APPLIED ISSUES

Macro-zooplankter responses to simulated climate warming in experimental freshwater microcosms

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SUMMARY

1. We report data collected from 48 replicated microcosm communities created to mimic plant-dominated shallow lake and pond environments. Over a 2-year period, the microcosms were subjected to warming treatments (continuous 3 °C above ambient and 3 °C above ambient during summer only), a nutrient addition treatment and the presence or absence of fish. We tracked macro-zooplankter dynamics, censusing cladoceran populations at the species level, copepods at the order level and ostracods as a class.

2. Responses to warming were subtle. Cladoceran diversity and overall abundance were not significantly affected by warming, although measures of community evenness increased. Warming effects on patterns of population trajectories tended to be strongly seasonal and most apparent during periods of pronounced increase. Populations of the prevalent cladocerans, *Chydorus sphaericus* and *Simocephalus vetulus*, displayed idiosyncratic patterns, with evidence in the case of *S. vetulus* for a negative relationship between warming and body-size at maturity. Copepod populations were reduced in size by warming, but those of ostracods increased.

3. The effects of the nutrient addition and fish treatments were strong and consistent, interacting little with warming effects in statistical models. Zooplankter abundance tended to be the highest in the fish-free microcosms receiving additional nutrient inputs and lowest when fish were present and no nutrients were added. Both treatments reduced cladoceran diversity and community evenness.

4. We suggest that warming, independently, is unlikely to supplant the effects of changing nutrient loading and fish predation as the major driver of zooplankter dynamics in shallow lakes and ponds. Moreover, in the situations where warming was of significant influence in our experiment, the distinction between summer-only warming and year-around warming was blurred. This suggests that warming effects were most pervasive during the summer, at the upper end of the temperature spectrum.

Keywords: climate warming, eutrophication, fish predation, shallow freshwater, zooplankton

Introduction

Zooplankters are common constituents of many shallow lake and pond communities. Importantly, they contribute to the mechanisms maintaining the dynamic balance between the ecosystem states of plant-dominated clear-water and of phytoplankton-dominated turbid-water (Moss, Madgwick & Phillips, 1996; Scheffer, 1998). The grazing of free-swimming Cladocera and copepods can regulate phytoplankton population growth (Sterner, 1989). Moreover, plant-associated crustacea may help to stabilise plant...
communities through the removal of periphyton and other organic material, thus improving light conditions at the plant surface (Moss et al., 1996). Plants provide spatial habitat complexity and refuge potential from predation for zooplankters (Timms & Moss, 1984).

Under increasing nutrient loading, often in association with intense fish planktivory, zooplankton community diversity and size-structure can be progressively reduced (Irvine, Moss & Balls, 1989; Jeppesen et al., 1990). A threshold of ecosystem stress may eventually be crossed whereby plants are completely lost from the system and phytoplankters become dominant (Scheffer et al., 1993; Moss et al., 1996; Scheffer, 1998). Under this extremity of eutrophication, conditions are unfavourable for most zooplankton species. Climate warming may enter the stress equation because shallow lakes and ponds have a large surface area to volume ratio and hence closely track prevailing climatic temperatures. Thermal stratification is normally absent and temperature refuges minimal. Intense interplay at the sediment–water interface means that warming has a strong potential to exacerbate chemical changes involved with eutrophication. Some species may be entirely excluded from warmed systems because of changes in their absolute thermal niche requirements (Schindler, 1997). Through more indirect and interactive mechanisms, by affecting temperature sensitive nutrient mineralisation and fish feeding behaviour, warming may alter zooplankter food resources, competitive relationships and risk of predator attack (Wooton, Allen & Cole, 1980; Beisner, McCauley & Wrona, 1997). Viewed as a functional aspect of the working of shallow lakes and ponds, reduction in zooplankter efficiency contributes to the suite of positive feed-back mechanisms which favour the forward switch between alternative ecosystem states.

Starting in the autumn of 1998 we conducted a 2-year study to examine some of the potential impacts of climate warming on shallow lake and pond ecosystems. Forty-eight microcosms mimicking unstratified, plant-dominated, environments were created. Using a factorial design, the microcosms were subjected to warming (continuous 3 °C above ambient and 3 °C above ambient during summer only), ‘eutrophication’ (addition or no addition of a nutrient solution) and fish (presence or absence of sticklebacks, Gasterosteus aculeatus). There were four replicate microcosms per treatment, and we report here the response of the microcosm zooplankton communities to these simulations of environmental change. In particular, we address the following predictions: (i) community diversity will be reduced by warming, nutrient addition and the presence of fish, with movement towards prevalence of copepods and smaller individuals in general; (ii) the remaining population sizes will be reduced by the presence of fish, but will show individualistic responses to warming and nutrient addition; (iii) population sizes will correlate positively with plant biomass and negatively with phytoplankton biomass.

