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Effects of mineral nutrients on the growth of bacterio- and phytoplankton in two southern reservoirs

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Abstract

Nutrient limitation of bacterio- and phytoplankton was studied simultaneously in two warm-water lakes in the southern United States—Joe Pool Lake (JPL) and Eagle Mountain Lake (EML). Lakes were sampled approximately biweekly between March 1998 and December 1999 from a single station. Nutrient limitation was assessed through dilution bioassays in which nitrogen (N, 50 μM above ambient), phosphorus (P, 10 μM above ambient), carbon (C, bacterioplankton only, 83 μM above ambient), and trace nutrients (Tm, phytoplankton only) were supplied. In both lakes, growth of bacterio- and phytoplankton was stimulated by nutrient additions. Multiple nutrient limitation was common. P alone and in combination with N and C or Tm most frequently limited growth of both bacterio- and phytoplankton in JPL. N alone and in combination with P and C or Tm most frequently limited growth of both bacterio- and phytoplankton in EML. Comparison of in situ growth rates to growth rates under potentially nutrient saturating conditions revealed that both bacterio- and phytoplankton in both lakes were growing well below maximum potential during warm months but near maximum potential during cooler months. This result was due to a combined effect of low temperature in winter and restricted nutrient availability in summer. Phytoplankton was generally more strongly limited by nutrient availability than was bacterioplankton, but there were occasions when the intensity of limitation shifted between communities.

Considerable effort has been invested in characterizing the nutrient demands of phytoplankton, motivated in part by the need to understand anthropogenic eutrophication. Such work ranges from nutrient acquisition in individual species (Droop 1983) to identifying sources of individual nutrients supporting entire communities (Andersen et al. 1991). While focusing attention on phytoplankton, we have frequently overlooked the fact that phytoplankton draw nutrients from the same dissolved pool as do bacterioplankton. Two views of microplankton nutrient dynamics seem to have developed. In one, phytoplankton is largely regulated by nutrient availability and supply reduced carbon (as photosynthate, Cole et al. 1982) to bacterioplankton, which is thought to be regulated by organic carbon supply. This view stems largely from correlations between algal biomass and bacterial abundance (Bird and Kalff 1984; Currie 1990; Pace and Cole 1994*a*) and, to a lesser extent, from findings that bacterial production across a wide range of marine and freshwaters seems to amount to between 10% and 30% of net primary production (Cole et al. 1988; White et al. 1991). Underlying this view is the implication that bacterioplankton is not limited by mineral nutrients.

In another view, bacterio- and phytoplankton are considered to be competitors for dissolved mineral nutrients. This view stems largely from data that indicate that bacterial phosphorus uptake kinetics are superior to those of phytoplankton (Currie and Kalff 1984*a,b;* Currie 1990). This scenario finds support in a collection of recent studies, clearly demonstrating limitation of bacterial growth by mineral nutrients (Toolan et al. 1991; Coveney and Wetzel 1992; Thingstad et al. 1998; Brett et al. 1999; Vrede et al. 1999), a requisite condition for competition.

Broad ecosystem surveys (such as Currie 1990; Pace and Cole 1994*b*) have been used to point out that the apparent correlation between bacterial abundance and chlorophyll concentration, and the apparent coupling between phytoplankton and bacterioplankton, may actually stem from regulation by common factors (such as temperature and nutrients). Several models have been considered that incorporate temperature, common nutrient demands, and competition into microplankton nutrient dynamics (Currie 1990).

Temperature and attendant summer stratification are often considered primary mechanisms through which physical forces interplay with biological activities to regulate nutrient dynamics in lakes and some marine environments. In many natural lakes and some marine habitats, periods of thermal stratification produce hydrodynamically stable environments. An emerging view is that during summer stratification, mineral limitation of both bacterio- and phytoplankton occurs, accompanied by accumulation of dissolved organic carbon resulting from reduced mineralization by bacteria (Morris and Lewis 1992; Thingstad et al. 1993, 1998; Thingstad and Rassoulzadegan 1995). In contrast, reservoirs are often hydrodynamically complex systems and may lack strong persistent stratification during summer.

In the present article, we consider the effects of nutrient additions on the growth of phytoplankton and bacterioplankton simultaneously, in two warm-water reservoirs of the southern United States. We ask whether microbial communities in these systems compete for nutrients, explore seasonal patterns of nutrient limitation when water columns are

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well mixed, and assess whether a warm water-column results in elevated metabolic rates that drive communities into nutrient limited states. Our study adds to a growing literature on such water bodies, whose physical limnology differs from well-studied natural lakes and marine systems in cooler climates.

Methods

Environmental setting—Samples were collected from two reservoirs in north Texas; Joe Pool Lake (JPL) and Eagle Mountain Lake (EML). JPL (32°53′N, 97°30′W) was impounded in 1986 and has a surface area of 3,620 ha and a mean depth of 7.2 m. The lake is mesotrophic, and peak algal abundance occurs during midsummer (mixed-species bloom of the diatom *Aulacoseira granulata* and several cyanobacteria). Additional information about this lake may be found in Sterner (1994). EML (32°35′N, 97°0′W, filled in 1932) has a surface area of 3,638 ha and a mean depth of 6.2 m. The lake is eutrophic, and peak algal abundance occurs during late summer, with abundant filamentous cyanobacteria. Additional information about EML may be found in Sterner and Grover (1998).

Sampling—Each lake was sampled between March 1998 and December 1999 at a single station near the deepest part. The lakes were sampled about every 2 weeks when water temperature was >16° and monthly at other times. Photosynthetically active radiation was measured with a Li-Cor model LI-185B coupled to a spherical sensor. At the surface, the sensor was shrouded to eliminate reflected light with a 37 × 32 × 14 cm dishpan having a roughened black interior and a hole cut in the bottom to fit over the sensor. Below the surface, measurements were made at 1-m intervals. Depth profiles of temperature and dissolved oxygen were taken (YSI model 33), and, from these, the depth of the surface mixed layer was determined. Both lakes stratified only occasionally and weakly during the period of sampling. When lakes were not stratified, the bottom was taken as the limit of mixing. Samples were taken with a 6 L Van Dorn bottle at discrete depths near top, bottom, and middle of the mixed layer, screened through 153 μm Nitex, and combined in 20-liter polyethylene carboys to create a pooled mixed layer sample (PML). Three such pooled samples were taken during each sampling, thereby creating true triplicate samples.

In the laboratory, samples were removed from each PML and preserved in formaldehyde (2% final concentration) for enumeration of bacterioplankton, and aliquots were filtered (Whatman GF/F), immersed in saturated $MgCO_3$ (1 ml), and frozen for later determination of chlorophyll *a* (Chl *a*) concentration.

Dilution assays (DA)—The effect of nutrients on growth of bacterio- and phytoplankton was assessed by use of a dilution-growth approach. Equal portions of each PML were combined and diluted 1 : 9 (whole : filtered) with equal portions of each PML that had been combined and filtered (0.2 μm). Filtered water was prepared by sequential filtration through Gelman ''extra thick'' filters (127 mm) and Gelman (12117) filter capsules. Samples of bulk-diluted water were taken for determination of bacterial abundance and Chl *a* (N_0, see below). Diluted water (500 ml) was dispensed to each of 36 700-ml clear-polycarbonate bottles. Nutrient spikes, consisting of nitrogen (N), phosphorus (P), carbon (C), and trace minerals (Tm) were added in a factorial design. N was added (as NH_4Cl) to 50 μM above ambient, P was added (as NaH_2PO_4) to 10 μM above ambient, C was added (as equimolar portions of glucose and sodium acetate) to 83 μM above ambient, Tm (formulated according to Sterner [1994] but without vitamins) were added, and controls received no supplements. Growth rates of bacteria or algae were determined in selected bottles. Bacterial growth rate was determined in bottles receiving, alone or in combination, N, P, and C, whereas algal growth rate was determined in bottles receiving, alone or in combination, N, P, and Tm. The experimental design thus consisted of two 2^3 factorial experiments with several overlapping treatments. In presenting results, combination treatments and the corresponding interaction effects are denoted by combined abbreviations (e.g., ''NP'' for additions of both N and P).

