Global Change Biology

Global Change Biology (2010) **16**, 1954–1965, doi: 10.1111/j.1365-2486.2009.02134.x

Water temperature and stratification depth independently shift cardinal events during plankton spring succession

STELLA A. BERGER[1], SEBASTIAN DIEHL[2], HERWIG STIBOR[3], GABRIELE TROMMER and MIRIAM RUHENSTROTH

Department Biologie II, Ludwig-Maximilians-Universität München, Grosshaderner Strasse 2, 82152 Planegg-Martinsried, Germany

Abstract

In deep temperate lakes, the beginning of the growing season is triggered by thermal stratification, which alleviates light limitation of planktonic producers in the surface layer and prevents heat loss to deeper strata. The sequence of subsequent phenological events (phytoplankton spring bloom, grazer peak, clearwater phase) results in part from coupled phytoplankton–grazer interactions. Disentangling the separate, direct effects of correlated climatic drivers (stratification-dependent underwater light climate vs. water temperature) from their indirect effects mediated through trophic feedbacks is impossible using observational field data, which challenges our understanding of global warming effects on seasonal plankton dynamics. We therefore manipulated water temperature and stratification depth independently in experimental field mesocosms containing ambient microplankton and inocula of the resident grazer *Daphnia hyalina*. Higher light availability in shallower surface layers accelerated primary production, warming accelerated consumption and growth of *Daphnia*, and both factors speeded up successional dynamics driven by trophic feedbacks. Specifically, phytoplankton peaked and decreased earlier and *Daphnia* populations increased and peaked earlier at both shallower stratification and higher temperature. The timing of ciliate dynamics was unrelated to both factors. Volumetric peak densities of phytoplankton, ciliates and *Daphnia* in the surface layer were also unaffected by temperature but declined with stratification depth in parallel with light availability. The latter relationship vanished, however, when population sizes were integrated over the entire water column. Overall our results suggest that, integrated over the entire water column of a deep lake, surface warming and shallower stratification independently speed up spring successional events, whereas the magnitudes of phytoplankton and zooplankton spring peaks are less sensitive to these factors. Therefore, accelerated dynamics under warming need not lead to a trophic mismatch (given similar grazer inocula at the time of stratification). We emphasize that entire water column dynamics must be studied to estimate global warming effects on lake ecosystems.

Keywords: ciliates, clearwater phase, *Daphnia hyalina*, enclosure experiment, mesocosms, phytoplankton spring bloom

Received 23 June 2009; revised version received 26 October 2009 and accepted 28 October 2009

Introduction

Changes in the phenology of plants and animals linked to global warming occur in all marine, freshwater, and terrestrial systems (Parmesan, 2006). For example, the onset of spring is advancing in many terrestrial ecosystems (Parmesan & Yohe, 2003). Similarly, winter and spring warming correlates with advances in the seasonal development of temperate lake plankton (Müller-Navarra *et al*., 1997; Scheffer *et al*., 2001; Winder & Schindler, 2004a; Adrian *et al*., 2006; Shatwell *et al*., 2008). A fundamental difference between phenological events in terrestrial vs. pelagic aquatic systems is that the former are largely driven by individual development of long-lived organisms, whereas the latter result from rapid population responses of short-lived organisms to seasonal shifts in abiotic conditions. In pelagic systems, the underlying mechanisms are two-fold. First, increased spring temperatures accelerate physiological processes such as

Correspondence: Stella A. Berger, e-mail: Berger@zi.biologie.uni-muenchen.de

[1]Present address: Stella A. Berger, Department of Biology, University of Bergen, PO Box 7803, 5020 Bergen, Norway.

[2]Present address: Sebastian Diehl, Department of Ecology and Environmental Sciences, Umeå University, 90187 Umeå, Sweden.

[3]Present address: Herwig Stibor and Gabriele Trommer, GIS Europole Mer, European Institute for Marine Studies, Technopole Brest Iroise, Place Nicolas Copernic, 29280 Plouzane; France.

nutrient uptake, feeding, respiration, and growth (Goldman & Carpenter, 1974). Second, increased spring temperatures accelerate the process of thermal stratification of the water column which, in turn, governs the underwater light climate in the surface layer and, thus, the onset of the phytoplankton growing season (Diehl *et al*., 2002; Livingstone, 2003; Peeters *et al*., 2007a; Austin & Colman, 2008). Although stratification primarily affects light-dependent autotrophic processes, temperature is believed to be more strongly affecting heterotrophic processes, especially under the light-limited conditions prevailing in late winter (Sommer *et al*., 1986; Peeters *et al*., 2007a; Rose & Caron, 2007).

Time series analyses have indeed revealed that the onset of the phytoplankton spring bloom in deep lakes is correlated with the onset of stratification, whereas the timing of subsequent events related to grazing (e.g., the spring peak in zooplankton biomass and the grazing induced decline of phytoplankton towards the so-called clearwater phase) is correlated with temperature (Müller-Navarra *et al*., 1997; Straile & Adrian, 2000; Straile, 2002; Winder & Schindler, 2004b; Peeters *et al*., 2007a). Disentangling the separate effects of stratification and temperature on spring plankton dynamics from purely descriptive lake data is, however, difficult, because stratification depth and surface water temperature are usually strongly negatively correlated (Mazumder & Taylor, 1994; Berger *et al*., 2006). Moreover, the typical sequence of spring phenological events in temperate lakes (phytoplankton bloom, grazer peak, clearwater phase) is, in part, the result of a coupled interaction between phytoplankton and various micro- and mesograzers (Lampert *et al*., 1986; Sarnelle, 1993; Tirok & Gaedke, 2006; Peeters *et al*., 2007b). Thus, direct effects of stratification and/or temperature on one food web component will indirectly affect other components. Disentangling the direct effects of different, but correlated, climatic drivers from their indirect effects mediated through species interactions is therefore a major challenge, which calls for the increased use of experimental approaches (Stenseth & Mysterud, 2002; Berger *et al*., 2007).

Here, we report on a field experiment in which we independently manipulated water temperature and stratification depth to investigate their separate and interactive effects on spring plankton dynamics. We hypothesized that shallower stratification would directly speed up phytoplankton growth (by altering the light climate in the mixed surface layer and thus the rate of photosynthesis) and that warmer temperatures would directly speed up zooplankton metabolism. Both shallower stratification and warmer temperatures should then indirectly speed up all subsequent successional events through similar trophic feedbacks triggered by either increased primary production or increased zooplankton metabolism (i.e., faster zooplankton growth, earlier grazing induced termination of the phytoplankton bloom, earlier onset of the clearwater phase and, consequently, earlier onset of food limitation leading to an earlier zooplankton decline). We furthermore expected that increased primary production in shallower surface layers should translate into higher peak densities of phytoplankton and zooplankton per volume of surface layer. In contrast, we expected increased grazing and metabolic rates at higher temperatures to translate into lower peak densities of phytoplankton and zooplankton.

