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Ingestion and digestion of an autotrophic picoplankter, *Synechococcus,* by a heterotrophic nanoflagellate, *Bodo saltans*

Abstract—We investigated the process of digestion and estimated ingestion from digestion rate and food vacuole content in a planktonic freshwater bacterivore. Digestion was examined in feeding and nonfeeding *Bodo* flagellates previously exposed to *Synechococcus* and in "naïve" flagellates. Digestion of *Synechococcus* by *Bodo* was estimated for flagellates in different growth phases. Food vacuole contents declined exponentially, and digestion rate was relatively constant, averaging about 1% food vacuole content min^{-1} at 22°C, regardless of feeding history or growth phase. There was close agreement among ingestion rates based on digestion rate and rates estimated from the disappearance or direct uptake of *Synechococcus.* We also examined ingestion of *Synechococcus* by *Bodo.* Ingestion was similar in cells of different growth phases and in the presence or absence of fluorescent microspheres. In contrast, in the presence of *Synechococcus,* microspheres were ingested at lower rates in late-exponential-phase cells and not at all in mid- and late-stationary-phase flagellates. Results of a preliminary experiment with marine *Synechococcus* and mixed marine flagellates gave results similar to those obtained with *Bodo* in terms of an exponential decline in average cell contents and a digestion rate of about 1% cell contents h^{-1}. Our results suggest that food vacuole content can be used to estimate ingestion of autotrophic picoplankton by heterotrophic nanoflagellates.

Autotrophic picoplankton are often the dominant primary producers (e.g., Stockner 1988), and the fate of this primary production is not always clear. Viral lysis could be an important loss factor, but the major consumers of these small autotrophs are generally assumed to be flagellates and small ciliates (Weisse 1993). However, although many detailed studies have examined protistan consumption of heterotrophic bacteria and nano-sized algae (e.g., *see* Capriuolo 1990), relatively few studies have focused on the consumption of autotrophic picoplankton by protists (Dryden and Wright 1987; Caron et al. 1991).

In field studies, the loss rates of autotrophic picoplankton have most commonly been estimated using dilution grazing experiments (e.g., Kudoh et al. 1990; Landry et al. 1995; Veldhuis et al. 1997) or measurements of uptake of prey analogs (e.g., Christoffersen 1994; Simek et al. 1996, 1997*a*). The dilution technique is unsuited to investigating grazing over the short time scales that may be of interest. Many autotrophic picoplankters, such as *Synechococcus* and *Prochlorochococus,* reproduce synchronously (e.g., Fahnenstiel et al. 1991; Vaulot et al. 1995) and likely experience variable losses from grazers over a 24-h period (Liu et al. 1997). The direct uptake method, although suited to examinations of short time-scale changes, is not without drawbacks, the most serious of which is perhaps selective ingestion of prey analogs.

We recently established the feasibility of using a "gut contents (food vacuole) and digestion time" approach to estimate ingestion in planktonic oligotrichs (Dolan and Simek 1997). We were interested in extending this approach to heterotrophic flagellates and evaluating the use of food vacuole content to estimate instantaneous rates of grazing on autotrophic picoplankton. Such an approach is attractive because distinctive autotrophic picoplankters, such as cyanobacteria, are commonly seen in food vacuoles of heterotrophic flagellates; observations have been made in studies of diverse systems ranging from Lake Ontario (Caron et al. 1985) to the Arabian Sea (Reckerman and Veldhuis 1997). The method is of interest because it offers the possibility of detecting small time- and space-scale changes in ingestion rates.

Relative to ciliates, in flagellates little is known about the formation of food vacuoles (Hausmann and Radek 1993) or their processing (Radek and Hausmann 1994). Studies that provide estimates of digestion rates are, to our knowledge, limited to a few investigations using heat-killed, fluorescently labeled bacteria and undefined mixed cultures of flagellates (Sherr et al. 1988; Gonzalez et al. 1990, 1993). In contrast, a large number of studies have been devoted to elucidating factors that influence ingestion in flagellates. These studies have documented considerable variability in ingestion rates of flagellates linked to prey characteristics (e.g., Pace and Bailiff 1987; Landry et al. 1991; Simek and Chrzanowski 1992) as well as the physiological state of the flagellate (e.g., Choi 1994; Jürgens and DeMott 1995; Zubkov and Sleigh 1995).

