Phytoplankton nutrient limitation in Colorado mountain lakes

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SUMMARY. 1. Limiting nutrients for phytoplankton were studied experimentally in eight mountain lakes of central Colorado between May and November of 1984.

2. 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 phosphorus-limited, and four showed primarily combined limitation (concurrent or reciprocal). Switching between categories of limitation was also observed within lakes. Nitrogen was the most frequently limiting nutrient: N, either alone or in combination with P, accounted for 79% of all observed instances of limitation.

3. Nine indices were tested for effectiveness in predicting phytoplankton limitation by N and P. The best 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 actually available to the phytoplankton.

Introduction

Phosphorus limitation of phytoplankton growth has been demonstrated in many lakes (Schindler, 1978). Nitrogen limitation is also known, especially in middle to low latitudes (Talling, 1966; Lewis, 1974, 1983; Coulter, 1977; Zaret, Devol & Dos Santos, 1981; White, 1982; White *et al.*, 1985; Canfield, 1983; Vincent *et al.*, 1984). Nitrogen limitation is considered by some to be a transitory

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condition of minor importance to the productivity of aquatic ecosystems because nitrogen deficiency may be offset by the growth of nitrogen fixing blue-green algae (Schindler, 1977). However, some investigators have found that the variance in phytoplankton productivity and biomass explained by both nitrogen and phosphorus is much greater than that explained by phosphorus alone (Smith, 1979, 1982). Even though the overall importance of nitrogen in regulating phytoplankton productivity in freshwater remains unresolved; it is clear that the productivity of a phytoplankton community over the short term can be suppressed by lack of nitrogen during the

interval between the onset of nitrogen limitation and the final compensatory response that offsets nitrogen limitation.

Several studies indicate that limitation of a phytoplankton community by either N or P can change on a variety of time scales (Edmondson, 1972; White, 1982; White *et al.*, 1985; Lewis *et al.*, 1984; Setaro & Melack, 1984). Lewis *et al.* (1984) found that the phytoplankton community of Lake Dillon, a mesotrophic mountain lake in Colorado, alternated between nitrogen and phosphorus limitation during summer stratification. Because Lake Dillon lacked heterocystous blue-green algae during this study, it was assumed that the seasonal switching between N and P limitation was caused primarily by seasonal changes in the availability of these nutrients. Such seasonal variations in limitation may be important in regulating the production, biomass, and composition of the phytoplankton community of Lake Dillon and other lakes. Because the nutrient chemistry and phytoplankton community composition of Lake Dillon is similar to that of many lakes in the central mountains of Colorado, we hypothesized that switching of phytoplankton limitation by N and P would be found in other lakes of this region. We report here the results of a series of phosphorus and nitrogen enrichment experiments conducted *in situ* on eight Colorado lakes at regular intervals from May to November 1984 as a test of this hypothesis. We also tested nine indices potentially useful in predicting phytoplankton limitation by N and P.

Methods

The eight lakes included in this study are located in the mountains of central Colorado (Fig. 1); they vary greatly in size, elevation and morphometry (Table 1). The phytoplankton biomass of these lakes was comprised mostly of chrysophytes and chlorophytes; none of the lakes developed significant populations of heterocystous blue-green algae during the study.

The lakes were sampled twice per month between the end of May and the beginning of September. During the autumn, the frequency of sampling was progressively reduced until the study ended in early November. A single collection site was located near the point of maximum depth for each lake. Lake water collected with an integrating sampler from the top 3 m of the water column was utilized for both enrichment experiments and chemical analyses.

Nutrients and chlorophyll *a* were analysed in conjunction with each of the enrichment experiments. Water was filtered within 1 h of collection and all analyses were performed within 24 h. Particulate and dissolved fractions were separated by filtration through Whatman GF/C glass-fibre paper (effective pore size 0.7 μm; Sheldon, 1972). Soluble reactive phosphorus (SRP) in the filtrate was analysed by the ascorbic acid–molybdate method of Murphy & Riley (1962), ammonium was analysed by the method of Koroleff (1976), and nitrate and nitrite were analysed by liquid ion chromatography. Total dissolved phosphorus (TDP) and nitrogen (TDN) were determined simultaneously by a modification of the oxidation method described by Lagler & Hendrix (1982) and Valderrama (1981). Following oxidation, TDN was analysed as nitrate by cadmium–copper reduction to nitrate (Wood, Armstrong & Richards, 1967) and diazotization (Bendschneider & Robinson, 1952). Dissolved organic nitrogen and phosphorus fractions (DON and DOP) were calculated as the difference between the total dissolved fractions and the dissolved inorganic forms.

Particulate phosphorus (PP) was analysed according to the method of Solorzano & Sharp (1980). Suspended particulate matter was removed with precombusted, preweighed GF/C filters, which were dried to constant weight at 60°C and reweighed. After determination of the weight of total particulate matter, the same filters were analysed for particulate nitrogen (PN) and particulate carbon (PC) with an elemental analyser.

Chlorophyll *a* was analysed by the spectrophotometric method of Marker *et al.* (1980) and Nusch (1980), which is based on the extraction of chlorophyll with hot 90% ethanol (v:v), and correction for phaeophytin. Phytoplankton cells were collected on Whatman GF/C filter paper.

