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On the feeding ecology of ciliates: what size particles do they prefer?

Abstract

The maximum ingestible particle sizes for 8 microphagic ciliates species were studied, using maize and potato starch as model food sources. Particles up to 7 to 16 μm were species specific size selected and ingested.

Detritus uptake was investigated with glass-homogenized charcoal. Little or none of this material was ingested. The importance of fine particulate material for the feeding ecology of microphagic ciliates is discussed.

Keywords

ciliates, feeding ecology, particles size, model food source

1 Introduction

The maximum ingestible particle size is an important aspect of the feeding biology of phagotrophic organisms. If they can not comminute their food mechanically while feeding, the maximum ingestible particle size tells us the maximum possible size of potential food particles and the extent to which a given particle size range in their environment can be utilized, and therefore shared, among animals of different species.

Various methodological approaches have been used to study this problem. If the animals concerned contain a sufficient number of food particles, direct measurement of the particle size permits conclusions to be drawn regarding the ingestible particle size range (for instance FINLAY and BERNINGER 1984, FINLAY et al. 1988). On the other hand, model food sources of a defined size, such as algal strains or plastic beads, can be offered to the animals as food and their uptake recorded.

The gain in knowledge can be enhanced by offering particles with a known size range as a food source since this permits the simultaneous identification of several

food particle size groups. Studies of this kind were performed on zooplankters by GLIWICZ (1969), who offered mineral particles as a model food.

Various kinds of starch have also been used for this purpose (for instance BOZLER 1924, SPITTLER 1973, 1976, HEERKLOSS and GUTELMACHER 1980, SPITTLER et al., 1990). Experiments with organisms feeding on larger prey have also used Sephadex (LABARBERA 1978) and grains of sand (SPITTLER 1979) as model food sources.

The purpose of the work described here was to gain knowledge concerning the maximum ingestible particle size for various protozoan species using commercial maize and potato starch as a model food source. The experiments were also designed to show whether these organisms ingest detritus. It is known that about 90% of the seston dry weight in bodden waters skirting the Baltic Sea consist of detritus (GEORGI et al.1980) and may therefore be of considerable feeding biological importance.

Experiments have been performed in which protozoans were fed carmine, and according to MEISSNER (1888) they were carried out for the first time in 1777. In our earlier experiments (SPITTLER 1973, 1976) we used glass-homogenized charcoal as a model detritus because this substance is neither dissolved nor otherwise changed in sea water or fixative mixtures.

2 Material and methods

The species included in the study and further data are presented in Table 1. The species from the bodden chain south of the Darss-Zingst peninsula (except *Strobilidium spec.*) were isolated from cultures based on sample site water and boiled wheat grains (one or two per 50 ml) and then cultured in wheat grain infusions. The animals were transferred to fresh culture medium every three or four weeks.

The culturing fluid consisted of bodden water taken from the sample collection site, filtered through 56 μm gauze and heated to 80 μC in a water bath. The cultures were kept in the dark at room temperature. *Tetrahymena pyriformis*, a clon-culture donated by SCHÖNBORN, was also cultured under identical conditions (Table 1).

The *Strobilidium spec.* for the experiment were from samples taken during a mesocosm experiment in June, 1990. In the course of this experiment, *Strobilidium spec.* developed abundances of up to 200 ind. ml^{-1} in one of the enclosures.

The two starches for the feeding experiments were soaked in a little water in the experimental vessels for 14 to 18 hours prior to each experiment. The water used for these experiments was the same as that used for the cultures (see Table 1). The experimental animals were held separately in gas jars while the starch was soaking so that particulate matter could settle and the food vacuoles of the animals could empty.

The starved animals were then decanted into the experimental vessels.

Table 1 List of investigated species with details of origin, salinity under culture and experimental conditions and mean animal size after fixation in Lugol's solution.

1: Own single species culture; isolates from bodden chain south of Darss-Zingst peninsula; 2: Cloned culture donated by Dr. habil. SCHÖNBORN, Jena; 3: Cloned culture, isolated from bodden chain south of Darss-Zingst peninsula by A. PRENA, FB Biologie, Univ. Rostock; 4: Study of a mass development during mesocosm experiments at Zingst (Zingster Strom) in June, 1990.

