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Preliminary prey digestion in a predacious estuarine ciliate and the use of digestion data to estimate ingestion

Abstract—Digestion of ciliate prey (*Pleuronema* sp. and *Metanophrys* sp.) in *Euplotes woodruffi* was examined in laboratory experiments. Similar exponential declines in recognizable food vacuole contents were found in feeding and nonfeeding cells. Ingestion rates, based on digestion rates and food vacuole contents, yielded estimates of 0.4–1.6 *Pleuronema* and 3.7–4.2 *Metanophrys* consumed per *E. woodruffi* h^{-1} at 20°C, in agreement with rates obtained by other methods. Cells starved for 24 h showed faster digestion rates; temperature variation over a range of 15°–25°C gave a Q_{10} of ~1.5.

In Chesapeake Bay, following the onset of bottom water anoxia, two ciliate species are typically encountered at the top of the anoxic water mass, *Pleuronema* sp., a scuticociliate that blooms ephemerally, and *Euplotes woodruffi*, a large hypotrich ciliate (Dolan and Coats 1991*a*). The present study was stimulated by the observation of recognizable remains of *Pleuronema* inside protargol-stained specimens of *E. woodruffi*. To use food vacuole contents as a means of estimating feeding rates in *E. woodruffi*, we conducted laboratory experiments to determine rates of preliminary digestion (i.e. digestion to the point where ciliate prey are no longer recognizable as ciliates) in *E. woodruffi*.

Feeding rates of fish have been calculated from "gut passage time" and the amount of ingested material. This approach has recently become a popular means of inferring in situ ingestion rates in crustaceous as well as gelatinous zooplankton, but only two studies has been conducted on ciliates. The first, by Kopylov and Tumantseva (1987) estimated in situ grazing rates for tintinnids with the average food vacuole contents of field-caught cells and digestion data from individuals held in a solution of filtered water and detritus. In the second, Fenchel (1975) calculated ingestion rates of ciliates from an arctic tundra pond from food vacuole contents of wild populations and digestion rates from laboratory studies on cells held in particle-free water. However, these studies did not consider factors that may affect digestion rates such as whether cells are feeding, the feeding history of the ciliate, and the size of the prey item being digested.

We examined the digestion of *Pleuronema* in *E. woodruffi* with cultures grown on *Pleuronema* in feeding and nonfeeding cells, in cells grown on an alternate ciliate food source, at different temperatures, and after 24 h of starvation. Additional experiments examined digestion of a smaller ciliate prey item by *E. woodruffi*. Food vacuole contents were analyzed in protargol-stained specimens on slides made following the procedure of Montagnes and Lynn (1987). Staining with protargol yields permanent preparations that allow the enumeration of recently ingested prey such as ciliates and dinoflagellates and also precise taxonomic identification of ciliates (Montagnes and Lynn 1987) whether predator or prey.

Digestion rates with data on food vacuole contents were used to calculate ingestion rates for laboratory populations and for the field population that motivated our investigation. The results are compared with a previous study of ingestion in *E. woodruffi* that used other methods (Dolan and Coats 1991*b*).

Approximate areal sizes of the experimental organisms (in μm) are: *E. woodruffi*, 122 × 66; *Pleuronema* sp., 40 × 24; *Meta-*

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nophrys sp., 20 × 7; *Prorocentrum* sp., 15 × 15. All experiments used log growth phase *E. woodruffi* grown in 15‰ seawater. The dinoflagellate *Prorocentrum* sp. was grown in f/2 media. Details of culture protocols appear elsewhere (Dolan and Coats 1991*b*). Briefly, *E. woodruffi* was introduced into cultures of *Pleuronema* or *Metanophrys* at a concentration of 1–2 ml^{-1}; 24–48 h later, when *E. woodruffi* densities reached of ~10 ml^{-1}, the cells were harvested for experimentation by gentle screening over a 20-μm Nitex screen and washed with GF/F-filterd water, which takes ~5 min. Replicated experiments used different strains of *E. woodruffi* that were fed different strains of prey ciliates. *E. woodruffi* was grown at the planned experimental temperature and manipulations were performed in a temperature-controlled chamber.

