

# Regeneration of Phosphorus and Nitrogen by Four Species of Heterotrophic Nanoflagellates Feeding on Three Nutritional States of a Single Bacterial Strain

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**Three physiological states of a single bacterial strain, namely, balanced, phosphorus-rich, and nitrogen-rich bacteria, were obtained by culturing a bacterial strain in chemostats under three different nutrient regimens. Each was shown to be distinctly different in elemental composition with respect to C/N/P ratio. These bacteria were fed to four species of heterotrophic nanoflagellates in batch culture grazing experiments, and the percent regeneration efficiencies of bacterium-bound nitrogen and phosphorus by the flagellates were compared. All flagellate species regenerated comparable amounts of nitrogen, which was thought to be due to their similar internal C/N ratios. There was, however, interspecies variation with regard to phosphorus regeneration: the two faster-growing species (*Paraphysomonas imperforata* and *Bodo designis*) released significantly more phosphorus than the two slower-growing species (*Stephanoeca diplocostata* and *Jakoba libera*). The observed differences were thought to have been influenced by a combination of life cycle strategies and internal C/P ratios.**

The "microbial loop" exists at the base of the aquatic food web and traditionally comprises interactions among phytoplankton, bacteria, and small protozoa (3). In simple terms, phytoplankton cells utilize light energy for photosynthesis and the extracellular material released is utilized by bacteria for growth and reproduction (17). Phagotrophic protozoa such as amoebae (29), ciliates (26), mixotrophic flagellates (23), and colorless heterotrophic flagellates (13) feed on the bacteria and release certain elements in excess of their growth requirements as soluble nutrients. In particular, this applies to excess nitrogen, which is released principally as ammonium, and phosphorus, which is released as soluble phosphate. These nutrients may then be taken up by phytoplankton and bacteria (secondary uptake), although division of uptake between these two components of the plankton may be disproportionate since bacteria have been shown to outcompete phytoplankton for regenerated nutrients, especially when nutrient availability is low (10, 18, 33).

Bacteria are the major natural food source for heterotrophic nanoflagellates, which are known to be the most important bacteriovores in pelagic environments (14, 30). One role of nanoflagellates is therefore the utilization of organic particles and the regeneration of soluble inorganic matter. A major proportion of regenerated nutrient is excreted within short time intervals due to the rapid growth rates of nanoflagellates compared with larger zooplankton (8, 22, 24, 31). The quantity of regenerated nutrients has been shown to be related to the elemental composition of the prey, especially if it is bacterial (21, 24); many bacteria are able to take up phosphorus supplied in excess of their growth requirements and store it in their cells, thereby altering their C/N/P ratio (25). There is no doubt that bacteria can, and do, regenerate nutrients, but the extent to which this occurs is unknown at present. Regeneration by bacteria, however, has been shown to be dependent on the C/N/P ratio of available bacterial substrates (15, 19).

The overall theme of this work, of which this communication

is part, has been to determine the kinetic processes involved in heterotrophic flagellate-bacterium interactions. The kinetics of six marine nanoflagellate species feeding on a single bacterial prey (bacterium B1) in batch culture have already been compared (11). The aim of this study has been to compare four of these six nanoflagellate species with respect to their capabilities for remineralizing bacterium-bound phosphorus and nitrogen into total dissolved phosphorus (TDP) and ammonium, respectively.

## MATERIALS AND METHODS

**Organisms.** Bacterium B1, which has been previously described (11), was the sole bacterial strain used in this investigation. The four selected species of marine heterotrophic nanoflagellates were *Paraphysomonas imperforata* Lucas, *Bodo designis* Skuja, *Stephanoeca diplocostata* Ellis, and *Jakoba libera* (Ruinen) Patterson. Clonal cultures of each flagellate species were routinely maintained in monoaxenic culture with bacterium B1.

