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# Regulation of the relationship between phytoplankton *Scenedesmus acutus* and heterotrophic bacteria by the balance of light and nutrients

**Tek Bahadur Gurung, Jotaro Urabe*, Masami Nakanishi**

Center for Ecological Research, Kyoto University, Kamitanakami, 509-3, Hirano-cho, 520-2113 Otsu-shi, Shiga, Japan

ABSTRACT: Culture experiments were conducted with the alga *Scenedesmus acutus* and heterotrophic bacteria to examine if the nature of their relationship changes according to the balance of light and nutrient supplies. Mixtures of algae and bacteria were grown in various combinations of 6 light intensities and 4 phosphorus (P) concentrations at high N:P ratio (80:1). We used an artificial medium composed of inorganic nutrients so that bacteria relied on organic matter released by algae as carbon (C) source. Every 2 d, 25% of the culture suspension was replaced by fresh medium. At the end of incubation when both bacterial and algal densities were stabilized, bacteria were separated from algae. Bioassays with glucose and/or inorganic P enrichment were then performed to assess the extent to which bacterial growth rate was limited by organic C or inorganic P. The algal density in the semi-batch culture was low under the light intensity <55 μE $m^{-2}$ $s^{-1}$ regardless of P concentrations, while it was higher at higher light and P supply rate above that light intensity. The bacterial density was higher in the cultures where algal density was higher. The bioassay revealed that bacteria were C limited at the light intensity <55 μE $m^{-2}$ $s^{-1}$, indicating a commensal relationship between algae and bacteria. Above that light intensity, bacteria suffered from deficiency of organic carbon rather than P at lowest P supply rate, because of low algal biomass due to a shortage in P supply. At moderate P supply rates and light intensities ≥55 μE $m^{-2}$ $s^{-1}$, however, bacterial growth was limited by P rather than organic C, because supply of organic C from algae exceeded P supply relative to bacterial demand. Further increase in P supply released both algae and bacteria from P limitation. Thus, competitive interaction for P was most intense at a moderate P supply rate. These results demonstrate that there is a shift between commensalism for C and competition for P depending on light intensity and nutrient supply rate.

KEY WORDS: Light:nutrient limitation · Commensalism · Competition · Bacteria versus algae

## INTRODUCTION

Much attention has been directed towards the relationship between phytoplankton and bacteria in aquatic environments (Bratbak & Thingstad 1985, Cole et al. 1988, Ducklow & Carlson 1992, Thingstad & Rassoulzadegan 1995). A number of studies showed that bacteria can use dissolved organic carbon (DOC) originated from phytoplankton as an energy source (Coveney 1982, Pomeroy & Wiebe 1982, Riemann & Søndergaard 1986, Bjornsen et al. 1989, Baines & Pace 1991, Lyche et al. 1996). Bacterial production and/or biomass covary with primary production or phytoplankton biomass over various aquatic systems (Cole et al. 1988, White et al. 1991, Ducklow & Carlson 1992, Egli 1995). Such cross-system relationships have led to the traditional view that the growth rate of bacteria may rely on phytoplankton's activities and therefore be substrate limited. If this view is true, the relation of bacteria to phytoplankton can be regarded as one of commensalism because the bacteria benefit from phytoplankton which are not harmed from the relationship (Lawrence 1995).

However, experimental work involving enrichment experiments with organic and inorganic chemicals has demonstrated that bacterial growth is also limited by inorganic nutrients (Toolan et al. 1991, Coveney &

*E-mail: urabe@ecology.kyoto-u.ac.jp

Wetzel 1992, Morris & Lewis 1992, Wang et al. 1992, Chrzanowski et al. 1995). Since P is one of the important nutrients limiting phytoplankton growth, the relationship between bacteria and phytoplankton might be regarded as competitive. Bratbak & Thingstad (1985) pointed out a paradoxical situation in phytoplankton-bacterial interactions. At low nutrient concentration, an increase in organic C from phytoplankton can stimulate the growth of their potential competitor because bacteria have a high affinity for low nutrient concentrations (Currie & Kalff 1984, Suttle et al. 1990, Güde et al. 1992, Rothhaupt & Güde 1992). At a high nutrient concentration, however, phytoplankton can take up nutrients predominantly over the bacteria because phytoplankton have a higher maximum uptake rate (Suttle et al. 1990, Güde et al. 1992, Rothhaupt & Güde 1992). Thus, the relationship between bacteria and phytoplankton is not simple but changes under different environmental conditions.

