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Review Paper

Control of Lacustrine Phytoplankton by Nutrients: Erosion of the Phosphorus Paradigm

key words: algae, dissolved organic nutrients, eutrophication, lake ecosystems,
lake management, nitrogen

Abstract

Control of lacustrine phytoplankton biomass by phosphorus is one of the oldest and most stable paradigms in modern limnology. Even so, evidence from bioassays conducted by multiple investigators at numerous sites over the last three decades shows that N is at least as likely as P to be limiting to phytoplankton growth. A number of important flaws in the evidence supporting the phosphorus paradigm have contributed to an unrealistic degree of focus on phosphorus as a controlling element. These include insufficient skepticism in interpretation of: 1) the phosphorus:chlorophyll correlation in lakes, 2) the results of whole-lake fertilization experiments, and 3) stoichiometric arguments based on total N:total P ratios for inland waters. A new paradigm based on parity between N and P control of phytoplankton biomass in lakes seems more viable than the P paradigm. The new paradigm renews interest in the degree to which plankton communities are molded in composition by small differences in relative availability of N and P, the mechanisms that lead to a high frequency of N limitation in oligotrophic lakes, and the failure of aquatic N-fixers to compensate significantly for N deficiency under most conditions. A new N/P paradigm still must acknowledge that suppression of P loading often will be the most effective means of reducing phytoplankton biomass in eutrophic lakes, even if N is initially limiting.

From error to error one discovers the entire truth
SIGMUND FREUD

1. Introduction

Control of phytoplankton in lakes by phosphorus is one of the oldest and most stable paradigms in modern limnology. Phytoplankton biomass and production can be controlled by factors other than nutrients under some circumstances (*e.g.*, strong herbivory, hydraulic displacement, shading, and seasonal deep mixing), but nutrients often are limiting, and phosphorus is cited consistently as the dominant limiting nutrient (Table 1). Persistence of the phosphorus control paradigm in limnology has caused phosphorus dominance to be recognized in related fields, such as environmental engineering.

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Table 1. Textbook generalizations concerning the importance of phosphorus and nitrogen in limiting growth of phytoplankton in lakes.

Source	Phosphorus Limitation	Nitrogen Limitation
Limnology		
HORNE and GOLDMAN, 1994	Lakes in wet climates	Lakes in dry climates
WETZEL, 2001	Foremost limit	About 25%
KALFF, 2002	Oligotrophic temperate lakes	Eutrophic lakes
DODDS, 2002	P key in many systems	Nitrogen can limit
DODSON, 2005	P dominant limitation	N secondary limitation
LAMPERT and SOMMER, 2007	Predominant	Not addressed
Applied Limnology		
WELCH and LINDELL, 1992	P most limiting	Highly enriched lakes
COOKE et al., 2005	P most often limiting	Highly polluted lakes

Most textbook authors acknowledge the possibility that nitrogen can limit growth of phytoplankton in lakes, but the range of predictions for circumstances under which N limitation will occur is inconsistent or vague (Table 1). In reading multiple texts, one gets the impression that the importance of phosphorus is well established for freshwater lakes, but the role of nitrogen is uncertain or unusual (the prevailing view for hypersaline lakes favors N dominance: JAVOR, 1989). For this reason, transfer of the phosphorus paradigm to applied ecology has occurred primarily through deletion of the confusing possibilities for nitrogen limitation. Thus, when translated outside the discipline, the phosphorus paradigm is even stronger than it is within the discipline.

The accumulation of credible studies of nutrient limitation in numerous lakes, as reviewed below, now suggests that the phosphorus paradigm is not nearly so strong as it appeared to be two decades ago. Evidence that nitrogen limitation in lakes is common rather than exceptional suggests that some of the fundamental arguments supporting the phosphorus paradigm should be reexamined critically, which is the purpose of this paper.

2. History of the Phosphorus Control Paradigm

The phosphorus control paradigm finds its roots in NAUMANN's (1919) classification of lakes according to algal abundance by use of WEBER's (1907) terminology for wetlands. NAUMANN separated oligotrophic from eutrophic lakes on the basis of algal abundance, and attributed the difference between categories to the relative amounts of plant nutrients. Because he could not quantify nutrients, he was unable to move his nutrient-control concept toward identification of one or more specific critical nutrients. Instead, he focused his attention on identifying landscape factors that were consistent with either rich or poor supplies of nutrients known to favor growth of terrestrial plants (N, P, Ca).

JUSTUS LIEBIG (1803–1873) set the stage for identification of specific limiting nutrients in various ecosystem types through his development of the limiting nutrient concept (HUTCHINSON, 1973), which became a general ecological paradigm under which the phosphorus control paradigm for lakes subsequently developed. He postulated that the growth of a plant population at any given place, when constrained by nutrient insufficiency, can be stimulated only by the limiting nutrient, which is the nutrient in shortest supply relative to the needs of the plant. It follows that the limiting nutrient may vary through time and space, and that it must be diagnosed experimentally. While LIEBIG's context was agricultural, the limiting-nutrient concept and its mathematical counterparts (TALLING, 1979) consistently have been of great interest to ecologists for analyzing autotrophy in various kinds of ecosystems.

Given that phosphorus and nitrogen are consistently among the limiting nutrients in agricultural settings, it was natural that limnologists should test the possibility that either or both of these nutrients might be commonly limiting to autotrophs in lakes. JUDAY and colleagues (1931) showed that both N and P are scarce in the water columns of lakes, and PEARSALL (1930, 1932) linked measured N : P ratios to successional stages of phytoplankton development in lakes, thus extending earlier, more hypothetical ideas about control of phytoplankton populations by nutrient ratios (reviewed by HUTCHINSON, 1957). Extensive laboratory investigations were conducted on cultured populations of algae by CHU (1943) and others. Experiments showed limitation of growth for individual algal species at low concentrations of N or P. Subsequent experiments with algae obtained directly from lake water showed lower thresholds of limitation, however, thus undermining confidence in specific thresholds based exclusively on laboratory studies (HUTCHINSON, 1957; VOLLENWEIDER, 1968). Even so, as early as 1930 it was already evident that scarcity of N or P could supress algal growth. Silica was also recognized at this time as a potentially limiting element for diatom growth (PEARSALL, 1930, 1932). There was also a small but steady interest in limitations caused by micronutrients (VOLLENWEIDER, 1968), but such limitations never have achieved broad credibility.

While stoichiometric analysis of N and P was inherent in PEARSALL's work of the 1930s, HUTCHINSON was first to formulate an explicit phosphorus paradigm based upon stoichiometry: "Of all the elements present in living organisms, phosphorus is likely to be the most important ecologically, because the ratio of phosphorus to other elements in organisms tends to be considerably greater than the ratio in primary sources of the biological elements" (HUTCHINSON, 1957, page 727).

