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Grazing on phytoplankton prey by the heterotrophic microflagellate *Paraphysomonas vestita* in non-balanced growth conditions

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Summary

The predatory behaviour of a phagotrophic microflagellate, *Paraphysomonas vestita*, was studied in the transient conditions of a laboratory batch culture. Two phytoplankton species of the same size (*Isochrysis galbana* and *Pavlova lutheri*) were used as alternative prey items. The rate of ingestion of phytoplankton and the *P. vestita* cell yield were similar in the presence or absence of bacteria, indicating that phytoplankton were selected as prey in preference to bacteria by *P. vestita*. In terms of cell numbers, *P. vestita* ingested *I. galbana* more rapidly than *P. lutheri* although both species are prymnesiophytes of similar size and were available at a similar carbon:nitrogen ratio. The difference in ingestion rate was not the result of *P. lutheri* being a poor prey species but could be related to the higher biomass content (in terms of C and N) of *P. lutheri* compared to *I. galbana*. Maximum ingestion rates in terms of dry weight biomass were similar for the two prey species. Maximum rate of ingestion of prey and maximum rate of microflagellate division were related to the inoculum prey concentration in a hyperbolic manner for each prey species. As prey density decreased, the ingestion rate at a particular time was not correlated with prey density at that time. Rather, non prey saturated ingestion rate was found to be better represented by a hyperbolic function of the prey/predator ratio. A threshold prey concentration was observed in all experiments and was higher when *P. lutheri* was the prey species. The threshold prey concentration increased with increasing inoculum prey density. However, in terms of prey/predator ratio a single threshold value was observed for both prey species.

Key words: *Paraphysomonas vestita, Isochrysis galbana, Pavlova lutheri*, non-balanced growth, prey/predator ratio

Introduction

Predatory microflagellates are now recognised to be important parts of marine planktonic food webs (Laybourn-Parry, 1992). They are capable of ingesting both phytoplankton and bacteria and exhibit high weight specific rates of nutrient regeneration (Caron, 1991). These phagotrophic species play a pivotal role in the dynamics of marine ecosystems, providing a link for nutrients to pass up the food chain through their ingestion by metazoan grazers that are unable to graze small phytoplankton and bacteria.

Numerous studies have been conducted to investigate the predatory behaviour of heterotrophic microflagellates (see the review: Capriulo, 1990). Many relate to the ingestion of bacteria. Phytoplankton has a much greater size range and potential for variation in nutritional quality (in terms of C and N). If we are to understand the dynamics of microbial food webs it is important to determine the mechanisms that govern the ingestion of phytoplankton as well as bacteria by microflagellate predators.

Studies of predation by microflagellates on phytoplankton have indicated that the rate of ingestion of prey may be related to a variety of factors. These include: prey size (Andersson et al., 1986; Goldman and Dennett, 1990; Epstein and Shiaris, 1992), whether the prey are alive or dead (Landry et al., 1991), nutritional quality (Goldman et al., 1985, 1987; Peters, 1994; Jurgens and DeMott, 1995;

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Flynn et al., 1996) physical conditions (Peters and Gross, 1994; Shimeta et al., 1995; Peters et al., 1996) and chemosynthesis or sensory detection (Sibbald et al., 1988).

A number of studies, including Fenchel (1982), have calculated the maximum specific growth rate of microflagellate predators in individual experiments in which the prey density is sufficiently high that it does not change significantly during the course of the experiment. This allows the experiments to be conducted in conditions approximating to balanced (exponential) predator growth. By plotting specific growth rate versus prey density at a number of different prey densities a hyperbolic Holling type II functional response (Holling, 1965) is often obtained. Eccleston-Parry and Leadbeater (1994a, 1994b), found a similar functional response when plotting maximum specific growth rate versus prey inoculum concentration in their microflagellate grazing experiments.

However, in the oceans and shelf seas transient rather than balanced growth conditions often exist. Caron et al. (1990a) highlighted that protozoa do not experience a homogeneous environment and are therefore unlikely to achieve prolonged periods of exponential growth, and Parslow et al. (1986) suggested that the extrapolation of steady state results to transient conditions should be carried out with caution. Therefore, if we are to understand and potentially model microbial food web dynamics it is necessary also to study non-balanced growth conditions.

In a series of papers, Goldman and colleagues (see Goldman et al., 1985) studied the non steady-state ingestion by *Paraphysomonas imperforata* of both phytoplankton and bacteria. During these studies they observed that phytoplankton were ingested in preference to bacteria and that certain phytoplankton species were ingested in preference to others, a phenomenon that they accredited to differences in cell size (Goldman and Dennett, 1990).

Although prey size undoubtedly affects grazing rates, as noted above, other factors may also exert a significant influence. However, not least because of methodological problems, quantitative studies of other potentially important factors that determine grazing rates, particularly in transient conditions, are rare.

The aim of this study was therefore to extend the work of Goldman et al. to investigate grazing in non-balanced growth conditions, by the microflagellate *Paraphysomonas vestita*, on alternative phytoplankton species of similar size and C:N ratio. We sought to investigate if the grazing behaviour of the microflagellate is related to factors other than cell size and if the functional relationships commonly derived to represent prey ingestion and predator division in balanced growth were applicable in non-balanced growth conditions.

Material and Methods

The heterotrophic microflagellate *Paraphysomonas vestita* (average diameter 9µm), isolated from Southampton water by Dr S. Tong was used as the predator in our study. Stock cultures were maintained in batch culture in a medium of aged filtered seawater, previously sterilised by autoclaving. Axenic additions of the appropriate phytoplankton prey species (see below) were conducted regularly to maintain predator population density.

Phytoplankton prey species were *Isochrysis galbana* Parke CCAP 927/1 and *Pavlova lutheri* (Droop) Green CCAP 931/1. Both species are marine prymnesiophytes of similar spherical shape with a diameter of approximately 4.5µm. Phytoplankton stock cultures were grown axenically as batch cultures, inoculated by the addition of cells to a growth medium consisting of sterile aged filtered seawater. Nitrogen was available as nitrate at a concentration of 200mM, with all other nutrients available in excess. Cells were grown in continuous light (250 µmol m^{-2} s^{-1}) at 18°C until they had exhausted extracellular nitrate and had just reached stationary phase.

Grazing experiments were conducted using *P. vestita* as the predator and either *I. galbana* or *P. lutheri* as prey. Early stationary phase prey cells were taken from the stock flasks, added to the experimental flasks and diluted to the required density using sterile, aged, filtered seawater to which no nutrients had been added. *P. vestita* cells were generally taken from stock cultures that had reduced prey levels to a minimum and had hence ceased dividing, but had yet to show significant sign of cell loss through death and/or cannibalism.

In all experiments prey and predators were added aseptically to the experimental flasks at the required concentrations. Duplicate experiments (at each prey and predator inoculum density) were conducted in sterile 100ml flasks. The experiments were conducted in continuous darkness at 18°C to prevent prey growth (confirmed by observing predator free control cultures in identical conditions). Samples were removed from the flask for enumeration two or three times a day. The experiments were concluded when phytoplankton prey density had been reduced to a constant low threshold concentration and predator density had reached a maximum and had begun to decrease due to cell death.

