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Nutrient control of bacterioplankton and phytoplankton dynamics

Michael T. Brett[1], Fred S. Lubnow[2,*], Manuel Villar-Argaiz[2], Anke Müller-Solger[2] and Charles R. Goldman[2]

[1]*Department of Civil & Environmental Engineering, Box 352700, 301 More Hall, University of Washington, Seattle, WA 98195-2700, USA;* [2]*Department of Environmental Science and Policy, University of California, Davis, CA 95616, USA; *Present address: Post, Buckley, Schuh & Jernigan, Inc., 3535 Quakerbridge Road, Suite 400, Hamilton, NJ 08619, USA*

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Abstract

To determine whether positive correlations between phytoplankton and bacterioplankton growth in nutrient addition experiments are due to growth coupling or growth stimulation by the same nutrients, we examined phyto- and bacterioplankton growth in a series of eleven nutrient addition (N × P) and light/dark experiments. In mesotrophic Castle Lake, the phyto- and bacterioplankton growth responses to phosphorus (P) addition were strongly correlated ($r^2 = 0.59$), while only a weak correlation ($r^2 = 0.10$) was observed for the nitrogen addition treatments. After normalizing the N + P treatments for the growth stimulation observed in the respective P treatments, we found a substantial stimulation of the phytoplankton (e.g., costimulation by N + P) and no stimulation of the bacterioplankton. Bacteria growth rates were similar in both light and dark incubated P treatments. In these experiments, we found clear evidence suggesting the dynamics of bacteria and phytoplankton were correlated because they are often limited by the same resource (mainly inorganic phosphorus). We found only limited evidence that bacterioplankton growth coupling to algal dynamics was occurring in these experiments. However, we did not consider several factors such as dissolved organic nutrient availability, bacterivory, availability of physical substrates, and temperature which are also thought to influence the nature of bacterial/phytoplankton interactions. Based on the results of our experiments, we conclude the biomass of the bacterio- and phytoplankton covaried because they were stimulated by the same nutrients.

Introduction

Recent research suggests phytoplankton-bacterioplankton interactions play a major role in global marine, estuarine and freshwater biological carbon cycling (Del Giorgio et al., 1997). There is a classic debate in aquatic ecology as to whether bacterioplankton act as a sink or as a link to higher trophic level production in the classic food web (e.g., phytoplankton, zooplankton, and fish) (Ducklow et al., 1986; Sherr et al., 1987). Bacterioplankton can act as a link to herbivore production by recycling carbon lost from the classic food web as algal exudates, zooplankton feces or through 'sloppy feeding' by zooplankton. Bacterioplankton can also act as a sink to higher level production by competing with phytoplankton for limiting nutrients (Caron, 1994). If bacterivory rates and bacterial nutrient remineralization rates are high bacterioplankton can also have a positive impact on phytoplankton by resupplying nutrients (Chrzanowski et al., 1995). Thus, the nature of phytoplankton-bacterioplankton interactions is a central concern in aquatic microbial ecology (Caron, 1994; Pace & Cole, 1994; Del Giorgio et al., 1997).

Many studies of marine and freshwater ecosystems have noted that bacterioplankton cell abundance or production is positively correlated with phytoplankton biomass or production (Fuhrman et al., 1980; Azam et al., 1983; Linley et al., 1983; Bird & Kalff, 1984; Bjørnsen et al., 1989; Chrzanowski & Hubbard, 1989; Marvalin et al., 1989; Currie, 1990; Robarts & Wicks, 1990; Pace, 1993; Robarts et al., 1994). Several studies have interpreted this to mean that the growth of bacterioplankton is *coupled* to that of the phytoplank-

ton (Cole et al., 1988; Currie, 1990; White et al., 1991; Pace, 1993), that is, the growth of the bacterioplankton is directly stimulated by the phytoplankton. Coupling is presumed to occur because heterotrophic bacteria derive their energy by metabolizing external sources of organic carbon. Algal exudates are known to be an important source of these molecules in some systems (Cole et al., 1988; Sundh, 1992b). Several investigations have, however, found only weak correlations between bacterial growth and phytoplankton growth (Findlay et al., 1991; Wang et al., 1992; Le et al., 1994).

Many studies have also shown that the growth of bacterioplankton and phytoplankton can each be stimulated by additions of inorganic nitrogen (N) and phosphorus (P) (Toolan et al., 1991; Morris & Lewis, 1992; Wang et al., 1992; Pace, 1993). In some cases this has also been interpreted as evidence for coupling (Pace, 1993). According to this interpretation, inorganic nutrients stimulate the growth of the phytoplankton and the greater availability of algal exudates stimulates the growth of the bacterioplankton. However, it is also possible that phytoplankton and bacterioplankton are directly stimulated by the nutrient additions. If this were the case, it would be difficult to distinguish between direct mutual nutrient stimulation and metabolic coupling because both phenomena would result in a positive correlation between the growth of the bacterioplankton and phytoplankton.

