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Differential effects of warming and nutrient loading on the timing and size of the spring zooplankton peak – an experimental approach with hypertrophic freshwater mesocosms

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4 2 **Differential effects of warming and nutrient loading on the timing and**
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6 3 **size of the spring zooplankton peak – an experimental approach with**
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8 4 **hypertrophic freshwater mesocosms**
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Abstract

In shallow lakes, environmental warming and nutrient loading are important influences on the likelihood of a shift between clear and turbid ecosystem states. With temperatures and nutrient runoff predicted to increase within the next decades, climate change poses a threat to lake communities. However, current predictions on the effect of these environmental factors on the abundance and timing of peak zooplankton numbers are based on correlations rather than on experimental isolation of thermal from other confounding effects.

We present results of warming (4°C above ambient) and increased nutrient loading on plankton communities in 48 outdoor mesocosms, simulating fishless, hypertrophic ponds. The timing of the chlorophyll *a* peak and crustacean zooplankton peak abundance, dominated by *Daphnia pulex*, responded strongly to temperature and nutrient addition. *Daphnia* numbers reached peaks 22 to 24 days earlier in heated than in unheated mesocosms. The chlorophyll *a* peak abundance advanced by 15 to 19 days with heating. Phytoplankton, total zooplankton and *D. pulex* reached peak abundance 12 to 19 days later when doses of nitrogen and phosphorus were added; this finding contradicts predicted earlier phytoplankton and zooplankton spring peak abundances with nutrient enrichment. Peak zooplankton and *D. pulex* abundances did not differ with temperature treatment, contrary to our expectations, but peak abundances occurred at similar actual temperatures. Nutrient additions had no effect on the peak zooplankton and *D. pulex* abundances in our mesocosms.

Overall, climate warming is likely to advance plankton phenology in fishless ponds, however this advance could be dampened in systems with high nutrient concentration. We found very high zooplankton abundances with warming and high nutrient loadings, inducing a clear water state in all our tanks owing to heavy zooplankton grazing despite high nutrient concentrations.

1 Introduction

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Climate change is predicted to have huge impacts on the Earth's ecosystems through temperature increase, changed patterns of precipitation, more frequent extreme weather events, and combinations of these (IPCC, 2007). In temperate regions, temperature is predicted to increase 3 to 5°C within the next century. Freshwater lake communities will probably be affected both by temperature and changing hydrology.

Shallow lakes and ponds are likely to be particularly susceptible to climate change (Mooij et al., 2005), as they have a high surface area compared with their volume and thus are very vulnerable to temperature increase. Previous research has shown that warming could exacerbate the impact of eutrophication and that the resulting population and ecosystem changes can include loss of fish due to oxygen starvation (Feuchtmayr et al., 2009; McKee et al., 2003; Mooij et al., 2005; Mooij et al., 2007; Moran et al., 2010). Temperature increase has also been shown to favour the growth of toxic cyanobacteria in some eutrophic lakes, which may lead to problems for a range of organisms, including humans (Johnk et al., 2008).

Long-term datasets, sediment records, process-based modelling and space-for-time-substitutions along latitudinal or other climatic gradients suggest earlier phytoplankton spring population increases with increasing temperature. Examples are: Müggelsee (Gerten and Adrian, 2000), Loch Leven (Carvalho and Kirika, 2003), three large Swedish Lakes (Weyhenmeyer, 2001), Lake Erken (Weyhenmeyer et al., 1999), Bassenthwaite Lake (Elliott et al., 2006), Plußsee (Müller-Navarra et al., 1997), Lake Washington (Winder and Schindler, 2004), Windermere (Thackeray et al., 2008) and Lake Constance (Peeters et al., 2007). For the deeper of these lakes, however, secondary hydrodynamic factors, stratification periods and mixing depths, can play a major role in seasonal phytoplankton development (Diehl et al., 2002). Shallow lakes avoid these complications.

Further evidence of the influence of climate warming on freshwater systems can be found for zooplankton, a major component of freshwater food-webs, which influences water quality by consuming algae and providing food for fish. Zooplankters, mostly *Daphnia spp.*, generally become very abundant in spring in temperate lakes following peaks in algal abundance. In North American lakes, the timing of maximum *Daphnia* abundances is linearly related to latitude, with earliest peaks in southern lakes (Gillooly and Dodson, 2000). Berger et al. (2007) found that while experimental cooling of lake enclosures did not affect the timing of the phytoplankton peak, it delayed the peak of *Daphnia* abundance. However, advances in the timing of the clear water period (presumed to

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2 1 follow the zooplankton peak in abundance), observed in Dutch lakes over 17 years with a water
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4 2 temperature increase of around 0.5°C (Scheffer et al., 2001), may be difficult to attribute wholly to
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6 3 effects of climate warming because of concurrent reductions in nutrient loadings (Van Donk et al.,
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8 4 2003).

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11 6 An earlier peak abundance of zooplankton has been predicted in warmer years by Scheffer et al.
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13 7 (2001) in a single numerical application of a minimal model, with this model including predation by
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15 8 fish. More recently, Schilau et al. (2008) predicted, using a size-structured *Daphnia* population
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17 9 model, that temperature rather than food availability is likely to drive inter-annual variability of
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19 10 *Daphnia* population dynamics during spring: they specifically predicted an advanced timing of peak
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21 11 abundance with warming. In their model, *Daphnia* grazing and population growth were
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23 12 temperature dependent, but phytoplankton growth was not. In the present experimental study in
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25 13 systems that lacked fish, we predict that peak abundance should occur earlier in response to
26
27 14 warming because the growth and grazing of zooplankton is generally more thermally sensitive than
28
29 15 is primary production (Allen et al., 2005; Bissinger et al., 2008; López-Urrutia et al., 2006; Rose
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31 16 and Caron, 2007). Another idea, which derives from equilibrium models of populations at carrying
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33 17 capacity (Savage et al., 2004), but which has not yet been explored in models of transient dynamics,
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35 18 is that an increased *per capita* grazing rate relative to rate of algal production at increased
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37 19 temperatures may reduce the peak standing crop per mass of algal food.

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39 21 Timing of peak plankton abundances is also affected by the many other influences on grazer-prey
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41 22 dynamics, including their respective initial abundances at the end of winter, light, and nutrients (e.g.
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43 23 Jäger et al., 2008; Thackeray et al., 2008). Trends towards heavier precipitation events with
44
45 24 increased frequency will result in altered nutrient run-off into freshwater systems (Jeppesen et al.,
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47 25 2009). Thackeray (2008) concluded that eutrophication and/or climate change induces phenological
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49 26 shifts in phytoplankton and that effects are species dependent. The experimental approach used in
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51 27 the present study can avoid or account for these confounding effects of temperature and nutrients.
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53 28 Experimental approaches are desirable but are expensive to set up and maintain and are therefore
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55 29 rare. Here we present results from a replicated experiment that manipulated temperature and
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57 30 nutrient availability in the absence of planktivorous fish. Fish-free systems are not only likely to
58
59 31 become more common as a result of warming, they are already important components of many
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32 temperate landscapes as small ponds that are of particular conservation importance for invertebrates
33 and amphibians (Biggs et al., 2005; Werner et al., 2007). Using fish-free systems also allowed
34 elimination of a major top-down effect from the bottom-up effects.