Seeking realism that could not be obtained in a laboratory setting, EINSELE (1941) added phosphorus to a mildly eutrophic German Lake (Schleinsee), thereby doubling the amount of organic matter in the water column. This experiment later strengthened HUTCHINSON's conviction, based on stoichiometry, that phosphorus must have the strongest governing influence on autotrophic production in lakes.

After 1940, interest in the relationship between algal biomass or production and limiting nutrients was stimulated especially by recognition of anthropogenic eutrophication as a serious problem. In his thorough review of the subject, VOLLENWEIDER (1968) recorded 13 significant studies of lake eutrophication for 1940–1950, 28 for 1950–1959, and over 50 for 1960–1966. VOLLENWEIDER's coverage of eutrophication included both P and N, but led him to conclude that P provides better prediction of algal biomass. VOLLENWEIDER, following SAWYER (1947), noted that spring concentrations of "assimilable" phosphorus exceeding 10 µg/L or inorganic nitrogen (ammonium plus nitrate) exceeding 200–300 µg/L would suggest eutrophication.

VOLLENWEIDER's main goal in 1968 was to predict phosphorus concentrations from nutrient loading. The result was his now famous double logarithmic graph relating total phosphorus load to mean water depth (VOLLENWEIDER, 1968, Fig. 17). The diagram carries diagonal isolines of uniform concentrations, thus dividing the graphical space into sectors, with eutrophic lakes in the top sector and oligotrophic lakes at the bottom. Linkage of the water column to the watershed, which was inherent in this diagram, satisfied a basic need in the study of lakes, and was widely appreciated outside of limnology because of its usefulness in management. By the late 1960's, limnologists had become broadly committed to studying and controlling eutrophication, but some time elapsed before the key issues came clearly into focus (SCHINDLER, 2006). EDMONDSON (1970) generated great excitement by reducing algal biomass in Lake Washington through sewage diversion, thus validating nutrient-based theories of algal control.

In the late 1960's and early 1970's efforts were made to establish statistical relationships between phosphorus concentration and phytoplankton biomass (chlorophyll). By using a double logarithmic transformation, SAKAMOTO (1966) was able to show a close relationship

between the two variables for 20 Japanese lakes. Subsequently DILLON and RIGLER (1974), seeking predictive capability, measured total P in the spring, *i.e.*, before seasonal development of phytoplankton biomass, and measured chlorophyll *a* as an average over the growing season. They also excluded lakes showing a molar N : P ratio of 12 or less, on grounds that this precaution would allow them to assume a causal relationship between phosphorus and chlorophyll *a*. With double logarithmic transformation, their data for 19 Canadian lakes demonstrated a close statistical relationship between the two variables ($r^2 \approx 0.8$). Adding data from other sources, they found a similarity in relationship with their own study, and proposed a general equation for use when the N : P ratio is < 12 : 1, *i.e.*, log (chlorophyll *a*) = 1.449 log (P) − 1.136, where concentrations are given as μg/L. The arithmetically expressed confidence limits were disappointingly broad, however. For a total P concentration of 10 μg/L, the 95% limits were 0.8–5.6; for total P of 21 μg/L, they were 7.7–58.4. They argued nevertheless that a 50% confidence interval, whose limits differed by approximately by a factor of two, would be useful for managers wishing to know the most likely outcome of an increase or decrease in phosphorus concentrations. They also speculated that future studies would tighten the confidence limits.

A landmark study organized by OECD (1982) encompassing the entire spectrum of relationships between nutrient loading, nutrient concentration, chlorophyll *a*, and other trophic response variables, was in progress as DILLON and RIGLER published their important paper on nutrient–chlorophyll relationships, but was not published in final form until 1982. The OECD study, which was based upon carefully designed protocols for data collection, was international, but dealt only with temperate freshwater lakes and was focused on Europe and North America.

Authors of the OECD study found that concentrations of total nitrogen and total phosphorus in lakes were strongly correlated ($r = 0.75$), but that total phosphorus was better correlated with either chlorophyll or primary production than was total nitrogen. The authors identified a number of lakes that they considered to be nitrogen limited on the basis of N : P ratios. They removed from the data set these and a few lakes that were not strictly under nutrient control, because of suspended inorganic matter or other factors. For the screened data set, correlations between the logarithms of total phosphorus and mean chlorophyll *a* were near 0.9 for all lakes together or for any of the five groups of lakes that were included in the study. They concluded that phosphorus is the primary factor controlling algal biomass in most lakes, and consequently focused the remaining portion of their analysis, which dealt with the relationships between loading and concentration, on phosphorus alone.

Although statistically strong correlations were shown by the OECD study between the logarithms of total phosphorus and mean chlorophyll *a* across lakes, the inclusion of large numbers of lakes and standardized analytical and sampling procedures did not narrow the uncertainty of prediction for chlorophyll *a* from total phosphorus, contrary to the hope expressed by DILLON and RIGLER. The 95% confidence limits of predictions made for individual lakes were approximately one order of magnitude for the OECD study, as they had been for the DILLON and RIGLER study. The OECD results in large part were anticipated by several important studies that were published while the OECD work was in progress (VOLLENWEIDER, 1976; JONES and BACHMAN, 1976; CANFIELD and BACHMAN, 1981).

As the OECD data collection was beginning, Canada established an ambitious program of experiments on eutrophication in its Experimental Lakes Area (ELA), Ontario, under the direction of DAVID SCHINDLER (SCHINDLER, 2006). In 1971, SCHINDLER reviewed the state of knowledge on nutrient limitation of phytoplankton in lakes. He noted the willingness of most authorities to assume that phosphorus is the dominating nutrient limitation on phytoplankton biomass, but also acknowledged a number of observations suggesting limitation by nitrogen or trace elements. He characterized the phosphorus paradigm for nutrient limitation as overly comfortable, in that no study had demonstrated mechanisms of nutrient control experimentally in a realistic way (SCHINDLER, 1971).

Schindler's initial objective was to test the possibility that inorganic carbon might limit the phytoplankton biomass accumulation in a manner comparable to limitations observed through phosphorus or nitrogen (SCHINDLER, 1971; SCHINDLER et al., 1971). His experiments countered earlier work purporting to show limitation of phytoplankton growth by deficiency of inorganic carbon. SCHINDLER's work demonstrated that the combination of an inexhaustible atmospheric source of CO_2, along with efficient transfer of CO_2 across the air-water interface in lakes, restricts carbon deficiency to conditions when both biomass and solar irradiance are very high, i.e., a portion of each day under bloom conditions. Thus, while carbon deficiency can slow the growth of phytoplankton under extreme conditions, it does not establish a cap on total biomass accumulation and has only a weak effect on production (SCHINDLER et al., 1972).

