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# A study of feeding in predacious ciliates using prey ciliates labeled with fluorescent microspheres

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**Abstract.** Feeding in predacious estuarine ciliates was investigated in a series of laboratory experiments using a new method of prey labeling which facilitates microscopic indentification of ingested prey items. Ingestion rates of *Mesodinium pulex*, *Euplotes vannus* and *E.woodruffi* were estimated using the appearance, inside the predator, of bacteriovorous ciliates (*Metanophrys* sp., *Cyclidium* sp. and *Pleuronema* sp.) labeled with fluorescent microspheres. Prey remain motile and have presumably unaltered surface characteristics. Ingestion rates of log-growth phase predators increased with prey density. *Mesodinium pulex* ingested 0.15–0.32 cells $h^{-1}$ over a prey concentration of 60–2300 $ml^{-1}$. Maximum ingestion rates of *E.woodruffi* and *E.vannus* were 4.5 and 3.4 cells $h^{-1}$ respectively, estimated at prey abundances of 75 and 172 cells $ml^{-1}$ respectively. Comparisons of feeding rates on prey of different sizes, and the effects of starvation, indicated that ingestion is likely limited by different factors in 'raptorial' (*M.pulex*) and 'filter feeding' (*Euplotes* spp.) predators.

## Introduction

Predacious ciliates, used here to denote species that feed on other ciliates, are a very common component of ciliate communities. In the benthos, a large variety of predacious forms have been found in both temperate and tropical locales (Fenchel, 1968; Dragesco and Dragesco-Kerneis, 1986). Haptorid ciliates (which include the predacious genera *Mesodinium*, *Askanasia* and *Didinium*) are among the most common ciliate types found in freshwater pelagic ecosystems (Beaver and Crisman, 1989). In marine planktonic systems, easily recognized forms, e.g. *Didinium* and *Cyclotrichium*, have been reported from many areas: the arctic and subarctic Pacific (Taniguchi, 1983), the tropical Pacific (Beers and Stewart, 1971), the antarctic and subantarctic seas (Hada, 1970), the Black Sea (Zaika and Averna, 1969), the northern Atlantic (Faure-Fremiet, 1924), coastal and open waters of the Baltic Sea (Andersen and Sorensen, 1986; Leppanen and Bruun, 1986), from sedimenting material in the north Pacific (Taylor, 1989), as well as associated with marine snow particles of the deep sea (Silver *et al.*, 1984). Predacious ciliates could potentially have a large impact on ciliate communities. Since Gause's (1934) classic studies a good deal of work has shown that *Didinium* may efficiently control populations of *Paramecium* species (Huffaker, 1958; Luckinbill, 1973, 1974; Salt, 1974, 1975, 1979; Hewett, 1988).

There are some data on carnivory among phytophagous ciliates, specifically tintinnids (Robertson, 1983; Stoecker and Evans, 1985). But there are no quantitative data on the grazing rates of predacious forms, despite their potential importance in the microbial loop, where ciliates have been given a central role (Sherr *et al.*, 1986). This may be due primarily to difficulties involved with culturing these forms but also due to difficulties involved in

measuring grazing with organisms characterized by small absolute clearance rates; the methods developed for use with metazoan zooplankton do not easily lend themselves for use with microorganisms.

However, methods have been developed recently which allow the direct measurement of ingestion rates for phagotrophic microorganisms using the appearance of natural prey, which are fluorescently dyed, inside the grazer (Sherr *et al.*, 1987; Rublee and Gallegos, 1989). Fluorescent dyes, though, are not ideal labels; the techniques involve killing the prey items which affects prey motility, and the dyes may alter the surface characteristics of the prey as well.

For the present investigation a labeling protocol was developed which avoids the drawbacks of fluorescent dye techniques. Prey ciliates were labeled using their ingestion of fluorescent microspheres, a technique which yields motile labeled prey with presumably unaltered surface characteristics. With this method labeled cells are easily located in the predator and are distinguishable from previously ingested prey.

Ciliates used in the experiments, with the exception of one prey species, *Metanophrys* sp., are commonly found in the plankton during summer months in Chesapeake Bay, a large coastal plain estuary located on the eastern coast of the United States. The effects of prey density on ingestion rates were examined in log growth phase populations of *Mesodinium pulex*, *Euplotes vannus* and *E.woodruffi*. Additional experiments examined the effects of starvation in *E.vannus* and changes in ingestion with prey size in *E.woodruffi*. The results are interpreted within models of ciliate feeding which emphasize the importance of different steps in the feeding process for different ciliate types.

## Method

### *Culture of ciliates*

Prey ciliates (*Metanophrys* sp., *Cyclidium* sp. and *Pleuronema* sp.) and predator ciliates (*E.vannus*, *E.woodruffi* and *M.pulex*) were isolated from water samples taken from the mesohaline section of Chesapeake Bay or Parish Creek, a small inlet of the Chesapeake Bay. Ciliates were grown in Gulf Stream sea water diluted to 15 p.p.t. with distilled water at 20°C under constant illumination. All cultures were bacterized and, with the exception of *M.pulex*, were clonal cultures. *Metanophrys* and *Cyclidium* were grown using 2216 Difco Marine Broth media (0.5 g $l^{-1}$); *Pleuronema* was cultured in 20% cerophyll (BC) media (Soldo and Merlin, 1972) filtered through Whatman GFF filters. Predacious ciliates were maintained in cultures of *Metanophrys*.

### *Growth curves*

Growth curves were generated to establish protocols for producing log-phase ciliate populations and examine the suitability of prey ciliates to support growth of predacious ciliates. For each predacious species, paired cultures of *Metanophrys* alone and *Metanophrys* with a predacious ciliate were sampled over a 2–5 day period. Liter flasks with 500 ml of 2216 medium were inoculated at ~1000

*Metanophrys* $ml^{-1}$. Predator flasks were inoculated at the following densities: *E.vannus*, 10 $ml^{-1}$; *E.woodruffi*, 1 $ml^{-1}$; and *M.pulex*, 5 $ml^{-1}$. The suitability of *Cyclidium* and *Pleuronema* as prey for *E.woodruffi* was tested using similar time-course experiments. A pair of 1 l flasks with 500 ml of 2216 medium was inoculated with *Cyclidium* to give 1000 cells $ml^{-1}$ and one flask was inoculated with *E.woodruffi* at 0.5 cells $ml^{-1}$. Similarly, two flasks of 10% cerophyll containing 300 *Pleuronema* $ml^{-1}$ were prepared and *E.woodruffi* were added to one flask to yield 2 cells $ml^{-1}$. For all experiments cultures were gently mixed and sampled at 6–18 h intervals. Samples were preserved in Bouin's fixative (Coats and Heinbokel, 1982) and aliquots examined in Palmer–Maloney cells, Sedgewick–Rafter chambers or Zeiss settling chambers (2, 5 or 10 ml) depending on the cell density. Raw cell counts were generally over 100.

