Deleterious effects of thermal and water stresses on life history and physiology: a case study on woodlouse

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Abstract

We tested independently the influences of increasing temperature and decreasing moisture on life history and physiological traits in the arthropod *Armadillidium vulgare.* Both increasing temperature and decreasing moisture led individual body mass and reproductive success to decrease. While immune cells density decreased and β-galactosidase activity increased with increasing temperature and decreasing moisture, suggesting a negative impact of these stresses on individual quality, increased temperature and decreased moisture affected differently the other biomarkers conjuring different underlying mechanisms depending on the stress applied. Our findings demonstrate overall a negative impact of high temperature and low moisture on woodlice welfare. Changing temperature or moisture had slightly different effects, illustrating the need of testing further the respective role of each of these key components of climate change on organisms to predict more reliably the future of our ecosystems.

Key words

Abiotic stresses, life history traits, physiological traits, arthropods, climate change

Introduction

The Intergovernmental Panel on Climate Change (IPCC) forecasts an average increase in temperature between +1.5°C and +4°C in 2100 (Masson-Delmotte et al., 2021). Not only will average temperatures and the frequency and intensity of precipitation change, but extreme events will increase in frequency. Although the link between global warming and drought is still highly debated and may not be direct (Trenberth et al., 2014), droughts due to a decrease in rainfall and an increase in evaporation are expected to take place in the coming decades (Dai, 2013), and should be much more intense than current droughts (Trenberth et al., 2014). As deterioration of environmental conditions are known to impact life history traits such as growth rate, reproductive success or longevity (e.g. Chen et al., 2019; Johnson and Jones, 2016; Khadioli et al., 2014), identifying the potential implications of climate change for organisms is a research question of paramount importance.

Terrestrial arthropods are ectotherms that are particularly sensitive to temperature and moisture changes (Lister and Garcia, 2018; Maron et al., 2015). Global warming constitutes a threat for them, as increasingly reported (Johnson and Jones, 2016). Thus, in Lepidoptera, too

high temperatures prevent hatching (Khadioli et al., 2014). In both Lepidoptera and Hymenoptera, survival decreases with increasing temperature (Abou-Shaara et al., 2012; Khadioli et al., 2014). In some Coleoptera, egg viability decreases and hatching time increases for viable eggs when they are exposed to drought (Johnson et al., 2010). When facing the costs of increased temperature and drought frequency and intensity on life history traits, arthropods display different responses to resist or tolerate such changes (Strachan et al., 2015). For example, some arthropods can migrate to refugia, others can implement physiological resistance tactics (e.g. resistant eggs) and/or dormancy, and others are able to alter their life cycle and/or development (Strachan et al., 2015; Verberk et al., 2008). In organisms with limited movement capacity, increased temperature and decreased moisture are expected to induce pronounced physiological stresses. Studying how these stresses affect both life history traits and physiological traits would allow us to anticipate the effect of global warming on organisms with limited movement capacity.

The common woodlice *Armadillidium vulgare* is a key soil decomposer naturally exposed to a wide range of environmental conditions (Souty-Grosset et al., 1988) that provides major ecosystem services (David and Handa, 2010), notably in agrosystems and grassland habitats where it is used as an ecological indicator. In the course of its evolutionary history, the common woodlice had to adapt to terrestrial life. Consequently, it is still very sensitive to moisture and temperature variations, which can induce water loss (Smigel and Gibbs, 2008) and have major consequences in terms of distribution, behaviour and survival (Hassall et al., 2018; Paris and Pitelka, 1962). Moreover, their movement capacity is low (i.e. several hundred meters at the best during the entire lifetime, Durand et al., 2019) to allow them to migrate so to avoid the stress imposed by the environment. Our knowledge and ability to measure woodlice life history traits and the availability of molecular and cellular biomarkers of individual quality (Depeux et al., 2020a) make this species a highly relevant experimental model to study the influence of both temperature and moisture on life history and physiological traits.

In this study, we tested independently the effects of increased temperature (experiment 1) and of decreased moisture (experiment 2) on a selected set of key life history (i.e. growth, reproductive success, and survival) and physiological (i.e. immune cell parameters (cell viability, cell density and cell size) and β-galactosidase activity) traits in woodlice. For the

experiment 1 (testing the effect of increased temperature), we compared individuals maintained at 20°C and 80% of moisture (i.e. the standard temperature and moisture laboratory conditions) to individuals exposed at 28°C (simulating a temperature increase of 8°C) still at 80% of moisture. For the experiment 2 (testing the effect of decreased moisture), we compared animals in standard conditions to individuals exposed at 50% of moisture (simulating a moisture loss of 30%) still at 20°C. We predicted that a rise in temperature and a loss in moisture should be stressful and should induce changes in life history and physiological traits.