Bacterioplankton are important recyclers of autochthonously produced DOM in aquatic ecosystems. If bacterioplankton growth is metabolically coupled to that of the phytoplankton, then microbial production has an overall positive impact on the production of the autotrophic food web (phytoplankton, zooplankton, fish, etc.). This will be mediated by macrozooplankton consumption of bacterioplankton or organisms such as heterotrophic flagellates or ciliates which have themselves consumed bacterioplankton. If, however, the growth of the bacterioplankton and phytoplankton are each stimulated by the same nutrients, they would instead be competing for these nutrients. Thus bacterioplankton might inhibit phytoplankton production by reducing nutrient availability.

In an attempt to untangle the underlying mechanisms behind the commonly observed positive correlations between phytoplankton and bacterial biomass we conducted eight bioassays employing factorial N and P addition treatments, in mesotrophic subalpine Castle Lake, California. We also conducted three bioassays which utilized both nutrient additions and light and dark incubations. Finally, we conducted similar experiments in ultraoligotrophic Lake Tahoe, California–Nevada, and hypereutrophic Clear Lake, California. These experiments were designed to test two alternative hypotheses. The first is that the growth of bacterioplankton and phytoplankton in nutrient addition experiments are generally correlated because the growth of bacteria is metabolically coupled to that of the phytoplankton. The second hypothesis is, the dynamics of bacterioplankton and phytoplankton are generally correlated because they are stimulated by the same resources.

The basic experimental approach employed in this study has certain well known methodological limitations which make it difficult to infer whether nutrient stimulation observed in microcosms correspond with limitation by that nutrient in lakes (Hecky & Kilham, 1988). Elser et al. (1990), however, have shown that this type of nutrient stimulation experiment can provide valuable insights into the nature of nutrient limitation in lakes. In keeping with the uncertainty expressed by Hecky & Kilham (1988) we will only refer to our experimental results as evidence of nutrient stimulation. This problem does not, we believe, preclude us from addressing the main objective of this study; that is determining whether positive correlations between bacterioplankton and phytoplankton are due to growth stimulation by the same nutrients or to bacterioplankton metabolic coupling to the growth of phytoplankton.

Methods

Experimental design

The date, duration, design, replicates, volume, and study site is outlined in Table 1 for each of the experiments presented in this analysis. All experiments were conducted in Cubitainers®. These experiments were incubated at 4 m depth (approximately 40% surface irradiance) in Castle Lake. Equal portions of lake water were collected with an opaque Van Dorn sampler at 0, 3 and 5 m and mixed to form a composite. This water was passed through a 80-μm mesh to remove larger zooplankton. All Cubitainers® used for incubation were thoroughly cleaned with dilute acid and rinsed with double deionized water and treatment lake water. The final concentrations of nutrients added were 50 μg l^{-1} P–PO_4^{-3}, and 300 μg L^{-1} N–NH_4^+ or N-glycine. These concentrations have been shown

Table 1. List of specific conditions for each of the experiments summarized in this study

Exp.#	Date ended	Duration (days)	Design	Replicates	Microcosm volume (l)	Site
1	8 July 1991	4	N*P	3	1	Castle
2	21 July 1991	4	N*P	3	1	Castle
3	9 August 1991	5	N*P	3	4	Castle
4	27 August 1991	5	N*P	3	4	Castle
5	2 August 1992	4	N*P	3	1	Castle
6	21 September 1992	5	N*P	3	1	Castle
7	17 July 1994	4	P*Light	4	4	Castle
8	3 August 1994	4	N*P*Light	4	4	Castle
9	15 September 1994	4	N*P*Light	4	4	Castle
10	11 August 1992	4	N*P	3	4	Tahoe
11	9 August 1994	4	N*P*Light	4	4	Clear

to saturate the plankton community of Castle Lake in nutrient uptake experiments (Lubnow, 1994; Elser et al., 1994a). Many of these experiments were also run with a glucose addition axis (e.g., N∗P∗G), but by and large the glucose treatments produced no or only modest growth stimulation of the bacteria (Lubnow, 1994). Therefore we have decided to only present the results from the N∗P treatments (e.g., Control, +N, +P, +N+P).

Description of study sites

Castle Lake (mean depth: 11.4 m, maximum depth: 37 m, surface area: 0.21 km^2) is a mesotrophic subalpine lake located at 1657 m in the Klamath/Siskiyou Mountain range of north central California. Secchi depth typically averages 11 m, chlorophyll-*a* concentrations average 1.4 μg l^{-1}, and total phosphorus concentrations average 10 μg l^{-1}. Recent research on Castle Lake has shown the phytoplankton of this lake are roughly equally stimulated by additions of N and P with costimulation quite common (Elser et al., 1995). The basic limnology of Castle Lake has been described extensively by Goldman & de Amezaga (1984), Jassby et al. (1990), Elser et al. (1995a, b).

