Nutrient limitation of phytoplankton growth in central plains reservoirs, USA

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Historically, phosphorus has been considered to be the primary nutrient limiting phytoplankton growth in freshwater ecosystems. We tested this hypothesis in 19 Kansas reservoirs located within the Central Plains region, USA. Bioassay experiments were conducted to assess growth-rate limitation by nitrogen (N), phosphorus (P) and nitrogen and phosphorus (N and P). The addition of P alone rarely increased phytoplankton growth with only 8% of the total bioassay experiments indicating P-limitation. In contrast, limitation by N (16%) and co-limitation by N and P (63%) were more commonly observed. Results from the bioassay experiments were also used to test the hypothesis that nutrient limitation could be inferred from the water column total nitrogen : total phosphorus (TN : TP) ratio. We found that there was a classification range of TN : TP ratios that correctly predicted nutrient limitation in the majority of reservoirs. Generally, reservoirs that were N limited had water column TN : TP ratios <18 (molar); reservoirs that were co-limited by N and P had water column TN : TP ratios between 20 and 46; and reservoirs that were P limited had water column TN : TP ratios >65. Overall, these results suggest that management efforts should focus on both N and P decreases to control phytoplankton growth. Furthermore, the water column TN : TP ratio can be an effective tool for assessing potential nutrient limitation in the Central Plains region using the TN : TP classification values provided above.

INTRODUCTION

Eutrophication is one of the leading causes of pollution in lakes and reservoirs throughout the world (Smith, 2003). Excess nutrient inputs can stimulate algal blooms leading to decreases in light penetration and hypolimnion oxygen levels (Smith et al., 2001), decreases in lake aesthetics and shifts to algal taxa (i.e. cyanobacteria) that are associated with objectionable taste and odor events (Downing et al., 1999; Saadoun et al., 2001). Therefore, determining which nutrients limit phytoplankton growth is an important step in the development of effective lake and watershed management strategies (Dodds and Priscu, 1990; Elser et al., 1990; Smith et al., 2002).

Consistent relationships exist between total phosphorus (P) and phytoplankton biomass (as indexed by chlorophyll) in a variety of aquatic habitats (Schindler, 1977; Hecky and Kilham, 1988; Jones and Knowlton, 1993; Smith, 2003). As a result, P has been considered to be the primary nutrient limiting phytoplankton growth in freshwater ecosystems, and management efforts have generally focused on controlling P loading (Smith et al., 2002; Havens and Walker, 2002). However, other studies suggest that nitrogen (N) limitation and co-limitation by N and P occur more commonly than previously thought (Elser et al., 1990; Maberly et al., 2002). In a review of North American freshwater bioassay experiments, Elser et al. (Elser et al., 1990) reported that co-limitation by N and P was the most common response of phytoplankton communities to nutrient additions, occurring more frequently than limitation by either N or P alone. Similarly, Maberly et al. (Maberly et al., 2002) reported that 63% of 30 European lakes were co-limited by N and P compared to only 24% that were limited by P alone.

While bioassay experiments have often been used to determine which nutrients limit phytoplankton growth in a particular water body (Elser et al., 1990; Smith, 1998; Mallin et al., 2004), the water column ratio of total nitrogen to total phosphorus (TN : TP; dissolved

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nutrient plus seston) may provide an additional tool for assessing nutrient limitation (Smith, 1998; Schelske et al., 1999; Guildford and Hecky, 2000; Dodds, 2003). Several ranges of TN : TP ratios have been suggested for use in the classification of nutrient limitation within a lake or reservoir (Smith, 1998; Guildford and Hecky, 2000). These classification ranges were originally based on the Redfield Ratio (106 Carbon : 16 N : 1 P molar ratio; Redfield, 1958), but as pointed out by Smith (Smith, 1998) have been adjusted to account for variations in the nutrient requirements of different taxa within a community. For example, molar ratios < 20 have been used to infer N limitation, ratios > 50 have been used to infer P limitation and ratios between 20 and 50 have been used to infer co-limitation of N and P (Guildford and Hecky, 2000).

Bioassay experiments were conducted to assess both nutrient limitation and the relationships between nutrient limitation and water column TN : TP ratios in a series of eastern and central Kansas reservoirs. Water quality data was collected from 19 reservoirs, and laboratory bioassay experiments were conducted during the spring and autumn of 2002 and 2003. Specifically, we addressed several research questions: (i) which nutrient or combination of nutrients (N, P, or N and P) most commonly limits phytoplankton growth as measured as increases in relative fluorescence? (ii) are there seasonal differences in nutrient limitation within reservoirs? and (iii) can the water column TN : TP ratio be used to accurately infer nutrient limitation?

