EUROPEAN JOURNAL OF ENTOMOLOGY
ISSN (online): 1802-8829
http://www.eje.cz
Eur. J. Entomol. **121**: 303–309, 2024
doi: 10.14411/eje.2024.032
ORIGINAL ARTICLE

Does pre-exposure to mild drought enhance desiccation resistance in Collembola?

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Key words. Drought acclimation, phenotypic plasticity, osmoregulation

Abstract. Species of springtails (Collembola) have a stratified distribution along the soil/air profile and present an opportunity to study adaptive traits relating to a gradient of fluctuating humidity. The habitat of eu-edaphic species (found in deeper litter and soil layers) secures a relatively stable humidity, whereas hemi-edaphic (found in upper litter layers) and epigeic springtails (found on soil surface or vegetation) can experience an increased range of moisture fluctuation with much more rapid shifts between extremes. We hypothesized that the capacity for acclimation to drought conditions is better developed in species associated with the soil surface than in soil-dwelling species. To test this hypothesis, we measured the osmotic response of two epigeic, two hemi-edaphic and three eu-edaphic species to mild drought acclimation (3 d at –1.5 MPa; the average wilting point of plants) and compared the drought tolerance of drought acclimated animals with controls. Epigeic species had no or very little osmotic regulation during mild drought acclimation and unexpectedly became less tolerant to subsequent acute drought stress than controls. The hemi-edaphic and eu-edaphic species significantly increased body fluid osmolality in response to mild drought acclimation. However, none of these species became more drought-tolerant after exposure to mild drought. In conclusion, we found that the osmotic response to drought acclimation was indeed related to the vertical position of a species in the soil/air profile, however, the relationship was opposite to our hypothesis.

1. INTRODUCTION

Springtails are wingless hexapods closely related to insects but forming their own order (Collembola). Most springtails are small (their body length is typically 1 to 5 mm) and live in the air-filled pore space of soils, in litter on the soil surface, or in the vegetation (Gisin, 1943; Hopkin, 1997). Springtails have ecological significance for nutrient cycling in soils through grazing on roots, microorganisms and detritus, and as prey for soil-dwelling predators such as spiders (Rusek, 1998; Wise, 2004; Potapov et al., 2016).

Springtail species typically occupy distinct vertical strata of the soil profile (Hopkin, 1997; Berg et al., 1998; Potapov et al., 2016; Ferrín et al., 2023) and are often divided into species that live in the uppermost litter or on the soil surface (epigeic species), in vegetation (atmobiotic species), and truly soil-living species which live in deeper soil layers (eu-edaphic species). Intermediate to these, the hemi-edaphic species live in the deeper litter layers. The life forms of springtails (i.e. their preferred vertical distribution in the soil) are often inferred from morphology such as pigmentation, presence of eyes, body size, antennal length and presence or absence of a furcula (Gisin, 1943; Parisi et al., 2005; Potapov et al., 2016; Malcicka et al., 2017; Ferrín et al., 2023). Hence, epigeic life-forms are colourful, have eyes, long antennae, well-developed furcula and are often relatively large. Eu-edaphic species are often blind, without cuticular pigmentation, have short antennae and often have reduced or absent furcula. Hemi-edaphic species are intermediate in these traits. However, exceptions to this categorization may occur, and the vertical distribution of individuals within a species may temporarily shift according to light, temperature and soil humidity (Hågvar, 1983; Lagerlöf & Andren, 1991; Detsis, 2000; Tsiafouli et al., 2005).

Springtails, with their stratified distribution along the soil/air boundary, present an opportunity to study the occurrence of plasticity in drought tolerance as an adaptive trait in species along an extended gradient of fluctuating humidity similar to a climatic gradient, however, at a much smaller spatial scale. The habitat of the eu-edaphic species secures a relatively stable humidity (Willmer, 1982). On the other hand, epigeic springtails can experience an increased range of moisture fluctuation with much more rapid shifts between extremes (Geiger et al., 1995). Assuming that species are well adapted to the conditions in the soil strata where they are found, it may be hypothesized

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that the capacity for acclimation to drought conditions is better developed in species associated with the soil surface than in soil dwelling species.

Eu-edaphic springtails can actively regulate internal osmotic pressure and absorb water vapor in relatively dry soil by accumulating compatible osmolytes (sugars, sugar alcohols or free amino acids) in response to desiccation (Bayley & Holmstrup, 1999; Holmstrup et al., 2015). Hence, pre-acclimation at mildly desiccating conditions can stimulate upregulation of the animals' body fluid osmolality, reducing cuticular water loss and increasing survival chances when exposed to more severe drought (Sjursen et al., 2001). In contrast to the eu-edaphic species, epigeics primarily rely on a low cuticular permeability to survive drought and are not able to absorb water vapor by osmoregulation (Kærsgaard et al., 2004; Holmstrup et al., 2015).

In the present study, we compare seven springtail species and test the hypothesis that a species' habitat position along the vertical soil/air transect (i.e. the life form) determines its capacity to acclimate to drought conditions. To this end, we measured the responses in a species' body fluid osmolality when exposed to mild drought conditions and whether pre-acclimation to mild drought increases survival of subsequent exposure to severe drought conditions.