The method used for the nutrient enrichment experiments was similar to that described by Lewis *et al.* (1984). Water was collected from the top 3 m of the epilimnion, thoroughly
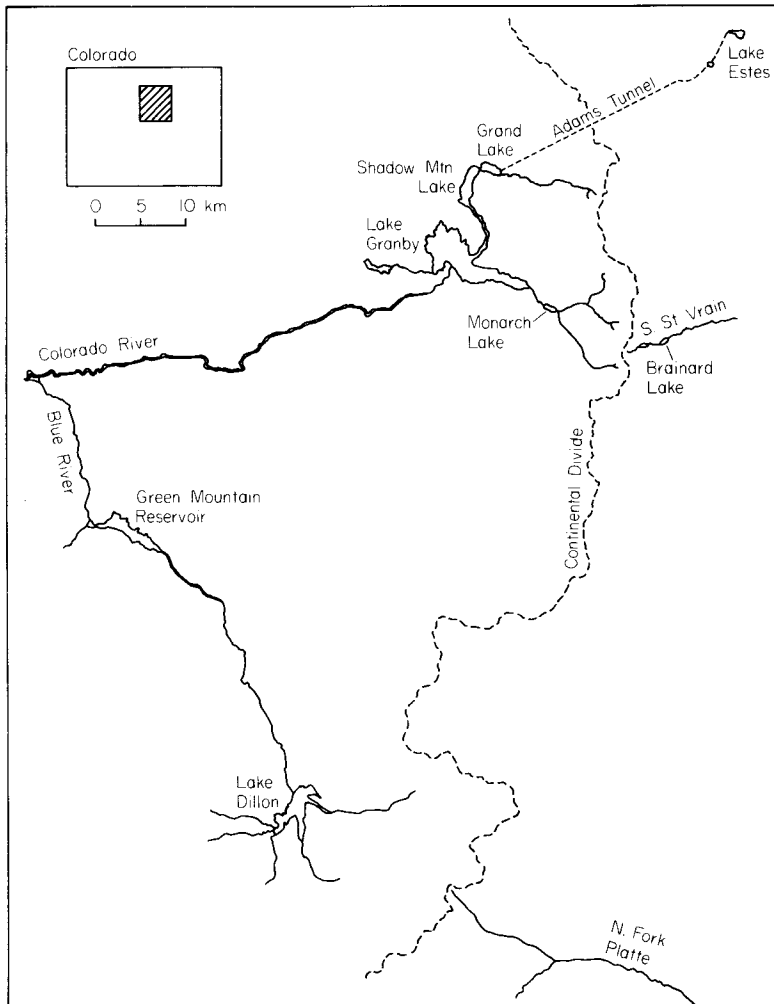
FIG. 1. Location of the lakes included in the study.

TABLE 1. A list of the lakes included in this study and their physical characteristics

Lake	Elevation (m)	Catchment area (km²)	Surface area (ha)	Maximum depth (m)	Average depth (m)
Lake Granby	2530	810	2960	61	23
Shadow Mountain Lake	2550	480	770	6	3
Grand Lake	2550	200	190	76	38
Monarch Lake	2550	110	53	4	2
Lake Dillon	2750	870	1300	57	24
Green Mountain Reservoir	2420	1550	850	80	23
Lake Estes	2310	355	75	15	5
Brainard Lake	3160	20	26	4	2

TABLE 2. Chemistry data for epilimnetic water on each of the sampling dates and the state of phytoplankton nutrient limitation as determined by nutrient enrichment experiments. States of limitation include, no limitation (none), nitrogen limitation (N), phosphorus limitation (P), concurrent limitation (N & P), and reciprocal limitation (N/P).

