Antarctic Science 2 (4): 313–320 (1990)

Growth of Antarctic and temperate terrestrial Protozoa under fluctuating temperature regimes

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Abstract: Population growth rates of Antarctic and temperate isolates of the microflagellate *Heteromita globosa* Stein and of the ciliate *Colpoda cucullus* Müller were determined during incubation both under constant 3.5° and 8.0°C, and under temperature regimes fluctuating on 24-hour cycles, between 0° and 15°C, to simulate Antarctic microclimates. Fluctuating temperatures did not inhibit growth. No growth of *Colpoda* occurred at 3.5°C. It is suggested that successful growth in nature depends upon the microclimate providing sufficient degree-hours per day above encystment/excystment threshold temperature (+1.5°C for *Heteromita*, about +4°C for *Colpoda*). The minimum number of degree-hours above threshold needed for growth of *Colpoda* is estimated to lie between 48 and 96. Monitored microclimates in the maritime Antarctic do not provide enough degree-hours per day, though subantarctic microclimates are more favourable, as may be exceptionally warm localities in the continental Antarctic where daily insolation is high in summer. These data are consistent with the recorded presence of *Colpoda* spp. in the subantarctic and rarely in the continental Antarctic, and their absence from the maritime Antarctic.

Received 31 April 1990, accepted 8 August 1990

Key Words: *Colpoda cucullus,* fluctuating temperatures, growth rates, *Heteromita globosa,*microclimates, protozoa.

Introduction

Early records of the occurrence of Protozoa in soils suggested that a small colourless bi-flagellate *Heteromita globosa* Stein was globally the most ubiquitous and cosmopolitan of soil Mastigophora (Sandon 1927, Robertson 1928). Similarly, species of the genus *Colpoda* Ehrenberg have been found to be the most abundant and widespread of ciliated Protozoa in soils (Sandon 1927, Bamforth 1969, 1980, Tirjackova 1988). Recent studies of the protozoan fauna of Antarctic fellfields, on Signy Island, South Orkney Islands (Smith & Tearle, 1985, Cowling *et al.* 1986, Smith 1987, Cowling & Smith 1987), have shown that *Heteromita globosa* is the dominant protozoan species in unvegetated fellfield fines soil. The temperature relations of both Antarctic and temperate isolates of *H. globosa* have been studied under constant temperature incubation (Hughes & Smith 1989). Trophozoites of both isolates showed psychrotrophy at sub-optimal temperatures down to +1.5°C, at which temperature cold-induced encystment occurred. Also in both isolates, rising temperature induced excystment at the same 1.5°C threshold. Differences between the two isolates were observed with the Antarctic isolate showing greater mobility, growth rates and synchrony of temperature-induced excystment at low sub-optimal temperatures. It was concluded that *H. globosa* has precise adaptations to the thermal regimes of terrestrial environments, ensuring survival during frozen conditions and rapid growth during favourable ones.

Abilities to survive long periods of desiccation and extreme temperatures in an encysted state and to grow rapidly under favourable conditions have also been well established for several isolates of *Colpoda* spp., outside the Antarctic zone, by Dawson & Mitchell (1929), Taylor & Strickland (1936), and Stout (1955). Their experimental results are consistent with numerous field observations of the great abundance of Colpodids in fluctuating, unpredictable and extreme environments (reviewed by Foissner 1987, pp 97–100).

Observations of the growth of *Heteromita* at sub-optimal temperatures (Hughes & Smith 1989, Table I) showed that the regression of the square root of maximum specific growth rate on temperature had a slope of 0.041 $\text{day}^{-1}\ ^{\circ}\text{C}^{-1}$ for both Antarctic and temperate isolates, substantially lower than those of two other temperate flagellate species: *Spumella elongata* and *Paraphysomonas* sp. These indicated significantly higher growth rates at low sub-optimal temperatures. The temperature relations of Colpodids have not been studied in such detail, but growth rate data are available for *Colpoda aspersa* Kahl, isolated from soil at 1 900 m in the Austrian Alps by Lüftenegger *et al.* (1985, Table I). Calculating the same regression for this isolate gives the identical slope of 0.041 $\text{day}^{-1}\ ^{\circ}\text{C}^{-1}$. In respect of both experimental and field observations therefore, both *Heteromita* and *Colpoda* may be described as **r**-selected (Lüftenegger *et al.* 1985) or even **a**-selected (Brunton *in press*).

