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Response of freshwater microcosm communities to nutrients, fish, and elevated temperature during winter and summer

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Abstract

Under conditions of stress, shallow freshwater ecosystems can undergo a state change characterized by the rapid loss of macrophytes and subsequent dominance of phytoplankton. Elevated water temperature may promote such change. Here we report the impact of two warming regimes (continuous 3°C above ambient and 3°C above ambient during summer only), with two nutrient loadings and the presence or absence of fish, on 48 microcosm ecosystems created to mimic shallow pond environments. We found that warming did not significantly encourage phytoplankton blooms, even in combination with increased nutrients and fish. Instead, macrophyte communities remained dominant. Macrophyte-associated invertebrates (gastropods and ostracods) increased in numbers in the warmed microcosms, potentially helping to stabilize the macrophyte communities. Nevertheless, warming produced trends in water chemistry that could be problematic. It increased phosphorus concentrations, total alkalinity, and conductivity. It decreased pH and oxygen saturation and increased the frequency of severe deoxygenation. These trends were largely independent of the other experimental treatments and support the suggestion that moderate warming has the potential to exacerbate existing eutrophication problems.

Under sufficient stress, shallow clear-water ecosystems can rapidly switch to turbid conditions with complete loss of macrophytes and their replacement with phytoplankton (Scheffer et al. 1993; Moss et al. 1996). From most perspectives this switch is undesirable because it involves a reduction of the diversity of macroorganisms and a decrease in amenity and recreation value. Throughout the world, therefore, common aims of resource management are to protect and restore water bodies from turbid conditions. The mechanisms by which these aims are achieved are reasonably well understood. However, increasing water temperature may add complications (Mulholland et al. 1997; Schindler 1997). In particular, warming-induced increases in rates of nutrient mineralization and intensity of predation on invertebrates by fish may compound already widespread eutrophication problems (Wooton et al. 1980; Carpenter et al. 1992; McDonald et al. 1996; Mulholland et al. 1997; Schindler 1997; Scheffer 1998).

In shallow freshwater ecosystems, macrophytes are of central importance. Through active processes (e.g., sediment stabilization, nutrient uptake and storage, production of phytoplankton inhibiting substances), as well as passive processes (e.g., increasing rates of sedimentation, provision of refuge from fish predation for invertebrates), they produce a suite of positive feedback mechanisms that help to maintain clear-water conditions (Scheffer et al. 1993; van Donk et al. 1993; Scheffer 1999; Engelhardt and Ritchie 2001). Nutrient concentrations are generally low when macrophytes are present. This is particularly the case for available nitrogen, although the occurrence of available phosphorus is less predictable (van Donk et al. 1993). By intermittently creating anoxia (by net respiration and microbial decomposition of their detritus) and conditions of high pH (intense photosynthetic activity), macrophytes can enhance phosphorus release from sediments. On the other hand, their reduction of turbulence limits diffusion and works in the opposite direction (Scheffer 1998). Invertebrates, whose impact comes mainly through their feeding habits, are often seen to increase in biomass and diversity with increasing macrophyte biomass. Large-bodied cladocerans (e.g., daphnids and simocephalids) have high filtering capacity and can strongly reduce phytoplankton populations (Sterner 1989; Scheffer 1999). Other invertebrate groups (e.g., gastropods and ostracods) consume periphyton and detritus and, in doing so, may improve light conditions and help to stabilize macrophyte communities (Carpenter and Lodge 1986; Underwood 1991; Moss et al. 1996; Scheffer 1998).

Stress to shallow freshwater ecosystems often involves eutrophication. With eutrophication, nutrient loadings and mineralization rates are altered by increased external input and/or by internal sediment release. Macrophyte-dominated communities are robust to these changes over a relatively wide spectrum of disturbance regimes (Scheffer et al. 1993; Moss et al. 1996). Nevertheless, a threshold may be crossed beyond which a new set of feedback mechanisms favoring phytoplankton becomes prevalent. Loss of macrophyte biomass leaves invertebrates exposed to higher rates of fish predation, reducing population sizes. In turn, herbivory pressure is eased and phytoplankton populations can increase unchecked (Timms and Moss 1984; Schriver et al. 1995). Eventually a point may be reached at which the density of

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

	Winter 1			Summer 1			Winter 2			Summer 2		
	C	h	H	C	h	H	C	h	H	C	h	H
Mean	5.8	5.8	8.8	16.4	19.5	19.5	6.8	6.8	9.8	15.5	18.8	18.8
Maximum	12.0	12.0	14.9	23.7	26.8	26.8	13.8	14.0	16.4	21.0	24.8	24.8
Minimum	3.0	2.9	6.0	7.9	10.9	11.1	2.6	2.6	5.5	6.4	9.9	9.5

phytoplankton can create shade sufficient to inhibit macrophyte growth, accelerating decline of the latter (Irvine et al. 1989). In this state, chlorophyll *a* (Chl *a*), total phosphorus, and soluble reactive phosphorus concentrations are high (>100 μg L^{-1}), and available nitrogen (NO_3-N, NH_4-N) may exceed several milligrams per liter (van Donk et al. 1993; Moss et al. 1996). One particularly problematic aspect of turbid, phytoplankton-dominated conditions is the commonly increased occurrence of nuisance cyanophytes (Berger 1975; Schindler 1975).

How might warming affect these processes? Laboratory studies demonstrate that small increases in temperature influence the growth, development, and feeding rates of many individual aquatic organisms (e.g., Wooton et al. 1980; McKee and Ebert 1996; Santamaria and van Vierssen 1997). This influence is usually positive below normally occurring optima. On the other hand, in simple microcosm communities increased temperature may be destabilizing, reducing the period of oscillation of populations and altering trophic relationships (Petchey et al. 1999; Grover et al. 2000). Under field conditions, temperature increases associated with moderate thermal pollution (power station discharge) often result in changed macrophyte community composition, increased productivity, and accelerated life cycles (Haag and Gorham 1977; Svensson and Wigren-Svensson 1992; Taylor and Helwig 1995). Positive correlation between macrophyte biomass (and phytoplankton) and increased interannual temperature has been demonstrated by Rooney and Kalff (2000) in five

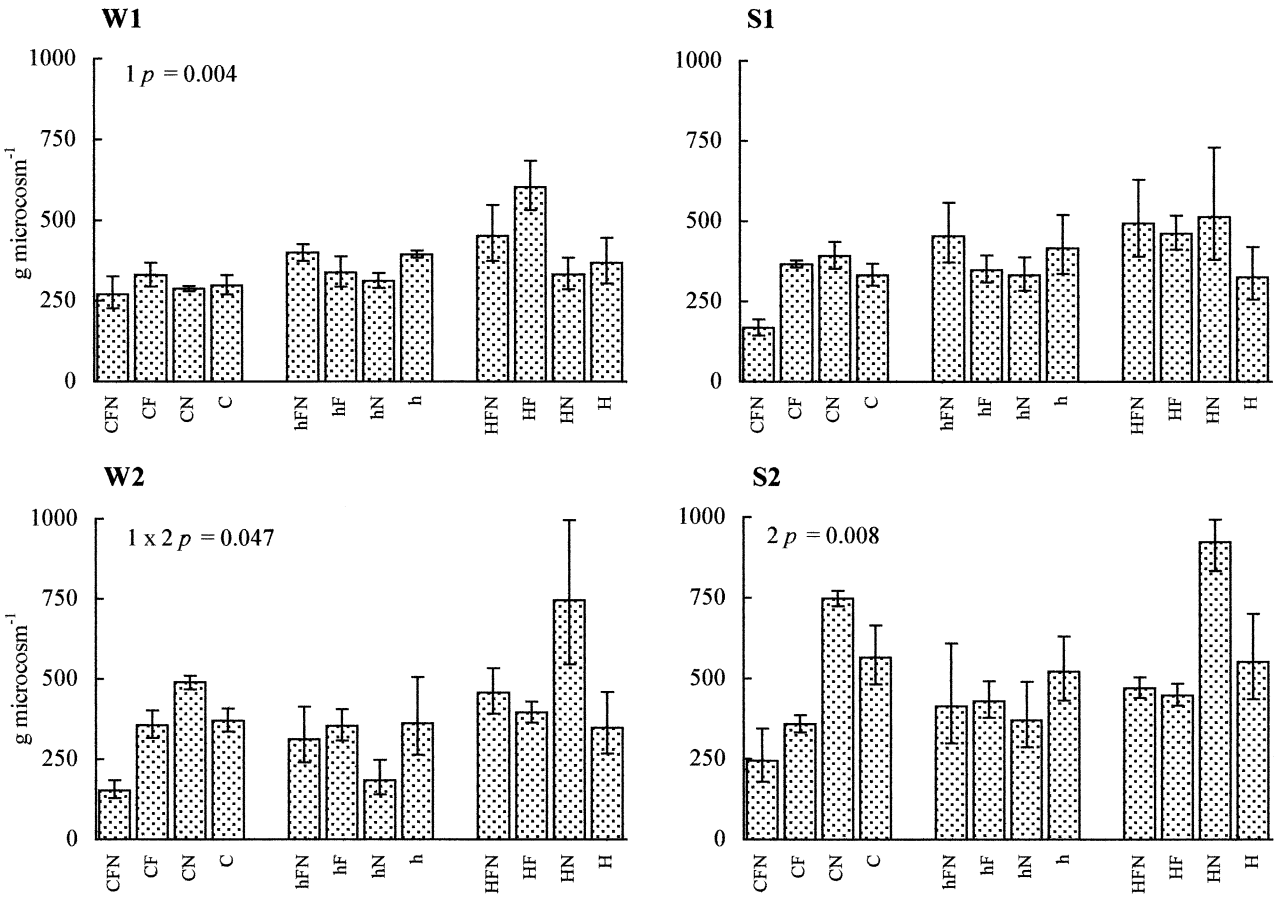

Fig. 1. Seasonal variation (W1, S1, W2, S2) of total macrophyte biomass (g dry weight per microcosm). Treatment codes are C = no warming, h = summer-only warming, H = continuous warming, F = fish present, N = additional nutrient input. *p* values indicate significant within-season ANOVA results (1 = warming treatment effect; 2 = fish treatment effect; 3 = nutrient treatment effect).

Table 2. *F* and *p* values from repeated-measures ANOVA of total macrophyte biomass, phytoplankton chlorophyll *a* concentrations, and total phytoplankton biovolume. Significant results are highlighted by asterisks.

	Macrophytes		Chlorophyll *a*		Biovolume	
Treatment effect	*F*	*p*	*F*	*p*	*F*	*p*
Warming (1)	2.5	0.099	0.7	0.525	0.2	0.799
Fish (2)	0.6	0.426	91.8***	<0.001	87.6***	<0.001
Nutrients (3)	1.0	0.316	74.1***	<0.001	48.4***	<0.001
Season (4)	9.0***	<0.001	112.0***	<0.001	93.0***	<0.001
1 × 2	2.9	0.066	2.0	0.147	2.8	0.073
1 × 3	1.0	0.387	2.5	0.098	2.5	0.096
2 × 3	1.4	0.243	18.1***	<0.001	31.9***	<0.001
1 × 4	2.6*	0.021	1.2	0.328	0.8	0.556
2 × 4	8.2***	<0.001	3.8*	0.012	1.9	0.128
3 × 4	0.1	0.952	5.5**	0.002	3.0*	0.032
1 × 2 × 3	2.5	0.098	2.8	0.072	1.1	0.329
1 × 2 × 4	2.6*	0.023	2.3*	0.041	0.2	0.964
1 × 3 × 4	3.0*	0.010	1.7	0.139	0.9	0.469
2 × 3 × 4	1.2	0.324	1.3	0.276	2.0	0.114
1 × 2 × 3 × 4	0.7	0.620	1.6	0.168	0.3	0.915

* $p<0.05$.
** $p<0.01$.
*** $p<0.001$.

