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Processing of ingested matter in *Strombidium sulcatum*, a marine ciliate (Oligotrichida)

Abstract—We examined the hypothesis that different types of ingested matter may be processed at different rates by a marine microzooplankter. Ciliates were exposed to a prey item and then diluted at 1 : 100. Disappearance or digestion of inert (fluorescent microspheres) and digestible prey items (heat-killed and fluorescently labeled *Synechococcus,* natural *Synechococcus,* and *Isochrysis galbana*) were determined from changes in cell contents using epifluorescence microscopy. Ingested prey declined exponentially, and prey analogues were processed like natural prey. There were no significant differences in rates of disappearance or decay constants for the different prey items or in feeding vs. nonfeeding ciliates or between ciliates cultured on (or naive to) natural *Synechococcus.* The overall average $t_{½}$ of cell contents at 22°C was 75 min. The exponential declines in cell contents indicated that some ingested chlorophyll *a,* whether in *Synechococcus* or *Isochrysis,* can be long-lived. The relatively invariant decay constant for ingested matter opens the possibility of using a gut passage approach to estimate instantaneous rates of grazing for natural populations of oligotrich ciliates.

Ciliate microzooplankton are generally considered an important component of planktonic food webs and are assigned the role of grazers on ultraplankton, i.e. cells between 0.5 and 5 μm in size (e.g. Sherr et al. 1991). In recent years, the grazing activity of ciliates has been evaluated, for the most part, using two major approaches: monitoring changes in phytoplankton pigment concentrations in dilution experiments or using prey analogues. An alternative technique, used occasionally in the past, is the gut contents approach; it relies on estimates of cell contents combined with data on digestion rates (Goulder 1972, 1973; Fenchel 1975; Kopylov and Tumantseva 1987; Dolan and Coats 1991). With regard to the dominant group of marine planktonic ciliates, oligotrichs, this particular approach has not been attempted and the process of digestion has received little attention. To our knowledge, the existing data concern disappearance rates of fluorescently labeled bacteria (Sherr et al. 1988). Although the need for digestion rate data is obvious if a gut contents approach is to be attempted, knowledge of how food items are processed may be useful regardless of the technique used to investigate grazing.

The use of fluorescently labeled bacteria (Sherr et al. 1987) or algae (Rublee and Gallegos 1989) as prey analogues has become common (Putt 1991). Grazing rates are estimated either via increases of prey analogues inside grazers (e.g. Simek et al. 1995) or via declines in bulk concentrations of prey analogues (e.g. Fuhrman and Noble 1995). When prey analogues are used to estimate ingestion, it is overtly assumed that analogues appear in the predator (via ingestion) at a rate similar to natural prey items (e.g. Simek et al. 1995), but there is an underlying assumption that prey analogues disappear (i.e. are digested or evacuated) at the same rate as natural prey. To our knowledge, this assumption has never been examined. There are reasons to suspect that some prey analogues could be digested at rates different from natural prey. For example, heat-killing the bacterium *Staphylococcus,* a process used in the preparation of fluorescently labeled bacteria (Sherr et al. 1987), coagulates cytoplasm, thereby rendering the cells resistant to digestion by the ciliate *Paramecium* (Mehlis et al. 1990). Thus, it is worth considering that heat-killed prey may be processed slower in the digestive vacuoles of ciliates compared to natural prey.

Here we report on the processing of ingested matter by *Strombidium sulcatum,* which is considered typical of marine planktonic ciliates and is the subject of previous studies concerning feeding and nutrient excretion (Allali et al. 1994). We examined in a set of laboratory experiments the disappearance of different types of ingested matter in *S. sulcatum:* inert fluorescent microspheres, heat-killed and fluorescently labeled *Synechococcus* cells, native marine *Synechococcus,* and cultured *Isochrysis galbana.* Our data support studies that reported an exponential decay of ingested matter in ciliates (Goulder 1972; Fenchel 1975; Berger and Pollock 1981; Fok and Schockley 1985; Fok et al. 1982;

Dolan and Coats 1991) rather than a fixed transit time (Sherr et al. 1988; Capriulo and Degnan 1991). We suggest that the decay constants for natural prey and prey analogues, whether inert or organic, are indistinguishable and represent cell evacuation rates for this oligotrich ciliate. A relatively constant digestion rate, for a given temperature, opens the possibility of using digestion rate data and cell contents as a means to estimate instantaneous grazing rates in natural populations of oligotrichs.