There are no records of *Colpoda* spp. from terrestrial habitats in the maritime Antarctic, despite extensive surveys of promising habitats and despite their ubiquity and abundance in the rest of the world, including southern cool-temperate and subantarctic islands (Smith 1978, p 66). There are records of rare occurrences in semi-aquatic and nunatak moss habitats in the continental Antarctic (Sudzuki 1964, Ryan *et al* 1989). It is possible that the absence of *Colpoda* from the maritime Antarctic is due to insufficient hours per day above the minimum temperature for trophozoite activity and growth in terrestrial habitats. Evidence that such threshold temperatures for *Colpoda* spp. (which presumably vary with species and provenances) are significantly higher than the 1.5°C for *Heteromita* comes from several observations of the lack of growth of *Colpoda* in culture below 5°C (Darbyshire 1972, Smith 1973, Buitkamp 1979). However, successful growth of the alpine isolate of *Colpoda aspersa* Kahl has been observed at 5°C by Lüftenegger *et al.* (1985, Table I & fig. 7).

Previous experiments on the temperature relations of Protozoa have utilized constant temperature incubation, whereas natural populations in terrestrial habitats experience diurnal temperature fluctuations. This discrepancy hinders attempts to relate the results of laboratory studies to the performance of populations in the field, since fluctuations may impose a stress upon the organisms, especially if they daily cross the species' cold-induced encystment thresholds.

The aims of the present experiments were to test the hypothesis, proposed by Smith (1973), that the summer microclimates of maritime Antarctic terrestrial habitats have too few hours per day at above-threshold temperatures for the successful growth of *Colpoda cucullus* Müller and also to extend the observations of Hughes & Smith (1989) on the effect of temperature on the growth of *Heteromita globosa*, including the effect of diurnally fluctuating temperature. A method of temperature cycling incubation was developed so that the temperature regimes experienced by the laboratory cultures could simulate those experienced by natural populations. Thus, the population growth rates of Antarctic and temperate isolates of *Heteromita* and also of *Colpoda* under both constant and cycling incubation temperature could be compared.

Materials and Methods

Source and maintenance of cultures

The Antarctic isolate of *Heteromita* was derived from Signy Island fellfield material by A. J. Cowling (Cowling & Smith 1987) and the temperate isolate from an Aberdeenshire farmland soil by J. F. Darbyshire (MacDonald *et al.* 1977). Both isolates were maintained in monoxenic culture in 5 cm petri dishes in Prescott & James' (1955) liquid medium. A bacterial isolate, supplied by D. D. Wynn-Williams and K. J. Richard (British Antarctic Survey), Pseudomonad strain SB3, was included as food. *Colpoda cucullus* was derived from a Coventry parkland soil and maintained in monoxenic culture in lettuce infusion liquid medium with *Klebsiella aerogenes*, NCIB strain 418, as food. Culture lines of the bacteria SB3 and NCIB 418 were maintained on tryptone soya agar. Details of isolation and sub-culture techniques have been given by Smith (1973) and by Cowling & Smith (1987).