Ingestion rate estimates were also made from growth curve experiments employing the set of equations devised by Frost (1972) as modified by Heinbokel (1978). Ingestion was calculated over the first time interval in which raw counts of predators exceeded 50 and prey counts were significantly different in control and grazer vessels. Measurements of cells were made using a calibrated ocular micrometer at ×400, and volumes estimated with formulae for the appropriate geometric shapes.

Additional grazing rate estimates were made in short-duration experiments using high predator concentrations. A log-growth phase population of *E.woodruffi* was removed from culture, washed free of prey and concentrated over 20 μm nitex screening. Aliquots of concentrated *E.woodruffi* and stationary phase *Metanophrys* were added to GFF-filtered water to yield two solutions of ~50 *E.woodruffi* and 107 or 12 *Metanophrys* $ml^{-1}$. Control solutions, lacking *E.woodruffi*, were prepared for each prey concentration. All solutions were prepared in duplicate. Samples were taken at time zero and after 15 min, preserved in Bouin's fixative and 5 or 10 ml aliquots examined in settling chambers using an inverted microscope. Grazing rates were calculated as in the growth-curve experiments.

*Labeling prey ciliates*

Preliminary experiments were used to determine the microsphere concentration needed to thoroughly label prey species and the label residence time in each species. For all experiments, fluorescent microspheres ~1 μm in diameter were used (Yellow-Green Fluoresbrite Plain Microspheres, Polysciences Inc., Warrington, PA, USA). Aliquots of log-growth phase cultures were exposed to a dilution series of microsphere stock solution ($1 \times 10^9$ microspheres $ml^{-1}$ in distilled water, briefly sonicated before each use) for 10 min. Label residence time was determined by examining a time series of samples of cells which has been exposed to $10^7$ microspheres $ml^{-1}$ for 10 min, washed over a 5 μm pore Nuclepore filter and then resuspended in GFF-filtered water. A relatively large filter area (i.e. 75 mm diameter) was required to wash a maximum of 50 ml of labeled prey using 3–50 ml filtered water rinses. Bouin's fixed samples were examined in Palmer–Maloney cells using a Leitz Diavert inverted microscope

equipped with a 100 W mercury lamp for epifluorescence and a 6 V 15 W tungsten lamp for transmitted phase contrast illumination.

*Predacious ciliate ingestion of free microspheres*

Preliminary experiments were run to examine microsphere ingestion by *Euplotes* spp. and *M.pulex*. Ciliates were removed from exponentially growing cultures and exposed to both high ($10^7$ ml$^{-1}$) and low ($10^2$ ml$^{-1}$) concentrations of fluorescent microspheres for 1 h and then examined following the protocol above used for prey species.

*Grazing experiments*

All experiments utilized predator and prey from exponentially growing cultures. Predacious ciliates were removed from cultures by gentle, slow (~100 ml min$^{-1}$) screening through Nitex screens (50–75 mm diameter) of 20 and 10 μm mesh for *Euplotes* spp. and *M.pulex* respectively, followed by gentle rinses with filtered water. Prey-free predators were then resuspended in GFF-filtered water at concentrations of 1–2 ml$^{-1}$ for *Euplotes* spp. and 35 ml$^{-1}$ for *M.pulex*. Except for experiments with starved *E.vannus*, where cells were held in filtered water for ~20 h, predators were kept in filtered water for 0.25–0.5 h before exposure to labeled prey.

Prey ciliates were exposed to $1 \times 10^7$ microspheres ml$^{-1}$ for 10 min, washed over 5 μm Nuclepore filters with GFF-filtered water for 5 min to remove excess microspheres, and mixed into the predacious ciliate solution for 30 s. This protocol yielded a solution with a residual microsphere concentration of $10^1$–$10^2$ microspheres ml$^{-1}$. The labeled-prey and predator solution was then quickly dispensed into six bottles, 1000 or 500 ml for *Euplotes* spp. and 20 ml vials for *M.pulex* experiments. At 4–5 min intervals the entire contents of a single container was preserved with Bouin's fixative.

For *Euplotes* spp., preserved fluid from containers was first concentrated to ~20 ml using 20 μm Nitex screens and 10 ml aliquots of the concentrate settled; in the *M.pulex* experiments a 10 ml aliquot from each experimental container was settled. Settling chambers were examined with the Lietz inverted microscope using epifluorescence and a low level of transmitted light. The entire surface of each chamber was examined at ×200 for *M.pulex* and ×100 for *Euplotes* spp., and the number of labeled prey in each cell recorded. Thus for each time series sample a minimum of 350 *M.pulex* or 500 *Euplotes* was examined.

Ingestion rates were calculated as the slope of the linear regression of average number of prey ingested predator$^{-1}$ versus time. Estimates of slopes and associated error statements were generated using the Biom PC program (Rohlf, 1983); values of prey ingested predator$^{-1}$, as estimates based on count data, were log $(x + 1)$ transformed before analysis. Clearance rates were calculated as number of prey ingested per hour divided by prey concentration.

## Results

### *Growth curves*

Predator and prey species growth curves are shown in Figures 1 and 2. In log-growth phase, *Metanophrys* showed generation times of 9–12 h; *Cyclidium* and *Pleuronema* doubling times were 16 and 42 h respectively. During exponential growth, predacious ciliates feeding on *Metanophrys* had generation times of 16 h for *E.vannus* and *M.pulex*, and 19 h for *E.woodruffi*. Log growth of *E.woodruffi* was also supported by *Cyclidium* and *Pleuronema* yielding generation times of 19 and 13 h respectively. Cell dimensions and calculated volumes are given in Table I.

