Acta Protozool. (2011) 50: 15–22
http://www.eko.uj.edu.pl/ap

ACTA
PROTOZOOLOGICA

Particle-associated Planktonic Naked Amoebae in the Hudson Estuary: Size-fraction Related Densities, Cell Sizes and Estimated Carbon Content

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Summary. Naked amoeba densities, sizes, biodiversity and carbon content were examined in relation to two particle size fractions (< 200 µm and > 200 µm) of suspended matter in the water column of the Hudson Estuary at a near-shore location south of the Tappan Zee, Palisades, New York. The densities varied markedly among the two particle fractions, and therefore the mean densities were not significantly different between the larger and smaller particle fractions. In contrast, the mean sizes and mean carbon content were statistically greater on larger size suspended particles compared to smaller size particles. There was a broader size range of amoebae on the larger particles, including very large *Cochliopodium*, *Vannella*, *Mayorella*, and *Hartmannella* species suggesting a larger biodiversity, also indicated by a larger diversity coefficient for the > 200-µm-particle fraction compared to the < 200-µm-particle fraction, 4.51 and 4.18, respectively. In conclusion, the size of suspended particulates in the water column of near-shore, estuarine habitats may have a significant influence on the composition of naked amoebae communities and their ecological roles, especially the organization of particle-associated microbial food webs.

Key words: Aquatic food webs, carbon budgets, microbial ecology, micro-habitats, protists.

INTRODUCTION

This is the first report of the densities, biodiversity and estimated carbon content of naked amoebae associated with different size fractions of suspended particles in an aquatic water column. Naked amoebae feed during locomotion by pseudopodial engulfment and enclosure of the prey in food vacuoles. Hence, amoebae must attach to surfaces in order to locomote and feed. Previous research on planktonic naked amoebae in coastal waters has shown that approximately 90% are particle-associated and can be deeply situated within loose floc particulates (Rogerson *et al.* 2003). Naked amoebae may reach relatively large densities (2,000 to 104,000/l) in highly productive habitats such as mangrove stands (Rogerson and Gwaltney 2000), Antarctic coastal water (Mayes *et al.* 1998), or in shallow, organically rich temperate ponds (Anderson 2007) – at times exceeding the densities of ciliates. High densities of particulates in the water column of coastal waters, and other productive aquatic habitats (e.g. Kiss *et al.* 2009, Zimmermann-Timm *et al.* 1998), support substantial communities of protists, and provide large surface areas for naked amoebae to attach and feed. Emerging evidence, based on modern experimental methods,

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indicates that particle-associated planktonic amoebae may be more significant bacterial predators and contribute more substantially to carbon flux in estuarine water masses than previously realized (e.g. Lesen *et al.* 2010), indicating the need for more intensive research on their ecology. The role of naked amoebae in aquatic habitats is less well documented than in terrestrial habitats (e.g. Adl and Gupta 2006). Although the diversity and densities of naked amoebae have been examined for some coastal locations, including estuaries, less information is available on the ecology of naked amoebae in relation to differences in suspended particle sizes. This research examined planktonic naked amoebae densities, biodiversity, and carbon content in relation to size variations of suspended particles in the Hudson Estuary. The research questions were: to what extent do floc and other suspended particles of varying size and composition support different assemblages of naked amoebae among two different size classes of particulates; and how does the size and carbon content of naked amoebae vary for particles of larger size compared to those of smaller size?

MATERIALS AND METHODS

Sampling sites and sample collection

Altogether seven, near-shore, surface water samples were obtained from the Hudson lower estuary USA at two locations (41°00 42.28 N, 73°54 11.14 W and 41°02 35.05 N, 73°53 46.94 W) during May, August and September of 2009. The samples were immediately returned to the laboratory and subsamples (200 or 400 ml, depending on the density of particulates) were removed and gently filtered through a 200-µm Nitex mesh filtration assembly (Fig. 1).

