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The impact of pH on interactions among phytoplankton algae, zooplankton and perch (*Perca fluviatilis*) in a shallow, fertile lake

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SUMMARY

1. The combined effects of increasing pH (< 9–11) and increasing densities of perch (0, two, four per enclosure) have been investigated in polyethylene enclosures of volume about 1100 l in a shallow, fertile lake in Cheshire.
2. Increasing pH decreased carbon dioxide concentrations, chlorophyll *a* concentrations and diatom biomass. It led to increases in the proportions of Cryptophyta (to pH 10) and Chlorophyta (to pH 11). Although ample inocula were present, significant growths of cyanophytes were not recorded, contrary to expectation.
3. Increasing pH led to increases in numbers of *Daphnia hyalina* particularly at pH 10 but collapse at pH 11. This persistence at pH 10, independent of perch number, is attributed to reduced feeding of the fish, which survived at this pH. Other zooplankters (*Ceriodaphnia* spp., *Bosmina longirostris*, *Polyphemus pediculus*, *Cyclops* spp.) all declined with increasing pH.
4. Increasing fish density resulted in an increase in chlorophyll *a* concentration and in the biomass of Chlorophyta but had no effect on cyanophyte or cryptophyte biomass.
5. Increasing fish density led to declines in *Daphnia* in the untreated lake-water controls (pH < 9) and at pH 9, but had no effect at pH 10 where *Daphnia hyalina* was very abundant. Numbers of *Ceriodaphnia* spp. and *Bosmina longirostris* increased with increasing fish density, whilst numbers of *Polyphemus pediculus* and *Cyclops* spp. were independent of it.
6. The results are discussed in the light of contemporary knowledge of the factors resulting in large cyanophyte growths and of the factors controlling the stability of macrophyte-dominated and phytoplankton-dominated states in shallow lakes. The interaction between pH and fish density and consequent non-linear response of *Daphnia hyalina* is particularly notable because of the significance of zooplankton grazing in shallow lakes.

Introduction

Major changes in hydrogen ion concentration have far-reaching effects on the communities of low-conductivity, poorly buffered waters. A large literature exists on the problem of recent acidification in upland igneous and metamorphic rock catchments (e.g. Hall *et al.*, 1980). Very high hydrogen ion concentrations (pH < about 4.5) cause large reductions in diversity through a variety of mechanisms. Equally, very high pH values in soda lakes (pH > 10) also create extreme conditions associated with low diversity (Jenkin, 1936).

In aquatic systems within these extremes, pH receives less attention, possibly being eclipsed in the interpretation of mechanisms and consequences in such waters by strong influences of nutrient loading and predation. However, there is a myriad of ways in which pH may interact with these agents of acknow-

ledged importance. It determines the speciation of inorganic carbon, leading to differential proportions of free CO_2 and bicarbonate (Hutchinson, 1957), which may influence the specific composition of algal (Moss, 1973 ; Shapiro, 1973; Talling, 1976; Peslova, Pokorny & Komarek, 1990) and plant (Madsen & Sand-Jensen, 1991) communities; it influences the solubility of trace metals (Stumm & Morgan, 1981); it may determine the behaviour of invertebrates (O'Brien & de Noyelles, 1972; Hansen, Christensen & Sortkjaer, 1991) and the habitat preferences of fish (Serafy & Harrell, 1993). Moreover, large diurnal changes in pH associated with the photosynthetic activity of plant beds may produce a mosaic of pH domains within a water body that might determine patterns of predation and zooplankton grazing. Superimposed on these complexities may be pH changes consequent on long-term changes such as eutrophication or lake restoration. Although there is an increasing literature on the interactions between nutrient loading, zooplankton grazing and fish predation on zooplankters and their part in determining the operation of plankton-based systems, there remain many details to be explained, perhaps by factors interacting with these processes. Ultimately this paper concerns the potential role of pH in these interactions in shallow lakes. It also concerns the potential links between incidence of cyanophytan blooms and the speciation of inorganic carbon mediated through pH in both shallow and deep lakes. Nowhere is the complexity of community interactions greater than in shallow, macrophyte-dominated freshwaters, where the structural complexity is great and where nutrient loads have often changed markedly as a result of eutrophication. Macrophyte-dominated lakes appear to be able to resist the effects of increased nutrient loading through a variety of buffering mechanisms (Timms & Moss 1984; Balls, Moss & Irvine, 1989; Irvine, Moss & Balls, 1989; Moss, 1991), which include provision of refuges for crustacean grazers of open-water phytoplankton against predation by fish, and allow maintenance of clear water and a suitable light climate for plant growth, despite phosphorus and nitrogen concentrations capable of supporting dense algal populations. Over a similar range of nutrient concentrations, phytoplankton communities can dominate as an alternative stable state with few or no macrophytes and little zooplankton grazing potential (Moss, 1991; Scheffer *et al.*, 1993). pH may have a major role to play in influencing the behaviour of both fish and zooplankton, through pH changes induced by plant uptake in macrophyte-dominated lakes and algal uptake in phytoplankton-dominated ones. The possible stimulation of cyanophytan growth by increasing pH (Shapiro, 1973, 1990) may be particularly germane to the outcome of fish–grazer–phytoplankton interactions.

Many shallow lakes in Europe have lost their macrophyte communities as a result of eutrophication and the process of restoring these communities is proving difficult (Sas, 1989). Reduction of nutrient load alone seems inadequate in most cases and additional measures prove necessary. Collectively these constitute restoration of the buffer mechanisms that stabilize the macrophyte community and often involve manipulation of the fish community (Moss, 1992). Sometimes they are successful for a time then fail. Current knowledge of the mechanisms is presently inadequate to predict the effects of a given management initiative with any certainty, and this underlines the need to investigate the mechanisms in much greater detail. Furthermore in lake systems of lesser structural complexity there is still considerable uncertainty about the mechanisms that lead to particular types of algal community at high nutrient concentrations because many potentially influential factors also change consequent to the increased nutrient loading and establishment of the symptoms of eutrophication. The reason for the occurrence of large crops of Cyanophyta in some situations, but not in others, is a case in point.