To progress in our understanding of community structure and material flow, it is crucial to identify the specific conditions promoting specific relationships between organisms, rather than just identify those relationships. Solar energy and nutrient input are essential external forces sustaining ecosystems but their balance differs among locations. Recently, Urabe & Sterner (1996) found that the efficiency of material flow from primary producers to consumers changed according to the balance of light and nutrient input. Sterner et al. (1997) hypothesized that the balance of light and nutrient input is also crucial in determining the relationship between bacteria and phytoplankton. According to their hypothesis, when light is abundant relative to nutrients, phytoplankton fix carbon in surplus and route it into exudation in large amounts (Lancelot 1983, Jensen 1984). In such a situation, bacterial growth will likely be nutrient limited because organic C availability is expected to be higher than nutrient availability, while at low light:nutrient ratios, phytoplankton are expected to excrete less organic C due to low photosynthetic rate. In such situations, bacteria will be energy limited because of a deficiency in organic C. If this hypothesis is true, competitive interactions between bacteria and phytoplankton should be more intense at higher light:nutrient ratios while commensalism should be promoted at lower light:nutrient ratios.

In the present study, such a possibility was examined by culture experiments, in which bacteria were grown with the phytoplankton species *Scenedesmus acutus* under different regimes of light intensities and inorganic P supply rates. The term 'nutrient supply rates' here refers to the concentration of nutrient input into experimental cultures. We conducted these experiments with a 'semi-batch culture' condition, where 25% of culture suspension was replaced with fresh medium every other day. The limiting resource and the magnitude of limitation for bacterial growth were then evaluated by the response of bacterial growth to nutrient enrichment after separating them from the cultured phytoplankton. Here, we focused on P as a limiting nutrient because this element is the most deficient one in many lakes.

## MATERIALS AND METHODS

Semi-batch culture experiments were conducted under 4 P concentrations (0.1, 0.25, 1.58 and 10 µM) in combination with 6 light intensities (5.5, 12, 25, 55, 130 and 260 µE $m^{-2}$ $s^{-1}$) using a green alga, *Scenedesmus acutus*, and lake bacteria. These P concentrations and light intensities are within the ranges found in freshwater lakes (Urabe & Sterner 1996). The experiment was made with COMBO (Kilham et al. 1998), an artificial growth medium which contains no organic compounds except the trace amount of vitamins and EDTA. Nutrient concentrations were adjusted by adding the desired concentration (see above) of phosphorus as $K_2HPO_4$ and nitrogen (N) as $NH_4Cl$. The N:P ratio was held at 80:1 (molar) so that N was sufficient relative to P. The experimental medium was autoclaved before use. Light intensity was adjusted by the number of cool-white fluorescent bulbs and by placing black window screens over the bulbs. The intensity was measured and determined by a Li-Cor (Lincoln, NB) quantameter (LI-1000, 2π sensor) placed just outside of the experimental flasks. All experiments were conducted at 20°C in an incubator.

*Scenedesmus acutus* were obtained from axenic stock cultures maintained for more than 4 yr under a constant lab condition. The bacteria were collected at 5.0 m depth from the north basin of Lake Biwa immediately before the experiment. The lake water was filtered through a pre-combusted (at 450°C for 2 h) GF/F filter and bacteria in the filtrate were used as the experimental inoculum. In this procedure, we used the precombusted GF/F filter to avoid any possible contamination of organic matter from the filter. Epifluorescence microscopic observation confirmed that there were no autotrophs (pico-phytoplankton) and bacterial predators (heterotrophic nanoflagellates etc.) in GF/F filtered lake water.

To initiate the experiments a small number (~$0.1 \times 10^4$ cells $ml^{-1}$ at final concentration) of *Scenedesmus acutus* from the stock culture and a known volume of the GF/F filtered lake water (~1.0 ml) with bacteria (<$1.0 \times 10^4$ cells $ml^{-1}$ at final concentration) were inoculated into 1 l Erlenmeyer flasks containing the artificial growth medium. No replication was made at each light and nutrient condition. At 2 d intervals, 25% of the flask volume was removed and replaced with auto-

claved fresh medium of appropriate nutrient concentrations. This procedure was made under a clean-bench (Showa Science, S-1300 PRV). All glassware was acid rinsed and sterilized by heat before use. Samples for algal and bacterial counts were collected from the replaced suspension at 2 d intervals. Each flask was hand shaken once a day. Since preliminary experiments indicated that both bacterial and algal densities were stabilized and not changed significantly after 10 d, the experiment lasted 14 d.

Samples for algal and bacterial counts were fixed with glutaraldehyde at 0.5% final concentration and stored at 4.0°C until enumeration. Algal density was determined using a haemocytometer. Bacterial density was estimated using the acridine orange direct count method (Hobbie et al. 1977): 3 subsamples were made by filtering 0.5 to 5.0 ml of experimental suspension onto Nuclepore filters (0.2 µm pore size). Bacteria were enumerated under an Olympus epifluorescence microscope (1250×) with a B-excitation system (50 W halogen lamp, IF 410-485 excitation filter). At least 300 bacterial cells were counted for each subsample. It should be noted that bacterial counts do not necessarily reflect the bacterial biomass because cell size is known to change according to different environmental conditions (Ferguson & Rublee 1975, Bjornsen et al. 1989).