2. MATERIALS AND METHODS

2.1 Animal material

Seven species of springtails were included in the study (Table 1). The species *Heteromurus nitidus* (Templeton), *Sinella curviseta* (Brook), *S. tenebricosa* (Folsom), *Folsomia candida* (Willem) and *Protaphorura fimata* (Gisin) were cultured in our laboratory and kept in Petri dishes with a base of moistened gypsum/charcoal mixture (8 : 1 w : w). These springtail species were held at a constant temperature of 20°C (12L : 12D cycle) and given dried baker's yeast as feed.

For the experiment, we randomly collected adult animals from mass cultures of these species. *Entomobrya nivalis* (Linnaeus) were collected in early September, 2023, from the canopy of *Juniper communis* bushes near the Mols Laboratory, Rønde, Denmark, and brought to the laboratory in plastic beakers containing moist *Juniper* litter. *Orchesella cincta* (Linnaeus) were extracted from mixed deciduous forest floor litter collected near Silkeborg, Denmark, in June 2021. We selected adults of the two field-collected species and initiated experiments after two days of acclimation to 20°C.

2.2 Drought tolerance experiments

The aim of the experiment was to investigate whether pre-acclimation to realistic mild drought would improve the tolerance of subsequent severe drought exposure. Laboratory experiments were designed to simulate the soil water potential (SWP) experienced by the springtails during a natural drought event. Considering that SWP at the permanent wilting point of plants is approximately –1.5 MPa (equivalent to 98.9% Relative Humidity; RH), and that SWP (even under severe drought) is rarely below –15 MPa (ca. 90% RH), we established a drought exposure system which was able to accurately create drought exposures in this range. To this aim, aqueous NaCl solutions were used to create controlled water potentials (and RH) in small vials where the springtails were kept (Holmstrup, 1997). Briefly, the springtails were placed in open-top (but covered with 100 µm nylon net preventing escape of the springtails) plastic sample vials (3 cm high, 1.6 cm diameter), which were glued to the floor of 300-mL plastic cups (4.5 cm high, 9.2 cm diameter) containing ca. 25 mL aqueous NaCl solution, sealed with tightly fitting plastic lids. The air in this small closed system rapidly equilibrates with the salt solution (following Raoult's law), and precise SWP can be established by adjusting the NaCl concentration and keeping the system at constant temperature (Lang, 1967).

As a pre-acclimation treatment representing mild desiccation stress for springtails, we chose exposure to the average wilting point of plants (–1.5 MPa or 98.9% RH; using 19.4 g NaCl L^{-1}) for three days at 20°C (Holmstrup et al., 2001; Kærsgaard et al., 2004). As a control treatment, we used exposure to –0.18 MPa (99.9% RH; 2 g NaCl L^{-1}). We did not use saturated conditions as a control treatment since this often causes some mortality due to the presence of liquid water in the test beakers (Holmstrup, unpubl. study). The haemolymph osmolality of springtails kept under humid conditions is in the range 250–400 mOsm (equivalent to –0.6 to –0.95 MPa) (Verhoef & Witteveen, 1980; Verhoef & Prast, 1989; Kærsgaard et al., 2004). Hence, the SWP in the pre-acclimation beakers resulted in potentially desiccating conditions for the exposed springtails, which could induce a physiological up-regulation of body fluid osmolality (Bayley & Holmstrup, 1999).

Table 1. Overview of the Collembola species used in the experiments and some traits associated with their vertical distribution in the soil profile.

Species	Maximal body length (mm)	Complex pigmentation	Colour	Antenna length (relative to body length)	Scales	Ommatidia	Furca	Vertical distribution [a]	Life form [b]	Vertical distribution rank [c]
Entomobrya nivalis	1.8	yes	intense/dark	0.52	no	8	fully developed	On bark and moss of live trees	Atmobiont/epigeic	1
Orchesella cincta	6	yes	intense/dark	0.5	no	8	fully developed	In surface litter and lower parts of tree trunks	Atmobiont/epigeic	2
Heteromurus nitidus	2	no	pale	0.45	yes	1	fully developed	Under rocks and pieces of wood, in surface litter	hemi-edaphic	3
Sinella curviseta	2	no	pale	0.6	no	2	fully developed	In litter	hemi-edaphic	4
Sinella tenebricosa	1.5	no	white	0.4	no	0	fully developed	In compost soil	eu-edaphic	5
Folsomia candida	2.5	no	white	0.25	no	0	fully developed	In thick organic litter and compost soil	eu-edaphic	6
Protaphorura fimata	2.5	no	white	0.16	no	0	absent	In compost soil	eu-edaphic	7

[a] Extracted information from Fjellberg (2007). [b] Based on morphological traits as outlined in Ferrin et al. (2023). [c] Ranking of species according to their most likely distribution along the vertical soil profile. This value is determined based on reported vertical distribution and life form, where a rank = 1 indicates the most superficial position, and rank = 7 the deepest position in the soil-air gradient.

Table 2. Concentrations of aqueous NaCl solutions (C_s) used in the drought exposure experiments. The soil water potential (SWP; -MPa) was calculated as: SWP = $0.0733 \times C_s + 0.0001 \times C_s^2$ (Eq. 1).