**Cultivation of bacteria.** In order to assess the effect of bacterial C/N/P ratio on the quantity of ammonium and orthophosphate regenerated by individual flagellate species in batch culture grazing experiments, it was necessary to obtain three quantitatively different states of bacterium B1, each containing different C/N/P ratios. Three cultures of bacterium B1 were maintained in chemostats (LH Fermentation; 1-liter vessels) under different nutrient regimens at 20°C. One culture (balanced bacteria) received an input of modified artificial seawater (MAS) (12) with a carbon/nitrogen/phosphorus ratio of 54:10:1 (Table 1). The second culture (nitrogen-rich bacteria) received MAS supplemented with additional nitrogen, while the third culture (phosphorus-rich bacteria) was supplemented with additional phosphorus. The carbon, nitrogen, and phosphorus sources were in the forms of glucose, NaNO<sub>3</sub>, and KH<sub>2</sub>PO<sub>4</sub>, respectively (Table 1). Each steady-state culture was maintained at a dilution rate (*D*) of 0.1 h<sup>-1</sup>, and the mechanical operation of the chemostats has been described elsewhere (12).

**Batch culture flagellate grazing experiments.** Batch culture flagellate grazing experiments were designed with the objects of (i) comparing the relative capacities of the different flagellate species for the regeneration of phosphorus and nitrogen and (ii) assessing whether the C, N, and P composition of the bacterial prey influenced the concentration of TDP and ammonium released by the different nanoflagellate species. A subsample of each of the three bacterial steady-state cultures and of each of the exponentially growing stock cultures of the four heterotrophic flagellate species was centrifuged to form a pellet of cells which was then washed three times in unsupplemented MAS. Each flagellate concentrate was then used as an inoculum for batch culture grazing experiments with each of the three bacterial states. Thus, each flagellate species was fed separately with balanced, phosphorus-rich, and nitrogen-rich bacteria. The experiments were performed in triplicate in 100-ml Erlenmeyer flasks containing unsupplemented MAS, which did not support bacterial growth. The initial concentration of each flagellate species was 1,000 cells ml<sup>-1</sup>, while the initial concentrations of the bacteria were 1.5 × 10<sup>7</sup> cells ml<sup>-1</sup> for balanced bacteria and 8 × 10<sup>6</sup> cells

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TABLE 1. Details of the composition of medium entering each chemostat vessel which contained each of three bacterial cell states

Vessel	Bacterial cell state	Supplementary additions to MAS (mg liter <sup>-1</sup> )			Elemental C/N/P of medium
		Glucose (C, mg liter <sup>-1</sup> ) <sup>a</sup>	NaNO <sub>3</sub> (N, mg liter <sup>-1</sup> )	KH <sub>2</sub> PO <sub>4</sub> (P, mg liter <sup>-1</sup> )	
1	Balanced	200 (80) <sup>a</sup>	93 (16)	7 (1.6)	50:10:1
2	N rich	200 (80)	186 (32)	7 (1.6)	50:20:1
3	P rich	200 (80)	93 (16)	35 (8)	10:2:1

<sup>a</sup> Equivalent element concentrations are given in parentheses.

ml<sup>-1</sup> for phosphorus-rich or nitrogen-rich bacteria. The flasks were incubated at 20°C until the flagellates reached the stationary phase of growth. Because of the difference in growth rate, experiments lasted longer for *S. diplocosata* and *J. libera* (6 days) compared with experiments involving *P. imperforata* and *B. designis*, which took 3 days. During the experimental period, the numbers of the flagellate cells and bacteria and the concentrations of TDP and ammonia were monitored by the methods described below.

**Control grazing experiments to determine gross and net regeneration efficiencies.** Four control experimental systems were set up with *B. designis* and *J. libera* feeding on balanced and phosphorus-rich bacteria in exactly the same manner as that described above. However, these grazing experiments were performed in the presence of the antibiotic polymyxin B sulfate at a final concentration of 100 µg ml<sup>-1</sup>. At this concentration, the antibiotic was bacteriostatic towards bacterium B1; that is, it inhibited growth and reproduction without lysing the cells. Triplicate flasks of the bacterium in the absence of the flagellate grazers, with and without the antibiotic, were also set up as bacterial controls. Total and "viable" bacterial counts were performed on subsamples taken from these flasks every day for 10 days. The experimental flasks containing the flagellate grazers were monitored for flagellate cells, bacteria, and TDP concentration in exactly the same way as for those flasks without the antibiotic, and the nutrient regeneration efficiency values (percentages) in the presence (gross values) and absence (net values) of the antibiotic were compared.