### *Prey labeling*

For all three prey species >90% of the cells were packed with microspheres when exposed to concentrations above $1 \times 10^7$ ml$^{-1}$ (Figures 3A–C and 4A). Microsphere retention varied from 40 to 120 min (Figure 4B) with *Metanophrys* showing the shortest retention (i.e. a sharp decline in the percentage of cells labeled after 40 min).

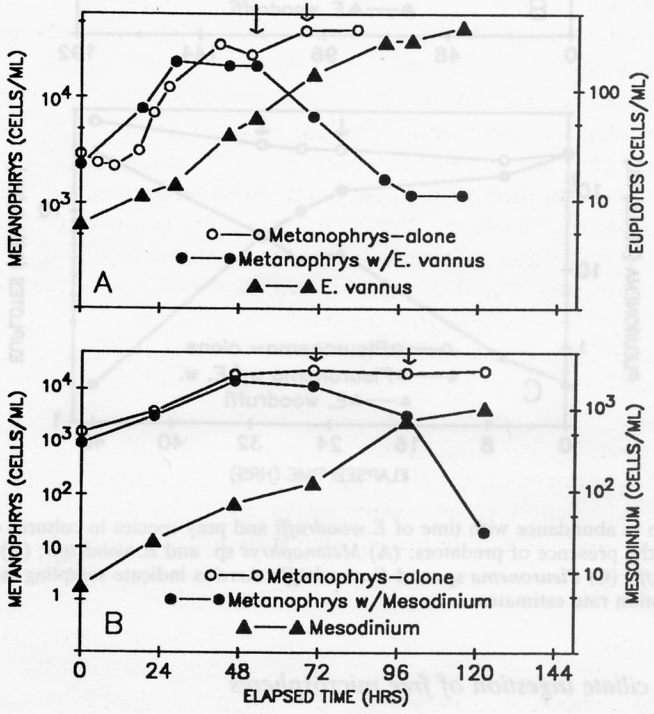


**Fig. 1.** Change in abundance with time of predator and prey species in cultures of prey ciliates alone, and in the presence of predators: (**A**) *Metanophrys* sp. and *E.vannus*; (**B**) *Metanophrys* sp. and *M.pulex*. Arrows indicate sampling intervals used to compute ingestion rate estimates.

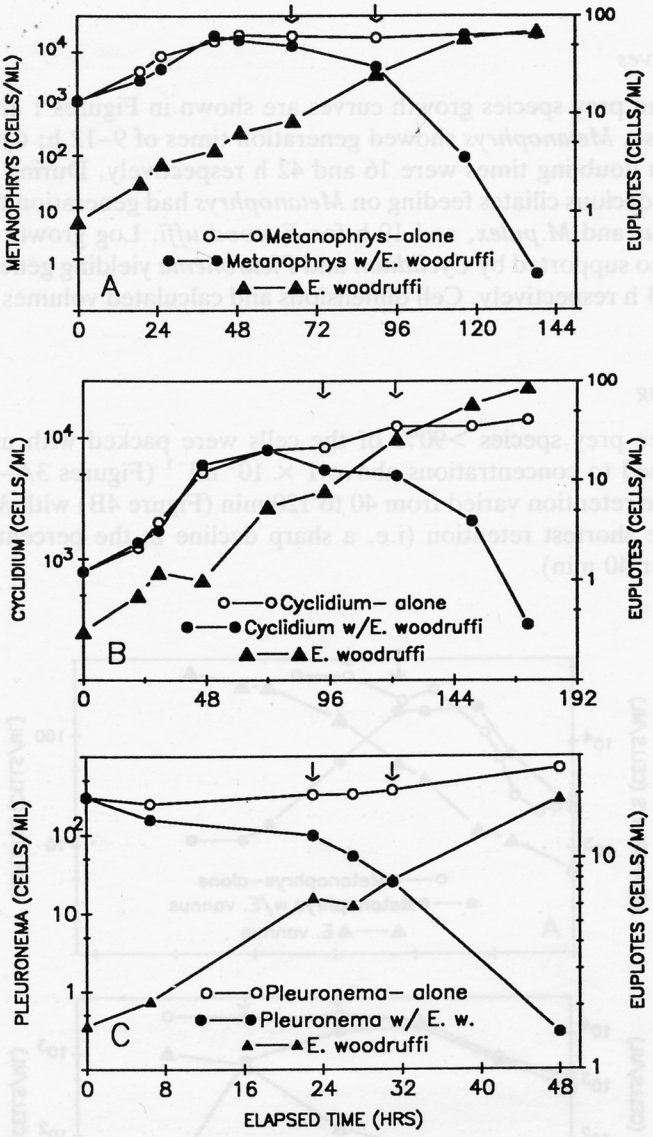


**Fig. 2.** Change in abundance with time of *E.woodruffi* and prey species in cultures of prey ciliates alone, and in the presence of predators: (**A**) *Metanophrys* sp. and *E.woodruffi*; (**B**) *Cyclidium* sp. and *E.woodruffi*; (**C**) *Pleuronema* sp. and *E.woodruffi*. Arrows indicate sampling intervals used to compute ingestion rate estimates.

## *Predacious ciliate ingestion of free microspheres*

*Mesodinium pulex* did not exhibit any free microsphere ingestion. Both *Euplotes* species ingested microspheres at high microsphere concentrations, but showed low ingestion rates (<2% of cells labeled) at microsphere concentrations equal

**Table I.** Dimensions and calculated volumes of ciliates used in experiments based on preserved specimens

| Species | Growth phase | *L* | *W* | Volume | *N* |
|---|---|---|---|---|---|
| Prey ciliates | | | | | |
| *Cyclidium* | log | 16 ± 1.5 | 9 ± 1.0 | $6.9 \times 10^2$ | 10 |
| *Cyclidium* | late log | 15 ± 1.1 | 8 ± 0.8 | $5.3 \times 10^2$ | 10 |
| *Metanophrys* | log | 27 ± 2.3 | 12 ± 1.1 | $1.9 \times 10^3$ | 25 |
| *Metanophrys* | stationary | 20 ± 1.8 | 7 ± 1.0 | $5.9 \times 10^2$ | 25 |
| *Pleuronema* | log | 39 ± 2.8 | 24 ± 3.6 | $1.2 \times 10^4$ | 10 |
| Predacious ciliates | | | | | |
| *Mesodinium pulex* | log | 21 ± 4.0 | 16 ± 1.4 | $2.8 \times 10^3$ | 10 |
| *Euplotes vannus* | log | 84 ± 5.2 | 52 ± 4.5 | $5.9 \times 10^4$ | 25 |
| *E.vannus* | 'starved' | 83 ± 5.2 | 45 ± 4.7 | $4.4 \times 10^4$ | 25 |
| *E.woodruffi* | log | 122 ± 10.3 | 66 ± 5.6 | $1.4 \times 10^5$ | 10 |

Length (*L*) and width (*W*) reported in microns ± standard deviation; volume in cubic microns; *N* = sample size.

to those occasionally present in the grazing experiments ($10^2$ ml$^{-1}$). Ingested free microspheres were easily distinguishable from ingested labeled prey as they were not found inside ingested prey.