Species	Concentration of NaCl solution (C_s; g L^{-1} solution)								
Entomobrya nivalis	2.0	19.4	31.6	52.0	80.0	124.0	166.0	191.0	242.0
Orchesella cincta	2.0	31.8	40.8	60.5	75.0	85.0	95.0	110.0	
Heteromurus nitidus	2.0	17.0	31.6	40.8	51.8	60.5	71.2	100.0	
Sinella curviseta	2.0	23.0	31.6	40.8	51.8	60.5	71.2	88.9	
Sinella tenebricosa	2.0	23.0	31.6	40.8	51.8	60.5	71.2	88.9	
Folsomia candida	2.0	17.0	31.6	40.8	51.8	60.5	71.2	100.0	
Protaphorura fimata	2.0	31.6	40.2	51.8	60.5	71.2	88.9	110.0	

A total of 32 beakers were dedicated to pre-acclimation to mild drought, and 32 beakers served as controls, with each beaker containing ten animals. After the pre-acclimation period, NaCl solutions were replaced by increasing NaCl concentrations (four beakers per concentration) ranging between 2 and up to 242 g L^{-1}, depending on the species (Table 2). Springtails were then exposed to drought for seven days, after which the animals of each beaker were transferred to separate Petri dishes with moist plaster of Paris/charcoal for re-hydration and recovery. Survival was scored after 24 h recovery. Animals that walked normally, either spontaneously or after light stimulation with a fine paint-brush, were scored as survivors. All other animals were scored as dead.

2.3 Differential scanning calorimetry

Body fluid osmolality was estimated using differential scanning calorimetry (DSC) as described by Holmstrup (2018). Calorimetric analysis of single springtails was done using a DSC4000 calorimeter (Perkin Elmer, Waltham, MA, USA). A single springtail was transferred to a pre-weighed 50 µL aluminium DSC pan, and its fresh weight was determined using a Sartorius SC 2 microbalance (Sartorius AG, Goettingen, Germany) accurate to 1 µg. The pan was immediately hermetically sealed, and samples were subjected to a DSC program consisting of three steps: (1) hold for 1 min at 5°C; (2) cool to −35°C at a rate of 5°C min^{-1} (causing the animal body fluids to freeze) (3) hold for 1 min at −35°C; (4) heat to 5°C at a rate of 1°C min^{-1}. The melt endotherm (enthalpy change during melting of ice formed in the animal) of the heating scan curve was recorded, and the melt onset temperature was estimated using Pyris Software (Perkin Elmer, Waltham, MA, USA) as described by Block (1994).

The melt onset temperature (determined by DSC) of intact springtails is not necessarily a true melting point of haemolymph. However, a good estimation of osmolality can be achieved using DSC when calibrating melt onset temperature with melting point determination of animal samples using vapor pressure osmometry as described by Holmstrup (2018).

Hence, osmolality was calculated based on melt onset temperature (M_{onset};°C) and fresh weight of the animal (FW; mg) following the equation:

$$\text{Osmolality} = -0.368756 \times M_{onset} - (0.331181 \times FW) - 0.185582 \quad \text{(Eq. 2)}$$

2.4 Statistical analysis

The effect of pre-acclimation on body fluid osmolality within each species was subjected to analysis of variance where data fulfilled the requirements of normal distribution and homoscedasticity. In other cases, a Kruskal-Wallis one-way analysis of variance on ranks was done.

The relationship between increasing drought and survival for each springtail species was analysed by fitting a dose-response curve using the model fitting function drm() in the R packages drc (Ritz et al., 2015). Two curves were fitted for each species, one for control and one for pre-exposed springtails. Appropriate two or three parametric log-logistic dose-response models were fitted, with binomial as the family type and logit as the link function, and the lethal SWP_{50} values (ED_{50}) were estimated from these models. Pairwise comparisons of the SWP_{50} values between control and pre-acclimated groups for each species were performed using the compParm function to assess statistically significant differences. Additionally, we compared the drought tolerance among six of the seven species (excluding *E. nivalis* due to its distinctive survival pattern) using a two-parameter log-logistic model fitted to the control data. The compParm function was again employed to identify significant differences in the SWP_{50} values among species. All statistical analyses were conducted using RStudio (version 2023.12.1) and R (version 4.2.2).

3. RESULTS AND DISCUSSION

3.1 Suitability of DSC analysis for the measurement of body fluid osmolality

The estimated osmolality of control animals ranged between 500 and 900 mOsm (Fig. 1). Previous research using more conventional methods such as melting point depression (e.g. Clifton nano-litre osmometer) or vapor pressure osmometry (Wescor) gave osmolality values for *O. cincta*, *F. candida* and *P. fimata* between 275 and 400 mOsm (Verhoef & Witteveen, 1980; Verhoef, 1981; Verhoef & Prast, 1989; Kærsgaard et al., 2004) which is 100–200 mOsm lower than estimated here by use of DSC (Fig. 1).