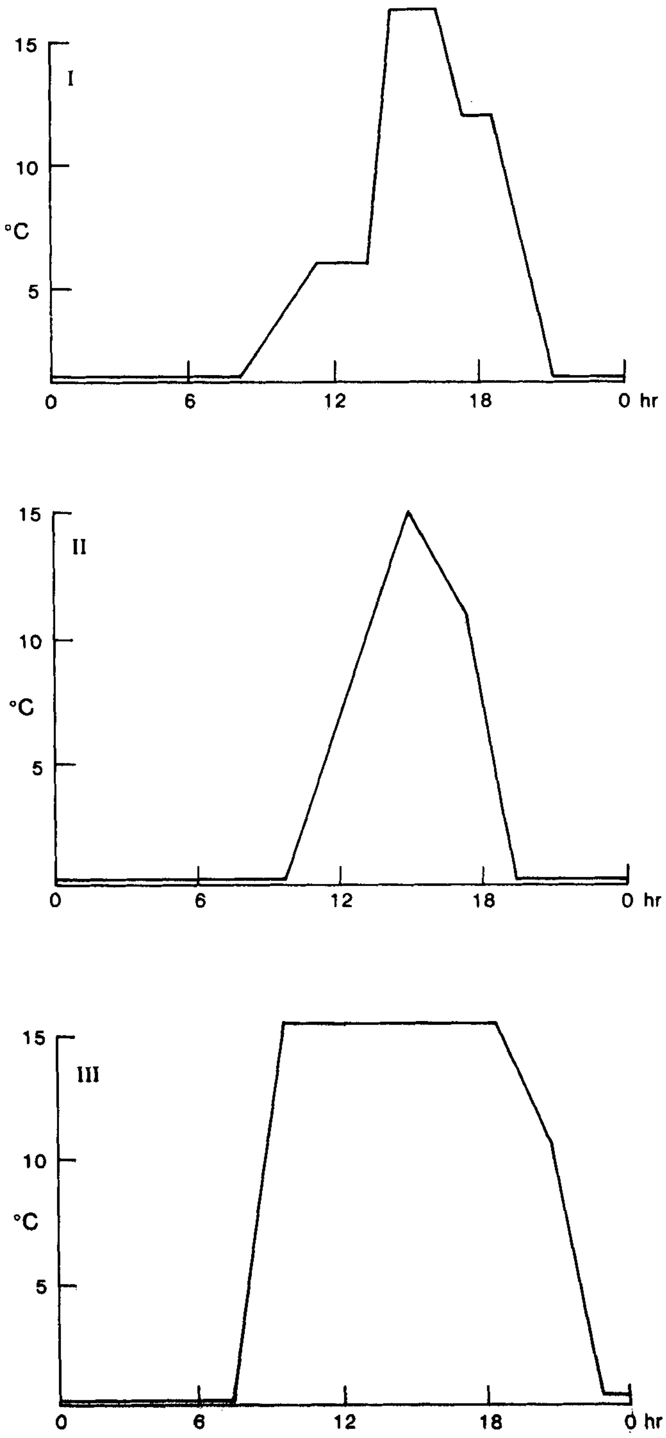

Fig. 1. Fluctuating temperature regimes I, II and III, designed to simulate Antarctic microclimates.

