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A Multi-year Seasonal Study of Amoeboid Protists in Surface Water at the Margin of a Hudson River Estuary Salt Marsh

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Abstract. Marshes bordering rivers and estuaries are productive ecosystems that interact dynamically with the adjacent water mass. This is a multi-year study (2019–2022) of seasonal changes in the density of naked amoebae in monthly samples from the surface water of the Hudson estuary near Piermont, N. Y. with relationships to key environmental variables (surface water temperature, salinity, Secchi depth representing turbidity, and enterococcus bacterial counts). During the colder months (November to March), when decayed leaves and litter from the deciduous marsh grass produced organic matter in the sediment surface, the mean abundance of active amoebae ± standard error of the mean (SEM) was higher ($3.07 \pm 0.99 \times 10^4$/ L, N = 7). In warmer months (May to September) the abundance of amoebae was lower ($1.35 \pm 0.29 \times 10^4$ / L, N = 10). A multivariate linear regression analysis was performed relating amoeba abundance to four major water mass variables, resulting in the following statistically significant equation ($p = 0.03$): $A_D = 0.121 \times T + 0.301 \times L - 0.047 \times S + 0.359 \times C$, where: A_D = active amoebae density ($\times 10^4$/L), T = temperature (°C), L = tide level (m), S = Secchi disc depth (cm) and C = bacterial enterococcus concentration (number/ml). In general, given the increasing evidence of the potential importance of amoeboid protists in aquatic ecosystems, further research is warranted on their role in food webs and the carbon biogeochemical cycle within heterotrophic estuarine and coastal waters.

Key words: Aquatic ecosystems, eukaryotic microbial communities, climate effects, microbial heterotrophic food webs, microbial productivity.

INTRODUCTION

The lower portion of the Hudson River (New York, U.S.A.) is an estuary extending 240 km from the New York Harbor at the south to the federal dam at Troy, N.Y. at the north. It is a tidally driven estuary, stratified vertically with a lens of less dense, lower salinity surface water and a denser, more saline basal layer. Due to the turbidity of the water and dynamics of vertical mixing, leading to limited penetration of light with depth, the Hudson River is largely heterotrophic with a net export of CO_2 to the atmosphere (Raymond et al. 1997). Patches of salt marsh, populated by tall marsh grass (*Phragmites australis*), occur at locations along the shoreline of the river. Due to the tidal influx and efflux of water from the marsh, considerable suspended organic matter is carried periodically from the surface water of the marsh into the Hudson River, especially when tide levels are moderate to high, and there is sub-

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stantial surface energy due to wind-driven waves (e.g. Kiviat et al. 2006). Suspended particulates and floc in the surface water near the river margin contain surface-dwelling microbes including bacteria and protists (Anderson 2007, 2011; Findlay 2006).

Considerable attention has been given to the role of floc and organic particulates in the ecology of microplankton in estuaries, particularly as hotspots of microbial activity as reviewed by Simon et al. (2002). Amoeboid protists are typically abundant and associated with suspended particulates in coastal and estuarine water (e.g. Rogerson and Laybourn-Parry 1992; Mayes et al. 1998; Rogerson and Gwaltney 2000; Lesen et al. 2010); and current evidence indicates that they may constitute an important link in the microbial carbon geochemical cycle based on estimates of their carbon content when associated with suspended particulates (Anderson 2011, 2016), among other microbiota inhabiting suspended estuarine particulates (Ploug et al. 2002; Turner and Millward 2002). Research on seasonal changes in the abundance and activity of microbial communities associated with suspended floc and particulates has provided a broad perspective on the role of climate and aquatic physio-chemical variables on the abundance, diversity and activity of bacterial and protist communities in estuarine systems (e.g. Zimmerman-Timm et al. 2002). However, more detailed studies of seasonal variations in abundance and activity of amoeboid protists associated with particulate-rich surface waters in estuaries has received less attention.