Experiment 1. Experiments were conducted at the same phytoplankton and microflagellate inoculum densities both with and without the presence of bacteria. In the flasks containing bacteria a known concentration of the bacterium *Vibrio natrigens* was added. Comparative experiments in which different concentrations of bacteria were present at inoculum were conducted. Bacterial stock cultures were grown in a medium prepared with 5% (w/v) of a stock solution containing 0.8g l^{-1} yeast extract and

$4g^{-1}$ proteose peptone (Eccleston-Parry and Leadbeater, 1994a).

Experiment 2. Subsequent experiments were conducted using a greater range of phytoplankton inoculum densities for both a) *I. galbana* and b) *P. lutheri*. The predator inoculum concentration was held approximately constant in all. Bacterial inoculum concentrations were either zero or constant for a particular set of experiments.

Experiment 3. The influence of changes in the predator inoculum density and "fitness" was investigated. In the above experiments the predator inoculum density was held constant and different prey inoculum densities similar to those that might be expected during a phytoplankton bloom (in the range of 1x 10^5 or 2 x 10^6 cells ml^{-1}) were studied. A set of experiments was therefore conducted with a single prey inoculum density (1 x 10^6 cells ml^{-1}) and various predator densities to determine if predator density influenced the conclusions drawn. Experiments were also conducted using predators of varying physiological state (more or less starved) at inoculum to investigate the possible effect of physiological stress on microflagellate grazers noted by Choi (1994).

In all experiments, phytoplankton and microflagellate cell number densities were determined by making triplicate counts using an electronic particle counter (Coulter Instruments Multisizer II fitted with a 50μm diameter orifice) which also gave volume information. A sub sample was removed from the experimental culture and diluted in sterile filtered seawater to reduce coincidence counts on the particle counter. The predator and prey could easily be distinguished in terms of cell size: the estimated spherical diameter of both prey being 4.5μm and that of the predator 8–10μm. Bacterial densities were determined by staining for five minutes with 5μg ml^{-1} of 4', 6'-diamidino-2-phenylinodole (DAPI). Stained cells were filtered onto black 0.2μm pore size, 25mm diameter polycarbonate filters (Osmonics inc) with a 0.2μm cellulose nitrate backing filter. The filter was mounted onto a slide and stored at –20°C prior to analysis. Cells were enumerated using a Zeiss Axiovert 100s microscope with a UV excitation block at 1000 times magnification. Bacterial density was determined by counting using a calibrated ocular grid. Bacterial densities were determined in experiments with and without added bacteria, the latter in order to check that bacteria free cultures remained axenic.

Determination of particulate C and N was carried out by collecting cells on pre-ashed 13mm Gelman A/E filters with blank filter controls. Samples were analysed using a Carlo Erba NA 1500 elemental analyser.

Results

Experiment 1: Influence of bacteria on ingestion of phytoplankton

The majority of the experiments were conducted in axenic conditions. However, experiments were also conducted to determine the influence of bacteria. These experiments allowed us to determine if the presence or absence of bacteria would influence the generality of our conclusion when extrapolated to natural conditions where both phytoplankton and bacterial prey items are available to microflagellate predators.

Batch culture experiments were conducted with *P. vestita* as predator (inoculum concentration ~ 8 x 10^3 cells ml^{-1}) and phytoplankton prey: *I. galbana* or *P. lutheri.* Phytoplankton prey densities at inoculum were either high (~ 1.2 x 10^6 prey ml^{-1}, or low (~ 1 x 10^5 prey ml^{-1}). Finite (either 2 x 10^6 cells ml^{-1} or 2 x 10^7 cells ml^{-1}) or zero concentrations of bacteria were also introduced at inoculum. Duplicate experiments were conducted at each permutation of inoculum cell densities. Phytoplankton alone, bacteria alone and phytoplankton/bacteria control cultures were conducted.

At both high and low phytoplankton inoculum densities, *P. vestita* actively ingested phytoplankton. When present, bacteria stayed constant or showed a slight increase and densities were similar to predator free control cultures. Furthermore, the rate of microflagellate division and cell yield was similar in the presence or absence of bacteria. The results therefore suggested that bacteria were rejected as prey in the presence of phytoplankton. The results of one set of experiments are shown in Fig. 1.

Experiment 2a: Experiments with *I. galbana* as the sole prey species

Replicate grazing experiments were conducted using *I. galbana* as the prey species, at each of seven prey inoculum densities, Table 1. In each experiment the prey C:N ratio was approximately 8 and the inoculum density of *P. vestita* was approximately $1x10^4$ cells ml^{-1}. *I. galbana* cell numbers decreased during the experiments and *P. vestita* numbers increased (Fig. 2). *I. galbana* was ingested to a low threshold density in all experiments. This threshold density decreased with decreasing prey inoculum density (Table 1a).

Control cultures indicated that no significant net change in prey numbers occurred due to processes other than grazing. It was therefore possible to calculate the rate of ingestion of prey from a knowledge of *I. galbana* and *P. vestita* cell densities using an equation of the form:

$$I = \frac{Iso_{to} - Iso_{t1}}{\frac{(Para_{t1} - Para_{to})}{\ln(Para_{t1}) - \ln(Para_{t0})}.(t_1 - t_o)}$$

where: I is the ingestion rate (*I. galbana*).(*P. vestita*)$^{-1}$. hr^{-1}, Iso: *I. galbana*, Para: *P. vestita* and t_0 and t_1 refers to the time of the two (consecutive) sampling points under consideration.

Specific division rate (μ, hr^{-1}) of *P. vestita* was also determined from:

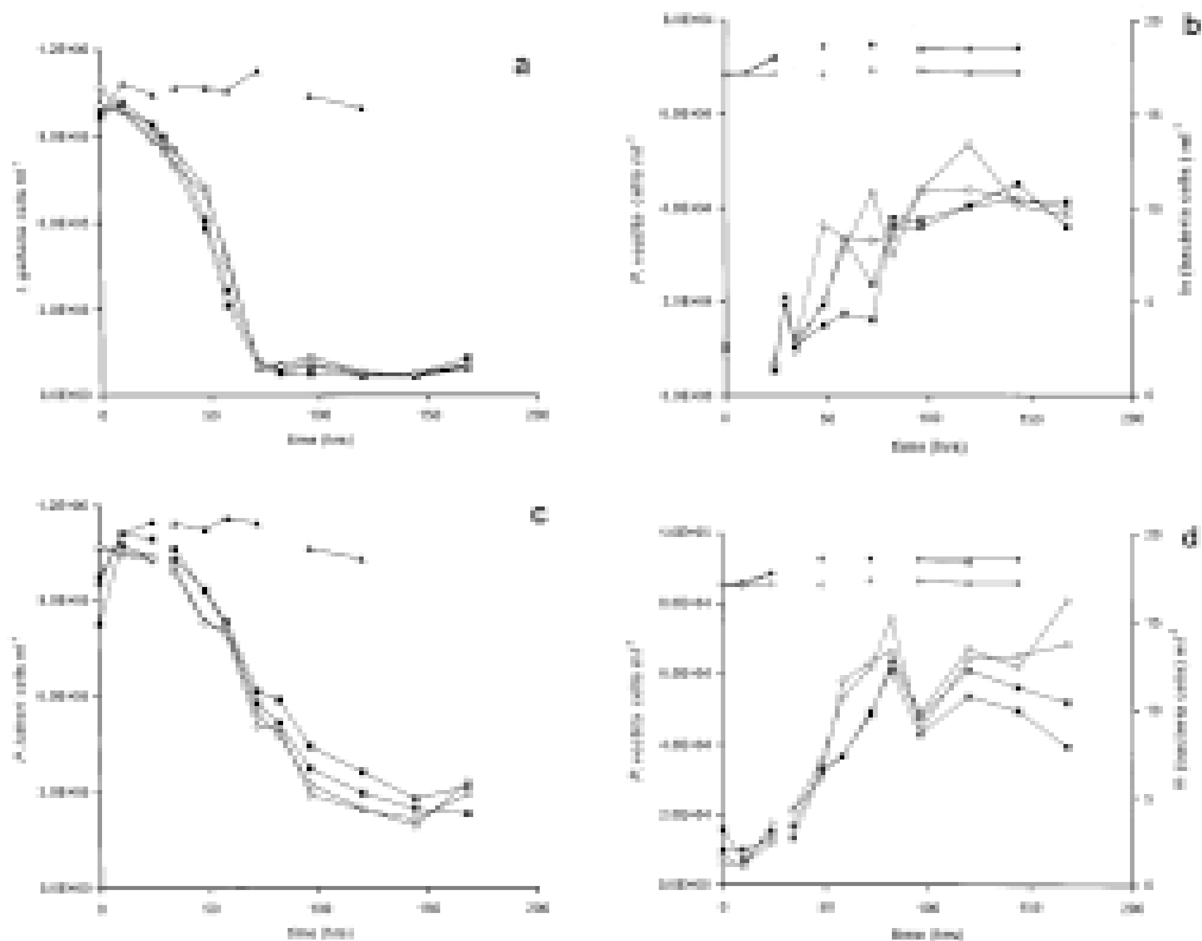

Fig. 1. Changes in cell density for the prey species, *I. galbana* (**a**) and *P. lutheri* (**c**), during predator/prey experiments (open circles – without bacteria, closed squares – with bacteria, triangles – prey density in predator free control cultures); changes in the predator, *P. vestita*, cell numbers and bacterial densities when *I. galbana* (**b**) and *P. lutheri* (**d**) was the prey species (open circles – without bacteria, closed squares – with bacteria, closed diamonds – bacterial densities in the predator/prey experiments that included bacteria, open diamonds – bacterial densities in bacteria alone control cultures).

$$\mu = \frac{\ln(Para_{t1}) - \ln(Para_{to})}{(t_1 - t_o)}$$

Ingestion rate and specific division rate were calculated for each of the experiments using the above equations. Use of these equations requires the assumption of exponential predator growth. Previously Fenchel (1982) highlighted the difficulties in determining ingestion and growth rates from batch style experiments in which extended periods of exponential growth may not occur. As we did not wish to make the a priori assumption of balanced growth in our experiments we also calculated the rates using a linear rather than an exponential approximation. Similar results were obtained for both I and μ as for the above equations with a maximum discrepancy of 6% and typically less that 1%.

Calculation of ingestion rates from such batch data in which the densities of two quantities (prey and predator) may be moving rapidly in the opposite direction unavoidably results in noisy data. Goldman et al. (1985) smoothed their data by drawing a best fit by eye through prey and predator density plots. We chose instead to apply a three point moving average to the derived values prior to their use in further calculations or relationships. Either approach allows us to use carefully controlled batch or stretch batch cultures in the study of non-balanced growth.

Balanced growth functional response

Functional response curves relating maximum ingestion or division rates to inoculum prey density were calculated for each set of experiments at different prey inoculum concentrations. Both maximum ingestion rate (I_{max}) and maximum specific division rate (m_{max}) were found, Table 1, to be related to prey concentration at inoculum (as opposed to prey concentration at the time I_{max} and μ_{max} occurred) in a hyperbolic manner (Fig. 3a, b), hereafter referred to as the prey density functional response. Such results are similar to those found in other studies

Table 1a. Parameter values observed in the 1st set of *I. galbana / P. vestita* experiments

I. galbana inoculum ml^{-1}	*I. galbana* threshold ml^{-1}	Prey/predator at inoculum	I_{max} *I. galbana. P. vestita*$^{-1}$ hr^{-1}	μ_{max} hr^{-1}
$2x10^6$	1.0 ± 0.1 $x10^5$	148	2.48 ± 0.04	0.056 ± 0.001
$1x10^6$	7.28 ± 1.51 $x10^4$	74	2.19 ± 0.45	0.039 ± 0.001
$5x10^5$	5.01 ± 0.35 $x10^4$	37	1.61 ± 0.50	0.035 ± 0.004
$1x10^5$	3.64 ± 0.05 $x10^4$	7	0.64 ± 0.15	0.020 ± 0.001
$8x10^4$	2.93 ± 0.26 $x10^4$	6	0.48 ± 0.19	0.018 ± 0.004
$5x10^4$	2.52 ± 0.01 $x10^4$	4	0.30 ± 0.04	0.020 ± 0.006
$3x10^4$	1.85 ± 0.07 $x10^4$	2	0.19 ± 0.01	0.018 ± 0.009

Table 1b. Parameter values observed in the 1st set of *P. lutheri* / *P. vestita* experiments

P. lutheri Inoculum ml^{-1}	*P. lutheri* threshold ml^{-1}	Prey/predator at inoculum	I_{max} *P. lutheri. P. vestita*$^{-1}$ hr^{-1}	μ_{max} hr^{-1}
2×10^6	$7.11\pm0.30x10^5$	36	0.72 ± 0.07	0.029 ± 0.004
1.25×10^6	4.08 ± 0.13 $x10^5$	23	0.85 ± 0.24	0.026 ± 0.001
6×10^5	1.86 ± 0.08 $x10^5$	11	0.73 ± 0.08	0.026 ± 0.001

Where I_{max} is the average maximum observed ingestion rate, μ_{max} the average maximum observed specific *P. vestita* division rate. Values presented are the average of the replicate experiments at the different prey inoculum densities ± the standard deviation.

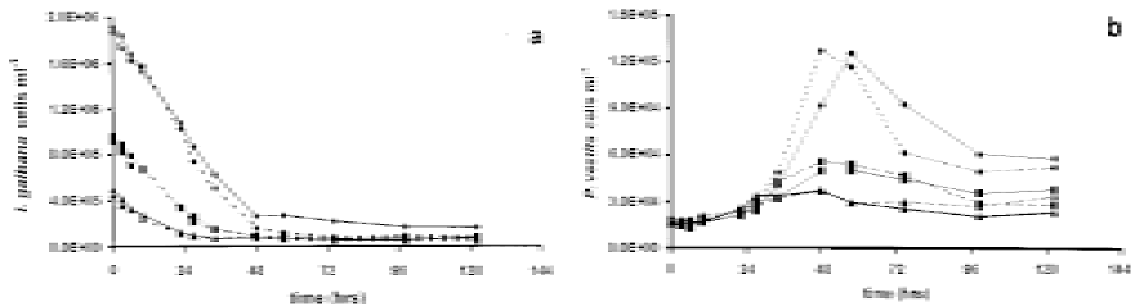

Fig. 2. Changes in cell density of prey (**a**) and predator (**b**) when *I. galbana* was the prey species (for clarity only the three highest of seven prey densities used are illustrated); duplicate experiments were conducted: denoted by solid lines and dashed lines respectively.