At the end of the semi-batch culture experiments, a bioassay test was performed to examine the factors limiting bacterial growth in the flasks placed at different light and nutrient conditions. A 400 ml sample was collected from the semi-batch culture and passed through a 1.0 µm Nuclepore filter to remove the algae. We filtered as gently as possible so as not to break algal cells, which might cause DOM contamination. Microscopical observation indicated that bacterial density was not significantly changed before and after the filtration. The 100 ml filtrate was then distributed into 4 flasks. One flask was used as control and did not receive any nutrients. The remaining 3 flasks were enriched with either $K_2HPO_4$ (final concentration 10 µM P), glucose (final concentration 100 µM C) or a mixture of the two. Nitrogen ($NH_4Cl$) was also added to the flask receiving P at a concentration of 800 µM to hold the N:P ratio at 80:1. Each flask was then incubated under the same light intensity at which bacteria were exposed during the semi-batch culture experiment. At the end of the 2 d incubation, 20 ml sub-samples were collected for bacterial enumeration.

The growth rate of bacteria ($\mu$, $d^{-1}$) during the 2 d incubation was estimated as follows:

$$\mu = \frac{\ln(C_{final}) - \ln(C_{initial})}{2} \quad (1)$$

where $C_{final}$ and $C_{initial}$ are the final and initial densities of bacteria in each flask. To examine the degree of carbon ($LI_C$) and phosphorus ($LI_P$) limitation for bacterial growth, a 'Limitation Index' was estimated as follows,

$$LI_C = \frac{\mu_{+C} - \mu_{control}}{\mu_{+P+C} - \mu_{control}} \quad (2)$$

$$LI_P = \frac{\mu_{+P} - \mu_{control}}{\mu_{+P+C} - \mu_{control}} \quad (3)$$

where $\mu_{+P}$, $\mu_{+C}$, $\mu_{+P+C}$, and $\mu_{control}$ are bacterial growth rates in +P, +C, +P+C and control treatments, respectively. An $LI$ value of 100% indicates that bacterial growth was limited by the given resource while an $LI$ of 0% indicates that the resource did not limit the growth rate.

Effects of light and P supply rate on bacterial and algal densities, and ratios of bacterial to algal densities were examined statistically by 2-way ANOVA without replication. The density ratios were transformed to log (n+1) before the statistical test to stabilize variances. The response of bacterial growth rate to organic C and inorganic P enrichment in the bioassays was examined by 2-way ANOVA with fixed model. In this analysis, data of the growth rate of bacteria cultured at each P supply rate was divided into 2 groups based on light intensity, ≤25 and >25 µE $m^{-2}$ $s^{-1}$, and the data within these groups were treated as replication. This criterion of light intensity is somewhat arbitrary but enables us to examine if the response of bacteria to C and P enrichment differs between high and low light conditions.

## RESULTS

In nearly all light and nutrient combinations, both algae and bacteria increased their densities for the first several days and reached saturation level within 10 d. The only exception was bacterial density in the 1.58 µM P treatment under high light intensities (Fig. 1). Therefore, we treated the average densities between Days 10 and 14 as equilibrium densities in the following analyses. The equilibrium densities of bacteria and algae differed significantly according to light and nutrient supply rate (Table 1) and increased with increasing light intensity and nutrient supply rate

Table 1. *F*-values of ANOVA for the densities of bacteria and algae at saturation levels, and their density ratio

| Source of variance | df | Bacteria | Algae | Bacteria:algae |
|---|---|---|---|---|
| Light intensity | 5,15 | 9.88*** | 5.91** | 13.97*** |
| P supply rate | 3,15 | 9.38*** | 5.34* | 4.84* |

*Significant at 5% level; **significant at 1% level; ***significant at 0.1% level

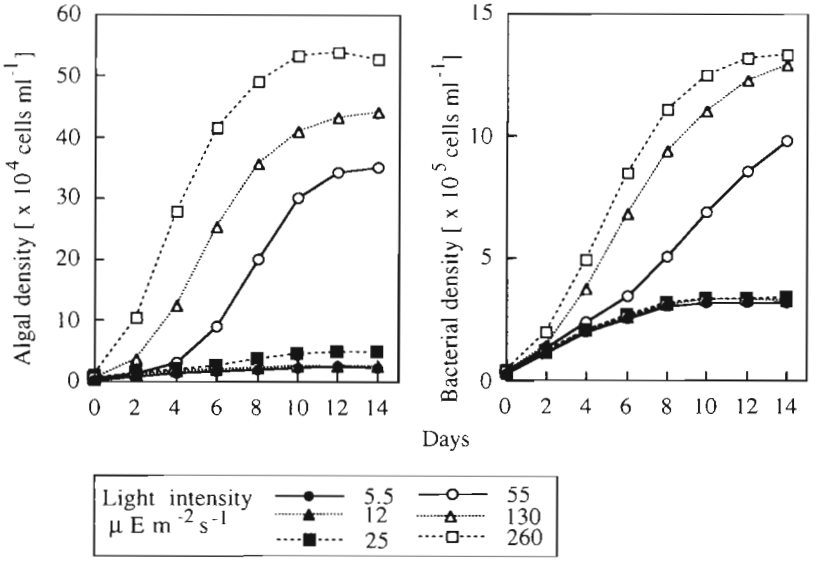


Fig. 1. Example of temporal changes in bacterial and algal densities in the semi-batch cultures. Results of 1.58 μM P treatments