*Grazing experiments*

Labeled prey ciliates were clearly visible inside predators and distinguishable from unlabeled, previously ingested prey (Figure 3D and E). The average number of labeled prey predator$^{-1}$ always increased linearly with time with a significant slope (for all experiments: $n = 6$, $P < 0.05$). Data from two typical experiments, *E.vannus* feeding on *Metanophrys* at 172 and 86 *Metanophrys* ml$^{-1}$, are given in Figure 5. Ingestion rates calculated from the slopes were 3.4 and 0.9 *Metanophrys* ingested h$^{-1}$ with 95% CIs of 2.08–4.71 and 0.44–1.38 feeding on 172 and 86 *Metanophrys* ml$^{-1}$ respectively.

For cells in exponential-growth phase (well-fed) feeding on *Metanophrys*, ingestion increased with prey concentration. *Euplotes vannus* ingested 0.2–3.4 prey h$^{-1}$ with prey concentrations from 16 to 172 ml$^{-1}$ (Figure 6A); clearing ~10 μl h$^{-1}$ ciliate$^{-1}$ regardless of prey density (Figure 6B). Ingestion by *M.pulex* increased from 0.15 to 0.32 cells h$^{-1}$ over prey densities from 60 to 2380 cells ml$^{-1}$ (Figure 7A); calculated clearance rates were inversely related to prey concentration, varying from 3.4 to 0.14 μl h$^{-1}$. For *E.woodruffi* feeding on *Metanophrys*, ingestion increased with prey concentration to a maximum value of ~4.5 cells h$^{-1}$ at 75 prey ml$^{-1}$ and clearance rates decreased with prey concentration from 93.6 μl h$^{-1}$ at 12 prey ml$^{-1}$ to 26.7 μl h$^{-1}$ at 152 prey ml$^{-1}$ (Figure 7B).

*Euplotes vannus* showed differences in ingestion and clearance rates when feeding shortly after removal from log-phase cultures (well-fed) versus after ~20 hours starvation (starved). The ingestion rates of starved cells did not increase regularly with prey density but averaged ~1 prey ingested h$^{-1}$ over a prey density of 15–150 *Metanophrys* ml$^{-1}$ (Figure 6A) and clearance rates decreased

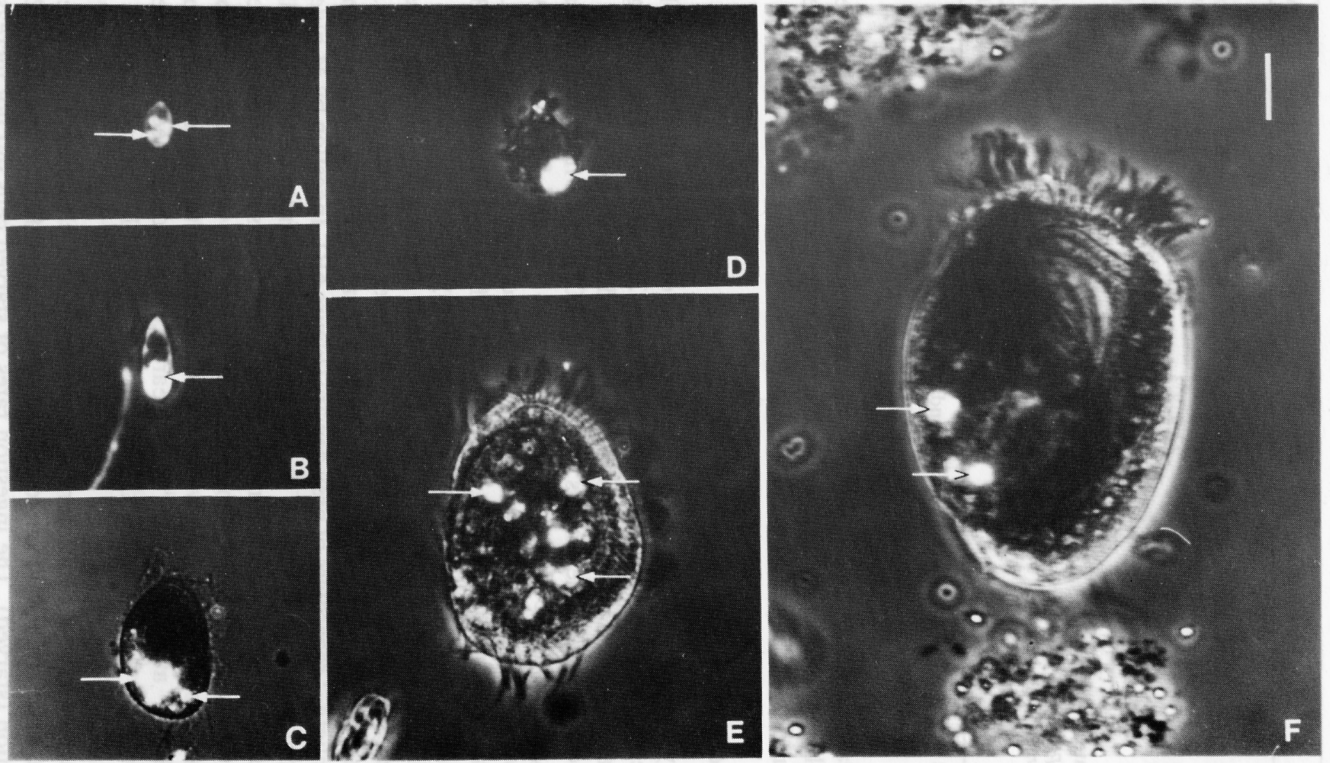


**Fig. 3.** Micrographs of prey and predator species taken with a combination of transmitted and epifluorescence illumination. The scale bar in (**F**) = 20 μm. (**A**)–(**C**) Prey ciliates labeled with fluorescent microspheres, *Cyclidium* sp. (**A**), *Metanophrys* sp. (**B**) and *Pleuronema* sp. (**C**); arrows indicate food vacuoles filled with microspheres. (**D**)–(**F**) Predacious ciliates containing labeled prey: *Mesodinium pulex* (**D**), *Euplotes vannus* (**E**) and *Euplotes woodruffi* (**F**); arrows indicate ingested labeled prey.