Canadian lakes. Also in Canada, analysis of longer term records showed positive correlation between increased climatic temperatures, greater concentrations of lake chemical solutes, and increased phytoplankton standing crop (Schindler et al. 1990). McDonald et al. (1996) suggest that young of year trout would need to increase their food consumption by more than tenfold when faced with a 3°C rise in seasonal Alaskan temperatures. Similarly, models looking at the predatory behavior of young of year perch indicate that feeding rates on *Daphnia* are substantially increased during simulated warmer springs (Mehner 2000). In European lakes, links between temperature, the North Atlantic Oscillation, and plankton dynamics have been made (George and Hewitt 1999; Weyhenmeyer et al. 1999; Straile 2000). Here, an underlying pattern seems to be the promotion of earlier seasonal successional events by milder climatic conditions. For Heiligensee (Germany), a strong association between decline in trophic state and a succession of mild winters is apparent (Adrian et al. 1995). During 1988 to 1992, mean winter air temperatures lay well above the long-term mean, with concomitant increases in Chl *a*, cyanophyte prevalence, and hypolimnetic soluble reactive phosphorus concentrations.

The above studies contribute valuable information to ideas about possible responses of shallow freshwater ecosystems

Table 3. *F* and *p* values from repeated-measures ANOVA of gastropod, large-bodied cladoceran and ostracod densities. Significant results are highlighted by asterisks.

	Gastropods		Cladocerans		Ostracods	
Treatment effect	*F*	*p*	*F*	*p*	*F*	*p*
Warming (1)	6.8**	0.003	2.2	0.129	10.6***	<0.001
Fish (2)	15.9***	<0.001	407.7***	<0.001	86.1***	<0.001
Nutrients (3)	30.4***	<0.001	27.5***	<0.001	7.1*	0.011
Season (4)	2.7	0.050	29.1***	<0.001	28.6***	<0.001
1 × 2	0.9	0.402	1.8	0.176	1.6	0.224
1 × 3	2.2	0.123	0.6	0.538	2.7	0.078
2 × 3	2.9	0.099	17.0***	<0.001	10.1**	0.003
1 × 4	2.5*	0.025	1.6	0.158	2.3*	0.042
2 × 4	2.3	0.087	33.6***	<0.001	12.8***	<0.001
3 × 4	3.1*	0.031	3.4*	0.020	1.7	0.172
1 × 2 × 3	0.6	0.543	0.7	0.518	1.2	0.311
1 × 2 × 4	0.7	0.629	0.7	0.618	1.0	0.433
1 × 3 × 4	1.5	0.196	0.5	0.829	0.9	0.530
2 × 3 × 4	1.3	0.274	3.4*	0.021	1.5	0.223
1 × 2 × 3 × 4	0.9	0.528	0.4	0.871	1.1	0.387

* $p<0.05$.
** $p<0.01$.
*** $p<0.001$.

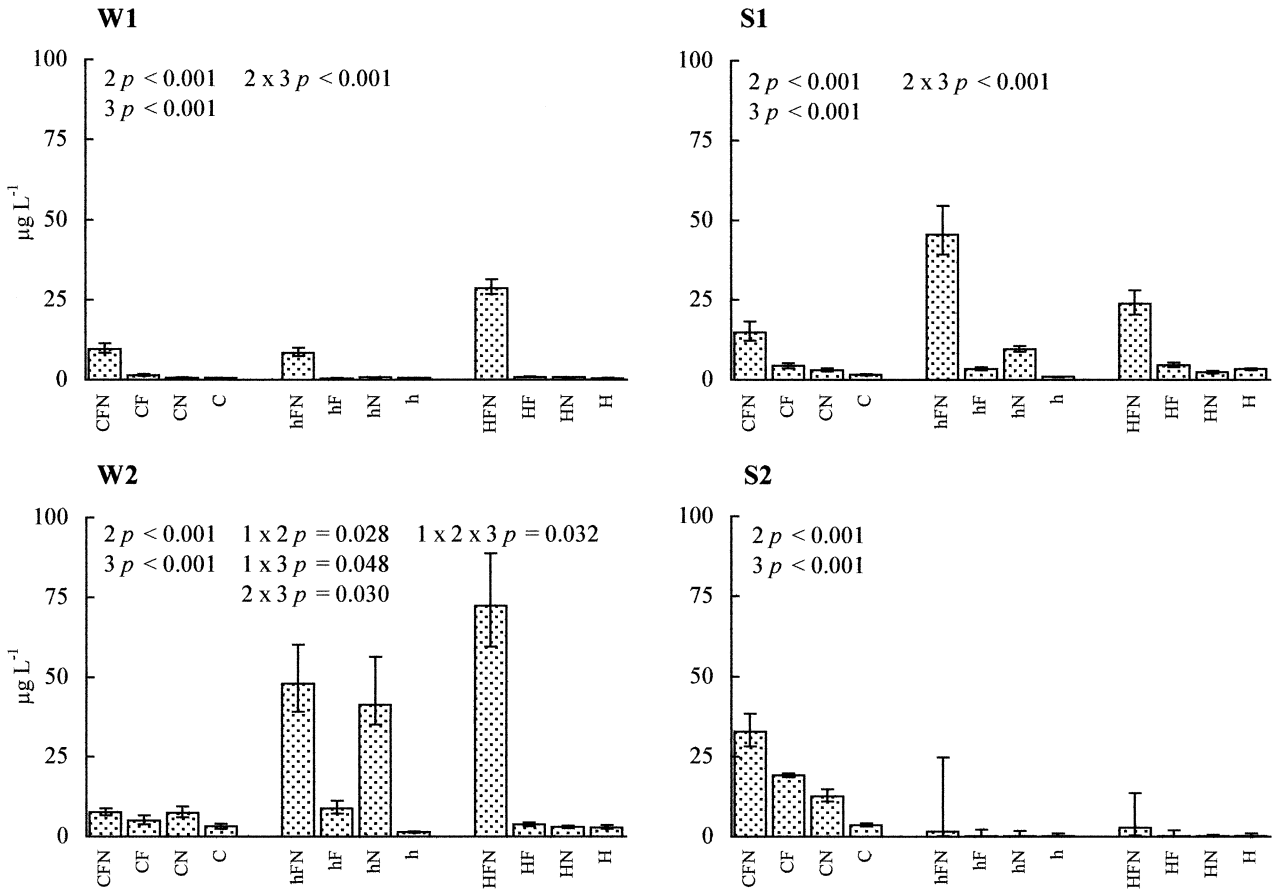

Fig. 2. Seasonal variation (W1, S1, W2, S2) of phytoplankton Chl *a* concentrations (μg L^{-1}). Treatment codes as in Fig. 1.

to warming. However, extrapolation of the behavior of isolated, individual species may not always be applicable to consideration of community and ecosystem dynamics (e.g., Davis et al. 1998). Furthermore, extrapolation of observations made on deep lake systems (the majority of studies) may not be fully applicable to consideration of shallow systems (e.g., Scheffer 1998; Rooney and Kalff 2000; Scheffer et al. 2001). There therefore remains a need for investigation of warming effects on shallow freshwaters, especially by the experimental approach and especially to test resilience of macrophyte-dominated systems to increased temperature.

To assess warming influences on shallow freshwater ecosystems, we created 48 microcosms, with sediments, plants, plankton, and invertebrates. The microcosms mimicked small ponds but were also representative of areas of shallow lakes, the littoral zone of deep lakes, and other marginal habitats. Climate change predictions for northern latitudes suggest an average temperature increase of around 3°C over the course of this century (Houghton et al. 1996; Conway 1998; Parry 2000; McCarthy et al. 2001). To keep our experiment in line with the final outcome of these predictions, we subjected the microcosms to three temperature treatments: no warming, continuous warming to 3°C above ambient, and warming to 3°C above ambient during summer only. The temperature treatments were combined with two nutrient treatments (addition or not of nitrate and phosphate salts) and two fish treatments (presence or absence of sticklebacks, *Gasterosteus aculeatus*). There were thus 12 treatment levels, each with four replicates. For 2 yr we followed the dynamics of our microcosms, focusing on the functional components anticipated as indicators of ecosystem change.

Methods

The microcosms were 3,000-liter fiberglass tanks (diameter 2 m, depth 1 m), sunk into the ground for insulation, at a field site near Liverpool, U.K. (3°03′W, 53°16′N). Each tank was given a layer of sediment (5-cm depth, 7 : 1 sand : loam), installed with a heating element, and filled with borehole water (pH 7.3, total alkalinity 3.4 milliequivalents L^{-1}, Mg^{2+} 38 mg L^{-1}, Ca^{2+} 103 mg L^{-1}). Plankton was then introduced using an inoculum from a local canal. Macrophytes (*Lagarosiphon major* Ridl. Moss, *Elodea nuttallii* Planch. H. St. John, *Potamogeton natans* L.) were locally collected and planted during spring 1998, using 400 g wet weight per species per microcosm. For each species, the stock was divided into three weighted net bags, which were randomly positioned in the bottom of each microcosm. Gastropods were not deliberately introduced but arrived incidentally with the plants. Communities were regularly cross-mixed and allowed to develop over the summer of 1998, before the start of experimental treatments.

The microcosms were warmed by pumping hot water from a boiler through to the heating elements in the bottom

