Estimation of Phosphorus Release Rate by Bacterivorous Flagellates in Lake Biwa

Shin-ichi NAKANO

Abstract

In situ phosphorus release rates of bacterivorous flagellates were estimated in Lake Biwa to clarify their contribution to P cycling. Density of the flagellates ranged from 0.67 to 11×10^6 cells l^{-1} , and their community ingestion rate, which was determined by the fluorescently labeled bacteria method, varied seasonally from 0.20 to 23×10^8 bacteria $\cdot l^{-1} \cdot d^{-1}$. Bacterial carbon content showed seasonal variation (0.53 to 15 fmol C cell $^{-1}$), but bacterial C : P molar ratio was relatively invariant (106 ± 25 , $n=24$). *In situ* P release rate by the flagellates was estimated, based on these data and a stoichiometric model. The validity of the estimates was confirmed by incubation experiments using bacteria with C : P ratios of 30 to 103. In four of five experiments, P release rates estimated from the model coincided well with those from another method. *In situ* P release rate ranged from 0.11 to 59 nmol P $\cdot l^{-1} \cdot d^{-1}$, accounting for 4.2 to 31% of P required for phytoplankton primary production. This result suggests that bacterivorous flagellates are important, if not major, P suppliers in the lake, and that other P sources are needed for the lake phytoplankton.

Key words : bacterivorous flagellates, P release rate, P limited bacteria, Lake Biwa

1. Introduction

Previous studies have demonstrated phosphorus limitation for phytoplankton in many lakes (SAKAMOTO, 1966 ; SCHINDLER, 1978 ; TEZUKA, 1985). Some biological processes can free phytoplankton from P limitation, *e.g.*, bacterial decomposition of organic matter and excretion of zooplankton.

However, lake seston are often poor in P (*cf.* TEZUKA, 1985 ; NAKANISHI *et al.*, 1990), so that P release through bacterial decomposition of the seston may be negligible (TEZUKA, 1990). Furthermore, P uptake by bacteria is swifter than that by phytoplankton when P exists in low concentrations (CURRIE and KALFF, 1984). Hence, bacteria are consumers rather than suppliers of P in lakes.

In addition, bacteria serve as large P pools, relative to P in other planktonic organisms (VADSTEIN *et al.*, 1988 ; WATANABE, 1990). Regeneration of P preserved in bacterial cells,

therefore, can furnish an important P supply for phytoplankton. Among bacterivory of zooplankton, that of heterotrophic flagellates accounts for a significant portion of bacterial biomass losses (NAGATA, 1988 ; SANDERS *et al.*, 1989). In addition to this, bacterivorous flagellates have higher P release rates per body weight than metazoan zooplankton (ANDERSEN *et al.*, 1986). For these reasons, flagellates have been considered to be important P suppliers for phytoplankton in lakes (VADSTEIN *et al.*, 1993).

However, there is circumstantial evidence that bacterivorous flagellates do not always serve as important P suppliers in lakes. Nutrient release by heterotrophs depends not only on the quantity but also the quality of their food. Recent theoretical studies suggest that the amount of P released by heterotrophs is closely associated with the C : P or N : P ratios of food relative to their stoichiometry (STERNER, 1990 ; HESSEN and ANDERSEN, 1992). URABE (1993) demonstrated that changes in N : P release

ratios by a zooplankton community dominated by *Daphnia* were consistent with those in N : P ratios of food seston. Similar regulation of assimilation and release of P was reported for bacteria, although bacterial elemental ratios were variable (TEZUKA, 1990). Phosphorus release by flagellates also depends partly on the quantity and quality of prey (GOLDMAN *et al.*, 1987; JÜRGENS and GÜDE, 1990). Interestingly, NAKANO (1994a) demonstrated that the inorganic P release rate by *Spumella* sp. was reduced to a negligible level when C : P molar ratios of bacteria were higher than 70. Since C : P molar ratios of lake seston in a bacterial sized fraction can, in fact, exceed 70 (WATANABE, 1990), P release by flagellates which ingest bacteria with such high C : P ratios may be low. Furthermore, nutrient release by an isolated flagellate is more efficient for N than for P in experimental systems (GOLDMAN *et al.*, 1987; NAKANO, 1994b).