Landry-Hassett grazing assays (LH)—A standard dilution approach was used to estimate losses of bacteria due to predation (Landry and Hassett 1982). Filtered water (0.2 μm, as above) was mixed with unfiltered water to create a series of four dilutions, prepared in duplicate. The actual dilutions were empirically determined by comparing bacterial abundance in diluted water to bacterial abundance in undiluted water at time zero and ranged between 0.1 and 1.0. Diluted water was dispensed into 700-ml clear-polycarbonate bottles and each bottle received N, P, and C enrichments as above.

Incubation, sampling, and determination of bacterio- and phytoplankton growth—All dilutions (DA and LH) were randomly placed on a rotary shaker held in a controlled environment room. Irradiance (fluorescent lamps) was adjusted to equal the depth-averaged irradiance in the mixed layer, given the average daytime surface irradiance expected at that time of year. This was computed from the exponential extinction coefficient (Li-Cor data) and mixing depth determined at sampling, by applying the equation for calculating mean light in the water column found in Sterner (1990). A photoperiod of 14 : 10 (light : dark) was used. Temperature was adjusted to equal the mean temperature of the mixed layer.

Samples for enumeration of bacteria (9 ml) were collected immediately after preparation of dilutions for the LH grazing assays (N_o for LH, see below, determination of growth rates), and all dilutions were sampled after 1 d incubation (N_f for DA and LH). Samples for enumeration of bacteria were preserved in formaldehyde (2% final concentration) and stored refrigerated. Samples for quantification of Chl *a* (DA only) were taken after 3 or 4 d of incubation (see below), filtered onto glass-fiber filters (Whatman GF/F), and frozen in saturated $MgCO_3$ (N_f for DA).

Bacteria were enumerated by epifluorescence microscopy, with use of 4,6-diamidino-2-phenyl-indole as the fluorochrome (Porter and Feig 1980). Chl *a* was determined by fluorometry (Turner model 10-AU) after an overnight freeze-

thaw extraction (without grinding) of pigments in 90% acetone (Glover and Morris 1979). The fluorometer was configured as in Welschmeyer (1994) to measure Chl *a* directly, without acidification to correct for phaeopigments.

Determination of growth rates—Growth rates of bacterio- and phytoplankton in each experimental replicate were calculated as $\mu_r = (\ln N_f - \ln N_o)T^{-1}$, where T is incubation time, N_f is concentration of bacteria or Chl *a* at the end of incubation, and N_o is the concentration of bacteria or Chl *a* at the start. Incubation times were always 1 d for bacteria and 4 d for algae when the temperature of the mixed layer was <25° at sampling and 3 d when the temperature was >25°.

Estimates of predation mortality from LH assays were used to correct the growth rate of bacteria in all treatments for this source of error. Thus, the corrected growth rate was determined from $\mu = \mu_r + g$ where g is the dilution-corrected grazing rate. Grazing corrections assume that only the level of dilution of whole water reduced grazing rate and that no other density dependent losses of bacteria occurred (Landry and Hassett 1982; Andersen et al. 1991). We estimated the resource-saturated growth rate (μ_{max}), following correction for mortality, see ''Discussion'') of bacteria from DA in which N, P, and C were supplied. For both LH and DA, we assumed no lag phase prior to exponential growth; thus, our growth rate estimates should be considered conservative.

For a subset of DA experiments, Chl *a* was determined daily. Preliminary analysis of algal growth dynamics demonstrated that reliable growth kinetics could be obtained from initial and end-points only (Grover unpubl. data), and only such estimates are reported here. We estimated the resource-saturated growth for phytoplankton (μ_{max}) from dilution assays in which N, P, and Tm were supplied. We made no correction for grazing mortality of phytoplankton, because lake water was prescreened, and DA volumes were small enough to ensure the probability of introducing large-bodied algal grazers into a DA bottle was low (g approaches 0 in the equation above). However, we cannot assure that this source of error was completely and always absent from DA.

Statistical analysis—Data were analyzed by use of ANOVA for factorial designs, with a separate analysis conducted for each sampling time. Bacterioplankton growth rates were analyzed as a 2^3 factorial design with three crossed treatments: N, P, and C; phytoplankton growth rates were analyzed as a 2^3 factorial design with three crossed treatments: N, P, and Tm. All treatments had three replicates. Significance of all main effects and interactions was tested ($\alpha = 0.05$).

Four additional ANOVAs were performed for bacterio- and phytoplankton within each lake. Growth rate data for each treatment from each experiment were pooled, and time was added as an additional factor to be crossed with treatments. Potential time-related effects consist of fixed effects (e.g., seasonality) and random effects (e.g., interannual meteorological variability), as well as serial correlations in residual errors. Results of these pooled analyses were qualitatively robust to treating time as a fixed or random factor, and thus we made no attempt to dissect fixed and random components. Analyzing serial correlations in the residuals of the pooled analyses would be quite difficult, given high dimensionality (a vector of 24 residuals per sampling time) and irregular sampling intervals, and was not undertaken. Graphical analyses did not suggest strong correlations between residuals at different sampling times but did suggest heteroscedasticity, with larger residuals during summer. Therefore, we treated the pooled analyses as guideline summaries of our data sets, rather than definitive analyses. In particular, we relied on the analyses conducted separately for each sampling time to identify changing patterns in treatment effects. Multiple linear regression was also used to characterize relationships between temperature and growth rates in specific treatments.

Results

Reservoir dynamics—JPL and EML are part of a complex series of reservoirs in north Texas that serve needs associated with water supply, flood control, power generation, and recreation. The two reservoirs are not hydrologically linked. Water levels may fluctuate dramatically as water demand rises or water is released to maintain downstream conditions. Water levels respond rapidly to rainfall in the watershed, particularly when the reservoirs are at normal capacity (JPL, 159.1 m above mean sea level (MSL); EML 197.8 m above MSL) (Fig. 1). There is a general seasonal pattern to water levels in these reservoirs. The systems recharge during winter months and are drawn down during the hot summers. During 1998, water levels in JPL fluctuated ~2 m, with a slow decline of ~1 m from late spring to early autumn. In 1999, the water level fluctuated ~1 m. Water levels in EML declined ~1 m from early summer to early autumn of 1998, remaining well below normal capacity through the end of the year. This reservoir did not follow the typical pattern of winter recharge during winter of 1999; however, water level increased ~0.6 m between March and June 1999.

EML and JPL were never strongly stratified during the sampling period. Average temperatures in the water column of both lakes ranged between 7° (winter) and ~30° (late summer).

Microbial community dynamics—Bacterioplankton abundance and Chl *a* concentration are variable in these reservoirs. In JPL, Chl *a* peaked during midsummer of 1998 and 1999, attaining levels as high as 25 μg L^{-1} (Fig. 2, top). After the midsummer peak, Chl *a* generally remained below 10 μg L^{-1}. Bacterioplankton abundance was variable and ranged between 2×10^9 and 5×10^9 cells L^{-1} (Fig. 2, top). In EML, Chl *a* attained maximum levels (~30 μg L^{-1}) in both years during the July–October period (Fig. 2, bottom). Bacterioplankton was most abundant during the early summer of both years, attaining levels $>5.0 \times 10^9$ cells L^{-1} (Fig. 2, bottom). Abundance declined steadily throughout the remainder of the summer and fall.

