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Nutrient cycling in a microflagellate food chain: IV. Phytoplankton-microflagellate interactions*

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ABSTRACT: The phagotrophic microflagellate *Paraphysomonas imperforata* was capable of grazing 2 marine phytoplankton species, the diatom *Phaeodactylum tricornutum* and the chlorophyte *Dunaliella tertiolecta*. The phytoplankton species, which are grossly different in size, shape and morphology, were first grown under different degrees of nitrogen and phosphorus limitation. Patterns of nutrient regeneration appeared to be a function of the physiological state of the prey: lags in NH_4^+ regeneration until the end of exponential growth occurred when the prey were N-limited and phosphorus regeneration was negligible during the entire growth cycle of the microflagellate when the prey were P-limited. There was, however, evidence for dark uptake of NH_4^+ by N-limited prey that were ungrazed, which could have biased the observed lags in nutrient regeneration. Regeneration efficiencies reached as high as 70 % for nitrogen and up to 50 % for phosphorus only after prolonged periods in the stationary phase. Because protozoa are able to convert prey nutrients to their own biomass with great efficiency during exponential growth, particularly when the prey are nutrient-limited, the size and complexity of the microbial food web may be related to the nutritional state of the phytoplankton.

INTRODUCTION

Recently, we delineated several important processes that control the rates and efficiencies of nitrogen and phosphorus regeneration by the phagotrophic microflagellate *Paraphysomonas imperforata*, which is able to graze either bacteria or phytoplankton (Goldman et al. 1985, Andersen et al. 1986). Most important, we found that regeneration efficiencies during exponential growth were low (< 25 %) and appeared to be strongly dependent on the prey physiological state. For example, if the prey was nitrogen-limited after extended batch growth, there was a lag in regeneration of NH_4^+ by the microflagellate until late in the exponential phase of growth (Goldman et al. 1985). Phosphorus limitation led to virtually complete conservation of ingested prey phosphorus well into the stationary phase (Andersen et al. 1986).

We thus hypothesized that an imbalance in the N:P ratio of the food supply, coupled with fairly rigid stoichiometric requirements for nutrients by the protozoan during unrestricted growth, resulted in conservation of the nutrient in shortest supply. We were, however, unable to control the chemical composition, and hence food quality of the prey, as precisely as we desired because both the prey and predator were grown in the batch mode. Additionally, we assumed that there was no uptake of nutrients by the phytoplankton prey once the cultures were darkened and the grazer was introduced. The accumulation of NH_4^+ and PO_4^{-3} during the course of grazing was thus considered to be due only to excretion by the microflagellate. In the current study we examine in closer detail these 2 factors that could affect the net regeneration of nutrients during microflagellate grazing. In a companion study (Caron et al. unpubl.) we focus on the coupling between respiration and nutrient excretion under these experimental conditions.

METHODS

Phytoplankton species and growth. Two marine phytoplankton species, *Phaeodactylum tricornutum* (clone TFX-1) and *Dunaliella tertiolecta* (clone Dun), both originally from the culture collection of R.R.L. Guillard, were grown to steady state in 1 l continuous cultures at 3 nominal dilution rates D: 0.2, 0.5, and 1.2 d^{-1}. All protocols for culture operation and synthe-

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tic seawater preparation were as described previously (Goldman & McCarthy 1978, Goldman & Peavey 1979). The cultures were grown under continuous lighting (636 $\mu E\ m^{-2}\ s^{-1}$) and the temperature was 20°C. Since the maximum specific growth rate $\hat{\mu}$ of both species at 20°C is about 1.5 d^{-1} (Goldman & Glibert 1982), the dilution rates employed represent relative growth rates $D:\hat{\mu}$ of about 0.15, 0.35, and 0.80 (see Table 1). Inorganic nitrogen and phosphorus were added to the medium in nominal ratios of 200 $\mu g\text{-at}\ l^{-1}$ NH_4^+ to 20 $\mu g\text{-at}\ l^{-1}$ PO_4^{-3} (N:P = 10:1) to insure nitrogen limitation or 240 $\mu g\text{-at}\ l^{-1}$ NH_4^+ to 4 $\mu g\text{-at}\ l^{-1}$ PO_4^{-3} (N:P = 60:1) to insure phosphorus limitation.

All combinations of species, dilution rate, and type of nutrient limitation were tested. The bulk of the experiments were performed under non-axenic conditions, except for a supplementary experiment in which comparisons of steady-state growth of N-limited *Phaeodactylum tricornutum* at 0.2 d^{-1} were made under axenic and non-axenic conditions. Additionally, dark NH_4^+ uptake by both species was measured. Steady-state, non-axenic cultures were first established at 0.2 d^{-1} under N limitation and then placed in the dark for 24 h under batch conditions. After this dark pre-conditioning period the cultures were pulsed with 65 $\mu g\text{-at}\ l^{-1}$ of NH_4^+ and time-course measurements of NH_4^+ disappearance were made over 24 h.

Microflagellate species and growth. The phagotrophic microflagellate *Paraphysomonas imperforata* (Lucas) (7 to 12 µm in diameter), which we isolated earlier from a local tidal channel and made bacteria-free (Goldman et al. 1985), was used in all batch grazing experiments. Grazing experiments were initiated by transferring the phytoplankton cultures to 1 l glass containers once steady state was attained, adding a small inoculum of *P. imperforata* to attain a starting microflagellate concentration of about 10^3 cells ml^{-1}, and then placing the containers in a water bath in the dark. The cultures were mixed continuously at a slow speed by circulating water from the bath through water-driven magnetic stirrers. Time-series measurements of a variety of biological and chemical parameters were made over a 5.5 d period. Samples were taken at approximate 8 h intervals over the first 2 d and less frequently over the remaining period. Enough sample was taken to perform all the analyses in this study and in the companion study of Caron et al. (unpubl.).

Particulate carbon and particulate nitrogen were measured with a Perkin Elmer 240 Elemental Analyzer on 25 ml samples retained on pre-combusted Whatman GF/F glass-fiber filters. Filtration pressure differentials were 25 mm Hg. Measurements of NH_4^+ (McCarthy & Kamykowski 1972) were made on the filtrate. We did not measure NO_3^- or urea because in earlier experiments (Goldman et al. 1985) NO_3^- concentrations were always below detection limits (0.03 $\mu g\text{-at}\ l^{-1}$) and urea excretion did not occur until after exponential growth of the microflagellate and then never exceeded about 15 % of total nitrogen excretion. Particulate phosphorus also was measured on 25 ml samples retained on pre-combusted and acid-washed Whatman GF/F filters. Both PO_4^{-3} and total dissolved phosphorus were measured on the filtrates. Dissolved organic phosphorus was calculated as the difference between these measurements. Both particulate and total dissolved phosphorus samples were first dry-combusted (Solorzano & Sharp 1980) and then analyzed for PO_4^{-3} (Murphy & Riley 1962). All microbial cells were counted by epifluorescence microscopy with acridine orange staining (Watson et al. 1977, Davis & Sieburth 1982). Samples were preserved in 1 % glutaraldehyde in filtered seawater.

