Feeding by *Euplotes mutabilis* (Ciliophora, Hypotrichida)*

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Abstract: Experiments on the capture and ingestion of microspheres and algal cells of different sizes presented at different concentrations, alone or in combination, showed that feeding activity and ingestion rates were dependent on the concentration and size of food particles; larger particles were preferred to smaller ones and algal cells to microspheres of similar sizes, at the same concentration. The membranelles of *Euplotes* create water currents carrying suspended particles, filter particles from the current and direct them to the cytostome. Specialised frontal membranelles may play some part in recognition of prey, but the principal selection site is probably the membrane lining the forming food vacuole at the cytostome.

Key words: Algal prey, ciliate food selection, lectin binding, membranelles, microspheres.

Introduction

Features of the ciliature of ciliates have long been used to characterise differences between them at all taxonomic levels from sub-phylum down to species. Ciliary patterns reflect the adaptations of the organism concerned to the particular mode of life, principally locomotion, including relevant aspects of behaviour, and feeding. In principle, a comparison between the ciliary patterns of two ciliates should allow an understanding of a major part of how they are fitted for their particular ecological niche. Our knowledge of the differences between the patterns of ciliature of ciliates is far in excess of our understanding of how cilia, and especially distinctive patterns of ciliature, are used in the life of these ciliates. One bold approach to studying the integrated use of cilia in ciliate locomotive behaviour was exploited by Ricci (e.g. 1990) in constructing 'ethograms' of moving ciliates, and ways of using cilia in collecting food have been studied by various workers.

If a dense suspension of particles is pipetted around a surface-dwelling motile ciliate, such as *Euplotes* or *Stylonychia*, then flow patterns are set up like those described by Machemer (1966). These are created by the membranelles of the adoral zone (AZM). Some of the particles are intercepted by the membranelles and are then seen to progress towards the cytostome at the rear end of the buccal area and may be taken into food vacuoles. We observed this feeding activity in a locally-isolated marine species, identified as *Euplotes mutabilis*, which was maintained on the bacterium *Vibrio natriegens*, and had been used in a study on the fate of ingested food materials by Zubkov & Sleigh (1995). This food bacterium is much smaller than the tracer particles used to observe flow patterns, and also smaller than the spacing between membranelles, which led us to take an interest in the feeding mechanism of this ciliate.

The results of two of our studies related to the feeding of *Euplotes mutabilis* have already been reported. In the first the relationship between the rate of ingestion of inert microspheres and the concentration and size of presented particles was investigated by Wilks & Sleigh (1998). It was found that microspheres of all five diameters from 0.57 to 10 μm were captured and ingested at rates that depended upon particle size and concentration, but that particles with diameters of 1.90 and 3.06 μm were taken in largest numbers at a particular concentration. However, the volume of 'food' ingested per cell per hour increased with microsphere diameter at any particular concentration, and a greater volume of 10 μm microspheres was ingested at a concentration of 10^4 particles ml^{-1} than of any smaller particle at a concentration of 10^6 ml^{-1}. It was interesting that in these experiments the proportions of cells that ingested particles decreased sharply as concentration was reduced from near 100 % at 10^6 ml^{-1}, to 75 % at 10^5 ml^{-1}, 25 % at 10^4 ml^{-1} and about 10 % at 10^3 ml^{-1}.

In the other study the patterns of lectin binding sites in *E. mutabilis* were determined by Wilks & Sleigh (2004). Living cells, including those of prey or-

* The authors dedicate this article to Prof. Dr. Wilhelm Foissner, on the occasion of his 60th birthday.

