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A comparison of the growth kinetics of six marine heterotrophic nanoflagellates fed with one bacterial species

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ABSTRACT: The growth kinetics of 6 species of marine heterotrophic nanoflagellates (5 to 13 µm) were compared. The maximum specific growth rates (μ_{max}) of the flagellates ranged from 0.035 to 0.21 h^{-1} and the half-saturation constants (K_s) ranged from 1.1 to 45 × 10^6 bacterial cells ml^{-1}. Theoretical threshold concentrations were calculated for each flagellate and the values of these generally ranged from 2 to 10 × 10^4 bacterial cells ml^{-1}. The flagellate yield values (Y), maximum uptake rates (U_{max}) and maximum clearance rates (F_{max}) for each flagellate species ranged from 0.3 to 13.2 × 10^{-3} cells bacterium^{-1}, 5 to 259 bacteria cell^{-1} h^{-1} and 1 to 58 nl cell^{-1} h^{-1} respectively while volume-specific clearance values ranged from 1.4 × 10^4 to 8.7 × 10^5 body volumes h^{-1}

KEY WORDS: Grazing · Batch culture Specific growth rate

INTRODUCTION

The heterotrophic nanoflagellates are ubiquitous in aquatic systems and comprise a mixed taxonomic assemblage (Patterson & Larsen 1991). They are known to be the major consumers of picoplankton, capable of consuming 60% or more of the bacterial population (Sherr et al. 1986). They are also important agents of remineralization (Sherr et al. 1983, Goldman & Caron 1985, Goldman et al. 1985, 1987, Andersen et al. 1986) providing a vital link between the 'microbial loop' (Azam et al. 1983) and the higher order food chain. Most studies have categorised all heterotrophic nanoflagellates as a single functional unit (HNAN) and they are continually referred to as a composite group whereas they actually comprise a mixture of very different species. It is therefore important to elucidate how and why so many species have managed to coexist with each other.

The persistence of a variety of heterotrophic nanoflagellate species within a very narrow size category (2 to 20 µm) will require them to occupy slightly different environmental niches in order to avoid exclusion from the particular habitat. Similar species are thought to compete more with each other than dissimilar species (Gause 1934) and so each nanoflagellate, being overall similar to the others in size, prey selection (i.e. bacterivorous) and prey utilization, has had to develop some form of survival strategy by which it is able to coexist with other species.

The physicochemical factors of an environment primarily govern the species composition of communities within a given location but the role of biotic factors such as inherent control mechanisms (intraspecies competition), interspecies competition and predator-prey interactions within the community are thought to be very important (Begon & Mortimer 1987). Information on this subject in the field of heterotrophic nanoflagellates is limited at present.

Five distinct phases are recognised within the feeding process of phagotrophic nanoflagellates, namely searching, capture, ingestion, digestion and assimila-

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tion of the prey. These 5 phases encompass the potential for a range of possible variations which may have led to the development of different feeding strategies by different flagellate species. Each species may have different preferences for prey types especially with respect to the size range of acceptable prey. Large prey are thought to be preferentially grazed by raptorial feeders (Fenchel 1982a, Ammerman et al. 1984, Andersen et al. 1986) while filter feeders, e.g. choanoflagellates, are thought to feed preferentially on smaller bacteria due to size limitations of their filtration apparatus (Fenchel 1991). Some flagellates can consume a range of different-sized prey, for example, *Paraphysomonas imperforata* is known to consume bacteria (0.5 μm^3) and large algal cells (200 μm^3) (Goldman & Caron 1985), while cell fusion is commonly seen in *Ciliophrys marina* cultures, providing a way in which cells can communally ingest prey larger than that which would be successfully attacked by individuals (Cienkowski 1870, Davidson 1982).

Of course, suitable prey may not always be available in the desired concentration or size range. Some flagellates can therefore resort to cannibalism (Fenchel 1982a, Goldman & Caron 1985, Jonsson 1986), photoautotrophy (Sanders et al. 1990, Jones et al. 1993), cyst formation (Sherr et al. 1983) or a state of low metabolic growth (Fenchel 1982a) in times of starvation.

The aim of this investigation was to compare the growth kinetics of 6 species of heterotrophic nanoflagellates, all of which have relatively simple life cycles in culture. They are each quite distinct and yet their characteristics overlap to some degree. This is the first part of a larger-scale study on the elucidation of possible niche segregation among the heterotrophic flagellates. Later studies include the evaluation of other survival strategies, e.g. growth at low prey density, but for this first part, an initial comparison of the potential ability of each species to avoid competitive exclusion by other species was made.

METHODS

Heterotrophic nanoflagellates. Six species were selected for this study based on their size (5 to 13 µm) and feeding mechanisms. They included *Paraphysomonas imperforata* Lucas, *Stephanoeca diplocostata* Ellis and *Codosiga gracilis* James-Clark. *Bodo designis* Skuja and *Jakoba libera* (Ruinen) Patterson were supplied by D. J. Patterson (formerly of Bristol University, UK) and *Ciliophrys infusionum* Cienkowski was supplied by S. Tong (Southampton University, UK) (Fig. 1).

Bacterial prey. A bacterial strain was isolated from a coastal seawater sample from Plymouth, UK. It is

