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RESEARCH ARTICLE

Long-Term Ecosystem Stress: The Effects of Years of Experimental Acidification on a Small Lake

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Ecologists believe that both natural and anthropogenic stresses cause changes in ecosystems that cannot be deduced from effects on individual species or populations because of the deterioration of ecosystem structure and function (*1*). The degree of such "ecosystem-level" responses to anthropogenic stresses, and the degree to which ecosystems can recover after the stresses are removed, are subjects of fundamental importance to natural resource management (*2*), yet only a few studies have been able to quantify the causes and effects of stresses on whole ecosystems (*3, 4*). Reasons for this include the following: (i) The ecosystems were too large or complex to study in their entirety. (ii) Documentation of ecosystem structure, function, and natural variation prior to anthropogenic stress was inadequate. (iii) Individual stresses could not be quantified, or effects of one perturbation on the ecosystem could not be isolated from other stresses (*5*).

We were able to overcome many of these problems in an ecosystem-scale experiment in Lake 223, a small Precambrian Shield lake surrounded by virgin boreal forest in the Experimental Lakes Area, and typical of poorly buffered small lakes of northwestern Ontario (*6–9*). Over a period of 8 years, the *p*H of the lake has been gradually decreased from 6.8 to 5.0 by the addition of sulfuric acid. Our studies revealed various apparent mechanisms of response of the lake's biota to increased acidity, ranging from direct toxicity of hydrogen ion to disruptions of normal food-chain relations, behavioral patterns of animals, and biogeochemical cycles in the lake. In some cases, synergistic interactions of several stresses appeared to be involved. Some of the adverse changes occurred much earlier in the acidification process (that is, at higher *p*H) than is commonly believed to cause ecosystem degradation. This suggests that early ecological damage from lake acidification may be more extensive than was previously believed, a result that should be of vital interest to agencies regulating the emission of acid precursors. Other effects were so subtle or so complex that they might have been undetected in a system less thoroughly studied. In this article, we summarize the biological results of the first 8 years

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of the Lake 223 experiment and compare our results with the responses that would be predicted if simpler methods were used, such as the laboratory toxicological or physiological tests that usually form the basis for regulating water quality standards or managing aquatic resources.

After a 2-year (1974 and 1975) background study, Lake 223 was acidified, from 1976 through 1983 (*10, 11*). Changes in the ecosystem caused by these additions are outlined below. Where possible, changes are compared with natural variability in nearby untreated lakes.

Abstract. *Experimental acidification of a small lake from an original* pH *value of 6.8 to 5.0 over an 8-year period caused a number of dramatic changes in the lake's food web. Changes in phytoplankton species, cessation of fish reproduction, disappearance of the benthic crustaceans, and appearance of filamentous algae in the littoral zone were consistent with deductions from synoptic surveys of lakes in regions of high acid deposition. Contrary to what had been expected from synoptic surveys, acidification of Lake 223 did not cause decreases in primary production, rates of decomposition, or nutrient concentrations. Key organisms in the food web leading to lake trout, including* Mysis relicta *and* Pimephales promelas, *were eliminated from the lake at* pH *values as high as 5.8, an indication that irreversible stresses on aquatic ecosystems occur earlier in the acidification process than was heretofore believed. These changes are caused by hydrogen ion alone, and not by the secondary effect of aluminum toxicity. Since no species of fish reproduced at* pH *values below 5.4, the lake would become fishless within about a decade on the basis of the natural mortalities of the most long-lived species.*

1976—Alkalinity decreased, but little apparent pH *change*. A mass balance budget for sulfate revealed that the addition of sulfuric acid had stimulated reduction of sulfate to sulfide in anoxic hypolimnion waters (*9, 12*). Dissolved inorganic carbon decreased as was expected because of transformation of bicarbonate to carbon dioxide and degassing to the atmosphere. No other chemical changes were distinguishable from background years or from nearby, unmodified reference lakes. An increase in chironomid emergence over 1975 was the only biological change observed, but the increase was within the range of natural variation. Differences in zooplankton sampling methods did not permit data to be compared directly with those from other years.

1977—Average pH *6.13, target* pH *6.00* (*13*). The relative abundance of chrysophycean species declined slightly from earlier years, but this group continued to dominate the phytoplankton. The abundance of chlorophytes increased (*14–16*) (Fig. 1A.). Phytoplankton production, biomass, and chlorophyll were within limits of natural variation for lakes in the area (Fig. 1, B to D). Emerging dipterans and rotifers, particularly species of *Polyarthra, Kellicottia,* and *Keratella,* were more abundant than in 1974, but probably still within the range expected on the basis of natural variation (*17, 18*) (Fig. 1, E and F). Populations of lake trout and white sucker remained within the range of natural variation (*4*), (Fig. 1G). None of the above factors was outside the normal range observed for reference lakes in the area.

1978—Average pH *5.93, target* pH *5.75*. Several key organisms in the lake's food web were severely affected. Between October 1978 and May 1979, the number of opossum shrimp, *Mysis relicta,* declined from 7×10^6 to only a few animals (*19*). The fathead minnow, *Pimephales promelas,* failed to reproduce (*4*). In the phytoplankton, different species of chlorophytes, cyanophytes, and Peridineae appeared at the expense of original diatom and chrysophycean species (*15*). Primary production was slightly higher than in any previous year, and chironomid emergence continued to increase relative to the reference lake 226S (Fig. 1, B and E). The copepod species *Diaptomus sicilis,* which had been rare in the lake, disappeared.

