

The role of bacterivorous flagellates in the phosphorus cycling in Lake Biwa, Japan

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INTRODUCTION

It is well known that biomass and activity of phytoplankton are limited by phosphorus in many oligo- to mesotrophic lakes. Bacteria are not only superior competitors for phosphorus to phytoplankton [3] but also large P pools relative to the P in other planktonic organisms [17]. Regeneration of P preserved in bacterial cells, therefore, may free phytoplankton from P limitation. Previous studies have demonstrated that grazing of heterotrophic nanoflagellates is one of the most important bacterial loss processes [7,12]. In addition to this, bacterivorous flagellates have higher P release rates per body weight than metazoan zooplankton [1]. The flagellates, therefore, could be important P suppliers for phytoplankton in lakes. However, there has been few studies on the importance of the flagellates as P suppliers [18].

In the present study, P release rate by bacterivorous flagellates was estimated in the north basin of P limited Lake Biwa, Japan (35° 10'N, 136° 00'E, altitude 85m). The importance of the flagellates as P suppliers for phytoplankton was examined by comparing the P supply by the flagellates with P demand for phytoplankton primary production.

METHODS

Laboratory experiments

The heterotrophic flagellate used was isolated from Lake Biwa by incubating the lake water with yeast extract (Y.E., 10 mg l⁻¹) after serial dilution. The culture of the flagellate was not free from bacteria. The flagellate was spherical (diameter 3-8 µm), non-pigmented and naked with two flagella of unequal lengths. Flagellates with these characteristics probably belong to the genus *Spumella*.

Experimental design, enumeration of organisms, chemical analyses and data analyses are detailed in Nakano (1994,[9,10]). The flagellate was fed on bacteria with different C:P molar ratios: the ratios were in a range of 32 to 130. To measure the carbon content of the flagellate, cells in the late exponential growth phase were retained on a precombusted Whatman GF/D glass fibre filter and analyzed with a CHN-analyzer after drying. The phosphorus content of the flagellate was determined by subtracting concentration of total P of the GF/D filtrate from those of unfiltered sample [5]. Dissolved inorganic phosphorus (DIP) concentration was

measured [6] and converted to DIP release rate of the flagellate as follows:

$$Pr = (C_2 - C_1) / (T \times F_b) \quad (1)$$

where Pr, DIP release rate of the flagellate; C_1 and C_2 , the initial and final concentrations of DIP; T, time of hours; F_b , mean biomass or cell density of flagellate.

Incubation experiments with natural assemblages of bacteria and flagellates in Lake Biwa were also carried out. A 10 liter of water sample was filter-sterilized through a Gelman capsule filter (Culture capsule, pore size 0.2 μm). Each 5 liter aliquot of the filtrate was poured into two 10 liter glass bottles. A 1 liter aliquot of the water sample was filtered through a Whatman GF/C filter (pore size 1.2 μm), and each 500 ml of the filtrate was added as inocula to the sterilized filtrate. Two cultures were prepared. One had no further addition of nutrients (control). The other was supplemented with glucose 5 $\mu\text{mol C l}^{-1}$, NH_4Cl 1 $\mu\text{mol N l}^{-1}$ and K_2HPO_4 0.5 $\mu\text{mol P l}^{-1}$ (C:N:P = 10:2:1). Cultures were incubated at 25 °C in the dark. Subsamples were withdrawn at various time intervals to determine densities of bacteria and flagellates and concentrations of DIP. DIP release rate of flagellates was determined using Eq. (1) and a model as described later. When the bacteria had attained the stationary phase, and flagellates had appeared (~ 1000 cells ml^{-1}), a portion of the culture was filtered through a precombusted Whatman GF/F filter to measure bacterial carbon content, and P content of bacteria was determined as the difference between concentrations of total phosphorus in the unfiltered culture and the GF/F filtrate.

Field surveys

Details for field surveys conducted at station Wani (35 11'N, 135 56'E, depth ca. 40 m) from July to November 1992 are written precisely in Nakano (1994c,[11]). Water sample was collected from 2.5m depth with a 3 liter Van Dorn water sampler. *In situ* ingestion rate on bacteria of flagellates was determined with fluorescently labeled bacteria (FLB,[13]) made of a minicell-producing mutant strain of *Escherichia coli*. To prepare samples for measuring C and P content of lake bacteria, two 8-10 liter portions of lake water were filtered through Whatman GF/C filters, and then the filtrate was filtered again through Whatman GF/F filters (pore size 0.6-0.8 μm). The amounts of C and P on the GF/F filters were analyzed.

