

Multiple stresses from a single agent: Diverse responses to the experimental acidification of Little Rock Lake, Wisconsin

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Abstract

A single stress, acidification with sulfuric acid, was applied to Little Rock Lake in a whole-ecosystem manipulation. We documented a wide range of responses to the acidification, including increases in the concentrations of various chemicals, shifts in microbial processes and a major increase in water clarity to UV-B radiation. Each of these changes could in itself be considered as a separate ecosystem stress that is distinct from the intended manipulation. Acidification in Little Rock Lake was accompanied by a number of substantial changes in the occurrence of organisms. A series of detailed investigations indicates that the mechanisms underlying these organismal changes are varied but cannot usually be tied to the direct effects of acidification. Overall, our results demonstrate how multiple stresses can arise from a single agent operating on an ecosystem and suggest that singly operating stresses may actually be quite rare.

Human activities have generated a wide variety of stresses, operating from local to regional scales, that have had major effects on ecosystems (Daily 1997). Substantial scientific efforts have evaluated the effects of many of these

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stresses on aquatic ecosystems and these have led to important basic insights in limnology and ecology (e.g., Schindler 1987; Charles 1991; Likens 1992; Williamson 1995; McKnight et al. 1996). Much of this work has emphasized particular single stresses despite the fact that several stresses are likely to influence an area simultaneously. Recently, there has been growing recognition of the need to identify the cumulative impacts of multiple stresses (e.g., Schindler et al. 1996; Yan et al. 1996; McKnight et al. 1996), but the widespread occurrence of different stresses requires the concomitant evaluation of multiple stresses for individual ecosystems. We illustrate this point here by reporting how a whole-lake experiment intended to evaluate the action of a single stress—acidification—revealed a more complicated array of secondary stresses operating through a variety of mechanisms. Further, over the course of the 7-yr experiment, regional fluctuations in climate imposed an additional external factor which interacted with the acidification. These sec-



Fig. 1. Aerial photograph of Little Rock Lake taken in 1990 showing its treatment (upper) and reference (lower) basins. The treatment basin has a surface area of 9.8 ha, a mean depth of 3.8 m, and a maximum depth of 10.3 m; the reference basin has a surface area of 8.1 ha, a mean depth of 3.1 m, and a maximum depth of 6.5 m. The reduced color in the treatment basin is visible relative to that in the reference basin. (Courtesy of C. Watras.)

ondary and external stresses illustrate the importance of considering the interacting actions of multiple factors, even in highly controlled experiments assessing the effects of a stress.

Little Rock Lake (LRL) has been the site of a whole-ecosystem acidification experiment since 1984 (Watras and Frost 1989; Brezonik et al. 1993; Sampson et al. 1995; Frost et al. 1998). To investigate the effects of acidification, we lowered the pH of LRL's treatment basin using sulfuric acid, the dominant anthropogenic acid form in deposition throughout much of the world (Galloway et al. 1984; Schindler 1988). Here we present data on the factors operating during the lake's manipulation that illustrate the importance of interacting forms of stress including several changes in lake biogeochemistry, food-web shifts, and increases in water transparency. Our results provide examples of responses to multiple stresses and illustrate some of the difficulties that could arise from evaluating a single stress in isolation from secondary stresses and the influence of external factors that can affect an ecosystem.

Methods

Detailed descriptions of LRL and the methods used for this paper have been reported previously. These references are cited below and techniques are only summarized here.

Little Rock Lake (45°59'55"N, 89°42'15"W) is a low-conductivity, low-acid-neutralizing-capacity, clear-water, seepage lake in the Northern Highland Lake District in northern Wisconsin. The treatment and reference basins were divided by a flexible, plasticized curtain in 1984 (Fig. 1). Following

a baseline year in 1984, the treatment basin was acidified with sulfuric acid in three stages from its original pH of 6.1 to target levels of 5.6, 5.1, and 4.7 (Fig. 2). Each target pH was maintained for 2 yr to accommodate phenomena like fish spawning that have response times longer than 1 yr. Our design in the experiment was to approach, but not to exceed, the target acid levels. We refer to the periods by their nominal target levels here even though these were not the mean values that occurred during those periods. The last acid was added just before the lake froze in fall 1990. Since ice-off in 1991, we have been evaluating recovery in LRL using many of the same approaches that we used during the acidification period (Sampson et al. 1995). This paper focuses on the period 1984 through 1991 except that we report data up through 1994 that bear on the UV-light field and responses to it.

Both lake basins were sampled every 2 weeks during ice-free seasons and approximately every 5 weeks while the lake was frozen. Chemical samples were collected on a subset of these dates at several depths at a central station in each basin. Major ions and most metals were analyzed with standard methods summarized by Brezonik et al. (1990) and Sampson et al. (1994). For mercury and methylmercury in water and organisms of the lower food web, sampling techniques and analytical methods are presented by Watras and Bloom (1992). For Hg in yellow perch, *Perca flavescens*, fish were sampled annually in April with minnow traps or trap nets fished in littoral habitat a few days after ice melt. We analyzed age-1 fish, which had hatched the previous spring and resided in the lake for 1 yr. Analytical techniques are described by Wiener et al. (1990) for samples obtained in 1986