(Fig. 2). In both algae and bacteria, the range of densities among light conditions was larger at a higher nutrient supply rate, indicating that response to changes in light intensity depends on nutrient supply rate. At the highest light intensity, the saturation density of algae showed a 7-fold increase from the lowest (0.1 μM) to the highest (10 μM) P supply rate, but that of bacteria showed only a 3-fold increase. The results imply that algae are more sensitive to changes in nutrient supply rate than bacteria are.

Throughout the experiments, the equilibrium density of bacteria correlated significantly with the algal density (Fig. 3). However, the ratio of bacterial to algal densities also substantially changed according to the light and nutrient supply rate (Table 1). In general, the ratio decreased with increasing light intensity (Fig. 4). At the light intensity ≤25 μE $m^{-2}$ $s^{-1}$, the ratio was around 100 regardless of nutrient supply rate, which was much higher than the ratio at the start of culture experiments (~10). However, at the light intensity above 25 μE $m^{-2}$ $s^{-1}$, the ratio of bacterial to algal densities tended to decrease with increasing nutrient supply rate.

To examine the relative importance between P and organic C as factors limiting bacterial growth rate, bioassays were performed by adding P and/or glucose to culture suspension after removing algae.

During the 2 d bioassay incubation, bacterial density changed little in control flasks (no nutrient addition), except for the bacteria from 10 μM P treatments (Fig. 5). Statistical analysis revealed that regardless of P supply rate during the semi-batch cultures, the growth rate of bacteria was significantly stimulated by glucose addition but not by P addition at light intensity ≤55 μE $m^{-2}$ $s^{-1}$ (Table 2). Above this light intensity, however, the response of bacteria to glucose and P addition differed according to P supply rate during the semi-batch cultures. At light intensity ≥55 μE $m^{-2}$ $s^{-1}$, both glucose and P addition stimulated the growth rate of bacteria from 0.1 and 1.58 μM P treatments. For bacteria from 1.58 μM P, the growth rate was much higher when both glucose and P were added than when either of these were added at light intensity ≥55 μE $m^{-2}$ $s^{-1}$, suggesting a dual limitation by C and P. The growth rate of heterotrophic bacteria from 0.25 μM P treatments was significantly stimulated by P addition alone. For the bac-

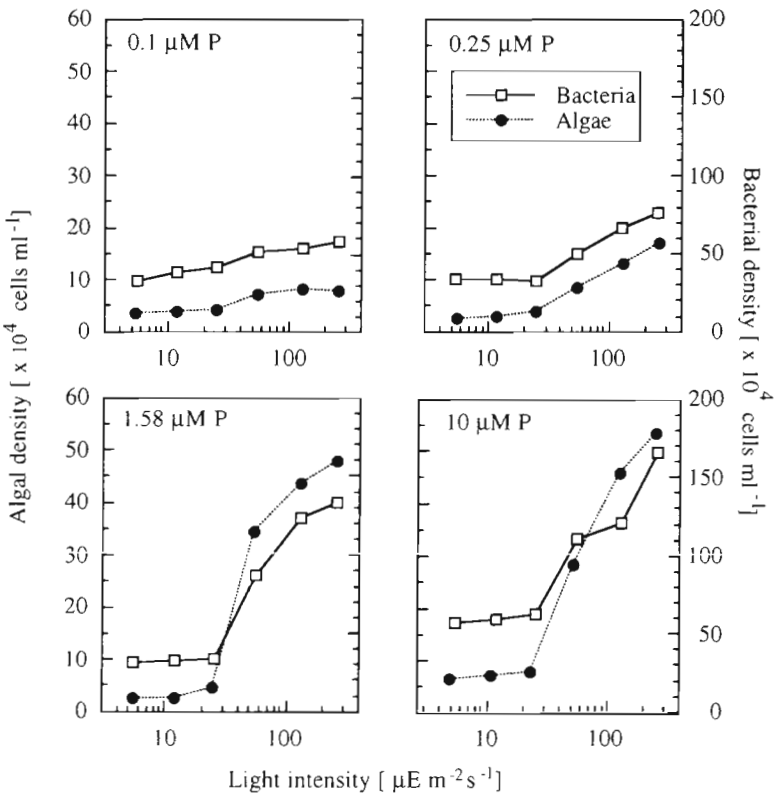


Fig. 2. Equilibrium densities of bacteria and algae at different light intensities and P supply rates. Scale of horizontal axis is logarithmic