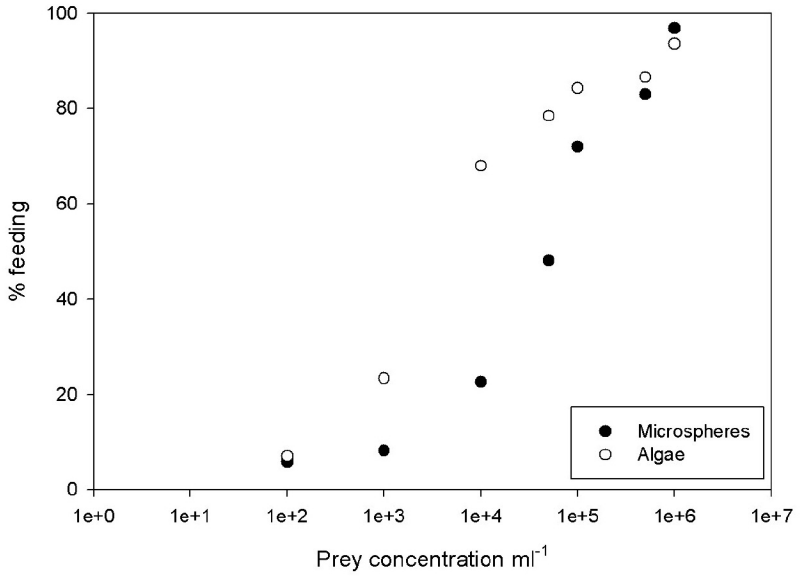

Fig. 1: The percentage of *Euplotes* observed to feed on microspheres or algae when these were presented at different concentrations from 10^2 to 10^6 ml^{-1}. For this figure the data for microspheres or algae of all sizes used were combined together at each concentration.

ganisms, expose carbohydrates of the glycocalyx on their surface which may be a useful property for the recognition of potential prey by a ciliate, using other carbohydrates on their own surface as binding sites. Carbohydrate recognition sites on the surface of a ciliate capable of binding to prey may be located by the use of lectins that specifically bind to those carbohydrates. Several fluorescent lectins were found to bind principally to the cytostome area and to food vacuoles; concanavalin A showed the strongest and most persistent labelling of this type. However, one lectin, wheat germ agglutinin (WGA), also rapidly labelled a group of 8–9 frontal membranelles of all *E. mutabilis* cells. Both frontal membranelles and the cytostome lining could be involved in positive or negative food selection.

The present report is concerned with further parts of the same study attempting to relate these results to the real life of the ciliates by seeking answers to two principal questions. 1. Will real prey be treated in the same way as inert microspheres? 2. How will the cell behave when presented simultaneously with prey of different sizes, as in nature? The findings of experiments are then combined into a discussion on the feeding of this ciliate.

Materials and methods

The ciliates, their preparative handling and the procedures for performing experiments were exactly as described for experiments on the uptake of microspheres by WILKS & SLEIGH (1998), except that 'algal' cells were provided as potential prey, and 0.5 % (v/v) glutaraldehyde was used as a fixative in place of TCA, since glutaraldehyde preserves ingested algal cells better. Each experiment was repeated three times and the number of ingested cells in at least 100 ciliate cells was counted in each replicate experiment and presented as a mean value with standard error, making these experiments exactly comparable with those on microspheres completed a few weeks previously.

Four types of photosynthetic cells were used as prey organisms. The algae *Chlorella* sp. (CCAP 211), nearly spherical and about 4.5 µm in diameter, *Isochrysis galbana* (Plymouth Culture Collection "I"), nearly spherical and about 3 µm in diameter, and *Rhodomonas* sp. (CCAP 995/2), whose elongate cells have an equivalent spherical diameter of about 9 µm, were all grown in 100 ml Nunclon culture flasks containing 40 ml of Guillard's f/2 medium (see GUILLARD 1975). The cyanobacterium *Synechococcus* sp. (strain WH7803 from Warwick University), with a diameter of about 2 µm, was grown in artificial seawater medium with added trace metals, boric acid and EDTA. All were cultured at a room temperature of 20 ± 5 °C in natural daylight. Although the cyanobacterium *Synechococcus* is not strictly an alga, it is a comparable photosynthetic cell, and will be referred to as an alga for brevity.