The experiment design was to monitor the average number of recognizable prey inside *E. woodruffi* in cells that had been removed from their food and placed in either filtered water (FW) or an alternate food solution. After manipulation, cells were distributed into a set (6–8) of 20-ml scintillation vials. At 15–60-min intervals over a 3–4 h period, contents of the vials were preserved in Bouin's fixative. Cell contents were examined in protargol-stained preparations following the procedure of Montagnes and Lynn (1987). After 48 h of preservation, the entire contents of each vial were drawn onto a single 25-mm, 0.42-μm HA Millipore filter, embedded in agar, run through the reagent series, mounted with a coverslip on a slide in Permount, and allowed to dry ~24 h before examination. For each time-course sample, the entire filter surface was scanned with a 63× (N.A. 1.25) oil immersion objective (total magnification = 788×).

Only cells lying flat on their dorsal or ventral surfaces and showing a well-stained macronucleus (dark with distinct edges) were examined for the presence of recognizable prey items with stained kinetal rows. Slides of samples with <50 well-stained and positioned *E. woodruffi* were discarded. The average experiment consisted of tabulating cell contents of ~300 individuals for each of seven time-course samples. We individually examined >29,000 *E. woodruffi* for these experiments.

Experiments 1, 2, and 3 investigated the length of time *Pleuronema* remains recognizable in feeding vs. nonfeeding cells. *E. woodruffi* cultured on *Pleuronema* was placed in FW or in another food source: a solution of *Metanophrys* sp. or *Prorocentrum* sp. at 1,000 ml^{-1}. The effect of feeding history was examined in experiments 4, 5, 6, and 7. In experiments 4 and 5 cells grown on *Metanophrys* were removed from culture, exposed to *Pleuronema* at ~300 ml^{-1} for 5.5 h, and digestion was followed in FW. The effect of starvation was tested in experiments 6 and 7. Cells grown on *Pleuronema* were held in FW for 24 h, re-exposed to *Pleuronema* at ~400 ml^{-1} for 45 min, placed in FW, and digestion followed. Temperature effects on digestion time were estimated in experiments 8, 9, 10, and 11. We grew *E. woodruffi* on *Pleuronema* at either 15° or 25°C and digestion was followed in FW. The length of time *Metanophrys* remains recognizable in *E. woodruffi* was examined in experiments 12 and 13. Cells grown on *Metanophrys* were removed from culture and placed in a solution of 200 *Metanophrys* ml^{-1} for 4 h, then placed in FW, and sampled with time.

Digestion rates were calculated as the slope of the linear regression of ln (% time-zero prey per predator) vs. time. Estimates of slopes and associated error statements were generated via the BIOM PC program (F. J. Rohlf unpubl.). Slopes were compared with the GT2 method (Sokal and Rohlf 1981). Multiplying the slope by 100 yields the exponential digestion rate constant K in units of % min^{-1} (Dam and Peterson 1988). K was used to generate ingestion rates by assuming steady state conditions, calculating the amount of material remaining after the first 15 min of digestion (Eq. 1), and equating the difference between this value and the cell contents at the beginning of the experiment to the amount of material ingested every 15 min and multiplying by 4 to yield an hourly rate.

