Vol. 61: 45–56, 2010
doi: 10.3354/ame01434

AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol

Published online September 9

Heterotrophic microplankton in the lower Hudson River Estuary: potential importance of naked, planktonic amebas for bacterivory and carbon flux

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ABSTRACT: The present study is the first to simultaneously document the contributions of bacteria, heterotrophic flagellates, ciliates, and naked, planktonic amebas to the carbon (C) budget of an estuarine water column, and is also the first study of protistan bacterivory in the lower Hudson River Estuary (HRE). Observations were collected at a single near-shore location between June 2006 and May 2009. Bacterial counts and biomass varied approximately 1 order of magnitude on different dates, but were comparable to previous studies of the HRE and other estuaries. Of the 3 heterotrophic protist groups enumerated, heterotrophic nanoflagellates were the least variable and generally had the highest biomass (on average equaling 38% of the bacterial biomass). Counts and biomasses of ciliates and amebas were highly variable, ranging over at least 3 orders of magnitude between sampling dates. Much of the variability in ameba abundance was consistent with previous observations of seasonality. Ciliate biomass averaged 8%, and ameba biomass averaged 15% of the bacterial biomass. Thus, at this location, the importance of amebas as micropredators may be comparable to that of the ciliates, a group generally receiving greater research attention. Ameba ingestion rates could not be measured directly but 3 indirect approaches for calculating ingestion rates produced mean values ranging from 1.2 to 2.5 ng C d^{-1} ng^{-1} ameba biomass. Each approach demonstrated that ameba C consumption at the study location was highly variable, but was at times high relative to the bacterial standing stock. Taken together, these data suggest that amebas may be more common and of greater importance in estuarine C-fluxes than generally appreciated.

KEY WORDS: Amoeboid protists · Bacterivory · Bactivory · Grazing · Microzooplankton · Microbial ecology · Ameba · Amoeba

INTRODUCTION

Heterotrophic protists are major members of the microbial community in aquatic and terrestrial environments that enhance nutrient cycling, can be the major source of mortality for bacteria and microalgae, and provide a critical link between lower trophic levels and higher-level consumers (e.g. Caron & Goldman 1990, Griffiths 1994, Calbet & Landry 2004, Adl & Gupta 2006). In aquatic systems, much of the research on the role of heterotrophic protists has focused on ciliates and flagellates (e.g. Capriulo 1990, Strom 2000, Ichinotsuka et al. 2006). However, current evidence suggests that amoeboid protists deserve greater attention given their abundance and diversity in a broad range of habitats.

Amoeboid protists are ubiquitous, single-celled eukaryotes characterized by amoeboid motion and the presence of ≥1 locomotory pseudopods (Page 1983, 1988). Current classification systems, based on fine

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structural and molecular genetic evidence, place them in 2 major groups: (1) the Amoebozoa, all amebas without a flagellated stage in their life cycle, and (2) Heterolobosea (within the eclectic supergroup 'Excavata'), including the amoebo-flagellates that possess a flagellated stage at some point in their life cycle (Adl et al. 2005). The term 'ameba' will be used here specifically to mean naked (non-testate), amoeboid protists, and we will be specifically focusing on free-living amebas, more typically found in aquatic environments, exclusive of the 'slime molds' and their relatives.

Naked amebas are considered the most important bacterial grazers in soils (e.g. Clarholm 1981, Bonkowski 2004), and thus, their role in terrestrial environments (especially agricultural soils) has received more attention than in aquatic habitats. The paucity of research on the role of amebas in aquatic systems may be partially attributed to a prevailing opinion that ameba abundance is very low compared to other protists, particularly in the water column (e.g. Laybourn-Parry 1992, Strom 2000). Because they are usually destroyed by commonly used field-collection and preservation methods, and are difficult to visualize and identify microscopically, amebas are rarely included in surveys of microbial standing stocks or in ecological studies of trophic fluxes in aquatic environments. However, advances in sampling and enumerating amebas from aquatic environments have provided substantial documentation that they can be abundant (see Table 1) and may therefore be important mediators of aquatic biogeochemical fluxes, at least in coastal and freshwater habitats. Far less is known about ameba abundance and activity in the open ocean (see Davis et al. 1978, Caron et al. 1982).

Bacteria are undoubtedly the main prey for the smallest ameba species. Some larger species can also ingest other protists, including algae and heterotrophic flagellates (e.g. Page 1977, Bovee 1985, Anderson 1994). However, many ameba species, including the larger ones, can be maintained long term in the laboratory using bacteria exclusively as prey. Electron-microscopic investigations of ultrathin sections of amebas consistently show bacteria in food vacuoles (e.g. Anderson 1977, 1994, Rogerson et al. 2003). Grazing rates of a wide size range of benthic marine amebas, measured by feeding fluorescently labeled bacteria to ameba cultures, varied between 10 bacteria h^{-1} for the smallest ameba (57.3 μm^3) to 1465 bacteria h^{-1} for the largest ameba (61 000 μm^3) (Rogerson et al. 1996). Consequently, there is good evidence that amebas are generally bacterivorous and, given evidence that they can occur at high concentration in the water column, they may exert significant predatory pressure.

