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The model high molecular weight DOC compound, dextran, is ingested by the benthic ciliate *Uronema marinum* but does not supplement ciliate growth

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ABSTRACT: Phagotrophic ciliates are capable of growth solely on dissolved compounds in laboratory cultures. Whether ciliates use dissolved compounds in the environment for growth is unclear. We investigated the ability of the marine benthic ciliate *Uronema marinum* to ingest a model high molecular weight dissolved organic carbon (HMW-DOC) compound, dextran, at concentrations typical for coastal salt marsh sediments (3 µM to 3 mM C). Ingestion was measured by incubating ciliates with fluorescein-labeled dextran (2000 kDa) and measuring the fluorescence signal of the labeled compound in cells via flow cytometry. Ciliates accumulated dextran (relative to formalin-killed controls) at concentrations as low as 0.1 mg l^{-1} dextran (3 µM C). Labeled dextran accumulated in food vacuoles and near the buccal cavity; thus, the ingestion of dextran appears to be a consequence of feeding activities rather than transport across the cell membrane via parasomal sac formation. Dextran accumulation did not increase with higher bacterial ingestion rates. Instead, dextran accumulation was greatest at intermediate bacterial concentrations and grazing rates. Ciliate growth rates were measured in treatments amended with model carbon compounds—soluble starch, acetate, and glucose (3 mM C, final concentrations). There was no significant increase in ciliate growth rates with these compounds in either bacteria-free or bacteria-enriched treatments. Rather, growth rates were significantly lower in treatments with DOC addition, indicating that processing of these DOC compounds may incur some energetic cost to these ciliates.

KEY WORDS: Ciliate · Microbenthos · Cell metabolism · Benthic food web · Carbohydrates · Pinocytosis

INTRODUCTION

Phagotrophic protists rely upon the ingestion of organic particulates (e.g. bacteria, algae) to satisfy their cellular metabolic requirements. However, some protists are capable of growth in axenic media through ingestion of dissolved materials (Soldo & Van Wagtendonk 1969, Hanna & Lilly 1974, Orias et al. 2000). Dissolved compounds can enhance growth of natural populations of bacterivorous protists, even among bacteria competing for these compounds. For example, planktonic estuarine flagellates exhibited increased growth when incubated with high molecular weight dissolved organic carbon (HMW-DOC) in the form of dextran, a linear carbohydrate composed of dextrose subunits with molecular weight approximately 2000 kDa (Sherr 1988). This result, along with another study (Tranvik et al. 1993), indicates that HMW-DOC supplements the energy obtained by flagellates via bacterivory, resulting in increased flagellate growth.

Although ciliates are capable of growth solely on dissolved compounds in cultures (Hanna & Lilly 1970, Thiele et al. 1980), the importance of DOC to metabolic requirements of natural populations of ciliates is not clear. Ciliates in carbon-rich environments, such as the rumen of cattle, use soluble carbohydrates but must also ingest bacteria for growth (Gutiérrez & Hungate 1957). Pelagic choreotrichous ciliates do not appear to

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directly assimilate fluorescently-labeled macromolecules (Tranvik et al. 1993). Protists adapted to high concentrations of humic substances appear more likely to ingest HMW-DOC (Legrand & Carlsson 1998). Thus ciliates adapted to high concentrations of HMW-DOC, as found in coastal sediments, may more readily ingest dissolved compounds to supplement energy obtained through bacterivory.

In shallow water marine sediments, HMW-DOC is released from the breakdown of plant matter and the production of exopolymeric substances by benthic microalgae (e.g. Wheeler 1976, Smith & Underwood 1998). Bacteria must hydrolyze large macromolecules extracellularly and, in some cases, the absence of specific hydrolytic enzymes can hinder organic matter decomposition (Arnosti 2004). The breakdown of HMW-DOC in ciliate food vacuoles, whether deliberate or fortuitous, would not require additional hydrolytic enzymes (and energy expenditures), allowing ciliates to effectively compete with bacteria for HMW-DOC. Indeed, DOC has been suggested as an energy source for benthic ciliates when the rate of particle ingestion appears lower than required for cell metabolism and growth (Epstein 1997). Ciliates are one of the most abundant groups of benthic bacterivorous protists (Fenchel 1967, Dietrich & Arndt 2000) and their role in processing sediment DOC could be significant.

