Estuarine, Coastal and Shelf Science 151 (2014) 45–53

Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: www.elsevier.com/locate/ecss

Geographic variability in amoeboid protists and other microbial groups in the water column of the lower Hudson River Estuary (New York, USA)

Andrew R. Juhl*, O. Roger Anderson

Lamont Doherty Earth Observatory of Columbia University, 61 Route 9W, Palisades, NY 10960, USA

ARTICLE INFO

Article history:
Received 18 February 2014
Accepted 24 September 2014
Available online 5 October 2014

Keywords:
amebas
bacterivory
protists
nanoplankton
turbidity
spatial distribution

Regional index terms:
USA
New York
Hudson River

ABSTRACT

In comparison to other groups of planktonic microorganisms, relatively little is known about the role of amoeboid protists (amebas) in planktonic ecosystems. This study describes the first geographic survey of the abundance and biomass of amebas in an estuarine water column. Samples collected in the lower Hudson River Estuary were used to investigate relationships between ameba abundance and biomass and hydrographic variables (temperature, salinity, and turbidity), water depth (surface and near bottom), distance from mid-channel to shore, phytoplankton biomass (chlorophyll fluorescence) and the occurrence of other heterotrophic microbial groups (heterotrophic bacteria, nanoflagellates, and ciliates) in the plankton. Although salinity increased significantly towards the mouth of the estuary, there were no significant differences in the abundance or biomass of any microbial group in surface samples collected at three stations separated by 44 km along the estuary's mid-channel. Peak biomass values for all microbial groups were found at the station closest to shore, however, cross-channel trends in microbial abundance and biomass were not statistically significant. Although ameba abundance and biomass in most samples were low compared to other microbial groups, clear patterns in ameba distribution were nevertheless found. Unlike other microbial groups examined, ameba numbers and biomass greatly increased in near bottom water compared to surface samples. Ameba abundance and biomass (in surface samples) were also strongly related to increasing turbidity. The different relationships of ameba abundance and biomass with turbidity suggest a rising contribution of large amebas in microbial communities of the Hudson estuary when turbidity increases. These results, emphasizing the importance of particle concentration as attachment and feeding surfaces for amebas, will help identify the environmental conditions when amebas are most likely to contribute significantly to estuarine bacterivory and C-flux.

1. Introduction

Amoeboid protists are single-celled eukaryotes characterized by amoeboid motion and the presence of one or more locomotory pseudopods (Page, 1983, 1988). This is a functional and morphological definition, representing many groups of organisms that are not necessarily closely related. Here, we will use the term 'ameba' specifically to mean naked (non-testate), amoeboid protists, and our focus is free-living amebas, more typically found in aquatic environments, exclusive of the "slime molds" and their relatives.

Amebas are considered the most important group of bacterial grazers in soils (e.g., Clarholm, 1981; Bonkowski, 2004; Anderson, 2012). In contrast, although ubiquitous in aquatic environments, amebas are often thought of as having only a minor role in planktonic ecological fluxes because their natural abundance is thought to be low compared to other heterotrophic protists, such as ciliates and nanoflagellates (e.g., Laybourn-Parry, 1992; Strom, 2000). While natural ameba abundance in the water column is often observed to be comparatively low, there have been numerous reports of high abundance and biomass (summarized in Lesen et al., 2010). For example, Murzov and Caron (1996) found high abundances of naked amebas in Black Sea plankton, peaking at 4×10^5 cells l^{-1}, when amebas dominated the biomass of heterotrophic nanoplankton (<20 μm). Other studies have also occasionally found planktonic ameba abundances as high as 10^5–10^6 cells l^{-1} in diverse near-shore environments (Anderson, 1997; Rogerson and Gwaltney, 2000; Rogerson and Hauer, 2002; Anderson, 2007). However, ameba abundance in offshore waters

* Corresponding author.
E-mail address: andyjuhl@ldeo.columbia.edu (A.R. Juhl).

http://dx.doi.org/10.1016/j.ecss.2014.09.020
0272-7714/

generally appears to be much lower; Davis et al. (1978) reported finding only 0.4 l^{-1} in subsurface water sampled from the North Atlantic. The localized abundance of amebas in particulates may nevertheless be high, even in the open ocean. For example, high ameba abundances were noted in association with pelagic *Trichodesmium* colonies in the Sargasso Sea (Anderson, 1997). Similarly, Caron et al. (1982) reported concentrations of planktonic protists (including amebas) in Sargasso Sea macroaggregates (marine snow, *Trichodesmium* tufts, and *Rhizosolenia* mats) that were four orders of magnitude greater than in samples of the surrounding water. Nonetheless, research on the distribution, abundance and biomass of naked amebas in open ocean and coastal waters is much less prevalent than research on other planktonic protists.

The relative rareness of such reports may be partly related to methodological biases. Amebas are usually destroyed by commonly-used field collection and preservation methods, and they are difficult to visualize and identify microscopically. Genomic tools have been used to track particular ameba species of interest (e.g., Mullen et al., 2005). However, the taxonomic diversity of amebas is too broad, and the sequence database remains too sparse, to currently apply genomic techniques to detect and enumerate all amebas in field samples (Nikolaev et al., 2004, 2006; Smirnov et al., 2011). Therefore, amebas must currently be enumerated separately from other planktonic microorganisms using specialized and labor-intensive, direct microscopic observational methods (e.g., Anderson and Rogerson, 1995).

Given the relatively sparse data on planktonic amebas in estuarine waters, Lesen et al. (2010) documented the temporal variability in abundance and biomass of amebas at a single, near-shore location in the Hudson River Estuary (HRE) in relation to other major groups of heterotrophic protists in the water column at that site. Mean ameba biomass exceeded that of ciliates, but was more variable than ciliate or heterotrophic nanoflagellate biomasses. Earlier work had also found that ameba biomass within the HRE, and in some highly productive freshwater habitats, often exceeded that of ciliates (Anderson, 2007). While in-situ growth and bacterivory rates for amebas are difficult to quantify, available evidence suggested that mean rates for amebas were comparable to those of other heterotrophic protists (Lesen et al., 2010), consistent with prior reports of surface grazing rates of some marine benthic amebas (e.g., Rogerson et al., 1996). Thus, at times when ameba biomass is a significant fraction of the total for heterotrophic protists, they likely contribute significantly to total bacterivory, phytoplankton grazing, and carbon fluxes. Nevertheless, ameba abundance and biomass seem to be characterized by much higher spatial and temporal variability than other heterotrophic protists in the plankton (Murzov and Caron, 1996; Lesen et al., 2010).

Given sporadic high ameba biomass coupled to high variability in estuarine environments, understanding the factors driving variability in planktonic ameba biomass would be valuable. In particular, predicting the conditions that most favor high ameba biomass would help in determining when the extra effort to enumerate this group would be most useful. In the few temperate locations that have been studied, ameba biomass varied seasonally, with peaks occurring in spring and summer (Anderson and Rogerson, 1995; Lesen et al., 2010), possibly related to more suitable temperatures for growth. Lower abundance and activity in colder conditions, as during winter in temperate regions, is consistent with observations from Antarctic waters (Mayes et al., 1997, 1998).