(Fenchel, 1982; Eccleston-Parry and Leadbeater, 1994a,b). Rectangular hyperbolae were fitted to these data using a least squares fitting procedure (that minimised least squares of residuals). The values of I_{max} and half saturation constant for ingestion K_I, m_{max} and half saturation constant for division Km were determined (Table 2a,b).

The maximum rate of ingestion or division predicted using such numerical fitting procedures is that which occurs at infinite substrate (in this case prey) concentration. This maximum calculated value therefore often exceeds the maximum observed value (see Table 1). The maximum rate of ingestion of prey occurred early in the experiments when prey density was high. However, maximum specific division rate of *P. vestita* was recorded some time later. There was therefore a poor correlation between ingestion rates and predator division rates (not shown).

Functional response in non-balanced growth phase

Functional response relationships were also determined during the unbalanced growth phase of the

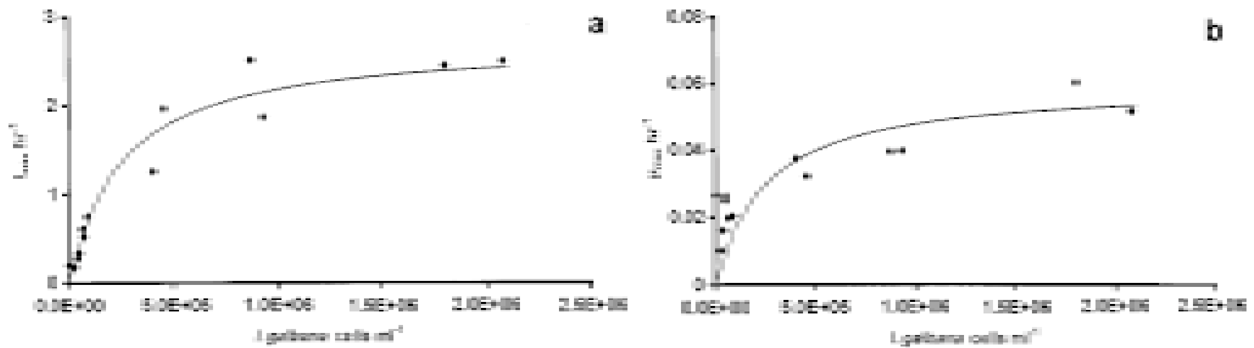

Fig. 3. Functional response curves for *P. vestita* ingesting *I. galbana*. Maximum observed ingestion rate, I_{max} (**a**) or predator specific division rate μ_{max} (**b**) plotted against inoculum prey density (solid lines are fitted rectangular hyperbolae. (r^2 = 0.95 and 0.79 respectively; see Table 2 for parameter values).

experiments (when prey and predator densities were changing). Ingestion rate was calculated (from the three point averaged data) at each time point during each of the experiments and was related to the prey density at the equivalent time. Plotting this data (Fig. 4a) we see that in the different cultures *P. vestita* ingested prey of a particular density at different rates.

Ingestion was therefore not a unique function of prey density in these transient conditions. Moreover, the prey density functional response derived for balanced growth (see Fig. 3a) was unable to simulate ingestion rates in these rapidly changing transient conditions. This is illustrated by superimposing the hyperbola derived from Fig. 3a on Fig. 4a.

Prey/predator ratio

The failure of the prey density functional response led us to seek an alternative method of relating ingestion rates during non-balanced growth conditions to some other readily measured quantity or quantities. In a series of papers (Berryman, 1992 and others) it was suggested that ingestion rate might be better related to the prey/predator ratio than simply to prey density. Relating per-individual consumption rates to prey density (as above) assumes there is no interference between consumers. However, should competition between consumers occur, then the resultant sharing of resources is better described by a functional response based on the prey/predator ratio. Based on the average swimming speed and hence volume cleared by heterotrophic flagellates, we estimated that the density of microflagellates in our experiments (and during phytoplankton blooms) was sufficient for competition between predators for prey to occur.

Ingestion rate was therefore plotted as a function of the prey/predator ratio for these experiments (Fig. 4b). A single functional response was found to be capable of describing the complete set of experiments carried out at different prey inoculum densities. A rectangular hyperbola was fitted to the data, this gave a maximum ingestion rate of 2.9 *I. galbana.P. vestita*$^{-1}$ hr^{-1}, a half saturation constant of 29 *I. galbana/P. vestita* and a threshold prey/predator concentration of 2 (Table 2).

Experiment 2b: Comparative grazing experiments using P. lutheri as prey

The grazing response of *P. vestita* on the alternative prey species (*P. lutheri*) was also investigated. To ensure that comparisons could be drawn between experiments with the two different prey species, parallel experiments were conducted with *I. galbana* and *P. lutheri* as prey, using predators obtained from the same stock flask. Replicate

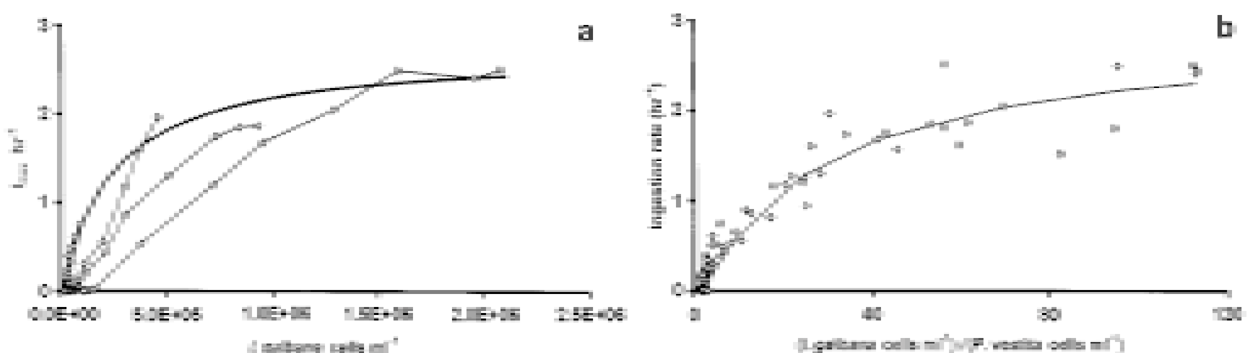

Fig. 4. The rate of ingestion of *I. galbana* by *P. vestita* as a function of prey density (**a**) and prey/predator ratio (**b**) at the equivalent time (in **a** each thin line relates to a different culture started from a different inoculum density, and the thick line is the hyperbola relating maximum ingestion rate to inoculum prey density (from Fig. 3a); in **b** the thin line is a fitted hyperbola relating ingestion rate to prey/predator ratio; r^2 = 0.94, parameters in Table 2).

