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SHORT COMMUNICATIONS

Numbers of naked amoebae inhabiting the intertidal zone of two geographically separate sandy beaches

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Numbers of naked amoebae (Gymnamoebae) inhabiting the lower intertidal zone of two sandy beaches were estimated using a novel enrichment cultivation method. Samples were collected between June and September, 1999. Beach sand at Kames Bay, Isle of Cumbrae, Scotland contained on average 2604 amoebae cm^{-3} while at Dania Beach, Florida, USA, sand harboured 4236 amoebae cm^{-3}. This is the first study to focus on the abundance of naked amoebae inhabiting a sandy beach. These numbers are higher than densities generally reported for shallow subtidal sands and show that amoebae must be considered in future studies on the dynamics of sandy beach communities.

Sandy beaches comprise the most widely distributed intertidal habitat dominating the world's temperate and tropical shorelines (Dexter, 1992). Fauré-Fremiet (1950) and Fenchel (1967) were among the first to describe and highlight the importance of protozoa in intertidal sediments by focussing on the conspicuous interstitial ciliates. In the 1980s, mainly because of an insightful paper by Azam et al. (1983), microbial ecologists were awakened to the grazing potential of the small heterotrophic flagellates in the water column. It soon became a matter of interest to understand the major grazers preying upon bacteria and algae in sandy sediments. Several papers have addressed this subject but virtually all overlook or ignore the naked amoebae. For example, Epstein (1997) recently commented that within the intertidal sandy community, microflagellates were the single most important group of bacterivores followed by nanoflagellates, ciliates and then the meiobenthos. This is despite early published work that suggests that amoebae might be worthy of consideration (e.g. Dye, 1979). Recent studies have indicated that amoebae can, on occasion, be numerically important in subtidal sandy substrates. Naked amoebae were found to average 874 cells cm^{-3} in subtidal sand of the Clyde Sea area (Butler & Rogerson, 1995) and data on the biomass (up to an annual mean of 9490 mg dry wt m^{-3}) of amoebae in sublittoral sand is given by Fernandez-Leborans & Novillo (1993). Anderson (1998) found high numbers of amoebae in permanently wet sand at two sites in Bermuda (~18,000 and 28,000 cells g^{-1} dry wt). The present study is the first to specifically enumerate naked amoebae in intertidal sand. By using a modification of an enrichment culture method, we show that amoebae are common and constitute an overlooked group of micrograzers that may be an important force controlling bacterial and microalgal dynamics in beach sands.

Recent counts of amoebae in the plankton and sediments have relied on an aliquot method (ALM) that dispenses small volumes of water (typically 10 μl) into media contained in the wells of tissue culture plates (for details see Anderson & Rogerson, 1995). For sediments, the sample is shaken vigorously with a known volume of seawater to dislodge amoebae. After allowing particulates to settle, aliquots are pipetted from the overlying water (Butler & Rogerson, 1995). However, shaking is unlikely to remove all amoebae, which are usually surface associated, hence the counts are probably underestimates.

In the present study, samples were not shaken and a novel sand grain method (SGM) was tested. Care was taken not to dislodge amoebae and a single, average sized sand grain was added to each of the 48 wells of two tissue culture trays. Each well contained 1 ml of sterile seawater and a small fragment of crushed rice to nourish the indigenous bacteria which in turn were prey to the inoculated amoebae. Any amoebae that grew up in the culture wells were from one or more amoeba(e) attached to the sand grain. The average number of positive scores from all 48 wells gave an estimate of the number of attached amoebae per individual sand grain. To convert amoebae per grain of sand to numbers cm^{-3} it was necessary to estimate the number of grains per cm^3. This volume of sand was washed with distilled water and dried until constant weight. Average sized sand grains (similar to those used in the amoebae counts) were counted and weighed (between 30 and 100 grains). The two weights were used to derive the number of grains cm^{-3}. The results are given as a footnote to Table 1. Subsequently, the sand grain method (SGM) and the aliquot method (ALM) were compared in a three-week trial. A surface sand sample (1 cm depth) was collected and 48 sand grains were removed for counting by the SGM. From the same sample, 1 cm^3 of sand was shaken vigorously for 1 min with 1 ml of sterile seawater. After sand grains settled, 10 μl aliquots of water were dispensed into the 48 wells of tissue culture dishes containing 1 ml sterile seawater and a fragment of rice grain. After incubation, the number of wells containing amoebae was used to estimate abundance (assuming one cell per 10 μl inoculum).

Amoebae that grew were subdivided into four morphological categories (Anderson & Rogerson, 1995). Type I amoebae were those with subspeudopodia usually emerging from the anterior hyaline edge i.e. genera like *Mayorella* and *Vexillifera* as well as unusual irregular forms like *Stereomyxa* that was relatively common at both sites. Type II were limax (slug-like) amoebae with steady locomotion e.g. *Hartmannella*. Type III were also limax but had eruptive locomotion such as found in *Vahlkampfia*. Type IV amoebae were without subspeudopodia and included, among others, the flattened fan or tongue shaped genera, *Vannella* and *Platyamoeba*, and the ovoid thecates such as *Thecamoeba*.