Because amebas attach to, move, and feed on surfaces (Pickup et al., 2007), they are associated with particles (Rogerson et al., 2003) and it is likely that particle characteristics are a major determinate of their abundance and biomass. For example, mean size, carbon biomass, and the diversity of ameba morphospecies increase with increasing particle size (Anderson, 2011), and high ameba abundance has been found in aquatic environments with high particulate load, such as ponds and mangroves (Rogerson et al., 2003; Anderson, 2007). Similarly, Murzov and Caron (1996) noted that the highest ameba counts in the Black Sea were from locations with abundant water-column particulates. However, quantitative relationships between ameba abundance and biomass with turbidity, or other direct estimates of particle load, have not been derived for any aquatic ecosystem.

In this study, ameba concentrations and biomass were estimated in multiple locations within the HRE. The objectives were to assess whether ameba abundance and biomass varied in relation to: 1) the estuarine salinity gradient; 2) water depth; 3) hydrographic variables such as temperature, salinity, and turbidity; and 4) other planktonic microbial groups within the estuary. In general, we hypothesized finding significant relationships between heterotrophic protists (including amebas) and their likely prey, heterotrophic bacteria or phytoplankton. With respect to amebas, given observations of high ameba abundance and biomass in water columns with high particle load (Murzov and Caron, 1996; Rogerson et al., 2003; Anderson, 2007) we hypothesized that ameba abundance and biomass would increase with turbidity. In addition, also due to their particle-associated lifestyle, we hypothesized finding higher ameba abundance near the bottom, compared to surface water samples.

2. Materials and methods

2.1. Sample collection and location

Surface water samples were collected at 5 locations within the salinity-stratified portion of the lower HRE on three dates: September 24, 2008, October 20, 2008, and May 12, 2009. This portion of the estuary has high particulate load from suspended sediments. In general, phytoplankton production in the HRE is strongly light limited due to high turbidity and vertical mixing, and by short residence time (Cole and Caraco, 2006; Howarth et al., 2006; Landeck-Miller and St. John, 2006). Surface station locations were chosen to allow several specific comparisons. Three mid-channel stations (1, 2, and 3, Fig. 1A) were chosen to provide data on potential trends along the estuarine salinity gradient. Northernmost station 1, in the Tappan Zee region of the estuary, is always the freshest of the three, while station 3, near the southern tip of Manhattan Island, is the most saline. Stations 1 and 3 are separated by approximately 44 km.

Station 1, together with two additional stations 4 and 5, represent a mid-channel to near shore transect (Fig. 1B). Data from these three stations were used to study potential cross channel trends. Station 5 has been the focus of several previous investigations of microbial abundance and activity in the HRE (Anderson and Rogerson, 1995; Anderson, 2007; Lesen et al., 2010). In addition to the surface sampling, on several dates, near bottom water samples were collected at mid-channel stations 1 and 2. These data were used to examine potential trends with depth.

Samples from near-shore station 5 were collected by wading out from shore to where water depth was approximately 0.5 m (3–4 m from the shoreline). All other samples were collected from the Riverkeeper survey vessel, R. Ian Fletcher (www.riverkeeper.org). For each surface sample, an autoclaved 250-ml polypropylene bottle was held several cm below the water surface until it was full. On four occasions, when we sampled a mid-channel station at the surface, we also collected a near bottom sample (from within the bottom meter). Three of these near bottom samples came from station 2, one from station 1. For near-bottom samples, a 250-ml sample bottle was filled from a 2.5-l Niskin bottle (General

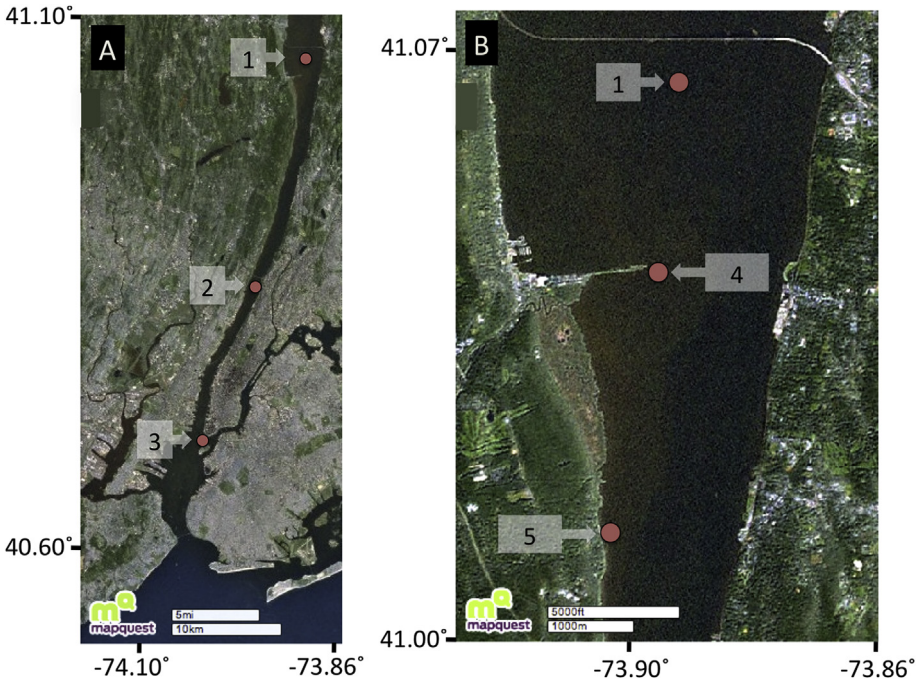

Fig. 1. Images of the lower Hudson River Estuary showing sampling station locations. A) Stations 1, 2 and 3 represent an approximately 44-km long, north to south, mid-channel transect along the estuarine salinity gradient. B) Image of the region near Station 1, showing the three stations, 1, 4, and 5, representing a cross-channel transect from mid-channel (1) to near shore (5).

Oceanics) that was tripped <1 m above the bottom. Samples were then held in a covered, insulated cooler filled with surface water until returning to the lab for further processing within 6 h after collection.

Water temperature, salinity, turbidity, and in-vivo chlorophyll fluorescence corresponding to each surface sample were measured using a Hydrolab DS-5 datasonde, calibrated prior to each sampling event according to the manufacturer's recommended procedure. In-vivo chlorophyll fluorescence (hereafter "chlorophyll fluorescence") is a proxy for phytoplankton biomass, providing an estimate of one possible prey field for the heterotrophic protists enumerated. For near-bottom water samples, temperature and salinity only, were measured by inserting a Hach IntelliCAL CDC401 conductivity probe into the Niskin bottle immediately after sample collection.

2.2. Microbial abundance and biomass

Water samples for planktonic heterotrophic bacteria and heterotrophic nanoflagellate (Hflag) abundance were preserved with electron microscopy-grade glutaraldehyde (2% final concentration) and prepared for epifluorescence microscopy using the double-stranded DNA-binding stain SybrGreen (Sigma Aldrich) following Suter et al. (2011), including treatment to dislodge attached cells from particles. Bacteria subsamples (0.5 ml) were filtered onto black, 0.2-μm pore diameter polycarbonate membrane filters; Hflag subsamples (0.5–1 ml) were filtered onto 1-μm pore diameter filters of the same type. Filters mounted on microscope slides were examined at 1000× magnification using an epifluorescence microscope with blue light excitation. At least 200 bacterial cells from at least 20 individual microscope fields per filter were counted. Autotrophic cyanobacteria could be distinguished from other bacteria by pigment fluorescence using this procedure, but they were not detected, therefore we assume our bacterial counts refer to heterotrophic bacteria only. Hflag cells were counted by scanning across two diametrical transects of each filter, arranged in a cross pattern. All Hflag cells along the scan were counted. The width of the microscope field and diameter of the filtered area were used to determine the fraction of the slide counted, which was used to calculate the original cell concentration. Generally <100 Hflag cells were counted per slide. During counting, each Hflag cell was assigned into one of four 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. Flagellates in the <8 μm size range with red chlorophyll autofluorescence were relatively rare and were considered autotrophic. Biovolume for the smallest Hflags (≤3 μm) was calculated as for a sphere, while for the 4–8 μm cells, biovolume was estimated as either a sphere or ovate spheroid, depending on their morphology.