Unlike some other water-column protists, amebas are largely surface-dwelling, and there is good evidence that they can feed only when attached to a surface (Pickup et al. 2007a). The supple locomotory behavior of amebas allows them to invade small and complex spaces, potentially exploiting microenvironments that are unavailable to other protists. Observations in the Hudson River Estuary (HRE) and in Florida coastal waters indicate that a majority (ca. 90%) of amebas in the water column are particle-associated and found deep within the interstices of flocs (Rogerson et al. 2003). Amebas do detach and float from time to time, particularly in response to mechanical disturbance or diminished quality of the environment (e.g. Page 1983). The floating forms typically have contracted bodies with stellate, radiating pseudopods that are usually rigid and are unlikely to capture and ingest prey (Rogerson et al. 2003, Pickup et al. 2007a). While one can question whether these amebas should be considered truly 'planktonic', many planktonic flagellates and ciliates are also actually particle-attached, or particle-associated (Rogerson & Laybourn-Parry 1992b, Wörner et al. 2000, Kiorboe et al. 2004). Similarly, particle-attached bacteria can comprise a large fraction of bacterial biomass in aquatic water columns, and the majority of planktonic bacterial production in some estuaries (Crump & Baross 1996, Crump et al. 1998, Hollibaugh & Wong 1999). Therefore, use of the term planktonic is consistent with the use for other microbial groups, and these planktonic amebas have the potential to access a large and dynamic fraction of the bacterial pool.

There is little reliable evidence on ameba growth rates in natural aquatic environments, but in culture, growth rates equivalent to 1 or more divisions per day are common (see Table 2). Likewise, knowledge of ameba growth efficiencies and their roles in ecological fluxes is limited and largely based on laboratory investigations. Estimated gross-growth efficiencies for amebas generally range from 20 to 50% (Heal 1967, Rogerson 1981, Butler & Rogerson 1996, Zubkov & Sleigh 1999), comparable to data for other heterotrophic plankton (Capriulo 1990, Caron & Goldman 1990, Straile 1997). Thus, the available evidence suggests that the abundance, growth rates, and growth efficiencies for planktonic amebas can be comparable to planktonic ciliates, for example. One might therefore expect amebas to be of comparable ecological importance, at least in some aquatic environments (Anderson 2007).

The HRE is a turbid, heterotrophic estuary with high inputs of organic material from terrestrial systems (Howarth et al. 1992). These characteristics make the HRE an ideal system in which to study the ecological dynamics of amebas. Further, understanding elemental fluxes through lower trophic levels may be helpful to resolving the many well-known environmental

problems this system has suffered (e.g. Bopp et al. 2006, Brosnan et al. 2006, Farley et al. 2006). Yet, relatively little is known about the eukaryotic microbiology of the HRE, particularly in the lower, euryhaline portion. Much of the work that has been done has focused on the freshwater portion of the Hudson and on the prokaryotic, as opposed to the eukaryotic, community (Sañudo-Wilhelmy & Taylor 1999, Findlay 2006). Only one study (Vaque et al. 1992) has quantified bacterivory (not including amebas) in the HRE, and that study was restricted to the tidal, freshwater portion of the upper estuary.

In summary, naked, planktonic amebas are common in many aquatic habitats, but their importance to estuarine bacterivory and carbon (C) flux remains underappreciated because of methodological constraints. There is a paucity of data documenting both: (1) the abundance of planktonic amebas and their role in aquatic ecosystems and trophic dynamics, and (2) the characteristics and dynamics of the microbial community in the lower HRE. Anderson & Rogerson (1995) have examined the seasonal abundances of amebas in the lower HRE, showing a seasonal pattern. Subsequently, Anderson (2007), using a new technique (Anderson 2006) for assessing ameba C content, demonstrated that in some seasons, the ameba biomass in the lower HRE exceeded the ciliate biomass. The work we present here extends those previous studies to include the contributions of bacteria, amebas, ciliates, and nanoflagellates to the microbial carbon budget at a site in the lower HRE. In addition, we apply several indirect methods to estimate the potential contribution of amebas to bacterivory and C fluxes. The present work represents the most comprehensive study to date of eukaryotic microbial stocks in the lower HRE, and additionally provides more evidence of the potential importance of planktonic amebas in aquatic systems.

MATERIALS AND METHODS

The present study is conceptually divided into 2 parts. For the first part, we microscopically enumerated bacteria, nanoflagellates, ciliates, and amebas in water samples from a near-shore site in the lower HRE. These data are presented together with previously published data collected from the same location. In the second part of the study, bacterivory and C-flux by the amebas quantified in the first part were estimated using 3 independent approaches, as follows: (I) total bacterivory was estimated using the serial dilution technique (Landry & Hassett 1982) and then apportioned to the amebas in relation to their contribution to total bacterivore biomass, (II) ameba bacterivory was calculated using a relationship between ingestion rate and ameba cell volume (Rogerson et al. 1996), and (III) ameba C-flux was calculated by applying an assumed mean growth rate and gross growth efficiency to the measured ameba biomass. Further details are provided below in 'Estimating ameba bacterivory and C-flux'.

Study site and study period. Samples were collected in the salinity-stratified portion of the lower HRE near Sneedens Landing, New York, USA (41° 00′ 42.7″ N, 73° 54′ 11.7″ W). This portion of the estuary has high particulate load from suspended sediments. In general, phytoplankton production in the HRE is strongly limited by insufficient light due to high turbidity and vertical mixing, and by short residence time (Howarth et al. 2006, Landeck-Miller & St. John 2006). We collected 12 samples between April 2006 and May 2009, by walking out from shore to approximately 0.5 m depth and filling bottles with water from a few centimeters below the surface. Care was taken to avoid sediment stirred up while walking out.