**METHOD**

Nineteen reservoirs were sampled once during the spring (April–June) and again in the late summer/autumn (August–October) of either 2002 (n = 9) or 2003 (n = 10). All of the reservoirs used in this study were small, shallow reservoirs located in eastern and central Kansas, USA (Fig. 1). The reservoirs ranged in surface area from 0.008 to 1.70 km² and had an average maximum depth of 4.6 ± 0.47 m (Table I). These reservoirs were chosen for this analysis because they provide many important services to their surrounding watersheds (i.e. drinking water, fisheries and recreation); however, they are also vulnerable to nutrient enrichment from their mainly agriculturally dominated watersheds.

**Lake water quality data**

Water quality data was collected from each reservoir during each sampling event. A 1-L surface water sample was collected from the main basin of each reservoir, roughly 50 m from the dam. This water was analyzed
for water column N and P (total and dissolved) and Chl a concentrations. Nutrient concentrations were determined colorimetrically (American Public Health Association, 1995) with a Lachat analyzer (Model 4200). Samples for dissolved nutrients (NO3-N, NH4-N and PO4-P) were filtered through Gelman Sciences ion chromatography acrodisc filters (0.45 μm) before analysis. TN and TP concentrations were determined using the automated colorimetric procedures after persulfate digestion of unfiltered samples (Ebina et al., 1983). All nutrient analysis was performed within 48 h of sample collection.

Chl a concentrations were determined by first filtering the algae from the water samples onto Whatman GF/F glass fiber filters. The filters were folded in quarters and frozen to disrupt the cells. Chl a concentrations were extracted in 90% basic methanol (10% saturated MgCO3) for at least 24 h, in darkness at 4°C. The concentration of Chl a, corrected for pheophytin a, was then determined by measuring the fluorescence of the sample with the Optical Technologies fluorometer before and after acidification (American Public Health Association, 1995). In situ measurement of dissolved oxygen, turbidity, specific conductance, pH, and air/water temperature were taken with a Horiba field water quality checker at the surface and then at 1-m intervals to the reservoir bottom. Water transparency was measured using a 20-cm Secchi disk (SD). Chlorophyll concentrations and SD depths were used to estimate the non-algal turbidity for each reservoir using: non-algal turbidity = (1/SD) – (0.025 × Chl) (Walker, 1996). This measure of non-algal turbidity was used to help assess the potential for light limitation within the reservoirs.

Bioassay experiments

Approximately 20-L of surface water were collected from the main basin of each reservoir during each sampling event. This water was returned to the Environmental Bioassay Research Facility (EBRF) where it was used in a series of bioassay experiments that were initiated within 24 h of collection. The water was not filtered prior to the start of the bioassay experiments. Three nutrient treatments and a control were established in triplicate 1-L incubation bottles for each lake during each of the two seasons: control (no nutrients added); TN addition (KNO3 added at a concentration of 57 μmol L⁻¹); TP addition (KH2PO4 added at a concentration of 6.5 μmol L⁻¹); and TN and TP addition (both KNO3 and KH2PO4 added at the same concentrations used in the single nutrient treatments). Each of these nutrient treatments was exposed to 200 μmole photons m⁻² s⁻¹ of light provided by a bank of fluorescent lights on a 12-h light/dark cycle. Because responses of algal production and biomass to nutrients are closely dependent on light conditions (Heyman and Lundgren, 1988), two additional light treatments were established to examine the potential effects of turbidity on phytoplankton growth: low light (LL) (no nutrients added; 400 μmole m⁻² s⁻¹); and high light (HL) (no nutrients added; 800 μmole m⁻² s⁻¹). After the nutrient and light treatments were established, the openings of the bottles were covered to minimize atmospheric exchange of gases, and the bottles were placed on shaker shelves that provided 12-h of gentle agitation and incubated in a growth chamber for seven to nine days at 20°C.

In order to measure phytoplankton responses to the four nutrient and two light treatments, in vivo fluorescence was measured using a Turner Model 10 Fluorometer. Fluorescence is often used as a surrogate for algal biomass and has been used to assess relative changes in algal growth in bioassay experiments (Elser et al., 1990; Maberly et al., 2002). Fluorescence measurements were taken at the initiation of each bioassay experiment, and then at daily intervals throughout the seven to nine day incubation periods.