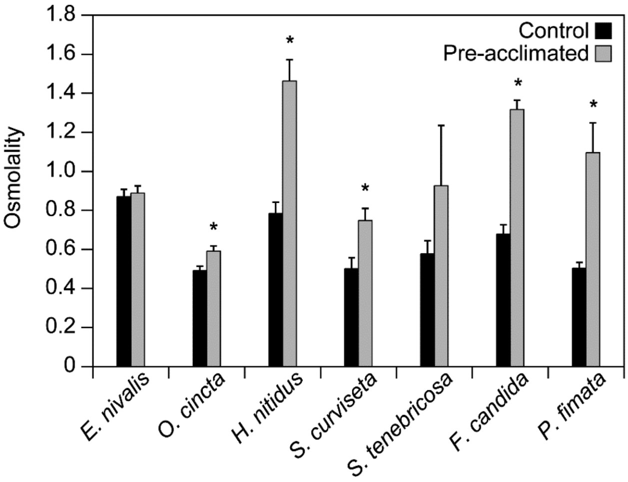

Fig. 1. Osmolality of body fluids in seven species of springtails kept at control conditions (–0.18 MPa) or acclimated to mild drought (–1.5 MPa) for three days. Values are mean ± SE (n = 5–8). An asterisk indicates that pre-acclimated animals had a significantly higher osmolality than controls ($P < 0.05$).

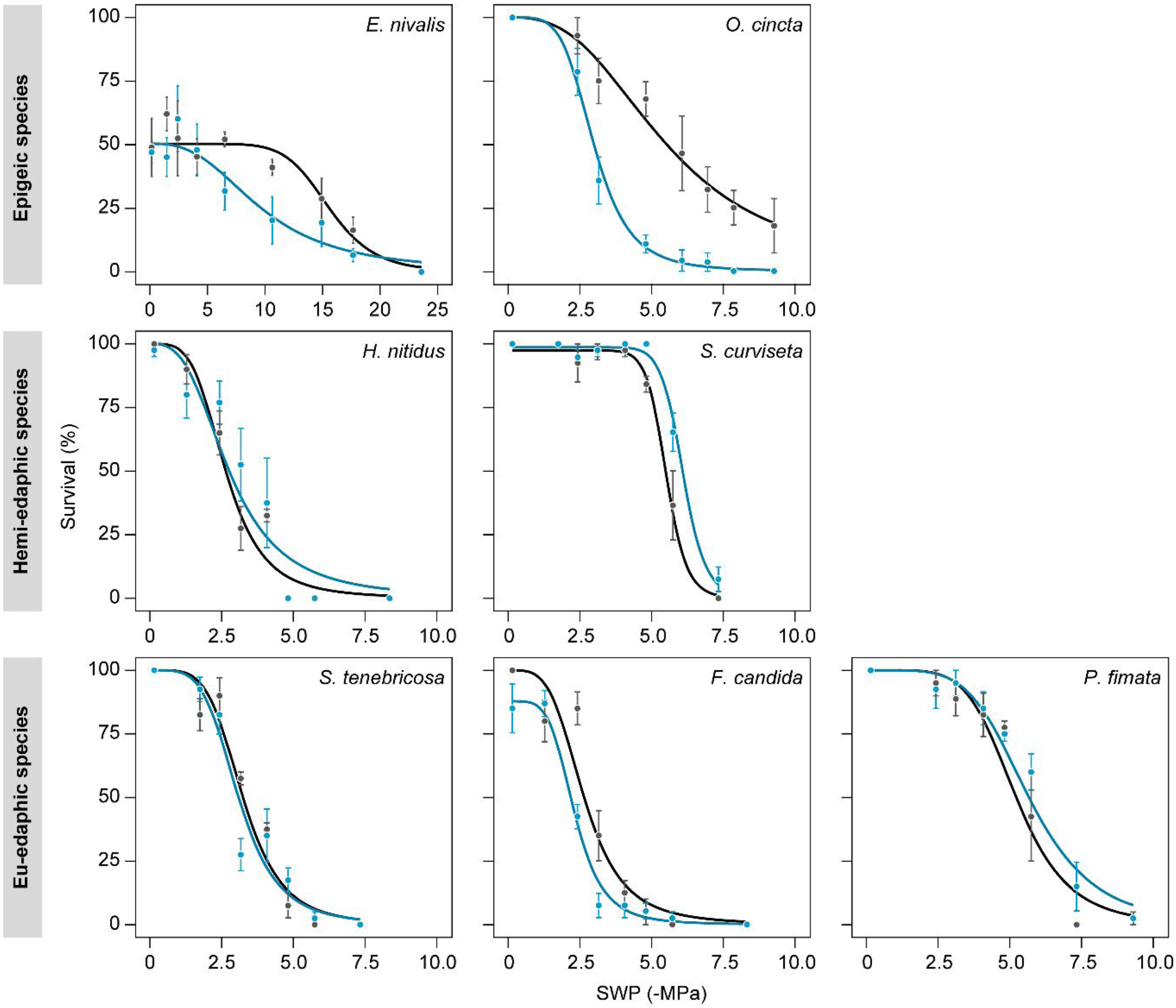

Fig. 2. Log-logistic dose-response analysis of drought survival in seven springtail species. Black symbols and lines indicate controls, Blue symbols and lines indicate animals pre-acclimated to mild drought (–1.5 MPa) for 3 days.

