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ECOLOGICAL HISTORY AFFECTS ZOOPLANKTON COMMUNITY RESPONSES TO ACIDIFICATION

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Abstract. The effects of ecological history are frequently ignored in attempts to predict community responses to environmental change. In this study, we explored the possibility that ecological history can cause differences in community responses to perturbation using parallel acidification experiments in three sites with different pH histories in the Northern Highland Lake District of Wisconsin, USA. In Trout Lake, high acid neutralizing capacity had historically buffered changes in pH. In contrast, the two basins of Little Rock Lake (Little Rock-Reference and Little Rock-Treatment) had experienced seasonal fluctuations in pH. Furthermore, the two lake basins were separated with a curtain and Little Rock-Treatment was experimentally acidified in the late 1980s. In each site, we conducted mesocosm experiments to compare zooplankton community dynamics in control (ambient pH) and acidified (pH 4.7) treatments. Zooplankton community responses were strongest in Trout Lake and weakest in Little Rock-Treatment suggesting that ecological history affected responses to acidification. In part, variation in community sensitivity to acidification was driven by differences in species composition. However, the results of a reciprocal transplant experiment indicated that changes in the acid tolerance of populations during past acidification events may make zooplankton communities less sensitive to subsequent pH stress. Our study highlights the role that ecological history may play in community-level responses to environmental change.

Key words: acidification; community dynamics; community structure; ecological history; freshwater zooplankton; Northern Highland Lake District, Wisconsin; rapid evolution.

INTRODUCTION

One of the challenges in developing a predictive understanding of community responses to perturbations is that relatively similar communities can vary substantially in their responses to a given perturbation. For example, effects of drought on old-field plant communities can vary strongly among sites with different species richness (Tilman 1996). Similarly, Menge et al. (1994) found that removal of the predator *Pisaster* had stronger effects on marine invertebrate communities in sites that were exposed to wave action than in more sheltered sites. Thus, within a particular type of ecosystem, among-site variation in community-level responses to natural and experimental perturbations can be substantial. In this study, we examine the role of ecological history in generating variation in community responses to perturbation by comparing zooplankton responses to experimental acidification in three sites with contrasting histories.

Historical events have been shown to have lasting effects on a wide variety of ecological systems includ-

ing shortgrass prairies (Jaramillo 1988), old-growth forests (Foster 1988), and coral reefs (Hughes 1989). However, the effects of ecological legacies on community and ecosystem responses to a changing environment are not widely recognized (Holt 1995, McPeck and Miller 1996). If historical conditions strongly influence community and ecosystem responses to environmental change, then explicit consideration of ecological history may improve forecasting of ecological responses to environmental change and guide identification of highly sensitive systems.

In some cases, stressful environmental conditions in the recent past can increase ecological sensitivity to subsequent perturbation (Peterson and Black 1988, Death 1996). For example, bivalve mussel communities that have been recently subjected to crowding exhibit increased sensitivity to subsequent physical stress (Peterson and Black 1988). Past environmental conditions may also reduce future community responses to the same perturbation by changing the species composition of a community toward a more tolerant assemblage. Examples where this mechanism plays a role in determining community sensitivity to perturbation include fish community responses to winterkill (Tonn and Magnuson 1982) and vegetation responses to fire (Muir 1993, Turner et al. 1997).

Natural selection caused by particular environmental perturbations may also alter responses of the com-

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PLATE 1. Aerial photograph of the Northern Highland Lake District of Wisconsin (USA) showing the study sites. Trout Lake is in the upper right, and Little Rock Lake is in the lower left.



munity to a repeated perturbation by increasing the tolerance of populations that persist. The literature contains numerous cases of evolution in field populations of a single species subjected to selection. Examples of adaptive variation within a species include plant responses to lead (Wu and Antonovics 1976), fish and aquatic plant responses to extreme thermal environments (Christy and Sharitz 1980, Smith et al. 1983), bird responses to drought (Price et al. 1984), oligochaete responses to heavy metals (Klerks and Levinton 1989), caterpillar responses to insecticide (Carriere et al. 1995), fish responses to predators (Reznick et al. 1997), and zooplankton responses to fish predation (Hairston and Dillon 1990). Interestingly, many of these examples involve responses to anthropogenic environmental changes that generated strong selective forces (Bishop and Cook 1981).

Despite extensive evidence for rapid evolution in some populations, the consequences of evolutionary changes for community- and/or ecosystem-level sensitivity to perturbation have not been demonstrated. Can evolutionary changes affect community- and ecosystem-level responses to a changing environment? If so, can these evolutionary changes occur on relatively short time scales that are relevant to ecological dynamics? Answers to these questions are important for determining whether ecologists need to incorporate rapid evolution in their predictions about the ecological impacts of environmental changes such as climate change, UV, and pollutants.

The goal of our study was to examine the role of history in controlling zooplankton community responses to acidification. Previous work conducted at a variety of spatial and temporal scales has demonstrated strong effects of acidification on zooplankton communities (e.g., Frost and Montz 1988, Schindler et al. 1991, Locke and Sprules 1993). To examine the effects of acidification history on zooplankton responses to current acidification events, we used a comparative experimental approach (*sensu* Menge et al. 1994) in

which identically designed, replicated mesocosm experiments were conducted simultaneously in three study sites. Our study sites included a highly buffered site, which was characterized by high and stable pH, a site that had been experimentally acidified during the 1980s, and a site that had intermediate levels of natural pH fluctuations representing a midpoint between the two extremes occurring in the other sites. In each study site, we contrasted zooplankton community dynamics in control (ambient pH) and acidified (pH 4.7) treatments. Comparison of zooplankton community responses in the three sites allowed us to examine the generality of community changes following acidification and to explore the role of history in determining community sensitivity to acidification. Specifically, we tested the hypothesis that acidification history affects community-level sensitivity to experimental acidification. We found that community-level sensitivity varied substantially among sites due to differences in species composition among sites, as well as population differences in acid tolerance.

We conducted two additional shorter term experiments to investigate the relationship between zooplankton sensitivity and acidification history in more detail. First, we conducted an experiment wherein zooplankton from each study site were exposed to a range of reduced pH conditions for one week. We expected zooplankton from each site to decline when exposed to conditions more acidic than historical conditions. Second, we examined the importance of zooplankton population differences and environmental factors in determining sensitivity to acidification using a reciprocal transplant experiment. Here, we present experimental evidence that history affects community-level responses to environmental change via changes in community composition and changes in population tolerance.

METHODS

Study sites

Our three study sites were located in the Northern Highland Lake District of Wisconsin, USA (see Plate

1). Despite their close proximity, the sites differed substantially in acidification history. Trout Lake (46°02' N, 89°40' W, area, 1608 ha; mean depth, 15 m) is relatively well-buffered (acid neutralizing capacity [ANC], 829 $\mu\text{mol/L}$) and maintains a pH >8.0. Located <4 km away, Little Rock Lake (45°59' N, 89°42' 15" W, area, 18 ha; mean depth, 4 m) is poorly buffered (ANC, 21 $\mu\text{mol/L}$), has a mean ice-free season pH of 6.1, and naturally experiences pH conditions <5.4 over the course of a year (Kratz et al. 1987). In 1984, the two basins of Little Rock Lake were divided with an inert vinyl curtain and, during the subsequent six years, the pH of the northern basin (henceforth Little Rock-Treatment) was reduced in three, 2-yr stages to 4.7 using sulfuric acid (Brezonik et al. 1986, 1993). The southern basin of Little Rock Lake (henceforth Little Rock-Reference) was maintained as an unmanipulated reference throughout the course of the experiment. After 1990, Little Rock-Treatment was allowed to recover naturally. Chemical recovery proceeded at roughly the same rate as the acidification (Sampson et al. 1995, Frost et al. 1998). Sampson et al. (1995) report the following mean ice-free season pH values during recovery: 1991, 5.1 ± 0.05 ; 1992, 5.2 ± 0.04 ; 1993, 5.3 ± 0.06 ; and 1994, 5.5 ± 0.11 . In 1995, at the time of the mesocosm experiment, the pH of Little Rock-Treatment (5.9) was similar to the pH (6.1) of the unacidified basin. In 1997, the year of the pH gradient and reciprocal transplant experiments, chemical recovery was virtually complete (mean surface pH was 6.2 ± 0.13 and 6.4 ± 0.27 during the ice-free season in Little Rock-Reference and Little Rock-Treatment, respectively).

While the three study sites exhibit striking differences in acidification history, other aspects of their water chemistry are quite similar. Nutrient concentrations in all three sites are characteristic of relatively unproductive lakes of the Northern Highland Lake District of Wisconsin (Trout Lake total nitrogen [TN], 235 $\mu\text{g/L}$; total phosphorus [TP], 16.9 $\mu\text{g/L}$;⁵ Little Rock-Reference TN = 291 $\mu\text{g/L}$, TP = 13.1 $\mu\text{g/L}$, Sampson 1992; Little Rock-Treatment TN = 226 $\mu\text{g/L}$, TP = 13.0 $\mu\text{g/L}$, Sampson 1992). Dissolved organic carbon (DOC) concentrations are >3 mg/L in all sites. When lake water was acidified in our experiments, DOC concentrations remained >2.8 mg/L in all sites. Estimates of the 1% attenuation depth of UV-B radiation based on published empirical relationships between DOC and UV-B penetration (Morris et al. 1995, Williamson et al. 1996) were always <0.7 m. Therefore, UV-B was probably not an important factor driving zooplankton responses to acidification in our experiments because our mesocosms were 4.5 m deep and the containers for our short-term experiments were incubated at a depth of 2 m.

