Responses of phosphorus limited Lake Michigan phytoplankton to factorial enrichments with nitrogen and phosphorus[1]

Claire L. Schelske
Great Lakes Research Division, The University of Michigan, Ann Arbor 48104

Edward D. Rothman
Department of Statistics

Eugene F. Stoermer and Mila A. Santiago
Great Lakes Research Division

Abstract

Factorial nutrient enrichment experiments were conducted with water containing natural phytoplankton assemblages collected from Grand Traverse Bay. Levels of phosphorus (phosphate) were 5, 15, 25, 40, and 60 μg P liter^{-1} and levels of nitrogen (nitrate) were 0.23, 0.84, and 1.12 mg N liter^{-1}. Nutrients were maintained at these levels by additions at 2- or 3-day intervals. Two different analyses of variance tests indicated that the responses were due to effects of phosphorus and not nitrogen. Production of chlorophyll *a* at the four highest levels of phosphorus was independent of concentration and could be predicted by

$$\ln \text{ Chl } a = XXX + 0.153t$$

where *XXX* was the ln of Chl *a* at day 4 and *t* was time in days. Phytoplankton growth, measured as chlorophyll production, fitted a Michaelis-Menten model when phosphorus concentrations were adjusted to correct for chemically measurable phosphorus apparently not available to phytoplankton. Assimilation numbers and species composition were not affected greatly by the nitrogen and phosphorus enrichments.

Phosphorus limits the growth of phytoplankton in Lake Michigan (Schelske and Stoermer 1972). Inputs of phosphorus have increased in the past 30 years, producing larger standing crops of algae and depleting the supply of silica required by phytoplankton assemblages dominated by diatoms (Schelske and Stoermer 1971). We predicted that continued silica depletion would limit the growth of diatoms and gradually cause a shift from assemblages dominated by diatoms to those dominated by blue-green and green algae, assuming that the effects of nitrogen were small, since silica was depleted by small additions of phosphorus.

Since the effects of nitrogen on growth of phytoplankton in Lake Michigan had not been investigated directly, we designed a factorial experiment to study the effects and interactions of nitrogen and phosphorus. Because the effects of nitrogen additions were small relative to those for phosphorus it was possible to use a Michaelis-Menten model to calculate growth kinetics as a function of phosphorus levels. Although the Michaelis-Menten model has been used in kinetic studies of nitrogen limited natural phytoplankton assemblages in the oceans (Thomas 1970; MacIsaac and Dugdale 1969; Eppley and Thomas 1969), which have recently been extended to interactions of light and nitrogen (MacIsaac and Dugdale 1972), we have not seen published studies on growth kinetics for natural assemblages limited by phosphorus.

We would like to acknowledge C. Matthews, T. Ladewski, W. Moore, L. Feldt, S. Fielek, N. McLean, K. White, L.-F. Hwang, T. Scullin, and P. Gimotty for their assistance.

Methods

Water from Grand Traverse Bay, Lake Michigan, was collected on 9 November

[1] Contribution No. 175 of the Great Lakes Research Division, The University of Michigan. Support provided by The University of Michigan Sea Grant Program and United States Atomic Energy Commission Contract C00-2003-21.

Table 1. Fixed nutrient levels of phosphorus, nitrate, and silica. Silica was adjusted to 0.74 mg SiO_2 $liter^{-1}$.

Levels	Phosphorus (µg P/liter)	Nitrate nitrogen (mg N/liter)
1	5	0.225
2	15	0.839
3	25	1.12
4	40	
5	60	

1971 from the Municipal Dock at Traverse City. Water was pumped from a depth of about 1 m into 20-liter rectangular translucent polyethylene containers, commonly known as Jerri-cans. Sixteen of these containers (17.5 × 33.8 × 47.5 cm high) were filled. The hose was alternated among containers about five times to randomize each sample. After filling was completed at 1400 hours, containers were transported to Ann Arbor. During the 6-hr trip, water temperatures increased about 1°, to 10°C. This gradual increase did not adversely affect the phytoplankton assemblage.

In the laboratory, the 16 containers were lettered A–P, treated with three levels of N and five of P (Table 1), and by 2300 hours placed in the growth chamber in two even rows. Positions were changed daily in a random fashion. Water temperatures were maintained at 8 to 9°C. Light was programed for a 12-hr day and a 12-hr night cycle. Incandescent lamps of about 200 lux were used for 30 min at the beginning and end of each day cycle, and illumination during the remaining 11 hr was provided by fluorescent lights of about 12,000 lux. The walls of the containers reduced this by about 40%.

To approximate steady state conditions, nutrients were measured at frequent intervals, usually 2 days, and kept at the concentration of the initial designated levels. Silica was maintained at a constant level in all treatments to ensure that growth of diatoms would not be limited by it.