Also, the DSC-estimated osmolality of control *E. nivalis* (ca. 900 mOsm) was twice as high as the osmolality of summer acclimated specimens measured by use of melting point depression (Verhoef & Witteveen, 1980; Zettel, 1984). While the inferior accuracy of the DSC methodology for osmolality measurement must be carefully considered in the interpretation of our results, the precision of this method is sufficiently good (coefficient of variation was 10–20%) for comparison of treatments (Fig. 1).

3.2 Effect of pre-acclimation on body fluid osmolality

The hemi-edaphic and eu-edaphic species clearly responded to mild drought acclimation by increasing their osmolality, although this was not statistically significant in *S. tenebricosa* (Fig. 1). The highest relative increases in osmolality were seen in the eu-edaphic species *F. candida* (93%) and *P. fimata* (117%). This osmotic response to mild drought is unique to especially hemi-edaphic and eu-edaphic springtails and important for their ability to maintain their body fluids hyperosmotic to ambient humidity conditions in the soil, thereby facilitating water vapor absorption and avoidance of fatal dehydration during drought spells (Bayley & Holmstrup, 1999; Kærsgaard et al., 2004; Holmstrup & Bayley, 2013). Thus, all hemi-edaphic and eu-edaphic species raised their osmolality well above 600 mOsm (which is equivalent to the pre-acclimation regime of –1.5 MPa) and thus re-established their hyperosmotic status in this experimental environment. The osmotic response to drought acclimation in these species is mainly based on accumulation of sugars, sugar alcohols and certain free amino acids known as compatible osmolytes (Verhoef & Prast, 1989; Bayley & Holmstrup, 1999; Holmstrup et al., 2001; Holmstrup et al., 2015). Evaporative water loss during pre-acclimation would also contribute to an increased osmolality, however, the mild drought level and short duration are unlikely to have caused any substantial dehydration in this experiment (Kærsgaard et al., 2004; Holmstrup et al., 2015).

Opposed to the species living in the litter and deeper soil layers, pre-acclimation to mild drought did not change – or only slightly changed – the osmolality of the epigeic/atmobiotic species; *E. nivalis* did not show any response at all, and only a small (20%) increase from 500 to 600 mOsm was observed in *O. cincta* (Fig. 1). Hence, these epigeic/atmobiotic species were much less osmo-responsive to drought acclimation.

3.3 Drought tolerance

The log-logistic dose-response curves fitted survival data well for control and pre-acclimated animals of all seven species (Fig. 2). It should be noted that control survival in *E. nivalis* was only 50% in both control animals and pre-acclimated animals. However, it was still possible to calculate the effect of drought in both treatment groups as the lethal SWP_{50}. Lethal SWP_{50} values were estimated from the dose-response curves, and we tested inter-specific differences in acute drought tolerance (i.e. without pre-acclimation), and within each species, we tested if pre-acclimation caused a significant change in lethal SWP_{50}.

Considering only the control treatment, we observed significant differences in drought tolerance among the species. The most drought-tolerant species was *E. nivalis*, for which half of the animals survived SWP below –15 MPa for seven days (<90% RH; Figs 2 and 3). This reflected a remarkable desiccation resistance, probably due to low cuticular permeability for water since osmoregulation was completely absent in this species (Fig. 1). In support of this interpretation, Verhoef & Witteveen (1980) showed that the transpiration rate of *E. nivalis* is very low compared to other springtail species despite its relatively small size and large surface : volume ratio (Table 1). The species *O. cincta*, *S. curviseta* and *P. fimata* had almost the same drought tolerance with lethal SWP_{50} of approximately –5.5 MPa (Fig. 3). The species *H. nitidus*, *S. tenebricosa* and *F. candida* displayed similar and lower drought tolerance with lethal SWP_{50} at around –3 MPa (Fig. 3). The drought tolerance levels found in the present study (except *E. nivalis*, which was not tested before) are in good agreement with what has previously been reported in studies using the same methodology (Holmstrup et al., 2001; Kærsgaard et al., 2004).

In a natural setting, soil organisms will likely be subjected to slowly increasing drought rather than acute shifts to low humidity conditions since drying of soils is a gradual, buffered process occurring over days or weeks. Thus, free-ranging soil invertebrates have the opportunity to acclimate to a relatively mild desiccation pressure before severe drought takes effect. Indeed previous research has shown that drought acclimation can greatly improve tolerance of later and more severe desiccation stress in a number of soil invertebrates such as nematodes, eu-edaphic springtails, midge larvae and earthworms (Womersley & Ching, 1989; Sjursen et al., 2001; Hayward et al., 2007; Petersen et al, 2008).