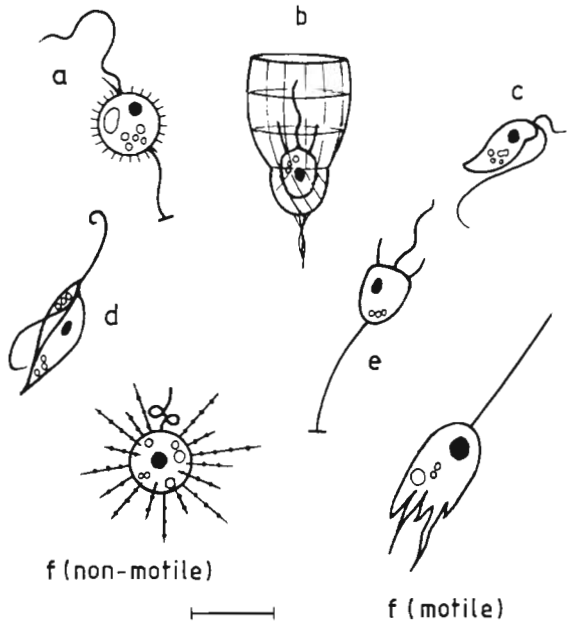

Fig. 1. The 6 species of heterotrophic nanoflagellate used in this study. (a) *Paraphysomonas imperforata*, (b) *Stephanoeca diplocostata*, (c) *Bodo designis*, (d) *Jakoba libera*, (e) *Codosiga gracilis* and (f) *Ciliophrys infusionum*. Scale bar = 10 µm

a Gram-negative, motile, rod-shaped bacterium (0.8 × 1.4 to 1.8 µm) with a bivolume of approximately 0.67 μm^3. It has been tentatively identified by the National Collections of Industrial and Marine Bacteria Ltd, Aberdeen, UK, as a strain of the genus *Alteromonas* or *Shewanella*. Throughout this study, the bacterium, designated 'B1', is the sole bacterial strain used in experiments.

Maintenance of flagellate cultures. Clonal cultures of each flagellate were obtained from single-cell isolations (Hamilton & Preslan 1969). Each species was serially diluted in artificial seawater (Harrison et al. 1980), microdrops from the higher dilutions were placed onto sterile slides and individual drops containing a single cell were enriched with 1% (w/v) of a yeast extract/proteose peptone mixture (0.8/4.0 g l^{-1}). After incubation at 20°C the individual drops containing numerous nanoflagellate cells were transferred to Erlenmeyer flasks containing a larger volume of enriched medium.

Indigenous bacteria in the 6 flagellate cultures were eliminated by the addition of 4 antibiotics which were active against Gram-negative bacteria, namely streptomycin-sulphate (final concentration 25 µg ml^{-1}), gentamycin (10 µg ml^{-1}), chloramphenicol (30 µg ml^{-1}) and tetracycline (100 µg ml^{-1}), and 1 antibiotic active against Gram-positive bacteria, namely penicillin G (100 units ml^{-1}). Antibiotic susceptibility profiles of the indigenous bacteria indicated that these would eliminate all the bacterial strains present. Individual anti-

biotic solutions were added separately to a stationary culture of each flagellate species with a few drops of the yeast extract-proteose peptone mixture and incubated at 20°C. The separate addition of antibiotics avoided possible antagonistic reactions known to occur between certain antibiotics, e.g. chloramphenicol/tetracycline with penicillin G (Singleton & Sainsbury 1981). Cultures were monitored for flagellate concentration using a haemocytometer and bacterial concentration, both viable, using the dilution plate technique, and total, using a haemocytometer. After 2 d incubation, those antibiotics active against Gram-negative bacteria, which appeared not to affect the flagellate concentration and motility but eliminated the bacteria, were prepared once again and added to the flagellate culture which had already been treated with penicillin G. This culture was reincubated at 20°C until no bacteria were detectable (2 to 3 d). Other workers who have also used mixed antibiotic treatments to remove indigenous bacteria include Daggett & Nerad (1982), Jonsson (1986), Sherr et al. (1986) and Geider & Leadbeater (1988).

A high density suspension of bacterium B1 was added to each flagellate culture and all were maintained monoxenically from then on. Every 5 d, subculturing was carried out in fresh medium supplemented with a standard volume of the yeast extract-proteose peptone solution (1% final concentration).

Flagellate grazing experiments. Kinetic data were obtained by monitoring the growth of each flagellate species in batch culture as a function of initial bacterium B1 concentration, using conventional experimental techniques. Bacterial suspensions were prepared by scraping off bacterium B1 in the stationary growth phase from Blood Agar Base plates (M.A.S.T. Laboratories, Bootle, UK) into artificial seawater. This stock suspension was enumerated with a haemocytometer using a relevant dilution which yielded 5 to 20 bacteria per small square (2.5×10^{-7} mm^3); 40 squares were counted. The bacterial suspension, and selected dilutions, were added to 7 × 30 ml artificial seawater in 100 ml Erlenmeyer flasks for each grazing experiment, to give initial bacterial concentrations ranging from 8×10^5 to 2.24×10^8 bacteria ml^{-1}. Triplicate flasks were employed at each initial bacterial concentration. The bacterium did not grow in this medium; instead, the concentration slowly declined, with a half-life of about 105 h.

A late-exponential culture of the flagellate was used to inoculate each flask. There was however some degree of carry-over of bacterium B1 from the flagellate inoculum into the experimental flasks. So, the actual initial bacterial concentration was corrected for, by further haemocytometer counts, after the addition of the flagellate culture to the Erlenmeyer flasks to give a starting concentration of 2000 flagellates ml^{-1}.

Flasks were incubated at 20°C without shaking and, at predetermined intervals, a 2 ml sample of each was fixed with 50 µl 4% glutaraldehyde solution and vortexed. Both the flagellate species and bacterium B1 were enumerated using a haemocytometer and 4 haemocytometer counts were performed for each sample. These experiments were performed twice.

Calculations. The natural logarithm of flagellate concentration was plotted against time for each flagellate species at each initial bacterial concentration, and from this relationship, 4 numerical values were obtained for each system: (1) the specific growth rate, μ (h^{-1}); (2) the highest concentration of flagellate achieved [in this paper referred to as peak abundance (cells ml^{-1}) (cf. 'maximum population abundance' in Sherr et al. 1983)]; (3) the overall increase in flagellate concentration; and (4) the corresponding overall decrease in bacterial concentration. These 4 values were then used to determine the yield value and maximal values of specific growth rate, bacterial uptake rate and clearance rate of the flagellate species studied.

Specific growth rate, μ, was calculated from regression analysis of the linear portion of the graph associated with the exponential phase of growth where the overall flagellate cell volumes remained constant and where there was only a slight decrease in bacterial concentration. This is viewed as the closest state to balanced growth obtainable in batch culture (Fenchel 1982a, Sherr et al. 1983, Andersen 1989).