This study examined the P release rate by bacterivorous flagellates in P limited Lake Biwa to clarify the contribution of flagellates to phytoplankton primary production. C : P ratios of bacteria and ingestion rates of flagellates were measured simultaneously. Based on these data, the P release rate by flagellates was calculated from a stoichiometric model established by CARON (1991). Incubation experiments were also carried out with enriched filtered lake water. P release rates estimated from the model were then compared with those from changes in dissolved inorganic P concentration during the incubation period to check the validity of the stoichiometric model.

2. Materials and methods

2-1. Sampling stations

Lake Biwa is located in the center of Honshu Island, Japan (35° 10' N, 136° 00' E, altitude 85 m). Samples were collected monthly at two stations from July 1992 to June 1993: Station NB (water depth, *ca.* 75 m) was located in a pelagic area of the mesotrophic north basin of the lake 4 km offshore (35° 13' N, 136° 00' E), and Station SB (water depth, *ca.* 4 m) was located in the area of the eutrophic south basin 2 km

offshore (35° 05' N, 135° 54' E). Additional experiments were also conducted monthly at Station Wani, which was in a pelagic area of the north basin 3 km offshore (35° 11' N, 135° 56' E), from July to November 1992. All water samples were collected at a depth of 0.5 to 1 m (Stations NB and SB) and 2.5 m (Station Wani) with a modified 10-liter Van Dorn water sampler.

2-2. Determination of microbial densities and bacterivory

On shipboard, a 50 ml portion of the water samples was fixed with glutaraldehyde (final conc. of 1%). Bacteria were counted by the acridine orange direct count method (HOBBIE *et al.*, 1977). Colourless flagellates were enumerated by the fluorescein isothiocyanate (FITC) staining method (SHERR and SHERR, 1983).

Ingestion rate of bacterivorous flagellates was determined with fluorescently labeled bacteria (FLB) (SHERR *et al.*, 1987) made of a minicell-producing mutant strain of *Escherichia coli*. This bacterium was cultured in a liquid medium, and minicells were separated using the method of CHRISTEN *et al.* (1983). These minicells are spherical with a diameter of 0.5 to 1 μ m.

FLB were added to water samples (100 ml) at a density of $3\text{--}5 \times 10^5$ cells $\cdot$ ml $^{-1}$ (<10% of *in situ* bacterial density). The FLB-spiked water samples were incubated at *in situ* temperature under dim light conditions. After 8 min of incubation, subsamples were fixed with 4% ice-cold, buffered glutaraldehyde, which is an effective fixative to reduce the egestion of bacteria ingested into a food vacuole of the flagellates (SANDERS *et al.*, 1989). The flagellates were stained with FITC, and FLB in food vacuoles of the flagellates were counted with an epifluorescence microscope. At least 200 cells of the flagellates were examined. Time-zero control was prepared to count for FLB adsorbed to the flagellates.

2-3. Elemental content and ratio of lake bacteria

Two, 8-10 liter portions of water samples were filtered through Whatman GF/C filters. The filtrates were then filtered again through

Whatman GF/F filters. These filters were precombusted at 450°C for 2h. I assumed any C and P of the seston retained on the GF/F filter was that of the bacteria. The amount of C on the GF/F filter ($C_{GF/F}$, nmol C) was determined with a CHN-analyzer (Yanaco, MT-3), and that of P ($P_{GF/F}$, nmol P) using the method of MENZEL and CORWIN (1965). Both GF/C and GF/F filtrates were fixed with glutaraldehyde to enumerate bacteria.

