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Separating effects of climatic drivers and biotic feedbacks on seasonal plankton dynamics: no sign of trophic mismatch

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SUMMARY

1. Climate change may impact most strongly on temperate lake plankton communities in spring, when light availability and water temperature change rapidly due to thermal stratification. Effects of changing light and temperature on one food-web component transfer to other components, producing a complex interplay between physical drivers and biotic feedbacks. Understanding this interplay is important, because altered climate regimes could result in phenological mismatch between the phytoplankton spring bloom and the timing of maximum food requirements of grazers.

2. To separate direct effects of light and temperature on spring plankton dynamics from effects mediated through micro- and mesograzer feedbacks, we manipulated water temperature, stratification depth and presence/absence of the mesograzer *Daphnia* in lake mesocosms.

3. In early spring, stratification depth and water temperature directly influenced the light supply to phytoplankton and the growth rates of all plankton groups. Subsequently, indirect effects, including light-dependent food supply to grazers and temperature-dependent grazing pressure, became increasingly important. Phytoplankton and *Daphnia* peaked earlier in warmer treatments and reached higher peaks when stratification depth was shallower. Ciliates responded positively to increased food density and higher temperature and subsequently affected the taxonomic composition, but not the total biomass, of phytoplankton.

4. In the absence of *Daphnia*, phytoplankton did not enter a distinct clear water phase. When present, *Daphnia* caused an extended clear water phase, maintaining phytoplankton and ciliates at low levels throughout early summer and suppressing all direct effects of physical drivers on these plankton groups.

5. Our *Daphnia* treatments mimicked the high and low fish predation settings of the largely descriptive, recently revised Plankton Ecology Group (PEG) model of seasonal plankton succession and explored their responses to climate change scenarios. The results largely support the PEG model, but attribute greater importance to early season temperature effects and later season grazing effects of *Daphnia*.

6. In warmer treatments, the timing of phytoplankton and zooplankton peaks tended to be more closely coupled, and temperature did not affect the height of zooplankton peaks. In line with other experiments, these results do not support the widely held concern that warming may create a trophic mismatch between phytoplankton and zooplankton and reduce spring zooplankton production.

Keywords: mesocosm experiment, plankton succession, stratification depth, trophic mismatch, water temperature

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Introduction

Global warming affects light-dependent and temperature-dependent processes differently and may have its strongest impact on the plankton of lakes and oceans in spring, when both light levels and water temperatures change rapidly with the onset of thermal stratification (Tirok & Gaedke, 2006; Peeters *et al.*, 2007a). The typical sequence of spring phenological events in temperate lakes begins with a phytoplankton bloom followed by an increase in grazer biomass and a subsequent clear water phase with low levels of phytoplankton. This sequence is, in part, the result of trophic interactions between phytoplankton and various micro- and mesograzers (Lampert *et al.*, 1986; Sarnelle, 1993; Tirok & Gaedke, 2006; Peeters *et al.*, 2007b; Sommer *et al.*, 2012). Differential effects of light and temperature on autotrophic and heterotrophic components of the planktonic food-web result in a complex interplay between abiotic drivers and biotic feedbacks, which creates the typical seasonal pattern in plankton dynamics (Aberle, Lengfellner & Sommer, 2007; Berger *et al.*, 2010). Understanding this interplay is important, because it is anticipated that the combination of seasonal light and temperature regimes may change in the future (Hondzo & Stefan, 1993; Stenseth *et al.*, 2002; Livingstone, 2003; Blenckner *et al.*, 2007; George, Hurley & Hewitt, 2007). It has been repeatedly suggested that climate change may cause a phenological separation of the phytoplankton spring bloom from the timing of maximum food requirements of their grazers, leading to a trophic mismatch of pelagic grazers and their algal food with negative consequences for secondary production (Winder & Schindler, 2004b; de Senerpont Domis *et al.*, 2007; reviewed in Thackeray, 2012).

In deep, temperate lakes, the onset of the spring phytoplankton bloom is correlated with the onset of thermal stratification, which establishes a well-lit mixed surface layer. In contrast, the timing of phenological events related to metazoan grazing such as the spring peak in crustacean zooplankton biomass and the clear water phase is correlated with temperature (Straile, 2000; Winder & Schindler, 2004a; Peeters *et al.*, 2007a; Straile, Adrian & Schindler, 2012). While water temperatures are often still very low at the onset of thermal stratification (Hondzo & Stefan, 1993), very little is known about trophic interactions among phytoplankton, microzooplankton and planktonic crustaceans at low temperatures (Weisse *et al.*, 1990; Rose & Caron, 2007). However, it has been proposed that protists, in particular ciliates, may be less sensitive to low temperatures than crustaceans in both fresh water and marine systems (Stoecker & Guillard, 1982; Weisse, 2006). Several lake studies have indeed suggested that ciliates have a role during the early phase of spring succession, whereas crustaceans, in particular the genus *Daphnia*, often dominate the zooplankton community at the higher temperatures in late spring (Straile, 2000; Tirok & Gaedke, 2006; Peeters *et al.*, 2007b; Berger *et al.*, 2010). Because of their short generation time, micrograzers tend to track phytoplankton biomass, whereas *Daphnia* populations respond with a time lag (Sommer *et al.*, 2012 and references in there). Once abundant, *Daphnia* can suppress phytoplankton and ciliates and cause a clear water phase (Lampert *et al.*, 1986; Sarnelle, 1993; Berger *et al.*, 2007, 2010; Sommer *et al.*, 2012).

Overall, available evidence thus suggests that climatic drivers affect plankton dynamics in temperate lakes primarily through direct pathways in very early spring, but that climate effects become increasingly dependent on indirect biotic feedbacks as the season progresses (Fig. 1). In this study, we hypothesise that earlier warming and shallower stratification have the following direct and indirect effects on plankton dynamics:

(i)In very early spring, water temperatures are still too low to allow for significant population growth of efficient crustacean grazers, in particular parthenogenetically reproducing large daphnids. Therefore, fast growing

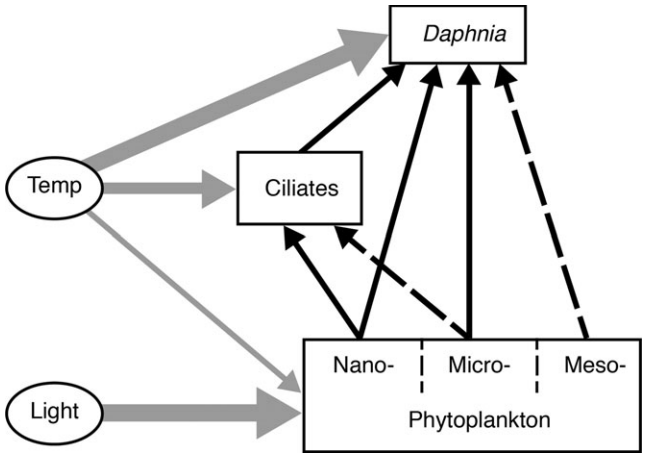

Fig. 1 Hypothesised pathways of direct and indirect effects of climate-dependent abiotic drivers on a simplified planktonic food web. Grey arrows indicate direct effects of abiotic drivers (oval boxes: temperature and light) on food-web components (rectangular boxes: *Daphnia*, ciliates and three size classes of phytoplankton), the width of the arrows indicating the relative strengths of the abiotic factors. Black arrows indicate pathways of energy flow from one food-web component to another. Dashed black arrows indicate partial pathways; that is, microphytoplankton is partly susceptible to ciliate grazing and mesophytoplankton is partly susceptible to *Daphnia* grazing.

small and mid-sized phytoplankton can respond to physical drivers largely unconstrained by grazing. Consequently, phytoplankton will reach an earlier spring peak in response to earlier warming, and algal biomass concentration at the spring peak will be higher at shallower stratification.

(ii)In contrast, we expect that at least some species of ciliates will respond to increased phytoplankton production at low temperatures. Consequently, tracking their phytoplankton prey, ciliate biomass will also reach an earlier and higher spring peak in response to earlier warming and shallower stratification, respectively. If ciliates are not exposed to significant competition and predation by crustaceans, their selective grazing on smaller phytoplankton will over time benefit more slowly growing larger phytoplankton taxa.

(iii)Once water temperature exceeds a critical threshold, *Daphnia* can respond to warming and increased phytoplankton production. *Daphnia* biomass will therefore also reach an earlier spring peak in response to earlier warming, and peak densities will be higher at shallower stratification. However, compared with phytoplankton and ciliates, these responses will occur with a considerable time lag.

(iv)As *Daphnia* becomes increasingly abundant over time, its influence will override direct effects of temperature and stratification on phytoplankton and ciliates. Effects of climatic drivers on these plankton groups will then be indirectly mediated by temperature-dependent *Daphnia* grazing pressure. During mid- to late spring, phytoplankton and ciliate biomass should therefore become increasingly depressed by *Daphnia*, the effect being stronger at higher temperatures.

To distinguish the direct effects of water temperature and thermal stratification on spring plankton dynamics from their indirect effects mediated by *Daphnia*, we performed a multi-factorial *in situ* lake mesocosm experiment. We independently manipulated water temperature and stratification depth with and without a seasonally developing *Daphnia* population. We tested the above suite of hypotheses by following the experiment from the end of winter to the end of spring. We analysed the data separately for three consecutive time windows defined by the phenology of *Daphnia*: an early spring phase when *Daphnia* population development was still strongly limited by low temperature; a late spring phase when all treatments with *Daphnia* had reached the clear water phase; and a transitory mid-spring phase in-between.