This is a multi-year (2019–2022) seasonal study of the abundance of amoeboid protists, and related key environmental variables, in the Hudson estuary surface water at the margin of a salt marsh located near Piermont, N. Y. The objective was to document the dynamics of the changing densities of the amoeboid protists in the surface water near the marsh across the multi-year seasons, particularly in relation to surface water temperature, salinity, tide level and enterococcus concentration (indicator bacteria for anthropogenic influence and a proxy for particulate suspension from the marsh surface sediments). Overall, the focus is on the functional ecology of active and encysted amoeboid protists in surface water near a salt marsh at the margin of the Hudson River estuary near Piermont, N. Y. across multiple seasons during a 19-month sampling program.

MATERIALS AND METHODS

Sample collection

Surface water samples for analysis of amoeboid protists were collected from the Hudson River at the margin of the Piermont Marsh (41° 02' 35.19" N, 73° 54' 57.61" W) using a 4-liter Nalgene plastic jar during morning or at mid-day, each month for 19 months beginning in November 2019 and ending in May 2022 (Table 1). Because of the COVID-19 pandemic, sampling and laboratory work were curtailed for six months (Nov. 2020 to April 2021) due to university rules limiting access to the laboratories. Sampling was resumed in May 2021 through May 2022.

The depth of the surface water at the marsh perimeter sampling site varied dynamically depending on the tidal cycle but averaged about 1 m depth. When each surface water sample was taken for the amoeboid protists, key environmental variables of the surface water were assessed at a pier near the marsh (41° 02'38.16", 73° 54' 50.12" W) where a river sampling station is maintained by Lamont-Doherty Earth Observatory of Columbia University. The depth of the water from surface to sediment at the pier sampling site was 5.2 m. During the course of this study, the tide level ranged from 0.3 to 3.7 m. A Hach HQ40 D Portable Multi Meter® (Hach Company, Loveland, Colorado) was used to measure the surface water temperature (°C) and salinity (p.p.t.). Turbidity of the water was estimated using a Secchi disc (Wildlife Supply Co., Yulee, Florida). The tide level at the time of water collection was determined by consulting an official tide level chart (https://tides.willyweather.com/ny/rockland-county/hudson-river--piermont-pier.html). Water samples collected at the pier were also analyzed for enterococcus bacterial counts (cocci/ml) using an Enterolert test kit® (IDEXX Laboratories, Westbrook, Maine). The enterococcus count was included as an indicator for possible anthropogenic effects due to output of sewage from local sewage treatment plants and also as a proxy for microbe-containing particulates from the salt marsh into the surface water of the Hudson. The salt marsh sediment is heavily populated by enterococcus bacteria at this site, and the suspended sediment particulates can carry the associated enterococci in the exported marsh water entering the Hudson estuary (O'Mullan et al. 2019). The enterococcus bacterial counts, combined with the Secchi depth data, were used as a proxy for suspended particulate matter likely derived from the marsh because it was more predictive of amoeboid densities than nephelometry-based estimates of suspended particulates.

Amoeboid protist analysis

The amoeboid protists were typically Amoebozoan and Heterolobosean amoeba species. Hereafter to be concise, the term amoebae will be used when describing them. A 100-ml portion of the surface water sample was taken for assessing the density of amoebae (number/ L) using a Culture Observation Method (COM) routinely used in our laboratory (Anderson and Rogerson 1995, Anderson 2007). A small aliquot (e.g. 20 µl) of the suspension from the 100-ml aliquot was deposited into each well of a 24-well, sterile Falcon® culture dish, where each well contained 2 ml of Millipore filtered Hudson River water (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.

Table 1. Hudson estuary environmental variables and surface water amoeboid protist densities.

