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Nutrient Flux between bacteria, bacterivorous nanoplanktonic protists and algae [1]

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Abstract

The fate of nitrogen and phosphorus supplied as viable bacterial cells in seawater stripped of inorganic and organic nutrients was followed in the presence of bacterivorous nanoprotists (a ciliate, *Cyclidium* spp., and an aplastidic chrysomonad flagellate) with and without the addition of a nanoplanktonic diatom, *Cyclotella* spp. Controls consisted of flasks containing algae and bacteria, and monocultures of algae and bacteria separately.

In all experiments, the presence of the nanoprotists increased algal growth over the controls indicating that the algae could effectively utilise P and N derived from nanoplankton grazing on intact bacterial cells. Some stimulation of algal growth also occurred in the presence of bacteria only. In the absence of algae, the grazing activities of nanoplankton increased the quantities of NH_3 and PO_4 which accumulated in the medium. No difference in the growth rates of flagellates or ciliates was observed in the presence or absence of the algae. These experiments support the idea that nanoprotist grazing on bacterial cells provides a flux of nutrients which enhances the growth of microalgae.

Key words : Nanoplanktonic Protists, Nutrient Cycling, Bacterivory.

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Résumé

Flux de nutriments entre les bactéries, les protistes nanoplanctoniques bactérivores et les algues

Le sort de l'azote et du phosphore fournis sous forme de cellules bactériennes viables dans l'eau de mer privée de nutriments minéraux et organiques a été suivi en présence de nanoprotistes bactérivores (un cilié, *Cyclidium* spp., et un flagellé chrysomonade aplastide) avec et sans addition d'une diatomée nanoplanctonique, *Cyclotella* spp. Les contrôles consistaient en flacons contenant des algues et des bactéries et des monocultures séparées d'algues et de bactéries.

Dans toutes les expériences, la présence des nanoprotistes augmentait la croissance algale vis-à-vis des contrôles, indiquant que les algues pouvaient effectivement utiliser P et N provenant du broutage de nanoplancton sur les cellules bactériennes intactes. Une certaine stimulation de la croissance algale se produisait également en présence des seules bactéries. En l'absence d'algues, le broutage du nanoplancton augmentait les quantités d'ammonium et de phosphate s'accumulant dans le milieu. Aucune différence dans le taux de croissance des flagellés ou des ciliés n'était observé en fonction de la présence ou de l'absence des algues. Ces expériences appuient l'idée que le broutage des cellules bactériennes par les nanoprotistes produit un flux de nutriments qui améliore la croissance des microalgues.

Introduction

Bacteria in aquatic environments have been implicated both as nutrient sinks because of their high affinity for and rapid uptake of organic carbon, nitrogen (N) and phosphorus (P) compounds (Johannes, 1965) and as mediators of nutrient release from detrital material. Moreover, if large numbers of bacteria are constantly cropped by microbial grazers (Williams, 1984), then a significant portion of the nutrients incorporated in the bacterial cells may be recycled (Fenchel, 1984; Sieburth, 1984; Sherr and Sherr, 1984). In recent years, much attention has been focused on the transfer of carbon and other major elements from primary producers through the "microbial loop" to higher trophic levels (Pomeroy, 1974; Sieburth and Davis, 1982; Taylor, 1982; Azam *et al.* 1983; Sherr and Sherr, 1984) and various proposals have been put forward to explain the functioning of such an apparently diversionary circuit of material flux in marine and freshwater ecosystems (Goldman, 1984a, b; Newell and Linley, 1984; Güde, 1985; Rheinheimer, 1984).

It has been a common observation that the numbers of bacteria found in the upper waters of the open ocean do not fluctuate greatly (ca.10^5-10^6 cells ml^{-1}) in time or geographic location, despite indications of active growth (Azam and Hodson, 1977; Williams, 1984; but see also Stevenson, 1978; Hobbie, 1979). These findings are consistent with the idea that increases in bacterial populations are offset by the active grazing of bacterivorous organisms. These grazers are presumed to be mastigophoran and ciliophoran protists (Fenchel, 1982) and probably also include myxotrophic algae (Porter *et al.*, 1985; Estep *et al.*, 1986). Recent evidence indicates that these protists

release nutrients as a result of their feeding and excretory activities (Taylor, 1982; Sherr *et al.*, 1983; Goldman *et al.*, 1985; Güde, 1985; Taylor *et al.*, 1985; Andersson *et al.*, 1985; Andersen *et al.*, 1986). It is still unclear, however, to what extent inorganic nitrogen or phosphorus are released by nanoplanktonic grazers and whether the larger phytoplanktonic primary producers can effectively exploit nutrients which derive from this source. Moreover, although it is a reasonable supposition that nanoprotist grazing can contribute to the pool of dissolved organic matter, information is lacking about the amounts and kinds of compounds which may be released.

