

# How important is climate? Effects of warming, nutrient addition and fish on phytoplankton in shallow lake microcosms

BRIAN MOSS, D. MCKEE, D. ATKINSON, S. E. COLLINGS, J. W. EATON, A. B. GILL, I. HARVEY, K. HATTON, T. HEYES and D. WILSON

School of Biological Sciences, Derby Building, University of Liverpool, Liverpool L69 3GS, UK

## Summary

1. Climate is changing. Predictions are for at least a 3 °C rise in mean temperature in northern Europe over the next century. Existing severe impacts of nutrients and inappropriate fish stocking in freshwater systems remain.

2. Effects of warming by 3 °C above ambient, nutrient addition and the presence or absence of sticklebacks *Gasterosteus aculeatus* were studied in experimental microcosms dominated by submerged plants, mimicking shallow lake ecosystems.

3. Warming had considerably smaller effects on the phytoplankton community than did fish and nutrients. It had very minor effects on chlorophyll *a* and total phytoplankton biovolume. However, it significantly decreased the biovolumes of Cryptophyceae (a major component in the controls) and Dinophyceae. Contrary to expectation, warming did not increase the abundance of blue-green algae (cyanophytes). Warming decreased the abundances of *Cryptomonas erosa* (Cryptophyceae) and *Oocystis pusilla* (Chlorophycota) and increased those of two other green algae, *Tetradion minimum* and *Micractinium pusillum*. It had no effect on a further 17 species that were predominant in a community of about 90 species.

4. Fish and nutrients, either together or separately, generally increased the crops of most of the 21 abundant species and of the algal groups. Exceptions were for diatoms and chrysophytes, which were very minor components of the communities. Fish, but neither nutrients nor warming, increased the number of species of phytoplankton detected. This was probably through removal of zooplankton grazers, and parallels terrestrial studies where the presence of top predators, by controlling herbivores, leads to increased plant diversity.

5. There was no particular pattern in the taxonomy or biological characteristics of those species affected by the treatments. In particular, there was no link between organism size (a surrogate for many important biological features of phytoplankton species) and the effects of warming, nutrient addition or presence or absence of fish. However, all species were relatively small and potentially vulnerable to grazing.

6. *Synthesis and applications.* The results suggest that fears of an increasing abundance of cyanophytes with current projections of global warming may be unrealized, at least in shallow unstratified lakes still dominated by macrophytes. However, they emphasize that eutrophication and fish manipulations remain very important impact factors that determine the abundance of phytoplankton and subsequent problems caused by large growths.

*Key-words:* bottom up, Chlorophyta, Cryptophyceae, Cyanobacteria, temperature, top down

*Journal of Applied Ecology* 2003 **40**, 782–792

## Introduction

Earth is steadily warming and predictions for the UK and much of Europe suggest a rise in temperature of 3–5 °C in the next half century (Houghton *et al.* 2001). Temperature increase, although only one of several related climate changes, including rainfall intensity and timing and evaporation, is the key variable of change and one that pervades almost all biological processes. All ecosystems will therefore be affected to some extent by global warming and there is much interest in predicting what its effects might be.

Because of their importance in natural economies, shallow wetland systems (Dugan 1994) are particularly important and already vulnerable. Shallow lakes, once dominated by submerged plants (macrophytes) in clear water, have often been converted to turbid, algal-dominated lakes by a variety of food web-linked mechanisms in an environment of increased nutrient loading (Moss, Madgwick & Phillips 1996) despite an array of mechanisms that to some extent can stabilize the plant-dominated state (Moss 1999). These mechanisms include nutrient competition between plants and algae and provision of refuges for invertebrate grazers on periphyton or phytoplankton.

A variety of switch mechanisms (Moss, Madgwick & Phillips 1996; Moss 1999), either directly damaging the plants (vertebrate grazing, mechanical damage, herbicide run-off or treatment) or indirectly influencing the effectiveness of invertebrate grazers (changes in fish community, toxins, increased salinity), may allow algal populations to suppress plant growth and become dominant. The thresholds at which these switches may act have not been precisely quantified but appear to be influenced by nutrient availability (Jeppesen *et al.* 1997). The greater the nutrient load, the more likely it is that a switch to phytoplankton dominance will take effect. Because of the pervasive influence of temperature on biological processes, it seems possible that warming might add to the list of switches and increase the risk of destabilization of submerged plant communities. This was our first hypothesis.

Phytoplankton ecology *per se* provides a valuable model for investigation of changing environments, with results possibly applicable to communities of larger organisms that are experimentally not so tractable over short periods (Reynolds 1997). Over just a few weeks phytoplankters undergo many generations, giving ample opportunity for the selection of forms more appropriately fitted to changed environments. Annually there is a periodicity of species that has reasonable predictability, at least in terms of overall groups of algae or life strategies (Hutchinson 1967b). Diatoms (Bacillariophyceae), for example, tend to be abundant in the well-mixed periods of spring and autumn in temperate lakes (Lund 1964, 1965); chrysophytes often follow in the short period, when nutrients depleted by diatoms have not yet been replaced by internal regeneration or new supplies from the catchment. Small 'weedy' species, often

small green flagellates (Chlorophyta) or cryptomonads (Cryptophyceae), have irregular cycles controlled by zooplankton grazing, itself linked to fish predation. In turn fish recruitment depends crucially on the weather during egg development and zooplankton abundance during maturation of the fry and young-of-the-year. In mid-summer, when previous intense biological activity has increased the chemical complexity of the water, a mixed community of green algae and dinoflagellates (Dinophyceae), often including mixotrophic species, tends to occur. Then, in late summer, larger forms of blue-green algae (Cyanophyta) and dinoflagellates may predominate, perhaps favoured by their abilities to obtain scarce nutrients from the bottom waters through vertical migration. They may be helped by removal of competition from smaller forms kept scarce by grazers. Along with late-appearing green algae, they may also simply divide less frequently and hence take more time to attain prominence (Lund 1971).

There is always a much larger pool of species available than the array that grows most abundantly in a given year (Hutchinson 1967a; Chorus & Schlag 1993). Weather can alter water temperature greatly in different years and different species have different temperature ranges and optima for growth (Moss 1973b; Seip, Sneek & Snipen 1994; Seip & Reynolds 1995), as they have for other environmental factors (Moss 1973a; Reynolds, Irish & Elliott 2001, Reynolds *et al.* 2002). Substitution of alternative species could thus maintain overall phytoplankton community function despite much warming. Because phytoplankton crops also appear to be controlled extensively by nutrient availability and grazer impact, we hypothesize that there might be no effect of warming on overall community size but some change in species composition.

