Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of a 37-year whole-ecosystem experiment

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Lake 227, a small lake in the Precambrian Shield at the Experimental Lakes Area (ELA), has been fertilized for 37 years with constant annual inputs of phosphorus and decreasing inputs of nitrogen to test the theory that controlling nitrogen inputs can control eutrophication. For the final 16 years (1990–2005), the lake was fertilized with phosphorus alone. Reducing nitrogen inputs increasingly favored nitrogen-fixing cyanobacteria as a response by the phytoplankton community to extreme seasonal nitrogen limitation. Nitrogen fixation was sufficient to allow biomass to continue to be produced in proportion to phosphorus, and the lake remained highly eutrophic, despite showing indications of extreme nitrogen limitation seasonally. To reduce eutrophication, the focus of management must be on decreasing inputs of phosphorus.

cyanobacteria blooms | Experimental Lakes | nutrient limitation | phosphorus

Eutrophication is the general term used by aquatic scientists to describe the suite of symptoms that a lake exhibits in response to fertilization with nutrients (1). Common symptoms include dense algal blooms causing high turbidity and increasing anoxia in the deeper parts of lakes from the decay of sedimenting plant material. The anoxia can in turn cause fish kills in midsummer. One of the most objectionable symptoms of eutrophication has been the appearance of floating algal "blooms" (Fig. 1). In freshwaters, these surface blooms are often of nitrogen (N)-fixing cyanobacteria (known popularly as blue–green algae) (2). Similar forms are also common in many eutrophied estuaries (3) although other types of nuisance algal blooms are also common (4).

The emphasis on controlling eutrophication in freshwater lakes has been focused heavily on decreasing inputs of phosphorus (P) (2, 5–7). Schindler (2, 7) noted that many lakes rendered eutrophic by the addition of P contained phytoplankton communities that showed signs of extreme N limitation in short-term bioassays such as N debt (8, 9) or nutrient addition bioassays (10), He concluded that N limitation was a symptom of overfertilization with P and proposed that short-term N limitation was not necessarily a reliable indication that N must be controlled to reverse eutrophication. Hecky and Kilham (11) also warned that short-term measures of N deficiency were not reliable indicators of ecosystem responses to N enrichment or removal. Despite these early warnings, many studies in lakes and estuaries still conclude that N must be controlled as well as, or instead of, P to reduce eutrophication (for review, see ref. 12). The subject is hotly debated with respect to reducing eutrophication in the Baltic Sea (3). Recently, there has been renewed advocacy of N control to mitigate eutrophication of both lakes and estuaries. N and P control is being proposed to halt the rapid increase in eutrophication in Lake Winnipeg (13) and the Baltic Sea (3). This is troubling because proponents of controlling N in lakes and estuaries are relying on the same bioassays or correlations with nutrient concentrations that we (2, 7, 11) found to

lead to the erroneous conclusion that N inputs must be controlled to reduce eutrophication. These bioassays and the related assumptions have led to very expensive mitigation programs in several countries.

Aquatic scientists have often relied on the Redfield ratio to gauge whether nutrient supplies are sufficient. Redfield (14) observed that the ratio of carbon:nitrogen:phosphorus in marine phytoplankton was quite constant, with mean ratios by weight of $\approx$40:7:1. The Redfield ratio has subsequently been accepted as a general indicator for balanced growth with potential for near optimum growth rates (8). In the Experimental Lakes Area (ELA), lakes rendered eutrophic by experimental additions of N and P at N:P ratios less than Redfield ratio (7:1 weight ratio) have had N concentrations increase to above Redfield ratios as the result of N fixation by diazotrophic heterocystous cyanobacteria (2, 9, 15, 16). Algal biomass and chlorophyll *a* have remained proportional to P inputs regardless of the ratio of N:P added as fertilizer. Here, we describe a deliberate and extreme long-term experiment to test the effectiveness of controlling N on eutrophication.

