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 the density of immune cells decreased and the β-galactosidase activity increased with increasing temperature and decreasing moisture, which suggests a negative impact of these stressors on individual performance, 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 woodlouse welfare. Changing temperature or moisture had slightly different effects, illustrating the need to test 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

Conflict of interest disclosure

The authors declare they have no conflict of interest relating to the content of this article.

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 (Johnson and Jones, 2016). In Lepidoptera, for example, too high temperatures prevent hatching (Khadioli et al., 2014). In both Lepidoptera and Hymenoptera, increasing temperature beyond the optimum can have detrimental effect on survival (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 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 and physiological traits would allow us to anticipate the effect of global warming on organisms with limited movement capacity.

The common woodlouse *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 woodlouse 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, behavior and survival (Hassall et al., 2018; Paris and Pitelka, 1962). Moreover, their movement capacity is low (i.e. several hundred meters during the entire lifetime at the best, Durand et al., 2019) to allow them to migrate so to avoid the stress imposed by the environment. Our knowledge and ability to measure woodlouse 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 woodlouse. In experiment 1 (i.e. 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. In experiment 2 (i.e. 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 hypothesized 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

97 the maintenance of the lineage over years, is performed in individual boxes (diameter x height:
98 9,8cm x 4,9cm), with reproductive pairs selected from their pedigree to avoid inbreeding. One
99 month after mating, offspring exit the female *marsupium* (i.e. female ventral pouch on which
100 the eggs develop) (Suzuki and Ziegler, 2005). We transferred these offspring a few days after
101 birth into a bigger box (length × width × height: 26.5 × 13.5 × 7.5 cm) with loam and food. After
102 3 months, once sexual characters have appeared but before sexual maturity, we placed young
103 males and females in separate boxes (length × width × height: 26.5 × 13.5 × 7.5 cm) in
104 laboratory conditions described above, enabling us to obtain virgin adults. For the
105 maintenance of the lineage, about 40 crosses have been performed following this protocol
106 each year. Each of the 40 broods provides at least one breeder for the next generation. The
107 animals used in the experiments of this study came from this controlled lineage.

108 ***Experimental Design***

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

110 The experiment 1 performed in January 2019 involved the comparison between two groups
111 of animals aged of 7 months old maintained at different temperatures in two climatic
112 chambers (Memmert HPP 256L with LED Light module cold white 6500K for HPP260 (15%) and
113 Interior IP68 socket (for temperature restriction)) during two months after standard
114 conditions of maintenance:

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

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

125 In each group, animals were fed *ad libitum* in 3 boxes (length × width × height: 26.5 × 13.5 ×
126 7.5 cm; standard laboratory density conditions) of 30 females and 3 boxes of 30 males from
127 15 different clutches (i.e. all treatments included animals with the same genetic background
128 (i.e. issued from 15 same clutches) to be comparable). For each condition, one box was used
129 to monitor survival and growth (mass gain over time) of animals from the beginning to the
130 end of the experiment, another was used to evaluate reproductive success and the last box
131 served to quantify physiological traits (i.e. immune cell parameters: cell viability, cell density,
132 and cell size) and β-galactosidase activity, see below). In this last box, the animals had to be
133 sacrificed (see 'Ethical statement' section below) because of the protein extraction on nerve
134 chains that was required to measure the β-galactosidase activity.

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

136 The experiment 2 performed in January 2021 involved the comparison between two groups
137 of 7 months old animals maintained under different conditions in our two climatic chambers
138 during two months after standard conditions of maintenance:

139 (i) The "Control Moisture" (CM) group of animals maintained in standard conditions
140 (i.e. at 20°C and 80% of moisture) in one of our two climatic chambers
141 (ii) The "Loss of Moisture" (LM) group of animals exposed at 50% of moisture
142 (simulating a moisture loss of 30% (i.e. water stress condition)) and at 20°C in the
143 second climatic chamber.

144 Similar to the experiment 1, in each group, animals were fed *ad libitum* in 3 boxes of 30
145 females and 3 boxes (length × width × height: 26.5 × 13.5 × 7.5 cm; standard laboratory density
146 conditions) of 30 males from 15 different clutches (i.e. all boxes to compare included animals
147 with the same genetic background (i.e. issued from 15 same clutches)). For each condition,
148 one box was used to monitor individual survival and growth from the beginning to the end of
149 the experiment, another was used to evaluate reproductive success and the last box served
150 to quantify physiological traits (see below).

151 In our two experiments, we aimed to compare individuals of the same age because age
152 negatively impacts both reproductive success (Depeux et al., 2020b) and physiological traits
153 (Depeux et al., 2020a). Having initially only two climatic chambers, we had to perform our
154 experiments 1 and 2 in different years (i.e. experiment 1 in 2019 and experiment 2 in 2021).

155 Thus, we systematically compared the effect of each stress against its own control condition
156 group (i.e. CT for HT and CM for LM). Moreover, at the beginning of each experiment (1 and
157 2), we selected individuals of the same size and we checked, at the end of the experiments,
158 potential statistical differences between the two control groups (CT and CM) on measures of
159 life history and physiological traits (Supp. File1).

160 **Ethical statement**

161 The Decree n°2003-768 from 01/08/2003 and the European Directive 2010/63/EU regulating
162 animal research does not require any ethical evaluation prior to research on arthropods.
163 However, we complied with the ethical 3R rules (Replace/Reduce/Refine). Even though it was
164 impossible to replace the use of animals in our study, we reduced the number of used animals,
165 optimizing this number to a minimum to ensure a reliable assessment of the effect of the
166 different stressors on life history and physiological traits. Although individuals were obviously
167 stressed during the experiments, we made sure that they were provided with optimal living
168 conditions throughout the experiments. In addition, when the tissue sampling required the
169 death of individuals (i.e. to measure physiological traits such as β-galactosidase activity), the
170 animals to be euthanized were frozen before protein extractions to take into account animal
171 welfare as much as possible.

172 ***Life history traits***

173 **Survival and growth**

174 One box of males and one box of females from each group (i.e. for the groups CT, HT, CM, LM)
175 were used to monitor and compare changes of survival and body mass over time. All
176 individuals in these boxes were monitored for 124 days (i.e. about 4 months). We sampled
177 individuals at 14, 28, 42, 69, and 124 days (i.e. 5 sampling points per box) and assessed
178 survivorship and change in body mass (in grams) of all surviving animals in each box (body
179 mass was measured with a precision balance 650g|1mg Sartorius™ BCE653-1S Entris™ II
180 Essential). Then, we compared these traits over time and between groups (CT vs. HT groups
181 and CM vs. LM groups) to test independently the effect of temperature and moisture changes
182 on these traits (see section on Statistical analyses). Due to regular moults, individual
183 identification among the 30 animals sharing in given box cannot be performed, leading our
184 measures to be average survival and growth instead of individual trajectories.

185 **Reproductive success**

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

200 ***Physiological traits***

201 At the end of the experimental treatments (i.e. after two months in our experimental
202 conditions), one box of males and one box of females were taken from each group (i.e. for the
203 groups CT, HT, CM, and LM) for measuring the level of our set of physiological traits (i.e.
204 immune cells parameters and β-galactosidase activity) developed in Depeux et al. (2020a).
205 These physiological traits were firstly described as senescence biomarkers because they allow
206 predicting the amount of cellular senescence in different organisms and are strongly age-
207 dependent in *A. vulgare* (i.e. older the individual, higher the decline of these biomarkers,
208 Depeux et al. 2020a). We performed these measures on each remaining animals (Table 1) and
209 we compared these metrics independently between CT and HT groups for experiment 1 and
210 between CM and LM groups for experiment 2 (see section Statistical analyses).

211 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

212

213 **Immune cells**

214 As immune cells are free-circulating, they can inform about a potential premature biological
215 aging. When an individual *A. vulgare* ages, its immune cells decrease in density and viability
216 while increasing in size (Depeux et al., 2020a). To measure these parameters, we collected 3µL
217 of haemolymph per individual and placed it in 15µL of MAS-EDTA (EDTA 9 mM, Trisodium
218 citrate 27 mM, NaCl 336 mM, Glucose 115 mM, pH 7, (Rodriguez et al., 1995)). We then added
219 6µL of Trypan Blue at 0.4% (Invitrogen) to discriminate live and dead cells. After, 10µL of this
220 solution was put in Invitrogen Coutness® counting slide and put in an automated Cell Counter
221 (Invitrogen) to quantify cell density (measured as the number of cells per mL of haemolymph),
222 viability (measured as the proportion of live cells) as well as cell size (in µm). These three
223 parameters of the immune cells are physiological traits that were found to be reliable
224 biomarkers of cellular senescence in *A. vulgare* (Depeux et al., 2020a).