An opportunity to investigate the interaction of pH with fish predation on zooplankton and on the consequent phytoplankton community has been presented by management carried out at a small shallow lake in Cheshire, called Little Mere (area 2.8 ha, z_{max} 2.6 m, z_{mean} 0.7 m). The lake lies below a larger lake, Mere Mere which delivers water to it and from which it is separated by a sluice. Mere Mere is a naturally fertile lowland lake with moderately high nutrient loading and an algal community in which cyanophytes are prominently represented in summer. Little Mere was, until 1991, considerably more nutrient-enriched by sewage effluent than Mere Mere and is more nutrient-rich still (Carvalho, Beklioğlu & Moss, in press).

Before 1991, a sewage-treatment works discharged effluent into Little Mere, whose water comprised little diluted effluent during the summer (Carvalho, 1994). It had a negligible fish population because of low

oxygen concentrations, although fish could move into the lake from Mere Mere upstream. Its zooplankton community was dominated by *Daphnia magna* Straus and the phytoplankton population in summer was negligible because of grazing by *Daphnia* (Carvalho, 1994). The water was very clear and there was a flourishing macrophyte community of nymphaeids, *Potamogeton berchtoldii* Fieber and *Elodea canadensis* Michaux. The algal growth potential of the water was very high, with mg l^{-1} quantities of ammonium and soluble reactive phosphorus. pH values were in the range 7.3–9.0, presumably kept lower than the potential created by the fertility of the effluent, by the decomposition of organic matter suspended in it. When phytoplankton algae grew in the spring they were diatoms. *Volvox* and cryptomonads constituted the negligible summer community. Cyanophytes were near absent.

After June 1991, when the sewage effluent was diverted elsewhere, the lake began to change (Carvalho *et al.*, in press). Oxygen concentrations rose, fish (predominantly perch, *Perca fluviatilis* L.), moved in from upstream, and although the N and P concentrations fell markedly they remained higher than in Mere Mere. In summer 1993, mean total phosphorus was 253 μg l^{-1} in Little Mere and 82 μg l^{-1} in Mere Mere. The former Little Mere environment had remained a clear-water macrophyte-dominated system because of the absence of fish predation. Now that fish were able to return and that pH was likely to rise as the residual organic matter completed its decomposition, we anticipated an increase in fish predation, reduced grazing and a move in the phytoplankton community towards the cyanophyte dominance of upstream Mere Mere.

In turn we expected the increasing predominance of cyanophytes to disfavour grazer control and events to lead to a breakdown of the buffers that had stabilized the macrophyte community, with consequent phytoplankton dominance. We have taken advantage of the lake situation to examine experimentally how the buffer mechanisms might be operating by creating, in mesocosms, conditions that the literature led us to expect would lead to cyanophyte dominance, and thus to test hypotheses summarized by Shapiro (1990).

We simulated increasing pH within a high-nutrient environment in which we created conditions of increasing predation on the zooplankters. Specifically, we hypothesized that increasing pH would increase the dominance of cyanophytes within the phytoplankton community, that increasing fish predation would increase the biomass of the phytoplankton community, with increasing predominance also of cyanophytes; and that number of *Daphnia* would decline with increasing fish predation, irrespective of pH.

Methods

Experimental design

The experiment was carried out between 16 July and 10 August 1993, in thirty-six polyethylene enclosures. Each enclosure (diameter 1 m, depth 1.5 m, volume 1100 l) was made of thin (125-μm wall thickness), clear and colourless polyethylene film formed into a cylindrical tube that was sealed at the bottom and open to the atmosphere at the top. The enclosures were placed in the middle of the lake, where the depth was 1 m, and were filled with water pumped from a depth of 0.5 m. They were suspended from a wooden raft, which was anchored to the lake bottom and buoyed up with air-filled plastic bottles, which kept the open ends of the enclosures 20 cm above the water surface so as to prevent exchange with the lake water. The placement of the treatments and replicates was randomized within the wooden frames.

The experimental design was 3 × 4 factorial with different fish densities and raised pH. Four pH values were employed, including the lake water pH as a control and pH 9, 10 and 11. The pH was increased using 1 M NaOH, the appropriate initial amount of NaOH being determined by titration of the lake water. In order to maintain the desired value, a pH check was carried out every other day throughout the experiment using a portable pH-meter and further amounts of NaOH were added if necessary. During this pH adjustment, the enclosure water was vigorously mixed with an oar. Perch, *Perca fluviatilis*, 9–10 cm total length, were caught by seining in Little Mere and immediately placed in designated enclosures in three different population densities, 0, two and four fish per enclosure. Each of the twelve treatments was carried out in triplicate, making a total of thirty-six enclosures.

Sampling methods

The first sample was taken immediately after the treatments had been initiated. Temperature and dis-

solved oxygen concentrations were measured using a WTW oxygen-meter to a precision of ± 1%. Sampling of the enclosures was carried out each week from the upper 1 m of water. Before sampling, the water in the enclosures was stirred using an oar. Samples for chemical analyses and phytoplankton were collected using a 1-m-long plastic-tube sampler. Water for chemical analyses was stored in acid-washed 1-l Pyrex bottles. Soluble reactive phosphorus (SRP), free-carbon dioxide concentrations and total alkalinity were determined according to Mackereth, Heron & Talling (1978) to precisions of ± 3%, ± 10% and ± 5%, respectively. Ammonium-nitrogen was determined according to Chaney & Morbach (1962) to a precision of ± 4%. Chlorophyll *a* was extracted in acetone, and concentration was calculated from the absorbance reading at 663 nm (Talling & Driver, 1961) to a precision of ± 5%.

Phytoplankton samples were preserved with Lugol's solution immediately after sampling and examined and counted to a precision of ± 20% with an inverted microscope (Wild M40) at a magnification of 400×. Biovolumes were determined from measurement of the linear dimensions of ten preserved cells of each taxon, using formulae for the appropriate geometric shapes (Wetzel & Likens, 1991). Biovolume density of each species ($\mu m^3\ l^{-1}$) was determined by multiplying average cell volume by cell population density. Community biovolume density was obtained by summing values for all species.