Lake	Date	Chl a (μg l^{-1})	PC (mg l^{-1})	Phosphorus (μg l^{-1})			Nitrogen (μg l^{-1})			Observed limitation
				SRP	DOP	PP	DIN	DON	PN	
Lake Granby	27 May	10.0	0.24	1.9	5.2	10.0	72	211	88	None
	12 June	6.5	0.63	1.2	6.2	11.3	33	156	91	None
	29 June	4.7	0.62	1.6	4.5	7.4	6	138	89	N
	20 July	4.7	0.44	1.3	7.7	5.6	10	201	66	None
	8 Aug	5.6	0.50	0.3	8.8	8.6	6	200	73	N
	28 Aug	3.3	0.40	0.9	8.0	4.8	16	214	66	N & P
	25 Sept	8.4	0.53	0.2	3.5	7.2	3	188	67	N
	3 Nov	4.8	0.30	0.0	8.9	6.7	44	152	27	None
Shadow Mountain	27 May	4.5	0.48	2.7	4.2	3.9	38	340	61	—
Lake	12 June	2.7	0.48	1.3	4.9	9.0	5	164	86	None
	29 June	6.2	0.69	1.6	6.0	6.8	4	125	67	None
	20 July	3.2	0.45	1.7	8.5	7.1	4	161	62	N
	8 Aug	2.8	0.30	1.0	8.9	7.1	3	200	36	N
	28 Aug	6.5	0.50	2.4	8.5	7.5	12	198	74	N & P
	25 Sept	12.1	0.59	1.9	13.5	12.6	4	188	112	N
	3 Nov	5.2	—	0.0	9.0	11.6	3	149	—	None
Grand Lake	27 May	1.9	0.33	0.3	4.4	9.8	83	222	35	None
	12 June	6.2	0.39	0.4	3.9	3.8	37	174	55	None
	12 July	2.1	0.23	4.1	3.1	2.4	30	142	16	None
	20 July	2.0	0.24	1.5	6.8	3.0	13	126	24	None
	8 Aug	3.6	0.31	2.4	7.3	5.5	5	188	30	N
	28 Aug	3.4	0.35	0.9	6.1	4.4	8	160	43	N & P
	25 Sept	7.5	0.40	0.2	3.1	6.4	4	176	72	N
	3 Nov	2.7	0.21	0.6	10.6	5.1	182	—	20	None
Monarch Lake	27 May	1.1	0.52	7.5	11.1	8.0	123	318	57	—
	12 June	0.2	0.16	0.1	2.4	5.0	4	178	18	N/P
	29 June	0.4	0.16	0.6	6.8	1.9	3	119	16	None
	20 July	1.0	0.25	0.9	6.7	2.5	40	76	4	N/P
	8 Aug	1.1	0.27	1.2	6.3	4.0	26	115	—	None
	28 Aug	0.8	0.23	1.3	5.5	2.6	55	113	22	None
	25 Sept	0.9	0.28	0.0	3.5	7.7	42	173	30	None
Lake Dillon	2 June	1.0	0.27	1.6	14.5	7.3	243	284	46	None
	22 June	4.5	0.48	1.0	2.4	5.7	188	—	73	None
	6 July	3.0	0.44	1.5	4.4	10.0	93	171	49	None
	26 July	7.6	0.42	1.0	5.1	10.7	57	147	139	P
	15 Aug	1.1	0.27	0.5	7.3	4.5	41	207	—	None
	4 Sept	1.2	0.35	0.4	5.5	3.6	32	149	52	N & P
	9 Oct	1.4	0.21	2.1	2.5	2.7	67	181	18	N & P
Green Mountain	2 June	1.4	0.31	4.3	18.6	16.1	271	380	56	None
Reservoir	17 June	11.8	0.79	2.4	8.1	14.7	241	194	110	None
	11 July	3.2	0.30	2.4	6.3	6.4	101	171	50	N & P
	26 July	3.4	0.30	1.0	4.5	4.4	154	99	44	P
	15 Aug	1.5	0.19	1.2	6.8	2.4	122	146	—	None
	14 Sept	2.0	0.27	3.1	6.5	3.1	122	184	38	None
	9 Oct	2.0	0.19	2.1	1.7	3.3	141	84	12	None
Lake Estes	7 June	1.2	0.37	2.4	6.5	7.4	93	192	57	None
	23 June	1.0	0.40	2.0	4.4	8.3	70	133	49	None
	13 July	3.8	0.43	2.8	3.1	7.5	60	157	38	None
	2 Aug	4.4	0.41	3.7	3.7	15.2	53	107	67	N & P
	21 Aug	3.9	0.34	3.9	8.9	11.5	66	104	51	N/P
	13 Sept	4.9	0.52	2.3	5.7	12.3	72	154	74	None
Brainard Lake	23 June	3.3	0.46	0.3	4.4	10.0	85	147	56	None
	13 July	1.8	0.21	2.5	1.7	2.6	89	106	16	P
	2 Aug	2.1	0.17	1.0	2.9	7.7	78	98	36	P
	21 Aug	3.0	0.40	3.6	2.1	5.1	68	84	41	P
	13 Sept	5.4	0.43	0.4	5.2	6.5	49	213	59	None

mixed in a 120-litre polyethylene mixing chamber, and then divided among twelve 10-litre polyethylene carboys. The experimental design incorporated four treatment groups, each with three replicates. The first group, which received no nutrient additions, was the control. The remaining three groups were enriched with either nitrogen (200 μg l^{-1} NH$_4$Cl–N), phosphorus (100 μg l^{-1} KH$_2$PO$_4$–P), or with a combination of the two nutrients. After the containers were filled and enriched, they were incubated *in situ* at 50% of the Secchi depth. This usually corresponded to a depth of 1–4 m, and provided light saturated conditions (a minimum of 200 μmol m^2 s^{-1} at mid-day). The duration of the incubations ranged from 4 to 7 days. The incubations were typically longer in the spring and autumn when the temperature of the epilimnion was below 10°C. The response of the phytoplankton to the treatments was determined by analysis of the chlorophyll *a* concentration of each of the carboys at the end of the incubation period.

Results

Chemical analyses

Table 2 gives the results of the nutrient analyses for each of the fifty-six enrichment experiments. Table 3 gives the mean concentrations over the period of stratification (mid-June to mid-October) for each of the major fractions of phosphorus and nitrogen, and for chlorophyll *a*. The apportionment of phosphorus among fractions was similar in all of the lakes during the period of stratification. On the average, total P was 12% SRP, 42% DOP and 46% PP.