Finally, we comprehensively discuss the results of this and two earlier studies (Berger *et al*., 2007, 2010) in relation to the canonical Plankton Ecology Group (PEG) model of seasonal plankton succession in temperate lakes (Sommer *et al*., 2012), explore the implications of these results for likely plankton dynamics under several future climate scenarios and evaluate the experimental evidence for a trophic mismatch between spring phytoplankton and grazer development in a warming world.

Methods

The mesocosm experiment was conducted in Lake Brunnsee (47°56′N, 12°26′E, 533 m a.s.l.) close to the Limnological Research Station of Ludwig-Maximilians-University Munich in Seeon, South-Germany. The general set-up and construction of the mesocosms has been described in detail earlier (Berger *et al*., 2010) and is here only briefly outlined. Mesocosms were cylindrical bags made from transparent Tricoron (RKW Wasserburg, Germany) of 0.95 m diameter and enclosed a total water column depth of 10 m. The mesocosm experiment lasted from late winter to the end of spring (20 March to 5 June 2007). We used a 3-factorial manipulation in a $2 \times 3 \times 2$ experimental design to separate the effects of zooplankton community structure, stratification depth and temperature. More specifically, we cross-classified two types of zooplankton communities (the ambient microzooplankton community of the lake in presence versus absence of stocked *Daphnia hyalina*) with three stratification depths (1.5, 3.5 and 6.5 m) and two temperature treatments ('ambient' versus 'cold'). Each treatment was replicated twice yielding a total of 24 mesocosms. At the beginning of the experiment, all mesocosms were filled with 30 μm filtered lake water including ambient phytoplankton and ciliates. Thereafter, all mesocosms were fertilised once at the beginning of the experiment with KH_2PO_4 from an ambient level of 8 μg L^{-1} total phosphorus (TP) to an initial concentration of 24.5 μg TP L^{-1} to simulate the nutrient pulse associated with spring overturn.

Treatment factors were manipulated as follows. The mesocosms were covered on the outside by black silage film to ensure that the light climate could respond to internal feedbacks rather than being set by the underwater light field of the surrounding lake. This also created a steeper vertical light gradient. Light levels at the experimental stratification depths thus corresponded to physical stratification depths in the lake of ca. 4, 9 and 18 m. Surface layers inside the bags were kept well mixed by intermittently releasing compressed air at the desired stratification depth. Because the lake stratified at ca. 4 m depth, temperature differences up to 2 °C developed between the 6.5 m and the 1.5–3.5 m 'ambient'

treatments (Fig. 2a). 'Cold' treatments were accomplished by enclosing all 'cold' mesocosms inside a 12-m deep, well-mixed outer bag that reduced temperatures by ca. 4 °C below 'ambient' over much of the experimental period (Fig. 2a). Finally, to mimic recruitment from overwintering ephippia, half of the mesocosms were stocked with small inocula of a mix of three *D. hyalina* clones from Lake Brunnsee that had been precultured in the laboratory and slowly acclimatised to *in situ* temperatures. The total inoculation was 1.75 *D. hyalina* per litre of mixed layer distributed on four occasions during the first 2 weeks.

A suite of physical, chemical and biological properties was measured in both the mixed surface layer and the deeper water. Similar to a previous mesocosm experiment (Berger *et al.*, 2010), very few *Daphnia* were caught below the mixed surface layer, and water chemistry and phytoplankton indicated that dynamics in the surface layer were unaffected by processes in the deeper water. We therefore only report data from the mixed surface layer.

Mixed surface layer temperature was continuously logged using temperature data loggers (Voltcraft K 204, Conrad Elektonik, Germany), and vertical temperature profiles from the surface to 10 m depth were taken once a week using a multiprobe LT1/T (WTW, Weilheim, Germany). On days 23, 37 and 65, we measured vertical profiles of photosynthetically active radiation (PAR) in 0.5 m steps down to the depth of stratification (z) with a spherical quantum sensor (LI-139SA, Licor, Lincoln, Nebraska, U.S.A.) while simultaneously measuring incident PAR (flat quantum sensor LI-190SA) above the water surface. We then calculated the light attenuation coefficient k by regression of ln-transformed PAR versus depth. Mean PAR intensity in the mixed surface layer (I_{mix} in per cent of incoming PAR) was then calculated using Lambert Beer's law as $I_{mix} = 100[1\text{-}\exp(\text{-}kz)]/(kz)$.

Water samples for the determination of plankton organisms in the mixed surface layer were taken twice per week and fixed with Lugol's solution. Phytoplankton/ciliate samples from the start of the experiment and from days 14, 21, 28, 35, 42, 49,63 and 77 were identified, enumerated and measured as previously described (Utermöhl, 1958) using an inverted microscope at ×40 magnification for large taxa and at ×200 magnification for small taxa.

Phytoplankton and ciliate biomass were estimated as biovolume based on approximations of cell shapes by simple geometrical bodies (Hillebrand *et al.*, 1999). Calculations of total ciliate biomass were based on the distinction of two size classes (<30 μm and >30 μm in largest dimension). Following (Sieburth, Smetacek & Lenz, 1978), we categorised phytoplankton taxa into three size classes according to their largest linear dimension as nanophytoplankton (<20 μm), microphytoplankton (20–200 μm) and large phytoplankton (>200 μm, subsequently referred to as 'mesophytoplankton'). These size classes differ in their grazing susceptibility, with nanophytoplankton being edible by both ciliates and *Daphnia*, microphytoplankton being partly resistant to ciliates but edible by *Daphnia*, and mesophytoplankton being resistant to ciliates, but partly edible by *Daphnia* (Burns, 1968). Mesozooplankton was collected once per week with vertical net hauls (mesh size ≤150 μm) through the mixed layer and counted under a dissecting microscope at ×25 magnification. Note that a mesh size of 150 μm will not retain smaller metazoans such as the majority of rotifer species, for which we therefore cannot provide any reliable estimates of population abundance.

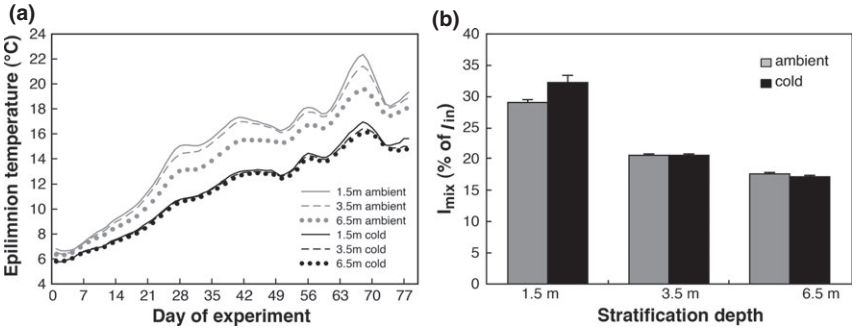

Fig. 2 (a) Temporal development of water temperatures and b) time- and depth-averaged light (photosynthetically active radiation, PAR) intensities (I_{mix} in per cent of incoming PAR) in the mixed surface layer as a function of stratification depth (1.5 m, 3.5 m, 6.5 m) and temperature treatments. Grey lines and bars = 'ambient', black lines and bars = 'cold' temperature. Shown are 5-day running means in (a) and means ±1 SE of measurements on days 23, 37 and 65 in (b).

Data analyses and statistics

In the majority of treatments, phytoplankton and ciliates established clear bloom dynamics, increasing from a low initial biovolume to a peak some time into the experiment and subsequently declining to a biovolume close to or below starting values (Fig. 3). *Daphnia* populations also always increased to a peak and subsequently declined, but never reached the very low initial starting densities (Fig. 3). We characterised these bloom dynamics by their timing (= day of peak) and magnitude (= peak height). A peak was defined as a maximum in biovolume (ciliates, phytoplankton) or population density (*Daphnia*) in a given mesocosm over the course of the experiment and could thus only be determined with weekly temporal resolution. In treatments where phytoplankton and ciliates showed two clearly separated peaks, we consistently used the first peak to calculate peak timing and magnitude.

In order to test statistically, our hypothesis that direct pathways dominate plankton responses to physical drivers early in the season at low temperatures, whereas indirect pathways mediated through the dynamics of *Daphnia* dominate late in the season at higher temperatures, we analysed the data separately for three consecutive time windows defined by the phenology of *Daphnia*. Phase I (day 0 to day 28) describes the early spring phase when *Daphnia* densities were <2 individuals L^{-1}, and *Daphnia* grazing was assumed to have negligible impacts on the plankton community. We defined the end of Phase I as the first sampling date on which *Daphnia* density exceeded two individuals L^{-1} in at least one treatment. In Phase I, phytoplankton or ciliate dynamics were not yet expected to respond to the *Daphnia* present versus absent treatments, but to be primarily driven by the temperature and light environment. Phase III (day 49 to day 77) spans the late spring period in which all treatments with *Daphnia* had reached the clear water phase. We defined the beginning of Phase III as the sampling date on which phytoplankton biovolumes in all treatments with *Daphnia* had returned to values <300 μm^3 mL^{-1} and thus were close to or below starting conditions. In this phase, we expected distinct differences between *Daphnia* present versus absent treatments. Phases I and III were thus defined by distinctive characteristics. In contrast, Phase II (day 28 to day 49) was pragmatically defined as the transition period in-between. Consequently, different treatments were expected to be in different states of succession, and physical and biological effects were expected to strongly interact with upcoming effects of *Daphnia* grazing.