Phytoplankton communities that develop when submerged plant communities are displaced from shallow lakes vary greatly, depending on the balance of nitrogen and phosphorus availability, the retention time of the water mass and the degree of mixing. Diatoms tend to be favoured by greater mixing (Moss & Balls 1989; Reynolds 1997), green algae by very high nutrient concentrations (Jensen *et al.* 1994) and cyanophytes by moderately high phosphorus levels, a scarcity of free carbon dioxide, stability of the water mass and long retention time (Shapiro 1990). However, there are two general trends in community composition that might be expected with warming. First, environmental impacts should favour pioneer or weedy species over those of more stable habitats. Very small species tend to have irregular annual cycles. They grow rapidly but are consumed easily by grazers. They are the equivalents of *r*-selected pioneer species with high birth and death rates, and are opportunistic and generalist in their habitat requirements. Larger species tend to have more specific needs and a more complexly defined habitat. Therefore, we predict that warming trends along with other experimental treatments will have fewer influences on smaller species and that there will be more

complex effects on larger ones. The nature of the community is, of course, important in the provision of domestic drinking water supplies. Small species pose greater filtration problems.

Secondly, we expect a greater representation of cyanophytes with warming. Cyanophytes evolved in the Precambrian period when temperatures were much higher than at present (Falkowski 2002) and, although widespread in the oceans and freshwaters, even in Antarctica, they show particular association with warm habitats. They tend to be most abundant in late summer in temperate lakes (Lund 1965; Hutchinson 1967b; Moss & Balls 1989) and frequently dominate tropical lakes (Talling 1986), desert soil crusts and hot springs (Brock 1967, 1978). Their temperature optima for growth tend to be higher than those of green algae and much higher than those of diatoms (Brock 1967; Reynolds, Irish & Elliott 2001).

In freshwaters, planktonic cyanophytes are associated with toxin problems and in recent years many freshwaters have been closed to public recreation because of the risk of cyanophyte blooms (Codd 1995). Multiple deaths occurred in a dialysis unit in Brazil as a result of use of water in which cyanophytes had grown (Pouria *et al.* 1998), despite their having been filtered out. In drinking water supplies they are also problematic because of odour and filtration problems (Uhlmann 1979). Global warming may therefore bring a greater incidence of cyanophytes and their associated problems in freshwaters. In shallow lakes where plants have been lost, circumstances leading to cyanophyte dominance also make restoration of the plant communities difficult because cyanophytes are often uningestible, indigestible or nutritionally poor for invertebrate grazers (Arnold 1971; de Bernardi & Giussano 1990).

Our overall prediction therefore is that increased warming will bring a greater risk of collapse of submerged plant communities in shallow lakes and that there will be a switch towards phytoplankton communities increasingly dominated by cyanophytes; that overall production will be unchanged by warming trends; that there will be some change in community species composition; and that there will be fewer effects on small species than on larger ones.

Although several lines of observational and modelling evidence provide pointers to eventual warming effects (Carpenter *et al.* 1992; Anderson, Robertson & Magnusson 1996; Melack *et al.* 1997; Hostetler & Small 1999; George 2002), long-term experiments at the whole-ecosystem scale perhaps provide the most reliable predictors (Schindler 1998). Such experiments have considerable logistic problems and replication of treatments may not be possible. A compromise, therefore, is the use of replicated microcosms, each containing a simplified, although still structurally complex, version of the whole ecosystem. Although microcosms cannot contain a complete food web (Carpenter 1996), they offer substantial increases in reality compared with laboratory systems (Petchey *et al.* 1999; Fox & Morin 2001). Experiments

at an ecosystem scale to examine the effects of warming on freshwater communities are as yet very rare.

To test our predictions we created 48 experimental, open-air microcosms (McKee *et al.* 2000) that could be heated to specified temperatures above ambient. We stocked them with a standard community resembling those of shallow lakes, with sediment, submerged plants, associated invertebrates and a plankton community. Because warming trends are occurring against a background of other major impacts, including eutrophication (Moss 1996) and changes in fish communities (Carvalho & Moss 1995; Williams, Moss & Eaton 2002; Williams & Moss 2003), we incorporated nutrient and fish treatments combined with warming treatments. We then examined the microcosm phytoplankton communities that resulted. Effects on water chemistry, zooplankton and plants have been reported elsewhere (McKee *et al.* 2002a,b, 2003).

## Methods

### MICROCOSM CONSTRUCTION

The microcosms were circular fibre-glass tanks, 1.02 m deep and 2 m in diameter, containing 3200 l of water and sediment. For insulation they were sunk into the ground on land in the University of Liverpool Botanic Gardens at Ness on the Wirral Peninsula, UK (53°16'N, 3°02'W). Each of the 48 tanks contained either a dummy heating element or a functioning element placed in its bottom. Hot water was circulated through the functional elements from a boiler at rates determined by a control sensor and system positioned in each tank. The control system allowed the tank water to be maintained at a predetermined temperature above the ambient sensed in the nearest unheated control tank. The control system operated with a sensitivity of +0.25 °C and functioned without any interruption or breakdown for 24 months. Evaporative losses in summer were replaced with deionized water generated on site. Full details of the system, specifications for the components and evidence of the precision of temperature control are given in McKee *et al.* (2000).

Before the experiment began, each tank was provided with a standard sediment (5 cm depth, 7 : 1 sand : loam) and filled with water from a bore hole installed on site (pH 7.3, alkalinity 3.4 mequiv l<sup>-1</sup>, Mg<sup>2+</sup> 38 mg l<sup>-1</sup>, Ca 103 mg l<sup>-1</sup>). Plankton was introduced as a net-concentrated inoculum from a local canal. Macrophytes were introduced as standard inocula of equal weights of plants from local sources held in weighted netting 'sandwiches' placed randomly on the bottom. *Potamogeton natans* L., *Elodea nuttallii* Planch. H. St John and *Lagarosiphon major* Ridl. Moss were used, with a deliberate choice of one native species, one naturalized species and one recent invader, respectively. Invertebrates were inevitably introduced with the plants and many others flew in. Invertebrate and plankton communities were regularly cross-mixed during the summer of 1998,

before the experiment began in September 1998, to ensure uniform presence. By the beginning of the experiment visually uniform, well-established plant and invertebrate communities were present in each tank.

#### EXPERIMENTAL DESIGN

Three temperature treatments, two nutrient treatments and two fish treatments (presence or absence) were used in a randomized block design with quadruple replication of each of the 12 treatment combinations from September 1998 until September 2000. Temperature treatments were: control (C) (no heating); heating to 3 °C above ambient year around (H); heating to 3 °C above ambient during summer, defined as April to September inclusive (h). These regimes were based on scenarios predicted for north-west Europe for the mid-twenty-first century by the International Panel on Climate Change (Houghton *et al.* 1996). Nutrient treatments included a control in which no additional nutrients were added and a treatment in which sodium nitrate and sodium phosphate were added every 3 weeks in winter and every 2 weeks in summer, to give instantaneous additional concentrations of 500 mg l<sup>-1</sup> N and 50 mg l<sup>-1</sup> P in winter, and 170 mg l<sup>-1</sup> N and 17 mg l<sup>-1</sup> P in summer. These values were determined following inspection of loading rates of local eutrophicated shallow lakes. Fish treatments included absence of fish and addition at the beginning of the experiment of 21 adult sticklebacks *Gasterosteus aculeatus* L. of both sexes. The fish freely bred and the final mean biomass per relevant microcosm was 27 ± 18.2 g m<sup>-2</sup>.