To facilitate data analyses the microflagellate growth curves were divided into 5 convenient phases, as described previously (Goldman et al. 1985). These phases – early and late exponential, transition, and early and late stationary – were established by inspection of the experimental curves. All microflagellate rate measurements (specific growth μ in d^{-1}, prey ingestion rates in cells microflagellate^{-1} d^{-1}, nutrient ingestion rates in pg N or P microflagellate^{-1} d^{-1}, clearance rates in ml microflagellate^{-1} d^{-1}, nutrient excretion rates in pg N or P microflagellate^{-1} d^{-1}), and prey cell quotas (in pg N or P $cell^{-1}$) were made as described previously (Caron et al. 1985, Goldman et al. 1985, Andersen et al. 1986). We assumed bacterial cell quotas of 10 fg N $cell^{-1}$ and 1 fg P $cell^{-1}$, based on unpublished data, to correct the total particulate nitrogen and phosphorus concentrations for calculating prey cell quotas and nutrient regeneration rates. Nutrient regeneration efficiencies (in %), summed through different growth intervals, were calculated as excretion rate × ingestion rate^{-1} × 100 for the exponential phase and as accumulated dissolved nutrient × particulate nutrient^{-1} × 100 for either the early or late stationary phases. Microflagellate clearance rates were determined for each interval and the maximum values for each experiment, which generally occurred at the end of exponential growth, were used in the data analyses.

RESULTS AND DISCUSSION

Characteristics of phytoplankton cultures

Steady-state cultures

We found large differences in the elemental chemical composition of the steady-state phytoplankton

cultures, which, for the most part, reflected the severity of N and P limitation (Table 1). These differences were most evident in the *Dunaliella tertiolecta* cultures; particulate C:N ratios (by atoms) in these cultures varied from 7.8:1 when D:$\hat{\mu}$ was 0.78 (slight N limitation) to 16.5:1 when D:$\hat{\mu}$ was 0.14 (severe N limitation) and C:P ratios varied from 218:1 when D:$\hat{\mu}$ was 0.79 (slight P limitation) to 521:1 when D:$\hat{\mu}$ was 0.13 (severe P limitation). These values are typical of N- and P-limited phytoplankton grown in continuous culture (Goldman 1980). Similar changes in C:N and C:P ratios of *Phaeodactylum tricornutum* occurred in the cultures maintained at the 2 higher relative growth rates (0.80 and 0.35), but, unexpectedly, the chemical composition of the diatom culture at the lowest relative growth rate (0.14) did not follow the expected trend for either N or P limitation. In this latter case the C:N ratio was 11.5:1 for N limitation and the C:P ratio was 196:1 for P limitation, values not representative of severe nutrient limitation (Table 1).

Possibly, the N-limited cultures of *Phaeodactylum tricornutum* did not reach steady state at the time of sampling and thus were not as nutrient-limited as presumed. Alternatively, the elemental ratios of at least the most N-limited *P. tricornutum* culture may have been biased downward by the relatively large bacterial biomass that was present in this culture ($>10^7$ cells ml^{-1} which, based on the assumed cell quota for nitrogen, represented 25 % of total particulate nitrogen) (Table 2). In contrast, bacterial nitrogen never exceeded 1 % of total particulate nitrogen in all cultures containing *Dunaliella tertiolecta* and was from 1 to 12 % of total nitrogen in the remaining cultures containing *P. tricornutum* (Table 2).

Not only were bacterial numbers highest in the *Phaeodactylum tricornutum* cultures maintained at the lowest growth rates, but there also were unexplainable large losses of total N and P in these cultures. These losses under N-limited growth varied from 22 % N of the original 200 μg-at l^{-1} NH_4^+ in the medium and 17 % P of the original 20 μg l^{-1} PO_4^{-3} when D:$\hat{\mu}$ was 0.34, to 49 % N and 37 % P when D:$\hat{\mu}$ was 0.14. Losses of about 25 % N and P were also observed in the most P-limited diatom culture (D:$\hat{\mu}$ = 0.14). No appreciable losses were found in any culture of *Dunaliella tertiolecta* (Table 2).

The nutrient losses were not bacterially-mediated because in subsequent experiments with this species we found similar losses of N and P in both axenic and non-axenic cultures at low dilution rates (Table 2). Although we did not measure the filtrates for total dissolved organic nitrogen, we found only negligible dissolved organic phosphorus (<0.6 μg-at l^{-1}) in the *Phaeodactylum tricornutum* cultures at the low dilution rates and we thus can rule out excreted organics as the source of the non-recovered N and P. We did, however,

Table 1. Summary of growth characteristics and chemical composition of phytoplankton prey and the microflagellate *Paraphysomonas imperforata* during grazing experiments

Prey growth conditions			Prey chemical compositions				Microflagellate growth characteristics[a]			
Limiting nutrient	Species	D:$\hat{\mu}$	Cell quota[b] (pg cell^{-1}) N	P	C:N:P ratio[c] (by atoms)	C:N ratio[c] (by atoms)	μ (d^{-1})	C:N:P ratio (by atoms)	C:N ratio (by atoms)	Ingestion rate (cells flag.$^{-1}$ d^{-1})
Nitrogen										
	Phaeodactylum tricornutum	0.80	1.26	0.28	116:13:1	9.2	2.67	59:9:1	6.9	41.0
		0.34	0.70	0.17	108:10:1	11.0	2.52	53:7:1	7.4	30.2
		0.14	0.46	0.15	89:8:1	11.5	2.50	64:9:1	7.2	38.4
	Dunaliella tertiolecta	0.78	6.02	1.44	72:9:1	7.8	1.97	40:6:1	6.3	4.0
		0.34	1.81	0.45	143:10:1	14.0	2.20	68:11:1	6.4	10.0
		0.14	2.21	0.59	176:11:1	16.5	2.08	102:12:1	8.9	10.0
Phosphorus										
	Phaeodactylum tricornutum	0.85	1.73	0.14	235:27:1	8.7	2.61	106:16:1	6.9	30.2
		0.38	1.39	0.08	359:34:1	10.7	1.98	180:26:1	7.0	38.4
		0.14	1.78	0.14	196:29:1	6.8	2.24	97:16:1	6.1	37.9
	Dunaliella tertiolecta	0.79	6.04	0.50	218:23:1	9.3	2.52	79:11:1	7.3	7.5
		0.40	6.53	0.33	459:45:1	10.2	2.07	149:24:1	6.4	9.4
		0.13	7.13	0.27	521:58:1	8.9	1.94	233:43:1	5.4	3.9

[a] Averaged during exponential growth phase except for C:N:P ratio which was calculated during stationary phase (3.0 d for grazing on *P. tricornutum* and 4.4 d for grazing on *D. tertiolecta*).
[b] Determined by assuming N and P cell quotas for bacteria: 10 fg N cell^{-1} and 1 fg P cell^{-1}
[c] Determined from particulate C, N, and P measurements at steady state

Table 2. Summary of growth conditions, nutrient mass balance and bacterial production of particulate nitrogen (PN) in steady-state continuous cultures of *Phaeodactylum tricornutum* and *Dunaliella tertiolecta*