1979—Average pH *5.64, target* pH *5.50*. Filamentous algae of the genus *Mougeotia,* which had not been noticed previously, formed highly visible, thick mats in littoral areas (*20*). These persisted throughout the remaining years of study. The exoskeletons of crayfish, *Orconectes virilis,* hardened more slowly after molting, and animals remained softer than in previous years or in reference lakes (*21*). *Pimephales* declined to near extinction because of its reproductive failure in 1978 and again in 1979, its short life expectancy, and possibly increased predation by lake trout (Fig. 1I). The slimy sculpin, *Cottus cognatus,* also declined in abundance (Fig. 1H), chiefly in the oldest and youngest age classes. Contrary to what we had expected from the reported data on acidification, phytoplankton production and chironomid emergence remained high, probably contributing to the increased abundance of young-of-the-year of both white sucker and lake trout (*4*). Changes in the species composition of phytoplankton described for 1978 became more pronounced. The large copepod *Epischura lacustris* became very rare (*17*).

1980—Average pH *5.59, target* pH *5.25*. Phytoplankton biomass again increased relative to previous years and reference lakes. Whereas, on average, chrysophyceans were still dominant, Peridineae and Cyanophyceae increased. The acidophilic diatom *Asterionella ralfsii,* which had previously been rare in the lake, appeared in large numbers, causing a decline in soluble silicate (Fig. 1J) (*22*). The copepod *Epischura lacustris* disappeared and has not been recorded since. The cladoceran *Daphnia catawba* was observed in the lake for the first time (*23*) probably competing with *Daphnia galeata mendotae,* which became rare in 1981. Other species were unaffected. As a result, there was no marked decrease in the biomass or total number of crustacean species (*17*). In fact, the average numerical abundances and biomass of both planktonic crustaceans and rotifers were the highest in the 8-year period of observation. Increases were noted for the previously recorded cladoceran species, *Bosmina longirostris*. Likewise, the number of pearl dace (*Semotilus margarita*) increased rapidly, apparently using resources that had supported *P. promelas* prior to acidification (Fig. 1K) (*24*). *Pimephales promelas* was now very rare in the lake. Chironomid emergence reached an all-time high, despite increases in pearl dace and young white sucker, which are both potential predators on chironomid larvae. The condition of lake trout was poorer than in 1977 and 1978, but similar to preacidification values (Fig. 1G).

Two new stresses were evident in *Orconectes* at this *p*H. In addition to the recalcification problem mentioned above, infestation of the population with a microsporozoan parasite of the genus *Thelohania* was higher than in background lakes (*25*). Egg masses were often infested with fungi, and empty egg capsules were often observed on ovigerous females. These phenomena were only rarely observed in four reference lakes. No young-of-the-year *Orconectes* were observed. There was no lake trout recruitment in 1980, although normal spawning had been observed in October 1979 (*4, 26*).

