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Dynamics of prey selection by an omnivorous flagellate*

Joel C. Goldman, Mark R. Dennett

Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA

ABSTRACT: The heterotrophic flagellate *Paraphysomonas imperforata*, a raptorial grazer, sustained maximum specific growth rates of ca 1.5 d^{-1} at 20 °C when fed 3 phytoplankton species of different sizes and shapes (the relatively small diatom *Phaeodactylum tricornutum* and haptophyte *Isochrysis galbana*, and the larger chlorophyte *Dunaliella tertiolecta*), either singularly or in combinations of 2 species. When prey combinations included *D. tertiolecta*, the chlorophyte was grazed only after a large fraction of the other species was first grazed. Diatom and haptophyte were grazed concurrently when offered in combination. Changing the relative proportions of starting biomass of the different species in combination had no effect on the order of grazing. However, in all cases the switch to the chlorophyte occurred rapidly and the maximum ingestion rate attained after the switch was proportional to the contribution of the chlorophyte to total starting biomass. From a hydrodynamic standpoint, specific clearance rate C′ increased as the ratio of predator radius to prey radius R:r decreased and C′ increased as R decreased for a given value of R:r. We suggest that the preference for the 2 smaller species is governed by the ability of the flagellate to adjust its own size downward to accomodate the smaller prey in order to maintain R:r at ca 2:1. When sized to graze these smaller species, the flagellate simply is too small to graze the chlorophyte. Thus, although there is clear evidence that the flagellate is a non-passive grazer and will not graze certain species at all, mechanoreception clearly plays a major role in the dynamics of grazing desired species. Raptorial grazers such as *P. imperforata*, by having the ability to graze prey almost as big as themselves, may be effective competitors with larger protozoa for nanoplankton-size food particles and also contribute to making the food chain (web) within the microbial loop long and complicated with high losses of energy and materials.

INTRODUCTION

In a series of recent studies, we have examined the grazing, growth, and nutrient cycling characteristics of a phagotrophic flagellate, *Paraphysomonas imperforata*, that is capable of both bacterivory and herbivory (Caron et al. 1985, 1986, Goldman & Caron 1985, Goldman et al. 1985, 1987, Anderson et al. 1986). We have observed the flagellate, a raptorial feeder, to change its own size about 5-fold by volume (ca 200 to 1000 μm^3) in order to accomodate a 400-fold range in prey sizes from bacteria (ca 0.5 μm^3) to numerous phytoplankton types and shapes, the largest of which in our experiments was the chlorophyte *Dunaliella tertiolecta* (ca 200 μm^3) (Goldman & Caron 1985). Grazing appeared to be distinctly non-passive since no growth was observed on several phytoplankton species in the same size range as those supporting growth (Goldman & Caron 1985). Judged by our results with non-axenic phytoplankton cultures, herbivory seemed to be the preferred mode of feeding by *P. imperforata* because bacterial numbers, which were as high as 10^7 ml^{-1}, remained relatively unchanged during the course of grazing on phytoplankton (Goldman et al. 1987).

The preference for herbivory may be related to the fact that *Paraphysomonas imperforata*, a fairly large flagellate (diameter 7 to 12 μm) was able to maintain a relatively constant predator to prey volume ratio of ca 7 to 9:1 by adjusting its own size when grazing individually on different phytoplankton species in the size range 15 to 200 μm^3; yet when much smaller bacteria were the sole prey it appeared that the flagellate could only reduce its size to a lower threshold volume of about 180 μm^3 so that the predator-to-prey volume ratio was elevated to ca 360:1. In all cases, however, as long as individual food sources were acceptable and presented in saturating concentrations, growth rates and gross growth efficiencies of the flagellate were maximal (Goldman et al. 1985, 1987).

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Fenchel (1986) has suggested, based on simple hydrodynamic considerations and empirical evidence, that raptorial feeding among protozoa is favored when the predator-to-prey length ratio is $\leq 10:1$ ($\leq 1000:1$ by volume). From our results it would appear that the optimal predator-to-prey volume ratio for a raptorial grazer such as *Paraphysomonas imperforata* may be far lower than 1000:1. In fact, the ability to engulf and ingest relatively large prey, in some cases prey even larger than themselves, seems to be widespread among flagellates that graze by direct interception (Goldman & Caron 1985, Suttle et al. 1986). Many heterotrophic dinoflagellates, in particular, are capable of feeding on prey much larger than themselves (Gaines & Taylor 1984, Jacobson & Anderson 1986). These grazers first capture prey through attachment of a thin stalk-like filament, followed by engulfment with an emergent pseudopod that advances along the capture filament until the prey organism is totally covered by a sheath-like membrane (Jacobson & Anderson 1986). *P. imperforata* similarly seems capable of engulfing prey of different shapes by extension of a very elastic outer membrane. For example, the pennate diatom *Phaeodactylum tricornutum,* which has a longitudinal dimension (ca 20 µm) more than twice the diameter of the flagellate (but a total volume only ca 15% that of the flagellate), once captured, is totally covered by this outer membrane, giving the odd appearance of a bulging pennate diatom (see Fig. 1A in Goldman & Caron 1985). Suttle et al. (1986) have observed an even more dramatic extension of the outer membrane of a freshwater species of *Paraphysomonas* (diameter 6 to 14 µm) in engulfing the pennate diatom *Synedra* sp. which is 40 to 85 µm long. Just how this capture and engulfment occurs is completely unknown.

Raptorial grazers entrain their prey in water currents passing over the outer surface of the flagellate with capture occurring after contact with the prey is made. Frequently, we have observed multiple prey being carried along behind a single swimming *Paraphysomonas imperforata* cell, presumably being held by a thin filament. This filament may be the same thin stalk-like structure that is used by *P. imperforata* to anchor itself to surfaces (Hibberd 1979), and, in fact, may be similar to the capture filament used by dinoflagellates.

Herbivory among raptorial-grazing flagellates (including dinoflagellates) is widespread (Goldman & Caron 1985, Güde 1985, Jacobson & Anderson 1986, Parslow et al. 1986, Suttle et al. 1986, Ramberg 1987, Barlow et al. 1988, Goldman et al. 1989). In fact, not only may herbivory at the flagellate level be an important component of the marine microbial food loop (Goldman & Caron 1985, Suttle et al. 1986, Sherr et al. 1988), but the size relationships between predator and prey may be far more complicated than the simple 10:1 length relationship previously envisioned (e.g. Azam et al. 1983). To explore further the dynamics of food selection by raptorial grazers, we have expanded our earlier studies with *Paraphysomonas imperforata* and have performed a series of time-course grazing experiments in which combinations of 2 acceptable phytoplankton prey of different sizes and shapes and varying biomass ratios were offered as food to the flagellate. We specifically address the question of whether or not mechanoreception is an important factor in prey selection by this raptorial grazer.