Zooplankton species composition was similar,

though not identical, in the three study sites. In our mesocosm experiment, more than half of the dominant zooplankton species were present in all three sites. Five out of eight total crustacean zooplankton species were common to all three sites (*Bosmina longirostris*, *Chydorus sphaericus*, *Holopedium gibberum*, *Sida crystallina*, and *Tropocyclops extensus*). Little Rock-Reference and Little Rock-Treatment differed only in the presence/absence of one species (*Diacyclops thomasi*). Community similarity, calculated for all pairwise combinations of sites using Jaccard's Index (Mueller-Dombois and Ellenberg 1974) on presence/absence data for the mesocosm experiment described below, was relatively high for all combinations of study sites (mean, 74%; range, 63–86%). Frost et al. (1998) report that species lists of crustacean zooplankton and rotifers in the two basins of Little Rock Lake were identical in 1995. However, Trout Lake contained some species that are typically absent in more acidic lakes. For example, the dominant daphnid in Trout Lake (*Daphnia mendotae*, formerly *Daphnia galeata mendotae*) is absent from Little Rock and is generally absent from other lakes with pH <5.5 (Keller et al. 1990).

Mesocosm experiment

To compare zooplankton community responses to acidification in Trout Lake, Little Rock-Reference, and Little Rock-Treatment, we conducted a mesocosm experiment from 19 June through 31 July 1995. In each site, two treatments were applied to in situ mesocosms containing zooplankton communities from the local site: (1) acidified, in which the pH was reduced to 4.7 ± 0.2 , and (2) control with ambient pH conditions.

We considered several alternative approaches for creating the acidified treatment in this experiment including (A) adding the same amount of acid to each site, (B) manipulating pH by the same amount in each site, (C) manipulating hydrogen ions by the same amount in each site, and (D) dropping pH to the same level in each site. We decided against approach A because the sites differed in acid neutralizing capacity (see *Methods: Study sites* description) and, therefore, adding the same volume of acid would lead to very different acidity levels in each site. We eliminated approach B because this design does not account for the fact that pH is calculated as the logarithm of the inverse of hydrogen ion concentration and, therefore, ignores the fact that the physiological response of zooplankton to acidification is a response to hydrogen ions, not pH units. Our calculations show that the change in hydrogen ion concentration using approach B would be several orders of magnitude greater in Little Rock-Treatment than in Trout Lake (Table 1). Although approaches C and D differ in principle, they are not different in practice because our accuracy during pH manipulations in the field was limited to target pH ± 0.2 . That is, the target pH for approach C where hydrogen ion concentration was changed by 2.00×10^{-5} in each site

⁵ URL: http://limnosun.limnology.wisc.edu/map/lter_lake/tr_text.html

TABLE 1. Comparison of alternative approaches (see *Methods: Mesocosm experiment* for explanation of approach A) for creating the acidified treatment in the mesocosm experiment.

Approach	Site	Target pH	Target [H ⁺]	ΔpH	Δ[H ⁺]
B) Manipulate pH by the same amount in each site	TR	6	1.00×10^{-6}	2	9.90×10^{-7}
	LR	4.1	7.94×10^{-5}	2	7.86×10^{-5}
	LT	3.9	1.26×10^{-4}	2	1.25×10^{-4}
C) Manipulate [H ⁺] by the same amount in each site	TR	4.70	2.00×10^{-5}	3.30	2.00×10^{-5}
	LR	4.68	2.08×10^{-5}	1.42	2.00×10^{-5}
	LT	4.67	2.13×10^{-5}	1.23	2.00×10^{-5}
D) Drop pH to the same level in each site	TR	4.7	2.00×10^{-5}	3.30	1.99×10^{-5}
	LR	4.7	2.00×10^{-5}	1.40	1.92×10^{-5}
	LT	4.7	2.00×10^{-5}	1.20	1.87×10^{-5}

Notes: Bold values indicate the factor held constant across sites in each design. Target pH([H⁺]) indicate pH([H⁺]) values in the acidified treatment, whereas ΔpH(Δ[H⁺]) indicate the difference in pH([H⁺]) between control and acidified treatments. Sites codes are TR, Trout Lake; LR, Little Rock-Reference; and LT, Little Rock-Treatment.

would range from 4.67–4.69 and we could not manipulate pH with that level of accuracy. Similarly, for approach D, our calculations reveal that the absolute change in hydrogen ion concentration was similar among study sites (Table 1). In fact, if we had chosen to change hydrogen ion concentration by 1.87×10^{-5} in all sites, the target pH for acidified treatments in Trout Lake would have been pH 4.73 (note that this value lies well within our target of pH 4.7 ± 0.2). Thus, our manipulations of pH to a final target 4.7 ± 0.2 are appropriate for comparing zooplankton community sensitivity among sites.

Mesocosms (2000 L, 0.8 m diameter, 4 m deep) were constructed of clear polyethylene cylinders, sealed at the bottom, and suspended from wooden rafts. Mesocosms were filled with lake water pumped from 2 m depth. Because pumping can impose high mortality for some zooplankton species, water was passed through an 80-μm mesh net to remove zooplankton, and mesocosms were then restocked with zooplankton at ambient lake density using net tows (80-μm mesh) taken through the entire water column at dusk.

Zooplankton were allowed to acclimate for 1 wk and initial samples were collected before treatments were imposed. Water was acidified to the target level of pH $4.7 (\pm 0.2)$ using sulfuric acid. Each treatment (control and acidified) was replicated three times. During the experiment, pH was monitored twice a week and adjusted to the target level when necessary. Following each acid addition, all mesocosms (including controls) were mixed thoroughly.

Zooplankton were sampled weekly using a 12-L Schindler Patalas trap equipped with an 80-μm mesh net. Samples were collected at four depths (0.5, 1.5, 2.5, and 3.5 m), pooled to form a single sample, and preserved in cold 5% sucrose-buffered formalin. One quarter of each sample was counted at 50× on a dissecting microscope. Due to the difficulties of accurately identifying copepod nauplii and early stage copepodites to species, these stages were not included in our analyses. Later stage copepodites and adult copepods were lumped for analyses. Here, we report on the dy-

namics of the dominant crustacean zooplankton taxa. We considered dominant taxa to include any species that constituted >5% of the total crustacean zooplankton community by biomass or abundance on any date in either treatment. Although rotifers can also exhibit interesting responses to acidification (e.g., Gonzalez and Frost 1994), we have excluded rotifers from our analyses in order to simplify the presentation of responses of multiple zooplankton species from several experiments in three study sites.

Zooplankton population densities were converted to biomass using measured lengths and published length–mass regressions (McCauley 1984, Peters and Downing 1984). Total zooplankton biomass was calculated as the sum of the biomass of the dominant species. For each sample date, we assessed differences in zooplankton community composition between control and acidified treatments using a proportional similarity index:

$$1 - 0.5 \sum_{i=1}^S |p_{ci} - p_{ai}|$$

(after Schoener 1970) where S is the total number of dominant species, p_{ci} is the mean proportion of species i in the control treatment, and p_{ai} is the mean proportion of species i in the acidified treatment. Biomass data were used to avoid bias due to small, but numerically abundant taxa.

Zooplankton responses to acidification were analyzed using univariate repeated-measures ANOVA. Population densities were square root($x + 1/2$) transformed, and total zooplankton biomass was $\ln(x + 1)$ transformed to normalize data and homogenize variance. To assess time effects and time by treatment interactions, we used the Huynh-Feldt adjustment for departure from sphericity. Departure from sphericity arises in time series data because adjacent time points are more tightly correlated than distant time points (von Ende 1993). For zooplankton population responses, we applied a sequential Bonferroni correction for the number of species analyzed in each study site to assess the significance of ANOVA results. We excluded one con-

trol mesocosm in Trout Lake from our analyses due to dramatic declines in all large-bodied zooplankton species suggesting contamination by fish.

pH gradient experiment

To explore further the relationship between acidification history and zooplankton sensitivity to acidification, we conducted a short-term experiment in July 1997 where zooplankton from each study site were exposed to a range of reduced pH conditions for one week. Because the generation times of most crustacean zooplankton species in north temperate lakes exceed one week, we assumed that changes in density during this experiment primarily reflected direct effects of acidification (i.e., effects of acidification that were independent of species interactions among zooplankton). We expected sensitivity to exhibit the following pattern: Trout Lake > Little Rock-Reference > Little Rock-Treatment. For each site, experimental treatments included ambient pH, pH 5.3, pH 4.7, and pH 4.0. Each treatment was replicated three times.