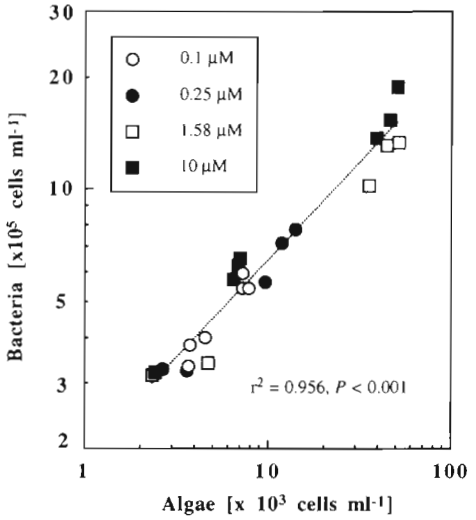


Fig. 3. Equilibrium densities of bacteria plotted against that of algae. The regression line was fitted for data from all experiments

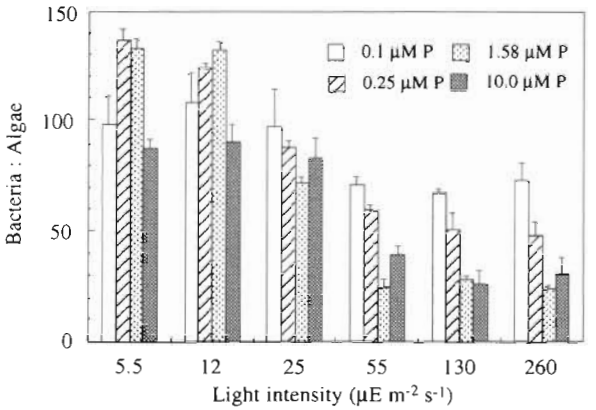


Fig. 4. Ratio of bacterial to algal densities at various light intensities and P supply rates. The vertical bars are ±SD on the mean

teria from 10.0 µM P treatments, neither P nor glucose additions stimulated their growth rate.

To quantify the degree of growth limitation of heterotrophic bacteria, a limitation index for P and organic C (glucose) was calculated (Eq. 1, Fig. 6). The index showed that at light intensities ≤25 µE $m^{-2}$ $s^{-1}$, bacterial growth was limited by organic C alone regardless of P supply rate. Above this light intensity, the degree of C limitation decreased with increasing P supply rate, while P limitation was most intense at moderate P supply rate (0.25 µM P treatment).

Table 2. *F*-values of ANOVA for bacterial growth rate in bioassays. Source of variance is effects of organic carbon (C) and inorganic phosphorus enrichment (P) and their interaction effect (C × P)

| P supply | Source of variance | Low light intensity <55 µE $m^{-2}$ $s^{-1}$ | High light intensity ≥55 µE $m^{-2}$ $s^{-1}$ |
|---|---|---|---|
| 0.1 µM | C | 900.58*** | 244.12*** |
| | P | 1.17 | 30.83*** |
| | C × P | 0.41 | 5.50* |
| 0.25 µM | C | 755.44*** | 3.76 |
| | P | 0.15 | 11.66** |
| | C × P | 0.11 | 0.66 |
| 1.58 µM | C | 6621.09*** | 22.85** |
| | P | 0.77 | 28.63*** |
| | C × P | 0.47 | 22.38** |
| 10.0 µM | C | 402.45*** | 0.34 |
| | P | 1.17 | 0.77 |
| | C × P | 2.52 | 0.32 |

*Significant at 5% level; **significant at 1% level; ***significant at 0.1% level

## DISCUSSION

The present study demonstrates that changes in light and nutrient supply alter the limiting resource not only for algae but also for bacteria. At light intensities ≤25 µE $m^{-2}$ $s^{-1}$, the density of *Scenedemus acutus* in the semi-batch cultures was relatively low regardless

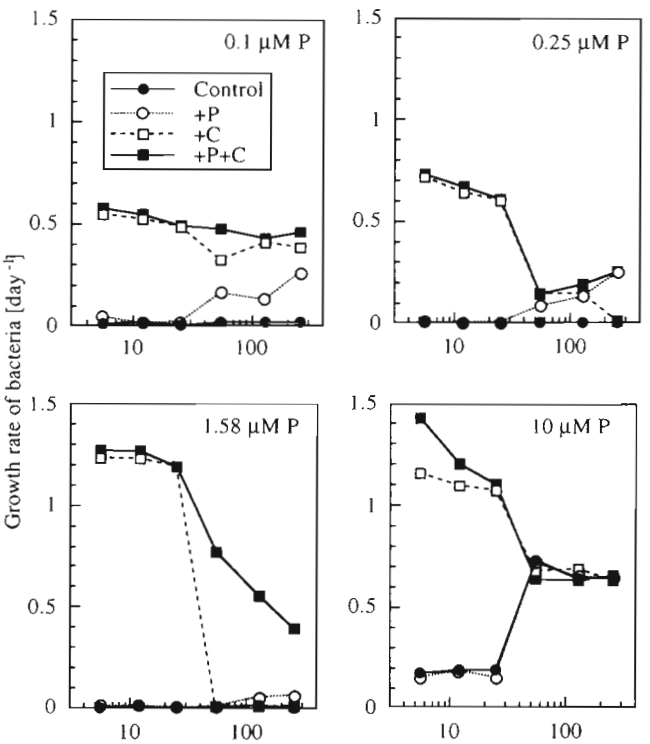


Fig. 5. Growth rate of bacteria in each treatment combination in factorial enrichments of organic carbon (+C) and inorganic phosphorus (+P). Response of the growth rate was examined after removing algae under the same light intensity where bacteria were grown with algae in the semi-batch cultures

