

## **Suspension Feeding in Ciliated Protozoa: Functional Response and Particle Size Selection**

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**Abstract.** The quantitative uptake of latex beads of different sizes and of live cells by 14 species of ciliates was studied. The functional response (uptake rate as function of food particle concentration) can be fitted to a hyperbolic function and this can be explained in terms of the function of the mouth apparatus. Each species shows a distinct size spectrum of particles which are retained and ingested. These size spectra may be explained by mouth morphology, and particle size selection may play a role for niche separation of coexisting ciliates. Most bacterivorous holotrich ciliates retain particles down to  $0.2 \mu\text{m}$  and in one case down to  $0.1 \mu\text{m}$ ; they retain particles between  $0.3$  and  $1 \mu\text{m}$  most efficiently. The spirotrich ciliates investigated do not retain particles smaller than  $1-2 \mu\text{m}$ .

### **Introduction**

The understanding of protozoan feeding is of interest in several contexts. A mechanistic explanation of the particle concentration carried out by suspension feeders in general and by ciliates in particular is still incomplete [14]. The ecological significance of numbers of bacteria and of eukaryote photosynthetic microorganisms in nature can be interpreted only when the turnover of these populations is known, and the phagocytosis of bacteria by protozoa has been conjectured and in some cases demonstrated to be quantitatively important. During the last years, several studies on the feeding of ciliates have appeared. However, most of these studies are quite restricted in their scope and do not allow any integrated picture of the physiological, morphological, evolutionary, and ecological aspects of the subject.

The present paper is one in a series which offers a comparative approach to the study of suspension feeding in ciliates. Other papers [6-8] will treat morphological, functional, and evolutionary aspects of ciliate feeding. In this paper, a description of the functional response to environmental food concentration and food particle selection will be given. In a sequel [6], ecological energetics and the role of ciliates will be discussed and compared to that of metazoan suspension feeders.

Suspension feeders are organisms which feed on freely suspended, relatively small food particles. Suspension feeders possess special morphological adaptations which

serve to concentrate the food particles. Further, the feeding of such organisms is in general characterized by a high degree of "automatization"; that is, they have a constant feeding rate and lack the ability to discriminate between different kinds of particles except for their mechanical properties [13]. Among ciliated protozoa, suspension feeding is far from the only feeding mode represented. Many groups contain parasites (including forms which depend exclusively on dissolved organics), forms feeding on microorganisms attached to surfaces, forms feeding on large microphytes and predators of other protozoans or small metazoa [5]. The functional response (that is, the ability to catch or concentrate food items as a function of food concentration in the environment) can be described in similar terms irrespective of the type of feeding. Thus included in the present study are some species which would normally be referred to as raptorial carnivores, since they depend on food particles which are large relative to their own size.

## Materials and Methods

The present study is based on 14 species of ciliates. *Glaucoma scintillans* Ehrb., *Colpidium campylum* (Stokes), *Paramecium caudatum* Ehrb., *P. trichium* Stokes, *Cyclidium* sp., *Lembadion* sp. [most likely *L. lucens* (Maskell)], *Blepharisma americanum* Suzuki, *Bursaria truncatella* OFM, and *Euplotes moebiusi* Kahl were all isolated from various ponds and streams in the surroundings of Woods Hole, Mass., or, in the case of the marine *Euplotes*, from "Eel Pond." *Colpoda steini* Maupas and *C. cucullus* OFM were isolated from soil of potted plants in the author's laboratory in Denmark; and *Colpidium colpoda* (Ehrb.), *Strylonychia mytilus* Ehrb., and *Stentor coeruleus* Ehrb. were isolated from ponds in the botanical garden, Aarhus, Denmark. The *Lembadion* and the giant form of *Blepharisma* were grown on *Glaucoma*; *Bursaria* was fed *Colpidium campylum*; and *Stentor* and *Strylonychia* were grown on *Chilomonas*. The remaining species were cultured in infusions of rice or wheat grains.

The cell volume of individual ciliates was determined by pressing live or formaldehyde-fixed cells under the cover glass until they had plane parallel sides, drawing the outline of the squeezed cell with a camera lucida and measuring the thickness of the preparation with the micrometer screw of the microscope.

