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Cold tolerance in Tardigrada from Dronning Maud Land, Antarctica

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Abstract Survival at low temperatures was studied in three species of Tardigrada from Mühlig-Hofmannfjella, Dronning Maud Land, Antarctica. Both hydrated and dehydrated specimens of *Echiniscus jenningsi*, *Macrobiotus furciger* and *Diphascon chilenense* had high survival rates following exposure to −22°C for ca. 600 days, and dehydrated specimens following 3040 days at this temperature. In hydrated *E. jenningsi*, mortality increased with the duration of exposure from 7 to 150 days at −80°C, while mortalities of the two other species did not change. Hydrated specimens of all species were rapidly killed at −180°C, but all species exhibited good survivorship in the dehydrated state after 14 days at −180°C. In conclusion, hydrated tardigrades are able to survive extended periods at low temperatures, and dehydrated specimens are even better adapted to survive overwintering on Antarctic nunataks.

Introduction

As described in a recent review by Wright et al. (1992), tardigrades may adapt to physiological stress by entering a quiescent state where metabolism is reduced to undetectable levels. This state of quiescence was termed cryptobiosis by Keilin (1959) and is reversible at the onset of favourable conditions. Cryptobiosis in tardigrades and other organisms may be induced by different environmental factors. Following desiccation the tardigrades enter a state of anhydrobiosis, which is the most studied form of cryptobiosis. In their dehydrated state, tardigrades are able to survive for years in dry moss, tolerating exposures to high temperatures, intensive UV radiation and freezing temperatures of liquid gases (Keilin 1959; Wright et al. 1992).

Thus, Rahm (1924) was able to maintain dehydrated tardigrades in liquid air for up to 20 months. Rahm (1921, 1924) also reported that *Ramazzottius oberhaeuseri*, *Milnesium tardigradum* and *Macrobiotus* sp. can survive exposures to −253°C in their hydrated state. Ramløv and Westh (1992) found that hydrated *Adorybiotus coronifer* survived exposures to −196°C for 15 min, but longer periods were not tested.

Although these low temperatures do not exist in terrestrial ecosystems, the experiments cited are interesting in relation to cryptobiosis in tardigrades. Little is known about the relation of tardigrades to low temperatures that are more representative of those of their natural environments. Westh and Kristensen (1992), however, found that *Adorybiotus coronifer* and *Amphibolus nebulosus* from Greenland overwinter in a frozen state. Hydrated specimens of both species have crystallization temperatures of ca. −7°C, but winter temperatures of their natural habitat may be much lower.

The ability to enter anhydrobiosis is of vital importance for many invertebrates under changing moisture conditions. Tardigrades in the Antarctic may survive long periods in the dehydrated state and continue development during short periods when melt water is available. They must also be able to tolerate long exposures at low temperatures, either in anhydrobiosis or in a hydrated frozen state.

Several species of tardigrades have been reported from the Antarctic, and reviews of previous literature have been presented by Jennings (1976a,b), Block (1984) and Dastych (1984). According to Usher and Dastych (1987) a total of 23 species are known from South Georgia and the maritime Antarctic, and Ottesen and Meier (1990) added 2 new species from South Georgia to the list. Lists of species from the Antarctic Continent include the tardigrade fauna of Vestfold

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Hills and Langhovde (Sudzuki 1979; Utsugi and Ohyama 1989), the Schirmacher Oasis (Ingole and Parulekar 1987, 1990) and western Dronning Maud Land (Ryan et al. 1989; Dastych et al. 1990).

Although there is a rich and widespread fauna of tardigrades in the Antarctic, nothing is known about the tolerance of Antarctic species to low temperatures. The purpose of the present study was to investigate the cold hardiness of tardigrades from the Antarctic Continent. Material was collected during the Norwegian Antarctic Research Expedition (NARE) 1984–85. Samples of vegetation and mineral soil from several locations in the Gjelsvikfjella and Mühlig-Hofmannfjella were originally intended for faunistic studies. A list of tardigrades from this area is in preparation (T. Meier, unpublished). On return to the laboratory in Oslo, it was realised that some of the samples contained sufficiently high numbers of tardigrades to make it possible to set up long-term experiments on tolerance to low temperatures.