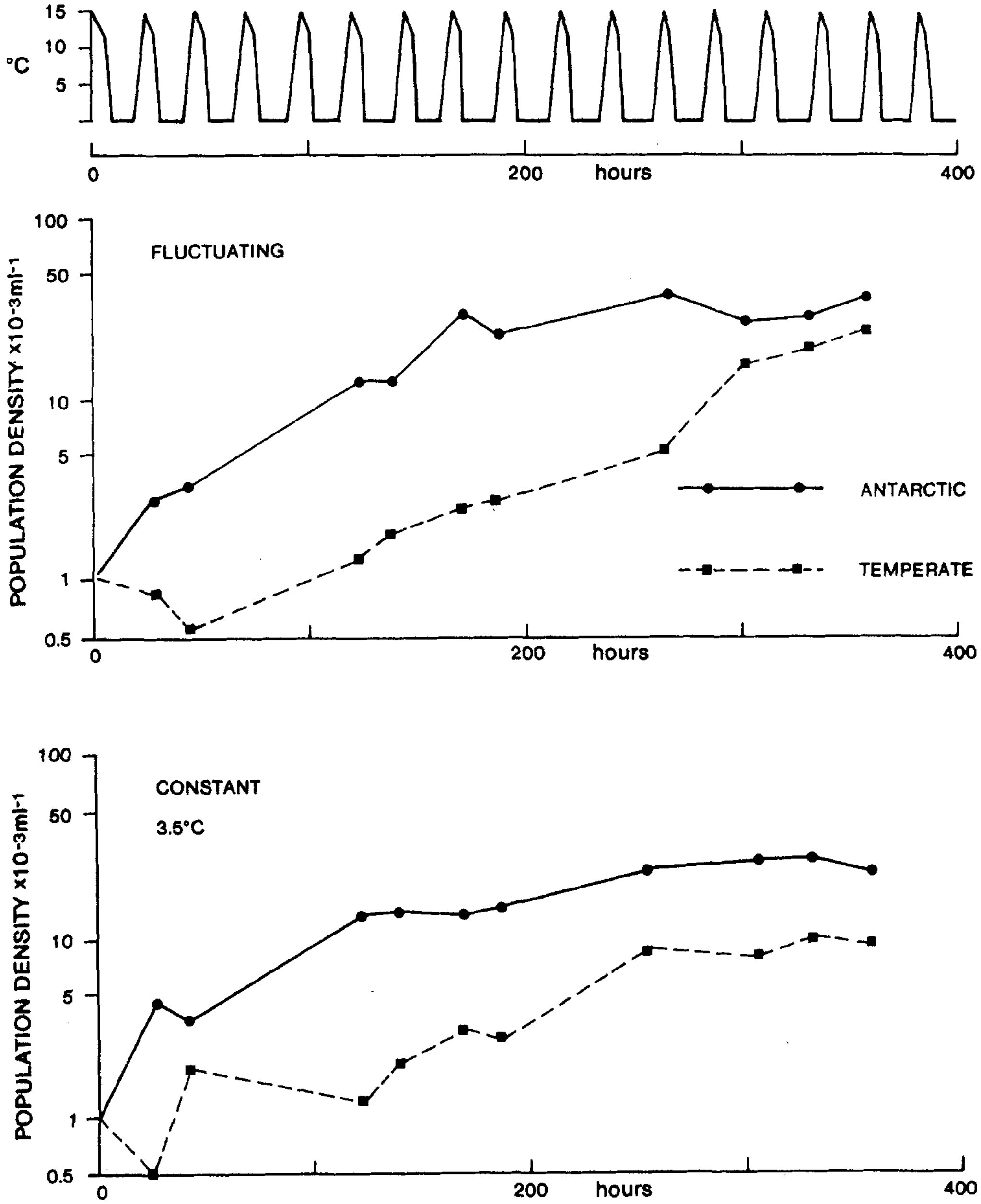

Fig. 2. Population growth of Antarctic isolates of *Heteromita globosa* under fluctuating temperature regime II and at a constant 3.5°C.

Incubation regimes

A cooled incubator was used with a programmable temperature controller, C.R.L. (Control Instrumentation) Limited, CRL 455, which allows up to 9 sets of ramp and dwell rates and hold lengths, and these may be cycled up to 999 times. The temperatures actually achieved were monitored with a thermistor connected to a Rustrack recorder, the signal being relayed continuously to a JJ chart recorder.

The first temperature cycle design was regime I (Fig. 1). This attempted to simulate the temperatures of Antarctic fellfield fines at 5 cm depth on a sunny, warm day on Signy Island, in January 1986 (Hughes & Smith 1989, fig 5). The diurnal range was between 0° and 15°C, with a mean of 3.5°C. The monitored output showed regime I to be unsatisfactory. It had too unsteady a temperature during hold periods and too sudden steps in temperature for successful establishment of cultures. The CRL 455 controls were therefore adjusted to produce regime II (Fig. 1) which on monitoring, proved to give a closer simulation of the field conditions. The population growth of Antarctic and temperate strains of *Heteromita* and of *Colpoda* was observed under regime II, on a repeating 24-hour cycle, and also at a constant 3.5°C, for 350 hours.

In order to determine the effect on population growth of more favourable temperatures (as may occur at lower subantarctic latitudes, or in a future ameliorated Antarctic climate), regime III (Fig. 1) was designed. This had the same diurnal temperature range and similar ramp rates, but a longer hold at 15°C and a mean of 8.0°C. The population

