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ORIGINAL ARTICLE

Field and laboratory studies on drought tolerance and water balance in adult *Pergalumna nervosa* (Acari: Oribatida: Galumnidae)

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Abstract. We studied the water balance, body fluid osmolality and survival of the oribatid mite, *Pergalumna nervosa*, when exposed to drought in field and laboratory experiments. In a replicated field experiment we artificially lowered the soil water content by putting roofs over selected plots, which reduced soil water potential to levels well below the permanent wilting percentage for plants (i.e. below –1.5 MPa). Even though a slight decrease in the abundance of *P. nervosa* (only found in the 0–5 cm soil layer) was recorded during the most severe drought stress (ca. –3.5 MPa), the majority of adult mites clearly survived these conditions for 3 weeks in the field without migrating to deeper soil layers. Exposing field collected adults in laboratory experiments simulating even more severe drought conditions revealed that *P. nervosa* can survive several weeks of gradually increasing drought stress (down to –7 MPa) with moderate water loss. The osmolality of body fluids increased as dehydration progressed, but apparently as a result of simple up-concentration of solutes and not the de novo synthesis of protective osmolytes. We compare and discuss these results in the light of what is known about other arthropods.

INTRODUCTION

Oribatid mites can be present at densities from 10^4 to more than 10^5 m^{-2} and biomasses approaching 1 g dry weight m^{-2} (Petersen & Luxton, 1982). Compared to other groups of mesofauna, orbatids often dominate the microarthropod community in soil and are therefore important decomposers in these environments (Petersen & Luxton, 1982). Oribatid mites and soil fauna in general, play significant roles in terrestrial ecosystems, by influencing decomposition and nutrient recycling processes in the soil (Bardgett, 2005). Thus, it is important to understand how these animals respond to scarcity of water in the soil, which is one of the most important environmental factors determining population dynamics of soil invertebrates (Rapoport, 1967). Recent syntheses and predictions of climate change suggest that summer droughts are increasing in frequency and intensity (IPCC, 2013), making an understanding of the effects of these climatic extremes ever more important (e.g. Siepel, 1996; Convey et al., 2003).

Laboratory studies show that Oribatid mites are generally quite desiccation resistant (i.e. able to reduce evaporative water loss) because of their sclerotized integument (Madge, 1964; Vannier, 1987; Wauthy & Vannier, 1988). Despite their resistance to desiccation, some field experiments show that oribatid populations decline in abundance during periods of drought (Lindberg & Bengtsson, 2005; Tsiafouli et al., 2005); whereas other studies suggest that oribatid mites are less responsive to drought than other groups of mesofauna (Andresen et al., 2011; Holmstrup et al., 2013; Vestergård et al., 2015). The outcome of such studies is likely to depend on the actual drought stress occurring in the soil. As pointed out by Vannier (1987) drought stress is best quantified by measuring the soil water potential (SWP) because this allows for a mechanistic understanding of the water balance and osmotic relationships of organisms in relation to their environment. However, in field experiments SWP is rarely documented (e.g. Gerard, 1967; Holmstrup & Bayley, 2013).

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Few studies have investigated desiccation resistance and water balance of oribatid mites exposed to drought. Siepel (1996) exposed a range of species of oribatids to drought stress (70% relative humidity at 20°C; 48 h) and reports that several species can tolerate acute desiccation stress, but their water balance or other physiological aspects were not investigated. To our knowledge, only one study has addressed water balance and osmotic relations in an oribatid mite (*Phauloppia* sp.) exposed to realistic desiccation conditions (Sjursen & Sømme, 2000).

The species *Pergalumna nervosa* (Berlese, 1914) (Oribatida: Galumnidae) is a semicosmopolitan species distributed in the Holarctic Region and South Africa, where it mainly inhabits the superficial litter layers of soils (Subias, 2004). Hence, this species is exposed to desiccating conditions during spells of drought. In the present study, we used field and laboratory studies to determine how *P. nervosa* adapts to drought conditions by measuring drought tolerance (survival), water loss rates, cuticular permeability and changes in body fluid osmolality in a simulated natural, but extreme drought situation.