$$N_{15} = N_0 e \exp[-K(15)] \quad (1)$$

where N_0, N_{15} are the *E. woodruffi* contents

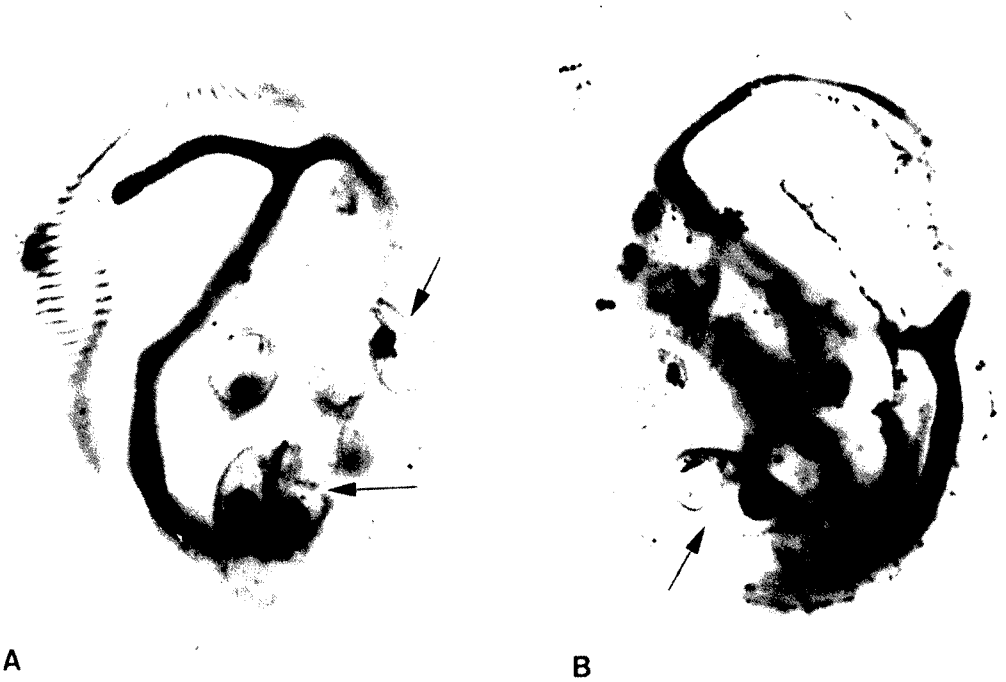

Fig. 1. Examples of protargol-stained specimens of *Euplotes woodruffi* showing the remains (arrows) of ingested *Pleuronema*: a cell from a log growth phase culture (A), and a cell collected on 5 August 1986 from station 845 in Chesapeake Bay (B).

at time-zero and after 15 min in units of prey cells per *E. woodruffi.*

Two field samples preserved in Bouin's fixative in which *E. woodruffi* and *Pleuronema* sp. were relatively abundant were processed with the Montagnes and Lynn (1987) protargol procedure. The samples were taken from a station (38°45′N, 77°32′W) in mesohaline Chesapeake Bay on 5 August 1986. Detailed descriptions of the study site, sampling protocol, and processing were given elsewhere (Dolan and Coats 1990). Briefly, CTDFO$_2$-Niskin bottle casts provided physical and chemical data (conductivity, temperature, O_2, and Chl *a* concentrations) and material for determinations of ciliate, microflagellate, and bacterial abundances. Samples for protargol staining were concentrated over 20-μm Nitex and preserved with Bouin's fixative. Whole-water samples were preserved in Bouin's fixative for ciliate enumerations and in glutaraldehyde for microflagellate and bacterial counts. Ciliate abundances and community composition were determined with the inverted microscope method (Utermöhl 1958).

Staining with protargol clearly revealed recently ingested ciliates inside *E. woodruffi* (Fig. 1). In our preparations very few broken cells or cell fragments were found, and consistent high-quality stains were evident. The apparent course of preliminary *Pleuronema* digestion by *E. woodruffi,* as revealed by the disappearance of structures stained by protargol, was a sequential digestion of macronuclei first, then somatic ciliature, and finally digestion of the distinctive buccal ciliature. It is assumed that these changes in prey morphology correlate with stages of biomass digestion.

Experimental results are summarized in Table 1. In all the digestion experiments, an exponential decline in prey per predator was noted; the average number of ln prey per predator decreased linearly with a significant slope ($P < 0.05$). Typical data from experiments 1, 2, and 3 are shown in Fig. 2.

The rate at which *E. woodruffi* digested *Pleuronema* to an unrecognizable state was

Table 1. Digestion of *Pleuronema* (exp. 1–11) and *Metanophrys* (exp. 12, 13) by *Euplotes woodruffi*. Details of feeding given in text. Digestion conditions: filtered water—FW; solutions of *Metanophrys*—Met; or *Prorocentrum*—Pro. Cell contents were initial (time-zero) average food vacuole contents in ciliates per *Euplotes woodruffi*, *N*—number of time-course samples, *K*—digestion rate in % per minute.