#### PHYTOPLANKTON METHODS

Phytoplankton samples were taken with a tube from the entire water column samples from each microcosm every 3 weeks in winter and every 2 weeks in summer, mixed and preserved with Lugol's iodine solution. A total of 2064 samples was subsequently examined and all the component species counted following sedimentation in counting chambers and examination with a Leitz inverted microscope at ×400 magnification. Biovolumes were obtained by measurement of a random sample of 50 individuals of each organism and, for calculation, approximation of the species' shape to the most appropriate geometric solid. Chlorophyll *a* was determined spectrophotometrically after filtration through glass-fibre filters and extraction with 90% acetone. For statistical analysis mean values were calculated, log-transformed where appropriate and submitted to repeated-measures analysis of variance, with time as the repeated-measures variable and warming, fish and nutrients as factors. For some comparisons, where seasonality was examined, means were calculated for the two winters (W1 and W2) and the two summers (S1 and S2) of the experiment. Significant results from the analyses of variance were subjected to Tukey honest

significant difference tests to reveal the specific effects of treatments.

## Results

#### OVERALL COMMUNITY

Eighty-nine photosynthetic planktonic taxa were recorded in the samples, together with a further 24 diatom species sporadically displaced from the surfaces of the plants by sampling or wind disturbance (Table 1). Thirty-six of the 89 taxa were flagellates; many of the remainder were small coccoid or colonial forms. There were few large colonies, all of them flagellate or, in the cases of *Botryococcus braunii* Kutz. and *Gomphosphaeria* sp., buoyant through oil storage and gas vesicles, respectively. Overall the community was that expected from weedy, shallow waters where turbulence is minimal and motility and the low sinking rates of small forms have selective advantage. Planktonic diatoms, which have relatively high sinking rates, were very infrequent.

The phytoplankton community developed in abundance during the first year (Table 2). Most algal groups and species showed changes between seasons with increases in biovolume, particularly during the second summer. This did not reflect availability because all abundant species were recorded within the first year. It may rather reflect accumulation of nutrients in the system with continued nutrient loading. Cryptophyceae were predominant in the first winter and remained a major component in the second winter. Chlorophyta became predominant in the first summer and remained predominant thereafter. Other than Euglenophyta in the second summer, other algal groups, including Cyanophyta, accounted for very small relative (and absolute) contributions to the total biovolume.

#### CHLOROPHYLL *a* AND BIOVOLUME OF MAJOR GROUPS

Phytoplankton chlorophyll *a* concentrations were generally low (overall mean per microcosm = 14 µg l<sup>-1</sup>, SD = 25, *n* = 192). There were significant increases in chlorophyll *a* and total algal biovolume with either fish presence or nutrient addition, but almost no significant effects of warming (Table 3). The major interaction effect was between fish and nutrients, with the combined effects of the two treatments significantly increasing chlorophyll *a* or total biovolume over the values obtained with the individual treatments.

There was a significant warming × fish treatment × season interaction in the repeated-measures ANOVA suggesting statistically significant variation overall in chlorophyll concentrations dependent on warming, although the effect was ecologically only minor. It was conditioned particularly by results from the second winter, when chlorophyll concentrations in the continuously warmed microcosms containing fish were unusually high. Within-seasons analyses yielded



**Table 1.** Composition of the phytoplankton community in a set of experimental microcosms

Division	Number (%) of taxa	Major genera
Chromophyta	21 (23.6)	
Cryptophyceae	4 (4.5)	<i>Cryptomonas</i> , <i>Rhodomonas</i>
Chrysophyceae	8 (9.0)	<i>Mallomonas</i> , <i>Chrysococcus</i> , <i>Synura</i> , <i>Chrysochromulina</i> , <i>Dinobryon</i>
Bacillariophyceae	6 (6.7)	<i>Aulacoseira</i> , <i>Nitzschia</i> , <i>Cyclotella</i> , <i>Asterionella</i> , <i>Stephanodiscus</i>
Dinophyceae	3 (3.4)	<i>Peridinium</i> , <i>Gymnodinium</i>
Chlorophyta	46 (51.7)	
Conjugatophyceae	6 (6.7)	<i>Cosmarium</i> , <i>Staurastrum</i> , <i>Closterium</i>
Chlorophyceae (Volvocales)	11 (12.4)	<i>Chlamydomonas</i> , <i>Gonium</i> , <i>Haematococcus</i> , <i>Pyramimonas</i> , <i>Pandorina</i> , <i>Eudorina</i> , <i>Chlorogonium</i> , <i>Carteria</i>
Chlorophyceae (Chlorococcales)	29 (32.6)	<i>Scenedesmus</i> , <i>Crucigenia</i> , <i>Oocystis</i> , <i>Tetradron</i> , <i>Schroederia</i> , <i>Ankyra</i> , <i>Micractinium</i> , <i>Pediastrum</i> , <i>Sphaerocystis</i> , <i>Golenkinia</i> , <i>Monoraphidium</i> , <i>Ankistrodesmus</i> , <i>Closteriopsis</i> , <i>Dictyosphaerium</i> , <i>Botryococcus</i> , <i>Asterococcus</i> , <i>Lagerheimia</i> , <i>Pseudococcomyxa</i>
Euglenophyta	10 (11.2)	
Euglenophyceae	10 (11.2)	<i>Euglena</i> , <i>Phacus</i> , <i>Lepocinclis</i>
Cyanophyta	10 (11.2)	
Chroococcaceae	4 (4.5)	<i>Dactylococcopsis</i> , <i>Chroococcus</i> , <i>Merismopaedia</i> , <i>Aphanothece</i>
Oscillatoriaceae	4 (4.5)	<i>Oscillatoria</i> , <i>Phormidium</i>
Nostocaceae	2 (2.2)	<i>Anabaena</i> , <i>Gomphosphaeria</i>
Anoxyphotobacteria	2 (2.2)	<i>Lamproedia</i>

**Table 2.** Changes in mean seasonal biovolume of the major algal groups ( $\mu\text{m}^3 \text{ ml}^{-1} \times 10^{-3}$ ). The standard deviation (SD) and percentage composition by season (%) are also given

Algal group	First winter			First summer			Second winter			Second summer		
	Mean	SD	%	Mean	SD	%	Mean	SD	%	Mean	SD	%
Bacillariophyceae	8.7	9.5	0.4	169	362	2.1	18.4	27	0.27	60.6	88.6	0.5
Chlorophyta	301	676	13.2	7480	13 200	92.3	4800	9200	69.5	8650	11 400	64.3
Chrysophyceae	19.5	30.4	0.9	0.55	1.6	0.01	26	36	0.4	120	111	0.9
Cryptophyceae	1950	3650	85.5	418	567	5.2	2000	1630	29	2970	2320	22.1
Cyanophyta	0.93	1.9	0.04	1.23	1.62	0.02	1.94	4.1	0.03	103	238	0.8
Dinophyceae	0	0	0	26.4	43	0.33	51	90	0.7	255	298	1.9
Euglenophyta	0.03	0.1	0.001	12	36.8	0.15	8.7	26	0.1	1300	2390	9.7

significant warming treatment effects only during this second winter (McKee *et al.* 2003). Warming significantly reduced (Table 4) the biovolume of the Cryptophyceae, a group that was proportionately more abundant in the winters than in the summers. The biovolume of Dinophyceae was also reduced by warming although the absolute change was very small. Warming had no effect on the biovolume of the predominant Chlorophyta or on any of the remaining groups (Tables 3 and 4).