Uronema marinum is a ubiquitous bacterivorous ciliate found in salt marsh surface sediments (First & Hollibaugh 2008). We used *U. marinum* as a model ciliate to test the hypotheses that (1) bacterivorous ciliates are capable of direct ingestion of HMW-DOC at concentrations typical of salt marsh sediments, (2) the rate of HMW-DOC accumulation is dependent upon the rate of bacterial grazing (i.e. the amount of particulate material ingested), and (3) DOC ingestion can increase the growth rate of *U. marinum*. The primary route in which DOC enters the food web is through bacterial production; bacterial production supports the growth of bacterivorous protists (Azam et al. 1983). Understanding alternative routes of carbon flow will provide insight into food web efficiency and microbial trophodynamics in marine sediments.

MATERIALS AND METHODS

Culture establishment. *Uronema marinum* was isolated from sandy subtidal sediments on Sapelo Island, Georgia, USA (31.389° N, 81.264° W), in November 2005. Proteose peptone (PP, 1 g l^{-1}) was added to the sediment to stimulate bacterial growth, which led to a bloom of scuticociliates. A small volume (0.5 ml) of the ciliate cultures with the natural bacterial assemblage was transferred into 30 ml of particle-free (0.22 µm filtered) 30 ppt artificial seawater (ASW; McLachlan 1964) enriched with 1 g l^{-1} of PP. Fresh cultures were started every 1 to 3 d using this procedure. The concentration of ciliates in culture was monitored by counting formalin-preserved cells using a hemocytometer. Ciliate concentrations stabilized after 1 to 2 d at approximately 10^5 cells ml^{-1}, at which point the population was considered to be in stationary growth. Ciliate cultures were incubated at 22 ± 1°C in tissue culture flasks.

Microscopic analysis was performed to verify that the cultures were composed of a single protist. Live cultures were observed using light microscopy (differential interference contrast, 1000×). Cultures were also observed using epifluorescence microscopy. Briefly, the culture was preserved with formalin (2% final concentration), stained with the DNA label DAPI, and collected on a 0.22 µm black polycarbonate filter. These filters were viewed on a Lecia DMX RA microscope at 1000× using the standard light filter set for DAPI fluorescence. Only one ciliate morphotype was identified in the cultures.

The ciliate was identified based on cell morphology. Ciliate morphology was observed by Protargol staining, which was performed following standard protocols (Skibbe 1994). Protargol staining allows for the visualization of key morphological features, such as the distribution patterns of cilia on the cell surface and the structure of the oral apparatus. These characteristics were used to identify the ciliate in culture using a taxonomic guide (Carey 1992).

Ciliates used in experiments were incubated for at least 1 d after inoculating new cultures, when the population was at or near stationary growth. The culture media was screened through a 37 µm Nitex mesh to remove aggregates. Ciliates were washed by concentrating the media on a 5 µm Nuclepore polycarbonate filter and rinsing with several volumes of ASW. The filter was always covered with >1 cm of water during the rinsing process to avoid drying the filter. The washing process removed carbon-rich media and reduced the standing stock of bacteria to an average concentration of 8.1 ± 0.5 (SE) × 10^5 cells ml^{-1} (determined by flow cytometry, described in 'Grazing and dextran ingestion'). This bacterial concentration is below the grazing threshold for *Uronema* sp. (10^6 cells ml^{-1}; Kujawinski et al. 2000). This washing procedure was performed prior to all experimental manipulations. Ciliates were held for 2 h prior to experimental manipulations to allow for digestion of intracellular bacteria.

Antibiotics (0.1 mg ml^{-1} gentamicin sulfate) were added to prevent further bacterial growth. This antibiotic treatment was effective at preventing the growth of bacteria in the enriched media described above.

Gentamicin was used because it does not penetrate eukaryotic cells (Vaudaux & Waldvogel 1979). We tested whether this antibiotic treatment affects ciliate growth (see 'Effect of antibiotics on ciliates').

Heat-killed bacteria were prepared for DOC ingestion and grazing experiments to assure that bacterial prey were uniform across treatments. Bacteria were isolated from *Uronema marinum* cultures on ASW agar plates (10 g l^{-1} peptone). One colony was suspended in 1 ml seawater and killed by heating to 95°C for 10 min. Bacteria were centrifuged (10 min at 10 000 × *g*) and the supernatant was discarded. Cells were then resuspended in ASW containing antibiotics. This washing procedure was repeated a total of 3 times. Suspended heat-killed cells were then filtered through a 5 µm pore size syringe filter to disperse or remove large aggregates. Microscope observations assured that the cells were well dispersed and intact. Treated bacteria did not grow on agar plates, indicating that this treatment was sufficient to kill bacteria.