The lakes can be divided into two categories on the basis of nitrogen fractions. In Grand Lake, Shadow Mountain Lake, Lake Granby and Monarch Lake, total nitrogen was, on the average, 5% DIN, 73% DON, and 22% PN. The lakes in this group showed a sharp temporal trend in epilimnetic nitrogen; total nitrogen often reached a minimum in mid-summer, nitrate was often undetectable throughout stratification, and DIN frequently fell below 10 μg l^{-1}. By contrast, Lake Dillon, Green Mountain Reservoir, Lake Estes and Brainard Lake had a much higher mean proportion of DIN (31%), a lower proportion of DON (52%), and a slightly lower proportion of PN (17%). While these lakes also showed mid-summer declines in DIN, concentrations rarely fell below 50 μg l^{-1}.

Mean epilimnetic concentrations of chlorophyll *a* ranged between 0.7 and 5.6 μg l^{-1} across all of the lakes during the period of stratification. Lake Granby, Shadow Mountain Lake and Grand Lake had maximum chlorophyll *a* concentrations in early summer and again in the fall. By contrast, chlorophyll *a* concentrations in Lake Dillon peaked in mid-summer, then fell precipitously and remained low for the duration of the study. Temporal chlorphyll trends in the remaining lakes were less obvious. Monarch Lake remained unproductive for the duration of this study; chlorophyll *a* was near the limit of detection on two occasions. We attribute this low phytoplankton biomass to a low hydraulic residence time.

TABLE 3. Mean concentrations of chlorophyll *a* and fractions of epilimnetic phosphorus and nitrogen for the period of stratification (mid-June to mid-October) in the eight lakes

Lake	Chlorophyll *a* (μg l^{-1})	Phosphorus (μg l^{-1})				Nitrogen (μg l^{-1})			
		SRP	DOP	PP	TP	DIN	DON	PN	TN
Lake Granby	5.5	0.9	6.5	7.5.	14.9	12	183	75	270
Shadow Mountain Lake	5.6	1.6	8.4	8.4	18.4	5	172	73	250
Grand Lake	4.1	1.6	5.1	4.2	10.9	16	61	40	217
Monarch Lake	0.7	0.7	5.2	4.0	9.9	16	129	18	163
Lake Dillon	3.1	1.1	4.5	6.2	11.8	80	177	66	323
Green Mountain Reservoir	4.0	2.0	5.6	5.7	13.3	147	146	51	344
Lake Estes	3.6	2.9	5.2	11.0	19.1	64	131	56	251
Brainard Lake	3.1	1.6	3.3	6.4	11.3	74	130	42	246

Nutrient enrichment experiments

The concentrations of chlorophyll *a* at the end of each enrichment experiment were analysed by one-way ANOVA. When a significant difference ($P \leqslant 0.05$) was detected between mean chlorophyll *a* concentrations of treatment groups, the groups were compared *a posteriori* with the Duncan multiple range test (Sokal & Rohlf, 1981). This analysis ordered the treatments with respect to the amount of chlorophyll *a* present at the conclusion of the incubation. In every case in which enrichment produced statistically distinguishable subsets, mean concentrations of chlorophyll *a* increased by at least 100% relative to the control.

Chlorophyll *a* concentrations may increase without a corresponding increase in phytoplankton biomass (Meeks, 1974). However, cell counts and analysis of particulate carbon from several of the enrichment experiments indicate that increases in chlorophyll *a* were indeed proportional to increases in phytoplankton biomass. ·

The results of the nutrient enrichment experiments are presented in Table 2. A limiting nutrient or a combination of limiting nutrients is designated for dates on which the mean chlorophyll *a* concentrations of enriched treatments were significantly higher than those of controls.

Five distinct and interpretable responses to enrichment are possible: (1) no significant difference in mean concentrations of chlorophyll *a* between treatments and control (no limitations); (2) significantly greater chlorophyll *a* only in treatments including phosphorus (P limitation); (3) significantly greater chlorophyll *a* only in treatments including nitrogen (nitrogen limitation); (4) significant increase in chlorophyll *a* only following simultaneous additions of nitrogen and phosphorus (concurrent limitation, symbolized N & P); and (5) significant increases of chlorophyll *a* following separate applications of either nitrogen or phosphorus alone, *and* following additions of both nutrients together (reciprocal limitation, symbolized N/P).

As indicated by Table 2, there was some form of nitrogen or phosphorus limitation of the enclosed phytoplankton community in twenty-four of the fifty-six enrichment experiments (43%). In general, limitation occurred during middle to late stratification. Nitrogen limitation was the most common, followed by phosphorus limitation, and concurrent limitation (N & P). Three cases of reciprocal limitation (N/P) were observed. Substantial variation was found in phytoplankton limiting nutrients within as well as between lakes. Temporal switching between the five categories of limitation was observed in each of the lakes.