Nutrient addition significantly increased the biovolumes of Chlorophyta, Cryptophyceae and Cyanophyta and significantly reduced that of the dinoflagellates. It had no significant effect on the scarce diatoms, chrysophytes and euglenophytes (Tables 3 and 4). Presence of fish had more widespread effects, with increases in all groups showing significant change. There were no effects on diatoms or chrysophytes. Interaction effects (Table 3) were mostly associated with the mutually sup-

portive combination of fish and nutrients in increasing the biovolumes.

#### EFFECTS ON INDIVIDUAL SPECIES

The mean number of species recorded from each microcosm over the entire course of the experiment was 32.6 (SD = 4.3,  $n = 48$ , range 22–48). To investigate diversity further, we calculated Hill's numbers (Ludwig & Reynolds 1988): number of species recorded ( $N_0$ ), number of abundant species ( $N_1$ ) and number of very abundant species ( $N_2$ ). These values (untransformed) were then subjected to analysis of variance. Only the fish treatment significantly changed  $N_0$ , more species being recorded where fish were present (Table 5). There were no two- or three-factor interaction effects on  $N_0$ . Nor were there any main or two-factor effects on  $N_1$  and  $N_2$ , although there were marginally significant three-factor

**Table 3.** Statistical significance of treatment effects as elucidated by repeated-measures analysis of variance of chlorophyll *a* concentrations, total phytoplankton biovolume and biovolumes of the major component groups of the phytoplankton. For each variable, the analyses used log-transformed mean values calculated for seasons when these groups were present (W, winter; S, summer; 1, first year; 2, second year). Significance is indicated as: NS, not significant, \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$

Treatment	Chlorophyll <i>a</i>	Total biovolume	Bacillariophyceae	Chlorophyta	Chrysophyceae	Cryptophyceae	Cyanophyta	Dinophyceae	Euglenophyta
Warming (W)	NS	NS	NS	NS	NS	*	NS	*	NS
Fish (F)	***	***	NS	***	NS	**	***	***	***
Nutrients (N)	***	***	NS	**	NS	***	**	**	NS
Season (S)	***	***	NS	***	NS	***	***	***	***
W × F	NS	NS	NS	NS	NS	NS	NS	NS	NS
W × N	NS	NS	NS	NS	NS	NS	NS	NS	NS
F × N	***	***	NS	**	NS	***	NS	NS	NS
W × S	NS	NS	NS	NS	NS	NS	NS	NS	NS
F × S	*	NS	NS	NS	NS	**	NS	NS	NS
N × S	**	*	NS	NS	NS	NS	NS	NS	NS
W × F × N	NS	NS	NS	NS	NS	NS	NS	NS	NS
W × F × S	*	NS	NS	NS	NS	NS	NS	**	NS
W × N × S	NS	NS	NS	NS	NS	NS	NS	NS	NS
F × N × S	NS	NS	NS	NS	NS	*	NS	NS	NS
W × F × N × S	NS	NS	NS	NS	NS	NS	*	*	NS
	W1, S1, W2, S2	W1, S1, W2, S2	W1, S1, W2, S2	W1, S1, W2, S2	W1, W2, S2	W1, S1, W2, S2	W1, S1, W2, S2	S1, W2, S2	S1, W2, S2

interaction effects on both (Table 5). The probable cause of this was the unusually high  $N_1$  and  $N_2$  values obtained from the summer-only warmed microcosms containing fish but not receiving additional nutrient input. However, the effects were not significant according to subsequent Tukey honest significant difference testing.

Many of the 89 species recorded were patchy in distribution either in time or microcosm, as is normal for phytoplankton communities. However, a suite of 21 species that occurred more consistently was identified and for which statistical analysis was likely to be informative. The criterion was that a species should have constituted at least 10% of the numbers or biovolume in at least one treatment in at least one season. The list included one diatom, 15 chlorophytes, two cryptophytes and one each of chrysophytes, dinoflagellates and euglenophytes (Table 6). No cyanophyte was sufficiently abundant to be listed. Further analysis of treatment effects on these species was carried out.

Data are shown in Table 6, with species arranged in order of the biovolume of the organism. Two species, *Tetraedron minimum* and *Micractinium pusillum* (both Chlorophyta), showed significant increases in their populations with warming, while *Oocystis pusilla* (Chlorophyta) and *Cryptomonas erosa* (Cryptophyceae) showed a significant decrease. The other 17 species were unaffected by warming. In the four cases of significant change, increases with warming were proportionately much greater than decreases (Table 7). Nutrient addition, perhaps surprisingly, was associated with about equal numbers of increases (six species), decreases (eight species) and lack of effect (seven species). In contrast, the presence of fish increased the populations of the majority of this suite of species, was associated with decreases in four and had no effect on only two. There was no particular pattern of main effects associated with organism size, nor any pattern of summed effects (main plus all interactions) (Table 6). This was true even after application of all conventional transformations.

## Discussion

We had predicted that increased warming would bring a greater risk of collapse of submerged plant communities, that there would be a switch towards phytoplankton communities increasingly dominated by cyanophytes and with some changes in species composition but with overall production unchanged by warming trends, and that there would be fewer effects on small species than on larger ones.

The plant community proved resilient (McKee *et al.* 2002b, 2003). There was no switch to phytoplankton dominance, even at the highest nutrient levels in the presence of fish. The stabilizing mechanisms that preserve submerged plant communities in the face of increased nutrient loading have thus been shown to be powerful. The phytoplankton community, measured either by chlorophyll *a* or biovolume, did not change significantly

**Table 4.** Mean seasonal values for biovolume ( $\mu\text{m}^3 \text{ ml}^{-1} \times 10^{-6}$ ) of the algal groups in the phytoplankton in a microcosm experiment investigating the effects of warming (C, ambient temperature; h, summer warming; H, all-year warming), fish (F) and nutrient (N) treatments. W, winter; S, summer; 1, first year; 2, second year. Values in parentheses are the appropriate mean square error estimates (from the repeated-measures analyses of variance, data log ( $x + 1$ ) transformed) for attaching back-calculated error intervals to each mean. A dash indicates that the group was not detected in that season