Bacterioplankton abundance was not correlated to Chl *a* concentration in either lake. The average concentrations of bacteria and Chl *a* in the reservoirs place them within the

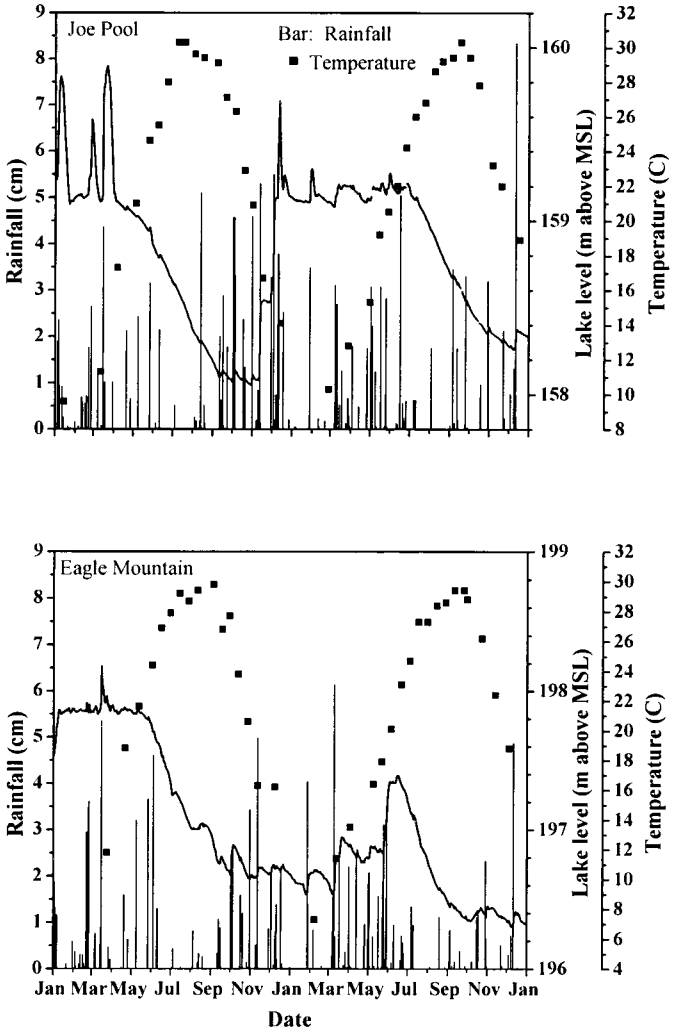

Fig. 1. Lake level, rainfall, and mean water column temperature at JPL and EML. Lake level and rainfall data are from the US Army Corps of Engineers (JPL) and Tarrant Regional Water District (EML). In this and following figures, the study period begins with January 1998.

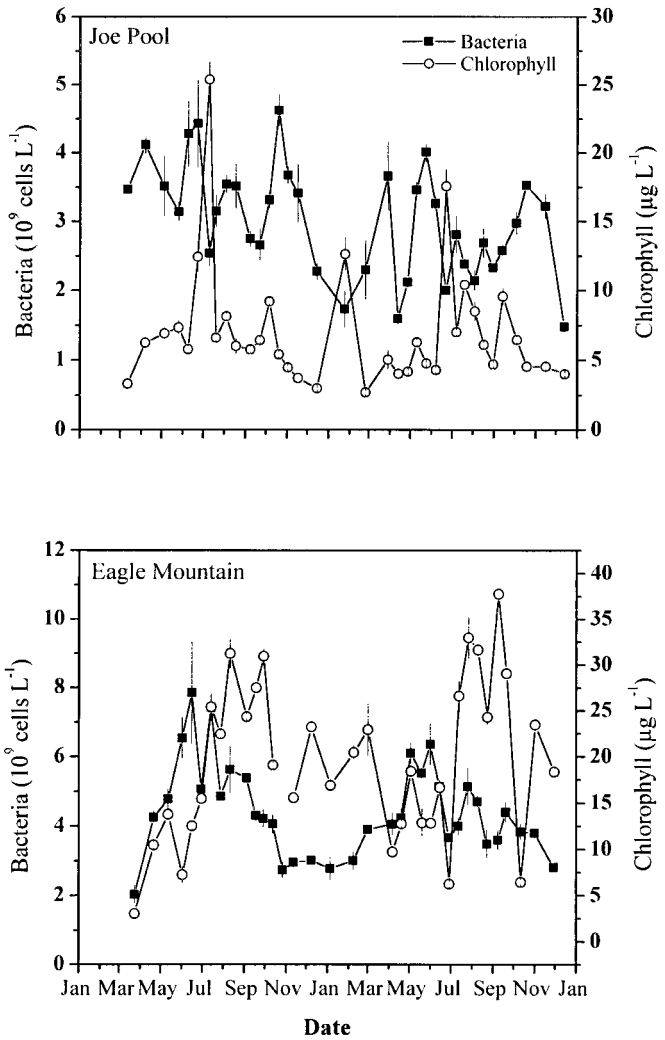

Fig. 2. Abundance of bacterioplankton and Chl *a* concentration in JPL and EML. Points represent the means of triplicate samples and bars equal ± SE. In this and following figures, missing error bars are within the confines of the symbol.

general relationship of bacteria and Chl *a* described by Bird and Kalff (1984).

Effect of nutrient additions: overview—Nutrient additions, alone or in combination, generally stimulated growth of both bacterio- and phytoplankton (Web Appendix 1: Tables 1–4, http://www.aslo.org/lo/toc/vol_46/issue_6/1319a1.pdf, Fig. 3). The responses of bacterio- and phytoplankton to nutrient additions were found to be very similar in both lakes when data for the entire study were pooled. Four-way ANOVA with time and three experimental factors showed all factors and interactions to be significant ($P < 0.05$) for bacterioplankton in EML and JPL and for phytoplankton in JPL. For phytoplankton in EML, all factors and interactions were significant except the NTm and NPTm interactions. These results held whether time was treated as a fixed or a random factor in the analysis and reinforce the impression that nutrient supplements generally produced significant growth stimulation. Importantly, all interactions with time were significant in these analyses, showing that, as expected in a seasonal climate, nutrient additions have differing effects on growth rates over the course of time. The pooled summary data (Fig. 3) suggest that bacterioplankton in EML responded more strongly to combined additions of N and C than did bacterioplankton in JPL, that phytoplankton responded more strongly to additions P, PTm, and NPTm in JPL than did phytoplankton in EML, and that NP and NPTm additions strongly stimulated growth of phytoplankton over that of controls in both lakes. However, there is much time-related variance in these general patterns, and we rely on analyses conducted separately for each sampling time to document changing patterns in treatment effects (discussed below).

Occasionally, individual nutrient additions suppressed growth rate; however, the majority of these instances were not statistically significant and/or the depressed growth rates were small in comparison to stimulation effects. In several experiments, we found net negative growth rates in controls