MATERIAL AND METHODS

Field experiment

The experimental site was situated at Brandbjerg, Denmark (55°53´N, 11°58´E), on a hilly nutrient-poor sandy deposit with a dry heath/grassland ecosystem dominated by grass (*Deschampsia flexuosa*) and an evergreen dwarf shrub (*Calluna vulgaris*). The mean annual temperature was 8.0°C and the mean annual precipitation about 610 mm (Mikkelsen et al., 2008). In 2008 we established the drought experiment described in Holmstrup & Bayley (2013). Briefly, the experiment consisted of 10 plots (1.0 × 1.0 m) laid out in a randomized block design with two treatments (drought and control) each replicated five times. Drought conditions were achieved in 5 plots from 21st April to 16th June (2008) by preventing precipitation reaching the ground by placing transparent plastic roofs (approximately 1.5 × 1.5 m) 1 m above the ground. Control plots were not covered at any time. The plastic roofs were removed at the end of the drought period, and the plots watered with tap water equivalent to 20 mm of rain. Thereafter, natural precipitation moistened the soil. SWP was measured at a depth of ca. 5 cm once per week using permanently installed Wescor soil psychrometers connected to a Wescor HR33T Microvoltmeter operated in the dew-point mode (Wescor, Logan, Utah). The output from the soil psychrometers (in µV) was transformed to SWP (MPa) using a calibration curve obtained using standard NaCl solutions (Lang, 1967). The SWP of all plots was measured, and mean values for control and drought plots were calculated.

Soil microarthropods were sampled, as described earlier (Holmstrup & Bayley, 2013), immediately before drought was applied (16th April 2008), on two occasions during the drought period, and about two months after the drought was terminated and the soil was again moist. One soil core (diameter 6 cm; depth 10 cm; 28.3 cm^2) was collected in each plot. Immediately after collection each soil core was divided into two layers of 5 cm thickness with a knife. Microarthropods from these soil cores were extracted over one week in a high gradient extraction apparatus (Krogh & Pedersen, 1997). The microarthropods were collected in benzoic acid and subsequently preserved and stored in glycerol until identification.

Collection of live specimens

Specimens of *P. nervosa* were collected at Brandbjerg in October 2012 and again in March 2014. Microarthropods were extracted from soil cores (only upper 0–5 cm) as described above with the exception that the animals were collected live on a layer of moistened plaster of Paris and subsequently sorted. The animals were stored under moist conditions at 15°C for two weeks until used in experiments.

Desiccation tolerance

Survival and water content were determined during a 43-day exposure to increasing water stress. Fully hydrated specimens were placed in small plastic vials (3 cm high, 1.6 cm diameter), with five specimens in each vial. The vials were covered with a 100 µm nylon mesh to prevent the mites from escaping. Each vial was then placed in the centre of a 160-ml plastic beaker (4.2 cm high, 7 cm diameter) containing an aqueous NaCl solution. Aqueous NaCl solutions were used to establish a range of increasing levels of desiccation, starting with 99.2% RH on day one and ending at 94.9% RH on day 43 (Table 1). Table 1 also shows the equivalent SWP at each concentration of NaCl. The controls were kept at a constant 99.6% RH, which is equivalent to a body fluid osmolality of ca. 250 mOsm (slightly lower than normal arthropod haemolymph osmolality) and therefore should not have caused evaporative water loss. The experiment was conducted at a constant temperature of 20 ± 0.5°C.

The experiment was repeated on two occasions. The first in October to December 2012, and the second in March to May 2014. In the first experiment the water content and survival during the experiment was determined on day 8, 15, 22, 28, 36 and 43. In the second experiment water content and survival was determined on day 0, 21 and 43. In addition we performed Differential Scanning Calorimetry (DSC) analysis on single animals (i.e. intact live specimens) in order to compare melting points (equivalent to body fluid osmolality) of mites kept under moist and dry conditions, respectively.