Stock cultures of *S. sulcatum* were maintained on a bacterized wheat-grain media at 15°C as described in Rivier et al. (1985). Ciliates for experiments were transferred into a bacterized yeast extract media (0.03 g liter^{-1}) and grown at the experimental temperature of 22°C in a temperature-controlled incubator. The first five experiments used late log-phase cultures of ciliates (cultures at 300–400 cells ml^{-1}; stationary phase concentrations of 600–800 cells ml^{-1}). The following prey items were used: fluorescent microspheres (FMS), 1-μm-diameter Yellow-Green Fluoresbrite Plain Microspheres (Polysciences Inc.); fluorescently labeled *Synechococcus* (FLS), ~1 μm in diameter, originally isolated from a reservoir (Simek et al. 1995) and heat-killed and fluorescently labeled according to the protocol of Sherr et al. (1987); native marine *Synechococcus* (Syn), ~1 μm in diameter, present in surface waters from Villefranche Bay (northwestern Mediterranean); and *Isochrysis galbana,* ~5 μm in diameter, from a late-log phase culture grown in f/2 medium. For the sixth experiment we grew *Strombidium* on a solution of *Synechococcus* concentrated from 3 liters of seawater using size-fractionation. Ciliates were inoculated into an 80 ml solution of 1×10^6 *Synechococcus* ml^{-1}. Over a 4-d period the concentration of ciliates doubled to ~100 ml^{-1} and *Synechococcus* declined to 7×10^3 cells ml^{-1}.

Preliminary experiments with dilution series of FMS and FLS were used to determine a particle concentration (5×10^4 ml^{-1}) that yielded optimally labeled ciliates (3–7 items ciliate^{-1} for ease in microscopic enumeration) and the concentrations that gave negligible prey uptake after 120 min (≤500 particles ml^{-1}). To examine prey processing, the basic experimental design consisted of exposing ciliates to a prey item for 45–120 min and then halting prey uptake by diluting the ciliate–prey mixture at 1 : 100 and sampling with time. For the sixth experiment, 40 ml of the ciliate culture grown on natural *Synechococcus* was diluted into 2 liters of GFF-filtered seawater. Time-course samples were taken for 2 h from the diluted ciliate mixture, fixed with alkaline Lugol's solution (final concn 1%), and cleared by adding a few drops of 3% sodium thiosulfate. For experiments with native *Synechococcus* and cultured *Isochrysis,* samples were settled and stored in a dark refrigerator. Aliquot samples (100 ml) were settled and individual ciliates examined by using an inverted microscope equipped with epifluorescence (Zeiss Axiovert, with a 50 W mercury lamp). For each time-series sample, 50–150 ciliates were examined at 400× magnification. For a given experiment, all samples were analyzed within a week.

All incubations were conducted in a temperature-controlled incubator at 22°C. Experiment 1 examined cell passage time of an inert particle (FMS) in nonfeeding cells. Ciliates were exposed to FMS, held in GFF-filtered seawater, and samples

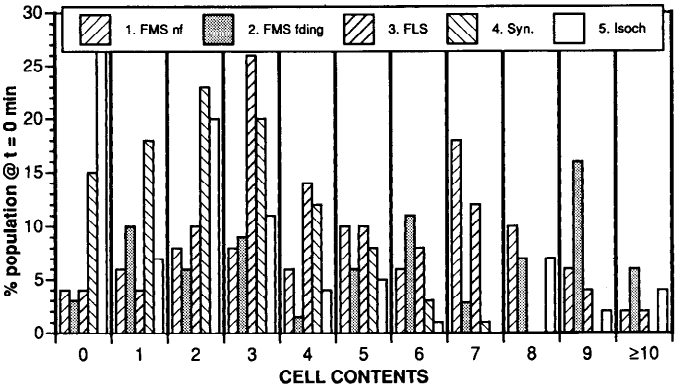

Fig. 1. Distribution of prey items in *Strombidium sulcatum* at the start of digestion experiments. Numbers in the key refer to the experiment number (for details see text). Note that most cells were labeled in all experiments and that prey items per cell seemed to follow a Poisson distribution with the possible exception of the *Isochrysis* experiment.