**Analyses.** Bacterial and flagellate counts were performed on glutaraldehyde-fixed samples (final concentration, 0.5%), using a hemocytometer. Viable counts of the bacteria in the control flasks were determined by the dilution plate technique, using nutrient agar (Oxoid). TDP was analyzed by means of the molybdenum blue reaction (36) after wet oxidation of a filtered sample (0.2 µm) following the method of Jürgens and Güde (24), while concentrations of ammonia were measured according to Strickland and Parsons (32).

The carbon and nitrogen content of bacteria was measured with a Perkin-Elmer CHN analyzer after drying washed bacterial cells onto acid-washed glass petri dishes and removing the resultant powder. The carbon and nitrogen content of flagellates was determined with a Carlo Erba model 1106 CHN analyzer on samples filtered through precombusted Whatman GF/F filters. A known concentration of bacterium B1 was also present in the flagellate samples, but this was corrected for when the C/N ratio relating to the flagellates alone was calculated. The average volumes of bacterial and flagellate cells were determined after fixation with glutaraldehyde, using the equation  $V = \pi(s^2l)/6$ , where  $s$  is the shortest length of the cell and  $l$  is the longest. Following this, carbon conversion values (femtograms of C per cubic micrometer) for bacteria and flagellates were estimated. Particulate P concentrations were determined from wet oxidation of an unfiltered culture by autoclaving for 1 h in the presence of potassium peroxodisulfate (0.15% [wt/vol] final concentration) and 10 N H<sub>2</sub>SO<sub>4</sub> and assaying with the molybdenum blue reaction after neutralizing the sample with NaOH.

**Presentation of results.** The increase in flagellate concentration, decrease in bacterial concentration, and increase in either TDP or ammonium were recorded for each experimental system, and calculations were made following the method of Jürgens and Güde (24). The initial bacterial concentration was expressed as a function of initial phosphorus concentration (this also applied to the calculation of initial nitrogen concentration), where

$$\text{initial bacterial concentration (bacteria liter}^{-1}\text{)} \times \quad (1)$$

$$\text{P content of bacterial cell (}\mu\text{g)} = \text{initial P (}\mu\text{g liter}^{-1}\text{)}$$

This initial P is not detectable in the medium at the beginning of the experiment as it is all bound within the bacteria (TDP = 0). However, once flagellates in the culture begin feeding on the bacteria, they release phosphorus into the surrounding medium, which manifests itself as an increase in TDP concentration in the medium. The percentage of the initial bacterium-bound phosphorus (P-bound) released at each sampling time can then be calculated as

$$\frac{\text{TDP}_{t_1} - \text{TDP}_{t_0} (\mu\text{g liter}^{-1}) \times 100}{\text{initial P} (\mu\text{g liter}^{-1})} = \% \text{ P-bound released} \quad (2)$$

TABLE 2. Particulate carbon, nitrogen, and phosphorus contents and the corresponding C/N/P ratios of bacterium B1 after culture in three nutrient regimens

Bacterial cell state	Elemental composition (fg cell <sup>-1</sup> ) ± SEM			C/N/P ratio
	Carbon	Nitrogen	Phosphorus	
Balanced	80.5 ± 15.4	19.1 ± 1.9	3.30 ± 0.4	26:6:1
N rich	88.9 ± 8.3	42.1 ± 1.6	1.72 ± 0.8	53:25:1
P rich	88.6 ± 1.3	20.9 ± 0.3	10.0 ± 1.2	9:2:1

where TDP<sub>t<sub>0</sub></sub> and TDP<sub>t<sub>1</sub></sub> are the concentrations of TDP at the beginning and at some point in the experiment, respectively.