Species	Origin	Salinity ‰	Mean size (μm) L = length D = diameter
<i>Coleps hirtus</i>	1	1.6	L = 40
<i>Paramecium multimicronucleatum</i>	1	1.8 ... 2.2	L = 200
<i>Tertahymena pyriformis</i>	2	8.2 ... 8.6	L = 40
<i>Colpidium colpoda</i>	1	1.7	L = 90
<i>Spirostomum minus</i>	1	1.7	L = 150
<i>Blepharisma salinarum</i>	3	9.2	L = 110
<i>Strobilidium spec.</i>	4	7.9 ... 8.5	L = 30
<i>Euplotes affinis</i>	3	8.6 ... 9.6	L = 50

When the starch was offered in suspended form, the experiments were performed in 20 ml glass vessels with ground glass stoppers. The final starch concentration was adjusted to $2 \text{ mg} \cdot \text{ml}^{-1}$. The vessels were inverted at regular intervals to keep the starch in suspension.

For the experiments with non-suspended starch, 1 g of starch was weighed into dishes (6 cm diameter), and the experiment was started by adding 15 ml of water containing protozoa.

The experiments were continued for up to 7 h. The animals were then fixed in Lugol's solution, only animals from the supernatant being fixed in the case of the sediment-feeding experiments.

For the experiments with *Strobilidium spec.*, 5 g of soaked starch were placed in 1 liter of site water and kept in suspension by regularly inverting the experimental vessels.

The animals were removed by pipette under a dissecting microscope, and the ingested starch grains measured using an ocular micrometer. Since the grains were not spherical, the smallest diameter was measured in each case. Where necessary, the cover slip was cautiously moved to ensure that the grains had actually been ingested and were not adhering to the exterior of the animal.

In the case of *Paramecium multimicronucleatum*, the number of grains ingested per unit time was also calculated. The animals in this case were crushed by the pressure of the cover slip.

IVLEV's (1961) formula was then used to calculate the selectivity:

$$s = (g\% - e\%) * (g\% + e\%)^{-1}$$

In this formula, e% is the proportion of a given size group offered as a model food source and g% is the proportion of that size group among the ingested particles. The formula permits s to take values between -1 (complete avoidance) and +1 (complete preference), while 0 means that the particles are ingested according to the grain size frequency.

The e% values are based on the measurement of 1,000 grains, and g% was based on the measurement of at least 500 grains. In the case of ellipsoid grains, only the smallest diameter was measured.

Glass-homogenized charcoal with a grain size ranging from less than 1 to about 30 μm was used to study detritus uptake. The experimental animals were transferred directly from the culture vessels to block dishes by pipette, whereupon a droplet of charcoal suspension was added. Starved animals were also studied in these experiments. In the case of *Strobilidium spec.*, the charcoal suspension (2 g . l⁻¹) was added to a water sample, and the experimental vessel was inverted at regular intervals. The feeding experiment lasted for one to two hours.

3 Results and discussion

Commercial starch is a cheap and easily obtainable product and, as a model food source, has the additional advantages of being uniform in chemical composition, taste and surface consistency. Its particles move only passively in suspension. The particle size ranges used in our experiments were:

- maize starch 3 to 26 μm
- potato starch 5 to about 80 μm .

For the present study it is necessary only to consider particles of up to 20 μm in size. The particle size distributions of these starches are shown in Fig. 1 a,b, and their cumulative frequency in Fig. 1c. As can be seen, the proportion of small particles size groups is much larger in maize starch than in potato starch.