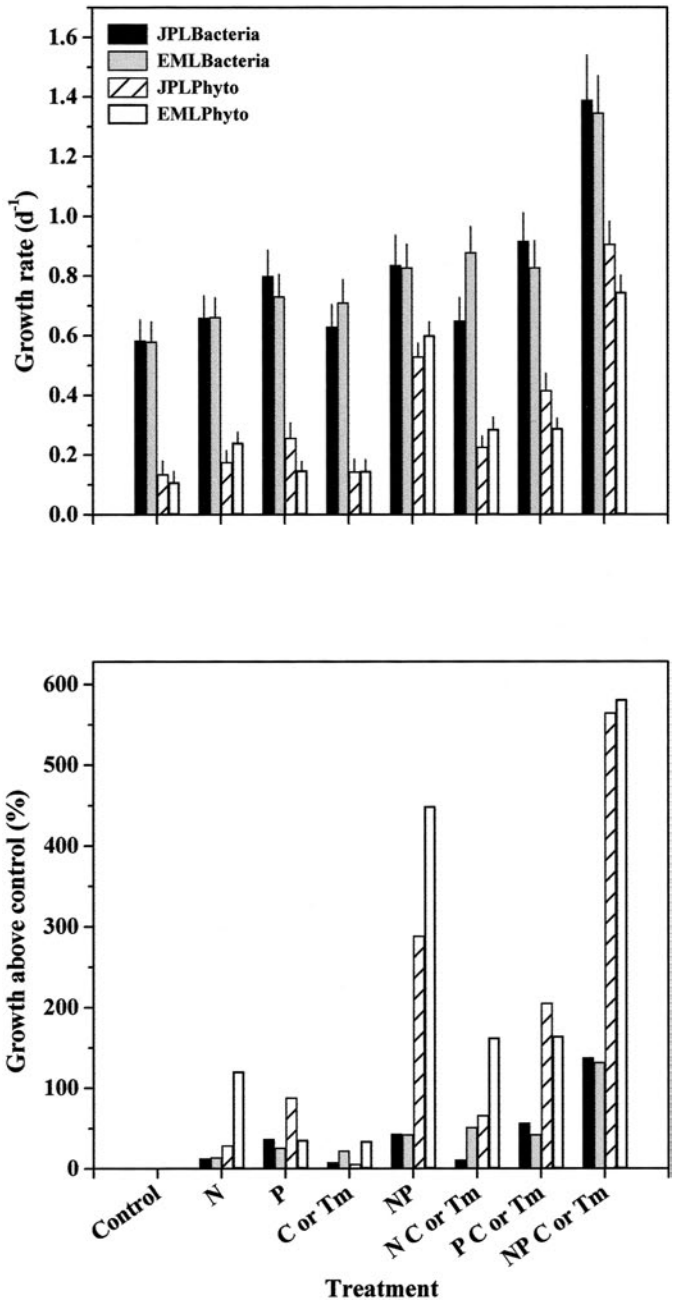

Fig. 3. The effects of nutrient additions on growth of bacterio- and phytoplankton averaged over the two-year sampling period ($N > 33$) (top panel). Bars equal ± SE. The average effects of nutrient additions on growth of bacterio- and phytoplankton as a percentage of growth rate in controls (bottom panel).

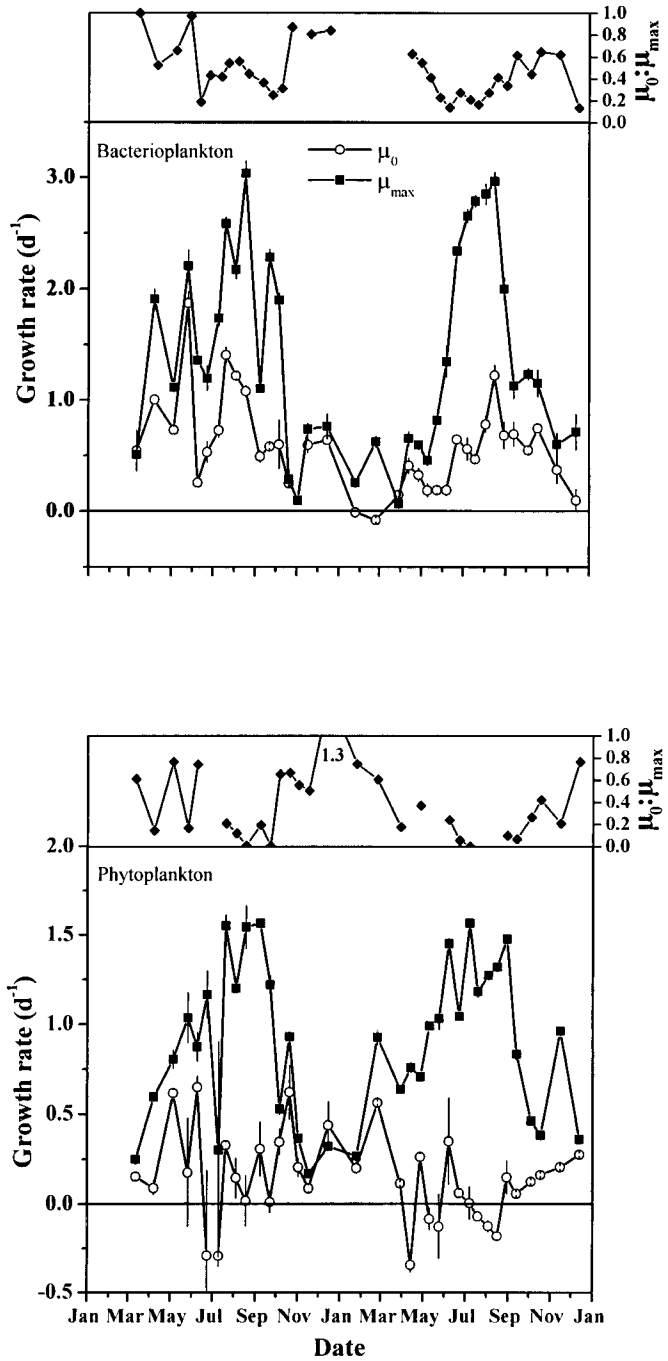

Fig. 4. Growth rate of bacterio- and phytoplankton in JPL dilution assays. Controls (μ_0); bottles supplemented with NPC or NPTm (μ_{max}). Each point is the mean of triplicate determinations and the bars represent ± SE. The ratio of $\mu_0 : \mu_{max}$ for each assemblage is shown in the upper section of each panel.

(3 for bacterioplankton, 21 for phytoplankton; see Figs. 4 and 7, below). These negative growth rates were usually close to zero and probably reflect low in situ growth and analytical error rather than effects of mortality uncontrolled by the experimental design.

Effect of nutrient additions: JPL—Growth rates of bacterio- and phytoplankton in bottles receiving NPC or NPTm almost always exceeded growth rates in control bottles (Web Appendix 1: Tables 1 and 2, Fig. 4). We interpret growth rates with three nutrients added as indicating growth potential under nutrient-saturated conditions ($\mu_{max,}$ see ''Discussion'') and consider that growth rates in bottles receiving no nutrients (μ_0, controls) reflect growth under in situ conditions. During cooler months, bacterioplankton under nutrient saturated conditions grew only slightly faster than those