The mesh was contained in the base of a 3-cm diameter, cylindrical filter assembly. The hand-held cylindrical filter assembly was grasped between the thumb and forefinger, and the 5-cm diameter watch glass was supported beneath the filter assembly by grasping its rim between the lower edge of the thumb and the third finger of the same hand. The base of the filter assembly was positioned such that a small pool of filtrate was collected beneath the filter screen to reduce impact of the filtered particles against the mesh surface during the filtration process. As the sample was poured into the filter assembly, the filtrate overflowed out of the watch glass and was collected in a 500-ml beaker positioned beneath the watch glass (Fig. 1). The small volume of filtrate remaining in the watch glass at the end of the filtration was combined with the total filtrate when the filter assembly was removed. Thus, the filtrate, containing the < 200-µm fraction, was collected in a separate beaker placed beneath the filtration apparatus. The > 200-µm particulates on the Nitex filter were immediately back washed gently into a second graduated beaker using 0.45 µm micropore-filtered water (MFW) from the collection site and resuspended to the original volume of the water sample using MFW. Thus, the volume of the filtrate containing < 200-µm fraction and that of the final volume of the resuspended > 200-µm particulates collected on the filter was identical.

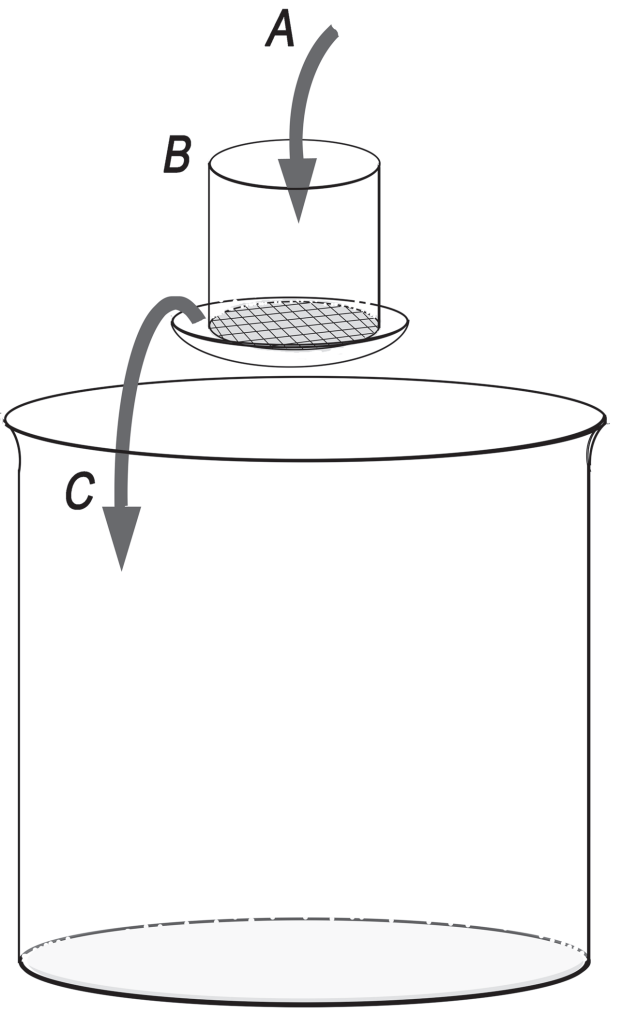

Fig. 1. Schematic diagram of the filtration apparatus used to collect suspended particulates. A water sample is poured gently **(A)** into the cylindrical filter assembly **(B)** containing a 200-µm mesh at the base; and the entire assembly is nestled loosely within a concave watch glass to provide a pool of residual water to reduce impact of the filtered particles against the mesh during filtration. The overflow **(C)** of the filtrate is collected in a large beaker situated beneath the filter assembly. The collected, filtered particles remain suspended in the base of the filtration apparatus after completion of filtration due to the small pool of residual water in the watch glass beneath the filter assembly.

Amoeba counting and size-estimation methods

Amoeba densities in the < 200-µm and > 200-µm particle suspensions were assessed by a Culture Observation Method (COM) routinely used in our laboratory (e.g. Anderson and Rogerson 1995;