Discussion

Categories of limitation

The restricted scope of phosphorus limitation of these phytoplankton communities was unexpected. Phosphorus limitation comprised only 21% of all observed cases of limitation. With the exception of Brainard Lake, extended periods of phosphorus limitation were not observed. Nitrogen was at least as important as phosphorus in limiting phytoplankton production in the remaining lakes. Limitation by nitrogen alone was found in 33% of all cases, while combined limitation (N & P, N/P) was found in 46% of all cases. Thus nitrogen, either alone or in combination with phosphorus, was significant in 79% of all cases of limitation. Grand Lake, Lake Granby and Shadow Mountain Lake were primarily nitrogen limited during 1984. In these lakes, the period of stratification was characterized by low epilimnetic concentrations of DIN which resulted in prolonged nitrogen limitation. Nitrogen limitation of phytoplankton communities has previously been recognized mostly in eutrophic or hypereutrophic temperate lakes (Maloney, Miller & Shiroyama, 1972; Cleasson & Riding, 1977; Kanninen, Kauppi & Yrjaha, 1982; Canfield, 1983), or in tropical lakes (Talling, 1966; Lewis, 1974, 1983; Coulter, 1977; Zaret *et al.*, 1981; Setaro & Melack, 1984; Vincent *et al.*, 1984). However, recent examples of nitrogen limitation have also been found in less productive temperate lakes (White & Payne, 1977; White, 1982; Lewis *et al.*, 1984; White *et al.*, 1985), suggesting that at least short-term nitrogen limitation of phytoplankton communities may occur frequently on a global scale.

Concurrent limitation (N & P, characterized by a response only to combined additions of both N and P) can be interpreted as simul-

taneous limitation of the phytoplankton assemblage by nitrogen and phosphorus. This phenomenon is not widely recognized because it is generally believed that algal growth rate and yield are determined by the abundance of the single nutrient that is in the shortest supply relative to the requirements of the cell. The validity of the single limiting nutrient concept has been demonstrated for single species in chemostat studies with nitrate and phosphate (Rhee, 1978; Terry, 1978, 1980). However, for any pair of nutrients for which this relationship is valid (e.g. N and P), a critical ratio (Terry, 1978, 1980) may exist at which growth is simultaneously limited by both nutrients. Concurrent limitation may be indicative of extreme shortages of both nutrients, and may thus represent a situation in which cellular N:P ratios are near critical values for a large proportion of the assemblage. While the single limiting nutrient concept may not be valid in mixed assemblages of phytoplankton (Tilman, Kilham & Kilham, 1982), concurrent limitation could still occur if most of the assemblage was composed of a relatively small number of closely related species with similar N and P kinetic abilities. Responses that may be interpreted as concurrent limitation have also been reported in previous studies (White & Payne, 1977; Zaret *et al.*, 1981; Setaro & Melack, 1984; Lewis *et al.*, 1984; White *et al.*, 1985).

Reciprocal limitation (N/P) can be interpreted as limitation of part of the assemblage by nitrogen and part of the assemblage by phosphorus. For this reason, reciprocal limitation does not violate the concept of single nutrient limitation. The substantial differences observed for critical N:P ratios among phytoplankton species (Rhee, 1978; Terry, 1978, 1980; Rhee & Gotham, 1980; Tilman *et al.*, 1982) could easily provide the variability necessary to produce reciprocal limitation.

Variability of limiting nutrients

Within the narrow geographic region encompassed by this study, we found wide variation in phytoplankton nutrient limitation. Substantial variation of phytoplankton limitation by N and P was also found between lakes by White *et al.* (1985) in a survey of twelve New Zealand lakes. This phenomenon may be common, but has not been widely reported in the literature.

We also found extensive temporal variation of nitrogen and phosphorus limitation of phytoplankton within lakes. Periods of limitation by N and P were frequently interrupted by periods of N and P sufficiency, and frequent transitions were observed between phosphorus limitation, nitrogen limitation, and combined limitation (concurrent or reciprocal). Temporal variation in N and P limitation may be caused by: (1) changes in the N:P supply ratio brought about by variations in the thickness of the mixed layer, (2) by differential fluxes of N and P within the mixed layer, (3) by changes in the N:P critical ratio associated with changes in phytoplankton growth rate (Terry, Laws & Burns 1985), or (4) by changes in the phytoplankton assemblage. Temporal variation in phytoplankton N and P limitation is not widely reported in the literature but, like regional variation among lakes, may be quite common. Switching between N and P limitation of phytoplankton has been reported by Edmondson (1972), White *et al.* (1977, 1985), Lewis *et al.* (1984) and Setaro & Melack (1984).

Rationale for indices of nutrient limitation

We tested nine indices (Table 4, top half) for accuracy in predicting limitation. Seven of these indices were chosen because they have appeared previously in the literature. We devised the other two indices on the basis of known principles of nutrient uptake and storage by phytoplankton.

The use of seston composition ratios (PN:PP, PC:PP, PC:PN, PC:Chl *a*) to predict nutrient limitation is common in the literature and is based on the premise that these ratios are strongly affected by nutritional status (Ketchum, 1939; Gerloff & Skoog, 1954, 1957; Redfield, 1958; Healey, 1978; Healey & Hendzel, 1980). One disadvantage with the use of composition ratios in natural assemblages is that the particulate fraction frequently includes detritus as well as live cells. Indices of external nutrient supply (e.g. DIN:SRP) have also been widely used and are reliable predictors of limitation in chemostat studies (Rhee, 1978; Rhee & Gotham, 1980; Terry *et al.*, 1985). However, extrapolation of these results to natural assemblages is difficult because N and P are present in lakes in a variety of forms, some of which are not so readily available to