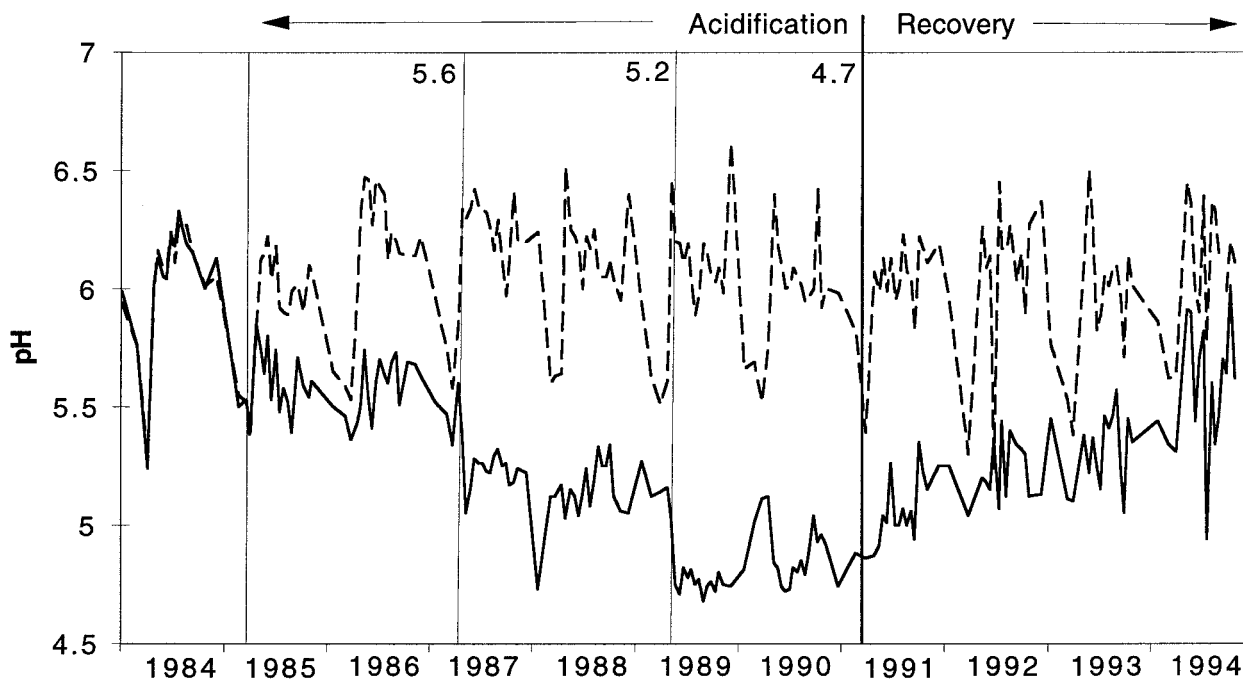


Fig. 2. Surface pH values in Little Rock Lake's treatment (solid line) and reference (broken line) basins from 1984 through 1994. The target pH levels for the three manipulation periods are illustrated.

and 1987 and Wiener et al. (in press) for samples obtained in 1988–1991. Mercury burden, defined as the total mass of mercury accumulated in a whole fish, was calculated as the product of body weight and whole-fish concentration. We used within-year statistical contrasts (*t*-tests, $\alpha = 0.05$) of mean mercury concentrations and burdens in age-1 yellow perch between the treatment and reference basins to evaluate the effect of experimental acidification on mercury bioaccumulation.

UV light values in LRL were estimated (Williamson et al. 1996) from dissolved organic carbon (DOC) measurements following Morris et al. (1995). DOC was determined as reported by Williamson et al. (1996).

For zooplankton, we assessed the abundance and biomass of crustacean and rotifer species (Frost and Montz 1988). Animals reported here were sampled at 0, 4, and 8 m in the treatment basin and 0, 4, and 6 m in the reference basin with a Schindler-Patalas trap with a 53- μm mesh bucket, and estimates were made of the average population throughout the entire lake basin. Samples were preserved with 4% sugar-buffered Formalin and each was counted separately. Descriptions of the zooplankton assays evaluating pH and food effects are given by Gonzales and Frost (1994).

Mesocosm experiments were conducted during summer 1995 in 0.64-m-diameter plastic bags that were 1.35 m deep, open to the air at the lake surface, and about 309 liters in volume. Twelve mesocosms were placed in the reference basin and deployed in a two-way factorial design. Six were allowed to remain at the ambient pH of 6.1 and six were acidified to pH 4.7 with sulfuric acid. Three of each of the pH treatments were shielded just above the water with UV-opaque Mylar while the other mesocosms in each pH treatment were screened with UV-transparent plastic (after Wil-

liamson et al. 1994). The UV-screening characteristics of the Mylar were tested using a Kontron double-beam scanning spectrophotometer. Mesocosms were set up in late July and their zooplankton sampled from the surface every 3 or 4 d on 7 dates using a 1.2-m-long, 0.05-m-wide plastic tube (DeVries and Stein 1991). The tube was corked just below the surface and its contents were filtered through an 80- μm mesh net. The contents of three tubes were pooled each time a mesocosm was sampled.

Results and discussion

Multiple stresses with acidification—The stresses that took place in LRL can be thought of as operating on three levels. Most basic among these are the impacts of the increased acidity and related changes driven by the accompanying sulfate ions. Following directly on these are a series of chemical and biological changes that occurred as secondary responses to the direct chemical manipulation. Finally, broadly acting regional processes had the potential to influence a diversity of within-lake chemical and biological phenomena.

The pH of the treatment basin was successfully maintained near target levels during the three acid-addition phases of the experiment (Fig. 2). There were, in addition, numerous other changes in chemistry that accompanied the intended pH depressions (Brezonik et al. 1993; Sampson et al. 1994).