225 **β-galactosidase activity**

226 The β-galactosidase activity is a physiological trait commonly used as a marker of cellular
227 senescence (Lee et al., 2006). Its indirect activity in regards to the process of cellular
228 senescence increases with age in *A. vulgare* (Depeux et al., 2020a). To measure this enzymatic
229 activity, we dissected and removed the nerve cord of each individual after having collected
230 haemolymph for assessing the immune parameters. We put individual nerve cords in 300µL
231 of Lyse Buffer 1X (CHAPS 5 mM, Citric acid 40 mM, Sodium Phosphate 40 mM, Benzamidine
232 0.5 mM, PMSF 0.25 mM, pH = 6) (Gary and Kindell, 2005). We centrifuged the sample at 15
233 000g for 30 minutes at 4°C and then we collected and kept the supernatant at -80°C. We
234 quantified the protein concentration thanks to the BCA Assay and we homogenized all
235 samples at the 0.1mg/mL protein concentration. Then, 100µL of these protein extracts were
236 added to 100µL of reactive 4-methylumbelliferyl-D-galactopyranoside (MUG) solution. The
237 synthesis of the fluorescent 4-methylumbelliferone (4-MU), the result of the contact of MUG
238 reactive with β-galactosidase, was measured using the multimode microplate reader Mithras
239 LB940 133 HTS III, Berthold; excitation filter: 120 nm, emission filter 460 nm, for 120 minutes.
240 We included two technical replicates for each sample to obtain the measures.

241 ***Statistical analyses***

242 All statistical analyses were performed using the software R 4.2.1 (R core Team 2022).

243 The effects of the stress condition (control vs. high temperature, or control vs. low moisture)
244 on life history and physiological traits were tested using the following models. *(i)* Life history
245 traits. For the survival data, Cox proportional hazard models were fitted with stress condition,
246 sex and their interaction term as fixed variables, using the 'survival' package (Therneau, 2022).
247 For the growth data, the body mass was modelled using linear models with Gaussian
248 distribution with stress condition, sex, time (i.e. time after placing in climatic chamber, in days)
249 and their two-by-two interaction term as fixed variables. The female reproductive success was
250 modelled as binary data (*presence of at least one clutch or absence of clutch)* using linear
251 regression with a binomial distribution, with stress condition as fixed variables. *(ii)*
252 Physiological traits. The cell density, cell size, cell viability, and the β-galactosidase activity
253 were modelled using linear models with Gaussian distribution with stress condition, sex, and
254 their interaction term as fixed variables.

255 We proceeded to model selection starting with full (saturated) model. We ranked all nested
256 models according to their AICc using 'MuMIn' package (Barton, 2022). We selected the most
257 parsimonious models among the top ranked models (ΔAICc < 2) (Galipaud et al., 2017). The
258 tables summarizing the model selection procedure was presented in Supp. File2. To represent
259 the effect of the two environmental stresses in each variable, we presented our results with
260 indices of size effect. The effect of each stress on each measure of life history traits and
261 biomarkers of individual quality were measured using the standardized slopes (Schielzeth et
262 al 2010) and their SE calculated by rescaling the variable of the selected model. For survival
263 data, the effect size was the hazard ratio, calculated as the exponential of the regression
264 parameter (Collett 2003). When the selected model did not include the effect of the stress,
265 we took the model with the variable stress as fixed factor to obtain a size effect as done in
266 Depeux et al. 2020a.

267 ***Data, script and code availability***

268 All datasets and source code are available as electronic supplementary materials on public
269 repository: https://doi.org/10.5281/zenodo.7496837

RESULTS

As said previously, we checked, at the end of the experiments, potential statistical differences between the two control groups (CT and CM) on measures of life history and physiological traits (Supp. File1). Although β-galactosidase activity and cell density were higher in the CT group than in the CM group (Supp. File1), we observed the same dynamics in these measures in the face of their stressful condition (HT and LM, respectively) (see Results part). The body mass at day 14 was higher in the CM group than in the CT group (Supp. File1). Whatever the differences observed between the two control groups (CT group used in 2019 and CM group used in 2021), we compared the effect of each stress (HT in 2019 and LM in 2021, respectively) against its own control group (CT group in 2019 and CM group in 2021, respectively) for testing the effect of each stress.

Life history traits

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 water stress 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 in water stress condition survived at the end of the experiment.

In both thermal and water 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 thermal 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 experiment, the body mass was not affected by the sex ($F_{1,522}$=0.35, P=0.55), the water stress ($F_{1,522}$=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 in presence of thermal 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 in presence of water 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 (water stress: 55% in the control group vs. 20% in the stressed group; thermal stress: 65% in the control group vs. 30% in the stressed group).

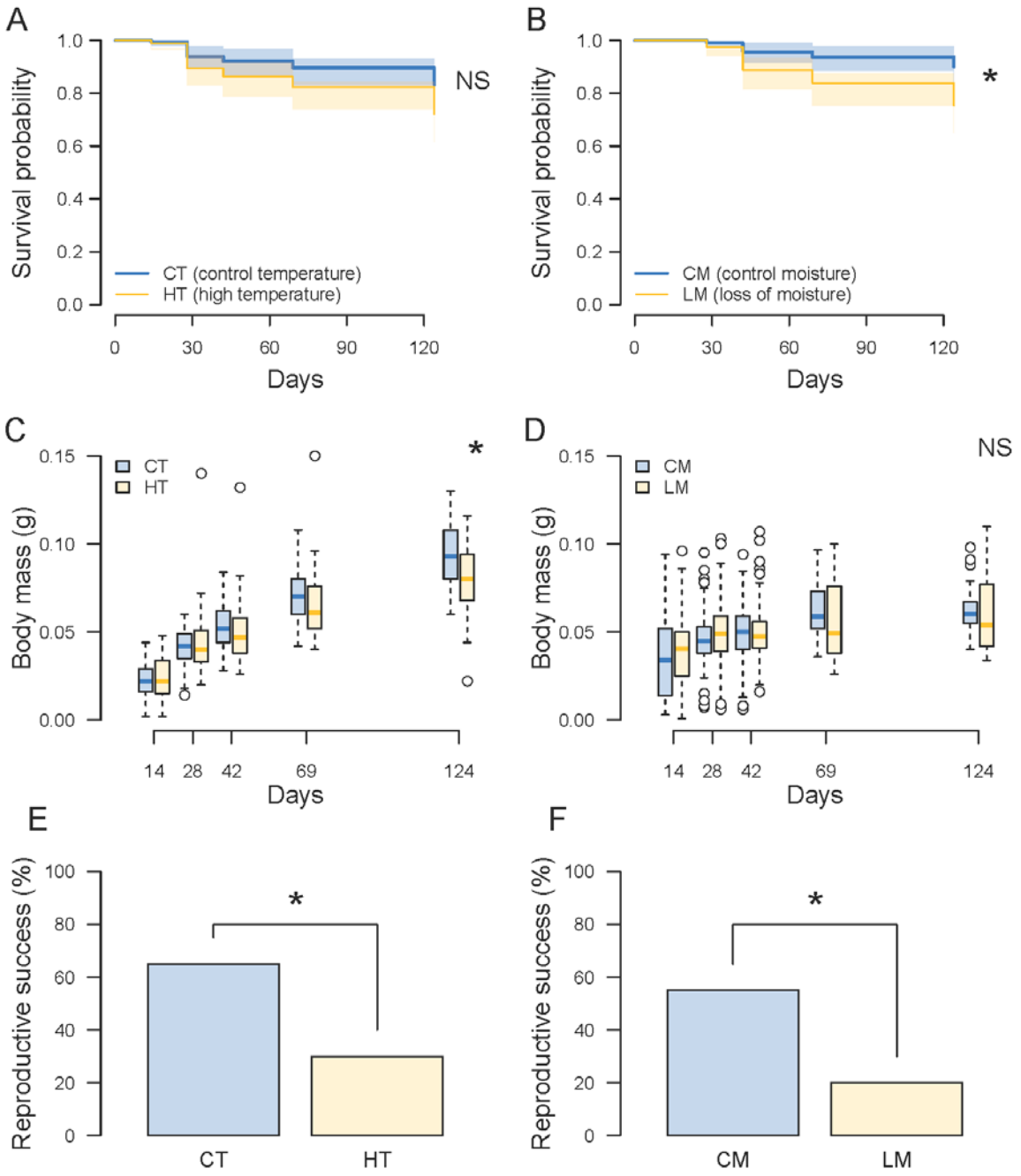

Figure 1. Effect of the two environmental stressors (Temperature (A, C and E) and Moisture (B, D and F) on Survival (A and B), Body mass (C and D) and Reproductive success (E and F). *Blue colour: control groups, orange colour: stress groups. CT: Control Temperature (20°C), HT: High Temperature (28°C),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