TABLE 4. The nine indices evaluated for their ability to discriminate states of limitation (N=N only, P=P only, combined=N & P or N/P) for cases with nutrient deficiency only, and for the entire data set

Index	Hypothesis tested	Significance‡
For cases with nutrient deficiency†		
PN:PP	N≥P	ns
	N≥ combined	ns
	Combined≥P	ns
PC:PP	N≥P	ns
	N≥combined	ns
	Combined≥P	ns
PC:PN	N≤P	ns
	N≤combined	ns
	Combined≤P	ns
PC:Chl *a*	N≥P	ns
	N=combined	ns
	Combined=P	ns
	Limited=nonlimited	ns
TN:TP	N≥P	**
	N≥combined	ns
	Combined≥P	*
DIN:SRP	N≥P	**
	N≥ combined	*
	Combined≥P	ns
[TN−DON]:[TP−DOP]	N≥ P	**
	N≥combined	ns
	Combined≥P	*
DIN:TDP	N≥P	**
	N≥combined	**
	Combined≥P	**
DIN:TP	N≥P	**
	N≥combined	**
	Combined≥P	**
All cases§		
TN:TP	N≥non N	ns
	P≤non P	ns
DIN:SRP	N≥non N	**
	P≤non P	ns
[TN−DON]:[TP−DOP]	N≥non N	**
	P≤non P	**
DIN:TDP	N≤non N	**
	P≤non P	**
DIN:TP	N≥non N	**
	P≤non P	**

† Number of cases is 14–18
‡ ns=not significant at $P=0.05$, * =significant at $P=0.05$, ** =significant at $P=0.01$.
§ Number of cases is 53–56.

phytoplankton as nitrate, ammonium and phosphate. Indices that combine seston composition and nutrient supply (e.g. TN:TP) have also been used in field studies. These indices are likely to incorporate problems that affect both nutrient composition and nutrient supply ratios.

Phytoplankton can obtain nutrients for metabolism and growth from cellular reserves or from the medium. The most reliable indices

of limitation should be those that best repre-
sent N and P available to the phytoplankton
from both sources. However, with one excep-
tion ([TN−DON]:[TP−DOP], White *et al.*,
1985), attempts have not been made to base
indices on physiologically relevant fractions of
N and P that can be readily measured. We
have attempted to do this on the basis of two
new indices, DIN:TDP and DIN:TP.
Although these indices have apparently not
been used, there is substantial theoretical justi-
fication for their use.

Phosphorus available to the cell in excess of
that which is currently in active use can best be
represented by TDP (externally available) and
PP (internally available). Total dissolved phos-
phorus (TDP) is significant because many
phytoplankton can obtain P not only from
SRP, but also from DOP via phosphatase-
mediated hydrolysis, performed either directly
by the algae or by other members of the
microbial community (Chrost & Overbeck,
1987; Nalewajko & Lean, 1980). Phosphorus is
also subject to substantial luxury consumption
by phytoplankton. For this reason, PP is an
effective index of phosphorus limitation in
chemostats (Fuhs *et al.*, 1972; Rhee, 1973;
Droop, 1974).

Available nitrogen can best be approximated
by DIN. Nitrogen in the medium is most
available to phytoplankton as ammonium and
nitrate, which together account for essentially
all DIN. Low molecular weight organic com-
pounds such as urea and amino acids are also
available (Bonin & Maestrini, 1981; Styrett,
1981), but these forms are not routinely mea-
sured, and are usually found only in very low
concentrations in inland waters. A large frac-
tion of DON is composed of high molecular
weight compounds (peptides, humic acids, ful-
vic acids) that are unavailable directly to the
phytoplankton. For this reason, TDN over-
estimates the availability of nitrogen. Unless
amino acids, urea and other low molecular
weight nitrogenous compounds are quantified
independently of other DON, the DIN fraction
will most closely approximate available nit-
rogen.

Particulate nitrogen (PN) is not such a
reasonable measure of internally available nit-
rogen as PP is for phosphorus. In phytoplank-
ton, nitrogen is stored as soluble ions, amino
acids, peptides, nucleic acids, or photosynthe-

tic pigments (Dortch *et al.*, 1985), and the
cellular capacity for these compounds, relative
to demand, is much less than for
polyphosphate-P. Thus, while PP has signifi-
cance in quantifying cellular reserves of phos-
phorus, PN lacks similar importance for nit-
rogen.

Tests for indices

For the subset of experiments (twenty-four)
in which some form of limitation was demons-
trated, we first tested the ability of the nine
indices to discriminate among three possible
states of limitation: nitrogen, phosphorus, and
combined (concurrent or reciprocal). Second,
for all experiments (fifty-six), we tested the
abilities of the indices to predict simultaneous-
ly the conditions under which limitation would
occur and the nature of the limitation. An
index that performed poorly in the first test
was not considered for the second test, which
is more demanding. In both sets of tests we
used the nonparametric Wilcoxon-Mann-
Whitney procedure (Sokal & Rohlf, 1981) in
comparing the values of each of the nine
indices across the three types of limitation.
The results are presented at the top half of
Table 4.

