

Seasonal and vertical difference in negative and positive effects of grazers on heterotrophic bacteria in Lake Biwa

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Abstract

Grazers affect prey abundance negatively through grazing and positively through releasing nutrients and substrates. In the present study, the relative importance of these positive and negative effects of grazers on bacterial abundance was examined seasonally at 2.5 m and 30 m depths in Lake Biwa. We used a method by Elser and Goldman to estimate in situ growth rate, grazing rate, and the fraction of growth rate that was stimulated by grazers smaller than 100 μm . At 2.5 m, the bacterial grazing rate showed clear seasonal changes with higher values in summer. At 30 m, bacterial grazing was significant but consistently low throughout the year. The fraction of the growth rate stimulated by grazers was changed with season and depth. At 2.5 m, bacteria compensated 30–40% of grazing loss by using deficient nutrients released by the grazers from May to October. During that period, bacterial growth rate was severely limited by phosphorus, and it is most likely that phosphorus release by grazers was stimulating bacteria. At 30 m, however, the bacterial growth rate was not stimulated by the grazers in the majority of the cases. Bacterial growth rate was limited by low temperature at this depth, so nutrient return from grazers does not have a beneficial effect on bacterial growth. These results indicate that the relative importance of positive and negative effects of grazers on bacterial abundance differs seasonally and vertically, and that positive effect becomes important when bacterial growth is limited by nutrients. In a given habitat, bacterial abundance changes less than one order of magnitude in spite of high growth rates. The present study suggests that grazers contribute to numerical stability of bacterial assemblages not only through grazing but also by regenerating deficient nutrients.

Heterotrophic bacteria in aquatic ecosystems depend on nutrients and dissolved organic matter as resources, and bacteria are in turn preyed upon by protozoan and metazoan plankton (Sherr et al. 1983; Caron et al. 1985; Azam 1998). As such, bacteria channel dissolved inorganic and organic matter into higher trophic levels. Among planktonic grazers, heterotrophic nanoflagellates (HNF) are known to be the most predominate consumers of heterotrophic bacteria (Güde 1986; Nagata 1988; Sanders et al. 1989; Nakano et al. 1998). Both laboratory and field studies have shown that grazing is an important loss factor for bacterial populations in aquatic ecosystems (Sherr et al. 1983; Bloem et al. 1988; Elser et al. 1995). Nonetheless, bacterial abundance is relatively stable across seasons within a habitat (Güde 1986; Pedrós-Alió 1989). This suggests that bacteria compensate their grazing loss by rapid growth. It is, however, not necessarily clear how their growth rate is related to grazers (Güde 1985).

It is known in algae–herbivore interactions that effects of herbivores on algae are not through exploitative consumption alone. They also affect the prey organisms positively by

altering the resource condition (Urabe 1995; Elser and Urabe 1999). Similarly, a part of bacterial biomass ingested by grazers is subsequently released as soluble forms available to bacteria. A number of studies have shown that association of protozoans with bacteria significantly increase nutrient regeneration as compared with bacteria alone (Güde 1985; Bloem et al. 1988; Eccleston-Parry and Leadbeater 1995). This suggests that bacterial grazers enhance bacterial growth by regenerating nutrients or supplying dissolved organic matter. Thus, bacterial grazers such as HNF affect bacterial abundance both negatively via consumption and positively via resource supply. However, few studies have examined quantitatively the importance of the positive and negative effects of bacterial grazers on bacterial abundance, although some studies suggest the importance of the positive effects of grazers on bacterial production (Güde 1986).

In the present study, we investigated both the effects of grazing and nutrient release on heterotrophic bacteria by grazers smaller than 100 μm in Lake Biwa, the largest lake in Japan. To quantify both negative and positive effects, in situ experiments were performed by manipulating grazer and nutrient conditions. The experiments were conducted seasonally at warm surface and cold deep layers to examine whether the relative importance of positive and negative effects differed temporally and vertically in the lake.

Methods

Lake Biwa is a mesotrophic lake, situated at 80 m above sea level, with a surface area of 688 km² and a maximum depth of 104 m. Field sampling and in situ experiments were carried out 10 times during the period from June 1997 to June 1998 at a pelagic site within the north basin of Lake Biwa, where the maximum depth was about 53 m. During

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sampling, temperature within the column was measured by a conductivity, temperature, depth (CTD) profiler (Sea Bird).

Experimental protocol—In situ experiments were performed at 2.5 m and 30 m, where a 2×2 factorial design with two replications was applied. In these experiments, we manipulated grazer's abundance and resource level as main factors. To initiate the experiments, a water sample was collected by a modified 10-liter Van-Dorn sampler. The lake water from 2.5 m depth was passed through a clean 100- μm mesh net to remove large zooplankters. The water was then filtered through either precombusted GF/F filters or 0.2- μm pore-size capsule filters (Gelman 12140) to remove bacterial grazers. These filters were prerinse with pure water, and filtration of the lake water was carried out as gently as possible to avoid contamination of dissolved organic and inorganic matter resulting from breakage of planktonic organisms. The GF/F filtrate was diluted by <0.2- μm lake water to 1:6.6 and poured into a total of four 165-ml transparent polycarbonate bottles. These bottles were used as treatments without grazers. A previous study showed that 70–90% of bacteria but no bacterial grazers passed through GF/F filters (Gurung et al. 1999). For treatments with grazers, the other four bottles were filled with <100- μm lake water. In each treatment, two bottles were enriched with KH_2PO_4 , NH_4Cl , and glucose to a final concentration of 2.5 μM P, 18 μM N, and 50 μM C. The remaining two bottles were treated as unenriched treatments. Aliquots of GF/F and <100- μm filtrate were collected and fixed with glutaraldehyde (0.5% final concentration) to determine initial abundance of bacteria and protozoan grazers. The same procedures were followed for lake water from 30 m.