Initial concentrations of nutrients, determined from samples taken the morning after the experiment was started, were different from those assumed in the preliminary experimental design. Silica averaged 0.74 mg $liter^{-1}$ instead of the 1.5 we had assumed would be present, based on known seasonal trends in the near-surface waters of Lake Michigan (Fee 1971). We had planned to run levels of nitrate-N, the principal form of combined nitrogen, of 0.20, 0.40 and 0.60 mg $liter^{-1}$. The lowest concentration, in unenriched lake water, averaged 0.225 ppm; but due to an error in calculating the amount of nitrate added, the two higher concentrations averaged 0.839 and 1.12 ppm. Nutrient solutions were made from analytical reagent grade chemicals: KH_2PO_4 for phosphorus, KNO_3 for nitrate, and $Na_2SiO_3 \cdot 9H_2O$ for silica.

Container 16, designated P, was used to evaluate the effects of trace metals and vitamins, when combined with the highest treatment levels of nitrogen and phosphorus, on phytoplankton growth. The effects on phytoplankton assemblages from Grand Traverse Bay were similar to those with phytoplankton assemblages from Lake Superior (Schelske et al. 1972); since these results do not apply directly here, they are not presented.

Statistical analyses were based on seven samplings from days 4–20 inclusive of the 30-day experiment; earlier samples were omitted due to an apparent lag in response by the assemblages and later ones because of increasing responses and associated difficulty in maintaining nutrient levels. Containers were shaken to mix the contents and subsamples taken for phytoplankton species composition, rates of ^{14}C fixation, specific conductance, pH, nutrients, and chlorophyll *a*. Water for silica, nitrate-N, and total soluble phosphorus (TSP) analyses was filtered through a HA Millipore filter, which was used for chlorophyll analysis. Water for chemical analyses was collected in polypropylene filter flasks and transferred to plastic test tubes, as we had found measurable amounts of silica dissolving from glass containers in as little as 1 hr. Containers were sampled alphabetically and then returned to the growth chamber. The procedure was repeated three times to obtain

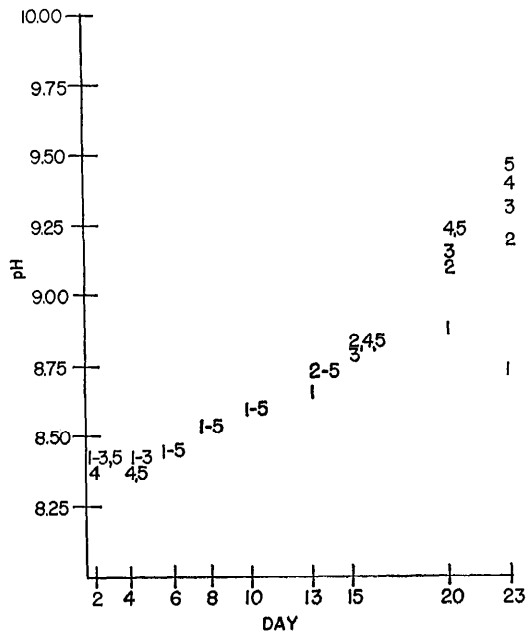

Fig. 1. Effect of phosphorus level on pH. Phosphorus levels in μg P liter^{-1} designated by numerals 1–5: 1 = 5, 2 = 15, 3 = 25, 4 = 40, 5 = 60. Measurements made on days shown on abscissa.

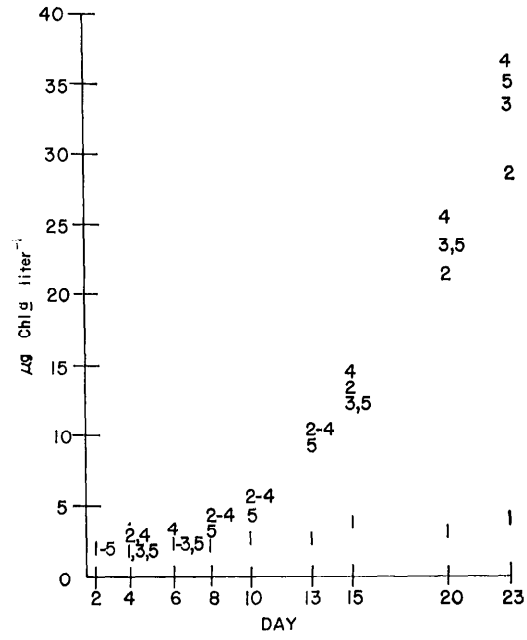

Fig. 2. Effect of phosphorus level on production of chlorophyll *a*. Symbols as in Fig. 1.

triplicate samples. Five or six persons participated in the initial sampling and measurement; each was assigned a specific task, enabling the entire sampling effort to be completed within 3 hr.

Water samples for silica and nitrate-N were analyzed within 4 hr of collection and concentrations of these nutrients were adjusted on the same day. Samples for TSP were concentrated overnight and digested for 1 hr at 110°C with persulfate; concentrations could not be adjusted until analyses were completed on the day after sampling.

A digital meter was used to measure pH. Chlorophyll *a* was measured with a fluorometer 24–26 hr after extraction with 90% acetone (Strickland and Parsons 1968). Samples for rates of carbon fixation were incubated in 60-ml glass-stoppered Pyrex bottles for 4 hr, filtered on 25-mm HA filters, and counted with a thin-window gas-flow Geiger counter. Nitrate-N, silica, and TSP were measured colorimetrically with an AutoAnalyzer (Schelske et al. 1972). A preliminary analysis of phytoplankton assemblages was made by counting 100 cells from each replicate sample taken on days 2 and 23 of the experiment; thus 300 cells were counted for the initial and final assemblages in each treatment.