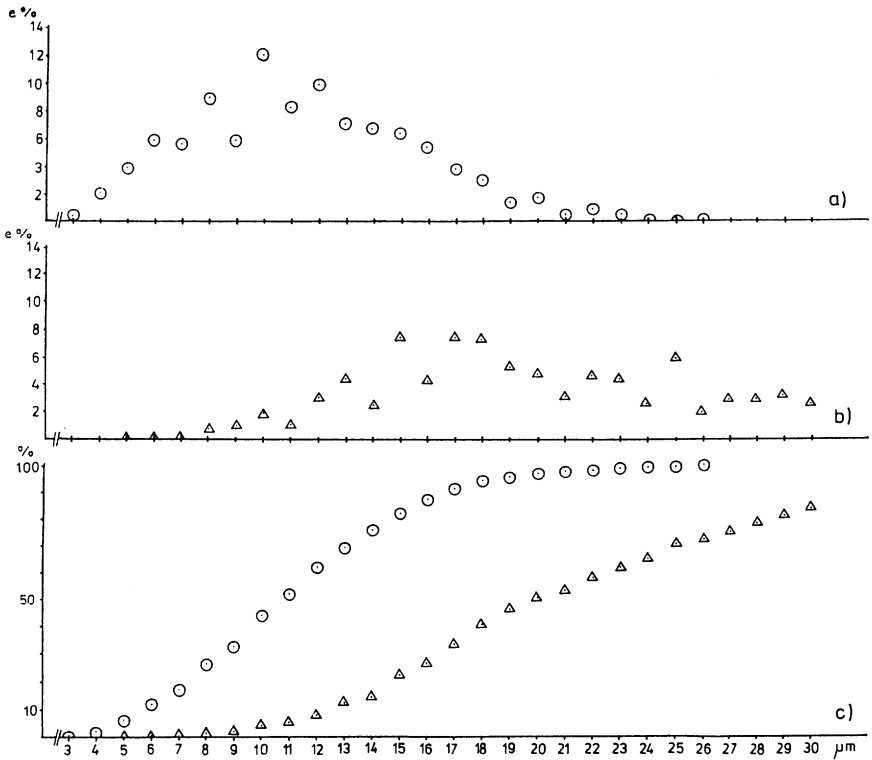


Fig. 1 Particle size distributions of a) maize starch, b) potato starch and c) cumulative maize and potato starch frequencies.

Only the smallest diameter of the irregularly shaped starch particles was measured in each case because we assumed on the basis of earlier experiments (SPITTLER 1976) that particles close to the ingestibility size limit would be taken up with their longitudinal axis aligned with the vestibulum.

Later examination showed that fixation in Lugol's solution had not affected the particle size even after months.

No starch that might have stemmed from the wheat grains in the cultures were found in the starved animals.

The results with *P. multimicronucleatum* (Fig. 2) showed that more maize starch than potato starch particles had been ingested. However, consideration of the differ-

ent feeding modes (suspension or sediment feeding) revealed no major differences between the uptake of the same starch in either mode, but distinct differences between maize and potato starch uptake (Fig. 3).

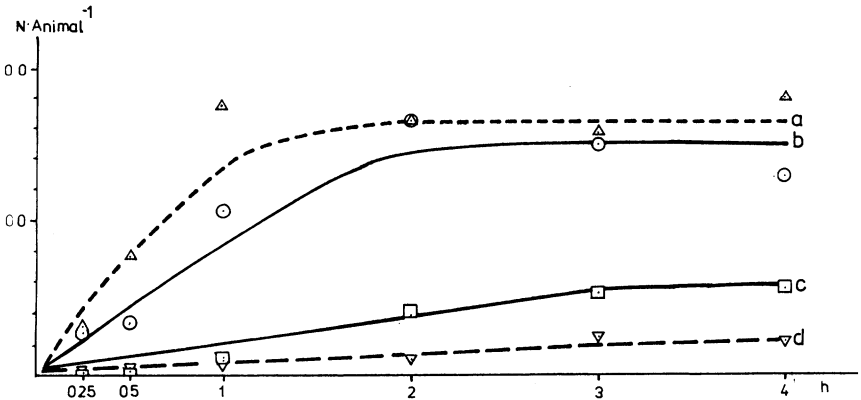


Fig. 2 Feeding rates of *Paramecium multimicronucleatum* on maize and potato starch in suspension and as sediment. Curves eye-fitted. a) Maize starch non-suspended; b) maize starch suspension; c) potato starch suspension and d) potato starch non-suspended. N = number of particles

Therefore, all other species investigated except *Strobilidium spec.* received only starch in the form of sediment as a model food source. The experimental animals could also be observed moving among the starch grains under the dissecting microscope.