Exp.	Feeding history	Digestion conditions	Cell contents	*N*	Cells per sample	*K* (SE)	*R* value
1	*Pleuronema* culture	FW, 20°C	1.80	8	193	1.7(0.24)	0.846
2	*Pleuronema* culture	Met, 20°C	0.77	8	249	1.7(0.15)	0.862
3	*Pleuronema* culture	Pro, 20°C	0.42	7	444	1.8(0.23)	0.837
4	*Metanophrys* culture	FW, 20°C	0.40	7	213	1.8(0.32)	0.816
5	*Metanophrys* culture	FW, 20°C	0.21	7	330	1.2(0.33)	0.764
6	Starved	FW, 20°C	1.31	8	339	1.9(0.32)	0.837
7	Starved	FW, 20°C	0.82	8	318	5.1(1.20)	0.803
8	*Pleuronema* culture	FW, 15°C	0.34	7	581	1.5(0.21)	0.830
9	*Pleuronema* culture	FW, 15°C	0.54	8	249	1.5(0.25)	0.841
10	*Pleuronema* culture	FW, 25°C	0.74	7	387	2.1(0.36)	0.818
11	*Pleuronema* culture	FW, 25°C	1.50	8	382	2.3(0.13)	0.869
12	*Metanophrys* culture	FW, 20°C	1.48	6	110	8.1(1.86)	0.772
13	*Metanophrys* culture	FW, 20°C	2.43	6	138	3.2(0.50)	0.803

nearly identical in nonfeeding cells (1.7% min^{-1}) compared to cells feeding on *Metanophrys* (1.7% min^{-1}) or cells feeding on *Prorocentrum* (1.8% min^{-1}). In both experiments where *E. woodruffi* was digesting *Pleuronema* in the presence of another food item, cells were packed with the alternate food within the first 15 min of exposure.

Replicate experiments which estimated the digestion time of *Pleuronema* in *E. woodruffi* grown on *Metanophrys* gave rate constants of 1.2 and 1.8% min^{-1}, which were not significantly different ($P < 0.05$) from those found in experiments 1, 2, and 3 with *E. woodruffi* grown on *Pleuronema*. For cells that had been starved for 24 h and then allowed to feed for 45 min, higher digestion rates of 1.9 and 5.1% min^{-1} were estimated with only the latter rate significantly different ($P < 0.05$) from rates determined in well-fed cells grown on either *Pleuronema* or *Metanophrys*.

At 15°C, rate constants of 1.5% min^{-1} were estimated compared to average rates of 1.73% min^{-1} at 20°C and 2.2% min^{-1} at 25°C; changes in digestion rate with temperature (Fig. 3) yielded a calculated Q_{10} estimate of ~1.5 over a range of 15°–25°C. Results from experiments with the smaller *Metanophrys* indicated that small prey are digested to an unrecognizable state more rapidly than larger prey. *E. woodruffi*, grown on *Metanophrys*, showed a decay rate of *Metanophrys* per *E. woodruffi* of 3.2 and 8.1% min^{-1}.

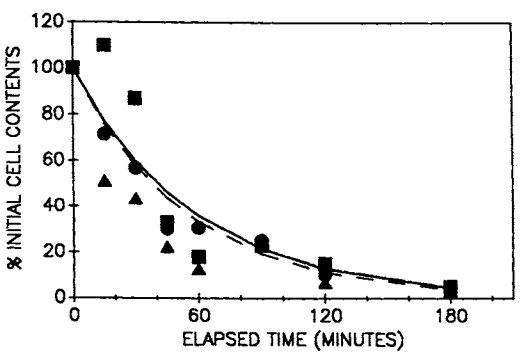

Fig. 2. Temporal declines in relative numbers of recognizable *Pleuronema* found inside *Euplotes woodruffi* among nonfeeding cells (■) held in filtered water and cells feeding on *Metanophrys* (●) or *Prorocentrum* (▲). Lines represent $N_0 = N_t e \exp[-Kt]$ with K calculated from linear regressions of ln-transformed data.

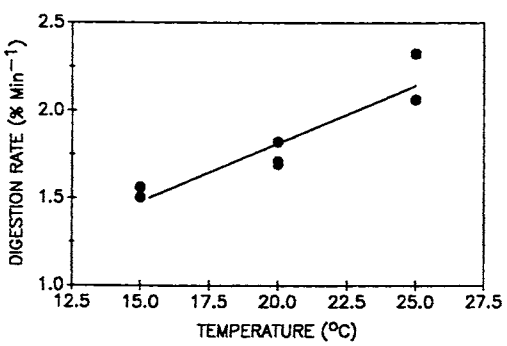

Fig. 3. Digestion rates of *Euplotes woodruffi* digesting *Pleuronema* plotted vs. temperature.

