Rationale for Control of Anthropogenic Nitrogen and Phosphorus to Reduce Eutrophication of Inland Waters

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ABSTRACT: Concentrations of phosphorus and nitrogen in surface waters are being regulated in the United States and European Union. Human activity has raised the concentrations of these nutrients, leading to eutrophication of inland waters, which causes nuisance growth of algae and other aquatic plants. Control of phosphorus often has had the highest priority because of its presumed leading role in limiting development of aquatic plant biomass. Experimental evidence shows, however, that nitrogen is equally likely to limit growth of algae and aquatic plants in inland waters, and that additions of both nutrients cause substantially more algal growth than either added alone. A dual control strategy for N and P will reduce transport of anthropogenic nitrogen through drainage networks to aquatic ecosystems that may be nitrogen limited. Control of total phosphorus in effluents is feasible and is increasingly being required by regulations. The control strategy for nitrogen in effluents is more difficult, but could be made more feasible by recognition that a substantial portion of dissolved organic nitrogen is not bioavailable; regulation should focus on bioavailable N (nitrate, ammonium, and some dissolved organic nitrogen) rather than total N. Regulation of both N and P also is essential for nonpoint sources.

INTRODUCTION

The United States and European Union are simultaneously moving toward nutrient regulation for inland waters with the goal of controlling eutrophication. The primary symptom of eutrophication is excessive growth of aquatic autotrophs, including suspended algae (phytoplankton), attached algae (periphyton), and aquatic vascular plants (macrophytes). Secondary symptoms include deep water anoxia in lakes, increased risk of harmful algal blooms, impairment of water treatment (taste and odor, filtration problems), and changes in the composition of aquatic communities. Nutrient pollution has raised global algal biomass and photosynthesis in lakes by approximately 60% over background conditions; streams and rivers are similarly affected. Within populated or agriculturally productive regions aquatic primary production and biomass often are many times greater than background. Two elements, phosphorus (P) and nitrogen (N), explain most of the experimentally diagnosed nutrient limitation of algal growth in inland waters under natural or human-modified conditions. Some research also suggests the potential for deficiencies of other elements such as iron in inland waters, but this type of limitation is likely confined to special situations.

Although the scientific basis of nutrient regulation seemingly was settled in the 1970s with emphasis on phosphorus control, strong controversy now has emerged about the alternative possibilities for controlling one nutrient preferentially (P) or two nutrients with equal emphasis (P, N). We provide here a perspective on nutrient control as it applies to algae, first for lakes and then for flowing waters.

Regulation of total P concentrations is a well established practice. Regulation of nitrogen for control of eutrophication has been a lower priority, but has developed in a few places by control of total nitrogen concentrations (e.g., New Zealand). National and international organizations (U.S. Environmental Protection Agency, Organisation for Economic Co-operation and Development)
recognize the significance of both elements, but current regulatory practice emphasizes phosphorus control. We describe lines of evidence showing that nutrient control based on both P and N offers a broader range of strategies and reduces the potential for corollary damage caused by anthropogenic mobilization of N.

I COMPARISONS OF PHOSPHORUS AND NITROGEN AS LIMITING NUTRIENTS IN LAKES

The limiting nutrient concept (Liebig’s Law of the Minimum$^{9,10}$) holds that nutrient deficiency at any given time in a photosynthetic organism can be traced to a single element, which is the element available in the least amount relative to the needs of the organism. Therefore, in controlling excessive algal growth, it is important to know which element limits the expansion of algal populations when their growth stops because of nutrient depletion.