It can be seen in Fig. 3 that a preference was shown for potato starch (c and d) particles up to 13 μm , whereas maize starch particles (a and b) of this size were definitely avoided. The preferred maize particle size range was only up to 7 μm . No ingested maize starch particles were found above 13 and 15 μm respectively or potato starch particles above 16 μm .

Both the more intensive uptake of maize starch (c.f. Fig. 2) and the preference for potato starch size groups (Fig. 3) which were already being definitely avoided in the case of maize starch can only be explained in terms of the different particle size distributions of the different starches (c.f. Fig. 1). Since ingestible potato starch particle sizes are relatively rare, the larger particles are taken up instead.

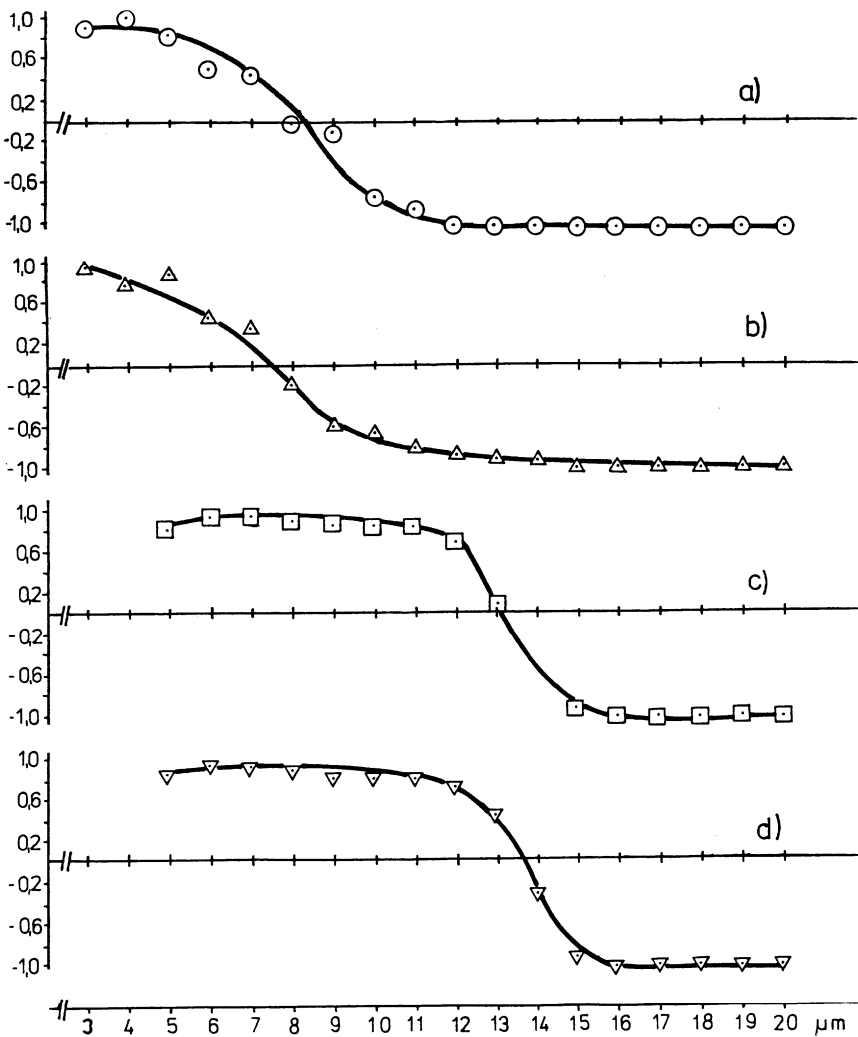


Fig. 3 "s" values for *Paramecium multimicronucleatum* fed maize starch in suspension and as non-suspended.
 a) Maize starch suspension; b) maize starch non-suspended; c) potato starch suspension and d) potato starch non-suspended.
 "s" = calculated selectivity.

According to the present results, only particles with diameters up to 15 μm can serve *P. multimicronucleatum* as food. BOZLER (1924) also used starch in his feeding experiments with *Paramecium caudatum* and found that this species ingested particles up to 11 μm in size. Some of the other species that were investigated also showed a preference for larger particles of potato starch, the maximum particle size ingested in each case being shown in Table 2, I.