Dextran ingestion. Fluorescein isothiocyanate (FITC)-labeled dextran (average molecular weight 2000 kDa, Sigma-Aldrich) was used as an analog for HMW-DOC. The FITC-dextran was prepared in 10X stock solutions and each concentration was syringe-filtered (0.22 µm) immediately before use. The FITC-dextran was added to triplicate *Uronema marinum* cultures at final concentrations ranging from 0.1 to 100 mg l^{-1} (3 µM to 3 mM C). ASW was added to one triplicate set as a negative control. One formalin-killed control (2% final concentration) was run at each concentration to account for the background fluorescence of the media and passive adsorption of dextran on ciliate cell bodies. The samples (1 ml) were incubated for 90 min in the dark at 22°C on a shaker table (~50 rpm). Incubations were stopped by formalin addition (2% final concentration).

Accumulation of FITC-dextran leads to an increase of the fluorescence signal in the ciliate relative to the background fluorescence. The fluorescence of individual ciliates was measured using a flow cytometer (Dako CyAn) with a 488 nm laser excitation. Ciliates were identified by their forward and side angle light scattering signals. The FITC fluorescence emission signals (FL1, 530/30 nm emission filter) for approximately 5000 ciliates were recorded for each treatment replicate and control. Epifluorescence and laser scanning confocal microscopy were used to verify the accumulation of FITC-dextran in ciliate food vacuoles. Cells were imaged at 1000× with a combination of fluorescence emission in the green light spectrum and transmitted light from a 488 nm argon/krypton laser.

The relative rate of HMW-DOC accumulation in *Uronema marinum* was measured by incubating ciliates at 3 concentrations of FITC-dextran. Triplicate ciliate cultures were prepared in 50 ml conical centrifuge tubes as described above and incubated with 1, 10, and 100 mg l^{-1} FITC-dextran for a total of 300 min. Samples (1 ml) were collected from each replicate periodically throughout the incubation period (at 0, 15, 30, 60, 90, 120, 180, 240, and 300 min) to determine the rate of FITC-dextran accumulation. Formalin-killed controls were also run to measure the background fluorescence of the solution. The fluorescent emission signal was recorded for approximately 5000 ciliates as described above, subtracting the mean fluorescence of the killed control. To measure the relative accumulation of dextran over time, the final ciliate fluorescence was normalized to the initial concentration. The relative accumulation (RA) of FITC-dextran was calculated as the mean fluorescence at each time point (T_i) normalized to the mean fluorescence at the initial time point (T_0) according to the formula:

$$RA_{T_i} = FL1_{T_i}/FL1_{T_0} \quad (1)$$

Thus an RA of 1 indicates that there was no change in fluorescence over the incubation period (i.e. no ingestion of dextran). We used this relative measurement because there were large differences in background fluorescence due to the extracellular dextran in the solutions. For example, absolute fluorescence of T_0 treatments of 100 mg l^{-1} dextran was greater than final (T_{300}) fluorescence in 10 mg l^{-1} solutions (See Fig. 1).

Grazing and dextran ingestion. We also tested the hypothesis that DOC accumulation increases concurrently with higher bacterial grazing rates. In theory, higher grazing rates can lead to increased food vacuole formation (Parker 1976). Alternatively, DOC ingestion may become more important when bacterial concentrations are low. Ciliate cultures were prepared as above and the washed culture was transferred into 50 ml conical centrifuge tubes. Two variables (bacteria and dextran concentration) were analyzed at 3 levels: low, medium, and high bacteria concentrations and 0, 10, and 100 mg l^{-1} dextran. Heat-killed bacteria were prepared as described above and added to the cultures to yield approximately 8.1, 15, and 110 × 10^5 cells ml^{-1} for low, medium, and high treatments, respectively (concentrations were determined by flow cytometry). The volume of sample analyzed was determined by weighing the sample before and after analysis (Rose et al. 2004). Treatments were immediately dispensed into 2 ml Eppendorf tubes. The samples were incubated for 90 min as described above. Ciliate and bacteria concentrations and ciliate fluorescence were measured at both initial and final time points by flow cytometry. Additional samples were incubated for 26 h to assess long-term ciliate growth in these treatments as described in 'Starch, glucose, and acetate amendments'. Per capita grazing rates were calculated as

the loss of bacterial cells during the 90 min incubation per the mean ciliate concentration (C_M):

$$C_M = C_{T_0} e^{(\mu \Delta t/2)} \quad (2)$$

where C_{T_0} is the initial ciliate concentration (cells ml^{-1}) (Gallegos et al. 1996), μ is ciliate growth rate, and t is incubation time. Ciliate growth rate was determined by the equation:

$$\mu = \ln(C_{T_f}/C_{T_i})/t \quad (3)$$

where C_{T_f} is the final ciliate concentration at the end of the incubation time (t). Ciliate growth rate (d^{-1}) was calculated for samples incubated for 26 h.