The limiting nutrient concept is more complex for an entire community or ecosystem than it is for a single organism. For example, species may differ, even among organisms of similar type (e.g., algae), in their optimal internal N: P ratios$^{11–13}$ and their ability to store critical nutrients or to take up a nutrient at low concentrations.$^{14,15}$ Thus, it is possible in a mixed community of algae for some species to be limited by phosphorus and others to be limited by nitrogen. In addition, it is possible for an environment to be very near the nutrient limitation thresholds for N and P simultaneously. Thus, a slight enrichment with one element could cause the other element to become limiting (e.g., refs 16–18). A third possibility is that seasonal or spatially heterogeneous changes may occur in the relative availability of potentially limiting nutrients (19). All of these circumstances have been documented experimentally and in nature.$^{20}$

Much more attention has been given to P limitation than to N limitation in inland waters for three reasons:20 (1) phosphorus is more easily removed from anthropogenic sources than nitrogen, (2) N$_2$ fixation by cyanobacteria (also known as blue-green algae) has been assumed to make N control ineffective, and (3) the correlation between chlorophyll (an index of algal abundance) and total P among lakes is stronger than the correlation between chlorophyll and total N.3

A high proportion of total phosphorus can be removed (to concentrations as low as 30 μg/L) from waste streams by flocculation and sedimentation.21 Thus, phosphorus limitation can be induced even in a lake that is nitrogen limited by restricting the phosphorus supply to such an extent that phosphorus limitation overtakes nitrogen limitation.22–23 This is an effective strategy when the main source of phosphorus is wastewater effluent, which can be readily treated. It is less feasible where diffuse (non-point) sources are important, and may be entirely infeasible where background phosphorus concentrations are high.24–27

Nutrient enrichment experiments (bottle bioassays, mesocosms, whole lakes) for lakes from all parts of the world now show that nitrogen limitation is globally as common as phosphorus limitation (Figure 1, refs 28,18, and 20). The occurrence of nitrogen limitation in lakes generally raises questions about the presumption that nitrogen limitation is self-correcting through the growth of N$_2$-fixing cyanobacteria.29 Studies of the nitrogen fixation rates for cyanobacteria show that they are unable to compensate fully for nitrogen limitation in lakes,30,31 most likely because the process of N$_2$ fixation can be influenced by factors other than nitrogen and phosphorus, including turbulence coupled with low transparency, trace metal or iron deficiency, or organic matter availability.32 Eutrophic lakes that are nitrogen limited may even be dominated by cyanobacterial taxa that cannot fix N$_2$.33 Another important factor that works against N accumulation in lakes is microbial denitrification that converts nitrate, which is bioavailable, to N$_2$ or N$_2$O which are not. Denitrification is stimulated by nitrate enrichment of lakes.34 Thus, nitrogen fixation and nitrogen limitation can coexist in lakes, and suppression of N availability may suppress total algal biomass even when cyanobacterial N$_2$-fixers are present.

N$_2$ fixers may become a larger portion of the algal community if nitrogen availability is suppressed sufficiently to cause N limitation, even if total biomass is reduced.35 The risk of inducing a shift in community composition favoring N$_2$ fixers is a possible undesirable byproduct of induced nitrogen limitation. Presence of N fixers at moderate abundances is common over a wide trophic range,36 however, and is not exclusively a symptom of impairment.

The correlation between phosphorus and mean or peak chlorophyll among lakes has been erroneously interpreted as showing cause and effect. In fact, the correlation reveals little about nutrient limitation because phosphorus is a mandatory component of algal biomass, as is chlorophyll.20 Therefore, chlorophyll and phosphorus will always be present together (as will all other biomass components), whether phosphorus is limiting or not (Figure 2). Nutrient limitation cannot be inferred from such correlations.