MATERIALS & METHODS

Biological Material – Routine Breeding

All specimens of *A. vulgare* used in this study descend from individuals sampled in Denmark (Helsingör) in 1982. Since then, animals were reared under laboratory conditions under the natural photoperiod of Poitiers (France) (46°35'N; 0°20'E), at 20°C and about 80-85% of moisture, in plastic boxes (length × width × height: 26.5 × 13.5 × 7.5 cm) containing humid loam, and fed ad libitum with carrot slices and dried linden leaves. Controlled breeding, for the maintenance of the lineage over years, is performed in individual boxes (diameter x height: 9,8cm x 4,9cm), with reproductive pairs selected from their pedigree to avoid inbreeding. One month after mating, the offspring exit the female *marsupium* (i.e. female ventral pouch on which the eggs develop) (Suzuki and Ziegler, 2005). We transferred these offspring a few days after birth into a bigger box (length × width × height: 26.5 × 13.5 × 7.5 cm) with loam and food. After 3 months, once sexual characters have appeared but before sexual maturity, we placed young males and females in separate boxes (length × width × height: 26.5 × 13.5 × 7.5 cm) in laboratory conditions described above, enabling us to obtain virgin adults. For the maintenance of the lineage, about 40 crosses have been performed following this protocol each year. Each of the 40 broods provides at least one breeder for the next generation. The animals used in the experiments of this study came from this controlled lineage.

Experimental Design

Experiment 1: effect of increased temperature on life history and physiological traits

The experiment 1 performed in January 2019 involved the comparison between two groups of animals aged of 7 months old maintained in different temperature conditions in two

108 climatic chambers (Memmert HPP 256L with LED Light module cold white 6500K for HPP260
109 (15%) and Interior IP68 socket (for temperature restriction)) during two months after classical
110 conditions of maintenance:

111 (i) The "Control Temperature" group (CT) of animals maintained in standard
112 conditions (i.e. at 20°C and 80% of moisture) in one of our two climatic chambers.
113 (ii) The "High Temperature" (HT) group of animals exposed at 28°C (simulating
114 increased temperature by 8°C) and at 80% of moisture in the second climatic
115 chamber.

116 Eight degrees (i.e. difference in temperature between the two groups) corresponds to a
117 temperature increase close to daily variations observed in Poitiers during some summers,
118 which could chronically induce stress. Moreover, we had observed the stressful effect of this
119 temperature increase in a preliminary experiment in which we did not control the moisture
120 variation (Depeux et al., 2019).

121 In each group, animals were kept with fed *ad libitum* in 3 boxes (length × width × height: 26.5
122 × 13.5 × 7.5 cm; classical laboratory density conditions) of 30 females and 3 boxes of 30 males
123 from 15 different clutches (i.e. all compared conditions contained animals with the same
124 overall genetic background (from 15 same clutches) to be comparable). For each condition,
125 one box was used to monitor survival and growth (mass gain over time) of animals from the
126 beginning to the end of the experiment, another was used to evaluate reproductive success
127 and the last box served to quantify physiological traits (i.e. immune cell parameters (cell
128 viability, cell density and cell size) and β-galactosidase activity) (see below).

129 **Experiment 2: effect of a loss of moisture on life history and physiological traits**

130 The experiment 2 performed in January 2021 involved the comparison between two groups
131 of animals also aged of 7 months old maintained in different conditions in our two climatic
132 chambers during two months after standard conditions of maintenance:

133 (i) The "Control Moisture" (CM) group of animals maintained in standard conditions
134 (i.e. at 20°C and 80% of moisture) in one of our two climatic chambers
135 (ii) The "Loss of Moisture" (LM) group of animals exposed at 50% of moisture
136 (simulating a moisture loss of 30%) and at 20°C in the second climatic chamber.

137 Similar to the experiment 1, in each group, animals were fed *ad libitum* in 3 boxes of 30
138 females and 3 boxes (length × width × height: 26.5 × 13.5 × 7.5 cm; standard laboratory density
139 conditions) of 30 males from 15 different clutches (i.e. all boxes to compare contained animals
140 with the same overall genetic background (from 15 same clutches)). For each condition, one
141 box was used to monitor survival and growth of animals from the beginning to the end of the
142 experiment, another was used to evaluate reproductive success and the last box served to
143 quantify physiological traits (see below).