We had expected that the autofluorescence of chlorophyll in these cells would be sufficient for them to be easily distinguished for counting within the food vacuoles of *Euplotes*, but this was not the case for three of these types, particularly *Synechococcus*. It was therefore necessary to use fluorescent labeling of the prey, which has been common practice in such feeding studies (e.g. SHERR et al. 1987). It was decided to stain algae with DTAF (5(4,6-dichlorotriazinyl)aminofluorescein, obtained from Sigma), following the method used by RUBLEE & GALLEGOS (1989). This involved gentle centrifugation to concentrate the cells, which were re-suspended in 0.02 M TRIS-NaCl buffer (2.42 g l^{-1} TRIS, 8.5 g l^{-1} NaCl) containing 0.2 mg ml^{-1} DTAF (Sigma) and incubated for 2–3 hours in a water-bath at 54–60 °C, before being washed three times in the TRIS buffer and re-suspended using a vortex mixer. Stained cells can be stored in this medium for months if kept frozen. Before use in experiments the cells were thoroughly washed three times in artificial seawater (ASW) and then re-suspended in ASW. *Isochrysis galbana* was too fragile to survive the heating involved in labelling by this method, but fortunately had sufficient autofluorescence to be used live in experiments.

The interplay between relative sizes and relative concentrations of even two different particles may be investigated by varying the parameters in many combinations. Two of the simpler patterns were tested experimentally. In the first one type of particle was presented

Table 1: Comparisons of the numbers of algae and the volume of algal material ingested at typical ambient concentrations for algae of various sizes, compared with numbers and volumes of microspheres of the nearest equivalent sizes ingested at the same concentrations. Confidence limits of the numbers of algae and microsphieres ingests were never greater than 10%.

Algae	Concentration	Number ingested per hour		Volume (µm³) ingested per hour	
		Algae	Microspheres	Algae	Microspheres
Synechococcus	1 x 10^6	217	185	911	664
Isochrysis	1 x 10^5	44	19	621	280
Chlorella	1 x 10^3	4	0.36	184	5.4
Rhodomonas	1 x 10^2	0.2	0	76	0

at the same concentration in all tests, but the concentration of a particle of a different size was varied. Such a test can be performed with microspheres of two different sizes labeled with different fluorochromes, or with a microsphere and an alga. It was not possible to use two different algae together because of the difficulty of distinguishing between their fluorescences. In the second type of test two types of particle were presented at the same concentration as one another, with the concentration varied in different tests, again using either or both of microspheres and algae. The same fluorescent microspheres and stained algae were used as before, with the addition of the DTAF-stained alga *Phaeodactylum tricornutum* (CCAP 1052/6), whose cells measured 8 × 4.5 µm on average, the spines being generally broken off during ingestion.

Results

The feeding of *Euplotes mutabilis* on four sizes of algal cells at different concentrations

It was quickly found that many *Euplotes* did not feed if the concentration of algae was too low, but the percentage of ciliates that ingested algal prey was greater at the lower prey concentrations than when inert microspheres were presented at the same concentrations (Fig. 1).

Comparisons of the numbers of algal cells and of microspheres of different sizes ingested at different concentrations, for which two examples are presented in Figure 2, showed that the general trends were the same for algae as for microspheres, but that algae were ingested by *Euplotes* at somewhat higher rates than microspheres of equivalent sizes and concentrations. Although fewer larger cells than smaller ones may be taken up at a particular concentration, the ingested volume of these larger cells is generally considerably higher. However, in life, small prey cells will generally be present in much higher concentrations than larger ones, so it is instructive to compare the numbers and volumes of algal prey of different sizes ingested at concentrations likely to be met in nature (Tab. 1), where the numbers and volumes of the nearest equivalent sizes of ingested microspheres are included for comparison. Clearly, both the numbers and volumes of algal prey ingested are larger than those of similar microspheres at the same concentration, but the ciliate may ingest larger volumes of smaller prey than of larger prey at concentrations commonly found in planktonic environments because of great differences in the frequency of encounters with particles of different sizes.