METHODS

Three marine phytoplankton species, the diatom *Phaeodactylum tricornutum* (clone TFX-1), the chlorophyte *Dunaliella tertiolecta* (clone Dun), and the haptophyte *Isochrysis galbana* (clone T. Iso = tropical strain), all grossly different in taxonomy, size, shape and morphology, were grown in batch culture under conditions identical to those used previously (Goldman et al. 1985). Culture medium was artificial seawater (Goldman & McCarthy 1978) with nominal additions of 200 µg-at. l^{-1} NH_4^+ and 20 µg-at. l^{-1} PO_4^{3-}.

The heterotrophic flagellate *Paraphysomonas imperforata* (Lucas) (diameter 7 to 12 µm) was used in all grazing experiments. The time-course grazing experiments were designed as follows: Expt A involved control experiments with single phytoplankton species and Expts B to D were, respectively, with different mixtures of *Dunaliella tertiolecta* and *Phaeodactylum tricornutum, D. tertiolecta* and *Isochrysis galbana,* and *P. tricornutum* and *I. galbana.* The effect on prey selection (if any) of preconditioning the flagellate on a particular food source was examined by duplicating each experiment involving mixed food sources and using inocula preconditioned on each of the prey species. Flagellate inocula for the control experiments (Expt A) were preconditioned on the respective prey species.

Cultures of each phytoplankton species (1.5 l) were grown to late exponential phase in 3 l Erlenmeyer flasks under a 14:10 h light-dark cycle at 20°C. At the designated time the cultures were split according to the requirements of each experiment. Subsamples also were taken for cell counts and particulate carbon and particulate nitrogen. Each grazing experiment was performed in the dark at 20°C with 75 ml of culture in 125 ml Erlenmeyer flasks. The flasks were mixed only when sampled. Initial mixtures of phytoplankton species used in the different experiments were estimated on the basis of nitrogen biomass by the product of initial cell counts and estimated nitrogen cell quotas Q_n (pg N $cell^{-1}$) based on previous experience. The desired initial phytoplankton mixtures were 33:67%

and 67:33 % for each pair of prey species. However, some of the actual starting mixtures were different and were determined only after Q_n was calculated for each species in the control experiments. These results were adjusted for small increases in cell numbers occurring during the first hours after cultures were placed in the dark and before grazing was measurable. Phytoplankton and flagellate cell counts were made with a Spencer Bright-line hemacytometer on samples fixed with Lugol's solution and particulate carbon and particulate nitrogen were analyzed with a Perkin-Elmer® 240C elemental analyzer on samples retained on precombusted glass fiber filters (Whatman® GF/F).

Monocultures or mixtures of phytoplankton were inoculated with the flagellate and sampled at 6 to 8 h intervals for cell counts of individual prey and predator species during the first 3 d and daily thereafter until the experiments were terminated at 4 to 5 d. Measurements of flagellate specific growth rates μ (d^{-1}), and ingestion rates on a cell basis I_p (cells flag.$^{-1}$ d^{-1}) and nitrogen basis I_n (pg N flag.$^{-1}$ d^{-1}) were calculated as described previously (Goldman et al. 1989). Specific growth rates were based on regression analyses of the linear portion of plots of the natural log of cell counts versus time and ingestion rates were calculated for each interval of sampling. Curves of prey decrease or flagellate increase were drawn by visual inspection and where individual data deviated significantly from the curves, ingestion rates for that interval were calculated using the adjusted values falling directly on the curve. In most cases, this involved minor adjustments of the data. Ingestion rates on a nitrogen basis I_n were calculated by multiplying I_p by a constant Q_n for each species, determined as described above, and, assuming that once cell numbers reached a maximum in the dark, Q_n did not change while grazing occurred over the next several days. Although *Paraphysomonas imperforata* is not a filter feeder, equivalent clearance rates C (ml flag.$^{-1}$ d^{-1}) for each species were determined from the slopes of the linear portion of the curves of I_n versus prey N concentration.

RESULTS

Phytoplankton characteristics

Maximum phytoplankton cell concentrations, occurring during the initial dark phase, ranged from 6.3×10^5 ml^{-1} for the largest species, *Dunaliella tertiolecta* (200 μm^3), to 3×10^6 ml^{-1} for the smallest species, *Phaeodactylum tricornutum* (40 μm^3) (Table 1). Corresponding particulate nitrogen concentrations varied from a low of 83 μg-at. l^{-1} for *Isochrysis galbana* (indicating that less than half of the enrichment NH_4^+ was consumed) to ca 185 μg-at. l^{-1} for the other 2 species (close to the nominal enrichment of 200 μg-at. l^{-1}). This led to >4-fold range of Q_n values among the species from 0.84 pg N cell^{-1} for *P. tricornutum* to 4.17 pg N cell^{-1} for *D. tertiolecta*. The amount of nitrogen per unit cell volume V_n remained relatively constant at ca 21 fg N μm^{-3}, however. The particulate C:N ratios were consistent with the fraction of available NH_4^+ consumed by the 3 species, varying from 8.2:1 (by atoms) for *I. galbana* (representing none to slight N-limitation) to 11.7:1 for *D. tertiolecta* (representing moderate N-limitation).

