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Growth Phase and Elemental Stoichiometry of Bacterial Prey Influences Ciliate Grazing Selectivity

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ABSTRACT. Protozoa are known to selectively graze bacteria and can differentiate prey based on size and viability, but less is known about the effects of prey cellular composition on predator selectivity. We measured the effect of growth phase and elemental stoichiometry of *Escherichia coli* on grazing by two ciliates, *Euplotes vannus* and *Cyclidium glaucoma*. Bacterial cells of a single strain were transformed with green and red fluorescent protein and harvested from culture at differing growth stages. Cells in exponential growth phase had low carbon:phosphorus (39) and nitrogen:phosphorus (9) ratios, while cells from stationary phase had high carbon:phosphorus of 104 and nitrogen:phosphorus of 26. When offered an equal mixture of both types of bacteria, *Cyclidium* grazed stationary phase, high carbon:phosphorus, high nitrogen:phosphorus cells to 22% of initial abundance within 135 min, while *Euplotes* reduced these cells to 33%. Neither ciliate species decreased the abundance of the exponential phase cells, lower carbon:phosphorus and nitrogen:phosphorus, relative to control treatments. Because protozoa have higher nitrogen:phosphorus and carbon:phosphorus ratios than their prokaryotic prey, this study raises the possibility that it may be advantageous for protozoa to preferentially consume more slowly growing bacteria.

Key Words. Bacteria growth phase, microbial loop protozoa grazing, selective grazing.

PROTOZOA play a major ecological role in aquatic environments due to their effectiveness in consuming a wide range of prey size classes and types, which include other protozoa, phytoplankton, and bacteria (Azam et al. 1983; Fenchel 1980a; Porter et al. 1985; Sherr and Sherr 2002; Sherr, Sherr, and Pedros-Alio 1989). Grazing by bacterivorous protozoa is of considerable importance given that protozoa not only constitute a major source of mortality to both heterotrophic and autotrophic bacteria (Cole 1999; Pace 1986), but they are also food for other zooplankton, serving an important link in the microbial web (Kiorboe 1998; Sherr and Sherr 2002, 2007; Stoecker and Capuzzo 1990). Bacterivory has been demonstrated to strongly influence prokaryotic diversity and alter genotypic, phenotypic, and metabolic composition of bacterial communities (Hahn and Hofle 2001; Hahn, Moore, and Hofle 1999; Jurgens and Matz 2002; Matz and Jurgens 2003; Matz et al. 2002b; Simek et al. 1997); viruses also play an important role in bacterial community composition (Fuhrman 1999; Fuhrman and Noble 1995; Herndl et al. 2005). While we do not address viruses in this study, an understanding of the mechanisms that influence grazing by phagotrophic protozoa is important in determining how bacterivory influences microbial food webs.

Grazing is most often quantified by measuring changes in bacterial abundance over time (Enzinger and Cooper 1976; McCambridge and McMeekin 1980) or by monitoring grazing rates on bacterial analogs, mainly fluorescent microspheres or fluorescently labeled bacteria (Borsheim 1984; McManus and Fuhrman 1988; Sherr, Sherr, and Fallon 1987). The use of bacterial analogs in microbial grazing studies has multiple limitations. Fluorescently labeled bacteria are often non-viable cells that have been chemically modified by the staining process in ways that have unknown effects on their detection by protozoa and their palatability as prey. Also, microspheres are commonly composed of plastic microbeads with no food value to consumers. This could be problematic as predators have been reported to discriminate between motile vs. non-motile (Gonzalez, Sherr, and Sherr 1993) and live vs. dead prey (Landry et al. 1991). Molecular approaches, such as length heterogeneity analysis by PCR (Suzuki 1999), have also been used to assess protistan bacterivory of filtered and non-filtered seawater samples.

There are multiple factors that can influence grazing. Growth rates of both ciliates (Taylor and Berger 1976) and flagellates (Sherr, Sherr, and Berman 1983) have been demonstrated to vary depending on the species of bacteria grazed. Different bacterial species are grazed at varying efficiencies (Mitchell, Baker, and Sleigh 1988). In addition, protozoa actively select bacteria based on various prey attributes, such as cell size and cell surface properties (Gurijala and Alexander 1990; Monger, Landry, and Brown 1999; Sanders 1988; Tso and Taghon 1999).