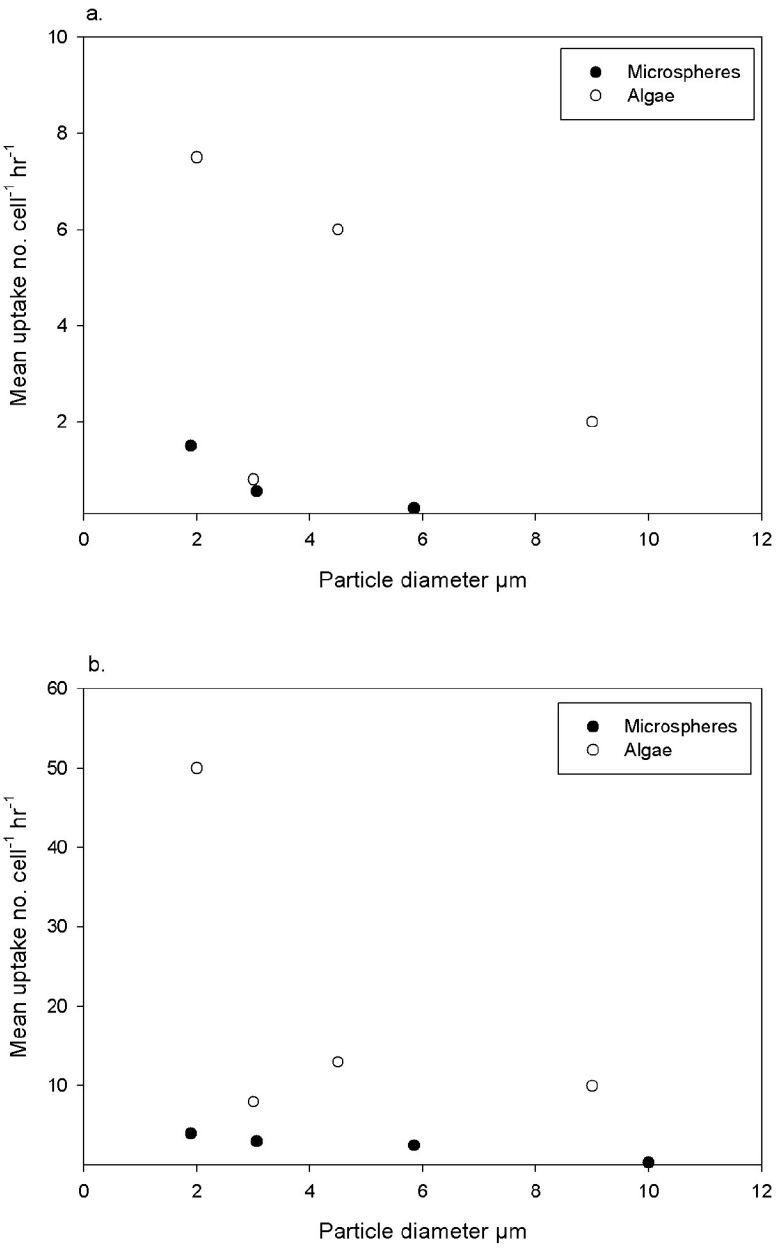

Fig. 2a, b: Comparison of the mean numbers of algae or microspheres of different sizes taken up in 10 minutes when the "prey" are presented at 10^3 particles ml^{-1} (**2a**) and at 10^4 particles ml^{-1} (**2b**).