The Lake and Its Experimental Treatments. Lake 227 in the ELA of northwestern Ontario, Canada, has a surface area of 5.0 ha, a mean depth of 4.4 m, and a maximum depth of 10.0 m (17). In June 1969, fertilization of the lake began with P and N to test the hypothesis then popular in North America that C could limit eutrophication of lakes (18). For the first five years (1969–1974), the ratio of N to P in fertilizer was added at 12:1 by weight, well above the Redfield ratio, to ensure that phytoplankton had adequate N and P supplies during the period when we were testing the C limitation hypothesis. Lake 227 became highly eutrophic, producing phytoplankton blooms in proportion to P supplies, despite phytoplankton showing symptoms of extreme C limitation for most of the summer months. C deficiency reduced daily rates of photosynthesis, but phytoplankton biomass increased until limited by P (19). In a second experiment in nearby Lake 226, we deliberately tested the effects of N limitation, by adding N and C to two isolated basins, but phosphorus only to one basin (North). N:P ratios in North Basin fertilizer were 4.6

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Fig. 1. Photograph of Grand Beach on the southern basin of Lake Winnipeg, August 2006. Photo by Lori Volkart.

Table 1. Summary of annual fertilizer additions to Lake 227, 1969–2005

Year	N, kg per year	P, kg per year	N:P by weight
1969	249	20.7	12.1
1970–1974	308	24.8	12.4
1975–1982	110	23.6	4.66
1983	110	19.8	5.54
1984–1989	110	23.6	4.66
1990–1997	0	23.6	0
1998	0	31.9	0
1999–2005	0	24.5	0

of fertilization averaged 42 μg/liter (Fig. 2A). Concentrations of total dissolved phosphorus (TDP) averaged 11 μg/liter (Fig. 2B). There was no significant long-term trend in either form. Soluble reactive P was not routinely measured, but it was generally well below the limit of detection except by radioactive P bioassays, i.e., in the nanogram per liter range (21).
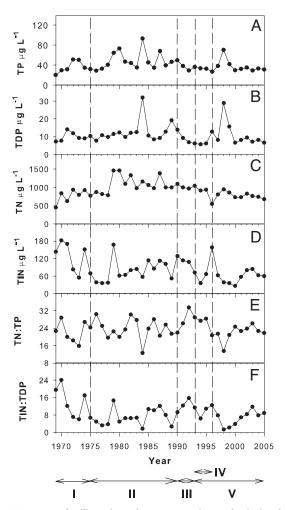
to 5.5:1 by weight, well below the Redfield ratio. Large algal blooms were again in proportion to P additions, but the responding species were primarily N-fixing cyanobacteria (2, 7). To test further the hypothesis that low N:P favored N-fixing species, the ratio of N to P in fertilizer added to Lake 227 was decreased to 4:1 beginning in 1975. The hypothesis was supported, and N fixation was high in subsequent years (2, 15, 16). Lake 227 continued to be fertilized at this N:P ratio through 1989. By that time there were signs that the lake was becoming both C- and N-sufficient because of slowly increasing concentrations of these elements as the result of several years of atmospheric invasion and net fixation and retention of N_2 and CO_2 (15). As nutrient balance was approached, the domination of phytoplankton by N-fixing cyanobacteria was decreasing (16), and short-term N limitation was less pronounced (9). From 1990 onward, no N fertilizer has been added to the lake. P continues to be added, and P inputs have remained relatively constant throughout the 37 years of fertilization (Table 1).

Superimposed on the nutrient fertilization was a short-term (4-year) food web manipulation (20). In 1993–1994, pike *Esox lucius* were added to the lake, which had contained only large numbers of forage fish, including fathead minnows (*Pimephales promelas*) and several species of dace (*Semotilus margarita*, *Phoxinus eos*, and *Phoxinus neogaeus*). By 1996, predation by pike had extirpated all forage fish. They have remained absent, and the lake fishless after all pike were removed in 1996 (ref. 20 and K. Mills, unpublished observation).

Nutrient Concentrations and Ratios. Concentrations of total phosphorus (TP) in the epilimnion during ice-free season in all years

Fig. 2. Mean annual epilimnetic nutrient concentrations and ratios in Lake 227, 1969–2005. Periods separated by vertical dashed lines represent: I, the period of fertilization at high N:P (12:1 by weight) 1969–1974; II, the period of fertilization with low N:P (4:1), 1975–1989; III–V, the period when no N fertilizer was added to the lake. IV, the years (1993–1996) that pike were present in the lake. The lake was fishless after 1996. (*A*) Total P. (*B*) Total dissolved P. (*C*) Total N. (*D*) Total inorganic nitrogen (= $NH_4 + NO_2 + NO_3$). (*E*) Ratio by weight of total N to total P in the lake. (*F*) Ratio by weight of TIN to TDP.
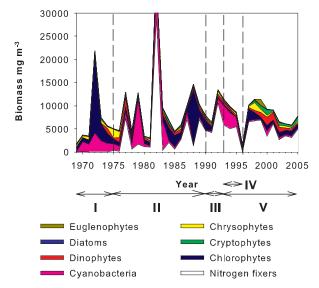
Fig. 3. Phytoplankton biomass in the epilimnion by algal group, 1969–2005. Vertical dashed lines were as in Fig. 2. In the Legend, "cyanobacteria" refers to cyanobacteria species that are not known to fix nitrogen. "Nitrogen fixers" refers to N-fixing species of cyanobacteria.