All bottles were incubated at the depth where water was collected. After 2-d incubation, bottles were transported to the laboratory within half an hour, where samples for determining final abundance of bacteria and HNF were collected and fixed with glutaraldehyde. These fixed samples were stored at 4°C until enumeration, which was done within 2 weeks of collection.

Enumeration—Bacterial abundance was estimated using acridine orange direct count method (Hobbie et al. 1977): three subsamples were made by filtering 0.25–3.0 ml of sample suspension onto Nuclepore filters (0.2 μm pore size). Bacteria were enumerated under an Olympus epifluorescence microscope (1250 \times) with a B-excitation system (50 W halogen lamp, IF 410–485 excitation filter). At least 300 bacterial cells were counted from more than 10 fields for each subsample.

Epifluorescence microscopic counts for HNF were also made with fluorescein isothiocyanate (FITC) according to Sherr and Sherr (1983). An aliquot of 10–30 ml was filtered onto a 0.8- μm pore-size Nuclepore filter and stained with FITC for 30 s. The organisms containing green-fluorescence with one or more flagella were counted as HNF. At least 90 eye fields were examined for each filter. We estimated only HNF abundance because of their ubiquitousness. In Lake Biwa, protozoans other than HNF and small metazoans <100 μm were found in limited numbers, especially during summer (Nakano et al. 1998; Yoshida et al. unpubl. data)

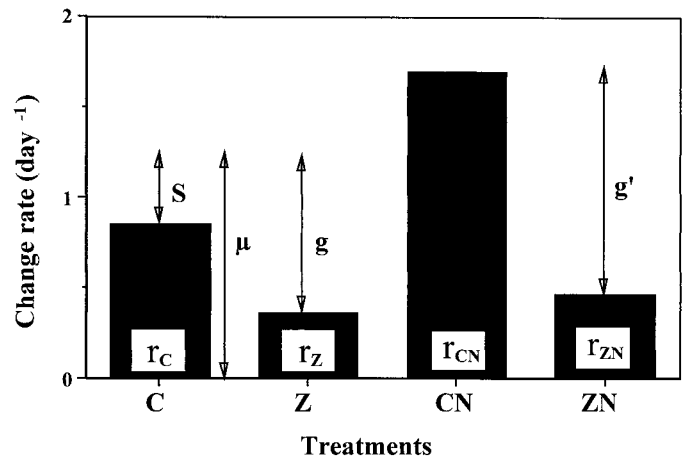


Fig. 1. Design of a factorial experiment illustrating the relation of grazing (g' and g), growth (μ), and stimulation rates (S) to net change rates of bacterial abundance in each treatment (r_C , r_Z , r_{CN} , and r_{ZN}). Treatments: C, unenriched treatment without grazers; Z, unenriched treatment with grazers; CN, enriched treatment without grazers; and ZN, enriched treatment with grazers. See details in the text.

Calculation—Intrinsic change rates (r : d^{-1}) of bacteria and HNF in each bottle were estimated as follows,

$$r = \ln(N_f/N_i)/2, \quad (1)$$

where N_f and N_i are the final and initial population density of organisms. This equation assumes exponential growth during the 2-d incubation. If their abundance reaches saturation level during the incubation or if there are initial lags in growth, the value estimated by this equation would be a minimal estimate of intrinsic change rate.

Grazing loss rate and a fraction of bacterial growth rate that was stimulated by grazers were estimated according to Elser and Goldman (1991). In enriched treatments, we assumed that nutrients and substrates were saturated for bacterial growth. Under this situation, nutrient and substrate supplies from grazers have no effect on bacterial growth rate. Thus, the difference in change rate of bacterial abundance between enriched treatments with (r_{ZN}) and without grazer (r_{CN}) reflects the mortality rate of bacteria due to grazing (Fig. 1). Mortality by grazing (g'), however, cannot be applied directly to unenriched treatments, if nutrient enrichment would increase grazers' abundance due to increased bacterial production. Therefore, we corrected the grazing rate estimated from enriched treatments (g') to that of unenriched conditions (g) assuming that the specific clearance rate of grazers did not differ between treatments as follows,

$$g = \text{HNF}_Z/\text{HNF}_{ZN} \times g', \quad (2)$$

where HNF_{ZN} and HNF_Z are mean HNF abundance in treatments with and without enrichments during the incubation. The mean HNF abundance was estimated by following equation,

$$\text{mean HNF} = (\text{HNF}_f - \text{HNF}_i)/\ln(\text{HNF}_f/\text{HNF}_i) \quad (3)$$

where HNF_f and HNF_i are final and initial abundance of HNF.

Table 1. Mean abundance of bacteria and HNF during 2-d incubation, and specific clearance rate for microspheres at the end of incubation in the preliminary experiment.