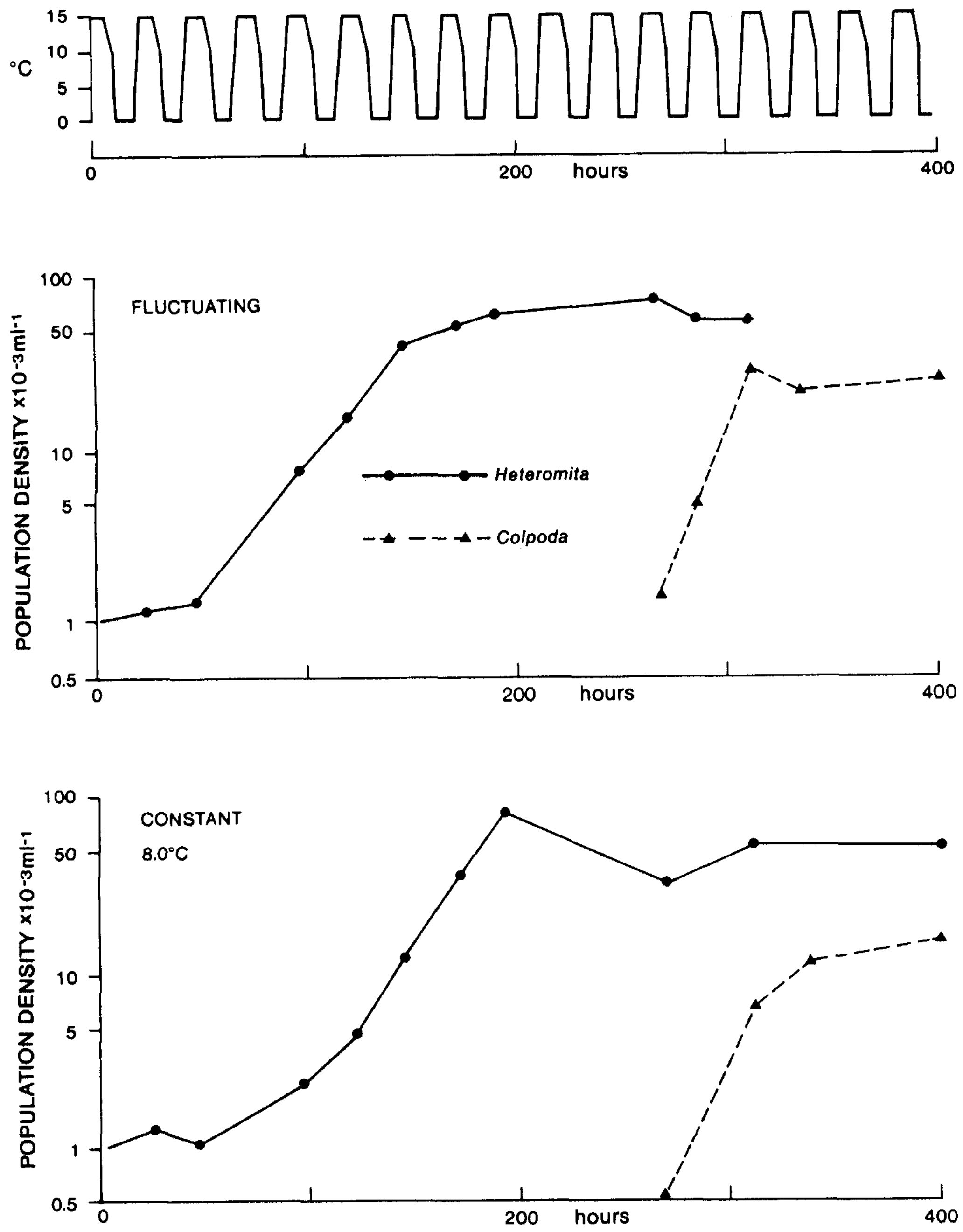

Fig. 3. Population growth of Antarctic isolate of *Heteromita globosa* and of *Colpoda cucullus* under fluctuating temperature regime III and at a constant 8.0°C.

growth of *Heteromita* (Antarctic isolate) and *Colpoda* was observed under regime III and at a constant 8.0°C for 400 hours.

Determination of growth rates

Duplicate cultures were established in 30 ml aliquots of Prescott & James' liquid medium, inoculated with each protozoan species and its bacterial food organism, from stock cultures acclimated to 5°C. Protozoan and bacterial inocula were adjusted to give initial densities of 10^3 and 10^8 ml^{-1} respectively in order to give adequate scope for the growth of protozoan populations. Protozoan population densities and specific growth rates were determined by the methods described by Hughes & Smith (1989, p.118). Haemocytometer counts were done at 24-hour intervals during the period of population increase and then at 3-day intervals up to 15 days. Triplicate counts were made on each of the duplicate cultures. Population specific growth rates were calculated between consecutive counts in the exponential phase of the growth curves.