Limiting nutrient	Species	D : $\hat{\mu}$	Total nutrient recovered[a]				Bacterial PN : total PN ratio	
			Concentration (μg-at l^{-1})		% of medium concentration		Initial	Early stationary[b]
			N	P	N	P		
Nitrogen	*Phaeodactylum tricornutum*	0.80	185.7	17.4	93	87	0.03	0.09
		0.34	156.9	16.5	78	83	0.02	0.05
		0.14	102.1	12.5	51	63	0.22	0.25
		0.14[c]	132.4	17.5	66	88		
		0.15[d]	139.6	15.4	70	77		
	Dunaliella tertiolecta	0.78	191.7	19.3	95	97	< 0.01	< 0.01
		0.34	192.9	18.9	96	95	< 0.01	< 0.01
		0.14	177.7	17.7	89	89	< 0.01	< 0.01
Phosphorus	*Phaeodactylum tricornutum*	0.85	215.1	4.1	90	103	0.05	0.12
		0.38	211.6	4.1	88	103	0.06	0.05
		0.14	179.6	2.9	75	73	0.06	0.06
	Dunaliella tertiolecta	0.79	225.3	4.4	94	110	< 0.01	0.01
		0.40	218.2	4.2	91	105	< 0.01	< 0.01
		0.13	229.7	4.2	96	105	< 0.01	< 0.01

[a] Sum of NH_4^+ + particulate N or PO_4^{-3} + particulate P + disolved organic P.
[b] After 3.0 d for *P. tricornutum* and 4.4 d for *D. tertiolecta*.
[c] Steady-state measurements from supplemental non-axenic culture
[d] Steady-state measurements from supplemental axenic culture

observe some wall growth in all the *P. tricornutum* cultures at the low dilution rates whether they were axenic or non-axenic. We thus suspect that adhered diatom cells (the adhesion seemed to increase dramatically under severe nutrient limitation independent of bacterial activity) were the source of the missing N and P since cell adhesion is known to be a common response of diatoms to nutrient stress (Myklestad 1974, Chamberlain 1976).

Dark nutrient uptake

In our earlier grazing experiments (Goldman et al. 1985, Andersen et al. 1986) we assumed that uptake of regenerated nutrients by ungrazed phytoplankton or bacteria that had been in the dark for long periods (days) was insignificant and that the observed patterns of nutrient regeneration were solely the result of microflagellate excretion. However, we found that cultures of both phytoplankton species, even after being kept in the dark for 24 h before they were supplemented with NH_4^+, had the potential for consuming virtually all the NH_4^+ excreted by the microflagellate during exponential growth. As seen from the NH_4^+ disappearance curves (Fig. 1), the most N-limited phytoplankton populations after 24 h in the dark without added NH_4^+ were capable after only 3 h of taking up 85 to 100 % of the 65 μg-at l^{-1} NH_4^+ added. Although dark uptake of NH_4^+ is known to occur in N-limited phytoplankton (Bongers 1956, Thacker & Syrett 1972), bacteria, which were present, may have also contributed to this process.

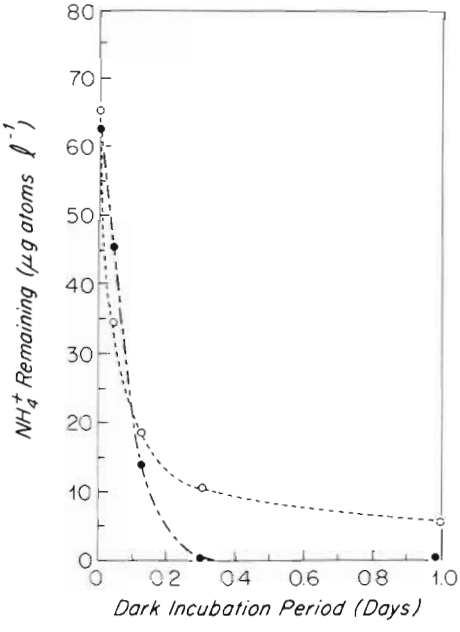

Fig. 1. Uptake of NH_4^+ over time by *Phaeodactylum tricornutum* (○) and *Dunaliella tertiolecta* (●) first grown to steady state at dilution rate of 0.2 d^{-1} and then pulsed with 65 μg-at l^{-1} of NH_4^+

Microflagellate grazing experiments

Growth and grazing characteristics

We were able to account for virtually all the starting total N (particulate + NH_4^+) and total P (particulate + dissolved organic + PO_4^{-3}) (Fig. 2 to 5). Generally when losses of N and P were observed they occurred during the late stationary phase when there was visible wall growth and aggregation of particulates. We attribute these losses, which are identical to those we observed in our earlier studies (Goldman et al. 1985, Andersen et al. 1986), to non-homogeneous sampling.

Growth rates of the microflagellate varied irregularly between 1.9 and 2.7 d^{-1} (Table 2) and were not a function of initial prey cell concentration or prey species (Fig. 2 to 5). The average μ in these experiments (2.3 d^{-1}) at 20>C appears to be maximal because it falls directly on the $\hat{\mu}$ versus temperature curve we established for this species previously (Caron et al. 1986). Since all of our grazing experiments have been performed in the batch mode with arbitrarily set initial prey biomass, we can only assume that these food levels were saturating for growth. Total biomass, rather than the type and nutritional quality of the food source, seems to be the major determinant of growth rate of a voracious predator such as *Paraphysomonas imperforata*. As we have observed earlier (Goldman & Caron 1985), *P. imperforata* can maintain $\hat{\mu}$ while grazing a wide array of phytoplankton and bacterial species.