In order to compare the nutrient regeneration properties of flagellates more effectively, the number of bacteria consumed during the experimental period must be taken into account as not all flagellate species will consume the same quantity of bacteria, thus giving biased results if percentage of P-bound was the parameter used for comparison. The percent regeneration efficiency (%RE) is a more meaningful way of comparing the regeneration efficiencies of different flagellate species:

$$\frac{\text{P released} (\mu\text{g liter}^{-1}) \times 100}{\text{P consumed} (\mu\text{g liter}^{-1})} = \% \text{RE} \quad (3)$$

where P released is the highest TDP value during the experiment and P consumed is the initial bacterial concentration (as P, equation 1) minus the lowest bacterial concentration recorded (also as P).

## RESULTS AND DISCUSSION

**Elemental composition of the three bacterial cultures and assessment of secondary uptake of regenerated nutrients.** Elemental analysis of the three bacterial states, that is, balanced, phosphorus-rich, and nitrogen-rich bacteria, indicated that their designated names satisfactorily described their respective C, N, and P contents after they had been cultured under different nutrient regimens in chemostats (Table 2). All three bacterial states contained a comparable amount of carbon (80.5 to 88.9 fg of C bacterium<sup>-1</sup>), giving a carbon conversion factor of 128 fg of C µm<sup>-3</sup>. This is lower than the factors of Bratbak (7) and Børsheim and Bratbak (6), which were 580 and 446 fg of C µm<sup>-3</sup>, respectively, but in the range of that reported by Bloem et al. (5), which was 221 ± 103 fg of C µm<sup>-3</sup>. Observed differences in conversion factors could be due to inaccurate measurements of cell volume due to cell shrinkage, inadequate calibration, etc.; precise measurements are often difficult to obtain (16). Nitrogen-rich bacteria contained significantly more nitrogen (42.1 fg of N bacterium<sup>-1</sup>) than the other two bacterial states, for which their nitrogen contents were comparable (19.1 and 20.9 fg of N bacterium<sup>-1</sup>) (Table 2). These were all within the range of 10 to 50 fg of N bacterium<sup>-1</sup> found by Goldman et al. (20, 21). Phosphorus-rich bacteria contained significantly more phosphorus (10 fg of P bacterium<sup>-1</sup>) than the other two bacterial states, which possessed 3.3 and 1.72 fg of P bacterium<sup>-1</sup> for balanced and nitrogen-rich bacteria, respectively. The latter values are within the range of other published data (1.3 to 6 fg of P bacterium<sup>-1</sup> [1, 5, 24]), but the value for phosphorus-rich bacteria is higher.

The extent of secondary uptake of phosphorus by bacterium B1 was examined during grazing experiments in the presence and absence of polymyxin B sulfate. This antibiotic did not lyse the bacterial cells and so they were available as a particulate food source for the flagellates. However, bacterial growth and division were inhibited and no nutrient uptake was apparent. In control flasks, containing bacteria only in MAS, the total numbers of bacterial cells in the presence and absence of

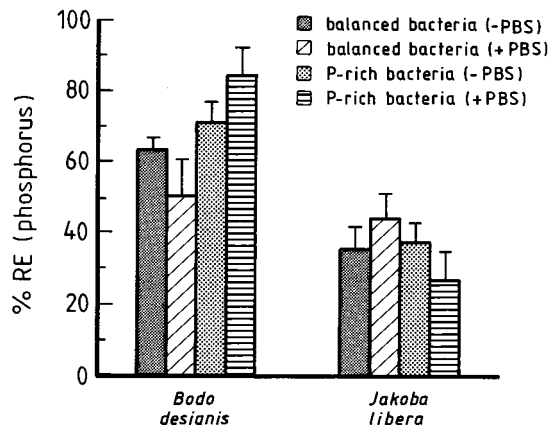


FIG. 1. Comparison of the percent regenerated bacterium-bound phosphorus by *B. designis* and that by *J. libera* after feeding on balanced- and phosphorus-rich bacteria in the presence and absence of polymyxin B sulfate (PBS).