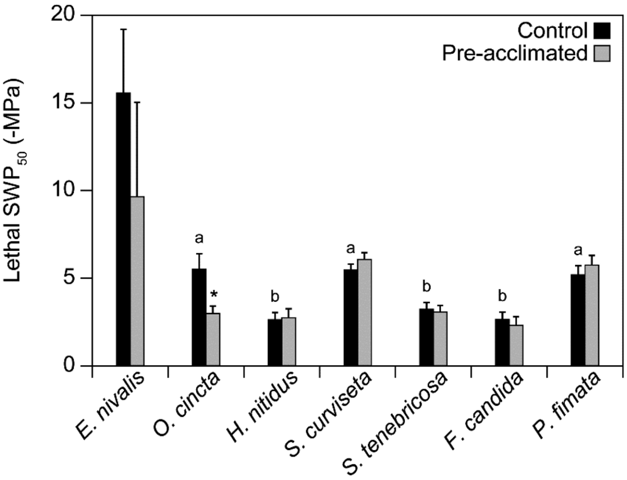

Fig. 3. The soil water potential causing 50% mortality (lethal SWP_{50}, 7-day exposure) of springtail species pre-exposed to mild drought (–1.5 MPa) or at non-stressing conditions (control; see text for further details). Error bars are SE, and asterisks indicate statistically significant differences between treatments (controls versus pre-acclimation to mild drought). Different letters above black bars indicate differences between species (only control treatments). Note that *E. nivalis* was not included in the inter-species comparison.

Pre-acclimation to mild drought reduced the drought tolerance by almost 50% of the two epigeic species (*E. nivalis* and *O. cincta*) although this reduction was statistically significant only in *O. cincta* (Fig. 3). In the remaining species we found no statistically significant effect of pre-acclimation. Combining the results of Fig. 1 and Fig. 3, we saw that species with the smallest osmotic response to drought acclimation (the two epigeic species; Fig. 1) became less drought tolerant, whereas those species having a distinct osmotic response to drought acclimation did not change their drought tolerance to any large degree (Fig. 4). This was surprising since it must be assumed that epigeic species in their natural habitat are exposed to harsher and more variable drought conditions than soil edaphic species simply because the upper part of the soil profile will desiccate more rapidly during droughts than the deeper soil layers. Hence, one might think that they, through evolution, were adapted to have higher plasticity in this regard. However, the epigeic species are not utilizing osmoregulation (Fig. 1), but rely on their ability to resist evaporate dehydration (Kærsgaard et al., 2004). Moreover, epigeic species have good abilities to migrate horizontally and find microsites with higher humidity (Joosse, 1970; Verhoef & Van Selm, 1983) and, therefore, seem to utilize different strategies to cope with drought than species from deeper soil strata. This idea was further elaborated by Ponge (2020) who highlighted the good dispersal abilities (‘move’ strategy) of epigeic species versus the phenotypic plasticity (‘change’ strategy) of eu-edaphic species (with poor dispersal ability) as fundamentally different adaptations to overcome environmental hazards. It is still unresolved, however, why the epigeic species suffer more from pre-exposure to mild drought than species deeper in the soil. These species cannot absorb water vapor as eu-edaphic species and may be unable to tolerate high water loss which might cause mortality due to thirst.

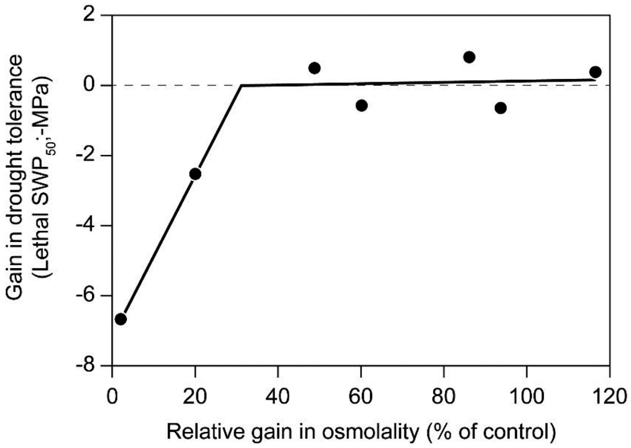

Fig. 4. The relationship between relative osmotic response to pre-acclimation and the following gain in drought tolerance of seven springtail species. Circles represent mean values and lines the result of piecewise linear regression ($R^2 = 0.96$; $P = 0.014$). Simple linear regression (not shown) also indicated a significant relationship ($R^2 = 0.624$; $P = 0.035$). Note that a negative value of Lethal SWP_{50} indicates a worsened drought tolerance after pre-acclimation.

3.4 Is plasticity related to life form?

In an attempt to show a possible connection between life forms and the adaptive ability to osmo-regulate during drought acclimation (i.e. plasticity), we plotted the relative increase of body fluid osmolality of a species (a measure of phenotypic plasticity) against its vertical position ('vertical distribution rank') in the soil profile (Fig. 5). Linear regression showed a clear relationship between vertical distribution rank and plasticity indicating that the species living above the ground displayed very little plasticity in osmoregulation whereas the eu-edaphic species had the highest plasticity in this trait. We stress that only seven species were included in our study, which limits the broadness of this observation, and further, we cannot rule out that phylogeny has a big influence on this relationship. Life form and vertical position of a species are to a large degree connected to phylogeny with most Entomobryidae being atmobiotics and most Isotomidae and Onychiuridae being eu-edaphic or hemi-edaphic (Potapov et al., 2016).