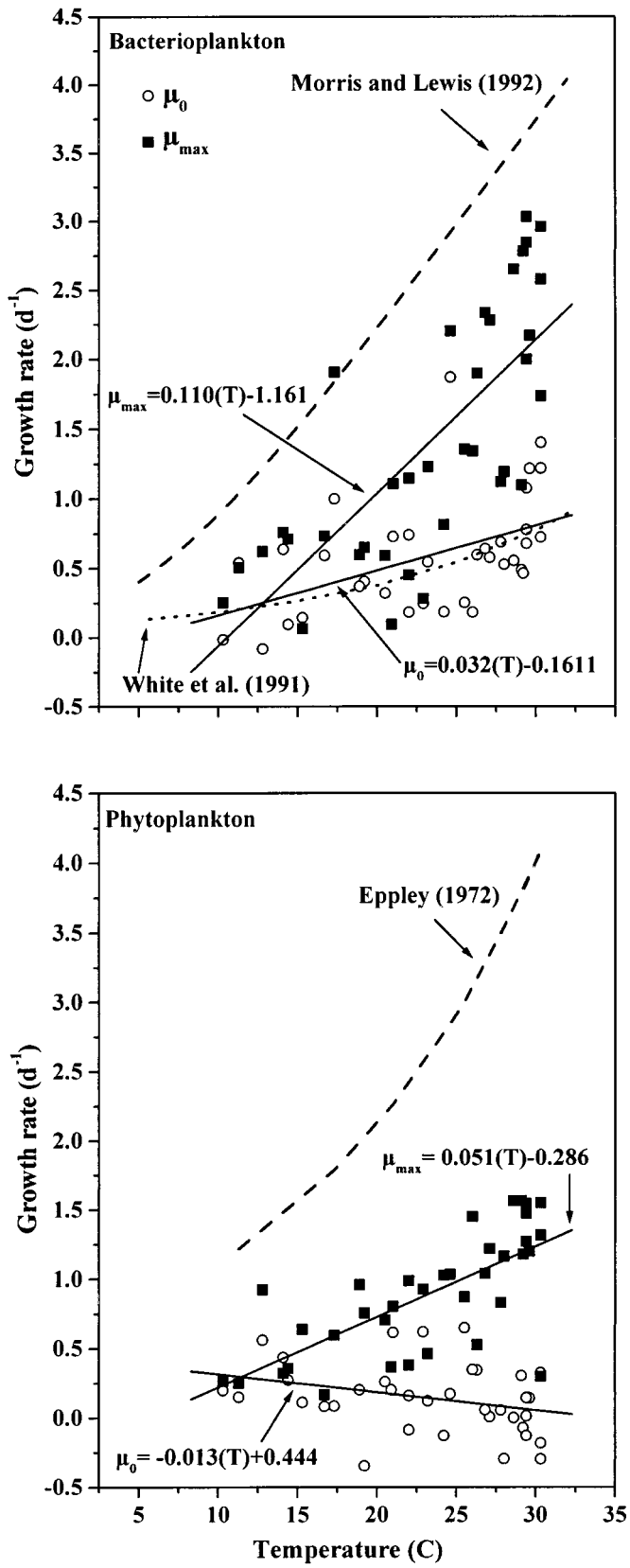

Fig. 5. Growth rate of bacterio- and phytoplankton in JPL as a function of temperature. Also shown are the relationships between μ_{max} and temperature described for bacterioplankton in Lake Dillon, Colorado (Morris and Lewis 1992), and between μ_0 of bacterioplankton and temperature for a wide variety of freshwater systems (White et al. 1991). For phytoplankton, the relationship between μ_{max} and temperature described by Eppley (1972) is shown.

growing under the in situ condition (see, for example, November 1998 through March 1999, Fig. 4). The ratio of μ_0: μ_{max} was generally >0.6 at these times. This ratio could not be calculated when μ_0 was negative, but it is clear that in situ growth rates were near maximum potential growth rates. Seasonal patterns of phytoplankton growth were similar, but rates were more variable (Fig. 4).

During warmer summer months, the difference between μ_{max} and μ_0 became more pronounced. Bacterioplankton in situ were growing below 50% of μ_{max}, whereas phytoplankton appeared to be more severely stressed. Relationships between temperature and μ_0 and μ_{max} are revealed by regression analysis (Fig. 5). For bacteria, both μ_0 and μ_{max} were positively correlated to temperature (μ_0: $N = 35$, $P < 0.004$, μ_{max}: $N = 36$, $P < 0.001$); however, the relationships were noisy (μ_0: $r = 0.48$, μ_{max}: $r = 0.74$). The slopes of the regression lines fitting μ_0 and μ_{max} to temperature were significantly different ($F_{1,67} = 15.14$, $P < 0.001$) and converge at ~13° as the $\mu_0 : \mu_{max}$ ratio approached unity. Also shown in Fig. 5 is the relationship between μ_{max} for bacterioplankton and temperature reported by Morris and Lewis (1992) for Lake Dillon, Colorado, and the relationship between bacterioplankton μ_0 and temperature for a wide variety of freshwater systems compiled by White et al. (1991). The maximum growth rates of bacterioplankton in JPL, although following a similar trend with temperature, were considerably less than that of bacterioplankton in Lake Dillon. The relationship between in situ growth rate and temperature in JPL was little different than that described by White et al. (1991).

For phytoplankton, μ_{max} was positively correlated to temperature (Fig. 5: $N = 36$, $P < 0.001$); however, μ_0 was weakly negatively related to temperature ($N = 36$, $P = 0.06$), and both relationships were noisy (μ_{max}: $r = 0.71$, μ_0: $r = -0.31$). The slopes of the regression lines fitting μ_0 and μ_{max} to temperature were significantly different ($F_{1,68} = 33.63$, $P < 0.001$) and converge at ~12°. Also shown in Fig. 5 is the relationship between μ_{max} and temperature reported by Eppley (1972) for marine algae growing in culture. Nutrient-saturated growth rates obtained here under field conditions lie well below the rates reached in cultures.

We adopted the scoring system used by Morris and Lewis (1992) to indicate the relative magnitude of statistically significant changes in growth rate brought about by nutrient supplements (Web Appendix 1: Table 1 and 2). It is apparent from these data that P is the element most frequently limiting growth of bacterioplankton in JPL (21 of 36 experiments); however, multiple nutrient limitation is also common. Additions of single nutrients had, for the most part, only a minor impact on growth of bacterioplankton (growth responses characterized as low were most common). In comparison, combinations of nutrients, with the notable exception of NC

additions for bacteria, tended to stimulate growth more strongly.

Nutrient limitation of the phytoplankton was complex. About 50% of the experiments indicated limitation by N, P or Tm alone and these additions frequently resulted in moderate to strong growth responses (Web Appendix 1: Table 2). As for bacterioplankton, limitation by multiple nutrients was common, and nutrients added in combination tended to have a larger impact on potential growth rate. The combination of NP stimulated phytoplankton growth in >60% of experiments and was the combination of elements that most strongly stimulated growth.

Bacterioplankton compared with phytoplankton—Growth responses to the additions of nutrients suggest that there are times when bacterio- and phytoplankton may compete for nutrients. For example, nutrient limitation of bacterio- and phytoplankton appeared to be more frequent during the warmer months than during the cool months. Further, comparisons of temporal patterns in the magnitude of the growth response suggest that the intensity of that competition may be variable.

To visualize the comparative intensity of nutrient limitation we calculated the "response index" (RI) as $(\mu_{x_{\text{bacterioplankton}}} - \mu_{0_{\text{bacterioplankton}}}) - (\mu_{x_{\text{phytoplankton}}} - \mu_{0_{\text{phytoplankton}}})$, where μ_x was the growth rate of bacterio- or phytoplankton, respectively, when nutrient x was added. We calculated the RI for N, P, and NP additions, because these were the treatments common to both suites of experiments. An RI of 0 indicates that a nutrient addition brought about a similar change in growth rate (relative to control) in both bacterio- and phytoplankton. A large positive value of RI indicates a strong growth response by bacterioplankton compared with that of phytoplankton, and a large negative value indicates a strong growth response by phytoplankton compared with that of bacterioplankton.

The trends in the RI suggest some patterns in responses of bacterio- and phytoplankton to N and P (Fig. 6). Bacterioplankton appear to respond more strongly than phytoplankton to nutrient additions in the early spring (responses to N, P, and NP in February to April) and late summer/early fall (primarily response to P in September). Phytoplankton appear to respond more strongly than bacterioplankton to N, P, and NP primarily in warm weather, with strong responses, particularly to NP, occurring from early summer to early fall. However, large episodic shifts in relative nutrient limitation between algae and bacteria occur throughout this warm season.