The physical and chemical properties of prey and their role in selective grazing have been extensively studied in phytoplankton and metazoan zooplankton (DeMott 1995), but less so in bacteria and protozoa (Matz, Deines, and Jurgens 2002a). Protozoan selective feeding appears to be an important mechanism determining community structure of planktonic food webs and influencing rates of organic matter remineralization and nutrient cycling. The purpose of this study was to examine if bacterivorous protozoa selectively graze prey based on the growth phase of bacterial cells within a population containing a single species of bacteria. We also measured C:N:P stoichiometry, cell dimensions, and protein content of the bacteria to determine what attributes may cause selection, so that inferences can be made regarding the nutritional value of bacteria at different growth phases. To accomplish this, we utilized bacterial prey that were labeled with either red fluorescent protein (RFP) or green fluorescent protein (GFP), to differentiate between growth phases. The use of GFP to label bacteria in grazing studies has been shown to be a successful method to measure bacterial grazing without using an external stain (Ishii et al. 2002), and this is the first study to employ several colored fluorescent proteins to examine selective protozoa grazing.

MATERIALS AND METHODS

Bacteria containing plasmids for RFP and GFP. A single *Escherichia coli* strain was transformed with a gene for RFP. This fluorescent protein was originally cloned from the corallimorph *Discosoma* sp. (Matz et al. 1999). The RFP coding sequence from pDsRed (Clontech, Palo Alto, CA) was excised by PvuII/StuI digestion (positions 55 to 1,041), digested with alkaline phosphatase, and the resulting 986-bp fragment was inserted into vector

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pCR4Blunt-TOPO (Invitrogen, Carlsbad, CA). Another *E. coli* clone was also transformed using the same vector, but containing a gene for GFP, instead of RFP. The GFP (Gp2) was cloned from the scleractinian coral, *Montastraea cavernosa*, as described by Kao et al (2007). To clone GFP from *M. cavernosa*, a conserved 5′-primer was designed corresponding to a region of the mRNA that included the start codon (5′-CTTACTTACGTCTACCATCATGAGTGTG-3′), and a 3′ primer that included the stop codon (5′-TTGGCTTTTCGTTAAGCCTTTACTTGGCC-3′). The corresponding PCR product (713 bp) was also cloned into pCR4-Blunt-TOPO.

Grazing experiments. Cultures of *E. coli*-RFP and *E. coli*-GFP were grown in sterile prefiltered Luria–Bertani medium with 10 μg/ml kanamycin. All cultures were grown at 37 °C (the optimal folding temperature for dsRED) on a rotary shaker at 50 rpm. We harvested cultures at different times because preliminary data showed cells in exponential growth phase have a lower C:N:P ratio than cells in stationary phase. We conducted two series of grazing experiments. In the first, *E. coli*-GFP was harvested after 8 h and *E. coli*-RFP after 72 h. In the second experiment, both *E. coli*-GFP and *E. coli*-RFP were harvested after 72 h. Cultures were filtered through a 5-μm mesh to remove large particulates and diluted using sterile, filtered seawater (salinity 22) to a concentration of 10^7 cells/ml according to direct cell counts. Equal volumes of *E. coli*-RFP and *E. coli*-GFP preparations were combined to make a mixed prey suspension.

Two species of ciliates were used as predators. *Euplotes vannus*, a large benthic hypotrich ciliate (82 μm long, 47 μm wide, 26 μm high), was obtained from the Culture Collection of Algae and Protozoa (Dunstaffnage Marine Laboratory, Oban, Argyll, UK) and *Cyclidium glaucoma*, a small planktonic scuticociliate (26 μm long, 10 μm wide, 10 μm high), was isolated from a New Jersey salt marsh. Both ciliates were maintained on natural populations of bacteria in seawater medium at salinity of 22. Before experiments, ciliates were harvested by passing cultures through plastic screens (105 μm mesh for *Euplotes* and 21 μm for *Cyclidium*) to remove larger particulates, then subsequently gravity drained twice onto polycarbonate filters (10 μm pore size for *Euplotes*, 5 μm pore size for *Cyclidium*), making the final preparations nearly axenic. Ciliates were kept in filtered seawater for 3.5 h to clear food vacuoles. In the first experiment, the abundances of *Euplotes* and *Cyclidium* were $\sim$ 2,000 and 1,000 ml^{-1}, respectively. In the second experiment, *Euplotes* abundance was $\sim$ 3,000 ml^{-1} and *Cyclidium* at 2,000 ml^{-1}.