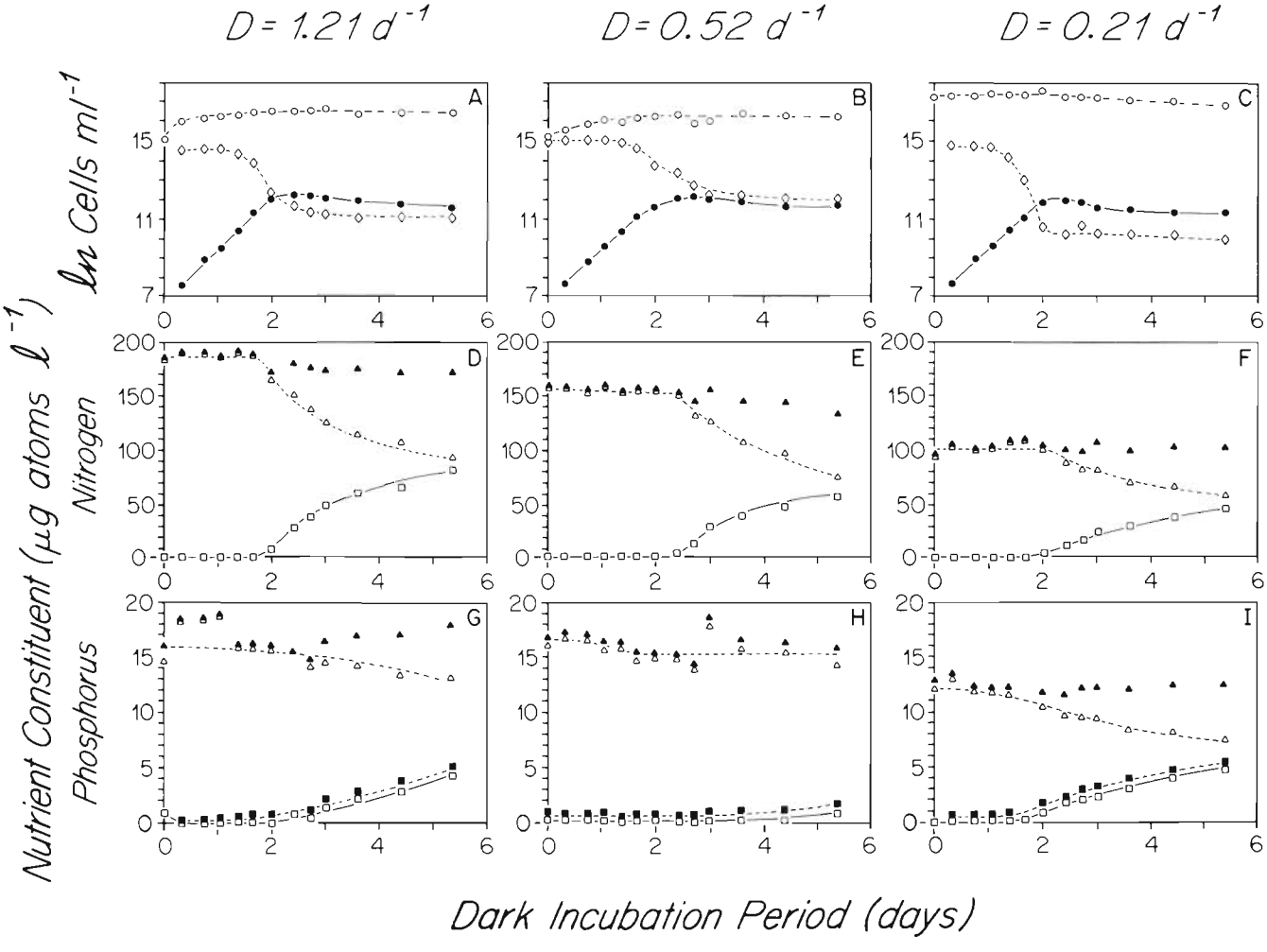

Fig. 2. Time course of grazing of *Phaeodactylum tricornutum* grown under nitrogen limitation at different dilution rates D by the phagotrophic microflagellate *Paraphysomonas imperforata*. (A–C) Changes in cell numbers of *P. tricornutum* (◇), *P. imperforata* (●), and bacteria (○). (D–F) Changes in concentrations of total nitrogen (particulate N + NH_4^+) (▲), particulate N (▲), and NH_4^+ (□). (G–I) Changes in concentrations of total phosphorus (particulate P + total dissolved P) (▲), particulate P (▲), PO_4^{-3} (□), and total dissolved P (■). All curves in Fig. 2 to 5 drawn by inspection

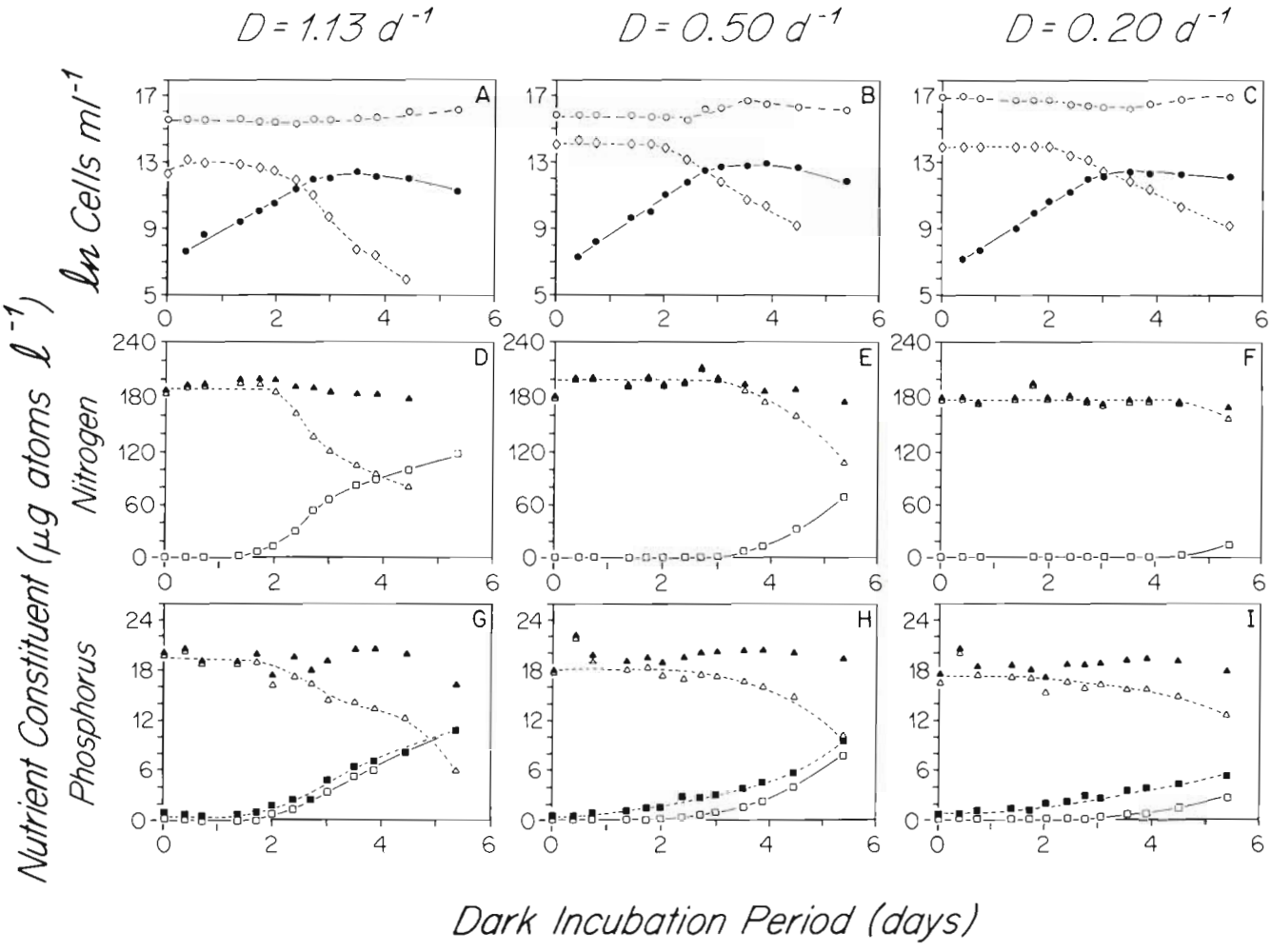

Fig. 3. Time course of grazing of *Dunaliella tertiolecta* grown under nitrogen limitation at different dilution rates D by the phagotrophic microflagellate *Paraphysomonas imperforata*. Panels and symbols as in Fig. 2

Cell ingestion rates during exponential growth, although variable between experiments, were more dependent on the type than on the physiological state of the prey species: ingestion rates varied from 4 to 10 cells ingested microflagellate^{-1} d^{-1} when the chlorophyte was grazed, to 30 to 41 cells ingested microflagellate^{-1} d^{-1} when the diatom was grazed (Table 1). When converted to a prey nutrient basis, however, nutrient ingestion rates were related more to the form of nutrient limitation than to the prey species (Fig. 6 & 7). For example, the average ingestion rates for nitrogen for each grouping of species and form of nutrient limitation varied from 33 pg N ingested microflagellate^{-1} d^{-1} under N limitation to 58 pg N ingested microflagellate^{-1} d^{-1} under P limitation when the diatom was the prey species, and from 22 pg N ingested microflagellate^{-1} d^{-1} to 45 pg N ingested microflagellate^{-1} d^{-1} under similar conditions for the chlorophyte (Fig. 6). A similar trend occurred for P ingestion (Fig. 7). The elimination of a species effect on grazing when ingestion rates were measured in terms of total nutrient in the food source, was obviously due to the much larger size of *Dunaliella tertiolecta* and correspondingly larger nutrient cell quotas than of *Phaeodactylum tricornutum* (Table 1).