Water content, survival and DSC analysis

The fresh weight (FW) of single mites was determined using a Sartorius SC 2 microbalance (Sartorius AG, Goettingen, Germany) accurate to 1 µg. After drying for 24 h at 60°C, dry weight (DW) was determined and water content (WC) calculated as g water g^{-1} DW. Mites were scored as survivors if they were able to move normally.

Thermal DSC analysis was conducted using a DSC4000 calorimeter (Perkin Elmer, Waltham, MA, USA). A single mite was transferred to a 50 µl aluminium DSC-pan, which was hermetically sealed. Samples were subjected to a program consisting of 5 steps: (1) held for 1 min at 20°C; (2) cool to –30°C at a rate of 5°C min^{-1} (mites froze at approximately –20°C); (3) held for 1 min at

Table 1. Concentration of NaCl in aqueous solutions with corresponding relative humidity (RH) in air equilibrated using these solutions and the corresponding water potential (WP).

Day	g NaCl L^{-1}	RH (%)	WP (MPa)
Control	6.2	99.6	–0.54
1	13.1	99.2	–1.01
8	31.6	98.2	–2.46
15	45.3	97.4	–3.56
22	63.3	96.5	–4.81
28	77.6	95.7	–5.93
36	91.7	94.9	–7.06
43	91.7	94.9	–7.06

–30°C; (4) warm to –10°C at a rate of 5°C min^{-1}; (5) warm to 5°C at a rate of 0.1°C min^{-1}. The melting endotherm (enthalpy change during melting of ice formed in the animal) of the latter thermal scan curve was analyzed and the melting onset temperature estimated using Pyris Software (Perkin Elmer, Waltham, MA, USA) as described by Block (1994). Estimated melting points were transformed to body fluid osmolality using the osmolal melting point depression of –1.86°C Osm^{-1}.

Water loss rate and cuticular permeability

The rate of water loss was determined for field collected specimens, which were kept in the laboratory under moist conditions for 14 days prior to the experiment to ensure that they were fully hydrated. FW of fully hydrated specimens were determined as described above and five individuals were placed in small plastic vials (3 cm high, 1.6 cm diameter), which were placed in the centre of a 160-ml plastic beaker (4.2 cm high, 7 cm diameter), containing ca. 5 g silica gel. Each beaker was sealed with Para film and a tightly fitting lid. The air in this small closed system quickly equilibrated with water vapour in the silica gel creating a RH of 16.3% as determined using a Tinytag Plus 2 (model TGP-4500) humidity datalogger with a precision of 0.1% RH (Gemini Data Loggers Ltd, Chichester, UK). Ten replicates, each with five mites, were exposed to 20 ± 1°C. The water loss was measured by recording the weight loss of groups of five mites after 5 h and 30 min of exposure to desiccation. The permeability was calculated as µg water lost per cm^2 surface per hour per unit saturation deficit (mm Hg) in the desiccation beakers. The saturation deficit was 14.68 mm Hg at 16.3% RH and 20°C. In a pilot study we found a good correlation between FW and surface area of the mites, at least in the range of FWs used in this experiment, using the approximation that *P. nervosa* roughly has the form of a sphere. We therefore used FW to estimate body surface area and assumed that permeability to water of all body parts is similar. Although this estimate of surface area and cuticular permeability is not perfect, it is sufficient for comparisons with other arthropods.

Statistical analysis

The abundance of mites in the field experiment was analyzed as a randomised ANOVA block design using the GLM procedure. Two-way ANOVA with treatment (control or drought) and time as fixed factors was used to test for effects of main factors and interactions between main factors. Abundance data passed the normality test (Shapiro-Wilk, $P = 0.105$) and equal variance test ($P = 0.178$). Pairwise comparisons were done using the Holm-Sidak method. Water content and body fluid osmolality was analyzed using one-way ANOVA. All these analyses were done using Sigmaplot for Windows Version 12.0 (Systat software Inc., Chicago, IL, USA). To assess if the survival of mites in the laboratory experiment changed with increasing desiccation level, we used the generalized linear model (glm) function in R (vers. 3.3.2) with the family type set as binomial (since survival is binomially distributed) and logit as the link function.