Separate grazing experiments with each predator were conducted in 50-ml-capacity flasks (not shaken, kept at 20 °C) by combining 8 ml of a ciliate preparation and 2 ml of the mixed bacterial prey preparation. A control treatment without protozoa was included to account for non-predatory changes in bacterial abundance and a control treatment with just protozoa, to ensure there was no mortality throughout the duration of the experiment. The main objective of this experiment was to get a robust statistical analysis of grazing selection, with an emphasis on the difference in consumption between two differing prey pools within a single species population. Therefore, each treatment was replicated 10 times, and samples for abundances of bacteria and ciliates were taken at 0 and 135 min and immediately fixed with glutaraldyhyde (1% [v/v] final concentration). For bacterial counts, samples were appropriately diluted and filtered onto 0.22 μm black Millipore membrane filters. Cell concentration was determined from averaging counts from 10 grids per sample with epifluorescence microscopy using an Olympus BH-2 microscope (Center Valley, PA) (100 W mercury lamp, BP490 excitation filter/17AFC+170,515 barrier filter). The abundance of ciliates was determined from direct counts in a Sedgewick Rafter counting cell (Graticules Ltd., Tonbridge, Kent, UK).

Measurement of bacterial attributes

Elemental composition of bacteria. Samples of each prey type were analyzed for carbon, nitrogen, and phosphorus. Samples were filtered onto precombusted 25-mm-diam. Whatman GF/F filters. The filters were dried and analyzed for carbon and nitrogen using a Carlo Erba Instruments NA 1500 series 2 elemental analyzer (Mortlock, and Froelich 1989). Separate samples were filtered and analyzed for phosphorus (Solórzano and Sharp 1980). Results for C, N, and P were corrected based on filters processed from sterile controls.

Cell dimensions. We measured the length and width of the bacteria for size comparison between strains at each growth stage. Samples of each prey type were taken and preserved with 1% (v/v) glutaraldehyde. Sub-samples were then diluted and filtered onto a 0.22 μm polycarbonate filter, rinsed, and air dried. Images were captured at 10,000X with a JEM-100CXII electron microscope (JEOL, Tokyo, Japan). An image measurement tool (Adobe Photoshop CS4) was used to measure cell dimensions. Cell dimensions were used to calculate cell volume.

Protein content. The protein content of each prey type, as a measure of nutritional value, was determined at each growth phase. One-milliliter samples of each replicate were transferred to microcentrifuge tubes, and spun at 11,905 *g* for 10 min. Supernatant was removed with a pipette and the samples were washed once with phosphate buffer solution, and subsequently centrifuged again. After removing the supernatant, the samples were dissolved with 1 ml of 1 M sodium hydroxide and stored at 4 °C until processing. A second sample of each replicate was fixed with 1% (v/v) glutaraldehyde to assess bacterial abundance. Abundance was determined by fluorescence microscopy. Total protein was assayed using a BCA protein assay reagent kit (Pierce Chemical Company, Rockford, IL), and the BCA microassay protocol (Walker 1994). These values were used to calculate protein content per cell.

Statistical analyses. Selective feeding was evaluated by two-sample *t*-tests of the change in prey abundance in the control vs. predator treatment (Peterson and Renaud 1989). When treatments had the same variances a pooled *t*-test was used, while the Satterthwaite *t*-test was used if treatments had unequal variances (Snedecor and Cochran 1980). Significance between bacterial attributes was tested using ANOVA (α—0.05). Statistical analyses were performed using Statistix v9 (Analytical Software, Tallahassee, FL).

RESULTS

The time of the experiments, 135 min, was chosen as it did not allow enough time to increase the ciliate population size, because it was well below the doubling time of both *Euplotes* and *Cyclidium* at these prey densities. Yet, the timeframe was ample for the grazers to make a significant impact on bacteria abundance (data not shown).