Starch, glucose, and acetate amendments. The role of dissolved carbon compounds commonly found in salt marsh sediments was examined by measuring the growth rates of *Uronema marinum* incubated with starch, glucose, and acetate in both the presence and absence of bacteria. Glucose constitutes over 80% of colloidal monosaccharides in diatom-dominated, intertidal sediments (Taylor et al. 1999). Acetate is an abundant, low molecular weight DOC in intertidal sediments, especially in anoxic zones (Holmer 1996). Soluble starch was used as a representative HMW-DOC compound. Soluble starch, sodium acetate, and D-glucose were dissolved in filter-sterilized ASW; the starch solution was heated (~60°C) to speed dissolution. All solutions were prepared in cleaned and baked (550°C, 6 h) glassware. Solutions were syringe-filtered through a 0.22 μm membrane filter prior to use in the experiment and diluted to yield a final concentration of 3 mM C in all treatments, which is in the range observed in salt marsh surface sediments (Weston et al. 2006).

Flow cytometric sorting was used to completely separate *Uronema marinum* from bacteria in cultures. The ciliates were identified based on forward and side angle light scattering signals on a Dako MoFlo high speed cell sorter. Ciliates were sorted into particle-free ASW and held for 2 h prior to the experiment. The process of cell sorting had no observable effect on the growth rate or activity of *U. marinum*, as verified by microscope analysis of live samples and incubating sorted ciliates with heat-killed bacteria. Sorted ciliates were motile and would reproduce when bacteria were added to the cultures.

Treatments were prepared in autoclaved, 2 ml Eppendorf tubes. Triplicates of each treatment were prepared by adding 100 μl of sorted *Uronema marinum* to 800 μl of starch, acetate, or glucose solutions. Filter-sterilized ASW was used for control treatments. For treatments with bacteria, 100 μl of the heat-killed bacterial suspension was added. Bacteria-free treatments received 100 μl of filter-sterilized ASW with antibiotics. The final antibiotic concentration for all treatments was 0.1 mg ml^{-1} of gentamicin sulfate. Initial (T_0) samples were chemically preserved with 0.22 μm filtered formalin (2% final concentration). Samples were incubated in the dark at 22°C for 26 h, at which point the incubation was stopped by adding formalin. Treatments were stained with DAPI (20 μg ml^{-1}, final concentration) and the concentrations of ciliates and bacteria in each treatment replicate were measured with a Dako CyAn flow cytometer using a 405 nm laser for DAPI excitation (FL6, 450/50 nm emission filter).

In addition to estimating ciliate growth by the change in cell concentration, ciliate growth was also calculated by the change in total biomass. With this approach, the biomass concentration (μg C ml^{-1}) rather than cell concentration is used in Eq. (3). Measuring ciliate biomass is potentially useful in calculating total ciliate carbon concentration, as the cell volume of *Uronema marinum* varies with ciliate growth stage (Parker 1976). Ciliates were preserved with formalin (2% final concentration), incubated with FITC for 10 min to stain the entire cell body, and filtered onto a 0.22 μm black polycarbonate filter. Cells were visualized via epifluorescence microscopy using standard filter sets for FITC fluorescence. The initial and final ciliate biomasses were determined by measuring microscope images of *U. marinum* captured at 400× on a Hamamatsu CCD digital camera. Image dimensions were calibrated using a stage micrometer and verified using 0.66 and 1.0 μm diameter fluorescent beads. The length and width of at least 30 non-dividing ciliates in each treatment were measured with image analysis software (Image Pro Plus 4.1) and used to calculate biovolume of ciliates (Wetzel & Likens 1991). Ciliate biovolume was converted to biomass using a published carbon:volume ratio that accounts for cell shrinkage due to formalin fixation (Putt & Stoecker 1989). The total biomass was the product of mean individual biomass and ciliate concentration.

Effect of antibiotics on ciliates. To verify that the antibiotics used to prevent bacterial growth did not affect ciliates, we compared ciliate growth in treatments with antibiotics (0.1 mg ml^{-1} gentamicin sulfate) versus treatments without antibiotics added. Triplicate treatments consisting of 0.5 ml of washed ciliates were added to 30 ml ASW containing antibiotics. Control treatments consisted of ASW without antibiotics. Heat-killed bacteria were added to all treatments to stimulate ciliate growth. These treatments were incubated for 1 d as described above. Ciliate growth was determined from initial and final cell concentrations using Eq. (3).

Data analysis. ANOVA was used to determine significant differences ($\alpha = 0.05$) in ciliate fluorescence, relative dextran accumulation, and ciliate growth rate. Linear regression analysis was used to determine the strength of the relationship between dextran concen-

tration and either ciliate fluorescence or dextran accumulation. The regression coefficient is shown for significant linear relationships. Measurements reported are the mean values for 3 replicate treatments shown with standard error.

