak Chlorophyll (-)	4	0.40	2.39	0.06	0.064
April temp (-), Chlorophyll GAM peak (+)	4	0.40	2.42	0.06	0.063
April temp (-), 50% peak Chlorophyll (-)	4	0.40	2.54	0.05	0.059
April temp (-), Chlorophyll Weibull onset (-)	4	0.40	2.76	0.05	0.051

*last model with AICc less than that of the null model

**the most highly ranked model was not an improvement on the null model

Supporting table 2 Models for Daphnia phenological change, using peak-type metrics. Model selection was based upon differences in Akaike’s Information Criterion adjusted for small sample size (ΔAIC_c) and the Akaike weight (w_i). Also shown are the adjusted R-squared values (Adj. R^2) and the number of parameters in each model, inclusive of the error variance (k). The most parsimonious model is displayed in bold. Where models judged less optimal than the most parsimonious model, by AIC_c, are nested within the most parsimonious model a likelihood ratio test was performed on the ΔAIC value. This was done to assess the significance of the difference in support for the two models. The P value associated with this test is given only for models nested within the top model (LRT P). DOM = day of maximum, CofG = centre of gravity. + or – is used to indicate the sign of the slope parameter estimated for each relationship.

Predictors	k	Adj. R^2	ΔAIC_c	w_i	LRT P
GAM peak					
Null model	2				
May temp (-)	3	0.54	0.00	0.18	
May temp (-), Chlorophyll DOM (+)	4	0.55	1.30	0.09	0.121
May temp (-), Chlorophyll GAM peak (+)	4	0.55	1.50	0.08	0.107
May temp (-), 50% cumulative Chlorophyll (+)	4	0.54	1.72	0.07	0.094
May temp (-), Chlorophyll Weibull onset (+)	4	0.54	2.13	0.06	0.073
May temp (-), 25% cumulative Chlorophyll (+)	4	0.53	2.47	0.05	0.059
May temp (-), Daph. Jan (-)	4	0.53	2.49	0.05	0.058
May temp (-), Chlorophyll Weibull peak (+)	4	0.53	2.59	0.05	0.055
Day of maximum abundance					

Null model	2				
May temp (-), Chlorophyll DOM (+)	4	0.30	0.00	0.14	
Chlorophyll DOM (+)	3	0.22	0.79	0.09	0.055
Weibull curve peak					
Null model	2				
May temp (-)	3	0.26	0.00	0.14	
May temp (-), Chlorophyll DOM (+)	4	0.31	0.40	0.11	0.110
May temp (-), 50% cumulative Chlorophyll (+)	4	0.28	1.32	0.07	0.124
May temp (-), Chlorophyll GAM peak (+)	4	0.28	1.36	0.07	0.121
May temp (-), 25% cumulative Chlorophyll (+)	4	0.25	2.20	0.05	0.072
May temp (-), Chlorophyll >0.1 mgC L^{-1} (+)	4	0.24	2.55	0.04	0.058
Chlorophyll DOM (+)	3	0.17	2.66	0.04	
May temp (-), 25% peak Chlorophyll (+)	4	0.23	2.76	0.03	0.051
50% cumulative Chlorophyll (+)	3	0.17	2.79	0.03	

Supporting table 3 Models for Daphnia phenological change, using growing season-type metrics. Model selection was based upon differences in Akaike's Information Criterion adjusted for small sample size (ΔAIC_c) and the Akaike weight (w_i). Also shown are the adjusted R-squared values (Adj. R^2) and the number of parameters in each model, inclusive of the error variance (k). The most parsimonious model is displayed in bold. Where models judged less optimal than the most parsimonious model, by AIC_c, are nested within the most parsimonious model a likelihood ratio test was performed on the ΔAIC value. This was done to assess the significance of the difference in support for the two models. The P value associated with this test is given only for models nested within the top model (LRT P). DOM = day of maximum, CofG = centre of gravity. + or – is used to indicate the sign of the slope parameter estimated for each relationship.

Predictors	k	Adj. R^2	ΔAIC_c	w_i	LRT P
Centre of gravity					
Null model	2				
May temp (-)	3	0.21	0.00	0.12	
May temp (-), Daph. Jan (-)	4	0.25	0.73	0.08	0.140
May temp (-), Chlorophyll DOM (+), Daph. Jan (-)	5	0.28	1.61	0.05	0.104
May temp (-), Chlorophyll Weibull onset (+)	4	0.20	2.25	0.04	0.067
May temp (-), Chlorophyll GAM peak (+), Daph. Jan (-)	5	0.26	2.30	0.04	0.124
May temp (-), Chlorophyll DOM (+)	4	0.19	2.35	0.04	0.064
May temp (-), Chlorophyll GAM peak (+)	4	0.19	2.56	0.03	0.056
May temp (-), Chlorophyll Weibull onset (+), Daph. Jan (-)	5	0.25	2.63	0.03	0.106
May temp (-), 25% cumulative Chlorophyll (+)	4	0.18	2.65	0.03	0.053

May temp (-), 50% cumulative Chlorophyll (+)	4	0.18	2.67	0.03	0.053
May temp (-), Chlorophyll >0.1 mgC L^{-1} (-)	4	0.18	2.71	0.03	0.051
Accrual of 50% of cumulative abundance					
Null model	2				
May temp (-)	3	0.33	0.00	0.22	
May temp (-), Chlorophyll GAM peak (+)	4	0.31	2.39	0.07	0.062
May temp (-), Chlorophyll CofG (+)	4	0.31	2.50	0.06	0.058
May temp (-), Chlorophyll DOM (+)	4	0.31	2.55	0.06	0.056