Date	Water temp. °C	Tide level m	Secchi depth cm	Surface salinity p.p.t.	Cocci density per ml	Active amoebae × 10^4/ L	Encysted amoebae × 10^4/ L	Percent active %
2019								
Nov. 20	10	0.52	10	2.0	1.7	1.55	1.88	45.5
Dec. 5	4.8	0.24	30	2.6	2.9	1.25	1.11	53.0
2020								
Jan. 15	5.0	0.61	40	0.9	8.6	2.84	1.46	66.1
Feb. 12	4.0	0.91	20	6.0	15.5	7.84	0.20	98.0
Aug. 25	25.4	0.09	85	10.0	1.06	0.00	4.17	0.00
Sept. 22	18	0.61	40	11.8	0.10	1.95	1.6	54.8
Oct. 27	15.6	0.70	35	9.9	0.52	1.92	0.90	67.6
2021								
May 25	17.3	1.01	25	7.5	0.01	0.91	4.65	83.6
June 29	38.0	0.73	65	6.3	0.20	2.15	0.35	86.0
July 27	28.2	1.04	35	0.6	0.20	1.26	0.21	85.6
Aug. 24	27.0	1.10	65	2.3	0.41	2.22	1.11	66.7
Sept. 27	21	0.52	70	1.9	0.01	0.34	4.02	8.0
Oct. 18	17.7	0.91	60	3.8	3.05	1.73	0.21	89.0
Nov. 8	13.3	1.13	40	1.5	5.73	0.00	1.74	0.00
Dec. 6	7.7	1.22	35	5.7	5.45	5.00	0.07	99.0
2022								
Jan. 25	1.6[a]	0.70	45	9.1	9.10	0.14	0.42	25.0
Feb. 15	1.1[a]	0.73	50	6.0	6.97	0.00	0.00	0.00
Mar. 15	4.8	0.73	45	2.7	13.8	3.00	0.90	77.0
May 17	16.9	1.04	30	8.94	0.75	1.02	0.30	77.0

[a] Note, extensive surface ice and ice rafting blocked surface water exchange between the marsh vegetation and the surface water of the river.

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). Presence of amoebae were identified based on morphospecies characteristics (Anderson and Rogerson 1995, Anderson 2007). 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 20-µl aliquot of sample deposited in the well. Based on the total number amoebae in the 24 wells, the density of amoebae per liter of sample was calculated on the proportional basis that the 24 wells contained all-totaled 240 µl of deposited sample. Therefore, a minimal estimate 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 20-µl aliquot of the water sample was deposited in each well of a 24-well 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 as was done for the non-dried COM method. This provided an estimate of the densities of encysted naked amoebae that were capable of withstanding the drying step. The amoebae were counted for COM (and dried COM) with a Nikon Diaphot inverted phase contrast microscope. The density of active amoebae (trophonts) was computed by subtracting the density of encysted amoebae from the density of total amoebae that grew out in the COM culture dishes. The percent active amoebae was calculated as follows: (density of active amoebae/ density of total amoebae) × 100.

Statistical analyses

Multivariate linear regression analysis (Vassarstats.net) was used to determine the relationship of amoeba density (dependent variable) with the four most predictive environmental variables as independent variables (temperature, tide level, Secchi depth and concentration of cocci). These variables were chosen based on prior research at the Piermont Pier sampling site that indicated these were likely significant environmental variables affecting the ecology of the estuary and amoeboid protists. Salinity concentration was not included in the multivariate regression analysis because it reduced the predictive power of the equation. The reasons for this lack of prediction are not known precisely, but environmental variables affecting surface water salinity are not very predictable. These include significant occasional precipitation events that reduce salinity through increased local freshwater runoff from the land, and also sporadic release of freshwater from wastewater treatment plants in communities along the Hudson River and its tributaries. Moreover, increased localized turbulence in the river water column can sporadically bring higher salinity water from depths into the surface, making it more saline. Therefore, in addition to its lack of predictability in the linear regression equation, salinity was not included in the multivariate regression analysis. To meet the requirements of a parametric multivariate regression analysis, the normality of the variables was confirmed using a Kolmogorov-Smirnov test.

RESULTS

The results of the multi-year, monthly assessment of amoeboid protist densities and the environmental variables (temperature, tide level above mean lowest level, Secchi depth, salinity, and concentration of cocci) are presented in Table 1.

The water temperature varied from 1.1 °C (Feb. 15, 2022), with considerable surface ice and shoreline ice rafting, to 38 °C (June 29, 2021). On the latter date in June, the tide level was relatively low (0.73 m) and the air temperature was also elevated, resulting in a shallow surface water layer overlying the mud flats of the marsh that became unusually warm due to insolation.