In each site, integrated water-column samples were collected from the epilimnion using a tube sampler (5 cm diameter, 2.25 m long) and passed through an 80- μ m mesh net to remove zooplankton. Water was acidified to target levels (± 0.1) using sulfuric acid. Transparent polyethylene containers (10 L volume) were filled with water of the appropriate pH and stocked with zooplankton at approximately lake densities. Zooplankton were collected from each site using net tows (80- μ m mesh).

After 1 wk of incubation in the lake at 2 m depth, containers were collected and the contents were filtered through an 80- μ m mesh net and preserved in 95% ethanol. Zooplankton were counted in toto at 50 \times on a dissecting microscope. Zooplankton population densities were $\ln(x + 1)$ transformed to normalize data and homogenize variance. Transformed data were analyzed using one-Way ANOVA to test for an overall pH effect. For species where ANOVA indicated a significant treatment effect, we used Dunnett's contrasts to test for significant decreases in population density in each acidified treatment compared to the control. We excluded 2 of the 36 containers from the analysis due to contamination with larval fish and vandalism. While we did not specifically quantify periphyton growth, we did not observe any periphyton inside the experimental containers at the end of the 7-d incubation.

Reciprocal transplant experiment

Differences in sensitivity to acidification such as those observed in the mesocosm and pH gradient experiment (see *Results*) could be caused by zooplankton population differences and/or environmental conditions that might differ among sites. For example, environmental conditions may play a role if food resources for zooplankton decline more with acidification in some lakes than in others. To examine the relative

importance of zooplankton population differences and environmental factors, it is necessary to conduct a reciprocal transplant experiment where the site of zooplankton origin and the environmental conditions are manipulated in a full factorial design (see Via 1994, Reznick and Travis 1996). We employed a $3 \times 3 \times 2$ factorial design where the factors were zooplankton source (Trout Lake, Little Rock-Reference, and Little Rock-Treatment), water source (Trout Lake, Little Rock-Reference, and Little Rock-Treatment), and pH (ambient pH and pH 4.7). The response variable in this experiment was the change in density during the 1-wk incubation. Change in density (ρ) was calculated as $\ln(N_f) - \ln(N_i)$ where N_f and N_i are final and initial densities. Three replicates were employed per treatment yielding 54 total experimental units. Methods and experiment duration for the reciprocal transplant experiment were the same as the pH gradient experiment. As in the pH gradient experiment, we assumed that changes in population density during the 1-wk incubation were driven primarily by direct effects. We excluded 7 of the 54 containers from the analysis due to contamination with the invertebrate predator *Chaoborus*.

For species that occurred in more than one study site, we used three-way ANOVA to evaluate effects of pH, zooplankton source, and water source on the change in zooplankton population density (i.e., $\ln(N_f) - \ln(N_i)$). The statistical model tested for main effects of zooplankton source, water source, pH, and all two- and three-way interactions between these variables. Our primary interest in interpreting this statistical model was in three effects: (1) water source \times pH, (2) zooplankton source \times pH, and (3) zooplankton source \times water source \times pH. These three effects allowed us to ask whether zooplankton responses to pH were affected by the site of population origins and/or local environmental conditions (Via 1994, Reznick and Travis 1996). Significant effects of zooplankton source \times pH and/or zooplankton source \times water source \times pH indicate zooplankton population differences in acid sensitivity. Similarly, significant effects of water source \times pH and/or zooplankton source \times water source \times pH indicate that local environmental conditions affect zooplankton responses to acid.

If the three-way ANOVA indicated a significant zooplankton source by pH interaction or a significant three-way interaction, we then used two-way ANOVA to test for pH and water effects for each zooplankton source separately to verify that patterns of sensitivity corresponded with acidification history as in our other experiments. The response variable in this experiment, $\rho = \ln(N_f) - \ln(N_i)$, is conceptually similar to the numerator of population growth rate (r); however, they are not equivalent because N_f and N_i in ρ only include stages that could be readily identified to species. For copepods, N_f and N_i include later stage copepodites and adults. Therefore, N_f in our experiment is determined

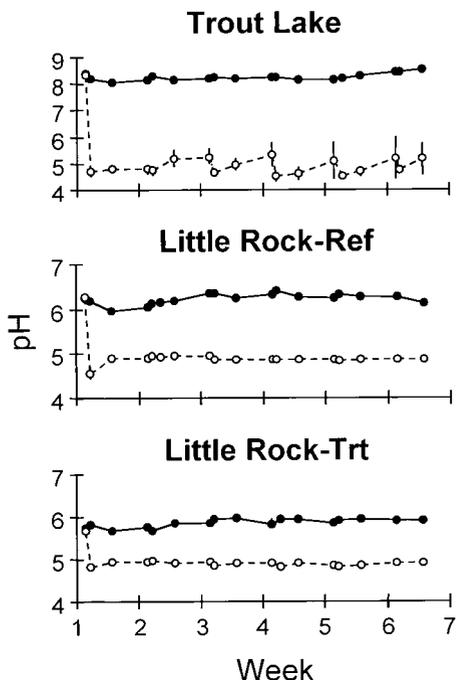


FIG. 1. The pH in the mesocosm experiment for Trout Lake, Little Rock-Reference, and Little Rock-Treatment. Means are plotted for control (solid circle) and acidified (open circle) treatments. Vertical bars indicate ± 1 SE. Ambient pH values in the study lakes during the same period were 8.4, 6.1, and 5.8 in Trout Lake, Little Rock-Reference, and Little Rock-Treatment, respectively.

by two factors: (1) survival of later stage copepodites and adults, which were present in the initial sample and (2) maturation of nauplii and early stage copepodites, which were present in the initial sample but not identified to species. For this reason, it is not appropriate to compare rho across different zooplankton sources because differences in rho may arise due to factors like variation in the age structure of populations at the time of stocking. To assess acid sensitivity of populations from different sources, we compared rho at ambient pH to rho at pH 4.7 for a given population. A population was considered to be acid sensitive if rho under acid conditions was significantly lower than rho at ambient pH.

Statistical analysis

Statistical analyses for the mesocosm experiment and the reciprocal transplant experiment were conducted using SAS for Windows, version 6.12 (SAS 1990), whereas analysis for the pH gradient experiment was conducted using SYSTAT, version 5.2.1, for the Macintosh (Wilkinson et al. 1992)

RESULTS

Mesocosm experiment

In all three study sites, target pH levels (± 0.2) were achieved in the acidified treatment while pH values in

control bags were similar to ambient lake pH (Fig. 1). Variability in the acidified treatment in Trout Lake was caused by drift toward higher pH in one of three replicate bags. We believe that this bag must have had a small hole, but we have retained this replicate in our analysis because zooplankton abundance in this bag was not different from the other two replicates.

Although zooplankton community dynamics were affected by acidification in all three study sites, zooplankton community responses were strongest in Trout Lake and weakest in Little Rock-Treatment. Over the course of the experiment, zooplankton community structure in control and acidified treatments diverged most in Trout Lake and least in Little Rock-Treatment (Fig. 2). It is notable that community similarity between control and acidified treatments declined over

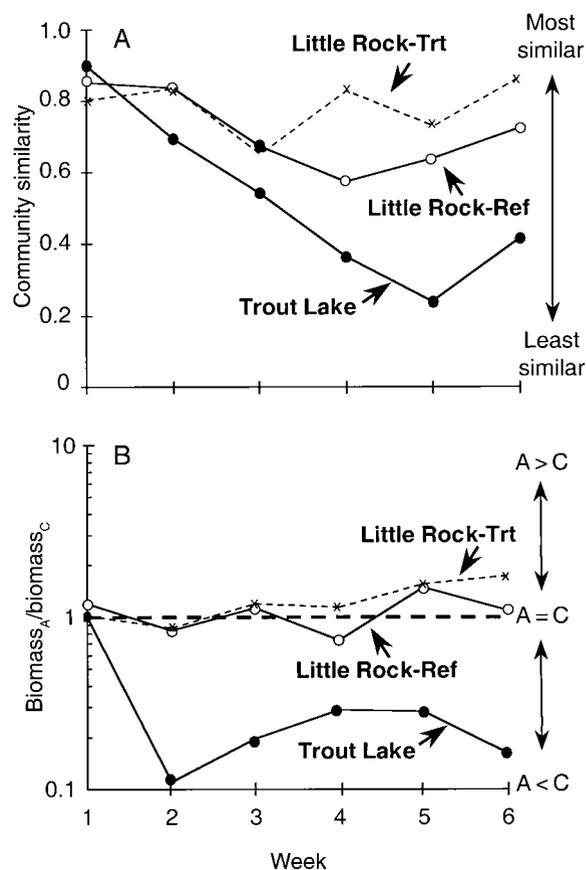


FIG. 2. Divergence in (A) community structure and (B) total zooplankton biomass between control (C) and acidified (A) treatments in the mesocosm experiment in Trout Lake, Little Rock-Reference, and Little Rock-Treatment. Community similarity was calculated using a proportional similarity index. We present differences in total zooplankton biomass as the ratio of mean biomass in the acidified and control treatments for each site. Because the ratio is presented on a log scale, the ratio represents a proportional difference in biomass, and ratios < 1 indicate that biomass was reduced with acidification compared to the control. Note that the raw biomass data were used in the univariate repeated-measures ANOVA presented in Table 2.