Growth and grazing characteristics of *Paraphysomonas imperforata*

A total of 13 grazing experiments were performed, 3 with the control prey species (Expt A), 4 each with prey mixtures of *Dunaliella tertiolecta/Phaeodactylum tricornutum* (Expt B), and *D. tertiolecta/Isochrysis galbana* (Expt C), and 2 with *P. tricornutum/I. galbana* (Expt D). Actual starting mixtures on a nitrogen basis in Expts B and C for each pair of prey species, as defined above, were ca 33 % *D. tertiolecta*:67 % *P. tricornutum* or *I. galbana* (Expts B-1 and C-1), ca 25 % *D. tertiolecta*:75 % *P. tricornutum* or *I. galbana* (Expts B-2 and C-2), ca 67 % *P. tricornutum* or *I. galbana*:33 % *D. tertiolecta* (Expts B-3 and C-3), and ca 75 % *P. tricor-*

Table 1. Characteristics of phytoplankton prey species used in mixed culture experiments

Prey species	Approx dimensions (μm)	Approx volume (μm^3)	Max cell number[a] (10^6 ml^{-1})	Q_n (pg N cell^{-1})	V_n (fg N μm^{-3})	C:N ratio (by atoms)
Isochrysis galbana	5[b]	70	0.81	1.43	20.4	8.2
Phaeodactylum tricornutum	2×20	40	3.00	0.84	21.0	9.0
Dunaliella tertiolecta	6×11	200	0.63	4.17	20.9	11.7

[a] Maximum cell number attained after cultures were placed in the dark
[b] Diameter

nutum or *I. galbana*: 25 % *D. tertiolecta* (Expts B-4 and C-4) (Table 2). In Expts D-1 and D-2 the mixtures were fixed at ca 55 % *P. tricornutum*: 45 % *I. galbana* (Table 2).

There was no indication that the preconditioning food source in any of the experiments (designated in Table 2) had any impact on the patterns of food selection that resulted. Thus, for illustrative purposes, the time-course results of only the experiments involving mixtures of 33 % *Phaeodactylum tricornutum* or *Isochrysis galbana*: 67 % *Dunaliella tertiolecta*, and 67 % *D. tertiolecta*: 33 % *P. tricornutum* or *I. galbana* in Expts B and C, and one of the replicate experiments of Expt D are represented in the figures, although data for specific growth rates and maximum ingestion rates obtained for all experiments are shown in Table 2.

Specific growth rates μ of *Paraphysomonas imperforata* were relatively constant and independent of either individual food sources (Expt A), combinations of food sources (Expts B to D), or the preconditioning food source, ranging between 1.41 and 1.83 d^{-1} and averaging 1.51 $\pm$ 0.255 d^{-1} ($\pm$ 2 SD) for 13 experiments (Table 2).

Because of the small measured changes in prey cell numbers very early in the exponential phase, estimates of I_p for this phase were unreliable and not recorded. Thus the measured maximum ingestion rates for all experiments are conservative. In the control experiments, patterns of grazing by the flagellate were similar regardless of the prey species (Fig. 1). Exponential growth of the flagellate continued for 2 to 3 d (Figs. 1A, D, G) and ingestion rates on a cell basis (Figs. 1B, E, H) followed a common trend: maximum I_p occurred early in the exponential phase and decreased to minimal values as the stationary phase was approached. The switch to the stationary phase usually was abrupt, occurring when flagellate numbers approached or exceeded those of the prey. Maximum I_p varied from 42 cells flag.$^{-1}$ d^{-1} when the prey was the large chlorophyte *Dunaliella tertiolecta* to 160 cells flag.$^{-1}$ d^{-1} when the much smaller diatom *Phaeodactylum tricornutum* was eaten. However, on a nitrogen basis, differences in the ingestion rate were less apparent (Figs. 1C, F, I), although the highest value of I_n (179 pg N flag.$^{-1}$ d^{-1}) was recorded for *D. tertiolecta* (Table 2).

In the mixed prey experiments, several important grazing patterns were evident. First, *Phaeodactylum tricornutum* (Fig. 2) and *Isochrysis galbana* (Fig. 3) clearly were preferred over *Dunaliella tertiolecta* as a food source by the flagellate. In both experiments, regardless of the relative proportions of *D. tertiolecta* and the other prey species, grazing on the chlorophyte did not begin until some time after grazing on the other species had begun and some fraction of that species was grazed. Except for Expts B-4 and C-2 (not represented in figures), a substantial fraction of *P. tricornutum* or *I. galbana* was grazed before the flagellate switched to grazing on *D. tertiolecta* (42 to 71 % for *P. tricornutum* and 82 to 91 % for *I. galbana*, as seen in Figs. 2A, D and 3A, D) and this switch took place

Table 2. Starting conditions for grazing experiments and growth (μ) and ingestion rates (I) of *Paraphysomonas imperforata* when phytoplankton prey were presented in different combinations of species

Expt	Prey	% of total prey N biomass			μ	Max I_p[a]			Max I_n[b]		
		D[c]	*P*[c]	*I*[c]	(d^{-1})	*D*	*P*	*I*	*D*	*P*	*I*
A-1[d](*D*)[e]	*Dunaliella* (Control)	–	–	–	1.54	42	–	–	179	–	–
A-2[d](*P*)	*Phaeodactylum* (Control)	–	–	–	1.48	–	160	–	–	135	–
A-3[d](*I*)	*Isochrysis* (Control)	–	–	–	1.49	–	–	66	–	–	94
B-1[d](*P*)	*Dunaliella-Phaeodactylum*	33	67	–	1.41	4	113	–	19	95	–
B-2 (*D*)	*Dunaliella-Phaeodactylum*	23	77	–	1.83	2	133	–	9	112	–
B-3[d](*P*)	*Dunaliella-Phaeodactylum*	64	36	–	1.39	11	66	–	47	55	–
B-4 (*D*)	*Dunaliella-Phaeodactylum*	76	24	–	1.49	31	62	–	130	53	–
C-1[d](*I*)	*Dunaliella-Isochrysis*	37	–	63	1.41	7	–	84	30	–	119
C-2 (*D*)	*Dunaliella-Isochrysis*	24	–	76	1.66	3	–	40	11	–	57
C-3[d](*I*)	*Dunaliella-Isochrysis*	66	–	34	1.42	13	–	86	55	–	123
C-4 (*D*)	*Dunaliella-Isochrysis*	76	–	24	1.41	8	–	37	35	–	53
D-1[d](*I*)	*Phaeodactylum-Isochrysis*	–	55	45	1.50	–	42	31	–	35	44
D-2 (*P*)	*Phaeodactylum-Isochrysis*	–	57	43	1.63	–	45	49	–	38	70

[a] Phytoplankton cells flag.$^{-1}$ d^{-1}
[b] pg N flag.$^{-1}$ d^{-1}
[c] *D*: *Dunaliella tertiolecta*; *P*: *Phaeodactylum tricornutum*; *I*: *Isochrysis galbana*
[d] Experiments represented in figures
[e] Species used to grow *Paraphysomonas imperforata* inoculum

