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Nutrient Enrichment Experiments in Tropical Great Lakes Malawi/Nyasa and Victoria

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ABSTRACT. *Enrichment experiments with and without zooplankton (> 50 μm) removed were conducted in Lake Malawi during three seasons (stratified rainy, deep mixing, and stratified dry) and demonstrated that when light is adequate phytoplankton in containers quickly become nutrient deficient. The response to enrichment was assessed using chlorophyll* a, *photosynthesis, particulate stoichiometric ratios, PO_4 turnover and N and P debt assays. The response to nitrogen (N), phosphorus (P), and iron (Fe) enrichments indicated that although N is the nutrient that becomes deficient most consistently, P deficiency is common as well. When Fe was added with N and P, the response by chlorophyll was four times the response to N and P without Fe. This suggests that, after N and P, Fe is the next most limiting nutrient in Lake Malawi. Light was a factor controlling phytoplankton growth* in situ *during the deep mixing season, and grazer removal experiments demonstrated that zooplankton > 50 μm are important in modifying the response of algae to light and nutrients. In Lake Victoria, experiments demonstrated that phytoplankton were primarily light-limited during the early-stratified season. Increased light levels resulted in N deficiency. Fe additions stimulated N uptake in both Lake Victoria and Lake Malawi and N_2 fixation in Lake Victoria.*

INDEX WORDS: *Lake Malawi, nutrient enrichment, zooplankton, nitrogen limitation, phosphorus limitation, iron limitation.*

INTRODUCTION

In their extensive reviews addressing the impact of accelerating land-use change on the N-cycle of tropical aquatic ecosystems, Downing *et al.* (1999) and Hecky *et al.* (2003) conclude that disturbances to pristine tropical lands will lead to increased primary production in fresh waters and potentially large changes in tropical freshwater communities. This change has already happened in tropical Lake Victoria, the world's second largest freshwater lake. Primary productivity doubled from the 1960s to the 1990s and N_2 fixing cyanobacteria have replaced the diatom, *Aulacoseira,* as the dominant phytoplankton (Mugidde 1993, Hecky 1993, Kling *et al.* 2001). The other African Great Lakes, Lake Tanganyika and Lake Malawi, are still oligotrophic. These deep lakes are dominated by diatoms, including *Aulacoseira*, *Stephanodiscus*, *Cyclostephanos,* and *Nitzschia*, during biomass maxima, with cyanobacteria accumulating only during the strongly stratified seasons. However, both are beginning to show signs that increasing human populations and associated land use are causing changes to the rivers and lakes. In Lake Tanganyika, primary production rates in water near the major city of Bujumbura in the north are 5 times the rates at the smaller centers of Kigoma and Mpulungu in the

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south (Sarvala *et al*. 1999) and higher than they were 25 years ago (Hecky and Fee 1981). In Lake Malawi, C, Fe, N and P concentrations in the Linthipe and Songwe, two major tributaries, have likely increased 5 to 10 fold from their pristine condition (Hecky *at al*. 2003). The consequences of this increased loading will depend on the degree to which the phytoplankton of these lakes are nutrient limited and by which nutrient.

Phosphorus regulation has been very successful in the recovery of Laurentian Great Lakes from eutrophication (Charlton *et al.* 1993). North American Great Lakes and north temperate lakes, in general, are P limited (Schindler 1977). Few studies have addressed the question of nutrient limitation in tropical great lakes (Hecky and Kilham 1988). Measurement of nitrate and phosphate concentrations by Talling (1966) led him to predict that nitrate might become limiting for algal growth in Lake Victoria. Enrichment experiments conducted by Moss (1969) in some Central African waters including Lake Malawi demonstrated that the addition of nitrate alone stimulated chlorophyll *a* and cell numbers consistently although in Lake Malawi the strongest response was to the combined addition of N, P, and sulfur (S). Lehman and Branstrator (1993) examined the effect of nutrient enrichment on waters from in-shore and offshore stations in Lake Victoria. They found that enrichment of inshore water with nutrients (either N alone or in combination with P and S) did not increase total chlorophyll *a.* The addition of nutrients did stimulate total chlorophyll *a* when added to water from their offshore station. Lehman and Branstator (1993) also examined the effect of manipulating the zooplankton concentration on chlorophyll *a*. Even at four times the ambient densites of grazers, no significant effect on chlorophyll *a* was observed at either their inshore or offshore station. They concluded N was the most limiting nutrient to the phytoplankton biomass of Lake Victoria, but light may be the ultimate control on algal growth (Mugidde 1993). Jarvinen *et al.* (1999) examined the stoichiometry of particulate nutrients and conducted nutrient enrichment bioassays in Lake Tanganyika. Similar to what was observed in Lake Malawi (Guildford *et al.* 2000, Guildford and Hecky 2000, Guildford *et al.* this study), they reported particulate C:N and C:P ratios indicative of moderate N and P deficiency and in their enrichment experiments observed a significant response to the combined addition of N and P but little response to either nutrient added alone. Given the already eutrophic condition of Lake Victoria and the threat of eutrophication in Lake Malawi and Lake Tanganyika, it is important to examine these remaining pristine tropical great lakes more closely to determine what factors are at present controlling algal growth and to determine what factors pose a threat to these lakes. An important issue is how applicable the North American experience with P control can be to these tropical great lakes.

It is not expected that lakes in general would be Fe limited as terrestrial material is Fe-rich, and most lakes have DOC concentrations in the range expected to chelate Fe and other micronutrients and maintain their availability to phytoplankton (Auclair 1995). However, because Lake Malawi is such a large deep lake with a long water residence time and low DOC (Ramlal *et al.* 2003), it may be possible that Fe could be limiting at times. Fe deficiency was demonstrated in Lake Erie during a period of strong thermal stratification (Twiss *et al.* 2000). Fe is a key cofactor in the enzymes and proteins involved in photosynthesis and N assimilation. Fe deficiency results in an inability to form functional electron transport components (Falkowski and Raven 1997). Nitrate and nitrite reductase have high Fe requirements and nitrogenase, the enzyme needed for N_2 assimilation, has an even higher Fe requirement (Price and Morel 1994). *Anabaena*, a N_2 fixing cyanobacteria, has been reported to have an Fe quota of 600 μmol Fe/ mol C which is 10 to 100 times greater than non-N_2 fixing cyanobacteria and eucaryotic algae (Price and Morel 1994). Fe limitation has been demonstrated in eutrophic lakes dominated by N_2 fixing cyanobacteria (Murphy *et al.* 1976, Wurtsbaugh and Horne 1983). Thus it is appropriate to test for Fe limitation in hypereutrophic Lake Victoria. Carbon limitation of algal growth has been demonstrated in hypereutrophic lakes (Schindler and Fee 1973), and Ramlal *et al.* (2001) observed diurnal depletion of DIC in Lake Victoria in the presence of high concentrations of cyanobacteria, thus the effect of C enrichment in Lake Victoria was also examined.

In this study, nutrient enrichment and grazer removal experiments conducted in Lake Malawi during 1997 to 1999 and nutrient enrichment experiments conducted in Lake Victoria during 1998 were used to assess factors controlling algal biomass and growth. Algal biomass was monitored by observing the response of chlorophyll *a* and particulate C to the various treatments. Information about growth rate limitation is inferred from measures of nutrient status (Healey and Hendzel 1979). Individual measures of nutrient status can give con-

flicting results (Hameed *et al*. 1999). To overcome this in the experiments, several measures were used (Healey and Hendzel 1980), and they were used in a relative sense to determine if nutrient status changes in a treatment relative to the control. In oligotrophic Lake Malawi, the objective is to determine what nutrient (N, P or Fe) or other factors (light or grazing) are controlling algal biomass. In hypereutrophic Lake Victoria, nutrients (C or Fe) or light are examined as factors controlling the growth of N_2 fixing cyanobacteria which now dominate the phytoplankton community (Kling *et al.* 2001).

METHODS

Study Areas

Lake Malawi lies in southern, central Africa between 9°30′S and 14°30′S. It is located in the western arm of the East African system of Rift Valleys. The surface area is 28,800 km^2 with a mean width of 60 km, mean depth of 292 m, maximum depth of 700 m and volume of 8,400 km^3 (Patterson and Kachinjika 1995). Lake Victoria is located on the plateau between the Eastern Rift Valley and the Western Rift Valley of East Africa spanning from 0°30′N to 2°30′S. Lake Victoria is roughly circular in shape with a surface area of 68,800 km^2, mean depth of 40 m, and volume of 2,760 km^3 (Spigel and Coulter 1996). In Lake Victoria samples were collected from an offshore station south of Bugaia Island (Station BG; 0°3.729′S, 33°16.249′E; depth 60 m) and from an inshore station located in Napoleon Gulf (Station NPL, 0°24.350′N, 33°15.183′E). In Lake Malawi, samples were collected from Station 928 (13°42.80′S, 34°40.45′E, depth 150 m). Both Stations 928 in Lake Malawi and Bugaia Station in Lake Victoria were chosen because they are relatively close to laboratories at Senga Bay and Jinja respectively, yet far enough from the mainland that they are representative of the deep, pelagic area of the lake.

Study Design

Six experiments were conducted in Lake Malawi spanning the time period from January, 1997, to July, 1999, allowing observing the response to nutrient enrichment and grazer removal during all three seasons (deep mixing, June to September, early stratified dry season, October to December, and deep stratified rainy season). In Lake Victoria the experiments were conducted only during the early stratified season of November, 1998.

For each set of experiments in Lake Malawi, water was collected from the upper mixed layer (20 to 30 m) using Niskin bottles. In Lake Victoria, water was collected with a submersible pump from 2.4 m at Bugaia, the off-shore station, and 0.75 m at Napoleon Gulf, the near-shore station. Enough water was collected to have 10 L for each of the two replicates for each treatment. In Lake Malawi, water was passed through a 200 μm nylon screen to remove large grazers. Details about the treatments and conditions in the lake at the time of sampling are given in Table 1. In Lake Malawi, temperature profiles were made using a Seabird CTD profiler. In Lake Victoria a YSI temperature probe and meter were used. Light attenuation was measured with a Li-Cor LI-185 underwater quantum sensor (flat plate, cosine-corrected collector) in the lakes. Mean water column light intensity was calculated from the mixed depth at the time of sampling and light extinction coefficient (Guildford *et al.* 2000). Lake water was kept in dark insulated boxes from the time of collection until replicates were set outdoors for incubation with the exception of when manipulations were performed under low light conditions in the laboratory. Water was usually obtained by noon and the various treatments administered and the replicate 10-L samples for each treatment were set out in acid-cleaned 10-L polyethylene containers. In Malawi, experimental containers were incubated floating in a large open polyethylene tank situated outside the laboratory. In Uganda, the experimental containers were incubated in open insulated boxes. In Malawi the incubation tank remained uncovered; however, the tank was shaded by a large tree. In Victoria it was necessary to cover the incubation boxes with one layer of black nylon window screen. The containers with the low light treatment were covered with six layers of screen. Sub-samples for biomass, nutrient status and photosynthesis were taken at the beginning of each incubation, at 24 h and after 48 h.