144 In our two experiments, we aimed to compare individuals of the same age because age
145 negatively impacts both reproductive success (Depeux et al., 2020b) and physiological traits
146 (Depeux et al., 2020a). Having initially only two climatic chambers, we had to perform our
147 experiments 1 and 2 in different years (i.e. experiment 1 in 2019 and experiment 2 in 2021).
148 Thus, we systematically compared the effect of each stress against its own control condition
149 group (i.e. CT for HT and CM for LM). Moreover, we selected, at the beginning of each
150 experiment (1 and 2), individuals of the same size and we checked, at the end of the
151 experiments, potential statistical differences between the two control groups (CT and CM) on
152 measures of life history and physiological traits (Supp. File1). Although β-galactosidase activity
153 and cell density were higher in the CT group than in the CM group (Supp. File1), we observed
154 the same dynamics in these measures in the face of their stressful condition (HT and LM,
155 respectively) (see Results part). The body mass at day 14 was higher in the CM group than in
156 the CT group (Supp. File1). Whatever the differences observed between the two control
157 groups (CT group used in 2019 and CM group used in 2021), we compared the effect of each
158 stress (HT in 2019 and LM in 2021, respectively) against its own control group (CT group in
159 2019 and CM group in 2021, respectively) for testing the effect of each stress.

160 ***Life history traits***

161 **Survival and growth**

162 One box of males and one box of females from each group (i.e. for the groups CT, HT, CM, LM)
163 were used to monitor and compare changes of survival and body mass over time. All
164 individuals in these boxes were monitored for 124 days (i.e. about 4 months). We sampled
165 individuals at 14, 28, 42, 69 and 124 days (i.e. 5 sampling points per box) and assessed
166 survivorship and change in body mass (in grams) of all surviving animals in each box (body

167 mass was measured with a precision balance 650g|1mg Sartorius™ BCE653-1S Entris™ II
168 Essential). Then, we compared these traits over time and between groups (CT vs. HT groups
169 and CM vs. LM groups) to test independently the effect of temperature and moisture changes
170 on these traits (see section on Statistical analyses). Due to their regular moults, individual
171 identification among the 30 animals sharing in given box cannot be performed, leading our
172 measures to be average survival and growth instead of individual trajectories.

173 **Reproductive success**

174 At the end of the exposure to different conditions, one box of males and one box of females
175 were collected from each group (i.e. for the groups CT, HT, CM, and LM). We formed 20
176 breeding pairs composed of one male and one female per group. Each breeding pair was
177 placed in a box, at 20°C, with food provided *ad libitum* and in a photoperiod of 16:8 (L/D)
178 stimulating the reproduction (Mocquard et al., 1989). We followed all these pairs for 5 months
179 during which each clutch produced was recorded. At the end of this period, the ability to
180 produce a clutch (i.e. the probability that one clutch or more is produced by a given pair) for
181 the 80 pairs (i.e. 40 pairs for experiment 1 and 40 pairs for experiment 2) was compared
182 independently between CT and HT groups and between CM and LM groups to test the effect
183 of temperature and moisture changes on breeding success. As we created groups from similar
184 clutches to have the same overall genetic background among boxes, we cannot exclude that
185 some crosses were composed of related individuals although we expect this event to be rare.
186 However, the probability of forming sibling pairs (8%) was similar among groups that were
187 exposed either at 20°C vs. 28°C or at 80% vs. 50% of moisture.

188 ***Physiological traits***

189 At the end of the experimental treatments (i.e. after two months in our experimental
190 conditions), one box of males and one box of females were taken from each group (i.e. for the
191 groups CT, HT, CM, and LM) for measuring the level of our set of physiological traits (i.e.
192 immune cells parameters and β-galactosidase activity) developed in Depeux et al. (2020a).
193 These physiological traits were firstly described as senescence biomarkers because they allow
194 predicting the amount of cellular senescence in different organisms and are strongly age-
195 dependent in *A. vulgare* (i.e. older the individual, higher the decline of these biomarkers,
196 Depeux et al. 2020a). We performed these measures on each remaining animals (Table 1) and

197 we compared these metrics independently between CT and HT groups for experiment 1 and
198 between CM and LM groups for experiment 2 (see section Statistical analyses).

199 Table 1. Numbers of individuals on which we measured quality biomarkers.

Groups	CT (Control temperature)	HT (High temperature)	CM (Control Moisture)	LM (Loss of Moisture)
Numbers of females	13	12	9	15
Numbers of males	17	17	15	15

200 **Immune cells**

201 As immune cells are free-circulating, they can inform about a potential premature biological
202 aging. When an individual *A. vulgare* ages, its immune cells decrease in density and viability
203 while increasing in size (Depeux et al., 2020a). To measure these parameters, we collected 3µL
204 of haemolymph per individual and placed it in 15µL of MAS-EDTA (EDTA 9 mM, Trisodium
205 citrate 27 mM, NaCl 336 mM, Glucose 115 mM, pH 7, (Rodriguez et al., 1995)). We then added
206 6µL of Trypan Blue at 0.4% (Invitrogen) to discriminate live and dead cells. After, 10µL of this
207 solution was put in Invitrogen Coutness® counting slide and put in an automated Cell Counter
208 (Invitrogen) to quantify cell density (measured as the number of cells per mL of haemolymph),
209 viability (measured as the proportion of live cells) as well as cell size (in µm). These three
210 parameters of the immune cells are physiological traits that were found to be reliable
211 biomarkers of cellular senescence in *A. vulgare* (Depeux et al., 2020a).