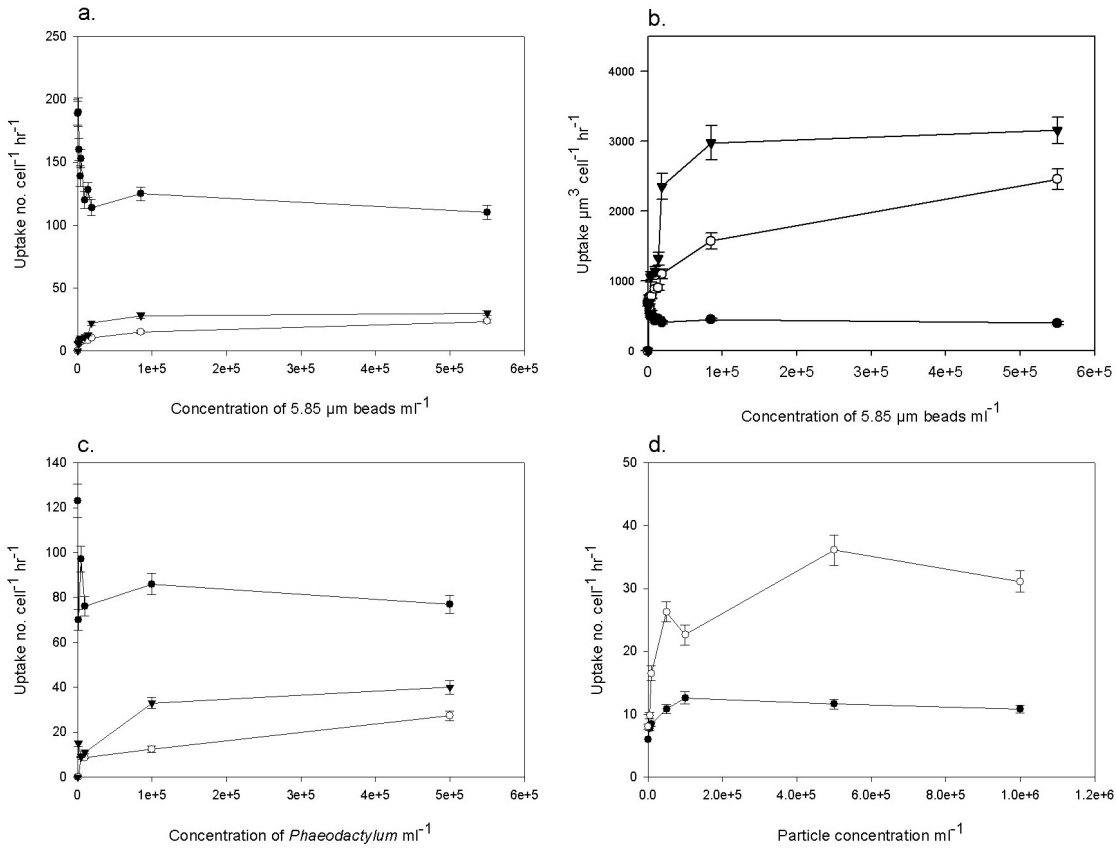

Fig. 3a-d: Examples of results of experiments in which two different particles are presented at the same time. **3a**: comparison of the numbers of microspheres taken up when microspheres 1.90 µm in diameter were always present at 5 x 10^5 ml^{-1}, while the concentration of 5.85 µm microspheres presented was varied between 1 x 10^2 and 5.5 x 10^5 ml^{-1} (numbers of smaller beads are shown by ●, and of larger beads by ○); the larger beads were presented alone in control experiments (▼). **3b**: volumes of microspheres taken up in the series of experiments shown in Fig. 3a, using the same symbols. **3c**: numbers of microspheres (●) and *Phaeodactylum* (○) taken up in a series of experiments in which 1.90 µm microspheres were always present at 4.7 x 10^5 ml^{-1} and the concentration of algae was varied between 1 x 10^2 and 5 x 10^5 ml^{-1}; the algae were presented alone in control experiments (▼). **3d**: numbers of microspheres 6.11 µm in diameter (●) and *Isochrysis* cells (○) taken up when both types of particle were presented together in equal numbers at concentrations between 10^3 and 10^6 ml^{-1}.

The feeding of *Euplotes mutabilis* on particles from mixtures

In life the ciliate will normally encounter mixtures of prey of different sizes and different concentrations, such as fewer larger flagellates and more numerous smaller bacteria. How does it handle such mixtures? Examples of several experiments designed to investigate this question will be described, although a wider diversity of combinations were examined experimentally with comparable results.