Results

Statistical analyses presented below show that the most significant effects of the treatments tested were due to phosphorus and that those of nitrogen were relatively small. Data for pH, chlorophyll *a*, and rates of carbon fixation therefore will be considered only from the standpoint of phosphorus concentrations.

In the containers with the lowest level of phosphorus, pH increased less than for the four higher levels (Fig. 1). Little difference in pH among the five levels of phosphorus was apparent during the first 10 days of the experiment, but average values for all treatments increased from 8.4 to 8.6. After day 20, the pH values generally in-

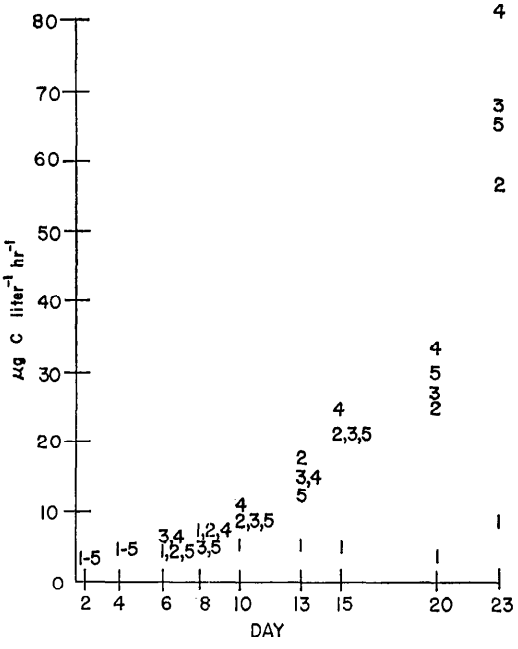

Fig. 3. Effect of phosphorus level on rate of carbon fixation. Symbols as in Fig. 1.

creased with increasing concentrations of phosphorus.

The values for pH up to day 10 are not high for Lake Michigan epilimnetic waters during summer when warm temperatures and photosynthetic activity reduce the free carbon dioxide (Schelske and Callender 1970). Values for days 20 and 23, above pH 9.0, are greater than normally encountered in Lake Michigan during summer (Schelske and Roth 1973; Great Lakes Res. Div. unpublished data). These high pH readings reflect increases in rates of carbon fixation and production of chlorophyll by phytoplankton, which after day 15 were also much larger than in the open waters of Lake Michigan and are characteristic of highly eutrophic areas of the Great Lakes including Green Bay, Saginaw Bay, and the western basin of Lake Erie (Schelske and Roth 1973; Glooschenko et al. 1973).

Concentrations of chlorophyll *a* increased at a greater rate in the four higher levels of phosphorus than at the lowest (Fig. 2). Differences in response due to phosphorus were obvious by day 10. These data also suggest an inhibition in chlorophyll *a* production at the highest level of phosphorus because, after day 6, concentrations were lower for level 5 than for level 4.

Rates of carbon fixation also were larger for the four higher levels of phosphorus than for the lowest, and carbon fixation too seemed to be inhibited slightly at level 5 (Fig. 3). In general, the results show the same trends as chlorophyll *a*.

Until day 23, there was no trend in variation among assimilation numbers (mg C mg Chl a^{-1} hr^{-1}). On that day, the assimilation number for the treatment of phosphorus level 1–nitrogen level 1 was lower than the other 14 treatments, but this seemed to be the only significant deviation. These results indicate that assimilation numbers may be a function of temperature (Eppley

Table 2. Three-way analysis of variance of the effects of nutrients on ln of chlorophyll a production.

Source	Sums of squares	df	Mean square	F-statistic
Mean	2,054.3	1	2,054.3	
N	0.0064984	2	0.0032492	2.61 *
P	2.7161	4	0.67903	546.0 †
t	24.729	6	4.1215	3,317.0 †
N,P‡	0.25903	8	0.032379	26.1 †
N,t‡	0.012536	12	0.0010446	0.841
P,t‡	1.9304	24	0.080432	64.7 †
N,P,t‡	0.12072	48	0.0025149	2.02 *
Error	0.26096	210	0.0012427	

* Significant at 0.10 level.
† Significant at 0.01 level.
‡ Interactions.

Table 3. Estimates of parameters for linear regression analysis of chlorophyll a production as a function of phosphorus level.

Phosphorus level	Slope estimate	Standard error of estimate	R-square
1	0.0525	0.00262	0.835
2,3,4,5	0.153	0.00121	0.980

1972; Glooschenko et al. 1973) and not of nutrients, as reported previously (Schelske and Roth 1973).

Statistical analyses of chlorophyll *a* production

Several statistical models have been developed for the effects of nutrients on production of chlorophyll. After examining several transformations, we transformed raw data to natural logarithms as the transformed data approximated a normal distribution with constant variances.