Anderson 2007). This method avoids some of the biases and limitations of serial dilution culture methods. Basically, a suspension of the water sample was deposited in a graduated 15-ml capped centrifuge tube and gently agitated to disperse particulates and promote dislodgement of amoebae from the particle surfaces. A small aliquot (e.g. 10 µl) of the suspension was deposited into each well of a 24-well, sterile Falcon culture dish, where each well contained 2 ml of MFW and a small cube of malt-yeast agar (MYA) to support food bacteria for amoeba predation and growth (Page 1988). After 10 to 14 days, each well was examined with a Nikon Diaphot inverted, compound phase contrast microscope using a 40 × objective. Amoebae as small as 5 µm were readily visible. Phase contrast is necessary to visualize the smallest amoebae and those with a thin, flattened morphology (e.g. Page 1988). The presence of each morphospecies of amoeba in each well was tallied as evidence that at least one individual of this morphospecies was present in the 10-µl aliquot of sample deposited in the well. Based on the total number of each morphospecies of amoebae in the 24 wells, its estimated density per liter of sample was calculated on the proportional basis that the 24 wells contained all-totaled 240 µl of deposited sample. Therefore, the number of amoebae per liter = (number counted/240 µl) × 4,166; the latter being the equivalent number of 240-microliter aliquots in one liter. A Gaussian correction for possible underestimation of the more frequently occurring morphospecies (e.g. Anderson 2007) was included in the Excel® program used to compute the amoeba densities. The COM method also includes a method of estimating the densities of encysted naked amoebae. A 10-µl aliquot of the water sample was deposited in each well of a Falcon plastic culture dish where the wells were dry; no MFW was added at this step. The small aliquot was completely dried gently under flowing air at ambient laboratory temperature. Only encysted naked amoebae survive the gentle drying step. Two ml of MFW were added to each well along with a small cube of MYA, and the number of morphospecies that emerged during the incubation period of 10 to 14 days was enumerated and sized as was done for the non-dried COM method. This provides an estimate of the densities of encysted naked amoebae that were capable of withstanding the drying step. While the amoebae were being counted for COM (and dried COM) with a Nikon Diaphot inverted phase contrast microscope, they also were measured using a reticule divided in 0.5-µm units. The amoeba size was used to estimate cell volume and carbon content based on a regression equation (Anderson 2006a, 2007). Because much of the carbon content is particularly related to amoebae of larger size and hence greater volume, the COM method is sufficiently sensitive to make these carbon content estimates. Larger amoebae are typically sparsely distributed among the 10-µl aliquots and thus, the counting method is likely to provide sufficient accuracy in detecting them to make a good estimate of the carbon content. The COM method has been shown to be reliable in many different applications, but it is important to comment that it provides, at best, an underestimation of total amoeba densities; because some species may not grow out readily in laboratory cultures. While it would be desirable to also count the number of naked amoebae on each particle by direct microscopic observation, many of the particles in these near-shore samples are opaque (brown to black) segments of decaying leaves and other plant material, thus preventing direct visualization of the amoebae. Also smaller amoebae, even in more transparent particulates, are not easily identified, and sometimes cannot be differentiated from similar-sized amoeboflagellates or other small heterotrophic protists. Thus, overall, the COM analysis appeared to be the best method of enumeration in this study.

Sedimented volume of particles in the two size fractions

The total sedimented volume of the particulates in the < 200-µm and in the > 200-µm size fraction was obtained by gently centrifuging (1,000 rpm) a subsample of each suspension in a graduated conical centrifuge tube and the volume of the resulting pellet was recorded. To calibrate the accuracy of the measurement, a fine line was made on the tube to mark the volume of the pellet. The pellet was withdrawn, and an equivalent volume of distilled water (filled to the same volume mark on the tube) was deposited in the graduated centrifuge tube. Then, the water was quantitatively removed with a Pasteur pipette and weighed on a Mettler precision balance. The volume of the introduced water was calculated based on the specific gravity of water (1 ml/g).

Bacterial densities

Bacterial densities (number/l) for each of the two particle size fractions were obtained by fluorescent counting adapted from the method of Hobbie *et al.* (1977), using SYBR green fluorescent stain (Lesen *et al.* 2010). The fluorescently stained preparations, collected on 0.5 µm pore-sized Teflon filters, were counted using a Leitz Laborlux 11, ultraviolet epiflourescent microscope with an oil-immersion lens. The estimates of bacterial densities in the < 200-µm particle fraction included those attached to the particles as well as those in suspension, because the smaller size fraction was obtained as the total filtrate recovered from the Nitex-filtration step. Data are presented as mean densities ± s.e. for three sample dates in May during the spring bloom (May 21, 22 and 23, 2009). An additional sample for bacterial counts was taken in August and one in September during the subsequent two summer sampling periods. During enumeration of the bacteria, they were also counted in three broad groups: bacilli, cocci, and other (vibrios, etc.). Carbon content of bacteria was estimated based on prior published regression equations relating carbon content to cell volume (e.g. Anderson 2008).