RESULTS

Dextran ingestion

There was no significant difference between ciliate growth in cultures with antibiotics versus cultures without antibiotics, indicating that the antibiotic treatment did not affect the ciliates and was appropriate to use to limit bacterial growth in these experiments. *Uronema marinum* incubated with FITC-labeled dextran were significantly more fluorescent than formalin-killed controls at all concentrations tested (Fig. 1). Negative controls (without dextran addition) exhibited only minor fluorescence, indicating that formalin was not a major source of the detected ciliate fluorescence. Ciliates were able to accumulate dextran in food vacuoles at concentrations as low as 0.1 mg l^{-1}, and the fluorescence signal emitted by ciliates was strongly correlated to the concentration of dextran in solution (r^2 = 0.99). The fluorescence signal was concentrated in structures consistent with food vacuoles in shape, size, and location in the cell (Fig. 2). Examination by epifluorescence, confocal microscopy, and flow cytometry revealed no fluorescent particles or aggregates that could introduce the fluorescence signal into the food vacuole via phagocytosis of particles, indicating that the source of the fluorescence signal was dissolved or colloidal (<0.22 µm) FITC-dextran.

The relative dextran accumulation was highly dependent on dextran concentration (Fig. 3). The slope of the relationship between relative accumulation and

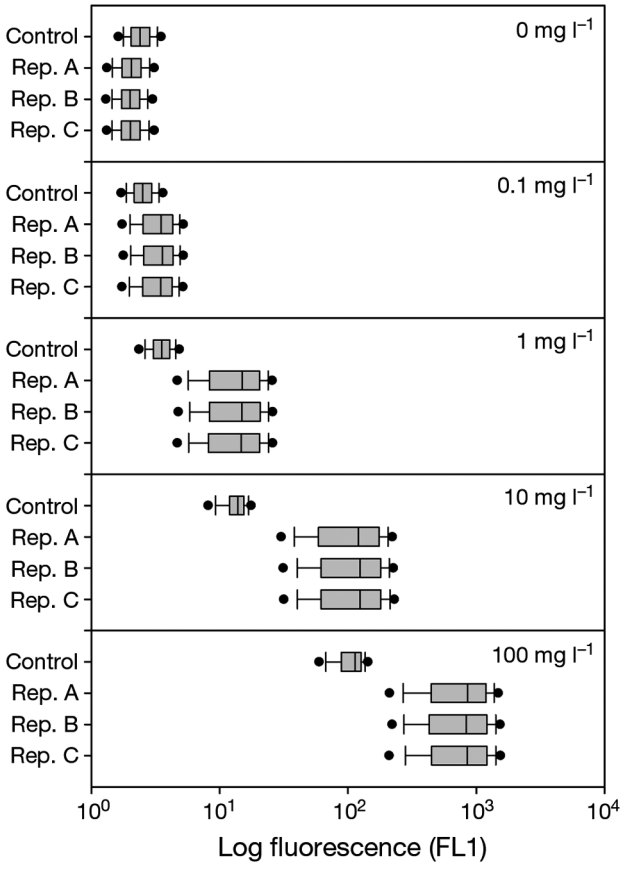

Fig. 1. Flow cytometric fluorescent signal (FL1) measured in ciliates incubated in several concentrations (0.1 to 100 mg l^{-1}) of fluorescein isothiocyanate (FITC)-labeled dextran. The box lines show the 25th, 50th, and 75th percentiles; bars and dots indicate the 10th and 5th percentiles of the data, respectively. Three replicates (Rep.) are shown separately

Fig. 2. *Uronema marinum*. Laser scanning confocal micrograph of representative *U. marinum* showing fluorescence from fluorescein isothiocyanate (FITC)-labeled dextran in food vacuoles located near the buccal cavity and the cell posterior. Scale bar = 10 µm

time was 0.003, 0.009, and 0.029 min^{-1} for 1, 10, and 100 mg l^{-1} FITC-dextran, respectively ($r^2 > 0.9$ in all cases). Ciliate fluorescence was significantly greater than initial background fluorescence after 60 min at all 3 dextran concentrations. The final percent enrichment after the 300 min incubation was 1.7 ± 0.07, 3.9 ± 0.16, and 8.7 ± 0.51% for 1, 10, and 100 mg l^{-1} dextran, respectively. In all 3 dextran concentrations, the rate of change in mean fluorescence decreased after 180 min (Fig. 3).