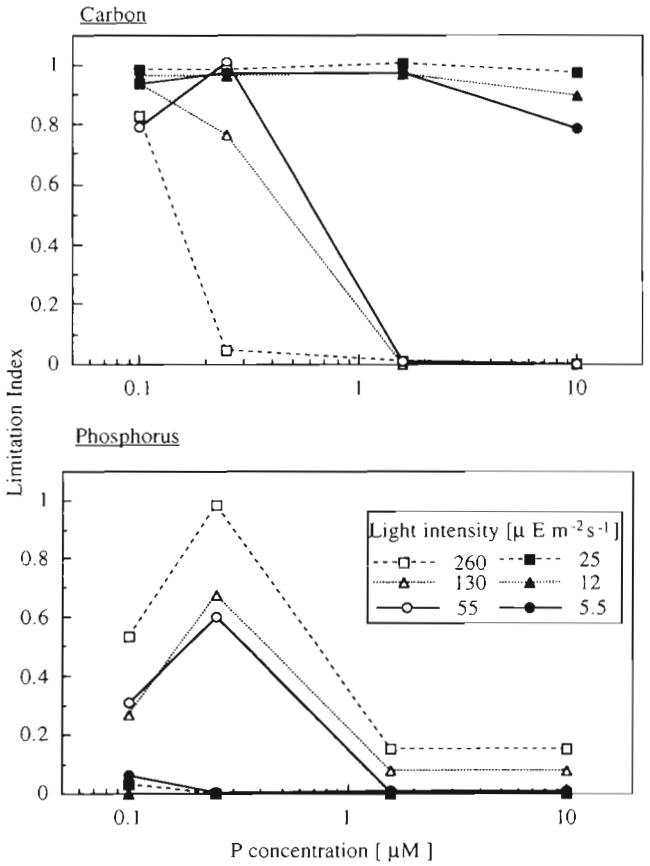


Fig. 6. Limitation index of C and P for the bacteria grown with algae in the semi-batch cultures under different light intensities and P supply rates

of P supply, indicating that algal growth rate was energy limited. However, above that light intensity, algal density increased with increasing light intensity and with increasing P supply rate. The response of algal density to changes in light intensity was larger at a higher P supply rate. Thus, at light intensities >25 μE $m^{-2}$ $s^{-1}$, P concentration was the primary factor limiting algal density with light intensity jointly affecting algal growth.

In parallel with algal density, bacterial density increased with increasing light intensity and P supply rate. At low light intensities, bacterial density was relatively low regardless of P supply rate. Under these light intensities, production of organic C by algae is expected to be low because of low photosynthetic activity. Indeed, growth rate of bacteria from the semi-batch cultures with algae under low lights was stimulated when glucose was added in the bioassay. This result implies that at low light intensities, both algae and bacteria are energy limited and their relationship is one of commensalism because bacterial growth rate depends on organic C produced by and released from algae which are not harmed from the relationship (Fig. 7A).

Under low light conditions, one might expect bacterial density to be lower relative to algal density because production of organic carbon per algal cell should be lower. However, the ratio of bacterial to algal densities was higher at lower light conditions regardless of P supply rate. At low light conditions, algal survival rate might be low. If this is true, the ratio of dead to live