In the experiment where *E. coli*-GFP was harvested after 8 h and *E. coli*-RFP after 72 h, the C:N:P (mean ± 95% confidence level) of *E. coli*-GFP cells was 39 (± 10):9 (± 3):1 (± 0.1), significantly lower than that of *E. coli*-RFP cells, 104 (± 9.8): 26 (± 2.5):1 (± 0.3). Average initial abundance (± 95% confidence level) was 1.24E+06 (± 6.4E+04) ml^{-1} for *E. coli*-GFP and 1.11E+06 (± 6.5E+04) ml^{-1} for *E. coli*-RFP. After 135 min there was no significant difference between the change in abundance of *E. coli*-GFP in the control treatment and in the *Cyclidium* treatment ($t_{17\,df} = 0.07$, $P = 0.94$) or in the *Euplotes* treatment ($t_{16} = -0.01$, $P = 0.99$) (Fig. 1). The abundance of *E. coli*-RFP declined significantly in the *Cyclidium* treatment ($t_{11.7} = 5.81$, $P = 0.0001$), to 22% of initial abundance, while *E. coli*-RFP also

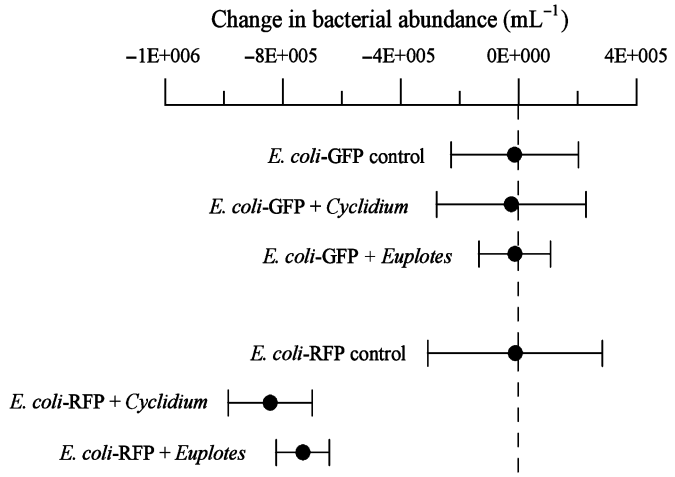

Fig. **1.** Change in bacterial abundance in the grazing experiment where *Escherichia coli*-GFP was harvested after 8 h and *E. coli*-RFP after 72 h. Symbols are the average for each treatment, error bars are 95% confidence levels. Vertical dashed line denotes no change in abundance. GFP, green fluorescent protein; RFP, red fluorescent protein.

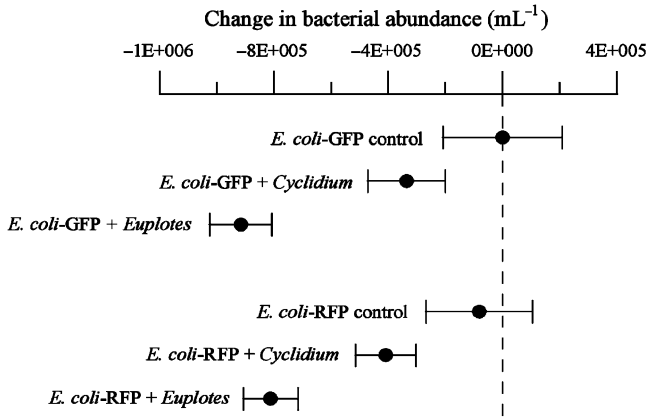

Fig. **2.** Change in bacterial abundance in the grazing experiment where both *Escherichia coli*-GFP and *E. coli*-RFP were harvested after 72 h. Symbols are the average for each treatment, error bars are 95% confidence levels. Vertical dashed line denotes no change in abundance. GFP, green fluorescent protein; RFP, red fluorescent protein.

declined significantly in the *Euplotes* treatment ($t_{9.5} = 5.36$, $P = 0.0004$), to 33% of initial abundance (Table 1).