Dextran accumulation by ciliates was highest at intermediate bacterial concentrations (Fig. 4A) and was significantly greater than accumulation at both low and high bacteria concentrations. There was no significant difference in relative accumulation of dextran between high and low bacteria treatments at either 10 or 100 mg l^{-1} dextran. Ciliate grazing rates increased with bacteria concentration, with the greatest per capita grazing rates occurring in the high bacteria concentration treatments (Fig. 4B). The growth rate of *Uronema marinum* measured over 1 d was greatest in high bacterial treatments (Fig. 5). There was no significant difference in ciliate growth rate between low and medium bacteria treatments, nor was there any significant effect of FITC-dextran on ciliate growth rate at these bacteria concentrations. The highest ciliate growth rate was measured in high bacterial concentrations without dextran addition. This growth rate was significantly greater than in the 10 mg l^{-1} FITC-dextran treatment, but not the 100 mg l^{-1} treatment.

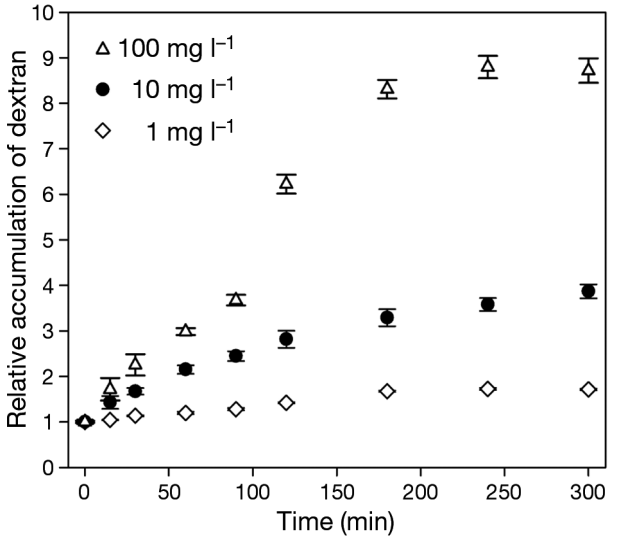

Fig. 3. *Uronema marinum*. Relative dextran accumulation rate in ciliates at 3 fluorescein isothiocyanate (FITC)-labeled dextran concentrations measured over a 300 min incubation period. Relative accumulation is the mean fluorescence at each time point normalized to initial mean fluorescence. Error bars are SE (n = 3)

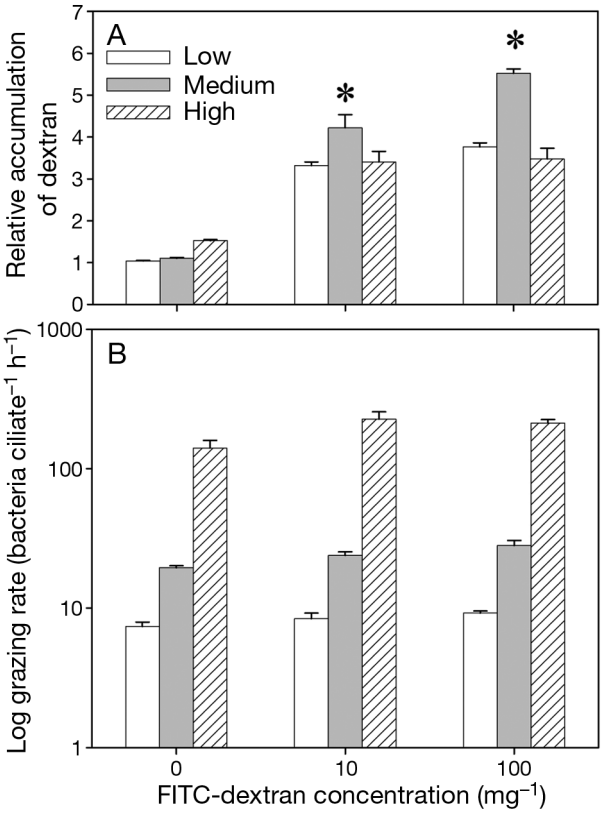

Fig. 4. *Uronema marinum*. (A) Relative dextran accumulation rate in ciliates treated with 0, 10, and 100 mg l^{-1} of fluorescein isothiocyanate (FITC)-labeled dextran and low, medium, and high bacterial concentrations. (B) Per capita ciliate grazing rates in all treatments. Error bars are SE (n = 3). Asterisks indicate a significantly higher dextran accumulation than measured in other treatments (ANOVA, $p < 0.05$)