Laboratory Procedures

Measurements to determine the nutritional status of algae consisted of four seston composition ratios and three metabolic indicators. The seston ratios were carbon:nitrogen (C:N), carbon:phosphorus (C:P), nitrogen:phosphorus (N:P), and carbon:chlorophyll *a* (C:chl). Particulate N and P, and chlorophyll *a*, change in relation to C in algae that are deficient in nutrients and /or light (Healey 1975). The metabolic nutrient status indicators were

TABLE 1. Initial conditions and treatments for experiments. Exp = experiment; S.D. = Secchi disk; Ext = vertical light extinction coefficient; Z_{max} = maximum depth; Z_{mix} = mixed layer depth; Z_{sam} = depth sampled; Temp Surf = temperature at the surface; Mean Light Lake = mean water column light intensity as a percent of surface irradiance; 928 = station 928 in Lake Malawi; BG = Bugaia station; NPL = Napoleon Gulf station.

Exp	Date	Lake	Season	S.D. m	Ext m^{-1}	Z_{max} m	Z_{mix} m	Z_{sam} m	Temp Surf °C	Mean Light Lake[1]	Treatments
1	28 Jan 97	Malawi 928	Rainy	11.5	0.13	150	55	20	27.5	15.2	(1) Control, no treatment (2) Grazers > 50 µm removed (3) N, P (NH_4Cl_3 and KH_2PO_4 final conc. 15 µM N and 5 µM P) (4) Grazers > 50 µm removed and N, P
2	19 Jun 97	Malawi 928	Windy	11.8	0.14	125	75	20	25.9	9.7	(1) Control (2) Grazers > 50 µm removed (3) N, P (4) Grazers > 50 µm removed and N, P
3	28 Nov 97	Malawi 928	Dry	15.8	0.11	137	25	20	29.5	33.1	(1) Control (2) Grazers > 50 µm removed (3) N, P (4) Grazers > 50 µm removed and N, P
4	22 Oct 98	Malawi 928	Dry	15.5	0.11	150	22	25	25.5	36.6	(1) Control (2) Grazers > 50 µm removed (3) N, P (4) Grazers > 50 µm removed and N, P (5) Fe ($FeCl_3$ final conc. 11 µM and EDTA final conc. 11 µM)
5	27 Oct 98	Malawi 928	Dry	16.5	0.07	150	38	30	25.3	34	(1) Control (2) N, P, Si ($NaSiO_3$ final concentration 0.18 mM) (3) Fe ($FeCl_3$ final conc. 5 µM and EDTA final conc. 5 µM) (4) N, P, Fe, Si
6	2 Jul 99 928	Malawi	Windy	15.0	0.12	150	75	25	24.5	11.1	(1) Control (2) N, P (3) Fe
7	3 Nov 98	Victoria BG	Early Stratified	2.4	0.56	60	12.5	2.4	25.8	14.4	(1) Control (2) C ($NaHCO_3$ final conc. 0.15 mM) (3) Fe ($FeCl_3$ final conc. 11 µM and EDTA final conc. 11 µM) (4) Low Light (1.5% o f surface irradiance)
8	4 Nov 98	Victoria NPL	Early Stratified	1.1	0.92	17	5	0.75	27.7	22	(1) Control (2) C (3) Fe

[1]Light in the incubation tanks was 50% of incident light in Lake Malawi and 25% in Lake Victoria.

nitrogen debt (N debt) and phosphorus debt (P debt). PO_4 turnover time was measured using ^{33}P-PO_4 in Experiments 1 and 3. Algae growing at low growth rates because they are deficient in either N or P will take up more of that nutrient per unit chlorophyll *a* than algae not deficient in that nutrient (Healey and Hendzel 1980). This uptake by N or P deficient algae was termed "N or P debt" by Healey and Hendzel (1980). Similarly, high demand for PO_4 relative to supply will cause faster phosphate turnover times (Lean and Nalewajko 1979). Particulate C, N and P samples were filtered through pre-ignited GF/F filters and were kept frozen until analyzed (Stainton *et al.*1977). For the N debt assay, 100 mL of unfiltered sample was enriched with ammonium chloride to yield a final concentration of 5 µM. Ammonium was measured on triplicate sub-samples (Stainton *et al*. 1977) at the beginning and end of incubation in the dark at room temperature for 24 h. Nitrogen debt was calculated as the N removed over a 24-h period per unit of chlorophyll *a* (Healey 1977). Phosphorus debt was measured in a similar way to N debt except that KH_2PO_4 was added (final concentration 5 µM). Soluble reactive P (SRP) was measured on triplicate sub-samples (Stainton *et al*. 1977). Phosphate turnover time was estimated from the rate of removal of carrier-free ^{33}P-PO_4 from the dissolved phase (Bentzen and Taylor 1991). Values indicative of nutrient deficiency for the seston and metabolic indicators are given in Table 2.

Photosynthesis was measured using the incubator method described by Fee *et al.* (1992). Water was incubated with ^{14}C labeled $NaHCO_3$ for approximately 3 hours in a water-filled incubator at *in situ* temperatures. For each sample, 2 dark and 10 light bottles were incubated. During the incubation, photosynthetically active radiation (PAR) was measured at each bottle position using a Biospherical QSP-200 spherical quantum sensor (400 to 700 nm) (Biospherical Instruments Inc., San Diego, California). Carbon uptake was obtained by filtering the sample at the end of the incubation and fuming the filter over HCl before adding fluor and counting on a liquid scintillation counter. P_{opt} is the rate of photosynthesis at PAR optimal for photosynthesis. Chlorophyll *a* was measured fluorometrically (Stainton *et al*. 1977). N_2 fixation was measured using the acetylene reduction technique (Flett *et al*. 1976)

RESULTS AND DISCUSSION

Physical Conditions—Stratification and Light

The temperature profiles (Fig. 1) show that sampling in Lake Malawi occurred during the deep mixing period (Experiment 2, June 1997 and Experiment 6, July 1999) and during varying degrees of stratification in the remaining experiments. Experiments conducted in the early stratified period (Experiment 4 and 5, October 1998 and Experiment 3, November 1997) exhibit shallower mixed depths than the experiment conducted later in the stratified period (January 1997). The temperature profiles in Lake Victoria demonstrate that the water columns at offshore Bugaia station and inshore Napoleon Gulf were weakly stratified. The Secchi disk depths (11.5 to 16.5 m) and light extinction coefficients (0.07 to 0.13/m) in Lake Malawi (Table 1) are typical of optically clear water, oligotrophic lakes and oceans. There was no seasonal pattern in Secchi disk or extinction. The water can be as optically clear in the windy season as in the stratified season because

TABLE 2. Values indicative of presence or absence or degree of nutrient deficiency for nutrient status indicators used in this study. C = particulate carbon; N = particulate nitrogen; P = particulate phosphorus; chl = chlorophyll* a. *Values are based on Healey and Hendzel (1979).

Indicator	Nutrient	No Deficiency	Moderate Deficiency	Extreme Deficiency	Deficient
C:N[a]	N	< 8.3	8.3–14.6	> 14.6	
C:P[a]	P	< 129	129–258	> 258	
N:P[a]	P	< 22			> 22
C:Chl[b]	N or P	< 4.2	4.2–8.3	> 8.3	
N debt[c]	N	< 0.15			> 0. 15
P debt[d]	P	< 0.075			> 0.075

[a] atomic ratio
[b] µmol C/µg chl
[c] µmol N/µg chl
[d] µmol P/µg chl

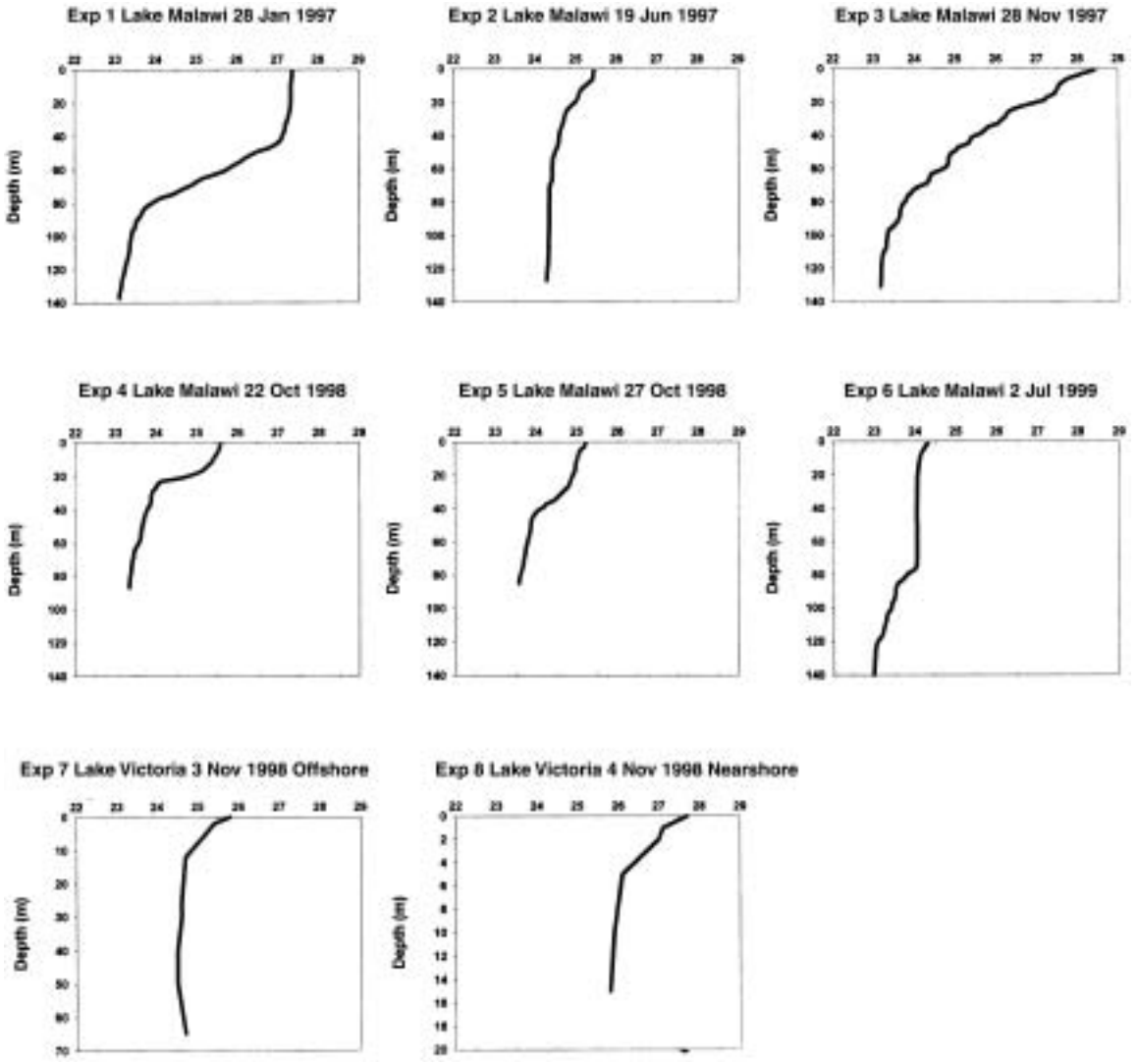

FIG. 1. Temperature profiles in Lake Malawi and Lake Victoria at the time of sampling for the enrichment experiments.

chlorophyll *a* is always low (Guildford *et al.* 2000). However, the mean water column light intensity within the mixed layer is lower in the mixed period as this is determined by the mixed layer depth as well as the light extinction coefficient. During the deep mixed periods and in the deep stratified period, the mean water column light intensity as a proportion of surface light is about one third of the mean water column light intensity in the early stratified period (Table 1). In Lake Victoria, high light extinction coefficients due to high chlorophyll *a* concentrations reduce light penetration, but because the mixed depths are shallow, mean water column light intensities are not as low as those in Malawi during the deeply mixed periods (Table 1).