		CFN	CF	CN	C	hFN	hF	hN	h	HFN	HF	HN	H
Bacillariophyceae (5.102)	W1	21	1.2	1.3	2.3	13.2	3.83	13.8	0.5	12.7	3.83	30.1	0.67
	S1	7.1	5.73	8.2	420	90	65.5	30.6	1260	32.7	68.2	0	43.6
	W2	15.6	2.3	86.7	0.094	48.5	6	0.188	0	5.06	0.75	19.8	35.2
	S2	58.8	292	1.25	2.81	168	9	607	8.34	12.6	98.1	9.94	6.25
Chlorophyta (0.741)	W1	66.2	26.3	35	4.94	494	195	716	7.40	2398	255	38.4	25.5
	S1	12 200	3641	517	780	46 100	4515	521	265	16 500	2453	612	1657
	W2	1280	1022	130	90.3	28 000	544	5080	359	19 800	576	26.9	180
	S2	10 700	2860	3587	1335	21 100	4750	9220	665	40 000	5840	665	3190
Chrysophyceae (6.580)	W1	0.07	102	0.19	333	0.032	35.6	0.357	14.5	0.017	41.4	4.97	1.15
	S1	—	—	—	—	—	—	—	—	—	—	—	—
	W2	56.6	18.5	4.0	126	0	19.3	12.1	35.8	0	22.5	0	17.4
	S2	170	333	64.4	30.3	6.25	127	27.4	186	0	232	18.5	239
Cryptophyceae (0.228)	W1	6390	156	154	175	4160	65.1	104	78.0	11 600	149	120	248
	S1	1689	118	197	430	207	108	387	123	1520	77.6	74.6	92.0
	W2	3730	724	2322	1730	5190	616	1465	576	4660	942	1262	824
	S2	6810	985	497	2246	7701	904	3131	1354	1760	1090	1740	2900
Cyanophyta (1.705)	W1	0.3	0.022	0.31	0.012	0.771	0.671	6.82	0.014	0.148	1.75	0.368	0.022
	S1	0.59	0.58	0.06	0.136	1.82	212	4.15	0	4.60	0.45	0.007	0.292
	W2	0.85	0.69	0.03	2.49	4.26	0.153	0	0.42	14.3	0.127	0.013	0
	S2	15.4	140	47.6	6.12	847	76.5	4.2	25.9	38.9	3.02	3.77	23.8
Dinophyceae (2.937)	W1	—	—	—	—	—	—	—	—	—	—	—	—
	S1	0	127	0	0.245	0	80.8	0.245	0	72.9	32.7	0	2.2
	W2	2.03	254	1.35	3.38	9.96	37.8	0	2.36	229	44.7	10.6	17.6
	S2	180	895	2.25	5.63	386	573	18.9	10.1	501	447	1.13	46.8
Euglenophyta (2.602)	W1	—	—	—	—	—	—	—	—	—	—	—	—
	S1	1.53	0.29	0	0	3.77	0.573	0	0	2.46	129	5.73	0.82
	W2	0	0.96	0	0.16	2.1	0.788	0	0.39	90.9	9.08	0	0
	S2	683	783	34.2	11.8	1850	602	470	60.5	2690	8420	12.3	33.0

with warming either throughout the year or in summer only. Both increased with nutrient addition and in the presence of fish. The mean increase in chlorophyll *a* was about 10-fold to 35  $\text{mg l}^{-1}$  (from a control value of 3.3  $\text{mg l}^{-1}$ ) when nutrients were added and fish were also present, but this is not a very large value compared with turbid phytoplankton-dominated systems where sometimes 300  $\text{mg l}^{-1}$  is achieved.

The fish predation intensity, judged from the bio-mass and its impacts on both zooplankton and benthic invertebrate communities (McKee *et al.* 2003), was comparatively high. This should have increased chlorophyll concentrations to a greater extent than might be expected in whole-lake systems with piscivores present. However, chlorophyll concentrations remained moderate, underlining the resilience of the plant-dominated system. It seems unlikely that the predicted warming trend alone will act as a switch in these systems, and even with the support of nutrients and fish predation on zooplankton a switch was not achieved. The effects of already known switches are thus likely to remain more important than the effects of warming.

Warming did change the phytoplankton community but only by modestly decreasing the cryptophyte and dinoflagellate components, particularly the former, which was most abundant in the controls in winter.

Neither of these changes is likely to be ecologically very important. The anticipated increase in blue-green algae did not occur, despite the presence of several cyanophyte species. Cyanophytes are important components of turbid, shallow lake communities and it may be that warming will increase the proportion of these algae in lakes where aquatic plants have been lost and phytoplankton dominance has been established. It may also increase their extent in stratified lakes if the hypolimnion becomes increasingly deoxygenated at higher temperatures. But for plant-dominated lakes our experiment suggests that the risk is small. None of the blue-green algae even entered our list of relatively abundant species.

The phytoplankton community that established was as diverse as those of natural lakes and the assemblage was typical of shallow lakes. Of those phytoplankton species that were sufficiently widespread within the treatments for statistical analysis to be used reliably, only a minority was influenced by warming. Two chlorophyte species increased with warming, one declined. Within groups, different species have different temperature optima and most grow very well over wide ranges. The range and optima of the three species have not been established in culture but what has been observed seems most likely to be a simple species substitution with warming, such as is common in year-to-year

**Table 5.** Effects of warming, fish and nutrient treatments on mean phytoplankton diversity. The first part of the table shows Hill's numbers: number of species recorded ( $N_0$ ), number of abundant species ( $N_1$ ) and number of very abundant species ( $N_2$ ). The second part of the table shows results of analysis of variance of these indices. C, ambient temperature; h, summer warming; H, all-year warming. Mean number of species in each treatment was calculated for each microcosm so that there were four replicates per value calculated. ANOVAS ( $n = 16$  (warming) or 24) were carried out without transformation. Probabilities below 0.05 are given in bold type

(a) Mean species richness per treatment

Warming	Fish	Nutrients	$N_0$		$N_1$		$N_2$	
			Mean	SD	Mean	SD	Mean	SD
C	+	+	32.8	5.1	6.3	1.1	4.4	1.3
C	+	–	36.5	1.0	5.3	2.6	3.3	1.8
C	–	+	28.8	5.9	4.7	0.7	3.6	0.7
C	–	–	28.5	6.2	6.2	0.8	4.8	0.6
h	+	+	41.5	5.8	4.4	0.3	2.8	0.5
h	+	–	34.5	2.5	8.7	2.9	5.9	2.2
h	–	+	27.5	3.1	5.9	1.7	4.4	1.0
h	–	–	28.3	3.6	4.9	1.2	3.6	1.3
H	+	+	39.8	4.2	5.1	1.2	3.4	1.1
H	+	–	36.0	4.2	5.5	3.2	3.7	2.1
H	–	+	26.3	3.3	4.5	1.2	3.3	0.9
H	–	–	31.5	3.4	4.7	0.9	3.5	1.0

(b) Results of ANOVA

Effect	$N_0$		$N_1$		$N_2$	
	F	P	F	P	F	P
Warming (W)	0.72	0.50	1.35	0.28	1.33	0.28
Fish (F)	45.7	<b>&lt; 0.001</b>	2.15	0.15	0.02	0.89
Nutrients (N)	0.03	0.87	2.19	0.15	1.77	0.19
W × F	0.99	0.38	0.20	0.82	0.27	0.77
W × N	1.44	0.25	0.81	0.45	0.85	0.43
F × N	2.90	0.10	0.90	0.34	0.50	0.48
W × F × N	2.79	0.07	5.09	<b>0.01</b>	5.90	<b>0.006</b>

