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Responses of zooplankton and zoobenthos to experimental acidification in a high-elevation lake (Sierra Nevada, California, U.S.A.)

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SUMMARY. 1. During the summer of 1987 we conducted an acidification experiment using large enclosure at Emerald Lake, a dilute, high-elevation lake in the Sierra Nevada, California, U.S.A. The experiment was designed to examine the effects of acidification on the zooplankton and zoobenthos assemblages of Sierran lakes.

2. Treatments consisted of a control (pH 6.3) and pH levels of 5.8, 5.4, 5.3, 5.0 and 4.7; each treatment was run in triplicate. The experiment lasted 35 days.

3. The zooplankton assemblage was sensitive to acidification. *Daphnia rosea* Sars emend. Richard and *Diaptomus signicauda* Lilljeborg decreased in abundance below pH 5.5–5.8, and virtually disappeared below pH 5.0. *Bosmina longirostris* (Müller) and *Keratella taurocephala* Ahlstrom became more abundant with decreasing pH, although *B. longirostris* was rare in the pH 4.7 treatment. These species might serve as reliable indicators of early acidification in lakes such as Emerald Lake.

4. The elimination of *D. rosea* in acidified treatments probably allowed the more acid-tolerant taxa to increase in abundance because interspecific competition was reduced. Even slight acidification can therefore alter the structure of the zooplankton assemblage.

5. In contrast to the zooplankton, there was no evidence that the zoobenthos in the enclosures was affected by acidification.

Introduction

Acidification of lakes and streams by atmospheric deposition can seriously affect the aquatic biota (Schindler *et al.*, 1985). Responses of zooplankton to acidification commonly include a reduction in species diversity and biomass and a shift in the dominant species (Yan & Strus, 1980; Marmorek, 1984; Havens & DeCosta, 1985, 1987; Malley & Chang, 1986). The zoobenthos also includes taxa that are sensitive to acidification, although most experimental studies have focussed on streams (Hall *et al.*, 1980; Burton, Standford & Allan, 1985; Hall & Ide, 1987; Ormerod *et al.*, 1987; Hopkins, Kratz & Cooper, 1989) rather than on lakes (Schindler *et al.*, 1985).

In the Sierra Nevada of California, acidic deposition has been reported in the Tahoe basin

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and on both the eastern and western slopes (Leonard, Goldman & Likens, 1981; Dozier *et al.*, 1987; Stohlgren & Parsons, 1987; California Air Resources Board, 1988). The lakes and streams of the Sierra Nevada are among the most weakly buffered in the world (Landers *et al.*, 1987), and are thus potentially susceptible to acidification by atmospheric deposition, although chronic acidification of Sierran lakes has not been documented (Melack, Stoddard & Ochs, 1985).

The biological effects of lake acidification have been studied primarily in eastern North America and Europe. Application of the results of these studies to the high-altitude lakes of the Sierra Nevada may not be appropriate because of differences in climate, hydrology and biotic assemblages. Consequently, *in situ* experimental investigations are necessary to predict the consequences of increased acid loading to Sierran lakes. Study of the pH tolerances of aquatic biota may also reveal particularly sensitive species that could serve as early-warning indicators of acidification in future monitoring programmes (Mills & Schindler, 1986).

Emerald Lake (36°35′N, 118°40′W) is a small (2.7 ha), cirque lake located at an elevation of 2800 m in the Kaweah River drainage of Sequoia National Park, California. Precipitation in the area is strongly seasonal and falls mostly as snow (Dozier *et al.*, 1987). Detailed information on the limnology of Emerald Lake is given by Melack *et al.* (1987, 1989). Most of the biological production in the lake occurs during the ice-free season, which is typically from June to November. Much of the annual flux of water through the lake occurs during snowmelt, which normally lasts from April to June or July. The lake water is extremely dilute in ions (specific conductance at 25°C, *c.* 3.5 μS cm^{-1}), and typically ranges in pH from 5.8 to 6.6 during the ice-free season. Phytoplankton production in the lake is very low, and is limited by phosphorus availability during the ice-free season. Zooplankton species peak in abundance during the summer, showing predictable sequences of appearance and dominance. The composition of zooplankton in Emerald Lake is affected by a resident population of brook trout, *Salvelinus fontinalis* (Mitchill).

Melack *et al.* (1987) acidified large enclosures in Emerald Lake to examine the responses of phytoplankton and zooplankton. Additions of various combinations of strong acids and neutral salts showed that the effects of acid addition on zooplankton were caused by the hydrogen ion rather than by indirect effects of fertilization with nitrate or sulphate. These experiments suggested that the crustaceans *Daphnia rosea* Sars emend. Richard and *Diaptomus signicauda* Lilljeborg and the rotifer *Conochilus unicornis* (Rousselet) were adversely affected by acidification below pH 5.5, while the cladocerans *Bosmina longirostris* (Müller) and *Holopedium gibberum* Zaddach and the rotifer *Keratella taurocephala* Ahlstrom were tolerant of acidic conditions until the pH dropped below 5.0.

We here report results of an enclosure experiment designed to determine the responses of the zooplankton and zoobenthos of Emerald Lake to decreased pH. The purpose of this experiment was to corroborate the findings of earlier experiments and to define more precisely the pH tolerances of key species of crustacean zooplankton in Emerald Lake. In contrast to earlier experiments, we included the lake sediments in the enclosure, thereby making the environmental conditions of the experiment more realistic. The inclusion of sediments also permitted us to examine the responses of the zoobenthos to experimental acidification of the overlying water column.

Methods

Design and installation of enclosures. Eighteen cylindrical enclosures made of clear polyethylene (4 mil) were suspended vertically through the water column from floating platforms anchored in the east-central part of the lake. Each enclosure was kept taut by hoops of PVC pipe (1.3 cm diameters) secured at 1 m intervals, and a circular frame of PVC pipe (2.5 cm diameters) at the base of the enclosures facilitated their insertion into the sediments. The enclosures were 1 m in diameter and extended from above the lake surface through the 9.7 m water column to the sediments, enclosing approximately 7.6 m^3 of water.

The enclosures were allowed to leach underwater for 7 days while tied near the surface of the floating platforms. On 29 July 1987, SCUBA divers pulled the bottoms of the enclosures down from the surface to the sediments, taking care to enclose the water column as evenly as possible, and gently implanted the bases of

the enclosures in the soft mud to a depth of 0.2–0.5 m. We sampled the enclosures twice (30 July and 4 August) before acidification.