In a three-way ANOVA, time and phosphorus account for most of the variance (Table 2). Interactions between nitrogen and phosphorus and between phosphorus and time are also statistically significant at the 0.10 level, but account for a relatively small proportion of the total variance. From a practical standpoint, it seems obvious that increases in the production of chlorophyll with time can be largely attributed to the additions of phosphorus.

Simplified models of the results as functions of phosphorus and time are suggested. Data were fitted by least squares linear regression into two sets:

$$\ln X_{ijtk} = \alpha_j + \beta_j + \epsilon_{ijtk} \text{ for } j = 1; \quad (1)$$

$$\ln X_{ijtk} = \alpha_j + \beta_j + \epsilon_{ijtk} \text{ for } j = 2,3,4,5. \quad (2)$$

The first set consists of all data from the lowest level of phosphorus, while the second set consists of the remaining data from the other four levels (Table 3). Throughout this statistical discussion the subscripts i, j, t, k, unless specified otherwise, are indices for the nitrate level, phosphorus level, time, and replicate number.

The close fit of the data to equation 2 initially surprised us since it suggests that the production rate of chlorophyll is the same for phosphorus levels 2–5. However, the analysis of covariance discussed below was then conceived and carried out in two stages to obtain a more detailed description of the regression slope β in terms of the effects of different levels of phosphorus and nitrate.

*Table 4. Estimates of slopes from regression analysis of ln chlorophyll a production in replicate samples from 15 phosphorus-nitrate treatments.**

Phosphorus levels	Nitrate levels 1	2	3
1	0.0405	0.0416	0.0656
	0.0547	0.0466	0.0609
	0.0585	0.0443	0.0598
2	0.140	0.151	0.148
	0.143	0.147	0.143
	0.146	0.140	0.152
3	0.154	0.166	0.146
	0.157	0.154	0.148
	0.163	0.153	0.151
4	0.153	0.160	0.157
	0.155	0.161	0.154
	0.153	0.157	0.157
5	0.159	0.159	0.152
	0.154	0.164	0.149
	0.150	0.159	0.154

* R-square for 43 out of the 45 regressions $\geq$ 0.91.

The first stage was based on the linear regression of ln chlorophyll versus time, according to

$$\ln X_{ijtk} = [\mu_{ij} + \epsilon_{ijk}^{(1)}] + [\beta_{ij} + \epsilon_{ijk}^{(2)}] (t_t - \langle t \rangle) + \epsilon_{ijtk}^{(3)}. \quad (3)$$

A separate regression line was fitted to the data for each replication of the 15 nitrate-phosphorus treatments, resulting in three independent estimates of the intercept μ_{ij} and the slope β_{ij} for each treatment or 45 estimates (Table 4). The errors $\epsilon_{ijk}^{(1)}$ and $\epsilon_{ijk}^{(2)}$ represent the individual replicate deviations from the "true" intercepts and slopes, respectively; we believe these errors to be very small.

If ω_{ijk} denotes the least squares estimate of β_{ij} for the kth replication of the ijth treatment, algebraic manipulations of the formula for this estimate yield

Table 5. Two-way analysis of variance on slope estimates of growth rates.

Source	Sums of squares	df	Mean square	F-statistic	% point
Mean	0.79526	1	0.79526		
N	0.20320×10^{-4}	2	0.10160×10^{-4}	0.504	$F^{0.90}_{(2,30)} = 2.49$
P	0.073490	4	0.018372	910.0 *	$F^{0.995}_{(4,30)} = 4.62$
N,P†	0.86251×10^{-3}	8	0.10781×10^{-3}	5.35*	$F^{0.995}_{(8,30)} = 3.58$
Error	0.60560×10^{-3}	30	0.20187×10^{-4}		

* Significant at the 0.005 level.
† Interaction.

$$\omega_{ijk} = \beta_{ij} + \epsilon_{ijk}^{(2)} + \frac{\sum_t \epsilon_{ijtk}^{(3)} (t_t - <t>)}{\sum_t (t_t - <t>)^2} \quad (4)$$

so this shows ω_{ijk} receives two components of error from equation 3. The mean value of ω_{ijk} is, of course, β_{ij} since the means of the errors are assumed to be zero.

The second stage of the analysis of covariance consisted of a two-way analysis of variance which treated the slope estimates from equation 3 as the dependent variable. This model of growth rate, β_{ij}, portions effects due to the nitrate level, phosphorus level, and the interaction between the two:

$$\omega_{ijk} = \mu + \alpha_i + \delta_j + \gamma_{ij} + v_{ijk}. \quad (5)$$

Here v_{ijk} is the error term derived from pooling the errors in equation 4. F-tests of the three classes of effects revealed that the phosphorus and interaction effects were significant at the 0.005 level but that the nitrate effect was not significant at the 0.10 level (Table 5). This corresponds to our earlier conclusion that although nitrate effects were statistically significant (Table 2) they are not practically significant.