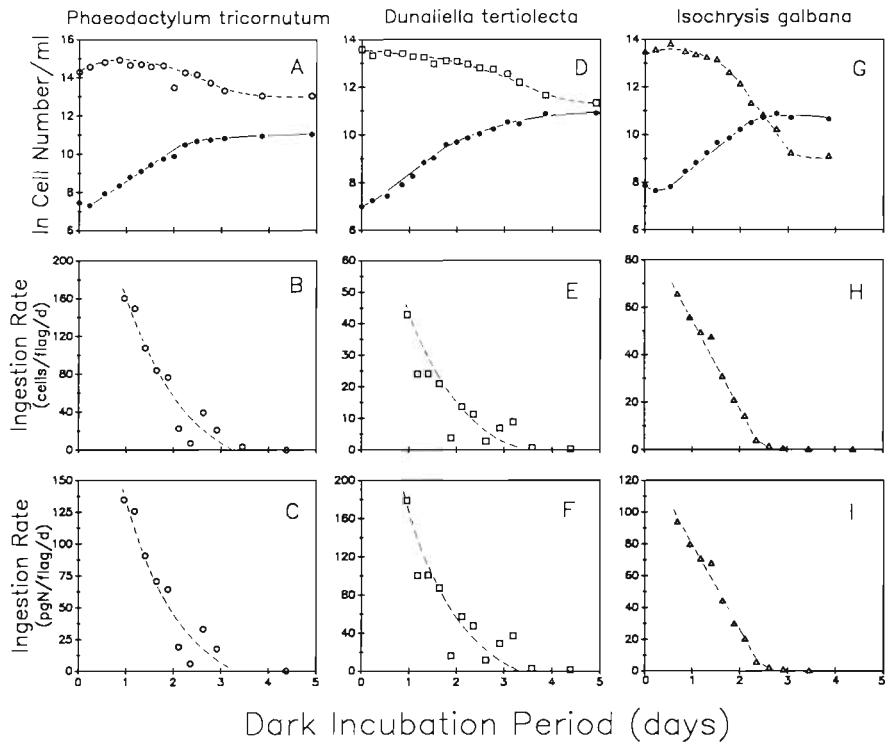

Fig. 1. Time course of grazing and ingestion of *Paraphysomonas imperforata* (●) on individual prey *Phaeodactylum tricornutum* (○), *Dunaliella tertiolecta* (□), or *Isochrysis galbana* (△). (A to C) Expt A-1. (D to F) Expt A-2. (G to I) Expt A-3. (A, D, G) Change in cell numbers. (B, E, H) Changes in ingestion rate on cell basis. (C, F, I) Changes in ingestion rate on a nitrogen basis. Symbols same in all panels

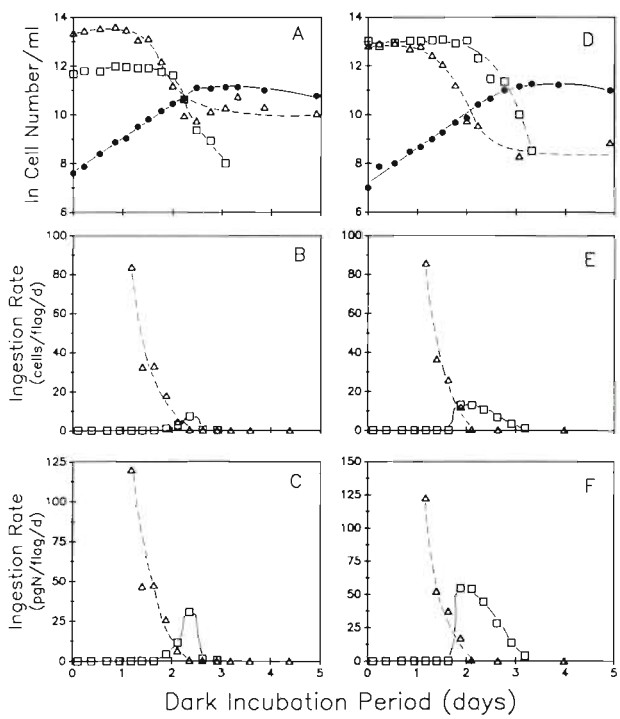

Fig. 2. Time course of grazing and ingestion of *Paraphysomonas imperforata* (●) on combined prey *Phaeodactylum tricornutum* (○) and *Dunaliella tertiolecta* (□). (A to C) Expt B-1. (D to F) Expt B-3. (A, D) Changes in cell numbers. (B, E) Changes in ingestion rate on cell basis. (C, F) Changes in ingestion rate on nitrogen basis. Symbols same in all panels

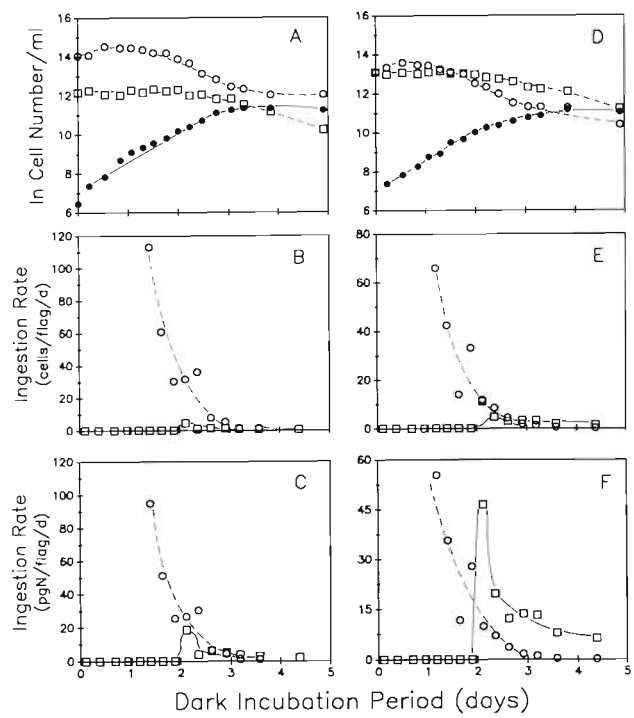

Fig. 3. Time course of grazing and ingestion of *Paraphysomonas imperforata* (●) on combined prey *Isochrysis galbana* (△) and *Dunaliella tertiolecta* (□). (A to C) Expt C-1. (D to F) Expt C-3. (A, D) Changes in cell numbers, (B, E) Changes in ingestion rate on cell basis. (C, F) Changes in ingestion rate on nitrogen basis. Symbols same in all panels

between 1.5 and 2.5 d after the experiments began (Table 3). Grazing on *D. tertiolecta* began after 22 to 23% of either *P. tricornutum* (Expt B-4) or *I. galbana* (Expt C-2) was grazed and this switch occurred after only 0.8 to 1.1 d. However, since Expt B-4 represents the largest fraction of the chlorophyte mixed with the diatom, whereas Expt C-2 represents the smallest fraction of the chlorophyte mixed with the haptophyte, it is difficult to attribute any special meaning to these results.