For studying particle uptake, suspensions of latex beads (Dow Chemical Company) were used. These are sold in suspensions which contain 10% dry matter. The following diameters were used: 0.09, 0.11, 0.23, 0.36, 1.09, 2.02, and 5.7  $\mu\text{m}$ . Larger particles cannot be used as they settle too rapidly (and the suspensions of 5.7  $\mu\text{m}$  beads had to be continuously shaken), whereas particles  $< 1 \mu\text{m}$  cannot be used in seawater as they tend to agglutinate to form complexes of two or more beads making it impossible to obtain suspensions with a defined particle size. In order to study feeding on large particles, suspensions of live ciliates were used: *Cyclidium* (average diameter  $\sim 10 \mu\text{m}$ ), *Glaucoma* ( $\sim 20.5 \mu\text{m}$ ), *Colpidium campylum* ( $\sim 34.4 \mu\text{m}$ ), and *Paramecium caudatum* ( $\sim 54 \mu\text{m}$ ). Finally, commercial bakers yeast (average diameter: 4.35  $\mu\text{m}$ ) was used for some experiments.

When ciliates are placed in suspensions of particles (within a size range which the organisms will ingest), a linear uptake with time is found (Fig. 1). In a typical experiment, ciliates were placed in a suspension for exactly 5 min. Individual cells (15–20) were then rapidly picked up with a capillary pipette and transferred to a drop of formaldehyde on a slide. Larger particles ( $> 1$  or  $2 \mu\text{m}$ ) were counted individually within each ciliate. Smaller particles cannot be resolved under the microscope. Instead, food vacuoles containing the highly refracting latex beads were counted and their volume determined from the diameter of their usually perfectly spherical shape. Since the beads show hexagonal packing in the vacuoles (unpublished EM observations, see also [15]), the total volume or numbers of beads could be calculated. All the mentioned species were tested for different particle sizes at different concentrations. All experiments were carried out at room temperature (20–22°C).

Much evidence shows that the ciliates do not discriminate between different particles on the basis of properties other than size or shape. However, a number of precautions must be taken to avoid unreproducible or erratic results. During several minutes to hours after cells have been centrifuged or transferred to water with other properties (temperature, pH, ionic strength), they may refuse to feed altogether or only a fraction of the cells will feed at a normal rate. This has previously been observed [17] for *Tetrahymena*. In the present

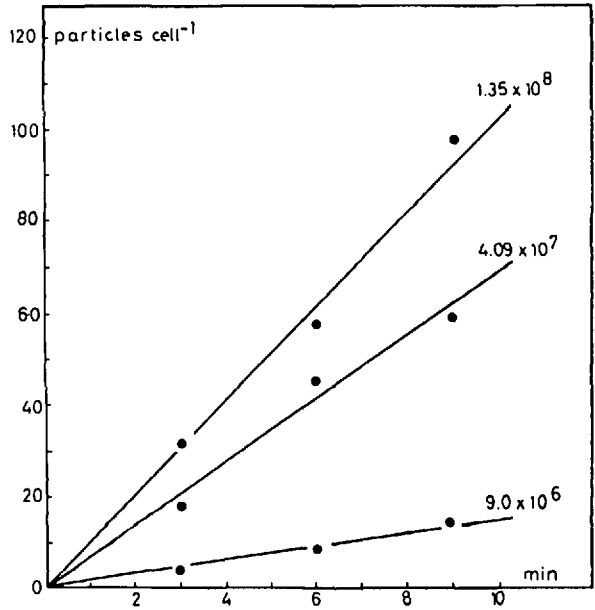


Fig. 1. The uptake of 1.09  $\mu\text{m}$  latex beads by *Glaucoma scintillans* as function of time and at 3 different particle concentrations (particles  $\text{ml}^{-1}$ ).

experiments, it was, of course, important that particle suspensions were made with particle-free water. Usually filtered culture fluid was used. Even so, a strict absence of foreign particles could not be obtained since some bacteria or other particles followed when the ciliates were transferred to the experimental dishes. The presence of such particles, however, was negligible compared to the concentration of particles tested, while a rigorous cleaning of individual ciliates immediately prior to an experiment often yielded erratic results. Curds and Cockburn [3] showed that in *Tetrahymena* feeding is a decreasing function of cell density, presumably because the organisms are disturbed when they bump into each other. This effect was negligible in the present study since I used relatively low numbers of cells (mostly  $\sim 100$  in 2 ml). The effect described in *Tetrahymena* is important only when concentrations exceed  $10^3 \text{ ml}^{-1}$ .

In a few species high concentrations of latex beads lead to a total clogging of the mouth or induce excessive mucus production. These effects, which lead to erratic results or failure to feed, are considered pathological, and in these cases the estimate of the maximum uptake rate is inaccurate and unrepresentative for natural food items. This reservation is of particular importance for the considerations in [7].