Our results on the plasticity of osmoregulation have bearings to observations of plasticity of heat tolerance as well as plasticity of cold tolerance as a result of thermal acclimation in springtails. Thus, van Dooremaalen et al. (2013) found that epigeic springtail species were more plastic than eu-edaphic species in their adjustment of membrane fluidity and that this adaptation was part of a more rapid gain of heat tolerance during acclimation to mild heat. Similarly, Bahrndorff et al. (2009) found that epigeic springtails more rapidly gained cold tolerance during low-temperature acclimation than eu-edaphic species did, possibly through similar membrane adjustments as for heat tolerance. Interestingly, epigeic springtails, which by definition live most of their life in a thermally fluctuating environment (i.e. on the soil surface or in vegetation), have adapted by being physiologically more plastic towards thermal stress, whereas for drought tolerance, the relationship seems to be opposite. The conclusion we draw with the present study is in line with experimental drought treatments of field communities of springtails across the European continent (Ferrín et al., 2023). In that study, it was shown that the springtail communities in shrubland became more dominated by eu-edaphic species at the expense of epigeic species after 8 years of repeated summer drought. The eu-edaphic species are likely living in a more stable and protected environment, but at the same time, they are also benefitting from adaptive osmo-regulation capabilities.

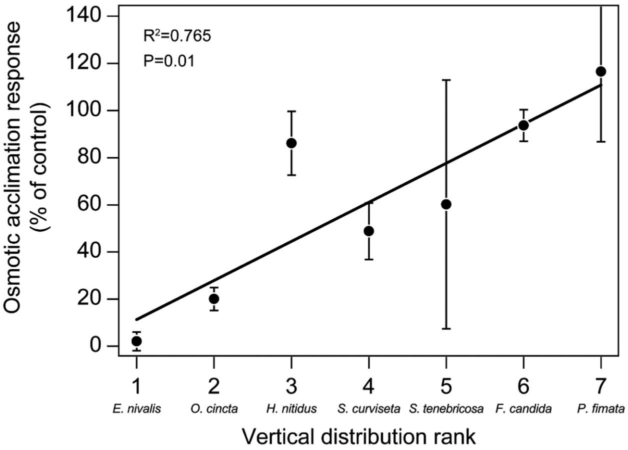

Fig. 5. The relationship between a species' vertical position and the response of body fluid osmolality to acclimation at –1.5 MPa.

ACKNOWLEDGEMENTS. The authors are indebted to E. Jørgensen and Z. Gavor for their excellent technical assistance in Collembola extraction and identification. M.H. was supported by a grant from the Danish Research Council (Grant no. DFF-4002-00384).

REFERENCES

Bahrndorff S., Loeschcke V., Pertoldi C., Beier C. & Holmstrup M. 2009: The rapid cold hardening response of Collembola is influenced by thermal variability of the habitat. — *Funct. Ecol.* **23**: 340–347.

Bayley M. & Holmstrup M. 1999: Water vapor absorption in arthropods by accumulation of myoinositol and glucose. — *Science* **285**: 1909–1911.

Berg M.P., Kniese J.P., Bedaux J.J.M. & Verhoef H.A. 1998: Dynamics and stratification of functional groups of micro- and mesoarthropods in the organic layer of a Scots pine forest. — *Biol. Fertil. Soils* **26**: 268–284.

Block W. 1994: Differential scanning calorimetry in ecophysiological research. — *Acta Oecol. / Int. J. Ecol.* **15**: 13–22.

Detsis V. 2000: Vertical distribution of Collembola in deciduous forests under Mediterranean climatic conditions. — *Belg. J. Zool.* **130**: 57–61.

Ferrín M., Márquez L., Petersen H., Salmon S., Ponge J.F., Arnedo M., ... Peguero G. 2023: Trait-mediated responses to aridity and experimental drought by springtail communities across Europe. — *Funct. Ecol.* **37**: 44–56.

Fjellberg A. 2007: *The Collembola of Fennoscandia and Denmark. Part II: Entomobryomorpha and Symphypleona. Fauna Entomologica Scandinavica, Vol. 42.* Brill, Leiden, 265 pp.

Geiger R., Aron R.H. & Todhunter P. 1995: *The Climate Near the Ground.* Vieweg, Braunschweig, 528 pp.

Gisin H. 1943: Ökologie und Lebensgemeinschaften der Collembolen im Schweizer Exkursionsgebiet Basels. — *Rev. Suisse Zool.* **50**: 131–224.

Hayward S.A.L., Rinehart J.P., Sandro L.H., Lee R.E. & Denlinger D.L. 2007: Slow dehydration promotes desiccation and freeze tolerance in the Antarctic midge *Belgica antarctica*. — *J. Exp. Biol.* **210**: 836–844.

Holmstrup M. 1997: Drought tolerance in *Folsomia candida* (Collembola) after exposure to sublethal concentrations of three soil-polluting chemicals. — *Pedobiologia* **41**: 354–361.

Holmstrup M. 2018: The springtail *Megaphorura arctica* survives extremely high osmolality of body fluids during drought. — *J. Compar. Physiol. (B)* **188**: 939–945.

Holmstrup M. & Bayley M. 2013: *Protaphorura tricampata*, a euedaphic and highly permeable springtail that can sustain activity by osmoregulation during extreme drought. — *J. Insect Physiol.* **59**: 1104–1110.

Holmstrup M., Sjursen H., Ravn H. & Bayley M. 2001: Dehydration tolerance and water vapour absorption in two species of soil-dwelling Collembola by accumulation of sugars and polyols. — *Funct. Ecol.* **15**: 647–653.