All response variables were analysed by ANOVA in SPSS 19.0 using stratification depth (1.5/3.5/6.5), temperature (ambient/cold) and absence/presence of *Daphnia* as treatment factors. We used a fixed effects model since we fully controlled either the absolute treatment levels (stratification depth) or their relative ranking (temperature, *Daphnia*), and treatment levels encompassed a desired, predetermined range of conditions (Quinn & Keough, 2002). Specifically, because of high light attenuation by the mesocosms walls, light levels in the three stratification treatments corresponded to stratification depths of 4–18 m in the lake (see *Methods*), which span most of the range typically observed in deep lakes in central Europe (Kunz & Diehl, 2003; Berger *et al*., 2006). Similarly, we were interested in studying the effects of moderate differences (≤4 °C) in late winter to late spring water temperatures and of presence versus absence of a seasonally developing *Daphnia* population under an otherwise natural seasonal temperature regime.

Response variables included the timing and magnitude of the biomass/population peaks of phytoplankton, ciliates and *Daphnia*, as well as the average biovolumes of phytoplankton and ciliates, the contributions of nano-, micro- and mesophytoplankton to total phytoplankton biovolume, and the average population densities of *Daphnia* in Phases I-III. Exploratory analyses indicated that no transformation of biovolume/abundance data was required prior to their statistical analysis. In contrast, the proportional contributions of nano-, micro- and mesophytoplankton to total phytoplankton were *arcsin* transformed prior to statistical analyses. Note that there is redundancy in the statistical tests of the size class contributions to total phytoplankton biomass, since the latter must add up to 100%. Nevertheless, because three size classes can add up in various, qualitatively different, ways, we decided to report separate statistical tests for all three size classes, keeping in mind their non-independence.

Results

Effectiveness of treatments

The *Daphnia* treatments were intended to create one treatment level exhibiting the typical seasonal increase and spring peak in *Daphnia* population density and a second (control) treatment that lacked crustaceans altogether. These goals were accomplished except that no significant *Daphnia* population established in one replicate of the '*Daphnia*, 1.5 m, cold' treatment. In that replicate, *Daphnia* barely reached a density of 20 ind. L^{-1} at

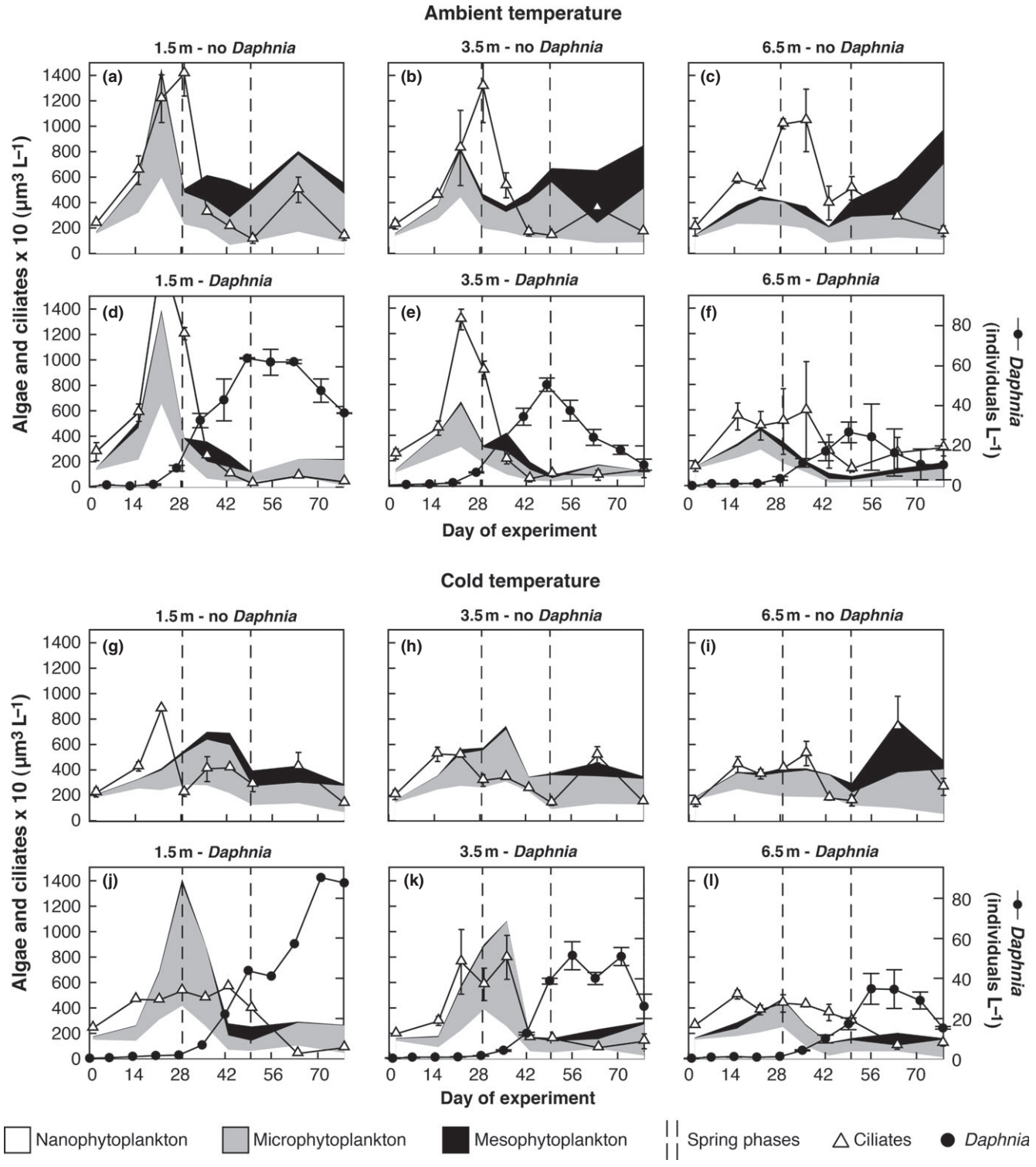

Fig. 3 Temporal development of the biomass of ciliates, nano- micro-, meso- and total phytoplankton, and of the population density of *Daphnia* in the mixed surface layer as a function of stratification depth (1.5, 3.5 and 6.5 m), temperature and *Daphnia* treatments: (a–c) 'ambient, no *Daphnia*'; (d–f) 'ambient, *Daphnia*'; (g–i) 'cold, no *Daphnia*'; and (j–l) 'cold, *Daphnia*'. White areas = nanophytoplankton; grey areas = microphytoplankton; and black areas = mesophytoplankton. Vertical dashed lines separate the 'early', 'mid-' and 'late' phases of spring succession. For clarity, error bars (±1 SE) are only shown for ciliates and *Daphnia*.

a single point in time and quickly dropped to levels below 5 ind. L^{-1}. This deviates strongly from all other shallow (mixed layer ≤2 m) mesocosms in this and similar experiments (Berger *et al.*, 2007, 2010), where *Daphnia* populations reached peak densities >35 ind. L^{-1} (typically 60–100 ind. L^{-1}) and stayed above 20 ind. L^{-1} for at least three consecutive weekly samplings. We therefore removed that replicate from all analyses. In the remaining *Daphnia* treatments, *Daphnia* population densities peaked at values between 24 and 90 ind. L^{-1}, whereas the no *Daphnia* treatments consistently had fewer than 1 ind. L^{-1}. Other crustaceans were rare at all times in most mesocosms, with the exception of three ambient *Daphnia* treatments, where small cladocerans of the genus *Bosmina* appeared after day 42 and reached maximal abundances of 14–82 ind. L^{-1}. We found no indication that these sporadic occurrences of *Bosmina* had detectable effects on other response variables. Merging *Bosmina* (weighted by their smaller individual biomass) with *Daphnia* counts made no difference to the reported patterns. We therefore focus entirely on responses of *Daphnia*.

Mixed layer temperatures were approximately 6 °C at the beginning of the experiment, exceeded 10 °C around day 18 in 'ambient' and around day 25 in 'cold' treatments and reached maxima of 16–17 °C in 'cold' and 20–23 °C in 'ambient' treatments in late spring (Fig. 2a). Mixed layer temperatures in 'cold' treatments were always lower than in 'ambient' treatments and differed by approximately 0.5–4 °C in Phase I, 2.5–4 °C in Phase II and 3–5.5 °C in Phase III (Fig. 2a). Averaged over the entire experimental period, mixed layer temperatures were weakly negatively related to stratification depth in 'ambient', but not in 'cold' treatments (ZxT interaction, $P < 0.0001$). Finally, the vertical temperature profiles indicated that water columns were well mixed to the target depths (no temperature difference) and stratified below (data not shown).

Averaged over the measurements on days 23, 37 and 65, mean PAR intensity in the mixed layer decreased with stratification depth from 31 to 18 per cent of incoming PAR (Fig. 2b, $P < 0.001$). Temperature and light in the mixed layer were not affected by the *Daphnia* treatments (ANOVA, $P \geq 0.12$).

General successional patterns

Phytoplankton, ciliates and *Daphnia* (where present) showed typical spring dynamics of increase to a transient peak followed by a decrease in all treatments (Fig. 3). Phytoplankton biovolume during the spring bloom was almost completely dominated by nano- and microphytoplankton (Fig. 3). The peak of the phytoplankton bloom occurred earlier at 'ambient' than at 'cold' temperatures and the magnitude of the peak decreased with stratification depth (Fig. 3; Table 1). All *Daphnia* treatments reached a distinct and extended clear water phase where phytoplankton biomass declined below 300 μm^3 L^{-1} and remained below 400 μm^3 L^{-1} throughout the experiment. In contrast, in treatments without *Daphnia*, phytoplankton showed a less pronounced clear water phase and often increased again towards the end of the experiment, the second increase usually consisting to a larger extent of mesophytoplankton (Fig. 3). Bloom dynamics of ciliates appeared to respond both to their food (small and mid-sized phytoplankton) and to temperature, as indicated by a tendency for the magnitude of the ciliate peak to decrease with stratification depth and to increase with temperature (Fig. 3; Table 1). In the latter half of the experiment, ciliate biomass was suppressed in the presence of *Daphnia* (see next section). The dynamics of *Daphnia* also responded to algal food and to temperature, as indicated by a negative effect of stratification depth on the magnitude of the *Daphnia* peak, and a positive effect of temperature on its timing (Fig. 3; Table 1).