Hence, we conducted a series of experiments to explore the possible influence on digestion rate of both prey quality and the physiological state of the grazer. We used a simple system of a single defined grazer, *Bodo saltans,* and two prey items, *Synechococcus* and inert latex fluorescent microspheres (FMS). The experimental approach was based on a "cold-chase" method of monitoring cell contents in labeled cells held in water in which the label, or prey of interest, had been diluted to a negligible concentration.

We examined digestion in feeding flagellates and those held in filtered water. Disappearance of *Synechococcus* in *Bodo* was monitored in cells previously exposed to *Synechococcus* and in naïve flagellates. We estimated the digestion of *Synechococcus* in cells containing only digestible prey and in those also containing FMS. Digestion and ingestion were measured in flagellates of different growth phases. Finally, for *Bodo,* we compared estimates of ingestion made with food vacuole content, direct uptake of prey, as well as prey disappearance. We also examined the kinetics of digestion of marine *Synechococcus* (m-Syn) in a mixed assemblage of marine heterotrophic nanoflagellates. Our results indicated that digestion rate, or disappearance of *Synechococcus,* in *Bodo* was invariant and, when combined with food vacuole contents, can provide an estimate of in-

gestion that agrees closely with estimates made from direct uptake and prey disappearance.

Cultures of *B. saltans,* isolated from the plankton of Lake Constance, were kindly supplied by Doris Springman (Limnological Institute). Stock cultures of *B. saltans* (cell dimensions: length = 6–7 μm; weight = 3.5–4.5 μm; and volume = 40–50 μm^3) were maintained on wheat-seed infusion (one seed autoclaved in 100 ml of tap water) with a mixed assemblage of bacteria. For experiments, 2 ml of the *Bodo* stock culture was inoculated into 100 ml of tap water supplemented with 20 mg liter^{-1} of a mixture of 90% bactopeptone (w/w) and 10% yeast extract (w/w). In the late-exponential phase, a 50-ml inoculum of the preconditioned flagellate culture was transferred into 950 ml of tap water supplemented with the same amount of the organics (20 mg liter^{-1}) and grown for 5–10 d at a temperature of 22°C in a temperature-controlled incubator.

We used the following prey items: (1) FMS with a diameter of 1 μm (Yellow-Green Fluoresbrite Plain Microspheres, Polysciences); (2) a rod-shaped freshwater *Synechococcus* (f-Syn) with a length of 1.02 ± 0.24 μm and width of 0.62 ± 0.1 μm (volume = 0.26 ± 0.12 μm^3) isolated from a reservoir (Simek et al. 1995); (3) m-Syn 1 μm in diameter, concentrated from surface waters of Villefranche Bay (northwestern Mediterranean Sea) by size fractionation of waters samples screened through 2-μm filters and concentrated on 0.6-μm filters. The filters were then briefly sonicated in glass fiber filter (GFF)-filtered seawater to free *Synechococcus* from the filter.

The experiment consisted of first exposing flagellates to a prey item for 60–240 min, followed by a cold chase. For several experiments, uptake was monitored by sampling at time zero and over the first hour at 15-min intervals. After exposure, the cold chase consisted of halting prey uptake by diluting the flagellate–prey mixture in a ratio 1 : 100 and sampling with time (n = 6–8) over an interval ranging from 120 to 240 min. All incubations were conducted at 22°C. All samples were fixed with alkaline Lugol's solution (final concentration = 3%), then formalin (final concentration = 2%), and decolorized by adding a few drops of 3% sodium thiosulfate. Cell contents were determined microscopically. Preliminary experiments with dilution series of prey items were conducted to determine a particle concentration (~10^6 ml^{-1}) that yielded optimally labeled flagellates (2–8 items cell^{-1}) and nondetectable ingestion (~10^4 ml^{-1}).

The first set of experiments (E1–E4) was performed to examine digestion, or prey residence time, in nonfeeding (E1) and feeding (E2) cells, in *Bodo* naive to *Synechococcus* (E3), and in cells exposed to *Synechococcus* for 24 h (E4). For these experiments, we used a *Bodo* culture in the stationary phase, except for E4, in which the flagellates were exposed for 24 h to *Synechococcus* by the addition of 1 × 10^6 f-Syn ml^{-1} to an aliquot of the stationary-phase *Bodo* culture.