Subsamples for ciliate abundance 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. Instead, samples were settled in 50-ml plastic centrifuge tubes for ~24 h. The supernatant was then removed down to ~5 ml and this remaining volume was resuspended and transferred to a 15 ml centrifuge tube. After, sedimenting the sample again, the supernatant was removed down to ~1 ml. This remaining volume was examined aliquot by aliquot (ca. 20 μl each) using an inverted microscope (400× magnification). All ciliates were counted and individually sized; most were naked ciliates, though some may have been tintinnid ciliates that escaped their loricas during the sampling and fixation steps. Ciliate counts were quite variable, with 10–150 individuals counted per sample. The volume of ciliates was estimated by measuring the radius of spherical morphotypes and computing the volume of the sphere. For spheroidal morphotypes, the major and minor axes were measured and used to compute the volume of the spheroid based on solid geometric formulas for spheroid volume. Ciliate biovolume was then 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, abundance of naked amebas was estimated using the well-established culture observation method (COM) (e.g. Anderson and Rogerson, 1995; Lesen et al., 2010). A freshly collected sample of water (0.1–0.9 ml) was pipetted into each well of a 24-well Falcon tissue culture plate as inoculum. 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 heterotrophic bacterial prey. Triplicate plates were prepared for each sample, each with a different initial inoculum volume. After 10–14 d incubation at the in-situ water temperature, the two "most-countable" plates (i.e. with ameba frequency neither too low nor too high) were selected in preliminary microscope scans for full enumeration of amebas. Each well was examined (with a Nikon Diaphot™ inverted compound microscope using phase-contrast optics) to determine the presence or absence of a given ameba morphospecies, indicating if present, that at least one individual of that morphospecies had been in the original sample inoculum. 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, although there is a Poisson, statistical correction factor in the computation algorithm to adjust for the likelihood of underestimation, by incorporating an adjustment in the final densities based on the total counts of each ameba morphotype (see Anderson et al., 2001 for further details).

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 the diameter of the sphered-up cell, allowing calculation of biovolume and biomass from specific length measurements (Anderson, 2006).

Heterotrophic bacterial biomass (expressed as C content), was estimated using a mean cellular C content of 2×10^{-14} g C cell^{-1} (Ducklow, 2000). Protist biomasses were calculated using the general C:biovolume relationship for taxonomically diverse protist plankton given in Menden-Deuer and Lessard (2000). Error estimates associated with microscopic enumeration of different microbial groups have been discussed previously (Juhl, 1991; Lesen et al., 2010). Expected coefficients of variation for replicate subsamples (i.e., samples from the same sample bottle) are in the following ranges: heterotrophic bacteria 10–15%, Hflag 10–20%, ciliates and amebas 20–30%.

2.3. Data analyses

Nonparametric statistical tests were used for most analyses. Friedman's repeated measures test was used to determine whether patterns of hydrographic and microbial variables varied significantly along the mid-channel and cross-channel transects. Comparing surface and near bottom samples paired by date used the Wilcoxon signed-rank test. Spearman correlations were used to compare the abundance estimates and biomasses of each of the four microbial groups counted microscopically (all stations and depths) relative to the hydrographic variables. In addition, the abundance and biomasses of the heterotrophic protist groups were correlated to the biomass of potential heterotrophic bacterial and phytoplankton prey. The relationships of ameba abundances and biomass with turbidity were analyzed in more detail using parametric regression analyses. Regression analyses were first conducted using only the data from this study. The relationships were then reanalyzed after adding in four additional data points that derived from the only samples reported in Lesen et al. (2010) that had concurrent measurements of ameba abundance and biomass with turbidity (using the same instrumentation as in this study). These four additional data points were from station 5. Relationships between amebas and turbidity were not discussed in Lesen et al. (2010), nor were the turbidity data shown, because at the time the number of turbidity observations were too sparse. Given additional observations, those data can now be included in the analyses of this study.

3. Results

The results are presented for the two sets of transects beginning with data from the mid-channel transect, followed by results from the cross-channel transect. Finally, some synoptic statistical analyses are made relating microbial variables from all stations with hydrographic variables, and to each other, to investigate possible ecological patterns.

Stations 1, 2 and 3 represent a mid-channel transect along the estuarine salinity gradient, north to south toward the New York Bight. Surface observations collected along this transect (summarized in Table 1) could be directly compared on the three sampling dates to determine whether there were significant differences in hydrographic (temperature, salinity, and turbidity) or microbial variables (chlorophyll fluorescence, abundance and biomass of planktonic heterotrophic bacteria, Hflag, ciliates, and amebas) among stations along the transect. Salinity was significantly different among the three mid-channel stations ($P = 0.03$, Fig. 2A). However, no significant differences were found along the mid-channel transect for any of the remaining variables: hydrographic or microbial. Fig. 2B highlights the ameba biomass results for each day and location.

Stations 1, 4 and 5 represent a transect from mid-channel to near shore. Similar to the analysis described in the preceding paragraph, surface observations along this transect (summarized in Table 2) were compared on three dates to identify significant differences in hydrographic water characteristics and microbial variables. Temperature and salinity did not vary significantly among

Table 1
Summary of environmental variables measured at the surface along Hudson River Estuary mid-channel stations 1, 2, and 3 on three dates ($n = 9$ in each case). SD = 1 standard deviation, CV = coefficient of variation, Hflag = heterotrophic flagellate. An asterisk indicates significant differences between stations ($\alpha = 0.05$). See also Fig 2 for more details of mid channel salinity and ameba biomass observations.

	Minimum value	Maximum value	Mean ± 1 SD	CV × 100
Temperature (°C)	15.4	24.4	18.3 ± 3.3	18.0
Salinity	1.3	25.4	11.7 ± 8.2*	70.1
Turbidity (NTU)	2	22	11.2 ± 7.0	62.5
In-vivo chlorophyll fluorescence (RFU)	1.3	3.5	1.8 ± 0.7	38.9
Heterotrophic bacteria abundance (cells l^{-1})	2.0×10^9	8.9×10^9	$5.5 \pm 2.1 \times 10^9$	38.2
Heterotrophic bacteria biomass (μg C l^{-1})	41	177	109 ± 42	38.5
Hflag abundance (cells l^{-1})	1.0×10^7	2.5×10^7	$1.6 \pm 0.5 \times 10^7$	31.3
Hflag biomass (μg C l^{-1})	11	35	23 ± 9	39.1
Ciliate abundance (cells l^{-1})	730	2800	1500 ± 740	49.0
Ciliate biomass (μg C l^{-1})	1	2	1.3 ± 0.5	38.5
Ameba abundance (cells l^{-1})	71	325	154 ± 97	63.0
Ameba biomass (ng C l^{-1})	0.4	13	3.2 ± 4.2	131.3

