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Growth potential, production efficiency and annual production of marine benthic naked amoebae (gymnamoebae) inhabiting sediments of the Clyde Sea area, Scotland

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ABSTRACT: A substantial population of naked amoebae is present in marine sediments, but little is known about their role in benthic microbial food webs. Central to elucidating this role is an understanding of their growth potential, and the present study measures growth rates of 10 species of naked amoebae isolated from benthic sediments in the Clyde Sea area, Scotland, UK. Across the range of species and temperatures examined (5 to 20°C), generation times varied from 11 to 130 h. Temperature had a marked effect on growth rate, with the slowest rates at the lowest temperature. Temperature also generally influenced the mean cell volume of cells, with many species showing increased cell size at lower temperatures. Consequently, it was possible to compute a significant regression of $\log_{10}$ generation time (G, h) against $\log_{10}$ cell volume (V, μm^3) using the combined data regardless of temperatures [$\log G = 0.231 \log V + 1.010$ ($p = 0.004$)]. This relationship may have application in the estimation of generation times of naked amoebae with known cell volumes. Gross growth efficiencies ranged between 11.7 and 79.7% with an overall mean of 36.1%. The growth data were combined with published information on the abundance of marine benthic amoebae to provide a first estimate of annual production by this group in fine marine sediments. Annual production estimates were between 7.60 and 15.8 kJ m^{-2} yr^{-1}, implying that annual consumption of bacteria by naked amoebae is of the order 21.1 to 43.8 kJ m^{-2} yr^{-1}

KEY WORDS: Benthos · Carbon cycling · Microorganisms · Protozoa

INTRODUCTION

At the present time our knowledge of the ecological importance of naked amoebae with lobose pseudopodia, the gymnamoebae, in marine benthic sediments is extremely limited. Recent sudies have demonstrated that naked amoebae are abundant in the Clyde Sea (Scotland, UK) area, numbering up to 15600 ml^{-1} in the plankton and 15000 cm^{-3} in the benthos (Rogerson & Laybourn-Parry 1992, Anderson & Rogerson 1995, Butler & Rogerson 1995). To elucidate the potential contribution of the naked amoebae to carbon flow in the sea, it is crucial to understand their growth potential.

There are few published studies on the growth rates of free-living naked amoebae, particularly for marine species, the only study being one by Bunt (1970) on an algivorous amoeba. Published experiments on soil amoebae include those of Heal (1967) on *Acanthamoeba* sp., Cutler & Crump (1927) on *Hartmannella hyalina*, and Rogerson & Berger (1981) on *Naegleria gruberi*. Experiments on freshwater amoebae have focused on the large *Polychaos fasciculatum* and *Amoeba proteus* and on 6 smaller species (Baldock & Baker 1980, Baldock et al. 1980, Rogerson 1980). In addition, Arndt (1993) has estimated the growth rate of a community of freshwater amoebae in a lake.

Other relevant studies have looked at the growth rates of species of testate amoebae (Laybourn & Whymant 1980) and heliozoa (Tobiesen 1991). There is far more literature on the growth rates of ciliates and flagellates (e.g. Phelps 1946, Fenchel 1969, Taylor &

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Berger 1976, Caron et al. 1986, Darbyshire et al. 1993), however, rates may not be comparable for all types of heterotrophic protists, given recent evidence that the energetic costs of locomotion in amoebae are far greater than those incurred by ciliates and flagellates (Crawford et al. 1994).

In the present study, the growth rates of representative naked amoebae spanning a wide size range were measured. Morphotypes were isolated from the benthos of the Clyde Sea area and cultured under defined laboratory conditions. Experiments were carried out at 5, 10, 15 and 20°C, to encompass the range of *in situ* temperatures experienced in marine sediments in temperate regions. This is the first study to consider growth and production in marine bacterivorous gymnamoebae.