Initial Biological Conditions

Chlorophyll *a* concentrations at the outset of the six enrichment experiments in Lake Malawi ranged from 0.4 to 1.5 µg/L (Figs. 2–7). There was no pattern with respect to seasonal stratification. Particulate carbon ranged from 15 to 20 µM and was even less sensitive to changes in season (Figs. 2–7). Indicators of N deficiency, particulate C:N and N debt, indicate that the algal community was rarely strongly N deficient. About one half the time, C:N and N debt indicated no N deficiency although the particulate ratio and N debt indicators were not always consistent with each other, i.e., the C:N ratio could indicate no N deficiency, while the N debt

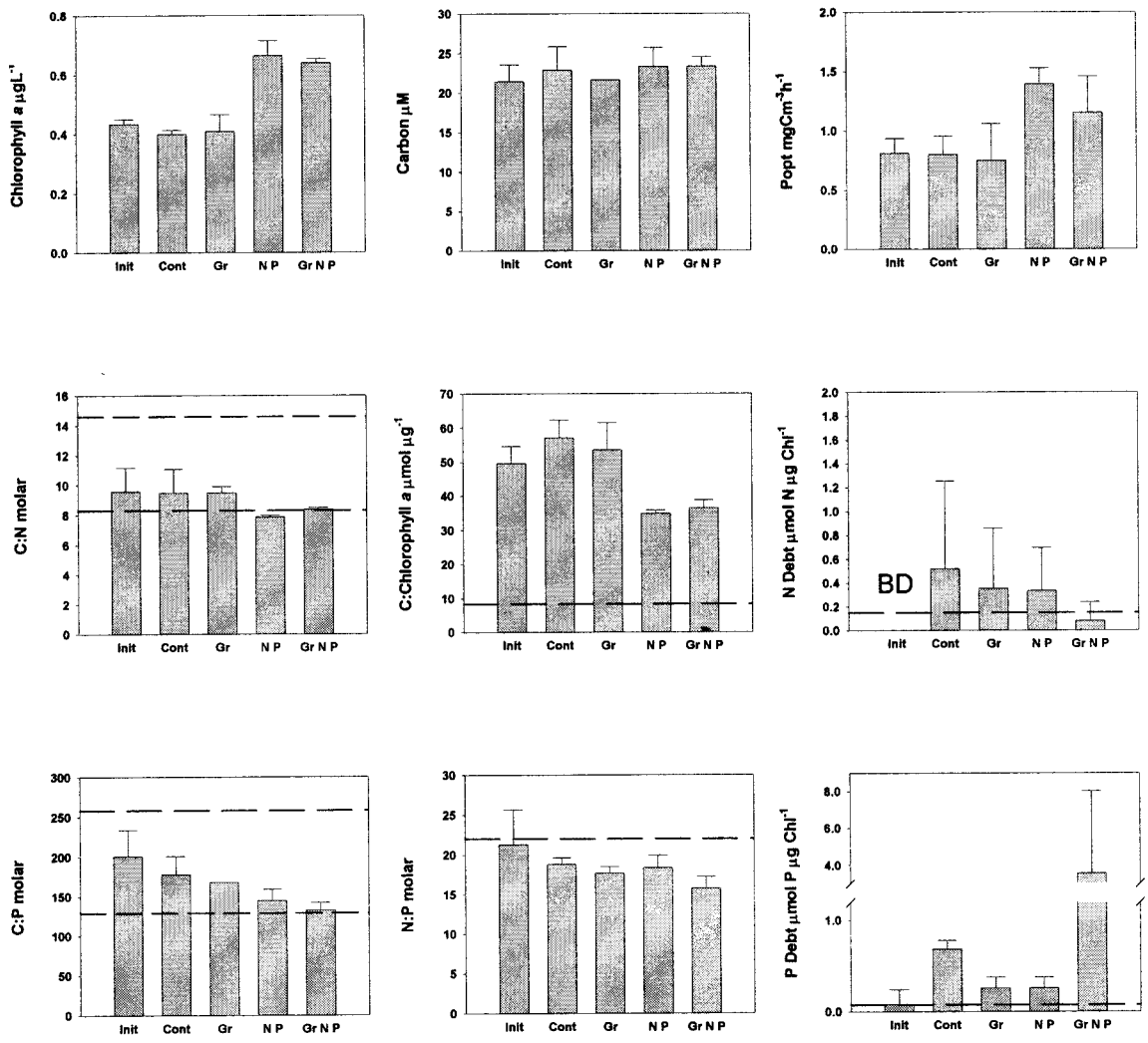

FIG. 2. Experiment 1, Lake Malawi Station 928, 28 January 1997. Mean and range of replicates. Init = the initial sample taken immediately after treatments were added. The remaining bars are samples taken after 48 h. Cont = control, Gr = grazers > 50 µm removed, N P = N and P added (see Table 1 for final concentrations), Gr N P = grazers > 50 µm removed and N and P added. Each variable is described in the methods. For the nutrient status indicators values above the dashed lines are indicative of nutrient deficiency. When two lines are present, values between the upper and lower dashed lines are indicative of moderate nutrient deficiency and values greater than the upper dashed lines are indicative of severe deficiency. Table 2 describes these values and which nutrient is deficient for each indicator. BD = below detection.

might indicate some N deficiency. Indicators of P deficiency at the outset of each experiment were similar to indicators of N deficiency in portraying a system that did not often appear to be strongly N or P limited. C:P ratios were usually in the moderate P deficiency range, and N:P ratios indicated no P deficiency while P debt indicated P deficiency less than one half the time (Figs. 2–7). Initial ^{33}P turnover times were long and not indicative of P deficiency (Experiment 1 mean = 47 h, range = 42 to 53 h, n = 2 and Experiment 2 mean = 4,979 h, range = 3,056 to 6,664 h, n = 4). Initial samples at Bugaia and Napoleon Gulf in eutrophic Lake Victoria appeared even less N and P deficient than initial Lake Malawi samples according to C:P ratios (Figs. 8 and 9). P uptake in the initial P debt experiments

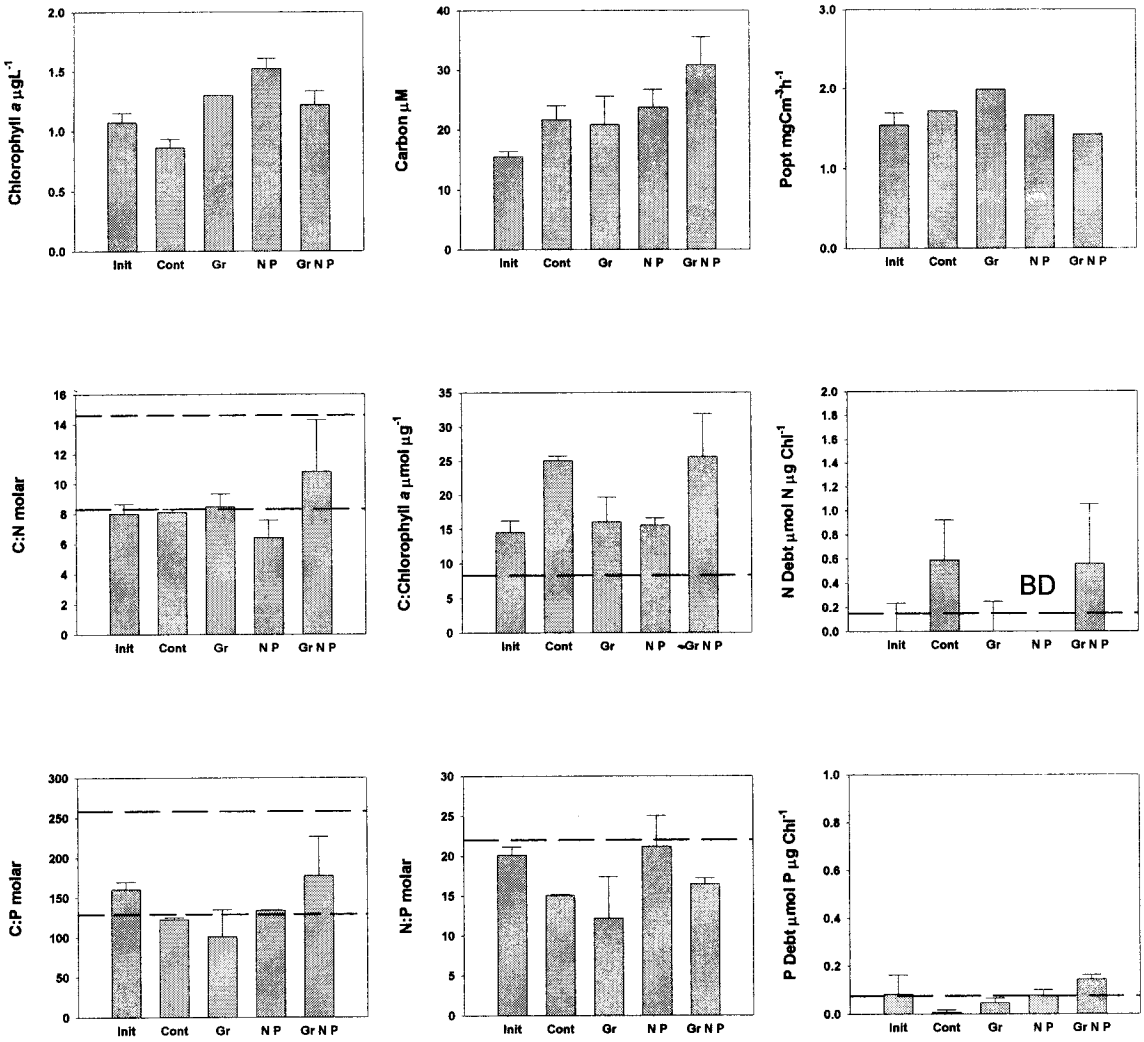

FIG. 3. Experiment 2, Lake Malawi Station 928, 19 June 1997. See Figure 2 caption for explanation of abbreviations and dashed lines.

was in the P deficient range at both Victoria stations; however, note the large difference in scale between the nutrient status indicators for Lake Victoria compared to Lake Malawi.