Materials and methods

Study site and experimental design

The experiment was carried out in lake Brunnsee (47°56′N, 12°26′ E), close to the University of Munich's Limnological Research Station at Seeon (S. Germany). Lake Brunnsee is small (area 5.8 ha) and deep (maximum depth 19 m). Its water is poor in total phosphorus ($<10\,\mu g\,L^{-1}$) but rich in silica and nitrate ($>2\,mg\,L^{-1}$). The lake usually freezes over in November or December. Ice-off, followed by the onset of stratification, occurs between February and April.

We used enclosures (diameter 0.95 m, total depth 10 m) to simulate stratified water columns consisting of a well-mixed surface layer (epilimnion) and an unmixed deep water layer (hypolimnion). The experiment was run in a 3×2 factorial design with three epilimnion depths (2, 4, and 8 m; and corresponding hypolimnion depths of 8, 6, and 2 m) cross-classified with two temperature treatments ('ambient' and 'cold'). Each treatment was replicated twice. Enclosures were cylindrical bags of transparent folia (Tricoron, RKW Wasserburg, Germany) surrounded by black silage film. The latter strongly increased the steepness of the vertical light gradient, thus creating a wide range of mixed layer underwater light levels (Fig. 1c) corresponding to physical stratification depths in the surrounding lake of ca. 5, 10, and 25 m (Berger *et al*., 2007). Epilimnia were kept well-mixed by intermittently blowing compressed air to the desired epilimnion depth.

The mixing was highly effective. Vertical temperature gradients *within* the epilimnia were always $<1\,°C$. However, as the lake stratified at 3–4 m depth, temperature differences between enclosures developed in the 'ambient' temperature treatments. Consequently, epilimnion temperatures decreased from 2 to 8 m 'ambient' enclosures (Fig. 1a and b), mimicking a natural situation where epilimnion depth and temperature are inversely related. 'Cold' epilimnia were accomplished by surrounding 'cold' enclosures with an outer, 12 m deep

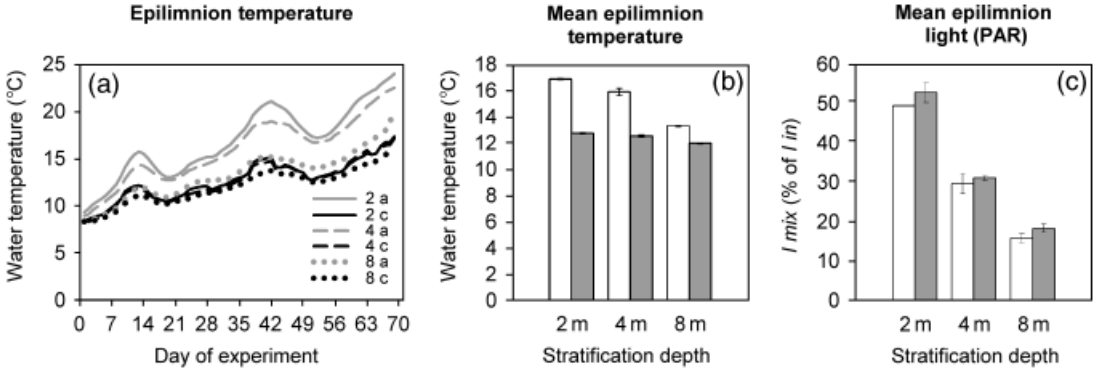

Fig. 1 (a) Temporal development of epilimnetic water temperatures, (b) time-averaged epilimnetic water temperatures, and (c) depth-averaged epilimnetic light intensities (I_{mix} in per cent of incident radiation I_{in}) as a function of stratification depth (2, 4, 8 m) and temperature treatment. Gray lines and white bars, 'ambient' ('a'); black lines and gray bars, 'cold' ('c') temperature. Shown are 5-day running means in (a), and means ± 1 SE in (b and c).

bag. The water inside this bag was intermittently mixed with compressed air, yielding an unstratified water bath of reduced temperature. The procedure was highly effective, i.e., epilimnion temperatures in the 'cold' enclosures were nearly independent of mixing depth and were considerably lower than in the 'ambient' treatments (Fig. 1a and b, Table 1). Furthermore, epi- and hypolimnion temperatures were similar in the 'cold' treatments, whereas hypolimnion temperatures in the 'ambient' enclosures followed the vertical profile of the surrounding lake (Appendix S1).

The experiment lasted for 10 weeks from 21 April to 28 June, 2005. In mid-April, the spring phytoplankton bloom is already underway in Lake Brunnsee, but a long lasting ice cover prevented us from setting up the experiment earlier. The taxonomic composition of the phytoplankton community was, however, still typical of early spring, and bloom dynamics could be easily triggered with a modest nutrient spike. On 20 April, we filled all enclosures with lake water filtered through 50 μm gauze. This excluded most crustacean zooplankton, but preserved microzooplankton and the natural phytoplankton community. Before the establishment of the mixing regimes, we fertilized all enclosures down to the bottom with 14.3 $\mu g\,L^{-1}$ phosphorus (as KH_2PO_4) to an initial total phosphorus content of 25 $\mu g\,L^{-1}$ to mimic the nutrient pulse associated with spring overturn. Small inocula of *Daphnia hyalina* were added to all enclosures once per week over the first 4 weeks of the experiment to simulate spring recruitment from an egg bank. All *Daphnia* were descendants of three clones that had been isolated from the lake and been precultured at 20 °C. The *Daphnia* were acclimated to 13 °C over night in a climate chamber and the clonal populations were carefully mixed in a 200 L container before stocking. Stocking densities (per volume of epilimnion) were 1.2 individuals (ind.) L^{-1} on April 21, 0.6 ind. L^{-1} on April 26, 0.15 ind. L^{-1} on May 3, and 0.3 ind. L^{-1} on May 11. Zooplankton counts indicated that initial mortality was approximately 90% leading to a simulated recruitment rate of *Daphnia* from resting eggs in the sediment between 0.002 and 0.017 ind. L^{-1} day^{-1}, which is close to observations by Càceres (1998).

Sampling program

Epilimnion temperatures in all enclosures were logged every 30 min (Voltcraft K 204; Conrad Elektronik, Munich, Germany). We also recorded vertical temperature profiles with a multi-probe (LT1/T; WTW-Wissenschaftlich-Technische Werkstätten, Weilheim, Germany) in 1 m steps once a week in each enclosure and several times a week in the outer water bath. Every 2 weeks, we measured vertical profiles of photosynthetically active radiation (PAR) in 1 m steps with a spherical quantum sensor (LI-139SA; Licor, Lincoln, NE, USA) while simultaneously measuring incident PAR (flat quantum sensor LI-190SA) above the water surface. For each enclosure, we calculated the depth-averaged intensity of PAR (I_{mix}) as percentage of incident PAR as described in Diehl *et al*. (2002).