Algae excrete phosphatases at the cell surface and into the surrounding water that allow them to assimilate phosphorus derived from cleavage of phosphorus from organic matter.32–33 Algae also can take up 10 or more times the minimum amount of P needed for synthesis of protoplasm and store the excess P as polyphosphate. Thus, toward the end of the growing season, most of the phosphorus in the upper water column of lakes is incorporated into algal biomass, except in lakes that are so strongly polluted with P as to exceed algal capacity for P uptake.34–35

For nitrogen, a significant portion of the dissolved fraction is refractory (not bioavailable, e.g., ref 39). Dissolved inorganic N (DIN, Table 1) typically is the main N source for algal growth in inland waters, but both unpolluted and polluted inland waters also contain substantial amounts of dissolved organic N (DON). Because DON persists even when phytoplankton show nitrogen stress, as indicated by very low concentrations of DIN, DON had until recently been considered entirely refractory, but experimental evidence now has shown that a significant portion of DON is

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<table>
<thead>
<tr>
<th>DIN (μg/L)</th>
<th>DON (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>400</td>
</tr>
<tr>
<td>100</td>
<td>600</td>
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</table>
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Figure 1. Growth response ratios (natural log of ratio of treatment to control, with standard error) of freshwater phytoplankton for worldwide bioassay studies (redrawn from ref 18; n = >500 for each treatment).
available to algal cells including not only DON from natural sources but also anthropogenic DON such as urea, which is widely used in agriculture. Some algal taxa have exoenzymes (amino acid oxidases, proteolytic enzymes) at the cell surface or excreted from the surface so that ammonium or small organic molecules can be released from large organic molecules and enter the cell; some taxa also are able to take up organic nitrogen by pinocytosis or phagocytosis. In addition, some components of DON are converted to DIN by photodegradation, but other components of DON resist photodegradation. Thus, the persistence of DON in the absence of DIN indicates fractional turnover of the DON pool rather than complete unavailability of DON over time scales ranging from days to months during a growing season.

Natural waters vary greatly in amount of refractory nitrogen in the DON pool. A study of rivers in the eastern U.S. showed two rivers with no detectable bioavailability and seven rivers with a mean of 23% ± 4% bioavailability as determined by change in DON concentrations in six-day incubations; an accompanying literature survey for 18 sites on rivers in Europe and the U.S. showed a mean of 30% ± 4 for the labile fraction as judged mostly by 14 day incubations. Thus, DON of natural waters must be viewed as potentially important nutritionally to algae under nitrogen stress, yet includes a significant refractory component.

Fractions of N and P differ in their potential to predict experimentally diagnosed nutrient limitation in lakes. For phosphorus, total P and total soluble P are equally accurate indicators. For nitrogen, dissolved inorganic nitrogen (almost entirely composed of nitrate plus ammonium) is an indicator superior to total nitrogen or total dissolved nitrogen. This is not surprising, given the unavailability of a substantial portion of DON to algae.

## CONTROL OF N, P, OR BOTH

Sole focus on phosphorus as a means of controlling algal biomass may seem advantageous because it is much less expensive than control of both N and P. Some researchers also continue to argue that nitrogen control does not work because N2 fixation can provide algae with labile nitrogen. According to this argument, lakes that are N deficient will accumulate N over time, thus eventually reaching P limitation. Lake 227 of the Canadian Experimental Lake Area, which offers the longest record of whole lake manipulation, is cited as an example of evolving N sufficiency under P enrichment but a contrary interpretation of the data has been proposed. Multiyear whole lake enrichment experiments with P only document persistence of N limitation in lakes with substantial P and populations of N2 fixers. For example, whole lake fertilization of several Swedish lakes with P only (multiple years), yielded no higher biomass or only slightly higher biomass than was found before fertilization. The same lakes developed biomass 15 - 60 times higher with P + N fertilization (refs 47 and 41 give other examples).

Focus on phosphorus control presumes that phosphorus loading of a lake can be reduced sufficiently to induce and sustain phosphorus control of algae. Where nonpoint phosphorus or background phosphorus sources are strong enough to sustain eutrophic conditions, phosphorus control measures may not provide enough phosphorus recovery to reduce algal biomass. In addition, allowing the balance between nitrogen and phosphorus to be strongly distorted over entire regions by selective control of phosphorus may change the species composition or diversity of aquatic communities which often reflect a close balance between nitrogen and phosphorus availability. Finally, because nitrogen limitation is quite common in fresh waters and even more common in coastal waters and saline lakes, allowing nitrogen to be released indiscriminately from one water body to another through the drainage network could cause widespread stimulation of algal growth by providing nitrogen to algal communities downstream that otherwise would be nitrogen limited. Thus, dual nutrient control has multiple advantages.