Physiological traits

Immune cells

Immune cell viability was not affected by the thermal stress ($F_{1,56}$=0.92, p=0.34, standardized slope β=-0.25; 95%CI=[-0.79;0.27], Fig. 2A, Supp. File2 Table S2g and Supp. File3-2.A.1) but

316 decreased during the water stress ($F_{1,50}$=4.17, p=0.046, standardized slope β=0.55;
317 95%CI=[0.01;1.09], Fig. 2B, Supp. File2 Table S2h and Supp. File3-2.A.2).

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

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

326 *β-galactosidase activity*

327 The *β*-galactosidase activity increased with the thermal stress ($F_{1,54}$=11.32, P=0.0014,
328 standardized slope β=0.82; 95%CI=[0.33;1.32], Fig.2G, Supp. File2 Table S2m, and Supp. File3-
329 2.D.1), but also with the water stress ($F_{1,50}$=10.50, P=0.002, standardized slope β=-0.83;
330 95%CI=[-1.31;-0.32], Fig. 2H, Supp. File2 Table S2n and Supp. File3-2.D.2).

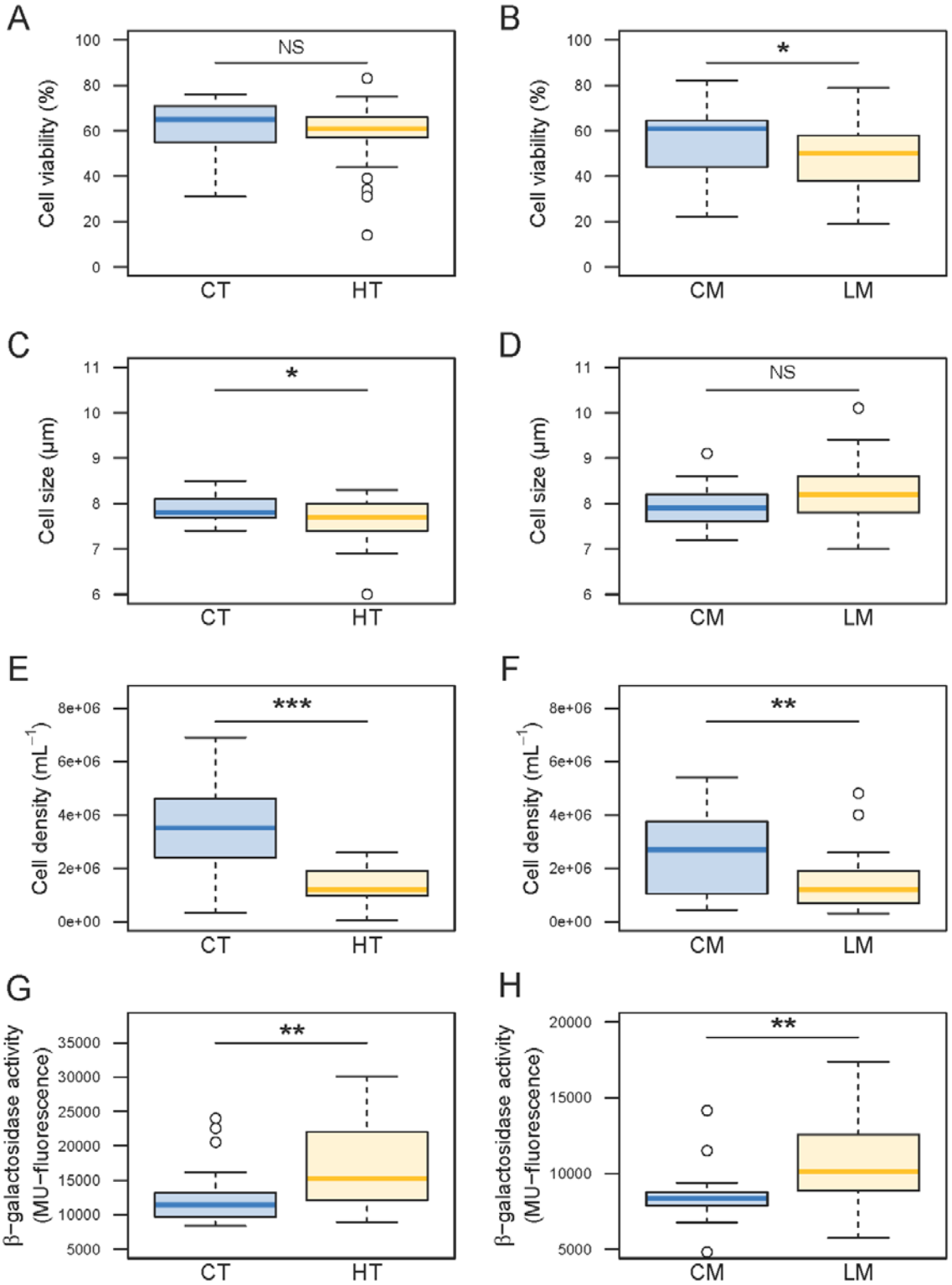

Figure 2. Effect of the two environmental stressors (Temperature (A, C, E, and G) and Moisture (B, D, F and H) on immune cell viability (A and B), immune cell size (C and D), immune cell density (E and F) and β-galactosidase activity (G and H). *Blue colour: control groups, orange colour: stress groups. 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.*

337 **DISCUSSION**

338 Our results highlight that life history traits were negatively impacted by the two environmental
339 stressors (thermal and water stresses) considered in this study. Moreover, the detrimental
340 effects of these stressors on our set of biomarkers of individual quality are consistent with an
341 overall premature ageing of stressed animals compared to unstressed ones. To briefly
342 summarize, an increase in temperature (thermal stress) negatively affects both the body mass
343 trajectory over time and the reproductive success of individuals. A decrease in moisture (water
344 stress) resulted in a decrease of both survival and reproductive success. Concerning our
345 physiological traits: (1) the density of immune cells decreases under both stresses, (2) immune
346 cell size decreases under thermal stress, but is not impacted under water stress, (3) the
347 viability of the cells decreases under water stress (but not under thermal stress) and (4) finally,
348 the β-galactosidase activity increases for the two stressed groups. In this context, our results
349 globally support marked negative effects of thermal and water stresses on woodlouse
350 performance, with some minor differences between the two stressors in their effects on life
351 history and physiological traits.

352 About the life history traits, if the thermal stress has no detectable effect on survival in *A.*
353 *vulgare*, contrary to what has been previously reported in arthropods studied so far such as
354 *Antestiopsis thunbergii*, *Calliphora stygia* and *Margaritifera margaritifera* (Azrag et al., 2017;
355 Hassall et al., 2017; Kelly et al., 2013), this stressor leads to a slowdown in woodlouse growth,
356 in line to what has been reported in three other isopods (Angilletta et al., 2004). In parallel,
357 the water stress leads to a decrease in reproductive success, as previously reported in females
358 of *Antestiopsis thunbergii* (Azrag et al., 2017). In *A. vulgare*, individual body size is positively
359 correlated with fecundity (Durand et al., 2018; Lawlor, 1976), meaning that the slowdown in
360 growth could explain, at least partly, the decrease in reproductive success for stressed animals
361 compared to non-stressed ones. Concerning the water stress, if the loss of moisture has no
362 detectable effect on woodlouse growth, it causes a decrease in both survival and reproductive
363 success. These findings suggest a high cost of drought on individual fitness in *A. vulgare*.