Although the density of active amoebae was relatively high (2.15×10^4/ L), the observed amoebae were likely warm water tolerant, heterolobosean species (e.g. *Vahlkampfia* spp.). As expected for a tidally-driven estuary, the tide levels varied markedly from 0.09 m (Aug. 25, 2020) to 1.22 m (Dec. 6, 2021). The Secchi depth varied from 10 cm (Nov. 20, 2019), indicating a relatively turbid surface water stratum, to 85 cm (Aug. 25, 2020) indicating a relatively less turbid state. As is typical for a mixed tidal estuary, there was considerable variation in salinity varying from 0.6 p.p.t. (July 27, 2021) to 11.8 (Sept. 22, 2020). Enterococcus (Cocci density) was markedly seasonal, being somewhat higher in late autumn and winter months and lower in summer months. The lowest value was 0.01/ml in May and Sept., 2021, and the highest was 15.5/ ml in Feb., 2020. Densities of active amoebae tended to be higher in the late autumn and winter months, except when there was ice cover or ice rafting (e.g. Jan. and Feb. 2022), which limited export of water from the marsh into the surrounding estuary water. Overall, the highest recorded density of active amoebae (7.84×10^4/ L) was in Feb. 2020 and the lowest values, aside from the ice-rafted dates, were in Aug. 2020 and Nov. 2021, when there were no detectable active amoebae. In general, the highest level of densities at 7.84×10^4/ L found in this study is consistent with the range of maximum densities of amoebae (ca. 10^4/L) reported previously in the Hudson River (e.g. Lesen et al. 2010).

The mean active amoebae ± standard error of the mean (SEM) for the colder months (Nov. to March) was $3.07 \pm 0.99 \times 10^4$/ L (N = 7) and for the warmer months (May to Sept.) was $1.35 \pm 0.29 \times 10^4$ / L (N = 10), excluding the two winter months that were ice-rafted. To examine the statistical relationship between the density of active amoebae and the four identified most predictive environmental variables (temperature, tide level, Secchi depth and concentration of enterococci), a multivariate linear regression analysis was performed, resulting in the following statistically significant (F = 3.82, p = 0.03, d.f. = 4) equation (1). The $R^2 = 0.52$, and the correlation coefficient (R) = 0.72.

$$A_D = 0.121 \times T + 0.301 \times L - 0.047 \times S + 0.359 \times C \quad (1)$$

Where: A_D = active amoebae density ($\times 10^4$/L), T = temperature (°C), L = tide level (m), S = Secchi disc depth (cm) and C = bacterial enterococcus concentration (number/ml).

DISCUSSION

Considerable research attention has been given to naked amoebae (gymnamoebae) in estuarine and coastal waters. Sawyer (1971) published some of the earliest taxonomic and ecological studies of amoebae in coastal waters of the Chesapeake Bay near Maryland. Further research has focused on the ecology and diversity of amoebae in various locations including estuarine rivers (Anderson 2007; Anderson and Rogerson 1995; Ettinger et al. 2003; Kiss et al. 2009; Lesen et al. 2010; Rogerson and Laybourn-Parry 1992; Zimmermann-Timm et al. 1998). Additional studies have been done in coastal waters of Virginia (Munson 1992) and near Antarctica (Mayes et al. 1998). Because active amoebae (trophonts) must attach to a surface to locomote and feed, they are particularly present on particulates in aquatic environments, and some attention has been given to the abundance and dynamics of their association with suspended aquatic particulates (Anderson 2011; Rogerson et al. 2003; Van Wichelen et al. 2006; Wörner et al. 2000).

Estuarine salt marshes are highly productive ecosystems and their ecological relationships to the biology and hydrology of the estuary are of increasing interest. This is especially the case because climate change and rising sea levels may have major effects on the health and survival of the marshes, especially if sedimentation leading to increased elevation and expansion of the vegetation cannot keep pace with the encroaching flooding and higher water mark from the estuary (Delgado et al. 2018; Dyer 1995; Mogensen and Rogers 2018; Richer et al. 2020). Under increasing climate stress pressures, less productive marshes may export less particulates and microbiota to the adjacent estuary, thus affecting the productivity and microplankton ecology of the estuary. The dynamic relationship of the marsh with the constituent estuary is particularly related to the nature of the vegetation, its productivity, and the ecology of the microbial communities in the marsh sediment. Organic matter from the roots and from shed vegetation contribute to the sustenance of the sedimentary prokaryotic and eukaryotic microbial communities, in this case particularly during late autumn and winter when decaying leaves and litter from the deciduous marsh grass provide increased sources of organic matter. In turn, tidal export of particulates and associated microbial communities into the estuary water provides substantial input to the standing stock of the aquatic microbial communities.