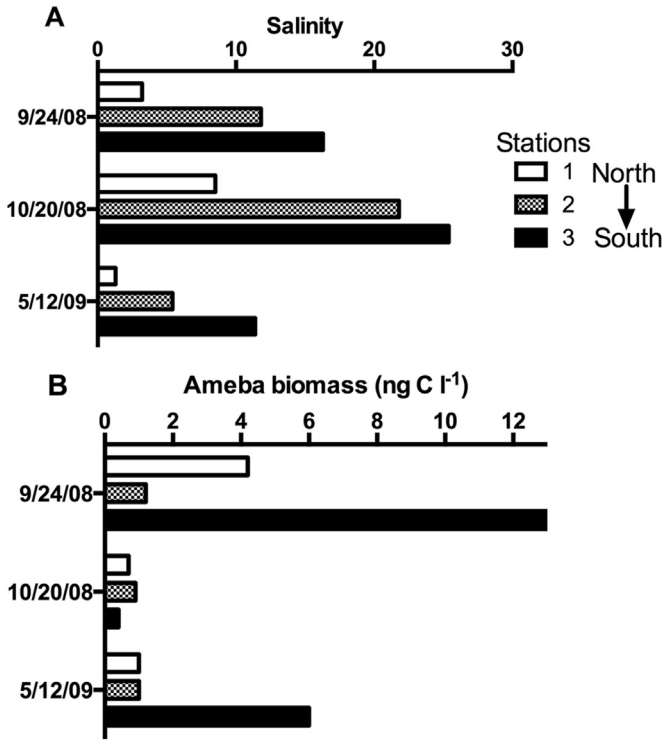

Fig. 2. Comparison of A) salinity and B) ameba biomass along a mid-channel transect heading towards the mouth of the Hudson River Estuary. Each bar represents the results for one of the three stations on the sampling dates shown.

stations. However, turbidity did increase significantly from mid-channel to near shore ($P = 0.03$, Fig. 3A). None of the microbial variables varied significantly. Fig. 3B highlights the ameba biomass results for each day and location. Comparing Tables 1 and 2, the coefficients of variation (standard deviation divided by the mean) for microbial variables were generally higher in Table 2. Thus, variability in microbial variables from near shore to the mid-channel was often higher than along the mid-channel salinity gradient over a distance of 44 km.

Comparing surface and near-bottom samples, temperature and salinity measurements (not shown) showed a salinity-stratified

Table 2
Summary of environmental variables measured at the surface along Hudson River Estuary cross-channel stations 1, 4, and 5 on three dates ($n = 9$ in each case). SD = 1 standard deviation, CV = coefficient of variation, Hflag = heterotrophic flagellate. An asterisk indicates significant differences between stations ($\alpha = 0.05$). See also Fig. 3 for more details of cross channel turbidity and ameba biomass observations.

	Minimum value	Maximum value	Mean ± 1 SD	CV × 100
Temperature (°C)	15.4	24.4	19.9 ± 2.9	14.6
Salinity	1.3	8.5	5.1 ± 2.8	54.9
Turbidity (NTU)	6	105	38.9 ± 30.2*	77.6
In-vivo chlorophyll fluorescence (RFU)	1.6	4.6	2.9 ± 1.1	37.9
Heterotrophic bacteria abundance (cells l^{-1})	4×10^9	1.3×10^{10}	$7.0 \pm 3.2 \times 10^9$	45.7
Heterotrophic bacteria biomass (μg C l^{-1})	80	257	140 ± 63	45.0
Hflag abundance (cells l^{-1})	1.0×10^7	3.5×10^7	$2.2 \pm 0.8 \times 10^7$	36.4
Hflag biomass (μg C l^{-1})	11	50	32 ± 13	40.6
Ciliate abundance (cells l^{-1})	750	7400	2740 ± 2040	74.3
Ciliate biomass (μg C l^{-1})	1	5	3 ± 2	66.7
Ameba abundance (cells l^{-1})	75	5990	1666 ± 2106	126.4
Ameba biomass (ng C l^{-1})	0.7	545	130 ± 226	173.8

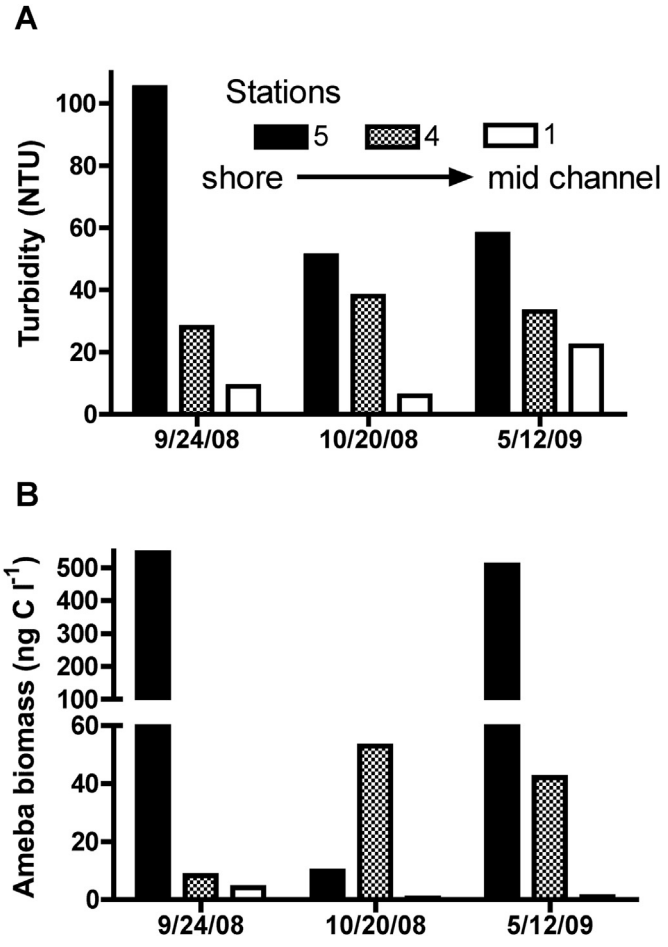

Fig. 3. Comparison of A) turbidity, and B) ameba biomass along a near shore to mid-channel transect in the lower Hudson River Estuary. Each bar represents the results for one of the three stations on the sampling dates shown.

water column that was nearly isothermal. The increase in salinity between the surface and bottom sample ranged from 0.6 to 13.4 on different dates, while temperature decreased by less than 1 °C with depth. Fig. 4A–D shows the mean (±1 SE) of surface and bottom biomass for each of the four heterotrophic microbial groups. There were no significant differences with depth for heterotrophic bacteria, Hflag or ciliates, either in terms of the biomass data, or in terms of abundance. In contrast, there was a significant increase in ameba abundance and biomass with depth ($P = 0.03$).

The hydrographic variables and microbial data from this study were also analyzed to determine environmental correlations ($n = 15$ in each case). Abundance and biomass of Hflag and amebas were positively correlated ($P < 0.01$) with chlorophyll fluorescence. Interestingly, ameba abundance and biomass were also highly significantly (positively) correlated with turbidity ($P < 0.001$). No other significant correlations were found between the abundance and biomass of the heterotrophic microbes and chlorophyll fluorescence, the hydrographic variables, or each other.