For zooplankton sampling, 10 l of water were taken using the tube sampler and filtered through a 63-μm-mesh net. The zooplankton samples were narcotized with chloroform water (Gannon & Gannon, 1975) and preserved in 4% formaldehyde solution. Samples were subsampled, and counted under a Kyowa stereomicroscope. When samples were subsampled at least 100 of the commonest species were counted (Bottrell *et al.*, 1976).

The demographic responses of *Daphnia hyalina* Leydig to increased pH and fish predation treatments were assessed using the egg-ratio method (Edmondson, 1971; Paloheimo, 1974). *E*, the egg ratio (total number of eggs per total number of females) was employed to calculate instantaneous per capita birth rate (*b*) as:

$$b = \ln (1 + E)/D \quad (1)$$

where *D* is the time required for eggs to develop, from the time an egg is laid to the time it is released as a free-swimming individual. *D* is a function of temperature (Downing & Rigler, 1984) and was calculated from measured temperature and a Krogh curve (Edmondson, 1971).

The instantaneous per capita rate of increase (*r*) was estimated from equation (2) where N_o and N_t are the population sizes at time 0 and time *t*, respectively.

$$r = (\ln_t - \ln_0)/t \quad (2)$$

The instantaneous per capita death rate (*d*) was estimated as:

$$d = b - r \quad (3)$$

r, *b* and *d* were calculated for each sampling interval (6–7 days).

Statistical analyses

The effects of treatments on water chemistry, phytoplankton and zooplankton populations were assessed using two-way ANOVA with repeated measures (Winer, 1971). All analyses used the Statistical Analysis System General Linear Model (GLM) routine (SAS Institute Inc., 1988). All dates except the initial sampling date were used in the analyses. To check for normality in the data, plots of fitted values in the ANOVA model against error terms were examined; when there was a noticeable heterogeneity of error variance, log transformations were employed. To assess whether initial conditions were similar among the groups of enclosures to be used as treatments, one-way ANOVA were performed.

Results

Initial status of enclosures

pH values in the enclosures remained close to the adjusted values (± 0.1–0.2 unit) throughout the experiment. In all pH 11 enclosures, the fish died soon after introduction (although the carcasses were left *in situ*) and zooplankton and algal growth were negligible until day 20. All fish survived in all other enclosures.

One-way ANOVA performed on the initial chlorophyll *a* concentrations, cyanophyte, chlorophyte, and diatom biovolumes and population densities of *Daphnia hyalina*, *Polyphemus pediculus* (L.) and *Bosmina longirostris* (O.F. Müller) s. str. revealed no significant differences among the group of enclosures.

Because of punctures made by a trapped duck in three of the polyethylene enclosures during the experiment, results from two of the pH 10 and one of the pH 11 enclosures were omitted from the subsequent analyses. All other enclosures remained intact throughout.

Response of chemical variables

Table 1 shows mean values (± SD) of major chemical and biological variables during the experiment. Repeated measures of ANOVA performed on the water chemistry showed that increasing pH, fish densities and pH−fish interaction treatments had no significant effects on the SRP concentration ($P = 0.06$, 0.5 and 0.2, respectively), and NH_4-N concentrations ($P = 0.66$, $P = 0.38$ and $P = 0.19$, respectively; Table 2). pH had significant effects on the free-CO_2 concentration ($P = 0.004$) which decreased with increasing pH value, and declined to zero in pH 10 and pH 11 enclosures. Increasing the number of fish had no significant effect on the CO_2 concentration ($P = 0.07$) whilst the interaction of pH and fish significantly decreased the free-CO_2 concentration ($P = 0.015$; Table 2).

Response of chlorophyll a *and phytoplankton biovolumes*

Repeated measures of ANOVA performed on chlorophyll *a* concentrations, revealed a highly significant effect of increasing pH and fish densities ($P = 0.0001$, $P = 0.0002$, respectively; Table 2). Concentrations increased especially in control, pH 9 and pH 10 enclosures at the highest fish densities (Table 1). There was a lesser effect at pH 11. The chlorophyll *a* concentration increased particularly after day 12 for control, pH 9 and pH 10 treatments at two- and four-fish densities (Table 1). In pH 11 enclosures, chlorophyll *a* concentration began to increase after day 20, giving a relatively low mean of 15 $\mu g\ l^{-1}$. There was a significant decline in chlorophyll *a* concentration with increasing pH (Table 2). No significant interaction effect of pH and fish density was found for chlorophyll *a* concentration (Table 2).

Cyanophytes, although present, did not dominate the phytoplankton community, either at the start or during the experiment. Increasing pH significantly decreased cyanophyte biovolume ($P = 0.02$; Table 2). The highest biovolume of cyanophytes was found in pH 9 enclosures on day 7 when the overall mean value was $1.4 \times 10^6\ \mu m^3\ ml^{-1}$ but overall (Table 3), it was a small percentage (17.3%) of the total phytoplankton biovolume. Cyanophyta biovolume was very low in control and pH 10 enclosures (Table 1). At pH 11, cyanophytes were not recorded during the experiment. *Anabena* spp., *Oscillatoria agardhii* (Gom.) and *Microcystis aeruginosa* Kutz. emend. Elenkin were the species of Cyanophyta variously present. Fish density and the pH−fish interaction had no significant effect on the biovolume of cyanophytes (Table 2).