Lake Tahoe (mean depth: 313 m, maximum depth: 505 m, surface area: 500 km^2) is a very large and deep ultraoligotrophic subalpine lake located at 1899 m in the Sierra Nevada mountain range on the border of California and Nevada. Lake Tahoe's Secchi depth typically averages 20–30 m, chlorophyll-*a* concentrations average 0.4 μg l^{-1}, and total phosphorus concentrations average 4 μg $^{-1}$L (Goldman, 1988). The phytoplankton in Lake Tahoe are presently primarily stimulated by P (Goldman et al., 1993). Clear Lake (mean depth: 10 m, maximum depth: 18 m, surface area: 170 km^2) is a large and shallow hypereutrophic lake located at 402 m in the coast range of northern California. Clear Lake's Secchi depth typically averages 0.8 m, chlorophyll-*a* concentrations average 130 μg l^{-1}, and total phosphorus concentrations average 350 μg l^{-1}. The cyanobacteria dominated phytoplankton in Clear Lake are N stimulated (Wurtsbaugh & Horne, 1983).

Analytical techniques

Immediately after each nutrient bioassay, 10–20 ml of sample water was preserved with 2% formalin for bacterioplankton cell counts and 100 ml of water was filtered unto GF/C filters for chlorophyll-*a* analysis. Epifluorescent microscopy of acridine-orange stained and formalin-fixed samples was used to count bacterioplankton (Hobbie et al., 1977). Sterile water was used to prepare the stain, and blanks were run for each counting session. Chlorophyll-*a* concentrations were determined using the fluorometric method with acid correction for degradation products (Strickland & Parsons, 1972) after freezing for 48 h and extracting with methanol in the dark at 4 °C for 24 h (Marker et al., 1980).

Statistical analyses

Bacterioplankton cell abundance was compared to chlorophyll-*a* concentration by regression analysis for each replicate of each treatment in the eight Castle Lake N × P bioassays. We then analyzed the

chlorophyll-*a* growth response in the eight nutrient bioassays with a three way ANOVA, including the nutrient treatments (N or P) and experiment number as independent category variables and normalized growth response as the dependent variable. The normalized growth response was calculated by dividing each observation of each experiment by the respective control treatment mean. The bacteria growth response in the eight nutrient bioassays was analyzed with a three way ANCOVA including the nutrient treatments and experiment number as independent category variables, chlorophyll-*a* concentration as a covariate, and normalized bacteria growth response as the dependent variable. These data were also analyzed using a regression analysis of the paired growth responses of the phytoplankton and bacteria in the N, P and N + P treatments. When conducting these regressions for the N+P treatments the data were further normalized by dividing by the mean growth response in the respective P treatments; this transformation was not used for the ANOVA and ANCOVA analyses discussed above.

The bacteria growth response data from the three P addition and light/dark incubation experiments were analyzed with a two way ANCOVA with P treatment and light/dark incubation as category variables, chlorophyll-*a* concentration as a covariate, and the growth response as the dependent variable. Since there was a possibility that phytoplankton would senesce in the dark treatments and stimulate bacterial growth, which would obscure the detection of coupling between bacterial and algal growth in these experiments, we conducted our statistical analysis both with and without accounting for this artifact. The data were 'corrected' by subtracting any stimulation of bacterial abundance from all dark treatment values. This stimulation was calculated by subtracting the mean bacterial abundance in the light control treatment from the mean bacterial abundance in the dark control treatment. However, we would emphasize that this transformation will not in and of itself account for all the potential pitfalls in this type of dark incubation. It is probably impossible to completely separate the metabolism of phytoplankton and bacterioplankton in natural lake water samples.

The Lake Tahoe N and P addition experiment was analyzed by ANOVA (chlorophyll-*a*) and ANCOVA (bacterioplankton) according to the procedures described for the Castle Lake N and P bioassays. The Clear Lake experiment utilized both N and P treatments and light/dark incubations. The chlorophyll-*a* light treatment data from this experiment were analyzed with a two way ANOVA with the N and P treatments as category variables and chlorophyll-*a* concentration as the dependent variable. The bacterioplankton data (both light and dark treatment) were analyzed with a two way ANCOVA with the nutrient treatments as independent category variables, chlorophyll-*a* concentration as a covariate, and bacterioplankton cell abundance as the dependent variable.

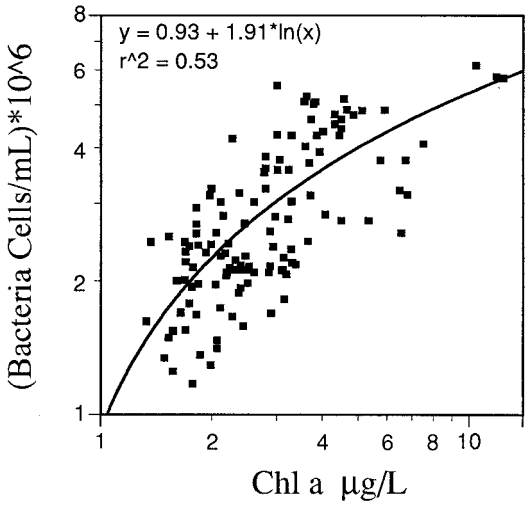

Figure 1. Phytoplankton chlorophyll concentration versus bacterioplankton cell abundance for all replicates of the eight Castle Lake N × P bioassays.

