

Respiration of the marine amoeba *Trichosphaerium sieboldi* determined by ^{14}C labelling and Cartesian diver methods

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ABSTRACT: Estimates of rates of respiration in the marine amoeba *Trichosphaerium sieboldi* were compared using 2 microtechniques; a single cell ^{14}C labelling method and Cartesian diver respirometry. Mean volume specific rates at 20 °C determined by the diver method were $5.35 \times 10^{-9} \mu\text{l O}_2 \mu\text{m}^{-3} \text{h}^{-1}$ for starved cells and $5.25 \times 10^{-9} \mu\text{l O}_2 \mu\text{m}^{-3} \text{h}^{-1}$ for cells fed on heat-killed prey. These were equivalent to carbon specific rates at 20 °C of 3.58 and 3.51 % cell C h^{-1} respectively, and compared favourably with results of the ^{14}C technique which gave 3.71 and 2.44 % cell C h^{-1} respectively. The greater precision of the ^{14}C technique revealed that carbon specific respiration rates of single cells displayed a strong inverse relationship with length of incubation, supporting the contention that the degree of starvation is an important factor in rate determinations. The ^{14}C technique also demonstrated that individual cell size distribution was non-normal (multimodal for *T. sieboldi*), and that carbon specific rates of respiration of individuals were strongly influenced by cell size (inverse relationship), as predicted by allometric considerations. Rates estimated from short incubations of around 15 min were of the order of 10 % cell C h^{-1} , while those from around 24 h or longer, under otherwise identical conditions, were 0.2 to 0.3 % cell C h^{-1} . Ability to decrease carbon specific rates with incubation time was inversely related to cell size. These observations suggest important limitations to the Cartesian and other respiration techniques when they involve relatively long incubations of several cells and the computation of specific rates from mean cell volumes.

KEY WORDS: Respiration · Amoeba · *Trichosphaerium sieboldi* · Marine protists · ^{14}C · Cartesian diver

INTRODUCTION

Respiration studies of aquatic protists are an important current field of research (e.g. Caron et al. 1990), but there has been little recent advance because of limitations in available methodology. The respiration rates of single species have traditionally been determined by measuring bulk oxygen depletion in cultures, using either Winkler titration or O_2 electrodes. However, these techniques require crowded cultures where stress effects can affect the rate of O_2 con-

sumption (e.g. Baldock et al. 1982) and frequently include microbial contaminants which also affect the measured respiration rate. Diver microrespirometry has been used to overcome these shortcomings, to some extent, in that a few cells can be isolated and washed free of contaminating organisms prior to placing in the diver. However, reviews by both Fenchel & Finlay (1983) and Caron et al. (1990) suggest that when cells become starved during lengthy incubations, they can show appreciably lower rates of respiration than well-fed cells. Consequently, physiological ecologists generally accept that during lengthy incubations what they are measuring are basal, or starvation, respiration rates which may underestimate the metabolic energy costs of growing cells in the natural environment. Given these limitations to interpreting the results of such respirometry in an ecological con-

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text, a method employing short incubations, with minimum starvation, is required. A sensitive method using single cell incubations could also provide new insights into studies in physiological ecology enabling, for example, empirical documentation of the effects of starvation on the respiration of single cells. Cartesian diver microrespirometry comes close to fulfilling these goals but still requires an experimental period of around 4 h and several cells per chamber unless large cells, such as the protozoan *Stentor* sp., are used (Laybourn 1975).

Stoecker & Michaels (1991) have recently modified a ^{14}C technique, initially used by Manahan (1983) for metabolic studies on bivalve larvae, for the determination of respiration rates of marine planktonic ciliates over several hours. The potential advantage of this method is that shorter incubations could be used in which the measured rate may be closer to that at time T_0 , if the rate does indeed vary with starvation. Fenchel & Finlay (1983) have already pointed out that shorter incubations of 10 to 15 min are preferable, although to date this has only been possible when using macrorespirometers or O_2 electrodes.

The present study uses the microtechnique of Stoecker & Michaels (1991) to measure the respiration rate of a marine amoeba over a range of incubation times and compares this rate with that derived by conventional Cartesian diver microrespirometry for a fixed incubation period under otherwise similar conditions. The advantages of the ^{14}C method are highlighted and the ecological implications of the findings discussed.

METHODS

Culture. The multinucleate marine amoeba *Trichosphaerium sieboldi* was isolated from the surface of the seaweed *Fucus* sp. from the intertidal zone adjacent to the Marine Station, Millport, UK. Cultures of amoebae were maintained in a dilute cereal leaf infusion made by boiling cereal leaves (0.1% w/v) (Sigma Chemical Co., UK) in 90% seawater for 5 min before filtering and diluting 1:1 with sterile filtered seawater (= C90S:SW medium). *T. sieboldi* was fed on the chlorophyte *Chlorella* sp. in the light at 18°C.