Methods

Microcosms were created using 48 circular fibreglass tanks (diameter 2 m, depth 1 m) sunk into the ground for insulation. The tanks were given a layer of sediment (5 cm depth, 7 : 1 sand and loam), installed with a heating element and filled with water from a bore-hole (i.e. ground-water). Frequent rain during the experimental period meant that microcosm water volume was most often at maximum capacity (slightly <3000 L because of wind generated overflow). During rare periods of excessive summer evaporation, volume was made good using deionised water produced onsite. Plants (Elodea nuttallii, Lagarosiphon major, Potamogeton natans) were locally collected and established from initial introduction of 400 g wet weight per species per microcosm during late spring 1998. The percentage volume of each microcosm occupied by each plant species, and also by filamentous algae, was monitored every 3 weeks, starting from January 1999, by two independent observers. Macroinvertebrates arrived incidentally with the plants (e.g. gastropods), or colonised of their own accord (e.g. odonates, notonectids, dipterans, ephemeropterans, corixids). They were censused live every 9 weeks, using netsweeps, and then returned to their respective microcosms. Plankton populations were established, and thoroughly cross-mixed, from a non-selective inoculum obtained from an area of slow flowing water with diverse plant beds in the Leeds/Liverpool canal (Aintree Racecourse, Liverpool), late summer 1998.
The water of warmed microcosms was heated by pumping hot water from a boiler through heating elements. Water temperatures were monitored continuously in each microcosm using data loggers. Full technological details of the design of the warming system, the site layout and the temperature characteristics of the microcosms are given in McKee et al. (2000). Continuous warming began in September 1998 and lasted uninterrupted until September 2000. Summer-only warming began at the beginning of April each year, and ended at the end of September, defining two ‘summer’ and two ‘winter’ seasons (W1, S1, W2, S2). Populations of sticklebacks were established in September 1998 after initial introduction of 21 individuals per relevant microcosm. Continuous visual observations and trapping sessions during the course of the experiment confirmed breeding in all populations. The nutrient treatment followed a 3-week cycle during the winter and a 2-week cycle during summer. Sodium salts were added in solution to the relevant microcosms to give instantaneous additional concentrations of 0.5 mg nitrogen L\(^{-1}\) and 0.05 mg phosphorus L\(^{-1}\) during winter, and 0.17 mg nitrogen L\(^{-1}\) and 0.017 mg phosphorus L\(^{-1}\) during summer.

Zooplankton samples were taken every 3 weeks during winter and every 2 weeks during summer (following the same cycle as nutrient addition), beginning on 15 October 1998 and ending 13 September 2000 (41 sampling occasions in total). Water samples for phytoplankton counts were taken with a similar frequency. Zooplankton samples were taken using a plastic tube (diameter = 64 mm, length = 1.2 m). Three vertical columns of water were taken from each microcosm on each sampling date (one column down the wall of the microcosm, two from amongst the plants, 9 L total volume). The water was mixed in a bucket and then filtered through a 50-μm nylon mesh screen. The animals were narcotised with chloroform and preserved in alcohol. Cladocerans were identified to species level; copepods and ostracods were routinely identified only to group level. All individuals were counted. From February of the second year, the number of gravid cladocerans and copepods in each set of samples (\(n = 15\)) was noted. During this same period, body-lengths of gravid cladocerans in every other set of samples (\(n = 8\)) were measured (up to five individuals per species per sample).

Cladoceran diversity (second year only) was examined according to methodology outlined by Ludwig & Reynolds (1988). Measures of community evenness, N1 (= \(e^{-H'}\), where \(H'\) is Shannon’s index) and N2 (= \(1/\lambda\), where \(\lambda\) is Simpson’s index), were adjusted for precision using bias estimates derived from 500 bootstrap samples for the data from each of the 48 microcosms (Dixon, 1993), although bias was consistently low (<1.5% in all cases). The data conformed well with the criteria necessary for analysis of variance (ANOVA) without transformation. Multivariate analysis of variance (MANOVA) was used to examine the overall frequency of zooplankter groups in the microcosm communities. To examine patterns in population sizes, and proportions of gravid cladocerans and copepods, we used repeated measures analysis of variance (RANOVA). Data were log\(_{10}\) transformed or arcsine square-root transformed (according to Anscombe, 1948). Post-hoc comparisons were done by Tukey honest significant difference tests. Randomisation tests (Manly, 1991) were used to test for differences between mean paired cladoceran body-length (4999 randomisations in each case). Correlation and multiple regression models used mean values calculated according to season (W1, S1, W2, S2). Backward elimination was used to reduce multiple regression models to their most parsimonious form. In 6 of 12 cases this process led to a single significant independent variable (i.e. ordinary regression models). Statistical significance is indicated throughout by asterisks (*\(P < 0.05\), **\(P < 0.01\), ***\(P < 0.001\), with ns indicating non-significance.