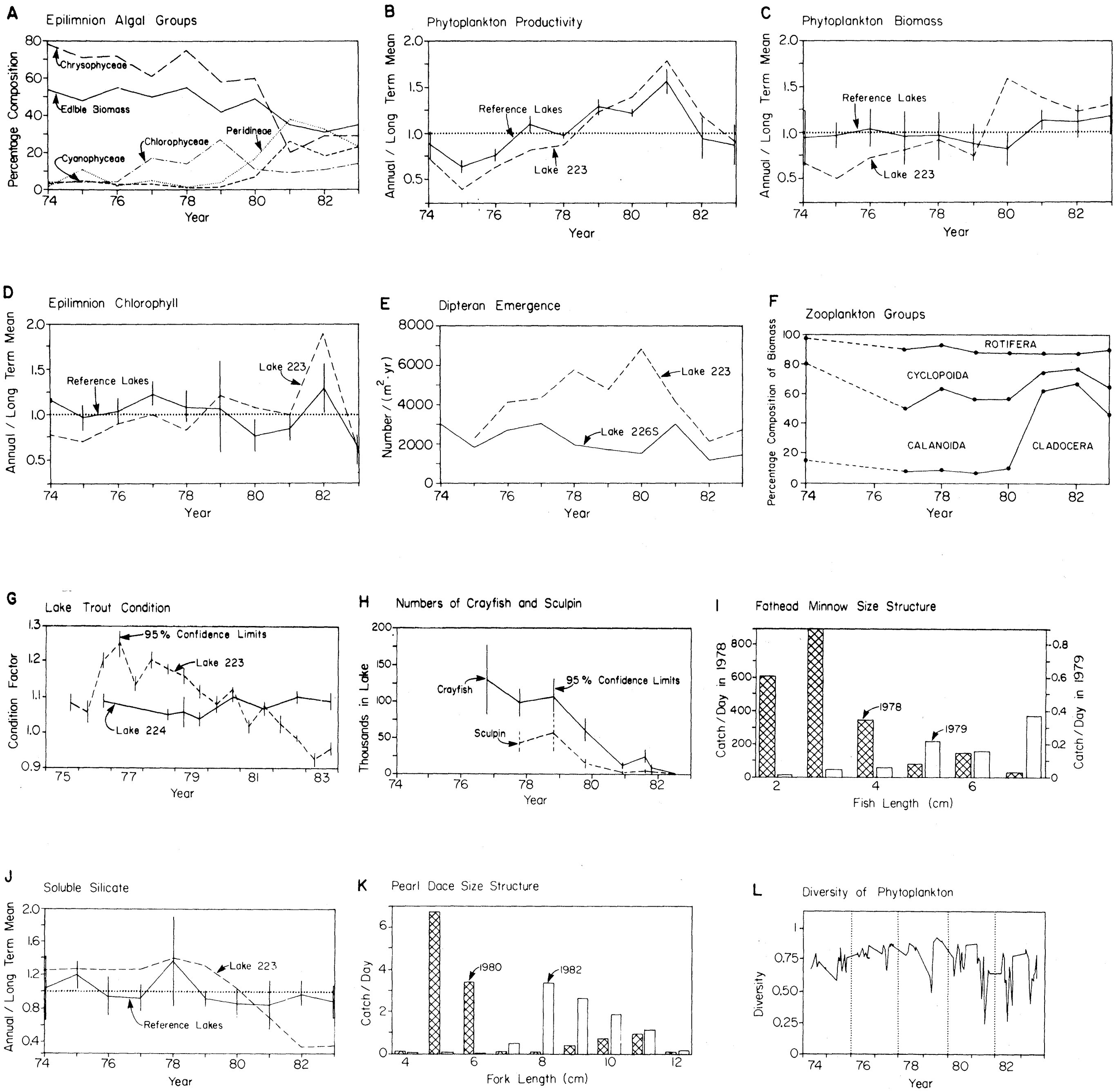

Fig. 1. Changes in several parameters in Lake 223 during acidification, including comparisons with pristine reference lakes in the area when this was possible. (A) Changes in the relative abundance of major phytoplankton groups and in small phytoplankton (species less than 30 μm in maximum dimension; roughly the size edible by zooplankton) in the epilimnion prior to and during acidification. Data shown are averages for the ice-free season. More detailed seasonal information on species composition and actual abundance is given in the text. (B) Phytoplankton production in the euphotic zone compared to several control lakes. Values in this and several subsequent panels are normalized to both control lakes and long-term means as described in (*43*). Vertical bars represent single standard deviations for the normalized annual means of all reference lakes. (C) Epilimnion phytoplankton biomass, normalized as in panel B. (D) Epilimnion chlorophyll a compared to control lakes as in panel C. (E) Emerging dipterans caught by transparent submerged funnel traps in Lake 223 and in unacidified Lake 226S. Values are morphometrically corrected averages calculated annually from catches of 8 to 27 emergence traps per lake, monitored weekly during the ice-free season. (F) Changes in relative biomass of zooplankton groups in the whole water column during the study period (except 1975 and 1976). Note the increases in Rotifera and Cladocera, and the steady or declining contribution of Cyclopoida and Calanoida. Mean ice-free season zooplankton biomass was 261 mg m^{-2} in 1974, 413 mg m^{-2} in 1980, and 250–384 mg m^{-2} in the 1981–1983 period. (G) The condition index for lake trout in Lake 233 and unmodified Lake 224, illustrating an initial increase in condition, followed by a dramatic decline during acidification. Condition = $10^5\ w/l^3$, where w is weight and l is fork length. A similar increase was observed in condition of Lake 223 white sucker in the first few years, but the decline after that date was not as pronounced as for lake trout. Vertical bars are 95 percent confidence limits. (H) The populations of crayfish (*Orconectes virilis*) and slimy sculpin (*Cottus cognatus*) during acidification, derived from areal counts done by scuba divers at night. Counts were made along eight transects and were stratified by depth and habitat type. Dashed lines indicate 95 percent confidence limits for population estimates. (I) Relative population size structure of fathead minnow (*Pimephales promelas*) during acidification. Numbers are from trapnet catches. The scarcity of new recruits in 1979 led to a total population collapse 2 years later. Note the difference in scale for the 2 years. Crosshatched bars represent 1978; and open bars represent 1979. (J) The concentration of soluble silicate in Lake 223, normalized as described in (*44*). (K) Relative population size structure of pearl dace (*Semotilus margarita*). Numbers are from trapnet catches. Note the high recruitment in 1980 as *Pimephales* declined, followed by recruitment failure in *Semotilus* by 1982, at lower pH. Crosshatched bars represent 1980; clear bars 1982. (L) Simpson's diversity index calculated over time for phytoplankton species in Lake 223 (*44*). Samples were taken every 2 to 3 weeks (*44*).

1981—Average pH *5.02, target* pH *5.00.* Chrysophycean abundance declined dramatically, with replacement by Peridineae and Cyanophyceae (Fig. 1A). Epilimnetic phytoplankton production and biomass remained slightly higher than normal (Fig. 1, B and C). *Diaphanosoma leuchtenbergianum* (= *D. birgei*) and *Daphnia galeata mendotae* disappeared early in the ice-free season. *Holopedium gibberum, Daphnia catawba,* and *Bosmina longirostris* as well as several species of rotifers continued to increase in abundance (*23*). Most of the remaining species of copepods declined in abundance. White sucker recruitment ceased, even though growth and survival of individuals over 1 year of age were similar to values in unperturbed lakes in the area. Numbers of white sucker were still abnormally high, due to the large recruitment in 1980. Spawning in May 1981 appeared normal, but no fry were present in the summer. In each of the previous years fry and fingerlings had been numerous and easily captured. Growth in length, survival, and abundance of lake trout remained normal as well, even though recruitment failed for the second consecutive year and condition continued to decline (*4*) (Fig. 1G). The crayfish population dwindled, reaching a few percent of preacidification values by late fall (Fig. 1H). No young-of-the-year were seen, and the exoskeletons of adults were far softer than normal. *Thelohania* infestation increased to 9 percent of the population (*25*). Despite its earlier positive response to acidification, very few young-of-the-year *Semotilus* were present, as illustrated by the decreases in small-sized fish. Sculpins were very rare (Fig. 1H).