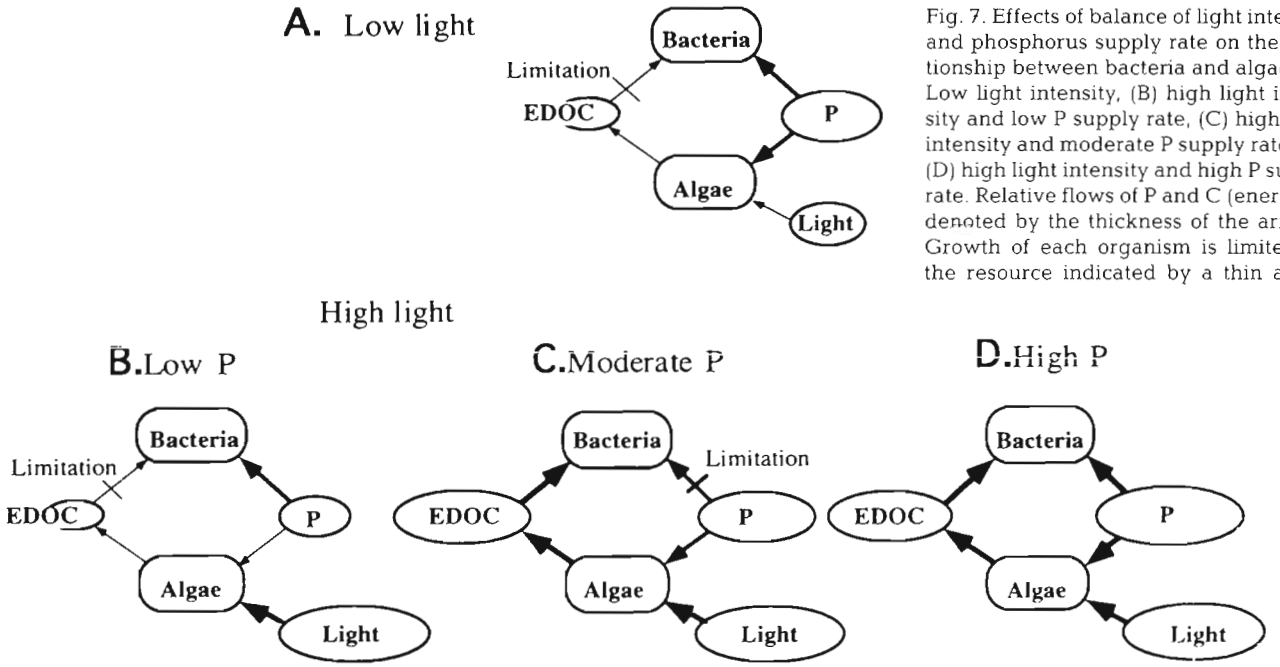


Fig. 7. Effects of balance of light intensity and phosphorus supply rate on the relationship between bacteria and algae. (A) Low light intensity, (B) high light intensity and low P supply rate, (C) high light intensity and moderate P supply rate and (D) high light intensity and high P supply rate. Relative flows of P and C (energy) is denoted by the thickness of the arrows. Growth of each organism is limited by the resource indicated by a thin arrow

algae should be higher under lower light conditions. At lower light conditions where algae are expected to release less extracellular organic C, lysis of algae may be a more important organic C source for bacteria.

At high light conditions, the relationship between algae and bacteria differed from that at low light conditions. The limitation index (Fig. 6) from the bioassays also revealed that with increasing light intensity and P supply rate, bacterial growth rate was less limited by organic C, indicating that algae supplied a higher amount of organic C than that required for the bacteria. Inorganic P was also sufficient for bacteria at high P treatment. However, bacteria from 0.1 and 0.25 µM P treatments showed a symptom of P limitation when they were grown under light intensity >25 µE $m^{-2}$ $s^{-1}$. The intensity of P limitation tended to increase with increasing light intensity and was higher for bacteria from 0.25 µM P treatments than that from 0.1 µM P treatments. Under these light and nutrient regimes, algal density was also limited by P as mentioned earlier. These results indicate that a competitive interaction between bacteria and algae was important at the moderate P supply rate.

The present study supports an idea by Sterner et al. (1997) that the relationship between bacteria and algae changes according to light and nutrient conditions. We first expected that 'intensity' of competitive interaction between bacteria and phytoplankton would increase with decreasing P supply at high light intensity because algae excrete more photosynthetically fixed carbon under high light:nutrient ratio. However, this was not the case because bacterial density was more limited by carbon than nutrient at the lowest P supply under the highest light intensity. In general, bacteria are competitively superior to algae for P because they have a higher affinity for lower P concentration (Currie & Kalff 1984, Güde et al. 1992, Rothhaupt & Güde 1992). At a high light intensity, nutrient deficiency promotes excretion of organic C by algae (Lancelot 1983, Jensen 1984), which is favorable for bacteria. If this were the case in the present study, we could expect that a single algal cell could support a larger number of bacterial cells at higher light and lower P (Bratbak & Thingstad 1985). Indeed, the ratio of bacterial to algal densities tended to increase with decreasing P concentration at light intensity higher than 55 µE $m^{-2}$ $s^{-1}$ (Fig. 4). However, bacteria could not exclude algae as long as this inferior competitor for P was the only source for energy resource (Bratbak & Thingstad 1985). Thingstad and his colleagues pointed out using theoretical models that, through competition for P, bacteria reduce algal biomass until the total supply of organic C from algae falls to a level where the bacteria become C limited (Bratbak & Thingstad 1985, Thingstad & Lignell 1997, Thingstad et al. 1997). The present study strongly supports their theory because bacteria were more limited by C than P when the P supply rate was very low relative to light intensity (Fig. 7B). It can be said that although bacterial growth is C limited, very low P supply 'indirectly' limits bacterial density through decrease in the amount of dissolved organic C from algae.