Table 3. Fraction of N biomass of either *Phaeodactylum tricornutum (P)* or *Isochrysis galbana (I)* grazed by *Paraphysomonas imperforata* before switch to *Dunaliella tertiolecta (D)* took place in Expts B and C

Expt	Percent *P* or *I* grazed (%)	Time when switch to *D* occurred (d)
B-1	42	2.0
B-2	71	2.5
B-3	59	2.0
B-4	22	0.8
C-1	91	2.0
C-2	23	1.1
C-3	82	2.0
C-4	82	1.5

The second grazing pattern we observed was that, when *Dunaliella tertiolecta* was the prey, the maximum value of I_n (which always occurred soon after the flagellate switched prey; Figs. 2C, F and 3C, F) appeared to be a direct function of how much the chlorophyte contributed to total initial prey biomass (Fig. 4). In fact, in Expt B-4 maximum I_n was 130 pg N flag.$^{-1}$ d^{-1} when *D. tertiolecta* was prey compared to 53 pg N flag.$^{-1}$ d^{-1} when *Phaeodactylum tricornutum* was eaten. Finally, there was no clear indication from visual inspection of the grazing curves representing Expt D (Fig. 5) that the

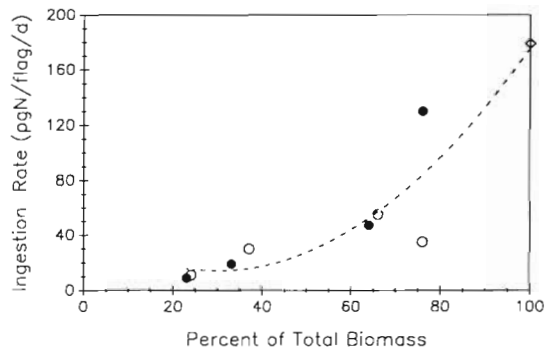

Fig. 4. *Paraphysomonas imperforata*. Effect of contribution to total biomass of *Dunaliella tertiolecta* in mixed prey cultures with *Phaeodactylum tricornutum* (●) and *Isochrysis galbana* (▲) and in control culture (○) on ingestion rate on nitrogen basis

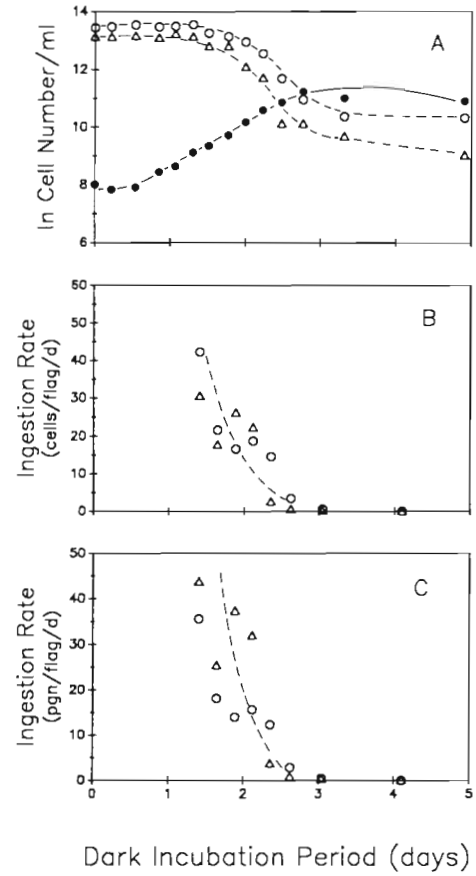

Fig. 5. Time course of grazing by *Paraphysomonas imperforata* (●) on combined prey *Phaeodactylum tricornutum* (○) and *Isochrysis galbana* (▲). (A to C) Expt D-1. (A) Changes in cell numbers. (B) Changes in ingestion rate on cell basis. (C) Changes in ingestion rate on nitrogen basis. Symbols same in all panels

flagellate displayed a food preference when the diet was a mixture of *P. tricornutum* and *Isochrysis galbana* (both prey species appeared to be grazed concurrently).

When the pooled data from Figs. 2, 3 and 5 were examined, there was a positive (and seemingly linear) response in I_n to increasing prey N concentration for the entire range of prey biomasses tested when either *Phaeodactylum tricornutum* (Fig. 6A) or *Isochrysis galbana* (Fig. 6C) was grazed and up to ca 2 mg l^{-1} prey N when *Dunaliella tertiolecta* was grazed (Fig. 6B). These responses occurred regardless of the combination of food sources. To analyze these relationships in a semi-quantitative way, we eliminated some outlying data points from each plot (open symbols in Fig. 6), and used linear regression analysis to determine the slope (= clearance rate C) and correlation coefficient (r) for each resulting curve. The resulting clearance rates as a function of prey species were 7.5×10^{-5} ml d^{-1} for *I. galbana* ($r^2 = 0.81$), 4.9×10^{-5} ml d^{-1} for *P. tricornutum*