364 About the physiological traits, our results of the thermal stress experiment show that although
365 cell density is negatively impacted by increased temperature, cell viability is not affected.
366 Moreover, contrary to the expectation when individuals are senescent, cell size decreases
367 instead of increasing. This last result supports our previous finding that cells decrease in size

368 when the temperature raises (without controlling moisture level, Depeux et al., 2019). That
369 smaller cells are associated with increased cell renewal in stressed animals might explain this
370 pattern. On the other hand, the increase of β-galactosidase activity seems to indicate
371 premature ageing (and thus a decrease in quality) in individuals exposed to thermal stress.
372 Concerning the water stress, the biomarkers of individual quality indicate a decrease in cell
373 density and viability, associated with an increase in β-galactosidase activity, which suggests an
374 acceleration of biological ageing in the individuals exposed to a water stress (Depeux et al.,
375 2020a).

376 We reported a global negative effect of the thermal stress in *A. vulgare* in our study, but our
377 results seem to show an even higher and clearer effect of the water stress on both life history
378 traits and biomarkers of individual quality. Although the woodlouse has become terrestrial for
379 a long time, the individuals of that species are still dependent on and require a substantial
380 water supply (Smigel and Gibbs, 2008). Thus, behaviours like aggregation that allow
381 individuals to resist to desiccation have been set up and thereby to maintain the rate of
382 moisture required for survival (Broly et al., 2013; Smigel and Gibbs, 2008). This can explain the
383 strong effect of water stress in our study. Under natural conditions, increase in temperature
384 and loss of moisture generally positively covary, leading to even higher negative consequences
385 on the woodlouse performance. Further work will be required to test the influence of more
386 extreme and maybe more realistic conditions by simultaneously increasing temperature and
387 decreasing moisture on life history and physiological traits. A study in the wild comparing life
388 history and physiological traits on *A. vulgare* collected across areas with different temperature
389 and drought gradient would also allow a better assessment of the combined effects of these
390 stressors.

391 Unlike what happens in nature, our experimental study on a laboratory line of woodlouse allowed us
392 to test the effect of the thermal and water stresses while controlling for potentially confounding
393 factors such as individual age. Indeed, it is highly challenging to control for individual age in the wild.
394 In this context, the use of our controlled laboratory line on which we developed our physiological
395 markers allowed us to account for the exact age of the animals (which is itself linked to life history and
396 physiological traits (Depeux et al., 2020b)) and for their genetic origin. We compared groups of the
397 same origin (and our controlled crosses guarantee the genetic diversity of our line) and of the same
398 age. This allows us to limit confounding effects as much as possible and to quantify the effects of the
399 two tested stressors independently.

Thanks to our experimental design that allowed us to test independently the influence of stressors that organisms are likely to face in the wild, we showed that thermal and water stress do not have the same impact. Although simulations based on mathematical models have predicted that both temperature and drought changes overall affect arthropods, experimental approaches such as reported in this work are required to quantify reliably the influence of changing conditions on life history and physiological traits (Johnson et al. 2010). Drought can have serious physiological consequences on invertebrates, involving e.g. protein denaturation and undesirable macromolecular interactions (Sano et al., 1999; Tang and Pikal, 2005) or oxidative damage (Lopez-Martinez et al., 2008), which are known to be associated with cellular senescence (Gilca et al., 2007) and thus in the decreased performance observed in stressed organisms. Due to the role of arthropods in services to many ecosystems (e.g. biochemical balance of ecosystems, agriculture, pest management…), and in the context of global warming, it is crucial to understand the effects of temperature and moisture changes on these organisms (Santos et al., 2021). As temperature increase is not the only environmental change expected to take place in the coming years, it is of paramount importance to assess also the impact of other stressors. Although many predictive models have been proposed so far, getting more accurate information on the expected responses of organisms facing with different kinds of stress would provide the required information to test these model predictions.

To conclude, *A. vulgare* is an important actor that delivers ecosystem services in many ecosystems because it actively impacts soil fertility (Souty-Grosset and Faberi, 2018) and it is also used as an ecological indicator of grassland habitats (Paoletti and Hassall, 1999; Souty-Grosset et al., 2005). This detritivorous species facilitates decomposition processes and nutrient cycling on which agricultural productivity and sustainability depend (Bredon et al., 2018; Paoletti and Hassall, 1999), and plays thereby a key role in ecosystem services (David and Handa, 2010). Extending knowledge in the response of soil biodiversity facing current global changes could promote sustainability by helping to the development of new tools and strategies for more efficient management of soils and associated crops, through more effective and targeted recolonisation and/or restoration of soil biodiversity. Also, to better understand what the future of the animal communities in the current context of global

warming will be, it is necessary to perform studies on models presenting particular ecological requirements, such as woodlouse.

Supplementary files

All supplementary files are available on public repository:
https://www.biorxiv.org/content/10.1101/2022.09.26.509512v1.supplementary-material
Supp. File1 Comparison of the two control groups
Supp. File2 Model selection
Supp. File3 Graphical representations of results per sex

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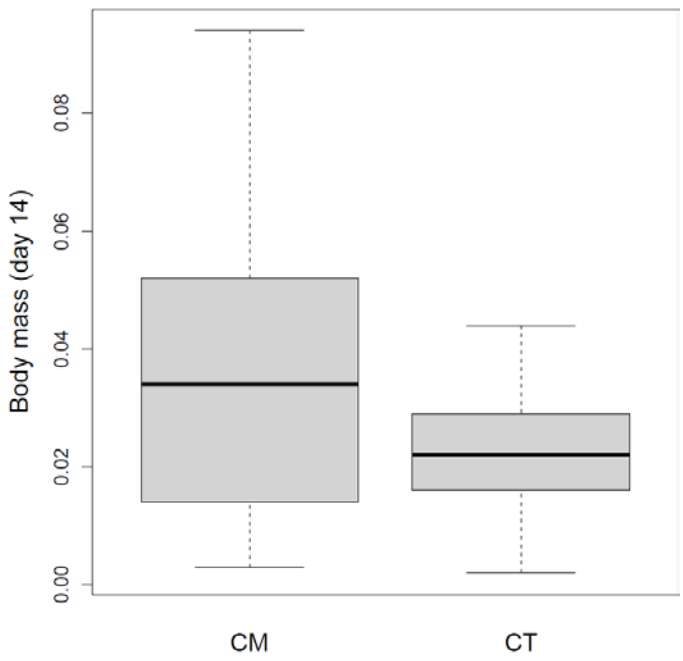
575 **Supplementary File 1: Comparison of the two control groups**

576

577 *Table 1: Comparison between the two control groups (CT (Control Temperature) and CM (Control Moisture)) of the two*
578 *experiments for each tested variable (in bold the variables with significant statistical differences with graphical associated*
579 *figures (Fig. 1, Fig. 2 and Fig. 3))*

Traits	Statistical value	P-value
Life history traits measures		
Survival	$\chi_1^2 = 1.25$	P = 0.26
Body mass **(day 14)**	**$F_{1,111} = 13.00$,**	**P < 0.001**
Reproduction	$\chi_1^2 = 0.417$	P = 0.52
Physiological traits measures		
Immune cells parameters		
Density	**$F_{1,50} = 6.21$**	**P= 0.016**
Viability	$F_{1,50} = 2.49$	P= 0.12
Size	$F_{1,50} = 0.029$	P = 0.86
β-galactosidase activity	$F_{1,49} = 17.0$	**P < 0.001**

580



581

582 *Figure 1: Body mass comparison between the two control groups*
583 *(CT (Control Temperature) and CM (Control Moisture)) P-value < 0.001*