Results

Mean protozoan population densities are shown in Figs. 2 and 3. Standard errors, which lay between 5% and 25% of the mean values, are omitted for clarity. Both isolates of *Heteromita* showed similarly successful growth at a constant

3.5°C and under fluctuating temperature regime II, with the Antarctic isolate showing the higher rate (Fig. 2). These results confirm those obtained under constant incubation by Hughes & Smith (1989). No growth at all of *Colpoda* populations was observed in this experiment.

The Antarctic isolate of *Heteromita* showed similarly successful growth at a constant 8.0°C and under fluctuating temperature regime III, showing higher growth rates than under regime II (Fig. 3). No growth could be detected in the *Colpoda* cultures for the first 11 days incubation, after which successful growth occurred both at a constant 8.0°C and under fluctuating temperature regime III (Fig. 3).

The maximum specific growth rates of the eight successful cultures under the four incubation conditions are shown in Fig. 4.

Discussion

Comparison of the growth of both protozoan species at constant temperatures with those under fluctuating regimes (Figs. 2 & 3) suggests that fluctuations in temperature do not constitute a stress inhibitory to growth for these species. In particular, *Colpoda* showed a higher maximum specific growth rate (Fig. 4) under fluctuating temperature regime II

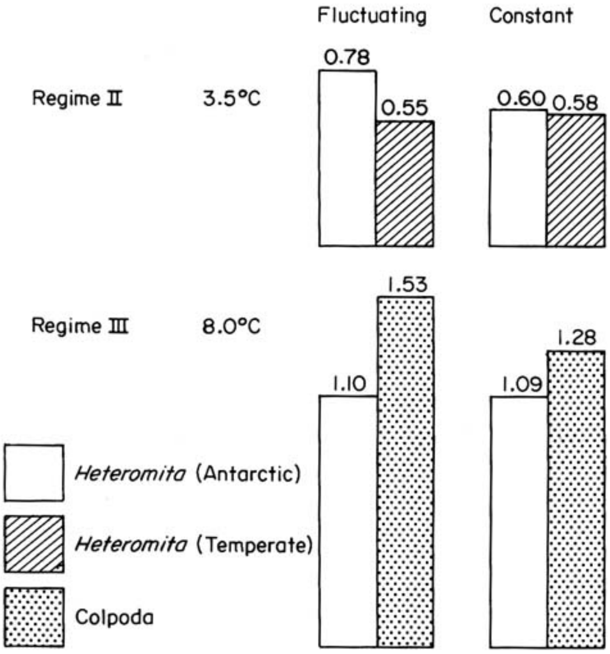

Fig. 4. Maximum specific growth rates per day of *Heteromita globosa* and *Colpoda cucullus* under fluctuating and constant temperature regimes.