Controls: Response to Enclosure and Response to Increased Light

In Lake Malawi, enclosure in 10-L containers for the 48 h incubations consistently resulted in severe N deficiency according to the N debt assay (Figs. 2–7) while particulate C:N ratios usually stayed constant in the moderate N deficiency range. Strong P deficiency was not observed as often in the control containers according to P debt or C:P ratios after 48 h. Turnover times of ^{33}P were much shorter after enclosure for 48 h (Experiment 1 mean = 1.1 h , range = 0.1 to 2.5 h, n = 4 and Experiment 2 mean = 241 h, range = 96 to 496 h, n = 4), these rates are still not in the range associated with P deficiency in North American Great Lakes; e.g. < 1 h (Lean and Nalewajko 1979). The C:Chl ratio was very sensitive to enclosure, increasing dramatically in the three experiments conducted during the early stratified dry season and to a lesser extent in the stratified rainy and the windy deep mixing seasons (Figs. 2–7). The C:Chl ratio is considered a general indicator of nutrient deficiency (Healey and Hendzel 1979) and also is indicative of the changed light conditions during the incubations (Healey 1985). In

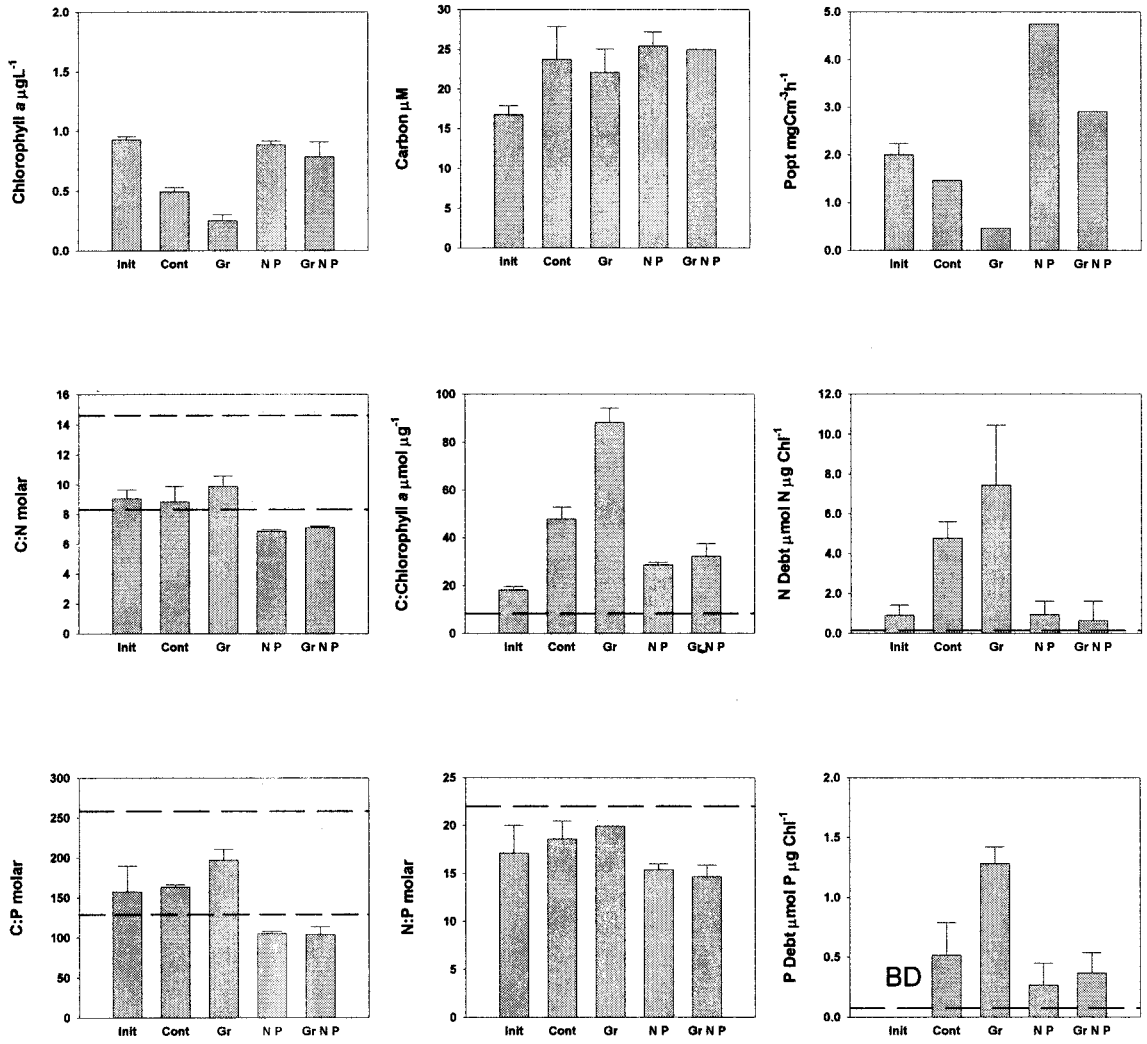

FIG. 4. Experiment 3, Lake Malawi Station 928, 28 November 1997. See Figure 2 caption for explanation of abbreviations and dashed lines.

the Lake Malawi experiments, an increase in the C:Chl ratio could indicate an increase in the severity of N or P deficiency or an adaptive change to higher light conditions or both.

In Lake Victoria, particulate C:P, C:N, and C:Chl ratios were initially at or below levels indicating P deficiency. These ratios all tended to increase as a result of enclosure for 48 h (Figs. 8–9). This shift in Lake Victoria from generally not N or P deficient to N and P deficiency, especially in the Napoleon Gulf experiment, suggests the *in situ* community was controlled by a factor other than N or P *in situ*.

Absence of nutrient deficiency *in situ* could mean light is limiting. It is expected that if phytoplankton were light limited *in situ*, either due to deep mixing depths in Lake Malawi or low transparency due to high chlorophyll *a* concentrations in Lake Victoria, then an increase would be seen in algal biomass as indicated by chlorophyll *a* and particulate C and possibly a development of nutrient deficiency when the water was incubated at higher light than *in situ* during the experiments. Because the containers were incubated at higher light than *in situ* light levels, each of the controls, compared to the initials, can be used to test for light deficiency. Because chlorophyll *a* per cell decreases with increased light (Healey 1985) and because particulate C, can have a non-living component, there is not an unequivocal indicator of algal biomass. However, in three of the six experiments in Lake Malawi, the changes in chlorophyll *a*, particulate C, and nutrient status suggest that light was at least partly controlling algal

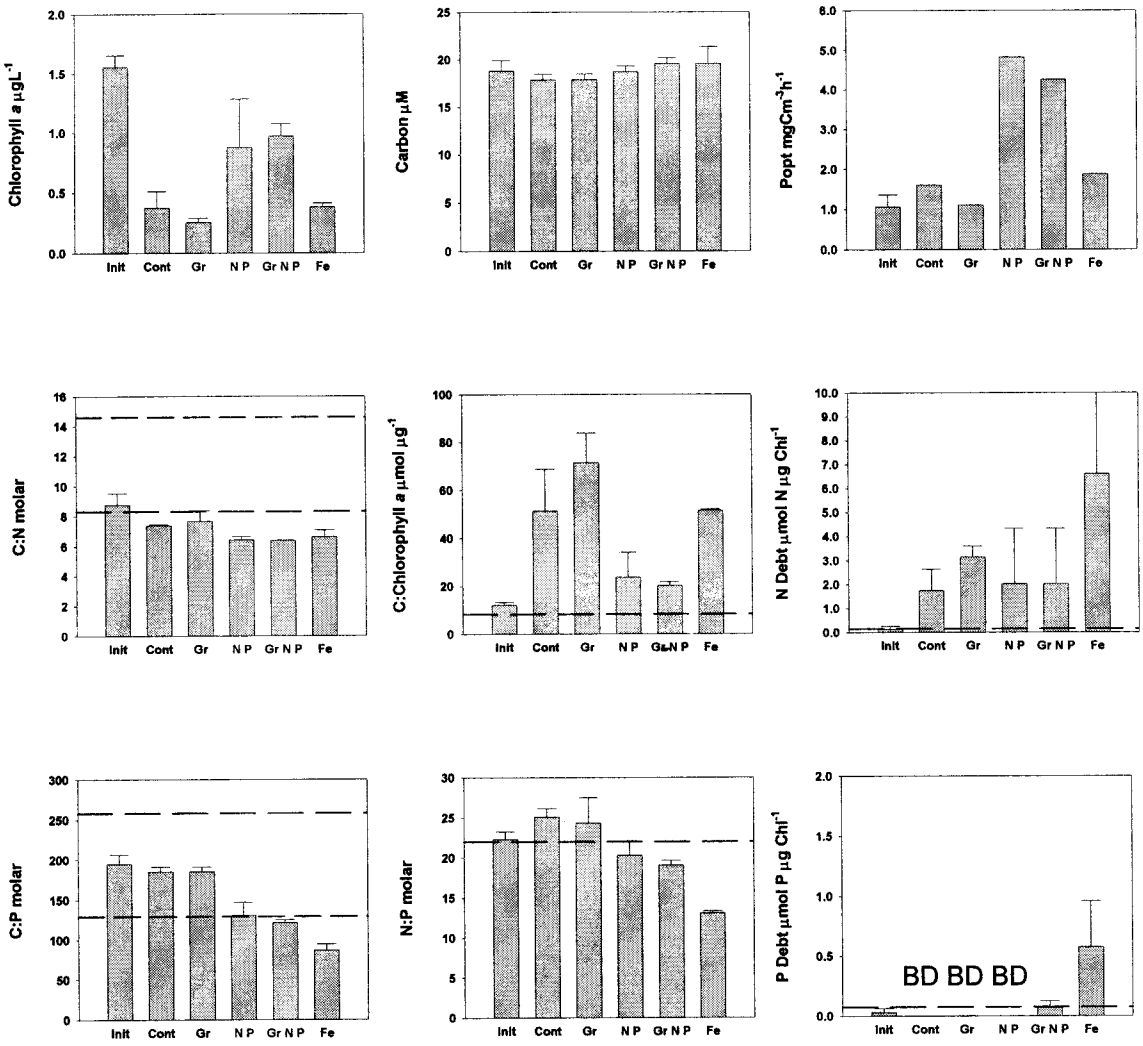

FIG. 5. Experiment 4, Lake Malawi Station 928, 22 October 1998. Abbreviations and dashed lines are as in Figure 2 except for Fe = Fe added (see Table 1 for final concentrations).

growth *in situ*. In Experiment 1, done in January 1997 the late stratified rainy season, and Experiments 4 and 6 done in June 1997 and June 1999, the windy, deep mixing season (Figs. 2, 4, and 7), chlorophyll *a* did not decline significantly. Particulate C increased or stayed the same as initial concentrations (Figs. 2, 4 and 7) and N debt developed or intensified (Figs. 2, 4 and 7). In the three other Lake Malawi experiments, which were conducted during November 1997 and October 1998, the early stratified, dry season, the response to light was a dramatic 50 to 75% reduction in chlorophyll *a* and intensification of N debt in the control treatments (Figs. 3, 5, and 6). These chlorophyll *a* and N debt responses in the different seasons can be interpreted as evidence that the light environment, which is primarily controlled by physical mixing depth in Lake Malawi, is an important factor which must be considered when identifying factors that potentially control algal biomass.