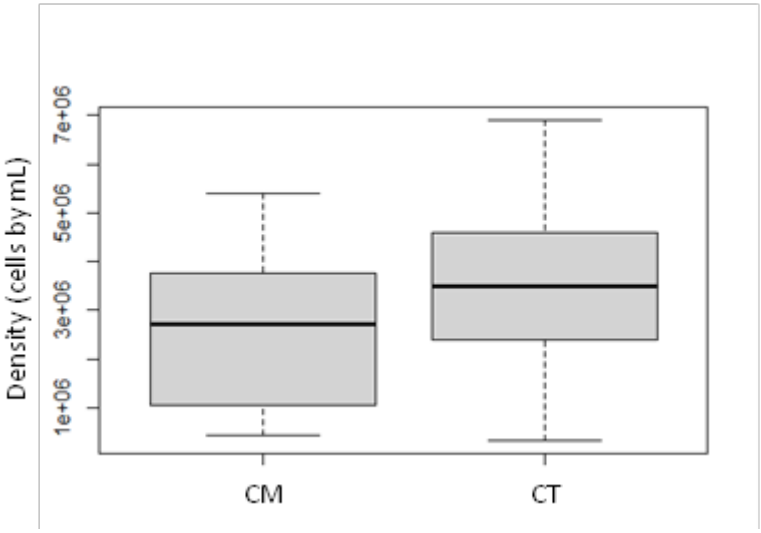


Figure 2: Immune cells density comparison between the two control groups (CT (Control Temperature) and CM (Control Moisture)) P-value=0.02

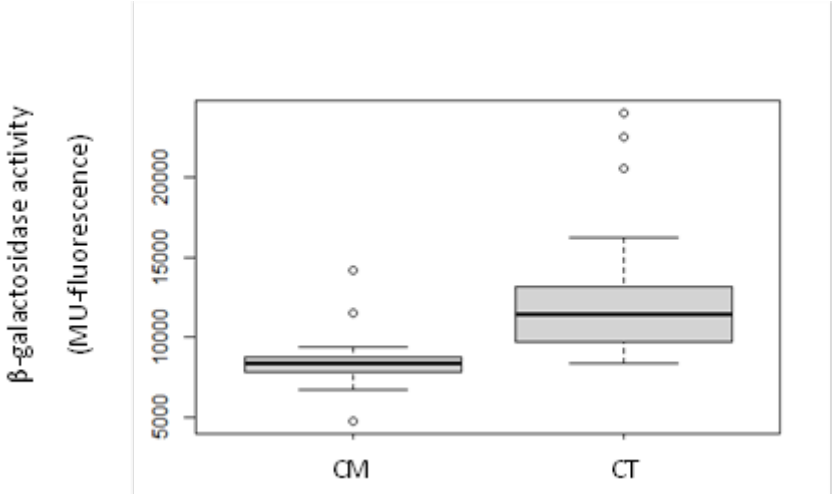


Figure 3: β-galactosidase activity comparison between the two control groups (CT (Control Temperature) and CM (Control Moisture)) P-value<0.001

Supplementary File 2: Model selection

Life history trait

Survival

Table S2a. Effect of the thermal stress condition and sex on the survival. For each model, we reported intercept of the regression, adjusted R^2 (adj.R^2), degree of freedom (df), Log likelihood (LogLik) values, Akaike information criteria values with a correction for small sample sizes (AICc), change in AICc (△AICc) from the best model, and model weight. The presence of the categorial variable (sex, stress condition, and their interaction term sex:stress) in the model is indicated by a "+" symbol.

Intercept	sex	stress	sex:stress	adj.R^2	df	logLik	AICc	△AICc	weight
+		+		0,08	1	-125,01	252,18	0,00	0,31
+				**0,00**	**0**	**-126,09**	**252,19**	**0,01**	**0,31**
+	+			0,02	1	-125,82	253,81	1,62	0,14
+	+	+		0,10	2	-124,70	253,89	1,71	0,13
+	+	+	+	0,17	3	-123,65	254,34	2,16	0,11

Table S2b. Effect of the water stress condition and sex on the survival. For each model, we reported intercept of the regression, adjusted R^2 (adj.R^2), degree of freedom (df), Log likelihood (LogLik) values, Akaike information criteria values with a correction for small sample sizes (AICc), change in AICc (△AICc) from the best model, and model weight. The presence of the categorial variable (sex, stress condition, and their interaction term sex:stress) in the model is indicated by a "+" symbol. The most parsimonious model is highlighted in bold font.

Intercept	sex	stress	sex:stress	adj.R^2	df	logLik	AICc	△AICc	weight
+		**+**		**0,20**	**1**	**-91,25**	**184,72**	**0,00**	**0,53**
+	+	+		0,22	2	-91,02	186,75	2,02	0,19
+				0,00	0	-93,52	187,04	2,32	0,17
+	+			0,02	1	-93,32	188,87	4,15	0,07
+	+	+	+	0,23	3	-90,94	189,39	4,67	0,05

Body mass

Table S2c. Effect of the thermal stress condition, sex and day on body mass. For each model, we reported intercept of the regression, adjusted R^2 (adj.R^2), degree of freedom (df), Log likelihood (LogLik) values, Akaike information criteria values with a correction for small sample sizes (AICc), change in AICc (△AICc) from the best model, and model weight. The presence of the categorial variable (sex, stress condition, and their two-by-two interaction terms) in the model is indicated by a "+" symbol. The regression parameter is only given for the corresponding continuous variable (day) when this variable is present in the model. The most parsimonious model is highlighted in bold font.

Intercept	day	sex	stress	day:sex	day:stress	sex:stress	adj.R^2	df	logLik	AICc	△AICc	weight
0,02	0,00	+	+	+	+	+	-0,01	8	1444,54	-2872,80	0,00	0,63
0,02	**0,00**	**+**	**+**		**+**	**+**	**-0,01**	**7**	**1442,55**	**-2870,90**	**1,91**	**0,24**
0,02	0,00	+	+	+	+		-0,01	7	1441,20	-2868,18	4,62	0,06
0,02	0,00		+		+		-0,01	5	1438,73	-2867,35	5,45	0,04
0,02	0,00	+	+		+		-0,01	6	1439,08	-2866,01	6,79	0,02
0,02	0,00	+	+	+		+	-0,01	7	1436,82	-2859,43	13,37	0,00
0,03	0,00	+	+			+	-0,01	6	1435,27	-2858,39	14,41	0,00
0,02	0,00	+	+	+			-0,01	6	1433,45	-2854,75	18,05	0,00
0,03	0,00		+				-0,01	4	1431,36	-2854,64	18,16	0,00
0,02	0,00	+	+				-0,01	5	1431,79	-2853,47	19,33	0,00
0,02	0,00	+		+			-0,01	5	1431,45	-2852,78	20,02	0,00
0,02	0,00						-0,01	3	1429,38	-2852,71	20,09	0,00
0,02	0,00	+					-0,01	4	1429,89	-2851,71	21,09	0,00
0,05			+				0,00	3	1179,76	-2353,47	519,33	0,00
0,05							0,00	2	1178,27	-2352,52	520,28	0,00
0,05		+	+				0,00	4	1179,85	-2351,62	521,18	0,00
0,06		+	+			+	0,00	5	1180,51	-2350,91	521,90	0,00
0,05		+					0,00	3	1178,39	-2350,74	522,06	0,00

Table S2d. Effect of the water stress condition, sex and day on body mass. For each model, we reported intercept of the regression, adjusted R^2 (adj.R^2), degree of freedom (df), Log likelihood (LogLik) values, Akaike information criteria values with a correction for small sample sizes (AICc), change in AICc (△AICc) from the best model, and model weight. The presence of the categorial variable (sex, stress condition, and their two-by-two interaction terms) in the model is indicated by a "+" symbol. The regression parameter is only given for the corresponding continuous variable (day) when this variable is present in the model. The most parsimonious model is highlighted in bold font.