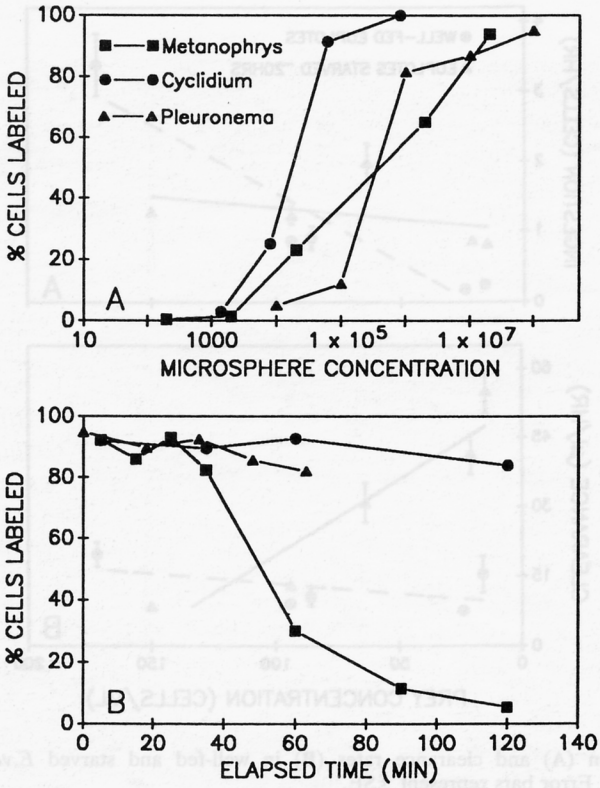


**Fig. 4.** Labeling of prey ciliates: (**A**) percentage of cells labeled as a function of microsphere concentration; (**B**) declines in percentage of cells labeled with time for labeled populations held in microsphere-free water; symbols as in (A).

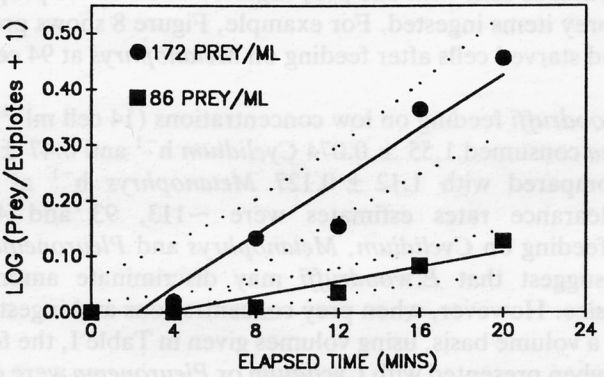


**Fig. 5.** Typical time-course series of increases in the appearance of labeled prey in a predator. *E.vannus*, feeding in solutions of 172 and 86 *Metanophrys* $ml^{-1}$. Dotted lines represent 95% CI for linear regression points.

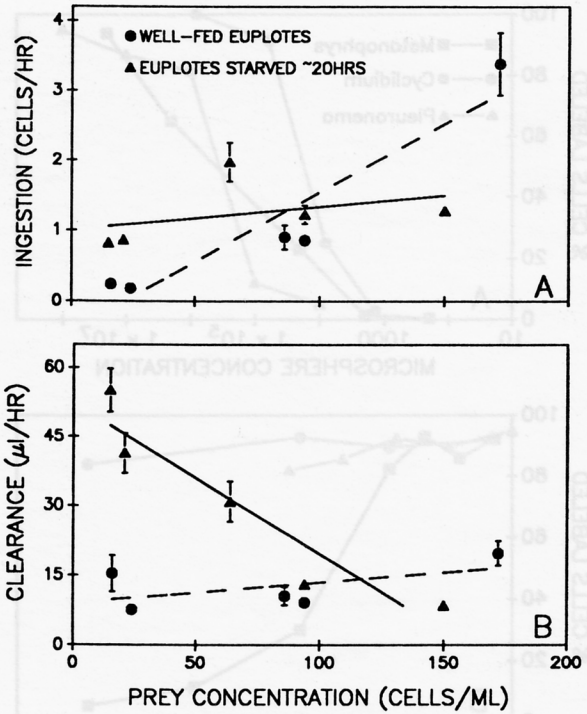


**Fig. 6.** Ingestion (**A**) and clearance rates (**B**) in well-fed and starved *E.vannus* feeding on *Metanophrys* sp. Error bars represent ±SE.

from 55 to 8.5 µl $h^{-1}$ with increasing prey concentration (Figure 6B). Differences were also noted in the distribution of prey between well-fed and starved *E.vannus*. In general, among starved cells the population showed a higher proportion of cells with one prey ingested and a lower proportion of cells with several prey items ingested. For example, Figure 8 shows prey distribution in well-fed and starved cells after feeding on *Metanophrys* at 94 cells $ml^{-1}$ for 20 min.