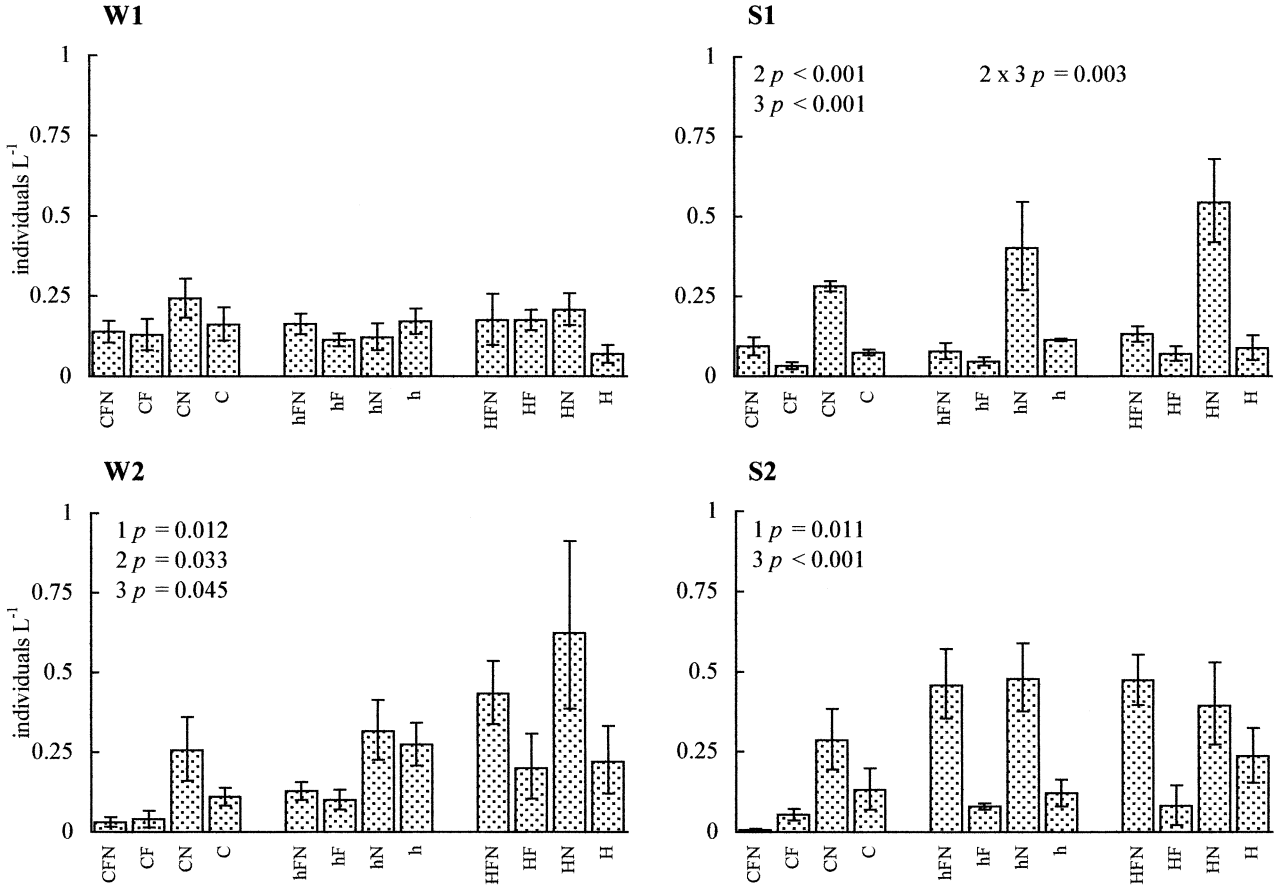

Fig. 3. Seasonal variation (W1, S1, W2, S2) of gastropod densities (individuals L^{-1}). Treatment codes as in Fig. 1.

of the relevant tanks. Full details of the technical specifications, the site layout, and the operating characteristics of the warming system are given by McKee et al. (2000). Continuous warming began in September 1998 and lasted uninterrupted until September 2000, defining a 2-yr study period. Summer-only warming was from the beginning of April to the end of September each year, defining two summer seasons (S1, S2) and two winter seasons (W1, W2). Stickleback populations were established after initial introduction of 21 individuals per relevant microcosm. Breeding was confirmed from visual observations and trapping (final census mean biomass per microcosm = 27.0 ± 18.2 g m^{-3}). The nutrient treatment followed a 3-weekly cycle during the winter and a 2-weekly cycle during the summer. Sodium salts were presented in solution to give added concentrations of 500 μg N L^{-1} and 50 μg P L^{-1} during winter and 170 μg N L^{-1} and 17 μg P L^{-1} during summer, representing a typical situation for natural ponds and lakes. The following codes identify the treatment combinations: C = no warming, h = summer-only warming, H = continuous warming, F = fish present, N = additional nutrient input.

Water chemistry and zooplankton sampling followed the same frequency as the nutrient addition cycle (seasonal sample sizes: W1 = 9, S1 = 12, W2 = 8, S2 = 12). On Monday mornings, prior to nutrient addition, a liter of water retained from a mixed column drawn from each microcosm was taken to the lab for immediate chemical analysis (total phosphorus, soluble reactive phosphorus, nitrate, ammonium, total alkalinity, methodology in Mackereth et al. 1978; pH meter readings; acetone extraction of Chl *a*; 50-ml subsample unfiltered water preserved with Lugol's solution for phytoplankton identification and counts). Oxygen and conductivity readings were taken directly in the microcosms using a meter (sample sizes: conductivity, W1 = 9, S1 = 12, W2 = 8, S2 = 12; oxygen, first year data unavailable, W2 = 8, S2 = 12). The following Thursday, zooplankton were sampled by using a tube to remove three vertical columns of water from each microcosm. The water was mixed in a bucket and then filtered through a 50-μm nylon mesh screen. All animals thus retained were narcotized with chloroform, preserved in 70% alcohol, identified, and counted. Gastropods were sampled live every 9 weeks using standard sweeps of a pond net (sample sizes: W1 = 3, S1 = 3, W2 = 3, S2 = 3). All individuals were identified, counted, and then returned to their respective microcosms.

Macrophyte biomass was assessed by two independent observers every 3 weeks beginning January 1999 (sample sizes: W1 = 4, S1 = 8, W2 = 8, S2 = 8). The percentage volume infested (PVI) by each macrophyte species in each microcosm was estimated by recording the two-dimensional area covered by the macrophytes (with the aid of a quadrat) and then multiplying by the mean depth of the macrophyte mass (measured with a graduated rod). Harvest of all macrophyte material at the end of the experiment allowed the

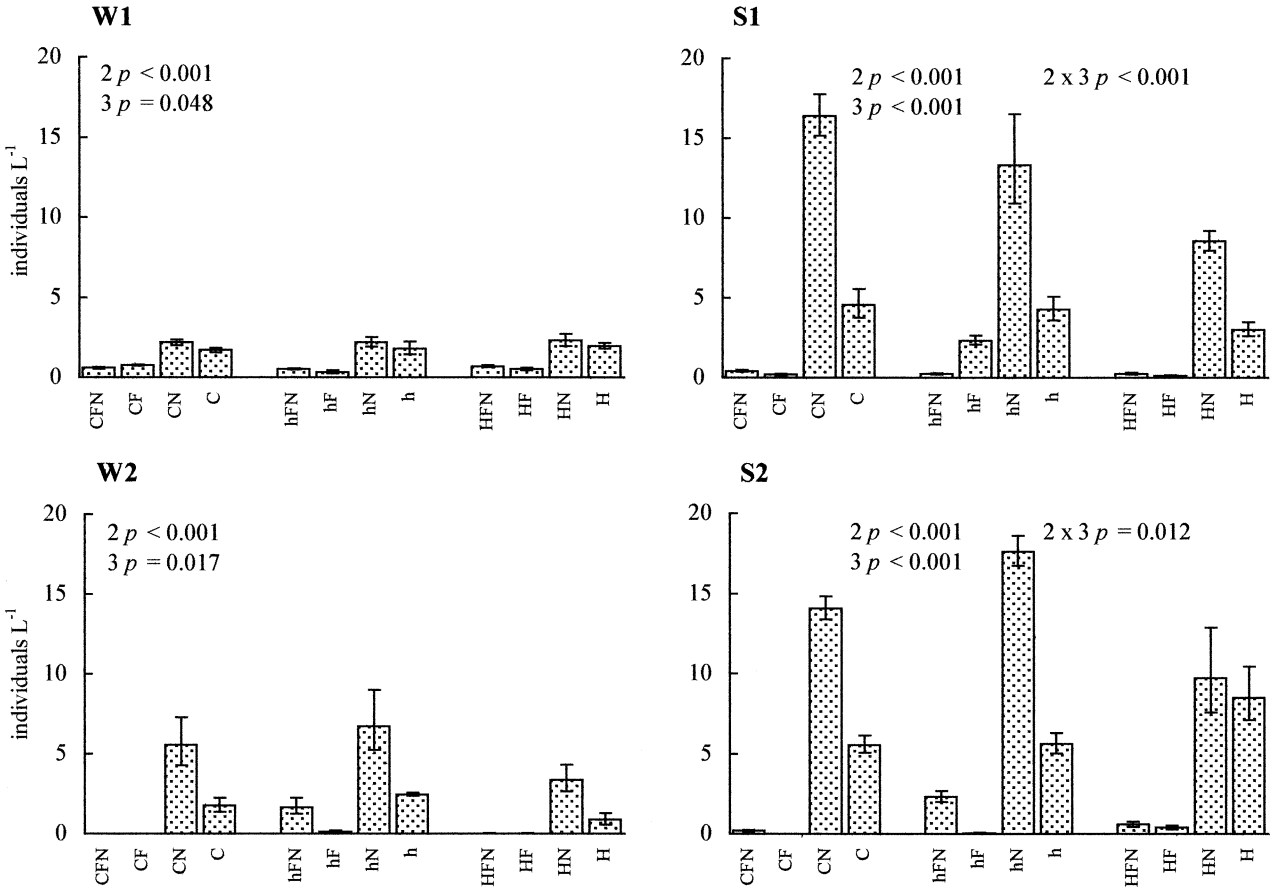

Fig. 4. Seasonal variation (W1, S1, W2, S2) of large-bodied cladoceran densities (individuals L^{-1}). Treatment codes as in Fig. 1.

demonstration of significant positive correlation between PVI estimates and grams dry weight per microcosm: *L. major*, $r = 0.70$, $n = 45$; *E. nuttallii*, $r = 0.77$, $n = 48$; *P. natans* (including roots), $r = 0.93$, $n = 25$; $p < 0.001$ for all correlations. Regression equations derived from these relationships were used to convert PVI estimates to estimate of total macrophyte dry weight on each sampling occasion.

For analysis, mean values for each response variable in each microcosm were calculated according to season (W1, S1, W2, S2). These values were then transformed (log-transformation in all cases except pH data, which was antilogged before manipulation, and oxygen saturation data, which was left untransformed) and submitted to a repeated-measures analysis of variance (ANOVA) model where season was the four-level repeated-measures variable and the treatments warming, fish, and nutrients were the three main factors. This procedure was used to explore major trends in our response variables over the entire course of the experiment. Four separate within-season ordinary analyses of variance for each response variable were then conducted. For post hoc comparison of warming treatment-level means we used Tukey honest significant difference tests (here, codes for each comparison are: C-h, no warming compared with summer-only warming; C-H, no warming compared with continuous warming; h-H, summer-only warming compared with continuous warming). Figures report seasonal treatment-level means calculated according to season, bounded by ± 1 SE back-calculated from repeated-measures ANOVA. They also show the p values of all significant treatment effects elucidated by within-season ANOVA.

Results

Warming—Warming was accurate and reliable. Warmed microcosms deviated from 3°C above ambient within narrow margins: daily mean positive deviation 0.2°C (SD = 0.2, $n = 697$); daily mean negative deviation 0.1°C (SD = 0.2, $n = 697$). The mean, maximum, and minimum seasonal water temperatures were typical of shallow bodies of standing freshwater at this latitude (Table 1).