Additional estimates of particle-size related amoeba densities

To further estimate the size of amoebae associated with suspended particles of different sizes, particles of representative sizes in the range of 0.5 to 4.0 mm were gently selected from samples of the estuary water using a fine pipette fitted with a rubber bulb. Particles included amorphous floc, small fragments of decaying plant matter, other suspended flake-like organic matter, and clumps or filaments of algae. Each particle was deposited in one well of a 24-well sterile Falcon culture dish, where each well contained 2 ml of MFW and a small cube of malt-yeast nutrient agar to support bacterial growth as prey for the amoebae, similar to the procedure used for COM. The maximum dimension (length) of each particle was measured using a magnifier and a millimeter scale. The size of amoebae that grew out from each particle was determined using the same measuring reticule and Diaphot microscope as for the COM. The size ranges in millimeters for each particle were determined to within 0.5 mm and the data were expressed as four particle size classes: < 1.0, 1.0–1.5, 2.0–2.5, and 3–3.5 mm.

Statistical methods

Diversity of amoebae associated with the < 200-µm and > 200-µm particles was estimated using the Shannon-Weaver diversity coefficient (H). $H = 1 - \sum p_i \log_2 p_i$ where p_i is the proportion of each morphospecies relative to the total number identified. Moreover, a bar graph comparing the numbers of amoebae per size class within each 10-µm range, for the < 200-µm particles and the > 200-µm particles, was plotted to visually present evidence of amoeba size diversity between the two particle fractions (see Fig. 2). Statistical significance of mean differences for amoeba densities, sizes and carbon content was determined by using a paired t-test, with a significance level (α of 0.05 or less. Values for each morphospecies were entered in the statistical data array. A paired t-test was used because the two sets of particle size fractions were not independently assorted, both were collected at the same site and on the same day.

RESULTS

Comparative data for the < 200-µm and > 200-µm particles

Amoeba densities, sizes and carbon content. The results (means ± s.e.) of the comparison of amoeba data for the < 200-µm- and > 200-µm-size particles, obtained for particles suspended in the water, are presented in Table 1. The mean densities of amoebae (number/l of water suspension) in the two size fractions of particles were not statistically different, given the errors of the means, i.e. < 200 µm: 636 ± 148 and > 200 µm: 541 ± 127 (t = 1.24, df = 54, p = 0.22). There was consid-

Fig. 2. Bar graph comparison of the percentage of naked amoebae expressed per total counted (ordinate) for each particle size fraction (abscissa) categorized by size ranges (µm) for the < 200 µm-size fraction (grey) and > 200 µm-size fraction (opaque).

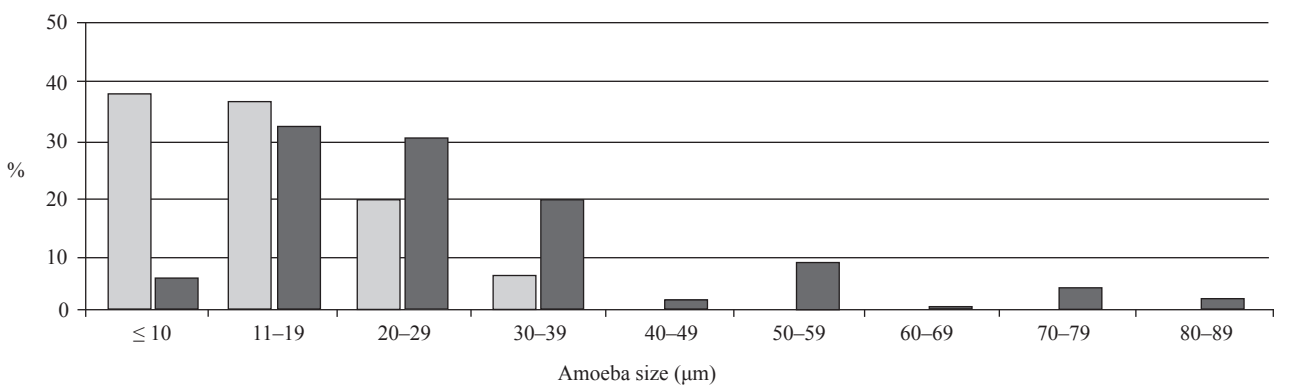

Table 1. Naked amoeba densities, size ranges and carbon content related to particle size fractions (< 200 µm and > 200 µm)[a]