Results

Microcosm temperature control was consistent and accurate (±0.25 °C), with appreciable stratification absent (<1 °C): mild wind generated mixing no doubt helped to maintain these conditions. Seasonal values for water temperatures are given in Table 1. Plants, plankton and macroinvertebrates rapidly established over the summer of 1998, before the onset of experimental treatments. Specimens of two unwanted plants (Potamogeton crispus and Myriophyllum spp.) occurred at very low frequencies (i.e. two to three individuals per year across all microcosms) and on detection were immediately removed. The volume of each microcosm occupied by plants (total PVI, %) varied little throughout the experiment across all microcosms:
in terms of biovolume (e.g. Cryptomonas erosa) prevalent, both in terms of number of individuals and temperature and light levels. Plankton blooms often associated with rising spring probably contributed to the dampening of phytoplankton blooms often associated with rising spring temperature and light levels.

Phytoplankton chlorophyll a concentrations remained consistently low (across all microcosms: W1 mean = 4.4 µg L⁻¹, SD = 13.3, n = 432; S1 mean = 9.8 µg L⁻¹, SD = 16.7, n = 576; W2 mean = 17.1 µg L⁻¹, SD = 31.2, n = 384; S2 mean = 23.1 µg L⁻¹, SD = 29.1, n = 576). Small cryptophytes and green algae were prevalent, both in terms of number of individuals and in terms of biovolume (e.g. Cryptomonas erosa, Rhodomonas minuta, Micractinium pusillum). Cyanophytes (e.g. Oscillatoria, Chroococcus, Merismopedia) were a minor component of all communities (mean frequency of biovolume across all microcosms and sampling dates = 0.3%, SD = 1.3, n = 1968), and filamentous algae consisted of a mixture of Spirogyra spp., Cladophora spp. and Ulothrix spp. Macroinvertebrates were continuously present. On termination of the experiment we were able to obtain direct estimates of live fish biomass (mean per relevant microcosm = 27.0 g⁻¹, SD = 18.2, n = 24).

Zooplankton communities were representative of those naturally found in plant dominated shallow lakes and ponds. Thirteen cladoceran species were recorded (Table 2), although Scapholeberis mucronata disappeared after the first few weeks of the start of experimental treatments, and Alonella excisa disappeared during the middle of the first experimental summer, apparently being replaced by Alona rect-angula. Most species occurred at low frequencies (Table 2), and daphnids in particular were rare when fish were present. However, Chydorus sphaericus and Simoecphalus vetulus were ubiquitous and relatively abundant, constituting 73 and 11%, respectively, of the total number of cladocerans recorded (346 026 individuals). All copepods were cyclopoid, predominantly Cyclops agilis and Cyclops viridis. Ostracods were predominantly Cypridopsis vidua and Herpetocypris chevreuxi. Totals of 136 822 copepods and 59 670 ostracods were recorded, constituting 25 and 11%, respectively, of the total number of zooplankters (Table 2).

Cladoceran species diversity (N0) was not significantly affected by warming (Table 3), but decreased significantly in the presence of fish, and in the microcosms receiving additional nutrient inputs (Table 3, Fig. 1a). The number of abundant species (N1) and very abundant species (N2) varied between fish and nutrient treatments in a similar manner, and both showed increasing trends with warming (Table 3, Fig. 1b). Fewer species dominated the

<table>
<thead>
<tr>
<th>Winter 1</th>
<th>Summer 1</th>
<th>Winter 2</th>
<th>Summer 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>h</td>
<td>H</td>
<td>C</td>
</tr>
<tr>
<td>Mean</td>
<td>5.8</td>
<td>5.8</td>
<td>8.8</td>
</tr>
<tr>
<td>Maximum</td>
<td>12.0</td>
<td>12.0</td>
<td>14.9</td>
</tr>
<tr>
<td>Minimum</td>
<td>3.0</td>
<td>2.9</td>
<td>6.0</td>
</tr>
</tbody>
</table>

**Table 1** Seasonal water temperatures (°C) in the microcosms according to warming treatment (C = no warming, h = summer-only warming, H = continuous warming).

<table>
<thead>
<tr>
<th>Year 1</th>
<th>Year 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acroperus harpae</td>
<td>0.440</td>
</tr>
<tr>
<td>Alona affinis</td>
<td>0.067</td>
</tr>
<tr>
<td>Alona rectangula</td>
<td>0.043</td>
</tr>
<tr>
<td>Alonella excisa</td>
<td>0.089</td>
</tr>
<tr>
<td>Ceriodaphnia reticulata</td>
<td>0.983</td>
</tr>
<tr>
<td>Chydorus sphaericus</td>
<td>36.161</td>
</tr>
<tr>
<td>Daphnia longispina</td>
<td>0.020</td>
</tr>
<tr>
<td>Daphnia magna</td>
<td>2.005</td>
</tr>
<tr>
<td>Graftoleberis testudinaria</td>
<td>1.590</td>
</tr>
<tr>
<td>Polyphemus pediculus</td>
<td>0.026</td>
</tr>
<tr>
<td>Peracantha truncata</td>
<td>0.015</td>
</tr>
<tr>
<td>Scapholeberis mucronata</td>
<td>0.004</td>
</tr>
<tr>
<td>Simoecphalus vetulus</td>
<td>3.173</td>
</tr>
<tr>
<td>Copepods</td>
<td>12.513</td>
</tr>
<tr>
<td>Ostracods</td>
<td>4.653</td>
</tr>
</tbody>
</table>