Cartesian diver microrespirometry. *Trichosphaerium sieboldi* cells for experimentation were taken from exponentially growing cultures and washed at least 5 times through sterile medium (C90S:SW) to remove attendant bacteria. Prior to experimentation, washed cells were placed in sterile medium for 4 to 5 h (i.e. starved conditions) or into medium containing heat-killed native bacteria (i.e. fed conditions). Mean cell volumes of the *T. sieboldi* populations were estimated

by measuring randomly selected cells fixed in Lugol's iodine (acid Lugol's, final concentration 1%). The diameters of rounded, semi-spherical cells were used to compute volume (cell volume = spherical volume/2). This approach may have introduced significant cell volume changes associated with preservation (see Choi & Stoecker 1989, Ohman & Snyder 1991), but since preservation induced a predictable semi-spherical shape, this was considered an advantage in the computation of cell volume. This approach was only necessary for computation of Cartesian diver data, and comparison with data of the ^{14}C technique. The ^{14}C method itself did not require cell volume for computation of carbon specific rates of respiration.

Cartesian divers (Klekowski 1971) with gas phases between 2.3 and 3.0 μl were loaded with between 4 and 10 *Trichosphaerium sieboldi* cells and after a 2 h acclimatisation period (at 18°C), readings were recorded over the next 2 h interval. A diver containing the washings of the final wash was used as a control against bacterial contamination. For fed cells, 12 replicate divers were used (total 75 amoebae) and for starved cells, 11 replicate divers were used (total 78 amoebae).

^{14}C labelling microtechnique. Batch cultures of ^{14}C labelled *Chlorella* sp. were prepared by inoculating C90S:SW medium containing ^{14}C bicarbonate ($\text{NaH}^{14}\text{CO}_3$; activity 1 $\mu\text{Ci ml}^{-1}$) with a small quantity of *Chlorella* sp. which were incubated in the light until cells had multiplied and reached early exponential growth. At this time, a small inoculum of early *Trichosphaerium sieboldi* culture was placed in the labelled prey culture and allowed to undergo several divisions before harvesting single cells for the respiration determinations. It was assumed that this procedure yielded evenly labelled amoeba cells, as was the case for ciliates (Putt & Stoecker 1989, Stoecker & Michaels 1991). That is, the specific activity of organic cell carbon (dpm per unit C), firstly of the *Chlorella* sp., and then of the *T. sieboldi*, eventually equals that of the labelled inorganic carbon in the medium. This was assumed to be achieved when the dpm cell $^{-1}$ of similar sized cells showed no further increase after several generations (Putt & Stoecker 1989).

Individual labelled cells were micropipetted through 5 changes of sterile, unlabelled medium. A single cell for experimentation was transferred in a 10 μl aliquot, dispensed with an automatic pipette, into 2 ml of sterile medium in a small glass shell vial. For the blanks, 10 μl aliquots of the final wash were transferred into identical shell vials with 2 ml medium. These vials were placed upright within 20 ml glass scintillation vials containing 1 ml 10% KOH as a CO_2 trap. The scintillation vials were capped with a rubber seal held in place with a plastic cap drilled to allow

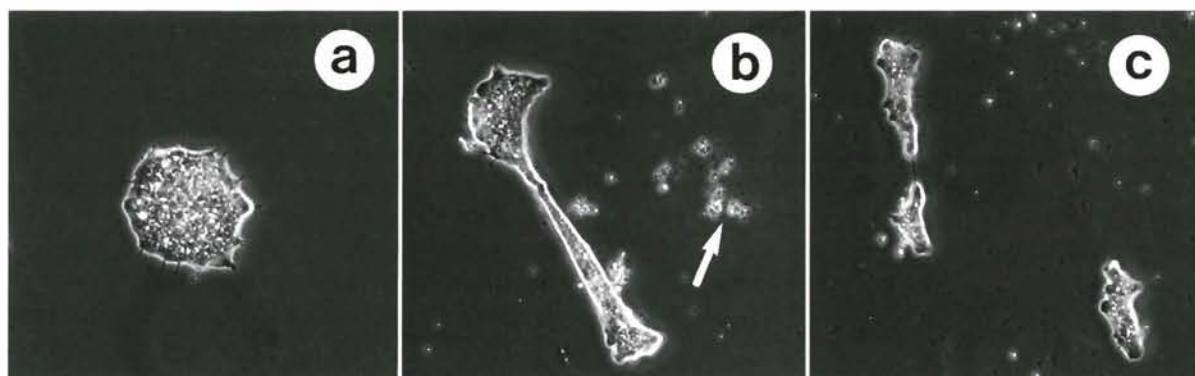


Fig. 1. *Trichosphaerium sieboldi*. Morphological configurations of cells over the time-course of the respiration experiments. (a) Stage I cells; (b) stage II cells; (c) stage III cells. Note the release of food vacuoles from stage II cells after about 5 to 6 h (arrow)