On each sampling date, vertical profiles of temperature and dissolved oxygen were measured in each enclosure, as well as in the open water near the enclosures, using a polarographic oxygen electrode and a thermistor. Samples for chemical analyses were collected with a Kemmerer sampler from depths of 1 and 7 m. A subsample was filtered in the boat through 24 μm Nitex mesh with the intent of size-fractionating the phytoplankton, removing large forms that are less likely to be consumed by zooplankton. We also sampled the zooplankton (see below).

To facilitate efficient mixing of acid we attempted to destratify the enclosures on 31 July and 4 August by pulling a mixer consisting of plastic baskets (0.4 m wide) on a weighted line through the water column twenty times in each enclosure. The temperature in the enclosures was 16.3°C at 1 m and 13.9°C at 8 m before mixing, and 15.9°C at 1 m and 15.2°C at 8 m immediately afterward.

Each enclosure was randomly assigned an experimental treatment. There were six treatments, each with three enclosures: control (pH *c.* 6.3), and pH 5.8, 5.4, 5.3, 5.0 and 4.7. These treatments were chosen on the basis of our earlier experiments to define precisely the pH tolerances of the zooplankton. The enclosures were acidified with a 0.5 N stock solution of nitric and sulphuric acids (1:1 by equivalents). The acid was added by pumping a measured volume of the stock solution through a 6 mm Tygon tube that was attached to the mixer; addition of acid as the mixer was raised ensured that the acid solution was mixed immediately and evenly into the enclosure. The acid addition was followed by another twenty mixings, after which samples were taken from depths of 1, 5 and 8 m for field measurement of pH. We added two-thirds of the required quantity of acid on 4 August and the remaining third on the following day, which was designated as Day 0 of the experiment.

The enclosures were sampled for chemistry and zooplankton on 7 August (Day 2), 2 days after pH adjustment, and again on 12 August (Day 7). Beginning on 12 August, we collected an additional water sample from 9 m in each enclosure to ensure that vertical differences in pH were adequately documented. Sampling continued at approximately weekly intervals until 9 September (Day 35). The pH of the enclosures was maintained within 0.1 unit of the target pH by adding acid after each weekly sampling; addition of base was never required. One of the pH 5.0 enclosures was lost when it was dislodged from the sediments by high winds; consequently, there were only two replicates for this treatment on the last two sampling dates.

Chemistry. In the laboratory, water samples were analysed for pH (electrode), acid neutralizing capacity (Gran titration), major cations (flame atomic absorption), major anions (ion chromatography), ammonium (indophenol method), phosphate (molybdenum blue method), silica (silico-molybdate method), and iron and aluminium (graphite furnace atomic absorption). Analytical methods are detailed in Melack *et al.* (1989). We first analysed samples from the control and pH 4.7 treatments for all major solutes from before acid addition on 3 August (i.e. Day −1) and Days 2, 14 and 35; additional treatments and dates were analysed only for variables that showed substantial differences resulting from acidification. To provide an indication of phytoplankton biomass, material collected on filters was analysed for particulate carbon and nitrogen with an Elemental Analyser, and for chlorophyll-*a* by fluorometric measurement after the filters were soaked overnight in 90% acetone. The sub-samples filtered through the 24 μm mesh were analysed for chlorophyll-*a* and particulate carbon and nitrogen.

Zooplankton. Zooplankton in the enclosures was sampled by taking a vertical tow from the bottom to the top of each enclosure using a 0.12 m diameter net with 64 μm mesh. Samples were immediately preserved with 5% formalin. In addition to the weekly samples from the enclosures, zooplankton was sampled fortnightly at five stations in the lake by taking vertical net tows from the bottom to the surface. These samples were compared with those taken from the control enclosures to determine whether zooplankton densities in the lake and enclosures were similar.

In the laboratory, zooplankton samples were diluted and subsampled with a Stempel pipette, and zooplankters were identified and counted at × 25 under a dissecting microscope. Micro-

crustaceans and rotifers were subsampled separately because of the generally higher numerical abundance of the latter. Subsample dilutions were adjusted so that at least 100 individuals were counted for each subsample. At least three subsamples were counted per sample, and each subsample usually comprised from 0.05% to 10% and from 0.05% to 5% of microcrustacean and rotifer samples, respectively. In several cases the entire sample was counted.

Zoobenthos. On 22 July, before the enclosures were pulled down through the water column, divers used a PVC core sampler (15 cm diameter, 30 cm long) to collect duplicate benthic samples from the future site of each enclosure, then marked the core sites with small plastic flags to ensure that identical spots would not be resampled later. At the end of the experiment, divers slit the enclosures near the sediments and again collected duplicate cores from within each enclosure. Samples were concentrated in the field using a 250 μm mesh sieve and preserved in 70% ethanol. In addition, zoobenthos at six sites in the lake was sampled with an Ekman grab (one sample per site) near the beginning and end of the experiment; these samples were compared with those from the enclosures. In the laboratory, Rose Bengal was added to stain the invertebrates, which were then sorted using dissecting microscopes, and identified to species when possible.

Data analysis. In order to compare the depth-integrated zooplankton samples to the chemical data for discrete depths, volume-weighted means of the chemical variables for each enclosure on each date were calculated. Thermal profiles in the enclosures were used to estimate the volumes of the epilimnion and hypolimnion during thermal stratification. Thermal layering inside the enclosures resembled that of the water column outside. During the first 3 weeks of the experiment there was a pronounced thermocline at 7–8 m; the enclosures were isothermal during the last 2 weeks (Fig. 1).

Analyses of variance (ANOVA) were performed on the response variables to check for significant differences amongst the enclosures assigned to the different treatments before acidification. For chemical data, only the samples from 4 August (Day −1) were included. Zooplankton data from both 30 July (Day −6) and 4 August (Day −1) were included. Statistical analyses of the zooplankton data were limited to the more abundant taxa. Heterogeneity of between-treatment variances was checked using the F_{max} test and, where appropriate, data were log-transformed and the analysis repeated.