Intercept	day	sex	stress	day:sex	day:stress	sex:stress	adj.R^2	df	logLik	AICc	△AICc	weight
0,04	**0,00**						**0,00**	**3**	**1309,82**	**-2613,60**	**0,00**	**0,29**
0,04	0,00		+		+		0,00	5	1311,34	-2612,57	1,03	0,17
0,04	0,00	+					0,00	4	1310,00	-2611,93	1,68	0,12
0,04	0,00		+				0,00	4	1309,98	-2611,89	1,72	0,12
0,04	0,00	+	+		+		0,00	6	1311,50	-2610,85	2,76	0,07
0,04	0,00	+	+				0,00	5	1310,18	-2610,24	3,37	0,05
0,04	0,00	+		+			0,00	5	1310,01	-2609,90	3,70	0,04
0,04	0,00	+	+		+	+	0,00	7	1311,79	-2609,37	4,24	0,03
0,04	0,00	+	+	+	+		0,00	7	1311,50	-2608,79	4,81	0,03
0,04	0,00	+	+			+	0,00	6	1310,48	-2608,79	4,82	0,03
0,04	0,00	+	+	+			0,00	6	1310,18	-2608,21	5,40	0,02
0,04	0,00	+	+	+	+	+	0,00	8	1311,79	-2607,30	6,30	0,01
0,04	0,00	+	+	+		+	0,00	7	1310,49	-2606,75	6,85	0,01
0,05							0,00	2	1272,47	-2540,92	72,68	0,00
0,05		+					0,00	3	1272,59	-2539,13	74,47	0,00
0,05			+				0,00	3	1272,57	-2539,10	74,50	0,00
0,05		+	+				0,00	4	1272,70	-2537,32	76,28	0,00
0,05		+	+			+	0,00	5	1272,79	-2535,47	78,13	0,00

Reproductive success

Table S2e. Effect of the thermal stress condition on the reproductive success. For each model, we reported intercept of the regression, adjusted R^2 (adj.R^2), degree of freedom (df), Log likelihood (LogLik) values, Akaike information criteria values with a correction for small sample sizes (AICc), change in AICc (△AICc) from the best model, and model weight. The presence of the categorial variable (stress condition) in the model is indicated by a "+" symbol. The value of regression parameter is only given for the intercept. The most parsimonious model is highlighted in bold font.

Intercept	stress	adj.R^2	df	logLik	AICc	△AICc	weight
0,62	**+**	**0,16**	**2**	**-25,17**	**54,66**	**0,00**	**0,80**
-0,10		0,00	1	-27,68	57,46	2,80	0,20

Table S2f. Effect of the water stress condition on the reproductive success. For each model, we reported intercept of the regression, adjusted R^2 (adj.R^2), degree of freedom (df), Log likelihood (LogLik) values, Akaike information criteria values with a correction for small sample sizes (AICc), change in AICc (△AICc) from the best model, and model weight. The presence of the categorial variable (stress condition) in the model is indicated by a "+" symbol. The value of regression parameter is only given for the intercept. The most parsimonious model is highlighted in bold font.

Intercept	stress	adj.R^2	df	logLik	AICc	△AICc	weight
-1,39	**+**	**0,17**	**2**	**-23,77**	**51,87**	**0,00**	**0,83**
-0,51		0,00	1	-26,46	55,03	3,16	0,17

651 # Individual physiological traits

652 **Immune cell viability**

653 **Table S2g**. Effect of the thermal stress condition and sex on immune cell viability. For each model, we
654 reported intercept of the regression, adjusted R^2 (adj.R^2), degree of freedom (df), Log likelihood
655 (LogLik) values, Akaike information criteria values with a correction for small sample sizes (AICc),
656 change in AICc (△AICc) from the best model, and model weight. The presence of the categorial variable
657 (sex, stress condition, and their interaction term sex:stress) in the model is indicated by a "+" symbol.
658 The value of regression parameter is only given for the intercept. The most parsimonious model is
659 highlighted in bold font.

Intercept	sex	stress	sex:stress	adj.R^2	df	logLik	AICc	△AICc	weight
60,31				**0,00**	**2**	**-231,04**	**466,30**	**0,00**	**0,47**
61,97		+		0,02	3	-230,57	467,58	1,28	0,25
61,00	+			0,00	3	-230,97	468,39	2,08	0,17
62,43	+	+		0,02	4	-230,53	469,81	3,51	0,08
63,67	+	+	+	0,03	5	-230,24	471,63	5,32	0,03

660

661 **Table S2h.** Effect of the water stress condition and sex on immune cell viability. For each model, we
662 reported intercept of the regression, adjusted R^2 (adj.R^2), degree of freedom (df), Log likelihood
663 (LogLik) values, Akaike information criteria values with a correction for small sample sizes (AICc),
664 change in AICc (△AICc) from the best model, and model weight. The presence of the categorial variable
665 (sex, stress condition, and their interaction term sex:stress) in the model is indicated by a "+" symbol.
666 The value of regression parameter is only given for the intercept. The most parsimonious model is
667 highlighted in bold font.

Intercept	sex	stress	sex:stress	adj.R^2	df	logLik	AICc	△AICc	weight
51,82	+	+		0,15	4	-212,23	433,32	0,00	0,48
47,45		**+**		**0,08**	**3**	**-214,36**	**435,21**	**1,90**	**0,19**
52,29	+	+	+	0,15	5	-212,20	435,70	2,39	0,15
55,52	+			0,06	3	-214,86	436,23	2,91	0,11
51,29				0,00	2	-216,44	437,13	3,81	0,07

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Immune cell size

Table S2i. Effect of the thermal stress condition and sex on immune cell size. For each model, we reported intercept of the regression, adjusted R^2 (adj.R^2), degree of freedom (df), Log likelihood (LogLik) values, Akaike information criteria values with a correction for small sample sizes (AICc), change in AICc (△AICc) from the best model, and model weight. The presence of the categorial variable (sex, stress condition, and their interaction term sex:stress) in the model is indicated by a "+" symbol. The value of regression parameter is only given for the intercept. The most parsimonious model is highlighted in bold font.

Intercept	sex	stress	sex:stress	adj.R^2	df	logLik	AICc	△AICc	weight
7,91	+	+	+	0,25	5	-29,17	69,50	0,00	0,32
7,99	+	+		0,20	4	-30,45	69,66	0,17	0,30
7,91		**+**		**0,15**	**3**	**-31,64**	**69,73**	**0,23**	**0,29**
7,87	+			0,07	3	-33,33	73,09	3,60	0,05
7,77				0,00	2	-34,79	73,80	4,30	0,04

Table S2j. Effect of the water stress condition and sex on immune cell size. For each model, we reported intercept of the regression, adjusted R^2 (adj.R^2), degree of freedom (df), Log likelihood (LogLik) values, Akaike information criteria values with a correction for small sample sizes (AICc), change in AICc (△AICc) from the best model, and model weight. The presence of the categorial variable (sex, stress condition, and their interaction term sex:stress) in the model is indicated by a "+" symbol. The value of regression parameter is only given for the intercept. The most parsimonious model is highlighted in bold font.

Intercept	sex	stress	sex:stress	adj.R^2	df	logLik	AICc	△AICc	weight
8,24		+		0,08	3	-44,46	95,42	0,00	0,45
8,10				**0,00**	**2**	**-46,36**	**96,97**	**1,54**	**0,21**
8,17	+	+		0,10	4	-44,10	97,06	1,63	0,20
8,04	+			0,01	3	-46,16	98,81	3,39	0,08
8,19	+	+	+	0,10	5	-44,06	99,42	4,00	0,06

Immune cell density

Table S2k. Effect of the thermal stress condition and sex on immune cell density. For each model, we reported intercept of the regression, adjusted R^2 (adj.R^2), degree of freedom (df), Log likelihood (LogLik) values, Akaike information criteria values with a correction for small sample sizes (AICc), change in AICc (△AICc) from the best model, and model weight. The presence of the categorial variable (sex, stress condition, and their interaction term sex:stress) in the model is indicated by a "+" symbol. The value of regression parameter is only given for the intercept. The most parsimonious model is highlighted in bold font.

Intercept	sex	stress	sex:stress	adj.R^2	df	logLik	AICc	△AICc	weight
3538275,86		**+**		**0,41**	**3**	**-898,33**	**1803,10**	**0,00**	**0,66**
3644762,62	+	+		0,41	4	-898,12	1804,99	1,89	0,26
3600666,67	+	+	+	0,41	5	-898,08	1807,31	4,22	0,08
2472758,62				0,00	2	-913,43	1831,07	27,98	0,00
2707777,78	+			0,02	3	-912,92	1832,29	29,20	0,00

Table S2l. Effect of the water stress condition and sex on immune cell density. For each model, we reported intercept of the regression, adjusted R^2 (adj.R^2), degree of freedom (df), Log likelihood (LogLik) values, Akaike information criteria values with a correction for small sample sizes (AICc), change in AICc (△AICc) from the best model, and model weight. The presence of the categorial variable (sex, stress condition, and their interaction term sex:stress) in the model is indicated by a "+" symbol. The value of regression parameter is only given for the intercept. The most parsimonious model is highlighted in bold font.