Finally, the relationships between turbidity and ameba abundance and biomass were examined in greater detail using regression analyses. Ameba abundance was found to increase linearly with turbidity ($r^2 = 0.75$, $P < 0.0001$, $n = 15$). Fig. 5A shows the relationship between ameba abundance and turbidity for the data from this study (open symbols). Four additional data points (closed symbols) from Lesen et al. (2010) are also shown. Adding these 4 data points had no significant effect on either the slope or intercept

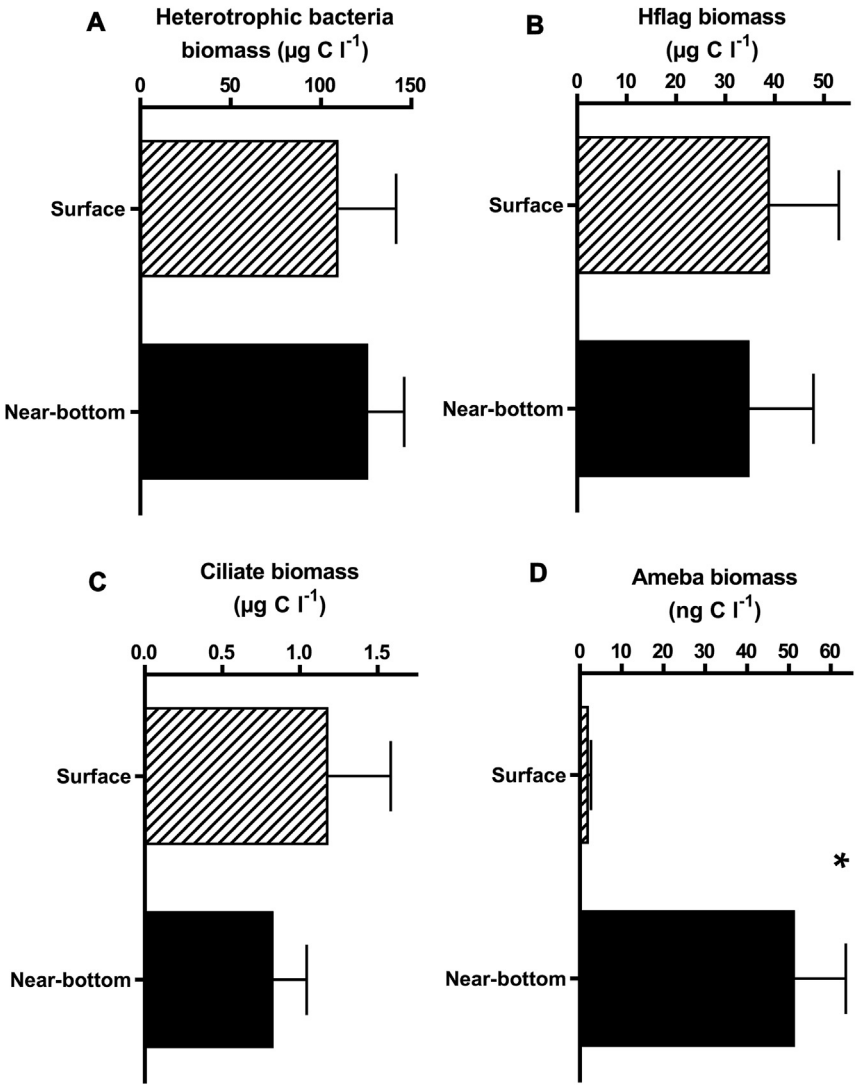

Fig. 4. Mean biomass (±1 SE, $n = 4$) of four microbial groups, A) heterotrophic bacteria, B) heterotrophic flagellates (=Hflag), C) ciliates, and D) amebas, in surface and near bottom-water samples from the lower Hudson River Estuary. The asterisk denotes a significant difference between surface and near bottom mean biomass. Note the difference in units for ameba biomass compared to the other microbial groups.

of the regression line, though the r^2 increased to 0.79 (as shown Fig. 5A). In contrast to ameba abundance, the relationship between ameba biomass and turbidity was best fit with a non-linear function (second-order polynomial, $r^2 = 0.70$). Adding the four data points from Lesen et al. (2010) to the regression, as shown in Fig. 5B, had no significant effect on any parameters of the regression equation ($P > 0.99$), though the r^2 increased to 0.89 (as shown Fig. 5B).

4. Discussion

Although limited in scope because of the labor-intensive methods needed to quantify amebas, this is the first study to survey the water column abundance and biomass of planktonic amoeboid protists at multiple geographic locations in an estuary. This is also the first geographic survey of planktonic heterotrophic protists (including amebas) in the water column of the lower HRE. Most previous work on planktonic protist ecology in the HRE has, because of logistical constraints, been based on sampling from a single near-shore station (Station 5 in this study, sampled by Anderson and Rogerson, 1995; Anderson, 2007, 2011; Lesen et al., 2010, and other studies). This study, therefore, provides valuable context for the earlier work. In addition to general observations of hydrography and microbial groups in the HRE, evidence supported the two main hypotheses of the study related to variability in ameba distributions. First, ameba abundance and biomass increased significantly from surface to near-bottom samples. Significant positive correlations between ameba abundance and biomass with turbidity were also found. Neither of these patterns were observed for the other protist groups enumerated.

Several strong environmental gradients in the HRE were demonstrated, particularly the expected north-south down estuary increase in salinity (Geyer and Chant, 2006) and a cross-channel gradient in turbidity. Two previous studies have found significant changes in heterotrophic bacteria counts along the salinity gradient in the HRE (Sañudo-Wilhelmy and Taylor, 1999; Taylor et al., 2003), though in one case there was a decrease from north to south, while in the other study the pattern was reversed. Thus, there is no consistent down-estuary trend in heterotrophic bacteria abundance in the HRE. In this study, there were no significant changes in the abundance or biomass of any planktonic microbial group along the mid-channel transect. The increase in turbidity from the mid

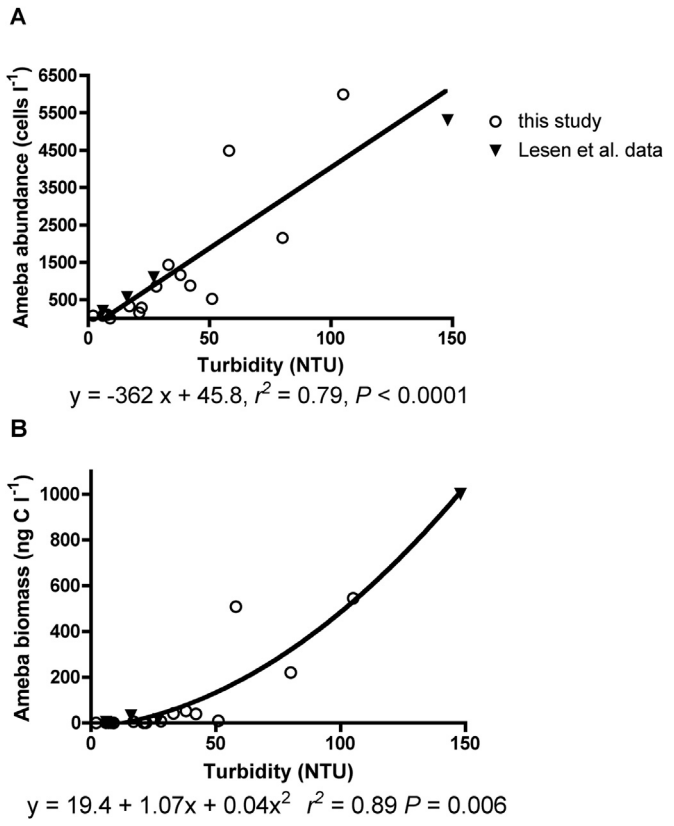

Fig. 5. Relationship between turbidity and A) ameba abundance and B) ameba biomass in the lower Hudson River Estuary. Four data points from earlier observations made at station 5 (Lesen et al., 2010) were included in the regression curves shown. In A, the *P*-value reflects the probability of a slope of 0. In B, the *P*-value reflects the probability that the x^2 coefficient is 0.