Extending through subsequent publications during the 1970's, SCHINDLER and colleagues presented and interpreted results of experiments with N and P at ELA. SCHINDLER's initial experiments were made with bottles and flasks as well as tubes of 1 m diameter in experimental Lake 227, and subsequent fertilization of Lake 227 with both N and P. The tube experiments showed that addition of phosphorus and nitrogen together could establish dense populations of phytoplankton, even though the CO_2 inventory of the water was the lowest on record for lakes in general (SCHINDLER et al., 1971). Addition of both N and P simultaneously to Lake 227 produced an algal biomass five times background and changed species composition. Overall, SCHINDLER gave an edge to phosphorus in his interpretation of control, but emphasized the strong combined effect of N and P.

SCHINDLER and colleagues conducted enrichment experiments on other lakes as well. The most striking and influential outcome of these experiments occurred through installation of a curtain subdividing Lake 226 into two nearly equal basins (SCHINDLER, 1974). Nitrogen, phosphorus, and carbon were added to one side, where a strong bloom of N-fixing cyanobacteria appeared, whereas the other side was fertilized with nitrogen and carbon but not phosphorus, and did not show the cyanobacterial bloom. Although the experiment continued for 8 years, results after year 4 (1976) were influenced by curtain failure and a forest fire in the watershed (D.W. SCHINDLER pers. comm., 2008). In addition, Lake 304 was fertilized with C, N, and P for two years after having been monitored with no experimental treatment for three previous years (SCHINDLER, 1974). Phosphorus addition was discontinued in 1973, but nitrogen and carbon enrichments were continued as before. Chlorophyll concentration declined in 1973 to concentrations that were typical of background conditions in earlier years. Based on these experiments, SCHINDLER concluded that phosphorus and nitrogen can cause rapid eutrophication, as indicated by drastic increases in phytoplankton biomass. His prescription for abatement of eutrophication was control of phosphorus.

SCHINDLER consolidated his conclusions in 1977. He noted a close relationship between the concentration of total phosphorus and standing crop of phytoplankton in a wide variety of lakes. He described the evidence, largely established by him and his colleagues, showing that carbon is very unlikely to limit phytoplankton biomass in lakes. For nitrogen, SCHINDLER introduced some new conclusions based on experiments with lakes in which amendments of N and P were made at low N:P ratios. Where only P was added to a lake, there was no phytoplankton bloom, but rather a proliferation of attached algae composed of nitrogen fixing taxa. Where nitrogen was added at a low ratio (5:1) with respect to phosphorus, strong blooms of nitrogen fixing cyanobacteria (bluegreen algae) developed. SCHINDLER concluded that the presence of phosphorus at relatively high ratios to nitrogen stimulates growth of N-fixing cyanobacteria, which compensate by fixation for deficiencies in nitrogen. In one of the lakes, compensation by nitrogen fixers for nitrogen deficiency averaged 29% of total N load over two years of fertilization with low N:P ratios.

SCHINDLER's (1977) paper marks the culmination of the formative period for the phosphorus control paradigm. Numerous important developments occurred subsequently, particularly

in the analysis of nutrient supply ratios for natural and anthropogenically perturbed lakes and in analysis of the phosphorus cycle within lakes (SCHINDLER, 2006).

The foregoing overview shows that much of the interest in the nutrient supply of lakes was motivated by a desire to show how cultural eutrophication could be controlled. The scientific question underlying the phosphorus control paradigm in its original form, as stated by HUTCHINSON, was the identity of the nutrient that limits development of algal biomass in lakes. In contrast, the need for a rational approach to the control of eutrophication gave rise to the concept of nutrient restriction, which at first involved simultaneous consideration of nitrogen and phosphorus, but quickly became focused on phosphorus. Initially, priority for phosphorus control was rationalized by the conclusion, based on statistical or experimental information, that phosphorus is the element most likely to limit algal growth in lakes. It became evident very early, however, that the possibility of nitrogen limitation was generally moot from the viewpoint of management, for two reasons (GOLTERMAN, 1975; RYDING and RAST, 1989): (1) control of phosphorus pollution typically is more feasible and cheaper than control of nitrogen pollution, and (2) phosphorus limitation can be induced by aggressive phosphorus control, even if algal populations are initially limited by nitrogen deficiency. Thus, the scientific question (identity of the limiting nutrient) was severed from the management question (most effective control of algal populations). Studies of eutrophication still are motivated by the need to control algal populations, which may be accomplished without any knowledge of the initially limiting nutrient. At the same time, interest in the identity of limiting nutrients in natural or perturbed systems has remained in the literature. Unfortunately, the phosphorus control paradigm for phytoplankton biomass and the eutrophication management principle based on phosphorus control often have been confused.

3. Flaws and Limitations in Evidence Supporting the Phosphorus Control Paradigm

The phosphorus control paradigm is supported by a cluster of mutually consistent types of evidence, including stoichiometric principles, statistical relationships based on sampling of lakes, and experiments. Use of this evidence contains a substantial number of flaws, most of which are related to interpretation or development of the evidence rather than the evidence itself.

3.1. The Phosphorus – Chlorophyll Relationship

The double logarithmic empirical relationship between phosphorus and chlorophyll *a* in lakes almost always appears when the subject of nutrient limitation is being presented for lakes. Use of this relationship is so commonplace that unwary readers may see in it substantial evidence for control of chlorophyll by phosphorus.

Phosphorus and chlorophyll both are essential components of phytoplankton biomass. Therefore, measurements of phosphorus and chlorophyll that are taken in a lake over the same span of time (*e.g.*, the growing season) are not independent variables; there must always be a correlation between the two variables, although the strength of the correlation will weaken if concentrations of phosphorus far exceed the need of phytoplankton for phosphorus. The inevitability of the relationship between phosphorus and chlorophyll *a* can be illustrated by use of a simulation, as shown in Figure 1. In this case, phytoplankton (seasonal mean) are assumed to have a cell quota (q_o) for particulate phosphorus equal to the amount of chlorophyll (the ratio varies; for present the ratio is set to $\mu = 1$; $\sigma = 30\%$, normal distribution), and to be capable of luxury consumption producing a cellular phosphorus concentration (q) up to a maximum of $18q_o$ (reviewed by REYNOLDS, 2006). All of the lakes
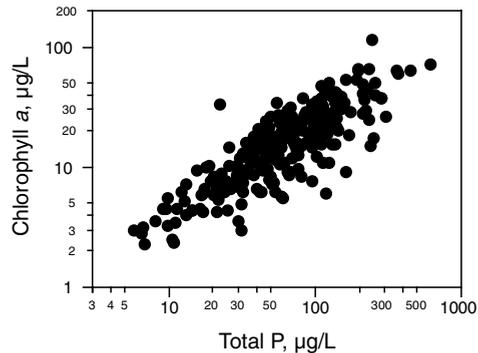
Figure 1.   Simulation of the relationship between P and chlorophyll *a* among a hypothetical population
of lakes (seasonal averages) when P is not limiting (see text; $r^2 = 0.70$).

in the simulated hypothetical population are phosphorus sufficient, in that all phytoplankton populations have phosphorus concentrations equal to q_o plus some amount $q - q_o$ that varies between 0 and $17q_o$. Chlorophyll *a* is a random variable with lognormal distribution across lakes (seasonal mean) $\mu = 18.4$ µg/L, $\sigma = 14.8$ µg/L. In this simulation, μ and σ were set at values that represent a full range of trophic categories.