**Table 6.** Significance of main and interaction effects of warming (W), fish (F), nutrients (N) and season (S) on the most abundant (by number or biovolume) phytoplankton species in an experiment carried out in experimental microcosms. Species codes are: a, *Chlamydomonas grovei* G.S. West; b, *Asterococcus limneticus* G.M. Smith; c, *Rhodomonas minuta* var *nannoplanktica* (Skuja) Javornicky; d, *Nitzschia* sp.; e, *Chrysochromulina parva* Lackey; f, *Chlamydomonas* sp.; g, *Tetradron minimum* (A. Braun); h, *Scenedesmus dimorphus* (Turpin) Kutzing; i, *Monoraphidium contortum* (Thuret) Komarkova-Legnerova; j, *Lagerheimia ciliata* (Lagerheim) Chodat; k, *Micractinium pusillum* Fresenius; l, *Cosmarium regnellii* Wille; m, *Pseudococcomyxa simplex* (Mainx) Fott; n, *Haematococcus pluvialis* Flotow emend. Wille; o, *Peredinium cinctum* (O.F. Muller); p, *Sphaerocystis planktonica* (Korshikov) Bourrelly; q, *Oocystis pusilla* Hangsgirg; r, *Cryptomonas erosa* Ehrenberg; s, *Trachelomonas volvocina* Ehrenberg; t, *Pandorina morum* (O.F. Muller); u, *Cosmarium botrytis* Meneghini ex Ralfs. Significance testing was carried out only for those seasons (W, winter; S, summer) when the species was detected. Presence of either NS or a significance value (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ) indicates that the species was present. Dashes indicate that it was not detected in all seasons. Sig. inter. is the total number of significant interactions (main, two-way, three-way and four-way) recorded for the species with all instances involving season ignored to preserve uniformity of comparison. The maximal number would be 7

Species Vol. ( $\mu\text{m}^3$ )	a 10	b 15	c 70	d 70	e 100	f 108	g 150	h 240	i 260	j 260	k 270	l 1370	m 730	n 1700	o 1800	p 2000	q 3700	r 4000	s 4200	t 17 500	u 27 000
W	NS	NS	NS	NS	NS	NS	***	NS	NS	NS	*	NS	NS	NS	NS	NS	*	***	NS	NS	NS
F	NS	**	***	NS	*	*	***	***	***	**	***	***	***	***	***	NS	NS	NS	***	***	***
N	NS	*	***	**	***	NS	NS	NS	NS	*	***	***	*	NS	***	NS	***	***	*	***	**
S	–	–	***	***	***	***	***	***	***	–	***	***	***	*	***	–	***	***	–	***	***
W × F	NS	NS	NS	NS	NS	NS	***	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	*	NS	NS
W × N	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
F × N	NS	*	NS	NS	NS	NS	*	*	NS	*	***	**	NS	NS	**	*	NS	***	*	***	NS
W × S	–	–	NS	NS	NS	NS	NS	NS	*	–	NS	NS	NS	NS	NS	–	NS	NS	–	NS	NS
F × S	–	–	***	NS	NS	NS	***	***	***	–	***	***	***	*	**	–	***	*	–	**	***
N × S	–	–	***	NS	NS	NS	*	NS	*	–	***	*	NS	NS	NS	–	**	NS	–	NS	*
W × F × N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
W × F × S	–	–	NS	NS	NS	NS	NS	NS	NS	–	NS	NS	*	NS	NS	–	NS	NS	–	*	NS
W × N × S	–	–	NS	NS	NS	NS	NS	NS	NS	–	NS	NS	NS	NS	NS	–	NS	NS	–	NS	NS
F × N × S	–	–	***	NS	NS	NS	*	*	*	–	**	NS	NS	NS	NS	–	NS	NS	–	NS	NS
W × F × N × S	–	NS	NS	NS	NS	NS	NS	NS	–	NS	NS	NS	NS	NS	–	NS	NS	–	NS	NS	NS
Sig. inter.	1	3	3	1	1	1	4	2	1	3	4	3	2	2	3	1	2	3	4	3	2



**Table 7.** Changes in population size (organisms per ml) of those abundant species (Table 6) showing significant changes in response to warming in a microcosm experiment investigating the effects of warming (C, ambient temperature; h, summer warming; H, all-year warming), fish (F) and nutrient (N) treatments. Seasonal means are shown (W, winter; S, summer; 1, first year; 2, second year). Values in parentheses are the appropriate mean square error estimates (from the repeated-measures analyses of variance, data log ( $x + 1$ ) transformed) for attaching back-calculated error intervals to each mean

		CFN	CF	CN	C	hFN	hF	hN	h	HFN	HF	HN	H
<i>Tetraedron</i> minimum	W1	—	—	—	—	—	—	—	—	—	—	—	—
	S1	—	—	—	—	—	—	—	—	—	—	—	—
	W2	2	6	1	1	34	38	5	2	3 970	681	1	0
(0.322)	S2	46	557	11	13	894	1960	25	9	6 120	7600	11	12
<i>Micractinium</i> pusillum	W1	71	2	1	4	1 660	5	5	2	8 110	2	134	1
	S1	10 200	3	0	46	78 800	190	1	15	4 300	105	240	295
	W2	929	0	0	1	26 500	0	17	2	19 340	0	25	1
(0.179)	S2	364	1	1	1	2 670	529	42	3	44 200	7	12	63
<i>Oocystis</i> pusilla	W1	—	—	—	—	—	—	—	—	—	—	—	—
	S1	10	56	0	78	3	9	2	2	12	10	0	12
	W2	1	4	24	19	0	1	1	1	0	6	0	1
(0.171)	S2	5	20	36	211	0	10	46	59	3	11	0	149
<i>Cryptomonas</i> erosa	W1	1 570	20	25	36	1 020	6	10	10	2 870	21	19	55
	S1	706	48	295	241	396	38	210	32	769	35	96	37
	W2	931	176	580	431	1 300	156	359	138	1 160	229	307	202
(0.287)	S2	1 700	242	1240	549	1 930	224	777	331	438	271	421	706

variations in phytoplankton communities dependent on weather. In a series of microcosm experiments carried out in 1998 and 1999 over a European transect from Finland to southern Spain, temperature differences in mid-summer between years averaged  $2.9 \pm 1.6$  °C, with a range from 0.3 to 4.3 (Stephen *et al.* 2004). The predicted temperature change from global warming during the twenty-first century is about the same as this inter-year difference. Not surprisingly, the change in phytoplankton community in the experiments with warming was slight. The fourth species that was significantly affected by warming was a cryptophyte, more abundant in general in winter in the controls than in the summer. Warmer winters are predicted for the future in north-west Europe. Changes in the winter flora of lakes might thus be more prominent than changes in the summer flora. However, no particular algal problems are associated with winter and it is unlikely that this effect will have great practical relevance. The extreme dispersability of algae (most genera and many species are ubiquitous) also means that the difficulties of dispersion, in colonizing new geographical ranges created by warming, that might be faced by larger organisms with longer and more specialized life histories are unlikely to be important.