In this paper we present data from experiments designed to examine some implications of grazing upon bacteria by nanoplanktonic protists. In a simple batch system, we measured the release of phosphorus, P (as soluble reactive P, SRP) and nitrogen, N (as NH_4 or NO_3 + NO_2) originally present in the form of intact bacterial cells. This release of nutrients resulted from the grazing activities of either a nanoplanktonic ciliate (*Cyclidium* spp.) or an aplastidic chrysomonad flagellate. Furthermore, we examined the ability of a nanoplanktonic diatom (*Cyclotella* spp.) to exploit these nutrients when in the presence of both bacteria and a nanoprotist grazer. *Cyclotella* was chosen as the test alga because preliminary work showed that this organism was itself not grazed by either of the experimental nanoplanktonic protists. The experiments were performed in seawater stripped of nutrients, so that the bacteria in the system provided the only significant source of N and P and the growth of the algae was limited by the availability of these nutrients. In some experiments we also looked for the appearance of dissolved ATP (dATP). Recent evidence indicates that there is a large pool of dATP in surface waters of the open ocean. The source of this material is unknown at present. Phagotrophic protists are known to exude a wide variety of organic compounds (Taylor *et al.*, 1985; Andersson *et al.*, 1985). We hypothesized that dATP may be released from nanoprotists as a result of excretion or exocytosis.

Recently Caron *et al.* (1985), Güde (1985), Andersson *et al.* (1985), Andersen *et al.* (1986), Pengerud *et al.* (1987) and Caron and Goldman (1987) have described experiments in continuous culture systems which have also elucidated details of nutrient recycling by nanoprotists feeding on bacteria or picoplanktonic algae.

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Experimental Methods

Low nutrient seawater (LNSW) was obtained from just below the sea surface outside of Kaneohe Bay, Hawaii. Before use, the seawater was extracted with activated charcoal and then passed through pre-combusted GF/F filters to reduce organic content. Initial concentrations of SRP, (NO_3 + NO_2), and NH_4 were 0.8 to 1.8 μM, 0.3 μM, and 1.0 to 1.5 μM respectively. When used as an experimental medium, the LNSW was supplemented with 200-300 μM SiO_3, trace metals and vitamins as per IMR medium (Eppley *et al.*, 1967).

1. *Bacteria*. As viewed by light microscopy, a morphologically diverse flora of small (<1 μm) bacteria was grown by inoculating 10 ml of 0.6 μm filtered seawater into 2 liters autoclaved LNSW to which Difco Casamino acids were added (0.1%, final concentration, w/v). These bacterial samples were washed by centrifugation and resuspension in 2 liters of LNSW four times and finally resuspended in autoclaved LNSW to give a concentrated bacterial source of ca. 5×10^9 cells ml^{-1}.

2. *Algae*. *Cyclotella* spp. was obtained from a culture maintained by J. Hirata and grown up on F/2 medium (Guillard and Ryther, 1962). Before use, the algae were washed 4 times with autoclaved LNSW and concentrated to about 6×10^7 cells ml^{-1}.

3. *Nanoplanktonic Protists*. Cultures of a small (7×14 μm) ciliate, *Cyclidium* spp., and an aplastidic chrysomonad, (about 4 μm in diameter, both organisms isolated from the North Central Pacific Ocean by G.T.T.) were maintained on LNSW with twice weekly supplements from the bacterial source.