**Table 2** Frequency of occurrence of cladoceran species, copepods and ostracods as a percentage of the total number of zooplankters recorded.
communities in the unwarmed microcosms compared with the continuously warmed microcosms (Tukey honest significant difference: N1, $P = 0.0213$; N2, $P = 0.0411$), with summer only warming intermediate. It is likely that N1 and N2 values largely reflected relative increases in numbers of A. rectangula, and Graptoleberis testudinaria under continuous warming (Fig. 1c).

**MANOVA** indicated that all three treatments had at least some significant influence in determining the proportions of zooplankter groups in each microcosm community (Table 4). Post-hoc examination of individual zooplankton group trends showed that only ostracods significantly changed in frequency with warming (compare Figs 2a,c,e). Ostracods made up larger, and similar, proportions of each community under both warming treatments (Fig. 2e). The effects of fish and nutrient treatments were warming-independent, but mutually interacted to produce complex patterns. Proportions of Cladocera increased significantly in the presence of fish and tended to increase with nutrient addition, although this nutrient related trend was only significant in the absence of fish (Fig. 2b). The proportion of copepods making up each community tended to decrease in the presence of fish and under nutrient addition. However, there was no significant difference between proportions when fish were absent but nutrients added, and when fish were present but no nutrients added (Fig. 2d). Proportions of ostracods decreased significantly particularly under nutrient addition in the presence of fish (Fig. 2f).

Mean numbers of Cladocera were the highest in the fish-free microcosms receiving additional nutrients (overall mean = 37 individuals L$^{-1}$, $n = 492$) and lowest in the fish containing microcosms not receiving additional nutrients (overall mean = 6 individuals L$^{-1}$, $n = 492$). The enhancement of numbers by nutrient addition on the one hand, and depression by fish on the other was a strong and consistent effect, illustrated by the population trajectories of S. vetulus and C. sphaericus (Table 5, Fig. 3). Warming did not influence overall cladoceran abundances (**MANOVA** warming main effect: $F_{2,36} = 0.01$, ns). More subtle warming effects on individual species depended strongly on sampling date (Table 5), and tended to be statistically significant only during periods of pronounced population increase. Thus, in the absence of fish, S. vetulus showed a tendency to occur in greater numbers in the unwarmed microcosms (Fig. 3b). Warming effects on C. sphaericus numbers were few and highly inconsistent between sampling dates and fish treatments (Fig. 3e,f).

Gravid individuals of C. sphaericus and S. vetulus occurred less frequently in the presence of fish (**MANOVA** fish main effect: $F_{1,36} = 5.8^*$ and $F_{1,36} = 103.6^{***}$, respectively). No other treatment effects (or interactions) displayed statistically significant influences on the occurrence of gravid individuals. Individuals of C. sphaericus were smaller in the presence of fish, and S. vetulus decreased in size with warming and with the addition of nutrients (Fig. 4). Comparison of the overall mean length of C. sphaericus in the absence of fish (0.36 mm, SD = 0.04, $n = 528$) with that when fish were present (0.32 mm, SD = 0.04, $n = 273$) yields a difference of 0.03 mm. This difference was significant according to a standard t-test ($t$-value = 11.99***). Similarly, significant differences in S. vetulus lengths were found between the unwarmed microcosms and the continuously warmed microcosms in the absence of nutrient addition (0.15 mm, $t$-value = 3.65***), and between the unwarmed microcosms and the continuously warmed microcosms in the presence of nutrient addition (0.10, $t$-value = 3.12**). Randomisation tests reinforced these assertions with significance levels of 0.0002, 0.0006 and 0.0028, respectively.

Adult copepod numbers increased with nutrient addition (Table 5, Fig. 5a), but decreased in the presence of fish (Table 5, compare Figs 5b,c). There was some tendency for significantly greater numbers of copepods to occur in the unwarmed microcosms, particularly during the summer in the absence of fish (Table 5, Fig. 5c). The proportion of egg-bearing
females was consistently low, and only significantly influenced by sampling date ($F_{14,504} = 3.63^{***}$). Around 2% of individuals were gravid during the winter and 7% during the summer. As might be expected, patterns in nauplii abundances generally reflected those of the adults; numbers tended to increase with nutrient addition, although this trend was not consistently significant across time or between fish treatments ($F_{40,1440} = 2.05^{**}$) and few individuals were found in the presence of fish. Warming produced occasional significant differences between nauplii numbers ($F_{80,1440} = 1.58^{**}$). As with the adults, there was some tendency for increased numbers to occur in the unheated ponds, particularly during the summer. Ostracod numbers were significantly (and similarly) increased by both warming treatments during the summer (Table 5, Fig. 1).
Fig. 5d). In the presence of fish, numbers were consistently reduced (Table 5, compare Figs 5e,f). When fish were absent, numbers were enhanced by nutrient addition (Table 5, Fig. 5f). Warming showed no significant interaction with either the fish treatment or the nutrient treatment in determining ostracod numbers.