Materials and methods

Specimens for some of the experiments (sample 1) were obtained from a sample of lichens (*Neuropogon* sp.), moss (*Grimmia lawiana*) and cyanobacteria on mineral soil. The sample was collected on 3 February 1985 from crevices in the rocks of an unnamed nunatak at 1661 m, situated at 71°45'S, 4°56'E in Hamarskaftet (see map published by the Norwegian Polar Research Institute 1993). A portion of approximately 250 ml was stored in a fresh condition in a plastic flask under freezing ambient conditions (−10 to −20°C). The date from the beginning of storage is considered as "day 0" in later experiments. After departure from the field camp, the sample was transferred to a deep freeze at the expedition ship, and finally stored at −22°C in the laboratory at the University of Oslo.

Another sample (sample 2) was collected at an unnamed nunatak at 1650 m in Plogskaftet, 12 km east of the previous location. This sample consisted mainly of moss (*Grimmia lawiana*) overgrown with lichens and cyanobacteria. The sample was spread out and dried for several days in the air inside a tent and appeared to be dried out after this treatment. Air temperatures in the tent reached 10–15°C during daytime. Subsequently, from 10 February 1985 the sample was stored outdoors and later treated in the same way as sample 1.

According to the different treatments, tardigrades from sample 1 were in the hydrated state, while those from the air-dried sample 2 were considered to be dehydrated.

Preliminary experiments showed that when the tardigrades were removed from their substratum and frozen in water, mortalities were higher than if they were frozen in the soil itself. For this reason, in subsequent experiments the tardigrades were not removed from the substratum before freezing.

Small portions of mineral soil and vegetation, approximately 0.4 g in weight, were transferred to different experimental temperatures in small petri dishes or plastic tubes. Soil from sample 1 was frozen at −22°C, −80°C and −180°C (liquid air) for different time intervals. Part of sample 2 was transferred to a temperature of −180°C after 605 days, and part of it maintained at −22°C for 3040 days. To ascertain that the tardigrades were in anhydrobiosis, the soil was dried at 5% r.h. and 3°C for 5 days prior to exposure to −180°C.

Following exposure to freezing temperatures for different time intervals, the Petri dishes were transferred to conditions of 3°C. Water was added after a few hours, and the samples examined under a binocular microscope the following day. Tardigrades unable to move were counted as dead, and moving ones as alive. The tardigrades were removed from the dish and stored in 70% alcohol for later identification. Three species, *Echiniscus jenningsi* Dastych 1984, *Macrobiotus furciger* Murray 1906 and *Diphascon chilenense* Plate 1888, were sufficiently numerous to be included in the experiments.

Combinations of two and two samples were carried out by the Mann-Whitney *U*-test, but this method is not suitable if the number of samples or observations within the two samples are too small. Linear regression analyses were used to test for significant differences in the series of exposure to −80°C (sample 1) and −180°C (sample 2).

Results

Hydrated tardigrades

The mean (± SD) percent survival following exposures to low temperatures in the hydrated tardigrades from sample 1 is presented in Table 1. For each treatment

Table 1 Mean percent (± standard deviation) survival in samples of Antarctic tardigrades following exposures to −22°C, −80°C and −180°C for various number of days (d). The tardigrades were collected from soil that was frozen without previous drying in the field (n = total number of specimens in the samples)