Acidification influenced major ion concentrations in the lake due to a shift in the sediment exchange capacities. Ca^{2+} increased by more than 80% during the pH 4.7 manipulation (Fig. 3). Mg^{2+} and K^{+} showed smaller increases relative to

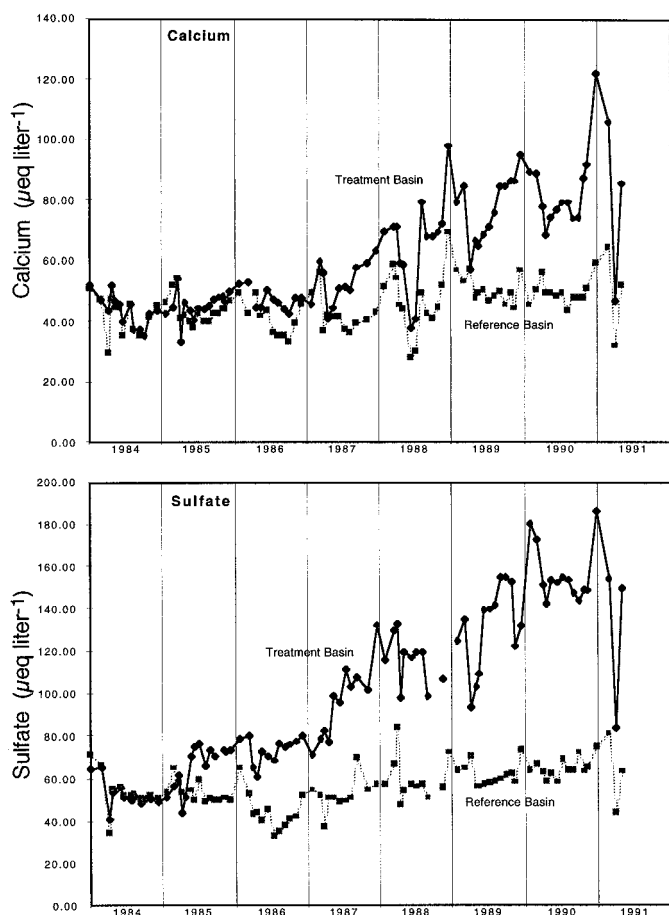


Fig. 3. Surface concentrations of Ca^{2+} and SO_4^{2-} in the treatment and reference basins of Little Rock Lake.

the reference basin and no detectable changes were noted in Na^+ , trends that were not unexpected and which generally followed predictions for the lake (Brezonik et al. 1993). The recorded increases in cation concentrations, particularly Ca^{2+} , may actually have buffered the ecosystem, ameliorating the effects of increasing acidity or other chemical changes (Brown 1982). At the same time, the magnitude of these increases begin to illustrate how adding sulfuric acid cannot be viewed simply as imposing a single chemical perturbation on the lake.

The concentrations of several minor and trace metals in lake water increased with acidification in the treatment basin. Increases were greatest for Al, Mn, Fe, and Zn (Fig. 4). Pb and Cd exhibited less substantial changes and no increase was noted in Cu (Brezonik et al. 1993). Unlike the major cations, several of the metals reached concentrations that have previously been associated with ecosystem stress (Schindler 1987). This was certainly the case for Al which was shown to influence fish populations at the levels recorded in LRL (Eaton et al. 1992). Increased metal concentrations provide a clear indication that several different stresses had been operating as LRL was acidified.

Microbial processes also showed responses to the pH changes that could have acted as stresses along with acidification. Elevated sulfate concentrations resulted from our

acid additions (Fig. 3) and stimulated higher rates of sulfate reduction in the treatment basin (Brezonik et al. 1993). In each of the three acid stages only about half of the sulfate ions added to increase the basin's acidity actually remained in the water column. This was associated with substantial internal alkalinity generation in the treatment basin. Increasing amounts of H_2S , iron-sulfur compounds and organic sulfur forms were produced by these processes, and each has the potential to generate significant changes in the lake for some organisms or processes. For example, in response to increases in reduced sulfur species, dense populations of phototrophic sulfur bacteria containing bacteriochlorophyll *d* developed in the hypolimnion of the acidified basin (Hurley and Watras 1991). Several aspects of the aquatic sulfur cycle in LRL were affected by the acid perturbation.

Particularly dramatic among the potentially, microbially mediated processes were high rates of methylmercury formation associated with acidification (Bloom et al. 1991; Watras and Bloom 1992). In 1990, the second year of the pH 4.7 stage of the experiment, methylmercury levels in water, phytoplankton, and zooplankton in the treatment basin were at least twice those in the reference basin (Table 1). In contrast, total Hg concentrations in surface waters were not distinguishable between the two basins (Watras and Bloom 1992). The observed increase in methylmercury could have resulted either from increased microbial activity or from increased $\text{Hg}(\text{II})$ as a substrate for methylation.

Within the hypolimnion of the treatment basin, a region of high methylmercury was located just below the oxic-anoxic boundary in profundal water (Watras and Bloom 1994). This is a region of intense sulfur cycling (see above) and subsequent field experiments indicated that microbial sulfate reduction, sulfide oxidation, and mercury methylation co-occur near the O-A boundary in the lake (Watras et al. 1995). Thus, changes in the sulfur cycle may have favored the microbial formation of methylmercury and its appearance in the planktonic food web.

Alternatively, increased methylmercury formation may have resulted from increases in the Hg_{II} pool that serves as substrate for microbial methylation. Fitzgerald et al. (1991) showed that the production of volatile Hg_0 species was lower in the acidified treatment basin than in the reference basin. Because less Hg_0 was presumably leaving the lake through gaseous evasion, the amount of Hg_{II} substrate may have increased.