Three regression models were used to further explore the distribution of zooplankters between microcosms. Data were split between the microcosms with and without fish. In model I, zooplankton abundance was related to the total volume of the microcosms occupied by plants (total PVI, %) and phytoplankton chlorophyll a concentrations (Table 6). Cladoceran abundances showed positive relationship with both total PVI and with chlorophyll a concentrations, but only in the absence of fish. Copepods showed a positive relationship with total PVI but, again, only in the absence of fish. Ostracod abundances increased with total PVI irrespective of fish, and increased with chlorophyll a concentrations when fish were absent. Model II incorporated the volume of each microcosm occupied by each individual plant species, phytoplankton biovolume, and the abundances of major potential zooplankter predators (odonates + notonectids + Chaoborus) and gastropods (predominantly Physa fontinalis) (Table 6). This model

### Table 4 Results of MANOVA of overall proportions of cladocerans, adult copepods and ostracods recorded from each microcosm community

<table>
<thead>
<tr>
<th>Effect</th>
<th>Wilks’ Lambda</th>
<th>Rao’s $R$</th>
<th>d.f. 1</th>
<th>d.f. 2</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warming (1)</td>
<td>0.33</td>
<td>8.48</td>
<td>6</td>
<td>68</td>
<td>***</td>
</tr>
<tr>
<td>Fish (2)</td>
<td>0.27</td>
<td>31.03</td>
<td>3</td>
<td>34</td>
<td>***</td>
</tr>
<tr>
<td>Nutrients (3)</td>
<td>0.45</td>
<td>14.02</td>
<td>3</td>
<td>34</td>
<td>***</td>
</tr>
<tr>
<td>1 × 2</td>
<td>0.86</td>
<td>0.89</td>
<td>6</td>
<td>68</td>
<td>n.s.</td>
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<tr>
<td>1 × 3</td>
<td>0.79</td>
<td>1.45</td>
<td>6</td>
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<tr>
<td>2 × 3</td>
<td>0.66</td>
<td>5.78</td>
<td>3</td>
<td>34</td>
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<td>1 × 2 × 3</td>
<td>0.86</td>
<td>0.90</td>
<td>6</td>
<td>68</td>
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**$P < 0.01$, ***$P < 0.001$, n.s. = not significant.**

Fig. 2 Treatment effects on overall proportions of zooplankters recorded from each microcosm: (a) Cladocera (b) copepods (c) ostracods. Warming treatment means are coded as $C =$ no warming, $h =$ summer-only warming, $H =$ continuous warming. Means according to fish × nutrient interaction are coded as circles = fish present, squares = fish absent, $N+ =$ nutrient addition, $N− =$ no nutrient addition. Pairs of means showing no significant differences according to Tukey honest significant difference tests are joined by lines. All other pairs were significantly different ($P < 0.02$).
Table 5 F-values from ANOVA of numbers of *C. sphaericus*, *S. vetulus*, adult copepods and ostracods

<table>
<thead>
<tr>
<th>Effect</th>
<th>Effect d.f.</th>
<th>Error d.f.</th>
<th>C. sphaericus</th>
<th>S. vetulus</th>
<th>Copepods</th>
<th>Ostracods</th>
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</thead>
<tbody>
<tr>
<td>Warming (1)</td>
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<td>0.12 n.s.</td>
<td>1.07 n.s.</td>
<td>1.35 n.s.</td>
<td>13.29***</td>
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<tr>
<td>Fish (2)</td>
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<td>34.82***</td>
<td>155.67***</td>
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<td>104.54***</td>
</tr>
<tr>
<td>Nutrients (3)</td>
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<td>45.57***</td>
<td>2.53 n.s.</td>
<td>13.37***</td>
<td>9.38***</td>
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<tr>
<td>Time (4)</td>
<td>40</td>
<td>1440</td>
<td>34.53***</td>
<td>14.48***</td>
<td>11.81***</td>
<td>22.41***</td>
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<td>1.11 n.s.</td>
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<td>2.62 n.s.</td>
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<td>1.55**</td>
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<td>1.82***</td>
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<td>2.41***</td>
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<td>36</td>
<td>1.04 n.s.</td>
<td>0.71 n.s.</td>
<td>0.47 n.s.</td>
<td>1.52 n.s.</td>
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<td>1440</td>
<td>2.22***</td>
<td>1.42*</td>
<td>1.49*</td>
<td>1.14 n.s.</td>
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<td>1440</td>
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<td>0.50 n.s.</td>
<td>0.94 n.s.</td>
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<td>1440</td>
<td>3.91***</td>
<td>1.56*</td>
<td>1.01 n.s.</td>
<td>1.50*</td>
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<tr>
<td>1 × 2 × 3 × 4</td>
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<td>1440</td>
<td>1.23 n.s.</td>
<td>0.81 n.s.</td>
<td>1.11 n.s.</td>
<td>1.23 n.s.</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01, ***P < 0.001, n.s. = not significant.