Macrophytes—Over the course of the experiment *L. major* was lost in three of the microcosms and *P. natans* in 23 of the microcosms. The particularly patchy distribution of the latter could not be attributed to the treatments at any level of design. On the other hand, total macrophyte biomass remained relatively stable during W1, S1, and W2 and then increased overall during S2 (Fig. 1; significant ANOVA season effect, Table 2). It was marginally affected through interaction between warming and the fish treatment and through interaction between warming and the nutrient treatment (Table 2). In the presence of fish, independent of the nutrient treatment, biomass was increased in the H microcosms relative to the C microcosms during W1, S1, and W2

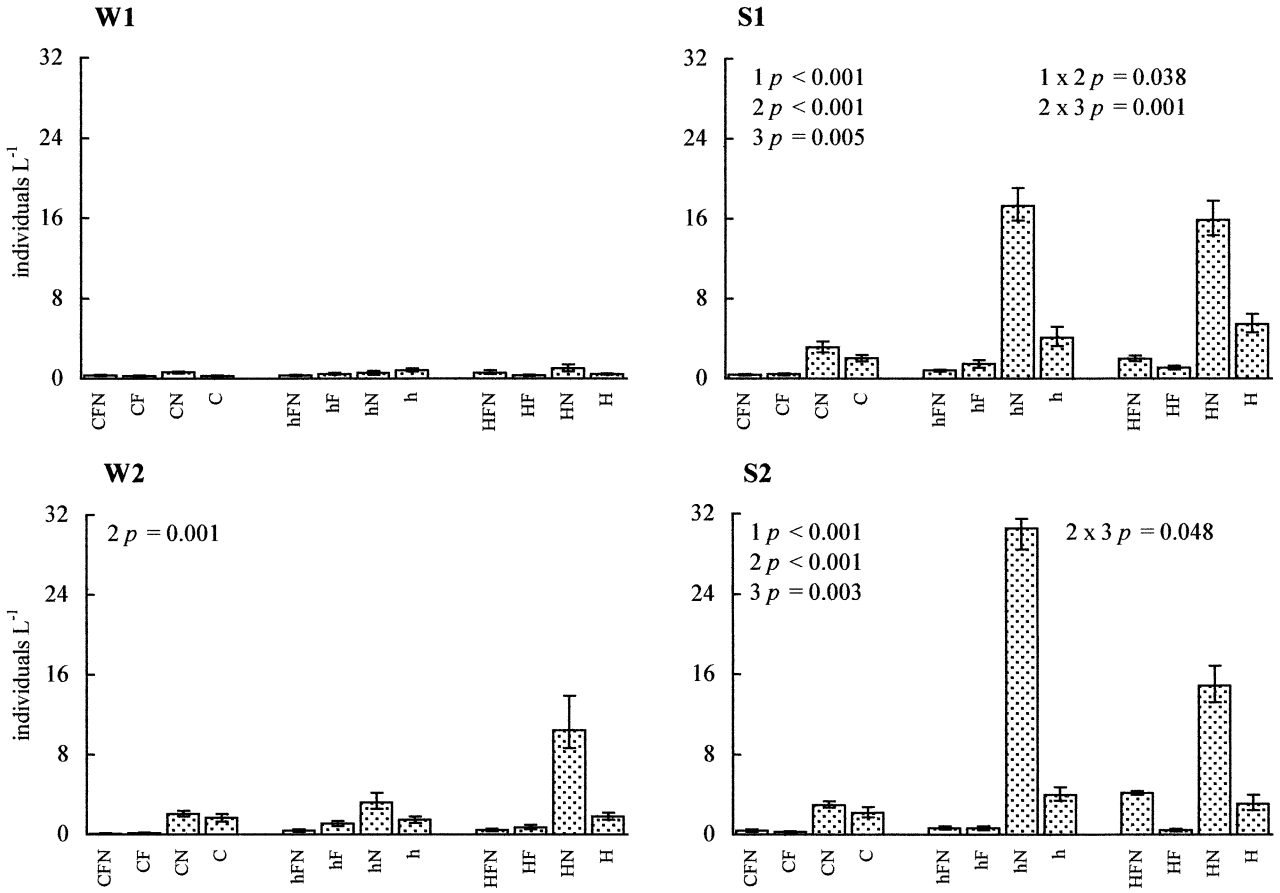

Fig. 5. Seasonal variation (W1, S1, W2, S2) of ostracod densities (individuals L^{-1}). Treatment codes as in Fig. 1.

Table 4. *F* and *p* values from repeated-measures ANOVA of total phosphorus concentrations (Tp), soluble reactive phosphorus concentrations (Srp), nitrate concentrations (NO_3), and ammonium concentrations (NH_4). Significant results are highlighted by asterisks.

	Tp		Srp		NO_3		NH_4	
Treatment effect	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Warming (1)	4.5*	0.018	6.4**	0.004	0.2	0.785	0.4	0.644
Fish (2)	3.3	0.077	46.6***	<0.001	2.2	0.145	0.001	0.974
Nutrients (3)	99.3***	<0.001	136.8***	<0.001	0.5	0.497	12.1***	0.001
Season (4)	74.5***	<0.001	152.3***	<0.001	2,378.6***	<0.001	10.1***	<0.001
1 × 2	1.4	0.271	1.0	0.379	1.9	0.168	2.0	0.144
1 × 3	2.9	0.069	2.9	0.068	0.6	0.535	0.8	0.437
2 × 3	0.9	0.354	19.4***	<0.001	0.001	0.981	0.1	0.782
1 × 4	2.4*	0.032	1.6	0.146	0.4	0.900	0.7	0.685
2 × 4	2.2	0.092	4.8**	0.003	1.9	0.139	0.7	0.561
3 × 4	14.2***	<0.001	5.5**	0.002	0.3	0.842	0.9	0.447
1 × 2 × 3	0.2	0.817	2.5	0.094	3.2	0.052	2.9	0.069
1 × 2 × 4	1.1	0.383	0.3	0.917	1.5	0.175	2.0	0.075
1 × 3 × 4	1.9	0.085	0.9	0.494	0.7	0.686	0.3	0.951
2 × 3 × 4	0.3	0.847	4.9**	0.003	0.02	0.997	0.2	0.863
1 × 2 × 3 × 4	1.6	0.147	0.7	0.653	4.7***	<0.001	1.1	0.348

* $p<0.05$.
** $p<0.01$.
*** $p<0.001$.

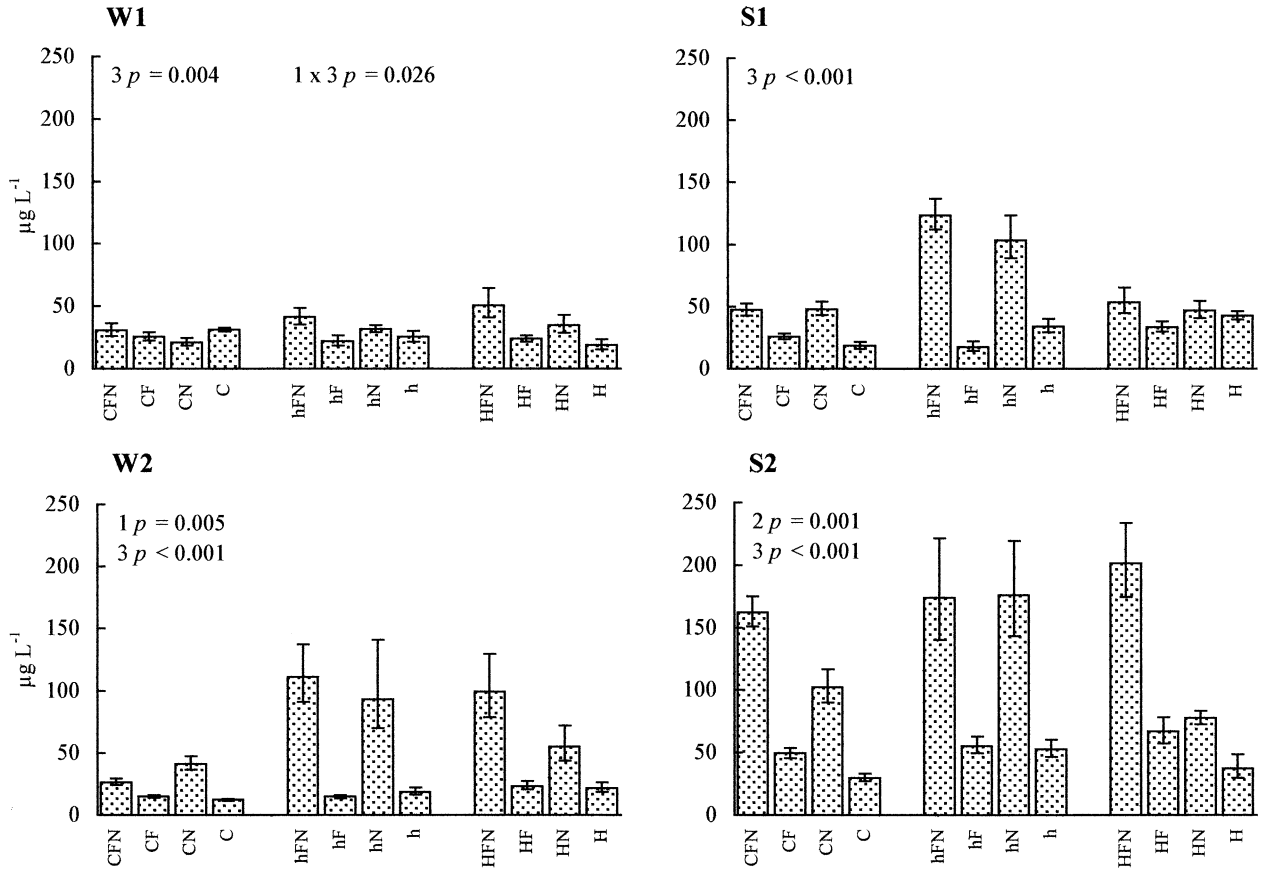

Fig. 6. Seasonal variation (W1, S1, W2, S2) of total phosphorus concentrations (μg L^{-1}). Treatment codes as in Fig. 1.

(compare HFN and HF means with CFN and CF means in Fig. 1). Under nutrient addition, independent of the fish treatment, biomass also showed some trend for increase in the H microcosms. When within-seasons analyses were performed, significant warming treatment effects largely disappeared (Fig. 1).

Phytoplankton—Phytoplankton Chl *a* concentrations were generally low (overall mean per microcosm = 14 μg L^{-1}, SD = 25, n = 192). Throughout the course of the experiment they varied significantly with nutrient addition and the presence of fish (Table 2), showing a relatively consistent trend across seasons for increase under nutrient addition, particularly in the presence of fish (Fig. 2). A significant warming × fish treatment × season interaction term in the repeated-measures ANOVA model suggested marginally significant variation overall in chlorophyll concentrations dependent on warming (Table 2). This is likely to have been mainly due to events during W2, when chlorophyll concentrations in the continuously warmed microcosms containing fish were unusually high (Fig. 2). Within-seasons analyses yielded significant warming treatment effects only during W2 (Fig. 2).

Small cryptophytes and green algae (e.g., *Cryptomonas erosa, Micractinium pusillum, Monoraphidium contortum, Rhodomonas minuta*) were prevalent in the phytoplankton communities, both in terms of number of individuals and in terms of biovolume. Cyanophytes (e.g., *Anabaena, Chroococcus, Merismopedia, Oscillatoria, Phormidium*) were a minor component (mean percentage of total algal biovolume across all treatments and seasons 0.3%, SD = 1.3, n = 192). Total algal biovolume (Table 2) and total cyanophyte biovolume varied significantly with the fish and nutrient treatments in a similar manner to the patterns displayed by Chl *a*. Neither was significantly affected by warming.