Fig. 4. Other measures of phytoplankton and nitrogen fixation, 1969–2005. (*A*) Chlorophyll *a*. (*B*) Ratio of chlorophyll *a*:phytoplankton biomass (μg/mm^3). (*C*) Nitrogen fixation calculated from heterocyst counts. (*D*) Heterocyst counts. Vertical dashed lines are as in Fig. 2.

Total nitrogen (TN) in Lake 227 averaged 825 μg of N per liter in 1969–1974, the period when high N fertilizer was added (Fig. 2*C*). It stayed constant for several years after N fertilizer was decreased, then increased in 1978, staying at ≈1,200 μg/liter through 1989. After termination of N fertilization in 1990, it decreased very slowly to ≈800 μg of N per liter after 1999. Dissolved inorganic N, the sum of nitrate, nitrite, and ammonium (TIN) was highest in the 1969–1974 period, when high N fertilizer was applied (Fig. 2*D*). It averaged 128 μg of N per liter. After 1975, there was no significant long-term trend. Mean values for 1975–1989, when N fertilizer was added, and 1990–2004, when no N was added were almost identical, 77 and 76 μg/liter, respectively. Concentrations of TIN were high in winter and spring but decreased to low concentrations in early summer when the phytoplankton exhibited strong N deficiency that was subsequently relieved as N-fixing cyanobacteria increased to high midsummer biomasses (9). An analysis of the data through 1984 revealed that the lake had attained steady-state with inputs of both N and C by that time (15).

Ratios of TN:TP in the lake were usually much higher than Redfield ratios (7:1 by weight; Fig. 2*E*). Average values for 1969–1974, 1975–1989, and after 1990 were 22–28 by weight, above the value of 20:1 where N limitation is usually inferred in freshwater or marine systems (22, 23). It is noteworthy that average TIN:TDP ratios were occasionally below Redfield ratios after 1975 (Fig. 2*F*). However, average TIN:TDP ratios equaled or exceeded Redfield ratios in all three fertilization periods, at 14, 7, and 10 by weight. The N:P in fertilizer was zero after 1989, so these mean ratios were maintained entirely by N fixation and nitrogen recycled within the lake.

Response of the Phytoplankton and N Fixation. From 1969–1974, all groups of phytoplankton increased as the result of fertilization with high N:P (Fig. 3). The algal assemblage was dominated by small unicellular desmids with large populations of *Limnothrix redekei* occurring from late June until early September. N-fixing cyanobacteria were not detected (24) as N was being added in excess of algal demand and TIN:TDP ratios were high (Fig. 2*F*). C necessary for algal biomass was supplied by invasion of CO_2 to the lake from the atmosphere (25). Short-term bioassays indicated that C-limited photosynthesis and algal growth and did
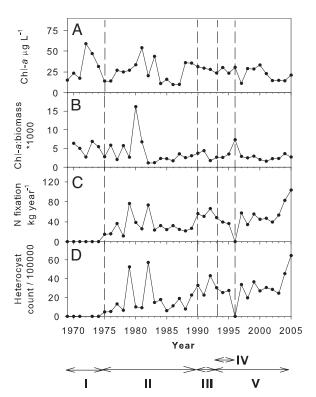
not predict the continued growth of algal biomass in proportion to P.

N-fixing cyanobacteria first appeared in significant numbers in 1975, within weeks of reducing the N:P ratio in loading (ref. 2 and Fig. 3). *Aphanizomenon schindlerii* appeared first, and by the early 1980s it dominated the summer assemblage. The average annual importance of N fixers varied considerably from 1975 through 1989. They were always the dominant part of the phytoplankton biomass in July and August. Annual *Aphanizomenon* biomass diminished slowly and became more variable during the late 1980s. After N fertilization ceased in 1990, N fixers (primarily *A. schindlerii*) always formed >50% of the total phytoplankton biomass, except in 1996, the terminal year of the food web manipulation (20). From 1996 when the lake became fishless to the present, the algal community became more diverse with increased representation by chrysophytes, diatoms, cryptophytes, and dinoflagellates. Nonfixing cyanobacteria, most notably *L. redekei*, remained prominent throughout the experiment but tended to increase after midsummer blooms of N-fixing cyanobacteria, suggesting a competitive advantage over the N fixers when recycled fixed N became available.