TABLE 2. Univariate repeated-measures ANOVA for zooplankton responses to acidification in the mesocosm experiment.

Species	pH treatment			Time			Time × pH treatment		
	df	F	P	df	F	P	df	F	P
Trout Lake									
<i>Bosmina longirostris</i>	1, 3	2.26	0.230	5, 15	6.53	0.009	5, 15	8.67	0.004
<i>Chydorus sphaericus</i>	1, 3	0.71	0.460	5, 15	5.56	0.012	5, 15	1.27	0.339
<i>Daphnia mendotae</i>	1, 3	83.74	0.003	5, 15	62.83	< 0.001	5, 15	31.21	< 0.001
<i>Holopedium gibberum</i>	1, 3	2.58	0.207	5, 15	7.04	0.003	5, 15	11.75	< 0.001
<i>Sida crystallina</i>	1, 3	0.82	0.433	5, 15	6.34	0.006	5, 15	2.15	0.138
<i>Diacyclops thomasi</i>	1, 3	19.34	0.022	5, 15	4.03	0.018	5, 15	1.61	0.219
<i>Tropocyclops extensus</i>	1, 3	129.24	0.002	5, 15	40.37	< 0.001	5, 15	15.95	< 0.001
Zooplankton biomass	1, 3	458.33	<0.001	5, 15	21.18	<0.001	5, 15	4.55	0.010
Little Rock-Reference									
<i>Bosmina longirostris</i>	1, 4	14.81	0.018	5, 20	7.82	0.003	5, 20	2.51	0.107
<i>Chydorus sphaericus</i>	1, 4	2.49	0.190	5, 20	29.00	< 0.001	5, 20	2.91	0.110
<i>Holopedium gibberum</i>	1, 4	8.03	0.047	5, 20	90.29	< 0.001	5, 20	3.34	0.031
<i>Sida crystallina</i>	1, 4	1.50	0.287	5, 20	16.69	< 0.001	5, 20	0.90	0.463
<i>Mesocyclops edax</i>	1, 4	2.75	0.173	5, 20	64.12	< 0.001	5, 20	2.27	0.107
<i>Tropocyclops extensus</i>	1, 4	29.08	0.006	5, 20	9.08	< 0.001	5, 20	4.48	0.007
Zooplankton biomass	1, 4	0.41	0.558	5, 20	10.09	<0.001	5, 20	1.00	0.442
Little Rock-Treatment									
<i>Bosmina longirostris</i>	1, 4	41.06	0.003	5, 20	42.48	< 0.001	5, 20	2.34	0.134
<i>Chydorus sphaericus</i>	1, 4	1.14	0.347	5, 20	18.14	< 0.001	5, 20	0.69	0.563
<i>Holopedium gibberum</i>	1, 4	0.43	0.550	5, 20	34.22	< 0.001	5, 20	1.27	0.321
<i>Sida crystallina</i>	1, 4	<0.01	0.952	5, 20	44.24	< 0.001	5, 20	1.03	0.412
<i>Diacyclops thomasi</i>	1, 4	0.01	0.930	5, 20	26.19	< 0.001	5, 20	0.49	0.629
<i>Mesocyclops edax</i>	1, 4	0.16	0.707	5, 20	11.56	< 0.001	5, 20	0.31	0.902
<i>Tropocyclops extensus</i>	1, 4	1.80	0.251	5, 20	29.26	< 0.001	5, 20	1.72	0.176
Zooplankton biomass	1, 4	7.79	0.049	5, 20	10.50	<0.001	5, 20	1.61	0.203

Notes: *P* values reported for Time and Time × pH Treatment interaction have been corrected for departure from sphericity using the Huynh-Feldt adjustment. For zooplankton population responses, bold *P* values indicate $P < 0.05$, while *P* values in bold italics were significant at $\alpha < 0.05$ after Bonferroni correction for the number of species tested per lake.

the course of the experiment in Trout Lake and Little Rock-Reference, whereas community structure became more similar in control and acidified treatments in Little Rock-Treatment. At the end of the experiment, community similarity was lowest in Trout Lake (0.42), intermediate in Little Rock-Reference (0.72), and highest in Little Rock-Treatment (0.88). In Trout Lake, total zooplankton biomass was significantly reduced with acidification (Fig. 2, Table 2). In contrast, acidification had insignificant or weak effects on total zooplankton biomass in Little Rock-Reference and Little Rock-Treatment (Fig. 2, Table 2).

Differences among sites in community-level sensitivity to acidification were caused by variation in population-level responses. Overall, zooplankton population responses were strongest in Trout Lake and weakest in Little Rock-Treatment (Fig. 3, Table 2). Seventy-one percent (5/7) of dominant zooplankton species in Trout Lake exhibited significant ($P < P_{\text{critical}}$ after Bonferroni correction for number of species tested) or marginally significant ($P < 0.05$) responses to acidification, compared to 50% (3/6) and 14% (1/7) in Little Rock-Reference and Little Rock-Treatment, respectively. We considered population responses to acidification to include significant main effects of treatment, as well as significant time by treatment interactions. Main effects of acid treatment test for differences in average densities, whereas the time by treatment in-

teraction tests for differences in population trajectories through time (Gurevitch and Chester 1986, Potvin et al. 1990, von Ende 1993). In rare cases where a significant time by treatment interaction was found in association with a nonsignificant treatment effect, we concluded that the population had responded to acidification only when ANOVA conducted for each sample date separately indicated significant treatment differences.

In Trout Lake, nearly all dominant zooplankton species were affected adversely by acidification (Fig. 3, Table 2). Average densities of *Daphnia mendotae* and *Tropocyclops extensus* were significantly lower in the acidified treatment than in the control. In addition, acidification caused a marginally significant reduction in average density for *Diacyclops thomasi* (i.e., $P_{\text{int}} < 0.05$ but $P_{\text{int}} >$ critical Bonferroni-corrected *P*-value). *Bosmina longirostris*, *Daphnia mendotae*, *Holopedium gibberum*, and *Tropocyclops extensus* followed significantly different population trajectories in control and acidified treatments, as indicated by significant time by treatment interactions for these species. Average densities of *Holopedium gibberum* differed significantly between control and acidified treatments during weeks 1–3 (week 1, $F_{1,3} = 45.69$, $P = 0.007$; week 2, $F_{1,3} = 45.25$, $P = 0.007$; week 3, $F_{1,3} = 69.49$, $P = 0.004$). Average densities of *Bosmina longirostris* differed significantly between control and acidified treatments dur-