Clearance rates during exponential growth also were variable between experiments. However, the general trend was for the clearance rate to decrease in a hyperbolic fashion with increasing concentration of prey biomass (represented by particulate carbon concentration), irrespective of prey species or nutritional state (Fig. 8). Particulate carbon concentrations of the prey were taken at the interval during grazing when the maximum clearance rate was measured during each experiment. The highest clearance rate (about 1.8×10^{-4} ml microflagellate^{-1} d^{-1}) occurred at a prey carbon concentration of 5 mg l^{-1} and the lowest rate of about 2.5×10^{-5} ml microflagellate^{-1} d^{-1} occurred when prey carbon concentrations exceeded about 20 mg l^{-1}. It is particularly noteworthy that clearance

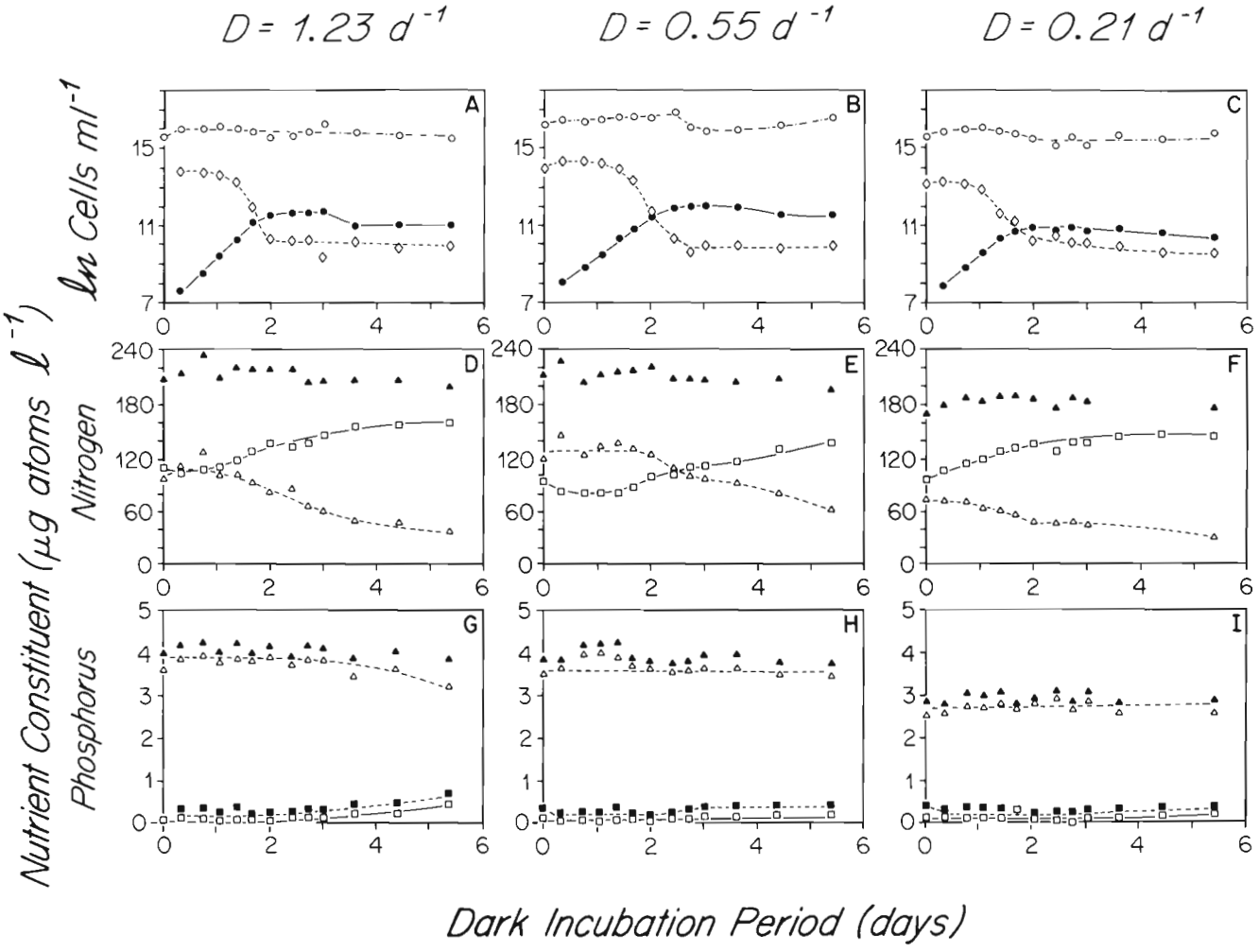

Fig. 4. Time course of grazing of *Phaeodactylum tricornutum* grown under phosphorus limitation at different dilution rates D by the phagotrophic microflagellate *Paraphysomonas imperforata*. Panels and symbols as in Fig. 2

rate was related to prey carbon concentration, given that *Dunaliella tertiolecta* is so much larger than *Phaeodactylum tricornutum* and microflagellate clearance rates are known to be functions of prey size and shape (Fenchel 1982a). By comparison, the shape of the curve in Fig. 8 is identical to that found by Sherr et al. (1983), who grew a phagotrophic microflagellate *Monas* sp. on different bacterial species and also found that prey biomass, and not species type, was the determinant of microflagellate clearance rate.

Although it has been convenient to gauge the effect of prey cell density on protozoan growth rates (Fenchel 1982b, Laybourn-Parry 1984), possibly the critical variable in controlling predator µ is not prey cell concentration, or necessarily prey type as long as it is an acceptable food source, but rather the rate with which the prey nutrient in shortest supply is assimilated. This rate depends on several factors including the capture mechanism and mode of ingestion of the microflagellate and the particulate nutrient concentration of the prey. For example, *Paraphysomonas imperforata* was capable of maintaining a constant µ when grazing on prey with grossly different taxonomic, morphological, size, and shape characteristics such as *Dunaliella tertiolecta* and *Phaeodactylum tricornutum* in this study and a variety of phytoplankton and bacterial species in previous studies (Goldman & Caron 1985); this occurred, most likely, because the rate of assimilation of the limiting nutrient always was in excess of that required for maximum biosynthesis even though the cell ingestion rate varied 300-fold from about 10 cells ingested microflagellate^{-1} d^{-1} when the large chlorophyte *D. tertiolecta* was grazed (Table 1) to 3000 cells ingested microflagellate^{-1} d^{-1} when much smaller bacteria were grazed (Caron et al. 1985).