To examine residence time of prey items in nonfeeding cells (E1), 5 ml of flagellate culture was exposed to FMS at a concentration of 2 × 10^6 FMS ml^{-1} for 1 h, then diluted into 500 ml of GFF-filtered culture medium. Processing time of FMS in feeding cells (E2) was estimated by adding *Synechococus* to the diluent, GFF-filtered culture medium, to yield a final concentration of 2 × 10^6 *Synechococcus* ml^{-1}. Digestion of *Synechococcus* by *Bodo* naive to the cyanobacteria (E3) was examined by adding *Synechococcus* to an aliquot of *Bodo* culture to yield a final concentration of 1 × 10^6 *Synechococcus* ml^{-1}, allowing *Bodo* to feed for 1 h and then diluting 5 ml of the *Synechococcus*-containing *Bodo* with 500 ml of GFF-filtered *Bodo* culture medium. The fourth experiment (E4) used *Bodo* that had been feeding on *Synechococcus* for 24 h (*see above*). Five milliliters of the *Synechococcus*-fed *Bodo* was diluted with 500 ml of GFF-filtered medium and sampled over time.

For the second set of experiments (E5–E7), designed to estimate ingestion and digestion in *Bodo* from different growth phases, we established a 1-liter *Bodo* culture. Samples were removed daily for counts of flagellates and bacteria. Prey processing in exponential-growth-phase cells was examined in E5. A 150-ml sample was removed 24 h after the culture was inoculated. Cell concentrations were 2 × 10^7 bacteria and 7 × 10^4 *Bodo* ml^{-1}. The sample was split in three with the subsamples receiving either FMS alone, *Synechococcus* alone, or both. Prey items were added to give samples for each time a final concentration of 1 × 10^6 ml^{-1} of each item. For each of the three treatments, subsamples for determination of ingestion rates were taken at times 0, 15, 30, 45, and 60 min; an aliquot was diluted 1 : 100 with GFF-filtered culture medium; and time-course subsamples for determinations of digestion rates were taken for 3–4 h. The same protocol was followed 3 d after culture inoculation in E6 to examine ingestion and digestion in stationary-phase cells. Cell concentrations were 1 × 10^7 bacteria and 9 × 10^4 *Bodo* ml^{-1}. However, because of the low uptake rates of FMS, only samples for determination of *Synechococcus* digestion were analyzed.

The last experiment with *Bodo* (E7) to investigate ingestion and digestion in late-stationary-phase cells (8 × 10^6 bacteria and 7 × 10^4 *Bodo* ml^{-1}) used a slightly different design because we wished to compare different measures of ingestion. The subsample to which *Synechococcus* alone was added was incubated and sampled over a 4-h interval before dilution. Samples were taken to monitor changes in the concentration of *Synechococcus* and to determine the time at which cell contents of *Bodo* approached a steady state. This approach allowed calculation of ingestion based on (1) direct uptake rates from the first 45 min, (2) decrease in bulk concentrations of *Synechococcus* over the 4-h interval, and (3) steady-state food vacuole contents and digestion rate established after dilution.

In the final experiment (E8), we examined the kinetics of digestion in natural flagellate assemblages from surface seawater of Villefranche Bay feeding on native m-Syn. Water from the bay was prescreened through a 8-μm filter (Nucleopore) to remove potential flagellate predators. The filtrate was supplemented with 20 mg liter^{-1} of yeast extract, and the flagellates were grown for 3 d. At the late stationary phase of flagellate growth (1.51 × 10^5 flagellates ml^{-1} and 1.16 × 10^6 bacteria ml^{-1}), a 20-ml subsample of the filtrate was incubated for 24 h with concentrated *Synechococcus* (final concentration = ~2.5 × 10^5 cells ml^{-1}), which yielded an increase in flagellate numbers of ~40%. An aliquot was diluted to a ratio of 1 : 50 by GFF-filtered seawater, and 40-

ml time-course subsamples were taken for 2 h to determine flagellate vacuole contents.

Subsamples with flagellates and bacteria were fixed with the Lugol's solution–formalin decolorization technique (*see above*) and with formalin alone (final concentration, 2%), respectively, stained with DAPI (final concentration, 0.2% w/v), and enumerated by epifluorescence microscopy at a final magnification of ×1,000 (Olympus BX60 or Zeiss Axiophot). Enumeration of prey items was based on the presence or disappearance of characteristic orange-red and bright yellow-green fluorescence of *Synechococcus* and FMS, respectively. For each time-course sample for ingestion or digestion, a minimum of 100 flagellates was examined; thus, for these experiments, >12,000 cells were examined for food vacuole content. The autofluorescence of phycobiliproteins of f-Syn was used to size 250 cells by an interactive image analysis system (Lucia, Laboratory Imaging).