1982—Average pH *5.09, target* pH *5.00.* Phytoplankton groups were similar to 1981. Phytoplankton production was slightly less than in 1981, but this was also the case for all reference lakes (Fig. 1B). While the *p*H in 1982 was nearly identical to that in 1981, a considerable increase in chlorophyll was observed. Spawning behavior of lake trout changed. In all years before 1982, these fish had spawned at the same locations along the shore of the lake. In 1982, these sites were partially covered with *Mougeotia*. This possibly explains why trout spawned in areas where spawning was not previously observed (*26*). Lake trout condition had become very poor. Crayfish had nearly disappeared. No young-of-the-year of any fish species were observed (*4, 26*). Although numerical data are lacking, leeches were rare. Zooplankton species, numbers, and biomass were similar to 1981.

1983—Average pH *5.13, target* pH *5.00.* Even though *p*H values were held nearly constant for the third consecutive year, changes in the food web continued to occur. Phytoplankton were now relatively stable in composition, with Peridineae and Chrysophyceae being codominant with unusually high proportions of Cyanophyceae and Chlorophyceae present, including numerous forms with tests or gelatinous sheaths (*22, 27*). Phytoplankton production was also stable relative to reference lakes. While lake trout were still numerous, their condition declined below the lowest levels observed for other populations in the area (Figs. 1G and 2) as might be expected from the severe disruption of the food web. Increased frequency of cannibalism put further stress on the remaining small trout, probably because of the scarcity of the minnows and benthic crustaceans that are normal prey for large trout. Lake trout spawned at locations different from those of other years. There was no successful recruitment by any species of fish in the lake. Crayfish, leeches, and the mayfly *Hexagenia,* previously abundant in the lake, were absent by fall (*25*).

Long-term trends. In addition to the changes documented above, a number of the factors being measured changed slowly or erratically in the course of acidification, becoming significantly different from background after several years. Although phytoplankton production increased for several successive years during acidification, there was a

Fig. 2. Appearance of lake trout in Lake 223 in 1979 (*p*H 5.4) and 1982 (*p*H 5.1), clearly illustrating the loss of condition as indicated by the factor calculated as in Fig. 1G.

reduction in the proportion of phytoplankton of the size that could be eaten by zooplankton. Small edible species were gradually replaced by large test- or mucilage-covered forms (Fig. 1A). A decrease in the mean body size of zooplankton, resulting from the increase in rotifers and small-bodied cladocerans, such as *Bosmina longirostris* relative to larger copepods and cladocerans, probably also favored the survival of larger phytoplankton and lowered the efficiency of energy transfer. Chironomid emergence increased through 1980, roughly in proportion to phytoplankton production; afterward it declined to values observed in the reference basin (*17, 18*) (Fig. 1E). Reduction of sulfate to sulfide increased continuously, in proportion to sulfate concentrations (*9, 12*). Concentrations of calcium, manganese, aluminum, and possibly zinc increased in the water (*28, 29*) while silicate decreased (Fig. 1J). There were no other significant changes in water chemistry (*29*). By 1983, it was obvious that the condition of lake trout had declined slowly from 1977 onward (Fig. 1G).

Results that contradict current beliefs. Many of our results were different from those deduced from laboratory and synoptic monitoring studies. Some effects of acidification were more dramatic than expected, and some were the opposite of what we had anticipated. We had expected a decrease in phytoplankton production and chironomid emergence during acidification, yet no decrease was observed. In fact, data show a possible increase (Fig. 1B) (*7, 9, 30*). After 8 years of acidification, periphyton production was similar to reference lakes in the area, despite the increased abundance of filamentous algae (*31*). No decrease in phosphorus was observed, as has been hypothesized to result from either interception of nutrients by benthic mats or by precipitation due to increased aluminum concentrations (*32*). The phytoplankton standing crop, chlorophyll, and production per unit of phosphorus were similar to those in other lakes in the area (*33*). Rates of decomposition in the lake were unaffected by acidification, apparently because the microflora at the sediment-water interface maintained a microenvironment with a higher *p*H (*34*).

Results that confirm current beliefs. As was expected from synoptic surveys made in Norway, benthic Crustacea and some cladoceran species were extremely vulnerable to acidification, whereas Diptera were not adversely affected (*35*). Dipteran emergence increased relative to a thoroughly studied control basin for the first several years of acidification (Fig. 1E). The epidemic of *Mougeotia* provides clear evidence that the appearance of this genus in the lakes of regions subjected to industrial pollution is caused by acidity rather than by some co-occurring stress unrelated to acidification. The sensitivity of *Pimephales* to acidification confirms laboratory studies of long-term toxicity (*36*), and the problems with calcium uptake suffered by *Orconectes* at low *p*H agree with the observations from physiological studies (*21*). Various mechanisms have been proposed to explain changes in fish populations due to acidification, including direct toxicity, reproductive or recruitment failure, disruption of food-chain relations, disappearance of spawning sites, and effects of aluminum (*37*). With the probable exception of aluminum, all of these changes have occurred in Lake 223. In that the watershed of the lake was not acidified, the range of aluminum concentrations observed in Lake 223 was only 7 to 36 μg per liter, far less than the concentrations that have caused mortality among fishes during episodic events. One or more of the other factors appeared to affect every species of fish that we studied (*4*).