The four indices based entirely upon seston
composition (PN:PP, PC:PP, PC:PN and
PC:Chl *a*) were not successful in discriminat-
ing between any of the three types of limita-
tion. The difference between nitrogen-limited
and phosphorus-limited groups was significant
($P{\leqslant}0.05$) for five indices: TN:TP, DIN:SRP,
DIN:TDP, [TN−DON]:[TP−DOP] and
DIN:TP. Only the two new indices based
upon physiological principles (DIN:TDP and
DIN:TP) exhibited highly significant differ-
ences ($P{\leqslant}0.01$) across all three categories of
limitation (nitrogen, phosphorus, combined).

Fig. 2 presents the results graphically for the
five indices that provided some discrimination
among the categories of limitation. Index
values associated with nitrogen limitation are
generally lower than those associated with
phosphorus limitation. Ratios for combined
limitation are intermediate between those for
N limitation and those for P limitation.

In order to quantify the relative accuracy of
the indices, we determined the minimum rela-
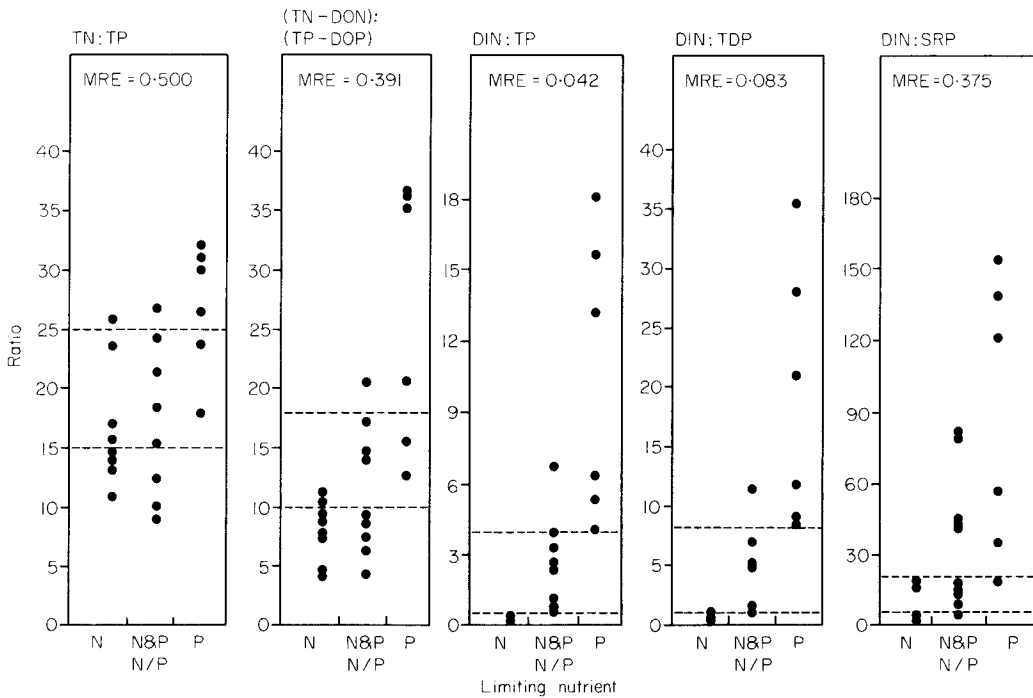tive error (MRE) for each index by a process

FIG. 2. Distribution of index values across three states of limitation for the twenty-four experiments in which the phytoplankton communities showed evidence of N or P deficiency (N=nitrogen limitation, P=phosphorus limitation, N & P and N/P=combined limitation). The dashed lines indicate boundary values corresponding to minimum relative error (MRE: see text).

of successive estimation. Two boundary values were selected arbitrarily for each index to delineate regions of nitrogen limitation, phosphorus limitation, and combined limitation. Only boundary values that correctly discriminated at least 50% of each of the observed states of limitation were considered. Relative error of the discrimination was then calculated as the number of errors divided by the total number of observations. Boundary values were then varied until the minimum relative error was determined. Boundary values associated with the MRE thus represent the optimal division of the index into regions predictive of nitrogen limitation, phosphorus limitation, and combined limitation for the eight lakes. Fig. 2 shows that three of the five indices (TN:TP, [TN−DON]:[TP−DOP] and DIN:SRP) have high MRE values (≥0.375). In contrast, the MRE values for DIN:TP and DIN:TDP are much lower (0.042 and 0.083).

The bottom half of Table 4 presents the outcome of the second set of tests, which included the entire data set of fifty-six observa-

tions. This analysis was restricted to the five indices that gave significant discrimination in the first set of tests. The use of the entire data set makes the discrimination of nutrient limitation more difficult because limitation is related not only to the nutrient ratios, but also to the nutrient concentrations. Given that these indices are based on ratios and not on the absolute concentration of nutrients, the category of combined limitation (N & P, N/P) must be considered inseparable from the category of no limitation.