Dates	Densities (No./l)		Mean sizes (µm)		Carbon content (µg/l)	
	< 200 µm	> 200 µm	< 200 µm	> 200 µm	< 200 µm	> 200 µm
5/21	390 (25)	647 (6)	15.3 (5–38)	25.9 (8–80)	0.03 (44)	0.49 (0.2)
5/22	200 (35)	129 (33)	15.7 (8–30)	23.8 (8–70)	0.02 (38)	0.15 (3)
5/23	347 (11)	1,110 (10)	17.1 (8–30)	24.3 (8–76)	0.01 (33)	0.29 (2)
5/27	1452 (8)	883 (13)	9.8 (5–20)	21.3 (8–50)	0.19 (10)	0.60 (1)
8/26	864 (37)	553 (32)	16.6 (10–38)	30.7 (13–50)	0.04 (45)	0.42 (9)
9/20	701 (39)	213 (55)	13.9 (5–20)	20.8 (15–38)	0.10 (21)	0.04 (54)
9/26	495 (<1)	261 (<1)	12.1 (8–28)	35.8 (10–80)	0.02 (<1)	0.42 (<1)
Means	636 ± 148	541 ± 127	12.9 ± 1.4	26.1 ± 2.0	0.06 ± 0.02	0.34 ± 0.07

[a] Entries for total amoebae densities and carbon content obtained by the COM include the percent of each entry that is contributed by encysted forms (in parentheses). Size data include the range (in parentheses) and the final mean is a grand mean. The means are reported as ± s.e. The error of the COM is ± 0.05 × mean density, based on prior published estimates of method reliability (Anderson 2002).

erable natural variability across the sampling dates as listed in Table 1. However, the mean sizes and mean carbon content were substantially different for the two particle size fractions. The overall mean amoeba size (μm) for the < 200-μm fraction was 12.9 ± 1.4 and for the > 200-μm fraction was 26.1 ± 2.0 ($t = 9.94$, $df = 219$, $p < 0.01$). Moreover, the amoeba carbon content (μg/l) was approximately six-fold greater in the > 200-μm fraction compared to the < 200-μm fraction (Table 1), and the mean difference was highly significant ($t = 3.06$, $df = 54$, $p < 0.01$). The differences in amoeba carbon content can be explained partially by the larger size of the naked amoebae in the > 200-μm fraction. Overall, the total estimated carbon content of the naked amoebae in the combined particle size fractions (< 200-μm and > 200-μm fractions) was 0.40 μg/l of estuary coastal water. The size range of naked amoebae for the < 200-μm particle fraction was 5–38 μm and for > 200-μm was 8–80 μm. The size distribution for the amoebae for the two particle fractions is presented as Fig. 2. For the < 200-μm particles, approximately 72% of the amoebae observed were in the less than 20 μm size range; whereas, for the > 200-μm particles, only 37% of the amoebae were < 20 μm, and some were considerably larger up to 70 or 80 μm. A visual inspection of the size distribution (Fig. 2) indicates there was a much broader diversity of size ranges for the amoebae on the large particles compared to the small. In addition to the data in Fig. 2, the total densities (number/l) of naked amoebae (combining data for all seven samples) in each particle size fraction (< 200 μm and > 200 μm), were reported for five size (μm) range categories (≤ 10, 11–19, 20–29, 30–39 and ≥ 40). The results were as follows: < 200 μm (2,010, 2,140, 1,325, 345, and 0) and > 200 μm (327, 1,456, 887, 919 and 590). This further supports the conclusion that the larger particle fraction was more biodiverse; and that over all it had a generally larger size range, compared to the smaller fraction. Moreover, the Shannon-Weaver diversity coefficient (H) was higher for the larger particle fraction compared to the smaller; i.e. 4.51 and 4.18, respectively. In total, 33 morphospecies were tallied in this study of Hudson River particulate-dwelling amoebae.