## STRATEGIES FOR LIMITING PHOSPHORUS AND NITROGEN IN THE ENVIRONMENT

Use of total P as an index of P availability in lakes is defensible for lakes because most of the phosphorus in the growth zone of lakes is available to algae; it consists of total dissolved P (TDP)

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**Figure 2.** Simulation of the relationship between P and phytoplankton chlorophyll a among a hypothetical population of lakes (seasonal averages) for which P is not limiting ($r^2 = 0.70$, from ref 20).

**Table 1.** Concentrations of Total N and P (μg/L) in a Representative Municipal Effluent with Secondary Treatment Plus 50% Nitrification and in Representative Unpolluted US Streams and Rivers

<table>
<thead>
<tr>
<th>nutrient</th>
<th>effluent</th>
<th>unpolluted streams</th>
</tr>
</thead>
<tbody>
<tr>
<td>range total P, μg/L</td>
<td>2000—4000</td>
<td>10—30</td>
</tr>
<tr>
<td>fractionation, %</td>
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<td>100</td>
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<tr>
<td>total P</td>
<td>96</td>
<td>63</td>
</tr>
<tr>
<td>total dissolved P</td>
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<td>30</td>
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<tr>
<td>dissolved inorganic P</td>
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<td>33</td>
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<tr>
<td>dissolving organic P</td>
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<td>37</td>
</tr>
<tr>
<td>particulate P</td>
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<td>50</td>
</tr>
<tr>
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<td>100</td>
</tr>
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<td>NO3−-N</td>
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</tr>
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<td>dissolved organic N</td>
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<td>50</td>
</tr>
<tr>
<td>particulate N</td>
<td>4</td>
<td>21</td>
</tr>
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</table>

* Unpolluted lakes will show lower DIP, DIN, PP.
with its two components, dissolved inorganic P (DIP, often designated soluble reactive P, SRP) and dissolved organic P (DOP) plus particulate P (PP), which consists mostly of phytoplankton with their internal phosphorus stores. In lakes the particulate fraction of N also consists mainly of phytoplankton, and can be counted as bioavailable, as can DIN and some DON. Thus, the concept of bioavailability suggests that water quality standards for P in lakes can be based on total P, but for N they should be based on total N minus refractory DON. Regulating total N without adjusting for unavailable DON would be equally effective, but would lower the feasibility and raise the cost of N control. For nutrient control we focus here on effluents as nutrient sources because regulation of effluents is feasible through established permitting processes and because the technological basis for regulation nonpoint of sources, which may be dominant nutrient sources in some cases, is weak.

**EFFLUENT REGULATION THAT IS CONSISTENT WITH STANDARDS BASED ON BIOAVAILABILITY**

Point source effluents, which are the main target for discharge permitting, are rich in bioavailable total dissolved P (Table 1). For the dominant treatment technologies (i.e., with the exception of oxidation ponds or ditches), particulate P is not a major concern because of the efficiency of particle removal during treatment. Thus, permits written on the basis of total phosphorus in effluent typically will translate well into a limitation on bioavailable phosphorus in lakes.

For nitrogen, the presence of dissolved organic N in effluent is a complicating factor. DON in municipal effluent is derived partly from the influent waste stream and partly from microbial metabolism that occurs during treatment. Effluents appear to be similar to inland waters and nearshore marine waters in having both refractory and labile components. One study of a domestic treatment effluent from a treatment facility with low nitrogen output attained by combined nitrification and denitrification showed a median labile component near 40% (range, 18–61%) based on 14-day bioassays. Other studies have shown a similar range for bioavailable N in municipal effluent.

