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Rates of benthic bacterivory of marine ciliates as a function of prey concentration

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ABSTRACT

Feeding rates of protozoa on bacteria attached to particles have been determined much less frequently than grazing rates on suspended bacteria, yet in sediments most bacteria are associated with particles. This study measured the ingestion rates of benthic bacterivorous ciliates over a range of attached bacteria concentrations. Using bacteria that were transformed to express green fluorescent protein as a natural tracer, bacteria were cultured in sediment microcosms to concentrations ranging from 10^8 to 10^{10} bacteria per milliliter of pore water (ml^{-1} PW). Ingestion rates of the marine ciliates *Euplotes vannus*, *Euplotes plicatum*, and *Uronema marinum* increased with attached prey density, ranging from 10^2 to 10^5 bacteria $ciliate^{-1}$ h^{-1}. Ingestion rates of attached bacteria were 10–1000 times greater than rates previously reported for benthic ciliates. Ingestion rates at a typical sediment prey concentration (10^9 ml^{-1} PW) were significantly higher for all grazer species when feeding on attached prey versus ingestion rates when grazing on suspended prey at similar concentrations. This, combined with species-specific responses to prey density when grazing attached v. suspended prey, leads to the conclusion that ciliates are capable of grazing a much larger portion of benthic bacterial biomass than has previously been measured.

1. Introduction

The importance of bacterivory in the microbial food web is well established. Protozoan grazing acts as an important top-down control over bacterial stock (Sherr and Sherr, 1994, 2002), has a stimulatory effect on bacterial activity (Barsdate et al., 1974; Caron, 1991; Johannes, 1965), and can alter the diversity of a bacterial community (Jurgens and Matz, 2002; Jurgens et al., 1999; Ronn et al., 2002). Additionally, bacterivory has been shown to accelerate the rates of decay of organic matter in detrital systems (Fenchel, 1970; Fenchel and Jorgensen, 1977; Patterson et al., 1989), where both the quantity of organic matter, as well as microbial abundances, typically occur at much higher concentrations than in the water column. Despite the potential for protozoan grazing to have a large impact on fate of benthic organic matter there remains a surprisingly limited number of studies quantifying the effects.

Early assumptions were that protozoan ingestion rates in a benthic habitat would be higher than in the water column due to the high density of bacteria that colonize particle surfaces (Fenchel, 1975, 1980a). Estimates thus far, however, have either been similar or low compared to measurements in suspension (Fischer et al., 2006; Konigs and Cleven, 2007; Starink et al., 1994). A possible explanation for these low estimates is that much of the research measuring benthic consumption rates has utilized variations of the monodispersed fluorescently labeled bacteria technique (FLB) (Parry et al., 2001; Posch et al., 2001; Shimeta et al., 2001; Wieltschnig et al., 2003), which does not include the consumption of naturally attached prey. This combined with the possible selection against artificial/manipulated food particles (Boenigk et al., 2001; Gonzalez et al., 1993; Landry et al., 1991) could result in underestimated feeding rates of benthic protozoa.

Alternatively, it may be that when protozoa feed on surface-associated particles they do not exhibit a density-dependent functional feeding response. It is well documented that ingestion rates of protozoa grazing in suspension increase with prey concentration (Fenchel, 1980b; Massana et al., 1994; Verity, 1985). We know much less about this dynamic in the benthic environment where protozoan grazing includes feeding on bacteria that are free (suspended within the pore water), as well as those attached to particles. Observations of ciliate feeding patterns while grazing on surfaces show that ciliates do not necessarily forage evenly over surfaces, but may continually feed in distinct patches, even when adjacent areas contain much higher prey densities (Artolozaga et al., 2002; Fenchel and Blackburn, 1999; Huws et al., 2005; Lawrence and Snyder, 1998).