Chlorophyta were not the predominant phytoplankters at the beginning of the experiment, but their biovolume increased during it. Repeated measures of ANOVA performed on Chlorophyta biovolumes revealed significant increases with increasing pH and fish density ($P = 0.0002$ and $P = 0.04$, respectively) (Table 2). After day 7, biovolumes of Chlorophyta increased consistently with increasing pH and fish densities. At pH 11 in the four-fish treatment after day 20, the biovolume of Chlorophyta started to increase and reached $1.3 \times 10^7\ \mu m^3\ ml^{-1}$, the highest recorded Chlorophyta biovolume. Interaction of pH and fish had no significant effects on biovolumes of Chlorophyta ($P = 0.11$; Table 2). In all treatments the increase in Chlorophyta biovolume was mainly due to *Chlamydomonas* spp., *Chlorella ellipsoidea* Gerneck, and *Gloeocystis major* Gerneck. Repeated-measures ANOVA employed on these species revealed that their biovolumes were significantly increased with increasing pH ($P = 0.01$, 0.0005 and 0.02, respectively) and fish densities ($P = 0.0035$, 0.003 and 0.05, respectively). The biovolume of *Chamydomonas* spp. showed a greater increase from day 20 in pH 11, four-fish-enclosures than in others ($1 \times 10^7\ \mu m^3\ ml^{-1}$). In pH 10, four-fish-enclosures, *Chlorella ellipsoidea* was the most abundant chlorophytan, with a biovolume of $1.4 \times 10^7\ \mu m^3\ ml^{-1}$, after day 12 which contributed much towards the chlorophyll *a* concentration of 255 $\mu g\ l^{-1}$, the highest recorded throughout the experiment. The highest biovolume of *Gloeocystis major* was recorded in pH 11, four-fish enclosures after day 20, and was $6.1 \times 10^6\ \mu m^3\ ml^{-1}$. The interaction effects of pH and fish were significant for *Chlamydomonas* spp. ($P = 0.033$) but not significant for either *Chlorella ellipsoidea* or *Gloeocystis major.*

The biovolume of Cryptophyta was proportionately high in the phytoplankton communities from the start of the experiment until day 20. Repeated-measures

Table 1 Mean values with standard deviations for variables measured across all dates after the initial sampling in an experiment carried out in enclosures in Little Mere, Cheshire in summer 1993. Algal volumes are given in millions of $\mu m^3 l^{-1}$ and zooplankton in numbers per litre

pH treatment	Control			pH 9			pH 10			pH 11		
No. of perch	0	2	4	0	2	4	0	2	4	0	2	4
No. of observations	12	12	12	12	12	12	12	8	8	12	8	12
pH	8.3 ± 0.6	8.9 ± 0.6	8.95 ± 0.4	9.2 ± 0.1	9 ± 0.2	9.1 ± 0.2	9.5 ± 0.2	9.9 ± 0.3	10.1 ± 0.2	11 ± 0.2	11 ± 0.2	11 ± 0.2
Chl *a* ($\mu g l^{-1}$)	5.2 ± 4.7	54 ± 64	57 ± 44	7 ± 11	28 ± 15	35 ± 34	4 ± 5	31 ± 43	78 ± 110	1 ± 1	7 ± 8	15 ± 21
Sol. reactive P($\mu g l^{-1}$)	103 ± 117	20 ± 45	6 ± 9	44 ± 25	131 ± 220	33 ± 50	28 ± 18	59 ± 54	93 ± 107	127 ± 132	272 ± 265	299 ± 327
NH_4-N ($\mu g l^{-1}$)	349 ± 499	60 ± 114	74 ± 102	52 ± 41	266 ± 227	159 ± 265	35 ± 33	146 ± 141	438 ± 748	134 ± 128	271 ± 265	398 ± 388
Free-CO_2($ng l^{-1}$)	39 ± 47	5.5 ± 7	4 ± 6	2 ± 1	3 ± 1	2.6 ± 1	0	0.25 ± 0.7	0	0	0	0
Cyanophyta	0.07 ± 0.2	0.2 ± 0.8	0.3 ± 0.7	0.004 ± 0.003	1.7 ± 4.4	2.4 ± 4.6	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0	0	0
Chlorophyta	0.7 ± 1	3 ± 4	9 ± 9	0.3 ± 3.6	1.2 ± 0.9	2 ± 3	0.5 ± 0.7	1.9 ± 1.5	7.8 ± 15	0.5 ± 1	13 ± 30	2.6 ± 4
Chlorella elipsoidea	0.8 ± 0.9	0.6 ± 1.7	0.5 ± 0.7	0	0.06 ± 0.09	0.1 ± 0.4	0.02 ± 0.04	0.2 ± 0.4	5 ± 10	0	0	0.3 ± 0.7
Gloeocystis major	0.03 ± 0.06	0.2 ± 0.4	0.03 ± 0.1	0.002 ± 0.003	0.01 ± 0.04	0.01 ± 0.03	0.001 ± 0.002	0.07 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.09 ± 0.01	0.23 ± 0.37
Cryptophyta	1.3 ± 2	1 ± 1	3 ± 2	4 ± 9	8 ± 16	3 ± 5	4 ± 8	14 ± 25	17 ± 30	0.1 ± 0.1	0.25 ± 0.5	0.7 ± 1.3
Cryptomonas ovata	0.7 ± 1	0.7 ± 1	2 ± 2	4 ± 8	8 ± 15	2 ± 5	3 ± 7	13 ± 25	16 ± 30	0.1 ± 0.1	0.2 ± 0.5	0.7 ± 1
Rhodomonas minuta	0.01 ± 2	0.3 ± 0.3	0.4 ± 0.4	0.3 ± 0.7	0.3 ± 0.4	0.3 ± 1	0.6 ± 0.4	2 ± 2	1 ± 2	0.01 ± 0.02	0.01 ± 0.01	0.002 ± 0.005
Bacillariophyta	0.8 ± 1	1.5 ± 2.7	2.6 ± 4	0.4 ± 0.9	0.8 ± 0.8	0.8 ± 0.8	0.06 ± 0.09	0.3 ± 0.4	0.3 ± 0.4	0.03 ± 0.05	0.02 ± 0.03	0.3 ± 0.7
Synedra ulna	0.02 ± 0.05	0.02 ± 0.05	0.09 ± 0.2	0.2 ± 0.4	0.1 ± 0.4	0.2 ± 0.3	0.03 ± 0.03	0.2 ± 0.3	0.2 ± 0.4	0	0	0
Aulacoseira granulata	0.4 ± 0.8	0.7 ± 1.9	1 ± 1.8	0.04 ± 0.01	0.4 ± 0.7	0.6 ± 0.1	0.01 ± 0.17	0	0.03 ± 0.06	0.01 ± 0.05	0	0
Daphnia hyalina	85 ± 112	4 ± 4	4.2 ± 11	101 ± 93	34 ± 69	8 ± 6	105 ± 69	181 ± 252	236 ± 333	14 ± 33	4 ± 5	9 ± 24
Polyphemus pediculus	66 ± 89	130 ± 141	104 ± 120	69 ± 84	96 ± 154	157 ± 154	19 ± 24	20 ± 11	20 ± 19	0.4 ± 1	0.6 ± 1	0.9 ± 1
Bosmina longirostris	2 ± 3	6 ± 10	64 ± 106	0.3 ± 0.7	1.3 ± 2.3	13 ± 31	4 ± 10	0	5 ± 11	0	0.1 ± 0.4	0.5 ± 1.4
Ceriodaphnia spp.	27 ± 70	66 ± 156	91 ± 169	0.3 ± 0.8	44 ± 63	41 ± 110	0.9 ± 1.8	1.5 ± 1.9	4.7 ± 6.8	0	0.1 ± 0.35	0
Cyclops spp.	11 ± 7	44 ± 73	99 ± 124	7 ± 5	32 ± 77	35 ± 39	9.5 ± 12	8.8 ± 8.8	8.9 ± 15	1.6 ± 2.9	1.3 ± 1.6	0.6 ± 1.2