MATERIALS AND METHODS

Species and culture. Ten species of naked amoebae were isolated during 1991 from the benthos of the Clyde Sea area (Fig. 1). The sediments at 4 sites ranged from mud to fine sand and all amoebae were isolated from the upper aerobic layer. Amoebae were maintained in culture at 5, 10, 15 and 20°C in Modified Erdschreiber medium (MErds, Page 1983) with the bacterium *Planococcus citreus* (NCIMB 1493, Aberdeen, UK) as the primary food source. The species of amoebae studied, with their mean lengths, were as follows: *Clydonella rosenfieldi* (17.4 µm), *Dactylamoeba* sp. (53.1 µm), *Paraflabellula reniformis* (26.9 µm), *Platyamoeba* sp. (5.6 µm), *Rhizamoeba* sp. (15.8 µm), *Stereomyxa ramosa* (199 µm), *Vahlkampfia baltica* (19.4 µm), *Vahlkampfia damariscottae* (12.0 µm), *Vannella caledonica* (19.9 µm) and *Vannella* sp. (8.1 µm).

Measurement of growth rates. Amoebae from exponentially growing cultures acclimatised at the appropriate experimental temperature were washed several times by decanting off the old culture medium and replacing with fresh MErds. Throughout this procedure most amoebae remained firmly attached to the base of the culture dish and the majority of bacteria were removed. Washed amoebae were suspended in a small volume of MErds and 100 µl of this concentrated cell suspension was added to each of 5 replicate 50 mm diameter petri dishes containing 10 ml of MErds. Knowing the exact number of cells in the inoculum was not critical, since growth rate is independent of the size of the initial inoculum (Baldock et al. 1980). In all cases, the bacterium *Planococcus citreus* was added as a food source, at a density similar to that found in local marine sediments (around 2.5×10^8 cm^{-3} sediment). Initial experiments showed that this prey concentration was within the range giving optimal amoebal growth rates (Butler unpubl.).

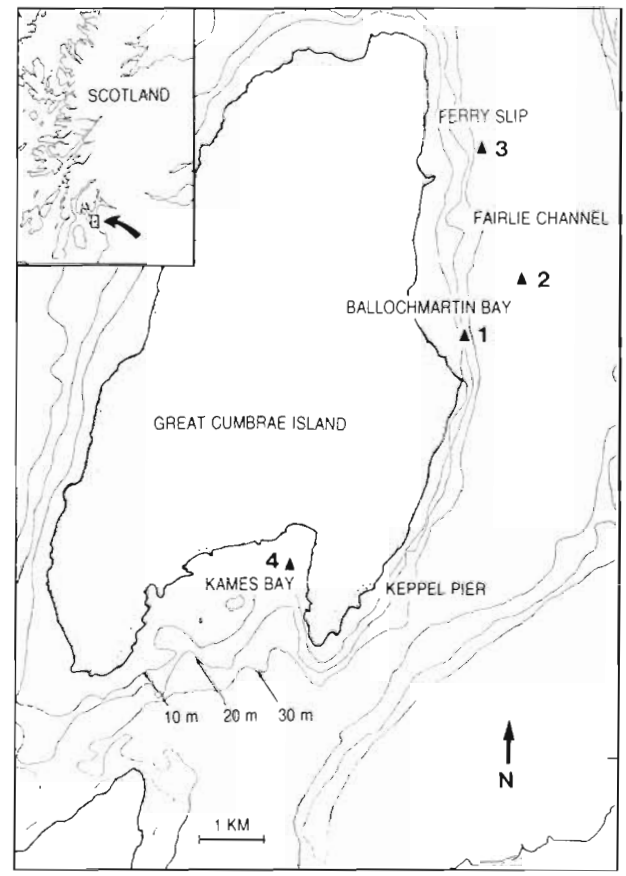

Fig. 1. Map showing the position of sampling sites (1 to 4) in the Clyde Sea area, Scotland, UK

Amoebae were incubated overnight at the required experimental temperature to allow cells to acclimate to the new culture conditions. The number of cells in 20 random fields of view was then determined for each replicate dish at t_0 and at appropriate time intervals thereafter, using an inverted phase contrast microscope with an overall magnification of 400×. Growth rates and generation times for each species were calculated from regressions of $\log_{10}$ mean number of amoebae against time extending over the period of exponential growth.