Growth kinetics

In attempting to fit our data for linear regression slope estimates (Table 4) to the Michaelis-Menten model, we discovered by plotting the replicate values for S_j/β_j versus S_j, a linear form of the Michaelis-Menten equation, that the points for the four higher levels of phosphorus fitted a straight line, but that the points for the lowest level did not (Fig. 4). This plot indicated that either the slope estimates or the phosphorus concentrations for the lowest level were too large to fit the model. Because our previous analysis of the chlorophyll data indicated little uncertainty in the slope estimate, we assumed the phosphorus values were too large, a tenable assumption since we measured TSP and not a fraction more available to algae. Using techniques developed for nonlinear least squares (Hartley 1961), we then determined the maximum likelihood estimates for the Michaelis-Menten parameters (Table 6). Results indicated that chlorophyll production could be described by the following equation:

$$\beta_j = \frac{\beta_{\max}(S_j - X)}{K_s + (S_j - X)} \quad (6)$$

where β_j is the slope or growth rate at the jth level of phosphorus; $\beta_{\max}$ is the maximum growth rate; K_s is the half saturation constant or the concentration where β equals ½ $\beta_{\max}$; S_j is the phosphorus concentration (μg P liter^{-1}) at the jth level; and X is the constant (μg P liter^{-1}) subtracted from all phosphorus levels. The equation including the calculated parameters (Table 6) is:

$$\beta_j = \frac{0.1596\ (S_j - 4.56)}{0.899 + (S_j - 4.56)}. \quad (7)$$

The largest relative error associated with these parameters is that for K_s which has a

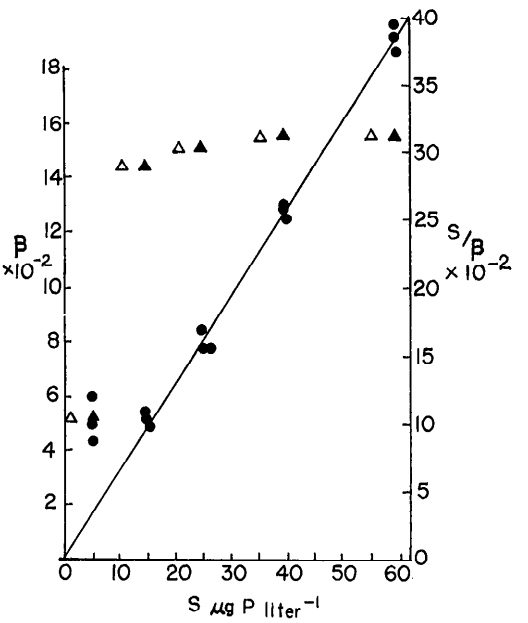

Fig. 4. Michaelis-Menten plots of chlorophyll data as slopes (β) of regression lines vs. phosphorus concentrations (S). Solid circles are raw data plotted as S/β vs. S and triangles are slopes estimates (β) plotted vs. phosphorus concentrations (S). Solid triangles are raw data and open triangles are data corrected for phosphorus unavailable to phytoplankton.

standard deviation of about 13% of the mean. Standard deviations for β_{max} and X are much smaller and are less than 1.0% of the mean (Table 6). To obtain better estimates of K_s would require replicates at intermediate concentrations between levels 1 and 2. A confidence interval for β_{max} was not determined since the marginal likelihood of the sample was not normally distributed.

Phytoplankton assemblage

It is obvious, even with limited population estimates, that the phytoplankton assemblage was affected by the treatments (Table 7). Only the major effects due to phosphorus will be considered here; additional study may show significant effects at the population level due to nitrogen and interactions between levels of nitrogen and phosphorus as indicated by ANOVA results (Tables 2 and 5).

Table 6. Maximum likelihood estimates and standard deviations of Michaelis-Menten parameters.

Parameter	Calculated value	Standard deviation
β_{max}	0.1596	0.00199
K_s	0.899	0.119
X	4.56	0.367

At the time of treatment, the phytoplankton assemblage was dominated by 10 taxonomic entities listed in Table 7 and by *Gleocystis* sp. and *Oocystis* sp., two green algae, whose population frequencies averaged 3.8 and 2.6% of the initial populations. These 12 entities comprised more than 90% of the initial assemblage. By the end of the experiment *Gleocystis* averaged about 3.0%, but *Oocystis* declined to 0.3% of the total counts for all treatments. These two species are not included in Table 7 because counts were not random within the treatments.

Two species of blue-greens, *Anabaena flos-aquae* (Lyng.) Breb. and *Anacystis cyanea* Drouet and Daily, comprised about 20% of the initial assemblage, but both declined during the treatment. No counts of

Table 7. Relative abundance of taxonomic entities in phytoplankton assemblage at the beginning and end of the experiment. Data are percentages of total cell counts except line labeled "assemblage," which indicates average relative changes in total cell counts. Final counts are samples from day 23.

	Phosphorus levels			
	1		2,3,4,5	
	Initial	Final	Initial	Final
Anabaena flos-aquae	5.0*	<0.1†	5.0*	<0.1†
Anacystis cyanea	15.6*	1.0†	15.6*	1.0†
Asterionella formosa	19.3	10.7	18.1	2.0
Fragilaria crotonensis	18.6	46.2	20.0	37.1
Nitzschia acicularis	0.2	1.0	0.2	5.1
Stephanodiscus minutus	1.2	0.8	1.2	38.0
S. subtilis	4.4	0.7	4.3	2.9
Synedra filiformis	0.6	5.2	0.3	2.7
Tabellaria fenestrata	0.4	5.4	1.6	1.0
Flagellates	21.6	5.0	18.5	1.9
Assemblage	1.0	4.3	1.0	28.3

* Average of all initial samples, levels 1-5.
† Average of all final samples, levels 1-5.