polymyxin B sulfate were not significantly different over a period of 10 days. However, bacterial viability tests revealed that the bacteriostatic effect of the antibiotic was only effective for 9 days, as on the 10th day a total of 5 bacteria ml<sup>-1</sup> were able to grow on agar plates, whereas previously there had been no detectable growth. This is really an insignificant concentration with respect to the overall number of bacteria present in the batch culture experiments, as 5 bacteria ml<sup>-1</sup> would not take up a significant proportion of regenerated nutrient to alter gross versus net %RE values. In addition, all experiments were complete within a 10-day period.

The %RE obtained for *B. designis* and *J. libera* feeding on either balanced or P-rich bacteria, in the presence and absence of the antibiotic, were not significantly different (Fig. 1), therefore indicating undetectable secondary nutrient uptake by the viable bacteria in the untreated flasks. The antibiotic did not appear to affect the kinetic behavior of any of the flagellate species; their specific growth rates and final flagellate concentrations were comparable in both treated and untreated systems.

TABLE 3. Specific growth rates calculated for each flagellate species in each experimental system

Flagellate species	Specific growth rate (h <sup>-1</sup> ) ± SEM		
	+ Balanced bacteria	+ P-rich bacteria	+ N-rich bacteria
<i>P. imperforata</i>	0.21 ± 0.005	0.17 ± 0.004	0.18 ± 0.008
<i>B. designis</i>	0.14 ± 0.004	0.10 ± 0.009	0.10 ± 0.010
<i>J. libera</i>	0.026 ± 0.0008	0.021 ± 0.008	0.022 ± 0.004
<i>S. diplocostata</i>	0.029 ± 0.001	0.028 ± 0.0009	0.02 ± 0.001

**Phosphorus and nitrogen regeneration by heterotrophic flagellate grazing.** The concentrations of the flagellate species and bacteria during grazing experiments followed a typical batch culture relationship (e.g., *P. imperforata* [Fig. 2]) whereby the number of flagellate cells increased to an asymptotic maximum at the onset of the stationary phase of growth while there was a corresponding decrease in bacterial numbers. There was no lag period evident, probably due to the flagellate inoculum being taken from a previously exponentially growing batch culture.

The specific growth rate values for each flagellate species appeared to be independent of the C, N, and P content of the bacterial prey; values were highly conserved (Table 3). Slight variations were due only to the different starting concentrations of balanced bacteria (1.5 × 10<sup>7</sup> bacteria ml<sup>-1</sup>) as opposed to N-rich and P-rich bacteria (8 × 10<sup>6</sup> bacteria ml<sup>-1</sup>), but all values were comparable to those obtained in other experiments (11). Other workers have sometimes recorded differences in specific growth rates between batch culture grazing experiments; although the underlying reasons for these differences have not been identified, they were known not to depend on the prey species (19, 21).

As a result of grazing by flagellates, bacterium-bound phosphorus and nitrogen were regenerated into the medium in soluble form during the exponential and stationary phases of flagellate growth. Immediate regeneration was recorded for all flagellates with all bacterial types. Although all flagellate species were similar in their regeneration responses, they were