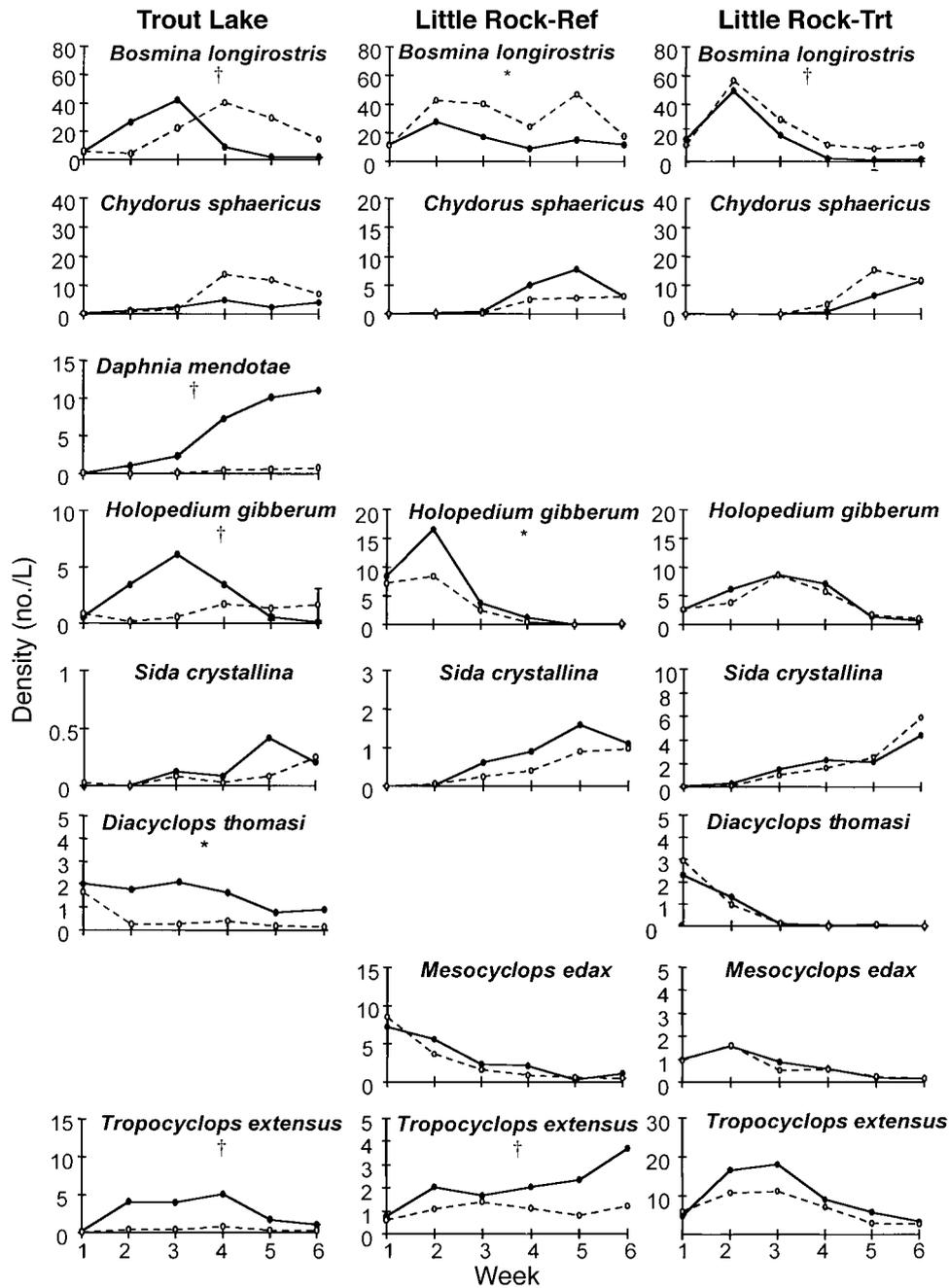


FIG. 3. Zooplankton population dynamics in the mesocosm experiment for Trout Lake, Little Rock-Reference, and Little Rock-Treatment. Means are plotted for control (solid circle) and acidified (open circle) treatments. Only dominant species (>5% by biomass or abundance in either treatment) are plotted for each site. In this figure, species are organized alphabetically within each major group (cladocerans followed by copepods). Asterisks below species names indicate significant responses to acidification as judged by a main effect of pH treatment and/or a time \times pH treatment interaction ($\dagger P < 0.05$ after Bonferroni correction for the number of species tested per site; $*P < 0.05$). Detailed statistics are presented in Table 2.

ing weeks 2 and 5 (week 2, $F_{1,3} = 11.86$, $P = 0.041$; week 5, $F_{1,3} = 15.76$, $P = 0.029$).

In comparison to the dramatic population responses in Trout Lake, responses to acidification by Little Rock-Reference zooplankton were relatively weak (Fig. 3, Table 2). Acidification resulted in a significant reduc-

tion in average density for *Tropocyclops extensus*. In addition, marginally significant treatment effects were detected for *Bosmina longirostris* and *Holopedium gibberum*. Interestingly, average *Bosmina longirostris* densities were higher in the acidified treatment than in the control. Finally, *Holopedium gibberum* and *Tro-*

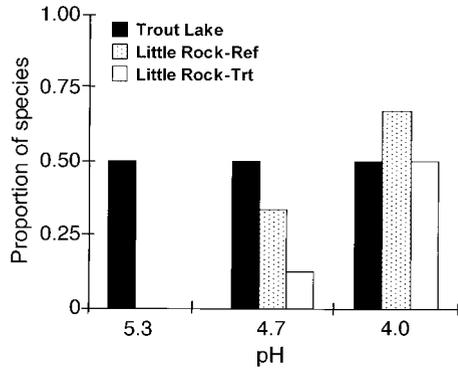


FIG. 4. Proportion of crustacean zooplankton species in the pH gradient experiment exhibiting significant decreases in density at reduced pH compared to the control for Trout Lake, Little Rock-Reference, and Little Rock-Treatment.

pocyclops extensus followed different trajectories in control and acidified treatments, as indicated by marginally significant or significant time by treatment interactions for these species.

In Little Rock-Treatment, zooplankton populations were highly resistant to acidification (Fig. 3, Table 2). In fact, only one species (*Bosmina longirostris*) responded significantly to acidification. Average *Bosmina longirostris* density was significantly higher in the acidified treatment (21.6 ± 0.9 individuals/L) than in the control (14.7 ± 2.4 individuals/L). Although this effect was statistically significant, the magnitude of the effect was relatively small (Fig. 3).

Because zooplankton species composition in mesocosms was similar among study sites, we could com-

pare population responses for the same species across sites. In several cases where a species occurred in more than one study site, significant or marginally significant responses to acidification were detected in Trout Lake and/or Little Rock-Reference, but not in Little Rock-Treatment (Fig. 3, Table 2; see *Holopedium gibberum*, *Diaicyclops thomasi*, and *Tropocyclops extensus*).

pH gradient experiment

The pH gradient experiment indicated that population sensitivity to acidification varied among sites consistently with our expectations based on acidification history. In all cases, detrimental effects of acidification on population densities were detected at all pH levels below a critical threshold pH. However, the threshold for population declines differed among study sites (Fig. 4, Table 3). At pH 5.3, 50% (2/4) of species from Trout Lake were detrimentally affected whereas none of the species from Little Rock-Reference (0/6) or Little Rock-Treatment (0/8) responded significantly to acidification. At pH 4.7 (the target acidification level in the mesocosm experiment), the ranking of responses paralleled the mesocosm experiment. That is, the Trout Lake assemblage was most sensitive and the Little Rock-Treatment assemblage was least sensitive. However, a large proportion of species responded in all sites when pH was reduced to 4.0, a level more acidic than historical conditions in all sites.

When we restricted our analysis to those species occurring in at least two of the study sites, we found that populations from the site with the mildest previous exposure to acidification tended to exhibit the most sensitive response to experimental reduction in pH

TABLE 3. One-way ANOVA for population responses to acidification in the pH gradient experiment.

Species	Lake	pH			Contrasts		
		df	F	P	5.3	4.7	4.0
<i>Bosmina longirostris</i>	Trout	3, 6	1.00	0.455			
	Little Rock-Trt	3, 8	2.77	0.111			
<i>Chydorus sphaericus</i>	Trout	3, 6	1.85	0.239			
<i>Daphnia catawba</i>	Little Rock-Trt	3, 8	10.02	0.004			*
<i>Daphnia dubia</i>	Little Rock-Ref	3, 8	14.38	0.001		*	*
	Little Rock-Trt	3, 8	9.59	0.005		*	*
<i>Daphnia parvula</i>	Little Rock-Trt	3, 8	3.57	0.067			
<i>Diaphanosoma birgei</i>	Little Rock-Ref	3, 8	3.31	0.078			
<i>Holopedium gibberum</i>	Trout	3, 6	5.47	0.037	*	*	*
	Little Rock-Ref	3, 8	4.67	0.036			†
	Little Rock-Trt	3, 8	2.07	0.183			
<i>Diaicyclops thomasi</i>	Trout	3, 6	50.36	<0.001	*	*	*
	Little Rock-Trt	3, 8	15.45	0.001			*
<i>Diaptomus minutus</i>	Little Rock-Ref	3, 8	60.73	<0.001		*	*
	Little Rock-Trt	3, 8	3.19	0.084			
<i>Mesocyclops edax</i>	Little Rock-Ref	3, 8	4.55	0.038			*
	Little Rock-Trt	3, 8	15.68	0.001			*
<i>Tropocyclops extensus</i>	Little Rock-Ref	3, 8	12.60	0.002			*

Notes: For species where one-way ANOVA indicated a significant pH effect, we performed Dunnett's contrasts to test for significant decreases in population density in each acidified treatment compared to the control. Asterisks in the contrasts column indicate acidified treatments where population densities were significantly lower than ambient ($\alpha < 0.05$).

† $P = 0.06$.