Fig. 3 Treatment effects on (log) numbers of individuals per litre over the course of the experiment (101 weeks, with x-axis delineation indicating our four seasons): (a) *S. vetulus*, warming × time (fish present); (b) *S. vetulus*, warming × time (fish absent); (c) *S. vetulus*, nutrients × time (fish present); (d) *S. vetulus*, nutrients × time (fish absent); (e) *C. sphaericus* warming × time (fish present); (f) *C. sphaericus*, warming × time (fish absent); (g) *C. sphaericus*, nutrients × time (fish present); (h) *C. sphaericus*, nutrients × time (fish absent). Population trajectories according to warming are: solid line = no warming, dashed line = summer-only warming, dotted line = continuous warming; and according to nutrient treatment, thick solid line = nutrient addition, thin solid line = no nutrient addition. The presence or absence of fish is indicated by F+ and F−, respectively.

indicated that cladocerans and copepods favoured *E. nuttallii* when fish were absent, and that copepods favoured *P. natans*, and ostracods *L. major*, when fish were present. Significant relationships between zoo- plankter abundance and the abundances of predators and gastropods occurred only in the absence of fish. Cladocerans were negatively influenced by predators, whereas ostracods increased with both predator and gastropod abundance. A series of individual regressions (model III) indicated that only cladoceran abundances, in the absence of fish, were significantly related to the percentage volume of each microcosm occupied by filamentous algae (positive relationship, Table 6). Correlation showed that when fish were present numbers of cladocerans and copepods increased together (*r* = 0.53***, *n* = 96; the relationship between cladocerans and ostracods, and between copepods and ostracods was not significant). When fish were absent, cladoceran numbers increased with both copepods and ostracods (*r* = 0.57***, *n* = 96, *r* = 0.24*, *n* = 96, respectively; relationship between copepods and ostracods not significant). Total PVI was

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**Fig. 4** Body-lengths of gravid *C. sphaericus* and *S. vetulus*. Values are means with 95% confidence intervals. Sample size is displayed in the appropriate bar. The warming treatments are indicated as follows: C = no warming, h = summer-only warming, H = continuous warming. Codes for the presence or absence of fish, and the addition or not of nutrients, are F+, F− and N+, N−, respectively.

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**Fig. 5** Treatment effects on (log) numbers of individuals per litre over the course of the experiment (101 weeks, with x-axis delineation indicating our four seasons): (a) copepods, nutrients × time; (b) copepods, warming × time (fish present); (c) copepods, warming × time (fish absent); (d) ostracods, warming × time; (e) ostracods, nutrients × time (fish present); (f) ostracods, nutrients × time (fish absent). Population trajectories according to warming are: solid line = no warming, dashed line = summer-only warming, dotted line = continuous warming; and according to nutrient treatment, thick solid line = nutrient addition, thin solid line = no nutrient addition. The presence or absence of fish is indicated by F+ and F−, respectively.

not significantly correlated with phytoplankton chlorophyll $a$ concentrations. However, there was a weak positive relationship between total PVI and phytoplankton biovolume in the absence of fish ($r = 0.26^*$, $n = 96$).

### Discussion

Climate warming is anticipated to influence the structure and functioning of most ecosystem types. Shallow freshwater ecosystems (ponds and lakes) are particularly susceptible to externally imposed change, and are therefore likely to be amongst the ecosystem types facing considerable perturbation under scenarios of climate warming. Much of this perturbation may come through alteration of hydrological regimes and possible increased exposure to ultra-violet radiation (Schindler, 1997; Pienitz & Vincent, 2000), phenomena, in some instances, which may be catastrophic (e.g. if a water body dries-out completely or is continuously flooded). Effects of increased water temperatures are likely to be more insidious and, whilst warming-induced changes in the abundance of species and individuals are in themselves cause for concern, they may or may not impact on primary ecosystem processes. However, changes to functionally important taxa clearly have the potential to reverberate throughout the ecosystem (Carpenter et al., 1993; Chapin et al., 2000). In shallow lakes and ponds, zooplankters fall into this category. Our initial predictions concerning the responses of zooplankton communities to warming, in combination with increased nutrient concentrations and fish predation, were only partially supported by our experimental results. Cladoceran diversity was reduced by the presence of fish and by additional nutrient input, but not by warming. However, there was a tendency towards greater community eveness under warming, with the prevalence of *A. rectangula* and *G. testudinaria* (both small-bodied) being increased. Ostracods increased their relative frequency under warming, but copepods and cladocerans did not. Moreover, there was no substantial shift in community size structure attributable to warming. The predominant ostracods and copepods were relatively large-bodied species and, although small-bodied cladocerans were numerically dominant, they were ubiquitously distributed between the experimental treatments. Body-size is a determinant of filtering capacity and in this context has important functional implications (Reynolds, 1994). Plasticity of body-size in individual zooplankters is well-described, depending on a range of covarying factors including temperature, exposure to size-selective predation and abundance and nutritional quality of food (Perrin, 1988; Demeester, Weider & Tollrnan, 1995; McKee & Ebert, 1996). Our evidence, from body-length measurements of mature *C. sphaericus* and *S. vetulus*, supports generally observed patterns (i.e. negative relationships between zooplankter body-size, tem-
temperature, the presence of predatory fish and food quantity/quality).