Once per week, we sampled water from the surface and from the mean hypolimnion depth of each enclosure which was immediately filtered through 250 μm gauze (to exclude mesozooplankton). Measurements of vertical chlorophyll *a* (chl *a*) profiles with a fluorescence probe (SCUFA; Turner Design, Sunnyvale, CA, USA) indicated that these sampling depths were representative of the epi- and hypolimnia, respectively. We determined chl *a* concentrations fluorometrically (TD 700; Turner Design) from aliquots of 100–300 mL after filtration onto glass fiber filters (Whatman GFF 25; Whatman International Ltd, Maidstone, UK) and extraction in acetone. Subsamples for the enumeration of phytoplankton and ciliates in the epilimnion were immedi-

Table 1 ANOVA of the effects of stratification depth (Depth), water temperature (Temp) and their interaction (Depth × Temp) on environmental conditions (temperature, light) in the surface mixed layer, on the timing of cardinal successional events, and on the magnitudes of ciliate, phytoplankton and *Daphnia* spring peaks in the surface mixed layer and in the entire water column

	Treatment effects (*P*-values)			
Dependent variable	Depth	Temp	Depth × Temp	Overall model R^2
Environmental conditions				
Average epilimnion temperature	< **0.001**	< **0.001**	< **0.001**	0.995
Average epilimnion light (PAR)	< **0.001**	0.11	0.84	0.99
Timing of cardinal events – epilimnion				
Onset of ciliate development	*	*	*	
Timing of ciliate peak	ns†	ns†	ns†	
End of ciliate development	0.69	0.13	0.087	0.66
Onset of phytoplankton bloom	< **0.001**	0.44	0.95	0.95
Timing of phytoplankton peak	**0.016**	**0.002**	0.42	0.88
End of phytoplankton bloom – clearwater phase	**0.010**	< **0.001**	0.93	0.96
Duration of phytoplankton bloom	0.87	< **0.001**	0.93	0.93
Onset of *Daphnia* development	**0.017**	**0.007**	0.71	0.85
Timing of *Daphnia* peak	0.17	**0.002**	**0.020**	0.86
End of *Daphnia* development	‡	‡	‡	
Magnitude of peaks – epilimnion				
Ciliate peak	< **0.001**	0.19	0.20	0.93
Phytoplankton peak	< **0.001**	0.99	0.065	0.98
Daphnia peak	**0.014**	0.70	0.36	0.78
Timing of cardinal events – entire water column				
Timing of phytoplankton peak	**0.016**	**0.002**	0.42	0.88
Timing of *Daphnia* peak	**0.040**	< **0.001**	**0.036**	0.93
Magnitude of peaks – entire water column				
Phytoplankton peak	0.57	0.24	**0.019**	0.77
Daphnia peak	0.052	0.82	0.73	0.64

*Biomass exceeded threshold for bloom development already on day 1.
†ns, no significant difference, ANOVA cannot be performed, because of zero variance.
‡Biomass did not decline below threshold for bloom development during experiment.
P-values in bold font indicate significant treatment effects on the 0.05 level ($P < 0.05$).

ately fixed with Lugols solution (to a final concentration of 2%) and subsequently counted and measured in an inverted microscope (Wild M 40; WILD Heerbrugg, Gais, Switzerland) at × 40– × 400 magnification. Once per week, we sampled zooplankton by means of two vertical hauls with a 55 μm mesh net, one taken through the epilimnion and one through the hypolimnion. We fixed the zooplankton samples with cold sugar formalin (250 g sugar in 1 L formalin) to a final concentration of 4%. All *D. hyalina* individuals of a haul were counted under a dissecting microscope at × 25 magnification.

Data processing and statistics

We analyzed the data separately for the mixed surface layer (on a volumetric basis) and for the entire water column (on an areal basis). In all treatments phytoplankton, ciliates, and *Daphnia* showed bloom dynamics; i.e., they increased from initial densities to a peak some time into the experiment to subsequently decline to densities close to or below starting values [Appendix S2, S4]. We characterized these cardinal successional events by their timing (onset, peak, and end of mass development) and by their magnitude (peak height). A peak was defined as an abundance maximum in a given enclosure over the course of the experiment and could thus only be determined with weekly temporal resolution. In contrast, onset and end of a mass development were defined by the transition of thresholds immediately before or after the peak. We determined the day of transition by linear interpolation between the sampling dates straddling the transition of the threshold. The thresholds themselves were set to approximately 10% of the highest observed peak, i.e., 30 $\mu m^3 L^{-1}$ of ciliate biovolume, 2 μg chl *a* L^{-1}, and 10 *Daphnia* L^{-1} in the epilimnion. The thresholds for depth-integrated values were set to 10 times these values, i.e., 20 mg chl *a* m^{-2} and 10^4 *Daphnia* m^{-2} (phytoplankton and ciliates were not counted in the hypolimnion; it was thus not possible to calculate depth-integrated biovolumes for these taxa). The results

were not sensitive to the exact choice of the threshold values. Also, an alternative approach to characterizing the timing and magnitude of cardinal events based on nonlinear curve fits to the observed temporal dynamics (see Berger *et al*., 2007) yielded very similar results (data not shown).

Following Sieburth *et al*. (1978), we categorized phytoplankton taxa according to their largest linear dimension as nanophytoplankton (<20 μm), microphytoplankton (20–200 μm), and mesophytoplankton (>200 μm). These size classes differ in their grazing susceptibility, with nanophytoplankton being edible by both ciliates and *Daphnia*, microphytoplankton being largely resistant to ciliates but edible by *Daphnia*, and mesophytoplankton being resistant to both grazers (Sommer & Lengfellner, 2008). We therefore show the dynamics of these three phytoplankton size classes separately. Total phytoplankton biovolume was highly correlated with chl *a* (Pearson's $r = 0.94$, $P < 0.0001$, $n = 132$). We therefore assessed treatment effects on total phytoplankton biomass only with chl *a* data.

Significant wall growth was not observed until day 49. At that time most cardinal successional events had already occurred, except for the *Daphnia* peak in some 'cold' enclosures. The periphyton biomass consisted mainly of benthic diatoms (*Achnanthes*, *Cocconeis*, *Cymbella*, *Diatoma*, *Navicula*, *Eunotia*) and the filamentous green alga *Mougeotia*. Although the former taxa made a minor contribution to pelagic biomass towards the end of the experiment, *Mougeotia* was excluded from the measurements of pelagic chl *a* and algal biovolume by screening with a 250 μm mesh.

To statistically test for effects of stratification depth and water temperature on the timing and magnitude of successional events, we performed two-way ANOVAs with stratification depth (2, 4, 8 m) and water temperature ('ambient' vs. 'cold') as fixed factors. All statistical analyses were performed with SPSS 16.0. Data of peak magnitudes were log-transformed to stabilize variances.

Results

Temperature and light

As the lake warmed up, epilimnion temperatures increased in all enclosures and diverged increasingly between 'ambient' and 'cold' treatments over the course of the experiment (Fig. 1a). Starting from 8.3 to 9.3 °C on 21 April, epilimnion temperatures increased to between 16.0 (8 m 'cold') and 24.0 °C (2 m 'ambient') on 28 June. On average, water temperatures in 'ambient' enclosures were higher in shallow than in deep epilimnia; in contrast, epilimnion temperatures in 'cold' enclosures were almost independent of stratification depth and considerably lower than in 'ambient' treatments (Fig. 1b; Table 1). Compared with the mean temperature observed at 2 m depth in Lake Brunnsee over the period 20 April to 30 June in the years 1987–2001 (Limnological Research Station Seeon, unpublished data), water temperatures in the 2 and 4 m treatments were 2–3 °C warmer in 'ambient' enclosures and 1.3 °C colder in 'cold' enclosures.