The gap between q_o and q is treated as a random variable with a lognormal distribution; $\mu = 2.4$, $\sigma = 2.1$ for seasonal averages across lakes. P that is not incorporated into biomass is assumed to equal P in biomass (ratio $\mu = 1$, with $\sigma = 30\%$, normal distribution). As shown by Figure 1, the bivariate distribution of chlorophyll *a* and total P under the conditions of simulation shows a pattern that is characteristic of log-log plots for field data based on total phosphorus and chlorophyll *a* in lakes. Because the two variables are not independent, probability statements regarding their joint distribution are not indicators of cause and effect. The graph only shows that chlorophyll cannot be present in the absence of organically bound phosphorus, *i.e.*, the correlation of chlorophyll *a* with P is a tautology.

DILLON and RIGLER (1974) recognized but did not completely avoid the problem of tautology in comparing phosphorus to chlorophyll *a*. They specified that phosphorus concentration must be measured in the spring, prior to the development of chlorophyll, from which they predicted mean chlorophyll for the growing season. In this case, the two variables are independent, but the outcome (uptake of P simultaneous with increase in biomass) is inevitable and therefore not indicative of nutrient limitation. The purpose of DILLON and RIGLER, however, was to produce a prediction to be used in management rather than to diagnose the cause of growth limitation, and for that purpose the tautological nature of the relationship could be considered admissible. The OECD log-log relationship followed the same pattern, except that the restriction on use of spring concentrations was dropped when it was determined that spring concentrations of total P did not differ much from growing season concentrations of total P in a given lake. The purpose of the OECD analysis, as in the case of the DILLON and RIGLER study, was not to diagnose the mechanism of limitation, but rather to find the most powerful predictor of chlorophyll development. The relationship does not diagnose the nutrient limitation of phytoplankton growth; it only shows what is self evident, *i.e.*, that P has the potential to be limiting, either naturally or by restriction of supply.

A second serious weakness in the quantitative relationship of phosphorus to chlorophyll is its enormous variation, even within a relatively homogeneous set of lakes such as the ones studied by DILLON and RIGLER (Fig. 2). The goodness of fit for the empirical relationship between P and chlorophyll *a* is due entirely to its presentation in double logarithmic form;
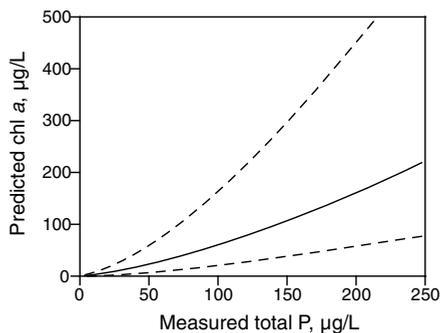
Figure 2.   Mean and 95% confidence limits for chlorophyll *a* as predicted from total P for the analysis by DILLON and RIGLER (1974).

in fact, predictions from the relationship are good only within about an order of magnitude for any given lake.

3.2. Uncritical Use of Stoichiometry

HUTCHINSON's (1957) succinct formulation of the phosphorus control paradigm was firmly grounded in stoichiometry, but the application of stoichiometry to N and P in lakes is very difficult and still mostly unresolved. HUTCHINSON's stoichiometric argument was extended by the publication of tables showing the relative availability of elements in world rivers as a ratio to the composition of algal biomass (VALLENTYNE, 1974, often reprinted in textbooks). The amount of an element in rivers, however, does not reflect its biotic availability to lacustrine phytoplankton. A relevant parallel example is iron, which shows a tremendously favorable ratio of biomass to source water (18:1 as compared with 2400:1 for P), but is nearly insoluble in water, and consequently is potentially a limiting or colimiting element for phytoplankton in marine waters (GREGG *et al.*, 2003) or lakes (NORTH *et al.*, 2007). Thus, ratios may be an interesting basis for a hypothesis, but are not a secure basis for judging the availability of elements for uptake by autotrophs.

The key defect in stoichiometric reasoning involving phosphorus and nitrogen derives from uncertainty about the differential availability of nitrogen and phosphorus fractions in lake water. The early emphasis on N:P ratios involved "assimilable" fractions, but this approach foundered on uncertainties about what is assimilable. In experiments, nutrients can be added in assimilable form, which insures their availability. On the other hand, in the statistical evaluation of N or P among lakes, total N and total P have typically been used in forecasting limitation without any consideration of availability.

Even if phosphorus in a lake is sampled prior to the growing season, at which time algal phosphorus may be only a small proportion of total phosphorus, interpretations of its availability are difficult. Soluble reactive P (SRP), which includes inorganic soluble P plus a fraction of small phosphorus-containing molecules, is most readily available, and shows extremely high rates of turnover (LEAN, 1973). Soluble organic phosphorus is not so readily available as SRP, but can become available through the synthesis of external phosphatases by phytoplankton cells when they experience P deficiency (HEALEY, 1973; RHEE, 1973). Particulate phosphorus that is not part of living biomass spans a tremendous range from potentially available (adsorbed phosphorus on clay or silt) to essentially unavailable (metallic precipitates, hydroxides, and complexes). Furthermore, the fractionation of phosphorus

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can be expected to change continuously in the mixed layer of a lake, and can be influenced by phosphorus entering the epilimnion from runoff, release of P from epilimnetic sediments, and exchange between the mixed layer and deeper waters. Thus, quantification of the availability of phosphorus in a lake is infeasible at present. While there has been some use of total soluble phosphorus as a direct indicator of availability (MORRIS and LEWIS, 1988), most statistical work on the importance of phosphorus has been based on total P.

Nitrogen presents problems similar to those of phosphorus. Nitrate and ammonium are highly available, and commonly are scrubbed from the mixed layer by algae and bacteria, as is SRP. Organic nitrogen compounds have a range of availabilities, as in the case of phosphorus. Urea, for example, is highly available (*e.g.*, BERMAN and CHAVA, 1999), as are some DON species previously thought to be unavailable (BRONK *et al.*, 2006), but nitrogen in large molecules may be weakly available or unavailable. Particulate nitrogen is likely to be directly available only to taxa capable of phagotrophy, but could become available by way of microbial degradation.