liquids to be syringed through the seal. Incubation time was recorded to the nearest minute for each vial and samples were incubated in the dark at 20°C, at least 25 replicates and 5 blanks being conducted for each time interval. After incubation 0.5 ml of 0.1 N HCl was injected into the inner shell vial to kill the cell and reduce the pH. Liberated CO₂ (including respired ¹⁴CO₂) was trapped in the KOH; vials were kept sealed for 24 h to ensure complete transfer of CO₂ (Manahan 1983). The inner shell vial was then removed and the outside rinsed into the KOH with 1 ml distilled water. 1 ml methanol was added to the KOH vial followed by 10 ml of scintillation cocktail (Ecoscint); the methanol prevented formation of a KOH/scintillation fluid precipitation (Manahan 1983). The organic fraction (remnant cell) in the shell vial was transferred to a second scintillation vial by rinsing with 1 ml distilled water. This vial and contents were then oven dried at 60°C to remove any trace of residual inorganic ¹⁴C. 1 ml distilled water was added to resuspend organic material, followed by 10 ml of scintillation fluid. Inner and outer blank vials were processed in an identical manner to that described above. Experiments on fed amoebae used the same protocol except that the sterile unlabelled C90S:SW medium, into which cells were placed, contained heat-killed labelled *Chlorella* sp.

Counts (¹⁴C dpm) were determined using a Packard Tricarb liquid scintillation counter with quench correction by external standard. Preliminary experiments with unlabelled samples showed that a delay of 48 h, prior to counting, was necessary to avoid chemiluminescence errors.

Carbon content of individual amoebae was not calculated from the dpm cell⁻¹ values and specific activity of total inorganic carbon of the medium (as in Putt & Stoecker 1989, Stoecker & Michaels 1991) because the diet of *Trichosphaerium sieboldi* was supplemented with unlabelled DOC from the organically rich

C90S:SW medium. Instead, when required, cell volume was estimated from a linear regression of activity (dpm cell⁻¹) against cell volume, from a subsample of 24 uniformly labelled, washed amoebae (fixed in acid Lugol's); cell volume was estimated microscopically as described for the Cartesian diver method above. The linear relationship relating activity (*a*) and cell volume (*v*) applicable in this study was:

$$v = 19752.7 + 29.6a$$

$$r = 0.694 \text{ (} n = 24; \text{ significant at } p < 0.001 \text{)}.$$

For computation of carbon specific rates of respiration, cell volumes were not required; respired dpm was simply expressed as a proportion of cell carbon dpm at *T*₀ (cell carbon dpm + respired dpm).

Behaviour of cells and respiratory cost of locomotion.

After *Trichosphaerium sieboldi* cells were washed in sterile medium prior to experimentation, they transformed from normal amoebae (stage I cells) to elongated stage II cells (Fig. 1) within 3 to 4 h regardless of culture medium (Table 1). After 5 to 6 h, these elon-

Table 1. *Trichosphaerium sieboldi*. Time (to nearest 1 h) for 80% (based on observation of at least 50 cells) of amoeba to transform to stage II and III cell configurations (see Fig. 1) after washing cells in sterile natural seawater (NSW), artificial seawater (ASW) and cerophyl-enriched seawater (C90S) with and without cytochalasin (µg ml⁻¹)

Culture conditions	Stage II	Stage III
NSW	3.0	4.5
ASW	4.0	5.0
C90S	4.0	5.0
C90S + 10 µg cytochalasin	4.0	6.5
C90S + 20 µg cytochalasin	4.0	5.5
C90S + 100 µg cytochalasin	4.0	6.0
C90S + 200 µg cytochalasin	24.0	nf ^a

^aNo fragmentation despite the appearance of elongated cells after 24 h; division only by binary fission

gated cells had expelled food vacuoles and fragmented to form 3 or 4 smaller *T. sieboldi* (stage III cells).

The respiratory cost of this locomotion was examined by comparing elongating cells with stationary cells. Amoebae were immobilized with cytochalasin B (Sigma Chem. Co., UK), a metabolite of *Helminthosporium dematioides*, which stops cell locomotion by affecting the state of actin polymerisation. A working concentration of 200 $\mu\text{g ml}^{-1}$ (in the presence of 1% dimethyl sulfoxide, DMSO) was found to be necessary to immobilize the majority of cells in stage I for up to 24 h (Table 1). The respiratory cost of motility was estimated by comparing the respiration of moving amoebae without cytochalasin (i.e. C90S:SW medium with DMSO) with respiration of nonmoving cells in the presence of inhibitor, using the ^{14}C method described. Experimental and control shell vials contained 3 washed ^{14}C labelled *Trichosphaerium sieboldi* of equivalent size. Samples were incubated for 2.5 h in the dark ($n = 15$) at 20°C.