taken to estimate time-course changes in FMS ciliate^{-1}. In experiment 2, uptake of FMS was monitored and then cell passage time for FMS in ciliates feeding in seawater was examined. Samples were taken during exposure to FMS and followed after dilution in 20-μm-filtered seawater where ciliates fed on native phytoplankton. In experiment 3, the disappearance rate of an organic prey analogue, FLS, was investigated by time-series of FLS-labeled cells held in GFF-filtered seawater. Digestion of native *Synechococcus* in *S. sulcatum* was examined in experiment 4 from disappearance of its characteristic orange fluorescence as detected by epifluorescence microscopy. A solution of cultured ciliates was mixed with a surface seawater sample that yielded a final *Synechococcus* concentration of 2.3×10^4 ml^{-1}. Ciliates were allowed to feed for 2 h, diluted in GFF-filtered seawater, and changes in *Synechococcus* ciliate^{-1} followed with time. Experiment 5 consisted of exposing ciliates to a solution of 2.5×10^4 *Isochrysis* ml^{-1} and following declines in numbers of recognizable (via Chl *a* autofluoresence) ingested *Isochrysis* per ciliate in cells held in GFF-filtered seawater. In experiment 6, we estimated the disappearance rate of *Synechococcus* inside ciliates exposed to *Synechococcus* over a period of days. A stationary-phase culture of *Strombidium* that had been growing on *Synechococcus* was diluted into GFF-filtered seawater and changes in cell contents with time were monitored.

Digestion rates were calculated as the slopes of the linear regressions of ln(% time zero prey per cell) vs. time. Regression parameters and associated errors and probabilities were determined with the program Statview (Abacus Concepts). Slopes were compared by the GT2 method (Sokal and Rohlf 1981). Multiplying the slope by 100 gives an exponential digestion rate constant, K, in units of % min^{-1}. Based on K, an expected half-life of cell contents, $t_{½}$ (Fok and Allen 1990), was estimated by calculating minutes required for a 50% decline in cell contents.

Figure 1 shows the distribution of prey items in the ciliate populations at time zero of prey item dilution for experiments 1–5. In each case, most cells contained the prey items

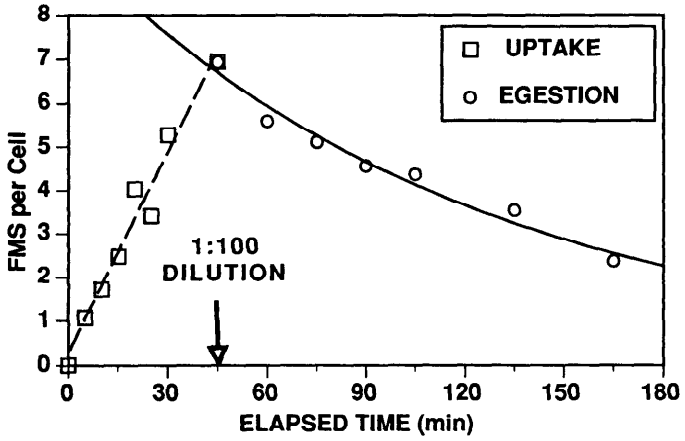

Fig. 2. Data from experiment 2 in which *Strombidium sulcatum* was exposed to fluorescent microspheres (FMS) (and uptake monitored), followed by dilution of the FMS-ciliate mixture and egestion and estimation of FMS per cell in postdilution time-course samples. Ciliates were diluted into seawater; ingestion of native *Synechococcus* and eukaryotic algae were noted after the first 15 min of dilution of exposure to seawater. Note the contrast between the linear uptake of FMS and their subsequent exponential decline.