Carbon content of bacteria (C_B , nmol C $\cdot$ cell⁻¹) was determined as

$$C_B = C_{GF/F} / (B_{GF/C} - B_{GF/F}) \quad (1)$$

where $B_{GF/C}$ and $B_{GF/F}$ were bacterial densities in GF/C and GF/F filtrates, respectively. The same equation was also used when the bacterial P content (P_B , nmol P $\cdot$ cell⁻¹) was calculated. In this case, $C_{GF/F}$ was replaced by $P_{GF/F}$ in the equation.

Whatman GF/F filters were sometimes unable to retain the lake bacteria efficiently; percentages of the bacteria retained on the filters to *in situ* bacterial density were 3.7–70% (mean 19%) at Station NB and 1.6–40% (mean 14%) at Station SB.

2-4. Lake water incubation experiments

A 10-liter water sample was filter-sterilized through a Gelman capsule filter (Culture capsule, pore size 0.2 μ m). Each 5-liter aliquot of the filtrate was poured into 10-liter glass bottles. A 1-liter aliquot of the water sample was filtered through a Whatman GF/C filter, and each 500 ml of the filtrate was added as inocula to the sterilized filtrate. Two cultures were prepared: one with no further addition of nutrients (control); another supplemented with glucose 5 μ mol C $\cdot$ l⁻¹, NH_4Cl 1 μ mol N $\cdot$ l⁻¹ and K_2HPO_4 0.5 μ mol P $\cdot$ l⁻¹ (C:N:P=10:2:1). Cultures were incubated at 25°C in the dark. Subsamples were withdrawn at various intervals to determine densities of bacteria and flagellates as well as concentrations of dissolved inorganic phosphorus (DIP), the last of which were measured using the method of MURPHY and RILEY (1962). For the experiments of August and September 1993, I also measured concentrations of total dissolved phosphorus (TDP) using the method of MENZEL and CORWIN (1965).

When the bacteria had attained the stationary phase, and flagellates had appeared (~ 1000 cells $\cdot$ ml⁻¹), 2-liters of the culture were filtered through a precombusted Whatman GF/F filter to measure bacterial carbon content on the filter with the CHN-analyzer. Bacterial P content was determined as the difference between the concentration of total phosphorus (TP) in the unfiltered culture and the GF/F filtrate, using the method of MENZEL and CORWIN (1965).

2-5. Estimation of release rate

From the experimental data, the phosphorus release rate by bacterivorous flagellates (E , fmol P $\cdot$ cell⁻¹ $\cdot$ h⁻¹) was calculated in two ways. One was as follows:

$$E = (P_2 - P_1) / (T \cdot D_{Fm}) \quad (2)$$

where P_1 and P_2 , concentrations of DIP at the onset and end of the exponential growth phase of the flagellates (fmol P $\cdot$ l⁻¹); T , time (hours) of the exponential growth phase of the flagellates; D_{Fm} (cells $\cdot$ l⁻¹), mean flagellate density determined according to HEINBOCKEL (1978) as

$$D_{Fm} = (D_2 - D_1) / (\ln D_2 - \ln D_1) \quad (3)$$

where D_1 and D_2 were densities of the flagellates (cells $\cdot$ l⁻¹) at the onset and end of the exponential growth phase, respectively.

The other was an estimation using the stoichiometric model proposed by CARON (1991):

$$E' = R \times (1 - Y)^{-1} \times \{ (C:P_{prey})^{-1} - Y \times (C:P_{flag})^{-1} \} \quad (4)$$

where E' , P release rate by flagellates (fmol P $\cdot$ cell⁻¹ $\cdot$ h⁻¹); R , respiration rate (fmol C $\cdot$ cell⁻¹ $\cdot$ h⁻¹); Y , growth yield in terms of carbon; $C:P_{prey}$ and $C:P_{flag}$, C:P molar ratios of the bacteria and flagellates, respectively. In the equation, I assumed that the growth yield and C:P molar ratio of the flagellates were, respectively, 0.32 and 83 (NAKANO, 1994a), and that all carbon ingested by flagellates was used for their production and respiration. The latter assumption means that the part " $R \times (1 - Y)^{-1}$ " in the equation equals the ingestion rate based on carbon (I_c , fmol C $\cdot$ cell⁻¹ $\cdot$ h⁻¹). Thus, the equation could be expressed as