Table 2a. Hyperbolic ingestion equation parameters

Parameter	Function of:	Units	*P. lutheri* as prey	*I. galbana* as prey
I_{max}	Prey density	Prey predator^{-1} hr^{-1}	0.7	2.7
K_I	Prey density	Prey ml^{-1}	2.6x10^5 *	2.5x10^5
I_{max}	Prey/predator	Prey predator^{-1} hr^{-1}	0.9	2.9
K_I	Prey/predator	Prey predator^{-1}	10	29
X_o	Prey/predator	Prey predator^{-1}	2	2

Table 2b. Hyperbolic *P. vestita* division equation parameters

Parameter	Function of:	Units	*P. lutheri* as prey	*I. galbana* as prey
μ_{max}	Prey density	hr^{-1}	0.04	0.06
K_μ	Prey density	Prey ml^{-1}	**	2.5x10^5
μ_{max}	Prey/predator	hr^{-1}	0.04	0.05
K_μ	Prey/predator	Prey predator^{-1}(t-τ)	**	26
X_o	Prey/predator	Prey predator^{-1} (t-τ)	**	2

Maximum ingestion (I_{max}) and division (μ_{max}) rates calculated by fitting hyperbolae to data (using a computer program that minimised least squares of residuals). The r^2 values for the fitted lines are presented in the legends of the appropriate figures.

K_I is the half saturation constant for ingestion (in terms of prey ml^{-1} or prey/predator ratio as appropriate) K_μ is the half saturation constant for division and X_o the threshold prey/predator concentration.

t-t indicates the value of prey/predator ratio used was calculated t hours previously.

* Due to the large variation in threshold prey densities that were observed with *P.lutheri* as prey (see Fig. 6a) this (or any single value) of the half saturation constant gave a poor fit to the data. This indicates the inadequacy of relating ingestion rate to prey density for this species.

** The range of μ_{max} values obtained in the *P.lutheri* experiments was insufficient to allow us to fit functional response curves.

predator/prey experiments were conducted at three prey densities (it was necessary to reduce the number of different inoculum prey densities studied as two prey species were now being sampled). C:N ratio was in the range 6–8 for both prey species.

The results for the experiments using *I. galbana* as prey coincided well with those detailed above. We therefore only report the results with *P. lutheri* as prey below. We found (Fig. 5, c.f. Fig. 2) that *P. lutheri* was ingested more slowly than *I. galbana* in terms of cell number. I_{max} was 0.7 prey predator^{-1} hr^{-1} compared to a value of 2.5 for *I. galbana*. This could be related to the marked difference in biochemical composition of the two prey species that is known to exist. Brown (1991) found that cultures of *P. lutheri* had a dry weight per cell approximately three times greater than that of *I. galbana:* 102.3 cf. 30.5 pg cell^{-1} (in both cases the cells were collected at the end of the expo-

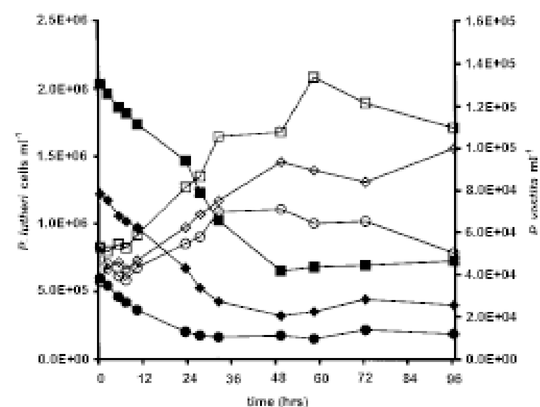

Fig. 5. *P. lutheri* (closed symbols) and *P. vestita* (open symbols) cell density in one of the replicate *P. lutheri* / *P. vestita* experiments (the same symbols types have been used depict prey and predator at each prey inoculum density).

nential phase of growth). Converting ingestion rates into terms of dry weight biomass we therefore find that the maximum ingestion rate is similar for the two prey species: 71.6 cf. 76.2 pg predator $^{-1}$ hr^{-1} for *I. galbana* and *P. lutheri* respectively.

As observed for *I. galbana*, a plot of ingestion rate versus *P. lutheri* density yields a different relationship, in transient conditions, for each different prey inoculum density (Fig. 6a). However if we plot ingestion rate versus the prey/predator ratio at that time, we find, as for *I. galbana*, a single hyperbolic function is generated (Fig. 6b).

***P. vestita* division (considering experiments with both prey species)**

As for ingestion rate, the specific rate of *P. vestita* cell division was plotted against both prey density and prey/predator ratio. A complex "humped" relationship was obtained for this quantity as a function of either prey density or prey/predator ratio for both prey species. This is illustrated for prey/predator ratio with *I. galbana* as prey in Fig. 7a. Such a relationship is not easily described mathematically with biologically meaningful parameters and is therefore of limited predictive value. Moreover, the relationship was not constant between experiments indicating that specific division rate was not a unique function of prey density or prey/predator ratio. This lack of correlation was a result of the time taken by the predator to digest a prey item (division rate increased as prey density decreased at the start of the experiment).

In order to identify a functional relationship it was assumed that prey digestion takes a time, t, of eight hours (based on the relative volume of prey and predator and the number of prey that must be ingested to produce a new predator). Specific predator division rate as a function of

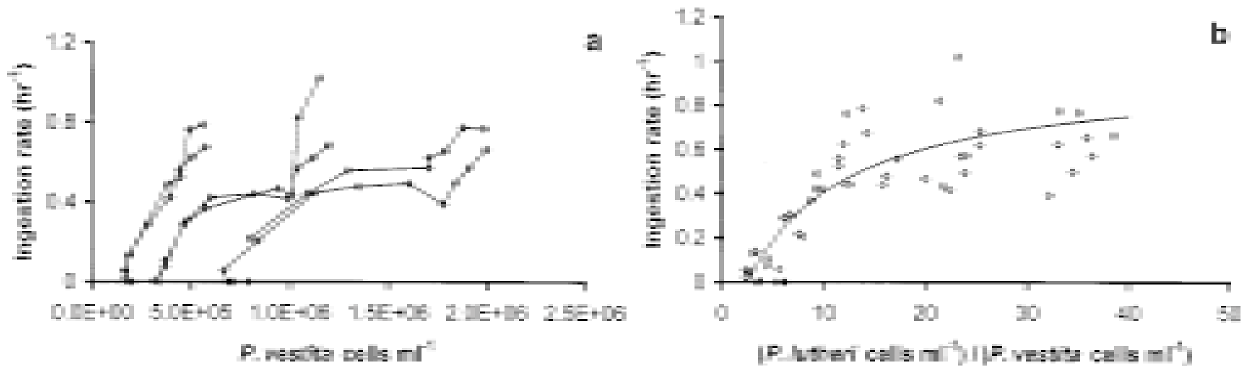

Fig. 6. The rate of ingestion of *P. lutheri* by *P. vestita* as a function of prey density (**a**) and prey/predator ratio (**b**) (the solid line is fitted rectangular hyperbola; parameters in Table 2, r^2=0.77).