In either case, the result was greater bioaccumulation of methylmercury throughout the food web. Mean concentrations of Hg in age-1 yellow perch were 20–100% higher in treatment-basin fish compared to reference-basin fish in 5 of 6 yr of acidification (Table 2). Only in the second year at pH 5.2 were Hg concentrations similar between the two basins.

The mean amount of mercury accumulated in age-1 yellow perch in the treatment basin exceeded that in the reference basin during 4 of the 6 yr of the acidification experiment (Table 2). These differences in accumulation cannot be attributed to differing growth rates, because the first-year growth of yellow perch was similar in both basins during 5 of the 6 yr of acidification, based on comparisons of mean fish weight (Table 2). The mean weight of age-1 fish differed

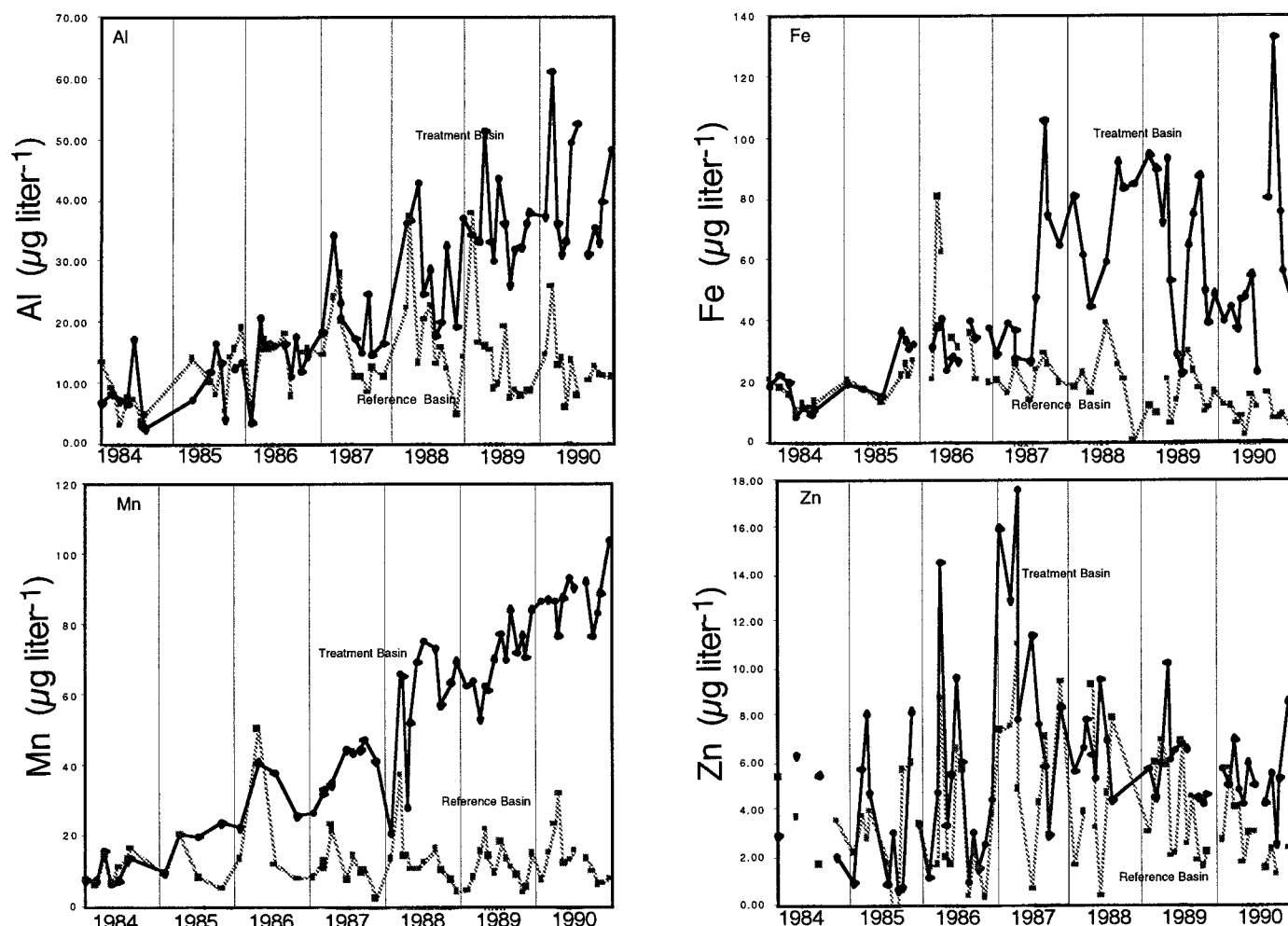


Fig. 4. Surface concentrations of Al, Fe, Mn, and Zn in the treatment and reference basins of Little Rock Lake.

between basins only after the first year of the pH 4.7 period, when growth was faster in the treatment basin than in the reference basin. Elevated Hg levels in yellow perch and elsewhere in LRL's food web represent another secondary stress linked with acidification.

Changes in microbial processing are the most likely source of increased Hg levels in the treatment basin. The lake has no identifiable on-site anthropogenic source of mercury. The annual atmospheric input of Hg to the lake (wet plus dry deposition), measured during 1988–1990, averaged about 0.1 g ha^{-1} (Fitzgerald and Watras 1989; Fitzgerald et

al. 1991). Analyses of sediment cores from nearby seepage lakes have indicated that the rate of atmospheric deposition of mercury in the area may have increased by a factor of three to four since about 1850 (Rada et al. 1989; Swain et al. 1992) but not during the period of the experiment. The technical-grade sulfuric acid used to acidify the treatment basin contained insufficient mercury to account for the changes observed during the manipulation (Wiener et al. 1990).