Table 2 Summary of the effects of pH, fish and pH/fish interaction on the water chemistry,algal biovolume and zooplankton density in a series of enclosures in Little Mere following repeated measures of 2-way ANOVA. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; NS = no significance. +,− signs show the direction of the effects as increase or decrease with increasing pH or fish density

	pH	fish	pH/fish interaction
Chlorophyll *a* ($\mu g\ l^{-1}$)	− ***	+ ***	NS
Soluble reactive P($\mu g\ l^{-1}$)	NS	NS	NS
NH_4-N ($\mu g\ l^{-1}$)	NS	NS	NS
Free-CO_2 ($\mu g\ l^{-1}$)	−**	NS	−*
Cyanophyta ($\mu m^3\ ml^{-1}$)	−*	NS	NS
Chlorophyta ($\mu m^3\ ml^{-1}$)	+***	+*	NS
Chlamydomonas spp. ($\mu m^3\ ml^{-1}$)	+**	+**	+*
Chlorella ellipsoidea ($\mu m^3\ ml^{-1}$)	+***	+**	NS
Gleocystis major ($\mu m^3\ ml^{-1}$)	+*	+*	NS
Cryptophyta ($\mu m^3\ ml^{-1}$)	+**	NS	NS
Cryptomonas ovata ($\mu m^3\ ml^{-1}$)	+*	NS	NS
Rhodomonas minuta ($\mu m^3\ ml^{-1}$)	+**	NS	NS
Diatoms ($\mu m^3\ ml^{-1}$)	−**	NS	NS
Synedra ulna ($\mu m^3\ ml^{-1}$)	−*	NS	NS
Aulacoseira granulata ($\mu m^3\ ml^{-1}$)	−**	NS	NS
Daphnia hyalina (ind. l^{-1})	+***	±***	± **
Polyphemus pediculus(ind. l^{-1})	−***	NS	NS
Ceriodaphnia spp. (ind. l^{-1})	−***	+**	NS
Bosmina longirostris(ind. l^{-1})	−**	+*	NS
Cyclops spp. (ind. l^{-1})	−*	NS	NS

Table 3 Composition of the phytoplankton community (Mean% of total biovolume ± SD) with increasing pH in experimental enclosures in Little Mere

	Control	pH 9	pH 10	pH 11
Cyanophyta	2.5 ± 5	17.3 ± 20	0.9 ± 0.6	0
Chlorophyta	55.0 ± 53	14.7 ± 11	22.0 ± 27	89.6 ± 92
Cryptophyta	21.2 ± 17	59.5 ± 62	76.2 ± 70	7.8 ± 5
Bacillariophyta	21.3 ± 24	8.5 ± 5	1.3 ± 1.1	2.4 ± 2.8

ANOVA showed a significant change in biovolume with pH whilst increasing fish densities, and the interaction of pH and fish were not significant (P = 0.01, 0.31 and 0.49; Table 2). The increase in Cryptophyta biovolume was greater at pH 9 and pH 10 than in the control and pH 11 (Table 1). Cryptophyta were represented largely by *Cryptomonas ovata* Ehrenberg and *Rhodomonas minuta* Lewis, whose biovolumes significantly increased with increased pH, except at pH 11 (P = 0.023 and 0.004, respectively; Table 2). Increasing fish density did not significantly change the biovolumes of these species, nor were the interaction effects significant.

Repeated-measures ANOVA revealed that increasing pH had significant negative effects on biovolume of diatoms (P = 0.0002; Table 2). The effect of increasing fish densities on biovolume of diatoms was not quite significant at the 5% level (P = 0.057). The biovolumes of *Synedra ulna* (Nitzsch) Ehrenb. and *Aulacoseira granulata* (Ehrenb.) Simonsen made the greatest contributions to the total biovolume of diatoms and increasing pH significantly decreased their biovolumes (P = 0.022 and 0.002, respectively; Table 2). In the control, pH 9 and pH 10 enclosures, biovolumes of *Synedra ulna* were similar but *Synedra ulna* was absent from pH 11 enclosures. There was a gradual decrease with increasing pH for *Aulacoseira granulata* biovolumes but neither the fish density nor the interaction effect of pH and fish density significantly changed biovolumes of *Synedra ulna* or *Aulacoseira granulata*.

In summary (Table 3), the effects of increasing pH on percentage biovolumes of the main phytoplankton groups were that throughout the experiment Cyanophyta, although present, were never prominent. The highest biovolume of Cyanophyta was recorded in pH 9 enclosures, but the biovolume decreased dramatically at pH 10 and declined to zero in pH 11 enclosures. Chlorophyta were predominant in the control and at pH 11. The biovolume of Cryptophyta was also considerable, and highest at pH 9 and pH 10, but sharply decreased at pH 11. Diatoms were never dominant in the algal community and their biovolume decreased gradually with increasing pH.