	Unenriched	Enriched	t-test (<i>df</i> = 2)	
			<i>t</i>	<i>P</i>
Mean abundance				
bacteria ($\times 10^6$ cells ml ⁻¹)	2.41	3.08	4.32	0.049
HNF ($\times 10^3$ cells ml ⁻¹)	1.93	3.01	9.96	0.009
Specific clearance rate (nl cell ⁻¹ h ⁻¹)	7.33	5.46	5.59	0.031

In unenriched treatments, change rate of bacterial abundance with grazers (r_z) reflects the bacterial growth rate (μ) minus mortality rate due to grazing (g). Therefore, the μ was estimated as

$$\mu = g + r_z \quad (4)$$

Finally, the change rate of bacterial abundance in unenriched treatments without grazers (r_c) is expected to underestimate the in situ bacterial growth rate because there was no resource return from grazers. A fraction of growth rate (S) that was stimulated by grazers was therefore estimated as

$$S = \mu - r_c \quad (5)$$

In this study, we described this fraction as stimulation rate due to grazers.

Significant differences in change rates of bacterial abundance among treatments was examined by 2-way analysis of variance (ANOVA) with fixed model. Mean abundance of HNF during the incubation between the enriched and unenriched treatments was compared with a *t*-test.

A preliminary experiment—The validity of μ and S estimates in the present study depends highly on the assumption made for Eq. 2. To examine whether specific clearance rates of grazers were the same between treatments without and with nutrient enrichments, a preliminary experiment was performed in early June 1997. Lake water collected at 2.5 m depth was filtered through a 100- μ m mesh net as in the in situ experiments and poured into four 1-liter flasks. Two of these flasks were spiked with C, N, and P as in the in situ experiments. The remaining two flasks were used as unenriched treatment. Both enriched and unenriched flasks were placed for 2 d in a constant temperature room (20°C) with cool-white fluorescent lighting under a 18:6 light:dark cycle. Ten minutes before the end of the 2-d incubation, 0.5- μ m diameter fluorescent microspheres (Fluoresbrite YG Microspheres, Polyscience) were added to each flask to a final concentration of 5×10^5 ml to estimate the cell specific clearance rate of flagellates. At the end of the incubation, aliquots of samples were fixed with ice-cold glutaraldehyde (2% final concentration) according to Sanders et al. (1989). Mean abundances of bacteria and flagellates during the 2-d incubation were determined as mentioned earlier. For samples collected at the end of the incubation, we separately counted flagellate cells with and without microspheres under the fluorescent microscope. In this enumeration, at least 150

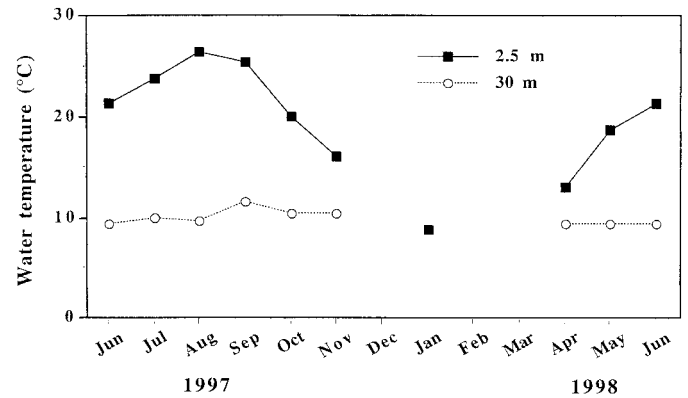


Fig. 2. Temporal changes in water temperature at 2.5 m and 30 m depths.

cells were counted. The specific clearance rate for microspheres was calculated according to Urabe et al. (1999).

Results

Preliminary check—When the preliminary experiment was conducted in early June 1997, abundance of bacteria and HNF at 2.5 m was 2.84×10^6 and 1.90×10^3 cells ml⁻¹, respectively. Nutrient enrichment stimulated growth of bacteria and HNF. As a result, mean abundance of bacteria and HNF during the incubation was higher in enriched than unenriched treatments (Table 1). In contrast, the specific clearance rate of HNF estimated at the end of the incubation was significantly lower in enriched than unenriched treatments. The result implies that Eq. 2 underestimates grazing rate (g) for unenriched treatments. If this were the case in the following in situ experiments, Eqs. 4 and 5 would underestimate a fraction of growth rate that was stimulated by the grazers.

Temperature and abundance of bacteria and flagellates—During the study period, water temperature at 2.5 m ranged from 8.8°C in January to 26.4°C in August, but it varied only between 8.8 and 10°C at 30 m (Fig. 2). At 2.5 m, bacterial abundance increased to 5.48×10^6 cells ml⁻¹ in late June 1997 and maintained at a relatively high level until November. In winter, it decreased to 1.91×10^6 cells ml⁻¹ (Fig. 3). In contrast, bacteria increased their abundance in winter at 30 m but the abundance was much lower than that at 2.5 m in other seasons. Compared to bacteria, temporal changes in HNF abundance were relatively small and ranged from 0.54 to 1.79×10^3 cell ml⁻¹ at 2.5 m (Fig. 3). At 30 m, HNF abundance tended to increase in winter, but always at lower values than 2.5 m except April 1998.