Results

Chlorophyll-a concentration versus bacterial abundance

The overall relationship between chlorophyll-*a* concentrations and bacterioplankton abundance for all replicates for the N × P treatments of the Castle Lake nutrient bioassays conducted was positive (Figure 1) and explained 53% of the variability in these data ($P = 0.0001$).

Castle lake response to N and P additions

The results for the eight factorial N × P experiments conducted in Castle Lake showed that the phytoplankton (as measured by chlorophyll-*a* concentrations) were strongly stimulated by N + P combined, as well as significantly stimulated by P and N individually (Figure 2 and Table 2). The strength of the stimulation afforded by the nutrient treatments varied significantly

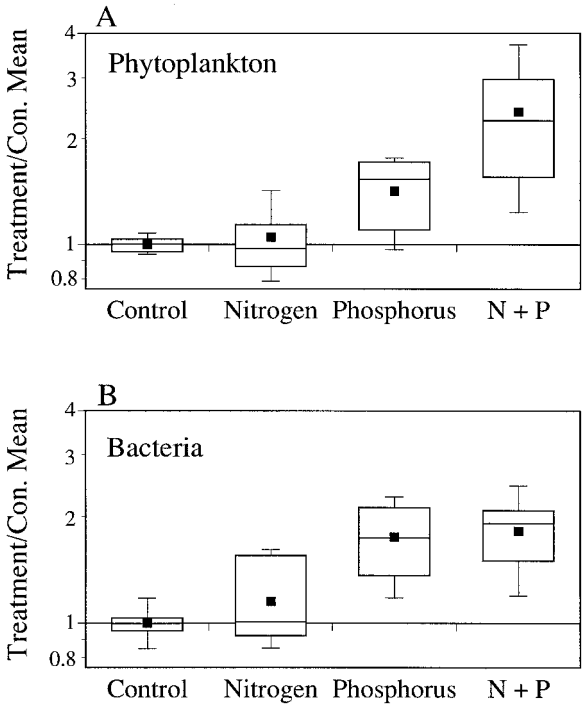

Figure 2. The phytoplankton (A) and bacterioplankton (B) treatment responses for the eight N × P bioassays conducted in Castle Lake. The treatment responses were calculated by dividing each replicate of each experiment by the respective control treatment mean. The line through the middle of the box shows the median and the dot shows the mean of the distribution. The outer edges of the box correspond to the 25th and 75th percentiles, and the 'whiskers' to the 10th and 90th percentiles.

Table 2. ANOVA results for the eight N and, P factorial experiments conducted in Castle Lake. The dependent variable is the normalized response to the nutrient additions

Source	df	SS	F-test	P	Variation explained
		Chlorophyll response			
Nitrogen	1	7.26	257.65	0.0001	13%
Phosphorus	1	20.02	710.86	0.0001	35%
N × P	1	5.68	201.85	0.0001	10%
Experiment	7	6.21	31.53	0.0001	11%
N × Exp	7	8.06	40.91	0.0001	14%
P × Exp	7	4.13	20.93	0.0001	7%
N × P × Exp	7	4.08	20.71	0.0001	7%
Error	72	1.97			3%
		Bacteria response			
Nitrogen	1	0.41	14.81	0.0003	2%
Phosphorus	1	13.17	478.53	0.0001	53%
N × P	1	0.05	1.79	0.1856	0%
Experiment	7	5.50	28.57	0.0001	22%
N × Exp	7	0.09	4.90	0.0001	0%
P × Exp	7	2.77	14.37	0.0001	11%
N × P × Exp	7	0.69	3.56	0.0024	3%
Chlorophyll	1	0.00	0.01	0.9285	0%
Error	71	1.98			8%

from one experiment to another both in terms of the overall extent of nutrient limitation (see experiment term) and by nutrient type (see interactions between experiment and nutrients).

In these same experiments, the bacterioplankton were strongly stimulated by additions of P and very weakly stimulated by additions of N (Figure 2 and Table 2). In addition, the magnitude of nutrient stimulation for the bacterioplankton differed significantly from one experiment to another. The bacterioplankton were not costimulated by N + P. Furthermore, the chlorophyll-*a* covariate was not significant.

When we compared the responses of chlorophyll-*a* and bacterioplankton to our nutrient additions by individual replicates, we found nitrogen had a positive effect on both groups but neither chlorophyll-*a* concentrations nor bacterioplankton cell abundance were markedly stimulated by additions of N alone (Figure 3a). These N results suggest phytoplankton and bacterioplankton were weakly stimulated by N in Castle Lake during these experiments.

Additions of P clearly stimulated both the phytoplankton and the bacterioplankton, and the degree of stimulation afforded by P was well correlated when comparing replicates for the individual experiments (Figure 3b). This relationship implies that P stimulates both the phytoplankton and bacterioplankton, and/or there is metabolic growth coupling between the phytoplankton and bacterioplankton in Castle Lake.