## Results and Discussion

### Form of the Functional Response

For any given particle size, the rate of ingestion gradually reaches a saturation value as the environmental particle concentration is increased. This relationship can be closely fitted to a hyperbolic function (Fig. 2) of the form:

$$U = U_m C_p / (K + C_p),$$

where  $U$  is the uptake rate (in numbers or total volume of particles per unit time),  $U_m$  is the maximum uptake rate,  $C_p$  the particle concentration, and  $K$  a half-saturation constant. This form of the Michaelis-Menten kinetics has previously been used to

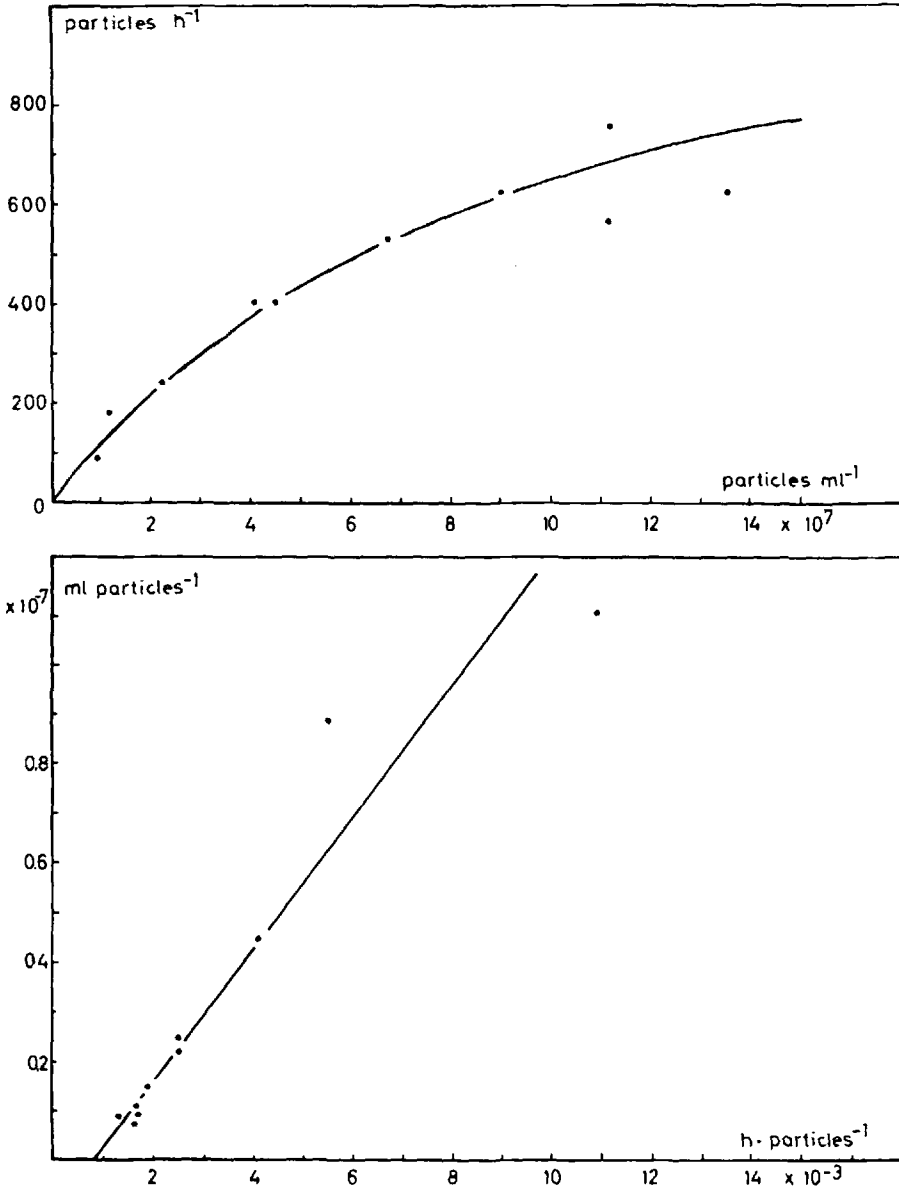


Fig. 2. The uptake of  $1.09 \mu\text{m}$  particles by *Glaucoma scintillans* as a function of particle concentration. Below the reciprocal data are plotted: The regression line intersects the horizontal axis at  $\tau$  and has a slope of  $F^{-1}$ .