Comparison of hypotheses (i)–(iv) with treatment effects in Phases I–III

Consistent with hypotheses (i) and (ii), *Daphnia* populations remained very low at temperatures ≤10 °C in early spring, allowing fast growing small and mid-sized phytoplankton and ciliates to respond directly to temperature and light, unconstrained by *Daphnia* grazing. The latter is supported by the following treatment effects in Phase I: a positive effect of higher water temperature on phytoplankton and ciliates, a negative effect of increased stratification depth on phytoplankton and no effect of *Daphnia* on phytoplankton and ciliates (Fig. 4d,g; Table 2). Ciliates were also negatively impacted by increased stratification depth in Phase I, suggesting that they profited from higher primary production in shallower mixed layers (Fig. 4d; Table 2). However, compared with 'ambient' treatments, phytoplankton and ciliates responded only weakly to stratification depth in the 'cold' treatments (Fig. 4d,g; ZxT interactions, Table 2), suggesting that low temperatures limited the positive responses of phytoplankton and ciliates to increased light availability in shallower mixed layers in Phase I.

Consistent with hypothesis (iii), *Daphnia* responded to treatment factors once temperatures exceeded a critical

Table 1 Summary of ANOVAs of the effects of stratification depth 1.5 versus 3.5 versus 6.5 m (Z), 'cold' versus 'ambient' water temperature (T), absence versus presence of *Daphnia* (D), and their interactions (ZxT, TxD, ZxD, ZxTxD) on the timing and magnitude of spring peaks in the population density of *Daphnia* and the biovolumes of ciliates and total phytoplankton in the mixed surface layer in spring. Shown are degrees of freedom, *P*-values, and the R^2 value of the overall model. Degrees of freedom are shown as (*n*, 11), where *n* = treatment degrees of freedom and 11 = error degrees of freedom, the total number of experimental units being 23. Significant relationships are in bold font. Black highlighting means that a higher level of the treatment factor was associated with an earlier peak timing and/or a smaller peak magnitude (negative relationships); light grey highlighting means the opposite (positive relationships) and dark grey highlighting indicates interactions among treatment factors

	Treatments and their interactions, degrees of freedom and *P*-values							
Dependent variable	Z (2, 11)	T (1, 11)	D (1, 11)	ZxT (2, 11)	TxD (1, 11)	ZxD (2, 11)	ZxTxD (2, 11)	R^2
Daphnia								
Timing	0.47	**0.023**	–	0.79	–	–	–	0.69
Magnitude	**0.015**	0.20	–	0.52	–	–	–	0.82
Ciliates								
Timing	0.91	0.38	0.59	0.44	0.22	0.28	0.44	0.46
Magnitude	0.072	**<0.001**	0.61	0.36	0.95	0.49	0.15	0.81
Phyto-plankton								
Timing	0.094	**<0.001**	**<0.001**	0.094	**<0.001**	0.094	**<0.001**	0.96
Magnitude	**0.013**	0.75	0.087	0.18	**0.036**	0.9	0.96	0.68

Table 2 Summary of ANOVAs of the effects of stratification depth 1.5 versus 3.5 versus 6.5 m (Z), 'cold' versus 'ambient' water temperature (T), absence versus presence of *Daphnia* (D), and their interactions (ZxT, TxD, ZxD, ZxTxD) on the population density of *Daphnia* and on the biovolumes of ciliates and total phytoplankton in the mixed surface layer in early, mid and late spring (Phase I, II and III, respectively). Shown are *P*-values and the R^2 value of the overall model as well as degrees of freedom (Df) and sampling size (N). Degrees of freedom are shown as (*n*, 11), where *N* = treatment degrees of freedom and 11 = error degrees of freedom, the total number of experimental units being 23. Significant relationships are in bold font. Negative relationships between dependent and independent variables are highlighted in black, positive in light grey and interactions in dark grey

	Treatments and their interactions, degrees of freedom and *P*-values							
Dependent variable	Z (2, 11)	T (1, 11)	D (1, 11)	ZxT (2, 11)	TxD (1, 11)	ZxD (2, 11)	ZxTxD (2, 11)	R^2
Phase I: early spring								
Daphnia	**0.14**	**<0.001**	–	0.41	–	–	–	0.94
Ciliates	**0.017**	**<0.001**	0.71	0.09	0.84	0.83	0.43	0.81
Phytoplankton	**<0.001**	**0.006**	0.84	**0.025**	0.23	0.52	0.34	0.86
Phase II: mid-spring								
Daphnia	**0.014**	**0.003**	–	0.21	–	–	–	0.93
Ciliates	0.51	0.45	0.47	0.32	**0.032**	0.64	0.91	0.54
Phytoplankton	**<0.001**	**0.002**	**0.013**	0.20	**0.016**	0.62	0.24	0.87
Phase III: late spring								
Daphnia	**0.025**	0.16	–	0.89	–	–	–	0.80
Ciliates	0.21	0.40	**0.002**	0.998	0.45	0.97	0.44	0.70
Phytoplankton	0.94	0.14	**<0.001**	0.57	**0.007**	0.34	0.34	0.81

threshold of approximately 10 °C. This is supported by an initially positive effect of increased water temperature on the population density of *Daphnia* (which was still weak in Phase I, but strong in Phase II) and by a negative response of *Daphnia* to increased stratification depth in Phases II and III (Fig. 4a–c; Table 2). The latter suggests that *Daphnia* profited from increased primary production in shallower mixed layers, but with a considerable time lag compared with ciliates. A temperature effect on *Daphnia* was no longer observed in Phase III (Fig. 4c; Table 2), when *Daphnia* populations were already decreasing from peak densities in 'ambient' treatments while still reaching their peaks in the 'cold' treatments (Fig. 3).

Consistent with hypothesis (iv), effects of *Daphnia* on phytoplankton and ciliates increased in strength over time as water temperatures increased. In the latter half of the experiment, effects of climatic drivers on these plankton groups appeared to be primarily mediated by temperature-dependent *Daphnia* grazing pressure. This is supported by negative responses of phytoplankton and ciliates to the presence of *Daphnia* at 'ambient'

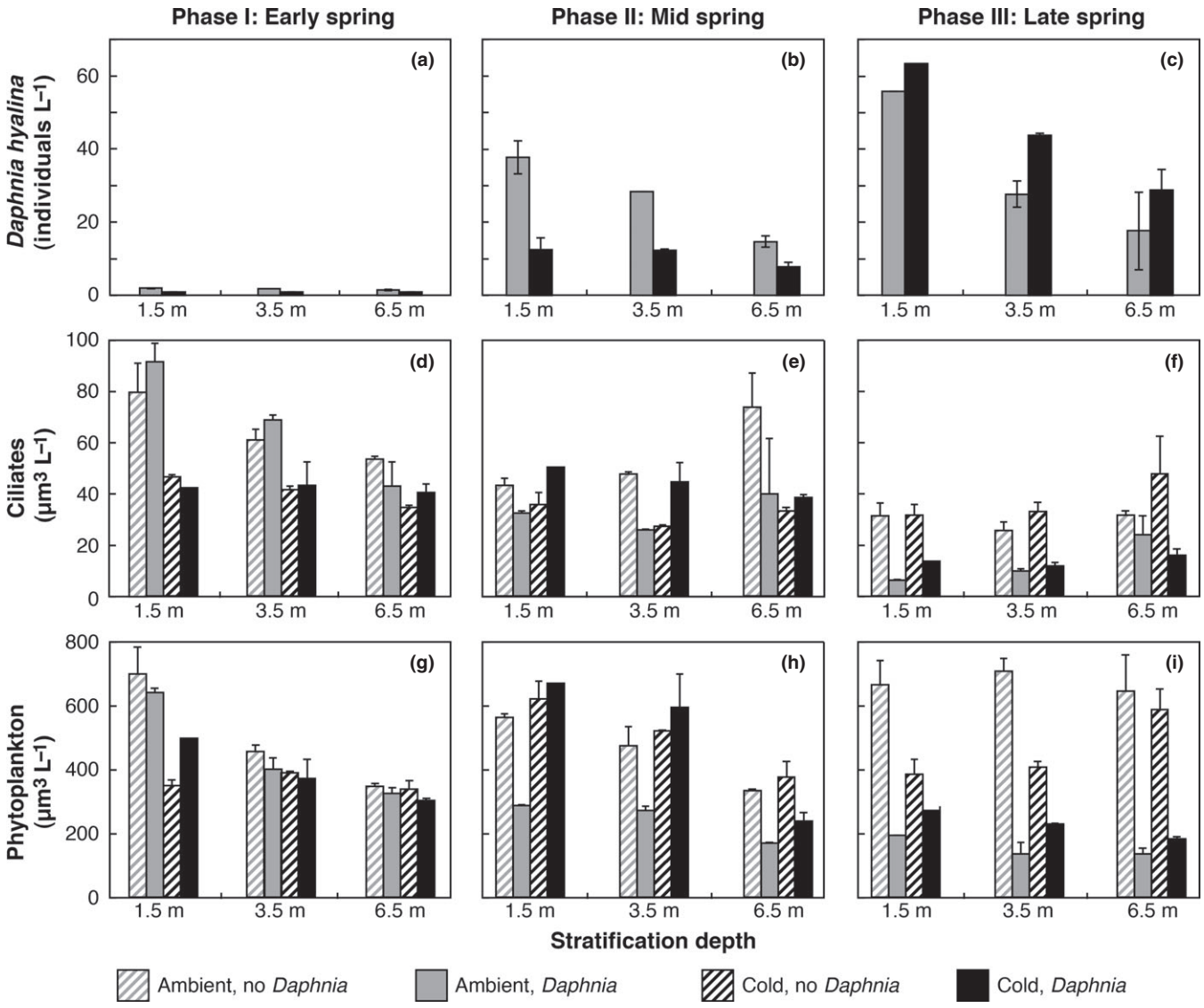

Fig. 4 Population density of *Daphnia hyalina* and biomass of ciliates and total phytoplankton in the mixed surface layer in early, mid and late spring as a function of stratification depth (1.5, 3.5 and 6.5 m), temperature and *Daphnia* treatments (see legend at bottom of figure). Shown are treatment means ±1 SE during (a, d, g) Phase I = day 1–28, (b, e, h) Phase II = days 28–49 and (c, f, i) Phase III = days 49–77.