Since this experiment was a split-plot or repeated measures design, post-acidification data were analysed using profile analysis, which is a multivariate alternative to repeated measures ANOVA. Profile analysis was used

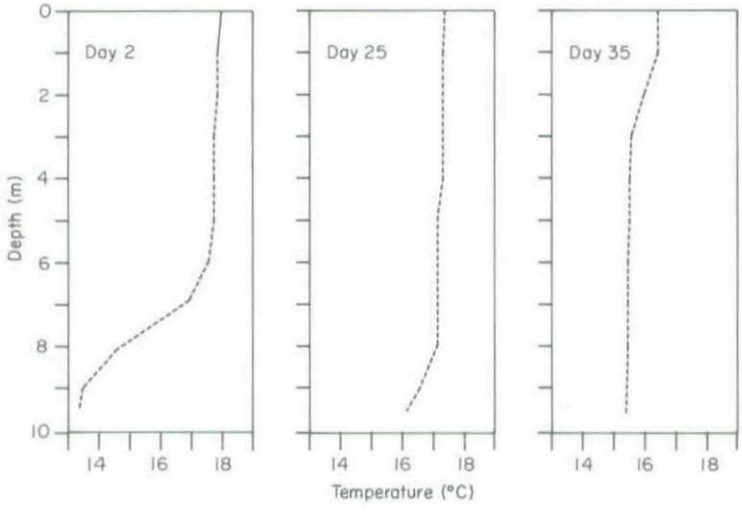

FIG. 1. Thermal profiles in Emerald Lake during the experiment. Thermal layering inside the enclosures resembled that of the water column outside.

because it has less restrictive assumptions than conventional univariate repeated measures ANOVA and is generally considered a more robust technique (Harris, 1985). This technique treats observations on each day as dependent variables in a multivariate analysis of variance (MANOVA) to test for effects due to the repeated factor (i.e. Day) and its interaction with pH, while the main effect due to pH is tested with a univariate ANOVA (Harris, 1985; Morrison, 1976). As in repeated measures ANOVA, a non-significant interaction indicates that the shape of the profile of the response variable over time is parallel between levels of pH, and permits valid testing for significant differences between days and between levels of pH. The *F*-ratios reported for the multivariate test statistics were calculated using Pillai's trace since this is among the most robust of the multivariate test statistics (Green, 1979; Harris, 1985).

Profile analysis was used on the zooplankton and chemical data to identify variables which responded consistently to manipulation after acidification. For those zooplankton species showing responses to acidification, the nature of their response was investigated using regression rather than by testing contrasts within the ANOVA design. This was done because the pH of replicate enclosures within treatments varied over the course of the experiment. The pH values were transformed to H^+ concentrations for calculation of means. The mean pH for each enclosure over each time interval was calculated from samples taken at the beginning and end of each interval (i.e. from the pH measured after adding acid in the previous week and from the pH measured immediately before readjustment of the pH by acid addition). The appropriateness of the regression models was checked by inspecting residuals, and the data were log-transformed where necessary.

Zoobenthic responses were examined using multivariate analysis of variance with the most abundant taxa as dependent variables. We also used exploratory techniques (multidimensional scaling and cluster analysis) using data on all of the taxa collected to see whether there were any changes in overall community composition that were not reflected by the common species alone. The latter techniques were based on the Kulczynski dissimilarity coefficient, which has been shown to be suitable for use with ecological data (Faith, Minchin & Belbin, 1987).

Results

Chemistry

For each treatment, the volume-weighted mean pH for each time period, as well as the overall means for the duration of the experiment, are presented in Table 1. Table 2 compares the concentrations of major solutes and trace metals in the control treatment with those in the most acidified (pH 4.7) treatment during the experiment. Only concentrations of magnesium ($P=0.04$) and ammonium ($P=0.02$) showed significant, but small (<0.3 μeq l^{-1}), differences among enclosures assigned to different treatments before acidification; these differences did not persist for the rest of the

TABLE 1. Volume-weighted means for pH in each treatment over the course of the experiment, and the overall time-weighted means for each treatment. Day numbers represent days since the initial acidification. The mean pH over each interval was calculated from samples taken at the beginning and end of the interval.

	Experimental treatment					
Interval	Control	pH 5.8	pH 5.4	pH 5.3	pH 5.0	pH 4.7
Day −1	6.37	6.41	6.35	6.32	6.34	6.32
Days 2–7	6.23	5.86	5.25	5.26	4.97	4.84
7–14	6.21	5.86	5.40	5.25	5.02	4.79
14–21	6.33	5.82	5.45	5.28	5.02	4.78
21–28	6.30	5.77	5.45	5.30	5.03	4.74
28–35	6.27	5.81	5.48	5.34	5.05	4.70
Mean (time-weighted)	6.27	5.82	5.39	5.28	5.01	4.77

TABLE 2. Concentrations of major solutes in the control and pH 4.7 treatments during the experiment. Data are volume-weighted means averaged over all post-acidification sampling dates. Units are μeq l^{-1} except Si, Fe and Al, which are μM. Results of the statistical analyses are given; 'before' denotes the ANOVA amongst enclosures prior to acidification. Significant effects ($P<0.05$) are in italics.

	Experimental treatment		Probabilities (*F*-test)			
Solute	Control	pH 4.7	Before	pH	Day	Day by pH
ANC	30.5	0.0	0.200	*0.002*	*0.047*	0.616
Ca^{2+}	23.6	23.3	1.000	0.947	*0.015*	0.079
Mg^{2+}	4.1	4.0	*0.036*	0.633	0.099	0.062
Na^{+}	17.7	18.2	0.165	0.147	0.804	0.225
K^{+}	3.7	3.8	0.212	*0.028*	*0.005*	*0.008*
Cl^{-}	2.5	2.4	0.098	0.898	0.119	0.706
SO_4^{2-}	7.1	30.6	0.495	*0.000*	0.101	0.097
NO_3^{-}	2.6	24.8	0.078	*0.000*	0.124	0.050
NH_4^{+}	0.7	0.4	*0.022*	0.165	*0.001*	0.147
PO_4^{3-}	0.0	0.0	N/A	N/A	N/A	N/A
Si	25.0	25.8	0.626	0.138	*0.004*	0.296
Total Fe	0.8	0.9	0.610	0.763	0.079	0.780
Total Al	0.6	1.0	0.946	*0.000*	*0.050*	0.093

experiment (Table 2). Addition of nitric and sulphuric acids directly affected pH, ANC and concentrations of nitrate and sulphate. Of the other solutes, only potassium and aluminium showed significant differences in concentration between treatments ($P=0.03$ and $P<0.001$, respectively). The concentration of potassium also showed a significant interaction; it was higher in the pH 4.7 enclosures on Day 2, but lower than the control at the end of the experiment. The concentration of aluminium was consistently higher (mean difference, 0.4 μM) at pH 4.7 throughout the experiment.

The data for nitrate and sulphate permit estimation of the importance of reduction reactions that might have decreased the concentrations of these anions in the enclosures. Such reactions are important because the reduction of nitrate or sulphate results in ANC generation, which in turn contributes to the recovery of water bodies from acidification (Schnoor & Stumm, 1985; Schindler, 1985). Nitrate and sulphate were added to the enclosures in a 1:1 ratio, by equivalents. Loss processes, which at a particular depth are unlikely to affect nitrate and sulphate equally, will change the ratio of these anions over time.