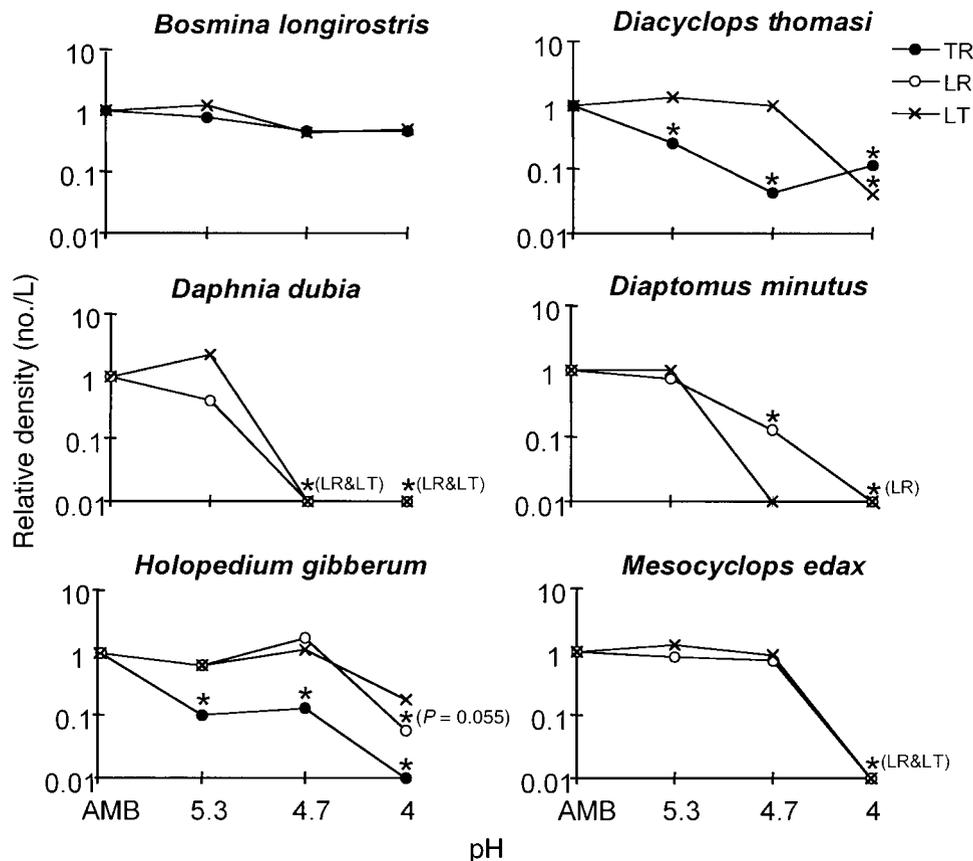


FIG. 5. Relative population densities of crustacean zooplankton at each pH level at the end of the pH gradient experiment. To facilitate comparisons among sites, we calculated relative densities (mean population density for each treatment was divided by mean population density in the control). Asterisks indicate acidified treatments where population densities were significantly lower than ambient pH, i.e., where the relative density was significantly < 1 . Site codes: TR, Trout Lake; LR, Little Rock-Reference; LT, Little Rock-Treatment.

(Fig. 5, Table 3). For example, *Diacyclops thomasi* from Trout Lake was detrimentally affected in all acidified treatments, whereas *Diacyclops thomasi* from Little Rock-Treatment tolerated acidification at pH 5.3 and 4.7 but declined at pH 4.0. In general, the threshold pH for population decreases occurred in the order Trout Lake \geq Little Rock-Reference \geq Little Rock-Treatment (Table 3). The only exceptions to this pattern involved two relatively acid-tolerant species (*Bosmina longirostris* and *Mesocyclops edax*), which did not respond differentially among sites and *Daphnia dubia*, which responded to acidification at pH 4.7 in both Little Rock-Reference and Little Rock-Treatment.

Reciprocal transplant experiment

The reciprocal transplant experiment indicated that intersite variation in zooplankton responses to acidification was primarily due to differences in acid sensitivity among zooplankton populations (Fig. 6). For three of the five species tested (*Diacyclops thomasi*, *Tropocyclops extensus*, and *Diaptomus minutus*), we detected a statistically significant zooplankton source

by pH interaction indicating that zooplankton response to pH depended on the site from which they were collected (Table 4). In addition, the three-way interaction between zooplankton source, water source, and pH was significant for two species (*Holopedium gibberum* and *Diaptomus minutus*).

In general, zooplankton populations from Little Rock-Treatment exhibited the weakest response to experimental reduction in pH, as in our other experiments. For example, *Diacyclops thomasi* from Trout Lake was detrimentally affected by acidification (pH $F_{1,12} = 89.67$, $P < 0.001$; Water Source $F_{2,12} = 6.44$, $P = 0.012$; pH \times Water Source $F_{2,12} = 5.12$, $P = 0.025$), whereas pH had no significant effect on *Diacyclops thomasi* from Little Rock-Treatment (pH $F_{1,7} = 0.52$, $P = 0.493$; Water Source $F_{2,7} = 2.05$, $P = 0.200$; pH \times Water Source $F_{2,7} = 0.89$, $P = 0.454$). Similarly, two-way ANOVA indicated *Tropocyclops extensus* populations from Trout Lake and Little Rock-Reference were adversely affected by acidification (Trout Lake, pH $F_{1,12} = 8.46$, $P = 0.013$; Water Source $F_{2,12} = 0.55$, $P = 0.591$; pH \times Water Source $F_{2,12} = 0.76$, $P = 0.488$;

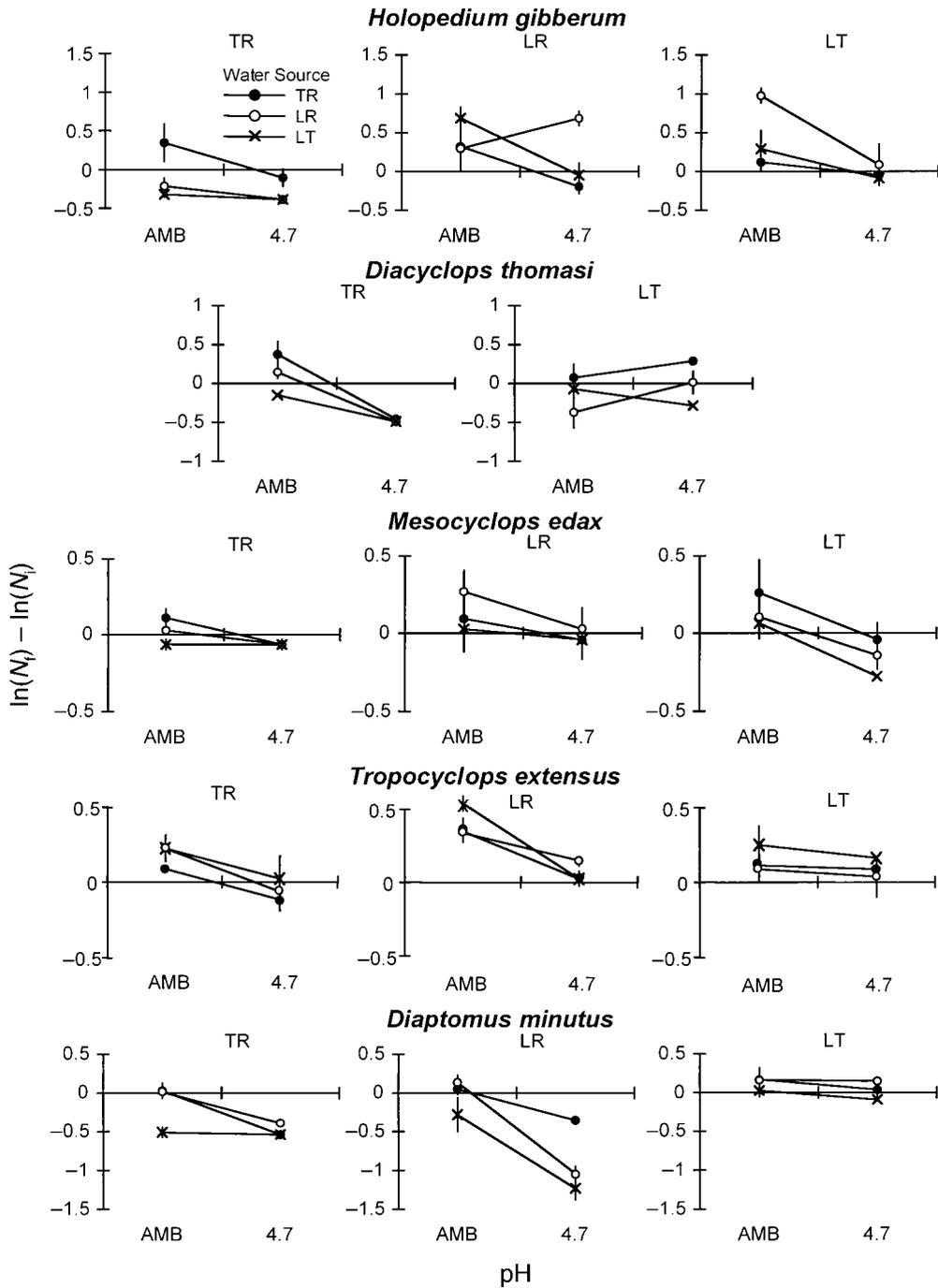


FIG. 6. Norms of reaction from the reciprocal transplant experiment. Change in population density during a 1-wk incubation [$\rho = \ln(N_t) - \ln(N_i)$, where N_t and N_i are final and initial densities] was plotted for each zooplankton source, water source, and pH treatment combination. Each panel represents a species collected from a particular site (i.e., zooplankton source). Site codes: TR, Trout Lake; LR, Little Rock-Reference; LT, Little Rock-Treatment. Treatment (pH) codes: AMB, ambient pH; 4.7, pH 4.7. Vertical bars indicate ± 1 SE.