RESULTS AND DISCUSSION

Previous studies have demonstrated that phosphorus release of heterotrophs is tightly coupled to the C:P or N:P ratios of grazer's food relative to their stoichiometry. For example, C:P and N:P ratios of

zooplankton are intraspecifically stable [4], thus the N:P release ratios of zooplankton community dominated by *Daphnia* were affected by those of the food [15]. Bacteria also show similar regulation of assimilation and release of P despite their variable C:P ratios [14]. However, there has been few data available for the relationship between elemental ratios and nutrient release of protozoa. I examined changes in dissolved inorganic phosphorus (DIP) release rate of the heterotrophic flagellate *Spumella* sp., when the flagellate was fed on bacteria with different C:P molar ratios (Table 1). C:P ratios of the flagellate were relatively stable against the large variations in bacterial C:P ratios, and the DIP release rate decreased with increase in the bacterial C:P ratios. These results suggest that P release rate of the flagellate is also dependent on C:P ratio of food relative to stoichiometry of the flagellate. From these results, I decided to take the stoichiometric approach to clarify the contribution of P release by bacterivorous flagellates to phytoplankton in a lake.

A stoichiometric model for estimating nutrient release rate of heterotrophs has been suggested by Caron (1991,[2]):

$$N_r = R \times (1 - Y)^{-1} \times \{(C:E_{\text{prey}})^{-1} - Y \times (C:E_{\text{graz}})^{-1}\}$$

where N_r , nutrient release rate of heterotrophs; R , respiration rate of heterotrophs in terms of carbon; Y , growth yield in terms of carbon; $C:E_{\text{prey}}$ and $C:E_{\text{graz}}$, carbon to element ratio of prey and grazer, respectively. In the present study, the model was applied to the estimation of P release rate of bacterivorous flagellates in Lake Biwa. I used growth yield 0.32 and C:P ratio 83 of flagellates both of which were obtained for *Spumella* sp. in the previous experiment (cf. [9]). Further, I assumed that all carbon ingested by flagellates was used for their production and respiration, so that the part " $R \times (1 - Y)^{-1}$ " in the model could be expressed as ingestion rate of flagellates based on carbon. To verify the model on these assumptions, in incubation experiments with enriched lake water filtrate, DIP release rate was estimated using the model and compared with the DIP release rate calculated from Eq. (1).

In those experiments, bacteria grew during the first 2 days, and growth of flagellates subsequently occurred with grazing on the bacteria in the nutrient-supplemented cultures. Bacterial C:P ratios in each experiment changed from 30 (August 1992) to 103 (July 1992). Concentrations of DIP increased consistently with the growth of flagellates in these cultures. Growth of bacteria and flagellates and increase in DIP concentrations were not detected in control experiments. P release rate estimated using the model agreed well with that using Eq. (1) in four of five experiments (Fig. 1). However, the estimation using the model was much higher than another when bacterial C:P ratio was the lowest (30, August 1992). The higher estimation could be attributable to dissolved organic phosphorus release of flagellates and/or the dominance of flagellates with a high growth yield and a low C:P ratio. From the results shown in Fig. 1, I regarded that the model is usable for estimating P release rate by flagellates when bacterial C:P ratios are higher than 50.

Finally, in field surveys, I examined the contribution of P release of bacterivorous flagellates in the north basin of Lake Biwa by comparing the P release rate with P required for phytoplankton primary production. Cell density of flagellates at station Wani fluctuated from 0.7 to 3.9×10^6 cells l^{-1} , and that of bacteria from 6.3 to 9.1×10^9 cells l^{-1} during this survey (Fig. 2A). *In situ* ingestion rate on bacteria of flagellates was in a range of 1.0 to 6.3×10^8 bacteria $l^{-1} d^{-1}$ (Fig. 2B). This range overlaps that at other stations in Lake Biwa [11] and falls within a range of that of previous studies using the FLB method [12]. The ingestion rate at station Wani, together with data for bacterial carbon content and C:P ratio (Table 2), was used for the estimation of P release rate of flagellates. C:P ratio of lake bacteria was relatively high (110, Table 2), suggesting that bacteria in Lake Biwa are P-limited.