Average epilimnetic light intensity (I_{mix}) decreased with stratification depth and was independent of water temperature (Fig. 1c; Table 1). I_{mix} ranged from ca. 51% to 17% of incoming light in the 2 and 8 m treatments, respectively. The euphotic zone (where PAR exceeds 1% of incoming PAR) reached down to between 6 and 6.5 m, indicating strong light limitation of algal growth in the lower parts of the 8 m deep epilimnia.

Plankton dynamics in the epilimnion

The starting density of ciliates was fairly high and already exceeded the threshold for bloom development. Epilimnetic ciliate density continued to increase sharply and peaked already after 1 week into the experiment (Fig. 2a) to subsequently decline more gradually to values below starting levels (Appendix S2). Ciliate taxonomic composition was only assessed during the bloom, when the community was completely dominated by oligotrich, algivorous ciliates of the genera *Rhimostrombidium* spp. and *Pelagostrombidium* spp. (S. Wickham, personal communication). In accordance with expectations, ciliate peak height was inversely related to stratification depth; peak height was unrelated to temperature treatment (Fig. 2d; Table 1). In contrast to expectations, the timing of the peak and of the end of the ciliate bloom were unrelated to both stratification depth and temperature (Fig. 2a; Table 1).

The early ciliate peak preceded the phytoplankton bloom. In consequence, the epilimnetic chl *a* concentration and total algal biovolume did not increase until the 2nd or 3rd week, following the progression of the ciliate decline (Fig. 3, Appendix S2). In agreement with expectations, the phytoplankton bloom was initiated earlier and peaked at higher densities in shallow than in deep epilimnia (Fig. 2b and e; Table 1). Moreover, the timing of the peak and the beginning of the clearwater phase were both accelerated by shallower stratification and by higher temperature (Fig. 2b; Table 1). In contrast to expectations, peak height of the phytoplankton bloom was unrelated to temperature (Fig. 2e, Table 1).

Changes in the phytoplankton community suggest that phytoplankton and grazer dynamics were tightly coupled. The initial phytoplankton community was dominated by highly edible, nanoplanktonic taxa (*Cyclotella* spp. <20 μm = 61%, *Rhodomonas minuta* = 14%, and other nanophytoplankters = 7% of total initial phy-

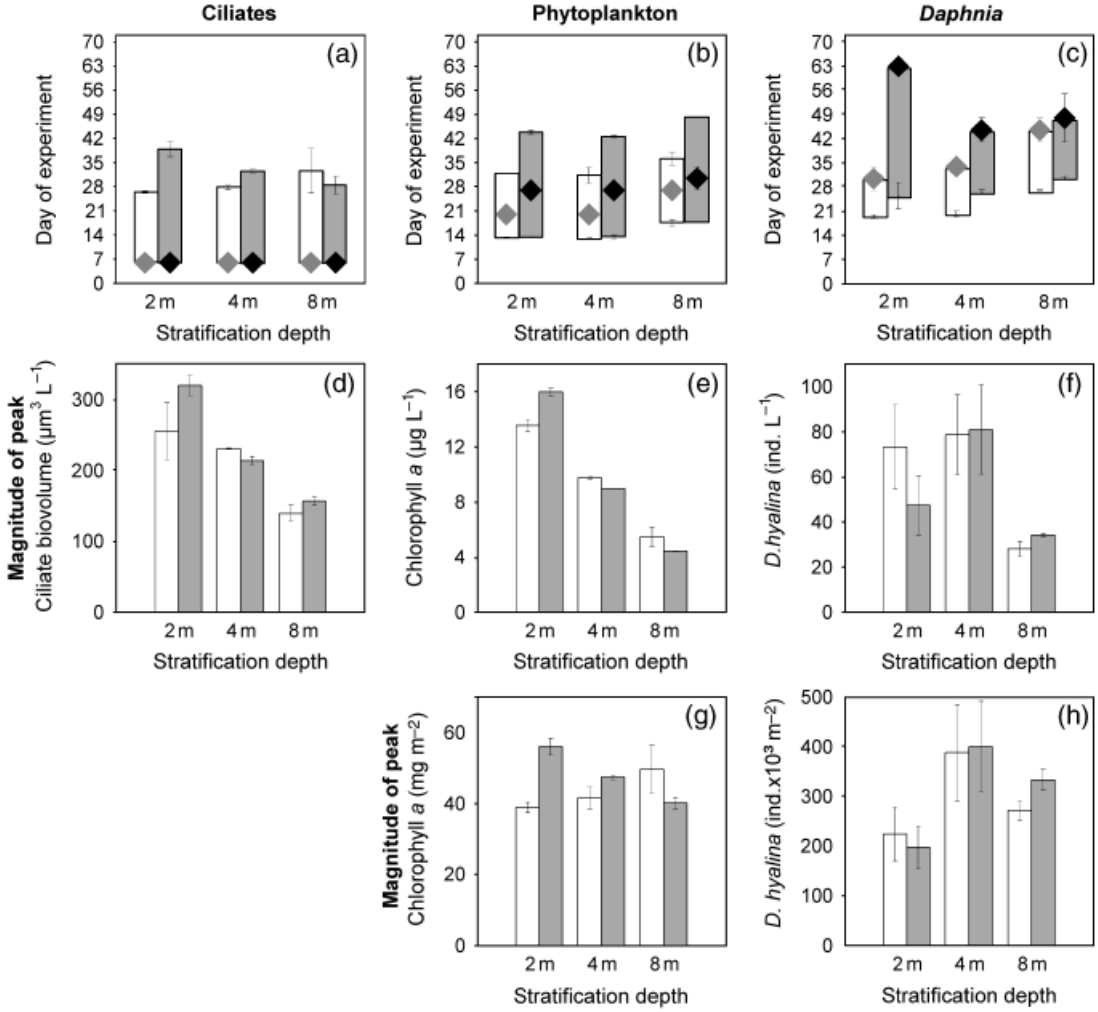

Fig. 2 (a–c) Timing of cardinal events in the seasonal, epilimnetic development of (a) ciliates, (b) phytoplankton, and (c) *Daphnia* as a function of stratification depth (2, 4, 8 m) and temperature treatment (white bars and gray diamonds, 'ambient'; gray bars and black diamonds, 'cold' temperature). Diamonds mark the timing of population peaks. For phytoplankton and *Daphnia* the lower end of a bar marks the onset of population development (± 1 SE). For ciliates and phytoplankton the upper end of a bar marks the end of the population decline (± 1 SE). The timing of the onset of ciliate development and of the end of the *Daphnia* decline are not shown, because densities were above the required threshold values in all or most treatments; (d–f) volumetric peak densities in the epilimnion of (d) ciliates, (e) phytoplankton, and (f) *Daphnia*; and (g–h) areal peak densities in the entire water column of (g) phytoplankton, and (g) *Daphnia* as a function of stratification depth (2, 4, 8 m) and temperature treatment (white bars, 'ambient'; gray bars, 'cold' temperature). Shown are means ± 1 SE.