Ingestion rates were calculated as the slope of the linear regression of average number of prey items per cell versus time. Slopes were calculated on the basis of four to five time points. Digestion rates were calculated as the slope of the linear regressions of log (% time zero prey per cell) on the basis of six to eight data points. Data were translated into percent time zero to standardize them, then log-transformed as variances increased with means. Based on the regression equation, an expected half-life of cell contents, $t_{½}$ (Fok and Allen 1990), was estimated by calculating the time (in min) required for a 50% decline in cell contents. For digestion rates, multiplying the back-transformed slope by 100 gives a digestion rate constant, K, in terms of percent per minute. Selected rates were compared by comparing slopes using the F-test. The ingestion rate of *Bodo* feeding on *Synechococcus* determined on the basis of declines in concentration of *Synechococcus* (E7) was calculated without correcting for the growth of either *Synechococcus* or *Bodo* over the short (4-h) incubation period. All rate estimates (±SE) are given.

We found exponential declines in cell contents in all experiments, as shown for E1–E4 in Fig. 1. The digestion rates or residence times for inert FMS were not significantly different for nonfeeding *Bodo* (held in filtered water) and *Bodo* feeding on *Synechococcus* (Table 1). Similarly, disappearance of *Synechococcus* in *Bodo* was not significantly different in cells exposed to *Synechococcus* for 24 h compared to naïve flagellates.

In E5, using late-exponential-phase *Bodo*, digestion of *Synechococcus* by *Bodo* processing only *Synechococcus* or both *Synechococcus* and FMS was identical (Fig. 2). Processing of ingested FMS appeared faster in cells containing only FMS than in flagellates with both FMS and *Synechococcus*, but the difference was not significant (Table 1). There were significant differences in ingestion rates. FMS uptake was lower than ingestion of *Synechococcus*. The uptake of FMS was lower in flagellates given both FMS and *Synechococcus* than in flagellates given FMS alone. In contrast, the ingestion of *Synechococcus* was similar among cells offered only *Synechococcus* and those offered both FMS and *Synechococcus*.

Bodo cells in the stationary phase (E6) digested *Synechococcus* at the same rate as exponential-growth-phase flagellates (Table 1). Uptake of *Synechococcus* was similar in

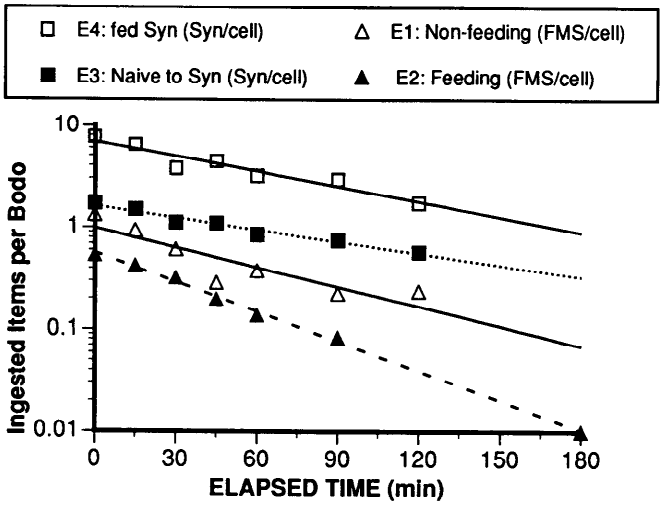

Fig. 1. Data from E1–E4 showing declines in food vacuole content of *Bodo* with time after dilution following exposure of a *Bodo* culture to FMS in E1 and E2 or *Synechococcus* (Syn) in E3 and E4. The slopes of the exponential declines of FMS in E1 and E2 in which *Bodo* was held in filtered water (nonfeeding) or supplied with 10^6 ml^{-1} *Synechococcus* (feeding) were not significantly different. Similarly, the rate of digestion of *Synechococcus* in cells fed *Synechococcus* for 24 h (fed Syn) was indistinguishable from the digestion rate calculated for *Bodo* exposed to *Synechococcus* for only 1 h (naive to Syn). Digestion rate parameters are given in Table 1.

Bodo offered only *Synechococcus* and in those given both *Synechococcus* and FMS (Fig. 3). Again, as in E5, FMS uptake was lower when FMS were offered with *Synechococcus* compared to FMS alone because the slope of the uptake curve for FMS offered with *Synechococcus* was not significantly different from zero.