212 **β-galactosidase activity**

213 The β-galactosidase activity is a physiological trait commonly used as a marker of cellular
214 senescence (Lee et al., 2006). Its indirect activity in regards to the process of cellular
215 senescence increases with age in *A. vulgare* (Depeux et al., 2020a). To measure this enzymatic
216 activity, we dissected and removed the nerve cord of each individual after having collected
217 haemolymph for assessing the immune parameters. We put individual nerve cords in 300µL
218 of Lyse Buffer 1X (CHAPS 5 mM, Citric acid 40 mM, Sodium Phosphate 40 mM, Benzamidine
219 0.5 mM, PMSF 0.25 mM, pH = 6) (Gary and Kindell, 2005). We centrifuged the sample at 15
220 000g for 30 minutes at 4°C and then we collected and kept the supernatant at -80°C. We
221 quantified the protein concentration thanks to the BCA Assay and we homogenized all
222 samples at the 0.1mg/mL protein concentration. Then, 100µL of these protein extracts were

223 added to 100µL of reactive 4-methylumbelliferyl-D-galactopyranoside (MUG) solution. The
224 synthesis of the fluorescent 4-methylumbelliferone (4-MU), the result of the contact of MUG
225 reactive with β-galactosidase, was measured using the multimode microplate reader Mithras
226 LB940 133 HTS III, Berthold; excitation filter: 120 nm, emission filter 460 nm, for 120 minutes.
227 We included two technical replicates for each sample to obtain the measures.

228 ***Statistical analyses***

229 The effects of the stress condition (control vs. high temperature, or control vs. low moisture)
230 on life history and physiological traits were tested using the following models. *(i)* Life history
231 traits. For the survival data, Cox proportional hazard models were fitted with stress condition,
232 sex and their interaction term as fixed variables, using 'survival' package (Therneau, 2022). For
233 the growth data, the body mass was modelled with linear model with Gaussian distribution
234 with stress condition, sex, time (in days) and their two-by-two interaction term as fixed
235 variables. The female reproductive success was modelled as binary data (*presence of at least*
236 *one clutch or absence of clutch)* using linear regression with a binomial distribution, with stress
237 condition as fixed variables. *(ii)* Physiological traits. The cell density, cell size, cell viability and
238 the β-galactosidase activity were modelled with a linear model with Gaussian distribution with
239 stress condition, sex and their interaction term as fixed variables.

240 We proceeded to model selection starting with full (saturated) model. We ranked all nested
241 models according to their AICc using 'MuMIn' package (Barton, 2022). We selected the most
242 parsimonious models among the top ranked models (ΔAICc < 2) (Galipaud et al., 2017). The
243 tables summarizing the model selection procedure was presented in Supp. File2. To represent
244 the effect of the two environmental stresses in each variable, we presented our results with
245 indices of size effect. The effect of each stress on each measure of life history traits and
246 individual quality biomarkers were measured using the standardized slopes (Schielzeth et al
247 2010) and their SE calculated by rescaling the variable of the selected model. For survival data,
248 the effect size was the hazard ratio, calculated as the exponential of the regression parameter
249 (Collett 2003). When the selected model did not include the effect of the stress, we took the
250 model with the variable stress as fixed factor to obtain a size effect as made in Depeux et al.
251 2020a. All statistical analyses and representations of results were performed using the
252 software R 4.2.1 (R core Team 2022).

All datasets and source code are available as electronic supplementary materials on public repository: https://gitlab.com/fxdm/armadillidium_stress

RESULTS

Life history traits

The survival was not impacted by an increased temperature (χ^2_1=2.16, P=0.14, Fig.1A, Supp. File2 Table S2a, Supp. File3-1.A.1) although mortality risk was almost twice as lower at low compared to high temperature. The hazard ratio was 1.78, with a 95%CI including 1 [0.81; 3.88]. By contrast, individuals exposed to a loss of moisture had a 2.5 times higher mortality risk (χ^2_1=4.54, P=0.03, Fig. 1B, Supp. File2 Table S2b, Supp. File3-1.A.2). The hazard ratio was 2.69, with a 95%CI excluding 1 [1.03; 7.01]. As a result, 90% of individuals placed at control moisture were still alive at the end of the follow-up, whereas only 75% of individuals placed at 50% of moisture survived at the end of the experiment.