Table 2 Maximum sizes of ingested starch particles (I) and carbon particles (II).
n. i. = not investigated; — = not ingested

Species	I	II
<i>Coleps hirtus</i>	16 μm (no Fig.)	n. i.
<i>Paramecium multimicronucleatum</i>	15 μm (Fig. 3c,d)	5 μm
<i>Tetrahymena pyriformis</i>	7 μm (Fig. 4a)	2 μm
<i>Colpidium colpoda</i>	9 μm (Fig. 5a)	2 μm
<i>Spirostomum minus</i>	16 μm (Fig. 6a)	5 μm
<i>Blepharisma salinarum</i>	12 μm (Fig. 7a,b)	--
<i>Strobilidium spec.</i>	10 μm (Fig. 8a)	--
<i>Euplotes affinis</i>	12 μm (Fig. 9a)	4 μm

Tetrahymena pyriformis avoided potato starch completely (Fig. 4). Particles with diameters up to 7 μm are present in potato starch with a relative frequency of only 0.6% (Fig. 1c) and are therefore obviously too rare. Only a few experiments could be performed with *Coleps hirtus* because the cultures died out.

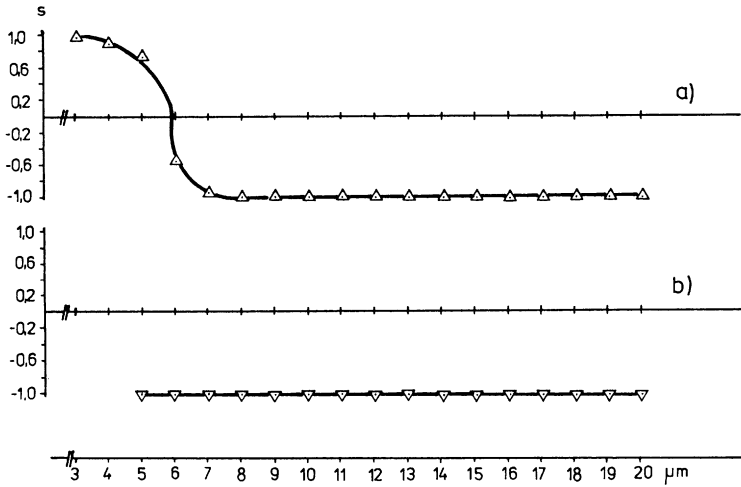


Fig. 4 "s" values for *Tetrahymena pyriformis* fed a) maize starch and b) potato starch non-suspended.

In the feeding experiments with *Strobilidium* spec. it was necessary to let the potato starch settle for an hour before fixing the supernatant. This permitted a sufficient number of animals to be separated from the starch serving as a food source for ingested particle measurement. This method could not be used in the case of maize starch owing to its smaller particle size and the correspondingly slow sedimentation rate.

There is obviously no direct link between ciliate size and size of prey. In the case of *Colpoda* spp., FENCHEL (1980) remarked: "It is surprising that the smaller species, *C. steini*, is specialized on somewhat larger particles than the larger *C. cucullus*... but this is in accordance with the mouth structure of the two species... The maximum size of particles ingested at all is determined by the dimensions of the mouth or vestibulum."

All of the investigated species must be regarded as microphagous because they are oriented towards relatively rare small particles in their selection of food. Their food source in culture consists of the microorganisms developing in the medium. The values obtained in our feeding experiments with potato starch represent the maximum ingestible size for the animals we used.

If sufficient smaller particles are present, whether as natural material in the water or as a model food, the species we investigated (and probably closely related species) prefer much smaller particles like bacteria and picophytoplankton as a food source (c.f. FENCHEL 1986, FINLAY et al. 1988).

This was confirmed by our experiments with homogenized charcoal. Initially there were no differences between starved animals and those from growing cultures. No

charcoal particles were ingested by *Blepharisma salinarum* or *Strobilidium* spec. The latter species therefore resembles the tintinnids in this respect (SPITTLER 1973). Some of the species ingested a few small particles of the size given in column II of Table 2.