The effects of acidification on LRL were also evident in the lake's water clarity (Fig. 1). Differences occurred in the color and in the total organic carbon of treatment-basin water relative to reference-basin water (Brezonik et al. 1993). Because of changes in the concentrations of DOC, there were ultimately shifts in the light field of the treatment basins particularly for UV-A and UV-B radiation (Williamson et al. 1996). By the second year of the pH 4.7 treatment, 1% of the UV-B radiation reached a depth of nearly 2 m in the treatment basin compared with a depth of $<0.8 \text{ m}$ in the reference basin (Fig. 5). Such levels have the potential to influence several phytoplankton and zooplankton species (Williamson and Zagarese 1994; Williamson 1995) as well as higher trophic levels such as fish and the predatory larvae

Table 1. Mercury species in the treatment and reference basins of Little Rock Lake. Data are for the final phase of the acidification experiment. Total mercury—HgT; methylmercury—MeHg. Modified from Watras and Bloom (1992).

Lake basin	Aqueous (ng liter^{-1})		Microseston (ng g^{-1} , wet wt)		Zooplankton	
	HgT	MeHg	HgT	MeHg	HgT	MeHg
Treatment	0.8	0.1	40	15	75	56
Reference	1.1	0.05	30	4	54	20

Table 2. Mercury concentration, mercury burden, and weight of age-1 yellow perch from the treatment (T) and reference (R) basins of Little Rock Lake, during the 6-yr acidification experiment. Standard error given in parentheses below each mean concentration and mean burden.

Sampling year	Mean pH of T basin [†]	Mean concn (ng ⁻¹ g wet wt) [‡]		Mean burden (µg fish ⁻¹) [‡]		Mean wt (g fish ⁻¹) [‡]	
		T basin	R basin	T basin	R basin	T basin	R basin
1986	5.6	131** (3)	117 (3)	0.72 NS (0.03)	0.68 (0.02)	5.4 NS	5.9
1987	5.6	138** (3)	111 (2)	0.79** (0.03)	0.68 (0.02)	5.8 NS	6.1
1988	5.2	148** (5)	81 (2)	1.12** (0.06)	0.60 (0.02)	7.6 NS	7.4
1989	5.2	127 NS (5)	134 (3)	1.01 NS (0.05)	1.01 (0.04)	8.1 NS	7.5
1990	4.9	133** (4)	66 (3)	0.85** (0.04)	0.27 (0.02)	6.4**	4.0
1991	4.9	116** (3)	98 (2)	0.79* (0.05)	0.66 (0.04)	6.7 NS	6.7

[†] Value is the mean pH to which fish, sampled from the treatment basin during April of the year indicated, were exposed during the preceding open-water period. The mean pH of the reference basin was ~6.1.

[‡] One asterisk—the means differed between basins at $P \leq 0.05$; two asterisks—the means differed at $P \leq 0.01$; NS—no significant difference ($\alpha = 0.05$), based on a t -test. The sample size (n) for each mean was 30 fish except during 1991, when n was 17 for the treatment basin and 28 for the reference basin.

of the *Chaoborus* (Williamson et al. 1999). The potential stress of UV radiation operating concomitantly with acidification has been described recently in several reports (Schindler et al. 1996; Williamson et al. 1996, 1999; Yan et al. 1996). Schindler et al. (1996) reported a relationship between DOC levels and UV-light transmittance that is somewhat different than the one that we have employed here (Morris et al. 1995), but the basic patterns predicted by the two models are largely the same.

Finally, the array of responses observed in LRL could have been substantially different had the experiment been conducted during a period of different climatological conditions. 1984 to 1990 included a period of drought in mid-western North America (Krug and Simon 1992). As a result of reduced precipitation and increased evaporation, the water level of LRL declined by 0.8 m and its volume declined by more than 20% (Rose 1993). Further, groundwater inflow, which averaged 3% of the total water input before the drought, ceased entirely during it (Rose 1993). If we had conducted the LRL experiment during a wetter climatic period, groundwater inputs, which occur only in the southeast corner of the reference basin, would likely have caused the temporal trends of solutes like Ca, Mg, pH, and ANC in the reference basin to move in different trajectories, potentially complicating interpretation of inter-basin differences during the acidification. Droughts not only alter local groundwater-flow patterns (Webster et al. 1990) but also influence other hydrologic connections between lakes and the landscape, such as with wetlands which can be major sources of DOC (Schindler et al. 1996; Yan et al. 1996). The nature of a lake's response to drought can be quite different depending upon its position in the landscape and its basic hydrology (Webster et al. 1996). Differences in such external factors further complicate predictions on the development of multiple stresses in different ecosystems.

Mechanisms underlying observed organismal responses—There were strong responses by zooplankton and fish to the acidification of the lake. For zooplankton, the abundance of many species declined at each pH stage and the number of affected species increased at each level of pH reduction (Brezonik et al. 1993). At the same time, some species were strongly favored by acid conditions. Their biomass increases compensated for species declines to the extent that total zooplankton biomass changed only during the pH 4.7 stage of the experiment (Frost et al. 1995). Despite these dramatic changes, however, small-scale manipulations have failed to reveal any simple, direct response to acidity (Gonzalez and Frost 1994; Fischer and Frost 1997).