Anabaena were recorded and *Anacystis* was only 1.0% of the final assemblage. These species were also obviously not randomly distributed in the small samples counted from each treatment, so the total counts for the initial and final treatments were averaged (Table 7).

Three species appeared to be affected directly by the phosphorus treatments. In the high levels of phosphorus, *Stephanodiscus minutus* Grun. et Cleve and Möll. increased from about 1.0% of the counts to 38% at the end of the experiment (Table 7). *Nitzschia acicularis* (Kütz.) Wm. Smith also increased a relatively large amount in the high levels of phosphorus, increasing in relative abundance about 25-fold, but comprising only 5% of the final assemblage. Only *Asterionella formosa* Hass. decreased significantly in relative abundance as the result of phosphorus treatment; it also seems likely that this species decreased in the low level of phosphorus.

Two species increased in relative abundance in all treatments. *Fragilaria crotonensis* Kitton became one of the major components of the final assemblage. *Synedra filiformis* Grun. also increased in relative abundance in all treatments; the relative increase was much greater than for *F. crotonensis* because this species initially comprised less than 1% of the assemblage.

Interpretation of these data on relative abundance should not be considered as estimates of population densities. Based on chlorophyll data, it seems reasonable to conclude that standing crops in all treatments increased during the experiment (Fig. 2). Also cell counts corrected for area counted increased fourfold in the lowest level of phosphorus and 28-fold in the other four levels (Table 7). A decrease in relative abundance, therefore, does not necessarily imply a decrease in standing crop. On the other hand, an increase in relative abundance does mean an increase in standing crop (i.e. an increase in the number of cells per unit volume). It is also apparent that increases in relative abundance in the high phosphorus levels represent a much greater increase in the population than would be represented by a similar increase in relative abundance in the low phosphorus treatment, due to the larger increases in cell counts and chlorophyll in the high phosphorus treatments.

Discussion

We expected these results confirming our previous conclusions that phosphorus limits phytoplankton growth in Lake Michigan (Schelske and Stoermer 1971, 1972). We did not expect nitrogen to be limiting, but did expect effects due to inhibition and larger interactions with phosphorus (Tables 2 and 5) since nitrate-N levels ranged from 0.23–1.12 mg liter^{-1}.

Our results agree with the conclusions of Ketchum (1939), who studied the absorption of phosphorus and nitrogen by *Nitzschia closterium*, particularly that growth rate is independent of phosphate and nitrate concentration in the medium, except for phosphorus concentrations <16 μg P liter^{-1} where it decreased. In our experiments, growth decreased at concentrations <15 μg P liter^{-1}, or 10 if we correct for the apparent unavailable phosphorus. Ketchum used nitrate-N concentrations ranging from 0.050–0.50 mg liter^{-1}.

Nitrate may not have been important in our experiments, because of the relatively high concentrations in the upper Great Lakes. Concentrations of epilimnetic nitrate-N are greatest in Superior—the most oligotrophic lake—and are inversely related to trophic state in Superior, Huron, and Michigan (Schelske and Roth 1973). Summer epilimnetic concentrations for open waters are not considered to be limiting and range from 0.25 mg liter^{-1} in Lake Superior to 0.10 in Lake Michigan. Small yearly fluctuations of nitrate are also associated with oligotrophy, since annual fluctuations in nitrate concentration with phytoplankton growth are directly related to trophic state in the three upper lakes (Schelske in press; Dobson 1974). The largest fluctuations in nitrate are found in Lake Erie where phosphorus concentrations are greatest. One would conclude that nitrate additions to the three upper Great Lakes would have little

effect on phytoplankton growth in the presence or absence of phosphorus. Nitrate increased in Lake Washington after sewage was diverted from the lake; this increase in nitrate was accompanied by decreases in phosphorus concentrations and by smaller standing crops of algae, providing evidence that large phosphorus supplies can produce nitrogen limiting conditions for phytoplankton in freshwater ecosystems (Edmondson 1970).

Phytoplankton growth, as measured by chlorophyll, could be predicted precisely with slope estimates from linear regression models. The equation for the four highest phosphorus levels (Table 3) is

$$\ln \text{ Chl } a = XXX + 0.153t, \qquad (8)$$

where XXX is the ln of chlorophyll concentration at day 4 and t is the time in days. It was obvious that slopes for combinations of nitrogen levels and the four higher phosphorus levels (Table 4) did not vary greatly from the slope used in equation 8. A different slope was obtained for the lowest phosphorus level (Tables 3 and 4).