In situ experiments—The intrinsic change rate of bacteria during the 2-d incubation differed largely among treatments at 2.5 m (Fig. 4). ANOVA showed that effects of nutrient enrichments, grazers, and their interaction on the change rate were significant ($P < 0.05$) in experiments performed at 2.5 m depth except in November 1997 and January 1998. In these months, significant effects were detected in main fac-

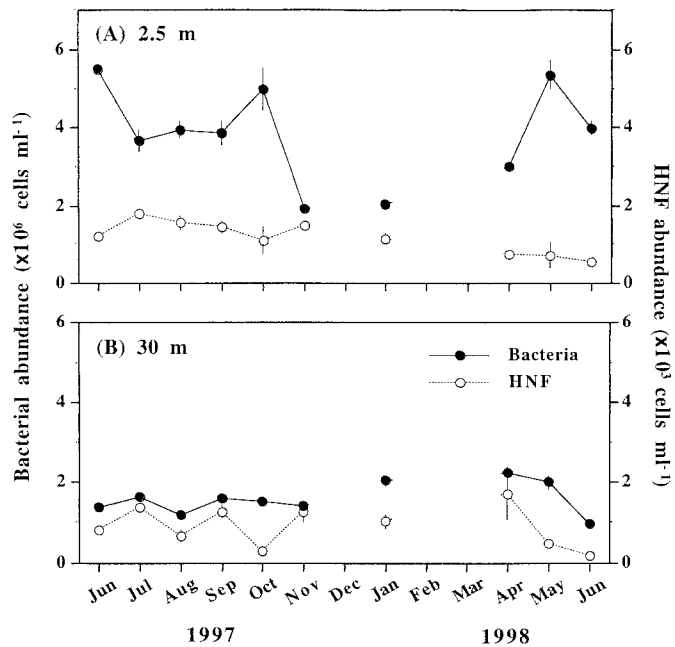


Fig. 3. Temporal changes in heterotrophic bacteria and HNF abundances at (A) 2.5 m and (B) 30 m. The vertical bars are standard deviations on the mean.

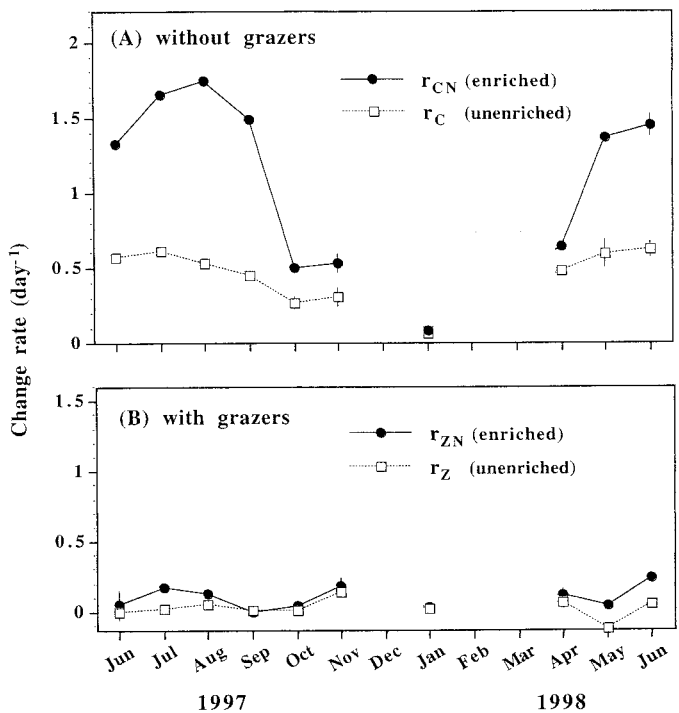


Fig. 4. Change rate of bacterial abundance at 2.5 m (A) without and (B) with grazers in unenriched and enriched treatments. The vertical bars are standard deviations on the mean.

tors (nutrient enrichment and grazers) but not in their interaction ($P > 0.05$). At 2.5 m, the change rate of bacterial abundance varied seasonally from 0.07 to 0.63 d^{-1} in unenriched treatments without grazers (r_C), whereas it was consistently lower than 0.14 d^{-1} with grazers (r_Z). Among treatments, the highest change rate was found in enriched treatments without grazers (r_{CN}) in most experiments, and the change rate exceeded 1.0 d^{-1} from May to September. In treatments without grazers, however, the bacterial abundance at the end of incubation was at most 4.5 times higher than that in the lake because we diluted initial bacterial abundance in treatments without grazers. Enrichment also increased bacterial growth in treatments with grazers (r_{ZN}) from May to August, but the response was much lower than without grazers. In contrast, at 30 m, enrichment did not significantly affect bacterial growth except in June and July 1997 (Fig. 5), although effects of grazers were always significant (ANOVA, $P > 0.05$). At this depth, the change rate of bacterial abundance varied from 0.12 to 0.66 d^{-1} in unenriched treatments without grazers (r_C) and was almost zero in most cases with grazers (r_Z).

In unenriched treatments, HNF increased 10 to 30% during the 2-d incubation at 2.5 m. When enrichment was made, the increase in HNF abundance was remarkable at this depth, especially in August and September when temperature was high. In these months, the mean HNF abundance was 2 to 3 times higher in enriched than unenriched treatments (Fig. 6). However, the mean HNF abundance did not differ between these treatments from October 1997 to January 1998 (t -test, $P > 0.05$). At 30 m, HNF decreased during the incubation and the mean HNF abundance did not differ between the treatments (t -test, $P > 0.05$).