temperatures already in Phase II (and III) but at 'cold' temperatures first in Phase III (Fig. 4e,f,h,i; main effects of T and TxD interactions, Table 2) and by the absence of any strong effect of temperature or stratification depth on these plankton groups in Phase III (Fig. 4f,i; Table 2). Phytoplankton, but not ciliates, still showed a negative response to increased stratification depth in Phase II (Fig. 4e,h; Table 2), suggesting that the direct positive effect of light on primary production was not yet completely suppressed by indirect grazing effects at that time.

Also consistent with hypotheses (iii) and (iv), differences in the community size structure of phytoplankton developed over time in the *Daphnia* versus no *Daphnia* treatments, paralleling the concomitant development of different size-selective grazing regimes. Specifically, in late spring (Phase III), the contribution of nanoplankton to total phytoplankton biomass was higher in the presence of *Daphnia*, reflecting the concomitant negative responses of ciliates and large mesophytoplankton to *Daphnia* (compare Fig. 5c with Fig. 4f, and *Daphnia* effects in Tables 2 and 3). This was expected since nanophytoplankton is the primary food of ciliates, whereas mesophytoplankton is largely resistant to ciliates but partially susceptible to *Daphnia* grazing.

Finally, a few observed treatment effects were unrelated to our hypotheses, but only very few were inconsistent

Table 3 Summary of ANOVAs of the effects of stratification depth 1.5 versus 3.5 versus 6.5 m (Z), 'cold' versus 'ambient' water temperature (T), absence versus presence of *Daphnia* (D), and their interactions (ZxT, TxD, ZxD, ZxTxD) on the per cent contribution of nano-, micro- and mesophytoplankton to total phytoplankton biomass in early, mid and late spring (Phase I, II and III, respectively). Shown are degrees of freedom, *P*-values, and the R^2 value of the overall model. Degrees of freedom are shown as (*n*, 11), where *N* = treatment degrees of freedom and 11 = error degrees of freedom, the total number of experimental units being 23. Significant relationships are in bold font. Negative relationships between dependent and independent variables are highlighted in black, positive in light grey and interactions in dark grey

	Treatments and their interactions, degrees of freedom, and *P*-values							
Dependent variable	Z (2, 11)	T (1, 11)	D (1, 11)	ZxT (2, 11)	TxD (1, 11)	ZxD (2,11)	ZxTxD (2, 11)	R^2
Phase I: early spring								
% Nano	**0.050**	0.61	0.09	0.47	0.085	0.17	0.49	0.66
% Micro	**0.008**	0.82	0.08	0.55	0.11	0.11	0.32	0.73
% Meso	0.15	0.65	0.91	0.94	0.66	0.78	0.58	0.37
Phase II: mid-spring								
% Nano	**0.001**	0.21	0.06	0.12	**0.014**	0.41	0.98	0.83
% Micro	**0.038**	**>0.001**	0.26	0.81	**0.002**	0.76	0.69	0.83
% Meso	**0.014**	**>0.001**	0.42	0.3	0.079	0.30	0.51	0.89
Phase III: late spring								
% Nano	0.25	0.6	**0.016**	0.44	**0.027**	0.32	0.077	0.74
% Micro	**0.047**	0.61	0.95	0.08	0.46	0.81	0.81	0.62
% Meso	**0.012**	0.45	**0.038**	0.25	0.27	0.81	0.1	0.75

with them. Specifically, phytoplankton community size structure appeared to be influenced by stratification depth in temporally shifting ways that were unrelated to our hypotheses (Fig. 5; Table 3). Also, we expected that negative effects of *Daphnia* on phytoplankton and ciliates in mid- to late spring should be stronger at higher than at lower temperatures (hypothesis iv). This was true for phytoplankton in both mid- and late spring and for ciliates in mid-spring (Fig. 4e,h,i; TxD interactions; Table 2), but not for ciliates in late spring (Fig. 4f; lack of a TxD interaction; Table 2).

Discussion

Summary of direct and indirect effects of physical drivers on spring successional dynamics

Our mesocosm experiment was designed to separate the direct effects of physical drivers on spring succession of the plankton in thermally stratifying temperate waters, from their indirect effects mediated through biological interactions. The experiment clearly demonstrated that stratification depth (affecting light supply to phytoplankton in the mixed surface layer) and water temperature (affecting growth rates of all plankton groups) drive plankton dynamics directly in very early spring. However, indirect effects such as light-dependent food supply to grazers and temperature-dependent grazing pressure become increasingly important as the season progresses.

In brief, decreased stratification depth enhanced the magnitude of the spring peaks of phytoplankton (by decreasing light limitation in the mixed surface layer) and of ciliates and crustaceans (by increasing production of their algal food). Increased temperature instead enhanced growth rates and shifted phytoplankton and *Daphnia* spring peaks forward in time. In the absence of higher, planktivorous trophic levels, *Daphnia* grazing eventually controlled phytoplankton and microzooplankton at very low levels in late spring/early summer and completely suppressed all other direct and indirect effects of physical drivers on these plankton groups (note the absence of any temperature and stratification depth effects in the *Daphnia* treatments in Phase III, Fig. 4f,i). *Daphnia* itself still responded to stratification depth at that time, that is, higher population densities of *Daphnia* were sustained in shallow, less light-limited mixed layers and were presumably also required to control phytoplankton at similar levels across the different depth treatments. As described elsewhere, *Daphnia* body size at first reproduction was also negatively related to water temperature throughout the experiment (Sebastian *et al*., 2012). Finally, phytoplankton and ciliates showed bloom dynamics also in the absence of *Daphnia*. However, no distinct clear water phase developed in the absence of *Daphnia* and larger phytoplankton size groups that are partially or fully resistant to ciliate grazing subsequently increased. While confirming that selective ciliate grazing can lead to size shifts in the phytoplankton towards larger taxa (Berninger, Wickham & Finlay, 1993; Hansen,

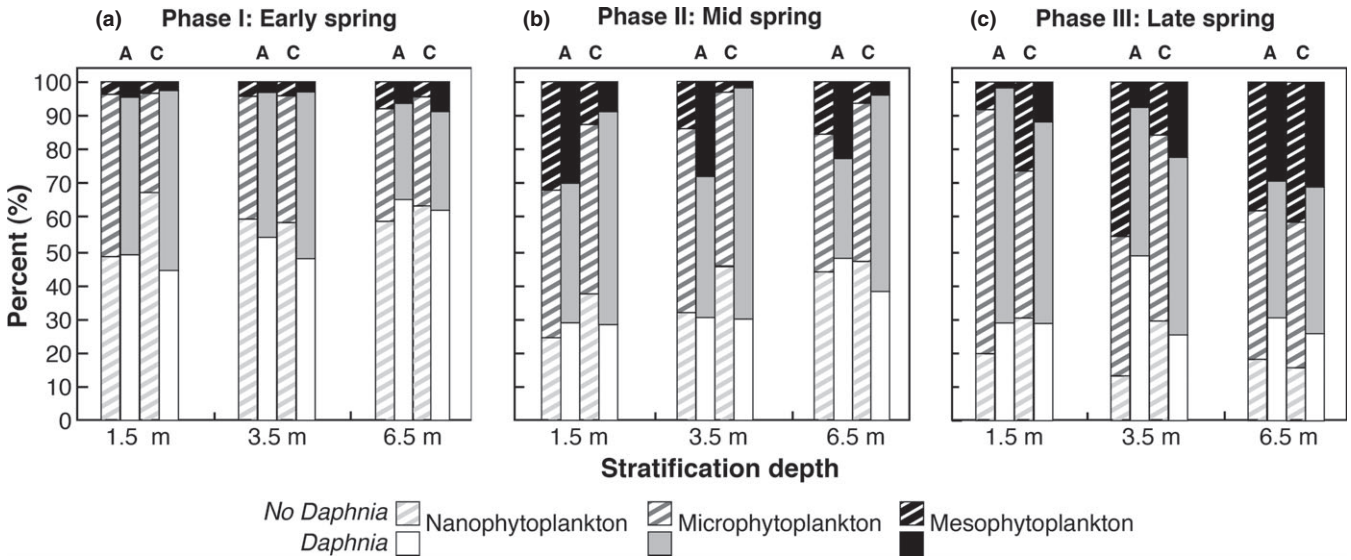

Fig. 5 Percentage contribution of nano-, micro- and mesophytoplankton to total phytoplankton biomass in early, mid and late spring (Phases I–III) as a function of stratification depth (1.5, 3.5 and 6.5 m), temperature (A = 'ambient', C = 'cold') and *Daphnia* treatments (see legend at bottom of figure).

Bjørnsen & Hansen, 1997), this also suggests that ciliates are unable fully to control phytoplankton biomass.