channel to the shore was consistent with general expectations related to shoreline inputs and resuspension, and previous observations of elevated near-shore turbidity (Suter et al., 2011). Some microbial variables, namely counts of fecal-indicator bacteria (Suter et al., 2011) and antibiotic-resistant bacteria (Young et al., 2013) have similarly been shown to increase significantly in near-shore, compared to mid-channel locations in the HRE. In this study, peak surface biomasses for all microbial groups were found at the near-shore station (station 5). However, neither the abundance, nor biomass of any of the microbial groups measured in this study varied significantly from mid channel to near shore. Nevertheless, it is worth pointing out that for many measurements relevant to aquatic microbes, variability over a few km across the channel equaled, or exceeded, the variability along the estuarine salinity gradient over tens of km. Further work on cross-channel variability of microbial parameters in estuaries may therefore be warranted. For this study, the high variability in microbial abundance throughout the estuary, and the relatively small number of samples examined, may account for the inability to detect statistically significant trends in microbial parameters along the two transects examined.

Abundances of heterotrophic bacteria, Hflag and ciliates reported here were similar to those reported in earlier studies in the HRE (Anderson, 2007; Lesen et al., 2010; Suter et al., 2011). For example, heterotrophic bacterial abundance estimates in this study all fall within one standard deviation of the average that Suter et al. (2011) described for surface samples in the lower HRE ($9.2 \pm 6.4 \times 10^9$ cell l^{-1}) and were also consistent with mean heterotrophic bacterial abundance in the HRE of ~8×10^9 cells l^{-1} reported by Findlay (2006). Similarly, abundance estimates of planktonic Hflag and ciliates in this study overlapped with data in Lesen et al. (2010). Our mean Hflag abundance of approximately 2×10^7 l^{-1} is comparable to expectations for estuarine environments (Davis et al., 1985; Capriulo, 1990) and also consistent with cross-system relationships between Hflag abundance and heterotrophic bacterial count (Berninger et al., 1991). Ciliate abundance in estuaries is highly variable, commonly ranging from 10^2–10^5 cells l^{-1} across different systems (Porter et al., 1985). We found an average ciliate abundance of 2–4×10^3 l^{-1} in this study, consistent with reports by Duecker and Sambrotto (2006) of ciliate abundances in the range of 10^3–10^4 l^{-1} for HRE samples taken in June and July.

Previous observations of planktonic ameba abundance and biomass in the HRE were all based on samples collected from the location designated in this study as station 5. Data in Lesen et al. (2010) show that ameba abundances near station 5 have ranged approximately 10^3–10^4 l^{-1} in September–October, and approximately 10^3–10^5 l^{-1} in May. Lesen et al. (2010) found a mean ameba biomass over multiple years of observations at this location of approximately 540 ng C l^{-1}, with a median of 90 ng C l^{-1}. As can be seen in Fig. 3B, the data in this study from station 5 fall within the range of those previous observations, although peak ameba abundance and biomass measured in this study were substantially lower than peak values described previously (Anderson and Rogerson, 1995; Lesen et al., 2010). Considering previous research and the observations in this study, near shore station 5 has often had higher ameba abundance and biomass than most other locations examined.

Among the 3 heterotrophic protist groups enumerated, Hflag were the only group whose biomass was comparable to the heterotrophic bacteria, suggesting that Hflag are responsible for the majority of bacterivory in the HRE (Lesen et al., 2010), as has also been described in other estuaries (e.g., Strom, 2000). Nevertheless, in this study, Hflag abundance and biomass were correlated with chlorophyll fluorescence, but not significantly with heterotrophic bacteria counts. In fact, although each of the protist groups enumerated likely contribute to bacterivory in the HRE, none of the groups were significantly correlated with heterotrophic bacteria concentrations. The evidence of ameba and Hflag correlation with heterotrophic bacteria in terrestrial environments (e.g., Anderson and McGuire, 2013; Anderson, 2014) may reflect the more limited availability of the bacteria in some terrestrial habitats compared to the highly productive HRE, where organic nutrients may be more abundant to support heterotrophic bacteria in suspension. The positive correlations between chlorophyll fluorescence and both Hflags and amebas could indicate that these groups are using phytoplankton as prey, even though both groups are generally considered to rely primarily on bacterivory (e.g., Sherr and Sherr, 1991; Anderson, 2012).

There were several other important relationships evident in the ameba data. Unlike the other microbial groups, ameba abundance and biomass increased significantly in near bottom compared to surface samples. In addition, ameba abundance and biomass also increased significantly with turbidity (in surface samples), another pattern that was absent for the other microbial groups. The ameba depth and turbidity patterns may be related to each other. Although we were not able to measure turbidity in the near bottom samples we collected, other work within this portion of the estuary has shown that turbidity generally increases with depth below the pycnocline (Orton and Kineke, 2001). The relationships of amebas with depth and turbidity could both be connected to resuspension from the sediment, or may simply be a reflection of the benefit of increasing particle concentration on density of amebas, because they are particle-associated, surface-grazing microorganisms.

Ameba concentrations in aquatic sediments can be relatively high (Butler and Rogerson, 1996; Rogerson et al., 1996; Butler and Rogerson, 1997), and resuspended sediment particles could carry attached amebas into the water column. Murzov and Caron (1996) similarly proposed that resuspension could explain spatial variability in ameba concentration observed in the Black Sea. In addition, amebas only feed when associated with particles (Pickup et al., 2007), therefore a positive relationship with particle concentration is not surprising, even if those particles did not originate in the sediments. Some species of Hflags and ciliates found in the water column are also known to be particle associated, feeding preferentially on attached bacteria (e.g., Caron, 1987; Wörner et al., 2000; Kiorboe et al., 2004). It is possible that these other particle-associated protists would have similar relationships with turbidity as amebas, however, we were not able to distinguish the particle-associated Hflag and ciliate species from the free-living ones in this study.

With respect to the increase in ameba abundance in deeper samples, it is worth noting that distance from the bottom, rather than depth per se, may be the key factor related to the locations we sampled that had higher ameba abundance and biomass. For example, both the near-bottom samples collected at mid-channel stations and the near-shore samples from station 5 were collected from <1 m above the local bottom. If resuspension from the bottom was a quantitatively important source of ameba cells, the distance from the bottom at these two locations may explain the higher abundance (relative to mid-channel surface) more than depth or distance from shore. As this was the first study of the depth distribution of amebas in an estuary, further research will be needed to determine the consistency and driving factors of these patterns. Nonetheless, the data suggest that turbidity and suspended particle concentrations are likely important indicators for ameba abundance and biomass.