Response of the zooplankton community

Several zooplankton species responded to the manipulation of pH and fish (Tables 1 and 2). In all treatments the filter-feeding component of the cladoceran community mainly comprised *Daphnia hyalina*, *Bosmina longirostris* and *Ceriodaphnia* spp. The copepods were solely *Cyclops* spp. Rotifers were rare and included *Keratella quadrata* Muller and *Keratella cochlearis* (Contd.). *Polyphemus pediculus*, an omnivorous raptorial cladoceran, was abundant in the zooplankton community.

The cladoceran, *Daphnia hyalina*, was the most abundant filter feeder, and probably of greatest importance with respect to grazing pressure. Repeated measures of ANOVA revealed that increasing pH, fish densities and interaction effects of pH and fish treatments all had significant effects on density of *D. hyalina* (P = 0.0001, 0.0001 and 0.005, respectively;

Table 4 Mean (± SD) instantaneous birth (*b*) and death (*d*) rates (per day) of *Daphnia hyalina* with respect to pH and fish treatments, and the effects of pH, fish and pH–fish interaction on birth and death rates of *D. hyalina* in enclosures at Little Mere. Results of repeated measures of ANOVA are shown as probability values. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; NS = not significant. +, − signs show the direction of the effects as increase or decrease with increasing pH and fish densities

	control			pH 9			pH 10					
Number of fish	0	2	4	0	2	4	0	2	4	pH	Fish	pH/fish interaction
Birth rate	0.26 ± 0.28	0.33 ± 0.50	0.05 ± 0.02	0.22 ±0.28	0.20 ± 0.25	0.29 ± 0.32	0.14 ± 0.20	0.15 ± 0.20	0.13 ± 0.14	NS	± *	± * *
Death rate	0.48 ± 0.2	0.31 ± 0.46	0.2 ± 0.07	0.3 ± 0.31	0.52 ± 0.29	0.32 ± 0.49	0.12 ± 0.21	−0.05 ± 0.17	0.25 ± 0.06	± ***	NS	± * * *

Table 2). With increasing pH, its density increased, except at pH 11 where *D. hyalina* was very sparse until day 20. However, even though it then increased, its numbers were low relative to the other treatments. *D. hyalina* numbers declined severely with increasing fish densities at the lowest pH values (Table 1) but in pH 10 enclosures the direction of the effect of increasing fish density on *D. hyalina* was reversed. Estimates of instantaneous per capita rate birth rate (*b*) and death rate (*d*) for all pH and fish treatments were made using the egg-ratio method (Table 4). Repeated measures of ANOVA on population variables of *D. hyalina* (Table 4) revealed that, while pH had no significant effect on birth rate, increasing fish densities and the interaction of pH and fish significantly decreased the birth rate in the control and pH 10 enclosures and significantly increased it at pH 9 ($P = 0.044$ and 0.005, respectively; Table 4). Increasing pH and the interactions of pH and fish density significantly decreased the death rate of *D. hyalina* between the control and pH 10 enclosures. Death rates due to pH and fish interaction increased significantly between the control and pH 9 ($P = 0.001$ and 0.0004), whilst the increasing fish densities had no overall effect on the death rate of *D. hyalina* (Table 4). In pH 11 enclosures, after day 22, *D. hyalina* began to increase, resulting in mean densities of fourteen, four and nine ind. l^{-1}, respectively, in the nominally increasing fish densities, although these were reflected only in the number of corpses present at pH 11.

Other cladocerans that responded significantly to the treatments were *Bosmina longirostris* and *Ceriodaphnia* spp. (Tables 1 and 2). Increasing pH significantly reduced *Bosmina longirostris* and *Ceriodaphnia* spp. numbers ($P = 0.003$ and 0.0001, respectively); increasing fish densities significantly increased the densities of these species ($P = 0.027$ and 0.016, respectively, Tables 1 and 2) but the interaction of pH and fish treatments had no significant effect on densities of these species (Table. 2).

Polyphemus pediculus was the predominant species in the zooplankton community throughout the experiment. Repeated measures of ANOVA revealed that increasing pH significantly decreased its density ($P = 0.019$; Table 2) but increasing fish density, and the interaction of pH and fish had no significant effect on *Polyphemus pediculus* (Table 2).

Repeated measures of ANOVA performed on the density of *Cyclops* spp. revealed that its density was significantly reduced by increasing pH ($P = 0.05$; Table 2). Fish density and the interaction between pH and fish had no significant effects on the density of *Cyclops* spp. (Table 2).

Discussion

The results of the experiment may be discussed in the contexts of the hypotheses initially posed. These were that increasing pH would increase the proportion of cyanophytes in the phytoplankton biomass; that increasing fish density would likewise increase the proportion of cyanophytes in the context of an increasing total phytoplankton biomass; and that numbers of *Daphnia* would decline with increasing fish density irrespective of pH change.

Increasing pH did not result in increasing proportions of Cyanophyta and resulted in decreasing total biovolumes of phytoplankton and decreasing concentrations of chlorophyll *a*. The reason for anticipation of increased amounts of cyanophytes arose from pro-

posals by Shapiro (1990). Shapiro outlined several current views relating sets of environmental factors to high biomasses of cyanophytes, including increased temperature, low light availability, low nitrogen to phosphorus ratios, ability to move between layers in stratified lakes, low vulnerability to grazing by zooplankters and the ability to thrive at very low carbon-dioxide concentrations. In all cases there was evidence of some relationship but the evidence pointed to the latter circumstance, the availability of carbon dioxide, as a master variable with which the others might be directly or secondarily linked. Cyanophytes in general have lower K_s values for carbon dioxide uptake than other algae, have demonstrated an ability to continue photosynthesis at the high pH values (to 11) found in some highly fertile lakes, have lower carbon dioxide compensation points than other algae, and a high ability to concentrate carbon dioxide from low concentrations in the water.