1
2 1 In an earlier investigation using the same mesocosm tanks as used here, modest influences of a 3°C
3
4 2 temperature increase were observed on phytoplankton, zooplankton, macroinvertebrates and
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6 3 macrophytes (Feuchtmayr et al., 2007; McKee et al., 2003; McKee et al., 2002; Moss et al., 2003).
7
8 4 The former experiments used a temperature slightly above the upper limit of predictions made for a
9
10 5 business-as-usual scenario by the Intergovernmental Panel on Climate Change in its Second Report
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12 6 published in 1995, on the grounds that there was a trend of increased warming in the predictions
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14 7 being made. These predictions were indeed revised upwards substantially in the Third Assessment
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16 8 of 2002 and the A2, business-as-usual, scenario predicted a 3°C mean global rise by 2100. For the
17
18 9 current experiments we thus chose a 4°C rise in line with the considered most likely A2 (regionally-
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20 10 orientated economic development) predictions (2.0-5.4°C) over the twenty-first century. These
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22 11 predictions have again been raised in the Fourth Assessment Report of 2007. Experiments have
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24 12 hitherto not been performed on open air plant-free shallow systems at very high nutrient levels that
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26 13 simulate the sort of lake or pond that has already been strongly affected by eutrophication. With
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28 14 richer organic sediment than in the previous experiment (McKee et al., 2003), we ensured high
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30 15 levels of phosphorus released to the water column, and with realistic nitrogen loadings we could
31
32 16 focus on plankton growth in turbid shallow lakes (Scheffer et al., 1993).
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36 18 The current experiment explored the effects of 4°C warming and realistically high external nutrient
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38 19 loading on 8-fold replicated plant-free shallow systems during spring. It allowed us to separate
39
40 20 temperature from nutrient impacts on zooplankton abundance and on any change in magnitude and
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42 21 timing of peak abundance. Our objectives were: a) to test the influence of warming and nutrient
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44 22 loading on zooplankton abundance, b) to quantify the extent to which the timing of zooplankton
45
46 23 peak abundance might be advanced by 4°C warming and nutrient additions, c) to test whether
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48 24 warming affects the peak abundance of zooplankton per unit chlorophyll, d) to test whether nutrient
49
50 25 addition increases the peak abundance of zooplankton and e) to describe the impacts of temperature
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52 26 and nutrients on shallow lake ecosystems.
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54 27

55 28 **Method**

56 29 57 30 **Experimental set-up and treatments**

58 31 Forty-eight mesocosm tanks, each 1 m deep and 2 m in diameter, sunk in the ground (see McKee et
59
60 32 al., 2000), are located at the University of Liverpool Horticultural and Environmental Research
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34 Station at Ness Botanic Gardens in North West England (53° 16' N, 3° 03' W), United Kingdom. In
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36 October 2005 the tanks were filled with a 20-cm deep sediment mixture containing (by volume)
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38 50% garden loam and 50% organic material (47.5% chopped organic oat straw and 2.5% rotted

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2 1 organic cow manure to simulate a sediment of a eutrophic lake or pond), before being filled with
3
4 2 around 3000 L of ground water pumped from a borehole.
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6 3

7 4 A heating element was placed on top of the sediment either as a “dummy” in 24 non-heated tanks or
8
9 5 connected to the heating system in 24 heated tanks. In heated tanks, 60°C hot water could be
10 6 pumped through submerged pipes to increase the water temperature. Water temperature was
11 7 measured continuously by sensors at a depth of 45 cm, and a computerised feedback system ensured
12 8 a 4°C higher water temperature in heated tanks compared with adjacent non-heated tanks. Thus,
13 9 heated tanks follow daily and seasonal temperature cycles of the ambient temperature tanks plus
14 10 4°C. Heating was switched on in October 2005 and tanks were allowed to settle until January 2006
15 11 when sampling and monitoring started.
16
17 12

18 13 Two different nutrient additions were superimposed on the heating treatments in a randomised
19 14 block design from January 2006, via a standard nutrient addition every 2 weeks. Sixteen tanks did
20 15 not receive nutrient additions (No), while the other two treatments, of sixteen tanks each, received
21 16 low (Low) and high (High) nitrogen loading ($17.9 \mu\text{M}$ or $250 \mu\text{g L}^{-1}$ and $178.5 \mu\text{M}$ or $2500 \mu\text{g L}^{-1}$
22 17 1), each combined with a constant phosphorus loading ($1.6 \mu\text{M}$ or $50 \mu\text{g L}^{-1}$) using sodium nitrate
23 18 (NaNO_3) and potassium dihydrogen orthophosphate (KH_2PO_4). Evaporation losses from the tanks
24 19 were compensated with deionised water to avoid interference with nutrient levels and ionic
25 20 composition. The study reported here represents the first, fishless phase (1st of January to 1st of June
26 21 2006) of a longer term experiment, which later included fish additions and macrophyte population
27 22 establishment.
28
29 23

44 24 **Inoculation**

45 25 Zooplankton, phytoplankton and macroinvertebrates were collected from four ponds within the
46 26 Botanic Garden and a nearby lake, Rostherne Mere, over several weeks and kept in a large storage
47 27 tank. In January 2006 the storage tank contents were well mixed and sub-samples added to the 48
48 28 experimental mesocosms. In addition, *D. magna* was added to each mesocosm as ephippia from a
49 29 nearby pond in October 2005, and 150 *D. magna* adults from the same origin on 20 March 2006.
50 30 Two and three weeks after inoculation, invertebrates were cross-mixed among tanks via
51 31 standardised sweep-net samples to ensure similar starting conditions.
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53 32

54 33 **Monitoring**

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2 1 Routine monitoring for water chemistry was based on Johnes & Heathwaite (1992) (total nitrogen
3 2 (TN), total phosphorus (TP)) and Mackereth et al (1989) (soluble reactive phosphorus (SRP), nitrate
4 3 (NO₃-N), ammonium (NH₄-N)), in addition to conductivity, pH (by electronic meter), chlorophyll-
5 4 *a* (by a submersible fluorometer Cyclops-7, Turner Designs), and zooplankton counts took place
6 5 every 2 weeks. Oxygen was measured at mid-water (35 cm) depth with a YSI Model 85
7 6 submersible system between 10.30 and 12.30 hr BST (termed “mid-day”). Temperature was
8 7 recorded automatically by the sensors and computer system every 15 minutes throughout the
9 8 experiment. The phytoplankton community was examined microscopically to determine major
10 9 components.

11 10
12 11 Zooplankton samples were taken after sunset to exploit a more equal distribution of the animals
13 12 within the water column. Ten-litre integrated water samples were taken with a 1-m long tube, mixed
14 13 in a bucket and concentrated for later transport to around 3 litres with 100- μ m mesh size gauze.
15 14 Using the same method, the samples were concentrated down to 50 ml and fixed with sugar-
16 15 formaldehyde the next morning. Sub-samples of at least 100 individuals (cladocerans and
17 16 copepods) were counted under a microscope. Copepod numbers include copepodid stages. Nauplii
18 17 were not included in the copepod counts nor were rotifers, nor *Daphnia* neonates containing egg
19 18 yolk reserves.

20 19
21 20 To estimate the time difference caused by treatment (heated vs. unheated and among nutrient
22 21 additions) for the phytoplankton and zooplankton peaks we applied two different methods. First, we
23 22 recorded the date when the greatest number in each tank was reached; secondly, for an estimate
24 23 between the fortnightly samplings we fitted a curve (Weibull-type function) to data from each tank,
25 24 providing a method for “peak” curve fitting following Rolinski et al. (2007), using the cardidates
26 25 package of the statistical data analysis system R (R Development Core Team, 2007). Data shown
27 26 for chlorophyll-*a* peak concentrations excludes seven mesocosms, distributed among the different
28 27 treatments, where chlorophyll-*a* peaks developed before the start of our 2-weekly sampling regime
29 28 preventing us from determining peak concentrations and fitting a curve to the data. For better
30 29 comparison of the timing of zooplankton, *D. pulex* and phytoplankton peaks, results determined
31 30 with fitted Weibull-type functions were standardised and Z-scores calculated (the difference in days
32 31 between peak occurrence in each tank and the average of all tanks, divided by the overall standard
33 32 deviation, and averaged for the different treatments). Hence, negative z-scores indicate earlier peaks
34 33 compared with the average time of peak occurrence, positive values indicate later peaks.