The relationship between the specific growth rate of the flagellate and the initial bacterial concentration was fitted to a hyperbolic function of a form $\mu = x\mu_{max}/(K_s + x)$ following Monod (1950), where μ = the specific growth rate as a function of initial bacterial concentration (x), maximum specific growth rate (μ_{max}) and the half-saturation constant (K_s). The parameters μ_{max} and K_s were calculated by the least-squares method to fit the line $x = \mu_{max}[x/\mu] - K_s$, where the gradient gave the μ_{max} value and the intercept on the y-axis gave the K_s value.

Ciliophrys infusionum was the only species to exhibit a measurable threshold level of bacterial concentration for positive flagellate growth and, therefore, the relationship between μ and initial bacterial concentration was fitted to a modified hyperbolic function of the form $\mu = \mu_{max}[x - t]/[(K_s - t) + (x - t)]$ following Taylor (1978), where t = the threshold concentration, the highest value of x where μ is zero, which was estimated at 2×10^7 cells ml^{-1} by visual inspection of the graphical data. Determination of μ_{max} and K_s were obtained by the least-squares method to fit the line $[x - t] = \mu_{max}[(x - t)/\mu] - [K_s - t]$ (Taylor 1978).

The highest concentration of flagellate within each of the flasks, the peak abundance, was recorded and

plotted against the initial bacterial concentration ($\log_{10}$ axes). A linear relationship was obtained for each flagellate species for which an equation of the line was calculated by regression analysis. The equation was used to calculate a theoretical threshold bacterial concentration that exists for each of the flagellate species with this bacterium. It was assumed that if the initial flagellate population (2000 cells ml^{-1}) exibited a zero growth rate, the final concentration would also be 2000 cells ml^{-1} ($\log_{10}$ 3.3) assuming a negligible death rate. By inserting 'y' at 3.3 into the equations of the lines, a theoretical threshold bacterial concentration ('x') for each flagellate was calculated.

Observed yield (Y) was calculated as the increase in flagellate concentration divided by the decrease in bacterial concentration (Y = highest minus initial flagellate concentration/initial minus lowest bacterial concentration; Pirt 1975) to give the number of flagellate cells produced upon the ingestion of 1 bacterial cell. This is similar to the method of Fenchel (1982a) whereby final flagellate concentration is divided by initial bacterial concentration, except that in our calculations, the initial flagellate concentration is taken into account and there is no assumption that all the prey are consumed. The method however assumes that all the energy obtained from ingested prey is being used for growth and reproduction, and not for maintenance (Fenchel 1982a).

Maximum uptake rate (U_{max}, bacteria flagellate^{-1} h^{-1}) was calculated as $U_{max} = \mu_{max}/0.5\,Y$ (following Fenchel 1982a), where Y = flagellate yield. Y had to be corrected to account for the number of divisions (usually 1) the flagellate underwent after the prey had fallen below the feeding threshold at the onset of the stationary phase (Fenchel 1982a).

The maximum clearance values (F_{max}, nl flagellate cell^{-1} h^{-1}) were calculated, following Fenchel (1982a), from the equation $F_{max} = U_{max}/K_s$. The volume-specific clearances (h^{-1}) were then calculated by dividing the maximum clearance rate by the volume (V) of the flagellate. Volumes were determined from the equation $V = \pi(s^2 l)/6$, where s is the shortest length of the protoplast and l is the longest.

RESULTS AND DISCUSSION

Description of the six heterotrophic nanoflagellates

Paraphysomonas imperforata, *Stephanoeca diplocostata* and *Codosiga gracilis* had been maintained in the Birmingham University (UK) culture collection prior to this study. *P. imperforata* (Chrysophyceae) is spherical (diameter 5 to 10 µm) and the cell is covered with siliceous spines (Fig. 1a). The volume of growing cells was approximately 212 μm^3. It possesses 2 flagella, the longer having a bilateral array of 'heterokont' tubular hairs, the shorter being smooth. *P. imperforata* feeds by direct interception of suspended prey including bacteria, microalgae and other protists (Goldman & Dennett 1990). Goldman & Caron (1985) have shown that the cell volume of acceptable prey for *P. imperforata* can vary from 0.5 μm^3 (bacterial cells) to 200 μm^3 (*Dunaliella tertiolecta* cells). *P. imperforata* can be found swimming at speeds up to 42 µm s^{-1} or may be attached in large clusters to detritus or bacterial aggregates by a thin stalk originating from the posterior end of the cell. This species is ubiquitous in both marine and freshwater habitats. The protoplasts of *S. diplocostata* and *C. gracilis* (Choanoflagellida) are both of the size range 5 to 8 µm with volumes of 35 and 83 μm^3 respectively. They possess a single flagellum encircled by a collar of tentacles (Fig. 1b, e). Both are filter feeders of bacteria in suspension (Sleigh 1964). *C. gracilis* is found attached by means of a stalk to bacterial clumps and detritus in marine environments. The protoplast of *S. diplocostata* is surrounded by a basket-like lorica composed of siliceous costae (Leadbeater 1979).

Bodo designis is elliptical in shape, measuring 5 to 10 µm with 2 flagella inserted sub-apically (Fig. 1c), and has a volume of ca 54 μm^3. It has a characteristic 'corkscrew' swimming behaviour, reaching speeds of 80 µm s^{-1}. The posterior flagellum is twice as long as the body and attaches to the substratum where bacteria are most commonly grazed. This species is known to occur in freshwater, marine and soil environments. The cell body of *Jakoba libera* (Ruinen) Patterson, formally *Cryptobia libera* Ruinen, is acutely obovate and measures 8 to 13 µm in length (Fig. 1d) with a volume of ca 75 μm^3. The anterior flagellum is inserted apically and is held in a characteristic hook shape while the posterior flagellum is inserted slightly sub-apically. The posterior flagellum beats in a ventral groove in which bacterial prey becomes trapped and ingested. Cells can be found free swimming at speeds up to 19.2 µm s^{-1} or exhibiting jerking movements backwards and forwards. *J. libera* feeds on bacteria although it is unknown whether it has a preference for prey in suspension or attached to surfaces. It is known to occur in both marine and freshwater sediments, intertidal benthic sites and surface waters (Patterson 1990).