If the total N limits are strict enough to be fully effective in protecting lakes from enrichment with labile N, wastewater treatment facilities will find that the limiting factor in their ability to produce low nitrogen effluent is DON, which is more difficult to remove than DIN. In fact, the ultimate baseline for DON concentration, as estimated by time course bioassays for a wastewater facility operating at low nitrogen output, may approach 1 mg/L. To regulate the refractory component of DON with stringency equal to that of DIN or labile DON overlooks the very different potential effects of the refractory and labile fractions of total dissolved nitrogen. A regulatory system that takes into account the relative abundance of refractory DON in setting effluent limits for nitrogen would require a standardized analysis of refractory DON. Bioassays could be used for this purpose according to a rationale very similar to the long accepted CBOD$_5$ (5 day) and CBOD$_u$ (ultimate) analyses for organic carbon. For both nitrogen and carbon, improved technology also offers new possibilities through the use of fluorescence spectroscopy which, if calibrated with bioassay, might allow rapid analysis of large numbers of samples for both DOC and DON.

![Figure 3. Response of attached algae in streams to experimental enrichments with N, P, or N+P (n = 237; redraw from ref 63).](Image)

<table>
<thead>
<tr>
<th>Table 2. Summary of Three Possible Effluent and TMDL-Related Regulatory Strategies for Nutrients</th>
</tr>
</thead>
<tbody>
<tr>
<td>basis of regulation</td>
</tr>
<tr>
<td>total P</td>
</tr>
<tr>
<td>total P, Total N</td>
</tr>
<tr>
<td>total P$^7$, total N</td>
</tr>
</tbody>
</table>

* TDP may be a better option for stream monitoring and lake loading limits where PP is mostly adsorbed onto mineral particles.

**STREAMS**

Although rivers and slowly flowing streams may produce phytoplankton populations comparable to those of lakes, periphyton (attached algae) also are important and may be dominant, especially in streams of small to intermediate size. Excessive growth of periphyton can be a byproduct of nutrient enrichment in streams or rivers. As in the case of lakes, extensive study at many sites has shown that phosphorus and nitrogen are about equally likely to be limiting to the growth of periphyton (Figure 3; refs 61–63). For stream periphyton, unlike lake phytoplankton, as much as half of experimentally tested locations show no nutrient limitation. As in the case of lakes, however the strongest responses to nutrient addition typically are for addition of both N and P. The stimulation threshold for nitrogen and phosphorus enrichment response in streams appears to be higher than in lakes. Thus, protective nutrient standard concentrations may justifiably be higher for streams than for lakes, but will differ among distinct categories of streams.

The arguments regarding fractions of phosphorus and nitrogen in lakes as given above are likely applicable to flowing waters as well. One exception is the consistently greater proportion of mineral particulate phosphorus (there is no significant mineral fraction for N) that is carried in suspension by flowing waters (Table 1). It may be preferable to use total soluble phosphorus rather than total phosphorus as a basis for regulation of P in flowing waters and for development of loading restrictions on lakes, given that mineral phosphorus is much less available to algae.

Assessment of eutrophication in streams and rivers has lagged behind that of lakes. Additional research will be necessary to
identify protective standards for them. Nevertheless, many of the issues surrounding nitrogen in streams and rivers are the same as for lakes. Regulation of eutrophication in flowing waters should be based on N and P controls and recognition of refractory DON as a regulatory consideration.

**CONCLUSION**

Restriction of the anthropogenic release of both N and P to inland waters is a means of controlling excessive algal growth. P regulation should be based on total P (for lakes) or total dissolved P (preferred for flowing waters). N regulation should be based on bioavailable N rather than total N; regulation of total N will likely be infeasible or will require unrealistically high standards (Table 2).

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**REFERENCES**