A *t*-test comparing the deviations of the observed ratios from 1:1 in the pH 4.7 enclosures was used to test the hypothesis that concentrations of nitrate and sulphate in this treatment were unaffected by differential loss processes. Before computing the ratios, the mean concentrations of the anions in the controls on each date were subtracted from the concentrations in the acidified treatment. There were no significant deviations from a 1:1 ratio ($P>0.10$) for any of the dates after acidification. There was no tendency for the difference in concentrations to be greater close to the sediments (at 9 m), as would be expected if dissimilatory reduction were important, or in the lower water column (at 7 m), as would be expected if nitrate assimilation by phytoplankton were important.

Concentrations of particulate carbon, particulate nitrogen, and chlorophyll-*a* over the course of the experiment are presented in Fig. 2. These variables were measured to assay phytoplankton biomass in the enclosures. Concentrations of particulate carbon and nitrogen did not differ significantly amongst the treatments at the beginning of the experiment ($P>0.25$) and, although these variables fluctuated over time, there were no consistent patterns attributable to pH treatment (Fig. 2). However, the volume-weighted mean concentrations of chlorophyll-*a* on Day −1 indicate that phytoplankton biomass differed significantly amongst the treatments at the beginning of the experiment ($P=0.022$). The profile analysis showed that these differences persisted throughout the experiment. *A posteriori* tests (Tukey's HSD) amongst the

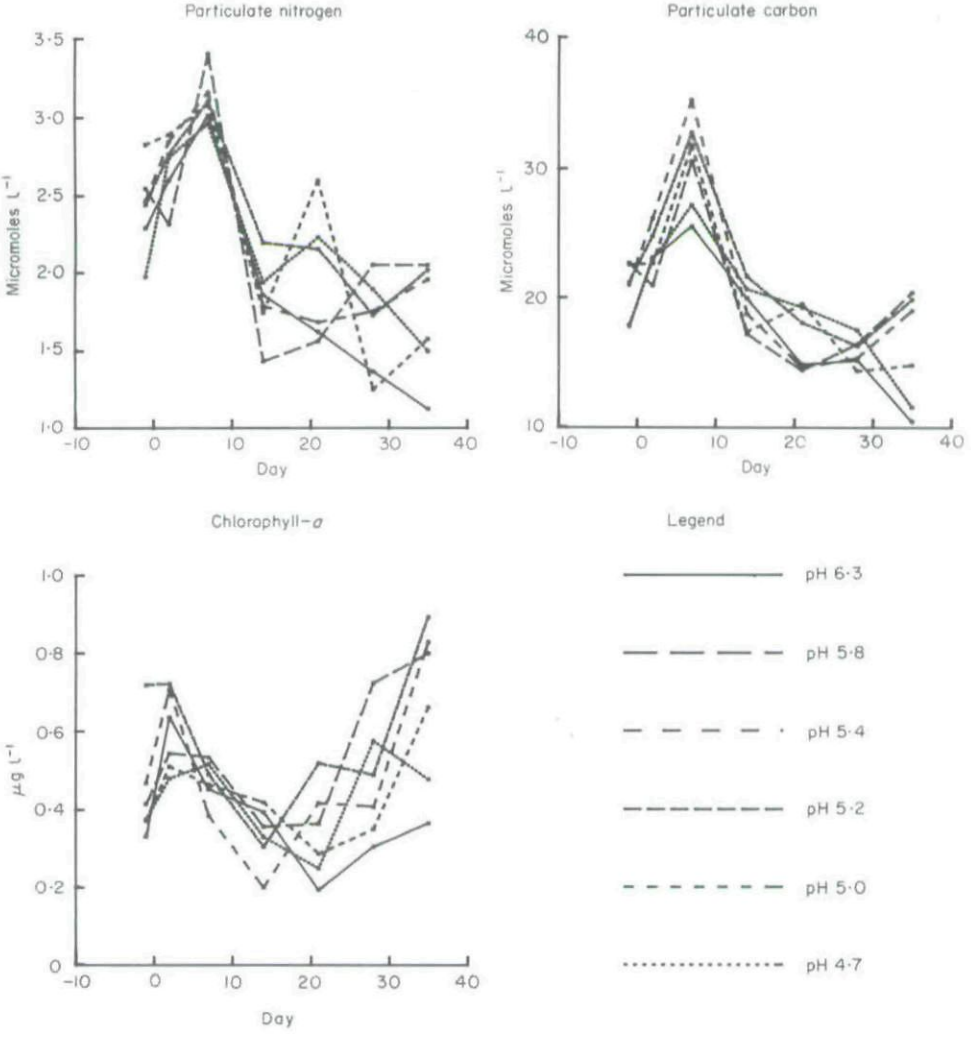

FIG. 2. Concentrations of chlorophyll-*a*, particulate nitrogen and particulate carbon in each treatment. Acidification was completed on Day 0.

treatment means show that only the pH 6.3 and 5.2 treatments differed significantly from each other, and that there was no direct correspondence between chlorophyll-*a* concentration and the level of acidification (Fig. 2).

Data for the samples filtered through a 24 μm Nitex mesh are not presented here. Chlorophyll-*a* concentrations for a representative subset of mesh-filtered samples (n=85) were compared with chlorophyll-*a* in the corresponding unfiltered samples using a *t*-test for paired comparisons. Filtration through the mesh had no significant effect (P>0.10) on chlorophyll-*a* concentrations. Algae large enough to be retained by a 24 μm mesh were evidently not a significant component of the phytoplankton in the enclosures. The *t*-test for particulate carbon and nitrogen in a smaller subset of samples (n=65) showed that filtration significantly reduced concentrations of these variables (P<0.001). We attribute this to the removal of zooplankton by the mesh; in the field, we observed that nearly all the material retained by the mesh was zooplankton.

Zooplankton

The major taxa of zooplankton were the cladocerans *Daphnia rosea*, *Bosmina longirostris* and *Chydorus* cf. *sphaericus*, the calanoid copepod *Diaptomus signicauda* and its nauplii, and three species of rotifers: *Trichocerca capucina* (Wierzejski), *Polyarthra vulgaris* Carlin and *Keratella taurocephala*. The latter species was incorrectly identified as *K. cochlearis* in Melack *et al.* (1987). Several taxa, including the cladoceran *Holopedium gibberum*, the cyclopoid copepod *Cyclops* sp. and the rotifer *Conochilus unicornis*, were also found in the enclosures, but they were not abundant enough for their responses to acidification to be quantified.

Prior to adding acid, *Diaptomus signicauda* was the only taxon that differed significantly ($P=0.005$) in abundance with respect to the pH treatments assigned to the enclosures; it was more abundant in the enclosures assigned to the pH 6.3, 5.0 and 5.4 treatments compared to the pH 5.8 and 4.7 treatments.