Ciliophrys infusionum (Helioflagellida) has 2 cell forms and measures 8 to 13 µm (Fig. 1f) and has a volume of ca 220 μm^3. The sessile form resembles a heliozoan, feeding on bacteria and small protists in suspension by means of 'granular' tentacles over the entire body surface. Little is known about the kinetic behaviour of *C. infusionum*. Laboratory observations have shown that motile cells move towards the edge of a cul-

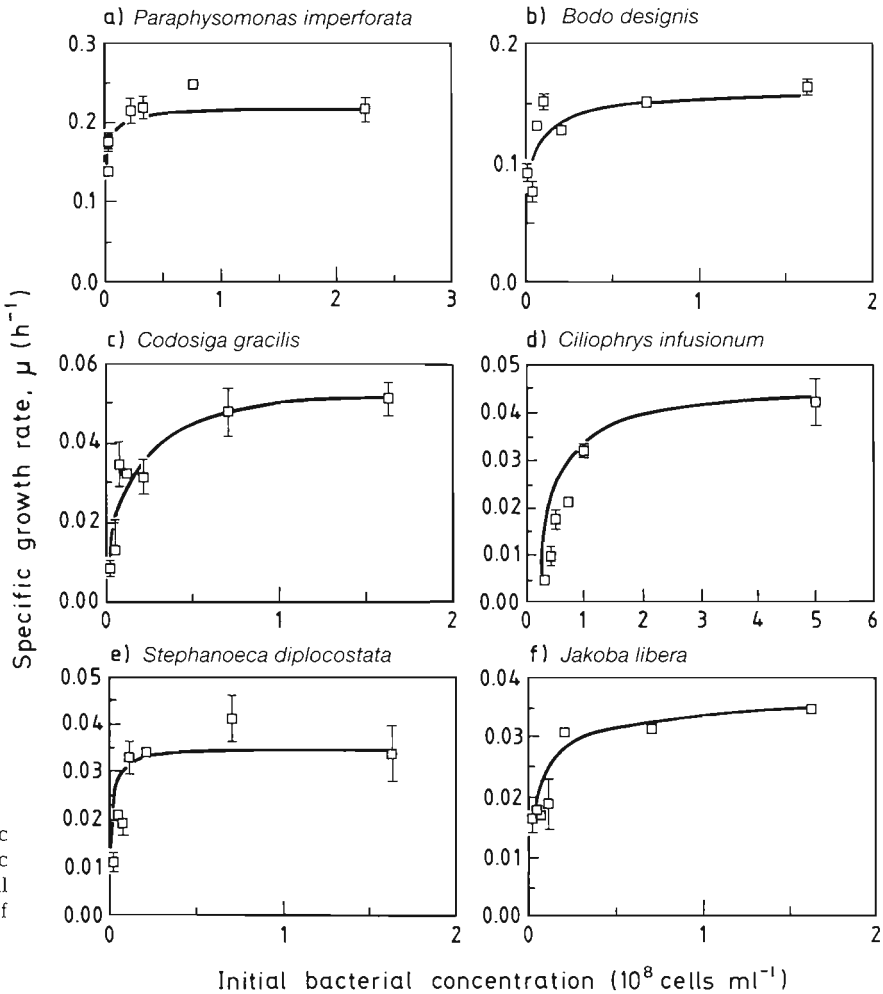

Fig. 2. A comparison of the hyperbolic relationship obtained from specific growth rate (μ) against initial bacterial concentration for each of the 6 species of heterotrophic nanoflagellate

ture drop where prey concentration is usually higher, before stopping and reforming into a heliozoan-like form. This organism may become mobile in order to relocate itself in an area of higher prey density than previously experienced. It is known to be common in marine environments.

Flagellate specific growth rates in relation to initial bacterial concentration

Each flagellate species was subjected to batch culture grazing experiments, in the presence of various initial concentrations of bacterium B1, from which the specific growth rates (μ) were determined. Data for all the flagellate species appeared to fit the hyperbolic relationship between μ and initial bacterial concentration (Fig. 2). The use of $x = \mu_{max}(x/\mu) - K_s$ for regression analysis gave estimates for μ_{max} and K_s which were more accurate than those obtained by a Lineweaver-Burk plot. The latter method bases linear regression calculations mainly on points associated with low values of μ and x, where statistical error is increased and therefore resultant K_s values, in particular, may be erroneous. The linear regression technique used here gave low standard errors for μ_{max} values but increased error for K_s values compared to the Lineweaver-Burk plot. It also complements the method used for *Ciliophrys infusionum*, whereby a threshold density was included into the equation (Taylor 1978).

The μ_{max} values (Table 1) ranged from 0.035 to 0.21 h^{-1} for the 6 species studied. *Paraphysomonas imperforata* had the highest value for μ_{max} (0.21 ± 0.006 h^{-1}), followed by *Bodo designis* (0.16 ± 0.003 h^{-1}). The values for the other 4 species were lower. The value for *Codosiga gracilis* (0.052 ± 0.002 h^{-1}) was greater than for *Ciliophrys infusionum* (0.045 ± 0.004 h^{-1}) which was in turn greater than those values for *Jakoba libera* (0.036 ± 0.0006 h^{-1}) and *Stephanoeca diplocostata* (0.035 ± 0.001 h^{-1}) which were themselves comparable. *C. infusionium* is misleading because it has a relatively high μ_{max} (0.045 h^{-1}) but this organism requires a high initial concentration of the prey bacterium (3 to 4 × 10^8 bac-

Table 1. A comparison of the values of maximum specific growth rate (μ_{max}, h^{-1}), half-saturation constant (K_s, 10^6 bacteria ml^{-1}) and yield (Y, 10^{-3} flagellate cells bacterium^{-1}) for various flagellate species and prey types. Temperature = 20 °C unless otherwise stated. Results of this study in **bold**