Paraphysomonas imperforata seems to prefer a herbivorous mode of nutrition when given a choice of food since bacterial numbers, although as high as 3×10^7 cells ml^{-1} in the most N-limited *Phaeodactylum tricornutum* culture (Fig. 2C), never changed appreciably

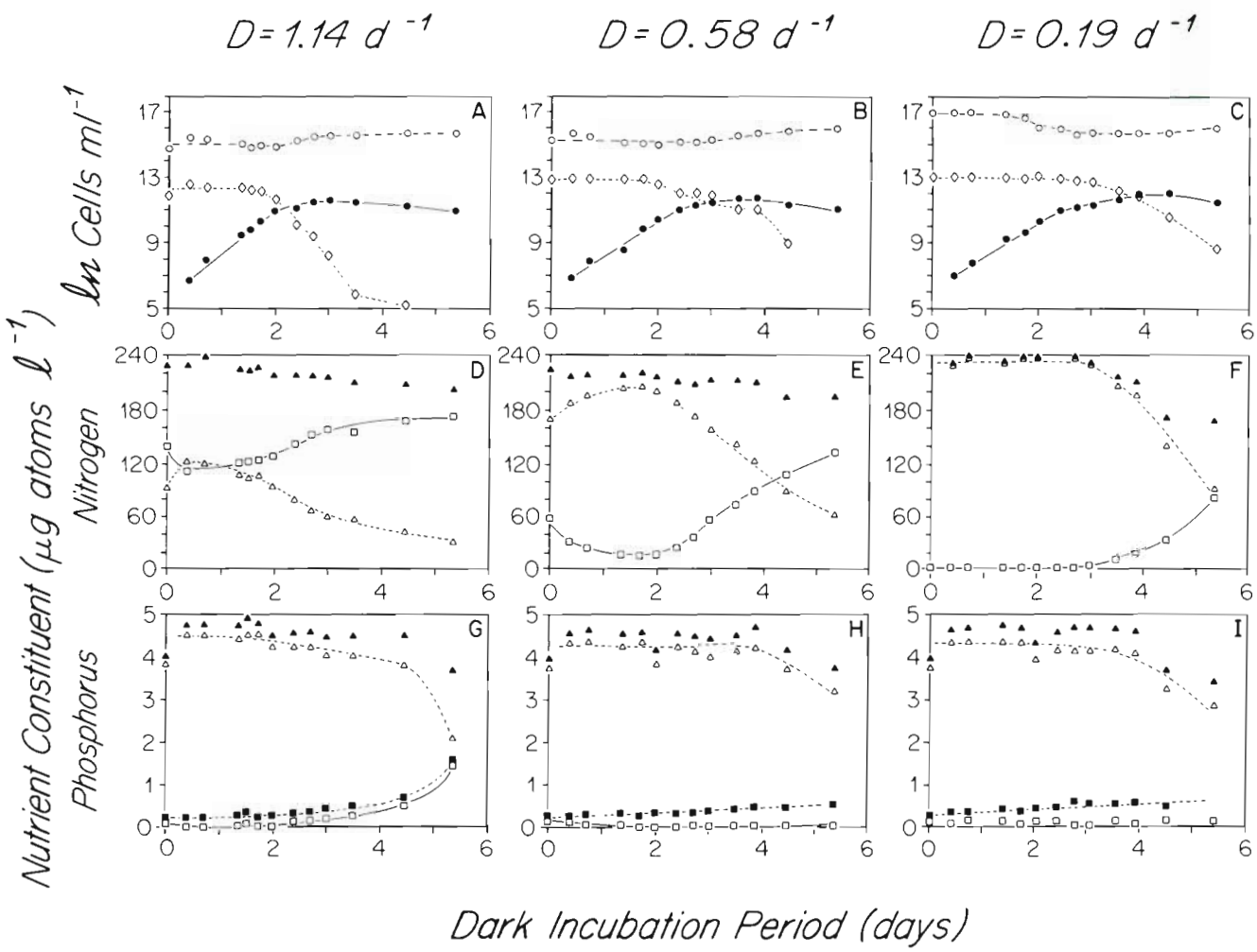

Fig. 5. Time course of grazing of *Dunaliella tertiolecta* grown under phosphorus limitation at different dilution rates D by the phagotrophic microflagellate *Paraphysomonas imperforata*. Panels and symbols as in Fig. 2

during the course of grazing on phytoplankton. The preference for phytoplankton may be linked to the way in which the microflagellate is able to alter its own size to accommodate phytoplankton prey of considerably different sizes and still maintain a predator-prey size ratio by volume of about 7 : 1 (Goldman & Caron 1985). Yet when growing on much smaller bacteria the predator-prey size ratio by volume is 360 : 1, which is suggestive that there is a lower limit to the size the microflagellate can attain when forced to graze on small particles. As Fenchel (1982a) has pointed out, clearance rate for a microflagellate of a given size decreases with decreasing size of food particle. Thus on this basis alone we would expect *P. imperforata* to graze preferentially on larger particles. When grazing on particles the size of bacteria, the microflatellate most likely is unable to reduce its own size sufficiently to optimize its clearance rate. However, the species obviously is highly adaptable to changing environments because it can grow as rapidly on bacteria as on phytoplankton if restricted to such a diet (Goldman et al. 1985), even though its filtering rate should decrease when grazing such small particles. Once again, the rate of assimilation of the prey nutrient in shortest supply may be the major determinant of microflagellate growth rate.

Threshold levels of both phytoplankton species, which occurred at the cessation of exponential growth of the microflagellate, were below 10^5 cells ml^{-1} (Fig. 2 to 5), and in the case of *Phaeodactylum tricornutum* leveled off to about 10^4 cells ml^{-1} before grazing ceased completely. As we have indicated earlier (Caron et al. 1985), only in eutrophic waters, or perhaps in microenvironments where local prey concentrations are elevated greatly above the ambient concentrations, would such high phytoplankton cell concentrations exist. Interestingly, reductions in *Dunaliella tertiolecta* cell populations continued throughout the stationary phase even though the microflagellate population

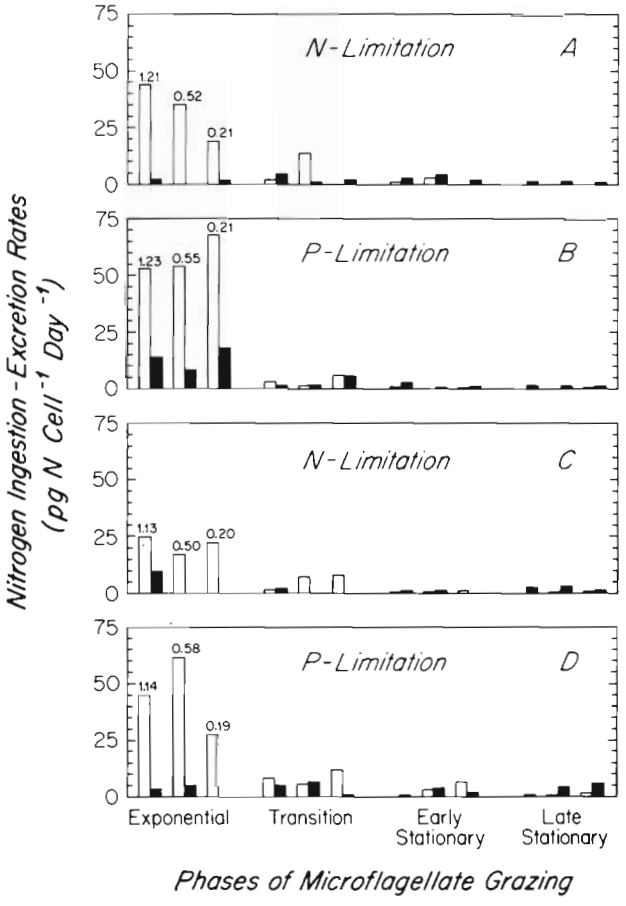

Fig. 6. *Paraphysomonas imperforata.* Rates of nitrogen ingestion (open bars) and regeneration (black bars) during different phases of grazing on (A, B) *Phaeodactylum tricornutum* and (C, D) *Dunaliella tertiolecta* grown under nitrogen or phosphorus limitation at different dilution rates (values in d^{-1} above bars)