Measuring the functional response of a consumer, or how consumption rate changes with prey concentration, provides an assessment of both feeding behavior and the impact that consumer might have on an ecosystem (i.e. ecosystem stability, prey population control, and biomass turnover) (Chow-Fraser and Sprules, 1992; Holling, 1966; Jurgens and Simek, 2000; Moigis, 2006; Solomon, 1949). The direct

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effects of prey density on rates of protozoan ingestion have not yet been quantified for a sedimentary system. It is inherently difficult to accurately simulate a natural sediment bacterial population that can be readily monitored. Recently, studies have shown that the use of bacteria transformed for expression of fluorescent protein as a natural prey tracer permit good estimates of grazing ability in suspension (Fu et al., 2003; Gruber et al., 2009; Parry et al., 2001).

The purpose of this study was to investigate the effect of prey concentration on protozoan bacterivory in a sedimentary environment. Bacteria expressing green fluorescent protein (GFP) were cultured in benthic microcosms, and the ingestion rates of three species of marine ciliates were measured as a function of sediment-attached prey concentration. Two separate suspended-prey ingestion rate trials were also performed at various prey concentrations. The first was to determine if the ingestion rates of the grazers might vary with feeding duration, and the second to act as a direct comparison to those when grazing attached prey.

2. Methods

2.1. Bacteria

A single strain of bacteria, *Vibrio* sp.-NAP (Tso and Taghon, 1999), was maintained in culture on a rotary shaker (20 °C) in a modified Zobell's media (0.2 μm filtered, aged seawater at a salinity of 23, 2.5 g l^{-1} soy peptone, 0.5 g l^{-1} yeast extract). A sub-set of *Vibrio* sp.-NAP was transformed for the expression of green fluorescent protein (GFP), and will hereafter be referred to as GFP-*Vibrio*. Briefly, a QLAprep DNA miniprep (Quiagen®) was performed to extract the plasmid DNA from a strain of *Escherichia coli* containing the GFP gene (Gruber et al., 2009). The plasmid was inserted into the *Vibrio* strain using standard calcium chloride competency techniques and cell transformation protocols (Sarkar et al., 2002). This resulted in the constitutive expression of GFP. GFP-*Vibrio* cultures were maintained as *Vibrio* sp.-NAP were, with the addition of kanamycin (0.1 g l^{-1}).

2.2. Ciliates

Two species of hypotrich ciliates, *Euplotes vannus* (length = 100 ± 11 μm, width = 57 ± 8 μm) and *Euplotes plicatum* (length = 51 ± 4 μm, width = 32 ± 4 μm), were acquired from the Culture Collection of Algae and Protozoa (CCAP, Dunstaffnage Marine Laboratory, UK). A species of scuticociliate, identified as *Uronema marinum* (18 s ribosomal RNA closest match, >99%; length = 26 ± 3 μm, width = 10 ± 2 μm), was isolated from the estuarine sediments of Piles Creek (Elizabeth, NJ). All protozoa were cultured in an Artificial Seawater for Protozoa medium (ASWP) adapted from a CCAP recipe (http://www.ccap.ac.uk/media/pdfrecipes.htm). Ciliate cultures were maintained on an uncharacterized assemblage of bacteria.

In preparation for all ingestion rate experiments, ciliate cultures were first passed through a mesh sieve (21, 80, and 105 μm mesh for *U. marinum*, *E. plicatum*, and *E. vannus* respectively) to remove large particles and detritus. Ciliates were then gravity filtered onto a 10 μm polycarbonate filter, rinsed three times with sterile-filtered seawater to remove as much extant bacteria as possible, and then re-suspended to the desired concentration.

2.3. Pulse-fed suspended prey ingestion

A "pulse-fed ingestion" experiment was designed to assess the variation in uptake of prey as a function of feeding duration. In all trials, ciliates were added to small test tubes at low densities ($<0.5 \times 10^3$ cells ml^{-1}) to limit the reduction of overall prey density throughout the course of an experiment. *Vibrio* sp.-Nap and GFP-*Vibrio* cultures were individually harvested by centrifuging (7500 ×g, 10 min), washing two times, and finally re-suspended in sterile sea water. Initial cell density was determined by direct counts and was subsequently diluted to desired concentrations. Two prey concentrations were used to assess whether prey uptake varied with either feeding duration or prey density: 10^6 and 10^9 bacteria ml^{-1} for *E. plicatum* and *E. vannus*, 10^7 and 10^9 bacteria ml^{-1} for *U. marinum*. Preliminary observations showed that uptake in this design were not easily discernible in *U. marinum* below 10^7 bacteria ml^{-1} (data not shown).