Data for primary production and sestonic C:P ratios were detailed in Urabe et al. (in press,[16]). P required for primary production was calculated by dividing a measured value of primary production by a sestonic C:P ratio. The P requirement thus calculated was from 11 to 40 $nmol P l^{-1} d^{-1}$, whereas P release rate of the flagellates was from 1.3 to 8.0 $nmol P l^{-1} d^{-1}$ (Fig. 3A). The contribution to the P requirement of the P release was in a range of 4.2 to 31% (Fig. 3B). Simultaneous measurements of P release rate by herbivorous macro zooplankton [16] demonstrated that 1 to 36% of P required for primary production was supplied by the zooplankton during this survey. Bacterivorous flagellates, therefore, are probably significant P suppliers for phytoplankton as well as the zooplankton.

In a Norwegian lake, Vadstein et al. (1993,[18]) estimated that P release of flagellates is twice of herbivorous macro zooplankton. Thus, flagellates may be the most important P suppliers in that lake. The difference between the importance of flagellates as P suppliers in Lake Biwa and the Norwegian lake is summarized in Fig. 4.

In Lake Biwa, P release through bacterial decomposition of organic matter may be restricted, because the lake seston are poor in P [8] and the lake bacteria are possibly P-limited (Table 2). Although bacterivorous flagellates were one of important P suppliers in Lake Biwa, the contribution of the P supply was small relative to that in a Norwegian lake (Fig. 4). This result can be ascribed to P limitation of bacteria in Lake Biwa. To elucidate the P source of phytoplankton, simultaneous surveys which examine P release of heterotrophic plankton, P upwelling from the hypolimnion and P input from the catchment area are needed.

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Table 1. Dissolved inorganic phosphorus (DIP) release rate of the flagellate *Spumella* sp., when the flagellate was fed on bacteria with different C:P ratios.

Bacteria	Bacterial C:P molar ratio	C:P molar ratio of the flagellate	DIP release rate (nmol P μ mol C ⁻¹ h ⁻¹)
An isolate*	32	77	5.40
<i>Pseudomonas fluorescens</i>	40	89	9.60
An isolate*	47	93	8.10
An isolate*	58	93	4.20
<i>Escherichia coli</i>	65	83	4.40
<i>E. coli</i>	65	90	5.10
<i>Flavovacterium ferrugineum</i>	66	78	0.86
An isolate*	79	70	0.48
An isolate*	79	69	0.50
An isolate*	120	109	0.98
An isolate*	130	80	0.64

* A bacterium isolated from Lake Biwa.

Table 2 Bacterial carbon content and C:P molar ratio at a station (35° 13'N, 136° 00'E) in the north basin of Lake Biwa.

	C content (fmol C cell ⁻¹)	C:P ratio
Bacteria	2.42*	110**

* A median of bacterial carbon content determined in Nakano (1994c,[11]).

** A mean value of bacterial C:P ratio determined in Nakano (1994c,[11]).

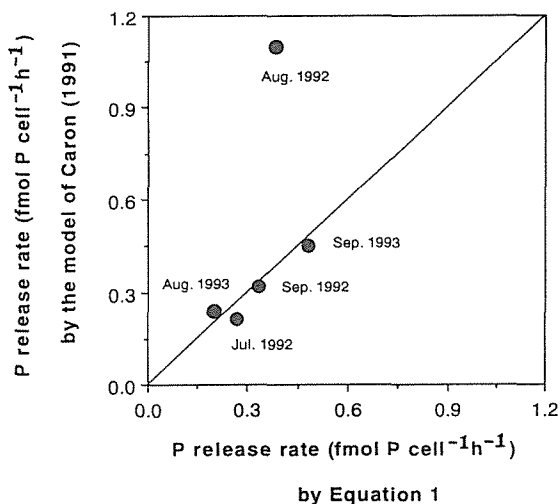


Fig. 1 A comparison between phosphorus release rates estimated from Equation (1) (see Methods) and the model of Caron (1991) in lake water incubation experiments.