Similar evidence of light limitation occurred in eutrophic Lake Victoria. The control containers from offshore Bugaia (Experiment 7, Fig. 8) and nearshore Napoleon Gulf (Experiment 8, Fig. 9) had higher particulate C, and increased C:N, C:P ratios after 48 hours and did not exhibit decreases in chlorophyll *a* concentration. The water from Bugaia that was incubated at low light did not have increased particulate C and did not develop N or P deficiency.

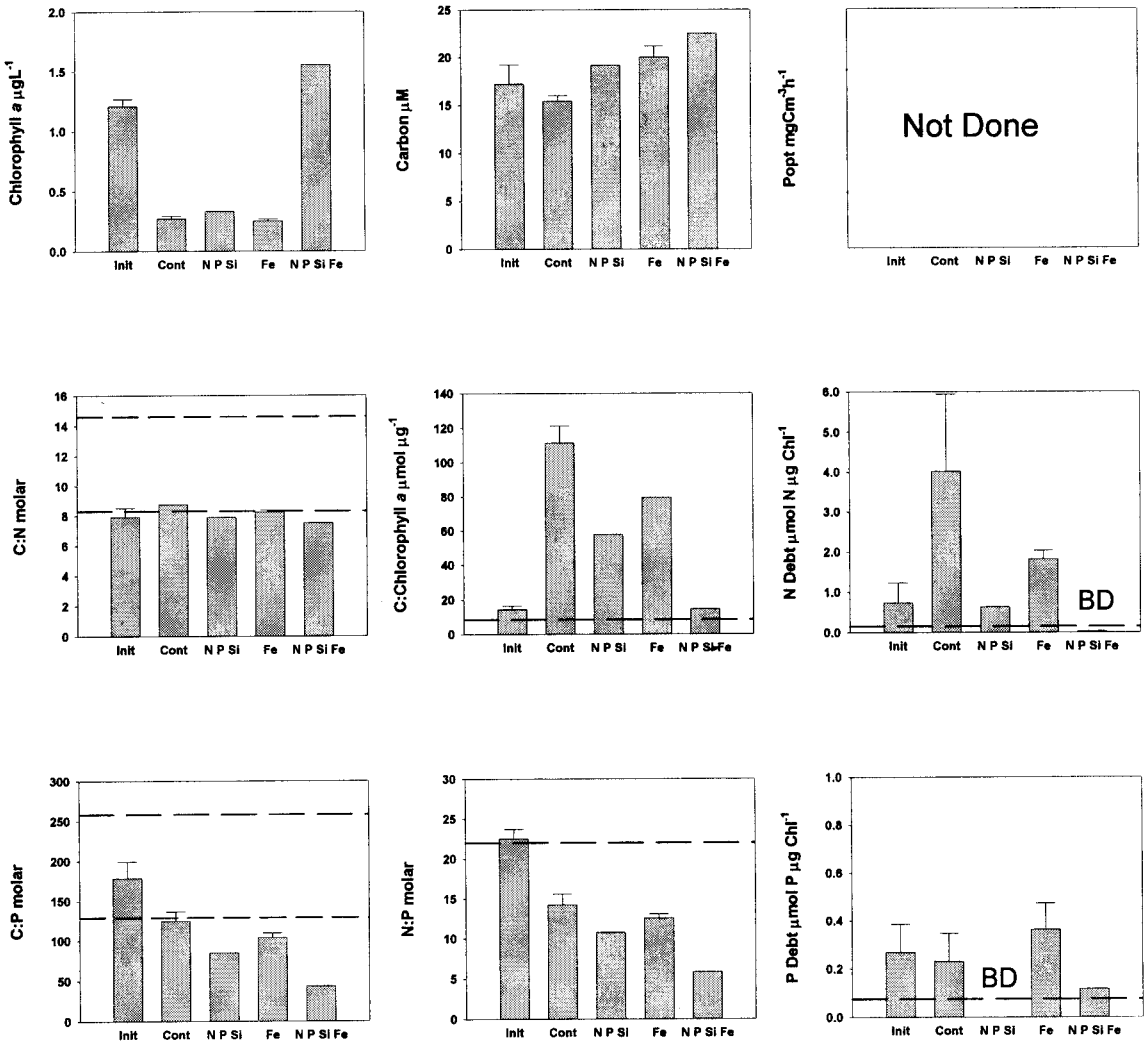

FIG. 6. Experiment 5, Lake Malawi Station 928, 27 October 1998. Abbreviations and dashed lines are as in Figure 2 except for Fe = Fe added, N P Si = N, P and silicon (Si) added and N P Si Fe = N, P, Si and Fe added. (see Table 1 for final concentrations).

Response to N, P, Fe, and C Enrichment

In Lake Malawi, the addition of N and P consistently resulted in reduction of N and P deficiency relative to controls and generally stimulated chlorophyll *a* and /or P_{opt} in the experiments conducted when the lake was stratified (Fig. 2, 4 to 6). The response to N and P enrichment was more variable in the windy deep mixing period (Figs. 3 and 7).

Fe enrichments were not done in the first three Lake Malawi enrichment experiments. In the experiments conducted in the stratified season (Figs. 5 and 6), the addition of Fe alone did not significantly increase the chlorophyll *a* concentration over the controls, but in the experiment conducted during the windy deep mixing season (Fig. 7), chlorophyll *a* was stimulated by the addition of Fe. The most striking effect of Fe enrichment was on the indicators of P nutrient status. After 48 hours, C:P and N:P ratios were lower in containers amended with Fe and rates of P uptake as indicated by the P debt assays were higher than the controls. In Experiment 5, where Fe was added in combination with N, P, and Si, there was a dramatic increase in biomass as indicated by chlorophyll *a* and particulate C as well as a completely consistent reduction in P and N deficiency as indicated by particulate C:N, C:P, N:P, and N and P debt assays (Fig. 6). In the container with N, P, and Si, but no Fe, N and P deficiency was reduced relative to the control, but there was no stimulation of chlorophyll *a*. Fe was always

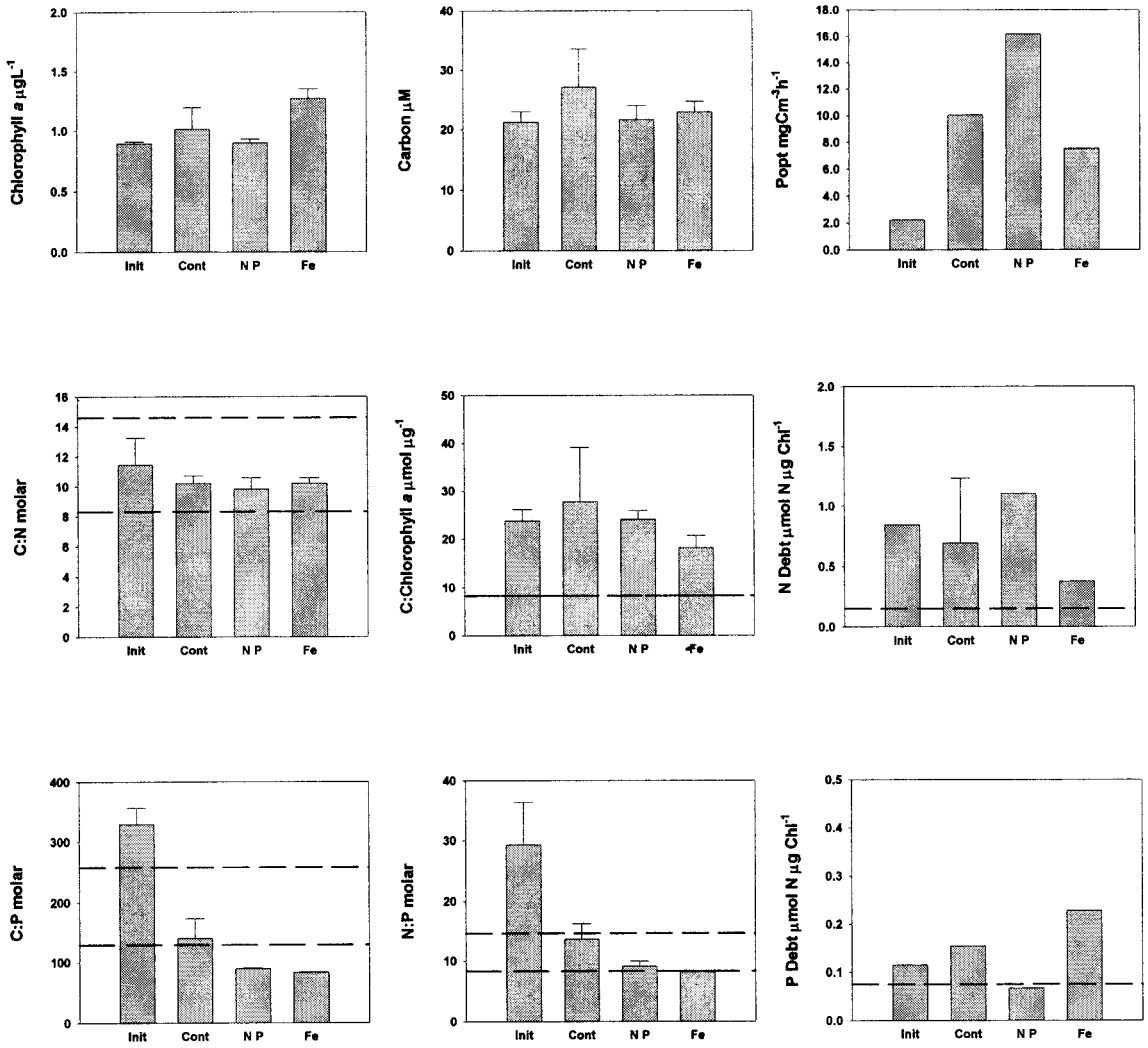

FIG. 7. Experiment 6, Lake Malawi Station 928, 2 July 1999. Abbreviations and dashed lines are as in Figure 2 except for Fe = Fe added (see Table 1 for final concentrations).

added with EDTA as a chelator so the response to EDTA without Fe was tested. Although the response to EDTA was not always identical to the control, EDTA did not stimulate P uptake or result in decreased C:P or N:P ratios relative to the controls as was observed in the Fe and EDTA additions (Guildford, unpublished data).