The profile analyses are summarized in Table 3. Five species showed significant effects due to pH, and four of these also changed in abundance over the duration of the experiment. Examples of the different sorts of responses to the treatments over time are shown in Fig. 3. In the case of *Daphnia rosea* the effect of time was significant, but there was no simple temporal trend; the same was true of *Diaptomus signicauda* and *Polyarthra vulgaris*. Nevertheless, the effects due to pH, where present, were consistent on each date as evidenced by the lack of significant interactions between time and pH (Table 3), although the effects were not fully apparent until after Day 7 (Fig. 3).

To summarize the relationship with pH, the mean abundances of each species in each enclosure over the period Day 7 to Day 35 inclusive were plotted against the mean pH of that enclosure over the same period (Fig. 4). *Daphnia rosea* and *Diaptomus signicauda* both declined with decreased pH, whereas *Keratella taurocephala* showed the opposite trend. *Bosmina longirostris* showed the most complex pattern of response, being least abundant in the control and most acidic enclosures, but increasing as pH dropped from 6.3 to *c.* 5.2. Copepod nauplii showed a significant response to acidification in the profile analyses, and Fig. 4 indicates that this was due to reduced abundances in the lowest pH treatments; above pH 5.0; however, there was no strong relationship.

TABLE 3. *F*-values from profile analyses of the responses of selected zooplankton taxa to acidification (see text for explanation); $^{***}P<0.001$, $^{**}P<0.01$, $^{*}P<0.05$, $^{ns}P>0.05$.

	Source		
Taxon	pH	Day	Day by pH
Daphnia	35.90^{***}	5.89^{*}	1.34^{ns}
Diaptomus	14.18^{***}	96.08^{***}	1.38^{ns}
Keratella	10.66^{***}	2.66^{ns}	1.35^{ns}
Bosmina	5.07^{*}	9.58^{**}	1.48^{ns}
Nauplii	4.41^{*}	29.86^{***}	1.22^{ns}
Chydorus	0.55^{ns}	9.58^{**}	1.02^{ns}
Polyarthra	2.43^{ns}	6.78^{**}	1.06^{ns}

The abundance of *Keratella* was log-linearly related to pH throughout the experiment; the regression was highly significant ($P<0.001$; $r^2=0.57$). The shape of the responses for *Daphnia* and *Diaptomus* suggest that there were threshold pH values above which response to pH was essentially linear. We used a simplex, least-squares model-fitting procedure to estimate these thresholds and the slopes of the responses (Wilkinson, 1988); this is similar to piece-wise linear regression (Neter, Wasserman & Kuter, 1985), except that the point at which the slopes changed was unknown *a priori*. This procedure allowed us to calculate confidence limits around the threshold as well as limits for the slope of the regression.

The models fitted were of the form:

$$Y = X_0 [b_1 (X_1 - b_0)] + \epsilon$$

where Y is the abundance of *Daphnia* or *Diaptomus*; b_0 is the threshold pH; X_0 is an 'indicator variable' that is zero if the pH (denoted by X_1) is below the threshold, and is one if the pH is above the threshold; b_1 is the slope of the regression of Y on X_1; and ϵ is the error.

Both regressions were highly significant. (Table 4), and both species had thresholds close to pH 5.0, below which they were virtually absent. Since the profile analyses showed significant effects due to time for these taxa, the regressions were repeated for each date separately. Day 2 was the only date to show sub-

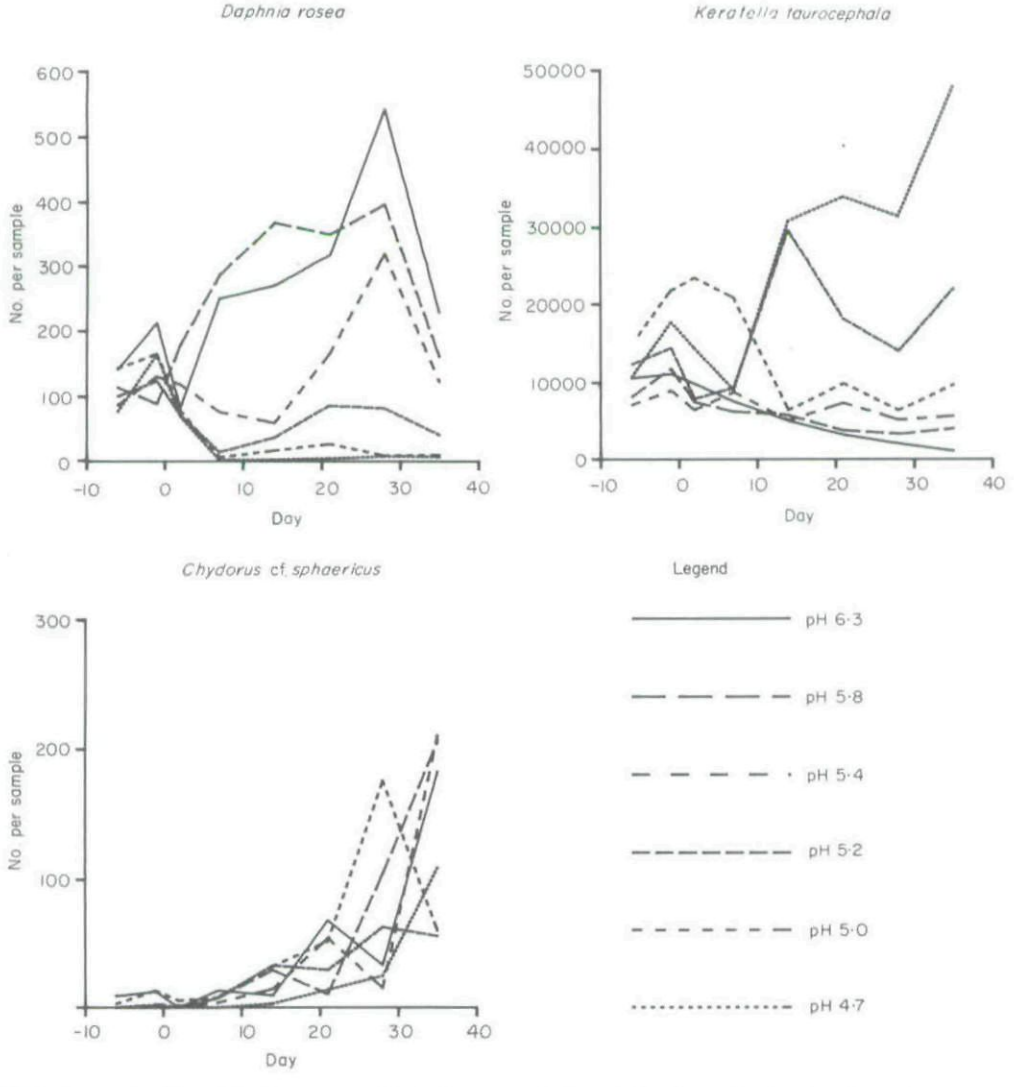

FIG. 3. Mean densities of *Daphnia rosea*, *Keratella taurocephala* and *Chydorus* cf. *sphaericus* in each treatment. Acidification was completed on Day 0.

stantial differences in the estimate of the threshold, indicating that the response to acidification may take a few days to manifest itself. The slope of the regression above the threshold also changed on the last day, reflecting lowered abundances in all enclosures at the end of the experiment relative to the preceding weeks.