Estimation of amoeba cell volumes. Cultures were fixed in Lugol's iodine at the termination of each experiment. This induced some amoebae to round-up and form spheres (Rogerson et al. 1994). The diameters of 50 rounded cells for each species at each temperature were measured using an eyepiece graticule and the average cell volume (µm^3) for each species calculated. It is important to note that these volumes may be slight underestimates because fixing protozoan cells can cause a degree of shrinkage (Choi & Stoecker 1989).

Estimation of growth efficiency. The percentage gross growth efficiency (GGE = Production/Consumption × 100) gives a measure of how much consumed biomass (μm^3) appears as production. Production over the cell cycle was approximated as 0.66 × measured mean cell volume of the population (μm^3). This assumes that newly divided cells double in volume over the cell cycle and that growth is linear, as has been shown for *Amoeba proteus* (Rogerson 1981). Total bacterial uptake (μm^3) over the cell cycle was estimated from mean specific rates calculated for 6 of the amoebae used in the present study: i.e. interspecific means of 0.042, 0.050, 0.084, and 0.131 bacteria h^{-1} μm^{-3} amoeba biomass for 5, 10, 15, and 20°C, respectively (Butler & Rogerson unpubl. data). In all cases, the volume of a single prey bacterium used in the consumption experiments was 0.8 μm^3. No attempt was made to convert the biomass (μm^3) data to energy equivalents (e.g. carbon or joules) since there is no clear consensus on appropriate conversion factors. For example, in the case of bacteria, factors range markedly from 0.12 to 0.56 pg C μm^{-3} (Bjørnsen 1986) while the value for protozoa (based on *Monas* sp.) is 0.22 pg C μm^{-3} (Børsheim & Bratbak 1987). By retaining the data in the form of biomass units (μm^3) the calculated efficiencies were within the expected range for protozoa (see 'Discussion'). This suggests that a bacterial conversion similar to that of *Monas* sp. may be appropriate for marine bacteria.

Estimation of annual production. Annual production was estimated from the growth rate data (present study) and from amoeba abundance data gathered throughout 1991 (Butler & Rogerson 1995). In this enumeration study, numbers of bacterivorous amoebae were estimated by an enrichment cultivation method. Sediment was diluted and 1 µl aliquots were dispensed into soil extract medium (Page 1983) contained in wells of tissue culture plates. These were incubated at 18°C in the dark. Based on the number of culture dish wells with populations of amoebae, the number of amoebae in undiluted sediment was calculated. Data on abundances were collected from 4 benthic sites in the Clyde Sea area (Fig. 1): Site 1 was fine sand at 18 m, Sites 2 and 3 were medium and coarse silt at 46 m and 38 m, respectively and Site 4 was fine sand at 8 m. In all cases, amoebae were counted in the surface, aerobic layer (usually the top 1 cm) and the numerical data were transformed to biomass equivalents (μm^3) using the cell volume data. Production was calculated by Calkorskaja's formula for continuously reproducing populations as reported in Kajak (1967). This method has been used previously for amoebae by Heal (1970) and Rogerson (1982) and for benthic protozoa (Finlay 1978). Kajak's formula is as follows:

$$P = [1/D][(B_0 + B_t)/2] \times t$$

where P is production during t time units, D is doubling time in days and B_0 and B_t are the initial and final biomass values with regard to time t. It is important to note that this expression assumes that growth is at a constant (maximum) rate over time. This is clearly an oversimplification of conditions in the field so the production value generated by this equation merely gives an order-of-magnitude estimate of amoeba production.

To facilitate comparisons with published production values, results were converted to energy and carbon equivalents. Unfortunately, few conversion factors are available in the literature and none are published for marine amoebae. At this time, the most appropriate values are the conversions relating biomass to dry weight (0.147 pg μm^{-3}) and dry weight to energy units (17.51 J mg^{-1} dry weight) derived for a freshwater amoeba, *Amoeba proteus* (Rogerson 1979). Production values (μm^3) were also converted to carbon equivalent units using a value of 0.22 pg C μm^{-3} derived for the flagellate *Monas* sp. (Børsheim & Bratbak 1987).