In the experiment where both *E. coli*-GFP and *E. coli*-RFP were harvested after 72 h, the C:N:P (mean ± 95% confidence level) of *E. coli*-GFP was 114 (± 13):28 (± 3.1):1 (± 0.2), not significantly different than that of *E. coli*-RFP, 104 (± 2.3):25 (± 0.6):1 (± 0.1). Initial abundance was 1.32E+06 (± 6.5E+04) ml^{-1} for *E. coli*-GFP and 1.25E+06 (± 5.7E+04) ml^{-1} for *E. coli*-RFP. The abundance of *E. coli*-GFP declined significantly in the *Cyclidium* treatment ($t_{18} = 3.05$, $P = 0.0068$) to 76% of initial abundance (Fig. 2). The abundance of *E. coli*-GFP also declined significantly in the *Euplotes* treatment ($t_{13.6} = 8.80$, $P < 0.00001$) to 27% of initial abundance. The abundance of *E. coli*-RFP declined significantly in the *Cyclidium* treatment ($t_{18} = 3.46$, $P = 0.0028$) to 68% of initial abundance. The abundance of *E. coli*-RFP also declined significantly in the *Euplotes* treatment ($t_{13.5} = 7.88$, $P < 0.00001$) to 29% of initial abundance.

There were no significant differences in cell length, width, and volume ($P = 0.79$, 0.52, and 0.67, respectively) between prey types at either growth stage (Table 2). Protein content was substantially higher after 8 h of growth compared with 72 h for both prey types (GFP, $P = 0.0001$; RFP, $P = 0.0023$). When comparing prey types, there was no significant difference in protein content between the two at 72 h ($P = 0.51$), while protein was significantly higher for *E. coli*-GFP than for *E. coli*-RFP at 8 h ($P = 0.0097$).

Table **1.** Initial and final abundance of bacteria (× 10^6 cells/ml) for both the grazer, as well as the control treatments in both experiments.

Treatment	*E. coli*-GFP		*E. coli*-RFP	
	Initial	Final 1	Initial	Final 1
E. coli-GFP harvested after 8 h and *E. coli*-RFP after 72 h				
Control	1.26 (0.209)	1.24 (0.156)	1.16 (0.214)	1.15 (0.332)
Cyclidium sp.	1.19 (0.163)	1.16 (0.346)	1.08 (0.173)	0.237 (0.088)
E. vannus	1.28 (0.137)	1.26 (0.116)	1.09 (0.121)	0.361 (0.123)
E. coli-GFP and *E. coli*-RFP harvested after 72 h				
Control	1.29 (0.205)	1.29 (0.169)	1.31 (0.165)	1.23 (0.183)
Cyclidium sp.	1.41 (0.154)	1.08 (0.136)	1.29 (0.185)	0.881 (0.184)
E. vannus	1.25 (0.124)	0.334 (0.065)	1.15 (0.125)	0.336 (0.044)

Data are means for n = 10 (SD).
E. coli, *Escherichia coli*; *E. vannus*, *Euplotes vannus*.

DISCUSSION

It is well documented that bacterivorous protozoa selectively graze based on cell size of bacteria (Gonzalez, Sherr, and Sherr 1990; Posch et al. 2001). While size-based selectivity has received the most attention, ciliates and flagellates use additional criteria to discriminate among prey, such as prey mobility (Gonzalez et al. 1993), cell surface hydrophobicity (Monger et al. 1999), and C:N ratio (John and Davidson 2001; Tso and Taghon 1999). Little research has been done on how growth-phase elemental stoichiometry of prokaryotic prey impacts protozoa grazing selection.

Generally, larger bacterial cells are more rapidly grazed by ciliates (Epstein and Shiaris 1992; Gonzalez et al. 1990; Simek and Chrzanowski 1992). Ciliates have a maximum, minimum, and optimal prey-size range depending solely on their anatomical feeding structure (Fenchel 1980b; Verni and Gualtieri 1997). A higher contact probability for larger particles has been assumed to be a primary mechanism for prey selectivity (Fenchel 1987) with morphological limitations of feeding also recognized as an important determinant (Boenigk and Arndt 2000). Sherr, Sherr, and McDaniel (1992) theorized that selective grazing of dividing cells may be an important factor in maintaining taxonomic and metabolic diversity within bacterioplankton assemblages. Because the larger-sized bacteria are under greater grazing pressure and the larger cells in the assemblage are mainly cells that are actively growing and dividing (Krambeck and Krambeck 1984), it has been indirectly surmised that protozoa are selectively grazing on the faster growing cells (Gonzalez et al. 1990; Krambeck 1988; Sherr et al. 1992) and are cropping the production rather than the standing stock of suspended bacteria. Cell dimensions were similar for all prey types in the present study. Therefore, size was not considered a selective factor.