toplankton biovolume). In parallel with ciliates, nanoplankton biomass showed a weak peak in week 1 and subsequently declined to very low densities in weeks 4 and 5 (Fig. 3, Appendix S2). The delayed development of total phytoplankton biomass therefore seemed to be related to high grazing pressure by ciliates on the initially dominating nanophytoplankton, whereas the early ciliate decline seemed to be related to a subsequent shortage of suitable nanoplanktonic food. The nanophytoplankton decline was, in turn, accompanied by a strong increase in microphytoplankton and a much weaker increase in inedible mesophytoplankton (Fig. 3). The phytoplankton bloom in weeks 3 and 4 was thus dominated by microplanktonic taxa (*Fragilaria* spp. = 47%–75%, *Gymnodinium* spp. and *Cryptomonas ovata* = 5%–14%, *Scenedesmus* spp. and colonial microchlorophytes = 1%–17%) that were inedible for ciliates but edible for *D. hyalina*.

Shallower stratification accelerated the onset and the termination of the phytoplankton bloom about equally. Consequently, the duration of the phytoplankton bloom (calculated as date of clearwater phase−date of bloom onset) was unaffected by stratification depth; in contrast, the duration of the phytoplankton bloom was extended by almost 2 weeks in all 'cold' treatments

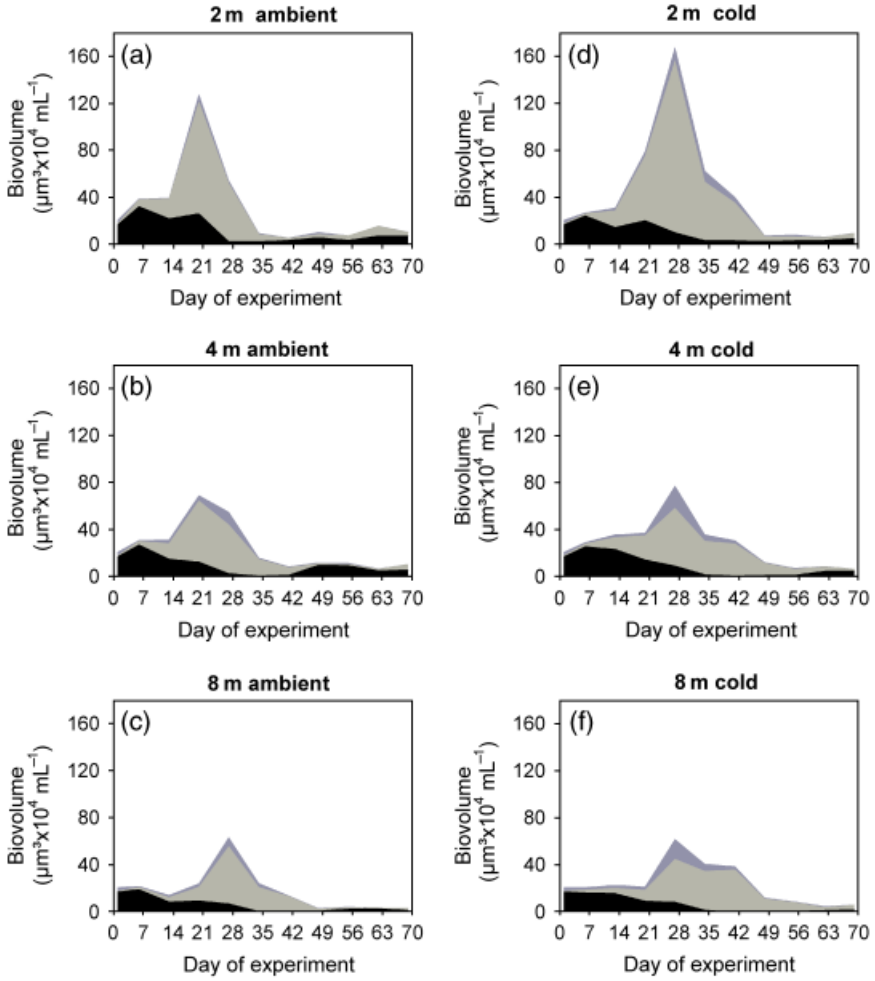

Fig. 3 Temporal development of the epilimnic densities of nano-, micro-, and inedible phytoplankton as a function of stratification depth (2, 4, 8 m) and temperature treatment: (a–c) 'ambient' and (d–f) 'cold' temperature. Nanophytoplankton, black area; microphytoplankton, light gray area; inedible phytoplankton, dark gray area.

(Fig. 2b; Table 1). This is consistent with the interpretation that the onset of the phytoplankton bloom was driven by light dependent, autotrophic processes, whereas the termination of the bloom was driven by temperature dependent, heterotrophic processes, i.e., grazing. In line with this, the timing of the phytoplankton peak corresponded almost exactly with the onset of the *Daphnia* development, and the timing of the clear-water phase occurred around the *Daphnia* peak (Fig. 2b and c; Pearson's $r \geq 0.72$, $P \leq 0.009$, $n = 12$), suggesting that *Daphnia* grazing contributed significantly to the termination of the phytoplankton bloom.

With the exception of the 'cold' 2 m treatment, epilimnetic *Daphnia* dynamics were also in close agreement with most expectations. That is, the height of the *Daphnia* peak was inversely related to stratification depth (Fig. 2f; Table 1). Moreover, the timing of both the onset and the peak of *Daphnia* mass development were accelerated by shallower stratification and by higher temperature (Fig. 2c; Table 1). Deviating from these patterns, however, the *Daphnia* peak height in the 'cold' 2 m treatment was lower than in the corresponding 4 m treatment and was reached several weeks later than in any other treatment (Fig. 2c and f; Table 1). Also deviating from expectations, the height of the *Daphnia* peak was unrelated to temperature treatment (Fig. 2f; Table 1). In many enclosures, *Daphnia* densities did not fall below the threshold of 10 ind. L^{-1} (Appendix S2). It was therefore not possible to assess treatment effects on the timing of the end and on the duration of *Daphnia* mass development.

Plankton dynamics in the entire water column

Hypolimnetic chl *a* concentrations were much lower than epilimnetic concentrations in the 2 and 4 m treatments, whereas the two were more similar in the 8 m treatments (Appendix S3). In all treatments, the temporal development of chl *a* in the hypolimnion followed epilimnetic

concentrations with a time lag of 1–2 weeks, suggesting that import of sinking algae from the epilimnion-dominated hypolimnetic phytoplankton dynamics (Appendices S2, S3). A similar pattern occurred in the vertical and temporal distributions of *Daphnia* suggesting that *Daphnia* stayed mostly in the epilimnion and that hypolimnetic *Daphnia* dynamics were largely driven by spill-over from the epilimnion (Appendices S2, S3). Consequently, with respect to timing, depth-integrated dynamics of phytoplankton and *Daphnia* in the entire water column followed closely the corresponding epilimnetic dynamics (Appendix S4, Table 1).