The estimation of production and production efficiency in this study allowed us to calculate an estimate of annual consumption by naked marine amoebae in benthic sediments. Again, because of the aforementioned limitations of the annual production estimate, this can only be considered to be an order-of-magnitude estimate. However, it does provide new information on the potential impact of this group on bacterial production in the benthos.

RESULTS

The relationship between $\log_{10}$ number of amoebae and time (h) was linear and in every case the fit of the regression line was significant ($p < 0.05$) with the exception of *Dactylamoeba* sp., which repeatedly failed to grow at 5°C. The growth rates (h^{-1}) and generation times (h) of all species of amoebae calculated from the regressions are given in Table 1.

Generation times varied with both species and temperature, ranging from 11.2 h for *Platyamoeba* sp. at 20°C to 130 h for *Rhizamoeba* sp. at 5°C. All species grew fastest at 20°C and slowest at 5°C, except *Stereomyxa ramosa*, which had the shortest generation time at 15°C. The 95% confidence intervals of the slopes of the regression lines showed that the growth rates of most species cultured at different temperatures were significantly different. Where they were not, the 95% confidence intervals of the y-axis intercept were also calculated. *Platyamoeba* sp., *Vahlkampfia baltica* and *Clydonella rosenfieldi* had growth rates at each temperature that were significantly different ($p < 0.05$) from rates at the other temperatures. However, no significant difference ($p > 0.05$) was found in growth rates of *V.*

damariscottae between 5 and 15°C, in *Vannella* sp. between 5 and 10°C and between 15 and 20°C, in *Paraflabellula reniformis* between 10 and 15°C and in *Dactylamoeba* sp. between 10 and 15°C. *Vannella caledonica* only showed a significant difference in growth rate when comparing results from the temperature extremes 5°C and 20°C.

Table 1. Growth rate constant (μ, $h^{-1} \times 10^{-2}$), generation time (G, h), mean cell volume (MCV ± SE, μm^3) and percentage gross growth efficiencies (GGE) for 10 species of marine amoebae cultured at 4 temperatures. ng: no growth; nd: no data

Species	T (°C)	μ	G	MCV	GGE
Clydonella rosenfieldi					
	5	0.94	74.0	700 (56)	26.5
	10	1.22	57.0	469 (30)	28.9
	15	2.62	26.5	308 (20)	37.0
	20	5.57	12.5	385 (36)	50.3
Dactylamoeba sp.					
	5	ng	ng	ng	ng
	10	0.67	103.2	3258 (645)	16.0
	15	0.83	83.7	2832 (164)	11.7
	20	2.02	34.3	1685 (141)	18.3
Paraflabellula reniformis					
	5	1.08	64.4	437 (35)	30.1
	10	2.48	28	392 (36)	58.9
	15	3.51	19.8	587 (53)	49.6
	20	3.99	17.4	387 (43)	36.1
Platyamoeba sp.					
	5	2.15	32.2	60 (9)	60.9
	10	2.87	24.2	26 (2)	68.8
	15	3.24	21.4	nd	nd
	20	6.21	11.2	28 (2)	56.0
Rhizamoeba sp.					
	5	0.53	130.0	430 (30)	15.1
	10	1.04	66.9	148 (12)	24.7
	15	1.27	54.8	57 (6)	17.9
	20	2.30	30.2	83 (5)	20.8
Stereomyxa ramosa					
	5	0.67	103.8	564 (48)	18.9
	10	0.80	87.0	835 (80)	18.9
	15	1.59	43.7	575 (58)	22.5
	20	1.52	45.8	1453 (140)	13.7
Vahlkampfia baltica					
	5	0.98	70.6	566 (40)	27.9
	10	1.10	63.0	361 (27)	26.2
	15	2.95	23.5	283 (20)	41.8
	20	4.38	15.8	301 (19)	31.9
Vahlkampfia damariscottae					
	5	1.09	63.4	68 (9)	31.0
	10	3.33	20.8	154 (9)	79.7
	15	3.22	21.5	83 (13)	45.7
	20	5.50	12.6	45 (4)	50.3
Vannella caledonica					
	5	1.83	38.0	525 (18)	51.6
	10	2.48	28.0	431 (23)	58.8
	15	2 60	26 7	352 (19)	36.7
	20	2.93	23.7	300 (18)	26.6
Vannella sp.					
	5	1.28	54.3	46 (3)	36.2
	10	1.71	40.4	37 (3)	40.7
	15	3.16	22.0	41 (3)	44.4
	20	4.53	15.3	38 (3)	41.1