The potentially confounded relationship between direct stimulation by N or P and metabolic coupling between the phytoplankton and bacterioplankton in Castle Lake, was teased apart by dividing the growth response for each replicate of the N + P treatments by the respective mean response for the P treatment (Figure 3c). The combination of N + P resulted in a marked stimulation of the phytoplankton above, and often far beyond that seen in the P treatment. However, the bacterioplankton were only marginally stimulated above values seen in the P treatment (Figure 3c). According to this relationship, a 250% increase in algal

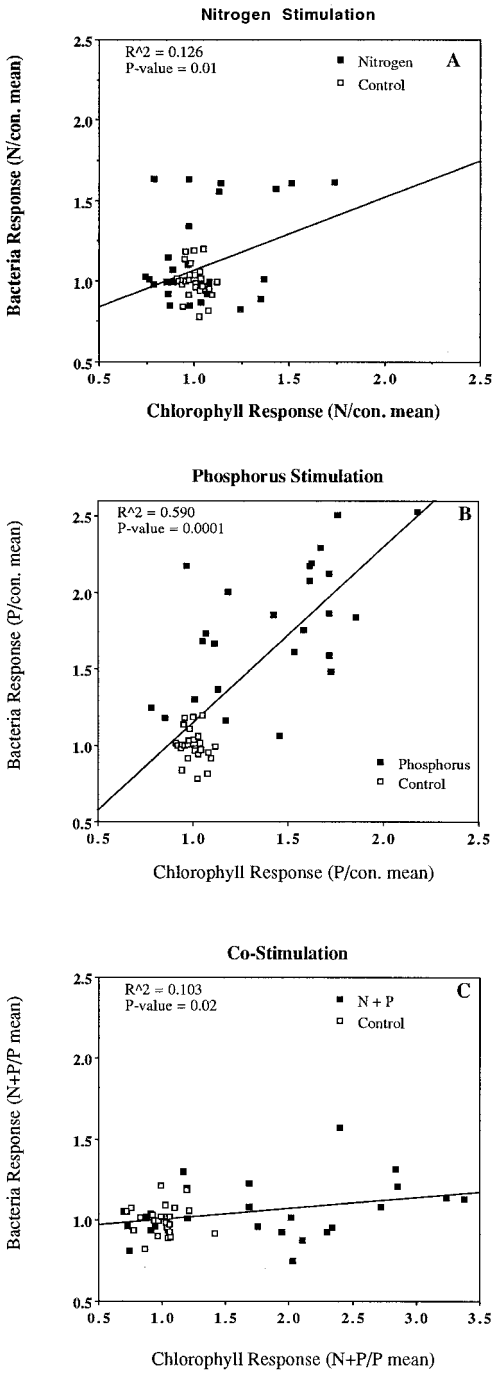

Figure 3. The relationship between the responses of the phytoplankton and bacterioplankton of Castle Lake to the nutrient bioassay treatments. See Methods section for explanation of units.

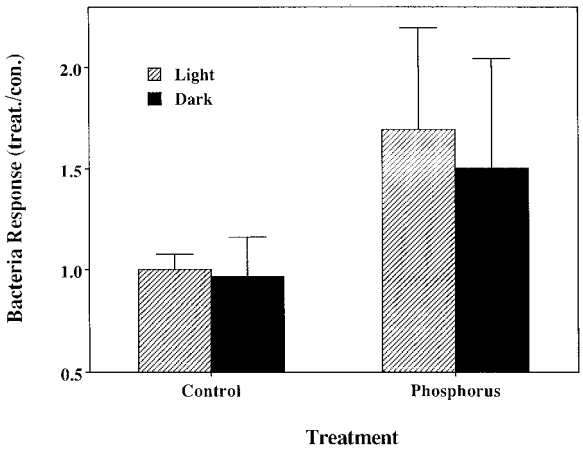

Figure 4. The treatment mean (± 1 SD) bacterioplankton responses to the three P and light/dark incubation bioassays conducted in Castle Lake.

Table 3. ANCOVA results for three light/dark and phosphorus addition bioassays preformed in Castle Lake. The dependent variable is the normalized bacteria growth response in these experiments

Source	df	SS	F-test	P	Variation explained
Phosphorus	1	4.16	76.60	0.0001	39%
Experiment	2	2.55	23.47	0.0001	24%
P × Exp	2	1.75	16.10	0.0001	16%
Chlorophyll	1	0.00	0.01	0.9804	0%
Error	40	2.17			20%

biomass would correspond to only a 17% increase in bacterioplankton cell abundance. These results are consistent with direct P stimulation of bacterial growth rather than metabolic coupling.

Phosphorus light/dark incubations

To gain further insights into nutrient stimulation and metabolic coupling between the phytoplankton and bacterioplankton in Castle Lake, we conducted three bioassay experiments utilizing P additions and light or dark incubations (Figure 4). If metabolic coupling between bacterioplankton and phytoplankton had a large impact on the growth of the bacterioplankton, then we would expect different bacterial growth patterns in the light and dark treatments due to the differences in phytoplankton growth and biomass between these treatments.