*Euplotes woodruffi* feeding on low concentrations (14 cell $ml^{-1}$) of *Cyclidium* or *Pleuronema* consumed 1.55 ± 0.074 *Cyclidium* $h^{-1}$ and 0.47 ± 0.049 *Pleuronema* $h^{-1}$ compared with 1.12 ± 0.127 *Metanophrys* $h^{-1}$ at 12 $ml^{-1}$. The associated clearance rates estimates were ~113, 93 and 47 µl $h^{-1}$ for *E.woodruffi* feeding on *Cyclidium*, *Metanophrys* and *Pleuronema* respectively. These data suggest that *E.woodruffi* may discriminate among prey items according to size. However, when prey concentrations and ingestion rates were computed on a volume basis, using volumes given in Table I, the feeding rates of *E.woodruffi* when presented with *Cyclidium* or *Pleuronema* were consistent with the pattern seen in the *Metanophrys* experiments (Figure 9). Similarly, in the growth-curve experiments, ingestion rate estimates for *E.woodruffi* cultured on the three prey species varied from 0.6 to 19 cells ingested $h^{-1}$ but when

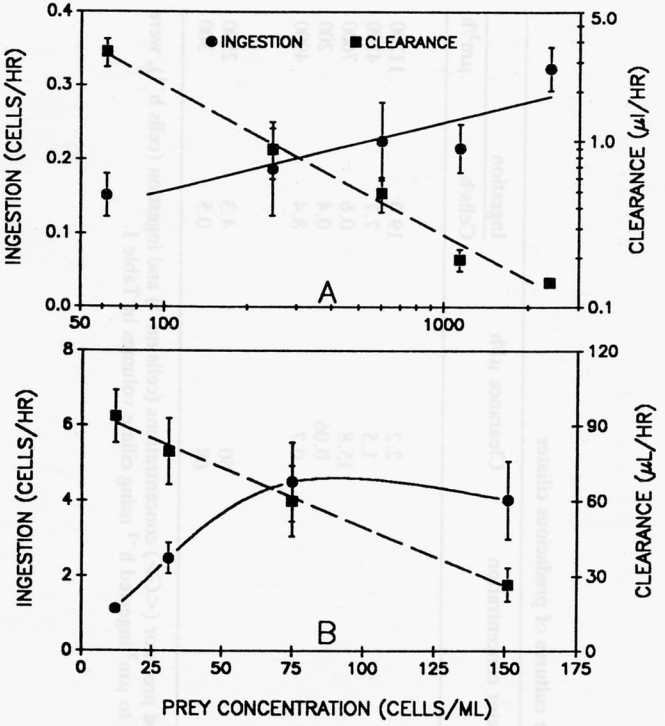


**Fig. 7.** Ingestion and clearance rates of *M.pulex* (**A**) and *E.woodruffi* (**B**) feeding on *Metanophrys* sp. Error bars represent ±SE.

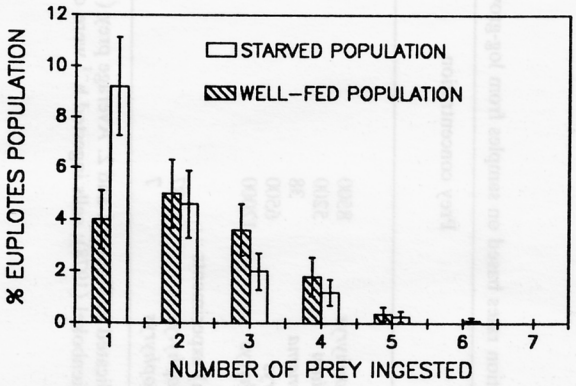


**Fig. 8.** Distribution of labeled prey in well-fed and starved *E.vannus* after feeding in 94 *Metanophrys* $ml^{-1}$ for 20 min. Error bars represent 95% CI.

computed on a volume basis varied only from 4.1 to 11.2 × $10^3$ $\mu m^3$ prey ciliate volume ingested $h^{-1}$ (Table II). The average volume ingested in the three growth-curve experiments agreed well with the maximum estimate obtained in the labeled prey experiments, 7633 and 8433 $\mu m^3$ ciliate volume ingested $h^{-1}$ respectively.

**Table II.** Calculated ingestion rates based on samples from log-growth phase cultures of predacious ciliates

| Predator and prey | Prey concentration | Predator concentration | Clearance μl/h | Ingestion | |
|---|---|---|---|---|---|
| | | | | Cells/h | $\mu m^3/h$ |
| A. Log-phase cultures | | | | | |
| *E.woodruffi* and *Metanophyrys* | 8500 | 15 | 2.2 | 19.0 | 11200 |
| *E.woodruffi* and *Cyclidium* | 5200 | 13 | 1.5 | 7.7 | 4100 |
| *E.woodruffi* and *Pleuronema* | 38 | 12 | 15.8 | 0.6 | 7600 |
| *M.pulex* and *Metanophrys* | 6500 | 339 | 0.06 | 0.4 | 200 |
| *E.vannus* and *Metanophyrys* | 12000 | 93 | 0.7 | 8.4 | 4900 |
| B. Short duration (15 min) experiments | | | | | |
| *E.woodruffi* and *Metanophyrys* | 87 | 39 | 50 | 4.3 | 2500 |
| *E.woodruffi* and *Metanophyrys* | 7 | 61 | 68 | 0.5 | 300 |

Sampling intervals are indicated in Figures 1 and 2. Average prey (<*C*>) and predator (<*G*>) concentrations (cells $ml^{-1}$) and ingestion (cells $h^{-1}$), were calculated according to Hienbokel (1978); cells ingested $h^{-1}$ were converted to $\mu m^3$ ingested $h^{-1}$ using ciliate volumes in Table I.

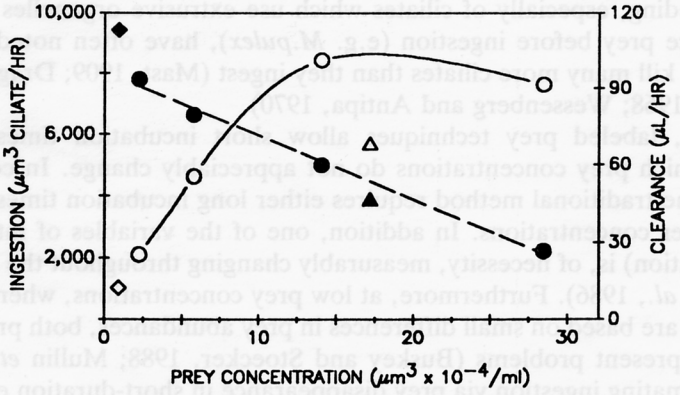


**Fig. 9.** Ingestion (open symbols) and clearance rates (filled symbols) of *E.woodruffi* calculated on a volume basis. Circles and joining lines represent *Metanophrys* data; diamonds and triangles represent *Cyclidium* and *Pleuronema* data respectively.