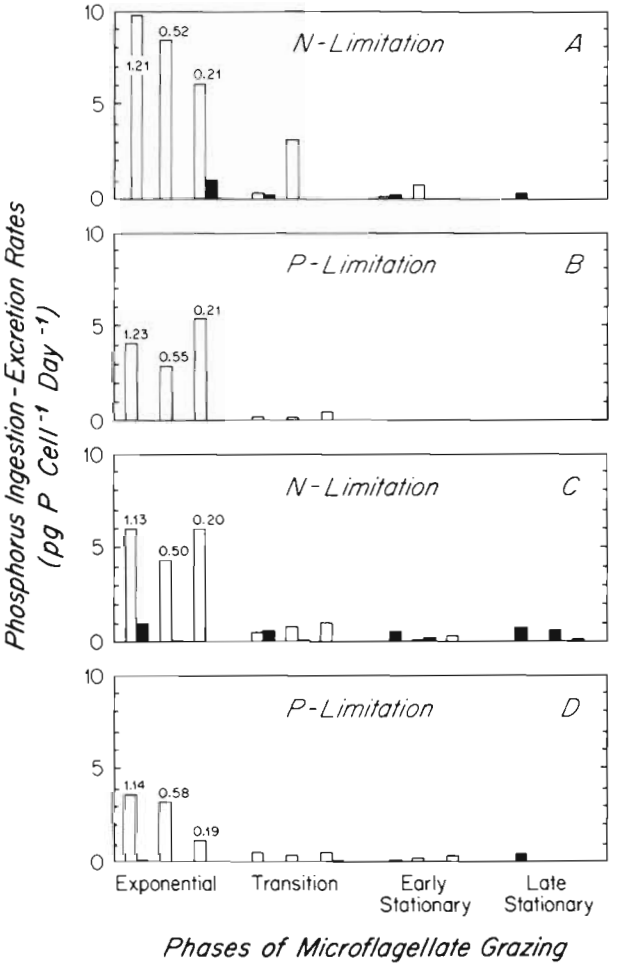

Fig. 7. *Paraphysomonas imperforata.* Same as Fig. 6, but for phosphorus ingestion and excretion

stopped growing. Cell ingestion rates decreased progressively during this period (down to <1 cell microflagellate^{-1} d^{-1} by Day 3) so that it seems likely that whatever food that was acquired below the threshold level was for meeting maintenance energy requirements.

Patterns of nutrient regeneration

The general patterns of NH_4^+ and total dissolved phosphorus regeneration by *Paraphysomonas imperforata* appeared to be linked to the physiological state of the prey phytoplankton, independent of which species was being grazed (Fig. 2 to 5). As in our previous experiments (Goldman et al. 1985, Andersen et al. 1986), the more severely N-limited the prey, the longer regeneration of NH_4^+ by the microflagellate was delayed until the transition and even stationary phases (Fig. 6). Excretion of NH_4^+ under P limitation, in contrast, generally commenced during the exponential phase and continued until the experiment was stopped at a point well into the stationary phase (Fig. 6). Phosphorus excretion was virtually non-existent during any phase of microflagellate growth in all experiments with P-limited prey, except that we observed some regeneration of total dissolved phosphorus during the entire grazing cycle in the experiment in which the slightly P-limited *Dunaliella tertiolecta* culture was grazed (Fig. 5G & 7). When the prey were N-limited, however, excretion of total dissolved phosphorus occurred at a relatively constant rate throughout the grazing cycle, regardless of prey species and physiological state (Fig. 7).

In general, the bulk of dissolved organic phosphorus present originated in the steady-state phytoplankton cultures. In fact, there were no apparent changes in dissolved organic phosphorus concentration throughout all the grazing experiments with *Phaeodactylum tricornutum* (Fig. 2G to I & 4G to I), and only minor

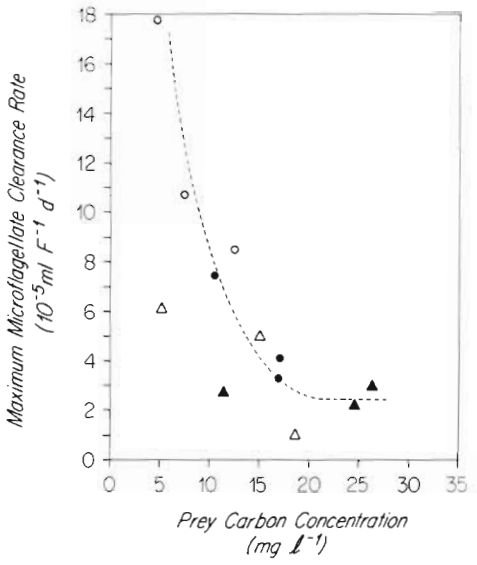

Fig. 8. *Paraphysomonas imperforata*. Effect of prey particulate carbon concentration on maximum clearance rate obtained during the course of grazing. (•) N-limited, (○) P-limited *Phaeodactylum tricornutum*; (▲) N-limited, (△) P-limited *Dunaliella tertiolecta*

increases when P-limited *Dunaliella tertiolecta* was the prey (Fig. 5G to I). When the chlorophyte was moderately to severely N-limited dissolved organic phosphorus accounted for up to 90 % of total phosphorus regeneration through the exponential phase before there was a switch to PO_4^{-3} excretion (Fig. 3G to I). In no case, however, did excretion of dissolved organic phosphorus exceed about 20 % of total algal P ingested by the microflagellate. These results are similar to our earlier findings that both urea (Goldman et al. 1985) and dissolved organic phosphorus excretion (Andersen et al. 1986) constitute only a small (15 to 20 %) fraction of nutrients regenerated by *Paraphysomonas imperforata*. Although bacterial uptake of excreted dissolved organic phosphorus may have biased our observations of small changes in this nutrient, in our earlier studies involving *P. imperforata* grazing phytoplankton we found no appreciable differences in dissolved organic phosphorus excretion with or without bacteria present (Andersen et al. 1986).