Abundance and biomass of microbial community. Water samples for bacteria and heterotrophic nanoflagellate (Hflag) counts were preserved with transmission electron microscopy-grade glutaraldehyde (2% final concentration) and prepared for epifluorescence microscopy (modified from Hobbie et al. 1977 and Porter & Feig 1980) using the DNA-binding stain DAPI. Bacteria subsamples were filtered onto black 0.2 µm pore diameter polycarbonate membrane filters; Hflag subsamples were filtered onto 1 µm pore diameter filters of the same type. The filters were mounted on microscope slides sandwiched between drops of immersion oil and frozen until examined. Using an epifluorescence microscope with UV excitation, individual bacteria and Hflag cells were counted at 1000× magnification. Each Hflag cell counted was placed into 1 of 4 size and shape categories for the purpose of estimating total Hflag biovolume. Only Hflag < 8 µm in longest dimension were numerous enough to count by this technique; however, the smallest flagellates are thought to be responsible for the majority of flagellate bacterivory, with larger flagellates feeding primarily on larger particles (Sherr & Sherr 1991). Flagellates in this size range with red chlorophyll autofluorescence were relatively rare and were considered autotrophic.

Subsamples for ciliate counts were preserved using Lugol's iodine/potassium iodide stain (4% final concentration). Because of high particle concentration, the common Utermöhl counting method (Utermöhl 1936) could not be applied for these ciliate counts. Instead, samples were settled in plastic centrifuge tubes for ~24 h. The supernatant was removed and then the entire settled volume was examined aliquot by aliquot using an inverted microscope. All ciliates were

counted and individually sized. Ciliate biovolume was corrected for fixation according to Montagnes et al. (1994).

Because amebas do not preserve well, and because they typically dwell deep inside of particles, they cannot be enumerated using microscopic techniques commonly applied to other aquatic protists. Therefore, counts of naked amebas were estimated using the well-established culture observation method (COM) (e.g. Anderson & Rogerson 1995, Anderson 2007). A freshly collected sample of water (0.1 to 0.6 ml) was pipetted into each well of a 24-well Falcon tissue culture plate. Filtered water (0.45 µm) from the collection site was added to bring the volume per well up to 2 ml and a small portion of malt/yeast agar was added as nutrient to support the growth of bacterial prey. Triplicate plates were prepared for each sample. After 10 to 14 d incubation at the *in situ* temperature, each well was examined (with a Nikon Diaphot^{TM} inverted compound microscope using phase-contrast optics) to determine the presence or absence of a given ameba morphospecies, indicating if present, that at least 1 individual of that morphospecies was in the original sample aliquot. Only presence/absence was noted for each well, not the number within each well. The total tally of wells containing each morphospecies was obtained and converted to the number per liter in the original sample. It should be pointed out that the COM provides a minimum estimate of ameba biomass because some ameba species may not grow well in culture and because some wells may have received >1 individual of a given type.

Ameba size is relatively constant within a species and is used for taxonomic identification (e.g. Page 1983). Therefore, the average size of each species originally present in a sample can be determined by measuring (with an ocular reticle) a representative sample of each ameba type present at the end of the COM incubation. Although ameba shape is plastic, the cell's motile length is linearly related to biovolume of the sphered-up cell (Anderson 2006), allowing calculation of biovolume from specific length measurements.

Bacterial C was estimated from the cell counts using a mean cellular C content of 2×10^{-14} g C cell^{-1} (Ducklow 2000). Cellular C content of protists was calculated from the measured biovolumes using standard conversion formulas (Pelegri et al. 1999, Menden-Deuer & Lessard 2000).

Ameba net growth rates. To demonstrate that the ameba biomass measured during this study represented active cells, their net growth rates were determined on 5 dates. After processing the water sample for the various counting methods, the remaining sample was used to fill a 250 ml polycarbonate flask. The flask was incubated at the *in situ* temperature for 24 h and then resampled for ameba counts using the COM described in 'Abundance and biomass of the microbial community'. The net exponential growth rate was calculated from the initial and final ameba concentrations.

Estimating ameba bacterivory and C-flux. Three independent approaches were used to estimate bacterivory and C-flux by amebas at the study site.

(*I*) *Serial dilution experiments:* We measured total bacterivory using the serial dilution technique (Landry & Hassett 1982) on 4 dates. This technique was originally developed to quantify microzooplankton grazing on phytoplankton, but has been applied to measuring bacterivory (e.g. Landry et al. 1984, Tremaine & Mills 1987). Water was screened with a 202 µm mesh to remove larger zooplankton. Half the water was then additionally filtered to 0.2 µm (dilution water). The dilution water was combined with whole water in 800 ml polystyrene tissue culture flasks to generate 2 replicate flasks each of 100, 40, 20, and 5% whole water (8 flasks in total). Subsamples from each flask within the dilution series were collected to determine initial bacterial concentrations. The initial concentration of Hflags, ciliates, and amebas were measured as described earlier in the 'Materials and methods' section. The 8 flasks were incubated in the dark for 24 h at *in situ* temperature and then resampled to determine final bacteria concentration. Net bacterial growth rates in each flask were plotted against their dilution factor. Significant and linear regression slopes were found for each experiment. The *y*-axis intercept of the regression line provided µ, the rate of bacterial growth without predation; the regression slope provided *m*, the bacterial mortality rate (Landry & Hassett 1982).

As a first approximation, we assumed that bacterivory by each bacterivore group was roughly equivalent to their contribution to total bacterivore C. Using this assumption, *m* was parsed into the mortality attributable to each bacterivore group. Thus, the fractional bacterial mortality caused by bacterivore group *i* is m_i and was calculated as $m_i = m \times (C_i/C_T)$, where C_i is the C content of bacterivore group *i* and C_T is the total C content of all bacterivores. The fractional bacterial mortality caused by bacterivore group *i* is also related to its clearance rate as $m_i = F_i \times D_i$, where F_i is the clearance rate and D_i is a measure of the bacterivore density (Landry 1981, Landry et al. 2000). In this case, we used the initial concentration of each bacterivore group (µg C l^{-1}) for *D*. Specific ingestion rate, I_i, relates to clearance rate as $I_i = F_i \times P$, where *P* is the geometric mean prey concentration (Frost 1972). From these calculations we derived a mean specific clearance and ingestion rate (based on C) for each bacterivore group.