Statistical methods

Wherever model residuals from analyses of untransformed data showed evidence of non normality and heteroscedasticity, data were log transformed before applying two-way ANOVAs with time as repeated measure (see Table 1). All analyses were examined for the influence of outliers by plotting Cook's Distances. One- way ANOVAs were applied to test treatment effects on the timing of peak abundances (see Table 2) along with all other comparisons using SPSS 15.0 for Windows®. The same package was used for pairwise *post hoc* Bonferroni-corrected tests.

Results

Water temperature in ambient tanks increased from 4°C in January to about 18°C during the first half of May 2006, with the greatest change in April 2006, while the temperature in the heated tanks was 4°C ± 0.1° C higher (Fig. 1). Throughout the period of observation, one heated mesocosm remained anaerobic with very low pH (by the end of May, pH mean ± SE 4.9 ± 0.17 compared with 7.1 ± 0.01 in the other 47 tanks); this outlier was not included in the analyses.

Mean oxygen concentration and saturation was significantly lower in heated tanks but increased with nutrient additions (Table 1). Among nutrient treatments, oxygen concentration and saturation were significantly higher in the High treatment, but did not differ between No and Low treatments (*post-hoc* test, $p=1.0$). Phosphorus released from the sediment resulted in high TP concentrations (154 to 707 $\mu\text{g L}^{-1}$). TP and SRP concentrations in both nutrient-addition treatments were higher than in tanks without experimental addition (Table 1). The *post-hoc* test showed a significant increase between No and Low treatments ($p=0.05$) for TP but no significant differences between other pairs.

TN concentrations were high, ranging from 1.35 mg L^{-1} to 4.42 mg L^{-1} , significantly increasing with nutrient loading treatments (Table 1). With warming, TN concentrations were high at the beginning of the experiment possibly due to release from the sediment, but decreased in February to the concentrations in unheated tanks, resulting in significant differences with temperature over time (Table 1). Added nitrate did not persist long in the water and two weeks after addition was generally only detected in low concentrations except in the high nutrient treatment (see Table 1). Nitrate and ammonium concentrations increased with nutrient addition and warming (Table 1) and among nutrient treatments, pairwise comparisons between Low and High and No and High were

1 significantly different. For TN, all *post-hoc* results of nutrient treatments were significantly
2 different.
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7 4 The concentration of chlorophyll *a* was significantly higher in mesocosms with high nitrogen
8 addition than in those with lower addition (pairwise Bonferroni-corrected comparison), but high
9 variation among replicated tanks prevented detection of significant differences with heating (Table
10 6
11 7 1, Fig. 2a). Phytoplankton biomass consisted mainly of Cryptophyceae (around 90%, averaged over
12 all tanks), followed by Chrysophyceae (around 8%), and minor amounts of all other groups.
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17
18 10 Crustacean zooplankton numbers consisted almost exclusively (84 to 96%) of *Daphnia pulex*.

19 *Daphnia magna* appeared in May, but in low abundance (mean 1% of total zooplankton numbers).

20 11 Cyclopoid copepods were relatively abundant until 4 April (mean 11% of total zooplankton
21 12 numbers), but declined from 18 April onwards (mean 5%) as *Daphnia pulex* abundances increased.
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23 14

24 Non- crustacean zooplankton was dominated by protozoa with little representation of other groups.

25 15 Total numbers of zooplankters and *D. pulex* alone increased with nutrient additions, increased

26 significantly with warming and showed strong and significant responses to temperature over time

27 16 when analysed by 2-way ANOVA with time as repeated measure (Table 1). Crustacean

28 17 zooplankton abundance continuously increased in heated mesocosms from January to 18 April, and
29 18 in unheated mesocosms from end of February to 16 May (Fig. 2b).
30 19
31 20

32 21 The timing of both measured and fitted peak abundances were significantly advanced with heating
33 22 for total zooplankton, *D. pulex*-only and for chlorophyll *a* (see ANOVA results in Table 2, Fig. 3).
34 23

35 24 The measured mean peaks were 24 days, 24 days and 15 days earlier and the fitted mean peaks

36 25 were 23 days, 22 days and 19 days earlier, respectively. There were also significantly delayed

37 26 peaks with nutrient addition: 12 days (measured) and 16 days (fitted) for *D. pulex* and 14 days

38 27 (fitted) for total zooplankton between No and High treatments (also see Fig 2d). Non-significant

39 28 delays in chlorophyll-*a* peak concentrations by the High treatment were 8 days (measured) and 19

40 29 days (fitted) (Table 2, Fig 2c). Z-scores in Figure 3 show earlier peaks (negative values) for

41 30 zooplankton, *D. pulex*-only and chlorophyll *a* with heating compared with later peaks (positive

42 31 values) in ambient treatments, that were delayed with increasing nutrient addition.
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44 33

45 34 To test whether warming or nutrient additions affect the peak abundance of zooplankton we

46 35 calculated mean plankton peak abundances for each treatment (Fig. 4.). Peak abundances of

47 36 zooplankton and *D. pulex* in heated tanks (mean \pm SE 1170 \pm 139 and mean \pm SE 1158 \pm 140)

48 37 were higher than in unheated tanks (mean \pm SE 940 \pm 80 and mean \pm SE 910 \pm 84), but not
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1 significantly different ($F_{1,45} = 2.02$, $p = 0.16$, $F_{1,45} = 2.28$, $p = 0.14$ for total zooplankton and *D.*
2 *pulex* respectively, Fig. 4a). For nutrient treatments, zooplankton and *D. pulex* peak abundance was
3 highest in tanks with high nitrogen addition (mean \pm SE 1223 \pm 174 and mean \pm SE 1190 \pm 170)
4 compared with low addition (mean \pm SE 952 \pm 145 and mean \pm SE 948 \pm 158) and no addition
5 (mean \pm SE 992 \pm 86 and mean \pm SE 968 \pm 86), but not significantly different between treatments
6 ($F_{2,44} = 1.08$, $p = 0.35$, $F_{2,44} = 0.87$, $p = 0.43$ for total zooplankton and *D. pulex* respectively, Fig.
7 4b). Chlorophyll *a*, however, measured on the morning after the zooplankton peak (sampled at
8 night), was lower in the unheated mesocosms than in the heated mesocosms (ANOVA, $F_{1,44} = 4.77$,
9 $p = 0.03$). Zooplankton per unit chlorophyll *a* at peak zooplankton abundance was similar in heated
10 and unheated mesocosms (ANOVA, $F_{1,43} = 1.71$, $p = 0.2$ for zooplankton, $F_{1,43} = 1.59$, $p = 0.22$ for
11 *D. pulex*, Fig. 4c) and in the different nutrient treatments (ANOVA, $F_{2,42} = 2.58$, $p = 0.09$ for
12 zooplankton, $F_{2,42} = 2.07$, $p = 0.14$ for *D. pulex*, Fig. 4d) when measured on the morning after the
13 zooplankton peak.