The data from our study indicate that prey size and species being equal, protozoa selectively graze the older standing stock (i.e. not actively growing), rather than the faster-growing cells. Removal of non-growing bacterial stock is possibly a mechanism that stimulates activity of the actively growing bacteria by lessening competition for resources, such as space and nutrients, and keeping the population in a state of perpetual youth. This is in

Table **2.** Comparison statistics and mean attribute values (± 95% confidence interval) for *E. coli*-GFP and *E. coli*-RFP after 8 and 72 h of growth.

Attribute	*E. coli*-GFP		*E. coli*-RFP		*P* value
	8 h	72 h	8 h	72 h	
Length (μm)	1.64 (± 0.09)	1.68 (± 0.07)	1.67 (± 0.07)	1.70 (± 0.07)	0.79
Width (μm)	0.76 (± 0.02)	0.78 (± 0.02)	0.77 (± 0.02)	0.77 (± 0.02)	0.52
Volume (μm^3)	0.64 (± 0.05)	0.67 (± 0.04)	0.66 (± 0.05)	0.67 (± 0.04)	0.67
Protein (fg/cell)	0.20 (± 0.05)	0.09 (± 0.01)	0.13 (± 0.02)	0.09 (± 0.01)	**0.0001**[a]
					0.0023[b]
					0.51[c]
					0.0097[d]

There was no significant difference in the cell dimensions (length, width, and volume) of either prey type at any growth stage. Protein was significantly higher at 8 h compared with 72 h for both *E. coli*-GFP[a] and RFP[b]. There was no significant difference in protein between the two prey types at 72 h[c], but *E. coli*-GFP was significantly higher than RFP at 8 h[d]; $n = 60$ for dimension measurements, $n = 10$ for protein measurements.

GFP, green fluorescent protein; RFP, red fluorescent protein; Bold, statistically significant.

agreement with several reports where protist grazing stimulates bacterial activity (Fenchel 1970; Jurgens and Sala 2000; Murase, Noll, and Frenzel 2006).

In the past, it has been difficult to differentiate and determine the growth state of the bacteria that protozoa are grazing upon. But, by using a single clonal species of bacterium, we were able to control for species-specific grazing preferences. In addition, by labeling bacteria cells in varying growth stages with different-colored fluorescent proteins, we were able to measure the grazing impact of ciliates in relation to bacterial growth phase. As the genetically coded fluorescent proteins act as in vivo tracers, this method reduced the risk of altering bacterial cell surface properties, which may occur when external staining techniques are used to make cells fluorescent (Epstein and Rossel 1995).

Originally, we also hypothesized that the protozoa would graze selectively on growing cells because growing bacteria have substantially lower C:P and N:P ratios and are assumed to have a higher nutritional value for protozoan predators. Optimal foraging theory predicts a generally positive selection for food of a higher nutritional value (MacArthur and Pianka 1966). But the results of our study demonstrate that both *Euplotes* and *Cyclidium* showed strong preferential removal of the cells that had been in stationary growth (higher C:P and N:P ratios) rather than the cells in exponential growth.

The lack of grazing on *E. coli*-GFP cells by either ciliate in the first experiment was surprising, and raised the concern that insertion of the plasmids with fluorescent protein genes caused other unknown changes in the cells independent of culture age, that might have affected their susceptibility to grazing. Measurements showed that the actively growing GFP bacteria had a significantly higher protein content compared with that of the stationary phase RFP prey. Therefore, in the second experiment, we used the RFP and GFP prey in which age, stoichiometry, and protein content of both prey types were equivalent, and they were grazed upon equally. This indicates that there were no significant effects of the different plasmids on prey properties that would effect preferential grazing.

Several studies have focused on prey nitrogen content in relation to its palatability as a food item. For example, John and Davidson (2001) show that the predatory microflagellate, *Paraphysomonas vestita*, presented with two phytoplankton species of similar cell size but differing C:N (ranging from 4.8 to 14), preferentially choose the lower C:N (N-replete) prey. Similar results were reported by Jones and Flynn (2005), who showed that prey with lower C:N support higher copepod production, but also that mixed prey diets are necessary for copepods as there are other growth-limiting factors (possibly differences in essential fatty acid or amino acid composition) when fed only one species of diatom. In our first experiment, C:N differed only slightly between the two prey types (4.3 for *E. coli*-GFP and 4.0 for *E. coli*-RFP, a 7% difference), while there was much more variation in C:P (62%) and N:P (65%). This suggests that nitrogen may not have been a selective factor. Protein and C:N measurements would lead us to assume that the bacteria with less nutritional value were being preferentially grazed.