Epifluorescent microscopy (Zeiss) was used for counting acridine orange stained bacteria and nanoprotozoans collected on irgalan-black treated 0.2 μm Nuclepore filters (Hobbie *et al.*, 1977). Between 10 to 20 microscope fields were counted for each sample. Determinations of SRP, NH_4, and $NO_3 + NO_2$ were made by standard Autoanalyser techniques (Strickland and Parsons, 1968). Concentrations of dissolved and particulate ATP (dATP and pATP) were measured using the methods of Karl and Craven (1980). Growth of algae in the experimental flasks was followed by *in vivo* chlorophyll *a* fluorescence (Perkin Elmer LS-5 spectrofluorometer excitation 485 nm, emission 685 nm) which was calibrated to cell numbers by direct microscope counts.

For each experiment, five flasks containing LNSW (100 ml for the preliminary series or 800 ml in later runs) were prepared as follows : a) algal inoculum only, A; b) bacterial inoculum only, B; c) algae and bacteria, AB; d) bacteria and ciliates or flagellates, BC or BF; e) algae, bacteria and ciliates or flagellates, ABC or ABF. Initial cell concentrations were approximately : bacteria 5×10^6 cells ml^{-1}, *Cyclotella* 4 to 6×10^4 cells ml^{-1}, *Cyclidium* 10^3 cells ml^{-1}, chrysomonad 3×10^3 cells ml^{-1}. (The numbers of bacterial cells added with the inocula of nanoprotists were 5-10% of the bacterial source addition).

The flasks were incubated in moderate continuous light (160 μEin m^{-2} s^{-1}) at 22 °C for 4 to 8 days. Supplements from the concentrated bacterial source to give 10^7 ml^{-1} cells initially and 5×10^6 cells ml^{-1} daily were added to flasks AB, ABC or ABF, and BC or BF. A similar volume of 0.2 μm filtrate from the bacterial source was also added to flasks A and B. Each day the flasks were monitored for changes of *in vivo* chlorophyll *a* fluorescence and 5 ml portions were removed for direct epifluorescent cell counting of nanoprotozoans and bacteria. In some experiments, samples were taken for determinations of SRP, NH_4, ($NO_3 + NO_2$), and particulate and dissolved ATP as indicated. Calculations of cell excretion rates were made as in Gast and Horstman (1983).

In order to estimate the potential amounts of nitrogen and phosphorus added in the form of bacteria, we measured the concentration of carbon and nitrogen in the bacterial source. Washed and concentrated bacteria were counted microscopically and 10 ml portions collected on precombusted Whatman GF/F filters were analysed using a

Hewlett Packard CHN Analyser. The bacteria contained 2.23×10^{-13} g C cell^{-1} and 4.9×10^{-14} g N cell^{-1}, with a C:N atomic ratio of 5.28. Particulate phosphorus was not measured; however if the atomic ration of N:P in these bacteria was 15:1 then the intracellular P content would have been 7.6×10^{-15} g P cell^{-1}.

Results and Discussion

The time course of growth for algae and nanoplanktonic grazers in two representative experiments is shown in Figure 1. No significant increase in cell numbers was observed in control flasks to which only algae or bacteria were added (A, B) although in the latter case some changes in cell morphology occurred. Therefore, we believe that the initial nutrient conditions in the flasks were truly limiting and that the only source for additional N and P was from the supplements of bacterial cells which were added to the experimental flasks.

In all experiments, the presence of either the ciliate or the flagellate (ABC or ABF) enhanced the growth rate and yield of *Cyclotella* in comparison to flasks without grazers. However, relative to control flasks with no supplements (A), growth rate of the algae was also stimulated in the presence of bacteria only (AB). No growth of algae resulted from additions of filtrate from the bacterial source indicating that no significant amounts of dissolved nutrients were added and that intact bacterial cells were required for a positive response. The activity of the phagotrophic protists facilitated the fastest growth and the highest yield of the phototrophic algae presumably by effectively increasing nutrient recycling from the bacterial cell material. The growth response was usually only perceptible after a lag period of 2 to 4 days, possibly because of the relatively small inoculum of nanoprotist grazers. The growth patterns of the nanociliates and nanoflagellates did not appear to be affected by the presence of the algae (Fig. 1). Maximum growth rates were 1.5 and 5.0 doublings day^{-1} for *Cyclidium* and the chrysomonad respectively.