Invertebrates—Gastropods were *Bithynia tentaculata* L., *Lymnaea peregra* Müll., *Lymnaea stagnalis* L., *Physa fontinalis* L., *Planorbis* sp., and *Potamopyrgus jenkinsi* Smith. Of these, *P. fontinalis* was predominant. Total densities were significantly affected by all treatments (Table 3). Overall, they tended to be reduced in the presence of fish and increased under nutrient addition (Fig. 3). After W1, densities were increased overall in the H microcosms and intermediate in the h microcosms, although within-seasons analyses yielded significant warming treatment effects only for the second year of the experiment (Fig. 3). Tukey tests comparing the warming treatment-level means showed that, during W2, gastropod densities under continuous warming were significantly higher than under no warming (H-C, p = 0.009) and that, during S2, densities under both warming treatments

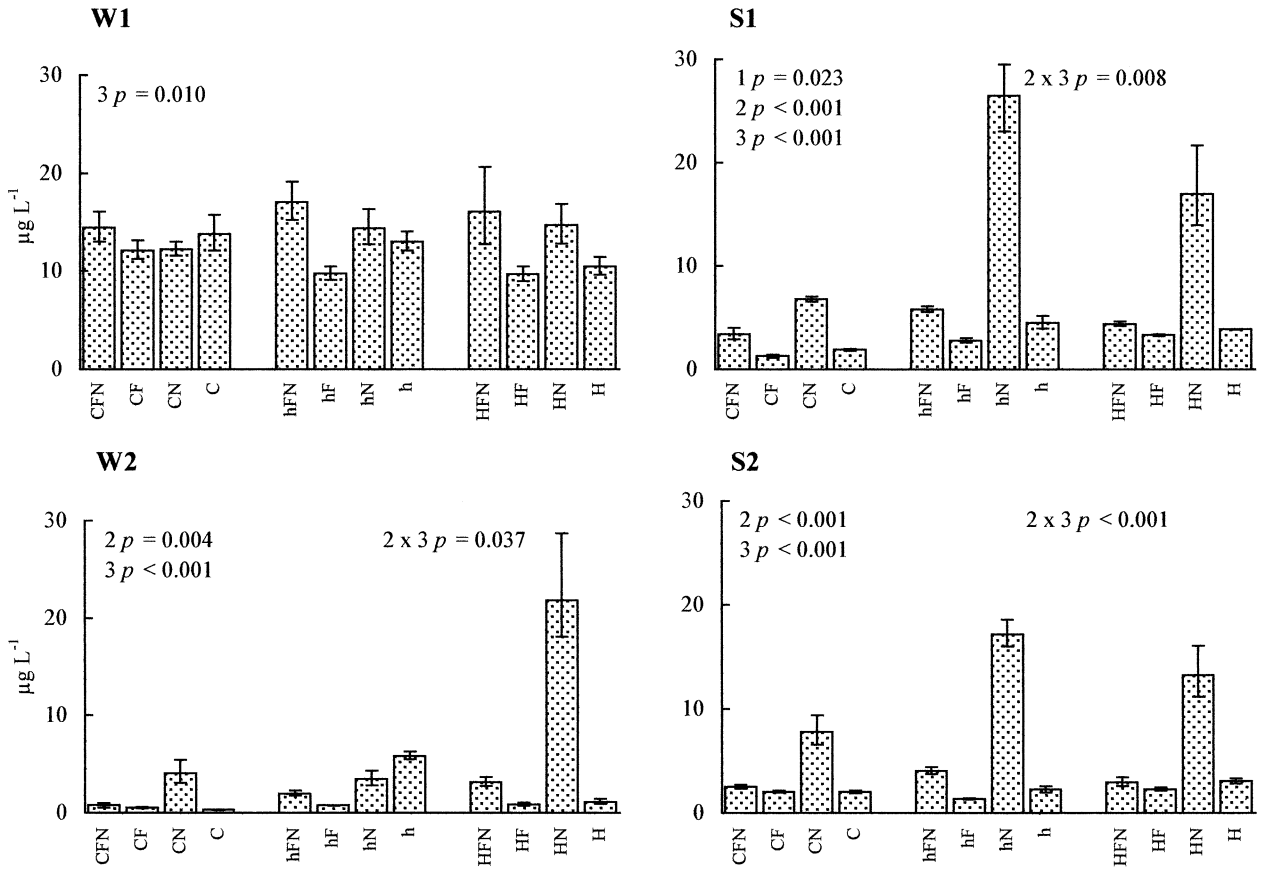

Fig. 7. Seasonal variation (W1, S1, W2, S2) of soluble reactive phosphorus concentrations (μg L^{-1}). Treatment codes as in Fig. 1.

were higher than under no warming (h-C, $p = 0.039$; H-C, $p = 0.015$).

Three species of large-bodied cladocerans, *Daphnia longispina* Müll., *Daphnia magna* Straus, and *Simocephalus vetulus* Müll., were recorded. Total densities of these animals were not significantly affected by warming (Table 3), but were always greatly reduced in the presence of fish (Fig. 4). In the absence of fish, densities tended to be increased by nutrient addition (Fig. 4). In a similar manner, ostracod densities (predominantly *Cypridopsis vidua* Müll. and *Herpetocypris chevreuxi* Sars) were reduced in the presence of fish and, in the absence of fish, showed a tendency to be increased by nutrient addition (Fig. 5). Unlike for cladocerans, however, warming also affected ostracod densities (Table 3). They were increased overall in the h and H microcosms (Fig. 5) although, within seasons, this trend was only statistically significant during the summers (Tukey tests comparing warming treatment-level means: S1, C-h, $p < 0.001$; C-H, $p < 0.001$; S2, C-H, $p = 0.023$).

Densities of all three invertebrate groups correlated positively with total macrophyte biomass: gastropods, $r = 0.22$, $p = 0.002$, $n = 192$; large cladocerans, $r = 0.16$, $p = 0.030$, $n = 192$; ostracods, $r = 0.24$, $p = 0.001$, $n = 192$.

Water chemistry—Total phosphorus concentrations varied significantly with warming and with nutrient addition (Table 4). The trends were for overall increase under both warming regimes and increase under nutrient addition (Fig. 6). The nutrient treatment effect was always significant within seasons. However, within-season differences between warming treatment-level means were only significant during W2 (Tukey tests: C-h, $p = 0.016$; C-H, $p = 0.011$; h-H, $p = 0.988$).

Soluble reactive phosphorus concentrations varied significantly with warming, the fish treatment, and the nutrient addition treatment (Table 4). The trends as shown by repeated-measures ANOVA were for overall increase under both warming regimes after W1 and increase under nutrient addition, particularly in the absence of fish (Fig. 7). However, within-season comparisons of warming treatment-level means indicated only one instance of significant difference (Tukey test: S1, C-h, $p = 0.017$). The nutrient addition $\times$ absence of fish effect was significant within all seasons after W1.

Nitrate concentrations declined throughout the experiment (Fig. 8). This, in combination with the fact that during W1 concentrations in the HF microcosms were unusually low, caused a significant four-way interaction term in the repeated-measures ANOVA model (Table 4). However, Tukey tests indicated no significant differences between pairs of W1 treatment-level means. After W1, concentrations remained unaffected by the treatments at any level of design. Ammonium concentrations were rather increased in the microcosms receiving additional nutrient inputs (Fig. 9) but were not significantly affected by the other treatments (Table 4).

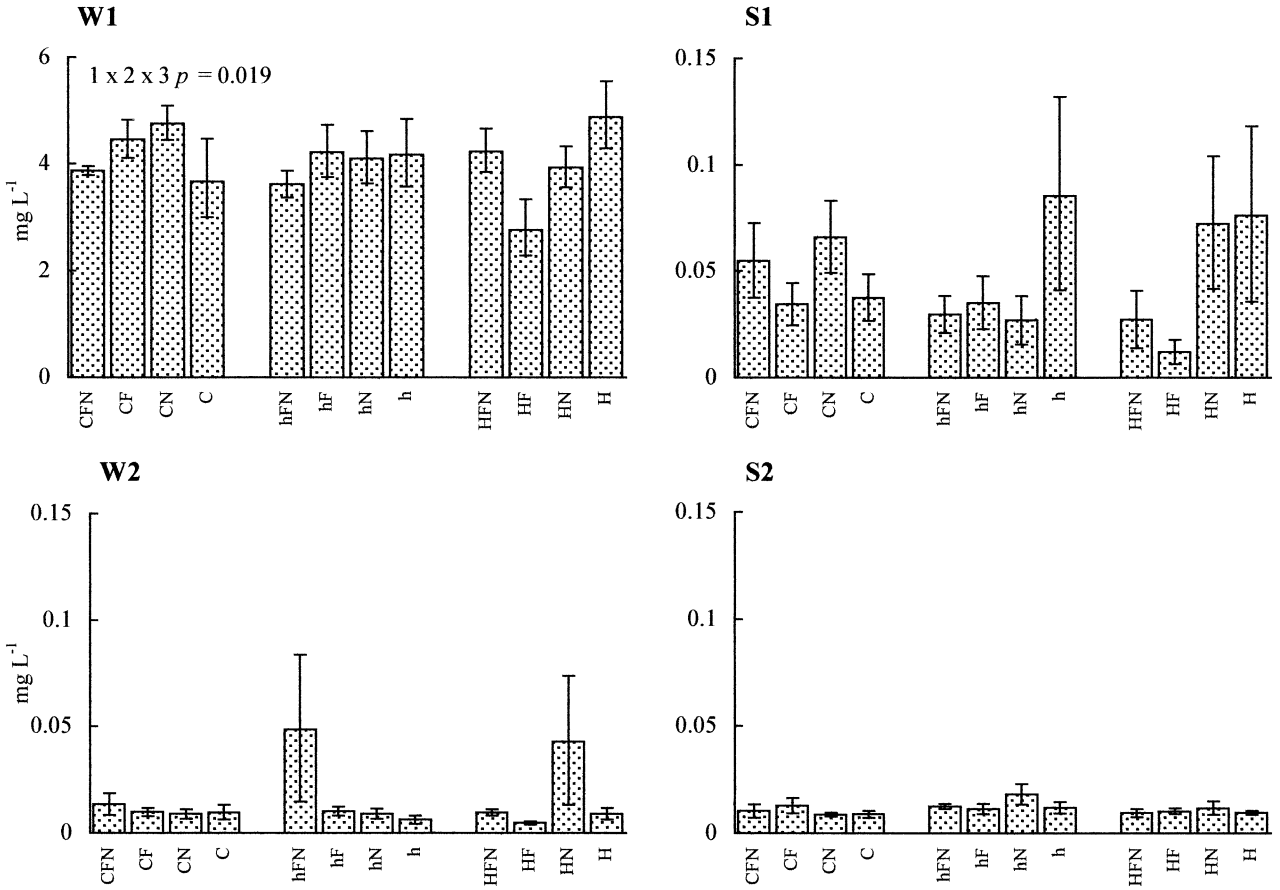

Fig. 8. Seasonal variation (W1, S1, W2, S2) of nitrate concentrations (mg L^{-1}). Note that *y*-axis scales are not the same in each panel. Treatment codes as in Fig. 1.

The pH of all microcosms was relatively high, although there was some tendency for decline throughout the experiment. Warming affected pH (Table 5), producing a trend for lowered levels (Fig. 10), although within-season treatment means were only significantly different during S1 (Tukey tests: C-h, $p = 0.061$; C-H, $p = 0.050$). There was a consistent trend for higher pH under additional nutrient input and a marginal trend for higher pH in the presence of fish (Fig. 10). In all microcosms conductivity decreased throughout the experiment and total alkalinity increased. Warming significantly affected both variables (Table 5). For conductivity, levels were highest overall in the H microcosms and intermediate in the h microcosms (Fig. 11). During the summer, levels of total alkalinity were similar and highest in the h and H microcosms; during the winter levels were similar in the h and C microcosms and higher in the H microcosms (Fig. 12). Within seasons, both these sets of trends were consistently significant after W1 (Tukey tests comparing warming treatment-level means: conductivity, W1, C-h, $p = 0.284$; C-H and h-H, $p < 0.001$; S1 all comparisons $p < 0.001$; W2, C-h, $p = 0.018$, C-H and h-H, $p < 0.001$; S2 all comparisons $p < 0.001$; total alkalinity, S1, C-h and C-H, $p < 0.023$, h-H, $p = 0.992$; W2, C-h, $p = 0.762$, C-H and h-H $p < 0.013$; S2, C-h and C-H, $p < 0.034$, h-H, $p = 0.967$). Under additional nutrient input both variables showed trends for increase (Figs. 11, 12). The fish treatment produced inconsistent trends. Conductivity showed some overall tendency for increase in the absence of fish during S1 (Fig. 11); total alkalinity was increased overall in the absence of fish during S1 and increased overall in the presence of fish during S2 (Fig. 12).