Compiling the available surface sample data, different relationships with turbidity were found for ameba abundance and ameba biomass; ameba abundance increased linearly, while ameba biomass increased non-linearly with turbidity. This difference indicates that as turbidity increased, larger amebas became disproportionally more abundant, contributing more biomass per cell. One possibility is that a greater number of larger particles are present as turbidity increases. Larger particles have been shown to carry larger amebas, higher ameba morphospecies diversity, and higher ameba biomass per particle (Anderson, 2011).

The concentration and biomass of amebas was low in most of the samples collected during this study compared to previous work, presumably indicating lowered biogeochemical importance than would be predicted from the shallow, near-shore sampling that was the basis of previous ameba research within the HRE. Nevertheless, several important patterns within the estuary were evident. Previous work demonstrated clear seasonality, despite high variability in ameba abundance (Anderson and Rogerson, 1995; Lesen et al., 2010). This study demonstrated that ameba abundance and biomass were higher near the bottom and in locations where turbidity increased. Thus, there is evidence that ameba distribution in the water column, though highly variable, is not random. Given that amebas can at times make a quantitatively important contribution to local biogeochemical processes and trophic food webs (e.g., Murzov and Caron, 1996; Lesen et al., 2010; Anderson, 2012) the increased ability to predict when ameba abundance in the water column is high will be of value for future research on estuarine microbial ecology. The culture-based method we necessarily used provides a conservative estimate of ameba abundance and biomass, and future improved methods of enumeration may show even greater contributions of amebas to ecosystem functions in other productive and turbid environments, such as the HRE.

5. Conclusions

As hypothesized, ameba abundance and biomass in the HRE water column were correlated with turbidity, and also increased near the bottom. These two patterns may be linked, either because amebas are favored when particle concentrations increase, or because of resuspension of amebas and particles from the bottom. Salinity in the HRE varied significantly along the mid-channel stations, and turbidity varied significantly from mid channel to near shore. However, neither the abundance nor biomass of any planktonic microbial group measured varied consistently along those gradients. We found that none of the heterotrophic protist groups were correlated with heterotrophic bacterial biomass, although all three groups (amebas, ciliates and Hflag) are known to prey heavily on bacteria, as well as smaller protists. Positive correlations between chlorophyll fluorescence and Hflag and ameba biomass suggested that phytoplankton may also serve as important prey for these groups in the HRE. By demonstrating strong environmental correlates of ameba abundance and biomass, the results of this study will aid future work to assess the biogeochemical and ecological importance of this diverse group of heterotrophic protists that are ubiquitous in aquatic habitats.

Acknowledgments

We thank Capt. John Lipscomb and Riverkeeper for their assistance in collecting and processing field samples. Comments from 2 anonymous reviewers greatly improved the clarity of the manuscript. This manuscript is based upon work supported by the US National Science Foundation under Grant No. OCE0845799. Additional critical support was provided by the Hudson River Foundation and the Brinson Foundation. This is Lamont-Doherty Earth Observatory Contribution No. 7837.

References

Anderson, O.R., 1997. The fine structure of a marine ameba associated with a blue-green alga in the Sargasso Sea. J. Protozool. 24, 370–376.
Anderson, O.R., 2006. A method for estimating cell volume of amoebae based on measurements of cell length of motile forms: physiological and ecological applications. J. Euk. Microbiol. 53, 185–187.
Anderson, O.R., 2007. A seasonal study of the carbon content of planktonic naked amoebae in the Hudson Estuary and in a productive freshwater pond with comparative data for ciliates. J. Euk. Microbiol. 54, 388–391.
Anderson, O.R., 2011. Particle-associated planktonic naked amoebae in the Hudson Estuary: size-fraction related densities, cell sizes and estimated carbon content. Acta Protozool. 50, 15–22.
Anderson, O.R., 2012. The role of bacterial-based protist communities in aquatic and soil ecosystems and the carbon biogeochemical cycle, with emphasis on naked amoebae. Acta Protozool. 51, 209–221.
Anderson, O.R., 2014. Microbial communities associated with tree bark foliose lichens: a perspective on their microecology. J. Euk. Microbiol. 61, 364–370.
Anderson, O.R., Rogerson, A., 1995. Annual abundances and growth-potential of gymnamoebae in the Hudson Estuary with comparative data from the Firth of Clyde. Eur. J. Protistol. 31, 223–233.
Anderson, O.R., McGuire, K., 2013. C-biomass of bacteria, fungi, and protozoan communities in Arctic tundra soil, including some trophic relationships. Acta Protozool. 52, 217–227.
Anderson, O.R., Gorrell, T., Bergen, A., Kruzansky, R., Levandowsky, M., 2001. Naked amoebas and bacteria in an oil-impacted salt marsh community. Microb. Ecol. 42, 474–481.
Berninger, U.G., Finlay, B.J., Kuuppoleinikki, P., 1991. Protozoan control of bacterial abundances in freshwater. Limnol. Oceanogr. 36, 139–147.
Bonkowski, M., 2004. Protozoa and plant growth: the microbial loop in soil revisited. New. Phytol. 162, 617–631.
Butler, H., Rogerson, A., 1996. Growth potential, production efficiency and annual production of marine benthic naked amoebae (gymnamoebae) inhabiting sediments of the Clyde Sea area, Scotland. Aquat. Microb. Ecol. 10, 123–129.
Butler, H., Rogerson, A., 1997. Consumption rates of six species of marine benthic naked amoebae (gymnamoebia) from sediments in the Clyde Sea area. J. Mar. Biol. Assoc. U. K. 77, 989–997.
Capriulo, G.M., 1990. Feeding-related ecology of marine protozoa. In: Capriulo, G.M. (Ed.), Ecology of Marine Protozoa. Oxford University Press, New York, pp. 186–259.