Effect of nutrient additions: EML—For most of the sampling period, bacterioplankton grew under in situ conditions at rates between 40% and 60% of the rate possible under nutrient-saturated conditions (Fig. 7). Nutrient-saturated growth rates during warmer summer months tended to be greater than those occurring during cooler months; however, growth rates of bacterioplankton in controls also increased during the warmer summer months. As in JPL, both μ_0 and μ_{max} were positively correlated to temperature (μ_0: $N = 33$, $r = 0.64$, $P < 0.001$, μ_{max}: $N = 33$, $r = 0.74$, $P < 0.001$). The slopes of the regression lines fitting μ_0 and μ_{max} to temperature were significantly different ($F_{1,62} = 8.21$, $P < 0.01$)

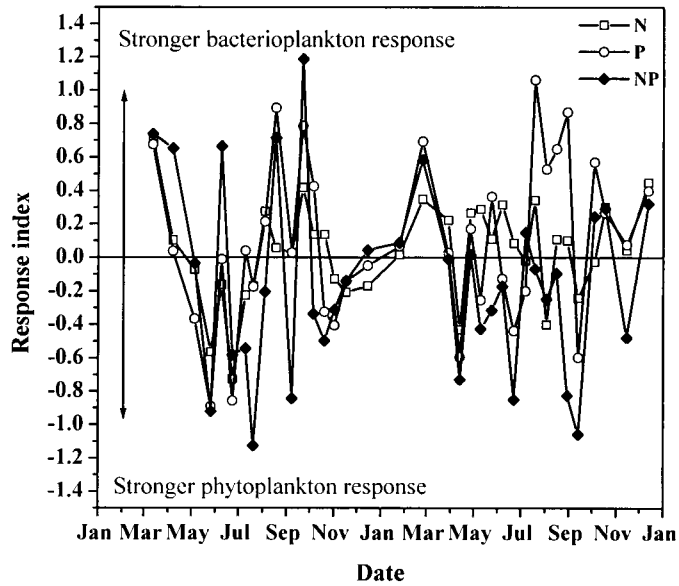

Fig. 6. Comparative responses (RI, d^{-1}) of bacterio- and phytoplankton to supplements of N, P, and NP in JPL. A value of 0 indicates an equivalent net response by both assemblages, a large positive value indicates a strong bacterioplankton response (change in growth rate) compared with that of phytoplankton, and a large negative value indicates a strong phytoplankton response compared with that of bacterioplankton.

and converge at ~8° (Fig. 8). The relationship between maximum growth rate and temperature for bacterioplankton in EML was not significantly different from that found in JPL ($F_{1,65} = 0.72$, $P > 0.05$), and the magnitude of μ_{max} was considerably less than that of bacterioplankton in Lake Dillon. The in situ growth rate of bacterioplankton in EML was also not significantly different from that found in JPL ($F_{1,64} = 0.53$, $P > 0.05$).

A pronounced seasonal signature characterized phytoplankton growth. Growth in the absence of added nutrients was essentially zero during warmer parts of the year (May through August) but was above zero during cooler sampling periods (Fig. 7). The $\mu_0 : \mu_{max}$ ratio cannot be calculated for much of the summer period because growth rates in the absence of added nutrients were often negative. Nevertheless, it is clear that phytoplankton were severely restricted by resource availability during this time frame. During cooler sampling periods, the $\mu_0 : \mu_{max}$ ratio often exceeded 70% (Fig. 7), suggesting an ample resource supply. As in JPL, μ_{max} was positively correlated to temperature ($N = 33$, $r = 0.55$, $P < 0.001$), whereas μ_0 was weakly negatively correlated to temperature ($N = 33$, $r = -0.39$, $P = 0.02$). The slopes of the regression lines fitting μ_0 and μ_{max} to temperature were significantly different ($F_{1,64} = 19.33$, $P < 0.001$) and converge at ~9° (Fig. 8). The relationship between maximum growth rate and temperature for phytoplankton in EML was not significantly different from that found in JPL ($F_{1,66} = 2.09$, $P > 0.05$), and the magnitude of μ_{max} was considerably less than that of marine phytoplankton in culture. The relationship between in situ growth rate of phytoplankton in EML was also not significantly different from that found for JPL ($F_{1,66} = 0.01$, $P > 0.05$).

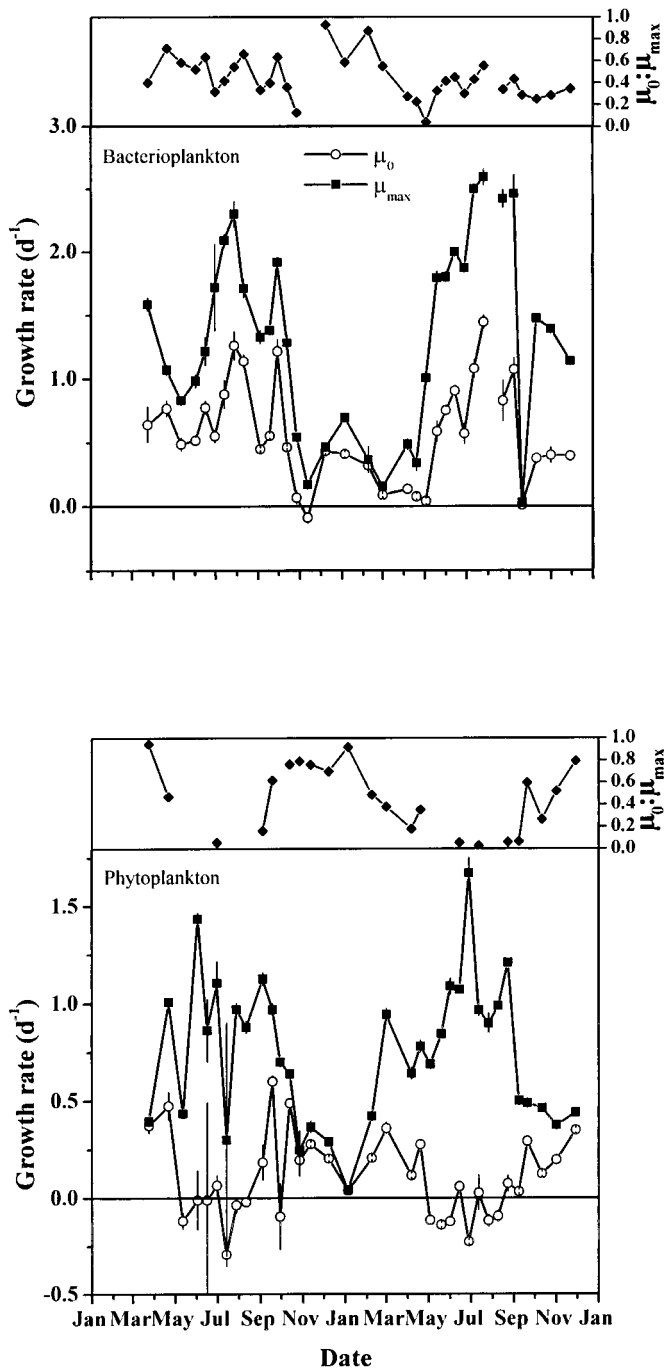

Fig. 7. Growth rate of bacterio- and phytoplankton in EML dilution assays. Controls (μ_0); bottles supplemented with NPC or NPTm (μ_{max}). Each point is the mean of triplicate determinations, and the bars represent ± SE. The ratio of $\mu_0 : \mu_{max}$ for each assemblage is shown in the upper section of each panel.

Responses of bacterio- and phytoplankton growth in dilution assay experiments revealed that single nutrient and multiple nutrient limitation was common in EML (Web Appendix 1: Tables 3 and 4), but the primary nutrient limiting growth in this lake appeared to be N. N limitation was found in ~60% of 35 experiments where N was the sole supplement or when N was added in combination with P or PC for bacteria. Tm alone or in combination with N seemed to suppress phytoplankton growth. Despite frequent (essentially constant) nutrient limitation by one nutrient or another, the intensity of nutrient limitation appeared to be weak for bacterioplankton (<100% increase over control at most times) but was stronger for phytoplankton, especially in summer (>500% stimulation over controls).