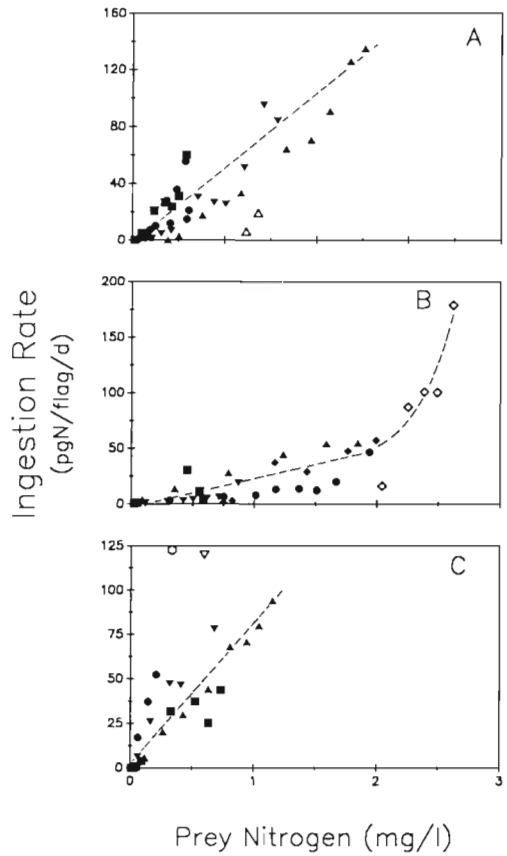

Fig. 6. *Paraphysomonas imperforata*. Effect of prey nitrogen biomass on ingestion rate. (A) *Phaeodactylum tricornutum* as prey: (▼) Expt B-1, (●) Expt B-3, (■) Expt D-1, (▲) Expt A-2. (B) *Dunaliella tertiolecta* as prey: (▼) Expt B-1, (●) Expt B-3, (■) Expt C-1, (▲) Expt C-3, (♦) Expt A-1. (C) *Isochrysis galbana* as prey: (▼) Expt B-1, (●) Expt B-3, (■) Expt D-1, (▲) Expt A-3. Open symbols represent data excluded from linear regression analyses summarized in Table 4

($r^2 = 0.88$), and 2.8×10^{-5} ml d^{-1} for *D. tertiolecta* ($r^2 = 0.64$) (Table 4). However, there was a sudden rise in the slope of the ingestion curve for the chlorophyte at prey N concentrations ≥ 2.5 mg N l^{-1}, indicative of a large increase in C at the higher prey biomass levels. Using estimates of flagellate volume V based on previous experiments when the prey were *D. tertiolecta* and *I. galbana* (Goldman & Caron 1985) and a revised estimate from unpublished Coulter® counter results when *P. tricornutum* was grazed, specific clearance rates C' ($= C\ V^{-1}$) were about equal (1.3 to 1.4 $\times 10^5$ d^{-1}) for grazing on *P. tricornutum* and *I. galbana* and considerably lower (0.3×10^5 d^{-1}) when *D. tertiolecta* was grazed (Table 4). These results are consistent with the order of food preference exhibited by the flagellate, as estimated by visual inspection of the grazing curves.

Also evident was a lower limit in food concentration below which grazing and growth on each prey species ceased. This effect is seen clearly in Figs. 1 to 3 and 5 where the growth curves for *Paraphysomonas imperforata* leveled off and ingestion rates went to zero while prey cell concentrations still were substantial. From visual inspection of the grazing curves (Figs. 1 to 3 and 5) we estimated these lower cell numbers T_c to be ca 4×10^4 to 6×10^4 cells ml^{-1} when *Isochrysis galbana* and *Dunaliella tertiolecta* were the prey to ca 1.2×10^5 cells ml^{-1} when *Phaeodactylum tricornutum* was grazed (Table 4). Corresponding values of T_n ($= T_c Q_n$) were 57 μg N l^{-1} for *I. galbana*, 101 μg N l^{-1} for *P. tricornutum*, and 250 μg N l^{-1} for *D. tertiolecta* (Table 4).

DISCUSSION

Factors influencing prey selectivity by *Paraphysomonas imperforata*

According to Fenchel (1986) raptorial grazers can be distinguished from filter feeders, not only in their ability to graze large prey relative to their own size, but also in that they can discriminate prey on the basis of properties other than size alone. Chemosensory behavior is known to be widespread among protozoa (Van Houten et al. 1981), although, frequently, it is not easy to separate responses to chemical cues from those involving mechanoreception (e.g. size and shape). In the current experiments *Paraphysomonas imperforata* clearly favored the 2 smaller phytoplankton species *Isochrysis galbana* and *Phaeodactylum tricornutum* over the larger species *Dunaliella tertiolecta*, even though the chlorophyte was as acceptable a food source as either of the other 2 species when offered by itself (Table 2). Although specific growth rates μ of the flagellate were unaffected by any of the combinations of prey, the rates (mean 1.51 d^{-1}), for reasons not fully explained, were lower than previously measured under similar experimental conditions (ca 2.5 d^{-1}) (Goldman et al. 1985, 1987).

We have observed the problem of variable μ before (Goldman & Caron 1985) and have suggested that cell aggregation seemed to be enhanced during the course of an experiment when the flagellate was preconditioned in the presence of bacteria, possibly leading to adhesion to surfaces and anomalously low cell count measurements and concomitantly low growth rates. No attempt was made to keep bacteria out of the preconditioning cultures, but since we did not observe any unusual cell aggregation during the experiments, cell aggregation does not appear to be a factor contributing to the lower growth rates observed. The fact that μ was internally consistent in the 13 experiments (Table 2), leads us to believe that the variability observed in μ for *Paraphysomonas imperforata* between these experi-

Table 4. Prey cell concentrations (T_c) and prey N biomass (T_n) for which ingestion rates were zero, and clearance rates for *Paraphysomonas imperforata* grazing on 3 phytoplankton species

Prey species	T_c[a]	T_n[b]	Clearance rate			
			C[c]	r^2[d]	V[e]	C'[f]
Phaeodactylum tricornutum	1.2	101	4.9	0.88	375	1.3
Isochrysis galbana	0.4	57	7.5	0.81	525	1.4
Dunaliella tertiolecta	0.6	250	2.8	0.64	1000	0.3

[a] Prey cell concentration (10^5 ml^{-1}) for zero ingestion rate based on visual inspection of grazing curves in Figs. 1 to 3 and 5
[b] Prey N biomass (μg N l^{-1}) for zero ingestion rate based on product of T_c and Q_n from Table 1
[c] Clearance rates (10^{-5} ml d^{-1}) equal to slopes of curves in Fig. 6 based on linear regression analysis.
[d] Correlation coefficient
[e] Approximate volume (μm^3) of *P. imperforata* when grazing designated species (from Goldman & Caron 1985 and unpubl. data)
[f] Specific clearance rate (10^5 d^{-1}) (= C V^{-1})

ments and our previous work is real and represents a physiological response to some unknown factor or perhaps a life cycle change. Although our strain of *P. imperforata* has been in culture since 1983, we do not believe the observed reduction in growth rate is the result of genetic drift. Specific growth rates have varied irregularly between the current values and ca 2.5 d^{-1} over this period (see Table 2 in Goldman & Caron 1985). Nonetheless, since μ of the control cultures was virtually identical to those of the mixed prey cultures, the lower μ values probably had no effect on the patterns of prey selection by the flagellate.