Growth kinetics could be predicted with a Michaelis-Menten model (equations 6 and 7) but only after measured phosphorus concentrations were reduced by 4.56 μg P liter^{-1} (Table 6). The original phosphorus levels, measured as total soluble phosphorus, undoubtedly included some quantity of phosphorus unavailable to phytoplankton. The small value for the half-saturation constant, K_s, of the order of 1.0 μg liter^{-1} (Table 6) is particularly significant because it indicates that phosphorus limited phytoplankton would grow rapidly at concentrations lower than ordinarily measured by routine methods for soluble reactive phosphate (Strickland and Parsons 1968). The presence of some phosphorus measured as total soluble phosphate that was not available for phytoplankton growth is not surprising since the method measures all forms of phosphorus. Paasche (1973) found that uptake of silica by marine diatoms fitted Michaelis-Menten kinetics only after a correction had been made for chemically reactive silicate that apparently was not utilized in growth.

Our study was concerned with growth kinetics in relation to supplies of added phosphate and not with forms or amounts of phosphate available to phytoplankton. Total additions of phosphorus during the first 20 days of the experiment averaged 1.8, 12.7, 25.9, 33.1 and 31.9 μg P liter^{-1} for phosphorus levels 1–5 respectively. It is assumed that the added phosphorus was present in the water as phosphate, a form readily available to phytoplankton, until taken up by the plankton. As shown by Lean (1973) one would expect a rapid turnover of added inorganic phosphate into plankton and excreted organic phosphorus. He calculated that the inorganic phosphate concentration was 0.09 μg P liter^{-1}, 0.21% of the total phosphorus, supporting the conclusion by Rigler (1968) that concentrations of soluble reactive phosphate measured chemically are much greater than the actual concentrations of inorganic phosphate. These data and our experimental results suggest the need for other than routine chemical methods for studying phosphorus uptake kinetics in lake water.

The adjustment of phosphorus levels by X (equations 6, 7) may be questioned because of our lack of information on forms of phosphorus. The observed effect may also be attributed to different phytoplankton assemblages resulting from phosphorus enrichment. Preliminary results indicate that the phytoplankton assemblage at the lowest level of phosphorus, the level that did not originally fit the Michaelis-Menten model (Fig. 4), was different from the assemblage at the other four levels (Table 7). It can be seen, however, from the plots of S_j/β_j versus S_j in Fig. 4 that deleting the lowest level would have little if any effect on the values of K_s or β_{max}.

It is also obvious that the Michaelis-Menten parameters obtained in nutrient enrichment experiments can be a function of the nutrient conditions, the phytoplankton assemblages, or both. In container P, treated with the highest level of nitrogen and phosphorus, as well as trace elements, vitamins, and a chelator, the growth rate of phytoplankton was twice as large as the

other containers. The assemblage was dominated by a small species of *Stephanodiscus* not abundant in the others (Table 7). Because of this variation in the phytoplankton assemblage with nutrient conditions, the causal relationships are not clear. It should be emphasized that this result includes effects of accessory growth factors and trace metals which could not be evaluated with our experimental design.

Other studies on growth kinetics of natural phytoplankton assemblages including analysis of species composition are not available. Results of experiments on nitrogen limited marine phytoplankton (MacIsaac and Dugdale 1969) did not include the study of species composition. Our experimental design apparently eliminates problems with "luxury consumption" of phosphorus which originally seemed to obviate the application of Michaelis-Menten kinetics in the analysis of plankton dynamics (Eppley and Strickland 1968) unless phosphorus was based on the amount per cell (Fuhs 1969).

Our data indicate that growth kinetics of phosphorus limited natural phytoplankton assemblages can be described by the Michaelis-Menten model. One set of parameters described growth kinetics over a wide range of phosphorus conditions with no great changes in the relative abundance of the dominant phytoplankton populations. In other studies of freshwater systems, primary productivity and standing crops of phytoplankton increased with increased nutrient supplies without greatly altering the qualitative character of the phytoplankton assemblages (Parsons et al. 1972; Schelske et al. 1972; Stoermer et al. 1972). Stoermer et al. (1972) also studied Grand Traverse Bay. Apparently with great perturbations of nutrient conditions, such as those in container P, species composition is altered, as has been suggested by studies of phytoplankton in Lake Michigan (Stoermer and Yang 1970; Holland 1968; Holland and Beeton 1972). Carpenter and Guillard (1971) have shown intraspecific differences in kinetic parameters for marine phytoplankton, with the highest values for clones isolated from nutrient rich estuarine areas. These data suggest that K_s values and maximum growth rates for the assemblages or populations responding to nutrient enrichment in perturbed nearshore areas are greater than those for less perturbed areas and provide additional evidence that environmental disturbance in the Great Lakes can be assessed from qualitative studies of phytoplankton.

References

CARPENTER, E. J., AND R. L. GUILLARD. 1971. Intraspecific differences in nitrate half-saturation constants for three species of marine phytoplankton. Ecology **52**: 183–185.

DOBSON, H. 1974. A summary and comparison of nutrients and related water quality in Lakes Erie, Ontario, Huron and Superior. J. Fish. Res. Bd. Can. **31**: In press.

EDMONDSON, W. T. 1970. Phosphorus, nitrogen, and algae in Lake Washington after diversion of sewage. Science **169**: 690–691.