The mean cell volumes for each species at each temperature are also given in Table 1. To determine the interspecific relationship between cell volume (V) and generation time (G), the $\log_{10} G$ on $\log_{10} V$ data were regressed for each temperature (Fig. 2). The regression was only significant ($p < 0.05$) at 20°C and the regression lines for each temperature were not significantly different from each other ($p > 0.05$). Therefore, a combined regression using data from all 4 temperatures was calculated as $\log_{10} G$ (h) = 0.231 $\log_{10} V$ (μm^3) + 1.010; $p = 0.004$.

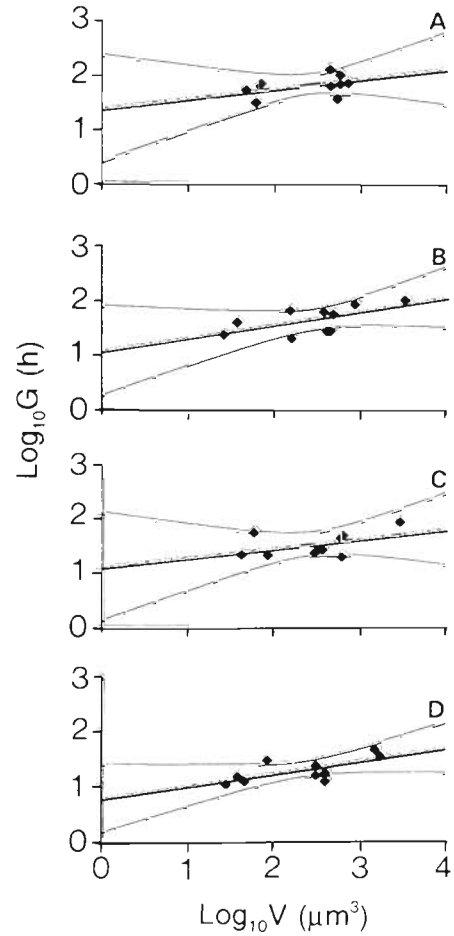

Fig. 2. Plots of log generation time (G) against $\log_{10}$ cell volume (V) for amoebae cultured at 4 experimental temperatures with 95% confidence limits (CL). Equations of the lines are as follows:
(A) At 5°C, $\log_{10} G = 0.187 \log_{10} V + 1.360$ (95% CL of slope and intercept are 0.306 and 0.747, respectively).
(B) At 10°C, $\log_{10} G = 0.245 \log_{10} V + 1.061$ (95% CL of slope and intercept are 0.254 and 0.632, respectively).
(C) At 15°C, $\log_{10} G = 0.168 \log_{10} V + 1.092$ (95% CL of slope and intercept are 0.302 and 0.747, respectively).
(D) At 20°C, $\log_{10} G = 0.221 \log_{10} V + 0.780$ (95% CL of slope and intercept are 0.153 and 0.451, respectively)

Growth efficiencies ranged from 11.7 to 79.7%, with an overall mean of 36.1% (Table 1). In most cases, efficiencies were lowest at 5°C and increased with temperature.

Annual production levels for naked amoebae inhabiting the aerobic layer of benthic sediments are shown in Table 2. Generally, the finest sediments contained higher numbers of amoebae, particularly in the top 0.5 cm layer. If this surface layer is considered, the site with the highest production is Site 2, where the sediments are 87% silt and clay. However, if the complete aerobic zone of the sediment at each site is considered, the total production is highest for the sandy Site 4. Although there are far fewer amoebae at each depth and a lower production in the surface 0.5 cm at Site 4, the aerobic layer extends to about 15 cm, whereas in the finer sediments it is restricted to the top 1 or 2 cm.