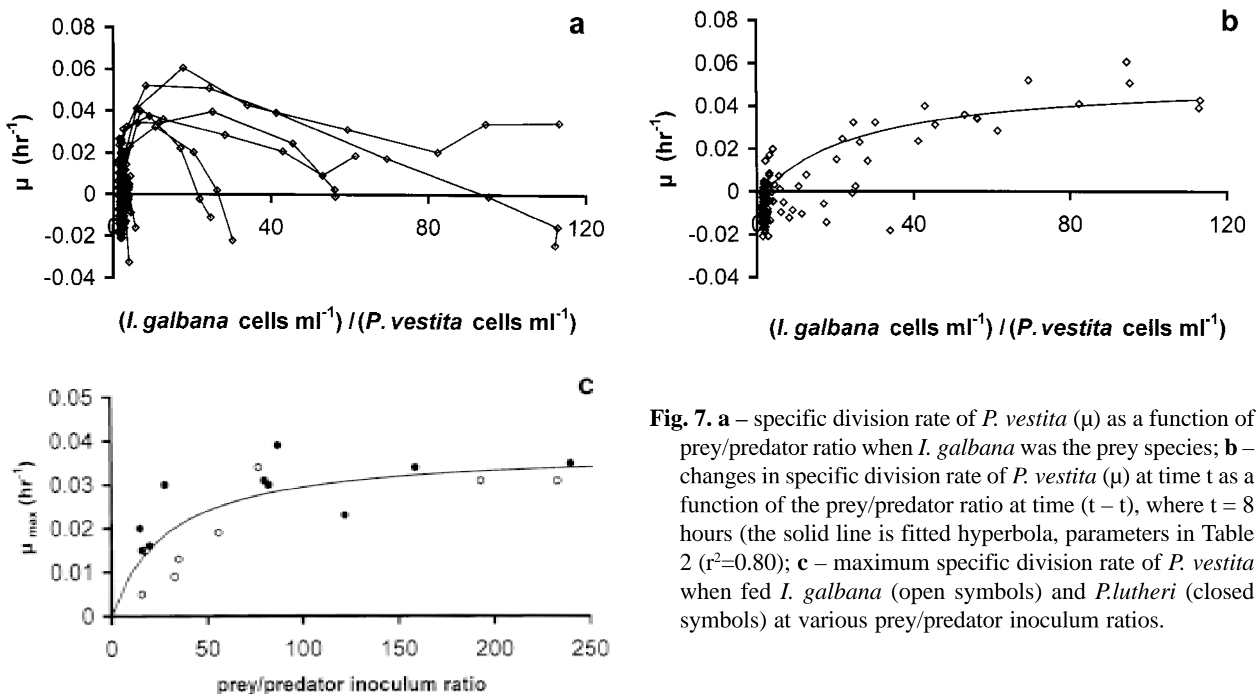

Fig. 7. a – specific division rate of *P. vestita* (μ) as a function of prey/predator ratio when *I. galbana* was the prey species; **b** – changes in specific division rate of *P. vestita* (μ) at time t as a function of the prey/predator ratio at time (t – t), where t = 8 hours (the solid line is fitted hyperbola, parameters in Table 2 (r^2=0.80); **c** – maximum specific division rate of *P. vestita* when fed *I. galbana* (open symbols) and *P.lutheri* (closed symbols) at various prey/predator inoculum ratios.

prey/predator ratio at (time – t) was then plotted and found to be a hyperbolic, and potentially predictive, function (Fig. 7b, Table 2b).

Maximum observed specific division rate of *P. vestita* when ingesting *P. lutheri* in experiment 2b was 0.03 hr^{-1}, somewhat less that the value of 0.056 hr^{-1} observed when *I. galbana* was the prey item. However, the specific predator division rates at similar prey/predator ratios were found to be similar for the two prey species. A higher predator inoculum density was used in experiment 2b resulting in a smaller range of inoculum prey/predator ratios. The predator division rate may therefore have not reached its maximum.

In order to determine if the maximum division rate of *P. vestita* was the same when feeding on the two different prey items, we conducted a further set of experiments using a wider range of *I. galbana* and *P. lutheri* prey/predator ratios. In these experiments, four (duplicated) inoculum prey densities ranging from $5e^4$ to $2e^6$ prey ml^{-1} were used. This resulted in prey/predator inoculum ratios from approximately 40 to 250. These experiments indicated (Fig. 7c) that the maximum specific division rate at saturating prey/predator ratios was similar for the two prey species. Furthermore, the relationship between maximum specific division rate and prey/predator ratio could be described by a single hyperbolic function for both prey species.

Threshold prey density (considering experiments with both prey species)

Prey threshold density was considerably higher at a particular prey inoculum density when *P. lutheri* was the prey species compared to *I. galbana* (Fig. 5, Fig. 2) for a particular prey inoculum density. For both prey species the threshold was not constant and increased with increasing prey inoculum density. However, a single threshold prey/predator ratio existed (Figs 4b, 6b) for each species. The threshold prey/predator ratio was similar for both species, at a value of approximately 2.

Experiment 3. Changes in predator inoculum density and physiological state

The above experiments were conducted with similar predator inoculum densities and with predators in similar physiological states (having just reached starvation). Therefore we next sought to investigate if either predator inoculum density or degree of starvation was capable of influencing the functional responses obtained.

Two sets of duplicated experiments were conduced. First in which a single prey density (1×10^6 prey ml^{-1}) was used but the predator density was varied (inocula of 4×10^3, 6×10^3, 8×10^3 and 1.5×10^4 *P. vestita* ml^{-1} were used). Second in which predator and prey density at inoculum were held constant between experiments (8×10^3 and 1×10^6 cells ml^{-1} respectively) but the *P. vestita* was subjected to greater (predator numbers decreasing in stock flask) or lesser (predators still actively ingesting prey in stock flask) degrees of starvation prior to the experiments. In both cases we found that the functional response in terms of ingestion versus prey/predator ratio was similar to that obtained above (data not shown).

Discussion

Balanced protozoan growth probably rarely occurs in natural planktonic assemblages (Caron, 1990b). Microflagellates will commonly find themselves in an environment in which prey density varies rapidly and is not saturating for ingestion and growth i.e. in unbalanced non-steady state conditions. This will be particularly evident following the peak of a phytoplankton bloom. At this time phytoplankton densities are relatively high (although not necessarily saturating for ingestion) and declining, while microflagellate densities are increasing. It is just such a scenario of non-balanced growth that our time course experiments best represent.

Two common approaches to determining microflagellate grazing and division rates are a) relatively short term experiments employing high prey densities b) experiments using the dilution technique of Landry and Hassett (1982). These approaches have been successful in allowing us to estimate the rates of grazing of microflagellates on their prey. However, both are strictly only applicable to the sphere of balanced growth. The former technique produces pseudo-steady state conditions in batch culture by ensuring prey densities are sufficiently high that they are saturating for ingestion. In the latter, nutrients are added to ensure exponential growth of the prey. Moreover, as noted by Landry and Hassett (1982), a critical assumption of the dilution technique is that the predators consume prey in direct proportion to their abundance. We have shown above that this was not the case in our experiments.