Intercept	sex	stress	sex:stress	adj.R^2	df	logLik	AICc	△AICc	weight
1465517,24		**+**		**0,13**	**3**	**-802,79**	**1612,09**	**0,00**	**0,52**
1827142,86	+	+	+	0,18	5	-801,23	1613,77	1,68	0,23
1575106,08	+	+		0,14	4	-802,60	1614,06	1,97	0,20
1892500,00				0,00	2	-806,49	1617,23	5,14	0,04
1960434,78	+			0,00	3	-806,44	1619,38	7,29	0,01

β-Galactosidase activity

Table S2m. Effect of the thermal stress condition and sex on β-Galactosidase activity. For each model, we reported intercept of the regression, adjusted R^2 (adj.R^2), degree of freedom (df), Log likelihood (LogLik) values, Akaike information criteria values with a correction for small sample sizes (AICc), change in AICc (△AICc) from the best model, and model weight. The presence of the categorial variable (sex, stress condition, and their interaction term sex:stress) in the model is indicated by a "+" symbol. The value of regression parameter is only given for the intercept. The most parsimonious model is highlighted in bold font.

Intercept	sex	stress	sex:stress	adj.R^2	df	logLik	AICc	△AICc	weight
12399,83		**+**		**0,17**	**3**	**-556,53**	**1119,51**	**0,00**	**0,60**
12988,70	+	+		0,18	4	-556,14	1121,06	1,55	0,28
12487,80	+	+	+	0,19	5	-555,81	1122,82	3,30	0,11
14534,95				0,00	2	-561,86	1127,94	8,42	0,01
15052,70	+			0,01	3	-561,62	1129,71	10,20	0,00

Table S2n. Effect of the water stress condition and sex on β-Galactosidase activity. For each model, we reported intercept of the regression, adjusted R^2 (adj.R^2), degree of freedom (df), Log likelihood (LogLik) values, Akaike information criteria values with a correction for small sample sizes (AICc), change in AICc (△AICc) from the best model, and model weight. The presence of the categorial variable (sex, stress condition, and their interaction term sex:stress) in the model is indicated by a "+" symbol. The value of regression parameter is only given for the intercept. The most parsimonious model is highlighted in bold font.

Intercept	sex	stress	sex:stress	adj.R^2	df	logLik	AICc	△AICc	weight
10694,60		**+**		**0,17**	**3**	**-477,96**	**962,43**	**0,00**	**0,67**
10867,90	+	+		0,18	4	-477,84	964,53	2,10	0,23
10955,89	+	+	+	0,18	5	-477,79	966,89	4,47	0,07
9725,79				0,00	2	-482,92	970,08	7,65	0,01
10087,84	+			0,01	3	-482,55	971,60	9,17	0,01

Supplementary file 3: Graphical representations of results per sex

1. Life history traits

1.A. Survival

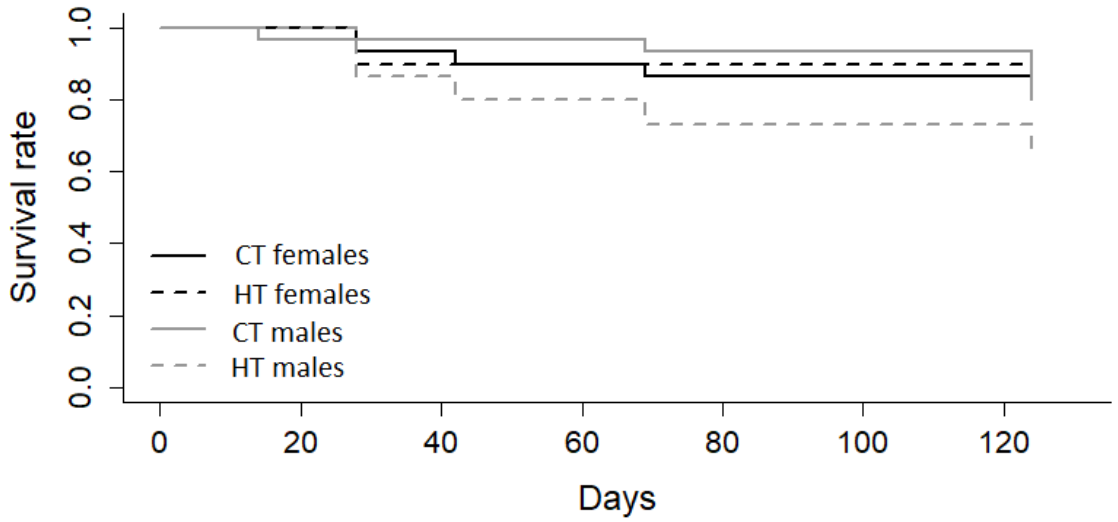


Figure 1.A.1: Effect of thermal stress on survival

CT females: control females in Control Temperature (20°C), HT females: stressed females in High Temperature (28°C), CT males: control males in Control Temperature (20°C), HT males: stressed males in High Temperature (28°C)

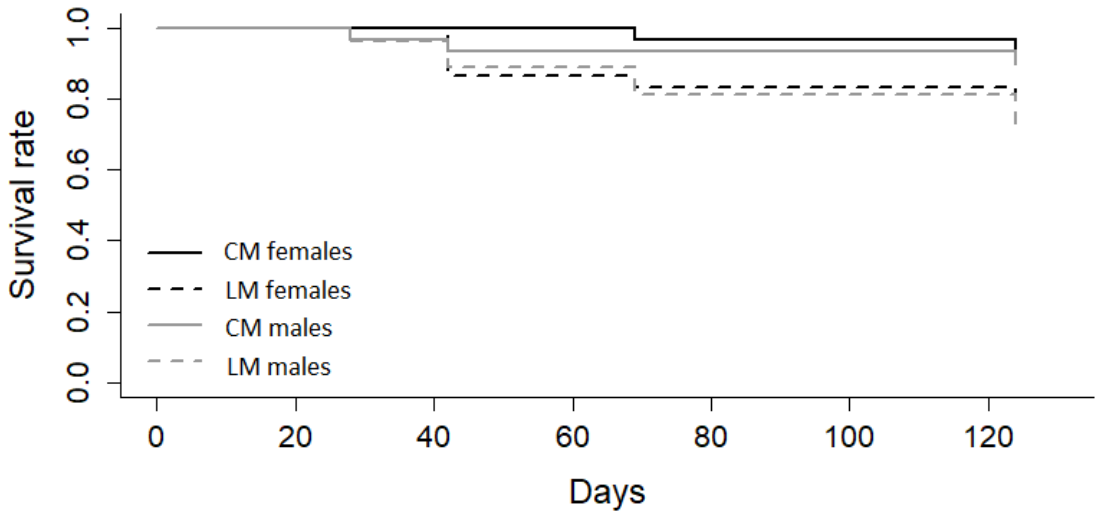


Figure 1.A.2.: Effect of water stress on survival

CM females: control females in Control Moisture (moisture 80%), LM females: stressed females in Loss of Moisture (moisture 50%), CM males: control males in Control Moisture (moisture 80%), LM males: stressed males in Loss of Moisture (moisture 50%)