14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 **Discussion**

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32 18 In a previous experiment using the same tanks, nutrients had a far greater impact on zooplankton,
33 phytoplankton and macrophytes than temperature (Feuchtmayr et al., 2007; McKee et al., 2003;
34 19 McKee et al., 2002; Moss et al., 2003). Our results, for a slightly greater temperature increase,
35 20 suggest that temperature is an important driver of zooplankton growth and its algal food during
36 21 spring. Our data allowed us: to quantify the extent to which the zooplankton peak abundance in
37 22 spring is advanced by a 4°C warming, affected by nutrient loading; to test whether high nutrient
38 23 loadings increase peak zooplankton abundance per unit of food; to test whether 4°C warming
39 24 reduces peak abundance per unit of food as expected from increased energetic demands; and to
40 25 explore the consequences for the aquatic system. Surprisingly, temperature and nutrient addition
41 26 had contrasting impacts on the timing of phytoplankton and zooplankton peak abundances during
42 27 this early stage of the growth season.
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45 30 Increased temperature induced an earlier peak of chlorophyll-*a* and zooplankton. As climate
46 31 warming continues, the evidence from our experiment and studies so far (Berger et al., 2007; Gerten
47 32 and Adrian, 2000; Scheffer et al., 2001) suggest an earlier zooplankton/*Daphnia* spring peak and
48 33 subsequent clear water phase. We calculated an advance in zooplankton and *Daphnia* peak of 22 to
49 34 24 days with 4°C warming. The advance in peak zooplankton and *Daphnia* abundance in the heated
50 35 tanks was consistent with findings from the Bautzen reservoir (23.2 days for 4°C warming (Wagner

1
2 1 and Benndorf, 2007)) and Plußsee (20 to 24 days earlier for a 4°C increase (Müller-Navarra et al.,
3
4 2 1997)), but was not as early as expected from the regression model of Scheffer et al. (2001) (33.6
5
6 3 days with 4°C warming) or from a linear extrapolation of findings from Lake Müggelsee (47 days
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8 4 for a 4°C increase (Adrian et al., 2006)). The advance of the zooplankton and *Daphnia* peak with
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10 5 warming was larger than that of the phytoplankton peak, supporting findings of Schalau et al.
11
12 6 (2008), a modelling study suggesting temperature and not food as the dominant factor for
13
14 7 determining the timing of spring *Daphnia* populations. This phenomenon could be explained by the
15
16 8 generally higher thermal sensitivity of growth and grazing of heterotrophs compared with primary
17
18 9 producers (Allen et al., 2005; Bissinger et al., 2008; López-Urrutia et al., 2006; Rose and Caron,
19
20 10 2007).

21
22 12 With climate change, winter precipitation is expected to increase, leading to higher nutrient runoff
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24 13 from the catchment into rivers and lakes (IPCC, 2007), especially in the agricultural lowlands. With
25
26 14 higher nutrient availability, phytoplankton growth is less likely to be limited by nitrogen or
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28 15 phosphorus which might result in high phytoplankton division rates leading to earlier peak
29
30 16 abundances (Thackeray et al., 2008). However, phytoplankton abundance is also expected to
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32 17 increase with higher nutrient loading, and similar division rates could lead to higher and thus later
33
34 18 peak abundances. Consequently, this might lead to higher and later peak grazer abundances. We
35
36 19 found evidence for this hypothesis in our experiment. Chlorophyll *a* increased with nitrogen
37
38 20 addition and the influence of nutrient addition on the date of *Daphnia* peak abundance was opposite
39
40 21 to the temperature effect (Table 2, Fig. 3); the higher the nutrient addition, the later the *Daphnia*
41
42 22 peak abundance occurred. In contrast, model predictions of nutrient effects on transient *Daphnia*
43
44 23 dynamics by Jäger et al. (2008) showed an advanced timing of the *Daphnia* peak biomass by
45
46 24 phosphorus enrichment. Phosphorus additions used in this study and in the experiment by Jäger et
47
48 25 al. (2008) were similar; however nitrogen enrichment was not studied by Jäger et al. (2008).

49
50 27 Jäger et al. (2008) predicted and observed an increase in *Daphnia* peak biomass with phosphorus
51
52 28 enrichment. A nutrient-enhanced increase in peak abundance of *Daphnia* is also consistent with the
53
54 29 prediction of Schalau et al. (2008) who proposed that the height of this peak should be mostly
55
56 30 controlled by phytoplankton carrying capacity, and hence may correlate with concentration of the
57
58 31 limiting nutrient. Our findings confirmed the predicted increased mean chlorophyll *a* concentrations
59
60 32 with nutrient addition (Table 1), but the heights of the zooplankton and *D. pulex* peaks did not differ
33
34 between nutrient treatments (see Fig. 4), possibly due to crowding effects.

35
36 The abundance of *Daphnia pulex* in the mesocosms was unusually high. Crowding effects of
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38 *Daphnia* are reported to occur at densities of around 150 individuals per litre (Burns, 1995) in the

1
2 1 laboratory. In the mesocosms, *Daphnia pulex* reached up to 10 fold higher abundances at peak
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4 2 times when food was plentiful. The simulation of Scheffer *et al* (2001, their Figure 3) suggested a
5
6 3 lower predicted peak of zooplankton abundance with 3 °C warming, in accordance with the model
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8 4 of Müller-Navarra *et al.* (1997). We found higher zooplankton and *Daphnia* mean abundances with
9
10 5 warming, but no difference in heights of the *Daphnia* abundance peaks with temperature. This lack
11
12 6 of a thermal effect could be explained by the occurrence of *Daphnia* peak abundance at similar
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14 7 temperatures (mean \pm SE of 15.3 ± 0.3 °C for unheated and 16.6 ± 0.3 °C for heated tanks)
15
16 8 irrespective of thermal treatment (also see Gillooly and Dodson, 2000).
17 9

18 10 In these organic-rich hypertrophic systems, oxygen concentrations were reduced to critical levels
19
20 11 for *Daphnia* survival in the heated tanks, due to respiration of open-water and sediment biota.
21
22 12 Weider and Lampert (1985) experimentally identified threshold oxygen concentrations for *Daphnia*
23
24 13 *pulex* of 0.5 to 1 mg L⁻¹, below which survival of the animals decreased significantly. In 20 out of
25
26 14 23 heated mesocosms, mean oxygen concentrations at mid-depth around the zooplankton peaks
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28 15 were lower than 1 mg L⁻¹, and *Daphnia* numbers were surprisingly high (see above). The
29
30 16 contribution of macroinvertebrates to total respiration is assumed to be small compared with that of
31
32 17 the zooplankton, phytoplankton and bacteria, as in this early stage of the mesocosm experiment,
33
34 18 general macroinvertebrate abundances were still low (R.J. Moran *et al.* unpublished data). Increased
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36 19 total community respiration with warming, along with decreasing phytoplankton is likely to have
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38 20 reduced the oxygen concentrations in heated mesocosms to levels that nonetheless probably limited
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40 21 *Daphnia* growth and reproduction.
41 22

42 23 Implications for freshwater systems of a temperature increase similar to that in our experiment are
43
44 24 three-fold. First, an increase of water temperature results in a lower dissolved oxygen saturation in
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46 25 shallow hypertrophic systems, and, together with high respiratory oxygen demands associated with
47
48 26 both high heterotroph biomass and warming, can lead to severe oxygen depletion. This can affect
49
50 27 not only survival of zooplankters but potentially also other invertebrates and fish (Moran *et al.*,
51
52 28 2010). With climate warming, shallow eutrophic lakes might increasingly face problems resulting
53
54 29 from very low dissolved oxygen concentrations. Secondly, while zooplankton can react rapidly to
55
56 30 changes within their environment, the extent to which taxa from higher trophic levels, with longer
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58 31 generation times, such as invertebrate predators and fish, can adjust and cope with the earlier
59
60 32 zooplankton peak needs to be addressed in further studies (match/mismatch hypothesis). As fish
33
34 larvae and planktivorous fish species are dependent on zooplankton as a food source, climate
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36 change could have a detrimental effect on the food web in hypertrophic lakes, not only through very
37
38 low oxygen concentrations but also because of an advanced timing of the zooplankton peak.