Contrasting changes by two rotifer species are typical of these complex responses (Gonzalez and Frost 1994). As *Keratella cochlearis* declined during the second year of acidification at pH 5.6, *Keratella taurocephala* exhibited increases sufficiently large that it eventually came to dominate rotifer biomass. The responses of these rotifers could not be linked solely to the direct effects of pH, however. *K. cochlearis* declined only when acid conditions were coupled with low food availability. In contrast with populations in the treatment basin, *K. taurocephala* exhibited reduced growth under acid conditions in the laboratory, suggesting that it too should have declined when the lake was acidified. Reductions in spine lengths that accompanied *K. taurocephala*'s increase suggest that its population shift is a response to reductions in predation pressure (Gonzalez 1992). In this case, then, a predation-driven stress has actually been removed due to altered food-web conditions resulting from the acidification. The mechanisms underlying changes in the dynamics of both these rotifers cannot be considered as direct reactions to acidification. Rather, they involve indirect responses to the acidification of the treatment basin and are typical of the mechanisms responsible for the changes ob-

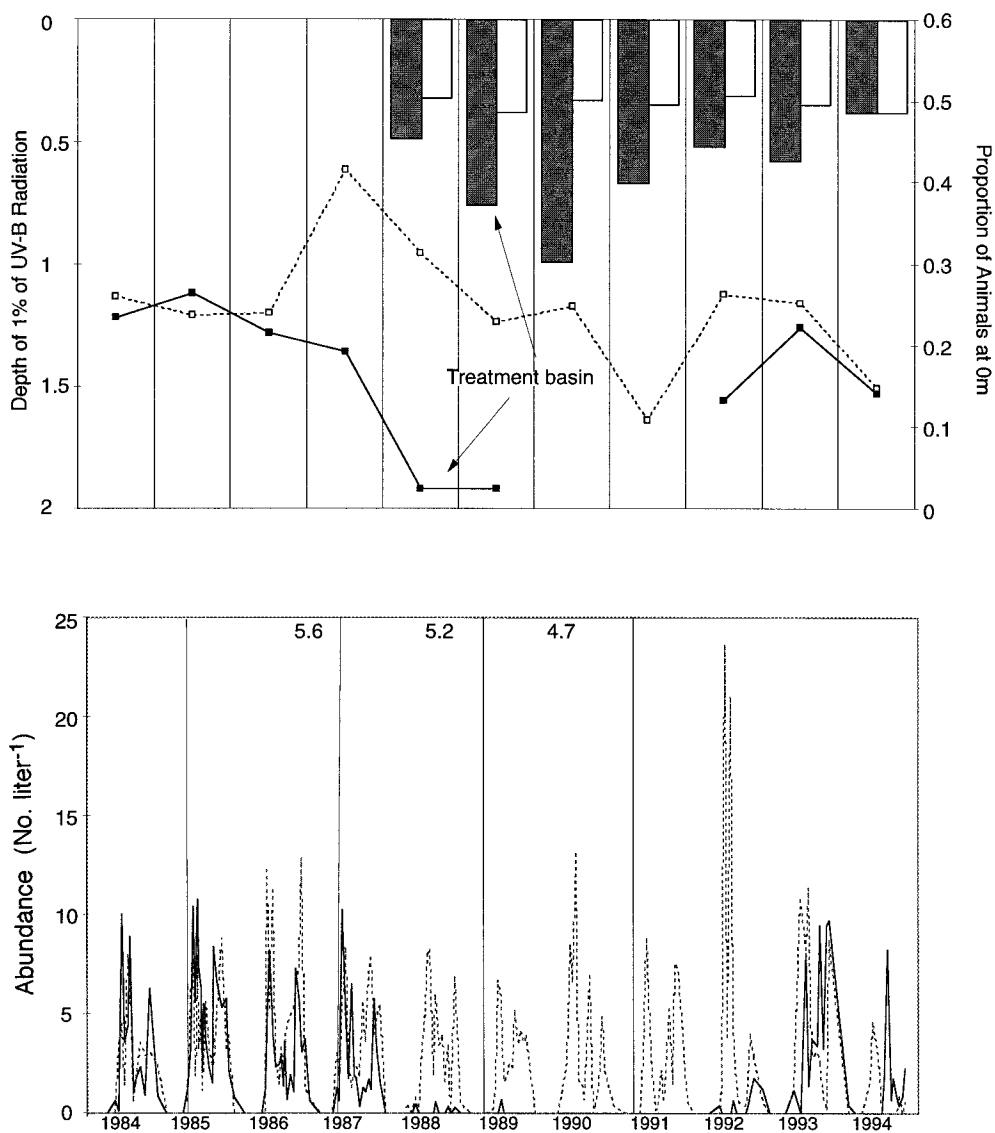


Fig. 5. Above—Proportional occurrence of *Holopedium gibberum* at 0 m and the depth to which UV-B light would be expected to penetrate in the treatment (solid lines, shaded bars) and reference (dotted line, clear bars) basins of Little Rock Lake. The annual average of the proportion of *H. gibberum* collected in a 0-m sample is shown at the bottom of the panel (■—■). The UV-B data represent the annual averages of the depths to which 1% of UV-B radiation would be expected to penetrate the lake basins, following Morris et al. (1995) and (Williamson et al. 1996). The 0 values in the treatment basin in 1990 and 1991 occurred when *H. gibberum* was not in that basin at all and do not represent years with reduced abundances in the surface waters. Below—Abundance of *H. gibberum* in the treatment (solid lines) and reference (dotted lines) basins is reported as the average number per liter throughout the water column on sampling dates during the period 1984–1994.

served in several other species during the LRL experiment (Webster et al. 1992).