The grazing loss rate of bacteria (g') also varied from

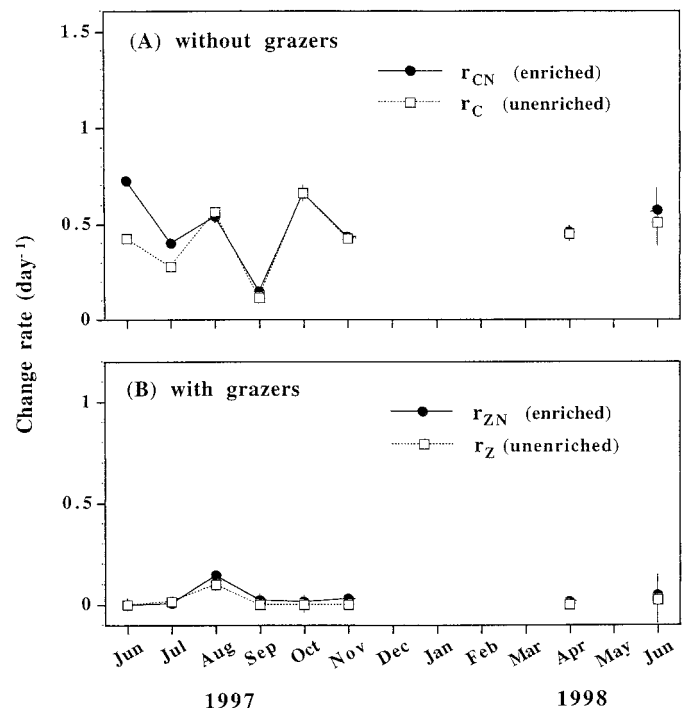


Fig. 5. Change rate of bacterial abundance at 30 m (A) without grazers and (B) with grazers. The vertical bars are standard deviations on the mean.

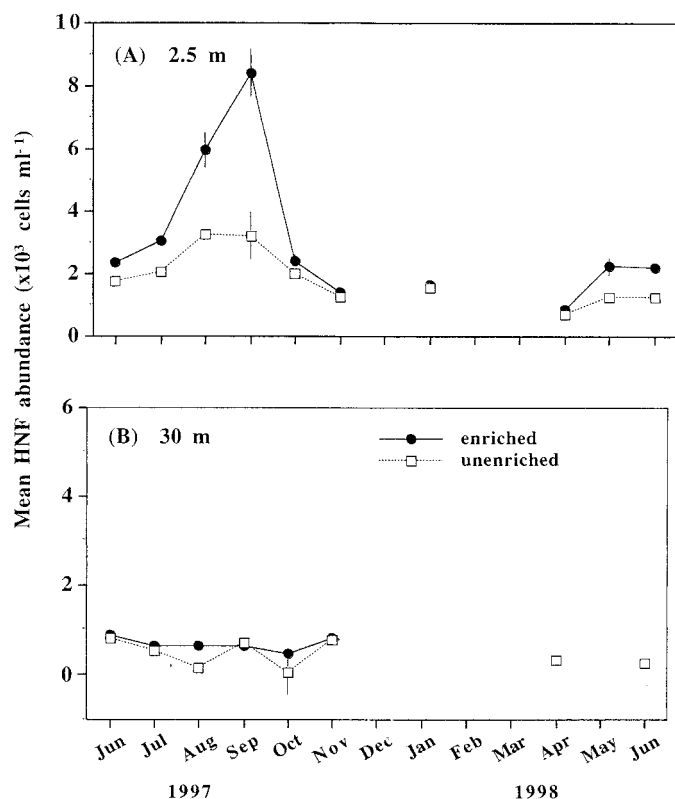


Fig. 6. Temporal changes in mean abundance of HNF during 2-d incubation with and without enrichment at (A) 2.5 m and (B) 30 m. The vertical bars are standard deviations on the mean.

almost zero to 1.61 d^{-1} in enriched treatments at 2.5 m. This value, however, apparently overestimates the in situ bacterial grazing rate because HNF abundance was stimulated by enrichment (Fig. 6). Therefore, we corrected the grazing loss rate to in situ level using Eq. 3. The corrected grazing loss rate (g) ranged from 0.053 to 0.99 d^{-1} and showed a clear seasonal trend with a summer maximum at 2.5 m (Fig. 7).

Bacterial growth rate (μ) at 2.5 m, estimated by Eq. 4, was balanced with the corrected grazing loss rate (g), because the change rate of bacterial abundance in unenriched treatments with grazers (rZ) was close to zero (Fig. 4). As expected, μ was always higher than the change rate of bacterial abundance in unenriched treatments without grazers at this depth, which indicates that grazers stimulated the bacterial growth rate. Stimulation rate (S), a fraction of the growth rate stimulated by grazer activities, was high from June to August and reached 10 to 40% of the growth rate. On average, S corresponded to 25% of bacterial growth rate throughout the study period.

At 30 m, there was no marked difference between g' and g because HNF abundance was not affected by resource enrichment. Again, the grazing loss rate (g) was balanced with the growth rate (μ) at this depth (Fig. 7). In contrast to 2.5 m, however, g and μ decreased in summer at 30 m and grazers did not stimulate bacterial growth rate at this depth except in June.

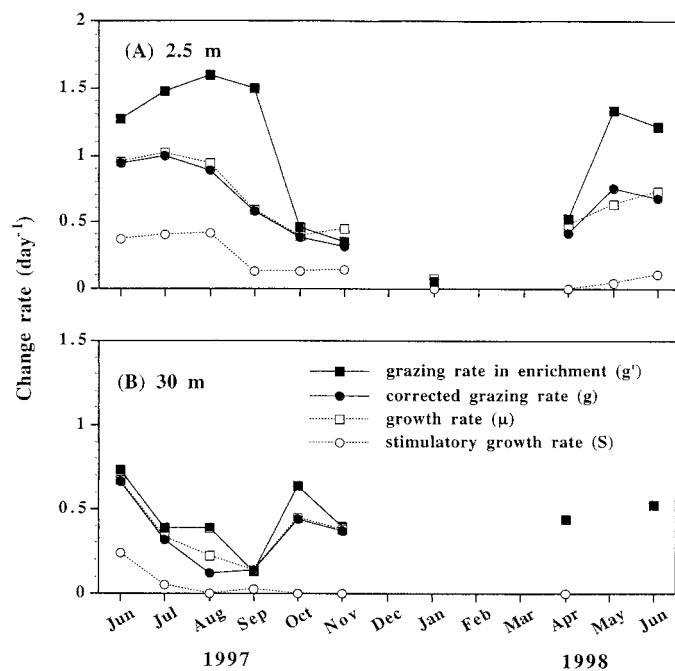


Fig. 7. Temporal changes in grazing rates in enriched treatments (g') and corrected grazing rate (g), growth rate (μ), and growth stimulation rate (S) at (A) 2.5 m and (B) 30 m depths.