The tighter log-log relationship between chlorophyll and total P as contrasted with chlorophyll and total N seems to support the idea that phosphorus controls chlorophyll *a*, whereas N typically does not. The weaker correlation for N may be explained, however, by a greater proportion of refractory N than refractory P for inland waters. Lakes commonly show virtual elimination of dissolved inorganic species of N and P from the mixed layer of unpolluted or mildly polluted lakes. Despite the strong demand for inorganic fractions of both N and P, however, particulate N and P tied up in phytoplankton biomass consistently coexists with significant amounts of both N and P in dissolved organic forms (WETZEL, 2001).

The present working hypothesis for P is that the dissolved pool consists of a broad mixture of chemical species having very different turnover rates based on their direct or indirect availability for phytoplankton uptake (LEAN, 1973). Thus, differential phytoplankton uptake of varied species of P maintains a dynamic equilibrium among DOP compounds.

DON also consists of a mixture of readily assimilated chemical species and more refractory chemical species (STEPANAUSKAS *et al.*, 1999; KAUSHAL and LEWIS, 2005). Higher analytical variability for N analyses may contribute to lower correlation between N and chlorophyll as compared with P and chlorophyll (D. W. SCHINDLER, pers. comm., 2008), although the differences between N and P may be expected for other reasons. An important difference between N and P is the ratio of the dissolved organic pool to the particulate pool, most of which is accounted for by phytoplankton. For phosphorus in waters that are unpolluted or mildly polluted, the ratio of dissolved organic P to particulate P is approximately 1 : 1 (WETZEL, 2001) or even less (VADSTEIN *et al.*, 1993), whereas for nitrogen the ratio can be as high as 10 : 1 (WETZEL, 2001). Thus, a higher fraction of the DON pool apparently is unavailable or weakly available at any given time.

The effect of a contrast in fractionation for N and P can be illustrated by simulation. The simulation shows the effect of differences in the ratio of unassimilable or weakly assimilable nutrient to the total nutrient pool for the same chemical species. For the simulation, chlorophyll *a* across a group of lakes (growing season mean) is assumed to have a lognormal distribution with $\mu = 18.4$ μg/L and $\sigma = 14.8$ μg/L (as in simulation 1, Fig. 3). Particulate phosphorus is set equal to chlorophyll *a* (ratio = 1) but with random variation ($\mu = 1$, $\sigma = 30\%$, normal distribution). Dissolved organic phosphorus is set equal to particulate phosphorus, also with $\sigma = 30\%$ and a normal distribution. Total phosphorus is the sum of dissolved and particulate phosphorus. Particulate nitrogen is set to 16 times (molar) particulate phosphorus (Redfield ratio), implying no overall bias in limitation between phosphorus and nitrogen (and no differential luxury uptake), but with variance ($\sigma = 30\%$, normal distribution). Mean assimilable dissolved nitrogen is set to 2 times particulate nitrogen with $\sigma = 30\%$ and a normal distribution. An additional component of dissolved organic nitrogen is assumed to be refractory, and is set to $\mu = 200$ μg/L, $\sigma = 150$ μg/L, lognormal distribution,
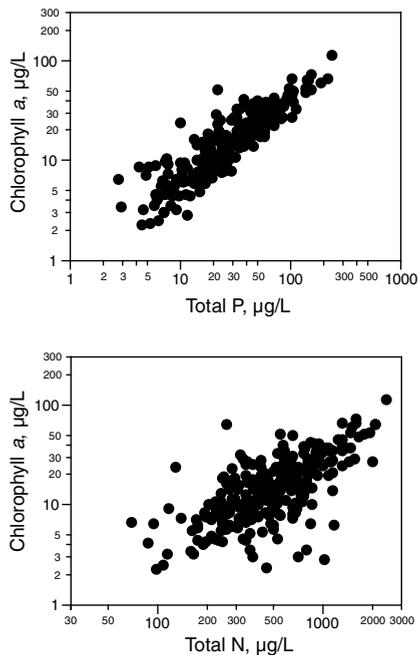
Figure 3.   Simulation of chlorophyll *a* in relation to total P ($r^2 = 0.75$) or N ($r^2 = 0.45$) when nitrogen and phosphorus are balanced according to demand (particulate N:P set to the Redfield ratio), and assuming that N has a higher dissolved: particulate ratio than P.

based on characteristic N composition of humic and fulvic acids, from THURMAN (1985), and the assumption that nonhumic refractory N is about as abundant as humic refractory N.

Figure 3 shows the expected effect of a substantial and variable component in the DON pool that is unavailable or weakly available to phytoplankton. Thus, if the assumptions underlying the simulation are reasonable, one would expect a looser correlation between total N and chlorophyll *a* than between total P and chlorophyll *a*, even when both have equal degree of control on biomass (*i.e.*, N:P of biomass set at the Redfield ratio).

3.3. Experiments: Categories of Evidence

Bioassay incubation is the key experiment for diagnosing nutrient limitation at a point in time. For small enclosures, long incubations (> 5 days) produce bottle effects, including changes in community composition of phytoplankton or growth of grazers, and may reveal only potential limitation rather than actual limitation. Very short experiments (<12 hours), which have been conducted with ^{14}C, can produce misleading results because the addition of nutrients may suppress algal growth temporarily (LEAN and PICK, 1981). For enclosures of small to intermediate size, incubation must extend beyond the interval of early response to the addition of nutrients but not last beyond the duration of a few cell divisions, *i.e.*, 2–5 days.

Mesocosms such as those used by SCHINDLER *et al.*, (1971), LEVINE and SCHINDLER (1992), as well as LUND (1975) and many others seem to provide more realism than chambers, but can produce anomalies. For example, the 1 m diameter mesocosms used by SCHINDLER *et al.*

(1971) produced contradictory signals about nutrient limitation, in that P alone showed very little influence on peak biomass, whereas P combined with C produced the highest biomass, even though it is clear from other work by SCHINDLER and colleagues that carbon in the open water of lakes cannot limit biomass accumulation except over very short intervals.

Manipulation of entire lakes is the most realistic measure of ultimate biomass production but experiments of shorter duration most clearly show the time course of differing limitation mechanisms. Also, replication often is weak with whole-lake manipulations.

3.4. Experimental Evidence: The Canadian Experimental Lakes Area

The early ELA experiments strongly supported the hypothesis that N and P in combination greatly stimulate algal biomass, and that N limitation can be induced by forcing a low N : P ratio. These points were important for management, but did not make a convincing case for P limitation in the ELA lakes.

HEALEY and HENDZEL (1980) evaluated physiological indicators of nutrient limitation for ELA lakes and other Canadian lakes. P was consistently indicated as limiting in the ELA lakes, but most of the lakes that were included in the study had been experimentally manipulated with nutrient additions.