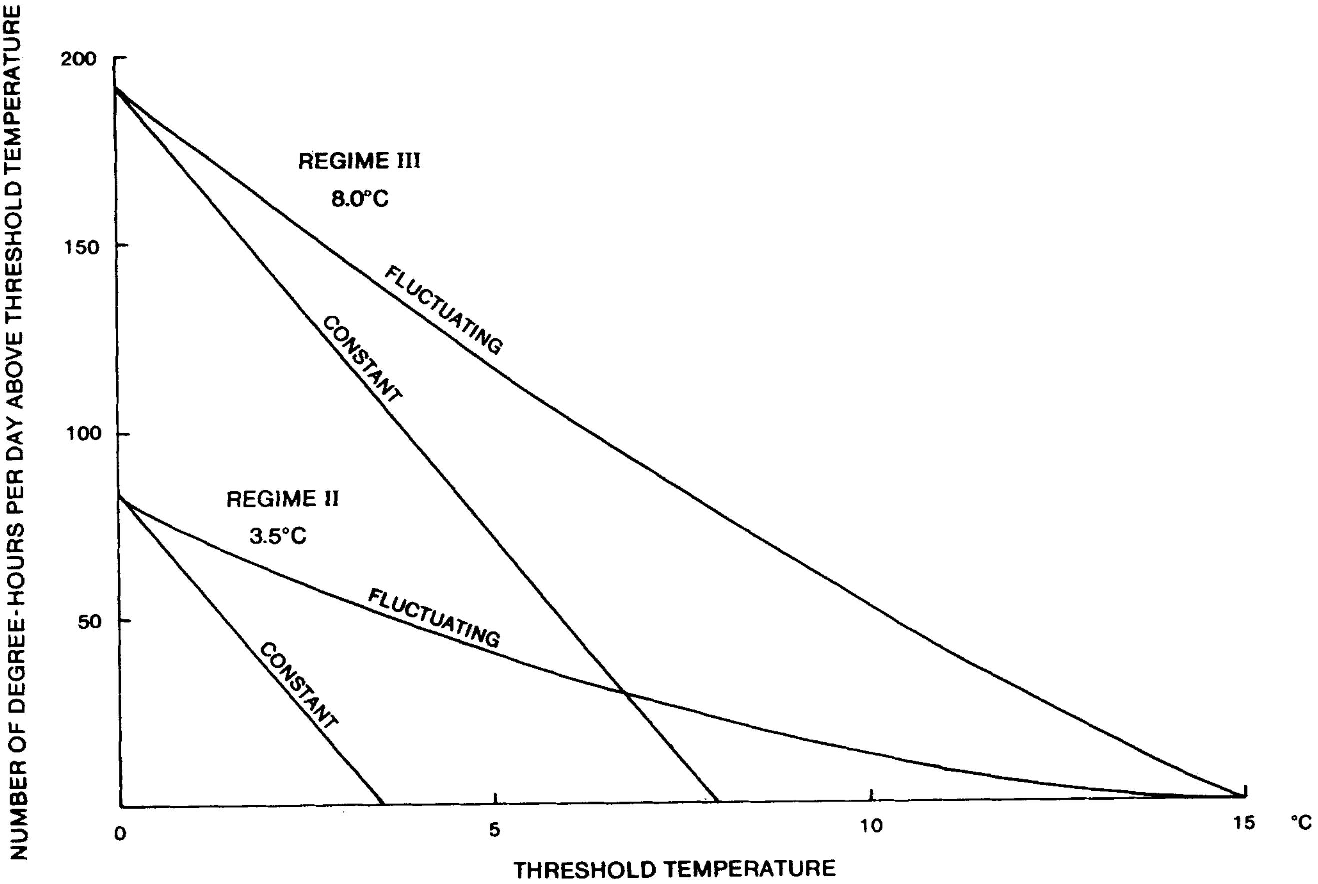

Fig. 5. Relationship between degree-hours per day above threshold temperatures and threshold temperature provided by fluctuating and constant temperature regimes.

than at a constant 8°C. This may be due to a greater number of degree-hours per day, above threshold temperatures greater than 0°C, being provided by the fluctuating than by the constant temperature regimes (Fig. 5).

The lack of growth of *Colpoda* under regime II confirms previous observations (Darbyshire 1972, Smith 1973, Lüftenegger *et al.* 1985) and suggests that the threshold temperature for population growth of *Colpoda cucullus* may be about + 4°C. This contrasts with the +1.5°C threshold for *Heteromita globosa* (Hughes & Smith 1989). The present results are consistent with the hypothesis that, in the presence of abundant moisture and food supply, successful growth depends upon sufficient degree-hours per day above the threshold temperature. On this basis, and taking the threshold temperature for *Colpoda cucullus* as 4°C, then the minimum number of degree- hours per day necessary for its successful growth, derived from the graphs in Fig. 5, lies between 48 and 96.

Smith (1973) advanced the hypothesis that the apparent absence of *Colpoda* from the maritime Antarctic was due to the low temperatures of terrestrial habitats during the summer. It is now possible to re-examine this hypothesis with the aid of the present experimental results and micrometeorological records from the Antarctic zone.