$$E' = I_c \times \{ (C:P_{prey})^{-1} - 0.003855 \} \quad (5)$$

I_c was calculated as a multiplication of the ingestion rate (I_g , bacteria cell⁻¹ $\cdot$ h⁻¹) by the

bacterial carbon content (C_B' , $\text{fmol C} \cdot \text{cell}^{-1}$). I_G was determined as

$$I_G = (B_2 - B_1) / (T \times D_{FM}) \quad (6)$$

where B_1 and B_2 , bacterial densities ($\text{cells} \cdot \text{l}^{-1}$) at the onset and end of the exponential growth phase of the flagellates, assuming no growth by bacteria in the stationary phase. C_B' was determined by dividing the concentrations of particulate carbon ($\text{fmol C} \cdot \text{l}^{-1}$) at the onset of the exponential growth phase of the flagellates by B_1 .

For field data, the P release rate by bacterivorous flagellates in Lake Biwa (E' , $\text{nmol P} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$) was also estimated using Eq. (5). In this case, $C : P_{\text{prey}}$ was replaced with the $C : P$ molar ratio of *in situ* bacteria for each month. Community ingestion rates of the flagellates determined by the FLB method were used and converted into the amount of carbon ($\text{nmol C} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$). For the conversion, I multiplied the ingestion rate by the *in situ* bacterial carbon content (C_B , see Eq.(1)) in the estimation of P release rates at Stations NB and SB. For Station Wani, I used a representative bacterial carbon content (median $2.42 \text{ fmol C} \cdot \text{cell}^{-1}$) and a mean $C : P$ ratio of bacteria at Station NB (110), since I was unable to determine carbon content and $C : P$ ratio of bacteria at Station Wani.

3. Results

3-1. Microbial densities, elemental ratios and bacterivory

Seasonal changes in bacterial density at Station NB were variable: 3.2 to $11 \times 10^9 \text{ cells} \cdot \text{l}^{-1}$ (Fig. 1A) but the density was relatively low from January to March 1993. Bacterial density at Station SB was relatively high from September to November 1992, ranging from 15 to $19 \times 10^9 \text{ cells} \cdot \text{l}^{-1}$. It decreased rapidly from December, was $< 5 \times 10^9 \text{ cells} \cdot \text{l}^{-1}$ until March, and increased again from April. The density of flagellates at both stations did not show clear seasonal trends, fluctuating between 1.0 to $4.0 \times 10^6 \text{ cells} \cdot \text{l}^{-1}$ at Station NB, and between 2.0 to $5.0 \times 10^6 \text{ cells} \cdot \text{l}^{-1}$ at Station SB (Fig. 1B). At Station SB, flagellate density reached $11 \times 10^6 \text{ cells} \cdot \text{l}^{-1}$ in April 1993. Densities of bacteria and

Fig. 1. Seasonal changes in densities of bacteria (A) and flagellates (B) at Stations NB, SB and Wani in Lake Biwa.

flagellates at Station SB were higher than those at Station NB, and those at Station Wani fell between the ranges from those two stations.

Bacterial carbon content showed large variations from 0.53 to $15 \text{ fmol C} \cdot \text{cell}^{-1}$ at Station NB, and from 0.68 to $9.3 \text{ fmol C} \cdot \text{cell}^{-1}$ at Station SB (Fig. 2A). At both stations, bacterial carbon was relatively low from January to March 1993, and variable during other period of the survey. $C : P$ ratios of the bacteria ranged from 72 to 153 at Station NB, and from 78 to 138 at Station SB (Fig. 2B). These ratios were relatively high from November to February at Station NB, indicating that bacteria were P limited during that period. However, bacterial $C : P$ ratios at Station SB did not show any

Fig. 2. Seasonal changes in carbon content per 1 bacterial cell (A) and bacterial C: P ratio (B) at two stations in Lake Biwa.

seasonal trends.