describe the functional response of suspension feeders, including ciliates, or the growth rate as a function of food concentration (e.g., 2, 3, 10, 11, and 19). Although this description cannot be proven to show a better fit to experimental data than a number of other equations (16), it can be rationalized to be functionally quite analogous to the model of Holling [12] for predation. Assume that the ciliates have a maximum clearing rate,  $F$ , which is the volume of water the organisms can clear of particles per unit time at

low particle concentrations. At low particle concentrations the uptake rate would then equal  $FC_p$ . Now, if each particle or unit volume of particles ingested blocks the mouth by  $\tau$  (time per particle) because it takes a finite time to phagocytize a particle or form a vacuole, then the uptake rate,  $U$ , equals  $FC_p(1 - \tau U)$ , which is identical to the Michaelis-Menten form with  $1/\tau = U_m$  and  $1/F\tau = K$ .

The above parameters are useful for comparing different species in ecological contexts. The maximum clearing rate,  $F$ , gives information on the minimum particle concentration at which the organisms can sustain themselves; the maximum uptake rate,  $U_m$ , relates to the maximum growth rate; and the half-saturation constant,  $K$ , gives information on the food concentration to which the organisms in question are adapted.

### *Evidence for the Biological Interpretation of the Parameters*

Much of our understanding of the function of the mouth of ciliates derives from direct observations on the mouth organelles and on the rate of vacuole formation and volume. These results are reported elsewhere (6, 8).

In the model for the functional response discussed above, it is implicit that the rate of water transport by the ciliate remains constant and independent of particle concentration. But as the particle concentration is increased, an increasing number of particles initially trapped by the filter apparatus is lost or clogs the mouth because the rate at which particles can be removed by phagocytosis is limiting. This could directly be observed in the large ciliate *Bursaria truncatella* feeding on suspended *Colpidium campylum*. From other experiments it was shown that for this prey, the maximum clearance is  $0.43 \text{ ml h}^{-1}$  and that a *Bursaria* can at most ingest about 500 *Colpidium* cells per hour. From these data it is easy to calculate the expected number of filtered but not ingested (lost) prey organisms as a function of density. These could also be counted directly by observing feeding *Bursaria* under the dissection microscope. With increasing prey density, an increasing number are drawn into the mouth. However, since it takes some time to form a vacuole around a *Colpidium* (about 7 sec on the average), an increasing number of prey cells blocks the inner, funnel-shaped part of the mouth and newcomers therefore tends to escape again. As seen in Fig. 3, there is a reasonable accordance between the expected and found number of prey lost from the mouth as a function of prey density. This observation supports the assumption of a constant water transport rate independent of food particle concentration.

Particles with diameters belonging to the upper range of those being ingested tend to have a low value of  $U_m$  since they are phagocytosed more slowly and also have a higher tendency to clog the mouth. If in mixtures of different particle sizes, all particles are drawn into the mouth proportional to their concentration and are assumed to interfere with each other in the same way as they interfere with particles of their own size, then the total uptake can be predicted from the above discussed model. If  $F', \tau', C_p'$  and  $F'', \tau'', C_p''$  are the relevant parameters for two different particle sizes, respectively, then the expected total uptake from a mixture of the two kinds of particles should be given by  $(F'C_p' + F''C_p'')/(1 + F'C_p'\tau' + F''C_p''\tau'')$ . Two such experiments are shown in Fig. 4. The uptake constants for 0.36 and 2.02  $\mu\text{m}$  particles were first determined for *Colpidium colpoda*. The smaller particles are the ones most efficiently taken up by this ciliate while the  $U_m$  for the larger particles is about 8 times lower (in terms of ingested total volume). In each of the two experiments, the concentration of 0.36  $\mu\text{m}$  particles was held