Flagellate	Prey	μ_{max}	K_s	Y	$1/Y$	Source
Actinomonas mirabilis	*Pseudomonas* sp.	0.25	1.4	2.3	435	Fenchel (1982b)
Bodo designis	**B1**	**0.16**	**3.4**	**2.0**	**500**	**This study**
B. designis	*Aeromonas* sp.	0.12	8.8	–	–	Hammond (1991)
B. edax	*Klebsiella* sp.	0.116[a]	–	6.7	150	Dagget & Nerad (1982)
Ciliophrys infusionum	**B1**	**0.045**	**45.0**	**0.3**	**3000**	**This study**
Codosiga gracilis	**B1**	**0.052**	**9.7**	**2.9**	**340**	**This study**
Diaphanoeca grandis (21 °C)	*Pseudomonas* sp.	0.12	2.4	–	–	Andersen (1989)
Jakoba libera	*Aeromonas* sp.	0.08	5.3	–	–	Hammond (1991)
J. libera	**B1**	**0.036**	**5.4**	**13.2**	**76**	**This study**
Monosiga sp.	*Pseudomonas* sp.	0.17	13.5	6.2	161	Fenchel (1982a)
Ochromonas sp.	*Pseudomonas* sp.	0.19	19.0	10.0	100	Fenchel (1982a)
Ochromonas sp. (18 °C)	Natural assemblage	0.016[a]	–	18.5	54	Ammerman et al. (1984)
Paraphysomonas vestita	*Pseudomonas* sp.	0.23	14.9	0.9	1235	Fenchel (1982a)
P. imperforata	**B1**	**0.21**	**1.1**	**6.6**	**152**	**This study**
P. imperforata	*Aeromonas* sp.	0.12	4.4	–	–	Hammond (1991)
P. imperforata	*Vibrio* sp.	0.22	13.0	1.7	606	Edwards (1989)
Pleuromonas jaculans	*Pseudomonas* sp.	0.16	38.6	3.0	333	Fenchel (1982a)
Pseudobodo tremulans	*Pseudomonas* sp.	0.15	8.4	1.8	555	Fenchel (1982a)
Stephanoeca diplocostata[s] (18 °C)	*Pseudomonas* sp.	0.076	6.8	7.5	133	Geider & Leadbeater (1988)
S. diplocostata[s]	*Pseudomonas* sp.	0.063	–	–	–	Leadbeater & Davies (1984)
S. diplocostata	*Pseudomonas* sp.	0.031	–	–	–	Leadbeater & Davies (1984)
S. diplocostata	**B1**	**0.035**	**2.3**	**1.9**	**528**	**This study**
Natural assemblage (15 °C)	Natural assemblage	0.006[a]	–	2.7	366	Bjørnsen et al. (1988)
Natural assemblage (15 °C)	Natural assemblage	0.013[a]	–	3.7	271	Bjørnsen et al. (1988)
Natural assemblage (15 °C)	Natural assemblage	0.027[a]	–	3.0	336	Bjørnsen et al. (1988)
Natural assemblage (June)	Natural assemblage	0.114[a]	–	28.5	35	Bloem & Bär-Gilissen (1989)
Natural assemblage (August)	Natural assemblage	0.068[a]	–	96.0	10	Bloem & Bär-Gilissen (1989)

[a]Not maximum value. [s]Stirred cultures

teria ml^{-1}) to attain this value due to a threshold concentration of 2×10^7 bacteria ml^{-1} (Fig. 2d). The half-saturation constant (K_s) (Table 1) was 5 to 40 times higher than those recorded for the other species ($45 \pm 2.16 \times 10^6$ bacteria ml^{-1}). *P. imperforata*, *B. designis* and *S. diplocostata* had comparable affinities for this bacterium (K_s values of 1.1 ± 2.1, 3.4 ± 1.3, $2.3 \pm 1.9 \times 10^6$ bacteria ml^{-1} respectively), followed by *J. libera* ($5.4 \pm 1.2 \times 10^6$ bacteria ml^{-1}) and *C. gracilis* ($9.7 \pm 3.7 \times 10^6$ bacteria ml^{-1}).

A comparison of these results with those of other workers for the same and other flagellate genera (Table 1) reveals that these kinetic values are variable, which is possibly due to inherent (genetic) differences, the different prey species used, in some cases due to different experimental protocols, for example the effect of stirring (Leadbeater & Davies 1984) and also due to different statistical analysis procedures. Our results are of the same order as most of the results obtained by others for the same taxa.

K_s is a parameter that can be used to compare the competitiveness of different flagellate species for the same limited resource in batch culture (Taylor 1978) but so too is the relationship between μ and initial bacterial concentration (Fenchel 1982a). Minimal variation in specific growth rates, at different initial bacterial concentrations, has been observed for the 6 flagellate species in subsequent experimentation; values are highly conserved. Comparison of the functional responses (Fig. 2) revealed that *Paraphysomonas imperforata*, *Bodo designis* and *Ciliophrys infusionum* did not have any overlapping specific growth rate values; the hyperbolic relationship for each species was quite distinct. The other 3 species, *Codosiga gracilis*, *Jakoba libera* and *Stephanoeca diplocostata*, did however have overlapping specific growth rates (Fig. 3). A crossover point, corresponding to the initial bacterial concentration at which the specific growth rate values of the 2 species involved were equal, was determined: $x = [(\mu_{max1} K_{s2}) - (\mu_{max2} K_{s1})]/(\mu_{max2} - \mu_{max1})$, where 1 and 2 represent species 1 and 2 respectively (Table 1).