Table 2. Annual production of the entire naked amoebae community inhabiting the aerobic layer of benthic marine sediments of the Clyde Sea area

Units	Site			
	1	2	3	4
kJ m^{-2} yr^{-1}	7.60	15.7	9.86	15.8
g C m^{-2} yr^{-1}	0.65	1.34	0.84	1.35

DISCUSSION

The generation times calculated for marine amoebae in the present study are similar to those reported in other studies on amoebae. For example, experiments on freshwater amoebae by Baldock et al. (1980), at temperatures between 10 and 25°C, found generation times to range from 4.5 to 33.3 h. Baldock & Baker (1980) reported doubling times of 23.5 h at 25°C to 323 h at 5°C for *Polychaos fasciculatum* and Heal (1967) calculated generation times of 17 to 240 h for *Acanthamoeba* sp. at 5 to 25°C. Comparable results, ranging between 2.4 and 46 h, have also been obtained for benthic cilates at 20°C (Fenchel 1968).

The importance of temperature in the regulation of growth rate of amoebae is clear. Over the range of temperatures used in the study, growth rate increased (i.e. generation time decreased) with temperature. Such a relationship has previously been found for other amoebae (Heal 1967, Baldock & Baker 1980, Baldock et al. 1980, Rogerson 1981).

The temperatures measured for the surface sediments in the Clyde Sea area, from which the amoebae were isolated, ranged from approximately 6 to 15°C over 1991. The results therefore indicate that all 10 species examined are capable of reproducing *in situ* throughout the year, apart from *Dactylamoeba* sp., which did not grow at 5°C. Attempts to grow 4 of the species at 1°C were only successful in the case of *V. damariscottae*. Normal growth over the range of temperatures found in the field is supported by the fact that 93% of the Q_{10} values were below 5.0 and 56% of the values were less than 3.0. Wieser (1973) speculated that low Q_{10} values indicate that an organism is in the optimum part of its temperature range, whereas high values of around 6 to 10 are found towards the limit of an organism's metabolic tolerance.

Nine of the 10 species of amoebae studied had their maximum growth rates at 15 or 20°C, suggesting that amoebae tend to grow at sub-maximal rates *in situ*. Optimum temperatures for growth above those encountered in the field have been demonstrated for other species of amoeba (Baldock & Baker 1980, Baldock et al. 1980) and for marine benthic ciliates (Fenchel 1968).

A significant regression of growth rate on temperature for protozoa was first described by Fenchel (1968) for marine benthic ciliates and similar relationships have been demonstrated for other protozoa (Fenchel 1974, Finlay 1977, Baldock et al. 1980, Montagnes et al. 1988, Muller & Geller 1993). In the present study, which is the first to consider marine amoebae, a linear relationship was found between $\log_{10}$ generation time and $\log_{10}$ cell volume, with a slope of 0.231 (±0.153), which is not significantly different ($p > 0.05$) from the slope of 0.311 obtained by Baldock et al. (1980) for a range of freshwater amoebae and ciliates. Their study also found that the regression lines at the separate experimental temperatures were not significantly different from each other. Such generalizations should be used with care in future studies on amoebal growth because of the difficulties of accurately estimating amoebal cell volume. In our study there is considerable variation in the data, and the inverse relationship between cell volume and temperature, which accounts for the temperature-independent generalized relationship, is not consistent across all species examined (Table 1).

Fenchel (1987) presented a relationship between log growth rate (h^{-1}) and log cell volume (μm^{-3}) for a wide range of protozoa at 20°C. Here, the growth rate constant decreased with around the 0.25 power of cell volume and showed that sarcodines appeared to have consistently lower growth rate constants than other protozoa. The data from the present study at 20°C showed similar results in terms of the slope of the line (−0.22) and the positioning of the amoeba data points below those of ciliates and flagellates. It is not clear why amoebae of similar size to flagellates and ciliates should have lower growth rates. A high respiratory

cost of locomotion (as suggested by Crawford et al. 1994) could account for slightly lower growth constants, with less energy being channelled to growth. It is more likely that lower growth potential in amoebae is an adaptive feature of the group. Fenchel (1987) speculated that protozoa living in relatively homogeneous environments, with excess food, would have no selective advantage in having very rapid growth.