In contrast, effects of stratification depth on the *magnitudes* of peaks differed markedly between the two spatial scales. Specifically, the strong negative effect of stratification depth on epilimnetic chl *a* concentrations almost disappeared at the scale of the entire water column (Fig. 2e and g, Table 1). Although there were still some minor influences of stratification depth, depth-integrated phytoplankton peak biomasses were nevertheless remarkably similar among different treatments (Fig. 2g; Table 1). This suggests that, integrated over the entire water column (epi- plus hypolimnion), stratification depth primarily affected the timing, but not the magnitude, of the phytoplankton spring bloom. Responses of *Daphnia* peak densities to stratification depth also differed between the two spatial scales. Integrated over the entire water column, *Daphnia* peak densities were lowest at the shallowest stratification; in contrast, epilimnetic peak densities were lowest at the deepest stratification (Fig. 2f and h; Table 1).

Discussion

Influence of temperature and light on the timing of seasonal events in the mixed layer

Our results support most of the hypotheses raised in the introduction. First, the onset of the phytoplankton bloom was accelerated by shallower stratification, but was independent of water temperature. Similarly, in a series of marine mesocosm experiments, the onset of the phytoplankton bloom was accelerated by higher external light supply (intended to mimic light conditions at shallower stratification), but did not respond to increased temperature (Sommer & Lengfellner, 2008). These results concur with an empirical modeling study of deep Lake Constance where Peeters *et al*. (2007b) concluded that direct temperature effects on phytoplankton production are weak under the light-limited conditions in the unstratified lake, and that stratification is required to trigger the onset of the phytoplankton spring bloom.

These results are consistent with ecophysiological measurements indicating that phytoplankton growth is hardly affected by temperature when production is limited by light dependent, photochemical reactions (Talling, 1957; Nicklisch *et al*., 2008). The same studies demonstrate, however, that temperature has a strong influence on phytoplankton growth under light-saturated conditions, when production is limited by the speed of enzymatic reactions. Furthermore, Tilzer *et al*. (1986) have described a positive effect of temperature also on light-limited growth when water temperatures are very low ($\leq 5\,^{\circ}C$). We therefore caution against generalizing our results too far beyond the experimental conditions. Rather, it seems plausible to expect that the onset of the phytoplankton bloom may be measurably affected by temperature in very shallow, high light environments and possibly also at water temperatures lower than in our experiment.

In further agreement with expectations, shallower stratification and warming independently shifted the onset of *Daphnia* growth, the phytoplankton peak, the clearwater phase, and (with the exception of the 2 m 'cold' treatments) the *Daphnia* spring peak forward. Our data thus indicate that not just the timing, but also the depth of stratification affects spring successional events. This agrees with the prediction from a recent population model that faster primary production in shallower (less light limited) water columns is, under nutrient replete conditions, converted into faster feeding and growth rates of *Daphnia*, leading to an acceleration of phytoplankton–*Daphnia* dynamics (Berger *et al*., 2007; Diehl, 2007; Jäger *et al*., 2008).

The question arises whether stratification depth (i.e., light availability) or temperature is a more important driver of seasonal succession. In an absolute sense, light is the ultimate driver in deep waters, because a shallowing of the mixed surface layer is absolutely required to trigger the phytoplankton bloom which all subsequent events depend on (Sverdrup, 1953; Bleiker & Schanz, 1997; Huisman *et al*., 1999). But how important is light vs. temperature once a critical stratification depth is passed? In our data, a huge, three-fold increase in average light availability from the 8 to 2 m enclosures advanced successional events by only 4–7 days (only 'cold' treatments with similar water temperatures considered), whereas a relatively minor temperature increase by 2–3 °C advanced all grazing related events by 7–12 days (only 2 and 4 m treatments considered). Our data therefore support Schalau *et al*. (2008), who concluded from a sensitivity analysis of a phytoplankton–*Daphnia* model that spring dynamics of *Daphnia* are primarily governed by water temperature and to a far lesser degree by (light-dependent) algal food production. The relative importance of temperature may even be stronger at lower overall temperatures, because development rates of most zooplankters decrease dramatically below 10 °C (Bottrell *et al*., 1976).

Timing of ciliate dynamics in the mixed surface layer

Contrary to our expectations and other experimental studies (Aberle *et al*., 2007; Sommer *et al*., 2007), the ciliate peak occurred before the phytoplankton bloom. This was probably a consequence of the relatively high initial density of ciliates, suggesting that an early ciliate bloom was already underway in the lake when the experiment was started. Although the timing of ciliate development in our experiment may thus not exactly reflect a typical spring situation, the observed dynamics yield nevertheless insight into the coupling of ciliates to other food web components. Specifically, the fast initial ciliate development appeared to be tightly coupled to the dynamics of highly edible nanophytoplankton. Given the initial nutrient spike, nanophytoplanktonic production was likely very high. Net population growth of nanophytoplankton was, however, only moderate in the 1st week and negative in the 2nd week, suggesting that most nanoplanktonic production was channelled into ciliate biomass.

These results are in line with descriptive studies suggesting that ciliates respond rapidly to increasing phytoplankton production in spring. In these studies the onset of ciliate net population growth was unrelated to water temperature, but closely linked to the decline in vertical mixing and the concomitant increase in the biomass of edible algae (Weisse *et al*., 1990; Mueller *et al*., 1991; Tirok & Gaedke, 2007). The studies furthermore suggest that the increase in ciliate production feeds back on their food. Weisse *et al*. (1990) assessed that in Lake Constance more than 50% of spring primary production was channelled through microzooplankton. Similarly, Peeters *et al*. (2007a) found that their empirical model of plankton dynamics in Lake Constance greatly overestimates the speed of the spring phytoplankton increase, if ciliate grazing is ignored.

In our experiment, ciliates started to decline 1–2 weeks before the peak of the phytoplankton bloom. This suggests that the microphytoplankters that were responsible for the phytoplankton bloom were inedible to most ciliates. This is consistent with Irigoien *et al*. (2005) who suggested that phytoplankton blooms are only formed by taxa that escape grazing by microzooplankton and thus are able to grow into predation 'loopholes' following a perturbation such as the onset of stratification. The termination of the ciliate bloom was then most likely caused by a shortage of appropriate nanoplanktonic food. In contrast, it is unlikely that the ciliate decline was induced by *Daphnia* predation as *Daphnia* densities were still very low (<1.5 ind. L^{-1} on day 12). A mix of predation and resource competition by *Daphnia* may, however, have suppressed a recovery of ciliates later in the experiment.