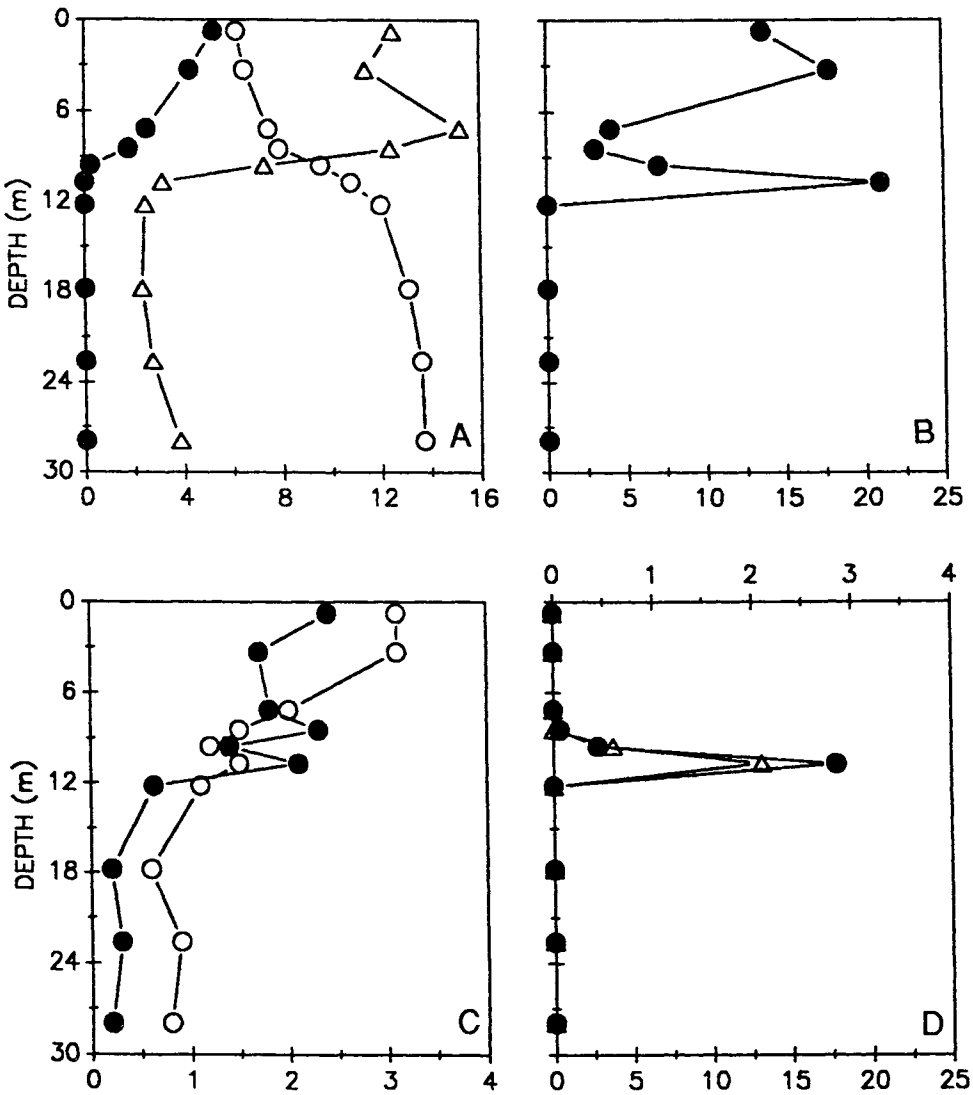

Fig. 4. Vertical profiles from Chesapeake Bay station 845 on 5 August 1986. A. Dissolved oxygen (●) in ml liter^{-1}, Chl *a* (△) in μg liter^{-1}, and σ_t (○). B. Ciliates in cells ml^{-1}. C. Heterotrophic microflagellates (●) in cells × 10^{-3} ml^{-1} and bacteria (○) in cells × 10^{-6} ml^{-1}. D. *Euplotes woodruffi* (△, upper scale) and *Pleuronema* (●, lower scale) in cells ml^{-1}.

Field data indicated that in Chesapeake Bay natural populations of *E. woodruffi* and *Pleuronema* sp. both occupy a narrow band in the water column that corresponds to the interface layer between oxygenated and anoxic waters and inversions of the bacterial and heterotrophic microflagellate distributions (Fig. 4). The temperature of the interface layer was 25.9°C. *E. woodruffi* and *Pleuronema* were present in peak abundances of 2.1 and 18 ml^{-1}. Protargol-stained specimens from this relatively dense population of *E. woodruffi* revealed an average content of 1.6 *Pleuronema* per *E. woodruffi* (N = 126).