Jakoba libera and *Codosiga gracilis* have a crossover value at 4.2×10^6 bacteria ml^{-1} (Fig. 3) and therefore below this initial bacterial concentration, *J. libera* has

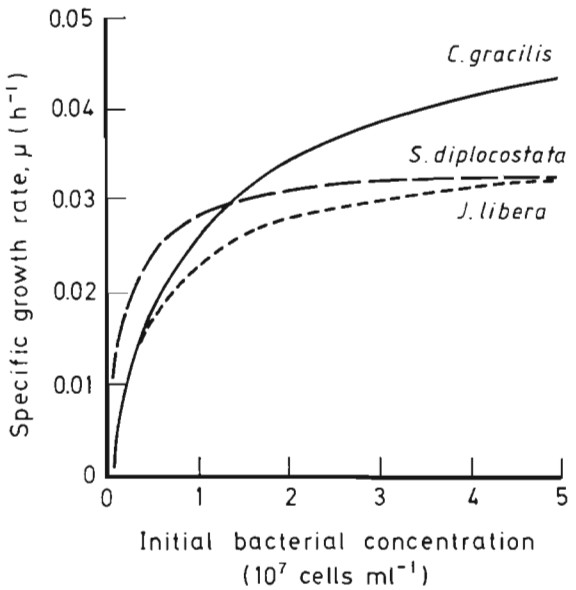

Fig. 3. *Jakoba libera*, *Stephanoeca diplocostata* and *Codosiga gracilis*. Overlapping characteristics in the relationship between specific growth rate and initial bacterial concentration

the higher value for μ whilst above this concentration, *C. gracilis* has the higher μ value. *Stephanoeca diplocostata* has a crossover value at 1.3×10^7 bacteria ml^{-1} with *C. gracilis*. Therefore, at natural bacterial concentrations, which normally range from 0.5 to 2×10^6 bacteria ml^{-1} (Hagström et al. 1979) the behaviour of each flagellate species in relation to another is quite different from that assumed by comparison of μ_{max} values only (Table 1). However, since the degree of error associated with the variable area of the hyperbolic function is relatively high, these predictions are not absolute but give an idea as to when different species would be likely to compete with each other.

Flagellate peak abundance and bacterial threshold levels

The linear relationships between $\log_{10}$ flagellate peak abundance and $\log_{10}$ initial bacterial concentration for each flagellate are shown in Fig. 4 while the equations of these lines are presented in Table 1. The actual data points in Fig. 4 have been omitted for reasons of clarity but all the data fitted well to a straight line relationship, with the standard error of the regresssion coefficient, i.e. the gradient, being within the acceptable limit of an order of 10 below the regression coefficient value (Table 1).

The determination of a threshold density for *Ciliophrys infusionum* was relatively easy as it was so large (Fig. 2d), but for other species, the threshold density may be too small to be observed and so these values were calculated mathematically. The theoretical threshold concentration for *C. infusionum* was calculated mathematically as 1.32×10^7 bacteria ml^{-1} (Table 1), which was close to the value estimated by visual inspection (Fig. 2d). Thresholds have also been determined by recording the minimum number of bacteria at the onset of flagellate stationary phase in batch culture, i.e. when the predator can no longer graze the prey (Caron et al. 1985, Goldman et al. 1985, Goldman & Dennett 1990). However, at this point in the growth cycle, the number of flagellate cells is increased and severe intraspecies competition for limited prey would be evident (Børsheim & Bratbak 1987, Geider & Leadbeater 1988). Some degree of partial refuge must be afforded to the prey in order for them to regenerate new cells and hence new food particles for the flagellate, and therefore a threshold level must exist. Size-selective predation amongst heterotrophic flagellates may also be an effective mechanism for allowing bacterial refuge from predation (Chrzanowski & Simek 1990) in addition to that obtained below the flagellate threshold feeding densities, although that was not investigated here.

The theoretical threshold values for 5 flagellate species ranged from 2.0 to 9.9×10^4 bacteria ml^{-1} (Table 2). These appear to be low values but the biovolume of bacterium B1 is approximately 0.67 μm^3 which is larger than natural bacterioplankton bio-

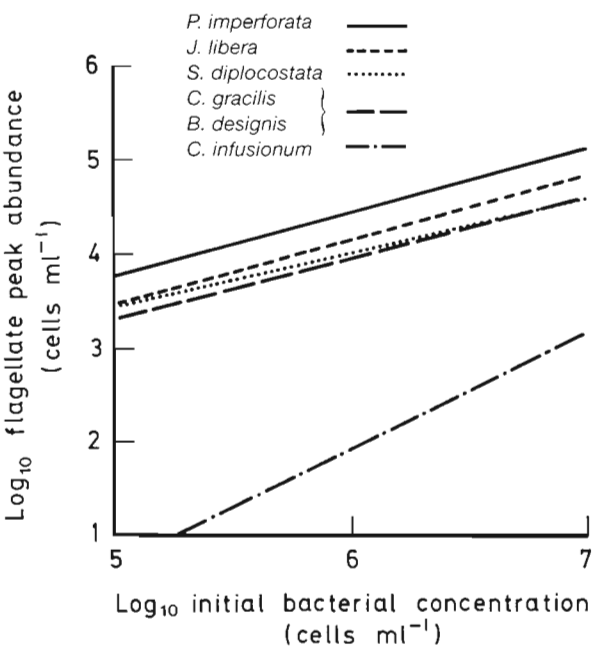

Fig. 4. Fitted lines for flagellate peak abundance against initial bacterial concentration for the 6 species of heterotrophic flagellate

Table 2. The equations of the lines for flagellate peak abundance against initial bacterial concentration, the theoretical threshold concentrations and volume-specific clearance

Flagellate	Equation of line (± SE regression coefficient)	Threshold conc. (bacteria ml^{-1})	Volume-specific clearance (10^5 h^{-1})
Paraphysomonas imperforata	$y = 0.4551 + 0.6625\,x$ (0.04178)	2.0×10^4	2.97
Bodo designis	$y = 0.0871 + 0.6432\,x$ (0.03828)	9.9×10^4	8.71
Codosiga gracilis	$y = 0.2232 + 0.6238\,x$ (0.0685)	8.6×10^4	0.45
Ciliophrys infusionum	$y = -5.5020 + 1.2360\,x$ (0.0750)	1.3×10^7	1.98
Stephanoeca diplocostata	$y = 0.5709 + 0.5729\,x$ (0.05846)	5.8×10^4	4.58
Jakoba libera	$y = -0.2825 + 0.7363\,x$ (0.0526)	7.3×10^4	0.14

volumes. Therefore, the threshold densities calculated here may be a misrepresentation and threshold densities pertaining to natural environments could be as much as an order of magnitude higher than those presented in Table 1. This would be in line with the results of Rivier et al. (1985) who observed thresholds for *Pseudobodo* sp. ranging from 9.5 to 187 $\times 10^5$ bacteria ml^{-1} using mixed bacterial assemblages as the prey source. However, a threshold values will always be overestimates because they are consistent with the fact that a minimum food level represents the point at which the energy required for growth balances basal metabolic activity (Goldman & Dennett 1990).