Shallow lakes will inevitably support a phytoplankton community fitted to their particular conditions. Water associated with plant beds is relatively calm, lacking the vigorous mixing of the pelagial zone of larger, deeper lakes. Life forms fitted to such conditions include small and motile species, which have low net sinking rates. Our communities epitomized this. There is no reason to think that such communities will be any more or less susceptible to the effects of warming than those of deep lakes, but they do differ in their susceptibilities to factors such as grazing. Effects of fish (and thus indirectly of the grazers on which the fish feed)

were generally more prominent than those of nutrients or warming. Fish allowed an increase in diversity as well as increased biovolumes of all the prominent algal groups. This is consistent with terrestrial experience where the loss of top predators tends to lead to an increase in grazing and a progressive loss of plant species (Terborgh 1992; Schmitz 2003). Analogies must not be drawn too far, however. In shallow lake communities diversity includes not only micro-organisms but also plants and macroinvertebrates. In such communities, the general principle still applies, predatory fish are associated with high diversity, clear water conditions in which cascading effects lead to an abundance of grazing zooplankters. These, by decimating the phytoplankton, lead to conditions where the macrophyte community and its associated invertebrates can flourish at high diversity.

Do these results suggest that there is little cause for concern as temperatures continue to rise in the next few decades from the approximately 0.6 °C rise (compared with the recent historic mean) at present to a predicted 3+ °C rise later this century? This is a difficult question. Microcosms inevitably do not replicate all the features of natural lakes. They cannot reflect extreme events of hydrology, for example. But, especially if sited in the open air and subject to natural rainfall, they do mimic quite well the conditions in small, shallow lakes and ponds, in which the macrophyte architecture strongly modifies many facets of the external environment (deadening of water movements, modification of water chemistry, for example). Macrophyte-dominated shallow lake communities are complex with many inherent stabilizers. Deep lake phytoplankton communities might be more vulnerable to temperature change, although their ability through high residual diversity to cope with between-year temperature variations as great as those predicted for global warming suggests that this is

unlikely. An increase in cyanophytes, because of their preference for the microaerophilic conditions associated with hypolimnia, which could become more severely deoxygenated with warming, is possible. However, the most severe effects of global climate change might come, not from temperature rise *per se*, but from the associated increasing incidence of extreme weather events and rising sea levels. Extreme flooding will remodel river systems and rising sea level will extend estuarine conditions at the expense of freshwater ones. Whether these changes will constitute boon or disaster from a conservation or ecological point of view is certainly not clear, but there can be no relent regarding the tackling of existing, perhaps more significant, problems of eutrophication (diminished diversity, reduced drinking water quality, toxic blooms, changed fish communities) and inappropriate fish stocking (removal of predators, introduction of favoured angling species such as rainbow trout *Onchorhynchus mykiss* and common carp *Cyprinus carpio*), irrespective of warming trends.

## References

- Anderson, W.L., Robertson, D.M. & Magnusson, J.J. (1996) Evidence of recent warming and El-Nino related variations in the ice breakup dates of Wisconsin lakes. *Limnology and Oceanography*, **41**, 815–821.
- Arnold, D.E. (1971) Ingestion, assimilation, survival, and reproduction by *Daphnia pulex* fed seven species of blue green algae. *Limnology and Oceanography*, **16**, 906–920.
- Brock, T.D. (1967) Life at high temperatures. *Science*, **158**, 1012–1019.
- Brock, T.D. (1978) *Thermophilic Microorganisms and Life at High Temperatures*. Springer-Verlag, New York, NY.
- Carpenter, S.R. (1996) Microcosm experiments have limited relevance for community and ecosystem ecology. *Ecology*, **77**, 677–680.
- Carpenter, S.R., Fisher, S.G., Grimm, N.B. & Kitchell, J.F. (1992) Global change and freshwater ecosystems. *Annual Review of Ecology and Systematics*, **23**, 119–139.
- Carvalho, L. & Moss, B. (1995) The current status of a sample of English Sites of Special Scientific Interest subject to eutrophication. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **5**, 191–204.
- Chorus, I. & Schlag, G. (1993) Importance of intermediate disturbances for species composition and diversity of phytoplankton in two very different Berlin lakes. *Hydrobiologia*, **249**, 67–92.
- Codd, G.A. (1995) Cyanobacterial toxins: occurrence, properties and biological significance. *Water Science and Technology*, **32**, 149–156.
- De Bernardi, R. & Giussano, G. (1990) Are blue green algae a suitable food for zooplankton? An overview. *Hydrobiologia*, **200/201**, 29–44.
- Dugan, P. (1994) *Wetland Conservation: A Review of Current Issues and Required Action*. IUCN, Gland, Switzerland.
- Falkowski, P. (2002) On the evolution of the carbon cycle. *Phytoplankton Productivity. Carbon Assimilation in Marine and Freshwater Ecosystems* (eds P.J. le B. Williams, D.N. Thomas & C.S. Reynolds), pp. 318–349. Blackwell Science, Oxford, UK.
- Fox, J.W. & Morin, P.J. (2001) Effects of intra- and interspecific interactions on species responses to environmental change. *Journal of Animal Ecology*, **70**, 80–90.
- George, D.G. (2002) Regional-scale influences on the long-term dynamics of lake plankton. *Phytoplankton Productivity. Carbon Assimilation in Marine and Freshwater Ecosystems* (eds P.J. le B. Williams, D.N. Thomas & C.S. Reynolds), pp. 265–290. Blackwell Science, Oxford, UK.
- Hostetler, S.W. & Small, E.E. (1999) Response of North American freshwater lakes to simulated future climates. *Journal of the American Water Resources Association*, **35**, 1625–1637.
- Houghton, J.E.T., Ding, Y., Griggs, D.J., Noguer, M., van der Linden, P.J., Dai, X., Maskell, K. & Johnson, C.A. (2001) *Climate Change 2001. The Scientific Basis*. Cambridge University Press, Cambridge, UK.
- Houghton, J.T., Meira Filho, L.G., Callander, B.A., Harris, N., Kattenberg, A. & Maskell, K. (1996) *Climate Change 1995. The Science of Climate Change. Contribution of Working Group I to the Second Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, UK.
- Hutchinson, G.E. (1967a) The paradox of the plankton. *American Naturalist*, **95**, 137–147.
- Hutchinson, G.E. (1967b) *A Treatise on Limnology*, Vol. 2. Wiley, New York, NY.
- Jensen, J.P., Jeppesen, E., Olrik, K. & Kristensen, P. (1994) Impact of nutrients and physical factors on the shift from cyanobacterial to chlorophyte dominance in shallow lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, **51**, 1692–1699.
- Jeppesen, E., Sondergaard, Ma., Sondergaard, Mo. & Christoffersen, K. (1997) *The Structuring Role of Submerged Macrophytes in Lakes*. Ecological Studies No. 131. Springer, New York, NY.
- Ludwig, J.A. & Reynolds, J.F. (1988) *Statistical Ecology. A Primer on Methods and Computing*. John Wiley and Sons, New York, NY.
- Lund, J.W.G. (1964) Primary productivity and periodicity of phytoplankton. *Verhandlungen Internationale der Vereinigung Theoretische und Angewandte Limnologie*, **15**, 37–56.
- Lund, J.W.G. (1965) The ecology of freshwater phytoplankton. *Biological Reviews*, **40**, 231–293.
- Lund, J.W.G. (1971) The seasonal periodicity of three planktonic desmids in Lake Windermere. *Mitteilungen Internationale der Vereinigung Theoretische und Angewandte Limnologie*, **19**, 3–25.
- McKee, D., Atkinson, D., Collings, S.E., Eaton, J.W., Gill, A.B., Harvey, I., Hatton, K., Heyes, T., Wilson, D. & Moss, B. (2003) Response of freshwater microcosm communities to nutrients, fish and elevated temperature during winter and summer. *Limnology and Oceanography*, **48**, 707–722.
- McKee, D., Atkinson, D., Collings, S., Eaton, J., Harvey, I., Hatton, K., Heyes, T., Wilson, D., Wolstenholme, L. & Moss, B. (2000) Heated aquatic microcosms for climate change experiments. *Freshwater Forum*, **14**, 51–58.
- McKee, D., Atkinson, D., Collings, S., Eaton, J., Harvey, I., Heyes, T., Hatton, K., Wilson, D. & Moss, B. (2002a) Macro-zooplankter responses to simulated climate warming in experimental freshwater microcosms. *Freshwater Biology*, **47**, 1557–1570.
- McKee, D., Hatton, K., Eaton, J.W., Atkinson, D., Atherton, A., Harvey, I. & Moss, B. (2002b) Effects of simulated climate warming on macrophytes in freshwater microcosm communities. *Aquatic Botany*, **74**, 71–83.
- Melack, J.M., Dozier, J., Goldman, C.R., Greenland, D., Milner, A.M. & Naimana, R.J. (1997) Effects of climate change on inland waters of the Pacific Coastal Mountains and Western Great Basin of North America. *Hydrological Processes*, **11**, 971–992.
- Moss, B. (1973a) The influence of environmental factors on the distribution of freshwater algae: an experimental study. II. The role of pH and the carbon dioxide–bicarbonate system. *Journal of Ecology*, **61**, 157–177.