In Lake Victoria at Bugaia station, the addition of Fe did not appear to affect biomass or nutrient status indicators although N uptake in the N debt assay was highest in the Fe enriched containers and C:P and N:P ratios were lowest in the Fe containers. At Napoleon Gulf, the Fe addition appeared to stimulate N fixation compared to the control. Carbon enrichment had no effect on the biomass or nutrient status in either the Bugaia or Napoleon Gulf samples (Figs. 7 and 8).

Response to Removal of Plankton > 50 µm

In four of the six Lake Malawi experiments, it was tested whether the removal of plankton > 50 µm, essentially rotifer and crustacean zooplankton, would affect the biomass and nutrient status of the phytoplankton community. Quite different responses were observed depending on the season. In the experiments conducted during the early stratified dry season, when mean water column light intensity is at its highest (Figs. 4 and 5), removal of plankton > 50 µm resulted in reduced chlorophyll *a* concentrations and increased P and N deficiency

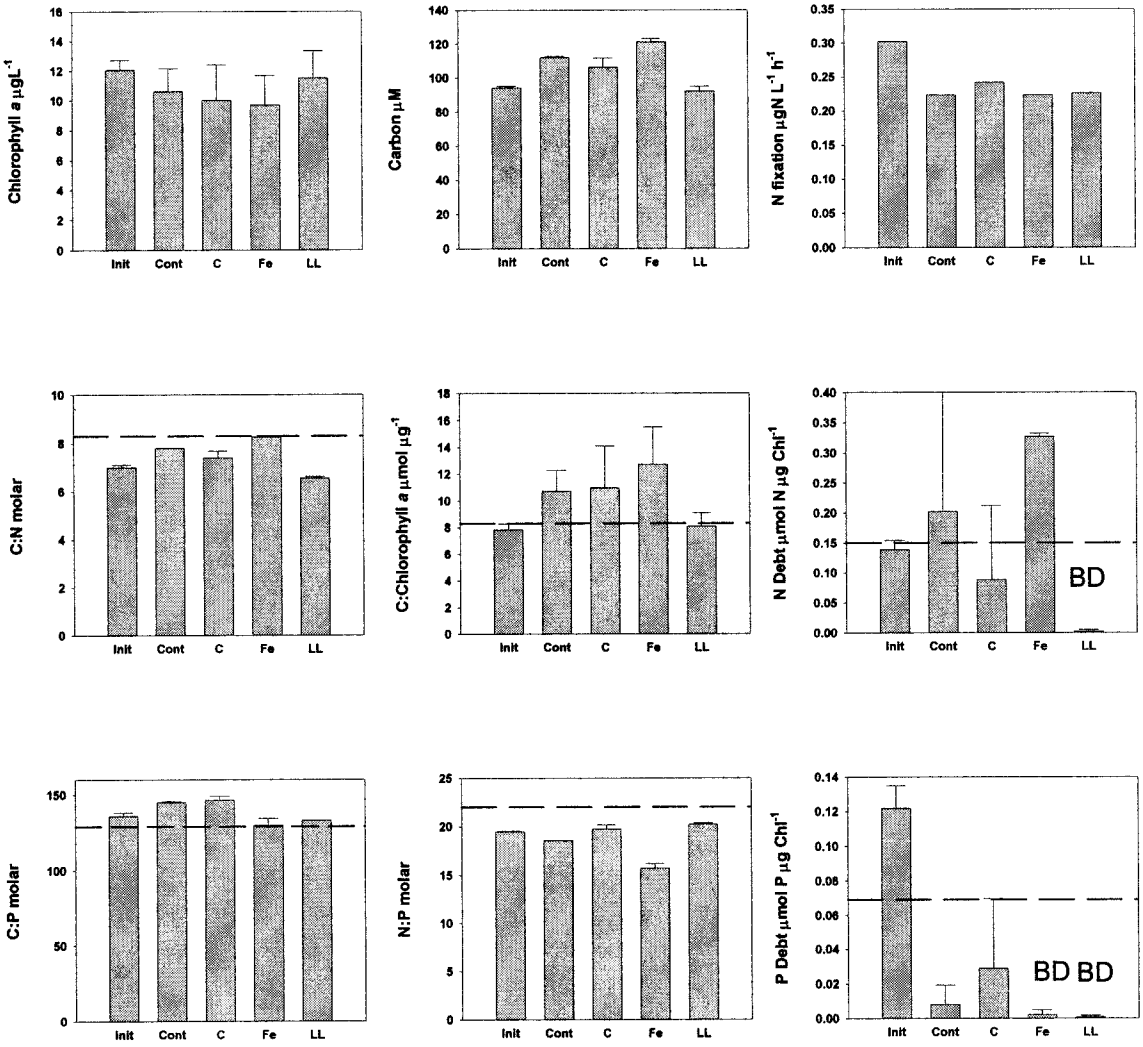

FIG. 8. Experiment 7, Lake Victoria Bugaia Station (offshore), 3 November 1998. Abbreviations and dashed lines are as in Figure 2 except for C = C added (see Table 1 for final concentrations), Fe = Fe added and LL = low light.

relative to the controls. Enrichment with N and P appeared to offset the effect of zooplankton removal on chlorophyll *a* concentration and nutrient deficiency (Figs. 4 and 5). In the experiments conducted during the late stratified rainy season and deep mixing season, when mean water column light intensity is lower than in the early stratified season, chlorophyll *a* in the containers with plankton > 50 µm removed either stayed the same as in controls or increased and N and P deficiency either stayed the same as the controls or deceased (Figs. 2 and 3).

Lake Malawi

The results or the nutrient enrichment experiments are consistent with earlier work. Guildford *et al.* (2000) found that phytoplankton in Lake Malawi were not as strongly N or P deficient as in Lake Superior and other temperate oligotrophic lakes (Guildford *et al.* 1994) with similarly low P concentrations. Guildford and Hecky (2000) found that the TN:TP ratio of Lake Malawi was in a range where either N or P could be limiting. Guildford *et al.* (2000) speculated that the moderate deficiency in Lake Malawi phytoplankton was a result of the balanced interplay of nutrient limited growth, light and grazing.

In Lake Malawi, light deficiency can only arise as a result of a deep epilimnetic layer. Light extinction coefficients would rarely be high enough to cause light limitation when there is shallow stratifi-

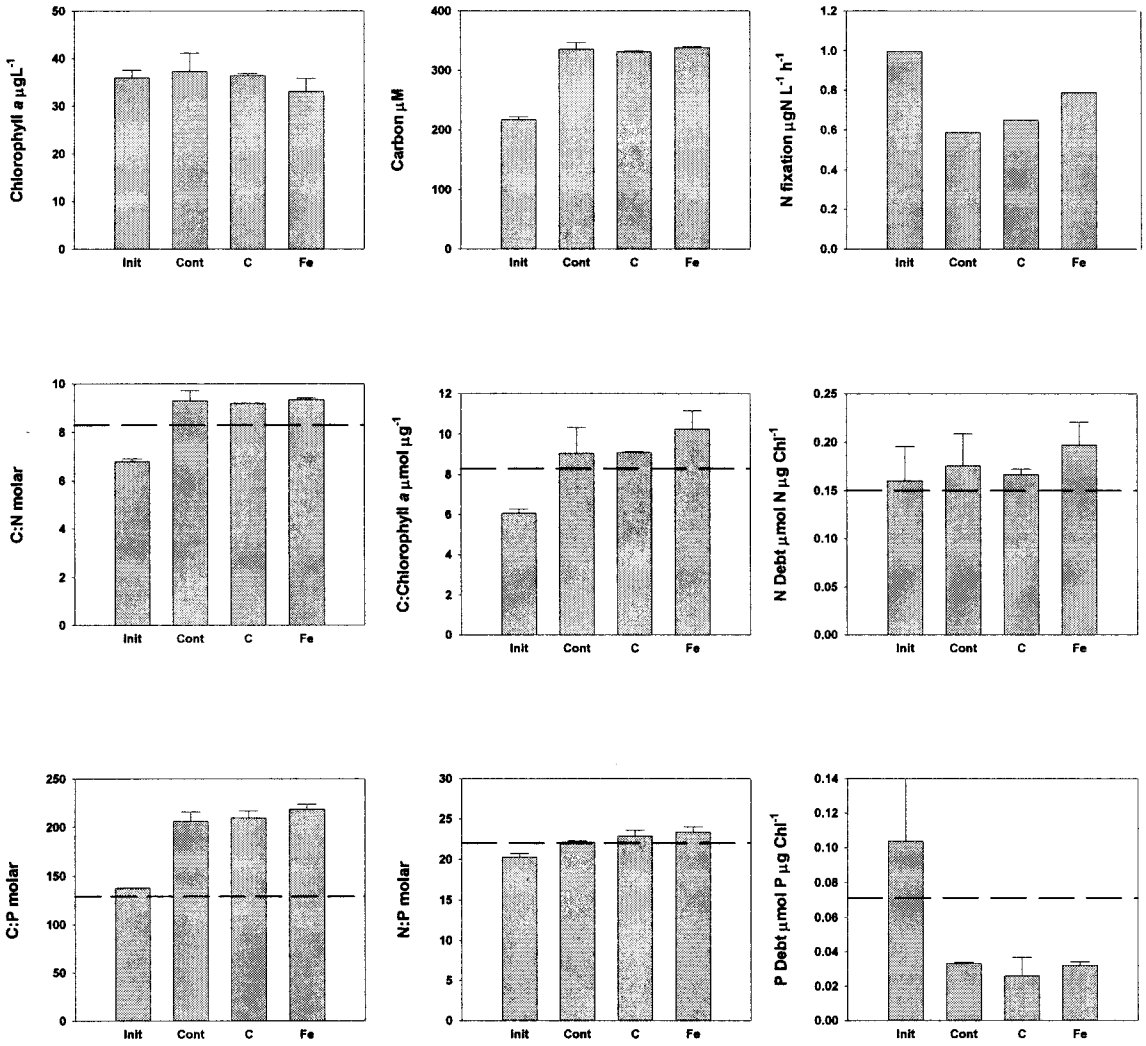

FIG. 9. Experiment 8, Lake Victoria Napoleon Gulf Station (inshore), 4 November 1998. Abbreviations and dashed lines are as in Figure 2 except for C = C added (see Table 1 for final concentrations) and Fe = Fe added.

cation of the surface layers. During the windy deep mixing season and the late stratified rainy season, the chlorophyll *a* and nutrient status response to increased light in the "control" treatments indicate that light is at least in part a factor controlling phytoplankton growth. The strongest indication of light limitation (stimulation of chlorophyll *a* above the initial concentration and above the N and P treatment) was observed in the experiment conducted in the windy deep mixing period of 1999 where the water column was mixing down to 75 m (Fig. 7). Guildford *et al*. (2000) predicted from comparison of mean water column light intensities that Lake Malawi phytoplankton would experience light limitation as a result of deep mixing very infrequently, especially in comparison to Lake Superior which experiences low solar radiation as well as deep mixing through much of the year.