Bacterioplankton compared with phytoplankton—Figure 9 shows the RI for bacterio- and phytoplankton in EML. Bacterioplankton were often more strongly limited by N and P in spring and fall than were phytoplankton. In general, phytoplankton appeared to be more stressed than bacterioplankton for nutrients during summer months, showing stronger responses to all nutrients in 1998 and to NP additions in 1999.

Discussion

Physical limnology and resource limitation—Reservoirs are dynamic systems with complex spatial and temporal heterogeneity. This dynamic nature may be seen by comparing JPL in 1991–1992 (Sterner 1994) with JPL in 1998–1999. In 1991–1992, JPL underwent summer stratification and oxygen depletion, whereas water level remained at, or above, normal reservoir capacity. In contrast, during 1998–1999, JPL rarely stratified, and the water level fell >1 m through spring and summer of each year. Similarly, EML was weakly stratified and also lost >1.5 m of water during the 2-yr period. These systems often do not express classic limnological features expected of temperate zone lakes, such as persistent summer thermal stratification and epilimnetic nutrient depletion associated with physical structuring of the water column. Even so, as in temperate lakes, nutrient limitation of bacterio- and phytoplankton occurred over much of the year in both lakes and was especially intense during the warmest part of the growing season. Although physical characteristics of the water column of these reservoirs appear to vary interannually, available evidence suggests that patterns of nutrient limitation may be more stable. Sterner (1994) found nearly the same patterns of phytoplankton nutrient limitation in 1991–1992 that we document here for 1998–99, despite differences in hydrodynamics and stratification. In 1991–1992, dissolved nutrients were reduced to low levels during warm weather in JPL (Sterner 1994), and this was also true during the time period reported here (Chrzanowski and Grover unpubl. data). Sterner and Grover (1998) found N to be an important limiting nutrient for phytoplankton in EML, although their study did not cover a full year. The strong nutrient limitation during summer that we document here was accompanied by reduced dissolved nutrient concentrations, compared with winter levels (Chrzanowski and Grover unpubl. data).

Measuring nutrient limitation: dilution bioassays—We applied a dilution bioassay approach to address the issue of nutrient limitation. This approach assumes that, in control treatments, ambient nutrient levels are unchanged from the in situ condition, whereas the number of organisms relying

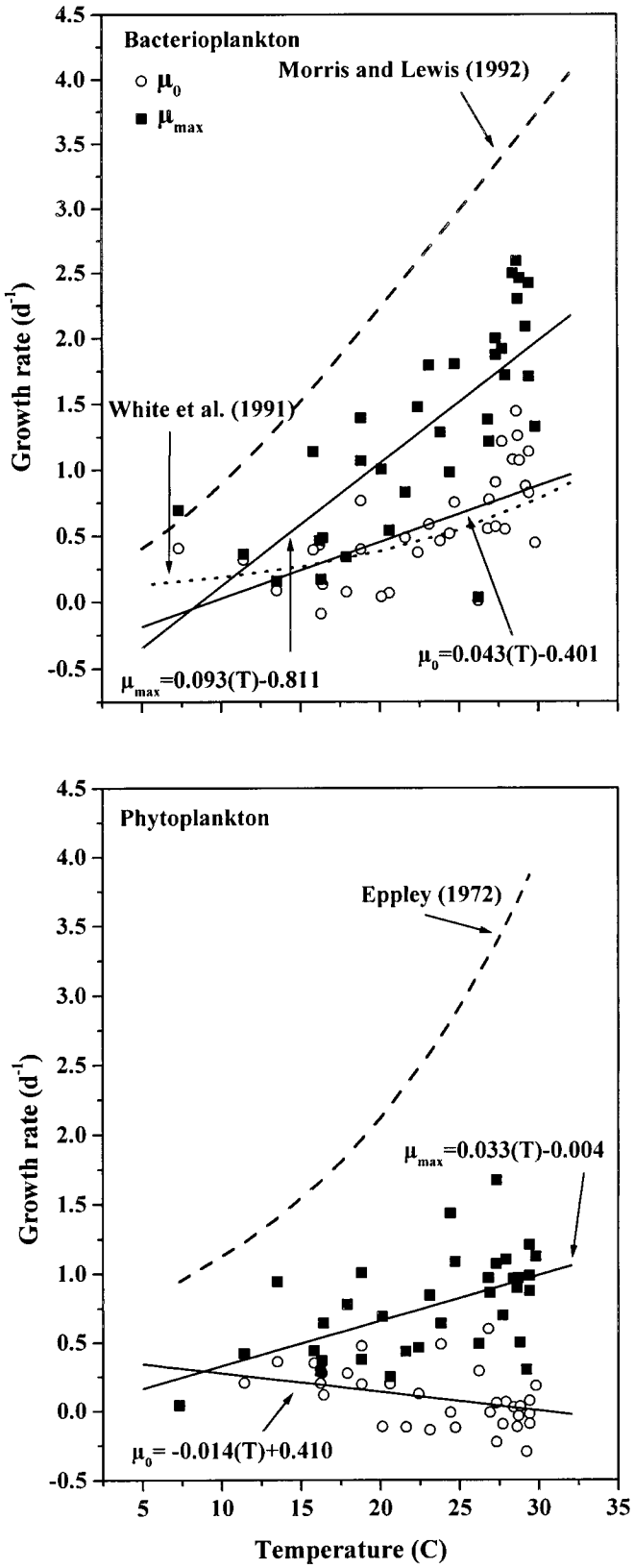

Fig. 8. Growth rate of bacterio- and phytoplankton in EML as a function of temperature. Also shown are the relationships between μ_{max} and temperature described for bacterioplankton in Lake Dillon, Colorado (Morris and Lewis 1992), and between μ_0 of bacterioplankton and temperature for a wide variety of freshwater systems (White et al. 1991). For phytoplankton, the relationship between μ_{max} and temperature described by Eppley (1972) is shown.

on those nutrients is decreased (by the dilution factor). Mortality due to predation is also assumed to be absent. Thus, the method should permit density-independent growth for some number of generations. For bacterioplankton, it is virtually impossible to remove small predators (such as protozoa) from the dilution assay without also removing a large portion of the phytoplankton. In our study, we sought to characterize the effect of nutrients on bacterio- and phytoplankton simultaneously, so rates of bacterioplankton mortality were accounted for in subsequent estimates of growth. Dilution bioassays reduce phytoplankton grazing by prescreening (153 μm Nitex) and preparation of a large dilution in a relatively small volume. However, in our experiments, negative growth rates occasionally occurred in control bottles, suggesting mortality of phytoplankton. In such cases, the impact of a nutrient supplement tends to be understated by an unknown amount (the phytoplankton mortality rate). Additionally, by removing large predators, the dilution bioassay removes a potential source of nutrients made available through regeneration processes involving predator-prey interactions (Chrzanowski et al. 1995). Because bacterioplankton and phytoplankton draw nutrients from the same dissolved pool, this source of variability would affect both communities.

It is also necessary to consider incubation times for bacterioplankton and phytoplankton growth. Samples to estimate bacterioplankton growth rates were drawn from bottles after 1 d, whereas samples to estimate phytoplankton growth were drawn after 3 or 4 d. Although this is appropriate timing on the basis of growth potential for each community, rapid bacterial uptake of nutrients and subsequent growth in control bottles could potentially reduce the pool of nutrients available to phytoplankton and suppress their growth. This would tend to magnify estimates of nutrient limitation for phytoplankton. It seems unlikely that this was ever a significant source of error. Daily time series of chlorophyll were examined for a subset of incubations, and results based on growth rates calculated over the last 2–3 d of incubation were nearly the same as those growth rates calculated over the entire incubation.

Potential for growth—Our approach also assumes that bacterio- and phytoplankton in bottles receiving NPC and NPTm, respectively, grew at nutrient-saturated rates. This assumption is probably valid for phytoplankton (NPTm), but the choice of two substrates to supply C to bacterioplankton may not accurately reflect the potential for nutrient-saturated growth. Because it is impossible to know the entire suite of substrates supplying C to bacterioplankton, the choice of glucose and acetate, both common metabolites, represented an expedient experimental compromise.