Although experiments in non-balanced growth conditions are inherently difficult to conduct, such experiments are necessary to shed light on the dynamics of microbial interactions in growth conditions that are outside the sphere of the above approaches.

Presence/absence of bacteria

Previously Goldman et al. (1985) found that *Paraphysomonas imperforata* ingested phytoplankton at the same rate in the presence or absence of bacteria. These results were confirmed by our experiments: *P. vestita* showed similar rates of ingestion (of phytoplankton) and division and a similar cell yield in the presence and absence of bacteria. The bacterial density behaving in a similar manner in the predator/prey experiments and predator free controls. We therefore concluded that at any of the bacterial densities studied, the presence of bacteria did not significantly influence the ingestion and growth dynamics of *P. vestita* on its phytoplankton prey.

Such results have implications for the dynamics of microbial food webs. Numerous authors have noted that microflagellates are the main grazers of bacteria in the sea and it has been demonstrated that *Paraphysomonas* sp. will graze actively on bacteria when it is the sole source of prey (Goldman et al., 1985; Landry et al., 1991; Davidson, personal observation). Earlier studies have indicated that *Paraphysomonas sp*. are able to discriminate between prey types. Our results confirm this and indicate that the presence of phytoplankton may result in grazing on these prey rather than bacteria. Such a response may alter the relative proportions of phytoplankton and bacteria in the microbial food web.

Ingestion rate and prey nutritional value

Our results indicate that the rate of ingestion of prey by *P. vestita* is a function not only of prey size as has been demonstrated previously for related species (by Goldman and Dennett, 1990) but also of prey type. Maximum observed ingestion rate of *P. vestita* was considerably greater when feeding on *I. galbana* compared to *P. lutheri* (2.9 c.f. 0.9 prey predator^{-1} hr^{-1}). The rate of ingestion is therefore not simply a function of prey size as both species are spherical and have an approximate diameter of 4.5mm. As the experiments were conducted in the dark, the prey was not actively dividing, and the average prey cell volume remained relatively constant throughout the experiments. Moreover, the two prey species are both prymnesiophytes and had similar C:N ratios.

Total carbohydrate, lipid and protein content of *I. galbana* and *P. lutheri* was studied by Brown (1991). These results indicate that *I. galbana* and *P. lutheri* differ markedly in composition, with *P. lutheri* containing per cell values of dry weight, chlorophyll a, protein, carbohydrate and lipid that were approximately three times greater than those for *I. galbana* when both species were growing exponentially. The maximum ingestion rates of the two species by *P. vestita* were related by an approximate 3:1 ratio (*I. galbana*:*P. lutheri*), similar to the inverse of the ratio of their cellular dry weight values. The lower ingestion rate of *P. vestita* on *P. lutheri* therefore does not indicate, as might initially be thought, that *P. lutheri* is a less preferred or less nutritionally acceptable prey species that *I. galbana*. Conversely, the more rapidly grazed *I. galbana* is considerably less nutritious and must be ingested at a greater rate than *P. lutheri* to maintain the division rate of the microflagellate grazer.

Functional response for ingestion

As noted above, it is unlikely that microflagellate predators will often experience the saturating prey densities that would allow them to maintain maximal ingestion and division rates. The predictive ability of functional relationships based on maximal rates may therefore be limited. This was confirmed by Fig. 4 in which we found the equation relating maximum ingestion rate to prey density to be unable to represent the temporal change in these quantities. It is therefore not possible to determine the ingestion rate simply from a knowledge of the prey density in conditions of non saturating prey concentrations.

Ingestion rate was, however, related to the prey/predator ratio in a hyperbolic manner for both prey species (Figs 4b, 6b). Relating ingestion to prey/predator ratios is conceptually and biologically appealing. Prey density is only of direct relevance to predators if they are sufficiently well spaced that they do not compete for prey. As discussed by Slobodkin (1992) the ratio of prey to predator is the quantity of importance to the predator, as it defines the number of prey in closer proximity to it than to another predator. These prey are more readily available for capture and ingestion. The prey/predator ratio is therefore more suitable than prey density to base a functional response for ingestion.

Our experiments use a range of prey and predator concentrations that are representative of bloom densities. The failure of the prey dependent, but the success of the predator/prey functional response indicates that significant interaction and competition between predators occurs at these densities. The fact that the prey threshold varied for different inoculum prey densities of the same species and between prey species but was approximately constant in terms of prey/predator ratio is further evidence that prey/predator ratio determines ingestion rates at these densities.

Predator division

The maximum specific division rate achieved by *P. vestita* was similar for both prey species, even though the alternative prey were ingested at different rates. The maximum rate observed in the initial *P. lutheri* experiments (that incorporated a lower maximum prey/predator ratio) was low. Only when the prey/predator density was sufficiently high, in the subsequent set of experiments, was the maximum specific division rate achieved. As inoculum prey density was similar in both cases, this lends further weight to the proposal that microbial predation is governed by prey/predator ratio rather than prey density.

Predictive modelling

The consequences of these results for our understanding of the behaviour of microbial food webs are particularly significant when considering the formulation of mathematical models. Succinct mathematical representations are desirable if we are to produce mathematical models of planktonic food webs that remain parameterisable, tractable and understandable. One of the major problems of model formulation is balancing these criteria against the need for the model to be biologically reasonable and accurate in its predictions.

Our results indicate that it is not sufficient simply to model grazing on the basis of cell size. Moreover, a functional group approach in which different ingestion rates

are allocated to different taxonomic groups may not be successful: both of our prey were prymnesiophytes and would probably be placed together in such an approach. Yet, it is obviously unrealistic to model microflagellate growth dynamics separately for all possible phytoplankton prey species. Detailed studies of the potential discrepancy in model predictions when using different model parameterisations within microbial food web models are therefore required. An approach that relates ingestion and/or division rate to available biomass may be the most successful. However, the preferential ingestion of phytoplankton over bacteria further complicates the situation.

As discussed by Ginzburg and Akcakaya (1992) ratio dependent and prey dependent models of trophic interactions make very different predictions about the steady state properties of a food chain. Prey dependent formulations predict alternating positive, negative and zero responses to increases in productivity. For example Thingstad and Sakshaug (1990) analysed a food web based on small and large phytoplankton and found the behaviour of the system to differ depending on whether the number of trophic levels was odd or even. Ratio dependent formulations, however, predict proportional increase in all trophic level biomasses to an increase in productivity. The implications for microbial food webs, which contain multiple grazing and nutrient recycling interactions are complex. The use of ratio dependent theory will potentially change the quantitative predictions that we make about carbon and nitrogen cycling near the base of marine food webs. In a subsequent study we shall investigate the effect on microbial food web model predictions of incorporating such a model structure.