Oxygen saturation was significantly affected by warming and by nutrient addition (Table 5). The overall trend was for consistent decrease by continuous warming and, during S2, disproportionate decrease by summer-only warming (Fig. 13). Nutrient addition lowered oxygen saturation, but this effect was only pronounced during the summer. Although within-season analyses indicated marginal interaction between the effects of warming and the other two treatments, Tukey tests picked out only one significant difference between these treatment-level means (oxygen saturation levels in the hFN microcosms were significantly lower than in the hN microcosms during W2, $p = 0.008$) and only one instance of significant difference between warming treatment-level means (S2, C-h, $p = 0.024$). A number of microcosms underwent brief periods of severe deoxygenation (<1 mg L^{-1} O_2) during S2. This was characterized by partial macroinvertebrate kills and, in some instances, the growth of purple sulfur bacteria. However, the effect appeared transitory and all microcosms recovered. Although no significant

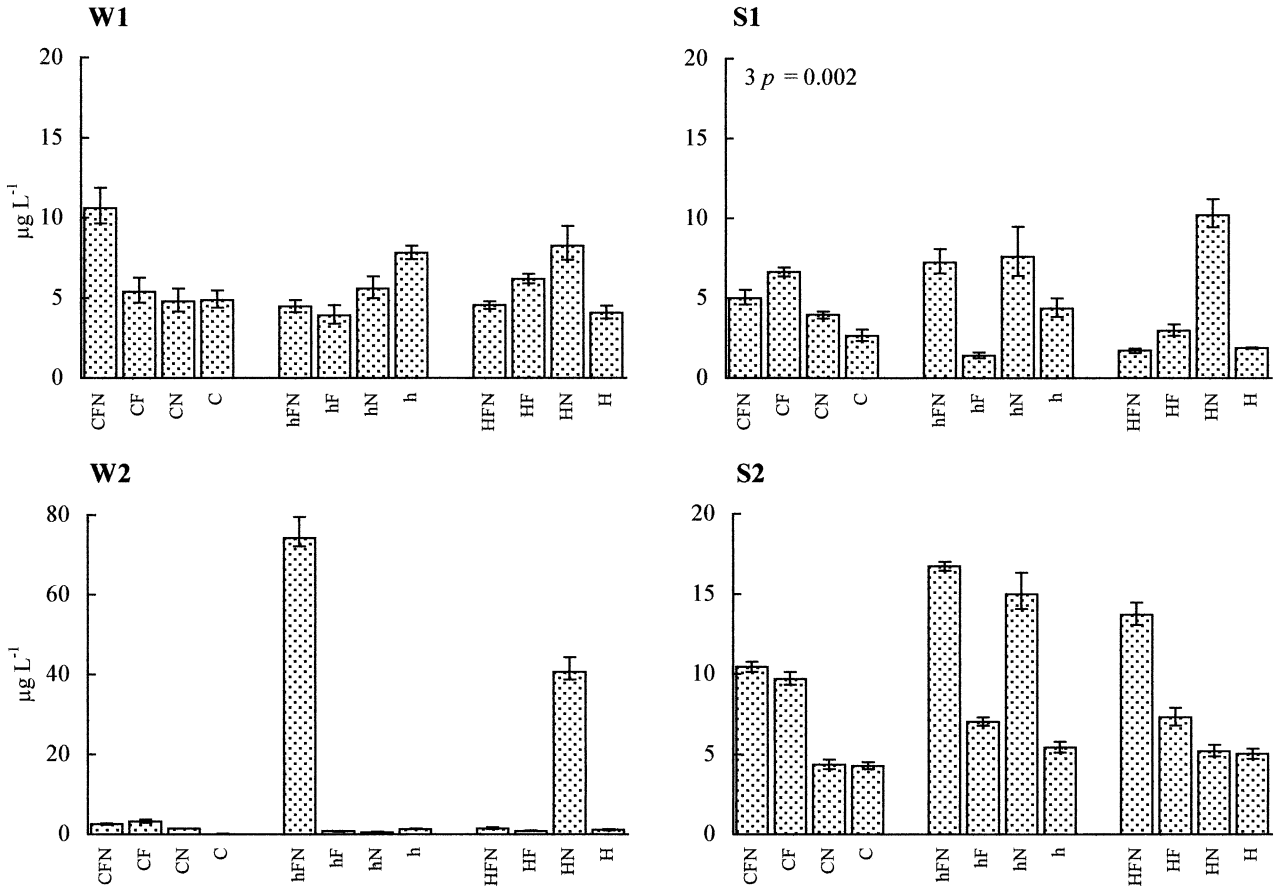

Fig. 9. Seasonal variation (W1, S1, W2, S2) of ammonium concentrations (μg L^{-1}). Note that *y*-axis scales are not the same in each panel. Treatment codes as in Fig. 1.

Table 5. *F* and *p* values from repeated-measures ANOVA of pH, conductivity, total alkalinity, and oxygen saturation (O_2). Significant results are highlighted by asterisks.

	pH		Conductivity		Alkalinity		O_2	
Treatment effect	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Warming (1)	4.6*	0.017	146.2***	<0.001	8.8***	<0.001	3.6*	0.036
Fish (2)	0.05	0.833	0.2	0.635	0.1	0.749	0.8	0.388
Nutrients (3)	59.6***	<0.001	21.4***	<0.001	56.4***	<0.001	9.0**	0.005
Season (4)	212.5***	<0.001	217.8***	<0.001	259.1***	<0.001	1.1	0.299
1 × 2	1.1	0.332	0.05	0.955	0.08	0.927	0.4	0.680
1 × 3	0.7	0.526	2.8	0.072	1.0	0.380	0.3	0.744
2 × 3	2.4	0.129	7.2*	0.011	2.5	0.121	3.2	0.082
1 × 4	1.1	0.386	18.1***	<0.001	3.9**	0.002	3.5*	0.040
2 × 4	3.4*	0.019	4.5**	0.005	7.1***	<0.001	0.2	0.632
3 × 4	9.1***	<0.001	2.6	0.059	8.5***	<0.001	10.5**	0.003
1 × 2 × 3	0.2	0.842	1.2	0.320	0.5	0.585	1.9	0.165
1 × 2 × 4	1.6	0.153	0.6	0.758	0.4	0.881	1.4	0.253
1 × 3 × 4	1.7	0.136	1.6	0.146	2.1	0.058	0.9	0.435
2 × 3 × 4	2.7*	0.047	2.6	0.056	1.1	0.355	0.01	0.907
1 × 2 × 3 × 4	1.3	0.273	0.5	0.792	0.4	0.900	0.02	0.976

* $p<0.05$.
** $p<0.01$.
*** $p<0.001$.

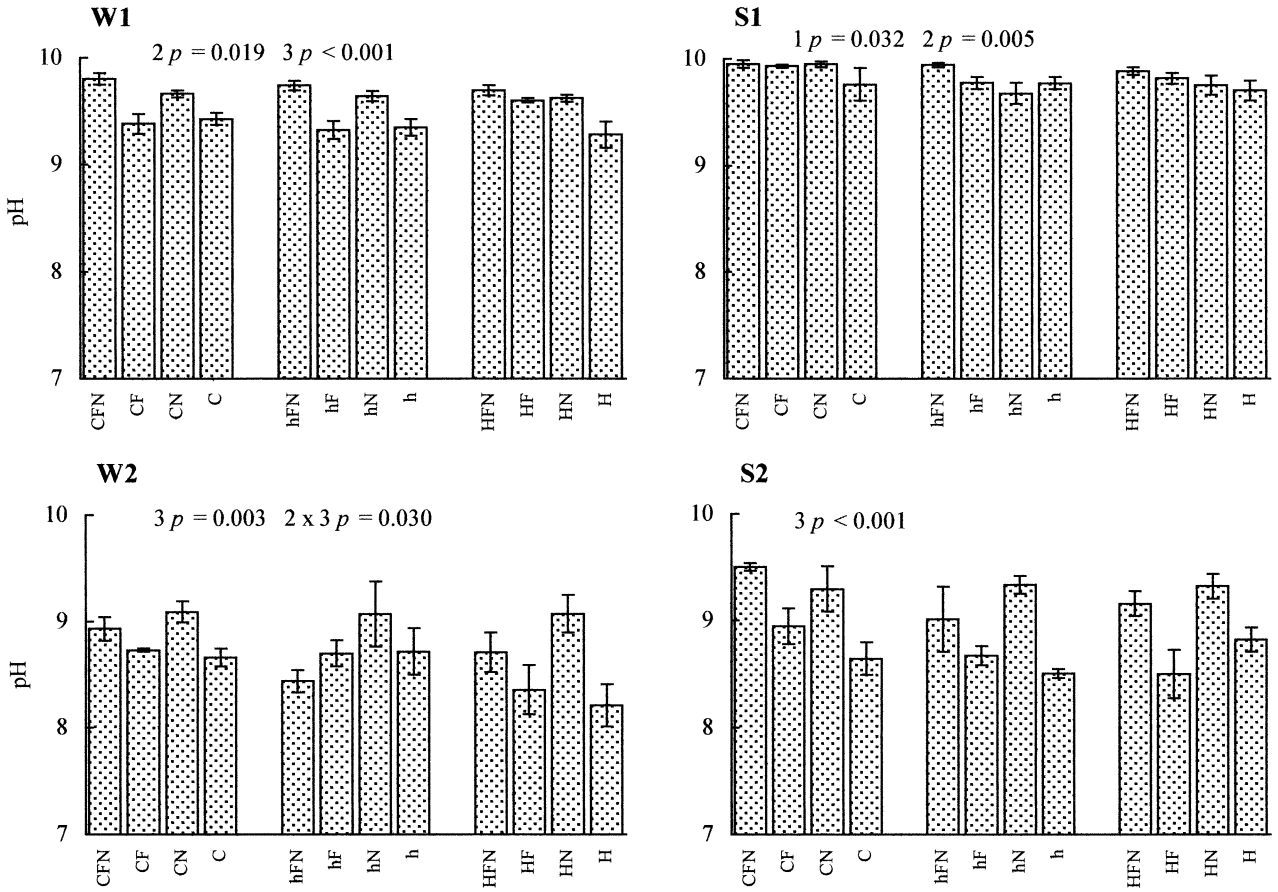

Fig. 10. Seasonal variation (W1, S1, W2, S2) of pH. Treatment codes as in Fig. 1.

interaction between warming and nutrient treatment effects was apparent during S2 (Table 5, Fig. 13), these deoxygenation episodes occurred only under nutrient addition and were most frequent in combination with summer-only warming. Six summer-only warmed microcosms, three continuously warmed microcosms and three no-warming microcosms, underwent deoxygenation (mean length of deoxygenation 4.3 weeks per microcosm, SD = 2.1, n = 12). Oxygen saturation correlated negatively with both total phosphorus concentration ($r = -0.56$, $p < 0.001$, $n = 96$) and soluble reactive phosphorus concentration ($r = -0.26$, $p < 0.05$, $n = 96$).