(*II*) *Predicting ameba ingestion rate from biovolume:* Rogerson et al. (1996) provided a relationship between ameba biovolume and maximum ingestion rate based on laboratory experiments using cultured benthic amebas. As no comparable studies have been conducted with water-column amebas, the Rogerson et al. (1996) study provides the best information currently available about likely ameba ingestion rates in the water column. To apply this function, we converted the relationship into units of C ingested per unit ameba biovolume per time, and then applied that relationship to the mean ameba biovolumes in our observations.

(*III*) *Predicting ameba C-flux from growth rate and gross growth efficiency:* A final way to estimate ameba C-flux required applying a mean growth rate (in C-units) and gross growth efficiency to the ameba abundances we measured. As mentioned in the 'Introduction', these parameters are poorly constrained for amebas, and may be highly variable, especially with respect to *in situ* populations. Nevertheless, based on the available literature (cited in the 'Introduction') and our measurements of ameba net growth rate (see 'Ameba net growth rates') we estimated ameba C-flux using a 'typical' growth rate of 1 doubling d^{-1} and a gross growth efficiency of 40%.

The estimates of ameba bacterivory and C-flux from these 3 approaches were compared to the bacterial standing stock for each day where sufficient data were available. The results of each approach were directly comparable on the 4 dates with dilution experiments (Approach I). Approaches II and III could be applied and compared to each other for all dates.

RESULTS AND DISCUSSION

Abundance and biomass patterns

This is the first study to document the abundance and biomass of planktonic amebas together with the abundance and biomasses of planktonic bacteria and other planktonic micropredators. Although all samples were from a single site within the HRE, it is worth noting that the abundances and biomasses within the microbial community at our study site were similar to data from other portions of the HRE, where comparable data are available, as well as to many other estuarine systems. Bacterial concentrations on different sampling dates ranged over approximately 1 order of magnitude (from 1.5×10^9 cells l^{-1} to 1.3×10^{10} cells l^{-1}) with a mean (±SD) bacterial concentration ($4.4 \times 10^9 \pm 3.9 \times 10^9$ cells l^{-1}) similar to values reported previously for the upper and lower estuary (Taylor et al. 2003, Findlay 2006). For complete bacteria and protist abundance and biomass data by date see Table S1 in the electronic supplement at www.int-res.com/articles/suppl/a061p045_supp.pdf. While Hflag concentrations have only been reported previously for the freshwater portion of the HRE (Vaque et al. 1992), the mean (±SD) value in the present study ($1.9 \times 10^7 \pm 9.8 \times 10^6$ cells l^{-1}) was close to expectations for other systems with similar bacterial concentrations (Berninger et al. 1991). Hflags are generally considered the most important planktonic bacterivores (e.g. Strom 2000). In the present study, Hflag biomass consistently exceeded the other microzooplankton and was always a large fraction of the bacterial biomass: mean (±SD) Hflag biomass was $3.3 \pm 1.2 \times 10^4$ ng C l^{-1}, and mean Hflag C biomass as percent of bacterial standing stock (C%) was 38 ± 18%. Ciliate abundance was highly variable and was more similar to open, coastal than to estuarine waters; however, total ciliate C was at estuarine levels (e.g. Sherr et al. 1986): mean (±SD) biomass and C% were $0.4 \pm 0.4 \times 10^4$ ng C l^{-1} and 7.8 ± 12% respectively (Fig. 1A,B). Thus, the ameba abundance and biomass patterns we report below exist within a fairly typical context for an estuarine water column.

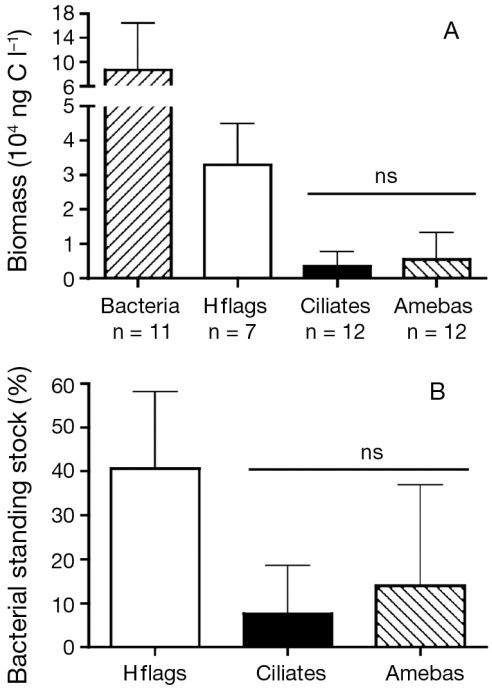

Fig. 1. (A) Mean carbon biomass for bacteria and the 3 groups of bacterivores at the Hudson River Estuary study site. (B) Mean carbon biomass as percent of bacterial standing stock for each bacterivore group. Hflags: heterotrophic nanoflagellates, ns: not significant. Error bars represent SD

Fig. 2 compiles all available counts of amebas for the study site including data from several previous studies (Anderson & Rogerson 1995, Anderson 2007). On an annual basis, ameba abundance at this location typi-