In both temperature and moisture stresses, body mass increased during the entire experiment duration (Fig. 1C and 1D, Supp. File3-1.B.1 and 1.B.2), as expected in an indeterminate grower as *A. vulgare*, but there was no detectable interaction between day and sex (Supp. File2 Table S2c and Table S2d). For the temperature experiment, interactions between sex and stress ($F_{1,528}$=6.90, P=0.0088, Supp. File3), and between day and stress ($F_{1,528}$=14.6, P=0.00015, Supp. File2 Table S2c) showed up, illustrating the impact of an increasing temperature on growth. By contrast, in the moisture stress experiment, the body mass was not affected by the sex ($F_{1,522}$=0.35, P=0.55), the stress condition ($F_{1,522}$=0.31, P=0.58), or any interaction between the variables (all P > 0.10).

The reproductive success markedly decreased in both experiments for the stressful condition: a fourfold increase of reproductive failure with temperature stress (χ^2_1=5.02, p=0.025, Odd-ratio=0.23, 95%CI=[0.057;0.83], Fig. 1E, Supp. File2 Table S2e, Supp. File3-1.C.1), and a fivefold increase of reproductive failure with moisture stress (χ^2_1=5.38, p=0.02, Odd-ratio=0.20, 95%CI=[0.045;0.79], Fig. 1F, Supp. File2 Table S2f, Supp. File 3-1.C.2). In both cases, it corresponds to halving the reproductive success in the stress groups (moisture stress: 55% in the control group vs. 20% in the stressed group; temperature stress: 65% in the control group vs. 30% in the stressed group).