The conditions created in the experimental bags should have strongly favoured cyanophytes; pH values were high, carbon dioxide concentrations were low, the water was supplied with inocula from the upstream lake, Mere Mere, where cyanophytes are a predominant summer feature and suitable inocula were present over a sufficiently long period for significant populations to have developed. In addition the phosphorus concentrations were high whilst nitrogen was present at low N : P ratios (because nitrate-N was undetectable during the periods of the experiment the NH_4-N to SRP ratio is essentially the available N : P ratio). The experiment was conducted at summer temperatures and provision was made for high grazing intensity. Growth of algae in some enclosures resulted in low underwater light intensity. Stratified conditions were not present but there are many examples of unstratified lakes with dense cyanophyte populations (see Reynolds & Walsby, 1975). Despite all this, cyanophytes remained relatively minor components of algal communities in the bags. There was a small increase at pH 9 where a mean allocation of biomass to cyanophytes of 17.5% was found but there was no trend upwards with pH and much greater proportionate biomasses of Cryptophyta (pH 9 and 10) and Chlorophyta (control and pH 11) were found. Cyanophyte mean biomass in upstream Mere Mere is at least 100 times greater than that found in the experimental enclosures whilst Mere Mere has pH values and carbon dioxide concentrations spanned by those provided in the enclosures.

There was also no relationship between cyanophyte biomass and fish density and hence it is difficult to see much support for any of the conventional hypotheses concerning factors favouring cyanophytes from the results of this experiment. The experimental results are consistent with events that have transpired in the lake itself. As fish have moved in, the expected loss of zooplankton grazing potential has not occurred, although there has been a switch from *Daphnia magna* to *Daphnia hyalina*, the water has remained clear, algal populations remain low and submerged plant populations have thrived. The buffer mechanisms that are purported to stabilize plant-dominated communities (Moss, 1991; Scheffer *et al.*, 1993) appear to be very effective.

Chlorophyta and Cryptophyta were favoured by pH, the former to pH 11, the latter to pH 10. Chlorophyta were also favoured by increasing fish densities although there were no fish effects on cryptophytes. Jeppesen *et al.* (1990) found abundant Chlorophyceae at pH 11.2 and Jensen *et al.* (1994) have found increasing dominance of Chlorophyta in shallow hypertrophic lakes (with pH values up to 10.1) and attributed it to high availability of nutrients and carbon dioxide released from the sediments. They suggest that high growth rates make Chlorophyta superior competitors to cyanophytes even when available nutrients are low and pH is high. The present results are consistent with those of the surveys carried out by Jensen *et al.* (1994) but suggest that the reasons for increasing Chlorophyta dominance must be more complex in that supply of nutrients from the sediments was prevented during the experiment and carbon dioxide concentrations were maintained at very low values. Because chlorophytes are small, edible and generally readily grazed, the increase in chlorophytan representation with increasing fish density (and hence declining grazer density, see below) was not unexpected but the similarly expected trend was not observed in the comparable cryptophytes. Nor was it in the trend of diatom biovolume where there was a strong decline with pH but no effect of fish (although the probability value of 0.057 was close to conventional significance). The apparent lack of grazer effects on cryptophytes and diatoms might be due to high growth rates of algae compensating for grazer losses but they may be due to subtle changes in feeding behaviour of zooplankters

at high pH. At pH 10 especially, large numbers of *Daphnia* were associated with large biomasses of edible algae, suggesting that feeding rates were in some way impaired by high pH. One of the few significant interaction effects detected was on *Daphnia* abundance.

Total phytoplankton biomass, predicted to increase with fish abundance, did indeed do so but declined with increasing pH. The former effect might be expected from the very large literature that now links decline in large filter-feeding Cladocera with increases in zooplanktivorous fish, but the links here are clearly more complex. *Daphnia* was the main filter feeder and decreased with fish number in the controls and at pH 9. It increased in number with increasing fish density at pH 10 and its numbers collapsed, as did the fish, at pH 11. The increase at pH 10 was not associated with major changes in birth or death rates and must be attributed, because all the fish survived, to changes in fish feeding behaviour at this potentially stressful high pH. Serafy & Harrell (1993) found that three species of shallow-water fish [banded killifish (*Fundulus diaphanus*, Lesueur), bluegill (*Lepomis macrochirus*, Rafinesque), juvenile striped bass (*Morone saxatilis*, Walbaum)] avoided pH values exceeding pH 9.5. Although *Daphnia hyalina* clearly thrived at pH 10, and indeed increased in density with pH up to 11, other, smaller, filter-feeding zooplankters (*Ceriodaphnia* spp. and *Bosmina longirostris*) showed conventional increases with fish density at all pH values in which fish survived but both significantly declined with pH.

Previous studies (Bogatova, 1962; Walter, 1969) have shown a generally deleterious effect of high pH on Cladocera but with some species-specificity. *Ceriodaphnia reticulata* Jurine survivorship markedly declined between pH 10.5 and 11 in experiments carried out in ponds in New York State and in the laboratory (O'Brien & De Noyelles, 1972) and the effect was apparently directly linked with pH. In experiments in enclosures from which fish were excluded in a Danish Lake, Hansen *et al.* (1991) found that with increasing pH from 9.0 to 10.6, numbers of *Daphnia longispina*, O.F. Müller, *Bosmina longirostris* and *Chydorus sphaericus*, O.F. Müller significantly decreased, whilst those of *Daphnia magna*, *Cyclops vicinus* Uljanin and *Cyclops strenuus* (s.str) (Fischer) were unaffected. Walter (1969) found pH 11 to be toxic to both *Daphnia pulex* De Geer and *D. magna*, whilst Bogatova (1962) found lethal limits between pH 10.6 and 11 for various Chydoridae. The marked reduction in Cladocera at pH 11 in the present experiment is consistent with an upper limit of about 10.5–11 for Cladocera in general. Below this there seems to be differential tolerance with considerable ability of *Daphnia* species to survive and increase at pH 10 and somewhat above. In the experiments of Hansen *et al.* (1991) although *Daphnia longispina* was less abundant at pH 10.6 than in controls at pH 9 and below, it still reached population densities of up to 110 animals per litre. To have increased to such densities it must have been actively feeding, as *Daphnia hyalina* must have been at pH 10 in the present experiment. There thus seems evidence that at pH values above 9.5 or 10, fish activity may be impaired (though fish survived) whilst the activity of large filter feeding Cladocera may continue to pH values about one unit higher.