In 1979, FEE published an analysis of relationships between lake morphometry and relative importance of nutrient recycling between epilimnetic sediments and the mixed layer during the growing season. FEE included a table of the results for ELA whole-lake nutrient enrichment studies, as summarized in Figure 4. FEE postulated that both primary production and chlorophyll are affected by the ratio of epilimnetic sediment exposure to epilimnetic volume (A_e/V_e) among unfertilized ELA lakes. If so, A_e/V_e would provide a prediction of expected production and chlorophyll to be used in quantifying degree of departure from expectations in response to experimental enrichment. He demonstrated the relationship of A_e/V_e to production, but found that A_e/V_e and chlorophyll were only weakly related (not significant, but reanalysis with logarithmic transformation shows a significant relationship: W. WURTSBAUGH, unpublished). In his analyses, FEE noted that the ELA experiments support a case for near equality in the importance of N and P among unfertilized ELA lakes, in apparent contradiction to SCHINDLER's evaluation of the same data (SCHINDLER, 1980).

The composite of ELA lake fertilization results indicates that addition of P alone did not markedly increase phytoplankton biomass (Fig. 4), nor did it stimulate the growth of nitrogen-fixing planktonic cyanobacteria, which could have potentially offset a deficiency of N. The results are uniformly consistent with SCHINDLER's observation that enrichment of ELA lakes with significant amounts of phosphorus plus nitrogen increased phytoplankton biomass by a large amount (5–10×). Figure 4 is not consistent, however, with phosphorus limitation of phytoplankton growth in ELA lakes. The results suggest that nitrogen had at least an equal stimulating effect on phytoplankton biomass and therefore may have been a dominant or codominant limiting nutrient in unfertilized ELA lakes.

LEVINE and SCHINDLER (1992) conducted experiments on mesocosms in a lake that had previous large additions of nitrogen in order to determine the degree of compensatory nutrient response in enclosed water columns of ELA lakes with and without contact between water and sediment. They found that compensatory mechanisms for nitrogen were stronger than those for phosphorus in the mesocosms, but the potential influence of the prior N addition was not addressed. Their results were taken as evidence for the dominance of phosphorus control on phytoplankton biomass. These findings were contrary, however, to the results of the whole-lake studies (Fig. 4), which showed that compensation (as indicated by accumulation of biomass above the baseline) for deficient phosphorus is at least as strong as compensation for deficient nitrogen. Of course it is possible that lakes as a whole have compensatory mechanisms that cannot be demonstrated by the use of mesocosms.
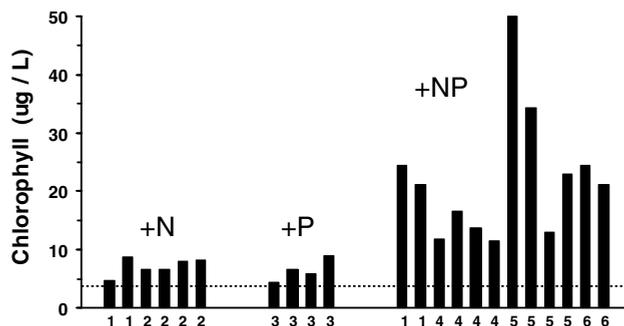
Figure 4.   Graphical summary of the results of lake fertilization experiments at ELA from 1973-1976 (mean chlorophyll *a* during ice-free seasons), as reported by FEE (1979, Table 1). The dashed line shows annual mean chlorophyll *a* in unfertilized ELA lakes (from FEE, 1979: 10 lakes, 22 lake years). The numbers below the histograms designate lakes. 1-Lake 304; 2-South basin of divided Lake 226 (data shown here are not affected by a curtain breech that occurred in 1977); 3-Lake 261 (Lake 261 was terminated prematurely and also had an unusual macrophyte flora: D.W. SCHINDLER pers. comm. 2008); 4-North basin of divided Lake 226; 5-Lake 227; 6-Lake 303. All of the lakes were analyzed for 2 or more years. The data from Lake 226N (#4) are frequently cited as evidence for phosphorus limitation in lakes.

3.5. Experiments Beyond the Experimental Lakes Area

Intense interest in N and P limitation, stimulated to a large degree by the ELA studies, led to a geographically broad proliferation of experiments. By short and large, these studies consisted of enrichment of phytoplankton in bottles or small chambers over relatively short durations and involving enrichment with N, P, N + P, and sometimes micronutrients. In addition, whole-lake fertilization studies were done according to the ELA pattern at a number of locations.

ELSER *et al.* (1990) reviewed the accumulated experimental literature on N and P limitation through the 1980's. Based on enclosure experiments for 60 lakes, they showed that a growth response (increase in chlorophyll *a* or increase in carbon fixation) is equally likely for P enrichment or N enrichment, contrary to the prevailing notion that P is much more likely to stimulate growth response than N. They also showed that fertilization with P + N was more likely to produce a response than fertilization with either P or N alone, and that the P + N response was quantitatively stronger (about 2.5×). They postulated that the degree of response often is very similar for N and P at a specific time and place.

In surveying 80 lake years of experimentation on whole lakes, ELSER *et al.* (1990) concluded that experiments almost always involved addition of N + P for comparison with a control, rather than experimentation with P and N separately. Only 12 lakes had been fertilized with P and N separately, and only two showed a significant response (both to P). The separate treatment of P and N at ELA, as shown in Figure 4, was not included in the survey by ELSER *et al.* (1990).

ELSER *et al.* (1990) also offered an explanation for the apparent contradiction between bioassays on ambient phytoplankton communities and the U.S. Environmental Protection Agency's test for algal growth potential (MILLER *et al.*, 1978), which involves addition of P, N, and N + P to filtered lake water used for growing cultures of *Selenastrum capicornutum*. Such bioassays show a strong trend toward phosphorus limitation, in contrast to studies of field populations of phytoplankton. The *Selenastrum* bioassay is not a reliable indicator of the status of field populations. As shown much earlier (HUTCHINSON, 1957), laboratory algal

populations differ from field populations in their nutrient requirements. Furthermore, as proposed by ELSER *et al.* (1990), field populations may consist of a balance of species that makes efficient use of both N and P. This concept is supported by experimental evidence that algal taxa differ in optimal TN : TP ratios (SMITH, 1982; SUTTLE and HARRISON, 1988).

DOWNING and MCCAULEY (1992) tabulated information on TN : TP ratios from multiple sources ranging from forests and croplands to sewage. They argued that TN : TP ratios of the predominant sources of N and P in a given watershed will determine the TN : TP ratio in lakes within that watershed, and provided confirmatory statistical evidence from the literature. From a list of nutrient enrichment experiments over a wide range of TN : TP ratios, DOWNING and MCCAULEY also determined that TN : TP molar ratios below 30 are consistently indicative of N limitation according to available experimental data. The presumption of a limiting TN : TP ratio in earlier work had been considerably lower (*e.g.*, 12). Finally, DOWNING and MCCAULEY showed that increasing TP is associated with a declining TN : TP ratio, probably because watershed sources providing the highest TP concentrations tend to have low TN : TP ratios.