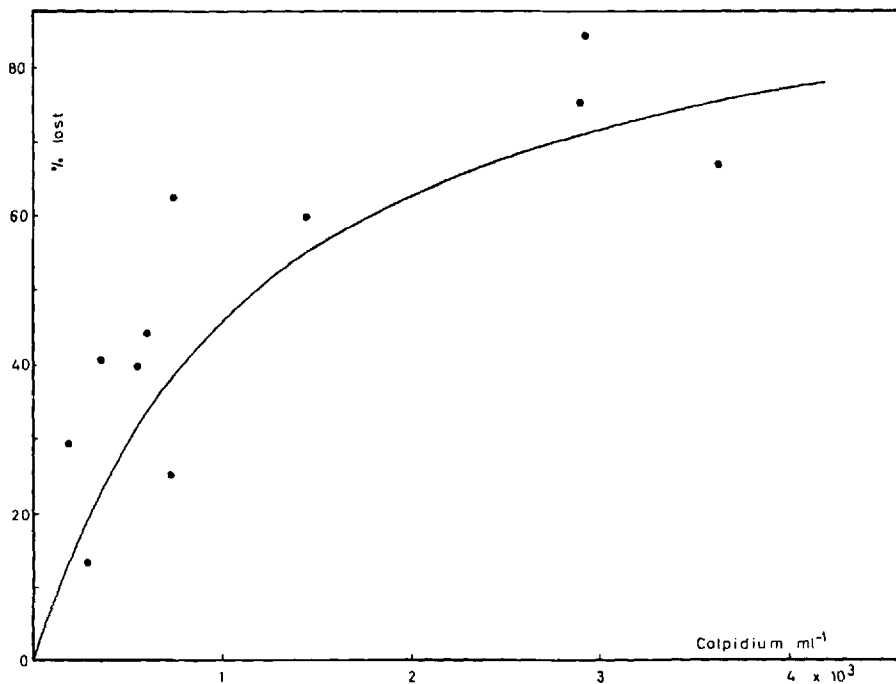


Fig. 3. The predicted and observed percentage of *Colpidium* cells sucked into the mouth and subsequently lost by *Bursaria truncatella* as function of prey concentration.

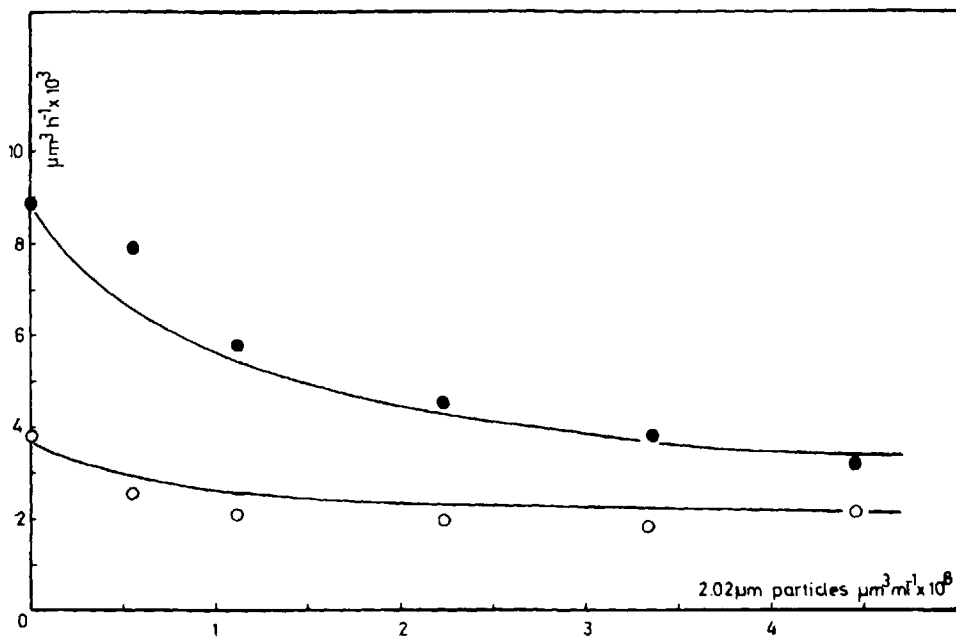


Fig. 4. The total uptake (volume) of mixtures of 0.36 and 2.02  $\mu\text{m}$  particles by *Colpidium colpoda*. The concentrations of 0.36  $\mu\text{m}$  particles were held constant at  $4.5 \times 10^7$  and  $1.8 \times 10^8 \mu\text{m}^3 \text{ml}^{-1}$ , respectively, in the two experiments. The solid lines represents the predicted uptake.