Magnitudes of ciliate, phytoplankton, and Daphnia *peaks in the mixed surface layer*

In close agreement with expectations, shallower stratification led to higher peak densities of phytoplankton, ciliates, and *Daphnia* in the surface layer. Consistent with similar results in previous experiments (Diehl *et al*., 2002; Berger *et al*., 2007) this suggests that peak densities of both producers and grazers are often limited by light supply. We believe that this relationship extends beyond the transient dynamics of spring blooms. Notably, a negative relationship between stratification depth and the mixed layer densities of both phytoplankton and zooplankton was also observed in a comparative study of German lakes conducted during summer stratification (Berger *et al*., 2006).

In contrast to the strong effects of stratification depth, peak densities of phytoplankton, ciliates, and *Daphnia* were unaffected by temperature. In the case of ciliates this might simply be related to the early timing of their peak and the small temperature differences ($<1.5\,^{\circ}C$) until then. In contrast, temperature treatments had diverged by 2.5–5 °C when phytoplankton peaked. Sommer & Lengfellner (2008) found that experimental warming by 2 °C reduced the phytoplankton spring peak by a factor of 2. They suggested that this was caused by temperature dependent grazing, arguing that heterotrophic processes increase faster with temperature than does primary production (Rose & Caron, 2007). Sommer and Lengfellner's marine experiments started out with fairly high densities of overwintering copepods, as taken from the field. In contrast, initial *Daphnia* densities in our experiment were very low, as is typical for temperate lakes in early spring. Grazing on bloom forming algae was therefore significant first after a lag phase of *Daphnia* population development.

These considerations highlight the importance of the overwintering strategy of zooplankton and point at a possibly general difference between marine and freshwater systems. Temperate marine systems (with significant populations of overwintering mesozooplankton) may experience both acceleration and reduction of the phytoplankton spring bloom with warming, whereas temperate freshwater systems (with mesozooplankton predominately recruiting from resting stages) may only experience acceleration. This scenario has theoretical support. In model simulations, the phytoplankton spring peak occurred earlier and at reduced height with increasing temperature if the grazer population recruited from overwintering individuals; in contrast, the phytoplankton bloom was also accelerated by warming but its height was largely unaffected by temperature if the grazers recruited from resting eggs (de Senerpont Domis *et al*., 2007).

The zooplankton overwintering strategy may thus determine the impact of temperature on the transfer of spring bloom primary production to higher trophic levels. High densities of overwintering zooplankton may reduce secondary production at higher temperatures through a tighter grazer control of the height of the phytoplankton bloom (Sommer & Lengfellner, 2008). In contrast, a temperature change may not affect the conversion of spring primary production into grazers (measured as grazer peak biomass) if zooplankton recruit from resting stages, as simulated in our experiment. The latter may, however, only hold when the spring inoculum from resting stages is sufficiently large. In de Senerpont Domis *et al.*'s model, the delay between the phytoplankton bloom and the subsequent grazer peak increases the smaller the inoculum from resting eggs. Winder & Schindler (2004a) found, in turn, that early summer *Daphnia* densities in Lake Washington were negatively related to the delay between the phytoplankton spring peak and the peak of a *Daphnia* population recruiting from eggs.

Timing and magnitudes of biomass peaks in the entire water column

Phytoplankton and *Daphnia* densities in the deep water layer were generally low and their dynamics seemed to be driven by sedimentation and spill-over from the mixed surface layer. Consequently, temporal dynamics in the surface layer and the water column as a whole were very similar. In contrast, the strong, negative influence of stratification depth on peak densities in the surface layer was not reflected at the spatial scale of the entire water column. Instead, depth-averaged peak biomass of phytoplankton was rather similar among treatments, suggesting that, once stratification is sufficiently shallow to allow a bloom in the first place, primary production *per area* of lake surface at the peak of the bloom may be relatively insensitive to a further shallowing of the mixed layer. Note that this phenomenon is transient. In the longer run feedback processes such as mixing depth-dependent nutrient depletion through algal sinking tend to decrease the areal standing stock of phytoplankton at shallow mixing depths (Diehl *et al.*, 2005; Jäger *et al.*, in press).

Our data also suggest that care needs to be taken in the comparison of descriptive studies of spring bloom dynamics. Different studies use different sampling regimes, e.g., taking a spot sample from a fixed depth, a satellite image representative of a depth depending on the optical properties of the water, or a depth-integrated sample over a fixed water column depth, irrespective of the actual mixed surface layer depth. Our study suggests that data taken at, or integrating across, different sampling depths should usually be comparable with respect to the timing of events, but probably not with respect to absolute biomass dynamics.

Conclusions and outlook

Our experiment has clearly demonstrated that increased temperature and shallower stratification independently accelerated spring dynamics of the plankton. We emphasize that an experimental approach was necessary to clearly separate the effects of temperature and stratification depth. Our approach furthermore allowed us to assess the relative contributions of temperature and stratification depth to the timing and magnitude of successional events. Within the range of environmental conditions covered, temperature had a greater influence on the speed of successional events, whereas stratification depth had a much greater influence on the amplitude of population fluctuations in the mixed layer. Integrated over the entire water column, bloom amplitude was, however, very similar across different stratification depths.

We want to caution that the relative influences of stratification depth and water temperature may differ earlier in the season, when a lower daily light dose (shorter day length, shallower solar angle) could exacerbate the importance of stratification for phytoplankton production and/or when temperatures below critical growth thresholds may limit zooplankton responses to warming. Future experiments should therefore be performed at lower water temperatures and shorter day length. Furthermore, to better understand how feedback processes in the pelagic food web mediate the direct effects of temperature and stratification on organisms belonging to different functional groups, experiments are needed in which the trophic structure of the community (e.g., presence/absence of *Daphnia* and/or microzooplankton) is manipulated. Understanding these feedbacks is important, because they mediate the consequences of global warming for ecosystem processes such as the export of biomass to deep waters and the sediment, the transfer of energy up the food chain, and the regeneration of nutrients within or below the mixed surface layer.

Acknowledgements

We thank Angelika Wild, Willi Brosch, Achim Weigert, Ellina Sycheva, Tobias Wiesmüller, Maren Striebel, Christoph Jäger, Sabine Gießler, Margit Feissel, and Mechthild Kredler for support in the field and lab, Steve Wickham for help with ciliate identification, two anonymous reviewers for helpful comments on the manuscript, and the DFG (German Research Foundation) for funding within the priority program 'Aquashift' (Grant DI 745/2).

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.

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.

Austin J, Colman S (2008) A century of temperature variability in Lake Superior. *Limnology and Oceanography*, **53**, 2724–2730.

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

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

Bleiker W, Schanz F (1997) Light climate as the key factor controlling the spring dynamics of phytoplankton in Lake Zurich. *Aquatic Sciences*, **59**, 135–157.

Bottrell HH, Duncan A, Gliwicz ZM *et al*. (1976) Review of some problems in zooplankton production studies. *Norwegian Journal of Zoology*, **24**, 419–456.

Càceres CE (1998) Interspecific variation in the abundance, production, and emergence of *Daphnia* diapausing eggs. *Ecology*, **79**, 1699–1710.

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

Diehl S (2007) Paradoxes of enrichment: effects of increased light versus nutrient supply on pelagic producer–grazer systems. *American Naturalist*, **169**, E173–E191.