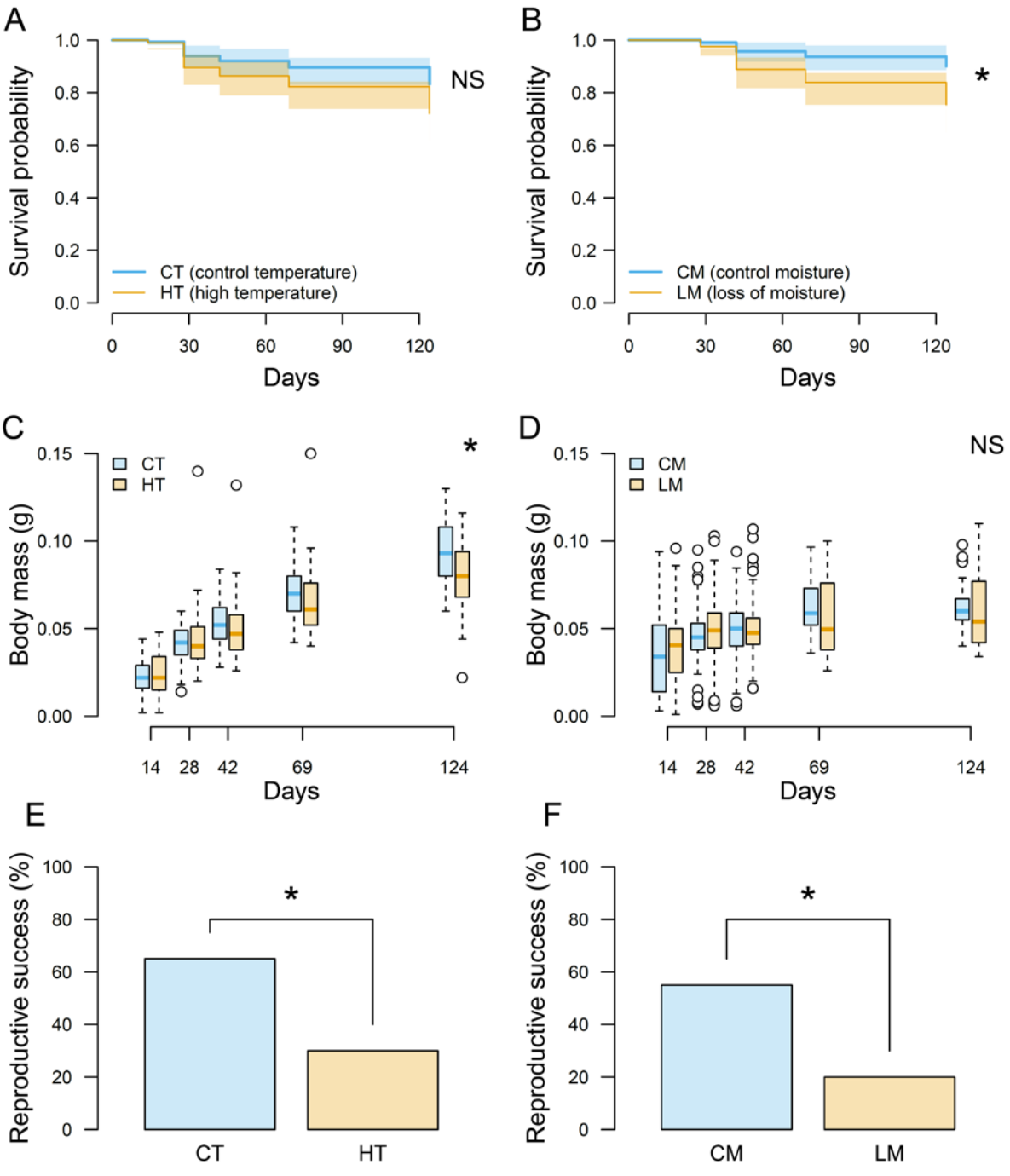

Figure 1. Effect of the two environmental stresses (Temperature (A, C and E) and Moisture (B, D and F) on Survival (A and B), Body mass (C and D) and Reproductive success (E and F). *Blue colour: control groups, orange colour: stress groups. CT: Control Temperature (20°C), HT: High Temperature (28°C), CM: Control Moisture (80%), LM: Loss of Moisture (50%). NS: No significant; * p<0.05.*

Physiological traits

Immune cells

290 Immune cell viability was not affected by increasing temperature ($F_{1,56}$=0.92, p=0.34,
291 standardized slope β=-0.25; 95%CI=[-0.79;0.27], Fig. 2A, Supp. File2 Table S2g and Supp. File3-
292 2.A.1) but decreased during a loss of moisture ($F_{1,50}$=4.17, p=0.046, standardized slope β=0.55;
293 95%CI=[0.01;1.09], Fig. 2B, Supp. File2 Table S2h and Supp. File3-2.A.2).

294 Immune cell size decreased during the temperature stress ($F_{1,55}$=5.72, p=0.02, standardized
295 slope β=-0.60; 95%CI=[-0.1;-1.1], Fig. 2C, Supp. File2 Table S2i and Supp. File 3-2.B.1) but not
296 under the moisture stress ($F_{1,50}$=3.79, p=0.057, standardized slope β=-0.55; 95%CI=[-
297 1.07;0.02], Fig. 2D, Supp. File2 Table S2j and Supp. File3-2.B.2).

298 Immune cell density decreased during the temperature stress ($F_{1,56}$=38.2, P<0.001,
299 standardized slope β=-1.26; 95%CI=[-1.67;-0.85], Fig. 2E, Supp. File2 Table S2k and Supp. File3-
300 2.C.1)) and the moisture stress ($F_{1,50}$=7.64, p=0.008, standardized slope β=0.72;
301 95%CI=[0.19;1.25], Fig. 2F, Supp. File2 Table S2l and Supp. File3-2.C.2).

302 *β-galactosidase activity*

303 The *β*-galactosidase activity increased when the temperature increased ($F_{1,54}$=11.32,
304 P=0.0014, standardized slope β=0.82; 95%CI=[0.33;1.32], Fig.2G, Supp. File2 Table S2m, and
305 Supp. File3-2.D.1), but also when the moisture decreased ($F_{1,50}$=10.50, P=0.002, standardized
306 slope β=-0.83; 95%CI=[-1.31;-0.32], Fig. 2H, Supp. File2 Table S2n and Supp. File3-2.D.2).