1
2 1 Concluding from our results that effects of warming and high nutrient load on timing of peak
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4 2 abundances compensate each other might be misleadingly comforting. Even though nutrient
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6 3 addition delayed the peak abundances, mean concentration of chlorophyll *a* in High treatments was
7
8 4 more than 2-fold higher than other treatments, and consequences of high nutrient loading to
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10 5 ecosystems are generally detrimental.

11 6
12 7 Thirdly, owing to increased temperatures and an earlier clear water phase, “non-grazeable” algae,
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14 8 such as cyanobacteria, might establish themselves earlier in the season and could then be able to
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16 9 dominate the community over the summer (Jeppesen et al., 2009). Results of the PROTECH model
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18 10 suggest that warming will increase phytoplankton biomass dominated by the cyanobacterium
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20 11 *Anabaena* during the summer bloom along with a loss of phytoplankton biodiversity (Elliott et al.,
21
22 12 2006). Together with heavy nutrient loads, either already in the system or entering via runoff from
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24 13 the catchment, this could result in a substantial change of the ecosystem. However, through an early
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26 14 zooplankton peak and subsequent clear water phase in shallow lakes, the probability of macrophyte
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28 15 development might increase, decreasing the probability of a high phytoplankton biomass because of
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30 16 competition mechanisms.

31 17
32 18 So far however, the general consensus is that establishment and stabilisation of a macrophyte-
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34 19 dominated system requires a relative increase in the piscivorous fish stock and a reduction in
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36 20 nutrient levels (Gonzalez Sagrario et al., 2005). In our experimental ecosystems, despite the high
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38 21 nutrient levels, and in the absence of fish, macrophytes established themselves in all our mesocosms
39
40 22 later that summer even in mesocosms in which planktivorous fish were introduced and became
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42 23 established for a subsequent experiment (Feuchtmayr et al., 2009). This indicates the establishment
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44 24 of a clear water state in our experimental mesocosms. However, the present study did not
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46 25 incorporate any influence of fish predation on zooplankton, including the effect of temperature on
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48 26 fish offspring and survival (Jeppesen et al., 2003) which might prevent such high zooplankton
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50 27 numbers from occurring and a clear water state from establishing.

51 28
52 29 Our findings that the timing of zooplankton peak abundance was advanced by 4°C warming and
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54 30 that oxygen was strongly depleted especially in heated tanks, together with evidence of differences
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56 31 in thermal sensitivity between autotrophic and heterotrophic rates (e.g. López-Urrutia et al., 2006;
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58 32 Rose and Caron, 2007) lead us to propose that an important component of future research on
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60 33 freshwater ecosystem responses to warming should include the difference in thermal sensitivities of
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35 34 rates of consumption, metabolism and production between autotrophs and heterotrophs. In contrast
to current predictions of earlier peak plankton occurrence based on phosphorus additions, our

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2 1 finding of a delay in the timing of peak zooplankton and *D. pulex* abundances with phosphorus and
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4 2 nitrogen addition in hypertrophic systems suggests that nitrogen might be a more important driver
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6 3 in freshwater systems than currently accepted. Further, the strong influence of nutrient loading on
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8 4 timing and peak abundances reported here suggests studies on climate warming should include the
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10 5 analysis of nutrient effects.
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12 6
13 7

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Table and Figure legends

Table 1: Effects of heating (H) and nutrient (Nu) loading (no addition No; addition of P and low addition of N Low; addition of P and high addition of N High) over time (t) on water chemistry, zooplankton abundance and chlorophyll *a* concentration, in 47 mesocosms from beginning of January 2006 to end of May 2006 (N=10 for all parameters except where N is given). Results are from a 2-way ANOVA with time (t) as repeated measure. Wherever results were significant, p values are indicated as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; ns indicates not significant results.

Table 2: Effects of heating (H) and nutrient (Nu) loading (no addition No; low addition Low; high addition High) on timing of zooplankton peak abundance, *Daphnia pulex* peak abundance and chlorophyll *a* peak abundance, all calculated as julian day with two different methods: 1) peak abundance sampled, and 2) fitting Weibull functions for 47 mesocosms from beginning of January 2006 to end of May 2006 wherever possible. ANOVA results are given, p values are indicated as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; ns indicates non-significant results.

Figure 1 Mean daily water temperatures averaged over 24 ambient (open circles) and 23 heated (filled circles) mesocosms from January to end of May 2006.

Figure 2 Mean \pm SE of a) chlorophyll *a* concentrations ($\mu\text{g L}^{-1}$) and b) crustacean zooplankton abundances (L^{-1}) from end of January to end of May 2006 in heated and unheated mesocosms. Mean \pm SE of c) chlorophyll *a* concentrations ($\mu\text{g L}^{-1}$) and d) crustacean zooplankton abundances (L^{-1}) from end of January to end of May 2006 in mesocosms with no nutrient addition, low nutrient addition (addition of P and low addition of N) and high nutrient addition (addition of P and high addition of N).

Figure 3 Z-scores for chlorophyll *a* (black bars), total zooplankton (white bars) and *D. pulex* (diagonally lined bars) peak abundance determined with Weibull-type functions (based on Rolinski et al. (2007)), in 47 mesocosms from February to end of May 2006. Negative z-scores indicate earlier peaks compared with the average time of peak occurrence, positive values indicate later peaks (Treatments: UNo – Unheated and No nutrient addition, HNo – Heated and No nutrient addition, ULow – Unheated and Low nutrient addition, HLow – Heated and low nutrient addition, UHigh – Unheated and High nutrient addition, HHigh – Heated and high nutrient addition).

1
2 1 **Figure 4** a) Boxplots of total zooplankton and *D. pulex* peak abundances in heated and unheated
3 mesocosms ($F_{1,45}=2.02$, $p=0.16$ for total zooplankton, $F_{1,45}=2.21$, $p=0.15$ for *D. pulex*) and b) in
4 mesocosms with no nutrient addition (No), low (Low) and high (High) addition ($F_{2,45}=1.08$, $p=0.35$
5 for total zooplankton, $F_{2,45}=0.87$, $p=0.43$ for *D. pulex*) c) Boxplots of peak zooplankton abundances
6 (L^{-1}) per chlorophyll *a* concentrations ($\mu g L^{-1}$) one day after the zooplankton peak in heated and
7 unheated mesocosms ($F_{1,43}=2.97$, $p=0.09$ for zooplankton, $F_{1,43}=1.80$, $p=0.19$ for *D. pulex*) and d)
8 in mesocosms with no nutrient addition, low (Low) and high (High) addition ($F_{2,43}=2.58$, $p=0.09$
9 for zooplankton, $F_{2,43}=2.07$, $p=0.14$ for *D. pulex*). Two chlorophyll *a* readings were negative and
10 were not included in the analysis; The large box represents data between the 25th and 75th
11 percentile; the line within the box represents the median. Vertical bars show the upper and lower 10
percentiles, dots represent outliers.