In E7, we used late-stationary-phase *Bodo*, when both flagellate and bacterial concentrations were in decline. The digestion rate estimated was similar to that found for exponential-growth- and mid-stationary-phase cells (Table 1). The ingestion rates (Fig. 3) closely resembled those found in E6 with mid-stationary-phase cells. FMS uptake was insignificant when FMS were offered with *Synechococcus* and measurable when FMS were offered alone. Ingestion rates of *Synechococcus* were not different in the presence or absence of FMS, and rates were similar to those estimated for exponential- and mid-stationary-phase *Bodo*.

Calculation of the ingestion rate of *Bodo* based on direct uptake over the first 45 min of incubation (Fig. 4) yielded an estimate of 2.4 ± 0.06 *Synechococcus* h^{-1}, compared with 2.5 ± 0.1 *Synechococcus* ingested h^{-1} based on a steady-state vacuole content of 4.5 *Synechococcus* per *Bodo* and a digestion rate of 0.92% vacuole contents min^{-1}. Both estimates agreed well with an ingestion rate (2.8 ± 0.4) based on the linear decline in concentration of *Synechococcus* over 4 h in the presence of 2.63×10^4 *Bodo* ml^{-1} (Fig. 4).

In our preliminary study of the digestion of marine *Synechococcus* by a mixed assemblage of marine flagellates, we found an exponential decline in *Synechococcus* per flagellate with time and a digestion rate very similar to that found for exponential- and stationary-growth-phase *Bodo* (Table 1).

Table 1. Summary of the results of E1–E8. Prey items were fluorescent microspheres (FMS), freshwater *Synchococcus* (f-Syn), and natural marine (*Synechococcus* (m-Syn). Digestion conditions for all experiments were flagellates held in filtered water (FW), except for E2, in which *Bodo* digestion of FMS was monitored in cells feeding on *Synechococcus*. The number of time points (n), associated r value, the back-transformed slope of the linear regression of log (% time zero cell contents) versus time (K), and calculated cell contents are given for each experiment. Note that E8 was conducted with a mixture of marine nanoflagellates. *** = significance level 0.001.

Experiment	Prey item	Growth phase	Digestion conditions	n	Adjusted r^2	K (% min^{-1} ± SE)	Prey $t_{1/2}$ (min)
1	FMS	Stationary	FW	7	0.763***	1.4 ± 0.23	50
2	FMS	Stationary	Feeding on Syn	7	0.967***	2.3 ± 0.51	30
3	f-Syn	Stationary	FW	7	0.958***	0.9 ± 0.07	75
4	f-Syn	Stationary, fed f-Syn	FW	7	0.894***	1.1 ± 0.23	60
5	FMS	Exponential	FW	8	0.815***	1.8 ± 0.23	38
5	FMS + f-Syn	Exponential	FW	8	0.900***	1.1 ± 0.23	60
5	f-Syn + FMS	Exponential	FW	8	0.960***	1.1 ± 0.09	60
5	f-Syn	Exponential	FW	8	0.981***	1.1 ± 0.06	60
6	f-Syn	Early stationary	FW	8	0.939***	1.1 ± 0.23	60
7	f-Syn	Late stationary	FW	6	0.991***	0.9 ± 0.04	73
8	m-Syn	Late stationary	FW	7	0.975***	1.1 ± 0.02	60

To estimate ingestion on the basis of digestion rate and food vacuole content, the digestion rate must be predictable and food vacuole contents must be assumed to be in steady state. For *Bodo,* digestion rate appears predictable, because our results suggest that at 22°C, digestion can be described by a rate constant that varies little with digestion conditions or the physiological state of the flagellate (Table 1). In contrast, the assumption of steady-state food vacuole contents may be problematic. In *Bodo,* reaching steady-state food vacuole contents took 4 h, beginning from zero, according to data from E7, Fig. 4. Calculation of ingestion rates using, e.g., cell contents after 1 h would have yielded a much lower (33%) estimate of ingestion. However, the example given here is perhaps the worst-case scenario and unlikely to occur in situ because it concerns a grazer beginning from nonexposure and encountering a saturating concentration of prey.