Phytoplankton biomass averaged 9,306 mg m^{-3}. There were no significant differences in annual average biomass among the periods when different N:P ratios were applied. Variability in phytoplankton biomass was high, ranging from 1,502 mg m^{-3} in 1996 to 39,000 mg m^{-3} in 1982.

Chlorophyll *a* is frequently used as a measure of phytoplankton biomass especially in short-term bioassays. Annual averages during the term of the experiment ranged from 17 to 59 μg liter^{-1}, with higher values occurring when the N:P ratio in fertilizer was high (Fig. 4*A*). Interannual variation in chlorophyll was higher during the periods when N fertilizer was added than after 1990. The chlorophyll *a*:biomass ratio was also less variable

when no N fertilizer was added (Fig. 4*B*). It fluctuated in a narrow range, except for high values in 1980 and 1996. In those 2 years, N-fixing cyanobacteria populations were low, and other taxa dominated the phytoplankton. Average chlorophyll *a*:biomass in the first several years of the experiment was somewhat higher than in the last 20 years. These results demonstrate that chlorophyll *a* per unit of biomass is higher when N is in excess of algal requirements. Conversely, if the algal community is made more N-deficient, chlorophyll per unit biomass declines. Consequently, nutrient enrichment experiments that rely only on chlorophyll *a* as a measure of phytoplankton abundance may give misleading results about changes in biomass.

N fixation was negligible before 1975, when fertilizer N:P ratios were high (Fig. 4*C*). From 1975 onward, fixation by the lake varied from 0.2 to 2.1 g m^{-2} year^{-1} with the exception of 1996, when no fixation occurred after food chain manipulation. By the late 1980s when indicators of N limitation were weakening (9), N fixation had stabilized at 0.5–0.6 g of N m^{-2} year^{-1}, approximately half of the highest values in earlier years. After fertilization with N was terminated in 1990, fixation increased again for several years. It decreased to zero in 1996, after which fixation returned to ≈1 g of N m^{-2} year^{-1} then increased again in 2004–2005 to >2 g N m^{-2} year^{-1}, the highest values recorded in the 37-year dataset.

Changes Caused by the "Trophic Cascade": 1996. The response of phytoplankton in 1996, after fish were extirpated, stands out. In that year, the zooplankton consisted primarily of large *Daphnia pulicaria*, as the result of decreased zooplanktivory by fish. Addition of pike in 1993–1994 and prior minnow trapping had extirpated populations of forage fish, as described earlier. Pike were removed in late 1995 and early 1996, rendering Lake 227 fishless. During that year, TIN and chlorophyll *a*:biomass were high and comparable with values observed before 1975, when ratios of added N:P were high. However, chlorophyll *a* and phytoplankton biomass were very low in 1996. N-fixing cyanobacteria and N fixation were unmeasurable. Increased sedimentation of P and excretion of TIN as the result of high grazing by *Daphnia* appeared to be responsible for the high TIN (20). N fixers returned to dominance in 1997 and in subsequent years as *Daphnia* populations returned to low abundances. Invertebrate predators such as *Chaoborus* have increased after fish removal and may now be the dominant predator on *Daphnia* and other zooplankton. Before 1996, predation by fish likely limited the abundance of invertebrate predators (26).

Interpretation. Although there was no increase in the abundance of N-fixing cyanobacteria after N fertilization ceased in 1990, heterocyst numbers and subsequent N fixation increased greatly (Fig. 3 *C* and *D*). Despite the reduction of N fertilizer to zero, P additions kept the lake eutrophic, with no substantial changes in biomass of phytoplankton. We suggest that in most freshwater lakes, attempts to manage N inputs will be equally futile. Indeed, addition of N to a hypereutrophic lake caused N-fixing cyanobacteria to be suppressed, allowing other species to thrive (27). In another case study, N addition was futile because it was rapidly denitrified. Water quality improved, but this was attributed to a fish kill and subsequent expansion of macrophyte cover (28). Unless N concentrations are of concern for human health, funds for eutrophication control are better spent on more complete removal of P sources. This work also demonstrates the necessity of using studies at whole-ecosystem scales for managing ecosystems. Bottle bioassays or mesocosm studies cannot properly account for important long-term processes such as atmospheric exchange, colonization by N-fixing cyanobacteria, changes in the grazer community, and increases in N as the result of N fixation and return from sediments that determine the long-term fate of eutrophied ecosystems.