- Moss, B. (1973b) The influence of environmental factors on the distribution of freshwater algae: an experimental study. III. Effects of temperature, vitamin requirements and inorganic nitrogen compounds on growth. *Journal of Ecology*, **61**, 179–192.
- Moss, B. (1996) A land awash with nutrients – the problem of eutrophication. *Chemistry & Industry*, 3 June 1996, 407–411.
- Moss, B. (1999) From algal culture to ecosystem, from information to culture. *European Phycological Journal*, **34**, 193–204.
- Moss, B. & Balls, H.R. (1989) Phytoplankton distribution in a temperate floodplain lake and river system. II. Seasonal changes in the phytoplankton communities and their control by hydrology and nutrient availability. *Journal of Plankton Research*, **11**, 841–869.
- Moss, B., Madgwick, J. & Phillips, G. (1996) *A Guide to the Restoration of Nutrient-Enriched Shallow Lakes*. Broads Authority, Environment Agency and EU Life Programme, Norwich, UK.
- Petchey, O.L., McPhearson, P.T., Casey, T.M. & Morin, P.J. (1999) Environmental warming alters food-web structure and ecosystem function. *Nature*, **402**, 69–72.
- Pouria, S., De Andrade, A., Barbosa, J., Cavalcanti, R.L., Barreto, V.T.S., Ward, C.J., Preiser, W., Poon, G.K., Neild, G.H. & Codd, G.A. (1998) Fatal microcystin intoxication in haemodialysis unit in Caruaru, Brazil. *The Lancet*, **352**, 21–26.
- Reynolds, C.S. (1997) *Vegetation Processes in the Pelagic. A Model for Ecosystem Theory*. Ecology Institute, Oldendorf/Luhe, Germany.
- Reynolds, C.S., Huszar, V., Kruk, C., Naselli-Flores, L. & Melo, S. (2002) Towards a functional classification of the freshwater phytoplankton. *Journal of Plankton Research*, **24**, 417–428.
- Reynolds, C.S., Irish, A.E. & Elliott, J.A. (2001) The ecological basis for simulating phytoplankton responses to environmental change (PROTECH). *Ecological Modelling*, **140**, 271–291.
- Schindler, D.W. (1998) Replication versus realism: the need for ecosystem-scale experiments. *Ecosystems*, **1**, 323–334.
- Schmitz, O.J. (2003) Top predator control of plant biodiversity and productivity in an old-field ecosystem. *Ecology Letters*, **6**, 156–163.
- Seip, K.L. & Reynolds, C.S. (1995) Phytoplankton functional attributes along trophic gradient and season. *Limnology and Oceanography*, **40**, 589–597.
- Seip, K.L., Sneek, M. & Snipen, L.G. (1994) How far do physical factors determine phytoplankton biomass in lakes? *Chemometrics and Intelligent Laboratory Systems*, **23**, 247–258.
- Shapiro, J. (1990) Current beliefs regarding dominance by blue-greens: the case for the importance of CO<sub>2</sub> and pH. *Verhandlungen Internationale der Vereinigung Theoretische und Angewandte Limnologie*, **24**, 38–54.
- Stephen, D., Alfonso, T., Balayla, D., Bécars, E., Collings, S.E., Fernández-Aláez, C., Fernández-Aláez, M., Ferriol, C., García, P., Goma, J., Gyllström, M., Hansson, L.-A., Hietala, J., Kairesalo, T., Miracle, M.R., Rueda, J., Romo, S., Ståhl-Delbanco, A., Svensson, M., Vakkilainen, K., Valentín, M., Van de Bund, W.J., Van Donk, E., Vicente, E., Villena, M.J. & Moss, B. (2004) Shallow wetland lake functioning along a climate gradient – introduction to a pan-European international mesocosm experiment. *Freshwater Biology*, in press.
- Talling, J.F. (1986) The seasonality of phytoplankton in African lakes. *Hydrobiologia*, **138**, 139–160.
- Terborgh, J. (1992) Maintenance of diversity in tropical forests. *Biotropica*, **24**, 283–292.
- Uhlmann, D. (1979) *Hydrobiology*. John Wiley, New York, NY.
- Williams, A.E. & Moss, B. (2003) Effects of different fish species and biomass on plankton interactions in a shallow lake. *Hydrobiologia*, **491**, 331–346.
- Williams, A.E., Moss, B. & Eaton, J. (2002) Fish-induced macrophyte loss in shallow lakes: top-down and bottom-up processes in mesocosm experiments. *Freshwater Biology*, **47**, 2216–2232.

Received 6 March 2003; final copy received 23 June 2003