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Table 1

	Mean value					Probability					
	Unheated	H	No	Low	High	H	Nu	HxNu	Hxt	Nuxt	HxNuxt
Oxygen concentration (mg L ⁻¹) (N=13)	4.8	2.4	3.2	2.9	4.8	***	***	ns	***	ns	ns
Oxygen saturation (%) (N=13)	40.5	21.9	27.6	24.8	41.6	***	***	ns	***	ns	ns
SRP (µg L ⁻¹)	81	64	65	78	73	*	**	***	***	ns	ns
TP (µg L ⁻¹)	319	326	257	349	357	ns	***	ns	**	ns	ns
Nitrate-N (µg L ⁻¹)	83	45	10	12	168	*	***	***	ns	***	ns
Ammonium-N (µg L ⁻¹)	274	466	136	268	685	ns	***	***	**	ns	ns
TN (mg L ⁻¹)	2.1	2.3	1.6	1.9	3.1	***	***	ns	***	**	ns
Conductivity (µS cm ⁻¹)	782	884	826	830	840	***	*	ns	***	ns	ns
Chlorophyll <i>a</i> (µg L ⁻¹)	37	37	21	26	63	ns	***	ns	***	ns	ns
Zooplankton abundance (Ind. L ⁻¹)	257	288	235	260	324	**	ns	ns	***	ns	ns
<i>D. pulex</i> abundance (Ind. L ⁻¹)	238	284	234	241	301	***	ns	ns	***	ns	ns

Table 2:

	Mean value					Probability		
	Unheated	H	No	Low	High	H	Nu	HxNu
1) Time of zooplankton peak	138	114	121	127	130	***	ns	ns
2) Time of zooplankton peak	140	117	120	130	134	***	*	ns
1) Time of <i>D. pulex</i> peak	138	114	119	128	131	***	*	ns
2) Time of <i>D. pulex</i> peak	139	117	120	128	136	***	*	ns
1) Time of chlorophyll <i>a</i> peak	54	39	45	41	53	*	ns	ns
2) Time of chlorophyll <i>a</i> peak	50	31	34	35	53	*	ns	ns

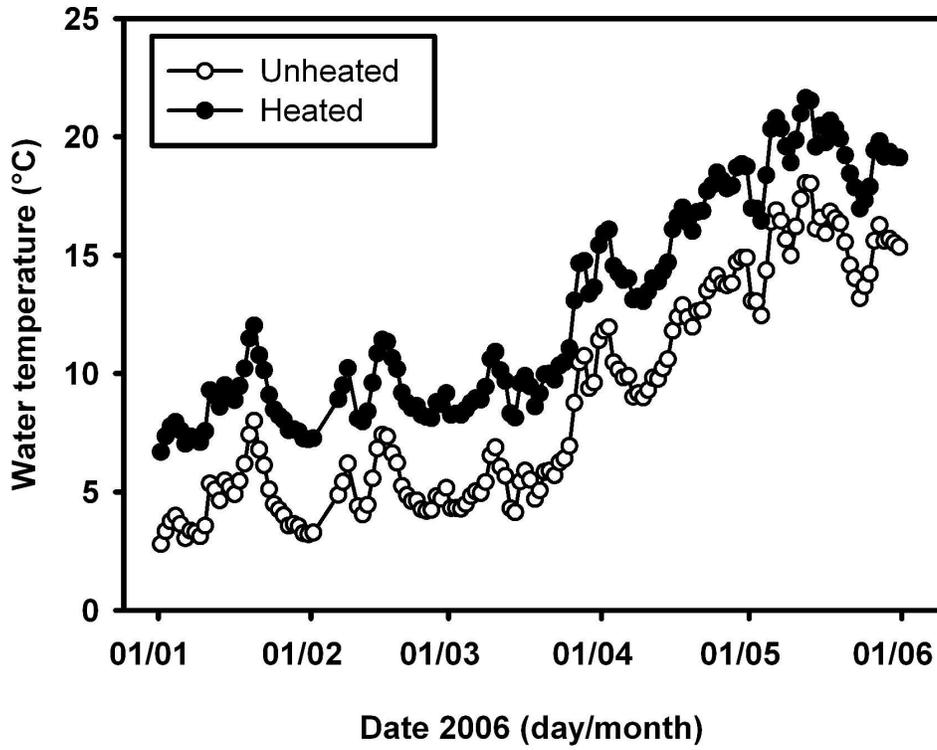


Figure 1
151x121mm (600 x 600 DPI)

Review

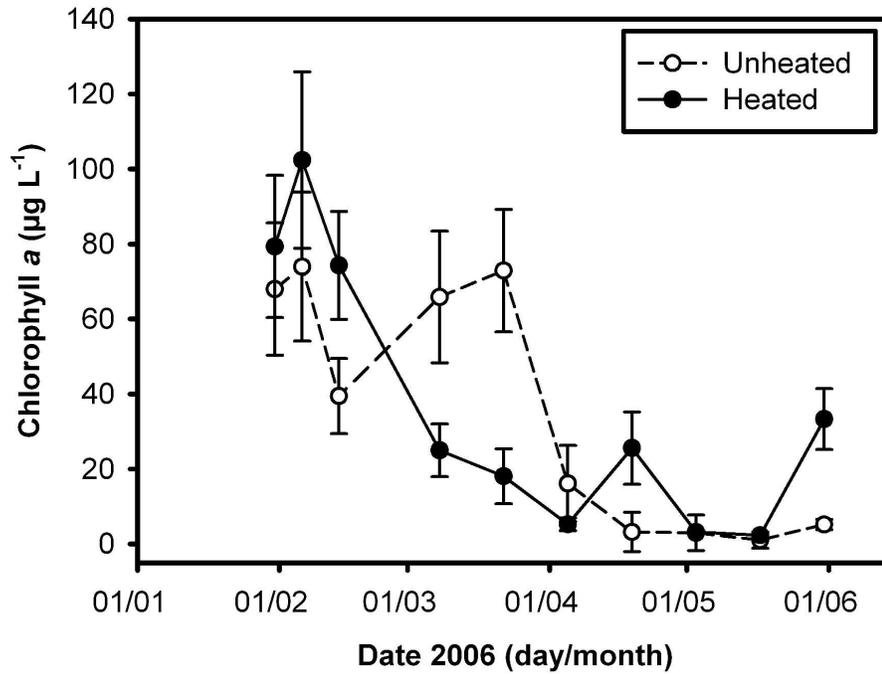


Figure 2a
164x122mm (600 x 600 DPI)

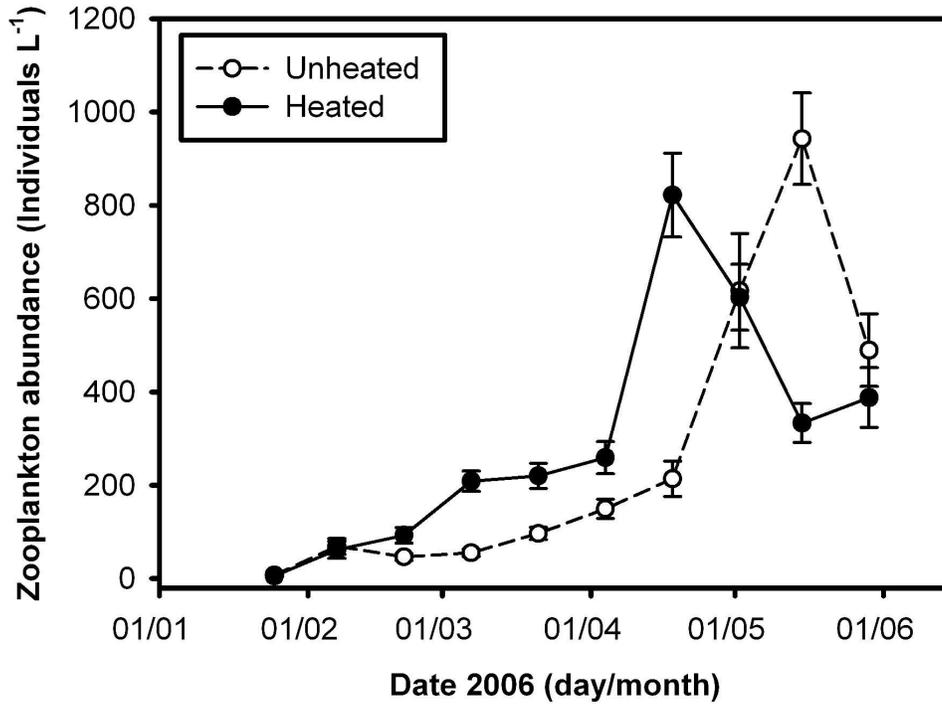


Figure 2b
157x122mm (600 x 600 DPI)