Are Our Findings Applicable to Estuaries? It is generally believed that coastal marine waters are N-limited and that N must be controlled to reduce algal abundance (for review, see 12). However, these conclusions are largely based on bioassays similar to those that we have found to give spurious results for freshwaters. In low-salinity estuaries such as the Baltic Sea, N fixers are abundant, and rates of N fixation are high (3, 29). In stratified eutrophic lakes and estuaries, both P and N are generally plentiful in spring, before stratification develops. Once warming allows thermoclines to become established, initial concentrations of N are depleted, and N-fixing species of phytoplankton are favored. N fixation allows phytoplankton standing crops to develop that are proportional to P, as indicated by the constant ratios for suspended N:P and total N:P in Lake 227 (Fig. 1). On a long-term basis, if annual N fixation does not exceed sedimentation plus denitrification, chronic N deficiencies favor N-fixing cyanobacteria every summer.

In at least one well documented case of an estuarine recovery from eutrophication (29), chlorophyll *a* in the Stockholm Archipelago estuary decreased from 30 μg/liter to <16 μg/liter in the early 1970s, after the control of P alone from Swedish sewage treatment plants. TP decreased from 75–90 to <30 μg/liter in 3 years. As in Lake 227, at the time, the waters of the Archipelago were dominated by N-fixing cyanobacteria species in summer and showed signs of extreme N limitation (3, 30). N fixation [measured in 1972 (30)] was 2.25 g/m^2 per year, higher than any year in Lake 227. P and phytoplankton in the Archipelago declined, initially rapidly with the imposition of P removal in waste treatments then more slowly since the mid-1970s (3)., The recent removal of N at waste treatment plants beginning in the mid-1990s has also led to somewhat lower chlorophyll concentrations. However, the improved waste treatment also removed more P and reduced biological oxygen demand (BOD from ammonia loading) (3). The reduction in BOD is important because it has decreased the intensity and duration of anoxia at the sediment surface. Preliminary data suggest that P release from sediments is beginning to decrease in response to increasing oxygen (3). Overall, it is difficult to tell whether the slight improvements in recent years are the result of reduced inputs of N, P, BOD, or all three. However, we suggest that as in Lake 227, N fixation and short-term indicators of N deficiency such as low TIN:TDP ratios or sensitivity to N enrichment bioassays are a sign of overfertilization with P and not necessarily evidence that N control will cause decreased eutrophication.

As we found in the 227 trophic cascade experiment described above, in large mesocosm experiments using near full-strength seawater from Narragansett Bay, RI, nitrogen fixers prospered, and nitrogen fixation occurred as long as the abundance of grazing zooplankton was low (31, 32). Normal concentrations of zooplankton were able to suppress cyanobacteria populations by grazing, keeping colonies too small to form heterocysts. These observations appear to be comparable with Lake 227 in 1996, when high-grazer populations after decreased zooplanktivory prevented N-fixing cyanobacteria from dominating despite low N:P inputs in fertilizer. These results suggest that under the right conditions, grazers may be able to prevent cyanobacteria blooms, in both freshwaters and estuaries.

There is, however, still need for caution when extrapolating our results to estuaries. It is conceivable that molybdenum, iron or other trace nutrients might limit the rate of colony growth by N fixers (31, 32), although the rates of fixation observed (29, 33, 34) are as high as we have observed in freshwaters. In some estuaries, such as the Baltic Sea, haloclines isolate productive epilimnions from deepwater nutrient sources even more strongly than during the temperature-driven summer stratification of most lakes. Persistent haloclines can restrict oxygen circulation to deeper waters and return of nutrients to the euphotic zone. Haloclines also promote hypoxia and release of P from sedi-

ments. The strength and duration of haloclines often depend on extreme weather events that are highly stochastic (3). High rates of denitrification in estuarine anoxic zones allow much of the accumulated fixed N to be returned to the atmosphere (34, 35). However, this would reinforce the chronic N deficiency that we have discussed above, which can only be relieved by reducing P loading. Also, unlike the Baltic, many estuaries have rather rapid flushing that would result in the continuous dilution of any N fixed by cyanobacteria (36), potentially keeping an estuary in a chronic N-deficient state despite high rates of N fixation. Finally, estuaries are often highly turbid, and light may limit N fixation.