Holmstrup M., Slotsbo S., Rozsypal J., Henriksen P.G. & Bayley M. 2015: Accumulation of free amino acids during exposure to drought in three springtail species. — *J. Insect Physiol.* **82**: 114–121.

Hopkin S.P. 1997: *Biology of the Springtails*. Oxford University Press, Oxford, 340 pp.

Hågvar S. 1983: Collembola in Norwegian coniferous forest soils II. Vertical distribution. — *Pedobiologia* **25**: 383–401.

Joosse E.N.G. 1970: The formation and biological significance of aggregations in the distributions of Collembola. — *Netherlands J. Zool.* **20**: 299–314.

Kærsgaard C.W., Holmstrup M., Malte H. & Bayley M. 2004: The importance of cuticular permeability, osmolyte production and body size for the desiccation resistance of nine species of Collembola. — *J. Insect Physiol.* **50**: 5–15.

Lagerlöf J. & Andren O. 1991: Abundance and activity of Collembola, Protura and Diplura (Insecta, Apterygota) in 4 cropping systems. — *Pedobiologia* **35**: 337–350.

Lang A.R.G. 1967: Osmotic coefficients and water potentials of sodium chloride solutions from 0 to 40°C. — *Aust. J. Chem.* **20**: 2017–2023.

Malcicka M., Berg M.P. & Ellers J. 2017: Ecomorphological adaptations in Collembola in relation to feeding strategies and microhabitat. — *Eur. J. Soil Biol.* **78**: 82–91.

Parisi V., Menta C., Gardi C., Jacomini C. & Mozzanica E. 2005: Microarthropod communities as a tool to assess soil quality and biodiversity: a new approach in Italy. — *Agric. Ecosyst. Environ.* **105**: 323–333.

Petersen C.R., Holmstrup M., Malmendal A., Bayley M. & Overgaard J. 2008: Slow desiccation improves dehydration tolerance and accumulation of compatible osmolytes in earthworm cocoons (*Dendrobaena octaedra* Savigny). — *J. Exp. Biol.* **211**: 1903–1910.

Ponge J.F. 2020: Move or change, an eco-evolutionary dilemma: The case of Collembola. — *Pedobiologia* **79**: 150625, 8 pp.

Potapov A.A., Semenina E.E., Korotkevich A.Y., Kuznetsova N.A. & Tiunov A.V. 2016: Connecting taxonomy and ecology: Trophic niches of collembolans as related to taxonomic identity and life forms. — *Soil Biol. Biochem.* **101**: 20–31.

Ritz C., Baty F., Streibig J.C. & Gerhard D. 2015: Dose-response analysis using R. — *PLoS ONE* **10**: e0146021, 13 pp.

Rusek J. 1998: Biodiversity of Collembola and their functional role in the ecosystem. — *Biodiv. Conserv.* **7**: 1207–1219.

Sjursen H., Bayley M. & Holmstrup M. 2001: Enhanced drought tolerance of a soil-dwelling springtail by pre-acclimation to a mild drought stress. — *J. Insect Physiol.* **47**: 1021–1027.

Tsiafouli M.A., Kallimanis A.S., Katana E., Stamou G.P. & Sgardelis S.P. 2005: Responses of soil microarthropods to experimental short-term manipulations of soil moisture. — *Appl. Soil Ecol.* **29**: 17–26.

van Dooremalen C., Berg M.P. & Ellers J. 2013: Acclimation responses to temperature vary with vertical stratification: implications for vulnerability of soil-dwelling species to extreme temperature events. — *Global Change Biol.* **19**: 975–984.

Verhoef H.A. 1981: Water balance in Collembola and its relation to habitat selection: Water content, haemolymph osmotic pressure and transpiration during an instar. — *J. Insect Physiol.* **27**: 755–760.

Verhoef H. & Prast J. 1989: Effects of dehydration on osmotic and ionic regulation in *Orchesella cincta* (L.) and *Tomocerus minor* (Lubbock) (Collembola) and the role of the coelomoduct kidneys. — *Comp. Biochem. Physiol. (A)* **93**: 691–694.

Verhoef H. & Van Selm A. 1983: Distribution and population dynamics of Collembola in relation to soil moisture. — *Holarctic Ecol.* **6**: 387–394.

Verhoef H.A. & Witteveen J. 1980: Water balance in Collembola and its relation to habitat selection: cuticular water loss and water uptake. — *J. Insect Physiol.* **26**: 201–208.

Willmer P.G. 1982: Microclimate and the environmental physiology of insects. — *Adv. Insect Physiol.* **16**: 1–57.

Wise D.H. 2004: Wandering spiders limit densities of a major microbi-detritivore in the forest-floor food web. — *Pedobiologia* **48**: 181–188.

Womersley C. & Ching C. 1989: Natural dehydration regimes as a prerequisite for the successful induction of anhydrobiosis in the nematode *Rotylenchulus reniformis*. — *J. Exp. Biol.* **143**: 359–372.

Zettel J. 1984: Cold hardiness strategies and thermal hysteresis in Collembola. — *Rev. Écol. Biol. Sol* **21**: 189–203.

Received June 24, 2024; revised and accepted August 8, 2024