RESULTS AND DISCUSSION

Response of *Pergalumna nervosa* to drought in the field

The spring in 2008 was characterized by low precipitation, which resulted in relatively low SWP in both control and drought plots until 21st May. The control plots received less than 16 mm of rain from the 21st April to 21st May (data not shown) and the SWP therefore fell below –1 MPa for a period of two weeks (Fig. 1). Nevertheless, SWP of drought plots was clearly affected by the roofs hindering precipitation. There was a clear treatment effect at a depth of 5 cm in the drought plots from 2nd June to 26th June (ANOVA, $F = 54.72–97.71$, $P < 0.05$). The SWP in this period was below –3.5 MPa and stayed there for at least three weeks and was significantly lower than in the control plots. Once the roofs were removed from drought plots (16th June) the SWP in drought plots rapidly increased to the same level as in control plots (Fig. 1). Our analysis of microarthropods extracted from soil cores (divided into 0–5 cm and 5–10 cm depths) showed that *P. nervosa* resided only in the upper 0–5 cm soil layers (no specimens were found in deeper soil layers). Thus, this species was indeed exposed throughout the experimental period to the drought conditions depicted in Fig. 1. We found that abundance of *P. nervosa* varied significantly over time (ANOVA, $F = 6.84$, $P = 0.001$) and was influenced by treatment (ANOVA, $F = 5.15$, $P = 0.03$). There was no significant interaction between time and treatment (ANOVA, $F = 2.83$, NS). On 19th May and 16th June, the abundance *P. nervosa* in drought plots was significantly lower than in control plots (Holm-Sidak, $P < 0.05$) indicating that drought had caused some mortality and/or reduced population growth. Microarthropods may seek to avoid dry conditions in the soil by migrating to deeper and moister soil layers (Usher, 1970). This apparently did not happen in the field experiment since there were no *P. nervosa* in the 5–10 cm layer of drought plots and therefore migration cannot account for the reduced abundance during the most severe drought conditions. In summary, the field study demonstrated that adult individuals of *P. nervosa* were able to survive a SWP of less than –3.5 MPa for at least 3 weeks. This level of drought is equivalent to the osmotic pressure exerted by haemolymph with an osmolality of about 1.5 Osm (Holmstrup & Bayley, 2013).

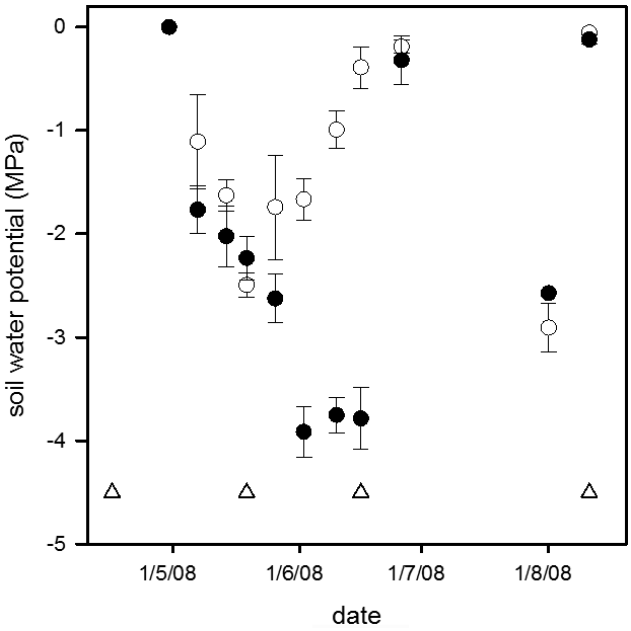

Fig. 1. Soil water potential (MPa; mean ± S.E.; *N* = 1–5) at a depth of 5 cm during the period 30th April to 11th August 2008. Control plots are indicated by open circles. Drought plots are indicated by black circles. Open triangles indicate dates when microarthropods were sampled.