In some experiments we monitored the ambient levels of SRP, (NO_3 + NO_2) and NH_4 in the flasks; results from a nanoflagellate and a *Cyclidium* run are given in Tables 1 and 2 respectively. After the first day, in the presence of algae, the concentrations of SRP and NH_4 decreased; there was then little subsequent increase in the levels of these nutrients in flasks A, ABC or ABF. In contrast large increases in SRP and NH_4 were observed in flasks with nanoprotozoans and bacteria (BC, BF). A significant rise in NH_4 and SRP concentrations was also found in the flasks containing stationary phase bacteria only (B). The release of these nutrients from stationary phase bacterial cultures could have been due to the endogenous utilisation of protein and other storage compounds (Caron and Goldman, 1987) or to cell autolysis. Levels of NO_3 + NO_2 (not shown) remained low in all flasks throughout the experimental period.

In experiments where the concentrations of released NH_4 and SRP were measured in BF or BC flasks we could estimate the efficiency of nutrient release by the nanoprotists relative to the input of N and P in the form of intact bacterial cells. The results (Table 3) indicate that both protists were capable of releasing a relatively high percentage of

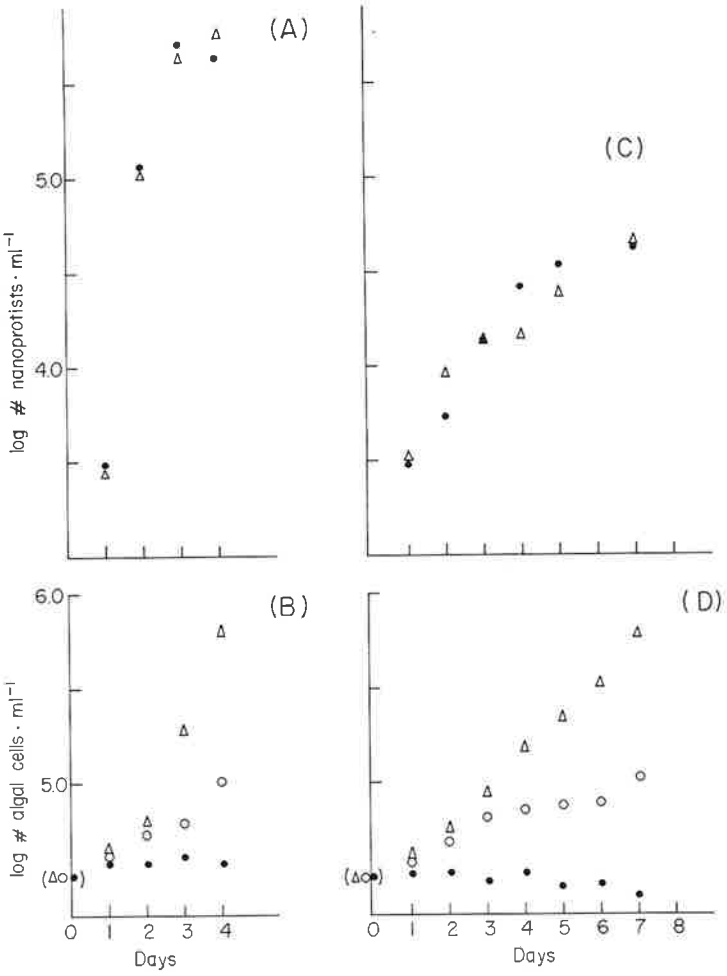


Fig. 1. *Growth curves of nanoprotists and* Cyclotella.
Nanoflagellate experiment :
A) Time course of nanoflagellate growth : Flask BF (•), Flask ABF (△).
B) Time course of Cyclotella *growth : Flask A (•), Flask AB (○), Flask ABF (△).*
Nanociliate experiment :
C) Time course of Cyclidium *growth : Flask BC (•), Flask ABC (△).*
D) Time course of Cyclotella growth : Flask A (•), Flask AB (○), Flask ABC (△).
For clarity, error bars are not shown. Coefficients of variation for bacteria and nanoprotist counts were generally 15-25% and 15-35% respectively. Algal cell numbers were determined by calibration with in vivo *chlorophyll* a *fluorescence.*

TABLE 1. — *Ambient concentrations (μM) of ammonium (NH_4) and soluble reactive phosphorus (SRP) in a grazing experiment with nanoflagellates.*

Day	Sample *	SRP	NH_4
1	LNSW +	0.81	1.0
2	A	0.56	0.26
	B	N.A.	2.73
	AB	0.18	0.16
	ABF	0.14	0.16
	BF	0.78	2.73
3	AB	0.23	0.51
	ABF	0.21	0.71
	BF	0.37	10.41
4	A	0.16	0.45
	B	1.05	14.65
	AB	0.26	0.21
	ABF	0.45	0.37
	BF	1.42	38.59

* Algae only; A; Bacteria B; Bacteria plus algae AB; Bacteria plus flagellates, BF; Bacteria plus flagellates plus algae, ABF. + Low nutrient sea water used as medium (see text). N.A. not available.