Data presented here show that preliminary digestion of ciliate prey items in *E. woodruffi* follows an exponential model (Fig. 2). Previous studies that followed the disappearance of prey items in herbivorous and

bacteriovorous ciliates have also noted exponential decay of food vacuole contents (Kopylov and Tumantseva 1987). For example, Kopylov and Tumantseva (1987) found that in the tintinnids *Eutintinnus franknoii* and *Epiplocycloides reticulata,* ~50% of ingested algae were digested in 1 h with 6% remaining after 8 h at 22°–27°C. For *E. woodruffi,* 50% of ingested *Pleuronema* were unrecognizable as ciliates after ~30 min and 7% after 3 h in experiments at 25°C. At 10°C, bacterial prey inside *Tetrahymena pyriformis* declined ~50% after 2 h; after 4 h ~35% of the original average cell contents remained (Fenchel 1975).

Other studies, which have focused primarily on the course of events involved in processing food vacuoles, have found exponential declines in labeled food vacuoles of ciliates (Berger and Pollock 1981; Fok and Shockley 1985; Fok et al. 1982). The exponential model of digestion found in *E. woodruffi* and apparently common in ciliates indicates that food items are not processed in a strict "first in, first out" manner but are mixed at some point(s).

A digestion process in which food items are continuously mixed is characteristic of the "continuous-flow, stirred-tank reactor" model (Penry and Jumars 1987). This model describes ciliate food processing more accurately than the alternative batch reactor (large, intermittent, sequential meals) or plug-flow reactor (constant-flow, items processed in order) models but still does not precisely reflect the process that occurs in ciliates.

Many ciliates may represent a collection of batch-flow reactor vessels. Whereas feeding can be nearly continuous or intermittent, food is always packaged intermittently in food vacuoles. These vacuoles can contain one or more prey items (Fig. 1) and be considered batch reactors. The question is why do the individual reactors appear to function at different rates? Long-lived food vacuoles did not show any obvious traits, such as the number of prey items contained.

Berger and Pollock (1981) hypothesized that a random element exists in egestion; their hypothesis was based on data showing that food vacuoles in various states of digestion circulate freely in *Parmecium* and that those in the cytoproct region are nonselectively egested. Our data on *E. woodruffi* show that preliminary digestion may have a random component, which suggests that fusion of primary lysosomes (*see* Nilsson 1979) with newly formed food vacuoles may be a random process in *E. woodruffi.*

However, a pattern in which food items are mixed during processing may only be characteristic of digestion when food is not limiting. Digestion theory points out that sequential processing is much more efficient than continual mixing of food items and predicts a longer throughput time under food-limited conditions (Penry and Jumars 1987). Yet, to our knowledge, no studies have examined ciliate digestion under food-limited conditions. Therefore, a hypothesis that the pattern seen in this and prevous studies is characteristic only of ciliates that have fed in a surplus of food cannot be rejected at this time.

Besides changing the pattern of digestion, feeding history appears to influence the magnitude of the digestion rate; in simple terms, more digestive enzymes may be available per food item at low ingestion rates compared to high ones. If this is true, cells digesting prey after a period of complete starvation would have higher digestion rates than well-fed cells. Evidence for this can be seen in the high rate recorded in one experiment (Table 1, exp. 7) with starved *E. woodruffi* relative to well-fed cells.

Temperature, not surprisingly, affected digestion in *E. woodruffi.* Our data were insufficient to distinguish between models of linear and exponential increases in rate with temperature, yet provide a Q_{10} value of ~1.5 over a temperature range of 15°–25°C. This value seems low and our data are variable (Fig. 3), but little comparative information exists on temperature dependance of digestion in ciliates. Fenchel (1975) presented data on the algivorous *Stylonychia mytilus* and bacteriovorous *T. pyriformis* which indicate Q_{10} values of 3 and 10 over a temperature range of 10°–25°C. Sherr et al. (1988) recently reported a Q_{10} of 2.05 over 12°–22°C for *Strombidium sulcatum* feeding on bacteria. It is unclear if these differences are real or the result of different methods. For example, our study only ex-

Table 2. Estimates of ingestion rates based on digestion and food vacuole contents compared to rates given by Dolan and Coats 1991*b*. Labeled prey rates are from direct counts of ingested prey labeled with fluorescent microspheres, and prey disappearance rates are based on volumes of water cleared of prey (both from Dolan and Coats 1991*b*). Prey digestion rates are from this study.