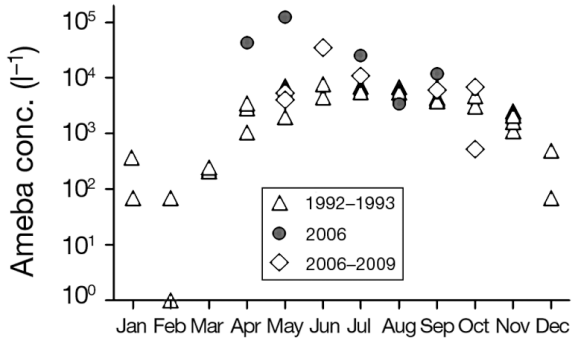

Fig. 2. Summary of ameba abundances at the Hudson River Estuary study site by month of observation, highlighting the seasonal cycle. Data compiled from observations made in 1992–1993 (Anderson & Rogerson 1995), 2006 (Anderson 2007), and 2006–2009 (the present study)

cally ranged 3 to 4 orders of magnitude, from <100 to 10^5 cells l^{-1}. However, much of the variability appeared to be seasonal, with spring and summer peaks, and very low ameba concentration in the winter and fall (e.g. the highest abundance in the present study was 1.2×10^5 cells l^{-1} in May 2006, while the lowest was 523 cells l^{-1} in October 2008). Similar seasonality in planktonic ameba count has been observed in the Clyde estuary (Rogerson & Laybourn-Parry 1992a, Anderson & Rogerson 1995). Low counts in winter are consistent with the strong effect of temperature on ameba growth rates (Baldock et al. 1980, Mayes et al. 1998). The peak ameba count reported in the present study (1.2×10^5 cells l^{-1} in May 2006) may be somewhat of an anomaly, as it was due almost entirely to a 'bloom' of *Cochliopodia* sp., a discoidal, granular-covered taxon, generally rare. Nevertheless, the observation suggests that there may be other periodic ameba blooms in the lower HRE, under appropriate environmental conditions. Similarly high concentrations of planktonic amebas have been reported in other aquatic environments (Table 1).

Table 1. Maximum planktonic ameba abundances from other studies. Location, habitat = all surface water unless otherwise noted

Location, habitat	Max. ameba abundance (l^{-1})	Source
Firth of Clyde (estuary, Scotland)	4.3×10^4	Anderson & Rogerson (1995), Rogerson & Laybourn-Parry (1992a)
Black Sea	3.8×10^5	Murzov & Caron (1996)
Freshwater pond	1×10^6	Anderson (1997, 2007)
Coastal East Antarctica	2.0×10^3 (water column) 2.6×10^3 (sea-ice interface)	Mayes et al. (1998)
Mangrove stand	1.0×10^5	Rogerson & Gwaltney (2000)
Salton Sea (saline lake, CA, USA)	2.4×10^5	Rogerson & Hauer (2002)

Temporal correlations between the biomasses of each of the 4 microbial groups (bacteria and the 3 micropredators) were tested for all possible pairings of the groups. However, no significant correlations were found (Pearson's correlation, $p > 0.11$ in all comparisons). Biomass variance differed significantly between the 3 bacterivore groups (Bartlett's test, $p < 0.0001$); therefore, group intercomparisons used non-parametric tests. The median biomasses of the 3 bacterivore groups were significantly different from each other (Kruskal-Wallis, $p = 0.0005$), with the HFlag biomass significantly greater than either the ciliates or amebas (Dunn's multiple comparison test, $p < 0.01$), which were not significantly different from each other (Dunn's multiple comparison test, $p > 0.05$). This supports the observation first described in Anderson (2007) that ameba and ciliate biomasses at this location were similar.

There are 2 levels of variation that need to be considered within the context of these microbial count data. The first is the counting error, i.e. how well a single count reflects the number of organisms (or their C content) in a sample. The second is the environmental variability in organism concentration (and C content) between samples across space and time. With respect to the first level of variation, the counting error can be assessed by the coefficent of variation (CV) between replicate subsamples (e.g. replicate slides for bacterial counts made from the same sample bottle). In earlier work, using counting approaches similar to those used in the present study, the CV between replicate subsamples for bacterial counts ranged from 2 to 25% with a mean CV of 12%, the CV for counts of small protists (<10 µm) ranged from 1 to 39% with a mean CV of 17%, and the CV for counts of larger protists (10 to 40 µm) ranged from 2 to 63% with a mean CV of 26.5% (Juhl 1991). These values are close to expectations for Poisson variables, given the actual number of organisms counted in each group (Lund et al. 1958), and also comparable to other studies that assessed this level of variation in direct counts (e.g. Kirchman et al. 1982, Kuosa 1988). Because the counting errors for these techniques have been previously established, replicate counts were not conducted for most samples used in the present study. However, we can calculate the CV for replicate subsamples of Hflags, because we produced and counted replicate slides on 6 of the sampling dates. The CV for

the Hflag count on those 6 dates ranged from 0 to 22% with a mean of 9%. The corresponding mean CV for C content was 12%. These CVs were somewhat better than expected (according to Lund et al. 1958, Kirchman et al. 1982, Kuosa 1988), but not unusual, given the relatively small sample size.