Discussion

The 3°C increase in temperature did not cause an ecosystem state change from macrophyte-dominated clear water (the pristine condition) to phytoplankton-dominated turbid water (the degraded condition) in our experiment. Indeed, the impact of warming was subtle and, even where of statistical significance, explained relatively low proportions of variance in our response variables. Continuous warming had the greatest influence. Nevertheless, effects of summer-only warming were often similar and provide some evidence to suggest that warming effects as a whole were most pervasive in the upper part of the seasonal cycle. The impacts of additional nutrient input and the presence of fish were relatively great, largely independent of warming and entirely consistent with conventional wisdom (e.g., Moss et al. 1996). As elucidated by ANOVA (Tables 2–5), significant interactions between warming treatment effects and the effects of the fish and nutrient treatments were rare and, where they occurred, always included the repeated-measures variable season.

In most microcosms, macrophytes occupied a large proportion of the available horizontal space and total macrophyte biomass remained fairly constant. Continuous warming showed some tendency to increase total macrophyte biomass, but only when fish were present. In contrast, phytoplankton populations were consistently low with a complete lack of prevalence of cyanophytes. Chl *a* concentrations were increased somewhat during the second winter in the continuously warmed microcosms containing fish. Although this trend was in a direction that might have been anticipated, statistically the effect was weak and was not reflected in estimates of biovolume. Populations of large-bodied cladocerans, *Simocephalus* and *Daphnia,* were not apparently affected by warming but were strongly reduced by the presence of fish and increased by additional nutrient input. Attack on these organisms by visual predators is es-

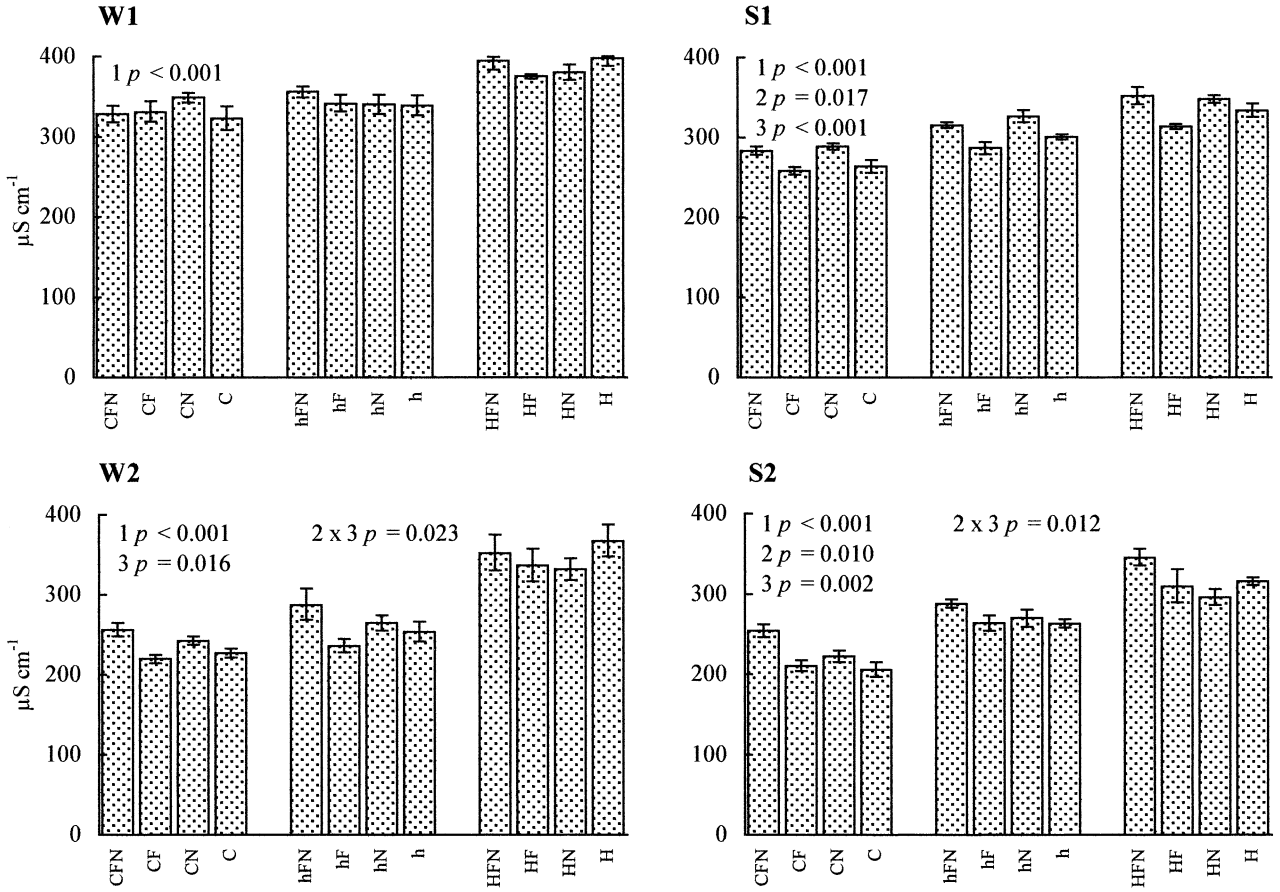

Fig. 11. Seasonal variation (W1, S1, W2, S2) of conductivity (μS cm^{-1}). Treatment codes as in Fig. 1.

pecially high. In the absence of fish, where additional nutrients were added, numbers were able to increase presumably because of greater availability and/or nutritional quality of food resources. Gastropods and ostracods were affected by the fish and nutrient treatments in a similar manner and showed trends for increase under both warming regimes. Increase in such invertebrates may help to stabilize macrophyte communities because, although this feeding guild can consume macrophyte material, net benefit is accrued through the additional removal of periphyton and detritus (Carpenter and Lodge 1986; Underwood 1991; Moss et al. 1996; Scheffer 1998). Regardless of warming, fish predation was intense and reduced invertebrate numbers to low levels. Perhaps as a consequence we were unable to detect significant interaction between the effects of warming and fish on invertebrate numbers.

Increasing nutrient levels eventually contribute strongly to the possibility of an ecosystem state change. Once a threshold is crossed, the change is rapid and difficult to reverse. While nutrient levels in our microcosms did not approach threshold levels, warming did create a trend in this direction. Total phosphorus, soluble reactive phosphorus, conductivity, and total alkalinity were increased by warming. On the other hand, pH was decreased (probably due to increased macrophyte respiration). Perhaps most worrying was the propensity for severe deoxygenation under warming and nutrient addition. This was particularly the case for the summer-only warmed microcosms where, we speculate, unusually large nutrient pulses were created by accelerated decomposition of winter-accumulated organic material as heating was switched on in conjunction with naturally rising spring temperatures. The general availability of nitrogen was consistently low, probably because of rapid macrophyte uptake and denitrification (Van Donk et al. 1993; Scheffer 1998).

Should we really expect to see prominent effects of warming? It could be argued, particularly at the organismal level, that greater adaptability to environmental variation will be encountered in ecosystems routinely faced with greater disturbance and change. Because of their large surface area to volume ratio, shallow freshwaters fall into this category, certainly relative to deep lake systems. Many aquatic poikilotherms are able to compensate for changes in habitat conditions by processes of acclimation. For example, changes in temperature optima due to acclimation are known to occur in some planktonic Crustacea (Halcrow 1963) as well as in sea grasses (Evans et al. 1986). Selection for plasticity has been postulated to contribute to the distribution and abundance of species between habitats with different levels of environmental variation (e.g., Leips et al. 2000). However, few studies have specifically examined the relationship between environmental variability and phenotypic plasticity,

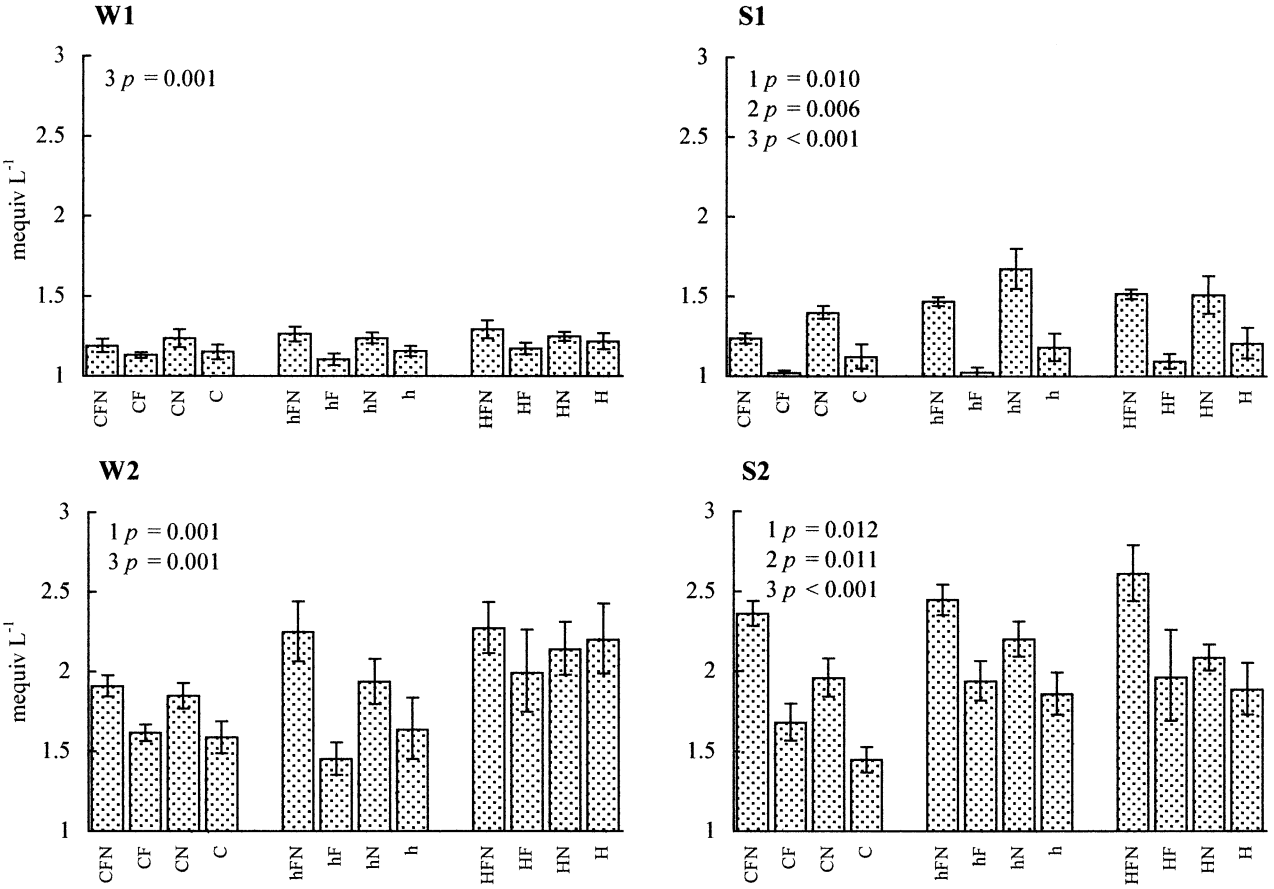

Fig. 12. Seasonal variation (W1, S1, W2, S2) of total alkalinity (milliequivalents L^{-1}). Treatment codes as in Fig. 1.

and strictly adaptive explanations are generally lacking (Leips et al. 2000).