Discussion

In the present study, we could detect positive as well as negative effects of grazers on bacterial abundance. Grazers negatively affect bacterial abundance by consumption, whereas they stimulate bacterial growth rate by regenerating inorganic nutrients or supplying organic substrates. Thus, bacteria can compensate for a substantial fraction of grazing loss by using organic and inorganic materials released by grazers.

It should be noted that there are several possibilities that the growth stimulation rates (S) estimated here are underestimated values. First, the specific clearance rate of flagellates depends on bacterial abundance (e.g., Capriulo 1990). Since mean bacterial abundance during the incubation was somewhat higher in the enriched treatments with grazers, the specific clearance rate might be lower in the enriched than unenriched treatments. Second, a recent study highlighted that nutrient and substrate enrichments to natural water select small flagellate species during incubation for more than 4 d (Lim et al. 1999). To avoid such an artifact, we incubated bacteria only 2 d. However, if small rapid-growing species dominated in the enriched treatment as in Lim et al. (1999), we can again expect that the specific clearance rate of flagellates was lower in the enriched than unenriched treatments because their specific clearance rate is expected to be lower due to smaller size. In accordance with these inferences, our preliminary experiment showed that the specific clearance rate of flagellates was lower in the enriched than unenriched treatments. Finally, we considered HNF abundance alone to correct grazing loss rate (g') of bacteria in the enriched treatment to in situ level (g) because the den-

sities of ciliates and other microzooplankton such as rotifers were limited in Lake Biwa (Nakano et al. 1998). However, ciliates and rotifers that prey on bacteria might exist in the incubation bottles. Since they are larger in body size, it is most likely that population increasing rates of ciliates and rotifers are slower than those of HNF even under favorable conditions. If these organisms were included, therefore, the abundance ratio of total grazers in the unenriched to enriched treatments would be higher than that of HNF alone. Thus, all of these artifacts, if any, results in an underestimate of the in situ grazing rate (g) and thus a fraction of the bacterial growth rate that was stimulated by grazers.

However, we would like to point out that the present method does not always underestimate the in situ grazing rate. If nutrient enrichment stimulates bacterial strains that are highly edible for grazers, HNF may increase their feeding activities. In addition, if grazers are starved in situ due to very low bacterial abundance, enrichment would overestimate the in situ grazing rate because of increased bacterial abundance. The growth rate (μ) in the present study, however, is well within the range of previous studies conducted in Lake Biwa with different methods (Nagata 1987, 1988; Nakano 1992). This agreement suggests that the grazing and growth stimulation rates estimated here are not far from in situ values.

A number of studies have examined seasonal changes in bacterial grazing rates mostly in surface water (McManus and Fuhrman 1986; Güde 1986; Nagata 1988). Similar to these studies, bacterial grazing at the surface layer in Lake Biwa showed a clear seasonal trend with a maximum in summer. In the deep layer, bacterial abundance was also held in check by grazers. However, in contrast to the surface layer, the grazing rate was relatively constant throughout the year at the deep layer where temperature was consistently low. These results suggest that the grazing rate depended on water temperature. Similar to the grazing rate, the growth stimulation rate changed seasonally and vertically. At the surface, 30–40% of bacterial growth was stimulated by the existence of grazers in summer. However, no stimulation was detected in the deep layer except for June. This is in contrast to the grazing rates that were always detected even at 30 m.

Under what environmental conditions is bacterial growth rate stimulated by the grazers? In treatments without grazers, change rate of bacterial abundance was much higher in enriched (r_{CN}) than unenriched treatments (r_C) at 2.5 m but was similar between these treatments at 30 m, which suggests that the bacterial growth rate is limited by resources at the surface but not at deep layers. The growth stimulation of bacteria at the surface was, however, not ascribed to release of organic carbon by grazers in the experimental bottles. Considering the fact that gross growth efficiency of HNF for carbon is generally 20–60% (Nagata 1988; Caron et al. 1990) and a substantial fraction of the assimilated carbon is released through respiration (e.g., CO_2), it is hard to imagine that bacterial grazers can supply labile organic carbon enough to recover 30–40% of bacterial biomass losses. There was probably excess organic carbon available to bacteria in the surface water due to supplies from other sources such as algae, large zooplankton, and allochthonous input, but the bacteria could not use the organic carbon due to the