Review

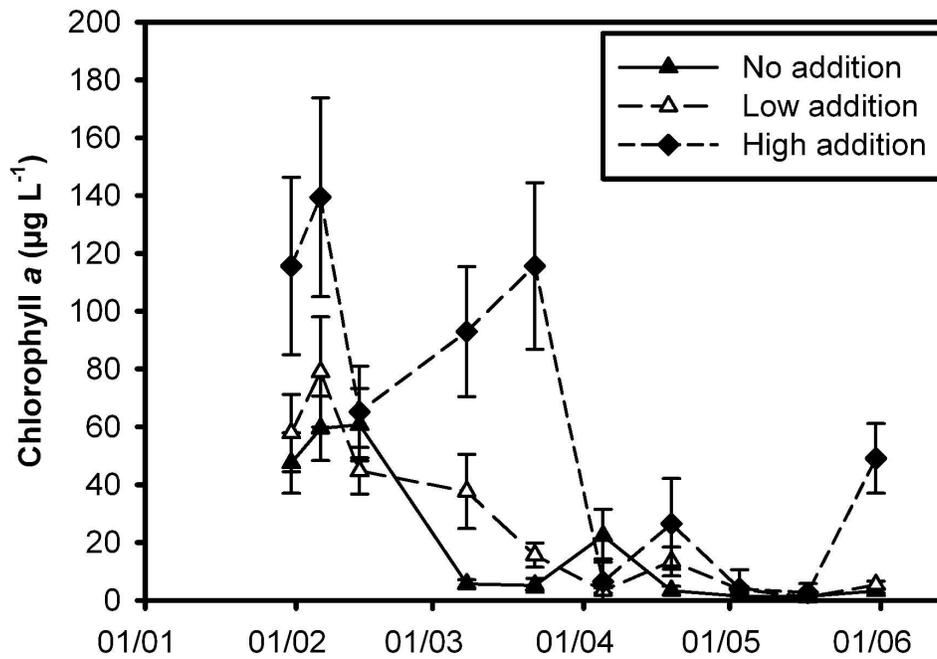


Figure 2c
154x117mm (600 x 600 DPI)

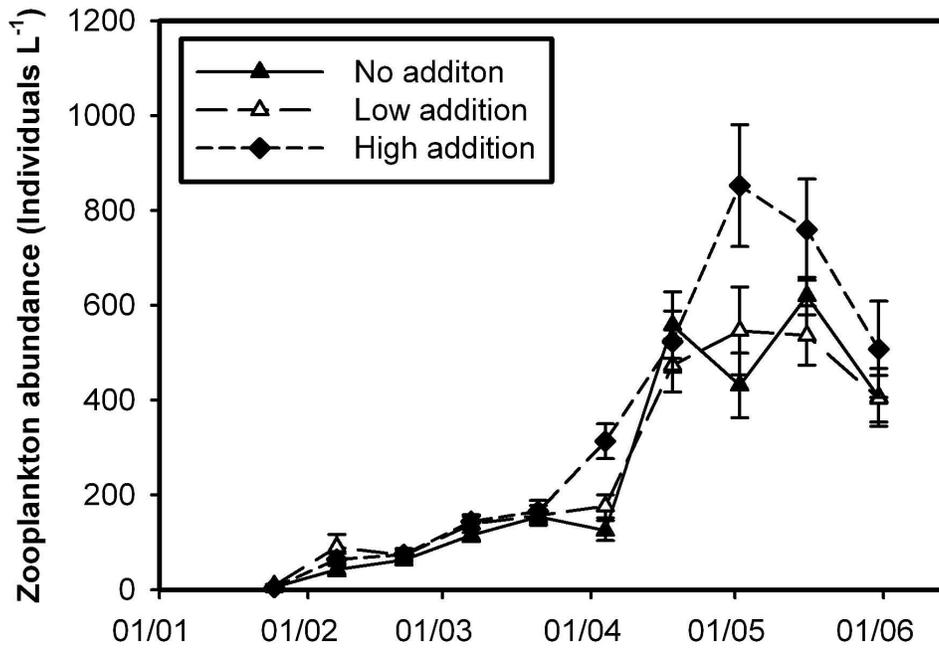


Figure 2d
157x117mm (600 x 600 DPI)

review

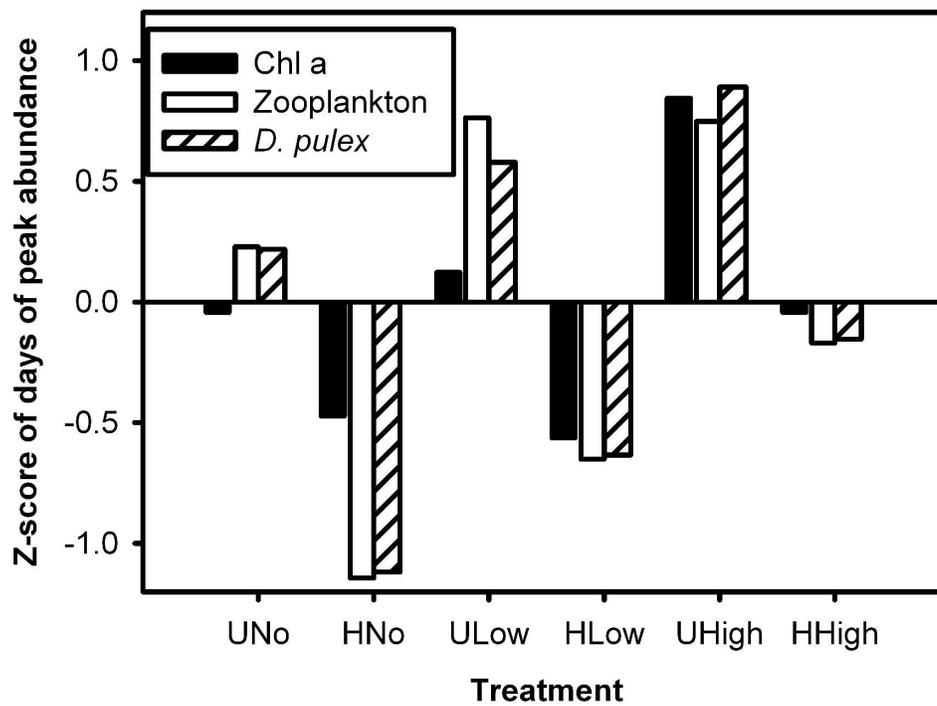


Figure 3
154x122mm (600 x 600 DPI)