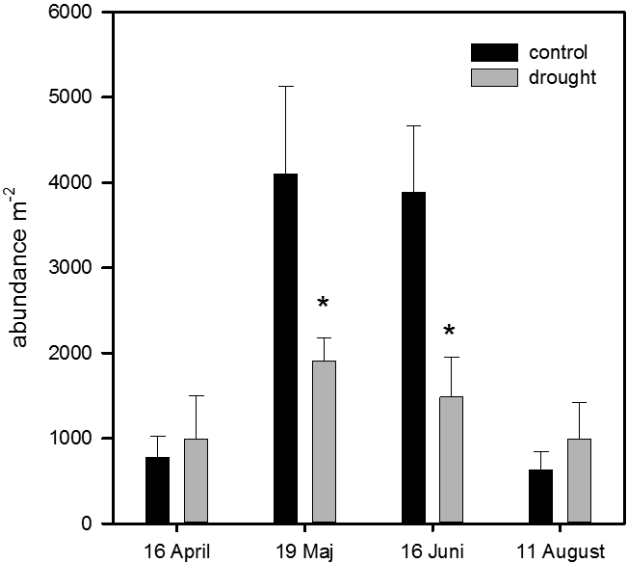

Fig. 2. Abundance of *Pergalumna nervosa* (mean ± S.E.; *N* = 5) in control and drought plots in the field on four dates during the experiment. An asterisk denotes that abundance in the drought plots was significantly different from that in the control plots (Holm-Sidak, *P* < 0.05).

In other words, soil animals with a normal haemolymph osmolality (ca. 400 mOsm) would potentially lose water to the environment due to the vapour pressure gradient across the body wall. Due to the technique used to extract microarthropods from soil cores, it was not possible to measure water content or haemolymph osmolality of field collected animals. Instead, we performed a laboratory experiment to simulate the desiccating conditions that *P. nervosa* was exposed to in the field.

Water balance and haemolymph osmolality

The average fresh weight of *P. nervosa* adults was 66 ± 3.5 µg (mean ± SD; *N* = 10). When exposed to 16.3% RH at 20°C for 5 h and 30 min mites lost on average 4 ± 0.8 µg water. The estimated average surface area of adult mites was ca. 1.05 mm^2 and the water permeability was thus estimated as 4.8 µg cm^{-2} h^{-1} mm Hg^{-1}. This places *P. nervosa* among some of the most desiccation resistant invertebrates with a cuticular water permeability similar to certain ticks and desert beetles (Hadley, 1994). Siepel (1996) subjected a range of oribatid species to 70% RH for 48 h and found that *P. nervosa* was among the most drought tolerant species with 72% survival. Cuticular water permeability of arthropods is especially determined by the hydrocarbon coating that water proofs the cuticle (Hadley, 1994). In line with this, Benoit et al. (2008) report that water permeability of three species of Antarctic oribatid mites is related to the thickness of the cuticle hydrocarbon layer. We may therefore speculate that cuticle hydrocarbons are also important for *P. nervosa*.

The low water permeability was also reflected in experiments in which we simulated gradually increasing drought stress that ended at –7 MPa, which is twice the drought intensity experienced in the field (Figs 3 and 4). In the first experiment the water content of adult mites dropped from