For ameba counts using the COM, we can calculate the CV between the separate multiwell plates prepared from each water sample as an estimate of subsample variability. For ameba count, the CV between plates prepared from the same water sample ranged from 0 to 76%, with a mean of 26%; for total C content the range was 2 to 89% with a mean CV of 40%. It should be noted that these are actually overestimates of the true variation between subsamples, because a full count would always be based on multiple plates. To properly assess variation between subsamples, one would have to at least double the total number of plates counted, a very labor-intensive proposition. Nevertheless, from this estimate, the COM results in counting errors that are comparable to other counting methods for protists. The estimated counting errors are much smaller than the variability observed over time for amebas (and other groups), providing confidence that the temporal patterns observed are related to the second, higher level of variation. Similarly, the SDs and SEs for mean organismal abundances (reported earlier in this section) primarily reflect temporal variation, rather than counting error. This is the level of variation that has true ecological relevance. Therefore, our discussion and conclusions are based on observations that were consistent across multiple dates, explicitly incorporating temporal variation, rather than focusing on results for specific dates.

Ameba net growth rates

The relative biomass comparison in the previous section suggests that amebas may at times be important micropredators and mediators of C flux at this site. The seasonality and shorter-term variability of ameba concentration also suggest that their populations can respond dynamically to environmental conditions, at least slowly. However, their actual predation rates in comparison to Hflags and ciliates are difficult to assess. We calculated the mean net growth rate for amebas in Hudson River microcosms based on both the change in ameba cell concentration and on the change in C over 24 h (Fig. 3). The means of the 2 measures of growth were not significantly different from each other (*t*-test, $p = 0.76$), and neither mean was significantly different from 0.693 d^{-1} (i.e. 1 division d^{-1}). For the complete net growth data set see Table S2 in the electronic supplement at www.int-res.com/articles/suppl/a061p045_supp.pdf.

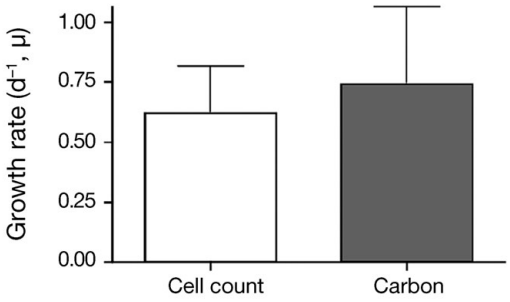

Fig. 3. Mean net growth rates (μ) for amebas in Hudson River microcosms based on the change in ameba cell numbers and on the change in carbon biomass over 24 h. Error bars represent +1SE

The net growth rates we measured under *in situ* conditions were within the range of gross-growth rates for various ameba species measured in culture (Table 2). With sufficient prey, free-living heterotrophic protists have an extremely high growth potential (e.g. Rose & Caron 2007). However, under prey concentrations comparable to estuarine water columns (and temperatures of 15 to 20°C), growth rates resulting in 1 to 2 divisions d^{-1} are typical for both Hflags (e.g. Capriulo 1990, Eccleston-Parry & Leadbeater 1994) and ciliates

Table 2. Ameba gross growth rates (μ) from culture studies

Amebas	Range of μ (d^{-1})	Mean μ (d^{-1})	Temp. (°C)	Source
4 marine benthic species	0.3–0.4	0.34	10	Cowie & Hannah (2006)
6 freshwater species	0.9–3.2	1.8	15	Baldock et al. (1980)
10 marine benthic species	0.2–0.8	0.6	15	Butler & Rogerson (1996)
6 freshwater species	1.5–3.6	2.3	20	Baldock et al. (1980)
10 marine benthic species	0.4–1.5	0.9	20	Butler & Rogerson (1996)
2 soil/freshwater species fed 6 bacterial prey types	0.5–1.9 (depending on prey type)	–	20	Pickup et al. (2007b)

(e.g. Montagnes 1996, Weisse et al. 2001). Thus, ameba growth rates appear to be comparable to expectations for other micropredators at this site.

Estimating ameba bacterivory and C-flux

Amebas predominantly reside deep within particles, their morphology is highly plastic and difficult to identify, they often do not preserve well, and they most likely feed only on the attached fraction of the bacterial pool. There are, thus, many challenges to directly measuring ameba bacterivory using available techniques. Therefore, we were limited to estimating potential ameba bacterivory at our study site using 3 indirect approaches.

(I) Serial dilution experiments

Each dilution experiment resulted in a measure of the bacterial gross growth rate μ and the total bacterial predation mortality *m* (Table 3). On 3 of the 4 dates, *m* was ≥90% of μ, which suggests a fairly typical balance between bacterial growth and mortality (e.g. Strom 2000). The total mortality rate was then parsed into a fractional mortality due to each of the 3 microzooplankton groups according to their relative biomass. From the fractional mortality attributed to each predator group, we also estimated mean specific clearance and ingestion rates (in C units) for each group. Total daily C consumption by amebas estimated from the dilution experiment data is shown in Fig. 4A (open triangles) as a function of total ameba biomass (see also Table S3 in the electronic supplement at www.int-res.com/articles/suppl/a061p045_supp.pdf).

The dilution technique was originally developed to quantify microzooplankton grazing on phytoplankton, but is also applicable to measuring bacterivory (e.g. Landry et al. 1984, Tremaine & Mills 1987). Use of the dilution technique for measuring bacterivory assumes that dilution itself did not change bacterial growth rate, a potential problem if substrate concentrations were near-limiting for growth. However, that potential concern is unlikely in the HRE. Dissolved organic C (DOC) concentrations in the system are high, generally 2 to 6 mg C l^{-1} (Findlay et al. 1996, Sañudo-Wilhelmy & Taylor 1999), and measurements of water-column bacterial production (Findlay et al. 1996, Sañudo-Wilhelmy & Taylor 1999) indicate that bacteria would use only <1 to 10% of DOC standing stock each day. In addition, bacterial production and growth rates do not correlate with DOC concentration in the HRE (Findlay et al. 1996, Sañudo-Wilhelmy & Taylor 1999, Findlay 2006), further indicating that substrates are unlikely to be limiting. Inorganic nutrient concentrations (N, P) are also high and therefore unlikely to limit bacterial growth. In fact, nutrient loading per area of the estuary is probably higher for the HRE than for any other major US estuary (Howarth et al. 2006).