Abundances of all zooplankter groups followed typically cyclic trajectories, with a general increase in numbers during summer and decreases during winter. In statistical models, we found no interactions between warming effects on population sizes and other treatment effects that did not also include time. This points to strong seasonality in any effect of warming. Indeed, population sizes were most affected by warming whilst undergoing periods of accentuated increase during the early and late summer. Therefore, differentiation between our summer-only and continuous warming regimes in affecting zooplankter abundances was minimal. Whilst numbers of *S. vetulus* and copepods showed some tendency to be reduced by warming, the most pronounced warming effect was on ostracods, whose numbers consistently followed the opposite trend. Both fish and nutrients strongly affected population sizes. Presumably through direct predation, fish reduced all populations to low densities, especially the large-bodied daphnids. In the absence of fish, nutrient addition tended to increase population sizes.

Is the subtlety of these results surprising? An alternative series of initial predictions may have suggested that the direct effects of small changes in temperature would be minimal for organisms inhabiting shallow bodies of freshwater. This is because such organisms might be expected to display potentially greater tenacity of response to fluctuating environmental conditions, certainly relative to those organisms routinely living in more stable habitats.

Selection for plasticity has been postulated to contribute to the distribution and abundance of species between habitats with different levels of environmental variation (Wilbur, 1980; Leips, McManus & Travis, 2000) However, few studies have specifically examined the relationship between environmental variability and phenotypic plasticity and strictly adaptive explanations are generally lacking (Leips et al., 2000).

A number of studies have related meteorological trends to variation in lake plankton dynamics (e.g. George & Harris, 1985; George et al., 1990; Adrian et al., 1995; Adrian & Deneke, 1996; Adrian et al., 1999; Weyhenmeyer, Blenckner & Pettersson, 1999; Gerten & Adrian, 2000; Straille, 2000). These studies have generally involved deeper lake systems (i.e. pelagic environments subject to stratification), with high thermal inertia and have tended to highlight patterns produced in phytoplankton communities by warming. Nevertheless, some notable observations regarding zooplankters are available. For Lake Windermere (U.K.), George & Harris (1985) showed that the biomass of crustacean zooplankters was inversely correlated with June water temperatures and the onset of stratification. Similarly, numbers of daphnids were correlated with thermocline stability, availability of edible algae and thence to temperature cycles in nearby Esthwaite Water (George et al., 1990). In Lake Constance (Germany), Straille (2000) related variation in daphnid growth rates during April/May to variation in mean water temperatures, which linked higher daphnid biomass during May to warmer conditions produced by the North Atlantic Oscillation.

Also in Germany, study of the Müggelsee has shown that periods of warmer weather (linked to the North Atlantic Oscillation) resulted in earlier seasonal zooplankton population development, but did not alter dynamics (e.g. net growth rates, height of population maxima) and only marginally influenced spring abundances of cladocerans (Gerten & Adrian, 2000). Indeed, water temperature in late spring alone had a high predictive power for the timing and magnitude of at least the spring daphnid peak, but further significant temperature correlation throughout the summer with zooplankter abundance was not found (Adrian et al., 1999; Gerten & Adrian, 2000). Two patterns are evident from these studies. First, the timing of population events is most likely to be influenced by temperature variation. Secondly, significant temperature effects tend to be detected only during periods of pronounced population increase during late-spring/early summer. Whilst perhaps not strictly comparable with the situation represented by our experiment (very shallow water, no stratification, year-around plant dominance), our results are supportive. It may be noted, however, that we do not provide detailed analysis or discussion of the phenology of our zooplankton populations. This is because basic time-series exploration indicated no pronounced trends in the timing of population peaks between warming treatments, a result that can be broadly surmised in visual comparison of population trajectories in Figs 3 and 5.

The cascading impacts of fish and nutrient addition in freshwater systems are well known (Reynolds, 1994; Moss et al., 1996; Scheffer, 1998). What we note from our experiment, was the lack of statistically

significant interaction between warming treatment effects and nutrient addition effects on zooplankter numbers and, to a lesser extent, between warming and fish effects (e.g. Table 5). In the cases where warming and fish did produce significant interaction, zooplankter numbers showed some tendency to be higher under warming in the presence of fish (compare Figs 3a, 3b, 3e, f & 5b, c), a trend contrary to that anticipated (e.g. Wooton et al., 1980). Of course, we recognise that the strength of our nutrient addition and fish treatments and/or the resolution of our sampling schedule, could have masked potentially significant warming effects. Nevertheless, the magnitude of our nutrient addition and fish treatments well represented the contemporary condition of many shallow lakes and ponds.

