

SURVIVAL OF ANTARCTIC SOIL METAZOANS AT -80°C FOR SIX YEARS

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Abstract

A sample of the liverwort *Cephaloziella varians* was collected on 1 January 1999 at Rothera Point on the Wright Peninsula, Adelaide Island, western Antarctic Peninsula and was partially dried and then frozen at -80°C . The sample was rapidly defrosted to *c.* 10°C after six years and two months of storage at this temperature. Nematodes, tardigrades and a bdelloid rotifer present in the sample were found to have survived. Of the 159 nematodes recovered from the sample, 49 (31%) were alive: of the tardigrades and rotifers, two of 15 (13%) and one of 48 (2%) had survived, respectively. A Chi-square test showed that there was a significant association between nematode taxon and survival: a greater proportion of *Coomansus gerlachei* individuals were alive than of *Rhysocolpus paradoxus*. A Chi-square test also showed that there was a significant association between phylum and survival: a significantly greater proportion of nematodes or tardigrades were alive than of bdelloid rotifers. We conclude that Antarctic soil metazoans are capable of surviving long-term exposure to low sub-zero temperatures and that there may be taxon-specific effects of freezing on survival.

Keywords: bdelloid rotifers, *Coomansus gerlachei*, freezing, nematodes, *Rhysocolpus paradoxus*, tardigrades

INTRODUCTION

The ability of Antarctic soil metazoans to tolerate exposure to sub-zero temperatures has important implications for their survival in soils and vegetation subjected to the daily freeze-thaw cycles that occur during austral summer in the Maritime Antarctic (2,9,10,11,14). Several studies have tested the tolerance of these animals to freeze-thaw cycles by examining their survival at sub-zero temperatures between -20 and -60°C for periods of ≤ 48 h (e.g. 2, 11). However, other than the report of Sømme and Meier (14), who found that three Antarctic tardigrade species survived for 8.3 years in dehydrated states at -22°C , no studies have apparently hitherto examined the ability of these animals to survive freezing for durations of several years. We therefore addressed this issue by testing the ability of Antarctic soil metazoans to tolerate exposure to -80°C for six years.

MATERIALS AND METHODS

A single sample (0.39 g air dry weight) of the leafy liverwort *Cephaloziella varians* was collected at Rothera Point on the Wright Peninsula, Adelaide Island, western Antarctic

Peninsula (67° 34' S, 68° 07' W) on 1 January 1999. As part of other analyses, the uppermost 2-3 mm of foliage was cut from the sample in a laboratory at the nearby research station on Rothera Point and *c.* 60% of free water was drawn from the plant tissues by blotting them on absorbent paper tissue. The sample was enclosed in aluminium foil within 2 h of collection and was placed in a -80°C freezer. Subsequent measurements showed that the sample cooled to -80°C within 5 min. It was held at this temperature for six years and two months, when it was rapidly defrosted on 9 March 2005 by adding 15 ml of cold (*c.* 10°C) distilled water. The sample was examined at × 40 magnification within 5 min. of wetting, was held at 4°C overnight in order to avoid desiccation, and was returned to room temperature. The sample was thoroughly sorted with a fine needle at × 40 magnification over the following 10 h. All metazoans observed were gently touched with the needle and those that moved in response to the stimulus were counted as alive. All of the live and dead metazoans were removed with the needle or a pipette and were fixed separately in hot (65°C) 4% formaldehyde solution with 2% glycerol. Nematodes were further sorted after fixation into adult males, adult females and juveniles. The metazoans were identified to genus or species levels at ≤ × 1000 magnification using appropriate taxonomic keys (4,6,7,12). Chi-square tests were used to test for the effects of taxon, and where possible, sex and age, on the survival of animals.