The overall ANCOVA results of these experiments show bacterial growth was stimulated by P additions,

Table 4. ANOVA and ANCOVA results for the light/dark and N and P factorial experiment conducted in Clear Lake

Source	df	SS	F-test	P	Variation explained
Chlorophyll concentration					
Nitrogen	1	33400	466.44	0.0001	98%
Phosphorus	1	2	0.03	0.8745	0%
N × P	1	14	0.20	0.6676	0%
Error	11	790			2%
Bacteria abundance					
Nitrogen	1	65.2	94.65	0.0001	72%
Phosphorus	1	3.3	4.83	0.0371	4%
N × P	1	0.0	0.01	0.9069	0%
Chlorophyll	1	4.0	5.87	0.0227	4%
Error	26	17.9			20%

however, chlorophyll-*a* concentration as a covariate did not have a significant impact on bacterial growth (Table 3). The chlorophyll-*a* concentration term was unaffected by adjusting the dark treatment values to account for the disparity in bacterioplankton abundance between the light control and dark control treatments. These results provide further evidence that the growth of bacterioplankton in Castle Lake was primarily directly stimulated by available P.

Clear Lake N and P, and light/dark bioassay

The results of this bioassay showed that the Clear Lake phytoplankton were markedly stimulated by additions of N, with no effect of the P treatment or costimulation (Figure 5 and Table 4). The Clear Lake bacterioplankton data showed a strong effect of N on bacterioplankton cell abundance, and weaker but significant effects of both P and chlorophyll-*a* concentration (Figure 5 and Table 4). When using uncorrected dark treatment values the chlorophyll covariate and the nutrient terms accounted for 5 and 85%, respectively, of the variability in the bacterioplankton growth responses. When the dark treatment values were 'corrected' to account for the disparity in bacterioplankton abundance between the light control and dark controls, the chlorophyll-*a* covariate term and the nutrient terms accounted for 15 and 75%, respectively, of the variability. In either case, these results suggest direct nutrient stimulation had the main impact on bacterial

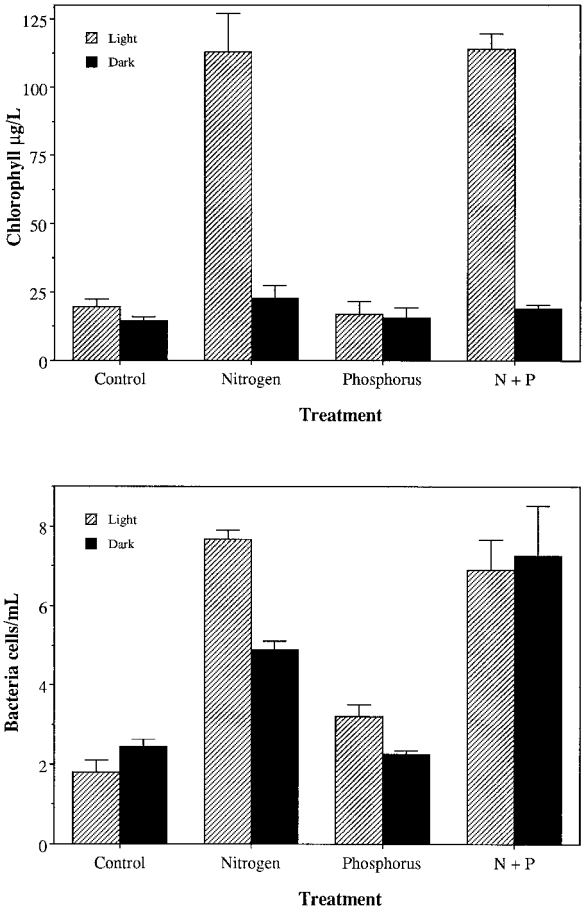

Figure 5. The treatment mean (± 1 SD) chlorophyll concentration and bacteria abundance for the N × P and light/dark bioassay conducted in Clear Lake.

abundance, but that some coupling between the bacterioplankton and phytoplankton probably occurred.

Lake Tahoe N and P bioassay

The results from the single nutrient bioassay conducted at Lake Tahoe showed phytoplankton to be mainly stimulated by additions of N, with some costimulation by N + P (Figure 6 and Table 5). In contrast the bacterioplankton were most stimulated by the P treatment and to a lesser extent stimulated by the N treatment (Figure 6 and Table 5).