TABLE 2. — *Ambient concentrations (μM) of ammonium (NH_4) and soluble reactive phosphorus (SRP) in a grazing experiment with nanociliates.*

Day	Sample *	SRP	NH_4
2	A	0.22	1.14
	B	1.04	15.6
4	AB	0.22	0.60
	ABC	0.31	0.35
	BC	3.82	38.36
5	AB	0.22	0.30
	ABC	0.49	0.37
	BC	N.A.	58.59
6	A	0.14	0.27
	B	1.83	10.62
	AB	0.55	0.30
	ABC	0.86	0.40
	BC	8.32	81.28
8	AB	0.23	0.30
	ABC	0.60	0.50
	BC	10.77	110.52

* Sample codes as in Table 1 except that ciliates (C) were used instead of flagellates (F). N.A. not available.

TABLE 3. — *Percentages of N (as NH_4) and P (as SRP) recycled by nanociliates and nanoflagellates grazing on bacterial cells* *.

Expt.	Day	% N	% P
1. Ciliates	4	87	97
2. Ciliates	4	44	62
Ciliates	5	56	—
Ciliates	6	66	97
Ciliates	8	70	97
3. Ciliates	8	70	97
1. Flagellates	4	44	23
2. Flagellates	4	56	67
Flagellates	6	82	79

* Based on concentrations of NH_4 and SRP measured in BC and BF flasks, and on the amounts of nutrients contained in the bacterial cells added (see text).

nutrients from the bacterial cells into the medium. The recycling efficiencies which we calculated are somewhat higher than those noted by Goldman *et al.* (1985) but, at present, our data are clearly insufficient to make any definitive statement. In natural systems, the protozoans will obviously tie up some portion of the available nutrients in their own biomass and this will limit the efficiency of the recycling process. Thus some of the high apparent recycling efficiencies calculated in Table 3 are probably overestimated. Also, as has been recently shown by Turley *et al.* (1986) different nanociliates (and presumably, nanoflagellates) may have different growth and survival strategies and may therefore function differently in recycling nutrients. More work is necessary to determine whether there are indeed any systematic differences in nutrient recycling efficiency between nanoflagellates and nanociliates.

In order to check if measurable quantities of dATP could be generated as a result of nanoprotist grazing on bacteria, the levels and turnover times of this compound were determined in selected experiments. Particulate ATP, which was also measured as an indicator of biomass, generally correlated well with observed changes in algal and nanoprotozoan cell numbers. Intercellular ATP concentrations calculated from determinations of ATP and cell numbers for *Cyclotella, Cyclidium* and the chrysomonad are given in Table 4. The nanoplanktonic alga and the chrysomonad averaged similar cellular ATP concentrations, the nanociliate cells which had about a 10 fold larger volume contained approximately 10 times as much ATP.

In Table 5 we show the amounts of dATP measured in the flasks during these experiments and also the ratios of dATP:pATP. Relatively greater concentrations of dATP and higher dATP:pATP ratios were only found occasionally in samples which contained nanoprotist grazers (BC, ABC, BF, ABF). Flasks with bacteria or algae separately or together (B, A, AB) had persistently low levels of dATP and low dATP:pATP ratios. Samples in which algae were growing rapidly in a fully supplemented medium (A^+) also had low dATP concentrations. From the limited time courses detailed in Table 5, it appears that there may be a particular phase during the

TABLE 4. — *Intracellular concentrations of ATP (ng ATP cell^{-1}) in* Cyclotella *spp. a chrysomonad manoflagellate and a nanociliate* (Cyclidium *spp.*).