Caron, D.A., 1987. Grazing of attached bacteria by heterotrophic microflagellates. Microb. Ecol. 13, 203–218.
Caron, D.A., Davis, P.G., Madin, L.P., Sieburth, J.M., 1982. Heterotrophic bacteria and bacterivorous protozoa in oceanic macroaggregates. Science 218, 795–797.
Clarholm, M., 1981. Protozoan grazing of bacteria in soil – impact and importance. Microb. Ecol. 7, 343–350.
Cole, J.J., Caraco, N.F., 2006. Primary production and its regulation in the tidal-freshwater Hudson River. In: Levinton, J., Waldman, J. (Eds.), The Hudson River Estuary. Cambridge University Press, New York, pp. 107–120.
Davis, P.G., Caron, D.A., Sieburth, J.M., 1978. Oceanic amoebae from the North Atlantic: culture, distribution, and taxonomy. Trans. Am. Microsc. Soc. 96, 73–88.
Davis, P.G., Caron, D.A., Johnson, P.W., Sieburth, J.M., 1985. Phototrophic and apochlorotic components of picoplankton and nanoplankton in the North Atlantic: geographic, vertical seasonal and diel distributions. Mar. Ecol. Prog. Ser. 21, 15–26.
Ducklow, H., 2000. Bacterial production and biomass in the oceans. In: Kirchman, D.L. (Ed.), Microbial Ecology of the Oceans. Wiley, New York, pp. 85–120.
Duecker, M.E., Sambrotto, R., 2006. Summer microbial populations in the lower Hudson River Estuary and their relationship to dissolved organic nutrients. Section II. In: Nieder, W.C., Waldman, J.R. (Eds.), Final Reports of the Tibor T. Polgar Fellowship Program, 2005. Hudson River Foundation, New York, p. 25.
Findlay, S.E.G., 2006. Bacterial abundance, growth, and metabolism in the tidal freshwater Hudson River. In: Levinton, J., Waldman, J. (Eds.), The Hudson River Estuary. Cambridge University Press, New York, pp. 99–106.
Geyer, R.W., Chant, R., 2006. The physical oceanography processes in the Hudson River Estuary. In: Levinton, J., Waldman, J. (Eds.), The Hudson River Estuary. Cambridge University Press, New York, pp. 24–38.
Howarth, R., Marino, R., Swaney, D., Boyer, E., 2006. Wastewater and watershed influences on primary productivity and oxygen dynamics in the lower Hudson River estuary. In: Levinton, J., Waldman, J. (Eds.), The Hudson River Estuary. Cambridge University Press, New York, pp. 121–139.
Juhl, A.R., 1991. The Biology of a Shallow-water Methane Vent. Appendix 1. Statistical Analysis of the Direct Count Method (MS thesis). Oregon State University.
Kiorboe, T., Grossart, H.P., Ploug, H., Tang, K., Auer, B., 2004. Particle-associated flagellates: swimming patterns, colonization rates, and grazing on attached bacteria. Aquat. Microb. Ecol. 35, 141–152.
Landeck-Miller, R., St John, J., 2006. Modeling primary production in the lower Hudson River estuary. In: Levinton, J., Waldman, J. (Eds.), The Hudson River Estuary. Cambridge University Press, New York, pp. 140–156.
Laybourn-Parry, J., 1992. Protozoan Plankton Ecology. Chapman and Hall, London, 231 pp.
Lesen, A.E., Juhl, A.R., Anderson, O.R., 2010. Heterotrophic microplankton in the lower Hudson River Estuary: potential importance of naked, planktonic amebas for bacterivory and carbon flux. Aquat. Microb. Ecol. 61, 45–56.
Mayes, D.F., Rogerson, A., Marchant, H., Laybourn-Parry, J., 1997. Growth and consumption rates of bacterivorous Antarctic naked marine amoebae. Mar. Ecol. Prog. Ser. 160, 101–108.
Mayes, D.F., Rogerson, A., Marchant, H.J., Laybourn-Parry, J., 1998. Temporal abundance of naked bacterivore amoebae in coastal East Antarctica. Estuar. Coast. Shelf Sci. 46, 565–572.
Menden-Deuer, S., Lessard, E.J., 2000. Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnol. Oceanogr. 45, 569–579.
Montagnes, D.J.S., Berges, J.A., Harrison, P.J., Taylor, F.J.R., 1994. Estimating carbon, nitrogen, protein, and chlorophyll-a from volume in marine phytoplankton. Limnol. Oceanogr. 39, 1044–1060.
Mullen, T.E., Nevis, K.R., O'Kelly, C.J., Gast, R.J., Frasca, S., 2005. Nuclear small-subunit ribosomal RNA gene-based characterization, molecular phylogeny and PCR detection of the neoparamoeba from western Long Island Sound lobster. J. Shellfish Res. 24, 719–731.
Murzov, S.A., Caron, D.A., 1996. Sporadic high abundances of naked amoebae in Black Sea plankton. Aquat. Microb. Ecol. 11, 161–169.
Nikolaev, S.I., Berney, C., Fahrni, J.F., Mylnikov, A.P., Petrov, N.B., Pawlowski, J., 2004. The twilight of Heliozoa and rise of Rhizaria, an emerging supergroup of amoeboid eukaryotes. Proc. Nat. Acad. Sci. U. S. A. 101, 8066–8071.
Nikolaev, S.I., Berney, C., Petrov, N.B., Mylnikov, A.P., Fahrni, J.F., Pawlowski, J., 2006. Phylogenetic position of *Multicilia marina* and the evolution of Amoebozoa. Int. J. Syst. Evol. Microbiol. 56, 1449–1458.
Orton, P.M., Kineke, G.C., 2001. Comparing calculated and observed vertical suspended-sediment distributions from a Hudson River Estuary turbidity maximum. Estuar. Coast. Shelf Sci. 52, 401–410.
Page, F.C., 1983. Marine gymnamoebae. Inst. of Terr. Ecol., Cambridge, 54 pp.
Page, F.C., 1988. A New Key to Freshwater and Soil Gymnamoebae. Freshwater Biol. Ass., Ambleside, 122 pp.
Pickup, Z.L., Pickup, R., Parry, J.D., 2007. A comparison of the growth and starvation responses of *Acanthamoeba castellanii* and *Hartmannella vermiformis* in the presence of suspended and attached *Escherichia coli* K12. FEMS Microbiol. Ecol. 59, 556–563.
Porter, K.G., Sherr, E.B., Sherr, B.F., Pace, M., Sanders, R.W., 1985. Protozoa in planktonic food webs. J. Protozool. 32, 409–415.
Rogerson, A., Anderson, O.R., Vogel, C., 2003. Are planktonic naked amoebae predominately floc associated or free in the water column? J. Plankton Res. 25, 1359–1365.
Rogerson, A., Hannah, F., Gothe, G., 1996. The grazing potential of some unusual marine benthic amoebae feeding on bacteria. Eur. J. Protistol. 32, 271–279.
Rogerson, A., Gwaltney, C., 2000. High numbers of naked amoebae in the planktonic waters of a mangrove stand in southern Florida, USA. J. Euk. Microbiol. 47, 235–241.
Rogerson, A., Hauer, G., 2002. Naked amoebae (Protozoa) of the Salton Sea, California. Hydrobiologia 473, 161–177.
Sañudo-Wilhelmy, S.A., Taylor, G.T., 1999. Bacterioplankton dynamics and organic carbon partitioning in the lower Hudson River Estuary. Mar. Ecol. Prog. Ser. 182, 17–27.
Sherr, B.F., Sherr, E., 1991. Proportional distribution of total numbers, biovolume, and bacterivory among size classes of 2-20 μm nonpigmented marine flagellates. Mar. Microb. Food Webs 5, 227–237.
Smirnov, A.V., Chao, E., Nassonova, E.S., Cavalier-Smith, T., 2011. A revised classification of naked lobose amoebae (Amoebozoa: Lobosa). Protist 162, 545–570.
Strom, S., 2000. Bacterivory: interactions between bacteria and their grazers. In: Kirchman, D.L. (Ed.), Microbial Ecology of the Oceans. Wiley, New York, pp. 351–386.
Suter, E., Juhl, A.R., O'Mullan, G., 2011. Abundance and particle association of *Enterococci* and total bacteria in the lower Hudson River Estuary, USA. J. Water Res. Prot. 3, 715–725.
Taylor, G.T., Way, J., Scranton, M.I., 2003. Planktonic carbon cycling and transport in surface waters of the highly urbanized Hudson River Estuary. Limnol. Oceanogr. 48, 1779–1795.
Utermöhl, H., 1936. Quantitativen Methoden zur Untersuchung des Nanoplanktons. In: Handb. Biochem. Arbeitsmeth, vol. 9, pp. 1879–1937.
Wörner, U., Zimmerman-Timm, H., Kausch, H., 2000. Succession of protists on estuarine aggregates. Microb. Ecol. 40, 209–222.
Young, S., Juhl, A., O'Mullan, G.D., 2013. Antibiotic-resistant bacteria in the Hudson River Estuary linked to wet weather sewage contamination. J. Water Health 11, 297–310.