Bacterial densities. The data from May 2009 are as follows: The mean ± s.e. bacterial density per liter of suspended particles for the larger size particle fraction was $0.56 \pm 0.14 \times 10^9$ while the densities in the smaller size fraction (including suspended free bacteria) was $1.59 \pm 0.33 \times 10^9$. Hence, the proportion of bacteria on the larger size particles relative to the total bacterial standing stock in the water suspension was c. 25%. The percentages of bacilli, cocci, and other bacteria (vibrios, etc.) relative to the total number enumerated in each particle size fraction were as follows: < 200-μm-particle size fraction, 21, 70 and 9%, respectively; and for the > 200-μm-particle size fraction, 32, 61 and 7%, respectively. Similarly, for the single sample in August, the density of bacteria on the large particle fraction was 0.43×10^9, and on the small particle fraction was 2.2×10^9. The percentages of bacilli, cocci and other bacteria relative to the total were as follows: < 200-μm-particle size fraction, 26%, 73%, and < 1%, respectively; and for the > 200-μm-particle size fraction, 24%, 75%, and < 1%, respectively. The sample taken in September had densities of 0.45×10^9 for the large particle fraction and 1.69×10^9 for the small particle fraction. The percentages of bacilli, cocci and other bacteria were as follows: < 200-μm-particle fraction, 22%, 77%, and < 1%, respectively; and for the > 200-μm-particle fraction, 28%, 71%, and < 1%. The estimated carbon content of bacteria was calculated using mean density data based on the entire suite of five samples (three in May and the two in late summer). The carbon content (mean ± s.e.) of bacteria from the > 200-μm-particle fraction was 32 ± 5.2 μg/l and for the < 200-μm-particle fraction was 109 ± 13.2 μg/l.

Sedimented volumes of particles. The sedimented volumes (mean ml ± s.e.) of suspended particles per liter of Hudson Estuary water, based on the seven samples, were comparable, i.e. 0.41 ± 0.15 (< 200-μm-size fraction) and 0.44 ± 0.15 (> 200-μm fraction). However, as may be expected, the larger particle fraction pellet was more loosely compacted than the smaller particle fraction pellet.

Comparative data for particles of different sizes pipetted from the water samples

Further analyses of the mean sizes of amoebae, based on the measurement of amoebae on particles pipetted from the estuary water samples, confirmed the trend for larger particles to support populations of larger amoebae. The data for particle size ranges in millimeters and mean amoeba sizes (μm ± s.e.) are as follows: 0.2–0.9 (22 ± 2.0), 1.2–1.5 (31 ± 1.8), 2.0–2.5 (36 ± 3.7) and 3.0–3.5 (39 ± 3.3). The amoebae on the larger particles included large-sized species of *Cochliopodium*, *Hartmannella*, *Mayorella*, vahlkampfiids, and *Vannella*. spp. Those greater than 30 μm were observed mainly in the larger-size particle fractions. The genera are similar to the large-sized genera observed in the >

200-µm-particle fraction collected from the water suspensions using the 200-µm-mesh filter as reported in the preceding section.

DISCUSSION

Overall, the results indicate that larger size suspended particles support more diverse size ranges of amoebae (including much larger ones) than smaller particles. The larger particles also have greater morphospecies diversity as assessed by the Shannon-Weaver diversity coefficient. Likewise, the carbon content of amoebae is greater on larger particles than smaller particles. However, the densities of naked amoebae (Table 1) in six of the seven samples were greater on the small size fraction compared to the large. The reason is not certain, but larger amoebae dwelling on the large particles are likely to be less abundant, being near the top of the food chain. Also, the presence of fewer smaller amoebae on the large particles may be due to competition for food by the larger species and/or predation of the larger amoebae on the smaller ones. The larger species of *Cochliopodium*, *Hartmannella*, *Mayorella*, vahlkampfiids, and *Vannella* were observed consistently in the larger particulate fraction. A comparative analysis of amoeba size distributions (Fig. 2) further supports a conclusion that the larger particles harbor a more diverse assemblage of naked amoebae, and may provide additional niches for the amoebae to occupy. The larger particles contain an overlapping size distribution with the smaller sized particles, but additionally the larger particles support a much larger size range of morphospecies than the smaller particles. Thus, the larger particles appear to support a more biodiverse assemblage of amoebae than the smaller particles in addition to evidence of greater biomass based on estimates of mean amoeba carbon content. The estimate of bacteria carbon content associated with each particle-size fraction was substantially larger than that of the naked amoebae (as much as two to three orders of magnitude, for large and small particulate fraction, respectively). This is to be expected, because amoebae are bacterial predators, and thus at a higher level in the food chain. Overall, the mean carbon content of the combined amoeba fractions was 0.40 µg/l, while that of the bacteria was in the range of 100 µg/l.