For *E.woodruffi*, grazing rates calculated via prey disappearance in the short-duration experiments (Table II) agreed relatively well with estimates from labeled prey experiments (Figure 7B) despite large differences in predator concentration, ~50 versus ~2 *E.woodruffi* $ml^{-1}$ respectively. At a time-averaged prey concentration (<*C*>) of ~7 *Metanophrys* $ml^{-1}$ the two replicates yielded calculated ingestion rates of 0.46 and 0.50 cell $h^{-1}$ compared with 1.12 cells $h^{-1}$ in the labeled prey experiment at 12 *Metanophrys* $ml^{-1}$. In the treatments which began with 107 *Metanophrys* $ml^{-1}$, the average prey concentration (<*C*>) was 87 $ml^{-1}$ and *E.woodruffi* ingested 3.7 and 4.9 cells $h^{-1}$ versus ~4.5 cells $h^{-1}$ at 75 labeled prey $ml^{-1}$.

## Discussion

### *Strengths and weaknesses of methodology*

The use of inert particles that are visible inside the consumer as prey analogs has a long history in protozoology (see Jennings, 1906). Recently, however, techniques for labeling natural prey organisms have been developed. Ingestion of bacteria by microflagellates and ciliates has been quantified using the appearance of fluorescently dyed bacteria (Sherr *et al.*, 1987) and ingestion rates of herbivores studied using fluorescently dyed algae (Rublee and Gallegos, 1989). These methods, and the one developed for the present investigation, have distinct advantages compared with the traditional methodology used in metazoan zooplankton grazing studies, i.e. measuring changes in prey concentration in grazer versus control containers (Frost, 1972).

First, ingestion is estimated directly by counting prey inside the consumer, rather than calculated indirectly based on a volume of water cleared of prey. The volume cleared yields an 'apparent filtering rate', an index of the predator's impact on a food resource (Conover and Huntley, 1980); this impact need not equal numbers of prey actually ingested. Descriptive accounts of predacious

ciliate feeding, especially of ciliates which use extrusive organelles to kill and immobilize prey before ingestion (e.g. *M.pulex*), have often noted that some predators kill many more ciliates than they ingest (Mast, 1909; Dragesco, 1962; Fenchel, 1968; Wessenberg and Antipa, 1970).

Second, labeled prey techniques allow short incubation times (minutes) during which prey concentrations do not appreciably change. In contrast, for protists the traditional method requires either long incubation times (hours) or high grazer concentrations. In addition, one of the variables of interest (prey concentration) is, of necessity, measurably changing throughout the experiment (Marin *et al.*, 1986). Furthermore, at low prey concentrations, where clearance estimates are based on small differences in prey abundances, both precision and accuracy present problems (Buskey and Stoecker, 1988; Mullin *et al.*, 1975). Thus estimating ingestion via prey disappearance in short-duration experiments necessitated the use of very high predator concentrations; ingestion rate estimates from the growth curve experiments could only be reliably made when both predator and prey concentrations were relatively high and so furnished only estimates of maximum ingestion rates.

Enumerating labeled prey in predators not only avoids the problems listed above but can, in addition, provide data on individual variability of ingestion (Rublee and Gallegos, 1989) and patterns of prey distribution within predator populations, such as the differences between well-fed and starved *E.vannus* (Figure 8).

A unique advantage of the method used in the present study, relative to fluorescent dye techniques, is that prey items are alive, their motility is unchanged, and the labeling is not likely to alter the surface properties of the prey items. There is evidence that, for both raptorial and filter-feeding ciliates, prey surface properties influence ingestion rates (Kiersnowska *et al.*, 1988; Sanders, 1988). Prey motility should theoretically affect feeding rates through changes in predator encounter rates (Gerritsen and Strickler, 1977) and perhaps through changes in capture efficiency or handling time as well. The use of radiolabeled prey (Lessard and Swift, 1986) could provide similar advantages, in that prey ciliates are essentially unaltered, but generating data similar to that presented here would involve relatively tedious single cell processing techniques (Rivkin and Seliger, 1981; Stoecker *et al.*, 1988). Prey selection could be investigated using prey items labeled with microspheres that fluoresce different colors. Possible prey items could include phagotrophic flagellates.

Labeling ciliates with microspheres also has some unique disadvantages. Different species retain microspheres for different amounts of time (Figure 4B). There was no obvious, simple, relationship between retention time and either size or growth rate of the prey ciliates. Thus some estimate of retention time is needed for each species, and the duration of experiments using more than one prey species is limited to the shortest retention time. Also, some difficulties were experienced in separating labeled ciliates from the microsphere solution: large filters were required and the volume of labeled ciliate culture which could be quickly processed was limited. It is also possible that some fragile ciliate species may not survive the filtration procedure. Some investigators may be tempted to

omit the filtration step, but we found that both predators and glassware tend to become coated with microspheres.

*Patterns in predacious ciliate feeding*

The data generated using microsphere-labeled prey ciliates indicate that *E.vannus*, *E.woodruffi* and *M.pulex* largely resemble other ciliates in their basic feeding parameters. The maximum size-specific clearance rates (body volumes $h^{-1}$) were $1.0 \times 10^6$ for *E.vannus* (starved cells), $6.7 \times 10^5$ for *E.woodruffi*, and $1.0 \times 10^6$ for *M.pulex*, assuming a 20% loss of cell volume due to preservation in Bouin's fixative (Gilron and Lynn, 1989). A general relationship of $10^5$ body volume $h^{-1}$ cleared is characteristic of suspension-feeding ciliates (Fenchel, 1986), hence these ciliates appear to be efficient predators. Patchiness of prey cannot be excluded as an alternative explanation for the relatively high maximum clearance rates reported here, although care was taken to thoroughly mix predator–prey solutions, and incubation times were kept short to minimize the possibility of patch formation. Direct comparison of the absolute clearance rates of *Euplotes* spp. and *M.pulex* with other studies of ciliate feeding is difficult as few studies have attempted to measure ciliate grazing rates under conditions of low prey abundance ($<100$ prey $ml^{-1}$) and using relatively large prey items.