In summary, the long-term experiment in Lake 227 and the early response of the Stockholm Archipelago to P control challenge the widely held belief that short-term N limitation in phytoplankton communities is evidence that external sources of N should be controlled to decrease eutrophication. N-fixing cyanobacteria cannot be limited by a shortage of dissolved N and instead are competitively favored. The increasing appreciation of the importance of N fixation to balancing the global ocean N budget (34, 37) demonstrates that salinity and marine geochemistry alone do not limit N-fixing species and N fixation has the potential to overcome N deficiencies in a wide range of aquatic environments. Our results suggest that controlling N inputs could actually aggravate the dominance of N-fixing cyanobacteria.

The adjustment of N deficiencies in Lake 227 required several

years (15), indicating that conclusions meaningful for nutrient management are unlikely to be obtained from short-term experiments. The responses of Lake 227 over almost 4 decades of fertilization indicate that experiments to guide nutrient management confidently must be full-ecosystem scale and carried out for at least several years (38).

Materials and Methods

Fertilization of the lake began on June 26, 1969 and was done weekly during the ice-free seasons of all years since that time. The lake was sampled from weekly to monthly during the ice-free seasons and from two to four times under ice in most years. On each date, nitrate plus nitrate, ammonium, total dissolved N, particulate N, total dissolved P, total P, chlorophyll a, base cations and strong acid anions, and pH were measured. Samples of phytoplankton were taken for identification and counting and for measurements of primary production. Zooplankton samples were also taken on each sampling date. A temperature profile was also measured.

Full details of methods are given in the supporting information (SI) *Materials and Methods*.