Oligotrophic lakes have been of special interest in the diagnosis of nutrient limitation because their TN : TP ratios are higher than ratios for polluted lakes. Experimental evidence has been inconsistent with this general conclusion. For example, MORRIS and LEWIS (1988) conducted repeated bioassays on eight montane lakes in Colorado. The results showed a close balance between N and P limitation in these lakes. Five categories of phytoplankton limitation were identified: no limitation, N limitation, P limitation, concurrent limitation (stimulation only by simultaneous additions of N and P), and reciprocal limitation (stimulation by addition of either N or P). The phytoplankton communities of three lakes were primarily N-limited, one was primarily P-limited, and four showed combined limitation (concurrent or reciprocal). Switching between categories of limitation was also observed within lakes, as it has been in other lakes (*e.g.*, EDMONDSON, 1972; WHITE *et al.*, 1977; 1985, LEWIS *et al.*, 1984; SOMMER, 1987; KÖHLER *et al.*, 2005). Nitrogen, either alone or in combination with P, accounted for 79% of all observed instances of limitation. Nine indices were tested for effectiveness in predicting phytoplankton limitation by N and P. The best chemical indices for discriminating all limitations were ratios of dissolved inorganic N : total P (84% accuracy) and dissolved inorganic N : total dissolved P (80% accuracy). The effectiveness of these indices may be explained by the degree to which they represent N and P fractions that are available to the phytoplankton. The observed parity of N with P limitation is consistent with other studies of oligotrophic lakes (*e.g.*, FEE, 1979; DODDS *et al.*, 1989), while the seasonal alternation of limiting nutrients as well as concurrent and reciprocal limitation are consistent with the concept that limitation by nitrogen and phosphorus often is closely balanced, especially in watersheds that are not strongly influenced by land use or nutrient disposal. High frequency of phytoplankton limitation by nitrogen in montane lakes also has been documented at other locations (DODDS *et al.*, 1989; LAFRANCOIS *et al.*, 2003; MCMASTER and SCHINDLER, 2005: WURTSBAUGH *et al.*, 1997; also see Fig. 5). Thus, while sparsely populated montane environments seemed the most likely prospect for predominance of P limitation, experimental evidence to date suggests that N and P are near parity in these environments. BERGSTRÖM and JANSSON (2006) have argued that significant atmospheric N pollution is reaching a large proportion of oligotrophic lakes (see also STODDARD, 1994). If many of these lakes were at one time N-limited, as seems likely now, some degree of change already may have resulted from modest N enrichment.

The range of algal responses to nutrient additions is shown in a compendium of experiments by WURTSBAUGH (Fig. 5). These experiments, conducted largely in relatively uncontaminated watersheds, demonstrate that nitrogen stimulated accumulation of chlorophyll more frequently than did phosphorus but, as expected, the addition of N and P together almost always provided the strongest stimulation. The frequent but small inhibition of chlorophyll concentrations by phosphorus additions in these experiments could be explained by
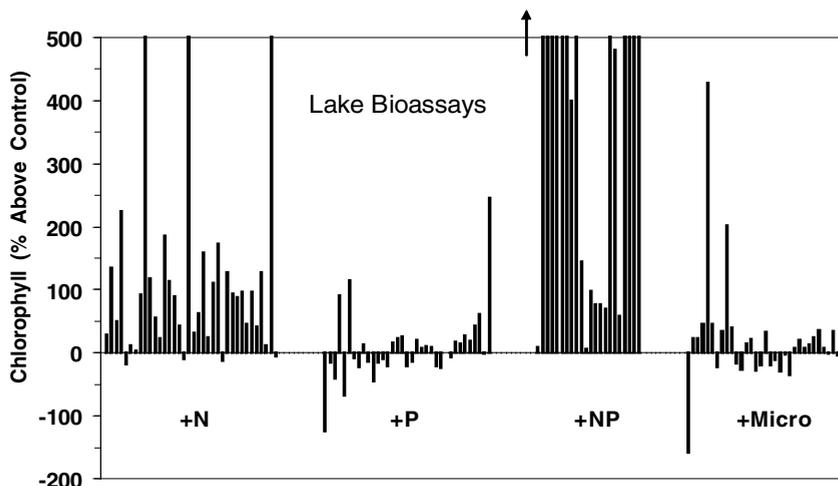
Figure 5. An example of geographically distributed laboratory and field bioassays for phytoplankton responses to nutrients in lake waters from western North America, Peru, and Spain. In these experiments, the laboratory incubations in flasks or field assays in 5–10 L mesocosms typically lasted 5–7 days. The micronutrient (+Micro) additions frequently tested iron limitation but mixed micronutrients were sometimes utilized. (Sources: WURTSBAUGH, 1988; WURTSBAUGH and HORNE, 1983; WURTS-BAUGH et al., 1985, 1997; CAMACHO et al., 2003; MARCARELLI et al., 2006 and some unpublished data of W. WURTSBAUGH).

the response of heterotrophic bacteria to phosphorus, and subsequent competition between the bacteria and phytoplankton for nitrogen or increases in phagotrophic predators on phytoplankton and bacteria (THINGSTAD et al., 2005). Micronutrient additions were as likely as P to stimulate chlorophyll accumulation.

3.6. Nitrogen Deficiencies and N Fixation Across Ecosystems

In lakes, the co-limitation of phytoplankton production by N and P may resemble limitations in terrestrial and marine systems, where production is not limited by a single nutrient across broad temporal and geographical ranges (ELSER et al., 2007). HEDIN et al. (2003), for example, showed that the nitrogen and phosphorus content of soils, which controls export of N and P to aquatic ecosystems, can show an increasing N : P ratio with age. Disturbances such as fires also can reset the N and P balance in forests by volatilizing accumulations of nitrogen in forest biomass and soils (LEWIS, 1974; MURPHY et al., 2006), so that some ecosystems prone to fires may not export excess nitrate to streams and lakes.

Terrestrial ecosystems typically are unable to ameliorate nitrogen deficiencies fully through nitrogen fixation (VITOUSEK et al., 2002). Similarly, there presently is no evidence for a consistently significant contribution of planktonic nitrogen fixation to oligotrophic and mesotrophic lakes. For eutrophic lakes showing N fixation in the plankton, the median contribution to total load that could be attributed to N fixation is near 22%, and the median fixation as a proportion of the total N necessary to support primary production is less than 5%, according to the data compiled by HOWARTH et al. (1988). Therefore, although HOW-ARTH et al. concluded that N-fixation offsets P deficiency, it seems clear that the potential offset is modest, in that N deficiency is likely to persist in most cases that lead to predomi-

nance of N fixers. For example, if a hypothetical lake had an N : P molar ratio of 10 : 1, implying nitrogen deficiency, a 25% boost in the N component would raise the ratio only to 12.5 : 1, which is unlikely to bring the lake into phosphorus deficiency. The reasons for the low compensatory potential of phytoplankton nitrogen fixers when phosphorus is abundant are unclear, but micronutrient limitation (WURTSBAUGH and HORNE, 1983), light limitation (LEWIS and LEVINE, 1984; VINER, 1985), and limitation related to the maximum sustainable ratio of heterocysts to vegetative cells (LEWIS and LEVINE, 1984) have been suggested. Nitrogen fixation by lake and stream periphyton appears to be less constrained than is fixation by phytoplankton (BERGMANN and WELCH, 1990; LEVINE and SCHINDLER, 1992; MARCARELLI et al., 2008), but the underlying reasons are not well understood.