RESULTS

The rates of respiration determined by the Cartesian diver technique over a 4 h experimental period (2 h acclimatisation and 2 h incubation) at 18°C are shown in Table 2. The mean rates of O_2 uptake per cell for starving amoebae ($1.48 \times 10^{-4} \mu\text{l h}^{-1}$; ± 0.82 SD, $n = 11$) are about 50% less than those of fed cells ($2.79 \times 10^{-4} \mu\text{l h}^{-1}$; ± 2.39 SD, $n = 12$), but when normalized for cell biomass on the basis of cell volume (starved = $31\,724 \pm 12\,348 \mu\text{m}^3$, fed = $61\,021 \pm 40\,782 \mu\text{m}^3$; $n = 20$) the specific respiration rates are equivalent at $4.66 \times 10^{-9} \mu\text{l O}_2 \mu\text{m}^{-3} \text{h}^{-1}$ for starved amoebae and $4.57 \times 10^{-9} \mu\text{l O}_2 \mu\text{m}^{-3} \text{h}^{-1}$ for fed cells. Assuming a carbon:volume ratio of 0.08 $\text{pg C } \mu\text{m}^{-3}$, a respiratory quotient (RQ) of 1.0 and a Q_{10} of 2.0 (Fenchel & Finlay 1983), then the mean respiration rates at 20°C translate to carbon turnover rates of 3.58% cell C h^{-1} for starved cells and 3.51% cell C h^{-1} for fed cells.

Mean carbon specific rates of respiration determined by the ^{14}C technique at 20°C are given in Table 3. Rates were calculated by expressing CO_2 dpm as a percentage of total dpm at T_0 (cell + CO_2 dpm), after first correcting both counts for blanks and then adjusting to rate h^{-1} . This gave carbon turnover rates of 3.71% cell C h^{-1} for starved cells and 2.44% cell C h^{-1} for fed cells. The precision of the ^{14}C method allowed an examination of both cell size distribution (Fig. 2) as well as elucidation of the influence of cell size on carbon specific respiration per individual (Fig. 3). In Fig. 2, cell sizes (dpm cell^{-1}) are expressed for time T_0 (by summation of cell C and respired C) and as such are not affected by the subsequent experimental treat-

ments of starvation or feeding. The cell size distribution of the *Trichosphaerium sieboldi* population was multimodal, probably as a result of cells fragmenting into a variety of cell sizes (see Fig. 1).

It is clear from Fig. 3 that cell size exerts an influence on carbon specific rates for individual cells. This can be predicted from allometric considerations; however, until now, methods have not been available to resolve this at the single cell, intraspecific level, with the exception of studies on large protozoa in Cartesian divers (eg. Laybourn 1975). It is likely that the influence of this wide size distribution upon carbon specific respiration rates accounted for much of the variance in

Table 2. *Trichosphaerium sieboldi*. Respiration rates ($\times 10^{-4} \mu\text{l O}_2 \text{ cell}^{-1} \text{h}^{-1}$) determined by Cartesian diver microrespirometry at 18°C for amoebae feeding on heat-killed bacteria and for cells in sterile C90S:SW. Numbers in parenthesis are amoebae loaded per diver

Feeding ^a	Starved ^b
9.60 (5)	0.56 (10)
4.81 (4)	0.66 (8)
3.50 (5)	2.06 (7)
1.92 (4)	3.35 (4)
2.39 (8)	1.55 (4)
1.19 (8)	1.64 (6)
1.71 (5)	1.87 (6)
1.47 (7)	1.26 (8)
2.35 (6)	1.75 (8)
1.08 (7)	1.02 (8)
1.53 (8)	0.59 (9)
1.96 (8)	
Mean = 2.79 ^c	Mean = 1.48 ^c

^aWith heat-killed native bacteria
^bStarved for 4 to 5 h before loading divers
^cEquivalent values at 20°C are 3.20 and 1.70, respectively, assuming a Q_{10} of 2.0
 (Note: these rates were converted in the text to C equivalents assuming a C:volume ratio of 0.08 $\text{pg C } \mu\text{m}^{-3}$ and a respiratory quotient = 1.0)

Table 3. *Trichosphaerium sieboldi*. Mean respiration rates at 20°C by the single cell ^{14}C labelling technique on individual starved cells ($n = 48$) or those fed heat-killed prey ($n = 46$). Also shown are mean (blank corrected) levels of radioactivity (dpm) per cell (inner vial) and mean dpm respired (outer vial). Incubation time approximately 160 min

Treatment	Resp. rate (% cell C h^{-1})	Cell dpm (T_0)	Cell dpm (T_{160})	CO_2 dpm
Fed (\pm SD)	2.44 (1.75)	3033 (1751)	2855 (1670)	178 (118)
Starved (\pm SD)	3.71 (1.91)	1975 (975)	1808 (930)	167 (80)