Treatment		No. of samples	*Echiniscus jenningsi* n	*Echiniscus jenningsi* $\bar{x} \pm$ SD	*Macrobiotus furciger* n	*Macrobiotus furciger* $\bar{x} \pm$ SD	*Diphascon chilenense* n	*Diphascon chilenense* $\bar{x} \pm$ SD
−22°C/185 d		5	238	33.2 ± 10.4	–	–	–	–
−22°C/235 d		4	170	39.1 ± 6.5	–	–	52	57.7 ± 9.9
−22°C/283 d		13	863	26.2 ± 16.7	124	45.6 ± 26.7	437	58.6 ± 21.5
−22°C/587 d		3	165	23.8 ± 12.4	28	34.7 ± 9.3	49	44.5 ± 8.6
−22°C/180 d:	−80°C/7 d	2	199	30.0 ± 3.8	13	87.5 ± 17.7	35	91.7 ± 2.3
	−80°C/14 d	2	128	38.3 ± 8.7	13	45.0 ± 7.1	45	37.8 ± 1.9
	−80°C/28 d	2	245	16.6 ± 11.2	22	68.2 ± 6.4	48	56.6 ± 11.5
	−80°C/60 d	2	224	10.8 ± 1.3	9	45.0 ± 7.1	49	63.4 ± 4.7
	−80°C/95 d	3	356	5.9 ± 2.7	15	75.8 ± 31.7	149	58.3 ± 6.6
	−80°C/150 d	5	741	6.9 ± 6.8	30	37.0 ± 27.1	188	49.5 ± 20.4
−22°C/290 d:	−180°C/1 d	4	205	0.0	31	5.6 ± 11.1	184	2.3 ± 2.8
	−180°C/2 d	3	228	0.0	22	0.0	77	0.0

and species the number of 0.4 g soil samples and total number of specimens in the samples are given. Usually, there was much variation in the mortalities between samples given the same treatment, as seen from the standard deviations. In general, higher rates of survival were recorded in *M. furciger* and *D. chilenense* than in *E. jenningsi.*

At −22°C there were no significant increases in mortalities in any of the species with increasing time, even when the most extreme values for *E. jenningsi* after 235 and 587 days were compared.

At −80°C there was a clear decrease in the survival rates of *E. jenningsi.* Linear regression analysis showed a significant negative correlation ($P < 0.01$, $r^2 = 0.55$) between percent survival and time of exposure. This relationship was not significant in *M. furciger* and *D. chilenense*, suggesting that there was no change in mortality during 150 days of exposure at −80°C. Although mean mortality apparently increased from 95 to 150 days in the two latter species, the Mann-Whitney *U*-test showed that the differences were not significant, probably as a result of the high variation between samples.

At −180°C all hydrated specimens of *E. jenningsi* were dead after 1 day, while small proportions of *M. furciger* and *D. chilenense* numbers survived. Following 2 days exposure at this temperature the mortality was 100% in all species.

Dehydrated tardigrades

The survival of dehydrated tardigrades (sample 2) at −22°C and −180°C is presented in Table 2. Samples of soil were removed from conditions of −22°C after 635 and 3040 days, and all three species survived these exposures. In *E. jenningsi* there was a significant decrease in survival from 635 to 3040 days (Mann-Whitney *U*-test, $P < 0.05$), while the survival of *M. furciger* and *D. chilenense* did not change. More than 50% of *M. furciger* and more than 40% of *D. chilenense* were alive after both periods at −22°C.

Large proportions of the dehydrated tardigrade numbers also survived exposures at −180°C. Apparently, the highest mortalities occurred in the control group, but due to the large variations between samples the differences were not significant from mortalities of those exposed to −180°C. Linear regression analysis showed no significant correlation between exposure time and survival in any of the three species. High survival rates, close to the level of those at −22°C for 635 days, were recorded for all species. Mean values for *E. jenningsi* varied from 44% to 53%, for *M. furciger* from 26% to 51% and for *D. chilenense* from 32% to 43%.

Discussion

Since samples used in the present study were originally collected for faunistic studies, cold hardiness experiments were not prepared in the field. In the field camp, it was also impossible to extract and identify tardigrades, although it would have been an advantage to know the proportion of live and dead specimens in fresh samples of soil and vegetation. In the laboratory, the percentage of live animals remained relatively constant under the less severe temperature exposures, suggesting that this was approximately the original proportion of live specimens.