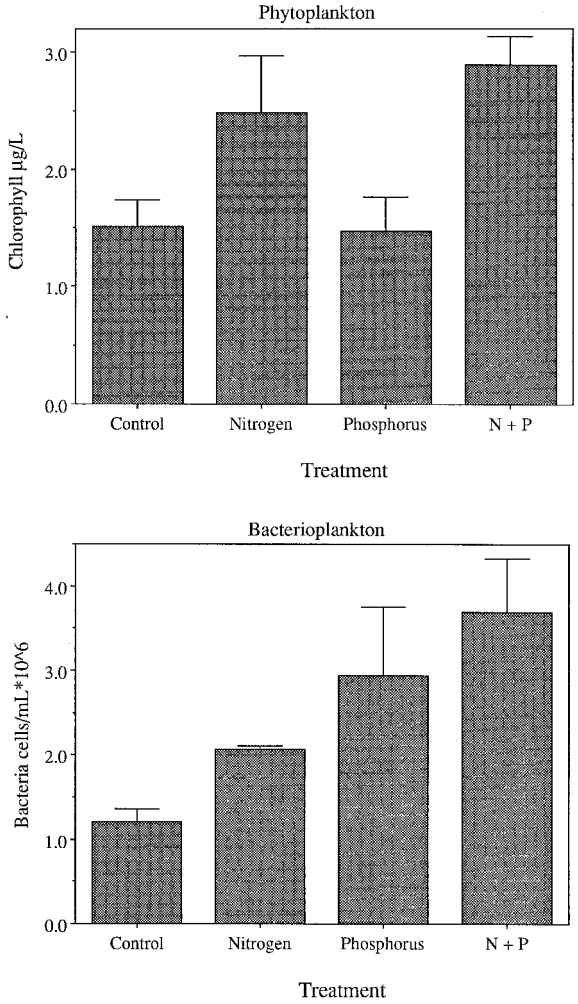

Figure 6. The treatment mean (± 1 SD) chlorophyll concentration and bacteria abundance for the N × P bioassay conducted in Lake Tahoe.

Table 5. ANOVA and ANCOVA results for the N and P factorial bioassay conducted in Lake Tahoe

Source	df	SS	F-test	P	Variation explained
Chlorophyll concentration					
Nitrogen	1	4.27	39.25	0.0001	79%
Phosphorus	1	0.11	0.96	0.5600	2%
N × P	1	0.15	1.42	0.2680	3%
Error	8	0.87			16%
Bacteria abundance					
Nitrogen	1	1.43	5.83	0.0465	13%
Phosphorus	1	8.84	35.99	0.0005	80%
N × P	1	0.03	0.14	0.7189	0%
Chlorophyll	1	0.47	1.91	0.2089	4%
Error	7	0.25			2%

Discussion

Coupling or direct nutrient stimulation?

We observed a correlation between bacterial abundance and chlorophyll-*a* concentration (Figure 1) and the growth responses of the bacterioplankton and phytoplankton to additions of P (Figure 2b). There are two obvious *and non-exclusive* explanations for this result (Currie, 1990). First, the phytoplankton and bacterioplankton may be stimulated by the same resources; and second, metabolic growth coupling between the phytoplankton and bacterioplankton may be occurring. So far most research examining bacterio- and phytoplankton interactions have focused on direct correlations between the biomass or dynamics of these biota or nutrient limitation by inorganic N and P, and simple forms of DOC such as glucose. However, it is becoming increasingly clear that other factors, such as the availability of dissolved organic N and P, protozoan bacterivory, availability of physical substrates, light and temperature, may also markedly influence the nature of bacterial/phytoplankton interactions (Björkman & Karl, 1994; Chrzanowski et al., 1995; Felip et al., 1996; Lind et al., 1997). Based on this research, we can not say that growth coupling does not occur in most cases. We can say, however, that it was not the most parsimonious explanation for the results of our experiments. In our experiments, the data suggest the dynamics of the bacterio- and phytoplankton were strongly correlated because both were strongly stimulated by additions of inorganic P. We did find some evidence of metabolic growth coupling between the bacterio- and phytoplankton in the Clear Lake experiment, but even there this affect appeared to be weaker than direct inorganic nutrient limitation.

Our N + P nutrient treatments and light/dark P addition experiments suggest that functional metabolic coupling of the growth rates of the bacterioplankton and phytoplankton in Castle Lake was weak at the short time scales used in our experiments. The results of our N + P treatments showed the phytoplankton were stimulated far beyond values seen in the P alone treatments (i.e., they were costimulated by N + P), while the growth of the bacterioplankton was only slightly stimulated above values seen in the P treatment (Figure 3c). This shows that even in the presence

of surplus P, the bacterioplankton did not to respond to a presumably increased supply of algal exudates. Several P addition and light/dark incubation experiments showed the bacterioplankton had similar growth rates in the light and dark treatments and chlorophyll-*a* growth did not explain a significant amount of variability within the nutrient treatments (Figure 4 and Table 3). Based on the above, we infer that the positive correlations between the abundance and growth responses of the bacterioplankton and phytoplankton in our experiments were a result of direct nutrient stimulation by P for both the bacterioplankton and phytoplankton.