Little Rock-Reference, pH $F_{1,10} = 59.67$, $P < 0.001$; Water Source $F_{2,10} = 1.42$, $P = 0.286$; pH \times Water Source $F_{2,10} = 4.43$, $P = 0.042$), whereas pH had no significant effect on *Tropocyclops extensus* from Little Rock-Treatment (pH $F_{1,7} = 0.28$, $P = 0.616$; Water

Source $F_{2,7} = 0.72$, $P = 0.520$; pH \times Water Source $F_{2,7} = 0.03$, $P = 0.975$). Finally, *Diaptomus minutus* from Trout Lake and Little Rock-Reference were detrimentally affected by acidification (Trout Lake, pH $F_{1,12} = 88.76$, $P < 0.001$; Water Source $F_{2,12} = 33.49$,

TABLE 4. Three-way ANOVA for main effects of pH, zooplankton source, and water source, as well as two- and three-way interactions between these factors in the reciprocal transplant experiment.

Species	Sites	Effect	df	F	P
<i>Holopedium gibberum</i>	TR, LR, LT	pH	1, 29	17.12	<0.001
		Water Source	2, 29	2.66	0.087
		Zooplankton Source	2, 29	14.98	<0.001
		Water Source by pH	2, 29	0.44	0.646
		Zooplankton Source by pH	2, 29	0.80	0.458
		Zooplankton Source by Water Source	4, 29	5.67	0.002
		Zooplankton Source by Water Source by pH	4, 29	4.38	0.007
<i>Diaptomus minutus</i>	TR, LR, LT	pH	1, 29	103.83	<0.001
		Water Source	2, 29	18.61	<0.001
		Zooplankton Source	2, 29	40.95	<0.001
		Water Source by pH	2, 29	1.53	0.234
		Zooplankton Source by pH	2, 29	27.55	<0.001
		Zooplankton Source by Water Source	4, 29	3.21	0.026
		Zooplankton Source by Water Source by pH	4, 29	7.94	<0.001
<i>Diacyclops thomasi</i>	TR, LT	pH	1, 19	8.63	0.008
		Water Source	2, 19	6.04	0.009
		Zooplankton Source	1, 19	2.26	0.149
		Water Source by pH	2, 19	0.52	0.602
		Zooplankton Source by pH	1, 19	20.69	<0.001
		Zooplankton Source by Water Source	2, 19	0.77	0.478
		Zooplankton Source by Water Source by pH	2, 19	3.34	0.057
<i>Mesocyclops edax</i>	TR, LR, LT	pH	1, 29	10.71	0.003
		Water Source	2, 29	1.63	0.214
		Zooplankton Source	2, 29	0.79	0.463
		Water Source by pH	2, 29	0.15	0.861
		Zooplankton Source by pH	2, 29	1.19	0.319
		Zooplankton Source by Water Source	4, 29	0.69	0.604
		Zooplankton Source by Water Source by pH	4, 29	0.23	0.922
<i>Tropocyclops extensus</i>	TR, LR, LT	pH	1, 29	23.80	<0.001
		Water Source	2, 29	2.09	0.142
		Zooplankton Source	2, 29	6.23	0.006
		Water Source by pH	2, 29	0.78	0.468
		Zooplankton Source by pH	2, 29	4.06	0.028
		Zooplankton Source by Water Source	4, 29	0.24	0.914
		Zooplankton Source by Water Source by pH	4, 29	0.94	0.456

Notes: The response variable was change in population density during a 1-wk incubation [$\rho = \ln(N_f) - \ln(N_i)$, where N_f and N_i are final and initial densities]. Sites codes: TR, Trout Lake; LR, Little Rock-Reference; LT, Little Rock-Treatment. Bold indicates $P < 0.05$.

$P = 0.001$; pH \times Water Source $F_{2,12} = 19.28$, $P < 0.001$; Little Rock-Reference, pH $F_{1,10} = 83.90$, $P < 0.001$; Water Source $F_{2,10} = 9.59$, $P = 0.005$; pH \times Water Source $F_{2,10} = 6.50$, $P = 0.016$); however, pH had no significant effect on *Diaptomus minutus* from Little Rock-Treatment (pH $F_{1,7} = 1.37$, $P = 0.280$; Water Source $F_{2,7} = 1.60$, $P = 0.268$; pH \times Water Source $F_{2,7} = 0.38$, $P = 0.695$).

Differences in population sensitivity among sites were not observed for *Mesocyclops edax* or *Holopedium gibberum*. For *Mesocyclops edax*, responses to acidification did not differ among sites, as indicated by the lack of a significant zooplankton source by pH interaction or a three-way interaction in Table 4. In contrast, *Holopedium gibberum* was affected by acidification in all three sites (Trout Lake, pH $F_{1,12} = 6.02$, $P = 0.030$; Water Source $F_{2,12} = 10.07$, $P = 0.003$; pH \times Water Source $F_{2,12} = 1.53$, $P = 0.255$; Little Rock-Reference, pH $F_{1,10} = 3.44$, $P = 0.093$; Water Source $F_{2,10} = 2.38$, $P = 0.142$; pH \times Water Source $F_{2,10} = 4.90$, $P = 0.033$; Little Rock-Treatment, pH $F_{1,7} = 7.40$,

$P = 0.030$; Water Source $F_{2,7} = 3.37$, $P = 0.094$; pH \times Water Source $F_{2,7} = 1.68$, $P = 0.254$).

In contrast to the strong effects of zooplankton source, effects of water source on zooplankton responses to acidification were relatively weak. We failed to detect a significant water source by pH interaction for any of the species tested, indicating that water source alone was not driving zooplankton responses to pH. However, for *Holopedium gibberum* and *Diaptomus minutus*, the effects of water source on responses to acidification were detected as complex three-way interactions, indicating that both zooplankton population differences and environment factors affected responses to acid for these species. In several cases (e.g., *Holopedium gibberum*, *Diacyclops thomasi*, and *Mesocyclops edax*), we noted that zooplankton collected from Trout Lake achieved higher ρ values at ambient pH in Trout Lake water than in water from Little Rock Lake. This result may indicate that Trout Lake zooplankton are intolerant to the pH conditions in ambient Little Rock Lake water (pH 5.8–6.1).

In sum, the reciprocal transplant experiment indicated that differential responses of populations of the same zooplankton species to acidification were not caused simply by differences in water chemistry, algal resources, or other site-specific environmental variables. Instead, variation in population responses to acidification was caused primarily by differences in acid sensitivity among the zooplankton populations.

DISCUSSION

Our study highlights the effects of ecological history on community-level responses to environmental change. In comparative experiments conducted in three study sites with different acidification histories, zooplankton exhibited widely divergent population- and community-level responses to acidification. Zooplankton populations responded most sensitively to acidification in Trout Lake, where high acid neutralizing capacity had buffered changes in pH historically. For most species, population densities were severely reduced in the acidified treatment compared to the control. Acidification was a novel perturbation for the Trout Lake community because pH manipulations changed the system outside of the range of historical variation. The results from Trout Lake indicate that ecological systems are likely to be highly sensitive to perturbations for which they have no history (e.g., novel temperature regimes caused by climate change, new chemical stressors associated with human activities, and exotic species).

In contrast to the novelty of acidification for the Trout Lake community, zooplankton in Little Rock Lake had varying degrees of prior exposure to acidification. In Little Rock-Reference, pH is routinely as low as 5.4 during the period just after ice-off due to natural processes associated with CO₂ buildup under the ice (Kratz et al. 1987). In this site, zooplankton responses were more moderate than responses in Trout Lake. In contrast, Little Rock-Treatment had a history of sustained acidification due to experimental acidification during the 1980s. We found that Little Rock-Treatment zooplankton were highly resistant in our acidification experiments.

As a result of variation in population-level responses to acidification, sites also differed in the sensitivity of community-level variables to acidification. In the mesocosm experiment, differences in community composition between acidified and control treatments were most dramatic in the highly buffered site (Trout Lake), intermediate in the site that experiences seasonal fluctuations in pH (Little Rock-Reference), and weakest in the experimentally acidified site (Little Rock-Treatment). Furthermore, total zooplankton biomass was significantly reduced with acidification in Trout Lake due to declines of many acid-sensitive populations. In contrast, effects of acidification on zooplankton biomass in Little Rock-Treatment were weak because zooplankton populations were highly resistant to acidifi-

cation. In Little Rock-Reference, zooplankton biomass was not significantly affected by acidification because decreases in the few sensitive species were matched by compensatory increases in other species (e.g., decreases in *Holopedium gibberum* and *Tropocyclops extensus* were coupled with an increase in *Bosmina longirostris*).

Community-level responses to acidification arise from direct effects of acidification on survival and/or reproduction, as well as indirect pathways operating through species interactions (Webster et al. 1992, Fischer 1997, Fischer and Frost 1997, Klug et al. 2000). Previous laboratory bioassays indicate that *Holopedium gibberum* is an acid-sensitive species (Havas and Likens 1985). The dramatic reduction in *Holopedium* abundance with acidification in Trout Lake mesocosms can, therefore, be interpreted as a direct response to acidification. In contrast, *Bosmina longirostris* is acid tolerant and often dominates the zooplankton community in acidified lakes (Yan and Strus 1980). In our experiment, *Bosmina longirostris* increased with acidification. This response was likely an indirect effect caused by competitive release following changes in the abundance of other species. Clearly, the magnitude and number of direct responses to acidification can play critical roles in determining the extent of indirect effects (Abrams et al. 1996), and both direct and indirect effects affect community-level responses to acidification.