In the absence of size as an influence, various prey-cell surface properties have been shown to effect grazing selection. Sanders (1988) showed that *Cyclidium* ingested microspheres coated in protein differently than carboxylated or amide-coated microspheres. The bacterial outer membrane is composed of phospholipids, proteins, and lipopolysaccharides (Prescott, Harley, and Klein 2002), and variations in stoichiometry within species may be a reflection of the different elemental allocation to these surface compounds under differing growth states.

Cell surface properties, such as hydrophobicity, have also been suggested to effect prey selection by phagotrophic protists (Jurgens and Gude 1994) due to effects seen during phagocytosis in the mammalian immune system (Ofek et al. 1995). Results from studies investigating the role of hydrophobicity in selective feeding have yielded inconsistent results, showing slightly positive (Monger et al. 1999) to no correlation (Matz and Jurgens 2001; Matz et al. 2002b). We did not detect cell surface hydrophobicity for both prey types used in this study using the traditional bacterial adherence to hydrocarbon test (Rosenberg 2006) (data not shown). For this reason, we assumed that hydrophobicity did not play a role. It is also probable that bacterial morphology and cell-surface attributes that constitute a selective factor for phagotrophic protists are species specific.

In most studies examining selective feeding and its implications for the elemental stoichiometry of prey, both prey and predator are eukaryotes. It is well established that prokaryotes have lower C:N, N:P, and C:P ratios than eukaryotes (Fagerbakke, Heldal, and Norland 1996; Sterner and Elser 2002). Therefore, it would be plausible for protozoa to select prey that match more closely their own cellular stoichiometry, such as the cells in our experiments in stationary phase, with higher C:P and N:P ratios. By doing so, protozoa could lower the metabolic costs of processing and eliminating ''extra'' nitrogen and phosphorus in their prey. It has been shown that flagellates regenerate more phosphorus when fed bacterial with low C:P (Nakano 1994) and when grazed by faster-growing flagellate species (Eccleston-Parry and Leadbeater 1995).

While our experiments were not designed to identify possible chemical cues that might influence selective feeding by the ciliates, it is likely that chemoreception plays a role. Evidence of considerable flexibility and complexity in chemoreceptive feeding

(Verity 1991) suggests that we have only glimpsed the more detailed features of protozoan feeding behaviors. For example, Sakaguchi, Murakami, and Suzaki (2001) demonstrates that glycoprotein produced by the heliozoan, *Actinophrys sol*, acts as an adhesive substance to immobilize flagellate prey. When this glycoprotein was coated to agarose beads, the beads adhered to flagellates and induced phagocytosis by *Actinophrys*. Chemically mediated grazing inhibition via metabolites produced by bacteria is also not well characterized. Matz et al. (2004) found that bacteria producing violacein had a toxic effect that reduced grazing. Perhaps the actively growing bacteria in the current study were producing a similar substance, while the stationary-phase prey pool did not. It is highly likely that the molecular means of prey selection is highly complex and relies on many additional factors that are still yet to be determined.

Overall, our results indicate that the effects of growth stage of the prey are substantial for the initial step of prey selection. This study was an experimental model in the strictest sense: *E. coli* were used as the model prey, and it must be understood that this species is representative of a narrow range of bacteria that are adapted to laboratory culturing. Also, *Euplotes* and *Cyclidium* are primarily associated with benthic and pelagic environments, respectively, and are not indicative of phagotrophic protozoa from all habitat types. However, these species are well characterized and are known to feed on both surface-associated bacteria, as well as suspended bacteria (Albright et al. 1987; Tso and Taghon 1999). Here we demonstrate that protozoa differentially ingest bacteria of varying attributes within a single-species prey population. The growth phase of bacteria is reflected in the cellular stoichiometric composition of each bacteria being consumed by bacterivorous protists. This selectivity of protozoa has implications for how organic matter assimilated in bacterial production might be regenerated, or otherwise cycled through the microbial food chain. As this study was performed using only one species of bacteria, experiments such as this should be performed using different bacterial and protist species to determine if this is a broad phenomenon.

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