The community ingestion rate of bacteria by flagellates fluctuated from 0.2 to 8.8×10^8 bacteria $\cdot$ l⁻¹ $\cdot$ d⁻¹ at Station NB, and from 0.9 to 23×10^8 bacteria $\cdot$ l⁻¹ $\cdot$ d⁻¹ at Station SB (Fig. 3). At Station SB, the community ingestion rate was relatively high from July to December 1992, decreased to $<3 \times 10^8$ bacteria $\cdot$ l⁻¹ $\cdot$ d⁻¹ from January to March 1993, and increased again from April. The rates at Station NB were much lower than those at Station SB except in December 1992, but the pattern of change was somewhat similar to that at Station SB. The rates at Station Wani were in the same range as those at Stations SB and NB, fluctuating from

Fig. 3. Seasonal changes in community ingestion rate on bacteria by flagellates at three stations in Lake Biwa. Perpendicular bars indicate the standard deviation.

1.0 to 6.3×10^8 bacteria $\cdot$ l⁻¹ $\cdot$ d⁻¹.

3-2. Lake water incubation experiments

To check the validity of the method of estimating the P release rate by bacterivorous flagellates, the rate of dissolved inorganic phosphorus (DIP) was estimated using the stoichiometric model of CARON (1991) (see Eq. (5)). This was then compared with the DIP release rate calculated with Eq. (2) in incubation experiments with enriched lake water filtrate. Figure 4 shows a representative result obtained in August 1992. Bacterial proliferation occurred during the first 2 days, followed by growth of flagellates which grazed on the bacteria in the nutrient-supplemented culture. No flagellates was detected in control cultures, and concentrations of DIP in the cultures were below the detection limit ($<0.10 \mu\text{mol P} \cdot \text{l}^{-1}$) throughout the experimental period (data not shown). DIP was utilized rapidly by bacteria during the first 2 days in the nutrient-supplemented culture, and thereafter concentrations of DIP increased with an increase in growth of flagellates. The increase in DIP concentrations in the culture was apparently due to P release by flagellates (Fig. 4). A similar pattern of growth and P release by flagellates was observed in all experiments conducted five times, although there were differences in growth rates of flagellates and changes in DIP concentrations. To

examine the release of dissolved organic phosphorus (DOP) by flagellates, I also measured concentrations of total dissolved phosphorus (TDP) in the experiments performed in August and September 1993. Although TDP concentrations increased consistently with the growth of flagellates, this increase was due to concentrations of DIP. Hence I concluded that DOP release by flagellates was negligible.

Fig. 4. Results of lake water incubation experiments conducted in August 1992. Changes in densities of bacteria and flagellates (A) and concentrations of dissolved inorganic phosphorus (DIP) (B) in the nutrient-supplemented culture.

Bacterial C : P ratios in the nutrient-supplemented culture ranged from 30 to 103 (Table 1). P release rate estimated with Eq. (2) ranged from 0.20 to 0.48 $\text{fmol P} \cdot \text{cell}^{-1} \cdot \text{h}^{-1}$, and that with Eq. (5) from 0.21 to 1.1 $\text{fmol P} \cdot \text{cell}^{-1} \cdot \text{h}^{-1}$. The estimates from Eq. (5) coincided well with those from Eq. (2) with one exception: a 2.9-fold difference was detected between the two in the experiment conducted in August 1992. Although the value 1.1 $\text{fmol P} \cdot \text{cell}^{-1} \cdot \text{h}^{-1}$ obtained in August 1992 was extremely high compared with other estimates from Eq. (5), the value was not rejected in the estimates from Eq. (5) by Smirnov test. Finally, I examined a difference between the estimates from Eq. (2) and Eq. (5) by Student's t-test, and found it to be insignificant ($p=0.5$). The results indicate that the model was applicable to an estimation of P release rate by flagellates grazing on bacteria.