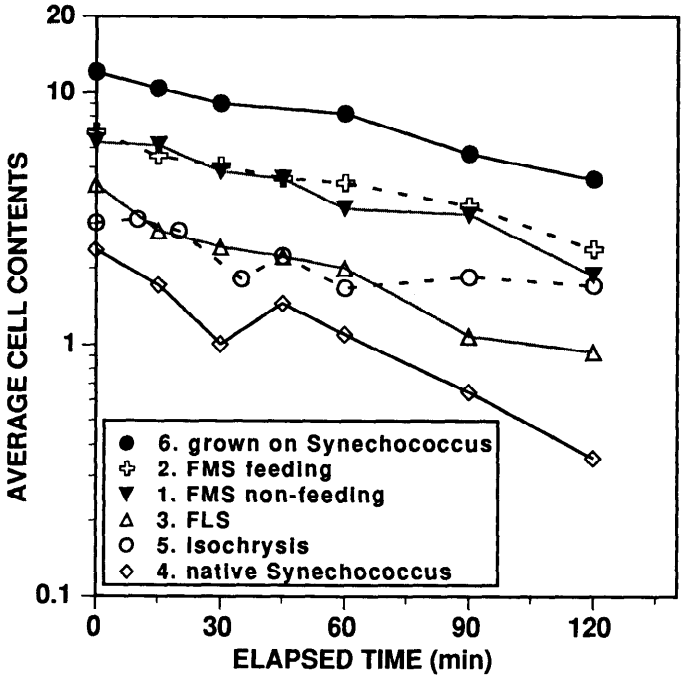

Fig. 3. Time-course changes in the average number of prey items per cell. For each prey type, a population of ciliates was exposed to the prey item, diluted at 1 : 100, and time-course samples were examined to estimate average cell contents. Numbers in the key refer to the experiment number. Estimated digestion parameters are given in Table 1.

of interest and prey per cell seemed to fit a Poisson distribution. In experiment 6, in which the ciliates had been growing on the prey of interest, prey per cell were relatively invariant with a SD of ~25% of the mean (n = 109, avg = 12.0, SD = 3.24).

The contrasts between linear uptake of prey and their exponential evacuation are shown in Fig. 2, which presents data from experiment 2. In the experiment, ciliates ingested FMS for 45 min and then were held in seawater where they fed on a natural phytoplankton community (native *Synechococcus* and eukaryotic algae were visible in the ciliates after 15 min incubation in seawater). Fig. 3 shows the time-course declines in average cell contents with time for the different prey items. In all the experiments, the declines in cell contents with time were best fit, in terms of maximum r value, with an exponential model.

Table 1 gives the calculated parameters describing rates of prey disappearance or digestion. There was no significant difference between the slopes of % t_0 prey content vs. time for the different prey types. However, plotting the apparent digestion rate, K, vs. the average volume of prey inside the ciliates indicated that the some of the variability found in K may have been related to the differences in the quantity of prey present in the ciliates at the beginning of the period over which digestion was followed (Fig. 4). Recall, however, that nonfluorescent cell contents were not quantified. Pooling the five estimates of cell content $t_{1/2}$ yields an average value of

Table 1. Summary of experimental results. Prey items were fluorescent microspheres (FMS), heat-killed, fluorescently labeled *Synechococcus* (FLS), native marine *Synechococcus,* and cultured *Isochrysis galbana.* Digestion conditions were incubation in GFF-filtered seawater (FSW); hence, cells were not feeding, except for experiment 2 in which ciliates were incubated in seawater (SW) containing natural algal prey. All ciliates were cultured on bacteria except for experiment 6 in which a stationary phase culture of ciliates grown on native *Synechococcus* were diluted and declines in *Synechococcus* per cell monitored. Cell contents are the average number of prey per cell at the beginning of the digestion period. N gives the number of time-course samples. Cells per sample indicate the average number of cells examined for each time-course sample. R value based on the linear regression of ln(% t_0 cell contents) vs. time; probability levels given as * = 0.05, ** = 0.001, *** = 0.0001. K is digestion rate in ln % min^{-1}. Prey $t_{1/2}$ is the estimated half-life in minutes of the ciliate food vacuole contents.