Implications for monitoring studies to detect acidification. Most monitoring programs now used for detecting lake acidification rely heavily on measurements of *p*H and abundance of adult sport fishes. Our study suggests that these factors are not sensitive reliable indicators of early damage due to acidification. For example, twice weekly *p*H measurements did not reveal the disappearance of 80 percent of the alkalinity from Lake 223 in 1976, the first year of acidification. Once bicarbonate alkalinity is eliminated, *p*H measurements become more useful, although electrode *p*H measurements in Precambrian Shield lakes may be in error by several tenths of a *p*H unit, as a result of interference by dissolved organic carbon and variations of the partial pressure of CO_2 from atmospheric equilibrium (*38*). While alkalinity is a far more sensitive early indicator of acidification, reliable measurements in poorly buffered waters were rare before the late 1970's.

Similarly, most large fishes are not sensitive indicators of early stages of acidification damage. Large populations of lake trout and white sucker still exist in Lake 223, even though there has been no recruitment into the lake trout population for 4 years and into the white sucker population for 3 years. The food web supporting lake trout has been so severely disrupted that continued acidification would cause almost complete disappearance of this species within the decade. Indeed, the paucity of suitable food organisms and the loss of younger year classes may make it easier for anglers to catch more and larger sport fish at this stage of the acidification process. If monitoring of fishes is to be useful, it must include long-term studies of reproduction, condition, and age structures of populations.

Both survey work in Scandinavia and our study suggest that benthic crustaceans are very sensitive indicators of acidification (*19*), but collection of these species is rarely incorporated in acidification monitoring in North America. *Pimephales*, an important forage fish, also appears to be sensitive (*4*). However, detailed distributions of these species are not known, and records of their occurrence in lakes prior to acidification are rare, so that it is difficult to tell whether their absence from a lake is due to acidification stress, to natural zoogeographical distribution patterns, or to year-to-year changes in abundance. The above species have much shorter life cycles than larger fishes so that they disappear rapidly after reproductive failure.

Phytoplankton production, rates of decomposition, and nutrient concentrations did not decrease in Lake 223, suggesting that these factors are not sensitive to acidification. There seems to be little point incorporating these insensitive variables in broad-scale monitoring programs. Phytoplankton species diversity and species numbers were also unaffected during early stages of acidification, although there was some evidence of a decline in diversity during midsummer from 1981 to 1983 when the *p*H of the lake was 5.0 to 5.2 (Fig. 1L). In contrast, shifts from a largely chrysophycean community to one where chlorophycean, cyanophycean, and peridinean species were often dominant in the phytoplankton, the predominance of acidophilic indicators such as *A. ralfsii* and certain species of *Gymnodinium* in the phytoplankton and of the genus *Mougeotia* in the periphyton, do not occur in circumneutral lakes. Such taxonomic changes appear to be reliable indicators of early acidification. Because most monitoring studies have rarely included the most sensitive indicators of acidification, it seems probable that they have underestimated the extent and degree of damage to lakes due to acid precipitation.

Implications for laboratory and microcosm studies. Laboratory tests of metabolic processes have usually overestimated the stresses of acidification on ecosystems. When samples of lake water or sediment are acidified rapidly, decreases in growth, photosynthesis, nutrient exchange, or decomposition normally occur (*39*). However, such experiments disregard the inherent resilience of natural ecosystems. For example, when some species of phytoplankton disappear as the result of acidification, others from the reservoir of species in a lake appear or increase, so that photosynthesis continues at a normal or even increased rate.

The magnitude of food-web disruption which occurred early in the acidification of Lake 223 could not have been predicted from small-scale studies. Redundant features of the Lake 223 ecosystem—such as the increase in *Semotilus* when *Pimephales* disappeared, and the increase in *Daphnia catawba* when *Daphnia galeata mendotae* declined—undoubtedly delayed the effect of food-web damage on upper trophic levels. For example, the condition of lake trout declined slowly over several years as the abundance of key species of its prey became rarer. The overall increase in *Daphnia catawba* could have resulted from the elimination of *Mysis relicta* by acidification because the latter species preys heavily on large Cladocera (*40*).

Likewise, laboratory or microcosm studies cannot predict declines or disappearances that result from the interaction of multiple stresses. The demise of *Orconectes virilis* appeared to involve disruption of recalcification, indicating problems in regulating ion balance, increased infestation with microsporozoan and fungal parasites, increased egg losses, decreased survival of young-of-the-year, and possibly increased predation by lake trout. Detrimental effects on lake trout included a host of food-chain, physiological, reproductive, and behavioral interactions. While it is difficult to isolate the effects of single stresses in an ecosystem-scale study, it is clear that the large herbivorous or carnivorous species which are of most concern to man cannot be realistically studied in an experimental vessel smaller than a whole ecosystem.