1. Hutchinson, GE (1973) Eutrophication. *Am Sci* 61:269–279.
2. Schindler DW (1977) Evolution of phosphorus limitation in lakes: Natural mechanisms compensate for deficiencies of nitrogen and carbon in eutrophied lakes. *Science* 195:260–262.
3. Boesch D, Hecky R, O'Melia C, Schindler DW, Seitzinger S (2006) *Eutrophication of Swedish Seas* (Final Report, Swedish Environmental Protection Agency, Stockholm Sweden).
4. Anderson DM, et al. (2002) Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. *Estuaries* 25:704–726.
5. Vollenweider RA (1968) *Scientific Fundamentals of the Eutrophication of Lakes and Flowing Waters, with Particular Reference to Nitrogen and Phosphorus as Factors in Eutrophication* (Tech Rep DAS/CS/68.27, OECD, Paris).
6. Vollenweider RA (1976) Advances in defining critical loading levels for phosphorus in lake eutrophication. *Mem 1st Ital Idrobiol* 33:53–83.
7. Schindler DW (1974) Eutrophication and recovery in experimental lakes: Implications for lake management. *Science* 184:897–899.
8. Healey FP, Hendzel LL (1980) Physiological indicators of nutrient deficiency in lake phytoplankton. *Can J Fish Aquat Sci* 37:442–453.
9. Hendzel LL, Hecky RE, Findlay DL (1994) Recent changes of nitrogen fixation in Canadian Precambrian Shield lakes. *Can J Fish Aquat Sci* 51:2247–2253.
10. Schindler DW (1971) Carbon, nitrogen and phosphorus and the eutrophication of freshwater lakes. *J Phycol* 7:321–329.
11. Hecky RE, Kilham P (1988) Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment. *Limnol Oceanogr* 33:796–822.
12. Howarth RW, Marino R (2006) Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: Evolving views over three decades. *Limnol Oceanogr* 51:364–376.
13. Lake Winnipeg Stewardship Board (2006) *Reducing Nutrient Loading to Lake Winnipeg and Its Watershed: Our Respective Responsibility and Commitment to Action* (Report to the Minister of Water Stewardship December 2006).
14. Redfield AC (1958) The biological control of chemical factors in the environment. *Am Sci* 46:205–221.
15. Schindler DW, Hesslein RH, Turner MA (1987) Exchange of nutrients between sediments and water after 15 years of experimental eutrophication. *Can J Fish Aquat Sci* 44(Suppl 1):26–33.
16. Findlay DL, Hecky RE, Hendzel LL, Stainton MP, Regehr GW (1994) Relationship between N₂-fixation and heterocyst abundance and its relevance to the nitrogen budget of Lake 227. *Can J Fish Aquat Sci* 51:2254–2266.
17. Brunskill GJ, Schindler DW (1971) Geography and bathymetry of selected lake basins in the Experimental Lakes Area (ELA), northwestern Ontario. *J Fish Res Board Can* 28:139–155.
18. Legge RF, Dingeldein D (March/April 1970) We hung the phosphates without a fair trial. *Can Res Develop* 3:19–42.
19. Schindler DW (1975) Whole-lake fertilization experiments with phosphorus, nitrogen, and carbon. *Int Ver Theor Angew Limnol Verh* 19:3221–3231.
20. Elser JJ, et al. (2000) Pelagic C:N:P stoichiometry in a eutrophied lake: Responses to a whole-lake food-web manipulation. *Ecosystems* 3:293–307.
21. Levine SN, Schindler DW (1980) Radiochemical analysis of orthophosphate concentrations and seasonal changes in the flux of orthophosphate to seston in two Canadian Shield lakes. *Can J Fish Aquat Sci* 37:479–487.
22. Smith VH (2006) Responses of estuarine and coastal marine phytoplankton to nitrogen and phosphorus enrichment. *Limnol Oceanogr* 51:377–384.
23. Guildford S, Hecky RE (2000) Total nitrogen, total phosphorus, and nutrient limitation in lakes and oceans: Is there a common relationship? *Limnol Oceanogr* 45:1213–1223.
24. Schindler DW, et al. (1973) Eutrophication of Lake 227 by addition of phosphate and nitrate: The second, third, and fourth years of enrichment 1970, 1971, and 1972. *J Fish Res Board Can* 30:1415–1440.
25. Schindler DW, Brunskill GJ, Emerson S, Broecker WS, Peng T-H (1972) Atmospheric carbon dioxide: Its role in maintaining phytoplankton standing crops. *Science* 177:1192–1194.
26. Ramcharan CW, et al. (1995) A comparative approach to determining the role of fish predation in structuring limnetic ecosystems. *Arch Hydrobiol* 133:389–416.
27. Barica J, Kling H, Gibson J (1980) Experimental manipulation of algal bloom composition by nitrogen addition. *Can J Fish Aquat Sci* 37:1175–1183.
28. Lathrop RC (1988) Evaluation of whole-lake nitrogen-fertilization for controlling blue–green algal blooms in a hypereutrophic lake. *Can J Fish Aquat Sci* 45:2061–2075.
29. Brattberg G (1986) Decreased phosphorus loading changes phytoplankton composition and biomass in the Stockholm archipelago. *Vatten* 42:141–152.
30. Brattberg G (1977) Nitrogen fixation in a polluted brackish water archipelago. *Ambio (Spec Rep)* 5:27–42.
31. Marino R, Chan F, Howarth RW, Pace ML, Likens GE (2006) Ecological constraints on planktonic nitrogen fixation in saline estuaries. I. Nutrient and trophic controls. *Mar Ecol Prog Ser* 309:25–39.
32. Chan F, Marino RL, Howarth RW, Pace ML (2006) Ecological constraints on planktonic nitrogen fixation in saline estuaries. II. Grazing controls on cyanobacterial population dynamics. *Mar Ecol Prog Ser* 309:41–53.
33. Voss M, Emeis K-C, Hille S, Neumann T, Dippner JW (2005) Nitrogen cycle of the Baltic Sea from an isotopic perspective. *Global Biogeochem Cycles* 19: GB 3001, doi:10.1029/2004GB002338.
34. Gardner WS, et al. (2006) Nitrogen fixation and dissimilatory nitrate reduction to ammonium (DNRA) support nitrogen dynamics in Texas estuaries. *Limnol Oceanogr* 51:558–568.
35. Seitzinger SP (1988) Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. *Limnol Oceanogr* 33:702–724.
36. Mahaffy C, Michaels AF, Capone DG (2005) The conundrum of marine N₂ fixation. *Am J Sci* 305:546–595.
37. Karl D, et al. (2002) Dinitrogen fixation in the world's oceans. *Biogeochemistry* 57–58:47–98.