We assumed that bacterivory for each bacterivore group was proportional to their C content. Omnivory is one potential caveat for this assumption. This caveat seems most likely for the ciliates observed. Given their small size, omnivory is probably unimportant for the observed Hflags. Similarly, while some larger amebas are known to supplement their bacterivory with algae and other protists, the relatively small size of most ame-

Table 3. Bacterial gross growth (μ) and total mortality rates (*m*) calculated from dilution experiments. These data were used to calculate ingestion and clearance rates for the 3 bacterivore groups in the lower Hudson River Estuary. Hflags: heterotrophic nanoflagellates

Date	Bacteria μ (d^{-1})	Bacteria *m* (d^{-1})	Hflags (±SE)	Ciliates (±SE)	Amebas
Jun 21, 2006	1.2 ± 0.3	1.2 ± 0.2	2.2×10^{-4} [a]	0.2[a]	0.05[a]
			2.3×10^{-9} [b]	2.5×10^{-6} [b]	5.1×10^{-7} [b]
			6.7×10^{4} [c]	4.2×10^{3} [c]	4.0×10^{4} [c]
			60.3[d]	3.8[d]	35.9[d]
Jul 10, 2006	0.9 ± 0.3	1 ± 0.1	2.0×10^{-4} [a]	0.04[a]	0.008[a]
			3.1×10^{-9} [b]	6.6×10^{-7} [b]	1.2×10^{-7} [b]
			4.2×10^{4} [c]	1.4×10^{4} [c]	2.0×10^{3} [c]
			72.0[d]	24.5[d]	3.4[d]
Oct 5, 2006	0.6 ± 0.06	1.3 ± 0.03	1.9×10^{-4} [a]	0.3[a]	0.008[a]
			1.5×10^{-9} [b]	1.9×10^{-6} [b]	6.3×10^{-8} [b]
			7.6×10^{4} [c]	0.9×10^{3} [c]	2.1×10^{3} [c]
			96.1[d]	1.1[d]	2.7[d]
May 7, 2007	1 ± 0.1	0.9 ± 0.06	2.6×10^{-4} [a]	0.0[a]	0.02[a]
			2.4×10^{-9} [b]	0.0[b]	2.0×10^{-7} [b]
			91.9[c]	0.0[c]	4.6×10^{3}[c]
			95.2[d]	0.0[d]	4.8[d]
Mean ± SE	0.93 ± 0.13	1.1 ± 0.09	2.2×10^{-4}	0.2	0.02
			2.2×10^{-3} [a]	0.09[a,e]	1.4×10^{-3} [a]
			2.3×10^{-9}	1.7×10^{-6}	2.2×10^{-7}
			3.3×10^{-8} [b]	5.4×10^{-5} [b,e]	1.0×10^{-7} [b]

[a] Specific ingestion rate (ng C predator^{-1} h^{-1})
[b] Clearance rate (l predator^{-1} h^{-1})
[c] Total ingestion rate per day (ng C d^{-1})
[d] Estimated contribution (%) to total daily bacterial mortality
[e] Only the 2006 dates were used because ciliates were not detected in the May 2007 samples

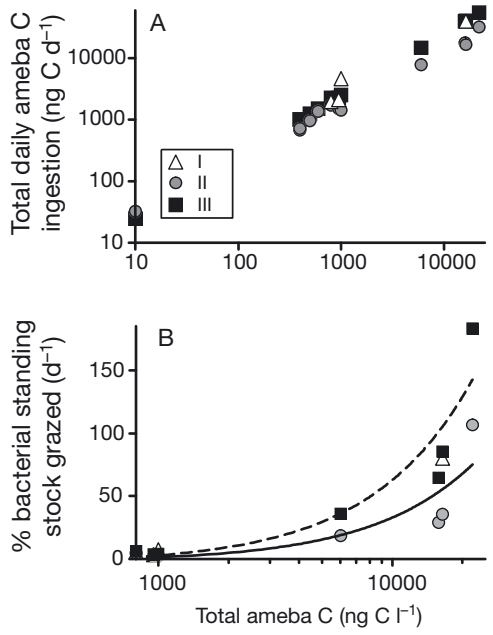

Fig. 4. (A) Total daily ameba carbon consumption as a function of total ameba biomass. Ameba carbon consumption was calculated using 3 different approaches: (I) estimated from the total bacterivory measured in dilution experiments, (II) calculated from the relationship of biovolume to ingestion rate of benthic amebas (Rogerson et al. 1996), and (III) calculated from the ameba biomass assuming a growth rate of 1 division d^{-1} and a 40% gross growth efficiency. (B) Percent of each sample date's bacterial biomass estimated to be ingested by amebas (for each of the 3 approaches) as a function of the total ameba biomass. Lines (which appear curved because of the log-scale *x*-axis) represent the best-fit linear regressions to the data. Solid line is fit to Approach II ($r^2 = 0.80$); dashed line is fit to Approach III ($r^2 = 0.89$). A regression line was not estimated for Approach I because there were only 4 data points. Note that data from when ameba biomass < 800 ng C l^{-1} are not shown

bas in the HRE (mean cell volume: ~1000 µm^3) indicates that they were also primarily bacterivorous. Moreover, the most commonly observed autotrophs at our sampling site were large diatoms, too big to be suitable prey for most amebas in our samples. It is also possible that our analysis missed certain bacterivores, e.g. many algal flagellates can be mixotrophic. However, the quantitative importance of both omnivory and mixotrophy are somewhat minimized for the HRE. Because of light limitation, high flushing, and high allochthonous C loading, bacterial production exceeds net primary production (gross production minus phytoplankton respiration) by ~2 to 6 times, the system is strongly net heterotrophic (Taylor et al. 2003, Howarth et al. 2006), and thus, fluxes through the lower food web are dominated by bacteria-based pathways.