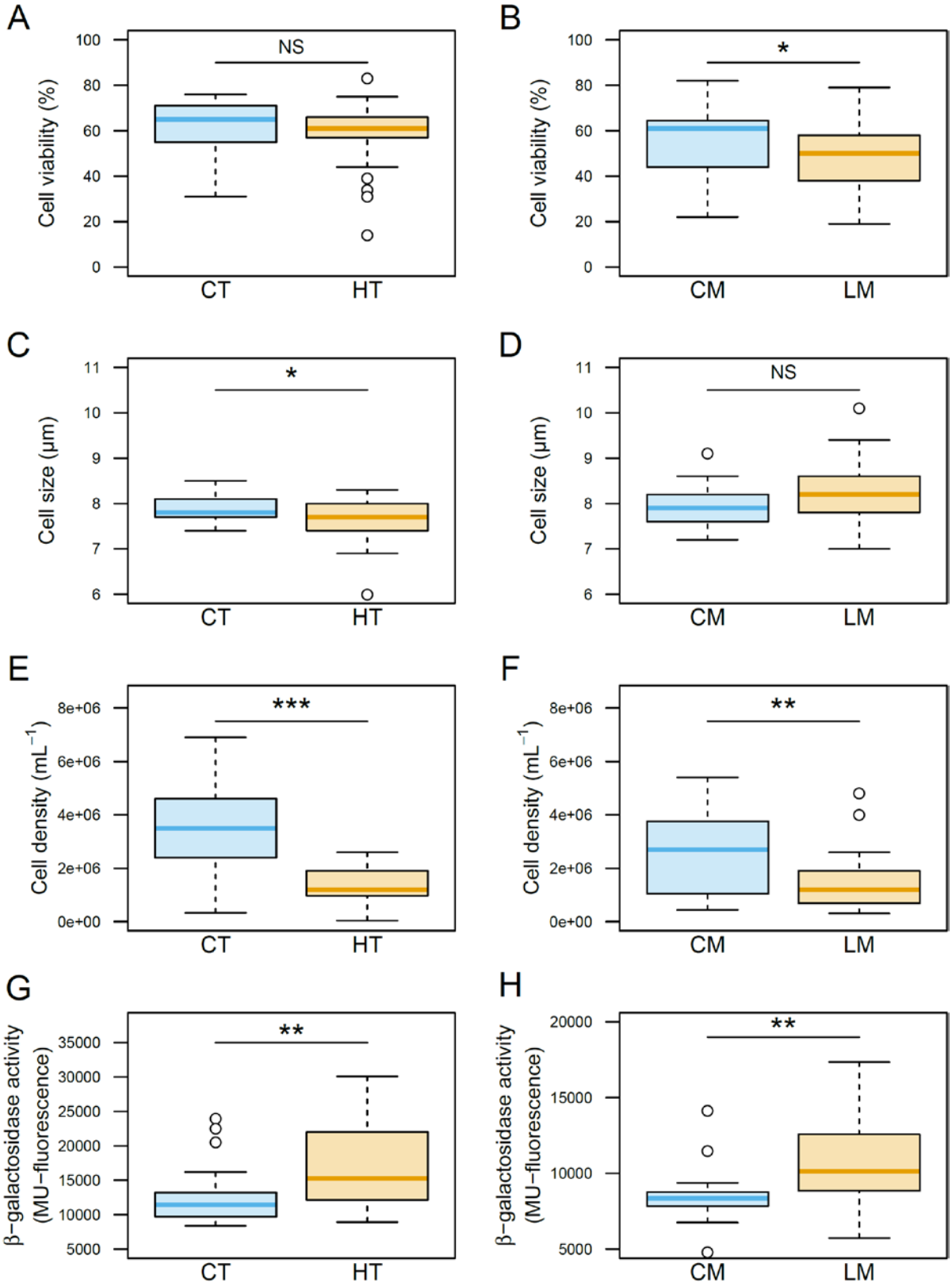

Figure 2. Effect of the two environmental stresses (Temperature (A, C, E, and G) and Moisture (B, D, F and H) on immune cell viability (A and B), immune cell size (C and D), immune cell density (E and F) and ß-galactosidase activity (G and H). *Blue colour: control groups, orange colour: stress groups. CT: Control Temperature (20°C), HT: High Temperature (28°C), CM: Control Moisture (80%), LM: Loss of Moisture (50%). NS: No significant; * p<0.05, ** p<0.01, *** p<0.001.*

313 **DISCUSSION**

314 Our results highlight that life history traits were negatively impacted by the two environmental
315 stresses (temperature and moisture) considered in this study. Moreover, the detrimental
316 effects of these stresses on our set of individual quality biomarkers are consistent with an
317 overall premature ageing of stressed animals compared to unstressed ones. To briefly
318 summarize, an increase in temperature negatively impacted the body mass trajectory over
319 time and the reproductive success of individuals. A decrease in moisture resulted in a decrease
320 of both survival and reproductive success. Concerning our physiological traits: (1) the density
321 of immune cells decreased under both stresses, (2) immune cell size decreased under
322 temperature stress, but was not impacted under moisture stress, (3) the viability of the cells
323 decreased when the moisture decreased (but not with temperature increase) and (4) finally,
324 the β-galactosidase activity increased for the two stressed groups. In this context, our results
325 globally support marked negative effect of higher temperature and lower moisture on
326 woodlice performance, with some minor differences between the two kinds of stresses in
327 their effects on the different measured life history and physiological traits.

328 About the life history traits, if the increase of temperature had no detectable effect on survival
329 in *A. vulgare*, contrary to what was previously found in arthropods studied so far such as
330 *Antestiopsis thunbergii*, *Calliphora stygia* and *Margaritifera margaritifera* (Azrag et al., 2017;
331 Hassall et al., 2017; Kelly et al., 2013), this stress led to a slowdown in woodlice growth, in line
332 to what has been reported in three other isopods (Angilletta et al., 2004). In parallel, we
333 observed that the increase in temperature led to a decrease in reproductive success, as
334 previously observed in females of *Antestiopsis thunbergii* (Azrag et al., 2017). In *A. vulgare*,
335 the animal size is positively correlated with fecundity (Durand et al., 2018; Lawlor, 1976),
336 meaning that the slowdown in growth could explain, at least partly, the decrease in
337 reproductive success for stressed animals compared to non-stressed ones. Concerning the
338 moisture stress, if the loss of moisture had no detectable effect on woodlouse growth, it
339 caused a decrease in both survival and reproductive success. These findings suggest a high
340 cost of drought on individual fitness in *A. vulgare*.