When the P supply rate is relatively low, a slight increase in the P supply rate favors algae. Increase in algal biomass is then accompanied by increase in total supply of dissolved organic C, which in turn relieves bacteria from C limitation. As a result, P directly limits bacteria as well as algae (Fig. 7C). This situation is, however, somewhat strange, because it is theoretically unlikely that 2 populations competing for 1 resource coexist at an equilibrium in a homogeneous environment (Thingstad & Lignell 1997). In our experiments, we supplied P every 2 d. Thus, the nutrient environment was not temporally homogeneous. This temporal heterogeneity might make it possible to stabilize the density of bacteria and algae which were competing for P (e.g. Rothhaupt & Güde 1992). Under conditions where nutrient is supplied continuously, like a chemostat, bacterial density at an equilibrium may be determined by organic C from algae even if P is supplied in moderate concentrations (Thingstad et al. 1997). However, present results indicate that if the nutrient environment is somewhat temporally heterogeneous as easily expected in nature, competition between algae and bacteria for P is most intense at a P supply rate with moderate concentrations. Probably, further increase in P supply rate weakens the competition for P between bacteria and algae as shown here (Fig. 7D).

Under high light conditions, bacteria from 1.58 µM P treatments showed much higher growth rate when both C and P were supplied than when either of these were supplied, suggesting a dual limitation, which is ubiquitous in bacterial growth (Egli 1995). At high P supply, P uptake rate is generally higher in algae than bacteria (Suttle et al. 1990, Güde et al. 1992, Rothhaupt & Güde 1992). In such a situation, bacteria may suffer from shortage in both C and P, if algae invest much photosynthetically fixed carbon in their sister cells with gained phosphorus. In accord with this possibility, the ratio of bacterial to algal densities was lowest at 1.58 µM P treatments under the high light conditions (≥55 µE $m^{-2}$ $s^{-1}$). This argument implies that whether bacterial growth is limited by C or P depends on the response of algae in growth rate and resource allocation to light and nutrient balance.

It should be noted that the growth rate of bacteria decreased with increasing light intensity in the bioassay even in +P+C treatments (Fig. 4). This decrease in +P+C treatments is probably due to deficiency of some inorganic elements other than P, N and C. In the bio-

assay, we used the medium from semi-batch cultures where bacteria and algae had been grown: P, N and C were then added in +P+C treatments in the bioassay after removing algae. Thus, it is likely that due to consumption both by algae and bacteria, concentrations of some inorganic elements except P and N were lower at the start of bioassay in the medium from semi-batch cultures under high light condition where their densities had been higher. However, this artifact would not affect our final conclusion, because the object of the bioassay was to examine which element was the more deficient for bacteria, C or P.

The present study was conducted in a system where organic C is supplied from algae alone. In natural habitats, there are other energy or C sources, such as allochthonous input and excretion and egestion by organisms of higher trophic levels, which may diminish the relative importance of a commensal relationship between the bacteria and algae for C regardless of light condition. In contrast, predators may mitigate competition between algae and bacteria for nutrients by reducing their densities much below the carrying capacity (Thingstad et al. 1997), regenerating nutrients (Urabe 1995, Rothhaupt 1997) and creating heterogeneity of nutrient concentrations (Suttle et al. 1990, Rothhaupt & Güde 1992). As such, allochthonous input and food web structure would modulate the relationship between algae and bacteria formed by light and nutrient balance in given habitats. Thus, precaution is needed to apply the present results to natural habitats.

Nonetheless, the present study provides potential trends in the relationship between algae and bacteria along spatial scales of aquatic habitats. With increasing spatial size of aquatic habitats, the surface mixing layer extends into the deeper layer due to various external and internal forces (Sterner 1990), where an algal cell may receive on average a smaller amount of solar radiation because of 'long distance' vertical mixing. Under such a condition, algae may excrete only a limited amount of organic carbon that is fixed through photosynthesis. In addition, allochthonous input is expected to be low in the pelagic area simply because of the distance from coastal sources. In this regime, the growth of bacteria seems to depend more on algal abundance and photosynthetic activity. On the other hand, in a small lake where the mixing layer is formed in a shallower depth and allochthonous input is expected to be high, competition for nutrients may be more important. In support of these arguments, several studies showed that the growth rate of bacteria is limited by organic carbon far from coast in the pelagic ocean (Kirchman 1990, Kirchman et al. 1990, Kirchman & Rich 1997), while nutrient limitation of bacterial growth is frequently detected in lakes and coastal areas (Toolan et al. 1991, Coveney & Wetzel 1992, Morris & Lewis 1992, Wang et al. 1992, Elser at al. 1995, Thingstad et al. 1998).

Finally, the present results indicate that cross-system correlation between algae and bacterial abundance (e.g. Cole et al. 1988) does not necessarily reflect a single universal relationship between them. The correlation has been traditionally explained by commensal relation of bacteria to algae. However, this type of covariation is also possible if correlated with nutrient supply (Currie 1990). In the present study, the nature of the algae-bacteria relationship changed according to light and nutrient regimes, although bacterial abundance correlated significantly with algal abundance across the experiments (Fig. 3). These results suggest that both competitive and commensal relations may contribute to the cross-system correlation between algae and bacteria.

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