Comparison with the PEG model

As described in the Results section, the responses to experimental treatments were in almost complete agreement with hypotheses (i)–(iv). Together with the results of previous experiments, this gives a detailed picture of direct and indirect climate effects on spring plankton dynamics in thermally stratifying, temperate lakes, which we discuss in relation to the recently updated PEG model (Sommer *et al*., 2012). The updated PEG model summarises empirical knowledge describing the temporal development of phytoplankton, protozooplankton and metazoan zooplankton over a seasonal cycle in archetypical eutrophic and oligotrophic waters, using three alternative scenarios of fish predation on metazoan zooplankton (Sommer *et al*., 2012). In this section, we synthesise our results with results from other warming and stratification experiments and compare them with appropriate scenarios of the updated PEG model, that is, a moderately eutrophic water (as achieved by mesocosm fertilisation) with either negligible or excessive fish predation, corresponding to our '*Daphnia*' and 'no *Daphnia*' treatments, respectively (Fig. 6a,b). Based on our synthesis of experimental results, we then use the PEG framework to illustrate empirically derived predictions of how two future climate change scenarios (warming combined with either shallower or deeper mixing) can be expected to affect spring plankton dynamics in thermally stratifying lakes (Fig. 6c,d).

According to the PEG model, light limitation is the overwhelming physical constraint on the entire plankton community at the end of winter, and thermal stratification is required to trigger the onset of the phytoplankton spring bloom in deep waters. In the PEG 'eutrophic – no fish' scenario, this is followed by a rapid increase in protist grazers and a slightly delayed increase in metazoan grazers as these groups experience a fast release from food limitation (Fig. 6a). As metazoan grazer biomass builds up, grazing pressure on both phytoplankton and protozooplankton increases strongly and causes both groups to decline to a mid-season minimum, the clear water phase. Metazoan grazing pressure subsequently declines as more resistant prey taxa increase in abundance, shifting metazoan control of phytoplankton and protozooplankton from control of total biomass to control of community composition (Sommer *et al*., 2012). In the PEG 'high fish' scenario, metazoan zooplankton is permanently suppressed. A clear water phase then does not arise because protozooplankton tracks rather than controls phytoplankton biomass (Fig. 6a).

The two described scenarios of the PEG model are largely supported by our experimental results (summarised in the 'ambient' scenario of Fig. 6b), with a few notable exceptions. Most importantly, the PEG model only considers light limitation as a controlling physical factor and contends that temperature is a subordinate driver of seasonal plankton succession (Sommer *et al*., 2012).

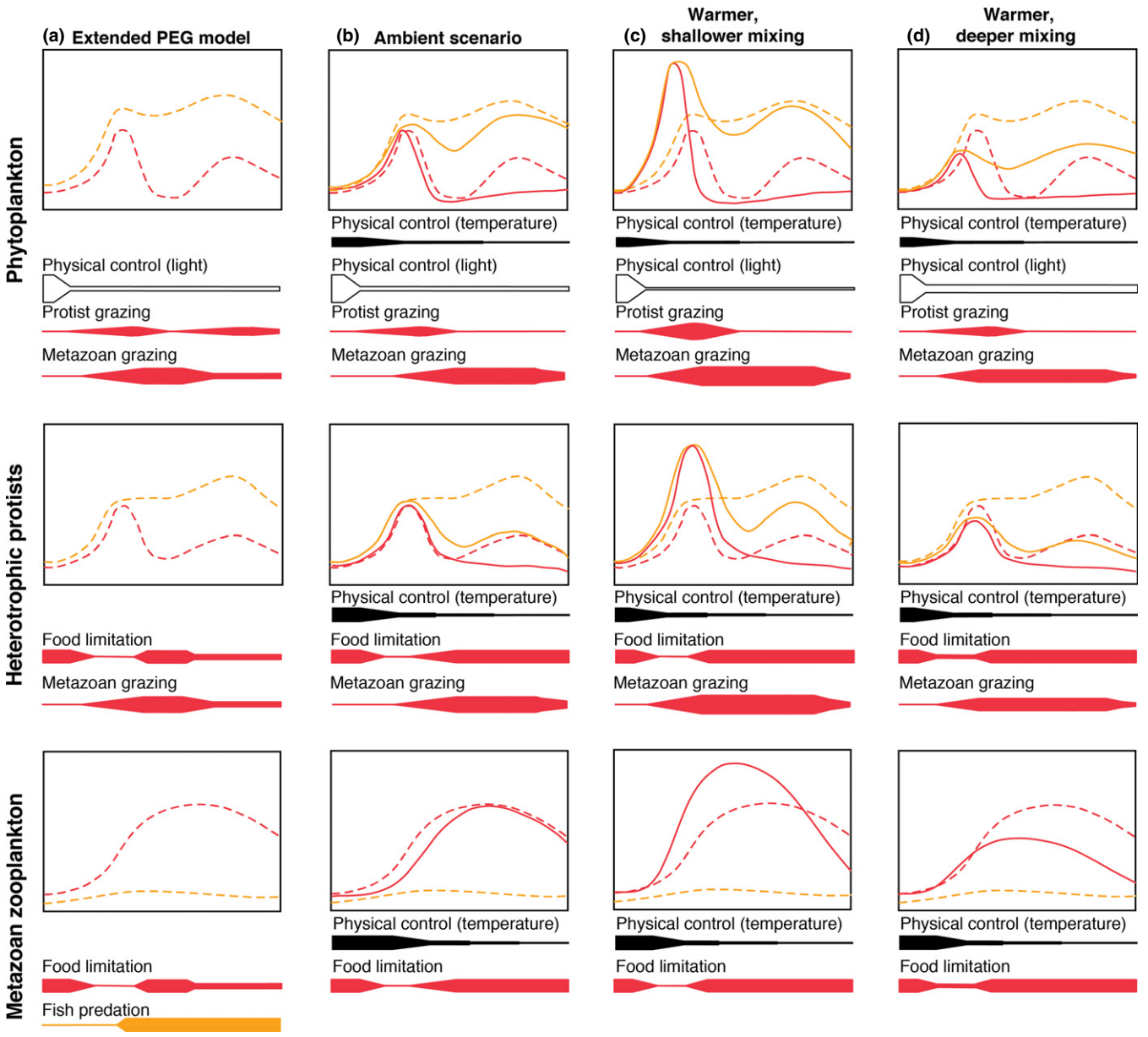

Fig. 6 Schematic representation of seasonal (winter through early summer) biomass patterns of phytoplankton (top), heterotrophic protists (middle) and metazoan zooplankton (bottom) in a moderately eutrophic waterbody. (a) The extended Plankton Ecology Group (PEG) Model (redrawn after Fig. 2 in Sommer *et al*., 2012) for two opposite scenarios: high metazoan density in the absence of fish (red broken line) and metazoan plankton suppressed by high fish predation (orange broken line). (b) The same scenarios as in (a), modified according to our experimental results for the *Daphnia* present treatment (red solid line; Berger *et al*., 2007, 2010, this study) and *Daphnia* absent treatment (orange solid line, this study). (c) and (d) Possible future scenarios of temperature and stratification patterns based on our experiments: (c) warmer, shallower mixing and (d) warmer, deeper mixing, again for no (red solid line) and high (orange solid line) fish predation on metazoan plankton. For comparison, the two PEG scenarios are shown in panels b–d as thin broken lines. The thickness of the horizontal bars indicates the seasonal change in the relative importance of potentially limiting physical and biotic factors. Physical control by temperature (black bars, not included in PEG model) and light (white bars) applies to both the no fish/*Daphnia* present and the high fish/*Daphnia* absent scenarios. Red bars for protist grazing, metazoan grazing and food limitation apply only to the no fish scenarios (red lines), and the orange bar for fish predation applies only to the high fish scenario (orange lines).

However, several experiments have shown that temperature can affect the timing of both the onset and the peak of the spring blooms of phyto- and zooplankton at both high and low light levels (Berger *et al*., 2007, 2010; Winder *et al*., 2012; Hansson *et al*., 2013; this study). An earlier onset of the phytoplankton bloom indicates a

direct positive effect of temperature on phytoplankton growth, and we believe this is particularly relevant at the low temperatures in very early spring. Note, for example, that average phytoplankton biomass had increased on day 15–437 $\mu m^3\ L^{-1}$ in 'ambient' treatments (when average ambient water temperatures were 9.1°C), but only to 302 $\mu m^3\ L^{-1}$ in 'cold' treatments (7.6 °C), (Fig. 3; ANOVA, effect of temperature, $P = 0.024$). In contrast, an earlier occurrence of the peak of the spring bloom need not reflect a direct phytoplankton response to higher temperatures, but could also be an indirect consequence of an earlier termination of the bloom by faster growing metazoan grazers. Accelerated population growth and grazing pressure of metazoan grazers have now been observed in several warming experiments (Berger *et al.*, 2007, 2010; Winder *et al.*, 2012; Hansson *et al.*, 2013, this study).

To indicate the proposed higher importance of water temperature compared with the PEG model, we have added horizontal bars below panels b, c and d in Fig. 6 indicating seasonal changes in temperature limitation of the respective functional groups. Nevertheless, while temperature clearly does influence phytoplankton growth, the strong transient responses of phytoplankton biomass to mixed layer depth in this and other experiments (Berger *et al.*, 2007, 2010; Jäger, Diehl & Schmidt, 2008) support the conclusion of Sommer *et al.* (2012) that light exerts considerably stronger direct control over the phytoplankton bloom than does temperature. We have indicated this by different thickness of the light versus temperature bars of phytoplankton limitation prior to the onset of stratification (Fig. 6b–d). In addition, temperature limitation of metazoan grazer growth clearly matters to spring dynamics (Straile *et al.*, 2012) and therefore must be included in climate change scenarios (Fig. 6c,d). Also, while many ciliate taxa quickly respond to the onset of the phytoplankton bloom even at low temperatures (Johansson, Gorokhova & Larsson, 2004; Rose & Caron, 2007; Berger *et al.*, 2010), our experiment in the ambient temperature scenario suggests that *Daphnia* requires water temperatures >10 °C for significant population growth and therefore shows a lagged response to increased food abundance compared with the PEG scenario (Fig. 6b). Our results thus support the proposition that different sensitivities of ciliates and *Daphnia* to low temperatures can create a loophole for an early season protist bloom (Tirok & Gaedke, 2006).