The experimental conditions imposed on small-scale experiments often are not a realistic simulation of those in natural ecosystems (*41*). For example, we have shown that decomposition in undisturbed surface sediments is not affected when overlying water is acidified because high pore water *p*H values are maintained by the action of microbes. In contrast, decomposition decreases when the *p*H of sediments is deliberately lowered in laboratory experiments, but such conditions do not occur in acidified lakes (*34*).

Experimental ecosystem manipulations can reveal which properties of ecosystems are likely to be sensitive to particular stresses. They can also elucidate interactive features of ecosystem organization that would aid in the interpretation of results from smaller scale studies and allow the calibration of paleoecological methods (*42*). Such studies can play a key role in the detection and interpretation of man's impact on natural ecosystems.

References and Notes

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2. For example, J. Cairns *et al.*, Ed., *Estimating the Hazard of Chemical Substances to Aquatic Life* (American Society of Testing Materials, Philadelphia, 1978); *Recovery and Restoration of Damaged Ecosystems* (Univ. of Virginia Press, Charlottesville, 1977); S. A. Levin, S. Kimball, K. Kimball, W. McDowell, Eds., *New Perspectives in Ecotoxicology* (Report 14a, Ecosystems Research Center, Cornell University, Ithaca, N.Y., 1983).
3. See, for example, G. E. Likens *et al.*, *Ecol. Monogr.* **40**, 23 (1970); G. M. Woodwell, *Ambio* **10**, 143 (1982); see also papers in *Can. J. Fish. Aquat. Sci.* **37** (No. 3) (1980) and *ibid.* **41** (No. 4) (1984).
4. K. H. Mills, in *Early Biotic Responses to Lake Acidification. Acid Precipitation Series*, G. R. Hendry, Ed. (Ann Arbor Science, Ann Arbor, Mich., 1984), vol. 6, pp. 117–121.
5. In particular, airborne pollutants subjected to long-range transport are numerous, difficult to isolate, and many have synergistic effects. For example, National Academy of Sciences, *Atmosphere-Biosphere Interactions: Toward a Better Understanding of the Ecological Consequences of Fossil Fuel Combustion* (National Academy Press, Washington, D.C., 1981); F. H. Bormann, "Factors confounding evaluation of air pollution stress on forests: Pollution input and ecosystem complexity," presented at the symposium, "Acid Deposition, a Challenge for Europe," Karlsruhe, Germany, 19 to 23 September 1983.
6. Lake 223 has an area of 27.3 ha, a mean depth of 7.2 m, and a maximum depth of 14.4 m. The Experimental Lakes Area and the whole-ecosystem experiments which are its mandate are described in (entire issues) *J. Fish. Res. Board Can.* **28** (No. 2) (1971); *ibid.* **30** (No. 10) (1973); *Can. J. Fish. Aquat. Sci.* **37**, No. 3 (1980).
7. Summary papers on the experimental acidification studies are D. W. Schindler in (*9*), Schindler and Turner (*10*), and Mills (*4*).
8. D. Drablos and A. Tollan, Eds., *Ecological Impact of Acid Precipitation* (SNSF Project, Oslo, Norway, 1980).
9. D. W. Schindler *et al.*, *Can. J. Fish. Aquat. Sci.* **37**, 342 (1980).
10. Sulfuric acid was added to the epilimnion of the lake during the ice-free season, by draining it slowly into the propeller wash of a moving boat powered by a 15-hp outboard, which crisscrossed the lake several times over a period of 1 to 2 hours while the acid was added. The resulting distribution of acid was relatively uniform horizontally. No acid penetrated the thermocline of the lake. Enough acid was added to the lake in May of each year to reach the "target" *p*H value, after which sufficient acid was added two to three times each week to maintain *p*H near to, but not below, the target value. The resulting temporal distribution of acid added to the lake was similar to that expected in the northern United States and southern Canada, with a spring "pulse" after snowmelt, followed by intermittent smaller additions during the rest of the ice-free season. In 1976, acidification reduced the alkalinity of the lake from preacidification values of 80 to 100 μeq per liter to less than 20 μeq per liter. Despite this enormous loss of alkalinity, lake *p*H values were similar to those measured in background years (time-weighted annual means for 1974 to 1976 were 6.71, 6.65, and 6.49, respectively), illustrating the relative insensitivity of *p*H as an indicator of early lake acidification (*11*). In 1977, we lowered the *p*H of the lake to about 6.0 to 6.1. In subsequent years, we added enough acid to lower the *p*H of the epilimnion from 6.0 to 5.0 to 5.1 in increments of about 0.25 *p*H unit per year. From 1981 to 1983, only enough acid was added to maintain the lake at *p*H 5.0 to 5.1. See D. W. Schindler *et al.* (*9*); D. W. Schindler and M. Turner, *Water Air Soil Pollut.* **18**, 259 (1982).
11. In natural ecosystems, *p*H values are affected not only by bicarbonate alkalinity, but by the partial pressure of CO_2, which can range from greatly undersaturated to greatly oversaturated, depending on biological and physical processes [D. W. Schindler *et al.*, *Science* **177**, 1192 (1972); A. Herczeg and R. H. Hesslein, *Geochim. Cosmochim. Acta* **48**, 837 (1984)].
12. R. B. Cook and D. W. Schindler, "Environmental biogeochemistry," *Ecol. Bull. (Stockholm)* **35**, 115 (1983).
13. The average and target *p*H values are for the epilimnion of the lake. Hypolimnion values were higher, due to the combination of sulfate reduction and precipitation of iron sulfides (*9*, *12*).
14. The phytoplankton changes described are for the epilimnion in the ice-free season only. The original chrysophycean species were still dominant early in 1977, but were replaced by Chlorophyceae (chiefly *Oocystis submarina* var. *lacustris*, and *Dictyosphaerium simplex*) and Cyanophyceae (*Merismopedia glauca*) in midsummer. Over the years, this shift became more and more pronounced with more new species appearing. Peridineae (*Gymnodinium* spp.) became abundant beginning in 1980 (*15*).
15. D. L. Findlay and G. Saesura, *Manuscr. Rep. Can. Fish Aquat. Sci. No. 1585* (1980).
16. D. W. Schindler, "Experimental acidification of a whole lake: A test of the oligotrophication hypothesis," in (*8*), p. 370; D. Findlay, *Can. Manuscr. Rep. Fish. Aquat. Sci. 1761* (1982).
17. D. F. Malley, D. L. Findlay, P. S. S. Chang, "Ecological effects of acid precipitation on zooplankton" in *Acid Precipitation: Effects on Ecological Systems*, F. M. D'Itri, Ed. (Ann Arbor Science, Ann Arbor, Mich., 1982), pp. 297–327.
18. I. Davies, *Can. J. Fish. Aquat. Sci.* **37**, 523 (1980); unpublished data. The total number of crustacean species present in the lake in a given year was from 8 to 11 before fertilization, increasing to 15 in 1980, then declining to 8 to 9 by 1983.
19. Approximately 7×10^6 *Mysis* were present in the lake in 1978 at a *p*H of 5.8 to 5.9. The population density and age structure were similar to those in reference lakes, and *Mysis* appeared to reproduce normally. Numbers were reduced by 96 percent by August of 1979, a year in which the *p*H averaged 5.6. The remaining few animals were eliminated during the fall of 1979. R. W. Nero and D. W. Schindler, *Can. J. Fish. Aquat. Sci.* **40**, 1905 (1983); R. W. Nero, thesis, University of Manitoba, Winnipeg (1981); several species of bottom-feeding crustaceans have also been identified as among the most acid-sensitive organisms in Norway [J. Økland and K. Økland, "*p*H level and food organisms for fish: Studies of 1000 lakes in Norway," in (*8*), p. 326] and elsewhere in Canada (R. W. Nero and L. Grapentine, personal communication).
20. This genus is common in the littoral areas of acidified lakes in eastern North America and Scandinavia; P. M. Stokes in *Effects of Acid Rain on Benthos*, R. Singer, Ed. (North American Benthological Society, Springfield, Ill., 1981), p. 119; more detailed studies of filamentous algae during acidification have been made in Lake 302, suggesting that filamentous algae begin to increase when alkalinity declines, prior to major changes in *p*H (M. A. Turner, unpublished data).
21. This observation was made both in laboratory studies by D. F. Malley [*Can. J. Fish Aquat. Sci.* **37**, 364 (1980)]; and on field samples by I. J. Davies, unpublished data.
22. D. L. Findlay, unpublished data; *Asterionella ralfsii* was not observed in the plankton before 1980; from 1981 to 1983, it constituted nearly 100 percent of the planktonic diatoms. There was no increase in total diatom biomass. *A. ralfsii* frustules are very resistant to dissolution in lake sediments and are commonly used by paleoecologists as an indicator of lake acidification. This increased resistance to dissolution would hinder recycling and has been suggested to have caused the decrease in silicate.
23. D. F. Malley and P. S. S. Chang, unpublished data; Malley *et al.* (*17*) referred to *D. catawba* as