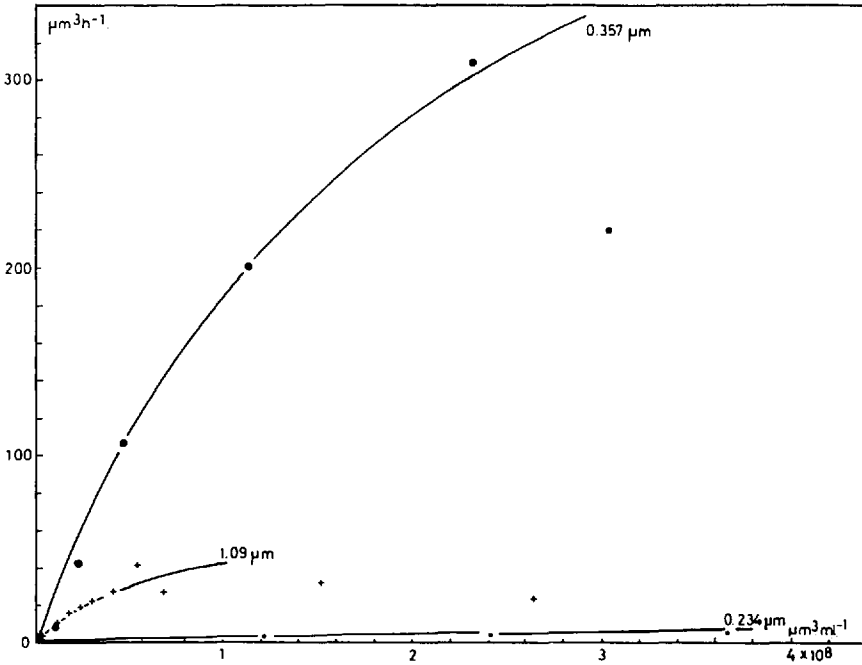


Fig. 5. The uptake of three different sizes of latex beads by *Cyclidium* sp. as function of particle concentration (measured as total volume  $\text{ml}^{-1}$ ).

constant, but different amounts of  $2.02 \mu\text{m}$  particles were added. The model predicts that the total uptake decreases with an increasing number of the larger particles, as shown on the figure. This experiment also supports an interpretation of the mouth as a purely mechanical filtering apparatus.

### Particle Size Discrimination

All investigated species show a distinct particle size spectrum. The minimum size of particles concentrated and ingested is sharply defined in many cases. For example, in *Cyclidium* sp. a decrease in the particle size from  $0.36$  to  $0.23 \mu\text{m}$  decreases the clearance by a factor of more than  $10^2$  (Fig. 5). This correlates closely with the distance between the cilia of the "haplokinety" (undulating membrane), which acts as a sieve for particles. In this species the free space between the cilia is about  $0.28 \mu\text{m}$  [6]. In *Glaucoma scintillans*, where the filtering is carried out by the third membranelle which is composed of several rows of cilia with varying mutual distances during the beat cycle [8], the lower particle size retained is less sharply defined (Fig. 6). This species shows some uptake of particles down to  $0.1 \mu\text{m}$  although the clearing rate is about two orders of magnitude lower than for the particle sizes most efficiently filtered. This species can therefore filter particles down to viral dimensions.

The maximum clearing rates for some species (expressed as multiples of the ciliates own volume per hour) are shown in Fig. 6 as function of particle size. Most bacterivorous holotrich ciliates can efficiently retain particles down to about  $0.2 \mu\text{m}$  and are most efficient for particles measuring between  $0.3$  and  $1 \mu\text{m}$ . There are, however,