Exp.	Prey item	Conditions (at 22°C)	Cell contents	N	Cells per sample	R value	K (SE)	Prey $t_{1/2}$
1	FMS	FSW	6.34	7	74	0.974***	1.0(0.10)	75
2	FMS	SW	6.95	7	80	0.984***	0.8(0.10)	83
3	FLS	FSW	4.28	7	57	0.981***	1.2(0.10)	48
4	*Synechococcus*	FSW	2.37	7	102	0.952**	1.4(0.20)	44
5	*Isochrysis*	FSW	3.02	8	65	0.816*	0.5(0.20)	126
6	*Synechococcus*	FSW	12.05	6	67	0.988***	0.8(0.10)	86

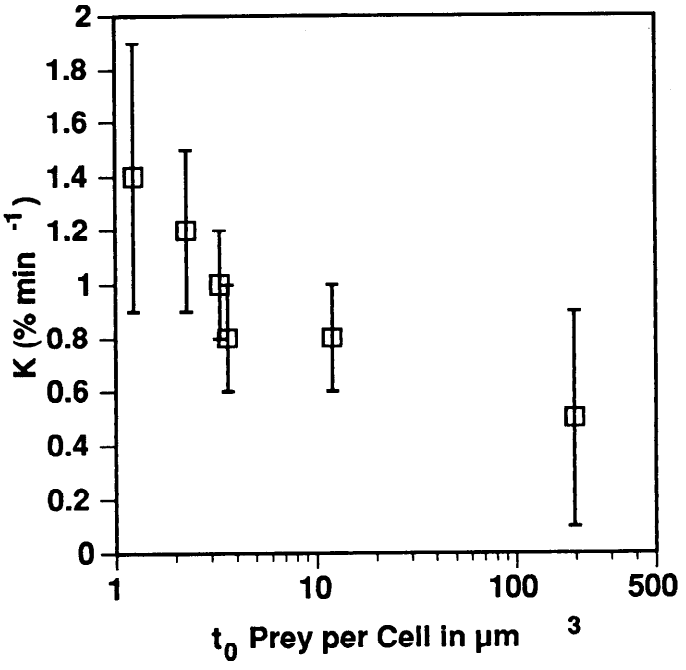

Fig. 4. Apparent digestion rate, K, as a function of the average volume of prey of interest contained at the beginning of each experiment. Prey volumes were calculated by assuming FMS, FLS, and native *Synechococcus* as 1-μm spheres and *Isochrysis* as a 5-μm sphere. Note that while rates are not significantly different (error bars represent 95% CI), K may have varied with the volume of prey processed.

~75 min. Note that the declines in FMS per cell represent evacuation rates because the microspheres are inert and retain their fluorescence. For the FLS, native *Synechococcus,* and *I. galbana,* detection relied on chlorophyll autofluorescence.

The exponential decline in prey within *S. sulcatum* corresponds with several reports on a large variety of ciliates: herbivorous freshwater benthic forms (Goulder 1972; Fenchel, 1975), the very well-studied bacteriovores *Paramecium* (Fok et al. 1982; Fok and Allen 1990) and *Tetrahymena* (Fok and Schockley 1985), predacious estuarine ciliates (Dolan and Coats 1991), and marine tintinnids (Kopylov and Tumantseva 1987). In contrast to a fixed transit time, an exponential decline indicates that ingested items are not treated in a first-in, first-out manner but rather are mixed at some point or points (Dolan and Coats 1991). In *Paramecium,* Berger and Pollock (1981) hypothesized that food vacuoles circulate randomly within the cell and are evacuated whenever they arrive at the cytoproct region, regardless of the digestive state of the prey. In contrast, Fok and Allen (1990) found that food vacuoles pass though a set of very well-defined stages before becoming "defecation competent."

Kaneshiro et al. (1992) proposed that fusion of food vacuoles, a phenomenon we have observed (Dolan unpubl. obs.), could reconcile the two disparate views of apparent random food vacuole elimination vs. well-defined sequential digestive stages. According to this explanation, there is a fixed sequence of steps though which most food vacuoles must pass before membrane as well as enzymes are recovered and recycled and undigested remains are expelled. However, not all food vacuoles follow the sequence because food vacuoles in different stages of the process can fuse, thereby short-circuiting the sequence. A given prey item enclosed in a vacuole may find itself in a fused combination late-stage, early-stage vacuole and be ejected only partly digested. Alternatively, nearly digested prey may be subjected to a second round of digestion.