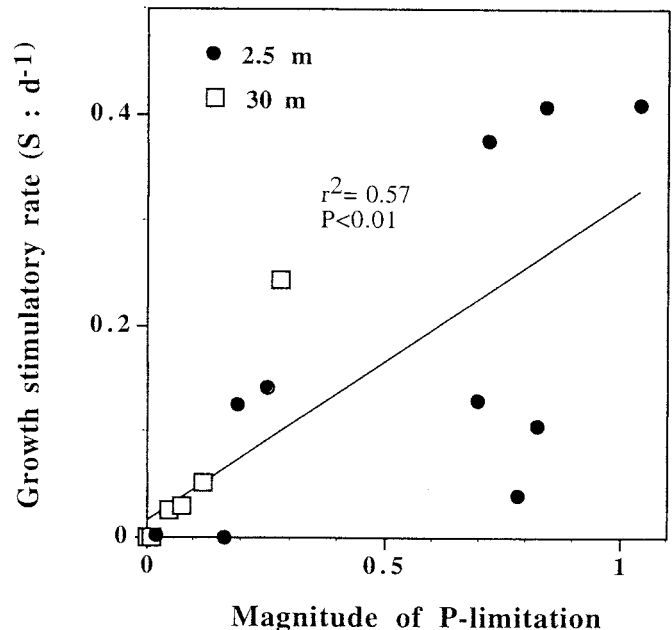


Fig. 8. Growth stimulation rate (S) plotted against magnitude of P-limitation for bacterial growth rate that is estimated in Gurung and Urabe (1999).

deficiency of some nutrients. In parallel with the present study, we performed dilution in situ bioassays at 2.5 and 30 m to clarify actual resources limiting bacterial growth rate in Lake Biwa. The bioassays revealed that bacterial growth rate was limited by phosphorous but not organic carbon and inorganic nitrogen, if ambient temperature was high (Gurung and Urabe 1999). Thus it can be hypothesized that the grazers stimulated the bacterial growth rate by regenerating P in summer. To examine this hypothesis, we plotted the growth stimulation rate against the magnitude of P limitation (Fig. 8). The magnitude of P limitation was estimated from the difference between bacterial growth rate in P enrichment and control treatments in the dilution bioassay (Gurung and Urabe 1999). The stimulation rate correlated significantly with the magnitude of P limitation ($r^2 = 0.57$, $P < 0.01$) and high stimulation rates were always found when the bacterial growth rate was severely limited by P. This result clearly supports the hypothesis that P released by grazers stimulated bacterial growth in summer within the surface layer.

As opposed to carbon, most phosphorus released by HNF is in the dissolved form. If we assume that gross growth efficiency of HNF for carbon is 20–60% (Nagata 1988; Caron et al. 1990) and the C:P ratio is similar in bacteria and HNF (Vadstein et al. 1993; Nakano 1994), 40–80% of ingested P in bacteria is released by HNF. Indeed, a large number of studies have shown that protozoans regenerate nutrients when they were fed bacteria (Güde 1985; Bloem et al. 1988; Eccleston-Parry and Leadbeater 1995). In the present study, we estimated that bacteria compensated for 10–40% of the grazing loss by using P released by grazers. The result implies that the substantial fraction of P released by grazers is quickly consumed by bacteria where bacterial growth is highly P limited.

At 30 m, the grazers did not stimulate bacterial growth rate in the majority of cases. Seasonal and vertical changes in the grazing rate suggest that grazers' activities depended on water temperature. However, no growth stimulation at 30 m, in general, is not due to a low nutrient return from the grazers. At this depth, bacterial growth was not limited by any nutrients and substrates, but limited by low temperature itself (Gurung and Urabe 1999). When bacterial growth is limited by temperature, it is apparent that increases in nutrients and substrate supplies have no effect on bacterial growth rate.

A large number of studies have shown that, in a given habitat, bacterial abundance varies <1.0 order of magnitude in a year in spite of their higher growth rate. Such a low variation in bacterial abundance is often attributed to the substantial grazing loss rate of bacteria (Pedrós-Alió 1989; Weisse and Scheffel-Möser 1991). Indeed, the present study also demonstrates high bacterial growth in close agreement with the grazing loss by grazers (Fig. 7). However, the low annual variation of bacterial abundance should not be attributed to direct grazing loss alone. Grazers contribute to a stability of bacterial abundance by regenerating nutrients as well. In the present study, we showed that bacteria can compensate for at most 30 to 40% of grazing loss by using nutrients released by grazers. If there was no nutrient return from grazers, bacteria could not compensate for grazing loss, and, therefore, their abundance would change more dramatically seasonally and spatially according to changes in abundance of grazers.

Finally, it is well known that the trophic chain or "loop" that is composed of bacteria and protozoans is an important component in material flow and cycling in aquatic systems (Azam 1998). However, few studies have examined how material flow and cycling are regulated. This study showed that HNF abundance increased when nutrients and substrates were enriched in summer. The result implies that resource input to bacteria can quickly channel into HNF biomass. In Lake Biwa, the bacterial growth rate is basically regulated by temperature, but high growth rates were not realized in summer at the surface layer because of low P relative to N and C supply rates (Gurung and Urabe 1999). In this lake, therefore, mass and carbon transfer efficiency along microbial chains seems to be regulated by P supply at the surface layer in summer, whereas it is regulated primarily by low temperature at the deep layer throughout a year.