Although both size fractions of particles had similar sedimented volumes, the smaller-size particles may have contributed a larger total surface area per unit volume compared to the larger-size particles. The larger surface area to volume expected for small size particles, compared to larger ones, may have provided greater surface area for attachment of the smaller amoebae. However, the smaller particles would likely be too small for effective colonization by larger amoebae. Thus, in this study of Hudson Estuary water samples, it appears that suspended particles of different size provide microhabitats supporting different communities of naked amoebae, and the larger particles may have more complex food web relationships. Prior research has shown that larger amoebae prey on bacteria as well as smaller protists, while smaller amoebae are largely bacterivorous (e.g. Anderson 1994; Bovee 1985). A total of 33 morphospecies was found in this study. This is comparable to the number found in other coastal sites, e.g. 37 in the planktonic waters of a mangrove stand (Rogerson and Gwaltney 2000).

The Hudson River, as with other estuaries, is hydrologically dynamic, and highly variable in major forcing functions such as: variations in salinity, surface water height, wind driven waves and tidal flushing. Variations in water column turbulence and efflux of freshwater output from marginal marshes, among other factors, are likely to contribute to large variations in the suspended particulates. As a result, the available particulate surface area available for attachment of naked amoebae can vary substantially, even from day-to-day and certainly seasonally (e.g. Zimmermann-Timm *et al.* 1998). This may account for the high variability in naked amoebae densities across the sampling dates in this study, in addition to possible variations in available organic nutrients, prey bacteria, etc. Given increasing evidence that naked amoebae can be a major link in the bacterial-based food web of estuaries, and account for a significant fraction of the carbon content of some protistan communities (e.g. Anderson 2007, Lesen *et al.* 2010), it is important to better document environmental conditions that may affect their abundance and diversity. Naked planktonic amoebae are known to be largely particle-attached. Therefore, the greater the number of habitable particulates in the water column, the greater the likely densities of amoebae. The study reported here provides additional evidence that particle size, in addition to particle densities, influences the diversity and mean size of naked amoebae associated with the particulates, especially larger particles supporting larger amoebae with greater diversity. This suggests that accounting for the particle size distribution in the water column may be a significant variable to consider in es-

tuarine and coastal plankton ecology, to account more fully for the naked amoebae standing stock and their likely predation pressure.

Although the COM enumeration method for amoebae, as with other microplankton counting methods, incurs some error, the larger amoebae, occurring less abundantly in the amoeba communities, are usually distributed sparsely among the 10-µl aliquots deposited in the wells of the Falcon culture dishes. Thus, it is unlikely that there is a major underestimation of their abundances due to more than one individual being present in each 10-µl aliquot introduced in the well. Moreover, the COM approach has now been used in many research studies and has consistently shown sufficient sensitivity to detect differences among samples from both aquatic and terrestrial environments (e.g. Anderson 2006b, 2007, 2008, 2010; Bischoff 2002; Bischoff and Wetmore 2009; Lesen *et al.* 2010).

The study reported here is limited to sampling sites on the Hudson lower estuary, and clearly additional research is needed at other locations to expand the database. Samples for this study were taken largely in summer and early autumn, based on existing evidence that these are among the more productive months for the Hudson lower estuary (e.g. Anderson 2007). In addition, the calculated carbon content of the amoebae, based on the regression equation of Anderson (2006a), is at best an approximation of the carbon-based biomass of the amoeba standing stock on the particles. Additional investigations are needed to document the occurrence of heterotrophic nanoflagellates, attached ciliates, and other protists in each particle size fraction to provide a better estimate of microbial community biomass associated with the suspended particles as has been done more generally for particle associated communities of protists (e.g. Kiss *et al.* 2009, Zimmermann-Timm *et al.* 1998). Prior research on planktonic naked amoebae has indicated the importance of considering suspended floc and other particle densities in the water column as a predictor of the presence of amoebae (e.g. Lesen *et al.* 2010, Rogerson and Gwaltney 2000, Rogerson *et al.* 2003). However, the evidence reported here suggests that it is also important to analyze the size fractions of the particles to more fully account for the diversity of amoebae and their potential role in microbial community structure and trophodynamics.

Acknowledgements. I thank Ms. C. A. Knudson who assisted in counting bacteria. Two anonymous reviewers made helpful recommendations to improve the manuscript. This research was supported partially by a U. S. National Science Foundation Grant No. OCE0845799. This is Lamont-Doherty Earth Observatory Contribution Number 0000.

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