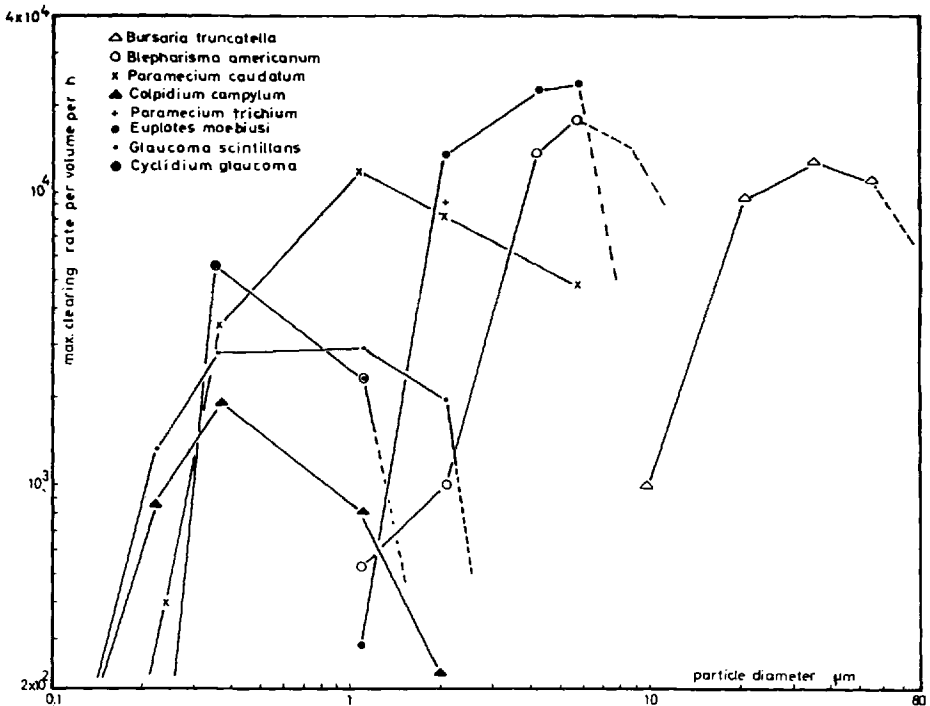


Fig. 6. Maximum clearing rate (in multiples of the ciliates own volume per h) for 8 species of ciliates and as function of particle size.

differences among the different species both with respect to the width of the spectrum of ingested particle sizes and with respect to the size of the most efficiently filtered particles. The trichostome *Colpoda* spp. are adapted to feed on somewhat larger particles. It is surprising that the smaller species, *C. steini*, is specialized on somewhat larger particles than the larger *C. cucullus* (Fig. 7), but this is in accordance with the mouth structure of the two species [6].

None of the spirotrich ciliates investigated can retain particles smaller than 1–2  $\mu\text{m}$ . The sieving action of these forms is carried out by the adoral row of membranelles. The free space between two neighboring membranelles varies from about 1.5  $\mu\text{m}$  in *Euplates moebiusi* to about 8  $\mu\text{m}$  in *Bursaria truncatella* among the species studied [6].

The upper size range of particles ingested is somewhat less well defined. Usually there is a size range of particles which are filtered and ingested fairly efficiently at low particle concentrations, but the maximum uptake capacity for them is low (Fig. 5). The maximum size of particles ingested at all is determined by the dimensions of the mouth or vestibulum. Some genera of ciliates contain polymorphic species. Under certain culture conditions and (in most cases conjecturally) under similar conditions in nature these species develop large individuals. Thus many *Blepharisma* spp. develop giant forms when their supply of bacterial food is exhausted [9]. In cultures they appear as cannibals. In general it may be considered as an adaptation to exploit larger food items such as other ciliates during later stages of successions based on decomposing organic material when



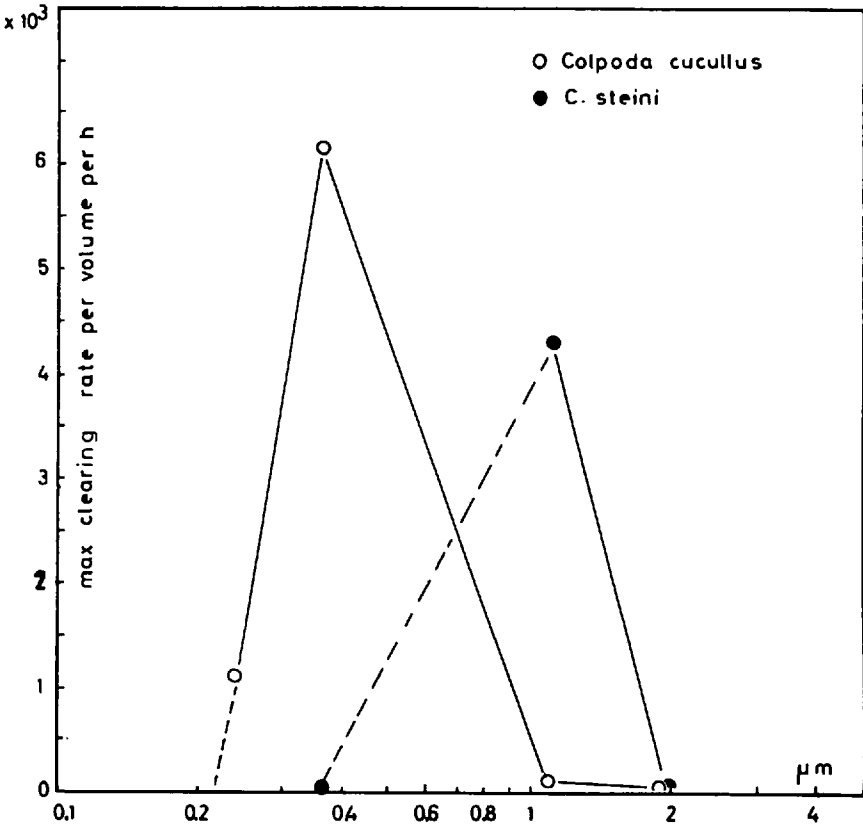


Fig. 7. Maximum clearance rate of 2 species of *Colpoda* as function of particle size.