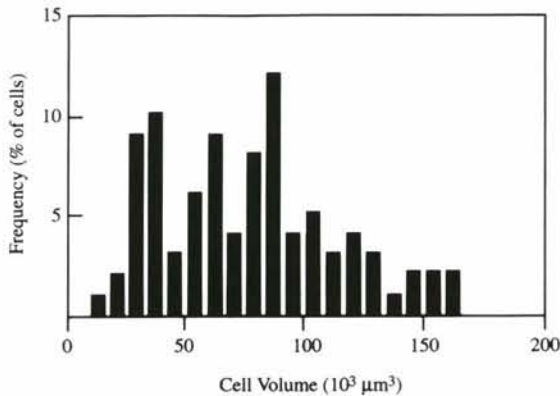


Fig. 2. *Trichosphaerium sieboldi*. Frequency-size distribution of the population used in ^{14}C respiration experiments. Cell volumes derived from the equation relating activity (dpm cell $^{-1}$) to cell size (see 'Methods')

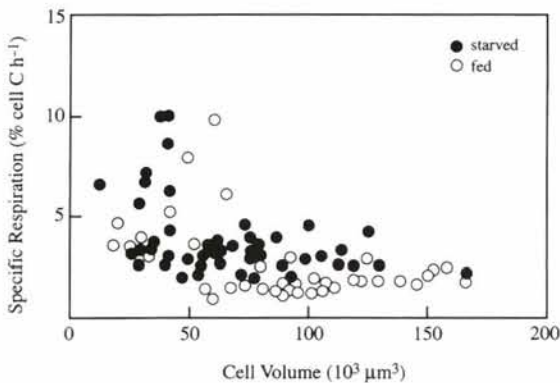


Fig. 3. *Trichosphaerium sieboldi*. Relationship between cell volume and specific respiration rate of starved and fed individual cells over an incubation of 160 min by the ^{14}C method. Cells were fed unlabelled, heat-killed *Chlorella* sp.

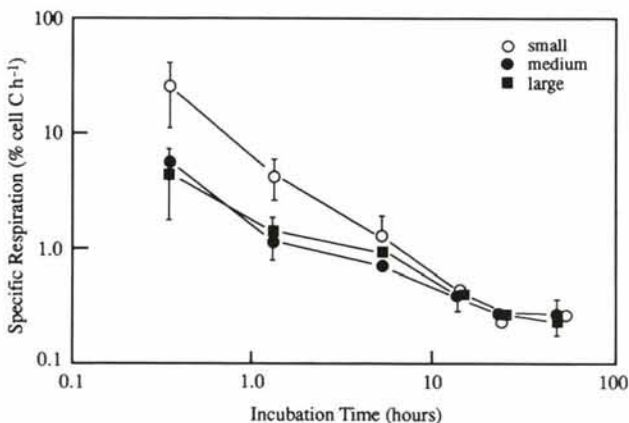


Fig. 4. *Trichosphaerium sieboldi*. Effect of incubation time on the specific respiration rate using the ^{14}C technique. Values represent means of sized groups comprising small (<50 000 μm^3), medium (50 000 to 100 000 μm^3) and large (>100 000 μm^3) individuals

mean rates shown in Tables 2 & 3. While it appears from Fig. 3 that starved cells have a slightly higher specific respiration rate than fed cells, this conclusion may not be valid because of possible dilution of labelled cell carbon by ingested prey during long-incubation experiments.

The effect of incubation time on individual carbon specific rates is shown in Fig. 4. Mean respiration rates are clearly inversely related to incubation time, with initial rates, determined by extrapolation to T_0 , being of the order of 10 % cell C h $^{-1}$, while rates after 24 h were less than 0.3 % cell C h $^{-1}$. A log:log transformation of the data on carbon specific rates against cell volume (Fig. 5) gave a significant negative slope for the shortest incubation (15 min) with a gradual levelling off with increasing incubation time; incubations of