These results suggest direct nutrient limitation is a plausible alternative explanation for the commonly observed positive correlations between the bacterioplankton and the phytoplankton biomass and growth responses to nutrient addition treatments (Cole et al., 1988; Currie, 1990; Robarts & Wicks, 1990; White et al., 1991; Pace, 1993). These correlations need not be *prima facie* evidence for functional coupling. Based on negative or insignificant correlations between the growth response, production, or biomass of bacterioplankton and phytoplankton, several recent studies have concluded that the growth of the bacterioplankton was weakly coupled or uncoupled to that of the phytoplankton (Findlay et al., 1991; Wang et al., 1992; Pace, 1993; Le et al., 1994). Clearly, DOC is the energy source for bacterial growth, and phytoplankton may supply a major fraction of the DOC, as well as DON and DOP. However, as Morris & Lewis (1992) explained 'it does not follow that algal [exudate] flux places a direct constraint on bacterioplankton growth. Inorganic nutrients could regulate bacterioplankton growth despite the ultimate reliance of bacterioplankton on organic matter'. This statement is supported by the findings of Reche et al. (1998) who found bacterial utilization of natural DOC was regulated by inorganic nutrient availability.

P stimulation of bacterial growth

We found that the bacterioplankton in Castle Lake were stimulated by additions of P (Figures 2, 3b and Table 2). This observation is consistent with the findings of several other studies which also found bacterioplankton to be stimulated by P additions (Toolan et al., 1991; Coveney & Wetzel, 1992; Morris & Lewis, 1992; Pace, 1993). Several studies have argued that the bacterioplankton's rapid uptake of P suggests bacterioplankton have the ability to acquire P when needed and therefore bacterioplankton should not be limited by P under most circumstances (Currie & Kalff, 1984; Currie, 1990). However, one could also use these data to argue that bacterioplankton have a high demand for P and thus have a high probability of being limited by P. If, as has been suggested by Cotner & Wetzel (1992) and Morris & Lewis (1992), the bacterioplankton have high P uptake rates and poor P storage and retention capabilities, then the question of whether bacterial P demand is actually met becomes less clear.

In our opinion, the theoretical line of reasoning regarding bacterial P demand which is the most persuasive ignores uptake rates and focuses on the elemental ratios (i.e., stoichiometry) of the bacterioplankton, phytoplankton, seston and dissolved nutrients. Studies have shown that bacterioplankton have high P:C ratios relative to phytoplankton and ambient lake water (Vadstein et al., 1988; Vadstein & Olsen, 1989; Vadstein et al., 1993). This suggests the bacterioplankton may be primarily stimulated by P, as opposed to labile DOC, and the bacterial demand for P will be greater than the algal demand for P. Bacterioplankton, phytoplankton and dissolved P:C ratios vary both within and particularly between lakes (Vadstein et al., 1993). Knowing this we may be able to predict that as the ratio of available P:C approaches the bacterial P:C ratio, bacterioplankton will become increasing stimulated by a resource other than P such as dissolved inorganic N and/or algal exudates (DOC).

Bacterial nutrient demands in N stimulated lakes

Our experiments in Clear Lake supported the prediction that bacterioplankton will be increasingly N stimulated in lakes where the phytoplankton are N stimulated (Figure 5 and Table 4). Since Clear Lake water generally has very high P concentrations the phytoplankton are generally N stimulated (Wurtsbaugh & Horne, 1983). Consistent with this we found the bacterioplankton were also N stimulated, but to a lesser extent than the phytoplankton. This is the second observation of substantial N stimulation of bacterioplankton growth in a freshwater lake, Wang et al. (1994) experiment #4 being the other. Other studies have found weak but significant stimulation of bacterioplankton in response to additions of inorganic N (Morris & Lewis, 1992; Pace, 1993; Wang et al., 1994; Le et al., 1994), but Wang's and our data show a N stimulation which is quite a bit stronger than the P effect. Notably, Clear Lake and Lake Hebgen (Wang's

study site) are the first cases where the nutrient demands of bacterioplankton were examined in lakes where the phytoplankton were primarily N stimulated.

Although a summary of numerous nutrient bioassays conducted over the last 30 years indicates Lake Tahoe has shifted from mainly N limitation to mainly P limitation (Goldman et al., 1993), the phytoplankton of Lake Tahoe were N and to a lesser extent co-stimulated by N + P when we conducted our bioassay (Figure 6 and Table 5). The bacterioplankton results from this experiment provide further support for the prediction that bacterioplankton will be increasingly N stimulated in lakes where the phytoplankton are N stimulated. Although bacterial abundance was most consistently stimulated by additions of P, the N treatment explained a substantial proportion of the variability for this experiment. In addition the interaction term of the statistical analysis was significant, indicating that extensive costimulation between N and P occurred.

Conclusions

Our results suggest that the bacterioplankton of Castle Lake were primarily stimulated by P, while the phytoplankton were stimulated by N and P. The correlation between the biomass and the growth responses of the bacterioplankton and phytoplankton of Castle Lake to P additions was due to a mutual demand for inorganic P. Metabolic coupling of bacterial growth to phytoplankton growth was not observed in Castle Lake during these experiments. In general the bacterioplankton's demand for P was greater than the phytoplankton's. In Clear Lake, where the phytoplankton were N stimulated, the bacterioplankton were also stimulated by P and evidence for some growth coupling was found. These results also suggest that at the short time scales on these experiments (4–5 d) competition for nutrients may be a critical aspect of bacterioplankton-phytoplankton interactions.

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