	Range	*x (+ S.D.)*	*n*
Cyclotella spp. [1]	$4.5\text{-}14.9 \times 10^{-5}$	$8.2\ (+3.6) \times 10^{-5}$	8
Cyclotella spp. [2]	$2.3\text{-}19.0 \times 10^{-5}$	$10.3\ (+5.3) \times 10^{-5}$	12
Nanoflagellate [3]	$1.7\text{-}14.0 \times 10^{-5}$	$7.3\ (+4.9) \times 10^{-5}$	9
Nanociliate [3]	$6.8\text{-}20.6 \times 10^{-4}$	$12.3\ (+5.4) \times 10^{-4}$	6

[1] *Cyclotella* cells under nutrient limitation.
[2] *Cyclotella* cells growing exponentially in complete medium.
[3] These concentrations were calculated from measurements of pATP and cell counts in BF and BC flasks. The pATP contributed by the bacteria in these flasks was taken to be not greater than 20% of the total pATP determination because these observations were made on day 2 of experiments when bacterial numbers were relatively low (ca. 3×10^5 cells ml^{-1}) as a result of grazing. (Two measurements of bacterial ATP gave 6×10^{-7} and 10^{-7} ng ATP cell^{-1} in a stationary culture after 2 and 6 days incubation, respectively).

TABLE 5. — *Nanoprotist grazing experiments : d'ATP concentrations (pg l^{-1}) and ratios of dATP:pATP (in parenthesis as %).*

Exp.	Flask	Day 2	3	4	5	6	7	8
9	A	0.04 (2)	—	0.05 (3)				
	B	0.12 (3)	—	0.66 (5)				
	AB	0.04 (1)	0.12 (1)	4.7 (28)				
	ABF	0.07 (1)	0.38 (4)	4.7 (28)				
	BF	0.23 (8)	0.92 (12)	14.0 (77)				
	A^+	0.19 (3)	0.48 (4)	0.46 (3)				
10	A	0.06 (1)	—	0.12 (5)	—	0.1 (6)		
	B	0.14 (3)	—	0.22 (13)	—	N.D.		
	AB	0.18 (2)	—	0.08 (1)	0.08 (1)	0.08 (1)	—	0.09 (1)
	BC	2.00 (33)	0.67 (8)	3.26 (18)	1.21 (7)	6.98 (25)	—	5.97 (10)
	ABC	5.47 (68)	4.1 (35)	30.48 (128)	27.04 (122)	67.3 (223)	—	33.28 (57)
	A^+	0.07 (2)		0.16 (1)	—	0.02 (1)		
11	BF		1.19 (5)	0.76 (3)	5.26 (14)	6.10 (12)	12.76 (28)	33.47 (58)

Values are average of duplicate measurements, N.D. not detectable.
A^+ = Algae in fully supplemented medium.

development of mixed bacterial-nanoprotist-algal systems when relatively large amounts of dATP and perhaps other intracellular organic compounds are released and accumulate in the medium (Parslow *et al.*, 1986). Several determinations of the turnover times for dATP in these experiments gave results ranging from 1.4 to 2.8 hours without any systematic pattern related to the biological components in the flasks. Our results, therefore, give some support to the idea that the persistent concentrations of dATP

observed in oceanic surface waters (Karl, 1986) might result to some degree from the grazing activity of nanoprotists. Future research will focus on this hypothesis.

Our results confirm that nutrients supplied in the form of bacterial biomass can be effectively transferred to planktonic microalgae, thus enhancing both the growth rates and yields of the phototrophs. Previous research has shown that the activity of nanoplanktonic grazers was responsible for extensive N recycling in ecosystems as diverse as Pacific coastal waters (Landry *et al.*, 1984), Western Baltic (Rheinheimer, 1984), Western Mediterranean (Van Wambeke and Bianchi, 1985), Antarctic waters (Koike *et al.*, 1986), Celtic Sea (Joint and Williams, 1985), English Channel (Newell and Linley, 1984), and freshwaters (Sherr *et al.*, 1982; Güde, 1986). We determined rates of NH_4 release (Table 6) ranging from 5.1 to 38.5 μg N mg dry wt^{-1} h^{-1} for the nanoflagellates and 2.4 to 7.4 μg N mg dry wt^{-1} h^{-1} for the nanociliates, assuming a dry weight of 0.2 pg μm^{-3} (Sherr *et al.*, 1983) and volumes of 40 μm^3 and 400 μm^3 for the chrysomonad and *Cyclidium* respectively. These rates may be compared to the range of NH_4 excretion rates (3-28 μg N mg dry wt^{-1} h^{-1}) given for various microprotozoans by Goldman *et al.* (1985). We did not measure the concentrations of dissolved organic nitrogen (urea, amino acids etc.). However, Goldman *et al.* (1985) found urea to comprise about 15% of total N excretion by a microflagellate *Paraphysomonas imperforata* feeding either on bacteria or diatoms. Andersson *et al.* (1985) measured that 7.5% of ingested bacterial N was released as amino acids by flagellate grazing and Antia *et al.* (1980) have reported that marine ciliates excrete N compounds such as hypoxanthine and guanine. If significant amounts of dissolved organic nitrogen compounds are indeed released as a result of nanoprotist grazing, this implies that present estimates of nitrogen recycling efficiencies are underestimated.