EPPLEY, R. W. 1972. Temperature and phytoplankton growth in the sea. Fish. Bull. **70**: 1063–1085.

———, AND J. D. H. STRICKLAND. 1968. Kinetics of marine phytoplankton growth. Adv. Microbiol. Sea **1**: 23–62.

———, AND W. H. THOMAS. 1969. Comparison of half-saturation constants for growth and nitrate uptake of marine phytoplankton. J. Phycol. **5**: 375–379.

FEE, E. J. 1971. A numerical model for the estimation of integral primary production and its application to Lake Michigan. Ph.D. thesis, Univ. Wis., Milwaukee. 169 p.

FUHS, G. W. 1969. Phosphorus content and rate of growth in the diatoms *Cyclotella nana* and *Thalassiosira fluviatilis*. J. Phycol. **5**: 312–321.

GLOOSCHENKO, W. A., J. E. MOORE, AND R. A. VOLLENWEIDER. 1973. Chlorophyll *a* distribution in Lake Huron. Proc. 16th Conf. Great Lakes Res. (Int. Assoc. Great Lakes Res.) **1973**: 40–49.

HARTLEY, H. O. 1961. The modified Gauss-Newton method for the fitting of non-linear regression functions by least squares. Technometrics **3**: 269–280.

HOLLAND, R. E. 1968. Correlation of *Melosira* species with trophic conditions in Lake Michigan. Limnol. Oceanogr. **13**: 555–557.

———, AND A. M. BEETON. 1972. Significance to eutrophication of spatial differences in nutrients and diatoms in Lake Michigan. Limnol. Oceanogr. **17**: 88–96.

KETCHUM, B. H. 1939. The absorption of phosphate and nitrate by illuminated cultures of *Nitzschia closterium*. Am. J. Bot. **26**: 399–407.

LEAN, D. R. S. 1973. Phosphorus dynamics in lake water. Science **179:** 678–680.

MACISAAC, J. J., AND R. C. DUGDALE. 1969. The kinetics of nitrate and ammonia uptake by natural populations of marine phytoplankton. Deep-Sea Res. **16:** 45–57.

———, AND ———. 1972. Interactions of light and inorganic nitrogen in controlling nitrogen uptake in the sea. Deep-Sea Res. **19:** 209–232.

PAASCHE, E. 1973. Silicon and the ecology of marine plankton diatoms. 2. Mar. Biol. **19:** 262–269.

PARSONS, T. R., K. STEPHENS, AND M. TAKAHASHI. 1972. The fertilization of Great Central Lake. 1. Effect of primary production. Fish. Bull. **70:** 13–23.

RIGLER, F. H. 1968. Further observations inconsistent with the hypothesis that the molybdenum blue method measures orthophosphate in lake water. Limnol. Oceanogr. **13:** 7–13.

SCHELSKE, C. L. In press. Silica and nitrate depletion as related to rate of eutrophication in Lake Michigan, Lake Huron, and Lake Superior. INTECOL Symp. "Land-water interactions," Leningrad, August 1971.

———, AND E. CALLENDER. 1970. Survey of phytoplankton productivity and nutrients in Lake Michigan and Lake Superior. Proc. 13th Conf. Great Lakes Res. (Int. Assoc. Great Lakes Res.) **1970:** 93–105.

———, L. E. FELDT, M. A. SANTIAGO, AND E. F. STOERMER. 1972. Nutrient enrichment and its effect on phytoplankton production and species composition in Lake Superior. Proc. 15th Conf. Great Lakes Res. (Int. Assoc. Great Lakes Res.) **1972:** 149–165.

———, AND J. C. ROTH. 1973. Limnological survey of Lakes Michigan, Superior, Huron, and Erie. Mich. Univ. Great Lakes Res. Div. Publ. 17. 108 p.

———, AND E. F. STOERMER. 1971. Eutrophication, silica depletion, and predicted changes in algal quality in Lake Michigan. Science **173:** 423–424.

———, AND ———. 1972. Phosphorus, silica, and eutrophication of Lake Michigan, p. 157–171. *In* G. E. Likens [ed.], Nutrients and eutrophication. Spec. Symp. 1, Am. Soc. Limnol. Oceanogr.

STOERMER, E. F., C. L. SCHELSKE, M. A. SANTIAGO, AND L. E. FELDT. 1972. Spring phytoplankton abundance and productivity in Grand Traverse Bay, Lake Michigan, 1970. Proc. 15th Conf. Great Lakes Res. (Int. Assoc. Great Lakes Res.) **1972:** 181–191.

———, AND J. J. YANG. 1970. Distribution and relative abundance of dominant plankton diatoms in Lake Michigan. Mich. Univ. Great Lakes Res. Div. Publ. 16. 64 p.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1968. A practical handbook of seawater analysis. Bull. Fish. Res. Bd. Can. 167. 311 p.

THOMAS, W. H. 1970. Effect of ammonium and nitrate concentration on chlorophyll increases in natural tropical Pacific phytoplankton populations. Limnol. Oceanogr. **15:** 386–394.

Submitted: 7 November 1973
Accepted: 15 February 1974