As summarized above, particle-bound amoebae are among some of the more abundant planktonic eukaryotic microbes in the estuary water column. Understanding the nature of the dynamic relationship between estuarine hydrology and the ecology of the salt marsh includes a better understanding of how seasonal changes in the marsh-estuarine ecosystem affect the abundance and composition of amoeboid protists inhabiting the suspended particulates exported from the marsh into the estuary water column. This study provides some of the first evidence of how the density of amoebae in an estuary water column at the verge between the marsh and estuary surface water changes seasonally and in relation to some key environmental variables at the estuary sampling site.

Given that the Hudson estuary ecosystem is largely heterotrophic, and that microplankton food webs are mainly based on heterotrophic prokaryotic and eukaryotic microbes, it is important to explore the sources and sinks of these microbes within the estuarine system, including relationships to the shore-based marsh communities. The multivariate linear regression analysis included here is an endeavor to make a mathematical analysis of the role of four environmental variables that may account for the density of suspended amoebae in the surface water of the estuary at the edge of the shoreline salt marsh. Microplankton amoebae, with densities in the range of 10^4/ L may be a significant component in the microplankton food web, especially for smaller invertebrates that prey on the microbial community associated with suspended particulates in the estuary. Bacterial abundances within the main channel of the Hudson estuary are estimated to be 1 to 10^6 cells/ml (i.e. within the range of 10^9/ L) with maximum values in summer, based on a 3-year study along a 158 km reach of the river (Findlay et al. 1991). As is typically the case in a heterotrophic-based aquatic ecosystem, the bacterial densities in the range of 10^9/L should be sufficient as a sustainable food source for the amoeba populations as well as other eukaryotic micrograzers within the river surface waters. However, physical environmental variables may exert far stronger forcing functions on the seasonal abundances of particle-bound active amoebae both directly and indirectly through interactions with other members of the microbial ecosystem.

In the multivariate regression equation (1), water temperature (T) in combination with concentration of enterococcus (C) had an appreciable contribution to the predicted abundance of amoebae, possibly as a proxy for seasonal influences on the ecology of the marsh and the subsequent effects on the export of amoebae from the flooded marsh sediments. Shed leaves and litter from the *Phragmites* plants provide substantial organic matter to support growth of amoebae and possibly increased loads of enterococcus during late autumn and throughout the winter months. Strong winds and stormy weather during winter, in combination with high tide (L), contribute to resuspension of the marsh surface sediments and particulates containing amoebae and enterococcus (in addition to other anthropogenic sources of these bacteria); thus, likely increasing export of the amoebae and enterococcus into the river surface water. The concentration of enterococcus (C) was a significant contributor to the multivariate prediction of amoeba abundance. Among other possible contributory factors, the presence of the enterococci in salt marsh sediments may make the concentration of enterococcus a good proxy for particulates released from the sediments when tidal forcing is high. Thus, tidal disturbance and the release of substantial amounts of the sedimentary particles in the tidal flood within the marsh may also account for release of suspended enterococci, making tide level (L) also a good proxy for suspended tidal particulates. The Secchi depth (S) has a negative regression coefficient (-0.047), which reflects the inverse relationship of Secchi depth with turbidity; i.e. Secchi disc depth (cm) is less when the water is more turbid, and may reflect increased density of suspended particulates with associated microbiota. Therefore, a negative regression coefficient for this variable is logically supported.

Additional research is needed to more fully explore the generality of the mathematical modeling used here, particularly at different locations in the Hudson estuary where there are similar tidal marshes, and more broadly at other geographical locations. Estuaries and some coastal locations are typically heterotrophic systems, largely attributed to a high load of suspended particulates that reduces light penetration in the water column. Therefore, the approach used here may also be appropriate in other locations with similar hydrological and biotic conditions, especially locations with substantial human populations. Overall, there is increasing evidence of the potential importance of amoeboid protists in aquatic ecosystems; and further research on their role in food webs and carbon biogeochemical cycle within heterotrophic estuarine and coastal water is warranted (e.g. Anderson 2007, 2016; Rogerson and Gwaltney 2000).

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