The model simulation of microflagellate division or growth rate is more problematic. Many successful mathematical models of microbial interactions simulate the growth of phytoplankton or microflagellates using a single model currency, e.g. nitrogen in the model of Ducklow and Fasham (1992). Such an approach allows the use of simple relationships that do not incorporate intracellular quantities. This considerably reduces model complexity. However, the above results indicate that neither a hyperbolic function of prey density nor a simple hyperbolic function of prey/predator ratio was adequate to represent specific division rates in transient growth conditions. Previously (Davidson et al., 1993) we used a time lag "the nutrient processing time" to relate phytoplankton division rate to cell nutrient content at a previous instant. Using a similar approach here (Fig. 7b) we were able to account for the time taken to digest prey cells and for this material to be available to promote growth by the introduction of a time lag between prey/predator ratio and specific division rate. The utility and robustness of this approach will be studied in our subsequent modelling paper.

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References

Andersson A., Larsson U. and Hagstrom A. 1986. Size-selective grazing by a microflagellate on pelagic bacteria. Mar. Ecol. Prog. Ser. 33, 51–57.

Berryman A.A. (Ed.) 1992. Special feature: ratio dependent predator-prey theory. Ecology 73, 1530–1566.

Brown M.R. 1991. The amino-acid and sugar composition of 16 species of microalgae used in mariculture J. Exp. Mar. Biol. Ecol. 145, 79–99.

Capriulo G.M. 1990. Feeding-related ecology of marine protozoa. In: Ecology of Marine Protozoa (Ed. Capriulo G.M.). Oxford University Press, Oxford. pp. 186–241.

Caron D.A. 1991. Evolving roles of protozoa in aquatic nutrient cycles. In: Protozoa and Their Role in Marine Processes (Eds. Reid P.C., Turley C.M. and Burkill P.H.) NATO ASI Series G: Ecological Sciences. Vol. 25. Springer-Verlag, Heidelberg, Berlin. pp. 387–416.

Caron D.A., Goldman J.C. and Dennett M.R. 1990a. Carbon utilisation by the omnivorous flagellate *Paraphysomonas imperforata*. Limnol. Oceanogr. 35, 192–201.

Caron D.A., Goldman J.C. and Fenchel T. 1990b. Protozoan respiration and metabolism. In: Ecology of Marine Protozoa (Ed. Capriulo G.M.). Oxford University Press, Oxford. pp. 307–322.

Choi J.W. 1994. The dynamic nature of protistan ingestion response to prey abundance. J. Euk. Microbiol. 41, 137–146.

Davidson K., Cunningham A. and Flynn K.J. 1993. Modelling temporal decoupling between biomass and numbers during transient nitrogen-limited growth of a marine phytoflagellate. J. Plank. Res. 15, 351–359.

Ducklow H.W. and Fasham M.J.R. 1992. Bacteria in the greenhouse: modelling the role of oceanic plankton in the global carbon cycle. In: Environmental microbiology (Ed. Mitchel R.) Wiley-Liss, New York. pp. 1–31.

Eccleston-Parry J.D. and Leadbeater B.S.C. 1994a. A comparison of the growth kinetics of six marine heterotrophic nanoflagellates fed with one bacterial species. Mar. Ecol. Prog. Ser. 105, 167–177

Eccleston-Parry J.D. and Leadbeater B.S.C. 1994b. The effect of long-term low bacterial density on the growth kinetics of three marine heterotrophic nanoflagellates. J. Exp. Mar. Biol. Ecol. 177, 219–233.

Epstein S.S. and Shiaris M.P. 1992. Size selective grazing of coastal bacterioplankton by natural assemblages of pigmented flagellates, colourless flagellates and ciliates. Microb. Ecol. 23, 211–225.

Fenchel T. 1982. Ecology of heterotrophic microflagellates II. Bioenergetics and growth. Mar. Ecol. Prog. Ser. 8, 225–231.

Flynn K., Davidson K. and Cunningham A. 1996. Prey selection and rejection by a microflagellate: implications for the study and operation of microbial food webs. J. Exp. Mar. Biol. Ecol. 196, 357–372.

Ginzburg L.R. and Akcakaya H.R. 1992. Consequences of ratio-dependent predation for steady-state properties of ecosystems. Ecology 73, 1536–1543.

Goldman J.C. and Dennett M.R. 1990. Dynamics of prey selection by an omnivorous flagellate. Mar. Ecol. Prog. Ser. 59, 183–194.

Goldman J.C., Caron D.A., Anderson O.K. and Dennett M.R. 1985. Nutrient cycling in a microflagellate food chain I. Nitrogen dynamics. Mar. Ecol. Prog. Ser. 24, 231–242.

Goldman J.C., Caron D.A. and Dennett M.R. 1987. Nutrient cycling in a microflagellate food chain IV. Phytoplankton-microflagellate interactions. Mar. Ecol. Prog. Ser. 38, 75–87.

Holling C.S. 1965. The functional response of invertebrate predators to prey density. Mem. Ent. Soc. Canada 48, 1–86.

Jurgens K. and DeMott M. 1995. Behavioural flexibility in prey selection by bacterivorous flagellates. Limnol. Oceanogr. 40, 1503–1507.

Landry M.R. and Hassett R.P. 1982. Estimating the grazing impact of marine micro-zooplankton. Mar. Biol. 67, 283–288.

Landry M.R., Lehner-Fournier J.M., Sundstrom J.A., Fagerness V.L. and Selph K.E. 1991. Discrimination between living and heat-killed prey by a marine zooflagellate, *Paraphysomonas vestita.* J. Exp. Mar. Biol. Ecol. 146, 139–151.

Laybourn-Parry J. 1992. Protozoan Plankton Ecology. Chapman and Hall, London.

Parslow J.S., Doucette G.J., Taylor F.J.R. and Harrison P.J. 1986. Feeding behaviour by the zooflagellate *Pseudobodo* sp. On the picoplanktonic prasinomonad *Micromonas pusilla*. Mar. Ecol. Prog. Ser. 29, 237–246.

Peters F. 1994. Prediction of planktonic grazing rates. Limnol. Oceanogr. 39, 195–206.

Peters F. and Gross T. 1994. Increased grazing rates of microzooplankton in response to small-scale turbulence. Mar. Ecol. Prog. Ser. 115, 299–307.

Peters F., Choi J.W. and Gross T. 1996. *Paraphysomonas imperforata* (Protista, Chrysomonadida) under different turbulence levels: feeding, physiology and energetics. Mar. Ecol. Prog. Ser. 134, 235–245.

Shimeta J., Jumars P.A. and Lessard E.J. 1995. Influences of turbulence on suspension feeding by planktonic protozoa; experiments in laminar shear fields. Limnol. Oceanogr. 40, 845–859.

Sibbald M.J., Sibbald P.R, and Albright L.J. 1988. How advantageous is a sensory prey detection mechanism to predatory microflagellates? J. Plank. Res. 10, 455–464.

Slobodkin L.B. 1992. A summary of the special feature and comments on its theoretical context and importance. Ecology. 73, 1546–1566.

Thingstad T.F. and Sakshaug, E. (1990) Control of phytoplankton growth in nutrient recycling ecosystems. Theory and terminology. Mar. Ecol. Prog. Ser. 63, 261–272.

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