D. catawba × *schoedleri* after J. L. Brooks, "The systematics of North American *Daphnia*," *Mem. Conn. Acad. Arts Sci.* **12** (1957); individuals in the population show features of species within the *Pulex* group but come closest to resembling *D. catawba*.
24. Detailed studies in nearby Lake 114 show a pronounced overlap in the diets of *S. margarita* and *P. promelas* [R. F. Tallman, K. H. Mills, R. G. Rotter, *Can. Manuscr. Rep. Fish. Aquat. Sci.* (No. 1756) (1984)].
25. R. L. France and L. Graham, *Water Air Soil Sci.*, in press; R. L. France, thesis, University of Manitoba, Winnipeg (1982); crayfish populations were estimated by sampling (SCUBA) known areas along transects at night. Numbers of leeches, sculpins, *Hexagenia*, and tadpoles seen were also recorded (I. J. Davies, unpublished data).
26. Spawning was observed each fall from 1979 to 1983 by K. H. Mills by patrolling the shoreline at night with a light-equipped boat.
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28. D. W. Schindler, R. Wagemann, B. deMarch, M. A. Turner, in preparation.
29. D. W. Schindler and M. A. Turner, *Water Air Soil Pollut.* **18**, 259 (1982).
30. E. J. Fee, D. Hayward, J. A. Shearer, *Can. Data Rep. Fish. Aquat. Sci.* (1982), p. 327; J. A. Shearer and E. R. DeBruyn, unpublished data.
31. M. A. Turner, unpublished data.
32. O. Grahn, H. Hultberg, L. Landner, *Ambio* **3**, 8 (1974); W. Dickson, "Properties of acidified waters," in (*8*), p. 75.
33. Natural lakes in the ELA are phosphorus-limited. The slight increases in phytoplankton production and biomass observed in Lake 223 appeared to be due to low water renewal of the lake caused by several years of lower than normal precipitation in the period of study; D. W. Schindler, *Science* **195**, 260 (1977); ——, E. J. Fee, T. Ruszczynski, *J. Fish. Res. Board Can.* **35**, 190 (1978).
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35. J. Økland and K. Økland, in (*8*), p. 326; L. Henrikson, H. G. Oscarson, J. A. E. Stenson, in (*8*), p. 316; J. P. Nilssen, in (*8*), p. 344.
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38. A. Herczeg and R. H. Hesslein, *Geochim. Cosmochim. Acta* **48**, 837 (1984).
39. For example, T. S. Traaen, "Effects of acidity on decomposition of organic matter in aquatic environments," in (*8*), p. 340. P. Ramlal and J. W. M. Rudd, in preparation; G. R. Hendrey, "Effects of *p*H on the growth of periphytic algae in artificial stream channels" (Report IR 25/76, SNSF Project, Oslo, Norway, 1976).
40. D. C. Lasenby and R. R. Langford, *J. Fish. Res. Board Can.* **29**, 1701 (1972); *Limnol. Oceanogr.* **18**, 280 (1973).
41. We have previously shown that by not incorporating atmospheric exchange and natural succession of algal species, laboratory experiments led to erroneous conclusions regarding the control of eutrophication; see D. W. Schindler in (*33*).
42. G. Davidson, thesis, University of Manitoba (1984).
43. In order to compare relative temporal variation in Lake 223 and reference lakes in panels B, C, D, and J of Fig. 1, the following procedure was used. (i) The long-term mean concentration or rate for a variable in each lake was calculated for the period 1974 to 1982. The time-weighted average annual mean for each open water year was calculated from weekly to monthly samples and then divided by the long-term mean, resulting in a group of numbers clustered about 1.0. These clusters did not depart significantly from normal distributions. For each year, the average ratio of the annual mean to the long-term mean was calculated for all control lakes, and 95 percent confidence limits were calculated. For the four examples shown, data from two to eight control lakes were used in any year, varying somewhat with the difficulty and cost of measurement. Lake 223 annual averages, normalized to the long-term mean as described above are shown for comparison with reference lakes. The following data allow conditions in lake 223 before acidification to be compared to long-term means for four to six reference lakes in the area, for the period 1974 to 1982. Means and standard deviations are given for reference lakes, followed by the preacidification value for Lake 223 in brackets. Phytoplankton production, as carbon, 164 ± 60 mg/m^2 per day (140 ± 56); phytoplankton biomass, 1.03 ± 0.41 mg/liter (0.69 ± 12); chlorophyll a, 3.00 ± 0.84 μg/liter (1.92 ± 0.14); dissolved inorganic carbon, 1.41 ± 0.61 mg/liter (1.60 ± 0.07); Ca, 2.43 ± 0.85 mg/liter (2.19 ± 0.02); SO_4, 4.09 ± 0.99 mg/liter (3.35 ± 0.20); Si, 1.20 ± 1.04 mg/liter (1.29 ± 0.01); total nitrogen, 314 ± 51 μg/liter (272 ± 25); and total phosphorus, 6.6 ± 1.2 μg/liter (6.8 ± 0.1). Mean depth and lake surface area hectare for Lake 223 and the reference lakes (mean and standard deviation, n = 6) were 7.2 m versus 7.2 m ± 3.3 m, and 27.3 ha versus 31.3 ha ± 16.6 ha, respectively.
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RESEARCH ARTICLE