3-3. Estimation of *in situ* phosphorus release by flagellates

At Station NB, the P release rate by bacterivorous flagellates estimated from Eq. (5) ranged from 0.11 to 24 $\text{nmol P} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$, and seasonal trends in the rate were not found (Fig. 5). The P release rate estimated at Station SB ranged from 1.2 to 59 $\text{nmol P} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$. Its seasonal changes were somewhat similar to those in the community ingestion rate of the flagellates: the P release rate was relatively high from July to October 1992, low from November 1992 to March 1993 and rose again from April. The range of P release rate by the flagellates at Station Wani was 1.3 to 8.0 $\text{nmol P} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$.

The contribution of P release rate by the flagellates was determined through a comparison with the calculated P requirement for phytoplankton primary production at Station

Table 1. A comparison between phosphorus release rates estimated from two methods in lake water incubation experiments.

		July 1992	Aug. 1992	Sep. 1992	Aug. 1993	Sep. 1993
Bacterial C:P ratio		103	30	75	53	76
P release rate	Equation(2)*	0.27	0.38	0.33	0.20	0.48
($\text{fmol P} \cdot \text{cell}^{-1} \cdot \text{h}^{-1}$)	Equation(5)*	0.21	1.1**	0.32	0.24	0.45

* See Materials and methods.

**The value was not rejected in estimates from Eq. (5) by the Smirnov test.

Table 2. Primary production, phosphorus requirement of phytoplankton and phosphorus supply by flagellates at station Wani.

	Primary production* ($\mu\text{mol C}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$)	Sestonic C:P ratio*	Required phosphorus** ($\text{nmol P}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$)	Phosphorus supplied by flagellates ($\text{nmol P}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$)	% supplied
July 1992	14	351	40	8.0	20
Aug. 1992	9.6	325	30	1.3	4.2
Sep. 1992	6.6	187	35	5.7	16
Nov. 1992	3.3	292	11	3.4	31

* The details are in URABE *et al.* (in press.). Primary production rate was measured at a depth of 2.5 m below the surface.

**Calculated by dividing a measured value of primary production by a sestonic C:P ratio.

Fig. 5. Seasonal changes in estimated phosphorus release rates of bacterivorous flagellates at three stations in Lake Biwa.

Wani. Primary production rates and sestonic C : P ratios 2.5m below the surface (Table 2) were detailed in URABE *et al.* (in press). Phosphorus required for primary production was calculated by dividing primary production by the sestonic C : P ratio, and was estimated as 11 to 40 $\text{nmol P}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$. The percentage of P supplied by bacterivorous flagellates of the P required for primary production was 4.2 to 31%.

4. Discussion

I assumed that any carbon and phosphorus collected in the <GF/C, >GF/F fraction were from the bacteria in this study (*cf.* Materials and methods). Considering the pore size of Whatman GF/C and GF/F filters, seston in the

fraction ranged in size from 0.6 to 1.2 μm . Bacteria, algal picoplankton and bacteria-sized detrital particles can be included in this size range. NAGATA (1986) has noted that the presence of bacteria-sized detrital particles can cause an overestimation of bacterial elemental content. However, when I observed microscopic specimens stained with acridine orange, I seldom found such particles in GF/C filtrates of lake water from Stations NB and SB. Also, the biomass of algal picoplankton in the <GF/C, >GF/F fraction is rather small compared with that of bacteria (NAGATA, 1986). For these reasons, I believe that the sestonic biomass in the <GF/C, >GF/F fraction can be regarded as that of bacteria. However, bacterial biomass in the bacterial fraction defined in this study represented only a small fraction of the total bacteria in Lake Biwa (*cf.* Materials and methods). It is necessary, therefore, to mention what bacteria was collected in the fraction. Previous studies have demonstrated that flagellates prefer to ingest particles about 1 μm in size (CHRZANOWSKI and SIMEK, 1990 ; BIRD and KALFF, 1993). The fraction in this study (0.6 -1.2 μm) overlaps that range. Hence, bacteria collected in the fraction may be the preferable prey for flagellates.