Increases in the amount of UV-B radiation in the acidified surface waters of LRL (Williamson et al. 1996) suggested another mechanism that may explain some of the observed declines in zooplankton species (Brezonik et al. 1993). One effect of such an increase in lethal radiation could have been a disproportionate decrease in the abundance of sensitive zooplankton species in the surface waters of the treatment

basin. The common cladoceran *Holopedim gibberum* declined substantially in the treatment basin during later stages of the experiment, a decrease that involved such a downward shift in its vertical distribution (Fig. 5). Consistent with such a UV-related mechanism, the reduction of *H. gibberum* was observed to be most pronounced in the surface waters of the treatment basin during 1988 and 1989. A decrease did not occur only in surface waters during 1990 and 1991, however, because *H. gibberum* had been completely extirpated from

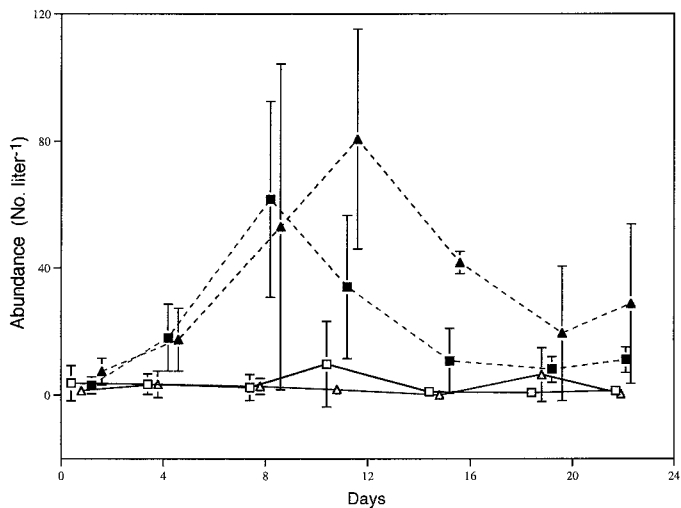


Fig. 6. Abundance of *Holopedium gibberum* during the UV-screen, mesocosm experiment. Time series are presented for the mean values ($\pm 95\%$ confidence intervals) recorded in mesocosms with natural (closed symbols, broken lines) and acidified (open symbols, solid lines) pH conditions. Mesocosms were screened with either Mylar that selectively limited the passage of UV radiation (squares) or plastic sheeting that was transparent to all radiation (triangles). A repeated-measure, two-way factorial ANOVA indicates that there was a significant pH effect ($F = 32.0$, $P = 0.0005$) but no significant Mylar-screening effect ($F = 2.285$, $P = 0.1691$) or pH–Mylar interaction ($F = 2.933$, $P = 0.1251$).

the treatment basin. Thus, the overall response of *H. gibberum*, cannot be attributed solely to a toxic UV effect. At the same time, the patterns of decline during 1988 and 1989 were consistent with a lethal UV effect in the surface water.

The patterns we observed for *H. gibberum* did not demonstrate that UV stress had actually operated in the lake, however. We tested directly for a radiation effect using UV-opaque Mylar screening over re-acidified mesocosms in LRL during 1995. These mesocosm experiments did reiterate a strong acid effect but comparisons between plastic-screened mesocosms that did not exclude UV-B radiation and Mylar-screened mesocosms did not demonstrate any direct UV effects for *H. gibberum* (Fig. 6). Other species likewise showed no evidence of response to UV radiation (Badillo and Frost unpubl. data). Our results do not exclude the possibility that UV effects operated at other times or for other species during the experiment. It does seem unlikely, however, that such effects are responsible for the *H. gibberum* declines that were observed with acidification. David Schindler (pers. comm.) has suggested that the *H. gibberum* decline may have occurred due to its increased susceptibility to predation with the increase of visible light that would have accompanied decreased DOC and increased UV radiation in the treatment basin.

Several fish species exhibited declines at various stages during the manipulation of the lake (Eaton et al. 1992). Age-1 largemouth bass, *Micropterus salmoides*, exhibited reduced overwinter survival as early as the pH 5.6 stage and certainly at pH 5.1 and 4.7. Laboratory assays suggested that these failures occurred because of the combined actions of at least two stresses: reduced pH and increased concentra-

tions of Al (Eaton et al. 1992). A decrease of young-of-the-year stages of yellow perch occurred later, only during the pH 4.7 stage of the experiment. Yellow-perch responses also appear to have been caused by the interactions of at least the same two stresses based on laboratory assays (Eaton et al. 1992). Yellow-perch population declines do not seem to be attributable to mercury effects, however. Equally high Hg levels were observed in yellow-perch body burdens during earlier years when no declines in fry survival were observed. Yellow perch did not exhibit detectable responses to the earlier LRL stress levels. Mercury levels were not evaluated in largemouth bass so the importance of this metal in this decline cannot be assessed, although a combination of pH stress and limited food availability seems likely to be responsible for the bass decline (Eaton et al. 1992).

Overall, our investigations suggest that several different mechanisms were operating to generate the changes that we observed in fish and zooplankton populations during acidification in LRL. Acid stress certainly provides part of the explanation for the changes, but other factors operating in conjunction with this primary stress have also had important influences.