Diehl S, Berger S, Ptacnik R, Wild A (2002) Phytoplankton, light, and nutrients in a gradient of mixing depths: field experiments. *Ecology*, **83**, 399–411.

Diehl S, Berger S, Wöhrl R (2005) Flexible nutrient stoichiometry mediates environmental influences on phytoplankton and its resources. *Ecology*, **86**, 2931–2945.

Goldman JC, Carpenter EJ (1974) Kinetic approach to effect of temperature on algal growth. *Limnology and Oceanography*, **19**, 756–766.

Huisman J, van Oostveen P, Weissing FJ (1999) Critical depth and critical turbulence: two different mechanisms for the development of phytoplankton blooms. *Limnology and Oceanography*, **44**, 1781–1787.

Irigoien X, Flynn KJ, Harris RP (2005) Phytoplankton blooms: a 'loophole' in microzooplankton grazing impact? *Journal of Plankton Research*, **27**, 313–321.

Jäger CG, Diehl S, Emans M Physical determinants of phytoplankton production, algal stoichiometry, and vertical nutrient fluxes. *American Naturalist*, in press.

Jäger CG, Diehl S, Matauschek C, Klausmeier CA, Stibor H (2008) Transient dynamics of pelagic producer grazer systems in a gradient of nutrients and mixing depths. *Ecology*, **89**, 1272–1286.

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

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

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

Mueller H, Schoene A, Pinto-Coelho RM, Schweizer A, Weisse T (1991) Seasonal succession of ciliates in Lake Constance Germany. *Microbial Ecology*, **21**, 119–138.

Müller-Navarra DC, Güss S, von Storch H (1997) Interannual variability of seasonal succession events in a temperate lake and its relation to temperature variability. *Global Change Biology*, **3**, 429–438.

Nicklisch A, Shatwell T, Kohler J (2008) Analysis and modelling of the interactive effects of temperature and light on phytoplankton growth and relevance for the spring bloom. *Journal of Plankton Research*, **30**, 75–91.

Parmesan C (2006) Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution and Systematics*, **37**, 637–669.

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

Peeters F, Straile D, Lorke A, Livingstone DM (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.

Rose JM, Caron DA (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.

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.

Scheffer M, Straile D, van Nes EH, Hosper H (2001) Climatic warming causes regime shifts in lake food webs. *Limnology and Oceanography*, **46**, 1780–1783.

Shatwell T, Kohler J, Nicklisch A (2008) Warming promotes cold-adapted phytoplankton in temperate lakes and opens a loophole for Oscillatoriales in spring. *Global Change Biology*, **14**, 2194–2200.

Sieburth JM, 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, Aberle N, Engel A *et al*. (2007) An indoor mesocosm system to study the effect of climate change on the late winter and spring succession of Baltic Sea phyto- and zooplankton. *Oecologia*, **V150**, 655–667.

Sommer U, Gliwicz ZM, Lampert W, Duncan A (1986) The PEG-Model of seasonal succession of planktonic events in fresh waters. *Archiv Fur Hydrobiologie*, **106**, 433–471.

Sommer U, Lengfellner K (2008) Climate change and the timing, magnitude, and composition of the phytoplankton spring bloom. *Global Change Biology*, **14**, 1199–1208.

Stenseth NC, Mysterud A (2002) Climate, changing phenology, and other life history and traits: nonlinearity and match-mismatch to the environment. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 13379–13381.

Straile D (2002) North Atlantic Oscillation synchronizes food-web interactions in central European lakes. *Proceedings of the Royal Society B – Biological Sciences*, **269**, 391–395.

Straile D, Adrian R (2000) The North Atlantic Oscillation and plankton dynamics in two European lakes – two variations on a general theme. *Global Change Biology*, **6**, 663–670.

Sverdrup HU (1953) On conditions for the vernal blooming of phytoplankton. *Journal du Conseil Permanent International pour l'Exploration de la Mer*, **18**, 287–295.

Talling JF (1957) Photo-synthetic characteristics of some freshwater diatoms in relation to underwater radiation. *New Phytologist*, **56**, 29–50.

Tilzer MM, Elbrachter M, Gieskes WW, Beese B (1986) Light–temperature interactions in the control of photosynthesis in Antarctic phytoplankton. *Polar Biology*, **5**, 105–111.

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.

Tirok K, Gaedke U (2007) The effect of irradiance, vertical mixing and temperature on spring phytoplankton dynamics under climate change: long-term observations and model analysis. *Oecologia*, **150**, 625–642.

Weisse T, Mueller H, Pintocoelho RM, 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.

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

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

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Temporal development of hypolimnetic water temperatures as a function of stratification depth (circles = 2 m, triangles = 4 m, squares = 8 m) and temperature treatment. Gray lines and symbols = 'ambient'; black lines and symbols = 'cold' temperature. Hypolimnetic temperatures were integrated from the depth of stratification to the bottom of the water column at 10 m.
Appendix S2. Temporal development of the epilimnetic densities of (a–c) ciliates, (d–f) phytoplankton, and (g–i) *Daphnia hyalina* as a function of stratification depth (2, 4, 8 m) and temperature treatment. Gray lines and open circles = 'ambient', black lines and filled circles = 'cold' temperature. Solid and broken lines mark the two replicates in each treatment.
Appendix S3. Temporal development of hypolimnetic densities of (a–c) phytoplankton and (d–f) *Daphnia* as a function of stratification depth (2, 4, 8 m) and temperature treatment (gray lines and open circles = 'ambient'; black lines and filled circles = 'cold' temperature). Solid and broken lines mark the two replicates in each treatment.
Appendix S4. Temporal development and timing of cardinal events in the seasonal succession of (a–d) total phytoplankton biomass and (e–h) total *Daphnia* abundance (integrated over the entire water column, i.e. epi- plus hypolimnion) as a function of stratification depth (2, 4, 8 m) and temperature treatment. Gray lines and open circles = 'ambient'; black lines and filled circles = 'cold' temperature. Solid and broken lines mark the two replicates in each treatment. White bars and gray diamonds = 'ambient'; gray bars and black diamonds = 'cold' temperature. Diamonds mark the timing of population peaks. The lower end of the bar marks the onset of population development (± 1 SE) and for phytoplankton the upper end of the bar marks the end of population decline (± 1 SE). The timing of the end of the *Daphnia* decline is not shown, because densities were above the required threshold values in most treatments.

Note that, with respect to timing, depth-integrated dynamics of phytoplankton and *Daphnia* in the entire water column followed closely the corresponding epilimnetic dynamics (compare Appendix S3 d, h with Figs. 2 b, c). This is indicated by close correlations between the timing of all cardinal events (onset, peak and end of phytoplankton bloom, onset and peak of *Daphnia* bloom) in the epilimnion and in the entire water column (all Pearson's $r \geq 0.76$, $p \leq 0.004$; $n = 12$, except for the onset of the *Daphnia* peak in the entire water column, where the 'cold' 2 m treatments were excluded as outliers and $n = 10$).