In the first series of experiments microspheres of two different sizes were presented. In one example 1.90 µm microspheres were always provided at a concentration of 5 × 10^5 ml^{-1}, while the concentration of 5.85 µm microspheres was varied between 1 × 10^2 and 5.5 × 10^5 ml^{-1} (Fig. 3a); the uptake of the larger microspheres at the different concentrations was recorded in the absence of the smaller ones as a control for comparison. In the absence of the larger particles about 180 of the 1.90 µm microspheres were ingested per cell per hour, but this number quickly fell when even a few of the larger particles were present, and the number of 1.90 µm microspheres ingested stabilized at about 120 cell^{-1} h^{-1} at the higher concentrations of the larger particles. The number of the larger particles ingested was also depressed in the presence of the smaller ones, in comparison with control values. The percentage of cells that ingested the larger particles was also somewhat depressed by the presence of the smaller ones, although the volume of the larger particles ingested was well above the volume of smaller ones ingested at all of the higher concentrations of the larger particles (Fig. 3b). Generally particles of different sizes were in different vacuoles, but occasionally a few of the smaller particles would be present alongside one or more of the larger ones.

In another experiment of the same basic design, 1.90 μm microspheres were presented at a constant concentration of 4.7×10^5 ml^{-1}, while *Phaeodactylum* was presented at concentrations between 1×10^2 and 5.5×10^5 ml^{-1} (Fig. 3c). The results mirrored those found with microspheres of different sizes. The presence of the larger algal cells depressed the ingestion of smaller particles, and the numbers of algae ingested were also depressed by the presence of the smaller particles. Usually microspheres and algae were seen to be ingested in different vacuoles.

When particles of two different types were presented together at the same concentration, the numbers of each type of particle ingested depended on their concentration and size. In the example shown in Figure 3d, the alga *Isochrysis* and microspheres of 6.11 μm in diameter were presented at the same concentration over a range from 10^3 to 10^6 ml^{-1}. The rate of ingestion of each type of particle increased with the concentration in the same way as if the other particle is not present, but the rate of ingestion of both types was depressed below the level expected in the absence of the other, particularly at the higher concentrations. The percentage of cells feeding on *Isochrysis* was considerably higher than that feeding on microspheres, but there is no evidence that this was anything more than a size effect since in a parallel experiment the percentage of cells feeding on *Isochrysis* was about the same as that feeding on 2.76 μm microspheres.

Discussion

We have observed, in confirmation of a report by FENCHEL (1987), that *Euplotes* feeds while attached to surfaces by its cirri, but not to any extent whilst swimming. The general pattern of water flow around a hypotrich like *Euplotes* 'standing' on its cirri on a substratum is very similar to that of the stichotrich *Stylonychia* described by MACHEMER (1966), and explained in the same paper on the basis of the metachronal motion of its membranelles. Waves of coordinated beating travel along the AZM from the posterior end near the cytostome, along the left side of the buccal area, and across near the anterior margin of the cell to the end of the membranelle row at the right. Although the beating activity of the dexioplectic waves is always directed so that it will propel water away from the buccal area, the frontal and lateral parts of the AZM have a different effect on the water flow, and hence different functions, at least in part. The frontal membranelles beat upwards and backwards around the anterior margin of the cell, drawing water from in front of the ciliate towards the anterior margin; most of this flow then passes over the dorsal surface of the cell, with a minor fraction being drawn beneath its ventral surface because of currents created by the lateral membranelles. These lateral membranelles propel water away from the buccal area towards the left of the cell. Particles in the water that is swept by the frontal membranelles can be redirected into the ventral flow by these frontal membranelles and then filtered on the 'upstream' side of the lateral membranelles from the current passing to the side. Posterior components of this lateral current convey the particles back to the cytostome area where they may be ingested. The constraints of the substratum on water flow beneath the cell in these low Reynolds number hydrodynamic conditions, as well as the contours of surfaces in the buccal area, are likely to contribute both to the flow lines of the water currents propelled by the membranelles and to the efficiency of filtration. This method of filtration of particles on the 'upstream' side of the propulsive ciliary band contrasts with the filtration seen in peritrichs like *Vorticella*, where the haplokinety cilia form the filter at the 'downstream' side of the propulsive polykinety ciliary band (SLEIGH & BARLOW 1976).