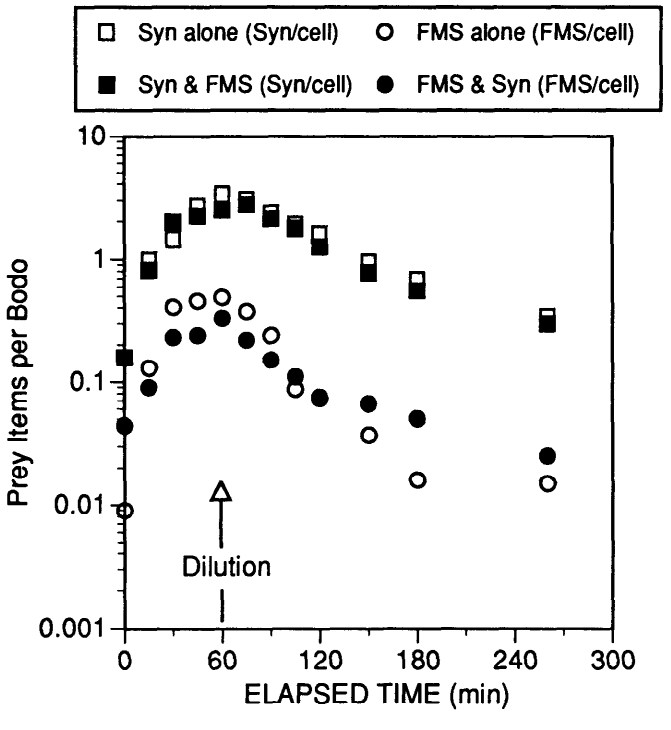

Fig. 2. Data from E5 showing temporal changes in food vacuole content of exponential-growth-phase *Bodo* offered either *Synechococcus* (Syn), FMS, or both. The dilution point shows the time of 1 : 100 dilution of the three treatments. Digestion rates were not significantly different for either prey item in the three treatments. Rate constants are given in Table 1. Note that uptake of *Synechococcus* alone was similar uptake with FMS. In contrast, uptake of FMS was markedly higher when given alone than in the presence of *Synechococcus.*

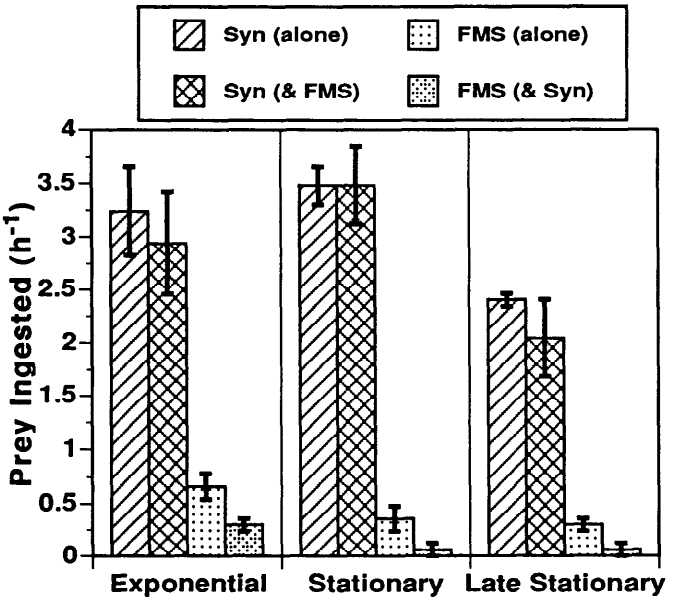

Fig. 3. Ingestion rate data from E5–E7. Error bars represent SEs. In all three experiments, both *Synechococcus* and FMS were presented in concentrations of ~1 × 10^6 ml^{-1}. Ingestion of *Synechococcus* was similar in exponential-growth-phase and mid- and late-stationary-phase *Bodo* regardless of the presence of FMS. FMS however, were ingested at lower rates in the presence of *Synechococcus* in exponential-phase flagellates. FMS ingestion was not significant in the presence of *Synechococcus* in mid- and late-stationary-phase cells.