Statistical analysis

Repeated Measures Analysis of Variance (RM-ANOVA) was used to determine whether there were significant treatment (nutrient and light) effects on phytoplankton production (i.e. fluorescence). For each site, during each of the two seasons, a separate RM-ANOVA was conducted for the nutrient and the light treatments. When significant treatment effects were observed, Tukey’s Honestly Significant Different (P= 0.05) was to determine which treatments were significantly different from the controls. Since significant increases in fluorescence were observed for several nutrient treatments relative to the controls, we used a hierarchical logic sequence similar to the one presented in Maberly et al. (Maberly et al., 2002) to determine which nutrient was most limiting to algal growth in a particular reservoir. Specifically, if N > Control and P > Control then both nutrients were determined to be limiting; if P > Control then P was determined to be limiting; if N > Control then N was determined to be limiting; if NP > N or NP > P then both N and P were determined to be co-limiting; and if Control > P, N or NP then no nutrient was determined to be limiting (Maberly et al., 2002). Furthermore, when (NP = N) > Control or (NP = P) > Control than the single nutrient (either N or P) was determined to be limiting.

All statistical analyses were completed using MINITAB version 12.0. Data are presented as the mean ± standard error unless otherwise stated.
RESULTS

Surface water temperatures averaged 23.3 ± 0.8°C in the spring and 23.3 ± 1.1°C in the late summer/early autumn (Table II). No distinct thermoclines were observed at any time during the course of the experiment. Turbidity and non-algal turbidity measurements varied widely among the reservoirs (Table II). The highest turbidity values were recorded in Sunflower and Mingenback reservoirs (133 and 416 nephelometric turbidity units, respectively), both of which also had the highest recorded concentrations of non-algal turbidity as well (7.14 and 14.39 m⁻¹, respectively).

The 19 reservoirs generally had high nutrient concentrations that were indicative of eutrophic or hypereutrophic conditions (Carlson, 1977; Table II). For example, surface TP concentrations ranged from 0.81 to 20.7 μmol L⁻¹, and surface TN concentrations ranged from 37.1 to 381.3 μmol L⁻¹ (Table II). The average TN : TP ratio of the 19 reservoirs was 34 ± 5, and the range of observed ratios was 9–200 (Table II).

Chl a concentrations in the reservoirs ranged between 1.6 and 110.6 μg L⁻¹ (Table II). Three categories of reservoirs were identified according to the trophic gradient presented by Carlson (1977). Two reservoirs (Mission and Bronson City Lakes) had average Chl a concentrations between 2.3 and 7.6 μg L⁻¹ (mesotrophic). Fourteen reservoirs were classified as eutrophic, with Chl a concentrations between 7.6 and 56 μg L⁻¹. The remaining three reservoirs (Edgerton, Gage Park and Hiawatha Lake) had Chl a concentrations ranging between 56 and 110.6 μg L⁻¹, indicating hypereutrophic conditions.

Significant relationships did not exist between nutrient (total or dissolved) and chlorophyll concentrations (linear regression; all P>0.05) in the reservoirs. However, when data from the reservoirs exhibiting light limitation (see Bioassays below) were removed from these analyses, there were significant relationships between chlorophyll concentrations and both TN (log chlorophyll = -0.62 + 1.05(log TN); \( R^2 = 0.35; P < 0.001 \)) and TP (log chlorophyll = 1.06 + 0.75 (log TP); \( R^2 = 0.31; P = 0.001 \)) (Fig. 2).

Bioassays

Phytoplankton growth, assessed as increases in fluorescence relative to control conditions, was co-limited by N and P in the majority (63%) of bioassay experiments conducted during both seasons (Table III; Fig. 3). In contrast, limitation by N or P alone occurred in only 16% and 8% of the total bioassay experiments, respectively. Nutrient additions did not stimulate algal growth relative to controls in 13% of the bioassay experiments (Fig. 3). There was also a greater occurrence of N limitation in the late summer/autumn—5% of the spring bioassays were N limited compared to 26% of the late summer/autumn bioassays. Co-limitation occurred in 68% of the bioassay experiments conducted in the spring, but only 38% of the late summer/autumn bioassay experiments. Phosphorus limitation occurred in only 11% and 5% of the bioassay experiments in the spring and late summer/autumn respectively.

Light limitation occurred in 16% of the bioassay experiments (Fig. 3). With the exception of Mingenback Reservoir, light limitation did not occur within a particular reservoir during both seasons. Mingenback Reservoir had the highest turbidity concentrations, and nutrient additions did not stimulate algal growth in this reservoir (Table III). The majority of the light-limited reservoirs, however, were also nutrient limited in the bioassay experiments (Table III). In support of these bioassay results, in situ non-algal turbidity was significantly greater (P= 0.047; Mann-Whitney) in reservoirs that were light limited (7.46 ± 2.56 m⁻¹) than in the reservoirs that were not light limited (1.53 ± 0.23 m⁻¹).