Human von Willebrand Factor (vWF): Isolation of Complementary DNA (cDNA) Clones and Chromosomal Localization

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The hemostatic system has evolved to minimize blood loss following vascular injury. In higher vertebrates, including man, the system is quite complex and requires the interaction of circulating platelets, a series of plasma coagulation proteins, endothelial cells, and components of the vascular subendothelium. The initial and critical event in hemostasis is the adhesion of platelets to the subendothelium. It occurs within seconds of injury and provides a nidus for platelet plug assembly and fibrin clot formation.

The factor VIII molecular complex is composed of two distinct protein components, the antihemophilic factor (AHF or VIIIC) and von Willebrand factor (vWF), and plays a major role in both platelet adhesion and fibrin formation (*1*). Two of the most common inherited clinical bleeding disorders are the result of a deficiency in the activity of one or the other of these components.

The VIIIC molecule is an important regulatory protein in the coagulation cascade. After activation by trace quantities of thrombin, it accelerates the rate of factor X activation by factor IX, eventually leading to the formation of the fibrin clot. Classic hemophilia (VIIIC deficiency) is an X chromosome–linked disorder that affects one in 10,000 males, and has been recognized as a major source of hemorrhagic morbidity and mortality since biblical times. Treatment consists of supportive measures and usually requires frequent transfusion with blood products. The latter results in a high incidence of infectious complications in this population, including various forms of hepatitis and acquired immune deficiency syndrome.

The vWF molecule is an adhesive glycoprotein synthesized by endothelial cells and megakaryocytes. It serves as a carrier in plasma for VIIIC and facilitates platelet-vessel wall interactions. By binding to subendothelial structures and to the platelet surface it promotes shear-dependent platelet adhesion to the vessel

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