For example, previous work with *E.vannus*, which yielded a maximum clearance rate estimate of 0.7 $\mu l\ h^{-1}$ (compared with $\sim 10\ \mu l\ h^{-1}$ estimated here for log-growth phase cells), utilized small prey items, 5.7 $\mu m$ diameter latex microspheres, and a high prey concentration, $9.9 \times 10^5$ microspheres $ml^{-1}$ (Fenchel, 1986). A recent exception is a study of the feeding behavior of *Favella* sp. (Buskey and Stoecker, 1988), a tintinnid with a cell volume presumably about equal to that of *E.woodruffi* since the lorica volume (Verity and Langdon, 1984) is about twice the calculated volume of *E.woodruffi*. Clearance rates of $>100\ \mu l\ h^{-1}$ were recorded at prey densities of 50 prey $ml^{-1}$ and clearance rates $\leq 20\ \mu l\ h^{-1}$ were reported with prey concentrations $>200$ prey $ml^{-1}$. The higher clearance rates were verified using a method similar to that employed here—direct counts of ingested dinoflagellates (Buskey and Stoecker, 1988). The lower clearance rates obtained with higher prey concentrations are typical of those commonly reported for large oligotrich and tintinnid species (see Jonsson, 1986).

For pelagic invertebrates there are two basic functional response models, reflecting different conceptions of the ingestion process: rectilinear and curvilinear (Mullin *et al.*, 1975). In the rectilinear model, ingestion increases linearly with prey density up to a constant maximum value corresponding to a saturating prey density. There is assumed to be no 'interference' between prey items until saturating densities are reached. In curvilinear models 'interference' between food items is postulated so that the rate of ingestion decelerates with increasing prey concentration. The two types of models are difficult to distinguish statistically and the choice is usually made on the perception of the mechanism of feeding (Heinbokel, 1978).

Ciliate feeding has generally been described with some component of

'interference' and ingestion modeled using Michaelis–Menten type curves (Stoecker, 1988). Three types of 'interference' have been identified, those relating to the finite amounts of time necessary to first capture and then process a food item and those relating to crowding effects of either predators or prey. Crowding effects, which may rarely occur in nature (Fenchel, 1986), appear to result from frequent temporary cessations of swimming when cells are continuously bumping into one another (Curds and Cockburn, 1968; Salt, 1967, 1974; Heinbokel, 1978). Feeding processes that should affect ingestion rates at all prey densities are: (i) time needed to capture prey (prey handling time); and (ii) the time necessary for phagocytosis of the prey (time needed for food vacuole formation) (Capriulo, 1982).

For raptorial ciliates that use extrusive organelles to capture prey (e.g. *M.pulex* and *Didinium*), there is a limit to the number of 'capture events' that can be made before extrusive organelles must be resynthesized (Dragesco, 1962). In *Didinium*, not only the extrusive organelles but the entire proboscis must be re-formed each time an item is ingested (Wessenberg and Antipa, 1970). Thus, times for both handling and phagocytosis are finite components of ingestion in *Didinium* (Hewett, 1980). For *M.pulex* prey handling may place an upper limit on ingestion. A close correspondence was found in the maximum numbers of *Metanophrys* ingested in the labeled prey experiments and the growth curve experiment, 0.32 and 0.38 cells $h^{-1}$ respectively, despite large differences in the sizes of the *Metanophyrs*, 1875 and 585 $\mu m^3$ respectively. This suggests that *M.pulex*, like *Didinium*, is capable of a finite number of prey capture events per unit time which may limit its ingestion rate.

In contrast, the filter-feeding *Euplotes* species ingested different numbers but similar volumes of different-size prey. For example, *E.vannus* in the labeled prey experiments with log-growth phase *Metanophrys* (1874 $\mu m^3$) ingested 3.4 (95% CI = 2.1–4.7) cells $h^{-1}$ equivalent to 6.3 (95% CI = 3.9–8.8) $\times 10^3$ $\mu m^3$, a volume which compares well with 4.9 $\times 10^3$ $\mu m^3$ $h^{-1}$ in the growth curve experiments calculated from an ingestion rate of 8.4 stationary phase *Metanophrys* (585 $\mu m^3$). Similarly, for *E.woodruffi*, the calculated ingestion rates from the growth experiments with different-size prey varied considerably, from 0.6 to 19 cells $h^{-1}$, but the associated volume estimates were much less variable, 4.1–11.2 $\times 10^3$ $\mu m^3$ and fell within the 95% CI of the maximum rate found in the labeled prey experiment, 2.8–14.4 $\times 10^3$ $\mu m^3$ $h^{-1}$. It should be recognized that the possibility of prey selection cannot be excluded with this limited data set. *Euplotes* spp. may have either passively or actively selected food items according to motility, shape, surface properties or size, and the product of ingestion probability and body volume found here was fortuitous.

However, these data support the idea that for filter-feeding ciliates, which do not expend cellular organelles to capture food, phagocytosis, specifically, food vacuole formation, is probably the rate-limiting step in the ingestion process (Fenchel, 1986). In ciliates membrane for food vacuoles is not synthesized *de novo* at the cytostome but rather is assembled from units recycled back to the oral region from pre-existing food vacuoles (Allan, 1974; Kloetzel, 1974; McKanna, 1973). For ciliates feeding in an excess of food, ingestion is limited by

the membrane-supply mechanism. Assuming that handling time is unimportant, that no 'selection' occurs, and that food items are packaged on a volume rather than unit basis, then the maximum ingestion rate should be an approximately constant volume value across the range of 'filterable' prey sizes. Data presented for *E.woodruffi* and *E.vannus* indicate that this is largely the case, but some discrepancies in maximum volume ingested were found, which are perhaps attributable to differences in food vacuole 'packing efficiency' with different-sized food items. Differential packing has been noted in other filter-feeders. For example, *Glaucoma scintillans* uses similar amounts of food vacuole membrane when feeding on 0.36 and 1.1 μm particles but packages 1.1 μm particles less efficiently, using relatively greater numbers of smaller vacuoles, which yields a lower volume ingested of 1.1 μm particles compared with 0.36 μm particles (Fenchel, 1986).

Relative to well-fed cells, starved *E.vannus* had higher clearance and ingestion rates at low prey densities but lower rates at high prey densities (Figure 6). Starvation-related changes in the supply mechanism of food vacuole membrane along with changes in swimming speed may account for these differences in ingestion rates between well-fed and starved *E.vannus*. Starvation is generally associated with increased swimming speeds (Dragesco, 1962; Fenchel and Jonsson, 1988; Salt, 1979) and therefore presumably increased apparent clearance rates. Increased swimming speed could then account for the relatively high clearance and ingestion rates of starved cells at low prey density. The differences in maximum ingestion may result from a reduced supply of membrane for food vacuole formation due to starvation.

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