In conclusion, our plant-dominated systems were broadly resilient to warming, even in combination with increased nutrient loading and the presence of fish. If shallow freshwater ecosystems are resilient to immediate moderate elevation of temperature, they may also be resilient to gradual increases in temperature (for example, those associated with scenarios of climatic change). However, warming did show a tendency to intensify the water chemistry processes involved with eutrophication. This could be problematic because ecosystems already pushed close to the threshold of a state change may be especially susceptible to further, unpredictable, stress events (forecast to increase in frequency with climatic change; Carpenter et al. 1992; Schneider and Root 1996). We highlight, in particular, year round increases in phospho-

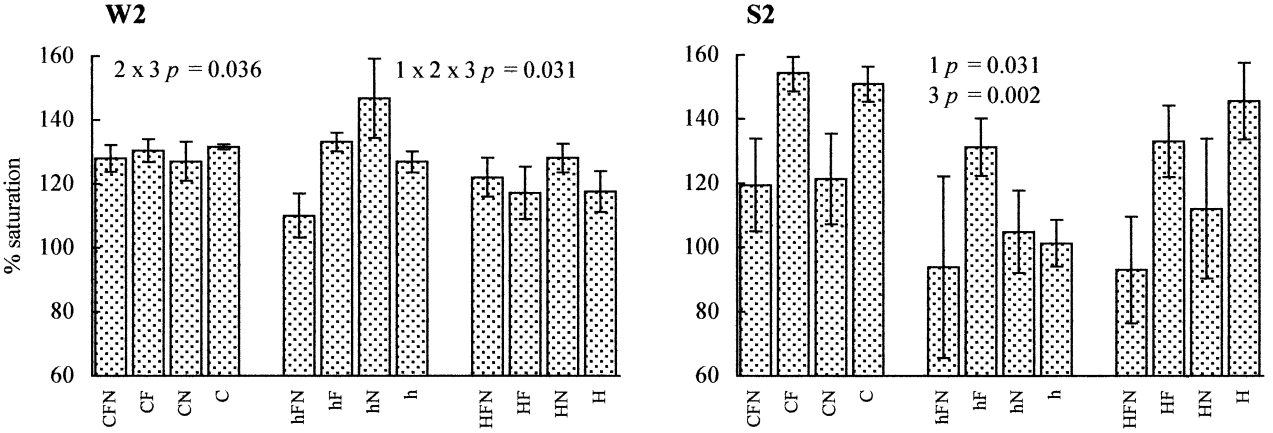

Fig. 13. Seasonal variation (W2, S2) of oxygen saturation (percentage saturation). Treatment codes as in Fig. 1.

rus concentrations under warming. In addition, given the idiosyncratic behavior of our summer-only warmed microcosms with regard to oxygen, we suggest that it is systems such as these, which routinely face greater seasonal extremes of temperature, that may be most at risk.

References

ADRIAN, R., R. DENEKE, U. MISCHKE, R. STELLMACHER, AND P. LEDERER. 1995. A long-term study of the Heiligensee (1975–1992). Evidence for effects of climatic change on the dynamics of eutrophied lake ecosystems. Arch. Hydrobiol. **133:** 315–337.

BERGER, C. 1975. Occurrence of *Oscillatoriacea agardhii* Gom. in some shallow eutrophic lakes. Verh. Int. Ver. Theor. Angew. Limnol. **19:** 2689–2697.

CARPENTER, S. R., S. G. FISHER, N. B. GRIMM, AND J. F. KITCHELL. 1992. Global change and freshwater ecosystems. Annu. Rev. Ecol. Syst. **23:** 119–139.

———, AND D. M. LODGE. 1986. Effects of submerged macrophytes on ecosystem processes. Aquat. Bot. **26:** 341–370.

CONWAY, D. 1998. Recent climate variability and future climate change scenarios for Great Britain. Prog. Phys. Geogr. **22:** 350–374.

DAVIS, A. J., J. H. LAWTON, B. SHORROCKS, AND L. S. JENKINSON. 1998. Individualistic species responses invalidate simple physiological models of community dynamics under global environmental change. J. Anim. Ecol. **67:** 600–612.

ENGELHARDT, K. A. M., AND M. E. RITCHIE. 2001. Effects of macrophyte species richness on wetland ecosystem functioning and services. Nature **411:** 687–689.

EVANS, A. S., K. L. WEBB, AND P. A. PENHALE. 1986. Photosynthetic temperature acclimation in two coexisting seagrasses, *Zostera marina* L. and *Ruppia maritima* L. Aquat. Bot. **24:** 185–197.

GEORGE, D. G., AND D. P. HEWITT. 1999. The influence of year-to-year variations in winter weather on the dynamics of *Daphnia* and *Eudiaptomus* in Esthwaite Water, Cumbria. Funct. Ecol. **13:** 45–54.

GROVER, J. P., D. MCKEE, S. YOUNG, H. C. J. GODFRAY, AND P. TURCHIN. 2000. Periodic dynamics in *Daphnia* populations: Biological interactions and external forcing. Ecology **81:** 2781–2798.

HAAG, R. W., AND P. R. GORHAM. 1977. Effects of thermal effluent on standing crop and net production of *Elodea Canadensis* and other submerged macrophytes in Lake Wabamun, Alberta. J. Appl. Ecol. **14:** 835–851.

HALCROW, K. 1963. Acclimation to temperature in the marine copepod, *Calanus finmarchicus* (Gunner). Limnol. Oceanogr. **8:** 1–8.

HOUGHTON, J. T., L. G. MEIRA-FILO, B. A. CALLANDER, N. HARRIS, A. KATTENBERG, AND K. MASKELL [EDS.]. 1996. Climate change 1996: The science of climate change. Cambridge Univ. Press.

IRVINE, K., B. MOSS, AND H. BALLS. 1989. The loss of submerged plants with eutrophication. II. Relationships between fish and zooplankton in a set of experimental ponds, and conclusions. Freshw. Biol. **22:** 89–107.

LEIPS, J., M. G. MCMANUS, AND J. TRAVIS. 2000. Response of tree frog larvae to drying ponds: Comparing temporary and permanent pond breeders. Ecology **81:** 2997–3008.

MACKERETH, F. J. H., J. HERON, AND J. F. TALLING. 1978. Water analysis: Some revised methods for limnologists. Freshwater Biological Association Publ. 36.

MCCARTHY, J. J., O. F. CANZIANI, N. A. LEARY, D. J. DOKKEN, AND K. S. WHITE [EDS.]. 2001. Climate change 2001: Impacts, adaptation and vulnerability. Contribution of working group II to the third assessment report of the Intergovernmental Panel on Climate Change. Cambridge Univ. Press.

MCDONALD, M. E., A. E. HERSHEY, AND M. C. MILLER. 1996. Global warming impacts on lake trout in arctic lakes. Limnol. Oceanogr. **41:** 1102–1108.

MCKEE, D., AND OTHERS. 2000. Heated aquatic microcosms for climate change experiments. Freshw. Forum **14:** 51–58.

———, AND D. EBERT. 1996. The interactive effects of temperature, food level and maternal phenotype on offspring size in *Daphnia magna*. Oecologia **107:** 189–196.

MEHNER, T. 2000. Influence of spring warming on the predation rate of underyearling fish on *Daphnia*—a deterministic simulation approach. Freshw. Biol. **45:** 253–263.

MOSS, B., J. MADGWICK, AND G. PHILLIPS. 1996. A guide to the restoration of nutrient-enriched shallow lakes. W. W. Hawes.

MULHOLLAND, P. J., AND OTHERS. 1997. Effects of climatic change on freshwater ecosystems of the south-eastern United States and Gulf Coast of Mexico. Hydrol. Proc. **11:** 949–970.

PARRY, M. L. [ED.]. 2000. Assessment of potential effects and adaptations for climate change in Europe: The Europe ACACIA project. Jackson Environment Institute.

PETCHEY, O. L., P. T. MCPHEARSON, T. M. CASEY, AND P. J. MORIN. 1999. Environmental warming alters food-web structure and ecosystem function. Nature **402:** 69–72.

ROONEY, N., AND J. KALFF. 2000. Inter-annual variation in submerged macrophyte community biomass and distribution: The influence of temperature and lake morphometry. Aquat. Bot. **68:** 321–335.

SANTAMARIA, L., AND W. VAN VIERSSEN. 1997. Photosynthetic temperature responses of fresh- and brackish-water macrophytes: A review. Aquat. Bot. **58:** 135–150.

SCHEFFER, M. 1998. Ecology of shallow lakes. Chapman & Hall.

———. 1999. The effect of aquatic vegetation on turbidity: How important are the filter feeders? Hydrobiologia **408/409:** 307–316.

———, S. CARPENTER, J. A. FOLEY, C. FOLKE, AND B. WALKER. 2001. Catastrophic shifts in ecosystems. Nature **413:** 591–596.

———, S. H. HOSPER, M. L. MEIJER, B. MOSS, AND E. JEPPESEN. 1993. Alternative equilibria in shallow lakes. Trends Ecol. Evol. **8:** 275–279.

SCHINDLER, D. W. 1975. Whole-lake eutrophication experiments with phosphorus, nitrogen and carbon. Verh. Int. Ver. Theor. Angew. Limnol. **19:** 3221–3231.

———. 1997. Widespread effects of climatic warming on freshwater ecosystems in North America. Hydrol. Proc. **11:** 1043–1067.

———, AND OTHERS. 1990. Effects of climatic warming on lakes of the central boreal forest. Science **250:** 967–970.

SCHNEIDER, S. H., AND T. L. ROOT. 1996. Ecological implications of climate change will include surprises. Biodiversity Conserv. **5:** 1109–1119.

SCHRIVER, P., J. BOGESTRAND, E. JEPPESEN, AND M. SONDERGAARD. 1995. Impact of submerged macrophytes on fish-zooplankton-phytoplankton interactions: Large-scale enclosure experiments in a shallow eutrophic lake. Freshw. Biol. **33:** 255–270.

STERNER, R. W. 1989. The role of grazers in phytoplankton succession. *In* U. Sommer [ed.], Plankton ecology: Succession in plankton communities. Springer.

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

SVENSSON, R., AND M. WIGREN-SVENSSON. 1992. Effects of cooling water discharge on the vegetation in the Forsmark Biotest Basin, Sweden. Aquat. Bot. **42:** 121–141.

TAYLOR, B. R., AND J. HELWIG. 1995. Submergent macrophytes in a cooling pond in Alberta, Canada. Aquat. Bot. **51:** 243–257.

TIMMS, R. M., AND B. MOSS. 1984. Prevention of growth of potentially dense phytoplankton populations by zooplankton grazing, in the presence of zooplanktivorous fish, in a shallow wetland ecosystem. Limnol. Oceanogr. **29:** 472–486.

UNDERWOOD, G. J. C. 1991. Growth enhancement of the macrophyte *Ceratophyllum demersum* in the presence of the snail *Planorbis planorbis:* The effect of grazing and chemical conditioning. Freshw. Biol. **26:** 325–334.

VAN DONK, E., R. D. GULATI, A. IEDEMA, AND J. T. MEULEMANS. 1993. Macrophyte-related shifts in the nitrogen and phosphorus contents of the different trophic levels in a biomanipulated shallow lake. Hydrobiologia **251:** 19–26.

WEYHENMEYER, G. A., T. BLENCKNER, AND K. PETTERSSON. 1999. Changes of the plankton spring outburst related to the North Atlantic Oscillation. Limnol. Oceanogr. **44:** 1788–1792.

WOOTON, R. J., J. R. M. ALLEN, AND S. J. COLE. 1980. Effect of body weight and temperature on the maximum daily food consumption of *Gasterosteus aculeatus* L. and *Phoxinus phoxinus* (L.): Selecting an appropriate model. J. Fish Biol. **17:** 695–705.

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