We classified the limiting nutrient status of each reservoir by comparing the results from the bioassay experiments and the water column TN : TP ratios. The majority of N-limited reservoirs had TN : TP ratios <20, the majority of reservoirs co-limited by N and P had TN : TP ratios between 20 and 50, and the three reservoir that were P-limited had TN : TP ratios >65. There was some overlap between reservoirs that were N-limited and reservoirs that were co-limited by N and P at TN : TP ratios near 20. However, the TN : TP classification ranges presented here correctly predicted the limiting nutrient in 88% of the reservoirs, excluding those reservoirs (n=5) where nutrient limitation did not occur (Table III).

DISCUSSION

Historically, P has been considered to be the primary nutrient limiting phytoplankton growth in freshwater ecosystems (Schindler, 1977; Hecky and Kilham, 1988). In the current study, the addition of P alone rarely stimulated algal growth rates (measured as increases in fluorescence), whereas growth rates were more often co-limited by N and P and to a lesser extent N (Fig. 3). The observed limitation of phytoplankton by both N and P is consistent with recent research from both oligotrophic and eutrophic systems and highlights the importance of both nutrients in regulating freshwater ecosystems (Dodds and Priscu, 1990; Elser et al., 1990; Levin and Whalen, 2001; Maberly et al., 2002; Lagus et al., 2004).

Elevated phosphorus concentrations are common in reservoirs within the Central Plains region (Wang et al., 2001; 2002; 2004; 2005).
Table II: Water quality data collected from each reservoir

<table>
<thead>
<tr>
<th>Lake</th>
<th>TN/TP</th>
<th>pH</th>
<th>Condition</th>
<th>DO (mg L$^{-1}$)</th>
<th>Turbidity (NTU)</th>
<th>Secchi disk (cm)</th>
<th>Water temperature ($^\circ$C)</th>
<th>NO$_3^-$ (µM)</th>
<th>NH$_4^+$ (µM)</th>
<th>NO$_3^-$ (µM)</th>
<th>PO$_4^{3-}$ (µM)</th>
<th>TP (µM)</th>
<th>Chl a (µg L$^{-1}$)</th>
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<td>Afton</td>
<td>20</td>
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<td>388</td>
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<td>33</td>
<td>1.47</td>
<td>65</td>
<td>22</td>
<td>4.3</td>
<td>5.1</td>
<td>55.0</td>
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<td>3.3</td>
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<td>11</td>
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<td>177</td>
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</table>

TN, total nitrogen; TP, total phosphorus.
Data for each variable was averaged for the two sampling events.
The majority of Kansas reservoirs in this study had TP and chlorophyll concentrations that were indicative of eutrophic or hypereutrophic conditions (Table II). Therefore, N limitation of phytoplankton growth rates (O’Brien, 1972) may occur when P concentrations are high. From a management perspective, however, it is important to stress that while P did not limit phytoplankton growth-rates, it likely plays an important role in determining the overall yield of phytoplankton within these eutrophic systems (O’Brien, 1972).

Furthermore, decreasing N inputs from watersheds alone will not necessarily curb production by cyanobacteria taxa, many of which can fix atmospheric N (Scheffer, 1998). Therefore, management efforts should focus on both N and P decreases to control overall phytoplankton biomass and cyanobacteria production in eutrophic systems.

Nitrogen limitation occurred in a greater percentage of Kansas reservoirs in the summer/autumn than in the spring. While it is difficult to determine seasonal patterns from two sampling dates alone, our data does show that seasonal differences exist in nutrient limitation. Phytoplankton taxa differ in their individual nutrient requirements, and seasonal shifts in species composition may have led to these differences in nutrient limitation (Hecky and Kilham, 1988; Elser et al., 1990; Maberly et al., 2002; James et al., 2003). For example, Lagus et al. (Lagus et al., 2004) reported that phytoplankton growth in the Baltic Sea was N limited in the spring, but P limited in the autumn. They attributed differences in nutrient limitation to shifts from dominance by a...
diatom species (Chaetoceros spp.) that was N limited, to dominance by taxa that were primarily P limited (Lagus et al., 2004). In some systems, N-limiting conditions correspond with dominance by cyanobacteria (Smith, 1982; Smith and Bennett, 1999; Wang et al., 2005). Furthermore, nutrient-limiting conditions may interact with a number of environmental variables including high pH, zooplankton grazing pressure, elevated water temperature and fluctuations in light intensity to impact phytoplankton species composition and dominance by cyanobacteria (Hyenstrand et al., 1998). Unfortunately, detailed phytoplankton community structure data was not collected from the reservoirs. To further evaluate seasonal patterns in nutrient limitation, research should focus on the responses of individual taxa to specific nutrient additions in relation to the environmental variables listed above (Elser et al., 1990; Lagus et al., 2004).