Flagellate yield, uptake rate and clearance rate

The yield values for the 6 flagellate species are presented in Table 1 and are of the same order except for *Ciliophrys infusionum* and *Jakoba libera*. *J. libera* had the highest yield value ($Y = 13.2 \pm 4.78 \times 10^{-3}$ flagellate cells $bacterium^{-1}$), requiring only 76 bacterial cells ($1/Y$) to produce 1 of its own whilst *C. infusionum* had the lowest yield value ($Y = 0.3 \pm 0.05 \times 10^{-3}$ flagellate cells $bacterium^{-1}$), requiring almost 3000 bacterial cells to produce 1 of its own. All the other results range from 1.9 to 6.6 $\times 10^{-3}$ flagellate cells $bacterium^{-1}$ or the requirement of between 153 and 526 bacterial cells to produce 1 flagellate cell. All the values, except for *C. infusionum*, fall into the range obtained by other workers, i.e. 0.9 $\times 10^{-3}$ (*Paraphysomonas vestita*; Fenchel 1982a) to 18.5 $\times 10^{-3}$ flagellate cells $bacterium^{-1}$ (*Ochromonas danica*; Ammerman et al. 1984) (Table 1). Values obtained for mixed flagellate cultures preying on mixed bacterial prey in natural systems also have a large range, 2.7 to 96.0 $\times 10^{-3}$ flagellate cells $bacterium^{-1}$ (Table 1).

Jakoba libera appears to be the most efficient feeder followed by *Paraphysomonas imperforata*, *Codosiga gracilis*, *Bodo designis* and *Stephanoeca diplocostata* and this bears no relationship to the other parameters discussed (Table 1). The term 'feeder' is an elusive term as we cannot determine at present which of the phases in the feeding process are determinants of efficiency. For example, little is known about the way in which *P. imperforata* captures and engulfs prey (Goldman & Dennett 1990) even though this species has been studied intensively. However, Seale et al. (1990) found, from video recordings, that *Spumella* sp. captured about 60 bacteria h^{-1} but only ingested half that number (20 to 30 bacteria h^{-1}).

Bacterial uptake per flagellate per unit time (U) is a hyperbolic function of initial bacterial concentration (Fenchel 1982a, Davies & Sieburth 1984, Goldman & Caron 1985, Geider & Leadbeater 1988, Andersen 1989) and the maximum uptake rate (U_{max}) effectively measures the maximum rate at which prey is ingested by the flagellate (Fenchel 1982a). *Ciliophrys infusionum* took up the most bacteria per hour (259; Table 3) followed by *Bodo designis* (160 bacteria h^{-1}) and *Paraphysomonas imperforata* (63 bacteria h^{-1}). Values for *Stephanoeca diplocostata* and *Codosiga gracilis* were comparable (37 and 36 bacteria h^{-1}) but greater than for *Jakoba libera* (5 bacteria h^{-1}).

Clearance (F) quantifies the ability to concentrate food particles. Fenchel (1982a) considers this the most meaningful measure of the competitive ability of an organism as a grazer of suspended particles in nature. *Paraphysomonas imperforata* had the highest maximum clearance value (Table 3) followed by *Bodo designis*, *Stephanoeca diplocostata*, *Ciliophrys infusionum*, *Codosiga gracilis*, then *Jakoba libera*.

There was no true relationship between the values of U_{max} and F_{max} and the size of the flagellate; the largest flagellate (*Ciliophrys infusionum*) had the largest U_{max} but not the largest F_{max}. The smallest flagellate (*Stephanoeca diplocostata*) did not have the smallest U_{max} or F_{max}. An appropriate comparison of flagellates can be made from volume-specific clearance rates (Table 2) (Fenchel 1982a, 1986) although the accuracy of these values is questionable since they are based on the volume of growing cells which is often difficult to calculate with a great degree of accuracy (Fenchel & Finlay 1983). However, comparison of the results shows that *Bodo de-*

Table 3. Comparison of specific growth rate (μ), uptake rate (U) and clearance rate (F) values of heterotrophic nanoflagellates. All experiments performed at temperatures of 20 to 24 °C unless otherwise stated. Results of this study in **bold**