General discussion

The range of responses to acidification observed in LRL were not surprising. Many of these responses were actually predicted based upon earlier investigations of acid stress (Brezonik et al. 1993), and pH is a controlling variable for many biogeochemical processes. Yet, we believe that it is worth highlighting the diversity of observed stresses in what was intended as an experiment to examine the actions of a single manipulation—the addition of sulfuric acid.

Multiple-stress effects in LRL are likely to be generalizable, at the least, to other acidified situations. A large number of responses have been observed to be common to LRL and several other lake acidification experiments that have been conducted in different areas at different times (Schindler et al. 1991).

Some of the chemical and biological changes generated by the acidification of LRL were sufficiently substantial that their effects could be detected easily throughout the lake ecosystem. Acid and Al effects are two examples (Eaton et al. 1992). Many other impacts were not as substantial, however, and their consequences are less straightforward to interpret.

The degree of stress imposed by elevated methylmercury levels is a prime example. Although increases of methylmercury up the food web to fish were well documented, there was no clear evidence that such increased levels influenced species or ecosystem functions in a deleterious way. For such evidence, it may be necessary to investigate more subtle physiological responses or to look beyond the in-lake biotic community to piscivorous wildlife. For example, Friedmann et al. (1996) exposed juvenile walleyes to dietary methylmercury and found that testicular development and immune function were inhibited by 100 ng Hg g⁻¹ in food, a dietary methylmercury concentration available to piscivorous fish in many North American waters. For comparison,

annual mean concentrations of mercury in whole, age-1 yellow perch from the treatment basin of LRL ranged from 116 to 148 ng g⁻¹ during the acidification experiment. Similarly, chicks of the common loon, *Gavia immer*, that have been fed fish from low-pH lakes in northern Wisconsin exhibit elevated concentrations of mercury in their blood (Meyer et al. 1995). In Ontario, Scheuhammer and Blancher (1994) estimated that 30% of the lakes had prey-size fish with mercury levels high enough to impair reproduction of common loons.

Our paper has emphasized responses only during the original acidification of LRL. We have also been evaluating recovery in the lake since the last acid additions to the treatment basin in 1990 (Sampson et al. 1995; Frost et al. 1998). These continuing investigations indicate interactions of multiple stresses during LRL's recovery processes parallel to those reported here.

Similarly, this paper focuses only on some of the changes that we have observed to accompany acidification. We observed a wide range of other patterns in zooplankton and in benthic organisms (e.g., Webster et al. 1992; Brezonik et al. 1993). Only total chlorophyll levels and primary production in the phytoplankton communities did not show strong patterns associated with acidification (Brezonik et al. 1993). Even among these organisms, it is likely that there were numerous species shifts accompanying pH changes as have been documented in other acidification experiments (Leavitt et al. 1999) and in other whole-lake manipulations (Cottingham 1999). Unfortunately, budgetary constraints limited any detailed evaluations of the phytoplankton community in LRL.

Some of the changes in LRL were driven by decreases in one species caused by single chemical stresses or combinations of them with subsequent effects propagating through the food web of the lake. It may be constructive to distinguish such indirect, food-web effects (sensu Abrams et al. 1996) from chemical stresses. The ubiquity of such propagating food-web interactions, combined with the secondary-stress effects illustrated here, suggest that there may actually be very few cases where a single stress acts alone without generating secondary stresses on ecosystems.

Several different stresses were clearly operating in LRL, but this is not to say that the full range of possible stresses have affected it. Several other human-caused factors are anticipated to operate in central North America such as heightened UV radiation, increased contaminant inputs, and climatic shifts (McKnight et al. 1996). Acidified lakes may be particularly sensitive to further environmental stresses because ecosystems that have been subjected to one stress may be more sensitive, in general, to additional stresses that follow the first. Although general ecosystem function appears to have been relatively insensitive to a stress in several different situations (e.g., Schindler 1987; Howarth 1991; Lawton and Brown 1993; Frost et al. 1995), this might not indicate that system processes are resistant to a sequence of different stresses or even to repeated impacts of the same stress. In LRL, ecosystem processes were unaffected by acidification despite the loss of several species because of complementary responses by remaining species (Frost et al. 1995). At the same time, the potential of the lake to maintain

system function may have been diminished by these species losses because the number of taxa that can serve as replacement has been reduced.

The complexity and diversity of stress effects in LRL are unlikely to be unique to our experiment. Responses during acidification largely paralleled patterns observed in several other acidification experiments and in lakes that were acidified by atmospheric deposition (Schindler et al. 1991). Recovery patterns exhibited less in common with other acidified systems particularly in that the rate of return to preacidified chemical conditions in LRL seemed particularly rapid (Frost et al. 1998). Delayed biological recovery in LRL, however, suggests that such recovery may be slow in most stressed systems. Multiple stresses are likely to be a common feature of many ecosystem responses to what initially appear as single stresses.

The LRL results indicate only some aspects of the complexity of responses to acidification. Some secondary stress effects may take longer than the 6-yr period of our acid additions to have detectable influences on lake populations. Such was the case with fish populations in Lake 223 at the Experimental Lakes Area in Canada (Schindler et al. 1985). The impacts of secondary stresses, such as increased Hg and UV levels, may have the potential to influence lake processes, even if they are difficult to detect against the background of natural variability. At the same time, it is interesting to consider that some of the earliest effects of some anthropogenic stresses may be exerted on humans at the top of the food chain rather than on other components of aquatic ecosystems. Self interest alone may be reason enough to focus attention on the early effects of anthropogenic stresses, before they propagate a multitude of effects.

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