Our rationale in investigating relationships between zooplankton abundance and macrophytes, phytoplankton and other macroinvertebrates followed conventional wisdom. Plants are thought to provide refuge for zooplankters against predation, as well as other resources related to spatial habitat complexity. Phytoplankters are the principal food resource of herbivorous zooplankters and therefore, on a seasonal basis, some relationship between zooplankton abundance and the abundance of edible phytoplankters may be expected (we did not distinguish between phytoplankton species in our analysis because of the overwhelming and persistent predominance of small, presumably edible, cryptomonads and green algae). Under eutrophication, a negative relationship between zooplankter abundance and phytoplankters is likely to occur, as phytoplankton communities begin to dominate the ecosystem and change in composition, often with higher prevalence of cyanophytes. A shallow lake or pond with high zooplankter diversity and abundance will often display similar patterns for macroinvertebrates, although through predatory activity (e.g. odonates feeding on cladocerans) and/or competitive interactions (e.g. between gastropods and other plant surface dwellers) positive or negative relationships between individual taxa may be found. However, it must be recognised that very little is currently known about the precise habitat use and feeding preferences of most littoral invertebrates. Therefore we treat our observations as indications only, without necessarily attaching direct cause and effect.

We did not find significant negative relationships between phytoplankton chlorophyll a concentration and zooplankton abundances, even in the presence of fish, perhaps because chlorophyll concentrations remained low, a condition frequently observed in plant-beds (e.g. Schriver et al., 1995). In fact, in the absence of fish, both cladoceran and ostracod numbers displayed marginal increase with chlorophyll a. Again, in the absence of fish, cladoceran numbers increased with the volume of filamentous algae. This increase is likely to reflect patterns in the abundances of strictly substrate-associated cladocerans and we may therefore have expected ostraocods to follow a similar trend, but they did not. Phytoplankton biomass was a non-significant predictor of zooplankter numbers. We anticipated positive relationships between total plant biomass and zooplankter abundances. This tended to be the case only in the absence of fish, and consistent patterns in zooplankton group preferences for individual plant species were not detected. The constancy of plant biomass throughout the experiment could be one explaining factor. Moreover, some evidence exists to suggest that larger free-swimming cladocerans and copepods prefer to seek daytime refuge from predation at the borders of vegetation stands (Scheffer, 1998). Such edge effects were minimal in the microcosms, where plants occupied a large proportion of the available horizontal space. For the smaller zooplankters, it also seems likely that the plant cover, although dense, was insufficient as a shield against intense predation, most probably from the young-of-year fish. Macroinvertebrate predators had a negative influence only on cladocerans, which are known to be included in the diet of at least odonates and Chaoborus (e.g. Paterson, 1994). Perhaps more surprising was the positive relationship between ostracod numbers and macroinvertebrate predators. This finding was unlikely to have been due directly to some release from competition for the ostracods because, in the absence of fish, cladoceran and ostracod numbers correlated positively and ostracod numbers increased with the number of gastropods (another group of assumed potential competitors).

Few studies have experimentally approached the potential impact of climate warming on shallow freshwaters at a large-scale community level. In laboratory microcosms, it has been demonstrated that simple communities of phytoplankters, daphnids and predatory flatworms were destabilised by increased temperature, with the outcome being extinction of the daphnids (Beisner et al., 1997). Extinctions were not a
prominent feature of our communities under any of
the treatments, although some species were undetect-
able for protracted periods. In an earlier study, Reed
(1978) showed that diversity of microcrustacea,
periphyton and benthic organisms was reduced under
nutrient addition. Diversity tended to increase with
physical heterogeneity, but was not affected by
stability of temperature. Under field conditions,
warming stream channels result in smaller adult
body-size of dipterans across various trophic groups
(Rempel & Carter, 1987). Using a more elaborate
approach, Hogg & Williams (1996) ascertained similar
results for a stream dwelling plecopteron and an
amphipod. They also found reduced total animal
densities under warming, but surmised that changes
in stream invertebrate taxon composition, species
richness, community biomass, or densities of indi-
vidual taxa are likely to be weak indicators of the
small and gradual shifts in temperature associated
with climate warming.

We conclude that the effects of small increases in
temperature caused by climate warming on the
zooplankton communities of shallow lakes and ponds
will most likely be subtle and, in the absence of other
forcing variables, probably of insufficient magnitude
to significantly altered ecosystem functional struc-
tures and processes. Regulation of the impact of
nutrient loading and fish activity on zooplankter
communities should remain the principal concern
surrounding the protection and restoration of shallow
lake and pond ecosystems.

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