Specializations of the ciliate membrane, detected by the binding of lectins, occur at two sites along the pathway followed by particles before ingestion. The first, detected only with WGA which binds to the basal halves of 8–9 membranelles at the left end of the frontal section, is situated in the region of strongest flow, where particles may first contact the cell. There is no clear evidence of the function of this specialized site. It could be used as a means of rejection of distasteful prey, or, perhaps more likely, as a means of positive selection by the triggering of a reverse beat of the membranelles to retain the prey, comparable with the mechanism of upstream retention of prey by ciliary bands of invertebrate larvae studied by STRATHMANN et al. (1972). The second site is at the cytostome, where the lining of the enlarging food vacuole binds a wide range of lectins. These binding sites may be essential to hold potential prey within the food 'cup' against forces of water flow, and could be selective by stronger binding (more sites for varied ligands) of larger or more attractive prey. Such bound prey items would then be ingested by closure of the food vacuole.

We have reported evidence here that algal prey are ingested in similar numbers to inert microspheres, according to their size and concentration, so algae and microspheres are in general handled in a similar way. However, there is some preference towards algae compared with microspheres of the same size and concentration which is evident in experiments on the uptake of particles from a homogeneous suspension and from a mixture of two types of particle. It is also found that the percentage of ciliates that feed on algal prey is greater than

the percentage feeding on microspheres of similar size and concentration. These expressions of preferential uptake may be the result of better retention of algae than microspheres at the cytostome through stronger binding (more occupied sites) to the food vacuole membrane. The number of occupied binding sites at the food vacuole membrane could be the factor which determines whether a ciliate feeds or not.

The finding that the presence of even a small number of larger particles in a mixture can depress the number of smaller particles that are ingested is more difficult to explain. It is clearly an advantage to the ciliate to ingest a few large particles than a larger number of small particles, because the former represent a greater food volume, but the mechanism for achieving this is more difficult to deduce, unless small particles are displaced by larger particles which could be bound to the food vacuole membrane at more sites.

It was remarked above that the volume of smaller prey ingested may be greater than the volume of larger prey when both are present at common ambient planktonic concentrations. Prey organisms tend to congregate in patches of higher concentration. This is even more true for bacteria or algae near to surfaces, where the availability of nutrients and/or light may encourage multiplication. The particle concentrations considered here may be thought of as rather high in a purely planktonic situation, but near a surface, where *Euplotes* feeds, higher concentrations are commonplace. *Euplotes* shows both avoidance behaviour that tends to keep it within a patch of food (JONSSON & JOHANSSON 1997) and chemosensory attraction that enables it to respond to concentration gradients of chemical attractants (FENCHEL 2004).

We conclude from these studies that *Euplotes* can and does feed on food particles of varied size and chemical nature. Its efficiency of feeding is broadly what might be expected of a filtration mechanism based on size and concentration of particles. Very small particles (e.g. average bacterial cells below 1 µm in diameter) are not filtered as efficiently as particles with diameters of 2 µm and above, but can still be captured in large enough numbers to provide the food needs of the ciliate when such bacterial food is sufficiently abundant. *Euplotes mutabilis* ingests food particles of sizes up to at least 10 µm in diameter, and potentially gets better food value from a few large particles than many small ones. There is some, but only limited, preference for real cells in comparison with inert microspheres. The feeding mechanism appears to involve physical filtration, chemical recognition and retention by binding of prey to the membrane of the forming food vacuole before ingestion by closure of the food vacuole.

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