We first made contrasts between index values representing nitrogen-limited (N) cases and all other cases (no limitation, P, N & P, N/P). We then made a second set of contrasts between phosphorus-limited cases (P) and all other cases (no limitation, N, N & P, N/P). Consistently high levels of discrimination ($P \leq 0.01$) were limited to three indices (DIN:TDP, [TN−DON]:[TP−DOP] and DIN:TP). Each of these indices discriminated well between both the nitrogen- and phosphorus-limited cases and the other cate-
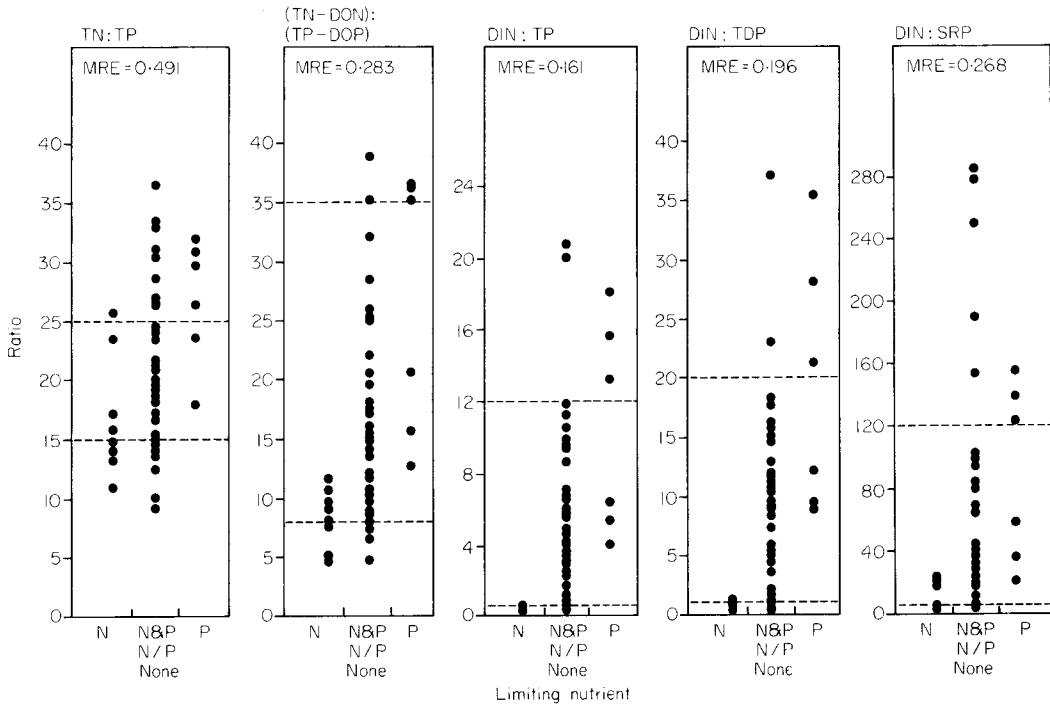
FIG. 3. Distribution of index values across three states of limitation for the entire data set. No limitation, concurrent limitation, and reciprocal limitation are considered as a single category.

gories of limitation (no limitation, N & P, N/P).

Fig. 3 illustrates the distribution of index values across the three categories of limitation (P limitation, combined and no limitation, and N limitation). Index values corresponding to nitrogen limitation were generally lower than those for phosphorus limitation, and values corresponding to concurrent limitation, reciprocal limitation, and no limitation were intermediate. We also calculated MRE for each of these indices using the entire data set. In several instances, boundary values differed from those found in the first set of tests. DIN:TP and DIN:TDP again provided the highest level of accuracy in identifying the observed state of limitation. The DIN:TP index correctly predicted the state of limitation in 47/56 cases (MRE=0.161), while DIN:TDP was correct in 45/56 cases (MRE=0.196).

Of the nine indices of nutrient limitation evaluated in this study, the four seston composition ratios were the poorest predictors of N or P limitation. These findings directly contrast with those of Healey & Hendzel

(1976, 1980) and Healey (1978). While these indices work well in predicting limitation in chemostat studies using a monoculture (e.g. Healey, 1973), they may be less effective in mixed natural assemblages. The particulate fraction of nutrients in these lakes may not accurately represent cellular levels of C, N or P because of the presence of seston.

The most effective indices of limitation were the ratios of DIN:TDP and DIN:TP. Dissolved inorganic nitrogen (DIN) was a highly effective predictor of nitrogen limitation in these assemblages; nitrogen limitation occurred exclusively at low concentrations of DIN (<6 μg l^{-1}). A high ratio of detrital N to cellular N could account for the poor performance of indices incorporating PN. The DON fraction is less useful in predicting limitation because a large proportion of DON is unavailable to the phytoplankton. This observation is supported by the fact that DON concentrations were high (138–200 μg l^{-1}) during periods of nitrogen limitation. A similar observation on the biological refractivity of DON has also been presented by White *et al.* (1985).

The interchangeability of TP and TDP in the most effective indices of limitation probably reflects the rapid turnover of phosphorus. The good performance of TDP in the DIN:TDP index may be explained by the importance of alkaline phosphatase in mediating the availability of DOP to phytoplankton under conditions of phosphorus stress (Chrost & Overbeck, 1987).

This study indicates that phytoplankton nutrient limitation can be a highly variable phenomenon both within and between lakes. Furthermore, the ratios of DIN:TDP and DIN:TP were excellent predictors of limitation by nitrogen and phosphorus. Since these indices represent the bulk of N and P available to phytoplankton, it is possible that these indices will be equally effective in a variety of other lakes.

Acknowledgment

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