Light limitation was observed in several of the reservoirs, although it was not as common as nutrient limitation (Table III). There also appeared to be an interaction between light limitation and nutrient limitation within the reservoirs—significant relationships were observed between water column chlorophyll and TN and TP concentrations only when reservoirs exhibiting light limitation were removed from the analyses (Fig. 2). Increased sedimentation and sediment loading are major disturbances to freshwater ecosystems within the United States (United States Environmental Protection Agency, 2000). Increases in turbidity due to sediment loading and sediment resuspension can lead to decreased light penetration, ultimately limiting algal growth (Scheffer, 1998; Schallenberg and Burns, 2004). With the exception of Mingenback Reservoir, light limitation did not occur during both seasons suggesting that the overall impact of light limitation was variable. Light-limited reservoirs had significantly higher levels of non-algal turbidity than non-light limited reservoirs indicating that they experience higher sediment loads. Turbidity concentrations fluctuate within midwestern reservoirs, likely as a function of sediment inflow and wind-induced sediment resuspension (Wang et al., 2005). Therefore, a better understanding of the environmental conditions that influence water column turbidity concentrations are needed to assess the overall importance of light limitation and its interaction with nutrient limitation within these reservoirs.

It should be pointed out that there are a number of potential problems associated with the use of bioassay experiments including the absence of environmental heterogeneity and the removal of natural sources of nutrient recycling and regeneration. In spite of these problems, bioassay experiments are one of the most commonly used methods to assess phytoplankton limitation in aquatic ecosystems (Elser et al., 1990; Dodds and Priscu (Dodds and Priscu, 1990) suggest that long-term bioassay experiments (several days duration) may provide more accurate information on phytoplankton growth limitation than short-term bioassays (particulate C : N : P ratios; nutrient uptake rates) or routine monitoring of in situ conditions. Furthermore, our bioassay results are consistent with in situ patterns observed within the reservoirs and additional studies conducted throughout the world (Dodds and Priscu, 1990; Elser et al., 1990; Levin and Whalen, 2001; Maberly et al., 2002; Lagus et al., 2004).

Several ranges of TN : TP ratios have been published for use in the classification of nutrient limitation within a lake or reservoir (Smith, 1982; Smith, 1998; Guildford and Hecky, 2000; Dodds, 2003). In the current study, a
classification range similar to the one presented in Guildford and Hecky (Guildford and Hecky, 2000) for both freshwater and marine systems correctly identified the limiting nutrient in the majority of reservoirs: N limitation occurred at TN : TP ratios <20; co-limitation by N and P occurred at TN : TP ratios between 20 and 50; and P limitation occurred at TN : TP ratios >65. Despite these results, however, others have shown that nutrient ratios do not always identify the correct limiting nutrient (Schelske et al., 1999; Maberly et al., 2002; James et al., 2003). Kobayashi and Church (Kobayashi and Church, 2003) reported that N : P ratios in an Australian reservoir correctly identified the limiting nutrient in only 33% of their bioassay studies. Similarly, Maberly et al. (Maberly et al., 2002) found that published data on nutrient ratios underestimated nitrogen limitation. Furthermore, TN : TP ratios reflect the potential for nutrient limitation only, and actual limitation will be determined by the concentrations of available dissolved inorganic nitrogen and phosphorus. Therefore, caution should be used when inferring nutrient limitation based on TN : TP ratios only. Classification ranges should be tested and modified with the results from bioassay experiments conducted for lakes and reservoirs within specific geographic regions that may be differentially impacted by environmental factors such as geology, climate and disturbance.

Determining which nutrients limit algal growth in freshwater ecosystems is an important step in the development of effective lake and watershed management strategies. Bioassay studies indicated that phytoplankton growth, measured as increases in fluorescence relative to control conditions, was co-limited by N and P in the majority of Kansas reservoirs included in this study. Similarly, co-limitation by N and P has commonly been observed in lake and reservoirs throughout the world, highlighting the importance of both N and P in regulating freshwater algal growth. As a result, management efforts targeting P inputs alone will not effectively control nuisance algal blooms and the associated negative impacts of eutrophication. Furthermore, our data suggests that the water column TN : TP ratio can be an effective tool for assessing nutrient limitation. However, caution must be used when developing and using TN : TP classification ranges for bodies of water within different geographic regions.

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