RESULTS

Locomotory movement of several nematodes was observed within 5 min. of adding water to the sample. Most nematodes observed at this time were loosely coiled and tardigrades and rotifers were contracted into tuns. Movement by many more metazoans, which were no longer coiled or contracted, was observed after the sample had been held overnight at 4°C. Of the 159 nematodes in the sample, 49 (31%) were found to be alive. The nematode species with the highest proportion of survivors was *Coomansus gerlachei* (de Man, 1904) Jairajpuri & Khan, 1977: of the 20 individuals in the sample, 16 (80%) were alive (Fig. 1). Of the 136 *Rhysocolpus paradoxus* (Loof, 1975) Andrassy, 1986 individuals present in the sample, 33 (24%) were alive (Fig. 1). All three of the *Plectus antarcticus* de Man, 1904 individuals in the sample were dead (Fig. 1).

Of the 15 tardigrade individuals present in the sample, two (13%), a *Diphascos* sp. and a *Hypsibius* *cfr.* *dujardini* Doyère, 1840, were alive (Fig. 1). One *H.* *cfr.* *dujardini* individual was dead, as were all 11 individuals of *Macrobotus furciger* Murray, 1906. The only *Echiniscus* sp. individual present was also dead (Fig. 1). Of the 48 bdelloid rotifers in the sample, only one was alive (Fig. 1). The tissues of the fixed nematodes and tardigrades showed no evidence of decomposition when examined microscopically, but those of most (*c.* 90%) of the rotifers showed signs of breakdown.

A Chi-square test applied to the data for *C. gerlachei* and *R. paradoxus* indicated that there was a significant association between nematode taxon and survival: a higher proportion of *C. gerlachei* individuals were alive than those of *R. paradoxus* ($\chi^2 = 25.1$, d.f. = 1, $P < 0.001$). It was not possible to include data for *P. antarcticus* in this analysis because the low expected counts for this species violated the assumptions of the Chi-square test. Low expected counts also precluded Chi-square tests on the effects of sex and age (Fig. 1) on the survival of *C. gerlachei* and *P. antarcticus*, but neither of these factors influenced the survival of *R. paradoxus* ($\chi^2 = 0.1$ and 2.8, respectively, both d.f. = 1, $P > 0.05$). A Chi-square test showed that there was a significant association between phylum and survival: significantly more individuals of nematodes or tardigrades were found alive than those of rotifers ($\chi^2 = 17.9$, d.f. = 2, $P < 0.001$).

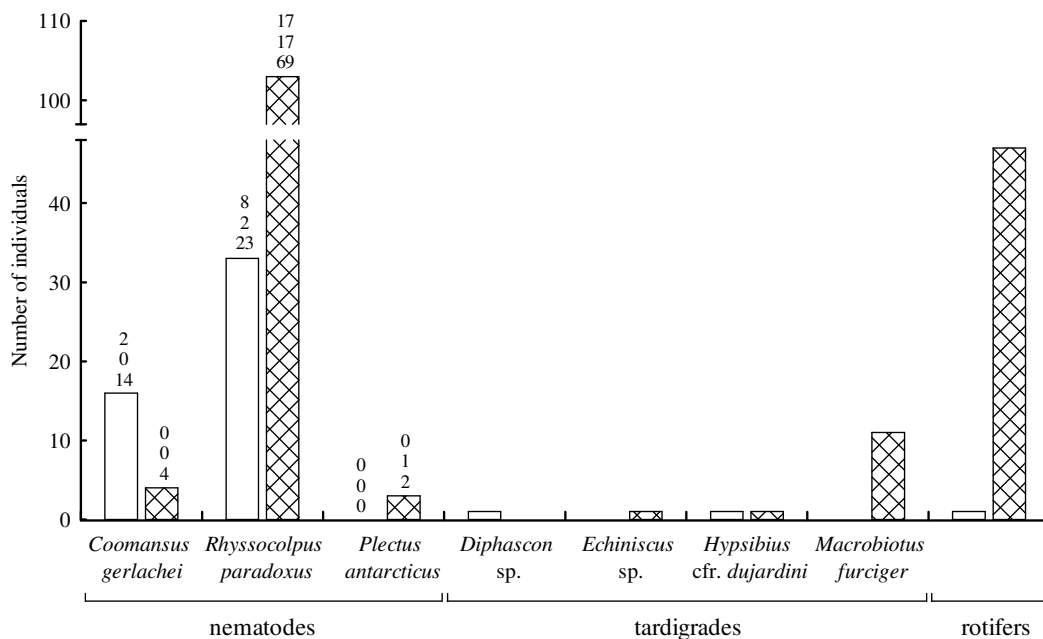


Figure 1. Numbers of live (□) and dead (▣) nematode, tardigrade and bdelloid rotifer individuals following storage at -80°C for six years and two months. Numbers above bars for nematodes are counts of adult males (top), adult females (middle) and juveniles (bottom) in each group. Note the split y -axis.