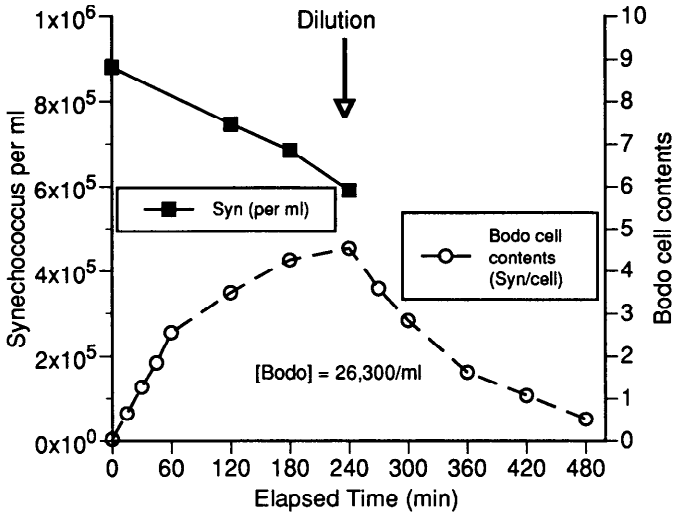

Fig. 4. Data from E7. Over the first 4 h of incubation, there were declines in the concentration of *Synechococcus* in the presence of *Bodo* and increases in *Synechococcus* inside the food vacuoles of *Bodo*. After dilution of the *Synechococcus–Bodo* solution, exponential declines in the number of *Synechococcus* contained in *Bodo* were recorded. Estimated rates of ingestion of *Synechococcus* by *Bodo*, calculated on the basis of (1) uptake over the first 45 min, (2) declines of *Synechococcus* over 4 h, and (3) digestion rate, were 2.4 ± 0.06, 2.8 ± 0.4, and 2.5 ± 0.1, respectively. Digestion rate constants appear in Table 1, and ingestion rate data are given in Fig. 3.

Overall, our results suggest that in situ rates may be estimated from cell contents and digestion rate, at least in *Bodo*.

Earlier studies on digestion of bacteria by nanoflagellates reported a linear decline in cell contents (Sherr et al. 1988; Gonzalez et al. 1990, 1993). The difference may be due to differences in the digestion of heterotrophic bacteria compared with the *Synechococcus* used here or to differences in the sampling intervals used. In our experiments, food vacuole content was followed for 2–4 h (Figs. 1, 2, 4), as opposed to 1–1.5 h in other studies (Sherr et al. 1988; Gonzalez et al. 1990, 1993). A linear model reflects processing of food vacuoles with no mixing or fusion of vacuoles such as that known to occur in ciliates (Dolan and Coats 1991; Dolan and Simek 1997) and certain flagellates (Radek and Hausmann 1994).

In contrast to our finding of similar rates for inert FMS and *Synechococcus*, differential digestion of distinct types of fluorescently labeled bacteria, gram-negative and -positive, has been reported (Gonzalez et al. 1990). Again, heterotrophic bacteria may be processed differently from larger food items. Alternatively, apparent differences in digestion rate may be due to differences in the stability of the marker used (fluorescent dye) in different bacterial types with distinctly different cell walls.

The general applicability of our results to flagellate consumers of autotrophic picoplankton is difficult to assess. Heterotrophic nanoflagellates are a diverse assemblage of organisms of different taxonomic groups and feeding strategies (Fenchel 1982). Bodonid flagellates are often considered more typical of benthic than planktonic habitats, although *Bodo* species are reported from both habitats (Zhukov 1991). We used *B. saltans* because it is a well-defined species, easily cultured, and covered abundantly in the literature. Data on the taxonomic composition of heterotrophic nanoflagellates are rare. However, bodonid flagellates may be a common and important component of the community of heterotrophic nanoflagellates. For example, bodonid nanoflagellates represented about 11% of total numbers of heterotrophic nanoflagellates in a recent study of a large reservoir (Simek et al. 1997*a*).

To our knowledge, no comparable data on digestion rates exist for other flagellate species. *Bodo* sp., however, do not appear unusual compared to other heterotrophic nanoflagellates. When feeding on heterotrophic bacteria, *Bodo* are about average in terms of maximum growth rates and average clearance rates (Eccleston-Parry and Leadbeater 1994). The feeding behavior of *Bodo* and bodonids is as complex as that described for other flagellates. For example, chemokinetic responses have been reported for *Bodo* (Mitchell et al. 1988). Selection of bacterial prey on the basis of size has been documented (Chzanowski and Simek 1990; Simek and Chzanowski 1992), as has apparent selection on the basis of cell wall characteristics (Simek et al. 1997*b*). Changes in feeding rates and selectivity with short-term changes in food concentration similar to those seen in the chrysomonad *Spumella* have been described for *B. saltans* (Jürgens and DeMott 1995).

We also found evidence of complexity in feeding behavior. Discrimination among prey items was evident and greater in stationary-phase cells relative to exponential-phase cells. FMS were ingested by exponential-growth-phase and mid- and late-stationary-phase *Bodo* when presented alone (Fig. 3). However, *Synechococcus* was ingested at higher rates than FMS, and *Synechococcus* ingestion was insensitive to the presence of FMS. In contrast, FMS, when offered with *Synechococcus*, were ingested at much lower rates by exponential-phase cells. In stationary-phase *Bodo*, uptake of FMS was insignificant in the presence of *Synechococcus*. It should be recalled that in models of direct-contact–feeding flagellates, ingestion of larger particles (in this case, FMS) would be expected to be higher than the smaller particles present in the same concentration (Monger and Landry 1992).