The enrichment experiments indicate that Lake Malawi is generally limited by N and/or P because an increase in chlorophyll *a* and photosynthesis and a reduction in N and P deficiency relative to the controls was obtained when both N and P were added in 4 of 6 experiments. N and P, when added in combination, more often result in a positive biomass response in enrichment experiments compared to N or P added alone. Elser *et al.* (1990) conclude the high frequency of response to N and P added in combination relative to N or P added separately is because both N and P are in short supply. Use of

nutrient status measurements specific to each nutrient as well as examination of chlorophyll *a* and photosynthesis should provide a more in-depth understanding of which nutrient may be most potentially limiting. Although N and P together stimulated chlorophyll *a* and reduced both N and P deficiency, the fact that N debt occurred more frequently than P debt in the controls after 48 h and that C:N ratios indicative of N deficiency persisted while C:P ratios declined suggest that, in Lake Malawi, N usually becomes limiting first in the containers.

Although N and P did stimulate chlorophyll *a* in most of the experiments in Lake Malawi, the chlorophyll *a* responses were rather modest compared to many enrichment experiments on other lakes (Elser *et al.* 1990). On average, based on the chlorophyll *a* concentration increase over 48 hours, the estimated growth rate in the N and P enrichments was 0.5 per d. This is not low for an *in situ* growth rate (Furnas 1990). However, if N and P are the only factors controlling growth, a higher growth rate in the N and P enrichments might be expected. When Fe was added in addition to N and P, the estimated growth rate increased to 1.3 per d. This response to the combined addition of Fe, N, and P suggests that the limited response to the additions of N and P is the result of other nutrients also being in short supply. Fe is often cited as the most likely micronutrient to become deficient (Twiss *et al.* 2000, Maldonado and Price 1999, Hutchins *et al.* 1998). Much progress has been made on methods to conduct experiments using trace metal clean techniques to measure Fe concentrations and development of physiological assays indicative of Fe deficiency (Twiss *et al.* 2000). The Fe enrichment experiments conducted in Lake Malawi and Lake Victoria were very simple and did not employ trace metal clean techniques, and used EDTA with $FeCl_3$ in much higher than natural concentrations. Although it is almost certain that all the treatments in the experiments had some level of Fe contamination, the fact that the Fe treatment elicited a response different from the other treatments supports the conclusion that Fe may be a potentially limiting nutrient in Lake Malawi. An alternative interpretation of the high rates of P uptake (Pdebt) and rapid expression of the P uptake as decreased C:P ratios in the enrichment treatments is that these results are an artifact caused by abiotic flocculation and precipitation of P with Fe. However, it was observed that Fe stimulated N uptake and there do not appear to be any abiotic process that would explain this. Hutchins *et al.* (1998) observed a similar stimulation of N and Si uptake (P was not measured) with Fe enrichment in moderately and severely Fe-stressed California coastal waters. The Fe enrichment experiments require corroboration with other indicators of Fe limitation. However, Fe alone stimulated N and P uptake and Fe when added with N and P clearly stimulated chlorophyll *a* compared to N and P alone. This strongly suggests that after N and P, Fe is the next most probable limiting nutrient for phytoplankton growth in Lake Malawi.

Zooplankton in the > 50 μm fraction may be grazers of nanoplankton and possibly picoplankton, predators of microzooplankton grazers, or both (Wickham 1998). Therefore, phytoplankton response to their removal may vary with both phytoplankton and zooplankton composition. In experiments conducted during the period of shallow stratification when nutrient deficiency is expected to be strongest, removal of zooplankton > 50 resulted in reduced chlorophyll *a* after 48 hours. One interpretation of these results could be that removal of larger zooplankton relieved predation pressure on micro-grazers allowing them in turn to graze the phytoplankton. However, the fact that the remaining phytoplankton in these grazer removal experiments developed extreme N and P deficiency relative to the control treatments suggests that the main role of zooplankton > 50 μm at this time of year is nutrient regeneration. Further evidence for the importance of zooplankton to nutrient regeneration during this period of stratification can be seen in the response in the containers where grazers were removed and N and P were added. Chlorophyll *a* did not decrease in these containers and N and P deficiency was reduced relative to the controls. The response to zooplankton removal during the deep stratified and deep mixing seasons indicates that, when mean water column light intensity is lower, zooplankton grazing is less critical to nutrient regeneration and perhaps more important in controlling phytoplankton biomass.

To summarize, in Lake Malawi, light can be an important control on phytoplankton growth during deep stratification and deep mixing. N is most likely to become limiting when light is adequate, however P and Fe are also in short supply. Grazing is an important factor modifying the response of phytoplankton to nutrients. The relative importance of these factors can vary seasonally with stratification.

Lake Victoria

Nutrient concentrations are much higher in Lake Victoria than in Lake Malawi (Guildford and Hecky 2000), and Mugidde (1993) found that algal populations are usually light limited because of self-shading due to high chlorophyll *a*. At the time of the experiments early in the stratified season the algal community was showing signs of moderate P deficiency at both stations despite the fact that soluble reactive P was 0.5 μM at Napoleon Gulf and 2.5 μM at Bugaia station. P debt uptake values indicative of P deficiency in Lake Victoria were detected. Particulate C:P ratios were barely indicative of P deficiency. In his study of the interacting effects of light and nutrient limitation on the growth rate of *Synechococcus linearis*, Healey (1985) showed P could compensate for low light. He suggested an increase in irradiance would result in a lower requirement for chlorophyll *a* and other photosynthetic pigments, and the membranes to accommodate them, freeing nutrients such as N and P from that role for other purposes. In his culture work, C:P ratios increased as light levels increased and the need for P was reduced. He also observed that saturated rates of phosphate uptake were very high and indicated P deficiency even in those low light situations where light limitation must have been important, which is similar to what we observed with P debt uptake in the initial samples. The two experiments cannot explain the possible interactions between light and P in Lake Victoria. However, in both experiments increased light resulted in stronger indications of N deficiency than P deficiency, suggesting the increased light level in the experiments would lead to further dominance by the N_2 fixers. It appears the availability of Fe may be an important factor affecting that increase. Although a dramatic biomass response to Fe enrichment in Lake Victoria was not observed, stimulation of N uptake at both stations and in N_2 fixation at Napoleon Gulf was observed. Reuter (1988) demonstrated Fe limitation of N_2 fixation by *Anabaena* in culture. Although no grazer manipulations were conducted in Lake Victoria, previous grazer manipulations in Lake Victoria by Lehman and Branstrator (1993) demonstrated that chlorophyll *a* did not respond to concentration or removal of grazers.

SUMMARY

Based on these experiments in Lake Malawi and Lake Victoria, it can be concluded that N is the nutrient most likely to limit phytoplankton biomass when light is adequate in both lakes. In Lake Victoria, N_2 fixers and P supply allow chlorophyll to increase to concentrations that result in increased turbidity and light limitation (Hecky 1993). Stimulation of N_2 fixation by the addition of Fe suggests that, if light and P are available, Fe may play a role in controlling N_2 fixing phytoplankton. In Lake Malawi, the fact that the control treatments became more consistently and severely N deficient than P deficient suggests that N would become limiting before P if stratification becomes more persistent or shallower. Although the effect of single N and P additions was not tested, concentrations of N and P are so low that both nutrients are likely required to produce an increase in phytoplankton biomass as observed by Moss (1969) and here. Fe added alone appeared to stimulate N and P uptake but did not result in a significant increase in chlorophyll *a*. The fact that, in combination with N and P, Fe caused the largest increase in chlorophyll *a* in all the experiments suggests that, after N and P, Fe is the next most likely nutrient to limit phytoplankton growth. In Lake Victoria it is clear that the N demand results in fixation of atmospheric N if light is available (Mugidde *et al.* 2003, Hecky *at al.*1996) and N_2 fixers now dominate the algal community for most of the year (Kling *et al.* 2001). In Lake Malawi, some of the N demand is already being met by fixation of atmospheric N (Higgins *et al.* 2001, Hendzel 1999). On time scales longer than these enrichment experiments, increased inputs of P would likely result in a shift to N_2 fixers followed by an increase in biomass as observed in temperate lakes (Findlay *et al.* 1994, Hendzel *et al.* 1994). More research is needed to determine whether Lake Malawi would be expected to become eutrophic as a result of increasing P or whether the requirement for Fe might constrain phytoplankton growth and eutrophication by P.

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REFERENCES

Auclair, J.C. 1995. Implications of increased UV-B induced photoreduction: iron(II) enrichment stimulates picocyanobacterial growth and the microbial food web in clear-water acidic Canadian Shield lakes. *Can. J. Fish. Aquat. Sci.* 52:1782–1788.

Bentzen, E., and Taylor, W.D. 1991. Estimating Michaelis-Menton parameters and lake water phosphate by the Rigler bioassay: importance of fitting technique, plankton size and substrate range. *Can. J. Fish. Aquat. Sci.* 48:73–83.

Charlton, M.N., Milne, J.E., Booth, W.G., and Chiocchio, F. 1993. Lake Erie offshore in 1990: restoration and resilience in the central basin. *J. Great Lakes Res.* 19:291–309.

Downing, J.A., McClain, M., Twilley, R., Melack, J.M., Elser, J., Rabalais, N.N., Lewis, Jr., W.M., Turner, R.E., Corredor, J., Soto, D., Yanez-Aranciba, A., Kopaska, J.A., and Howarth, R.E. 1999. The impact of accelerated land-use change on the N-cycle of tropical ecosystems; Current condition and projected changes. *Biogeochemistry* 46:109–148.