DISCUSSION

These data corroborate those from previous studies showing that metazoans can survive exceptionally stressful conditions for prolonged periods. Notable examples include the survival in dried plant material of the nematodes *Anguina tricolor*, *Ditylenchus dipsaci* and *Filenchus polyhyphus* for 9-30, 16-23 and 39 years, respectively, and the survival of *D. dipsaci* at -80°C for 5 years (3,5,15). Of the Antarctic nematodes, *Panagrolaimus davidi* is known to survive at -80°C for 28 days (16), a species of *Ditylenchus* survives exposure to -80°C for two days (11), and eight species, including all three of those reported here, survive momentary exposure to -60°C (2). The Antarctic tardigrades *Echiniscus jenningsi*, *M. furciger* and *Diphascoson chilense* all survive storage at -22°C for 8.3 years, and, after dehydration, -180°C for 14 days (14). The mortality of *M. furciger* is highest at the latter temperature (14), in part corroborating the data here.

Most cases of long-term survival of metazoans at sub-zero temperatures have been attributed to animals entering anhydrobiosis (8,13). It is unlikely, however, that all of the metazoans which survived in this study had entered this state: although immediately after defrosting most nematodes were coiled, and tardigrades were contracted into tuns, suggesting that they had responded to the drying process prior to freezing (8), recovery in some cases was almost immediate, indicating that animals were not in an anhydrobiotic state (8,13). These metazoans were hence most probably freeze-tolerant. It is unlikely that the animals underwent cryoprotective desiccation, as this process requires a slow ($0.1 - 0.2^{\circ}\text{C min}^{-1}$) rate of cooling (17), which did not occur in the present study.

The data on the differential survival of metazoans reported here should be treated with some caution, since we did not extract animals from replicate samples of material, which were not available in this case. Neither did we measure the numbers of live and dead animals in the liverwort sample before it was frozen. The poor condition of rotifer tissues suggests that many of these animals were dead when the sample was frozen, which would account for the higher

numbers of dead rotifers than of nematodes and tardigrades. However the observation that the tissues of nematodes and tardigrades were in an undecomposed state suggests that these animals were alive when they were frozen. If this was the case then the data here support those from previous studies showing taxon-specific effects of freezing on survival, possibly arising from interspecific differences in freezing tolerance (2,14). For example, lower survival of *P. antarcticus* following exposure to -15°C and -60°C compared with that of *Rhysocolpus* (= *Eudorylaimus*) spp. and *C. gerlachei* has been recorded (2). However there is apparently no difference between the survival of *C. gerlachei* and *Rhysocolpus* spp. following brief exposure to -60°C (2), which the data shown here do not support. The reason for the greater long-term survival of *C. gerlachei* relative to that of *R. paradoxus* is unclear, but could be related to the thicker cuticle possessed by the former species or the capacity to produce cryoprotectants such as trehalose, which stabilizes membrane phospholipids and proteins during dehydration (13).

Given that mean coastal and inland near-surface ice temperatures in Antarctica are respectively -10°C to -30°C and -30°C to -55°C (1), exposure to low sub-zero temperatures in the Antarctic natural environment, although it may alter community structure, clearly does not pose a significant threat to the survival of soil metazoan communities. It is apparent that the duration for which these animals can tolerate freezing is more than adequate for overwintering in dehydrated states in exposed habitats. At present it is not clear for how long Antarctic soil metazoans might tolerate exposure to low sub-zero temperatures, but it is quite plausible that they might withstand freezing in dehydrated states for periods of decades, with possible implications for the colonisation of terrestrial habitats following deglaciation.

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