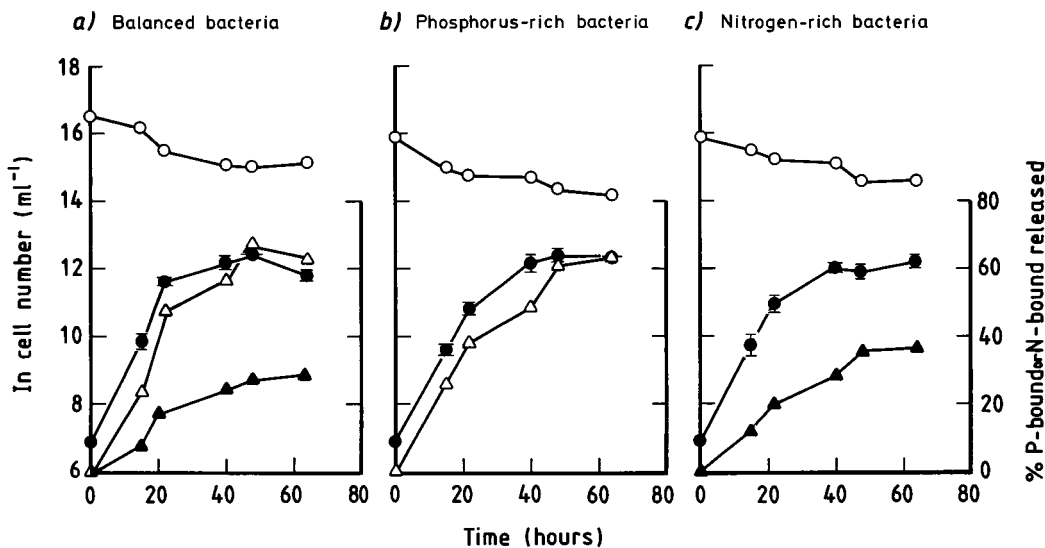


FIG. 2. Percent regenerated bacterium-bound phosphorus (Δ) and nitrogen (▲) by *P. imperforata* (●) fed with (a) balanced, (b) phosphorus-rich, and (c) nitrogen-rich bacteria (○) over time in batch culture grazing experiments.

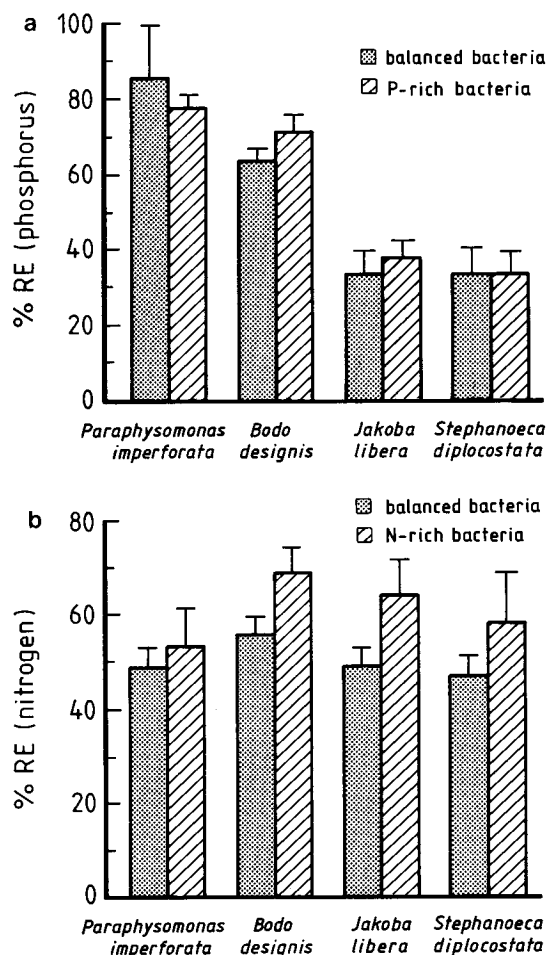


FIG. 3. Comparison of the %RE for each nanoflagellate species with respect to phosphorus (a) and nitrogen (b) after feeding on balanced, phosphorus-rich, and nitrogen-rich bacteria.

different with respect to the quantities of soluble phosphorus and ammonium they actually regenerated.

The %RE of phosphorus for the four flagellate species feeding on balanced and phosphorus-rich bacteria (Fig. 3a) reveal that they regenerated between 33 and 85% of bacterial-bound phosphorus into the surrounding medium, values which are within the extremely wide range of 4 to 91% found by other workers (1, 2, 24). A narrower range of 5 to 68% has been found with phytoplankton as prey (1, 19–21). Variations have previously been attributed to the C, N, and P content of the prey used in the experiments. However, in this study, variations appear to be related to the flagellate species rather than the C, N, and P content of the prey. This is evident when %RE values for each of the four flagellate species feeding on a comparable prey state are compared, whether it be balanced or P-rich bacteria (Fig. 3a). When balanced bacteria were used as the prey, the %RE values of the four flagellate species ranged from 33 to 85%, while with phosphorus-rich bacteria, a range of 33 to 77% was obtained.