Prey	Concn (cells ml^{-1})	Ingestion (cells h^{-1})	Method
Pleuronema	14	0.5	Labeled prey
Pleuronema	38	0.6	Prey disappearance
Pleuronema	100	0.4, 0.7, 1.6	Prey digestion
Metanophrys	75	4.5	Labeled prey
Metanophrys	87	4.3	Prey disappearance
Metanophrys	200	3.7, 4.2	Prey digestion

amined preliminary digestion; we did not measure time to total disappearance of prey or egestion of prey remains.

Exponential rates of preliminary digestion in *E. woodruffi* complicate calculations of "average food digestion time" and present problems in calculating ingestion rates, as has been noted in studies of copepods (Dagg and Grill 1980; Kiorboe et al. 1982). We have chosen to calculate ingestion rates by estimating cell contents present 15 min after the start of an experiment and taking the difference between this value and cell contents at time-zero, multiplied by 4, to yield ingestion h^{-1}. The choice of time period is somewhat arbitrary; however 15 min seems reasonable as it represents the minimum time between ingestion events (*see Table 2*) and therefore most realistically minimizes deviation from the assumption of steady state conditions. Calculation over a shorter time interval (which yields larger hourly ingestion rate estimates) implies that the consumer captures fractions of food items to maintain steady state contents, while using longer intervals involves larger deviations from the steady state assumption.

Ingestion rates based on digestion data agreed well with those obtained by other methods (Table 2). For example, at 20°C, based on cell contents and digestion time, log growth phase cells ingested 0.4–1.6 *Pleuronema* h^{-1} compared to 0.6 h^{-1} determined from a batch culture experiment in a previous study (Dolan and Coats 1991*b*).

Calculation of an ingestion rate for the field population based on the 25°C digestion rate of log growth phase *E. woodruffi* yields an estimate of 1.8 *Pleuronema* ingested h^{-1}, assuming steady state conditions. At densities of ~2 *E. woodruffi* ml^{-1} and 18 *Pleuronema* ml^{-1}, it is probable that *E. woodruffi* had a significant impact on the standing stock of *Pleuronema*. However, it should be noted that food vacuole contents were determined from cells concentrated before fixation, so the possibility of cod-end feeding cannot be excluded.

Use of digestion rates and cell contents to estimate grazing rates may answer many questions that are difficult to examine with standard methods, such as, are there small-scale spatial or temporal differences in feeding? It is encouraging to note that ingestion rates derived from digestion and "gut content" data for ciliates agree with rates obtained with other methods and that digestion in feeding and nonfeeding cells was similar. It should also be noted that dinoflagellate and diatom digestion could also be followed with this methodology. Considering that many ciliates consume a large variety of food items that could complicate digestion considerably, it is clear that further work, especially with field populations, is warranted.

John R. Dolan
D. Wayne Coats

Smithsonian Environmental Research Center
P.O. Box 28
Edgewater, Maryland 21037-0028

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Enhanced microbial methane oxidation in water from a deep-sea hydrothermal vent field at simulated in situ hydrostatic pressures

Abstract—Water from a hydrothermal vent field was incubated in the presence of $^{14}CH_4$ under conditions of both atmospheric (1 atm) and simulated in situ hydrostatic pressure (~200 atm). Methane oxidation rates measured in samples incubated at elevated pressures were 21–62% higher than those measured in replicate samples incubated at atmospheric pressure. The magnitude of the observed effect was consistent with that predicted to occur from changes in CH_4 activity with depth-dependent pressure, suggesting that microbial CH_4 oxidation is a functionally barophilic process. The data indicate that methane oxidation, as well as other microbial gas consumption processes, is likely to be affected by moderate increases in hydrostatic pressure and that the rates of these processes in the deep sea, based on measurements at atmospheric pressure, may be underestimated.

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Hydrostatic pressure, which increases ~1 atm for every 10 m of depth, is a major environmental factor that can affect microbial processes in the deep sea. The effect of intermediate hydrostatic pressures encountered in the marine environment (corresponding to depths <3,000 m) has been examined for several microbial processes. In situ or simulated in situ hydrostatic pressure