The numbers of amoebae were compared at two beaches; Kames Bay, Isle of Cumbrae, Scotland, UK and Dania Beach,

Table 1. *Comparison of counts of naked amoebae cm^{-3} on two beaches (Millport, Scotland and Dania Beach, FL, at two depths (1 cm and 10 cm). The types of amoebae are explained in the text. Values as means with standard errors in parentheses, (N=10). All counts based on the 'sand grain method'.*

	Numbers of amoebae cm^{-3}				
Locality/Types	I	II	III	IV	Total
Millport (1 cm)[1]	581	150	112	1496	2339
	(120)	(67)	(75)	(172)	(285)
Millport (10 cm)[2]	1029	416	197	1228	2870
	(368)	(158)	(89)	(225)	(684)
Florida (1 cm)[3]	1825	301	181	2446	4753
	(730)	(100)	(47)	(1,016)	(1,215)
Florida (10 cm)[4]	1317	183	256	1964	3720
	(349)	(55)	(50)	(643)	(739)

[1], number of sand grains cm^{-3} (1 cm)=8972; [2], number of sand grains cm^{-3} (10 cm)=10513; [3], number of sand grains cm^{-3} (1 cm)=9625; [4], number of sand grains cm^{-3} (10 cm)=5833.

Table 2. *Comparison of two counting methods; individual sand grain method (SGM) and aliquot method (ALM) (see text for methods). Samples from 1 cm depth. Standard errors in parenthesis (N=3).*

	Numbers of amoebae cm^{-3}				
Locality/Types	I	II	III	IV	Total
Millport (SGM)[1]	89	177	0	621	887
	(89)	(89)		(387)	(235)
Millport (ALM)	9	1	0	12	21
	(4)	(1)		(7)	(11)
Florida (SGM)[2]	468	0	1003	1070	2541
	(373)		(592)	(482)	(854)
Florida (ALM)	0	0	4	12	16
			(2)	(12)	(10)

[1], number of sand grains cm^{-3}=12768; [2], number of sand grains cm^{-3}=9625.

Florida, USA. Samples were collected on the receding tide, just above the low tide mark, at 1 cm and 10 cm deep. There was considerable variation throughout the sampling period (June–September, 1999) with total numbers of amoebae ranging between 438 and 13,035 amoebae cm^{-3}, irrespective of site or depth (equivalent to ~265–7900 cells g^{-1} dry wt). There was no significant difference between the counts at the surface (1 cm) and at 10 cm. However, there were significantly ($P<0.05$) more amoebae cm^{-3} in the Florida samples compared with the Millport samples (4236 and 2604 amoebae cm^{-3}, respectively, Table 1).

At both locations, flattened, fan-shaped amoebae predominated. A recent study (Armstrong et al., 2000) showed that these amoebae are well suited to surfaces and comprised 70.8% of all amoebae on seaweeds. On the other hand, limax amoebae, which are common in fine sediments (Butler & Rogerson, 1995) were rare. Amoebae with subpseudopodia averaged around 1000 cm^{-3} at both sites. It is interesting to note that larger amoebae were often observed to consume algae (e.g. diatoms). Given the high numbers of these types, it is likely that amoebae are important grazers of both bacteria and sand-attached algae. The counts of amoebae in this study are higher than densities in shallow subtidal sand. Butler & Rogerson (1995) found an average of 874 cells cm^{-3} throughout the year by an aliquot enrichment method and Fernandez-Leborans & Novillo (1993) found up to 462 ml^{-1} by direct counting. However, these counts might be significant underestimates because amoebae are not easily dislodged from sand particles. A comparison of the two counting methods showed that the shaking aliquot method (ALM) gave much lower counts than the sand grain method (SGM) (Table 2). The count was some 40 fold less for Millport samples and 156 fold less in the case of the Florida samples or around 100 fold less amoebae overall.

The densities of amoebae in this study (averaging 3420 cells cm^{-3}) are underestimates for three reasons. On occasion, some sand grains probably contained more than one amoeba cell, not all amoebae are amenable to laboratory cultivation and unattached amoebae within the interstitial space were not counted by the SGM method. Nevertheless, the study is important since it shows that naked amoebae are numerically important in wet beach sand and must be counted along with the more conspicuous flagellates and ciliates. The study also shows that amoebae are not easily dislodged from sediments implying that counts of amoebae in subtidal sands (often only in the hundreds cm^{-3}) might be significant underestimates. It is reasonable to assume that amoebae would be more abundant in the stable subtidal sands than in the 'high energy' tidal zone.

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