*P. imperforata* and *B. designis* released significantly more bacterium-bound phosphorus (85 and 63%, respectively) than *J. libera* and *S. diplocostata* (35 and 33%, respectively [Fig. 3a]). This was recorded when the flagellates were fed with either balanced or phosphorus-rich bacteria. *P. imperforata* and *B. designis* were the two faster-growing species, with specific

growth rates of around 0.2 and 0.14  $\text{h}^{-1}$ , respectively (Table 3), while *S. diplocostata* and *J. libera* grew at the slower specific growth rates of around 0.029 and 0.026  $\text{h}^{-1}$ . Reasons for the differences in regeneration capacities may possibly be related to life cycle strategies whereby the two faster-growing species may be described as having an opportunistic life cycle (r strategy) in which the species have a limited need to conserve elements in excess of their growth requirements. *S. diplocostata* and *J. libera* may be considered equilibrium populations (K strategy), in which case they may be more conservative with respect to remineralization processes (28).

%RE values have been based on the measurement of TDP, which is made up of soluble reactive phosphorus and dissolved organic phosphorus (DOP). The major proportion of phosphorus regenerated by protozoa has been shown to be in the form of soluble reactive phosphorus (24, 34). Other workers have found that DOP may be a component of regenerated phosphorus, but values vary considerably between experiments and between time intervals within a single experiment (1). Pomeroy et al. (27) found that DOP accounted for almost 50% of the TDP excreted by protozoa during the first 2 to 8 h of the grazing experiment. Andersen et al. (1) also found that DOP was most prevalent at the beginning of the batch culture growth cycle, and this depended on the prey type in that more DOP was released when *P. imperforata* was feeding on bacteria (70%) than when it was feeding on algae (15 to 25%). However, DOP never exceeded 15 to 20% of TDP at the end of any flagellate growth cycle. They concluded that DOP release can occur, but it is exceedingly variable and unpredictable.

Figure 3b shows that, overall, the four flagellate species regenerated between 46 and 69% of bacterium-bound nitrogen, which is higher than in previously published studies (13 to 41%) for heterotrophic flagellates feeding on bacteria (2, 20) but is within the range obtained when phytoplankton was used as prey (7 to 70% [19–21]). *P. imperforata* and *B. designis* had lower %RE values for nitrogen (Fig. 3b) than for phosphorus (Fig. 3a), while *S. diplocostata* and *J. libera* had higher values for nitrogen (Fig. 3b) than for phosphorus (Fig. 3a). There was no significant interspecies difference between the %RE values for nitrogen; all values were comparable (Fig. 3b). *P. imperforata* and *B. designis* therefore appeared to be more conservative with respect to the regeneration of nitrogen than they had been with phosphorus.

*P. imperforata* is known to have the ability to conserve nitrogen (20) independent of prey species used (19). Andersson et al. (2) thought that *P. imperforata* could either accumulate nitrogen (relative to phosphorus) or that other nitrogenous compounds were being excreted which were not being monitored. However, there is general agreement that nitrogen is excreted mainly as ammonium (20), but other nitrogenous compounds may also be excreted, though in much smaller amounts. Urea can be excreted at the onset of stationary phase, making up around 15% of the total nitrogen, with urea as a secondary nitrogenous excretion product (20). Bidigare (4) found that released amino acids contributed 10 to 15% of excreted nitrogen. However, Andersson et al. (2) found that only 0.02% was excreted as amino acids, and Goldman et al. (20) concluded that amino acid composition must be tiny, after they managed to equate total nitrogen with ammonium, urea, and particulate nitrogen.