One test of the approach is whether the estimated rates for the ciliates and flagellates are consistent with direct estimates from other studies (i.e. based on uptake of labeled prey). The mean clearance rates (±SE) calculated here for Hflags and ciliates, $2.3 \times 10^{-9} \pm 3.3 \times 10^{-8}$ and $1.7 \times 10^{-6} \pm 5.4 \times 10^{-5}$ l predator^{-1} h^{-1}, respectively (Table 3), are both near the center of the range of clearance rates of Hflags and ciliates reviewed in Capriulo (1990) and Eccleston-Parry & Leadbeater (1994). From this perspective, the results of our approach are therefore plausible, at least as a first approximation.

(II) Estimating ingestion rate from ameba biovolume

Rogerson et al. (1996) provided a functional relationship between ameba biovolume and the ingestion rate of bacteria based on culture experiments with marine benthic amebas grazing on surfaces. This function was converted into C units and applied to the mean ameba biovolume from each day to approximate a mean ingestion rate. After multiplying the mean ingestion rate by the ameba abundance, total daily C ingestion by amebas calculated by this method ranged from 3.3×10^1 ng C d^{-1} in October 2008 to 3.2×10^4 ng C d^{-1} in May 2006.

(III) Estimating ingestion from growth rate and gross growth efficiency

Fig. 4A also shows an estimated ingestion rate for the ameba population based on growing the biomass at 1 division d^{-1} with a 40% gross-growth efficiency. Both growth rates and gross-growth efficiencies can be highly variable and the use of different values would result in different calculated ingestion rates. However, these values were selected to represent a single, typical situation for a mixed population of amebas (Heal 1967, Rogerson 1981, Capriulo 1990, Caron & Goldman 1990, Butler & Rogerson 1996, Straile 1997, Zubkov & Sleigh 1999, Anderson 2007) for comparison to the other approaches. Total daily C ingestion by amebas calculated by this method ranged from 2.5×10^1 ng C d^{-1} in October 2008 to 5.5×10^4 ng C d^{-1} in May 2006.

The 3 approaches to estimating total ameba ingestion generally agreed within a factor of 2 (Fig. 4A). The mean slope for each approach in Fig. 4A provided the mean ingestion rate per unit ameba biomass. The Rogerson et al. (1996) function (Approach II) resulted in a mean ingestion rate of 1.2 ng C d^{-1} ng^{-1} ameba biomass, while the data from the dilution experiments (Approach I) and the gross-growth efficiency approach (Approach III) gave mean rates of 2.4 and 2.5 ng C d^{-1} ng^{-1} ameba C, respectively. In Fig. 4B, the daily total C ingestion by amebas (according to each approach) was

divided by each day's bacterial biomass to show the percent of the bacterial standing stock that would have been grazed by amebas. Because bacterial biomass was more consistent than ameba biomass, the percent of the bacterial standing stock grazed was mostly an increasing function of the ameba biomass. Note that the *x*-axis scale in Fig. 4B was changed relative to Fig. 4A because a negligible fraction (<3 %) of the bacterial standing stock was estimated to have been grazed on dates when ameba biomass was <800 ng C l^{-1}. Based on these plots, we suggest that the discrepancy between the 3 approaches is minor compared to the variability in ameba biomass over time. Each approach demonstrated that ameba C consumption at the study location was highly variable among sampling dates, but that at times ameba C consumption was high relative to the bacterial standing stock and to consumption by other more commonly studied micropredators.

CONCLUSIONS

This is the first study to document the contributions of amebas to the carbon budget of an estuarine water column together with contextual data on the biomasses of bacteria, Hflags, and ciliates. It is also the first study of protist bacterivory in the lower HRE. While the data are from a single study site, they demonstrate that naked, planktonic amebas can be abundant in the water column of the lower HRE, especially in the spring and summer, with a total biomass that is at times a large fraction of the bacterial biomass and comparable to the biomasses of other heterotrophic protist groups. Available evidence suggests that ameba growth rates and gross-growth efficiencies are comparable to other bacterivore groups. Independent approaches to estimating ameba ingestion rates during the present study consistently demonstrated that when abundant, amebas were capable of consuming a large fraction of the bacterial biomass. While Hflags appear to generally dominate bacterivory in the water column of the lower HRE, ciliates and amebas intermittently played import roles in C flux within the microbial food web. Greater recognition should be given to the potential importance of amebas to microbial trophic interactions and C fluxes in the water column of other systems. Ameba natural history suggests that their role as micropredators will be greatest for the important subset of particle-associated bacteria and other microbes.

Acknowledgements. We thank E. Suter for her assistance in collecting and processing field samples. The comments from 3 anonymous reviewers and the editor greatly improved the presentation of the present study. This material is based upon work supported by the US National Science Foundation under Grant No. OCE0845799. Additional critical support was provided by the Wallace Research Foundation and the Brinson Foundation. This is Lamont-Doherty Earth Observatory Contribution No. 7388.

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Editorial responsibility: Urania Christaki, Wimereux, France

Submitted: December 6, 2009; Accepted: June 16, 2010
Proofs received from author(s): August 26, 2010