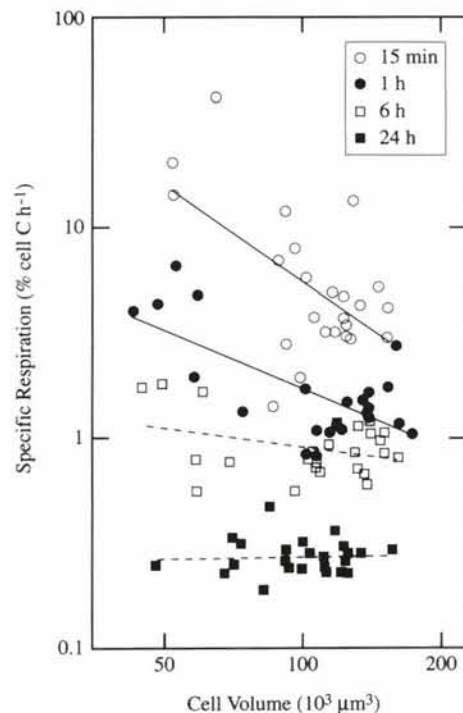


Fig. 5. *Trichosphaerium sieboldi*. Influence of cell size on specific respiration rate of individual cells incubated over a range of time intervals. Solid lines for 15 min and 1 h incubations represent significant fits for least squares linear regressions of log cell size (V) on log specific respiration rate (R). Dashed lines for the 6 and 24 h incubations represent nonsignificant fits. The linear regressions are described by the following equations:

$$\begin{aligned}
 15 \text{ min: } & \log R = -1.5643 \log V + 8.5614 \\
 & (r^2 = 0.3380; p < 0.005) \\
 1 \text{ h: } & \log R = -0.9450 \log V + 4.9562 \\
 & (r^2 = 0.4836; p < 0.0005) \\
 6 \text{ h: } & \log R = -0.2936 \log V + 1.4239 \\
 & (r^2 = 0.1172; \text{ nonsignificant, } 0.1 > p > 0.05) \\
 24 \text{ h: } & \log R = 0.0494 \log V - 0.8154 \\
 & (r^2 = 0.0054; \text{ nonsignificant, } p > 0.5)
 \end{aligned}$$

Table 4. *Trichosphaerium sieboldi*. Estimates of mean carbon specific respiration rate (95% confidence intervals in parentheses) for selected cell volumes and incubation times. Estimates were derived from the least squares linear regressions of log cell volume on log specific respiration rate shown in Fig. 5

Incubation time	Specific respiration rate (% cell C h ⁻¹) at a cell volume (× 10 ³ μm ³) of:		
	50	100	150
5 min	16.25 (7.58–34.81)	5.50 (4.16–7.26)	2.92 (1.86–4.56)
1 h	3.28 (2.28–4.71)	1.70 (1.43–2.03)	1.16 (0.92–1.47)
6 h	1.11 (0.83–1.47)	0.90 (0.79–1.03)	0.80 (0.67–0.96)
24 h	0.26 (0.21–0.32)	0.27 (0.25–0.29)	0.28 (0.24–0.32)

6 h or longer gave slopes not significantly different from zero. Both Figs. 4 & 5 and Table 4 suggest that cells of all sizes can decrease their carbon specific rates with incubation time but it is the smaller cells that clearly have a greater ability in this respect.

The energetic cost of locomotion was estimated by the ¹⁴C technique using cytochalasin (200 μg ml⁻¹) to immobilise cells (Table 1). Over a 2.5 h incubation, when cells were elongating and undergoing rapid locomotion, the mean % C respired by the total biomass of amoebae in the control vessels was 6.8% of total labelled carbon, while in the experimental vessels (i.e. cytochalasin immobilised cells) only 3.0% of labelled C was respired. While bearing in mind that other energy-demanding cell functions may have been inhibited by cytochalasin, the results suggest that for actively moving *Trichosphaerium sieboldi*, cell locomotion could account for around half of total cell respiration.

DISCUSSION

The estimates of cell specific rates (Table 2) obtained by the O₂ diver method (mean 0.32 nl O₂ cell⁻¹ h⁻¹ at 20°C) for fed cells fall within the wide range of values (0.0043 to 58.4 nl O₂ cell⁻¹ h⁻¹) reported for amoebae by Fenchel & Finlay (1983). There do not appear to be other published values for *Trichosphaerium sieboldi*, but taking similar sized amoebae for comparison, these rates agree well with those for growing *Amoeba proteus*, where rates range between 0.19 and 1.80 nl O₂ cell⁻¹ h⁻¹ (Korohoda & Kalisz 1970, Rogerson 1981), and for *Diffugia* sp. with its rate of 1.16 nl O₂ cell⁻¹ h⁻¹ (Zeuthen 1943). When the empirical formula of Fenchel & Finlay (1983) is used to predict the respira-