The major question posed by our results is whether *Paraphysomonas imperforata* grazes by chemosensory responses or by mechanical reception. Several lines of evidence lead us to conclude that both grazing mechanisms may be involved simultaneously. In an earlier study (Goldman & Caron 1985) we were able to show that when 11 different phytoplankton species were offered individually as prey to *P. imperforata*, 5 species spanning a size range from the small chlorophyte *Nannochloris* sp. (diameter 2 μm) to the large centric diatom *Thalassiosira weissflogii* (10 × 14 μm) were marginally ingested and did not support growth. The remaining 6 species along with bacteria, spanning a similar range of prey sizes as the unacceptable food sources and of different taxonomic groups and shapes, were readily grazed and all supported maximal growth rates. On this basis alone, it is evident that mechanoreception is not the only way in which the flagellate chooses food particles. Yet, in the current study, the evidence in favor of a chemosensory response to explain the preference for the smaller diatom and haptophyte over the larger chlorophyte is not so clear.

Flagellates feeding by direct interception can be likened to a spherical collector in a filter bed: the efficiency of grazing as measured by the specific clearance rate is proportional to the prey radius (r) and inversely proportional to the square of the predator radius (R), assuming spherical cells and $R \gg r$ (Fenchel 1986). Without the constraint that $R \gg r$ it can be shown that the specific clearance rate C' of a raptorial grazer is $0.75\ v\ (2Rr + r^2)\ R^{-3}$ where v is the predator's swimming speed. Although this type of approach is simplistic (Fenchel 1986), the relationship is instructive in highlighting some important general features of raptorial grazing. For example, from a resulting nomograph of C' vs R:r for *Paraphysomonas imperforata* of different sizes [representing the effective diameters attained when grazing on each of the 3 test species (Table 4), that is, $R \approx 9$ μm *(Phaeodactylum tricornutum)*, $R \approx 10$ μm *(Isochrysis galbana)*, and $R \approx 12.5$ μm *(Dunaliella tertiolecta)*], and assuming a swimming speed of 100 μm s^{-1}, 2 important points are easily visualized (Fig. 7). First, for each of the prey species, specific clearance rate C' is reduced asymptotically as R:r increases. On this basis alone, not only can we see how raptorial grazing becomes increasingly inefficient as the ratio R:r increases, but also that there is a dramatic increase in C' as R:r decreases below ca 4 1 And second, when R:r is constant C' increases as R decreases. Thus from a hydrodynamic standpoint alone, it pays for a flagellate to alter its own size when grazing on prey species of different sizes so as to minimize R:r and thereby maximize C'. Other protozoa, most notably the ciliates *Didinium nasutum* and *Blepharisma*, have been shown to possess a similar ability to alter their size so as to accomodate prey of different sizes (Giese 1973, Hewett 1980, 1988 and references cited therein).

Clearly, there must be a lower limit to R:r determined by the way in which engulfment takes place. For heterotrophic dinoflagellates it has been shown that $R:r \ll 1.1$ is possible, due to the unique way in which very large prey are captured and engulfed by the

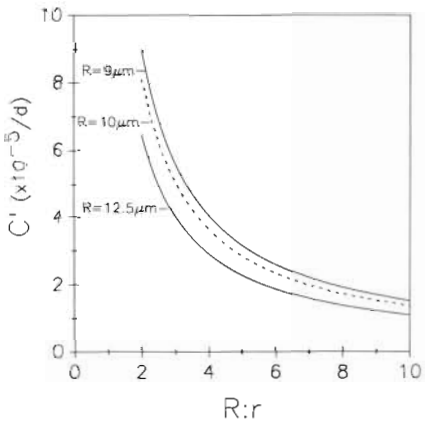

Fig. 7. Relationship between specific clearance rate C' and ratio of predator to prey radii R:r for a raptorial grazer as described in text for predators of different radii

extention of pseudopod-like structures (Gaines & Taylor 1984, Jacobson & Anderson 1986). For *Paraphysomonas imperforata* virtually nothing is known of the way in which capture and engulfment of prey take place. However, based on our earlier studies, it appears that the lower limit to R:r for this flagellate is ca 2:1 (Goldman & Caron 1985). Although we did not measure the size of *P. imperforata* in the current experiments, the preference for *Phaeodactylum tricornutum* and *Isochrysis galbana*, 2 species of intermediate size, over the larger *Dunaliella tertiolecta* is not only predicted by the curves in Fig. 7, since C' decreases as R increases at a fixed value of R:r, but is also consistent with the trend in our estimates of C' (Table 4).

One puzzling aspect of our results was that there was no apparent saturation of the nitrogen ingestion rate I_n at high levels of prey N (Fig. 6). The curves were generated by the combined data from each interval of grazing from the exponential through stationary phases, and not as is customarily done by measuring ingestion rate as a function of initial prey concentration during the exponential phase of separate experiments spanning a range of prey concentrations. Thus it may well be that ingestion rates at low prey N (concomitant with grazing well advanced into the exponential phase) were low due to a slowing of grazing resulting from factors related to the onset of unbalanced growth. If so, the resulting linearity in the ingestion curves for all 3 prey species (Figs. 6A to C) was fortuitous and the values of C' reported in Table 4 are underestimates, a point consistent with the fact that these values are considerably lower than estimates of C' derived from the nomograph for each prey species with R:r = 2:1 (Fig. 7). Alternatively, the values of C' depicted by the nomographs in Fig. 7 are unrealistically high because a capture efficiency of 100% was assumed in developing the relationship between C' and R:r. In reality, capture efficiencies of raptorial grazers may be considerably lower that 100%.

At the lower end of the size spectrum, the preference for a variety of phytoplankton species over bacteria may simply be due to the inability of the flagellate to reduce its own size below some lower limit (which we estimated to be ca 180 μm^3; Goldman & Caron 1985) so that specific clearance rates while grazing on bacteria always would be lower than when larger species were prey. Our earlier results showing higher C' for *Paraphysomonas imperforata* when grazing on *Phaeodactylum tricornutum* compared to bacteria (Caron et al. 1985) support this conclusion and provide an explanation for why the flagellate seems to prefer the herbivorous mode.