On the basis of our data (Table 1), native *Synechococcus* and heat-killed, fluorescently-labeled *Synechococcus* are digested to nondetectable states at similar rates. The apparent digestion rate of *Synechococcus* by *S. sulcatum* is faster than, but not significantly different from, the processing rate of inert latex microspheres. These findings indicate that inert or heat-killed prey analogues are processed at about the same rate as natural prey items. Although prey analogues may be ingested at rates different from natural prey items by some ciliates (e.g. Pace and Baliff 1987; Putt 1991), neither accumulation of the analogues inside cells nor rapid digestion should bias ingestion estimates. However, these results may not be extendible to flagellates. For example, the euglenoid flagellate *Peranema trichophorum* readily ingests latex microspheres but is incapable of egesting them; its accumulation in the cell leads to death (Allen et al. 1966).

For the oligotrich *S. sulcatum,* our data suggest that the disappearance of pigment detectable with epifluorescence microscopy may correspond closely with the final stages of digestion. Ingested algal pigments do not seem to be rapidly transformed into degradation products but rather are almost as long-lived as the food vacuole itself. In grazing experiments that rely on changes in bulk concentrations of algal pigments, or pigment degradation products, it may be desirable to compare grazer cell contents at the start and end of incubations to account for pigments inside ciliate grazers.

Most of our experiments used ciliates cultured on a food source different from those for which digestion rates, or residence times within the cells, were estimated. In one experiment we examined disappearance rates of *Synechococcus* in cells exposed to *Synechococcus* over a period of 4 d. The rate estimated was not significantly different from the disappearance rates in cells naive to *Synechococcus.* Clearly, our results are only directly applicable to *Synechococcus.* Furthermore, one might argue that ciliates raised on heterotrophic bacteria (Exp. 1–5) may not differ from those raised on autotrophic bacteria (i.e. *Synechococcus*; Exp. 6). However, we think that by using ciliates naive to the other food or food analogues offered probably did not bias our results because digestion time is largely determined by vacuole processing time, which appears independent of feeding history. For example, disappearance rates of FLB inside *S. sulcatum* feeding at two different food levels were reported to be sensitive only to temperature (Sherr et al. 1988). In *Fabrea salina,* digestion time was estimated via the disappearance of algal fluorescence and vacuole passage time by using the disappearance of fluorescent pigment particles (Capriulo and Degnan 1991). Both digestion time and vacuole passage time were reported to be unrelated to food level. From the data presented, average algal disappearance rates were shorter than for fluorescent particles, but the digestion time of the algal pigment and residence time of the inert particles seemed statistically indistinguishable. In *Euplotes woodruffi,*

digestion of ciliate prey to an unrecognizable state was independent of total vacuole contents and similar in feeding and nonfeeding cells; digestion rates were not consistently different in cells starved for 24 h compared to feeding cells (Dolan and Coats 1991). In contrast to feeding history, temperature effects on food vacuole processing times are well established and Q_{10} factors from 1.5 to 10 have been reported for ciliates (Dolan and Coats 1991).

A predictable decay rate or passage time of cell contents is a prerequisite for the estimation of grazing rates by means of cell contents and digestion data. Our data showed a relatively constant digestion rate in cells feeding or deprived of prey, processing inert latex microspheres or native marine cyanobacteria and digesting 1- or 5-μm-diameter cells. Nonetheless, there was some indication of longer processing times with increases in cell contents (Fig. 4). One might expect digestion rates to vary not with the volume of marked food items, but with total cell contents. However, in these experiments there was no formal attempt to control for variability in total volume of cell contents between experiments beyond using cells from late log-phase cultures. A possibility is that the food vacuoles with more than several *Isochrysis* cells represented giant food vacuoles with unusually long processing times. Our results are very encouraging, but clearly the experimental examination of wild populations is the next logical and necessary step before gut contents can be used to assess instantaneous grazing rates in oligotrich ciliates.

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