Elser, J.J., Marzolf, E.R., and Goldman, C.R. 1990. Phosphorus and nitrogen limitation of phytoplankton growth in the freshwaters of North America: a review and critique of experimental enrichments. *Can. J. Fish. Aquat. Sci*. 47:1468–1477.

Falkowski, P.G., and Raven, J.A. 1997. *Aquatic Photosynthesis*. Malden, Massachusetts: Blackwell Science.

Fee, E.J., Shearer, J.A., DeBruyn, E.R., and Schindler, E.U. 1992. Effects of lake size on phytoplankton photosynthesis. *Can. J. Fish. Aquat. Sci.* 49:2445–2459.

Findlay, D.L., Hecky, R.E., Hendzel, L.L., Stainton, M.P., and Regehr, G.W. 1994. Relationship between N_2-fixation and heterocyst abundance and its relevance to the nitrogen budget of Lake 227. *Can. J. Fish. Aquat. Sci.* 51:2254–2266.

Flett, R.J., Hamilton, R.D., and Campbell, N.E.R. 1976. Aquatic acetylene reduction techniques: solutions to several problems. *Can. J. Fish. Aquat. Sci.* 22:43–51.

Furnas, M.J. 1990. In situ growth rates of marine phytoplankton: approaches to measurements, community and species growth rates. *J. Plankton Res*. 12: 1117–1151.

Guildford, S.J., and Hecky, R.E. 2000. Total nitrogen, total phosphorus and nutrient limitation in lakes and oceans: Is there a common relationship? *Limnol. Oceanogr.* 45:1213–1223.

_____, Hendzel, L.L., Kling, H.J., Fee, E.J., Robinson, G.G.C., Hecky, R.E., and Kasian, S.E.M. 1994. Effects of lake size on phytoplankton nutrient status. *Can. J. Fish. Aquat. Sci.* 51:2769–2786.

_____, Bootsma, H.A., Fee, E.J., Hecky, R.E. and Patterson, G. 2000. Phytoplankton nutrient status and mean water column light intensity in Lakes Malawi and Superior. *J. Aquatic Ecosystem Health and Management* 3:35–45.

Hameed, H.A., Kilinic, S., McGowan, S., and Moss, B. 1999. Physiological tests and bioassays: aids or superfluities to the diagnosis of phytoplankton nutrient limitation? A comparative study in the broads and meres of England. *Eur. J. Phycol.* 34: 253–269.

Healey, F.P. 1975. *Physiological indicators of nutrient deficiency in algae*. Fish. Mar. Serv. Res. Div. Tech. Rep. 585.

_____. 1977. Ammonium and urea uptake by some freshwater algae. *Can. J. Bot.* 55:61–69.

_____. 1985. Interacting effects of light and nutrient limitation on the growth rate of *Synechococcus linearis* (Cyanophyceae). *J. Phycol.* 21:134–146.

_____, and Hendzel, L.L. 1979. Indicators of phosphorus and nitrogen deficiency in five algae in culture. *Can. J. Fish. Aquat. Sci*. 36:1364–1369.

_____, and Hendzel, L.L. 1980. Physiological indicators of nutrient deficiency in lake phytoplankton. *Can. J. Fish. Aquat. Sci.* 37:442–453.

Hecky, R.E. 1993. The eutrophication of Lake Victoria. *Verh. Internat. Verein. Limnol.* 25:39–48.

_____, and Fee, E.J. 1981. Primary production and rates of algal growth in Lake Tanganyika. *Limnol. Oceanogr.* 26:532–547.

_____, and Kilham, P. 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment. *Limnol. Oceanogr.* 33:796–822.

_____, Bootsma, H.A., Mugidde, R., and Bugenyi, F.W.B. 1996. Phosphorus pumps, nitrogen sinks, and silicon drains: Plumbing nutrients in the African Great Lakes. In *The Limnology, climatology and paleoclimatology of the East African lakes*, eds. T.C. Johnson and E. Odada, pp. 205–224. Amsterdam: Gordon and Breach.

_____, Bootsma, H.A., and Kingdon, M.J. 2003. Impact of land use on sediment and nutrient yields to Lake Malawi/Nyasa (Africa). *J. Great Lakes Res*. 29 (Suppl. 2):139–158.

Hendzel, L.L. 1999. Nitrogen Fixation. In *Water Quality Report*, eds. H.A. Bootsma and R.E. Hecky, pp. 183–190. Lilongwe, Malawi: Lake Malawi/Nyasa Biodiversity Conservation Project.

_____, Hecky, R.E., and Findlay, D.L. 1994. Recent

changes of N_2-fixation in lake 227 in response to reduction of the N:P loading ratio. *Can. J. Fish. Aquat. Sci.* 51:2247–2253.

Higgins, S.N., Hecky, R.E., and Taylor, W.D. 2001. Epilithic nitrogen fixation in the rocky littoral zones of Lake Malawi, Africa. *Limnol. Oceanogr.* 46:976–982.

Hutchins, D.A., DiTullio, G.R., Zhang, Y., and Bruland, K. 1998. An iron limitation mosaic in the California upwelling regime. *Limnol. Oceanogr.* 43:1037–1054.

Jarvinen, M., Salonen, K., Sarvala, J., Vuorio, K. and Virtanen, A. 1999. The stoichiometry of particulate nutrients in Lake Tanganyika—implications for nutrient limitation of phytoplankton. *Hydrobiologia* 407:81–88.

Kling, H.J., Mugidde, R., and Hecky, R.E. 2001. Recent changes in the phytoplankton community of Lake Victoria in response to eutrophication. In *The Great Lakes of the World (GLOW): Food-web, health and integrity*, eds. M. Munawar and R.E. Hecky, pp. 47–65. Leiden, The Netherlands: Backhuys Publishers.

Lean, D.R.S., and Nalewajko, C. 1979. Phosphorus turnover times and phosphorus demand in large and small lakes. *Ergeb. Limnol.* 13:120–132.

Lehman, J.T., and Branstrator, D.K. 1993. Effects of nutrients and grazing on the phytoplankton of Lake Victoria. *Verh. Internat. Verein. Limnol.* 25:850–855.

Maldonado, M.T., and Price, N.M. 1999. Utilization of iron bound to strong organic ligands by plankton communities in the subarctic Pacific Ocean. *Deep-Sea Research II* 46:2447–2473.

Moss, B. 1969. Limitation of algal growth in some central African waters. *Limnol. Oceanogr.* 14:591–601.

Mugidde, R. 1993. The increase in phytoplankton primary productivity and biomass in Lake Victoria (Uganda). *Verh. Internat. Verein. Limnol.* 25: 846–849.

_____, Hecky, R.E., Hendzel, L.L., and Taylor, W.D. 2003. Pelagic nitrogen fixation in Lake Victoria (East Africa). *J. Great Lakes Res.* 29 (Suppl. 2):76–88.

Murphy, T.P., Lean, D.R.S., and Nalewajko, C. 1976. Blue-green algae: Their excretion of iron-selective chelators enable them to dominate other algae. *Science* 192:900–902.

Patterson, G., and Kachinjika, O. 1995. Limnology and phytoplankton ecology. In *The fishery potential and productivity of the pelagic zone of Lake Malawi/Niassa,* ed. A. Menz. Chatham, UK: Natural Resources Institute.

Price, N.M., and Morel, F.M.M. 1994. Trace metal nutrition and toxicity in phytoplankton. In *Algae and Water Pollution,* eds. L.C. Rai, J. P.Gaur, and C.J. Soeder, pp. 79–97. Stuttgart: E. Schweizerbartsche Verlagsbuchhandlung.

Ramlal, P.S., Kling, G.W., Ndawula, L.M., Hecky, R.E., and Kling, H.J. 2001. Diurnal fluctuations in PCO_2, DIC, oxygen and nutrients at inshore sites in Lake Victoira, Uganda. In *The Great Lakes of the World (GLOW): Food-web, health and integrity*, eds. M. Munawar and R.E. Hecky, pp. 67–82. Leiden, The Netherlands: Backhuys Publishers.

_____, Hecky, R.E., Bootsma, H.A., Schiff, S.L., and Kingdon, M.J. 2003. Sources and fluxes of organic carbon in Lake Malawi/Nyasa. *J. Great Lakes Res.* 29 (Suppl. 2):107–120.

Reuter, J.G. 1988. Iron stimulation of photosynthesis and nitrogen fixation in *Anabaena* 7120 and *Trichodesmium* (Cyanophyceae). *J. Phycol.* 24:249–254.

Sarvala, J., Salonen, K., Jarvinen, M., Aro, E., Huttula, T., Kotilainen, P., Kurki, H., Langenberg, V., Mannini, P., Peltonen, A., Plisnier, P.-D., Vuorinen, I., Molsa, H., and Lindquist, O. 1999. Trophic structure of Lake Tanganyika: carbon flows in the pelagic food web. *Hydrobiologia* 407:149–173.

Schindler, D.W. 1977. The evolution of phosphorus limitation in lakes. *Science* 195:260–262.

_____, and Fee, E.J. 1973. Diurnal variation of dissolved inorganic carbon and its use in estimating primary production and CO_2 invasion in Lake 227. *J. Fish. Res. Board Can.* 30:1501–1510.

Spigel, R.H., G.W. Coulter 1996. Comparison of hydrology and physical limnology of the East African Great Lakes: Tanganyika, Malawi, Victoria, Kivu, and Turkana (with reference to some North American Great Lakes). In *The limnology, climatology and paleoclimatology of the East African lakes*, eds. T.C. Johnson and E. Odada, pp. 103–139. Amsterdam: Gordon and Breach.

Stainton, M P., Capel, M.J., and Armstrong, F.A.J. 1977. *The chemical analysis of freshwater*, 2nd ed. Can. Fish. Mar. Serv. Misc. Spec. Publ. 25.

Talling, J.F. 1966. The annual cycle of stratification and phytoplankton growth in Lake Victoria (E. Africa). *Int. Rev. Gesamten Hydrobiol.* 51:545–621.

Twiss, M.R., Auclair, J-C., and Charlton, M.N. 2000. An investigation into the iron-stimulated phytoplankton productivity in epipelagic Lake Erie during thermal stratification using trace metal clean techniques. *Can. J. Fish. Aquat. Sci.* 57:86–95.

Wickham, S.A. 1998. The direct and indirect impact of Daphnia and Cyclops on a freshwater microbial food web. *J. Plankton Res.* 20:739–755.

Wurtsbaugh, W.A., and Horne, A.J. 1983. Iron in eutrophic Clear Lake, California: Its importance for algal nitrogen fixation and growth. *Can. J. Fish. Aquat. Sci.* 40:1419–1429.

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