The high variation in survival rates within the subsamples is difficult to explain, but may reflect the fact that different proportions of dead and live tardigrades were present in different parts of samples 1 and 2 at the time of collection. Thawing and freezing of the subsamples in the laboratory may also have affected survival rates.

Since the moist sample 1 was placed directly in a closed plastic flask, the tardigrades were assumed to be in the hydrated state. The fact that they had higher mortalities at −180°C than dehydrated specimens supports this assumption. According to Wright et al. (1992), anhydrobiotic tardigrades contract into a compact tun. Anhydrobiosis may be achieved in a few hours, but too rapid dehydration is fatal. In this study, tardigrades from sample 2 were presumably in anhydrobiosis, since their substrate had been dried for

Table 2 Mean percent (± standard deviation) survival in samples of Antarctic tardigrades following long-term exposures at −22°C and subsequent exposures at −180°C after desiccation above silica gel. The tardigrades were collected from soil samples that were air-dried in the field before exposure to −22°C (n = total number of specimens in the samples)

Treatment		No. of samples	*Echiniscus jenningsi* n	*Echiniscus jenningsi* $\bar{x} \pm$ SD	*Macrobiotus furciger* n	*Macrobiotus furciger* $\bar{x} \pm$ SD	*Diphascon chilenense* n	*Diphascon chilenense* $\bar{x} \pm$ SD
−22°C/635 d		6	167	52.9 ± 10.9	65	54.3 ± 10.4	115	41.5 ± 10.8
−22°C/3040 d		10	90	14.9 ± 14.1	76	51.3 ± 28.7	45	41.8 ± 38.1
−22°C/605 d[a]	−180°C/0 d	5	173	37.0 ± 20.4	67	30.0 ± 18.4	161	28.7 ± 7.5
	−180°C/2 d	3	71	52.8 ± 21.0	39	47.8 ± 27.1	113	34.5 ± 18.1
	−180°C/7 d	3	139	44.2 ± 7.2	41	50.8 ± 23.4	123	42.3 ± 2.8
	−180°C/14 d	3	156	47.6 ± 3.6	39	25.7 ± 10.0	96	32.3 ± 1.9

[a] Dried 5 days at 5% r.h. and 3°C before exposure to −180°C

several days under field conditions. In addition, subsamples were desiccated in the laboratory before exposure to −180°C.

Tardigrades removed from the soil and frozen directly in water had higher mortality rates than those frozen in the soil and vegetation itself. Freezing within the porous soil and plant debris may be slower than in water and give the tardigrades time for physiological adjustment. Ramløv and Westh (1992) found that survival rates in *Adorybiotus coronifer* decreased with increasing cooling rates, to −196°C, and highest rates of survival were found in specimens with a high trehalose content.

On continental Antarctic nunataks, overwintering tardigrades are exposed to some of the most severe temperature conditions on earth. A minimum air temperature of −43°C was recorded in 1992 at the Troll Station in Dronning Maud Land (Hanssen-Bauer 1993), 80 km west of the collection sites of this study, but temperatures may be even lower in other years. It is not known if the tardigrades are protected from such extremes in their microhabitats, e.g. by a snow cover.

In the present study, all species were rapidly killed at −180°C in their hydrated state (Table 1), while high survival rates were found in dehydrated specimens (Table 2). At −22°C a proportion of both hydrated and dehydrated tardigrades survived exposure of ca. 600 days. Survival rates of hydrated *M. furciger* and *D. chilenese* did not decrease during 150 days at −80°C, while higher mortalities were recorded in *E. jenningsi.*

In conclusion, it appears that all three species survive extended periods at temperatures down to −80°C in the hydrated state. To judge from the differences in survival at −180°C, dehydrated specimens are even better adapted to extremely low environmental temperatures. Higher mortalities were found in *E. jenningsi* than in *M. furciger* and *D. chilenense*, but it appears that the cold tolerance of all three species is sufficient for survival during overwintering on Antarctic nunataks.

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