A final difference between the PEG scenarios and our experimental results concerns the recovery of phytoplankton and ciliate biomass after the clear water phase (Fig. 6a). Such a recovery occurred in the absence of metazoan grazers (Fig. 3), but has never been observed in any of our experiments with *Daphnia* present (Berger *et al.*, 2007, 2010; Fig. 3). Possibly, this discrepancy arises because the PEG 'no fish' scenario does not fully apply to our experiments, in which metazoan grazers were completely free from top-down influences (including invertebrate predators) and thus could exert extended control over their prey. Alternatively, the initial communities in our mesocosms may not have included a sufficient number of grazing-resistant taxa to enable a recovery of phytoplankton and ciliate biomass in the *Daphnia* treatments. Still, in the absence of firm experimental evidence to the contrary, we assume that phytoplankton and protozooplankton in 'no fish' systems cannot recover much above clear water densities over the successional periods considered in the climate change scenarios below.

Predicting the response of spring plankton dynamics to climate change scenarios

Coupled climate and hydrodynamic models predict that a warmer climate will result in changes of lake stratification patterns and water temperatures. The direction of change will depend on lake morphometry, transparency and radiative balance (Magnuson *et al.*, 1997). Wind-exposed lakes in regions experiencing an increase in wind velocity, which is a likely scenario for some coastal regions in the temperate zone, will probably see a deepening of the thermocline during the summer months (Hondzo & Stefan, 1993). In contrast, smaller temperate lakes with short fetch will experience higher epilimnetic temperatures, an earlier onset of stratification, a later overturn in the season and a decrease in mixed layer depth in the spring and summer (Hondzo & Stefan, 1993; DeStasio *et al.*, 1996; Peeters *et al.*, 2002; Livingstone, 2003). We therefore explore two climate scenarios that we believe are relevant as future drivers of spring plankton dynamics in thermally stratifying lakes: earlier, stronger warming combined with either shallower or deeper stratification.

To summarise how warming and altered stratification depth are expected to affect spring plankton dynamics based on our accumulated experimental evidence (Berger *et al.*, 2007, 2010; this study), we again use the scenarios of low versus high control of metazoan grazers and superimpose them on the corresponding PEG scenarios for ease of visual comparison (Fig. 6c,d). The following rather simple patterns emerge.

1. Warmer surface water at the onset of stratification is expected to speed up the development of all functional

plankton groups, leading to an earlier onset of the phytoplankton bloom and its earlier termination by metazoan grazing compared with the ambient scenario (Fig. 6c and d versus b).

2. Shallower mixing is expected to reduce light limitation of primary producers and food limitation of grazers, leading to higher bloom peaks of all functional groups; deeper mixing will produce the opposite patterns (Fig. 6c versus d).

3. High and low planktivore pressure scenarios are expected to diverge later in the season. At low planktivore pressure metazoan grazing produces a persistent clear water phase (which sets in earlier at higher temperature; Fig. 6c and d versus b) with strongly reduced phytoplankton and protozooplankton biomass. Continued physical control of primary production by the light environment is then expressed in a positive effect of stratification depth on the later season biomass of metazoan grazers, which in turn suppress any biomass responses of their food (Fig. 6c versus d, red solid lines). In contrast, at high planktivore pressure on metazoans, only a weak and transient decline in phytoplankton and protozoan biomass is expected after the spring bloom, which is followed by a shift in community composition and a recovery of total biomass. Physical control by the light environment is then expressed in a positive effect of stratification depth on the later season biomasses of both phyto- and protozooplankton (Fig. 6c versus d, orange solid lines).

Contrary to results of direct light and temperature manipulations in marine mesocosms (Lewandowska & Sommer, 2010; Winder *et al*., 2012), our data suggest that stratification-depth-dependent light supply exerts a much stronger influence on plankton biomass in spring than does temperature. However, temperature and stratification depth are not independent in natural systems, where deeper mixing is typically associated with lower surface water temperatures (Mazumder & Taylor, 1994; Berger *et al*., 2006). Still, because temperature and mixing depth appear to act independently on plankton dynamics, predictions for different combinations of these two factors should be easy to derive. For example, if deeper mixing offsets positive effects of warming on the mixed surface layer temperature of a wind-exposed lake, the resulting plankton dynamics should follow the 'ambient' scenario (Fig. 6b) for timing, but the 'deeper mixing' scenario (Fig. 6d) for amplitude. Note, however, that the scenarios in Fig. 6 do not include any potential effects of climate change on other external drivers of plankton dynamics such as the supply with nutrients and coloured dissolved organic carbon from the catchment (Schindler, 2009; Weyhenmeyer & Karlsson, 2009), which may either reinforce or counteract the discussed processes depending on the correlation structure of climatic drivers (Mazumder & Taylor, 1994; de Senerpont Domis *et al*., 2013).

Climate change and trophic mismatch

Our experiments lend little support to the hypothesis that climate change could lead to a trophic mismatch of pelagic grazers and their food (reviewed in Thackeray, 2012). In fact, in all our experiments (Berger *et al*., 2007, 2010), both higher temperature and shallower mixing had either no effect on or advanced the timing of zooplankton peaks compared with the timing of the phytoplankton peaks and thus tended to increase rather than decrease the degree of synchrony in population development of grazers and their food. Moreover, the height of the *Daphnia* peak was always unrelated to temperature (see also Feuchtmayr *et al*., 2010; Hansson *et al*., 2013). Both findings agree with predictions from a model of temperature- and light-dependent spring dynamics of *Daphnia* and phytoplankton (Schalau *et al*., 2008). Finally, where measured, the height of the ciliate peak was also either unaffected (Berger *et al*., 2010) or positively affected by temperature (this study).

Our experiments thus give no indication of a trophic mismatch or of reduced zooplankton production in response to likely climate scenarios (Fig. 6). While these results are in line with a recent review of long-term observational studies (Thackeray, 2012), they cannot be extrapolated unreservedly to field situations. For example, while our stocking procedure was intended to mimic the recruitment of *Daphnia* from ephippial resting eggs, it is still unclear which actual cues trigger the hatching of ephippia in the lake. The abiotic cues of photoperiod, temperature, light intensity, CO_2 and O_2 conditions have been found to affect hatching success (Pancella & Stross, 1962; Stross, 1966; Schwartz & Hebert, 1987; Vandekerkhove *et al*., 2005), but most of these experiments were conducted in the laboratory, while field experiments are rare (Wolf & Carvalho, 1989). More recent evidence has indicated temperature to be the most important cue governing hatching in *Daphnia* (Gyllstrom & Hansson, 2004; Rother, Pitsch & Huelsmann, 2010). However, the amplitude of the hatching rate might be triggered by light because hatching rates in the laboratory were found to be higher than in the field (Caceres & Schwalbach, 2001). Importantly, if these cues are desynchronised from environmental factors triggering the phytoplankton bloom, a trophic

mismatch could still arise (Winder & Schindler, 2004b). Also, our experiments have focused on only two physical factors influencing early seasonal succession, water temperature and depth of stratification. A third factor, the *timing* of the onset of stratification, is only partly coupled to the other two (Winder & Schindler, 2004a; Straile, 2005;
Peeters *et al*., 2007a) and therefore carries some potential for a decoupling of phytoplankton and zooplankton dynamics (Schalau *et al*., 2008). Future field experiments should therefore include manipulation of the timing of stratification as a treatment factor.

Acknowledgments

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References

Aberle N., Lengfellner K. & Sommer U. (2007) Spring bloom succession, grazing impact and herbivore selectivity of ciliate communities in response to winter warming. *Oecologia*, **150**, 668–681.

Berger S.A., Diehl S., Kunz T.J., Albrecht D., Oucible A.M. & Ritzer S. (2006) Light supply, plankton biomass, and seston stoichiometry in a gradient of lake mixing depths. *Limnology and Oceanography*, **51**, 1898–1905.

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.

Berger S.A., Diehl S., Stibor H., Trommer G., Ruhenstroth M., Wild A. *et al.* (2007) Water temperature and mixing depth affect timing and magnitude of events during spring succession of the plankton. *Oecologia*, **150**, 643–654.

Berninger U.G., Wickham S.A. & Finlay B.J. (1993) Trophic coupling within the microbial food web – a study with fine temporal resolution in a eutrophic freshwater ecosystem. *Freshwater Biology*, **30**, 419–432.

Blenckner T., Adrian R., Livingstone D.M., Jennings E., Weyhenmeyer G.A., George D.G. *et al.* (2007) Large-scale climatic signatures in lakes across Europe: a meta-analysis. *Global Change Biology*, **13**, 1314–1326.

Burns C.W. (1968) Relationship between body size of filter-feeding cladocera and maximum size of particle ingested. *Limnology and Oceanography*, **13**, 675–678.

Caceres C.E. & Schwalbach M.S. (2001) How well do laboratory experiments explain field patterns of zooplankton emergence? *Freshwater Biology*, **46**, 1179–1189.

DeStasio B.T., Hill D.K., Kleinhans J.M., Nibbelink N.P. & Magnuson J.J. (1996) Potential effects of global climate change on small north-temperate lakes: physics, fish, and plankton. *Limnology and Oceanography*, **41**, 1136–1149.