Within the limitations of our experimental approach, bacterio- and phytoplankton in JPL and phytoplankton in EML apparently grew well below μ_{max} during most of the summer

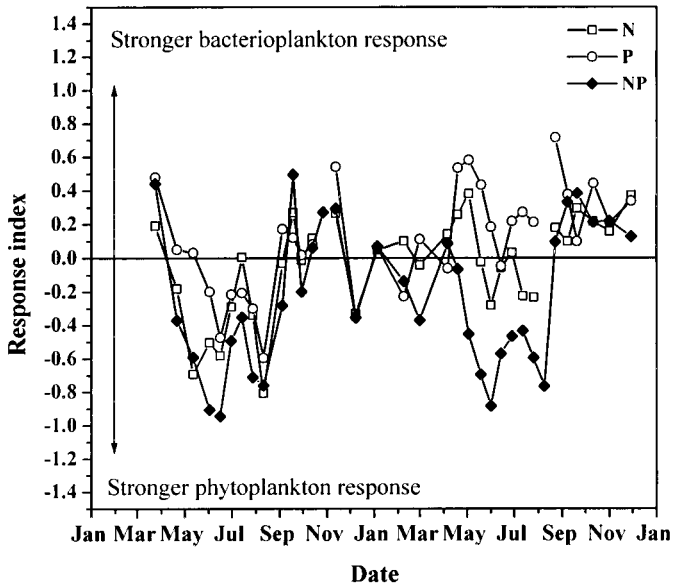

Fig. 9. Comparative responses (response index, d^{-1}) of bacterio- and phytoplankton to supplements of N, P and NP in EML. A value of 0 indicates an equivalent net response by both assemblages, a large positive value indicates a strong bacterioplankton response (change in growth rate) compared with that of phytoplankton, and a large negative value indicates a strong phytoplankton response compared wotj that of bacterioplankton.

months. The relative difference between growth under the in situ condition (μ_0) and growth at μ_{max} was much greater for phytoplankton than for bacterioplankton. Bacterioplankton in control bottles typically grew at rates that were 40% of μ_{max} or greater. Phytoplankton in control bottles grew at rates <40% of μ_{max}. This situation was similar during the cooler months in JPL, because bacterioplankton grew at rates near 80% of μ_{max}, whereas phytoplankton grew at rates typically ~60% of μ_{max}. There are insufficient data to make this comparison for samples collected during cool temperatures for EML (*but see Fig. 8*). The available data suggest that growth rates of both communities were constrained by nutrients during the entire sampling period, with bacterioplankton generally less constrained than phytoplankton.

Vertical temperature stratification is often cited as a primary mechanism driving warm-season nutrient dynamics. Sterner (1994), in his 1992 study of nutrient limitation of phytoplankton in JPL, found that vertical stratification did not delimit the onset of nutrient-limited growth. In the current study, JPL was rarely stratified, yet nutrient limitation became pronounced for both bacterio- and phytoplankton during warm-water time frames. We conclude that vertical temperature stratification has little impact on nutrient limitation of the microbial plankton in these systems.

We also note that μ_{max} for both bacterio- and phytoplankton was greater during warm months than during cooler months, although μ_{max} for both groups was lower than might be expected from previous studies (Figs. 5 and 8). Nevertheless, these patterns imply increased metabolic potential during warmer seasons. Even though bacterio- and phytoplankton had the potential for increased metabolic activity, neither community was able to fully realize this potential because of restricted nutrient availability. Thus, we suggest that the increased metabolic activity associated with higher water column temperatures brought about the nutrient limited conditions typical of summer months. Because the μ_0: μ_{max} ratio in both lakes was frequently close to 1 during the cool months, which is when we also found the lowest frequency of nutrient limitation, we conclude that the in situ growth rate at these times was primarily regulated by temperature. Our data add to the growing evidence that suggest that temperature is one of the primary factors limiting microbial growth in winter, whereas resource availability becomes one of the primary factors regulating growth in summer (Morris and Lewis 1992, Felip et al. 1996, Gurung and Urabe 1999).

Interestingly, although the estimated in situ growth rate (μ_0) for bacteria was positively related to temperature, the estimated in situ growth rate of phytoplankton was negatively related to temperature. Although both relationships are weak, this difference suggests that resource limitation (perhaps coupled with grazer mortality) more severely depressed phytoplankton than bacterioplankton growth during warm seasons. Under the assumption that warm seasons are characterized by low concentrations of dissolved nutrients, such a difference would be consistent with bacterial superiority in uptake kinetics.

Intensity of nutrient limitation—For both bacterio- and phytoplankton, multiple substrate limitation appears to be very common, and the strength of limitation by individual nutrients, or nutrients in combination, varies in time. Within this context, both bacterio- and phytoplankton in JPL seemed to be most restricted by P, whereas, in EML, N appeared to be the element most limiting to growth. In EML, the more eutrophic of the lakes, nutrient limitation was common but was not always strong for both the bacterio-and phytoplankton. In JPL, there was greater complexity in the relative strength of limitation particular when multiple nutrients were added.

Bacterio- and phytoplankton as competitors—There is a continuing, although somewhat confusing, evolution of our understanding of the interplay between bacterio- and phytoplankton in aquatic systems. Some of this confusion can be related to scale (data from single lakes vs regional lake series; see, for example, Currie 1990) and some to trophic status. For the most part, oligotrophic systems serve as the model for understanding the interplay of bacterio- and phytoplankton.

Three lines of evidence have been used to develop a working scenario in which bacterioplankton out-compete phytoplankton for nutrients but subsequently rely on phytoplankton for organic C: a strong linear relationship between bacterial and algal biomass (Bird and Kalff 1984; Cole et al. 1988), photosynthate uptake by bacteria (Riemann et al. 1982; Brock and Clyne 1984; Laird et al. 1986), and comparative nutrient uptake kinetics (Currie and Kalff 1984*a*). Bacterio- and phytoplankton draw inorganic nutrients from the same dissolved pool, and our data clearly indicate nutrient competition, in that both communities were limited by

the same nutrients (N and P) at the same time. Observations of competition and a strong influence of temperature are consistent with a model describing bacterioplankton-phytoplankton interactions proposed by Currie (1990), in which competition, temperature effects, and mutualistic provisioning and processing of organic exudates all occur. However, our data, from warm-water eutrophic systems, suggest that some refinements to the model may be in order.

We consider the response index to be a means of assessing the relative intensity of resource limitation between bacterio- and phytoplankton. The RI tends to focus attention on imbalances in responses to nutrient additions. For example, an RI of zero suggests that nutrient additions stimulated bacterio- and phytoplankton growth rates about equally; however, it is important to realize that the growth responses of both bacterio- and phytoplankton could have been large (implying strong nutrient limitation in both) or slight (implying weak nutrient limitation in both). With this understanding, consider that JPL and EML are very different lakes with respect to morphometry, age, drainage basins, and trophic status, yet there was a remarkable similarity to the timing in the strength of response to N, P, and NP additions, as well as in the overall growth responses in the lakes (compare Figs. 5 and 8). Bacterioplankton tended to respond more strongly than phytoplankton to these nutrients during early spring and fall in both lakes. Phytoplankton appeared to respond more strongly than bacterioplankton to N, P, and NP throughout the summer. Thus, although competition for nutrients could occur throughout much of the year, the intensity of competition between bacterio- and phytoplankton appears to be lessened by differences between these groups in their seasonal timing of periods of intense resource limitation. These seasonal differences in nutrient limitation between bacterio- and phytoplankton could be related to successional changes in population composition within these communities and are ripe for further exploration.

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