The bacterial carbon content fluctuated greatly during this study: 30-fold differences were detected at Station NB, and 14-fold differences at Station SB (Fig. 2A). However, the changes in bacterial C : P ratio were rather small: roughly two-fold differences between

the greatest and least values at both stations (Fig. 2B). VADSTEIN *et al.* (1988) have demonstrated that C : P ratios of bacteria in a Norwegian lake ranged from 7.2 to 76. A mean C : P ratio of bacteria in the present study was 106 ± 25 , and that in the Norwegian lake was 24 ± 28 . Thus, the changes in C : P ratio of bacteria in Lake Biwa are smaller than those in the Norwegian lake. Compared with a C : P ratio obtained experimentally from an isolated flagellate (83, NAKANO, 1994a), the bacterial C : P ratio was relatively high. The lake bacteria may serve as qualitatively stable but P limited prey for flagellates. Furthermore, as already mentioned in the Introduction, the DIP release rate of an isolated flagellate was negligible when C : P molar ratios of bacteria were > 70 (NAKANO, 1994a). This suggests that flagellates which ingest lake bacteria may concentrate P by reducing the release rate of P.

In all the lake water incubation experiments, the P release rate by bacterivorous flagellates estimated from the stoichiometric model of CARON (1991) corresponded well to that estimated from changes in concentrations of DIP and densities of the flagellates (Table 1). This result suggested that assumed values for growth yield and C : P ratio of the flagellates (0.32 and 83, respectively) were reasonable. Indeed, the growth yield is a common value compared with what was reported previously (FENCHEL, 1982; NAGATA, 1988). In addition, NAKANO (1994a) demonstrated that the C : P ratio of an isolated flagellate was invariant despite the large variation in bacterial C : P ratio. Further, since the model assumes that all ingested elements are completely assimilated by an animal, it can underestimate the elemental release rate of a particle-feeding animal when that animal releases indigested matter (LANDRY, 1993). If this is the case, not only DIP but also dissolved organic phosphorus (DOP) could be released in lake water incubation experiments. DOP release, however, was not detected in the experiments conducted in August and September 1993. For these reasons, I believe that the phosphorus release rate of bacterivorous flagellates can be successfully

estimated in this study using the stoichiometric model. However, the estimation from the model was much higher than that from another estimation in the experiment conducted in August 1992 (Table 1). This result could have been due to the DOP release of flagellates and/or the dominance of flagellates with a high growth yield and a low C : P ratio.

Phosphorus is not released during aerobic decomposition of seston in Lake Biwa (TEZUKA, 1986), but is released when C : P ratios of the seston are reduced through incubation with external nutrients at low C : P ratios (TEZUKA, 1989). Bacterivorous flagellates were responsible for P release during the decomposition of crustacean zooplankton with a C : P ratio of 132 in an *ex situ* experiment (MIYAJIMA, 1992). However, there is little information available on the *in situ* P release rate of bacterivorous flagellates in lakes. VADSTEIN *et al.* (1993) reported that the P supply of flagellates was twice as that of zooplankton in a Norwegian lake. The present study demonstrated that the P release rate of flagellates accounted for 4.2 to 31% of P required for primary production in Lake Biwa (Table 2). The contribution of the P release rate by crustacean zooplankton to the P required was 1 to 36% in Lake Biwa (URABE *et al.*, in press.). Thus, the contribution of the P supply by flagellates is almost equivalent to that of zooplankton. However, there was a large seasonal variation in the P release rate by the flagellates: 222-fold differences were detected at Station NB, and 51-fold differences at Station SB (Fig. 5). Although flagellates are one of the important P suppliers in Lake Biwa, it is likely that their significance as P suppliers in the lake varies seasonally.