Feuchtmayr H., Moss B., Harvey I., Moran R., Hatton K., Connor L. *et al.* (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.

George G., Hurley M. & Hewitt D. (2007) The impact of climate change on the physical characteristics of the larger lakes in the English Lake District. *Freshwater Biology*, **52**, 1647–1666.

Gyllstrom M. & Hansson L.A. (2004) Dormancy in freshwater zooplankton: induction, termination and the importance of benthic-pelagic coupling. *Aquatic Sciences*, **66**, 274–295.

Hansen P.J., Bjørnsen P.K. & Hansen B.W. (1997) Zooplankton grazing and growth: scaling within the 2–2,000-μm body size range. *Limnology and Oceanography*, **42**, 687–704.

Hansson L.-A., Nicolle A., Graneli W., Hallgren P., Kritzberg E., Persson A. *et al.* (2013) Food-chain length alters community responses to global change in aquatic systems. *Nature Climate Change*, **3**, 228–233.

Hillebrand H., Durselen C.D., Kirschtel D., Pollingher U. & Zohary T. (1999) Biovolume calculation for pelagic and benthic microalgae. *Journal of Phycology*, **35**, 403–424.

Hondzo M. & Stefan H.G. (1993) Regional water temperature characteristics of lakes subjected to climate-change. *Climatic Change*, **24**, 187–211.

Jäger C.G., Diehl S. & Schmidt G.M. (2008) Influence of water-column depth and mixing on phytoplankton biomass, community composition, and nutrients. *Limnology and Oceanography*, **53**, 2361–2373.

Johansson M., Gorokhova E. & Larsson U. (2004) Annual variability in ciliate community structure, potential prey and predators in the open northern Baltic Sea proper. *Journal of Plankton Research*, **26**, 67–80.

Kunz T.J. & Diehl S. (2003) Phytoplankton, light and nutrients along a gradient of mixing depth: a field test of producer-resource theory. *Freshwater Biology*, **48**, 1050–1063.

Lampert W., Fleckner W., Rai H. & Taylor B.E. (1986) Phytoplankton control by grazing zooplankton – a study on the spring clear-water phase. *Limnology and Oceanography*, **31**, 478–490.

Lewandowska A. & Sommer U. (2010) Climate change and the spring bloom: a mesocosm study on the influence of

light and temperature on phytoplankton and mesozooplankton. *Marine Ecology Progress Series*, **405**, 101–111.

Livingstone D.M. (2003) Impact of secular climate change on the thermal structure of a large temperate central European lake. *Climatic Change*, **57**, 205–225.

Magnuson J.J., Webster K.E., Assel R.A., Bowser C.J., Dillon P.J., Eaton J.G. *et al.* (1997) Potential effects of climate changes on aquatic systems: Laurentian Great Lakes and Precambrian Shield Region. *Hydrological Processes*, **11**, 825–871.

Mazumder A. & Taylor W.D. (1994) Thermal structure of lakes varying in size and water clarity. *Limnology and Oceanography*, **39**, 968–976.

Pancella J.R. & Stross R.G. (1962) Effectiveness of light on hatching of resting eggs of *Daphnia*. *American Zoologist*, **2**, 435.

Peeters F., Livingstone D.M., Goudsmit G.H., Kipfer R. & Forster R. (2002) Modeling 50 years of historical temperature profiles in a large central European lake. *Limnology and Oceanography*, **47**, 186–197.

Peeters F., Straile D., Lorke A. & Livingstone D.M. (2007a) Earlier onset of the spring phytoplankton bloom in lakes of the temperate zone in a warmer climate. *Global Change Biology*, **13**, 1898–1909.

Peeters F., Straile D., Lorke A. & Ollinger D. (2007b) Turbulent mixing and phytoplankton spring bloom development in a deep lake. *Limnology and Oceanography*, **52**, 286–298.

Quinn G.P. & Keough M.J. (2002) *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge.

Rose J.M. & Caron D.A. (2007) Does low temperature constrain the growth rates of heterotrophic protists? Evidence and implications for algal blooms in cold waters. *Limnology and Oceanography*, **52**, 886–895.

Rother A., Pitsch M. & Huelsmann S. (2010) The importance of hatching from resting eggs for population dynamics and genetic composition of Daphnia in a deep reservoir. *Freshwater Biology*, **55**, 2319–2331.

Sarnelle O. (1993) Herbivore effects on phytoplankton succession in a eutrophic Lake. *Ecological Monographs*, **63**, 129–149.

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 D.W. (2009) Lakes as sentinels and integrators for the effects of climate change on watersheds, airsheds, and landscapes. *Limnology and Oceanography*, **54**, 2349–2358.

Schwartz S.S. & Hebert P.D.N. (1987) Methods for the activation of the resting eggs of *Daphnia*. *Freshwater Biology*, **17**, 373–379.

Sebastian P., Stibor H., Berger S. & Diehl S. (2012) Effects of water temperature and mixed layer depth on zooplankton body size. *Marine Biology*, **159**, 2431–2440.

de Senerpont Domis L.N., Elser J.J., Gsell A.S., Huszar V.L.M., Ibelings B.W., Jeppesen E. *et al.* (2013) Plankton dynamics under different climatic conditions in space and time. *Freshwater Biology*, **58**, 463–482.

de Senerpont Domis L.N., Mooij W.M., Huelsmann S., Van Nes E.H. & Scheffer M. (2007) Can overwintering versus diapausing strategy in Daphnia determine match-mismatch events in zooplankton-algae interactions? *Oecologia*, **150**, 682–698.

Sieburth J.M., Smetacek V. & Lenz J. (1978) Pelagic ecosystem structure – heterotrophic compartments of plankton and their relationship to plankton size fractions – comment. *Limnology and Oceanography*, **23**, 1256–1263.

Sommer U., Adrian R., de Senerpont Domis L., Elser J.J., Gaedke U., Ibelings B. *et al.* (2012) Beyond the Plankton Ecology Group (PEG) model: mechanisms driving plankton succession. *Annual Review of Ecology, Evolution, and Systematics*, **43**, 429–448.

Stenseth N.C., Mysterud A., Ottersen G., Hurrell J.W., Chan K.-S. & Lima M. (2002) Ecological effects of climate fluctuations. *Science*, **297**, 1292–1296.

Stoecker D. & Guillard R.R.L. (1982) Effects of temperature and light on the feeding rate of Favella sp. (ciliated Protozoa, suborder Tintinnina). *Marine Pelagic Protozoa and Microzooplankton Ecology*, **58**, 309–318.

Straile D. (2000) Meteorological forcing of plankton dynamics in a large and deep continental European lake. *Oecologia*, **122**, 44–50.

Straile D. (2005) Food webs in lakes: seasonal dynamics and the impact of climate variability. In: *Aquatic Food Webs: An Ecosystem Approach*. (Eds Belgrano A., Scharler U.M., Dunne J.A. & Ulanowicz R.E.), pp. 41–50. Oxford University Press, New York, NY, U.S.A.

Straile D., Adrian R. & Schindler D.E. (2012) Uniform temperature dependency in the phenology of a keystone herbivore in lakes of the Northern hemisphere. *PLoS ONE*, **7**, 1–9.

Stross R.G. (1966) Light and temperature requirements for diapause development and release in *Daphnia*. *Ecology*, **47**, 368–374.

Thackeray S.J. (2012) Mismatch revisited: what is trophic mismatching from the perspective of the plankton? *Journal of Plankton Research*, **34**, 1001–1010.

Tirok K. & Gaedke U. (2006) Spring weather determines the relative importance of ciliates, rotifers and crustaceans for the initiation of the clear-water phase in a large, deep lake. *Journal of Plankton Research*, **28**, 361–373.

Utermöhl H. (1958) Zur Verfollkommnung der quantitativen Phytoplankton-Methodik. *Mitteilungen. Internationale Vereiningung für Theoretische und Angewandte Limnologie*, **9**, 1–38.

Vandekerkhove J., Declerck S., Brendonck L., Conde-Porcuna J.M., Jeppesen E. & De Meester L. (2005) Hatching of cladoceran resting eggs: temperature and photoperiod. *Freshwater Biology*, **50**, 96–104.

Weisse T. (2006) Freshwater ciliates as ecophysiological model organisms – lessons from Daphnia, major achievements, and future perspectives. *Archiv für Hydrobiologie*, **167**, 371–402.

Weisse T., Mueller H., Pintocoelho R.M., Schweizer A., Springmann D. & Baldringer G. (1990) Response of the microbial loop to the phytoplankton spring bloom in a large prealpine lake. *Limnology and Oceanography*, **35**, 781–794.

Weyhenmeyer G.A. & Karlsson J. (2009) Nonlinear response of dissolved organic carbon concentrations in boreal lakes to increasing temperatures. *Limnology and Oceanography*, **54**, 2513–2519.

Winder M., Berger S.A., Lewandowska A., Aberle N., Lengfellner K., Sommer U. *et al.* (2012) Spring phenological responses of marine and freshwater plankton to changing temperature and light conditions. *Marine Biology*, **159**, 2491–2501.

Winder M. & Schindler D.E. (2004a) Climatic effects on the phenology of lake processes. *Global Change Biology*, **10**, 1844–1856.

Winder M. & Schindler D.E. (2004b) Climate change uncouples trophic interactions in an aquatic system. *Ecology*, **85**, 2100–2106.

Wolf H.G. & Carvalho G.R. (1989) Resting eggs of lake-*Daphnia* II. *In situ* observations on the hatching of eggs and their contribution to population and community structure. *Freshwater Biology*, **22**, 471–478.

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