It is possible that the flagellate species used in our experiments were adjusting their internal C/N/P ratios, because no matter whether the bacterium was phosphorus rich or nitrogen rich, no significant increase in percent regenerated phosphorus or nitrogen occurred compared with that obtained with balanced bacteria (Fig. 3). Published data on the internal C/N/P

TABLE 4. Particulate carbon, nitrogen, and phosphorus composition and corresponding elemental ratios for four flagellates

Flagellate species	Biovolume ( $\mu\text{m}^3$ )	Particulate concn (pg cell <sup>-1</sup> ) $\pm$ SEM			Elemental ratio			fg of C $\mu\text{m}^{-3}$
		C	N	P	C/N/P	C/N	C/P	
<i>P. imperforata</i>	212	32.49 $\pm$ 5.44	7.42 $\pm$ 0.78	1.17 $\pm$ 0.14	27:6:1	4.5	27	149
<i>B. designis</i>	54	12.60 $\pm$ 1.87	3.10 $\pm$ 0.43	2.22 $\pm$ 0.09	6:1.5:1	4.1	6	233
<i>S. diplocostata</i>	35	5.42 $\pm$ 1.02	1.10 $\pm$ 0.13	0.12 $\pm$ 0.02	45:9:1	4.9	45	156
<i>J. libera</i>	75	14.15 $\pm$ 3.14	2.58 $\pm$ 0.71	0.20 $\pm$ 0.02	71:13:1	5.5	71	189

ratios of flagellates are limited to those for *P. imperforata*. Goldman et al. (21) found that particulate C/N/P ratios changed from 60:10:1 to 233:43:1 during batch culture grazing experiments. However, particulate C/N/P ratios tend to approach an optimal ratio of 75:12:1 (C/N, 6.2) in the stationary phase of growth regardless of the prey nutrient status (9). This value is lower than the Redfield ratio of 106:16:1 (35).

Table 4 shows the particulate carbon, nitrogen, and phosphorus contents of the four flagellates and corresponding elemental ratios. Carbon conversion values ranged from 149 to 233 fg of C  $\mu\text{m}^{-3}$ , which are not too dissimilar from those calculated by other workers: 180 and 300 (13) and 220 (6) fg of C  $\mu\text{m}^{-3}$ . However, our values show that no one conversion factor is suitable for every flagellate species, in agreement with Fenchel (13).

Calculated C/N ratios were quite conserved, ranging from 4 to 5.5 (Table 4), being close to the lower end of other published values, 5.4 to 7.0 (5, 9, 21). The similarity in C/N ratios exhibited by the four flagellate species may have influenced the comparability of their %RE with respect to nitrogen (Fig. 3b). Results pertaining to the differences observed in the regeneration of phosphorus (Fig. 3a) also appear to be somewhat related to the particulate phosphorus content of the flagellate. *J. libera* and *S. diplocostata* released significantly less phosphorus than *P. imperforata* and *B. designis*, and this may have been influenced by the two former species having higher C/P ratios (71 and 45, respectively) than the two latter species (27 and 6, respectively) (Table 4). However, it should be noted that the observed differences in C/P ratios may be spurious due to the method used for determining particulate phosphorus concentrations, which was not as accurate as the elemental analysis performed to determine particulate carbon and nitrogen concentrations.

This study has highlighted the possible occurrence of interspecies variation with respect to nutrient regeneration capability. Although the atomic constituents of the culture media in which the bacteria were grown were much higher than those known to occur in natural environments, it was necessary in a study of this kind. Measurable levels of bacterium-bound phosphorus and nitrogen were required so that any evidence of interspecies differences between flagellates could itself be assessed at a satisfactory level. Knowing that interspecies differences can exist, it is now necessary to determine whether they occur in natural environments where the C, N, and P contents of the bacterial prey are far different from those used in these laboratory experiments. This would help to elucidate whether heterotrophic nanoflagellates are a diverse biotic group in nature or whether environmental conditions force them to conform to a monofunctional heterotrophic nanoflagellate community.

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