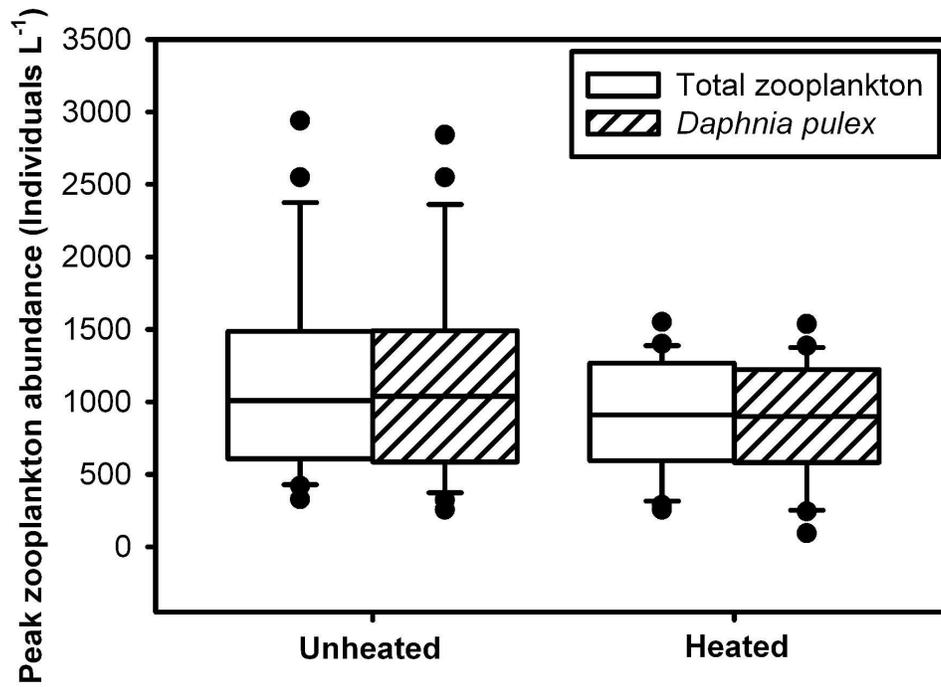


Figure 4a
156x116mm (600 x 600 DPI)

review

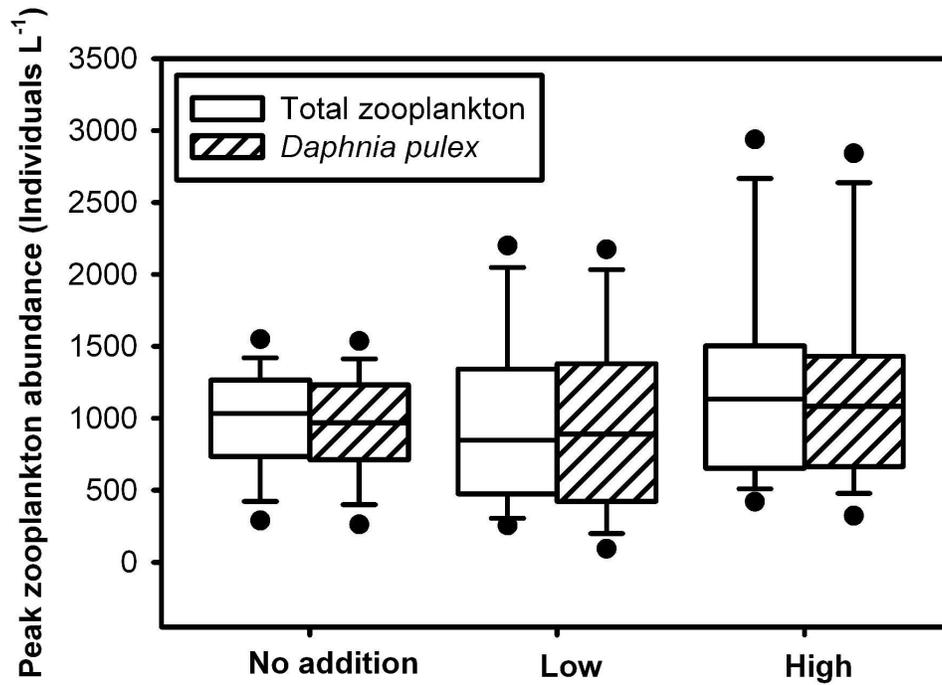


Figure 4b
157x113mm (600 x 600 DPI)

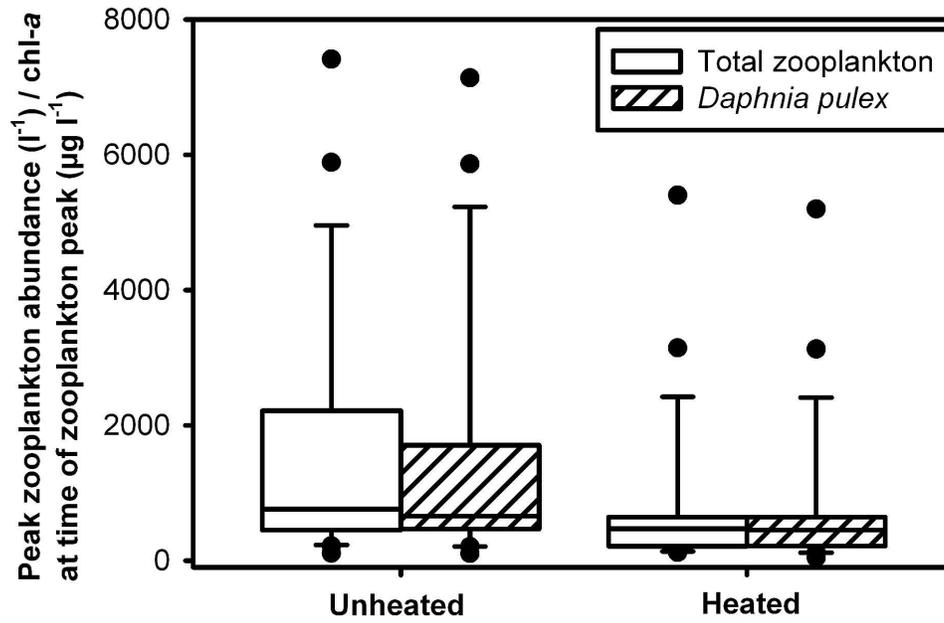


Figure 4c
163x113mm (600 x 600 DPI)

Review

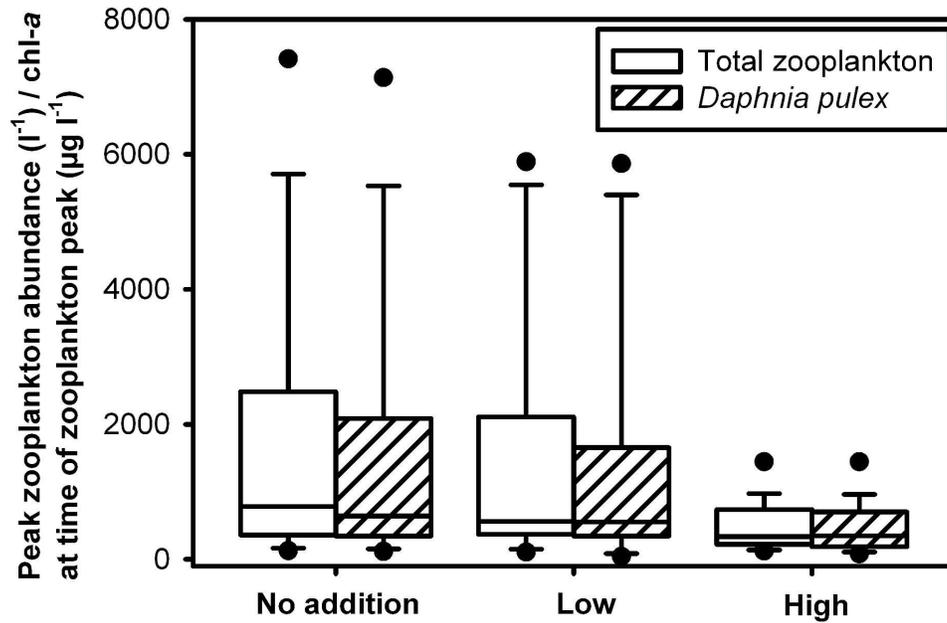


Figure 4d
163x113mm (600 x 600 DPI)

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Suggestion for front cover: Picture of the Liverpool University mesocosm facility.
144x108mm (180 x 180 DPI)

Review