Unfortunately, there is a problem in estimating the *in situ* P release rate by flagellates. Community ingestion rates presented here (Figs. 3 and 4A) are within the range of those in previous studies which used the FLB method (*cf.* SANDERS *et al.*, 1989). However, that method gives relatively low ingestion rates, compared with those obtained by other methods. For example, NAGATA (1988) reported the community ingestion rate of the flagellates in Lake

Biwa as 8.0 to 25×10^8 bacteria $\cdot l^{-1} \cdot d^{-1}$, using bottle incubation experiments. The low estimates by the FLB method may be due to the use of boiled and stained *E. coli* minicells as surrogates. As all methods for measuring the ingestion rate of flagellates have technical problems, there is an important need to develop methods for understanding microbial cycling in freshwater and marine ecosystems.

This study suggests that the importance of bacterivorous flagellates as P suppliers for phytoplankton varies seasonally in Lake Biwa. Thus, phytoplankton appear to be supported not only by flagellates but also by other P sources. Further studies should elucidate these P sources and their relative importance for phytoplankton.

Acknowledgements

I wish to thank Drs. Y. TEZUKA, M. NAKANISHI and other researchers at the Center for Ecological Research, Kyoto Univ. for their advice and encouragement throughout this study. Thanks are also due to Messrs. T. UEDA and T. KOITABASHI for their assistance in the field survey, and to Dr. Y. WATANABE for his gift of a minicell-producing strain of *E. coli*. Also, I greatly appreciate the many constructive comments on this manuscript by Drs. T. NAGATA and J. URABE, and correction of the English text by Dr. P. J. BELLINGHAM. This study was partly supported by Lake Biwa Museum Research Project 93-20, Shiga Prefecture.

琵琶湖における従属栄養鞭毛虫の細菌摂食 にともなうリン排出速度の見積もり

琵琶湖における従属栄養鞭毛虫のリン排出速度を見積もり、鞭毛虫の本湖のリン循環における重要性を検討した。鞭毛虫の細胞密度は 0.67 から 11×10^8 cells $\cdot l^{-1}$ の範囲にあり、蛍光色素ラベルされた細菌を用いる方法による細菌摂取速度は 0.20 から 23×10^8 bacteria $\cdot l^{-1} \cdot d^{-1}$ であった。細菌の炭素含量は季節的に大きく変化した ($0.53 \sim 15$ fmol C $\cdot$ cell $^{-1}$) が、その C : P 比は比較的安定であった (モル比で 106 ± 25 , $n=24$)。これらのデータとある数式モデルから、鞭毛虫によるリンの排出速度を見積もった。このモデルの信頼性を確認するために、C : P 比が 30 から 103 の細菌を

用いた培養実験を行った。この実験の 5 回のうち 4 回において、このモデルから見積もられた鞭毛虫のリン排出速度は、他の方法で見積もられたそれによく一致した。琵琶湖における鞭毛虫のリン排出速度は、 0.11 から 59 nmol P $\cdot l^{-1} \cdot d^{-1}$ と大きく変化した。一次生産に必要なリンの 4.2 から 31% に相当した。以上の結果から、琵琶湖の鞭毛虫は主要ではないが重要なリンの供給者であり、また植物プランクトンの一次生産には他のリン供給源も必要であることが考えられた。

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(著者：中野伸一*, 京都大学生態学研究センター,
〒520-01 滋賀県大津市下阪本4丁目1-23; *現在, 滋
賀県琵琶湖研究所, 〒520 滋賀県大津市打出浜1番10
号; Shin-ichi NAKANO*, Center for Ecological
Research, Kyoto University, Shimosakamoto, Otsu,
Shiga 520-01; *Present address: Lake Biwa
Research Institute, 1-10 Uchidehama, Otsu 520
Japan)

Received : 23 April 1994

Accepted : 29 July 1994