To determine whether there were any artefacts due to the experimental design, abundance data for the samples from the control enclosures and from the five monitoring stations were converted to volumetric densities and the results compared graphically, since the sampling dates did not coincide for the two series of samples. Fig. 5 indicates that the mean abundances of zooplankton were similar in the enclosures and in the lake at the beginning of the experiment. Thereafter, densities of *Daphnia* and *Diaptomus* increased in the lake relative to the enclosures, while *Trichocerca* disappeared more quickly in the enclosures than in the lake. Conversely, *Chydorus* became more numerous in the enclosures, while the other species generally behaved similarly in the two series of samples.

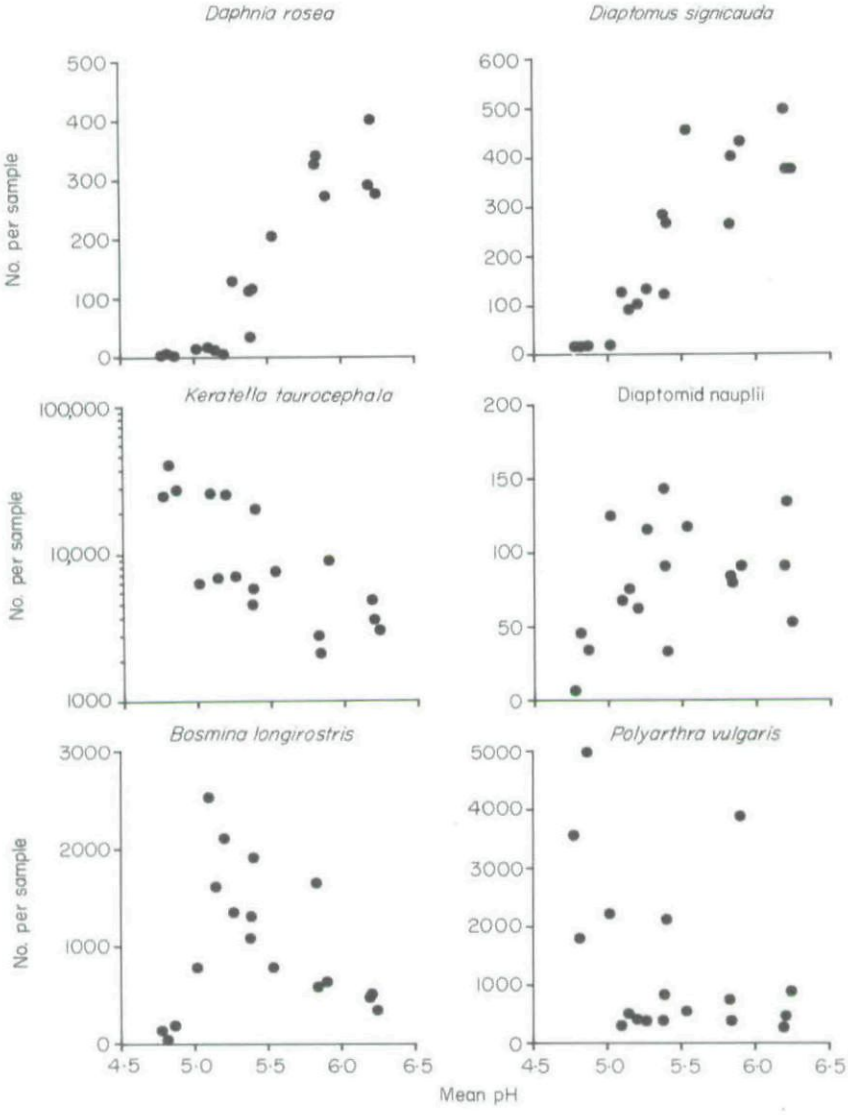

FIG. 4. Mean abundance of each species in each enclosure versus mean pH of each enclosure after acidification.

Zoobenthos

A total of thirty-three taxa were found in the benthic cores from the enclosures (Table 5). No additional species were found in the samples taken from soft sediments elsewhere in the lake using the Ekman grab. Because the two sampling techniques have different biases, direct comparison of densities is inappropriate. Instead, the rank abundances of all taxa in the two types of samples were compared using the Wilcoxon signed-ranks test. There was no significant difference ($P=0.82$) between rank abundances in the cores and in the Ekman grab samples. Overall, there were eight taxa collected in the cores that were not present in the Ekman grab samples (Table 5). Seven of these were the rarest taxa in the core samples, occurring in fewer than nine of the sixty-eight cores analysed. *Bosmina longirostris* was moderately abundant in the cores, but was absent from the Ekman samples.

Fourteen species were sufficiently abundant

TABLE 4. Results of regressions performed to define the responses of *Daphnia rosea* and *Diaptomus signicauda* to acidification (see text for explanation). The regression coefficients (r^2) were highly significant; 95% confidence limits for the estimated threshold pH's are given in parentheses.

	Daphnia	*Diaptomus*
r^2	0.869	0.794
$F_{(2,16)}$	118.116	10.583
Threshold pH (b_0)	5.01 (4.84, 5.17)	4.74 4.50, 4.97)
Slope of regression (b_1)	299 (228, 369)	311 (244, 399)

for statistical analyses. Two-way MANOVA (i.e. date by pH treatment) showed no significant interaction (Pillai's trace, $P>0.27$) or response to pH treatment ($P>0.40$). There was, however, a significant change in the abundances of several species between the two dates. To ensure that there were no species showing a significant response to pH treatment, separate one-way ANOVAs were performed on the data from the end of the experiment for each of the fourteen taxa. Only *Bosmina longirostris* showed a significant result ($P=0.04$). Variances for this species remained heterogenous even after transformation; this and the significant result of the ANOVA were due to its absence from the pH 6.3 and 4.7 treatments, a pattern which was also found for this species in the zooplankton samples.