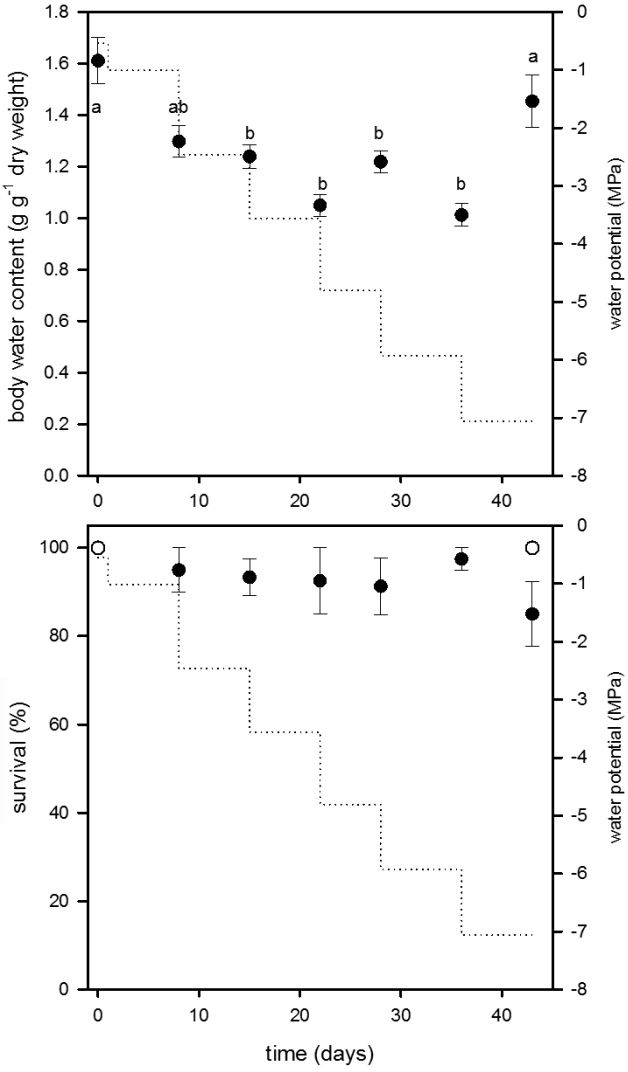

Fig. 3. Body water content (upper panel) and survival (lower panel) of *Pergalumna nervosa* (mean ± S.E.; *N* = 5) exposed to increasing drought stress over 43-days in the laboratory in 2012. Black circles indicate mites exposed to drought. Open circles indicate controls. The increasing drought stress (i.e. increasingly negative values; MPa) is shown as dotted lines with values shown on the axis to the right. Different letters indicate that mean values are significantly different (Holm-Sidak, *P* < 0.05).

1.6 to about 1.0 g g^{-1} dry weight over the 40-day experiment (Fig. 3; ANOVA, F = 9.27, *P* < 0.001). In the second experiment water content dropped from 1.3 to about 1.0 g g^{-1} dry weight (Fig. 4; ANOVA, F = 8.8, *P* < 0.001). Although these water losses indicate a substantial dehydration of adults this occurred over a prolonged period during which the mites had no access to free water. Moreover, drought-exposed mites did not suffer significant mortality during the experiment (Fig. 3; GLM, Z-value = –1.53, *P* = 0.13), which is in agreement with the high survival recorded in the field (Fig. 2). Previous studies have shown that oribatid mites typically tolerate loss of up to 35% of the initial body water content (Worland & Block, 1986; Benoit et al., 2008). Judging from the results shown in Fig. 1 it seems that this level of dehydration was reached at the