In the present experiment, numbers of *Cyclops* spp. decreased with rise in pH as did those of the raptorial cladoceran, *Polyphemus pediculus*, which was very abundant in the enclosures. Neither taxon was significantly affected by fish, presumably because of their rapid movements and, in the latter case, considerable visual abilities in helping to avoid attack.

Therefore the first hypothesis posed – that increasing pH would increase the proportion of cyanophytes in the phytoplankton – was rejected; the second – that increasing fish density would increase the biomass of phytoplankton – was supported although its rider, that there would be an increasing proportion of cyanophytes in this biomass, was not; and the third hypothesis – that *Daphnia* would decline with increasing fish density, irrespective of pH – was rejected. *Daphnia* became abundant at pH 10 irrespective of the number of fish. What are the implications of these results in the understanding of process that go on in shallow lakes and in the restoration of them?

First it would seem wise to stop generalizing about whole algal groups such as the cyanophytes and to accept that simple, single cause and effect relationships are unlikely to be helpful in explaining the functioning of complex systems. Shapiro's (1990) apparently very convincing arguments were based largely on reductionist studies of single species under laboratory conditions. Although the magnitude of K_s values, and similar indices, undoubtedly reflect real properties of the algae, their relevance under ecosystem conditions is perhaps hidden under the effects of more powerful environmental factors. In this experiment there was every reason for an upsurge of cyanophytes; but it

simply did not happen, for reasons that we do not know. Such reasons can only be adduced from more complex field experiments. However, experiments that cross classify just two major factors with graded treatments and even minimal replication are costly and logistically complex. This one involved thirty-six enclosures. Manipulation of a third factor at three levels with the same replication would increase this to 108. Some comfort may be drawn from the comparative shortage of interaction effects between pH and fish density. However, one of these effects concerned the numbers of *Daphnia hyalina*, an organism representative of a key link in shallow lakes.

Daphnia spp. are important phytoplankton grazers and may control the algal crops in shallow lakes (Moss, McGowen & Carvahlo, 1994). They appear to be one of the important buffers that maintain clear water and a suitable environment for submerged macrophytes in waters that are often nutrient rich and capable of supporting large algal crops (Moss, 1991; Scheffer *et al.*, 1993). Other Cladocera present in plant beds may also be important (Moss *et al.*, 1995) although they have been little investigated. Their absence through predation in waters that have lost their macrophyte communities seems to be an important factor in stabilizing the then established phytoplankton crops so that biomanipulation is consequently necessary for re-establishment of plants (Moss, 1992). pH may have an important role in operation of these functions.

In these experiments, pH values of 10 and 11 had important differential effects on both *Daphnia* and perch. At pH 10 *Daphnia* was abundant and perch survived but apparently did not consume many *Daphnia*. The fish died at pH 11 and *Daphnia* was greatly reduced in numbers. pH values in the region of 10.5–11 have been recorded elsewhere as lethal to various species of Cladocera, whilst some e.g. *Daphnia magna* (Hansen *et al.*, 1991) are unaffected at least at pH 10.6. In and around plant beds in the afternoon, pH values may easily rise to such values (Shutte & Elseworth, 1954; Mizuno, 1961; Stangenberg-Oporowska, 1966; Walter, 1969; O'Brien & De Noyelles, 1972) and potentially contribute to a discouragement of fish predation and an enhancement of the refuge role of macrophyte beds for grazing Cladocera (Timms & Moss, 1984). Serafy & Harrell (1993) did not find that fish avoided macrophyte beds in a species-rich North American shallow lake but the pH values recorded were less than 10. Small differences in the pH at these general levels represent very large changes in hydrogen ion concentrations. Venugopal & Winfield (1993) also found no reduction in the incidence of juvenile cyprinid fish within weed beds compared with open water in a hypertrophic pond and argued against the refuge function proposed by Timms & Moss (1984). However, if increased pH within the beds affects fish feeding behaviour, it would be possible to reconcile the separate evidence of large populations of Cladocera which build up in the plant beds, access of fish to the beds and the increased growth rates of fish in weedy lakes as a result of the fish feeding in the open water on Cladocerans drifting out of the beds.

In macrophyte-dominated systems the pH increase generated by macrophyte photosynthesis may be muted by the carbon dioxide generated by decomposition at the bases of plant beds. This may also be true in phytoplankton-dominated shallow lakes but perhaps to a lesser extent because much of the produced biomass is washed out of the lake rather than accumulated on the bottom. In such circumstances pH values may rise marginally higher to the point where pH becomes lethal to most Cladoceran species and may eliminate the possibilities of much grazing even where fish are absent or not feeding. The ponds of O'Brien & De Noyelles (1973) would appear to be in this category, as are sewage oxidation ponds (Verduin, 1971). Talling (1976) lists several large lakes in which pH values above 10 have been recorded. Such conditions are more likely, however, in shallow waters in lowland catchments with high populations and intensive agriculture. Where pH does rise to such values as a result of phytoplankton photosynthesis, it may act as an additional factor buffering the maintenance of the phytoplankton-dominated state.

The course of events in Little Mere following the diversion of sewage effluent in 1991 has so far been one of maintenance of the grazer-maintained, clear water, macrophyte-dominated conditions found prior to diversion and attributed to lack of fish through deoxygenation. Fish, perch in particular, have now colonized the Mere in considerable numbers but have not prevented the development of substantial *Daphnia hyalina* populations. We attribute this to the refuge role played by the plants, for the water remains extremely nutrient rich as a result of release of phosphate and ammonium from the sediment. The results of the enclosure experiments reported here support this interpretation because increasing fish densities in

the absence of macrophyte refuges in the enclosures led to markedly increased phytoplankton crops. They also hint that the increased pH predicted from the expansion of the plant beds that has so far occurred will help maintain plant dominance rather than result, with the operation of other factors, in an increasing predominance of cyanophytes. But the complexity of species-specific effects such as the differential effects of pH on *Daphnia hyalina* and other zooplankters advise caution in far-reaching prediction.

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