References

- AZAM, F. 1998. Microbial control of oceanic carbon flux: The plot thickens. *Science* **280**: 694–696.
- BLOEM, J., M. STARINK, B. M. BÄR-GILLISEN, AND T. E. CAPPENBERT. 1988. Protozoan grazing, bacterial activity, and mineralization in two-stage continuous cultures. *Appl. Environ. Microbiol.* **54**: 3113–3121.
- CAPRIULO, G. M. 1990. Feeding related ecology of marine protozoa, p. 186–260. *In* G. M. Capriulo [ed.], *Ecology of marine protozoa*. Oxford Univ. Press.
- CARON, D. A., J. C. GOLDMAN, O. K. ANDERSEN, AND M. R. BENNETT. 1985. Nutrient cycling in a microflagellate food chain. II. Population dynamics and carbon cycling. *Mar. Ecol. Prog. Ser.* **24**: 243–254.
- , ———, AND M. R. DENNETT. 1990. Carbon utilization by the omnivorous flagellate *Paraphysomonas imperforata*. *Limnol. Oceanogr.* **35**: 192–201.
- ECCLESTON-PARRY, J. D., AND B. S. C. LEADBEATER. 1995. Regeneration of phosphorus and nitrogen by four species of heterotrophic nanoflagellates feeding on three nutritional states of a single bacterial strain. *Appl. Environ. Microbiol.* **61**: 1033–1038.
- ELSER, J. J., AND C. R. GOLDMAN. 1991. Zooplankton effects on phytoplankton in lakes of contrasting trophic status. *Limnol. Oceanogr.* **36**: 64–90.
- , L. B. STABLER, AND R. P. HASSET. 1995. Nutrient limitation of bacterial growth and rates of bacterivory in lakes and oceans: A comparative study. *Aquat. Microb. Ecol.* **9**: 105–110.
- , AND J. URABE. 1999. The stoichiometry of consumer-driven nutrients recycling: Theory, observation, and consequences. *Ecology* **80**: 735–751.
- GÜDE, H. 1985. Influence of phagotrophic processes on the regeneration of nutrients in two-stage continuous culture systems. *Microb. Ecol.* **11**: 193–204.
- . 1986. Loss processes influencing growth of planktonic bacterial populations in Lake Constance. *J. Plankton Res.* **8**: 795–810.
- GURUNG, T. B., AND J. URABE. 1999. Temporal and vertical difference in factors limiting bacterial growth rate in Lake Biwa. *Microb. Ecol.* **38**: 136–145.
- , ———, AND M. NAKANISHI. 1999. Regulation of the relationship between phytoplankton *Scenedesmus acutus* and heterotrophic bacteria by the balance of light and nutrients. *Aquat. Microb. Ecol.* **17**: 27–35.
- HOBBIE, J. E., R. J. DALY, AND S. JASPER. 1977. Use of Nuclepore filters for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.* **33**: 1225–1228.
- LIM, E. L., M. R. DENNETT, AND D. A. CARON. 1999. The ecology of *Paraphysomonas imperforata* based on studies employing oligonucleotide probe identification in coastal water samples and enrichment cultures. *Limnol. Oceanogr.* **44**: 37–51.
- MCMANUS, G. B., AND J. A. FUHRMAN. 1986. Bacterivory in sea water studies with the use of inert fluorescent particles. *Limnol. Oceanogr.* **31**: 420–426.
- NAGATA, T. 1987. Production rate of planktonic bacteria in the north basin of Lake Biwa, Japan. *Appl. Environ. Microbiol.* **53**: 2872–2882.
- . 1988. The microflagellate picoplankton food linkage in the water column of Lake Biwa. *Limnol. Oceanogr.* **33**: 504–517.
- NAKANO, S. 1992. Changes in bacterioplankton production and dominant algal species in the north basin of Lake Biwa. *Jpn. J. Limnol.* **53**: 145–149.
- . 1994. Estimation of phosphorus release rate by bacterivorous flagellates in Lake Biwa. *Jpn. J. Limnol.* **55**: 201–211.
- , T. KOITABASHI, AND T. UEDA. 1998. Seasonal changes in abundance of heterotrophic nanoflagellates and their consumption of bacteria in Lake Biwa with special reference to trophic interactions with *Daphnia galeata*. *Arch. Hydrobiol.* **142**: 21–34.
- PEDRÓS-ALIÓ, C. 1989. Toward an autecology of bacterioplankton, p. 297–336. *In* U. Sommer [ed.], *Plankton ecology: Succession in plankton ecology*. Springer-Verlag.
- SANDERS, R. W., K. G. PORTER, S. J. BENNETT, AND A. E. DEBIASE. 1989. Seasonal patterns of bacterivory by flagellates, ciliates, rotifers, and cladocerans in a freshwater planktonic community. *Limnol. Oceanogr.* **34**: 673–687.
- SHERR, B. F., E. B. SHERR, AND T. BERMAN. 1983. Grazing, growth, and ammonium excretion rates of a heterotrophic microflagel-

- late fed with four species of bacteria. *Appl. Environ. Microbiol.* **45**: 1196–1201.
- SHERR, E. B., AND B. F. SHERR. 1983. Double-staining epifluorescence technique to assess frequency of dividing cells and bacterivory in natural populations of heterotrophic microprotozoa. *Appl. Environ. Microbiol.* **46**: 1388–1393.
- URABE, J. 1995. Direct and indirect effects of zooplankton on seston stoichiometry. *Ecoscience* **2**: 286–296.
- , T. B. GURUNG, AND T. YOSHIDA. 1999. Effects of phosphorus supply on phagotrophy by the mixotrophic alga *Uroglena americana* (chrysophyceae). *Aquat. Microb. Ecol.* **18**: 77–83.
- VADSTEIN, O., Y. OLSEN, H. REINERSTEN, AND A. JENSEN. 1993. The role of planktonic bacteria in phosphorus cycling in lakes—sink and link. *Limnol. Oceanogr.* **38**: 1539–1544.
- WEISSE, T., AND U. SCHEFFEL-MÖSER. 1991. Uncoupling the microbial loop: Growth and grazing rates of bacteria and heterotrophic nanoflagellates in the North Atlantic. *Mar. Ecol. Prog. Ser.* **71**: 195–205.

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