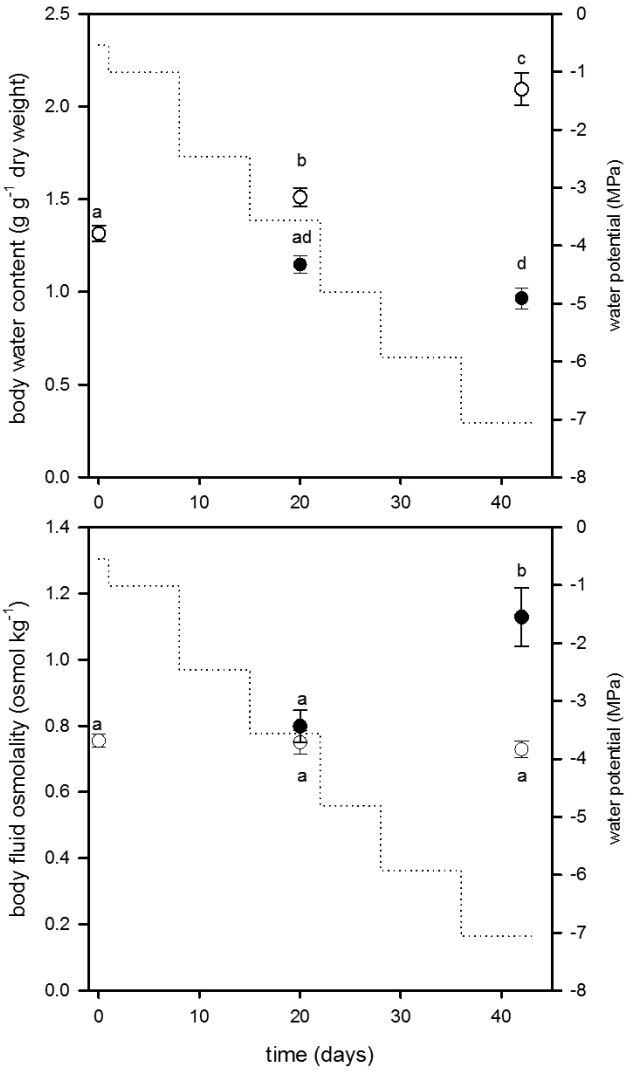

Fig. 4. Body water content (upper panel) and body fluid osmolality (lower panel) of *Pergalumna nervosa* (mean ± S.E.; *N* = 5) exposed to increasing drought stress over 43-days in the laboratory in 2014. Black circles indicate mites exposed to drought. Open circles indicate controls. The increasing drought stress (i.e. increasingly negative values; MPa) is shown as dotted lines with values shown on the axis to the right. Different letters indicate that mean values are significantly different (Holm-Sidak, $P < 0.05$).

highest drought stress where survival also tended to decrease slightly.

The estimated body fluid osmolality of *P. nervosa* held under control conditions was approximately 750 mOsm (Fig. 4), which is similar to that recorded for other oribatid species in summer (Sømme et al., 1993; Sjursen & Sømme, 2000). It should be noted that the melting onset temperature of DSC scans are not necessarily a true melting point of aqueous solutions, such as arthropod haemolymph. Ideally, a range of DSC scans should be run with decreasing heating rates and subsequent extrapolation to a heating rate of zero in order to derive a "true" melting temperature. In the present study we used the lowest possible scan rate during heating (0.1°C min^{-1}) and used melting onset temperature to derive an approximate osmolality. Our results are comparable to what is known from studies of other oribatids using the melting point depression technique (Clifton nanolitre osmometer) (Sømme et al., 1993; Sjursen & Sømme, 2000), and therefore useful for describing the changes in osmolality in our experiment. Here, we found that body fluid osmolality increased significantly when adult mites were exposed to increasing drought stress and consequent dehydration (Fig. 4; ANOVA, F = 7.13, $P = 0.003$). Osmolality increased from 750 mOsm to 1130 mOsm (i.e. an increase of 380 mOsm), whereas water content of mites decreased from 1.3 to 1.0 g g^{-1} dry weight during the same period (Fig. 4). It seems therefore that the increase in osmolality largely resulted from up-concentration of solutes in the body fluids during dehydration, and not to a de novo synthesis of carbohydrate osmolytes or free amino acids as occurs in e.g. Collembola (Bayley & Holmstrup, 1999; Holmstrup et al., 2015).

Terrestrial arthropods, including oribatid mites, living in the soil or on the soil surface are occasionally exposed to drought and have adapted to these conditions. As a supplement to the obvious benefits of behavioural avoidance of drought stress, two general morphological and/or physiological strategies have evolved to survive drought. One is to minimize water loss by reducing permeability as much as possible by having a thick cuticle covered by water proofing carbohydrates. As a supplement to this strategy, some oribatid species (and many other arthropods, see e.g. Hadley, 1994) have evolved the ability to absorb water vapour from the atmosphere against a water vapor pressure gradient (Benoit et al., 2008). The other strategy is to increase tolerance of water loss and consequent cellular dehydration, in its most extreme example known as anhydrobiosis (Crowe et al., 1992). In this context, *P. nervosa* clearly belongs to the first category; our studies have shown that it has a very low cuticular permeability for water and is therefore able to endure several weeks of severe drought under both field and laboratory conditions before dehydration becomes injurious.

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