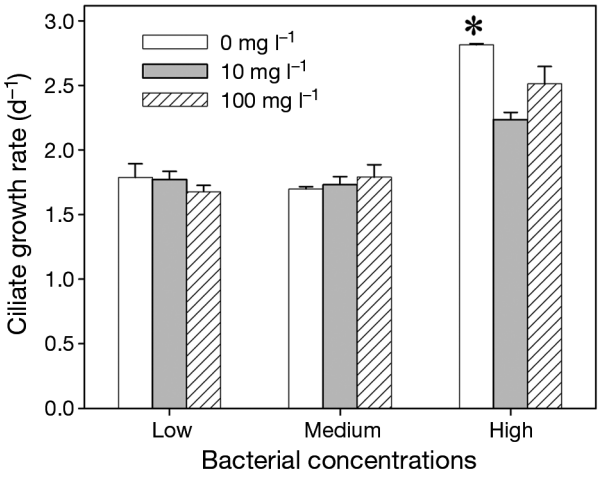

Fig. 5. *Uronema marinum*. Growth rate (d^{-1}) measured as the change in ciliate concentration over a 26 h incubation period. Ciliates were treated with low, medium, and high concentrations of bacteria and 0, 10, and 100 mg l^{-1} of fluorescein isothiocyanate (FITC)-labeled-dextran. Error bars are SE (n = 3). Asterisk indicates a significantly higher ciliate growth rate than measured in 10 mg l^{-1} dextran (ANOVA, $p < 0.05$)

Starch, acetate, and glucose amendments

Ciliate growth rate, based on the change in concentration of cells over the incubation period, did not increase with starch, acetate, or glucose treatments relative to the controls (Fig. 6). Rather, the highest ciliate growth rates were observed in control samples without DOC addition. In bacteria treatments, ciliate growth rates in the control treatments were significantly greater than in DOC treatments. Growth rates based upon changes in biomass were higher than those based only on cell concentrations in most treatments, indicating that the mean cell biomass increased over the incubation period (Fig. 6). However, the biomass growth rates measured in DOC treatments were still not significantly greater than the control treatment.

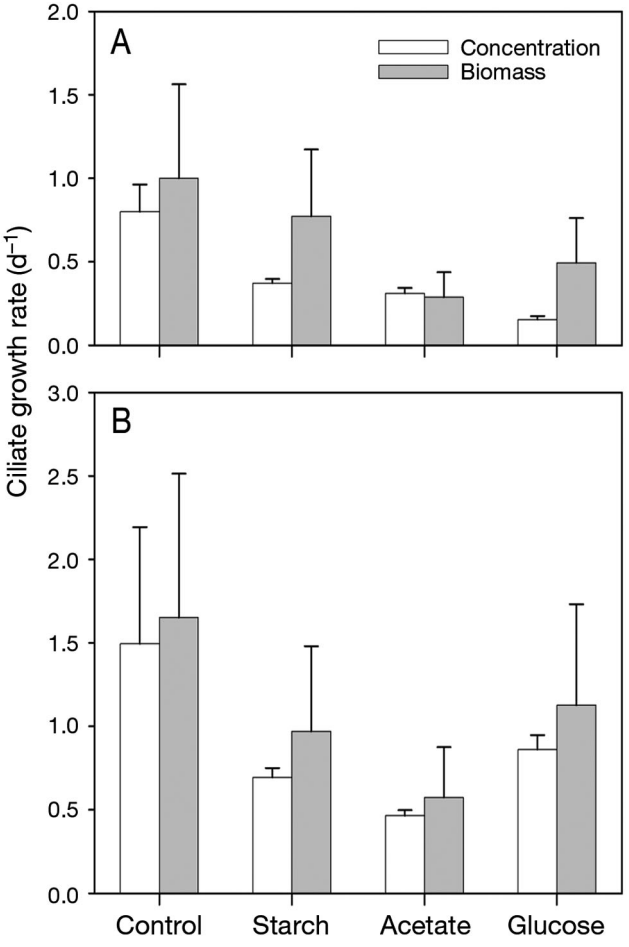

Fig. 6. *Uronema marinum*. Growth rate (d^{-1}) of ciliates determined by changes in cell concentrations (open bars) or ciliate biomass (gray bars). The ciliates were incubated in control (no dissolved organic carbon [DOC] addition), starch, acetate, and glucose treatments, with a final concentration of 3 mM DOC. Growth rates were measured (A) without and (B) with bacteria. Error bars are SE (n = 3)

DISCUSSION

Several ciliates, including *Uronema marinum*, have been cultured under axenic conditions, hence their ability to ingest and metabolize DOC is well established (Soldo & Van Wagtendonk 1969, Hanna & Lilly 1970). We hypothesized that ciliates are capable of ingesting HMW-DOC at concentrations typical of salt marsh sediments and that DOC ingestion could supplement ciliate growth. Phagotrophic protists adapted to high concentrations of HMW-DOC may more readily ingest dissolved compounds (Legrand & Carlsson 1998). Salt marsh sediments have high concentrations of HMW-DOC, such as lignocelluloses that originate from the breakdown of intertidal marsh grasses (Moran & Hodson 1990). Additionally, large quantities of algal-derived exopolymers are released by benthic microalgae in shallow water sediments (Smith & Underwood 1998). Recalcitrant carbon compounds are also produced by bacteria from labile materials, such as sugars and amino acids (Ogawa et al. 2001). Bacterial growth yield on these recalcitrant compounds is typically low (Newell et al. 1983), potentially allowing for competition by ciliates.