The other multivariate analyses performed on all taxa from all cores showed no discrete groupings or systematic patterns related to pH treatment. The only pattern to emerge from these analyses was that the two sampling dates were slightly different, as would be expected from the significant time effect in the MANOVA on the abundant taxa.

Discussion

Chemistry

The only solutes that changed significantly in concentration with the addition of acid were the hydrogen ion, nitrate, sulphate, potassium and aluminium. Although elevated levels of aluminium might be anticipated as a result of acidification, its concentration and that of potassium were both well within the annual range of concentrations in Emerald Lake (Melack *et al.*, 1989). The concentrations of aluminium in the enclosures were less than those found to have deleterious effects on *Daphnia magna* Straus and *D. catawaba* Coker in laboratory assays, and concentrations an order of magnitude greater than those found in Emerald Lake do not affect *Holopedium gibberum* and *Chironomus anthrocinus* (Zetterstedt) (Havas & Hutchinson, 1982; Havas, 1985; Havas & Likens, 1985). Consequently, aluminium toxicity was unlikely to be a proximal factor mediating faunal change in this experiment. The lack of deviation of the ratio of nitrate to sulphate from 1:1 indicated that there was probably little reduction of these anions. This is not surprising given the oxic conditions in the water column, which would preclude dissimilatory reduction reactions, and the strong phosphorus limitation of Emerald Lake phytoplankton (Melack *et al.*, 1987), which would limit assimilation of nitrate.

Before acid addition, there were some differences amongst the treatments with respect to chlorophyll-*a*, and these differences persisted for the duration of the experiment. The volume- and time-weighted mean concentrations of chlorophyll-*a* in the treatments varied from 0.36 to 0.53 $\mu g\ l^{-1}$.

There was no apparent relation between these differences and the observed responses of zooplankters sensitive to acidification.

Zooplankton

Most of the abundant taxa of zooplankton showed patterns of population change in the enclosures that resembled those in the lake, albeit at reduced densities in the enclosures for some species. We attribute the higher abundances of *Daphnia, Diaptomus* and *Trichocerca* in the lake to the opportunity for continued recruitment from resting eggs in the lake. *Chydorus*, which increased in abundance in the enclosures relative to the lake, is epibenthic and probably used the sides of the enclosures as an extension of its habitat. The scarcity of *Holopedium gibberum* in this experiment compared to our previous trials was most likely due to its normal seasonal decline towards the end of summer in Emerald Lake (Melack *et al.*,

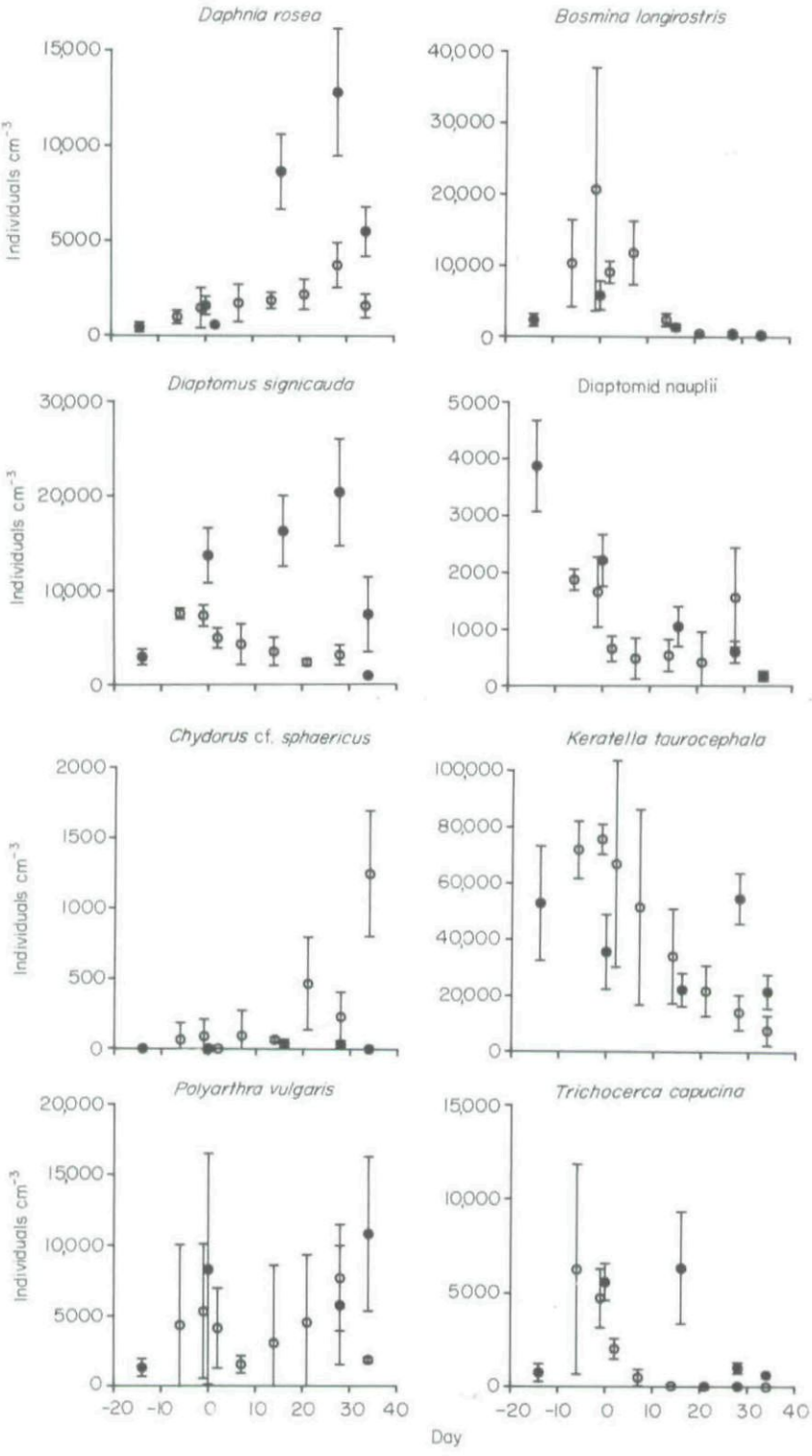

FIG. 5. Mean abundances of zooplankton species in the enclosures (open symbols) and in the lake (closed symbols); vertical lines are ±2 standard errors of the mean.

TABLE 5. List of benthic taxa found in core samples from inside the enclosures; a + indicates that the taxon was not also found in the Ekman samples taken outside the enclosures.