These particle sizes differ radically from those found with potato starch, and surface consistency obviously also influences selection. In view of these results and those reported by FENCHEL (1986) and FINLAY (1988), it can be assumed that the natural food particle size is between 1/4 and 1/3 the maximum ingestible potato starch particle size.

These results also confirm that the animals used for our experiments are microphagous.

Summary

The maximum ingestible particle sizes for 8 microphagic protozoa species were studied, using maize and potato starch as model food sources. Particles up to the following sizes were ingested: *Coleps hirtus* 16 μm , *Paramecium multimicronucleatum* 15 μm , *Tetrahymena pyriformis* 7 μm , *Colpidium colpoda* 9 μm , *Spirostomum minus* 16 μm , *Blepharisma salinarum* 12 μm , *Strobilidium* spec. 10 μm and *Euplotes affinis* 12 μm .

Detritus uptake was investigated with glass-homogenized charcoal. Little or none of this material was ingested, and ingested particle size was only 1/4 to 1/3 that of ingested starch.

The importance of fine particulate material for the feeding ecology of microphagic ciliates is discussed.

Zusammenfassung

Durch Fütterung mit Mais und Kartoffelstärke wurde die maximal aufnehmbare Partikelgröße bei 8 mikrophagen Ciliaten untersucht. Körner bis zu folgenden Größen wurden aufgenommen: *Coleps hirtus* 16 μm , *Paramecium multimicronucleatum* 15 μm , *Tetrahymena pyriformis* 7 μm , *Colpidium colpoda* 9 μm , *Spirostomum minus* 16 μm , *Blepharisma salinarum* 12 μm , *Strobilidium* spec. 10 μm , *Euplotes affinis* 12 μm .

Die Aufnahme von Detritus wurde mit glass-homogenisierter Aktivkohle untersucht. Dieses Material wurde nur spärlich oder gar nicht gefressen, wobei die größten Partikel nur 1/4 bis 1/3 der o. g. Korngrößen erreichten.

Die Bedeutung von feinem partikulären Material für die Nahrungsökologie mikro-phager Ciliaten wird diskutiert.

Appendix

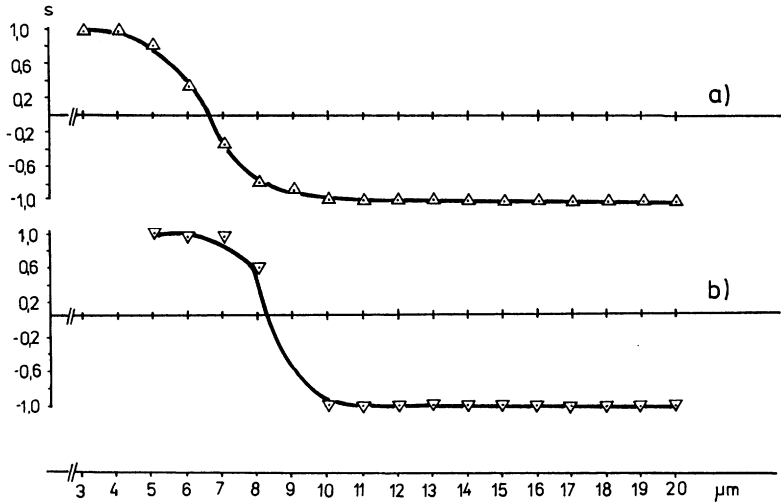


Fig. 5 "s" values for *Colpidium colpoda* fed a) maize starch and b) potato starch non-suspended.