Uronema marinum was capable of ingesting dextran, a model HMW carbohydrate. However, DOC in solution (whether in the form of dextran, starch, glucose, or acetate) did not increase ciliate growth. Rather, the processing of these compounds appeared to incur some energetic costs to ciliates, resulting in lower growth rates at high DOC concentrations. This energetic cost may be associated with the hydrolysis of nitrogen-poor carbon compounds. Marine bacteria (the typical prey for *U. marinum*) have a high relative contribution of nitrogen to their total biomass (Fukuda et al. 1998), providing nitrogen necessary for ciliate biomass production. Although DOC consumption does not appear to significantly increase the growth rate of *U. marinum*, other benthic ciliates (including larger taxa with lower particle ingestion rates) may depend upon DOC to supplement metabolic requirements (Epstein 1997).

Dextran accumulated in ciliates within 15 min, but the accumulation of labeled dextran changed little after 180 min. This suggests that the rate of food vacuole formation equals the rate of vacuole egestion after about 3 h. This vacuole passage time is much longer than determined by measuring the accumulation of bacteria in *Uronema* sp. food vacuoles (Sherr et al. 1988). This large difference suggests that ingesting dextran may slow the rate of ciliate food vacuole passage. While prey concentration did not affect the food vacuole passage time for other ciliates (Capriulo & Degnan 1991), it is possible that low bacterial abundance contributed to longer vacuole passage times in dextran accumulation rate experiments.

Because dextran accumulated in food vacuoles, we expected that greater rates of prey ingestion would lead to a higher accumulation of dextran. *Uronema marinum* cells contain more food vacuoles when prey are abundant (Kujawinski et al. 2000) and this should lead to greater dextran accumulation at high prey concentrations. However, high prey concentrations did not correspond to the greater uptake of dextran. One explanation for this unexpected result is that ciliate metabolism may vary depending upon prey concentration, leading to high degradation rates of dextran with few prey or rapid food vacuole passage rates when prey are abundant. However, ciliate digestion and food vacuole cycling times do not vary significantly with prey concentrations (Capriulo & Degnan 1991). If degradation of dextran is not dependent upon prey concentration, the observation that dextran accumulation is greatest at intermediate, rather than low, prey concentrations suggests that DOC ingestion does not contribute to ciliate metabolism when prey are scarce.

The accumulation of dextran in ciliate food vacuoles indicates that HMW-DOC enters the cell via the oral cavity where prey are ingested, and that this is an active process. There was no visible evidence of active transport of dextran across the cell membrane via the formation of parasomal sacs (e.g. Radek & Hausmann 1996). Thus, the mechanism by which dextran is concentrated and accumulated in the cell is different from other dissolved compounds. For example, *Uronema* sp. has been shown to accumulate chlorinated biphenyls through passive diffusion across the cell membrane (Kujawinski et al. 2000). The observation that dextran is ingested in the food vacuole suggests that cell-surface receptors with high affinity for carbohydrates near the feeding apparatus may play an important role in particle capture (Sundermann et al. 1986). It is important to note that although dextran was dissolved and filtered to remove particles >0.22 µm, it is probable that a portion of this dextran existed as colloids (Verdugo et al. 2004). However, even colloids are not expected to be concentrated via suspension feeding. In suspension feeding, particles are sieved as water is passed through the paroral membrane; mechanical filtration is considered to be the major mechanism of particulate concentration (Fenchel 1980b). Some suspension feeding ciliates, such as *Tetrahymena pyriformis*, do not show selectivity of prey based upon cell-surface properties, such as antigen structure (Wildschutte et al. 2004). However, chemical interactions increase the ingestion rates of particulate prey by some bacterivorous flagellates and ciliates (Monger et al. 1999, Wilks & Sleigh 2004). The ability to concentrate and ingest dissolved or colloidal matter, which would not be retained on the paroral membrane (Fenchel 1980a), suggests that molecular attraction may play a role in how *Uronema marinum* captures and ingests food items. HMW-DOC ingestion, although not beneficial to these ciliates, may be a consequence of cell-surface structures functioning to attract and retain bacterial prey.

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