tion rate of a hypothetical heterotrophic protist with similar volume to *T. sieboldi*, then the rate of respiration (i.e. 0.46 nl O₂ cell⁻¹ h⁻¹) is similar to that of fed cells in this study. The mean cell specific rate for starving cells, 0.17 nl O₂ cell⁻¹ h⁻¹, compares favourably to the rate for starving *A. proteus* (i.e. 0.25 nl O₂ cell⁻¹ h⁻¹; Brachet 1955) and for *A. proteus* of unspecified physiological state (i.e. 0.15 nl O₂ cell⁻¹ h⁻¹; Emerson 1930). Likewise, volume specific rates at 20°C for *T. sieboldi* (5.25 to 5.35 × 10⁻⁶ nl O₂ μm⁻³ h⁻¹) are within the range reported for amoebae in general (3.4 × 10⁻⁸ to 1.3 × 10⁻⁵ nl O₂ μm⁻³ h⁻¹; Caron et al. 1990), and comparable to rates for the larger protozoa *A. proteus*, *Diffugia* sp. and *Spiroloculina hyalini* which range from 3.8 × 10⁻⁷ to 5.7 × 10⁻⁶ nl O₂ μm⁻³ h⁻¹ (Zeuthen 1943, Korohoda & Kalisz 1970, Lee & Muller 1973, Rogerson 1981). These volume specific rates for *T. sieboldi* will be subject to errors caused by cell volume changes in the acid Lugol's fixative employed (Choi & Stoecker 1989, Ohman & Snyder 1991); however, these may in fact be less significant than the errors associated with approximation of amoebae to simple geometric shapes in previous studies. Preservation in Lugol's had the advantage of 'rounding' cells to an approximate semi-sphere.

The data reviewed by Caron et al. (1990) suggest that volume specific rates for starved cells are about 24% those of fed cells for *Amoeba proteus*, although the other amoeba mentioned, *Chaos carolinense*, showed the opposite, with starved cells giving higher volume specific rates than fed cells. The Cartesian diver data for *Trichosphaerium sieboldi* showed lower mean cell specific rates for starved cells (by a factor of 53%), but after normalizing for cell size, the volume specific rates (μm⁻³) were equivalent. As highlighted by the contradictory results for *A. proteus* and *C. carolinense*, the interpretation of respiration rates for starved versus fed cells is seldom straightforward. In the case of *T. sieboldi*, fed cells behaved sluggishly by comparison to the starved cells which were observed to move around the Cartesian diver chamber. Thus, a high energetic cost of locomotion in starved cells may have balanced the cost of digestion and growth in the fed cells which expended little energy searching for prey.

There is some experimental evidence to support a high energetic cost of locomotion in amoebae. By immobilizing *Trichosphaerium sieboldi* with cytochalasin it was estimated that locomotion could account for some 56% of total metabolic activity. This value is at odds with theoretical estimates by Fenchel & Finlay (1983) who maintain that the cost of locomotion in protists is less than 1% of their energy budget. This difference could reflect the different modes of locomotion among protozoa. In the case of amoebae, the continual

polymerization and depolymerization of a cytoplasmic actin skeleton may be less efficient than locomotion mediated by cilia or flagella. Alternatively, a purely theoretical consideration of locomotion may underestimate the true cost, as has been shown in studies on rotifers. The cost of ciliary movement was calculated based on drag, velocity and Reynolds number and found to be less than 1%; however, when measured directly, it appeared that some 62% of the energy budget could be devoted to locomotion (Epp & Lewis 1984). Moreover, Crawford (1992) has calculated that in contrast to previous theoretical estimates (such as Fenchel & Finlay 1983), fast-swimming protists could incur high locomotive costs (over 10% total rate of respiration).

The mean carbon specific rate for starved individuals of $3.71\% \text{ cell C h}^{-1}$ (Table 3) agrees well with the rates from the oxygen diver when converted to 20°C (i.e. 3.51 and $3.58\% \text{ cell C h}^{-1}$ for fed and starved cells respectively). However, the mean rate for fed cells of $2.44\% \text{ cell C h}^{-1}$ using the ^{14}C method was considerably lower. While this could be explained, in part, by the reduced motility of fed cells, a likely contributory factor is also the introduction and subsequent ingestion of non-radioactively-labelled prey gradually 'diluting' the specific activity of total cell carbon. Moreover, if recently digested metabolites were preferentially respired, then this would cause an underestimation of respiration rates. These effects could, however, be minimised by using incubation times less than the 160 min used in this experiment. A short incubation experiment over 15 min (Fig. 6) clearly shows that the influence of cell size on carbon specific rate is itself influenced by the presence of heat-killed prey; cells incubated in the absence of food had significantly higher respiration rates, and this was significantly size dependent. This could presumably be attributed to elevated locomotory costs in the absence of food.