Taxon	Mean abundance (per 0.02 m^2)
Nematoda	45.5
Nematomorpha	0.03
Gordius sp.+	
Oligochaeta	38.6
Mollusca	
Bivalvia	
Pisidium sp.	1.5
Acarina	
Trimalaconothrus sp.	1.0
Nanahermannia nana (Nicolet)	1.2
Crustacea	
Harpacticoida	6.8
Cyclopoida	
Macrocyclops albidus (Jurine)	47.2
Calanoida	
Diaptomus signicauda Lilljeborg	37.0
Amphipoda	
Hyalella azteca (Saussure)+	0.5
Cladocera	
Alona spp.	2.3
Bosmina longirostris (Muller)+	4.7
Chydorus sphaericus (Muller)	14.6
Daphnia rosea Sars emend. Richard	6.4
Eurycercus glacialis Lilljeborg	8.6
Ilyocryptus sordidus (Lieven)	6.9
Polyphemus pediculus (Linné)	1.5
Diptera (Chironomidae)	
Tanypodinae	
Ablabesmyia sp.	0.7
Procladius sp.	1.2
Chironominae	
Chironomus sp. A	5.3
Chironomus sp. B	7.3
?*Cladotanytarsus* sp.	3.1
Paracladopelma sp.	0.8
?*Phaenopsectra* sp.	0.3
Tanytarsus sp.	1.2
Orthocladiinae	
Corynoneura sp.	2.7
Cricotopus sp.+	0.09
?*Eukiefferiella* sp.+	0.5
Heterissocladius sp.+	0.5
Orthocladiinae sp. 4+	0.1
Psectrocladius sp.	4.3
Lepidoptera+	0.09

1987); furthermore, this species is known to be adversely affected by enclosures (Tessier, 1986).

Of the taxa examined in this experiment, three showed relatively simple responses to pH. *Daphnia rosea* and *Diaptomus signicauda* were both virtually eliminated below *c.* pH 5.0, whereas *Keratella taurocephala* reached much higher abundances in the most acidic enclosures. *Bosmina longirostris* also increased in abundance with increased acidity, but was almost

absent from the pH 4.7 treatment. These patterns are consistent with our earlier experiments (Melack *et al.*, 1987), and also resemble the patterns found in several studies that have compared the zooplankton assemblages in acidic and circumneutral lakes (Yan & Strus, 1980; Havens & DeCosta, 1987). The positive responses of *Bosmina* and *Keratella* to decreased pH in the enclosures probably resulted from decreased competition with *Daphnia*, which has been shown to be competitively dominant in similar zooplankton assemblages (Neill, 1984, 1985; Vanni, 1986; cf. Schaffner, 1989). Thus the increased abundances of *Keratella* and *Bosmina* were probably mediated by interspecific interactions rather than by direct physiological benefits of higher acidity.

Not all of the patterns observed in our earlier experiments were reproduced here, however. Previously we had found that *Keratella taurocephala* tolerated pH between 5.0 and 6.0, but declined at more acidic levels. We are unable to explain this discrepancy; however, the fact that *K. taurocephala* increased steadily in abundance in the most acidified treatment over the course of the experiment suggests that it is generally tolerant of low pH in Emerald Lake. Surveys in eastern Canada have showed that this species was more abundant in acidic lakes than its congeners *K. cochlearis* (Gosse) and *K. crassa* Ahlstrom, which were more common in circumneutral waters (Carter *et al.*, 1986).

As with all such analyses, however, care must be exercised when extrapolating either to wider ranges of pH or to different systems. Tonnessen (1984), for example, found that in laboratory microcosms, *Keratella* species were eliminated at very low pH (*c.* 4.0), while Havens & DeCosta (1985) found that *Bosmina longirostris* continued to be numerous at pH 4.2 in West Virginia. Marmorek (1984), working in British Columbia with a fauna similar to that of Emerald Lake, found that *Chydorus* cf. *sphaericus* also increased in abundance at lower pH, together with *Bosmina longirostris*, and attributed this to competitive release from *Daphnia*. In Emerald Lake, *Chydorus* showed no such behaviour. Inconsistencies in the relation between species abundances and pH have also been reported from studies based on surveys. For example, *Conochilus unicornis* is less abundant in acidic waters in Sweden (Almer *et al.*, 1974, 1978), but Siegfried *et al.* (1984) found that it was more abundant in acidic than in circumneutral lakes of the Adirondack Mountains, U.S.A. Such differences could be due to intra-specific variations in acid tolerance (e.g. different clones or sibling species predominating at different localities), faunal and physicochemical differences between localities, and variations in experimental technique.

We should also emphasize that the results of this experiment apply to the latter part of summer, when the crustacean zooplankton assemblage is normally dominated by *Daphnia rosea*. Seasonal differences in zooplankton community structure (Melack *et al.*, 1989) must be considered when designing monitoring programmes. Nevertheless, the results indicate that several zooplankters are sensitive and reliable indicators of acid stress in Emerald Lake.

Zoobenthos

In contrast to the zooplankton, there was no firm evidence to suggest that the zoobenthos was affected by acidification in this experiment. There are two possible explanations for this. First, the zoobenthos may have been so patchily distributed that two cores were insufficient to estimate reliably its abundance within each enclosure; thus, intra-treatment variation could have obscured any inter-treatment effects. Second, the benthic sediments may have buffered the effects of acid addition (Schindler *et al.*, 1985; Okland & Okland, 1986).

It would be premature to conclude that acidification would not affect the benthos at Emerald Lake. Congeners of species found in this study have been found to be detrimentally affected in other studies (e.g. *Heterissocladius*, Tanytarsini, reviewed by Okland & Okland, 1986), while other species of *Chironomus* have been recorded from waters with pH as low as 2.8 (Havas & Hutchinson, 1982). Nevertheless, the benthic taxa most frequently cited as being good indicator species (Mills & Schindler, 1986) are either absent from Emerald Lake (e.g. *Orconectes virilis* (Hagen), *Lepidurus arcticus* Pallas, *Asellus aquaticus* L.) or are found only occasionally (e.g. *Hyalella azteca*). Thus our results, combined with the extra effort of processing benthic samples compared with zooplankton samples, suggest that lacustrine benthos would make poor indicator species for acidification at Emerald Lake.

Amongst the zooplankton, *Daphnia rosea*, *Diaptomus signicauda*, *Bosmina longirostris* and *Keratella taurocephala* are evidently reliable indicators of acidification in late summer in Emerald Lake. If the pH remains above about 6.0, we predict the dominant crustaceans in August and September will be *Daphnia* and *Diaptomus*; moderate acidification will result in *Bosmina* dominating the crustaceans, with *Keratella* becoming increasingly numerous. Below pH 5.0 (but above pH 4.7), we expect both cladocerans and *Diaptomus* to be absent, with *Keratella* dominating the rotifers. This latter scenario, however, would only occur after serious and prolonged acidification.

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