A prolonged period of food limitation, which stationary-phase cells experience, could have increased selectivity. Such a conclusion complements the findings of Jürgens and DeMott (1995), who, using initially food-limited *Bodo*, documented short-term changes in selectivity relative to ambient food concentrations. They found that selection by *Bodo* against FMS was greater in the presence of bacteria.

Some support for the general applicability of the digestive pattern of *Bodo* can be found in the results of our experiment with marine nanoflagellates. The mixed assemblage of flagellates, digesting natural m-Syn, showed the same exponential decline in average cell contents as observed for *Bodo* and a surprising resemblance in rate constants (Table 1). Overall, we believe that the data presented here indicate that, with further studies on digestion in other representative flagellates, food vacuole content can be used to provide ingestion estimates in field populations. Ingestion could poten-

tially be determined for any prey detectable by microscopy or flow cytometry. More importantly, ingestion estimates could be made on very fine temporal and spatial scales, which are generally those of interest and relevance in microbial food webs.

John R. Dolan

Marine Microbial Ecology Group
CNRS URA 2077
Station Zoologique, B. P. 28
F-06230 Villefranche-Sur-Mer, France

Karel Šimek

Hydrobiological Institute of the Academy of Sciences of the Czech Republic
Na sádkách 7
CZ-37005 České Budějovice, Czech Republic

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Annual average abundance of heterotrophic bacteria and *Synechococcus* in surface ocean waters

Abstract—Global abundance of marine bacteria was investigated at the annual climatological scale. In surface waters of diverse marine habitats, the annual average abundances of heterotrophic bacteria and the photosynthetic cyanobacterium *Synechococcus* are directly related to annual average temperature below 14°C. Notably, average nitrate concentrations at the surface are never high where the temperature is above 14°C. These results suggest that, over the course of a year, temperature is the dominant factor affecting bacterial growth and loss in colder waters. Other factors, such as substrate supply, may be important in warmer waters.

Heterotrophic bacteria and the photosynthetic cyanobacterium *Synechococcus* are found almost everywhere in the upper ocean. In temperate waters, the annual cycle of their abundance may be quite regular. Generally, both groups are most abundant in summer and least so in winter. More particularly, cell abundance at individual locations can evidently track water temperature throughout the year. A broader issue, however, is whether temperature exerts a significant influence on cell abundance across a biogeographical range. In other words, is there a relationship between climatological averages of abundance and temperature in diverse marine habitats? If so, the large-scale distribution of these cells could conceivably be mapped by temperature. I addressed this question by compiling annual average abundances of bacteria and *Synechococcus* from seasonal cycles with wide geographic coverage reported in the literature, combined with new observations made in Bedford Basin, Canada.

My aim was to establish the annual average abundances of heterotrophic bacteria and *Synechococcus*, together with annual average temperature, from as many locations as possible using the published literature. For this purpose, the ideal data sets were multiyear records of frequently sampled sea surface temperature and cell abundance. Some data sets extended to slightly less than a full year; in these cases, I assumed that the annual cycle was symmetrical about the peak at mid-year. Studies in freshwater or hypereutrophic systems and those in which bacteria were assessed as colony-forming units were not included.

Where authors did not directly report average values from their time series data, they were computed as follows: Published figures of seasonal cycles were image-scanned and stored as bitmap files. Images were analyzed by Scion ImagePC software to compute annual areas representing the number of degree-days for temperature and the number of cells per milliliter-day for abundance. Annual averages were calculated by dividing the annual areas by 365 d. Where abundance was expressed in logarithmic units, the data were digitized, expressed as antilogarithms, and then replotted to calculate arithmetic averages. Where the results were presented as three-dimensional surface plots (i.e., abundance versus time versus depth), the data could not be extracted; such studies were left out of consideration. Data from the U.S. Joint Global Ocean Flux Study (JGOFS) were downloaded from the agency's World Wide Web site (http://usjgofs.whoi.edu).

Where temperatures were not reported, annual averages were taken from one of three sources: (1) a database from the Bedford Institute of Oceanography (http://dfomr.dfo.ca/science/ocean/ocean_data.html#oceansst); (2) the *World Ocean Atlas* (U.S. Department of Commerce et al. 1994) maintained by the International Research Institute for Climate Prediction and the Lamont-Doherty Earth Observatory at Columbia University (http://ingrid.ldgo.columbia.edu/sources/.levitus94/); and (3) other published papers describing areas such as Narragansett Bay (Karentz and Smayda 1984) and Villefranche Bay (Buecher et al. 1997).