Despite ambiguities in the comparisons between starved and fed amoebae, the estimations from the ^{14}C technique show good agreement with rates from the O_2 method and validate its application in microrespirometry studies. The ^{14}C technique has, however, distinct advantages over the Cartesian diver in that it can be used with single cells and can provide additional information on the biomass (total C) of each cell; initial cell carbon content can be derived from the summation of final cell carbon and total respired CO_2 carbon. This means that carbon specific rates can be calculated without reference to cell volumes, thus avoiding errors caused by variation in carbon:volume ratios, and cell volume changes associated with preservation. This is in line with the suggestion by Fenchel & Finlay (1983) that it is now important that exact biomasses be known when conducting respiration stud-

ies. Other microrespirometry methods require volume to be estimated from the best geometric approximation and appropriate conversions; an approach not well suited to precise studies on individual cells. To exemplify this advantage, the ^{14}C method was used to examine the influence of cell size on respiration rate. Although allometric considerations have been well documented at the interspecific level (e.g. Fenchel & Finlay 1983, Caron et al. 1990), few studies have attempted to examine this variable at the intraspecific level. Carbon specific rates of respiration of individuals are plotted against cell size in Figs. 3 & 5, and it is clear that size exerts a strong influence on this rate, even over the limited range of a *Trichosphaerium sieboldi* population. For short incubations, mean rates of respi-

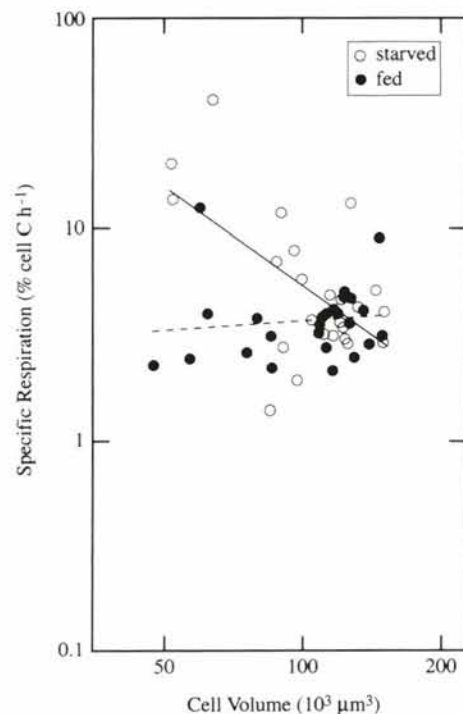


Fig. 6. *Trichosphaerium sieboldi*. Influence of cell size on specific respiration rate for starved and fed individual cells. Cells were fed with unlabelled, heat-killed *Chlorella* sp. The experiment was conducted over a short incubation time of 15 min using the ^{14}C method. The upper solid line represents a significant fit, using a least squares linear regression, of \log cell size (V) on \log specific respiration rate (R). This influence of cell size is removed when cells are fed, since the dashed line represents a nonsignificant fit, suggesting no influence of cell size on specific respiration rate. The linear regressions are described by the following equations:

$$\text{Starved cells: } \log R = -1.5643 \log V + 8.5614 \\ (r^2 = 0.3380; p < 0.005)$$

$$\text{Fed cells: } \log R = 0.1363 \log V - 0.1184 \\ (r^2 = 0.0111; \text{nonsignificant, } p > 0.5)$$

A t -test on the regression coefficients revealed a significant difference between the 2 treatments ($p < 0.01$)

ration of the smallest cells, reaching 16% cell C h⁻¹, were approaching an order of magnitude greater than rates for the largest cells (Fig. 5, Table 4). Moreover, the non-normal distribution of cell sizes at T_0 (Fig. 2) clearly negates the practice of using mean cell volumes to normalize respiration rates, at least for amoebae like *T. sieboldi*.

The ¹⁴C method also permitted examination of the effect of starvation time (equivalent to incubation time for cells without prey) upon individual carbon specific respiration. The specific respiration rates for cells incubated for different times without food are shown in Figs. 4 & 5. By extrapolation, it is evident that a mean respiratory loss of around 10% cell C h⁻¹ could apply at T_0 (presumably representing rate for fed cells); this is a value some 3 times higher than rates derived empirically in incubations of several hours using the Cartesian diver method. The combined effects of cell size and incubation time on measured rates are clearly apparent in Fig. 5 and Table 4. Small cells have a pronounced ability to decrease specific rate with incubation time; however, we are not in a position at this stage to discriminate between the relative contributions made to total rate by locomotion, growth and digestion.

The results of this study have important ramifications for modelling respiration rates of protists in the field. Since the specific respiration rates decline rapidly in response to starvation (in the first few hours of incubation), any microrespiration technique using incubation times of several hours in the absence of prey are going to seriously underestimate the respiration rate of growing cells. Cartesian diver microrespirometry requires an equilibration period of about 2 h and a minimum experimental run of 2 h and consequently underestimated respiration at T_0 by one-third.

Caron et al. (1990) have recently pointed out that the relative importance of protozoan respiration is unclear although it is likely that respiration, like growth, follows cycles dictated by the presence of prey and predators. If this is the case, then a clear understanding of the physiological state of populations is needed before meaningful respiratory costs can be assigned. We have shown a variation of carbon specific rate of greater than 60-fold (Table 4) for 1 species, just due to variation in cell size and duration of incubation; this approximates to the 50-fold variation attributed to physiological state of cells in the comprehensive review of protozoan respiration by Fenchel & Finlay (1983).

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