In part, the contrasting community-level responses to acidification in Trout Lake, Little Rock-Reference, and Little Rock-Treatment were due to differences in the species composition of the three communities. For example, the acid-sensitive cladoceran *Daphnia mendotae* was present in Trout Lake but does not occur in Little Rock Lake. We observed a dramatic reduction in *Daphnia mendotae* abundance with acidification in Trout Lake mesocosms. Previous laboratory bioassays and lake surveys indicate that *Daphnia mendotae* is an acid-sensitive species that does not persist in lakes with pH < 6 (Keller et al. 1990). Therefore, the dramatic response of *Daphnia mendotae* in Trout Lake and the absence of this species from Little Rock Lake illustrates how species composition can play a role in intersite variation in community responses to acidification. Thus, changes in zooplankton species composition during past acidification events can decrease sensitivity to subsequent acidification in the same way that changes in species composition reduce the effects of repeated winterkills in lakes (Tonn and Magnuson 1982) and repeated fires in forests (Muir 1993, Turner et al. 1997). Species composition in Little Rock-Reference and Little Rock-Treatment was virtually identical (except for *Diacyclops thomasi*, which occurred only in Little Rock-Treatment). Therefore, differential responses to acidification in these two sites could not be explained simply by the presence/absence of particular species.

We found evidence that variation in community responses to acidification can also arise due to popula-

tion-level differences in acid tolerance. In the six week long mesocosm experiment, those species that occurred across study sites were relatively less sensitive to experimental pH manipulations in Little Rock-Treatment than in Trout Lake and/or Little Rock-Reference. This pattern was also evident in the pH gradient experiment where the critical threshold for detrimental effects of acidification occurred at a lower pH level in Little Rock-Treatment than in the other sites. Furthermore, the reciprocal transplant experiment indicated that this pattern was driven primarily by population differences in acid tolerance, rather than other factors (e.g., water chemistry or food resources) that might differ among sites.

Our results indicate that changes in acid sensitivity of zooplankton can occur rapidly. We were particularly interested to find that the sensitivity of *Tropocyclops extensus* to acidification differed between Little Rock-Reference and Little Rock-Treatment (Fig. 6). Presumably, these two populations existed as a single well-mixed population before the lake basins were separated in 1983. *Tropocyclops extensus* became the dominant copepod species in Little Rock-Treatment during the most acidic phase of the whole-lake acidification (Frost et al. 1995). Our results suggest that this dramatic increase in *Tropocyclops extensus* may be attributable to increases in acid tolerance by *Tropocyclops* in Little Rock-Treatment.

Our results support the hypothesis that zooplankton population differences in acid tolerance can arise due to evolutionary processes. Zooplankton are likely candidates for rapid evolutionary responses to environmental conditions. Other studies have reported differences in life history characteristics for zooplankton populations from different environments, some of which are heritable (Tessier 1986, Wyngaard 1986a, b, Tessier and Consolatti 1989, Hairston and Dillon 1990, Ellner et al. 1999). Furthermore, a recent study by Hairston et al. (1999) has documented rapid evolution of tolerance of toxic bluegreen algae by *Daphnia* during the eutrophication of Lake Constance. Therefore, we believe that the relatively short generation times of crustacean zooplankton (weeks to months) and strong selective pressures exerted by six years of experimentally reduced pH in Little Rock-Treatment could have combined to cause rapid evolution of acid tolerance in some zooplankton species. Detailed genetic studies comparing populations would strengthen our interpretations, but these studies are beyond the scope of this study.

It is noteworthy that we detected population differences in acid tolerance in the pH gradient and reciprocal transplant experiments, which were conducted during the summer of 1997. At this time, abiotic and biotic conditions were virtually identical in Little Rock-Reference and Little Rock-Treatment (Frost et al. 1998; T. M. Frost, unpublished data). Furthermore, pH conditions in Little Rock-Treatment had recovered sev-

eral years earlier (see *Methods: Study sites* description). Therefore, it seems unlikely that our results reflect phenotypic plasticity, maternal effects, or effects of individual condition on acid tolerance because many generations of zooplankton had occurred in Little Rock-Treatment under the relatively benign pH conditions of 1994–1997. However, without additional experiments in which zooplankton are raised for several generations in a common environment, we can not rule out the possibility that maternal effects can influence acid tolerance.

Similarly, without additional experiments conducted repeatedly through time, we have no way to assess whether increased acid tolerance in Little Rock-Treatment populations will persist through time. If tolerance imposes costs in performance of other activities, we expect acid tolerance to be lost under recovered pH conditions. In that case, enhanced resistance to acidification would be a transitory effect in Little Rock-Treatment. However, we found enhanced resistance in 1997, which was several years into recovery, suggesting that the costs of acid tolerance may be low and that the return of Little Rock-Treatment zooplankton to pre-acidification levels of acid tolerance may not be rapid. It is also important to note that the Little Rock acidification is an example of a perturbation where selection pressure was imposed and then relaxed. In other cases where perturbations are maintained or repeated through time, we would expect these more constant selection pressures to maintain stress-tolerant populations.

Previous studies on the specific physiological mechanisms controlling acid tolerance have implicated neuromuscular dysfunction associated with hydrogen and aluminum binding at ion exchange sites as the cause of high mortality of zooplankton in acidic waters (Havas and Likens 1985, Havens 1990). It is unlikely that aluminum toxicity played a critical role in our experiments. When lakes are acidified as a result of acid rain, aluminum ion concentrations can reach high levels because aluminum is leached from watershed soils and sediments (Cronan and Schofield 1979). However, our pH manipulations were in bags and small containers and, therefore, independent of the watershed soils and sediments. Similarly, aluminum levels were low during the whole lake acidification of Little Rock Lake because acid was added directly to the lake rather than the watershed. In fact, aluminum concentrations increased only moderately from initial concentrations of $<10 \mu\text{g/L}$ to an average of $38 \mu\text{g/L}$ at pH 4.7 (Brezonik et al. 1993). Aluminum concentrations of this level are characteristic of unacidified lakes and are not toxic to zooplankton (Havas and Likens 1985). Typically, aluminum becomes toxic to zooplankton at concentrations of hundreds or thousands of micrograms per liter (Havas and Likens 1985, Havens 1990).

To our knowledge, only one previous study has reported laboratory bioassays showing interpopulation variation in acid tolerance by zooplankton. Price and

Swift (1985) used laboratory bioassays to demonstrate that *Mesocyclops edax* from an acidic pond were more acid tolerant than *Mesocyclops edax* collected from a more neutral pond. Our findings and their results suggest that variation in acid sensitivity among populations of the same species can be substantial. Nonetheless, it is common practice to infer the acid sensitivity of a species based on an assay of a single population (see review by Locke 1991). Gonzalez and Frost (1994) have cautioned against the use of laboratory bioassays to predict population responses to acidification at the whole-lake scale. Clearly, variation in population sensitivity to acidification may also contribute to a substantial difference between laboratory bioassays and population dynamics in the field.

Inter-site differences in the extent and nature of community-level responses to perturbation such as we have documented here may have important implications for predicting the ecosystem-level responses to stress. Previous studies have argued that sensitivity of ecosystem processes to perturbation decreases with increasing species diversity (Walker 1992, 1995, Tilman 1996). In these studies, variation in community responses to perturbation is caused by compensatory dynamics between perturbation-sensitive and perturbation-tolerant taxa. Species-rich communities may be more likely to contain perturbation-tolerant species, which can increase to compensate for the loss of perturbation-sensitive species.

Our study highlights another mechanism that can generate variation in ecosystem responses to perturbation. We found that historical conditions can lead to changes in perturbation tolerance of populations. Resistance and/or resilience of ecosystem processes to perturbation is likely to be greater in communities containing perturbation-tolerant populations than in communities dominated by sensitive populations. Furthermore, our results suggest that the effects of history on ecosystem responses to perturbation can operate independently of species richness. In our mesocosm experiment, the sensitivity of total zooplankton biomass (an aggregate measure often used to infer changes in ecosystem function) to acidification varied substantially among sites that contained a similar number of crustacean zooplankton species (range = 6–7 dominant species).

Overall, our findings demonstrate that ecological history cannot be ignored when attempting to predict ecological responses to environmental perturbations. Ecosystems may respond sensitively to environmental changes that fall outside of the range of historical conditions. Thus, disruption of ecosystem functions and services is likely following natural and anthropogenic disturbances that create extreme conditions. Our results also suggest that, under some circumstances, rapid evolution may buffer community responses against subsequent perturbations of the same type. The extent of rapid evolution and buffering of community responses

to future perturbations is likely to be system specific. Therefore, our findings reinforce previous calls for research integrating ecology and evolution (Antonovics and Levin 1980, Parsons 1989, Holt 1995, McPeak and Miller 1996, Thompson 1998), two disciplines that have traditionally been viewed as operating on different time scales. Such research may provide the basis for a predictive understanding of the ecological responses to a rapidly changing environment.

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