the initial peak of bacteria has been exploited. Figure 8 shows the size distribution of starch grains ingested by three size classes of *B. americanum*. It is seen that the larger dimensions of the ciliates allow the ingestion of larger food particles.

### General Conclusions

The literature contains many claims of the ability of ciliates to discriminate between different qualities of food particles. It is a well established fact that the growth rates of different species are strongly affected by the strains of bacteria or microalgae which are offered as food, and indeed some strains of bacteria are directly toxic to certain ciliates [4, 20]. This, however, does not necessarily imply any selectivity with respect to the type of particles which are retained and ingested, nor does it give any direct evidence of niche diversification of coexisting species. Earlier work [1], the present results, and other quantitative estimates of the uptake of inert particles that agree with estimates of the uptake rate of natural food items [7] give no indication that any qualitative discrimination takes place among suspension feeding ciliates. On the other hand, the

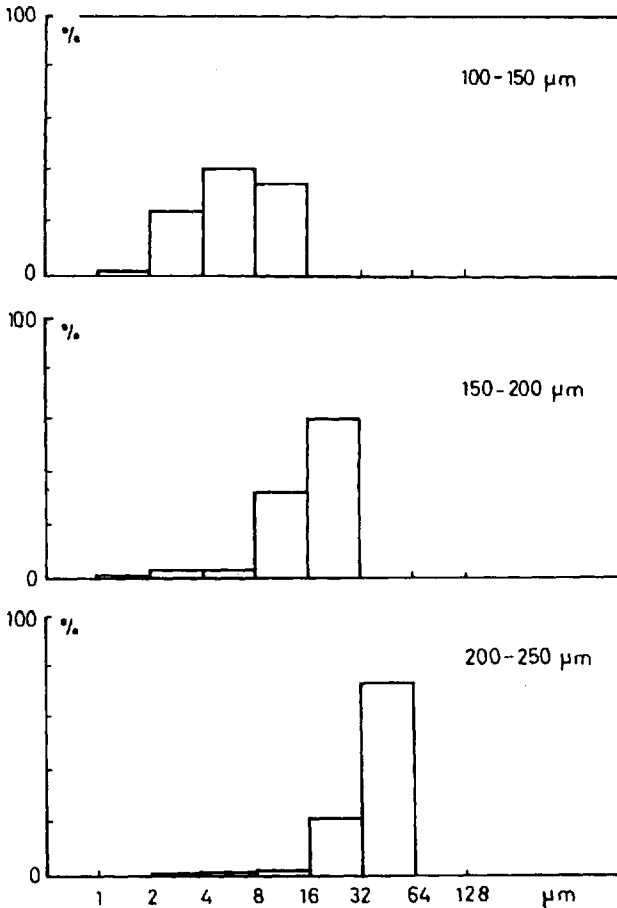


Fig. 8. The size spectra (% of total ingested volume) of 3 populations of *Blepharisma americanum* with different size spans placed in the same suspension of potato starch for 10 min.

results do indicate particle size discrimination as a function of the morphological properties of the mouth apparatus. This form of selection may explain earlier claims of preferences for food particles according to nutritive value or the rejection of inert particles (see 18 and references therein). Particle size selection may also under natural conditions lead to a selection for certain types of bacteria or other food particles. The general absence of selection mechanisms other than those considered here is conceivable. When, e.g., a *Paramecium caudatum* filters  $0.36 \mu\text{m}$  particles at a relatively high concentration, it will ingest about 400 particles per second and it is hard to suggest a mechanism which could sort individual particles sufficiently rapidly on the basis of properties other than mechanical ones.

The spherical latex beads employed in the present study do not in general mimic the shapes of bacteria. The size spectra shown in Fig. 6 do therefore not necessarily reflect the size spectra of particles ingested in nature precisely. Still the results do show that some ciliates can retain even the smallest prokaryote cells and that even within the food particle size range of  $0.2\text{--}2 \mu\text{m}$  size selection may be a significant niche separating factor for coexisting ciliates.

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