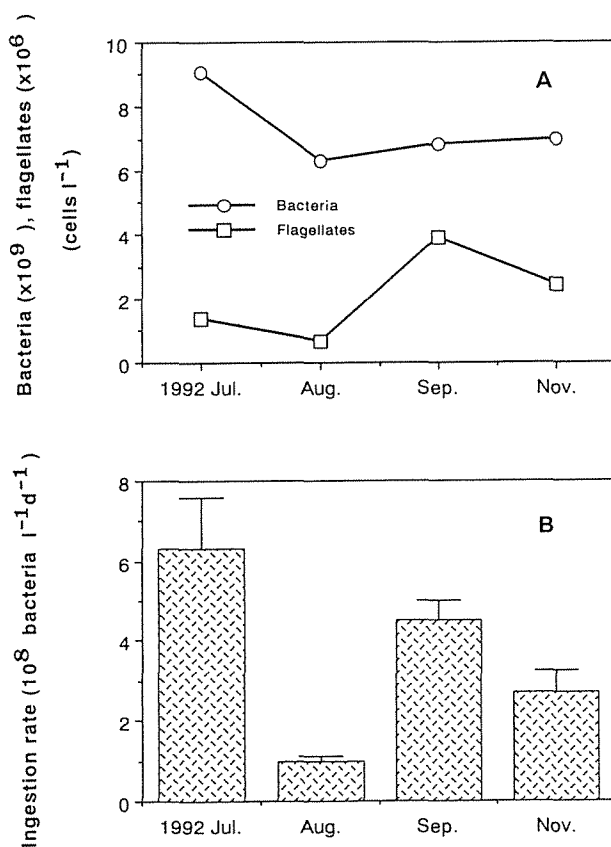


Fig. 2 Changes in cell densities of bacteria and flagellates (A) and ingestion rates on bacteria of flagellates (B) at station Wani (35°11'N, 135°56'E) in the north basin of Lake Biwa. Parpendicular bars indicate standard deviation.

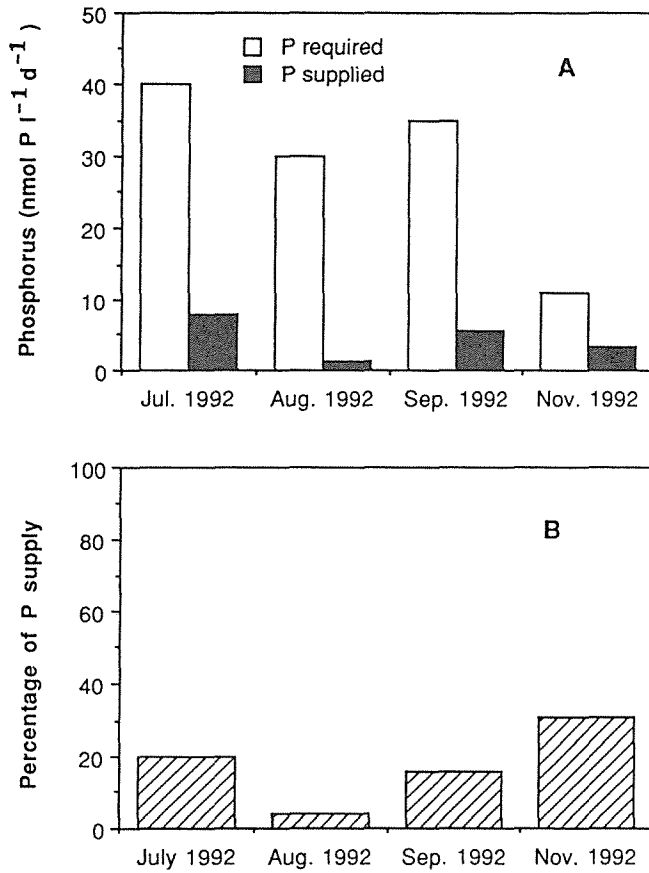


Fig. 3 Phosphorus required for primary production and supplied by bacterivorous flagellates in the north basin of Lake Biwa (A). The contribution of the supply to the requirement is also shown as percentages (B).

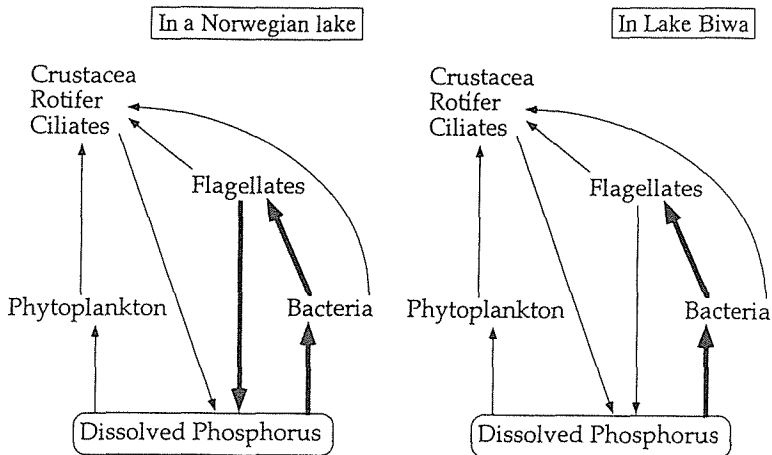


Fig. 4 A simplified scenario of phosphorus transfer among organisms in a Norwegian lake [18] and Lake Biwa.