341 About the physiological traits, the results of the temperature stress experiment showed that
342 although cell density was negatively impacted by increased temperature, cell viability was not
343 affected. Moreover, contrary to what was expected when individuals are senescent, cell size

344 decreased instead of increasing. This last result supports our previous finding that cells
345 decreases in small when the temperature raises (without controlling moisture level, Depeux
346 et al., 2019). That little cells came from a more important cell renewal in stressed animals
347 might explain this pattern. On the other hand, the increase of β-galactosidase activity seems
348 to indicate premature ageing (and thus a decrease in quality) in individuals exposed to
349 increased temperature. Concerning the moisture stress, the individual quality biomarkers
350 indicated a decrease in cell density and viability, associated with an increase in β-galactosidase
351 activity, which and suggest an acceleration of biological ageing in the individuals exposed to a
352 decrease in moisture (Depeux et al., 2020a).

353 We observed global negative effect of temperature increase in *A. vulgare* in our study, but our
354 results seemed to show an even higher and clearer effect of loss of moisture on both life
355 history traits and individual quality biomarkers. Although the woodlouse has become
356 terrestrial for a long time, they are still dependent on and require a substantial water supply
357 (Smigel and Gibbs, 2008). Thus, behaviours like aggregation allowing them to resist to
358 desiccation have been set up and allow them to maintain the rate of moisture required for
359 their survival (Broly et al., 2013; Smigel and Gibbs, 2008). This can explain the strong effect of
360 loss of moisture in our study. In natural conditions, increase in temperature and loss of
361 moisture generally positively covary, leading to even higher negative consequences on the
362 woodlouse performance. Further work will be required to test the influence of more extreme
363 and maybe more realistic conditions by simultaneously increasing temperature by 8°C
364 decreasing moisture by 30% on our measured life history and physiological traits. A study in
365 the wild comparing life history and physiological traits on *A. vulgare* collected across areas
366 with different temperature and drought gradient would also allow a better assessment of the
367 combined effects of these stresses.

368 Thanks to our experimental design that allowed us to test independently the stresses that the
369 organisms are likely to face, we showed that thermal and water stress do not have the same
370 impact on organisms. Although simulations based on mathematical models have predicted
371 that both temperature and drought changes overall affect arthropods, experimental
372 approaches (such as this work) are required to quantify reliably the effect of temperature
373 changes (Johnson et al. 2010). Drought can have serious physiological consequences on
374 invertebrates, such as protein denaturation and undesirable macromolecular interactions

375 (Sano et al., 1999; Tang and Pikal, 2005), or oxidative damage (Lopez-Martinez et al., 2008),
376 which are known to be involved in the process of cellular senescence (Gilca et al., 2007) and
377 thus in decrease in performance of stressed organisms. Due to the role of arthropods in
378 services to many ecosystems (e.g. biochemical balance of ecosystems, agriculture, pest
379 management…), and in the context of global warming, it is crucial to understand the effects
380 of temperature and moisture changes on these organisms (Santos et al., 2021). As
381 temperature increase is not the only environmental change expected to take place in the
382 coming years, it is of paramount importance to assess the impact of other stresses too.
383 Although many predictive models have been proposed so far, getting more accurate
384 information on the expected responses of organisms facing with different kinds of stress
385 would provide the required information to test these model predictions.

386 To conclude, *A. vulgare* is an important actor that delivers ecosystem services in many
387 ecosystems because it actively impacts soil fertility (Souty-Grosset and Faberi, 2018) and it is
388 also used as an ecological indicator in grassland habitat (Paoletti and Hassall, 1999; Souty-
389 Grosset et al., 2005). This detritivorous species promotes decomposition processes and
390 nutrient cycling on which agricultural productivity and sustainability depend (Bredon et al.,
391 2018; Paoletti and Hassall, 1999), and plays thereby a key role in ecosystem services (David
392 and Handa, 2010). Extending knowledge in the response of soil biodiversity facing current
393 global changes could promote sustainability by helping to the development of new tools and
394 strategies for more efficient management of soils and associated crops, through more
395 effective and targeted recolonisation and/or restoration of soil biodiversity. Also, to better
396 understand what the future of the animal communities in the current context of global
397 warming will be, it seems necessary to multiply the studies on models presenting particular
398 ecological requirements, like the woodlouse.

399 **Supplementary files**

400 Supp. File1 Comparison of the two control groups
401 Supp. File2 Model selection
402 Supp. File3 Graphical representations of results per sex
403

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