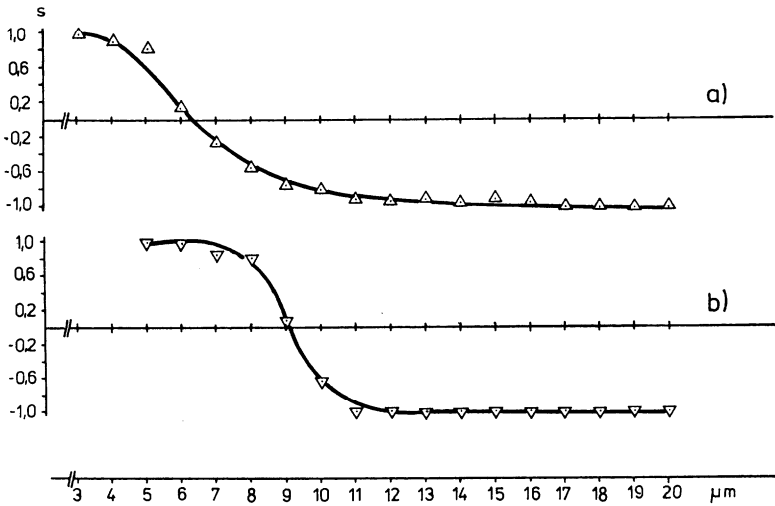


Fig. 6 "s" values for *Spirostomum minus* fed a) maize starch and b) potato starch non-suspended.

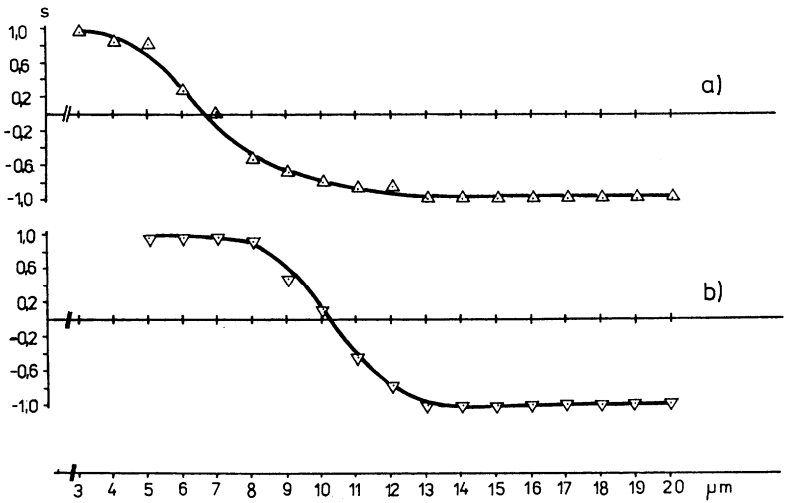


Fig. 7 "s" values for *Blepharisma salinarum* fed a) maize starch and b) potato starch non-suspended.

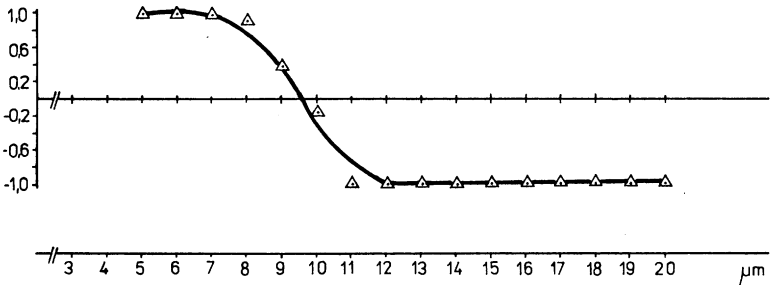


Fig. 8 "s" values for *Strobilidium spec.* fed suspended potato starch.

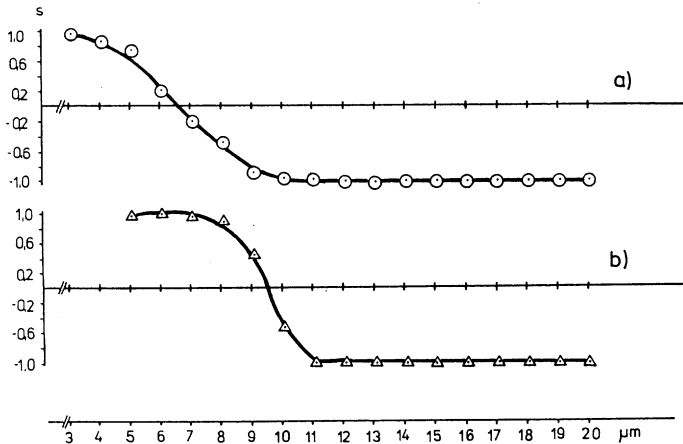


Fig. 9 "s" values for *Euplotes affinis* fed a) maize starch and b) potato starch non-suspend.

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