Table I gives examples of the numbers of degree-hours per day during summer at the surface or sub-surface of terrestrial habitats in three zones. They suggest that the microclimates of South Georgia are favourable for the growth of *Colpoda*, whereas those of the maritime Antarctic Signy Island are only rarely so. Similarly the majority of observations from Signy Island references site 1 during 1972–1974 indicate an unfavourable microclimate (Walton 1977, 1982). An exception occurred on 6th January 1972 (Table I). This was a day on which integrated daily radiation exceeded 700 cal cm^{-2} day^{-1}, a level exceeded on only three other days during the three years of records (Walton, 1977). More recent micrometeorological records from fellfields on Factory Bluffs (Pickup 1987) and on Jane Col (Smith *in press*) confrim the general unfavourability of Signy Island for *Colpoda.*

Interestingly, favourable microclimate conditions may occur more frequently at particular sites in the continental Antarctic, due to longer hours of insolation during the short summer period. Measurements by Smith (1988) indicate a highly favourable temperature regime on the north faces of

Table I. Examples of diurnal thermal regimes in terrestrial habitats

Site	Location	Date	Habitat	Number of Degree-Hours above 4°C day^{-1}	Source of data
Subantarctic					
South Georgia	54°17'S, 36°30'W	29 January- 8 February	*Polytrichum* turf:		M J Smith (1983 Appendix 3)
			surface	120	
			soil 10 cm depth	97	
			Festuca grassland		
			surface	83	
			soil 10 cm depth	80	
Maritime Antarctic					
Signy Island South Orkney Islands	60°43'S, 45°36'W		*Polytrichum-Chorisodontium* turf surface:		Walton (1982)
		2 December 1972	sunny day	77	
		4 December 1972	overcast day	9	
		6 January 1972	sunny day	172	
		7 January 1972	overcast day	43	
		6 March 1972	sunny day	27	
		8 March 1972	overcast day	0	
		January 1986	Fellfield fines 5cm depth		Hughes & Smith (1989)
			sunny day	44	
			overcast day	4	
		3-5 March 1989	Fellfield fines surface		R.I.L. Smith (*in press*)
			under experimental cloche	91,100,0	
			untreated control	11,0,0	
Continental Antarctic					
Bailey Peninsula Wilkes Land	66°17'S, 100°32'E	1-2 January 1986	*Ceratodon* turf: north-facing surface of ridge	133,125	R.I.L. Smith (1988)

convoluted hummocks of *Ceratodon* moss carpet at 66°S. It is possible that this effect could be even greater at still higher latitudes within, for example, moss cushions on the north-facing slopes of nunataks. Such occurrences could explain why *Colpoda inflata* (Stokes) Kahl has been recorded by Foissner (*in* Ryan *et al.* 1989) from three moss species at Robertskollen, 71°S, in west Dronning Maud Land. Thus there appears to be broad consistency of the present experimental results with the data available on the biogeographical distribution of terrestrial Protozoa and on the microclimates of terrestrial habitats in the Antarctic zone.

The influence of possible future changes in climate upon Antarctic terrestrial invertebrate fauna deserves consideration. Temperatures in the South Orkney Islands have been distinctly greater in the second half of the 20th Century compared with the first half, and show a continuing upward trend (Smith, 1990). The "most probable" scenario of further, CO_2-induced, warming predicts a 1.0° to 1.4°C increase per decade in summer temperatures in polar regions. Experimental manipulation of the microclimate of Jane Col fellfield on Signy Island by the use of clear polystyrene cloches (Smith *in press*) was sufficient to convert a totally inadequate temperature regime into a highly favourable one for the growth of *Colpoda* (Table I). It seems probable therefore that the geographical range of terrestrial species may be considerably extended in the Antarctic zone in the future. Since *Colpoda* spp. are amongst the easiest of soil Protozoa to extract and culture, its future presence or absence in the maritime Antarctic can be established with accuracy. It thus has useful potential as a sensitive indicator of biological response to climatic change.

Acknowledgements

This research was assisted by a grant from the British Antarctic Survey (Natural Environment Research Council), under the Special Topic Awards Scheme No. 1, and by the E.E.C. under the programme "Stimulation des cooperations et des échanges et techniques européens". For assistance with the practical work and preparation of the manuscript, we wish to extend grateful thanks to: Dr W. Block, Dr J. F. Darbyshire, Dr J. C. Ellis-Evans, Mr P. M. Harrisson, Mr K. J. Richard, Dr R. I. Lewis Smith and Dr D. D. Wynn-Williams.

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