While we can see that the food preference displayed by *Paraphysomonas imperforata* is explainable by consideration of hydrodynamic factors, there is still the unanswered question of how the flagellate makes a food selection as a mixed population of acceptable prey is being swept in flow lines that pass alongside of the organism's outer surface; for example, even though prey encounters with raptorial grazers are the result of random contacts, it was evident that the chlorophyte remained uningested in mixed populations with either of the other 2 species until these prey were grazed down to some lower level (Table 3; Figs. 2 and 3). Although we cannot discount the possibility of a chemosensory response, part of the reason for this avoidance may be due to the fact that the flagellate, by reducing its size to about 400 to 500 μm^3 in order to graze the smaller and preferred diatom or haptophyte (and thus increase its specific clearance rate), simply was too small to ingest the larger chlorophyte, which under these conditions was about half the volume of the flagellate. However, grazing on the chlorophyte commenced while there was still 30 to 60% of the diatom and 10 to 20% of the haptophyte cultures remaining (Table 3) and ingestion rates on these 2 prey, although falling rapidly, were still measurable (Figs. 2B, E and 3B, E). In fact, both smaller species continued to be grazed (albeit at slowing rates) along with the chlorophyte until grazing ceased. Thus if the small size of the flagellate alone was the only factor controlling the timing of the start of grazing on the chlorophyte, grazing on both of the smaller prey should have stopped first.

What triggered this change in the mode of grazing by the flagellate is impossible to determine with the data available. It would seem, though, that the flagellate, in some fashion (mechanical or chemical?), was able to sense the diminishing food supply of the smaller prey and the availability of the larger food source and make the switch to the latter. Possibly, this change in feeding strategy may be linked to the way in which ingestion

rates on a nitrogen basis for the flagellate varied in 2 distinct phases, first falling rapidly during late exponential growth in the current experiments even before grazing on the chlorophyte took place, and then increasing as a function of the availability of the alternate food source (Figs. 2C, F, 3C, F and 4). During this period of changing ingestion rate, cell division continued uninterrupted, suggesting that there were concomitant changes in cell volume of the flagellate, first decreasing as the smaller prey was depleted and then increasing as the larger and less preferred food source was grazed and that the initial decrease in cell volume, perhaps to a limiting value of R:r, played a role in the switch to the larger prey. This conclusion is consistent with the fact that even though many protozoa including *Paraphysomonas imperforata* become smaller and reduce their respiration rates as a means of coping with starvation conditions when a particular food source is depleted and growth becomes unbalanced (Fenchel 1982a, Caron et al. 1985), they are able to renew grazing and get larger almost immediately upon being exposed to additional food (Fenchel 1982b). Fenchel (1987) describes this type of feeding, which is common among many raptorial grazers, as an adaptation to a feast or famine existence where the predator is always poised to exploit available food sources in order to avoid starvation. Our earlier observation of *P. imperforata* resorting to cannibalism after grazing a single phytoplankton food source with an increase in the size of a segment of the flagellate population (Goldman & Caron 1985), is an extreme example of how the flagellate copes with changing food supplies.

While we can only speculate as to the mechanisms involved in the food selection patterns of *Paraphysomonas imperforata*, we can conclude that it, and probably many raptorial grazers, are tremendously opportunistic predators that, when faced with starvation conditions (e.g. depletion of a desired food source), can readily adapt to alternate food sources of varying sizes and shapes and maximize their grazing efficiency by adjusting the ratio R:r. Thus, while there is much evidence that food choices among raptorial grazers are influenced to some degree by chemosensory responses (e.g. Sibbald et al. 1987, Bennett et al. 1988), prey selection based on size (and probably shape) remains an important feeding strategy (e.g. Dubowsky 1974, Andersen et al. 1986).

Grazing thresholds

In our previous studies on grazing by *Paraphysomonas imperforata* (Caron et al. 1985, Goldman et al. 1987) and *Oxyrrhis marina* (Goldman et al. 1989) we concluded, based on our observations of what we believed to be threshold concentrations for grazing (ca 10^4 to 10^5 phytoplankton cells ml^{-1} or ca 10^6 bacterial cells ml^{-1}), that these 2 phagotrophs were restricted to grazing in productive waters or to microenvironments (e.g. marine snow) where prey concentrations were elevated considerably above ambient levels. This conclusion may not be entirely correct. Although threshold food levels for protozoan growth commonly have been observed (Taylor 1978, Rivier et al. 1985), and are consistent with the fact that a minimum food level represents the point at which the energy required for growth balances basal metabolic activity, the idea that actual grazing activity ceases when food levels reach a minimum level is not so easily explainable.

The concentrations of prey we have measured corresponding to zero ingestion rates always occurred at the end of exponential growth when predator and prey populations were about equal and not early in the growth phase under the general conditions of very low prey concentrations and far lower predator concentrations. The former conditions are similar to those found by Borsheim & Bratbak (1987) and Geider & Leadbeater (1988) for some bacterivorous flagellates and by Luckinbill (1973, 1974) and Salt (1974, 1979) in their studies on grazing of one ciliate *(Paramecium)* by another *(Didinium)*. These latter researchers concluded that severe competition among predators for the relatively small number of prey at the tail end of batch growth led to starvation conditions which, in turn, caused reductions in swimming speeds and, concomitantly, to massive reductions in prey capture efficiencies. Thus some caution must be exercised in viewing our cell concentrations corresponding to zero grazing as true grazing thresholds in nature where prey populations generally exceeded those of their protozoan predators by orders of magnitude. There are, however, indications from other studies on both flagellates and ciliates that true grazing thresholds do exist (Davis & Sieburth 1984, Rivier et al. 1985). Taylor (1978), in fact, did not discount the possibility that the thresholds for growth he measured for bacterivorous ciliates might be the net result of growth and grazing thresholds; he suggested that searching for food in a more enriched environment by protozoa might be an alternative to grazing very low food concentrations.

Ecological importance of herbivory among flagellates

Herbivory, as practiced by raptorial flagellates such as *Paraphysomonas imperforata*, may provide an important link in the microbial food loop first envisioned by Pomeroy (1974) and Azam et al. (1983). Because of their tremendous versatility in grazing a wide size range of prey and their ability to switch prey

rapidly once a particular food source become scarce, raptorial grazers, including a variety of larger chrysomonads and dinoflagellates, may be effective competitors with ciliates for the nanoplankton size class (2 to 20 µm) of phototrophs and heterotrophs. In fact, *P. imperforata* is capable of grazing smaller bacterivorous flagellates (Goldman unpubl.). This would not only leave the smaller flagellates to graze bacteria, but contributes to making the food chain (web) within the microbial loop long and complicated with high losses of energy and materials (Caron et al. 1985, Goldman et al. 1985).

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