The trends in delayed nutrient regeneration we observed are to be expected from a physiological standpoint even though our results are equivocal due to possible dark uptake of NH_4^+ and PO_4^{-3} by ungrazed phytoplankton and bacteria. For example, the microflagellate must divert the bulk of its energy towards protein and nucleotide synthesis to sustain maximum growth rates. Under such conditions the stoichiometric requirements for carbon, nitrogen, and phosphorus are fairly rigid and we would expect the lowest possible cell C:N and C:P ratios. Surprisingly, little information is available on the chemical composition of protozoa, but for phytoplankton growing at $\hat{\mu}$ we know that the C:N:P ratio (by atoms) typically is close to the Redfield proportions of 106:16:1 (C:N = 6.6:1) (Goldman et al. 1979). Bacteria, because of their high nucleic acid content, tend to have C:N ratios between 4:1 and 6:1 when nutritionally fit (Luria 1960). Assuming that protozoa have a chemical composition similar to that of bacteria and phytoplankton, we would expect excre-

Table 3. *Paraphysomonas imperforata*. Summary of nutrient regeneration efficiencies for nitrogen and phosphorus during the course of grazing on phytoplankton prey

Prey growth conditions			Nutrient regeneration efficiency (%)					
			Nitrogen			Phosphorus		
Limiting nutrient	Species	D:$\hat{\mu}$ (d^{-1})	Exponential	Early stationary[a]	Late stationary[b]	Exponential	Early stationary[a]	Late stationary[b]
Nitrogen	*Phaeodactylum tricornutum*	0.80	5.5	25.6	42.8	0.3	9.7	25.6
		0.34	0	18.3	37.0	0.3	2.1	6.7
		0.14	3.9	23.8	43.1	8.3	20.8	38.3
	Dunaliella tertiolecta	0.78	39.0	45.8	60.4	16.7	33.9	50.2
		0.34	0	6.3	33.5	2.3	21.8	48.9
		0.14	0	0	7.5	0	19.8	28.8
Phosphorus	*Phaeodactylum tricornutum*	0.85	24.6	39.0	55.0	0	2.9	12.6
		0.38	14.2	23.1	41.5	0	5.3	5.3
		0.14	25.6	58.1	69.5	0	4.4	7.8
	Dunaliella tertiolecta	0.79	8.2	42.5	51.7	0.4	8.9	30.4
		0.40	9.6	36.8	58.3	0	5.8	7.4
		0.13	0.4	6.8	35.1	1.2	7.2	7.7

[a] After 3.0 d for grazing on *P. tricornutum* and 4.4 d for grazing on *D. tertiolecta*
[b] After 5.5 d

tion of N or P by the microflagellate only when the C:N or C:P ratio of the predator was somewhat greater than that of its food source. The gross growth efficiency (on a carbon basis) of the microflagellate would determine how far apart these ratios would need to be before excretion would occur (Fenchel & Blackburn 1979, Caron & Goldman in press).

Excretion of NH_4^+ and PO_4^{-3} in the current experiments was paralleled by reductions in the particulate C:N:P ratios during the course of grazing. By early stationary phase when the microflagellate comprised the bulk of the particulate matter, C:N ratios were generally between 6:1 and 7:1 (by atoms), regardless of the original prey C:N ratios which varied from 6.8:1 to 16.5:1, depending on the prey nutritional state. These ratios are characteristic of microbes with high protein contents and growing at high relative growth rates. In contrast, C:P ratios of the microflagellate, although they were reduced approximately 2-fold over those of the prey phytoplankton in most of the experiments and up to 3-fold when severely P-limited *Dunaliella tertiolecta* cells were grazed, generally increased in magnitude with increasing degree of nutrient limitation from about 40:1 to 80:1 when the prey were only moderately nutrient-limited to 233:1 when the most severely P-limited *D. tertiolecta* cells were prey (Table 1). Possibly, undigested prey cells may have been retained in food vacuoles during the transition and early stationary phases, thereby biasing the particulate chemical composition towards the higher C:P and N:P ratios found in the prey even though the microflagellate was synthesizing C, N, and P into macromolecules in relatively constant proportions concomitant with a high relative growth rate. We did observe multiple *D. tertiolecta* cells stored in food vacuoles during these periods which is consistent with our observation of continued grazing on *D. tertiolecta* even after growth of the microflagellate stopped. Hence, at some point in the microflagellate growth cycle after all prey were consumed, the C:N:P ratio of particulate matter would be expected to equal that of the predator.

Given our uncertainty as to the role of dark nutrient uptake by ungrazed phytoplankton, we can only guess that nutrient regeneration efficiencies through the exponential phase really were negligible when the prey were either moderately to severely N-limited or P-limited at all physiological states (Table 3, Fig. 6 & 7). Our estimates of regeneration efficiency based on accumulated nutrient excretion through the stationary phases should not, however, have been influenced by dark uptake because prey biomass at that point made up a minute fraction of total biomass. Thus by the end of the experiments the efficiency of N regeneration increased substantially (up to 35 to 70 %) and, with the exception of the most N-limited *Dunaliella tertiolecta* culture for which the efficiency of N regeneration was only 7.5 %, this pattern of regeneration seemed not to be related to prey type, form of nutrient limitation, or physiological state. The efficiency of P regeneration, in contrast, although not influenced by prey species, generally was much greater when the prey were N-limited (up to 50 %) then when grazing on P-limited prey (5 to 8 % for moderate to severe P-limitation and 12 to 30 % for slight P-limitation) (Table 3). This latter observation is indirect evidence that a true lag in excretion of P occurred when the prey were severely P-limited.

Ecological significance

Considerable emphasis has been placed in recent years on characterizing pelagic surface waters as zones of nutrient impoverishment, low primary productivity, and containing an assortment of small (<10 µm size) microbes (bacteria, phytoplankton, and protozoa) that are tightly connected with respect to the flow of energy and nutrients. We now suspect that the bulk of nutrients in these waters (80 to 90+ %) are retained within this 'microbial loop' through regeneration processes (Eppley & Peterson 1979, Williams 1981, Azam et al. 1983), and that the speed by which nutrients cycle within the loop (the spinning wheel) is set by the maximum growth rate of the phytoplankton component (i.e. relative growth equal to unity) (Goldman et al. 1979, Goldman 1984, 1986).

Much of the evidence supporting rapid and efficient recycling of nutrients by the microbial (<10 µm) component comes from size-fractionation studies on labelled N and P uptake by natural phytoplankton populations (including isotope dilution measurements) (Harrison 1980, Glibert 1982, Herbland 1984, Harrison & Harris 1986). The microbial assemblages involved in this recycling generally have not been characterized or enumerated so that it has been impossible to describe quantitatively from these field studies the energetics and dynamics of these microbial interactions. From recent laboratory experiments (Taylor & Lean 1981, Goldman et al. 1985, Andersen et al. 1986, Taylor 1986), it is becoming increasingly clear that protozoa retain a sizeable (up to 50 to 70 %) fraction of the prey N and P that they ingest during balanced growth, and that modes of nutrient regeneration are closely linked to prey nutritional state.

To reconcile this high efficiency of nutrient assimilation in protozoa with field observations of efficient nutrient turnover at the microbial level, we invoked the possibility of a tightly knit and complicated microbial food web with, at least, several grazing steps (Goldman & Caron 1985, Goldman et al. 1985). Taylor & Lean (1981) and Taylor (1986) envisioned a dramatically

different system in which protozoa act as sinks, rather than as sources, of nutrients and thus contribute little to the overall nutrient regeneration process. Because there is no disagreement that protozoa are extraordinarily adaptable to changing environments and are highly efficient in conserving prey biomass, the resolution of these seemingly opposing views rests, to a large extent, on a critical re-evaluation of the accuracy of size-fractionation results.

Currently, there is some controversy as to how fast phytoplankton are growing in pelagic surface waters and whether or not growth rates are regulated by nutrient availability (Goldman et al. 1979, Sharp et al. 1980, Goldman 1986). Given that very high nutrient conversion efficiencies are possible when the prey are N- or P-limited, the microbial food web would have to be extraordinarily complicated with many grazing steps in order for regeneration efficiencies of 90 % to be achieved if relative growth rates of phytoplankton were greatly less than one (i.e. growth rate limited by severe N- or P-limitation) (Goldman et al. 1985). The size of the microbial food web thus may be an indicator of how fast phytoplankton in pelagic waters are growing relative to their maximal possible rates.

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