No significant relationship was found between cell volume and temperature, although 70% of the amoebae examined had greater cell volumes at the lowest temperatures at which they could be grown. For example, the cell volume of *Rhizamoeba* sp. was some 5× greater at 5°C than at higher temperatures. The extended generation times at these lower temperatures show that some cells can lay down new biomass and increase in size without undergoing cell division. An increase in cell volume at lower temperatures has been noted for other species of amoebae (Baldock & Baker 1980, Rogerson 1981).

The growth efficiencies varied from 11.7 to 79.7%, with a mean of 36.1%. The highest values may reflect problems in accurately measuring the cell volumes and consumption rates of amoebae. However, the majority of efficiencies in Table 1 are well within the range published for other protozoa, with most lying between 30 and 50% (Fenchel 1987), the variation being due to inherent differences among species and to the qualitative nature of the prey. In most cases, efficiencies increased with increasing temperature, suggesting that amoebae converted food into biomass with increased efficiency at the higher temperatures. The 2 morphotypes with the lowest efficiencies, *Dactylamoeba* sp. and *Stereomyxa ramosa*, had the largest cell volumes. It is possible that these species would have yielded higher efficiencies if fed prey other than bacteria, since both have been found by us to readily ingest protistan prey such as pennate diatoms.

Extrapolation of laboratory data to the field situation must be handled with care, the natural environment being more complex because of the many interacting environmental factors. However, the temperatures and food concentrations in our experiments were chosen to be as similar as possible to those in the sediments, and amoebae were surface-attached, as in the benthos. Laboratory growth rates were measured during exponential growth of the population, but this is a reasonable simplification since exponential growth of protozoa in the field has been observed (Fenchel 1968, Bick 1973). Additionally, Arndt (1993) found generation times of a community of naked amoebae from Lake Muggelsee to be similar when measured in the laboratory and *in situ*.

When applied to field data, the results of the present study provide first information on the production of benthic marine amoebae. The annual production values, ranging between 7.6 and 15.8 kJ m^{-2} yr^{-1}, depending on sediment type, demonstrate the potential ecological importance of this group in the benthos. It must be stressed, however, that these values can only be viewed as order-of-magnitude estimates, because of the assumptions that are inevitable in such calculations (Schönborn 1992). Even so, these levels are important since there are few comparable data in the literature. The only study for marine sediments is that of Fernandez-Leborans & Novillo (1993), who found that amoeba annual production in sublittoral beach sand ranged between 3.4 and 24.7 kJ m^{-2} yr^{-1}, a range that included all amoebae, not just naked forms.

Production values calculated in the present study are within the range quoted for naked amoebae from other habitats. Production was slightly lower for amoebae attached to plants in a chalk stream (6.2 kJ m^{-2} yr^{-1}; Baldock et al. 1983) and slightly higher for amoebae inhabiting *Sphagnum* moss (49.7 kJ m^{-2} yr^{-1}; Rogerson 1982). The value reported by Heal (1967) for soil amoebae is 2 orders of magnitude greater, reflecting the importance of naked amoebae in soils.

By using the average gross production efficiency value (36.1%) calculated for amoebae in this study, it is possible to estimate how much bacterial biomass amoebae crop each year in marine sediments. Annual consumption by amoebae was 1.80, 3.71, 2.33 and 3.74 g C m^{-2} yr^{-1} for Sites 1, 2, 3 and 4, respectively. Sander & Kalff (1993) compiled data from 25 studies on bacterial production in sediments. Using their data to calculate an average annual bacterial production value (350.4 g C m^{-2} yr^{-1}), amoebae therefore consume between 0.5 and 1.1% of the total bacterial production. However, if their lowest bacterial production value is used (0.4 g C m^{-2} yr^{-1}), then the entire benthic assemblage of naked amoebae could consume between 450 and 935% of bacterial production.

Clearly, without reliable data on bacterial production values and possible prey preferences of amoebae in marine sediments, it is impossible to draw firm conclusions about the exact ecological role of this group. Perhaps the major contribution of this paper is that it draws attention to a frequently overlooked group of protists in the benthos and demonstrates that they need to be considered in future studies of carbon flow.

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