The non-fluorescent *Vibrio* sp.-Nap was added to the ciliates at the desired concentrations to initiate feeding. The fluorescent GFP-*Vibrio* was added after ciliates had been feeding on the non-fluorescent prey for predetermined time durations (10, 60, and 120 min. for *U. marinum*; 10, 60, and 360 min. for *E. plicatum* and *E. vannus*). The fluorescent prey was added at a density equal to the non-fluorescent prey so that overall concentration was not altered. The pulse of fluorescent prey permitted the measurement of ingestion rate constancy as a function of feeding duration. Triplicate samples were taken at four different intervals, ranging from 5 to 30 s after the addition of fluorescent. At the end of each interval the sample was fixed with 1% (by volume) ice-cold glutaraldehyde. Immediately after fixation, samples were gravity filtered onto polycarbonate filters (5 μm pore size for *U. marinum*, or 10 μm pore size for *E. vannus* and *E. plicatum*). Filters were rinsed with a sodium borohydride solution (SBH: 0.2 ml of 1 mg ml^{-1} $NaBH_4$ in 2 ml cold buffer) to reduce auto-fluorescence which is caused by glutaraldehyde and otherwise hinders visualization of prey within vacuoles. A final rinse with 2 ml of buffer was applied to remove any excess SBH. The filters were air dried, placed on a glass slide between two drops of immersion oil, and a cover glass was placed over the filter. All samples were stored at −20 °C until analyzed.

Filters were scanned using an epifluorescence microscope at either 100 or 400× magnification to locate ciliates. The number of prey consumed per predator was determined by switching to 1250× magnification. The purpose of this study was to observe any changes in per capita ingestion rates with time, not rates of the whole population. Therefore, only ciliates containing one or more discernible fluorescent bacteria were included, and 15 ciliates per filter were analyzed for a total of 45 ciliates. The average number of bacteria per vacuole was determined, and ingestion rates were calculated by piecewise, rectilinear fit to the ingestion data.

2.4. Benthic prey ingestion rates

Sediment was collected from a tidal estuary on the Manasquan River, New Jersey. Mineral particles from the sediment were wet sieved, washed, and sorted to a size class of 177–210 μm. For each treatment, 0.5 g of sediment was added to 10 ml serum bottles (n = 3). This resulted in an average sediment depth of 1.0 mm (±0.1 mm). This sediment depth minimized the possibility of anoxia and allowed re-suspension of preserved ciliates and suspended pore-water bacteria during sampling, without physically disturbing the sediments. To achieve four successively higher concentrations of attached bacteria, 1 ml of Zobell's media was added at concentrations of 0, 1, 25, or 100% to bottles for treatments 1–4 respectively. All bottles were autoclaved, allowed to cool, and received a sterile addition of 0.5 g l^{-1} of kanamycin. A GFP-*Vibrio* inoculum was prepared as described above. All serum bottles were inoculated with 10 μl of the GFP-*Vibrio* preparation, covered with a foam stopper, and incubated in a humid chamber on a rotary shaker for 48 h. Following the incubation period the media was aspirated, and the sediment washed 4× by gentle addition of 2 ml of sterile seawater to remove both the remaining media and the majority of free bacteria.

All three ciliates were harvested as described above and adjusted to an approximate target concentration of ≥5000 cells ml^{-1}. Experiments began by inoculating all replicates with 0.2 ml of ciliate harvest. This resulted in complete saturation of the sediment pore space with no overlying fluid, allowing for all calculations to be normalized to units per milliliter of pore water (ml PW^{-1}). Time-zero samples were