4. The Emerging Paradigm for N and P Limitation in Lakes

Experimental information on nutrient limitation of phytoplankton now shows that phytoplankton biomass accumulation is as likely to be limited by N as P. Even in unpolluted oligotrophic lakes instances of N and P limitation appear to be equally likely. The hypothesis that N and P enrichment together provide a stronger growth response in phytoplankton than either P or N alone is consistently confirmed, but the hypothesis that phosphorus limitation is consistently offset by N-fixation through cyanobacteria is not valid as a generalization. Although N deficiency in fertile waters often shifts community dominance to N fixers, this shift does not occur in most N-limited lakes of low to moderate fertility. Where it does occur, lakes typically remain N limited despite the presence of fixers.

As recognized early in the study of nutrient limitation, enrichment of lakes with strongly biased N : P ratios can induce either P or N limitation. In particular, N limitation is induced by substantial amounts of treated domestic wastewater or animal waste, which have low N : P ratios and high concentrations of P. Similarly, some lakes in industrialized Europe and North America may have been driven to P limitation by atmospheric deposition of nitrogen and the subsequent N-saturation of watersheds (STODDARD, 1994; BERGSTRÖM and JANSSON, 2006). In addition, some instances are known of high ambient P concentrations that can be traced to geologic sources. In these instances, N limitation is very likely (e.g., WURTSBAUGH, 1988; JAMES et al., 2003; BUNTING et al., 2005; NRC 2004; LEWIS et al., 2008). N limitation of phytoplankton also may be especially important in tropical or subtropical waters (THORNTON, 1987; LEWIS, 1996) and in saline lakes, possibly because evaporation magnifies P concentrations (TALLING, 1992) or because high sulfate concentrations enhance phosphate release from sediments (BLOMQVIST et al., 2004), while denitrification is enhanced by warmth (DOWNING and MCCAULEY, 1992; LEWIS, 2002).

Evidence of parity between N and P in the limitation of phytoplankton growth across inland waters as a whole supports a new N + P control paradigm for nutrient limitation that is much more consistent with the accumulated data than the original P control paradigm. Table 2 sets forth the components of the emerging N + P control paradigm for limitation of phytoplankton growth in inland waters.

The new paradigm is essential for an accurate depiction of the manner in which phytoplankton growth is limited in natural lakes and in lakes that are selectively enriched with biased N : P ratios. Even so, the key management principle for control of eutrophication in individual lakes remains the same as it was under the phosphorus paradigm: the most promising management tool for control of phytoplankton growth in most situations is restriction of phosphorus supply. It is also true in some instances, including principally those involving high background P concentrations, that suppression of total phosphorus load by manipulation of anthropogenic sources cannot suppress phytoplankton growth because the background phosphorus load is capable of saturating the P demand of an N-limited phytoplankton community (LEWIS et al., 2008), or because internal loading of P offsets nutrient

Table 2.   Components of the emerging N+P control paradigm.

N + P Phytoplankton Control Paradigm for Lakes

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1. N and P are equally likely to explain nutrient limitation of phytoplankton in unpolluted lakes.

2. Temporal shifts in N and P limitation often occur, and concurrent or reciprocal limitation by N and P is common, especially among oligotrophic lakes.

3. Near parity between N and P control in many unpolluted or moderately polluted lakes influences development of phytoplankton communities with complementary N and P requirements, which explains concurrent and reciprocal limitation by N and P, as well as seasonal shifts in limitation.

4. Low N:P ratios combined with high P concentration often will shift phytoplankton composition to dominance by cyanobacterial N fixers.

5. Cyanobacterial N fixers do not fully offset N deficiency.

6. Significant anthropogenic bias in watershed sources of N and P can shift a lake toward N or P limitation.

7. Whether N or P limits biomass accumulation, restoration of individual polluted lakes is generally most feasible by restriction of P supply, which either tightens existing P limitation or induces P limitation in an N-limited lake. Interception of anthropogenic P may not be sufficient to induce P control of phytoplankton for lakes with naturally high P supplies or strong internal loading of P. Where atmospheric deposition of nitrogen increases the N : P ratios of large numbers of naturally N-limited lakes, reduction of N loading may be the only possible means of reversing changes in primary production or algal composition.

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control (KALFF, 2002). Thus, the feasibility of inducing P deficiency must be evaluated on a case-by-case basis. Also, the occurrence of widespread N enrichment of lakes through atmospheric transport of anthropogenic fixed N cannot be offset by P restriction, as it involves entire landscapes rather than individual lakes. Prevention of broadly distributed changes in lakes by N enrichment can only be achieved by suppression of the atmospheric source of fixed nitrogen.

There remain many issues to be studied as the new paradigm solidifies. Perhaps most interesting is the tendency of oligotrophic lakes in montane environments with low atmospheric nitrogen contamination to show high probability of N limitation for phytoplankton growth. Another is the common failure of heterocystous cyanobacteria to take full advantage of excess phosphorus in many cases, including lakes of high productivity but consistent N limitation and weak or erratic growth of heterocystous cyanobacteria (LEWIS et al., 2008). In addition, the question of micronutrient limitation remains unresolved. The most parsimonious explanation for nutrient limitation at present is achieved without any reference to micronutrient limitation. It is possible, however, that micronutrient limitation comes into play through the unequal requirements of individual species for specific micronutrients, which might explain small or sometimes substantial response of phytoplankton communities to micronutrient enrichment (Fig. 5). Nutrient limitation of periphyton in lakes has not been thoroughly studied; periphyton may influence nutrient availability to phytoplankton in small lakes (LEVINE and SCHINDLER, 1992; AXLER and REUTER, 1996). Finally, there is much yet to be known about the ability of natural phytoplankton communities to conform in species composition to specific N : P ratios, as foreseen by PEARSALL in the 1930s.

Continuing global mobilization of nitrogen and phosphorus by anthropogenic activities (VITOUSEK et al., 1997) and increasing demands on freshwater resources are causing changes in the balance of limiting nutrients in the world's lakes. Knowledge of the effects of N : P ratios on algal growth, species composition, and nitrogen fixation will be required for effective management of inland waters.

5. Acknowledgements

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