Measured rates of P release were 0.4-5.0 and 0.5-15.1 μg P mg dry wt^{-1} h^{-1} for the nanoflagellates and the nanociliates, respectively. Andersen *et al.* (1986) reported maximum phosphorus release rates ranging from 2.2 to 3.8 μg P dry mg wt^{-1} h^{-1} when *Paraphysomonas* was feeding on diatoms or on bacteria, respectively.

Less information is available concerning the role of nanoprotists in remineralization of P than N from bacterial biomass. Johannes (1965) and later Barsdate *et al.* (1974)

TABLE 6. — *Ammonium and soluble reactive phosphorus excretion rates, E (μg mg dry wt^{-1} h^{-1}), and Body Equivalent Excretion Times, BEET (day) for nanociliates and nanoflagellates.*

	NH_4		SRP	
	E	BEET	E	BEET
Cyclidium [1]	48-178	0.9-2.8	13-363	0.1-2.6
Nanoflagellate [1]	123-924	0.1-1.3	8-120	0.3-3.5
Paraphysomonas [2]	72-240	—	53-91*	0.6-2.0
Monas sp. [3]	240-408	—	—	—
Macrozooplankton [2]	48(+36)	—	12.0	—

1. This study.
2. Goldman *et al.*, 1985, Andersen *et al.*, 1986. (Note that the E_{SRP} here are the maximum reported.)
3. Sherr *et al.*, 1983.

suggested that, because bacteria appeared to act as a "sink" for P, protozoan and metazoan grazers of bacteria would be indispensable as agents of recycling. In contrast to some, but not all other studies (e.g. Güde, 1985), we observed an outgrowth of algae in the presence of bacteria only, which suggests that bacteria may not always be a very tight "sink" for P (and N) and that cell "leakiness" might depend on nutrient supply, physiological state and growth phase. Thus Caron and Goldman (1987) have shown that bacteria grown in a rich amino acid medium released large amounts of NH_4 when in stationary phase. Nevertheless, even if in certain situations bacteria may "leak" nutrients, the enhancement of remineralization caused by nanoprotist grazing on intact bacterial cells is probably a major factor in stimulating the growth of algae in many aquatic ecosystems.

Bacteria are generally assumed to have greater affinity and faster uptake rates for P and N than algae (Currie and Kalff, 1984; but see also Berman, 1985), and thus might be expected to outcompete and perhaps limit algal growth in low nutrient conditions. In our experiments, this did not occur. The bacterial populations in flasks with grazers and algae showed no evident increases in cell numbers with time. In fact, despite daily additions, the numbers of bacteria in flasks ABF, ABC, always decreased after 24 hours to relatively low levels (ca. 2-5 $\times$ 10^5 cells ml^{-1}) presumably because of active grazing by the nanoplanktonic protists.

Despite the recent spate of research interest in the nanoprotozoan grazers, their functions and impact in oceanic and freshwater systems are still far from clear. Of course, we realize that the conditions and concentrations of organisms in our experimental flasks were very different from those prevailing in the sea. Moreover, in our system we only used single species of nanoprotists and algae. Nevertheless, here we have provided experimental verification that, in low nutrient environments, nanoprotists can enhance nutrient flux to microalgae by grazing on intact bacterial cells. The next step is to quantify this phenomenon under more ecologically realistic conditions.

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