Heterotrophic flagellate Prey species	μ (h^{-1})	U (bacteria h^{-1})	F (nl $cell^{-1}$ h^{-1})	Source
Acanthoeopsis sp.				
Vibrio sp.	–	136	9	Davies & Sieburth (1984)
Actinomonas mirabilis				
Pseudomonas sp.	0.25[a]	107[a]	79[a]	Fenchel (1982b)
Vibrio sp.	–	224	160–203	Davies & Sieburth (1984)
Bodo sp.				
Vibrio sp.	–	50–250	19–120	Davies & Sieburth (1984)
Pseudomonas fluorescens	–	3–5	0.2–0.4	Chrzanowski & Simek (1990)
P. fluorescens	–	11–44	2–8	Chrzanowski & Simek (1990)
P. fluorescens	–	2–7	0.4–1	Chrzanowski & Simek (1990)
Bodo edax				
Klebsiella pneumoniae (?)	0.12	25	–	Daggett & Nerad (1982)
Bodo celer				
Vibrio sp.	–	137	39	Davies & Sieburth (1984)
Bodo designis				
Vibrio sp.	–	40–220	32–57	Davies & Sieburth (1984)
Aeromonas aerogenes	0.12	–	–	Hammond (1991)
B1	**0.16[a]**	**160[a]**	**47[a]**	**This study**
Ciliophrys infusionum				
B1	**0.045[a]**	**259[a]**	**6[a]**	**This study**
Ciliophrys sp.				
Inert fluorescent particles	–	–	5	Tobiesen (1990)
Codosiga gracilis				
B1	**0.052[a]**	**36[a]**	**4[a]**	**This study**
Diaphanoeca grandis				
Pseudomonas sp. (15 °C)	0.15[a]	40[a]	16[a]	Andersen (1989)
Pseudomonas sp.	0.19[a]	–	25[a]	Andersen (1989)
Jakoba libera				
Aeromonas aerogenes	0.08	–	–	Hammond (1991)
B1	**0.036[a]**	**5[a]**	**1[a]**	**This study**
Monas sp.				
Chlorobium phaeobacteriodes	0.11	11–44	0.3–1	Sherr et al. (1983)
Unidentified isolate	0.128	50–75	0.2–0.3	Sherr et al. (1983)
Escherichia coli	0.119	37–75	0.2–0.6	Sherr et al. (1983)
Salmonella typhimurium	0.089	22–25	0.07–0.5	Sherr et al. (1983)
Vibrio sp.	–	–240	14–63	Davies & Sieburth (1984)
Vibrio sp.	–	60–275	38–336	Davies & Sieburth (1984)
Vibrio sp.	–	173	20	Davies & Sieburth (1984)
Monosiga sp.				
Pseudomonas sp.	0.17[a]	27[a]	2[a]	Fenchel (1982a)
Ochromonas danica				
Pseudomonas fluorescens	–	1.8–33	0.1–2	Chrzanowski & Simek (1990)
Ochromonas sp.				
Pseudomonas sp.	0.19[a]	190[a]	10[a]	Fenchel (1982a)
Oikomonas sp.				
Vibrio sp.	–	50–300	28–65	Davies & Sieburth (1984)
Paraphysomonas imperforata				
Phaeodactylum tricornutum	0.062	7[a]	2[a]	Goldman & Dennett (1990)
P. tricornutum	0.10	–	5.4	Goldman & Caron (1985)
P. tricornutum	0.087–0.10	2.66[a]	3.96–10.4	Caron et al. (1985)
P. tricornutum	0.083–0.11	1–2	1–7	Goldman et al. (1987)
P. tricornutum	0.088–0.14	1–2[a]	–	Andersen et al. (1986)
Dunaliella tertiolecta	0.064	2[a]	1.17[a]	Goldman & Dennett (1990)
D. tertiolecta	0.082–0.10	0.2–0.4	0.4–2	Goldman et al. (1987)
D. tertiolecta	0.1	–	5	Goldman & Caron (1985)

(Table continued on next page)

Table 3 (continued)

Heterotrophic flagellate Prey species	μ (h^{-1})	U (bacteria h^{-1})	F (nl $cell^{-1}$ h^{-1})	Source
Paraphysomonas imperforata (continued)				
Isochrysis galbana	0.062	3[a]	3[a]	Goldman & Dennett (1990)
I. galbana	0.1	–	13	Goldman & Caron (1985)
Chlorella stigmataphora	0.1	–	4	Goldman & Caron (1985)
C. capsulata	0.096	–	11	Goldman & Caron (1985)
Porphyridium sp.	0.1	–	4	Goldman & Caron (1985)
Vibrio sp.	–	~180	18–145	Davies & Sieburth (1984)
Pseudomonas halodurans	0.10	23–118[a]	2–4[a]	Caron et al. (1985)
Pseudomonas sp.	0.10	21[a]	–	Andersen et al. (1986)
Mixed bacteria	0.14	70[a]	–	Andersen et al. (1986)
Aeromonas aerogenes	0.12[a]	–	–	Hammond (1991)
Vibrio sp.	0.21–0.22[a]	249–278[a]	19–21[a]	Edwards (1989)
B1	**0.21[a]**	**63[a]**	**58[a]**	**This study**
Paraphysomonas vestita				
Pseudomonas sp.	0.23[a]	254[a]	17[a]	Fenchel (1982a)
Pleuromonas jaculans				
Pseudomonas sp.	0.16[a]	54[a]	1.4[a]	Fenchel (1982a)
Pseudobodo tremulans				
Pseudomonas sp.	0.15[a]	84[a]	10[a]	Fenchel (1982a)
Vibrio sp.	–	10–300	20–165	Davies & Sieburth (1984)
Pseudobodo sp.				
Natural (15 °C)	0.08–0.16[a]	4.5–85.4	0.2–4	Rivier et al. (1985)
Rhynchomonas sp.				
Vibrio sp.	–	30–210	17–37	Davies & Sieburth (1984)
Spumella sp.				
Xenorhabdus luminescens	0.22	20–30	10–62	Seale et al. (1990)
Stephanoeca diplocostata				
Pseudomonas sp. (stirred) (18 °C)	0.079[a]	21[a]	3[a]	Geider & Leadbeater (1988)
Pseudomonas sp. (stirred)	0.063	–	–	Leadbeater & Davies (1984)
Pseudomonas sp. (unstirred)	0.031	–	–	Leadbeater & Davies (1984)
B1	**0.035[a]**	**37[a]**	**16[a]**	**This study**

[a]Maximum values

signis clears the largest number of body volumes and would therefore be expected to be an avid competitor for bacterial prey. *S. diplocostata* had the second highest value, even though it was the smallest flagellate, however, the existence of a basket-like siliceous lorica around the protoplast may in some way have increased the organism's efficiency of prey capture by increasing the surface area and overall volume of the cell. *Paraphysomonas imperforata* had a higher volume-specific clearance rate than *Ciliophrys infusionium*, followed by *Codosiga gracilis* and *Jakoba libera*.

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