1.B. Body mass across time

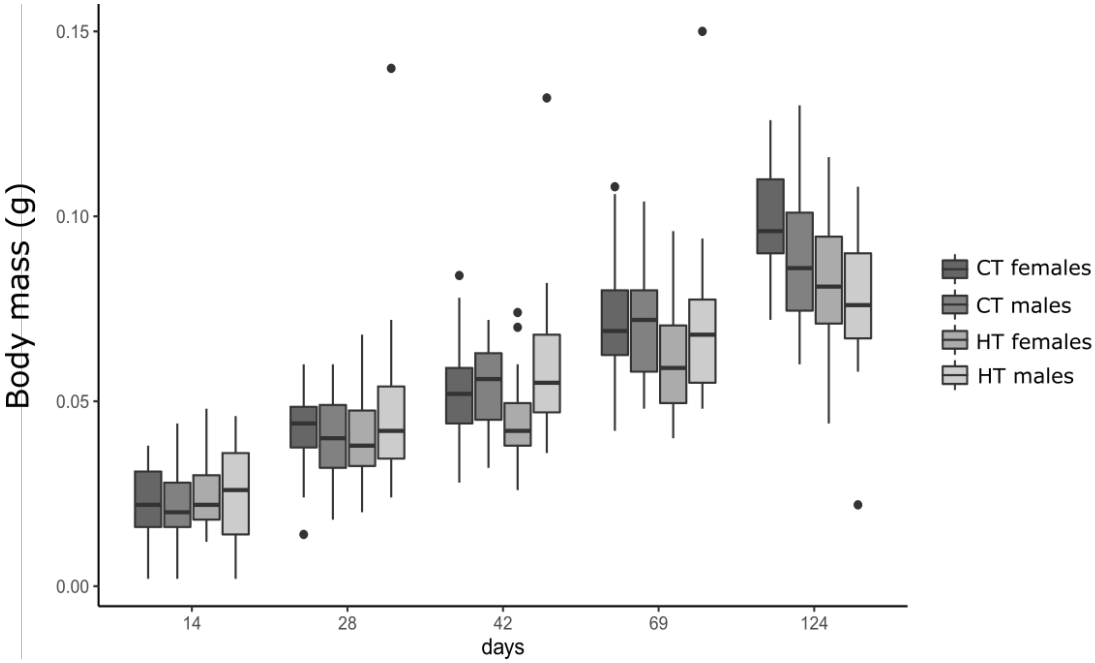


Figure 1.B.1.: Boxplot of the effect of thermal stress on body mass (measured in grams) over time

CT females: control females in Control Temperature (20°C), HT females: stressed females in High Temperature (28°C), CT males: control males in Control Temperature (20°C), HT males: stressed males in High Temperature (28°C)

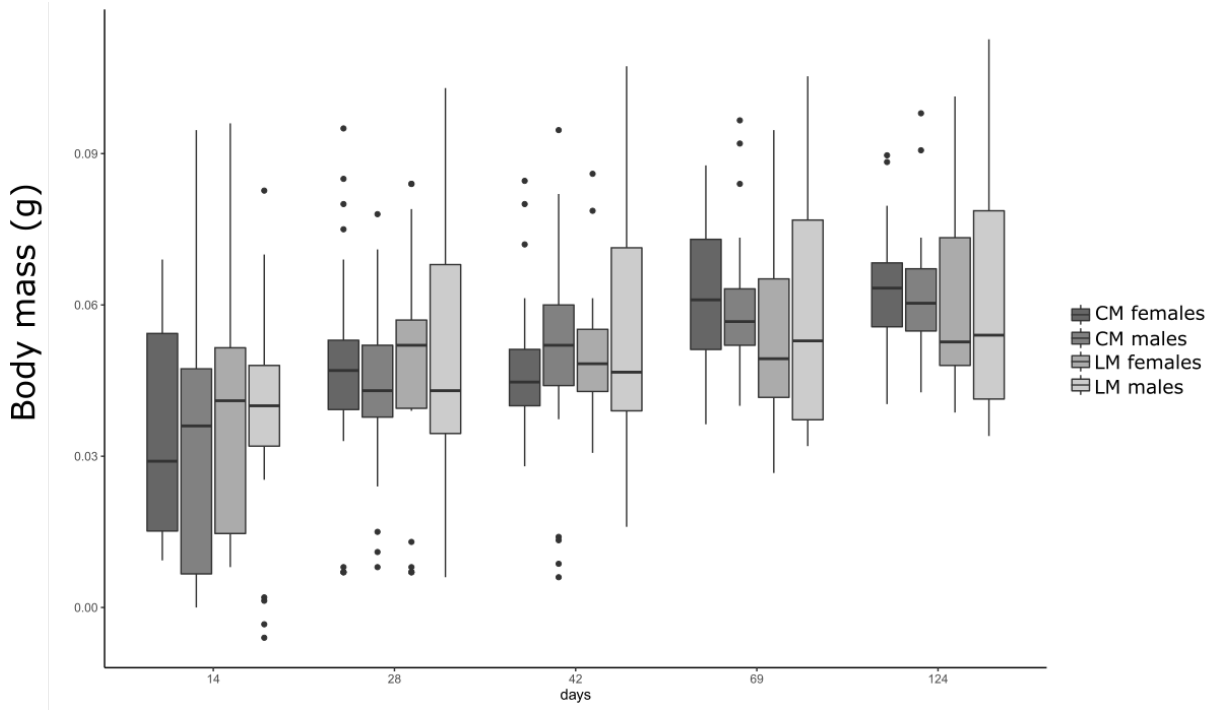


Figure 1.B.2.: Boxplot of the effect of water stress on body mass (measured in grams) over time

CM females: control females in Control Moisture (moisture 80%), LM females: stressed females in Loss of Moisture (moisture 50%), CM males: control males in Control Moisture (moisture 80%), LM males: stressed males in Loss of Moisture (moisture 50%)

1.C. Reproduction success

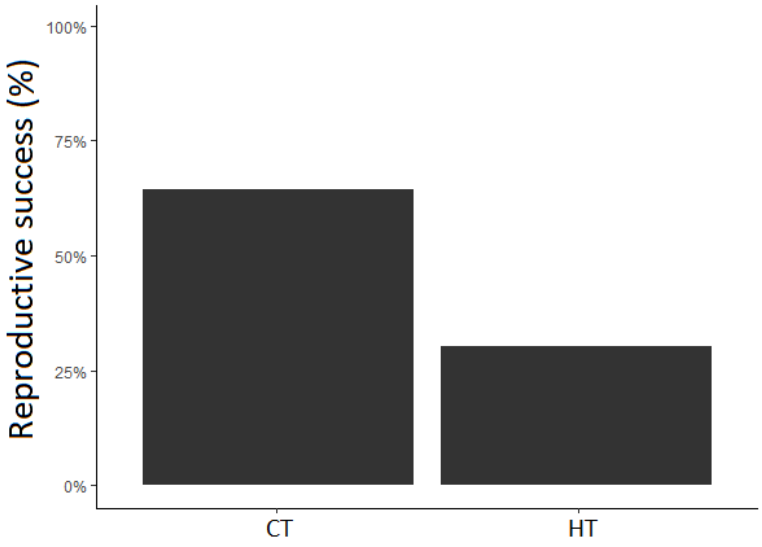


Figure 1.C.1.: Effect of temperature on breeding success *(0 = pairs that did not produce offspring; 1 = pairs that produced offspring; CT: control individuals in Control Temperature (20°C), HT: Stressed individuals in High Temperature (28°C))*

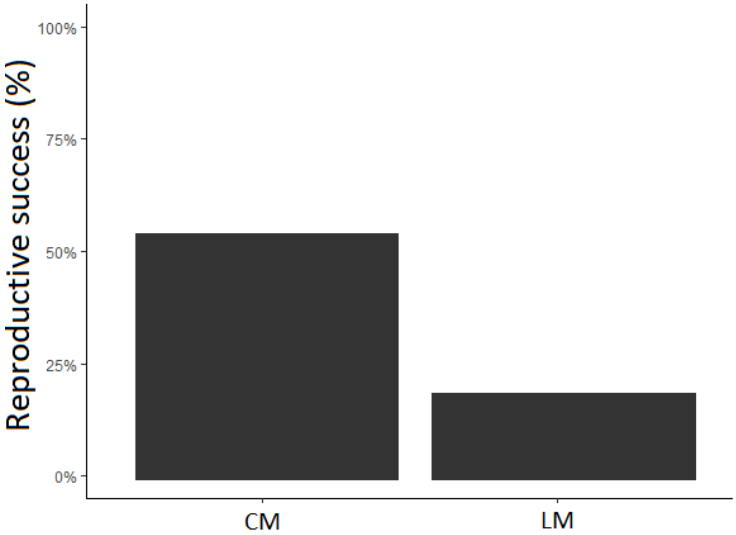


Figure 1.C.2.: Effect of moisture on breeding success *(0 = pairs that did not produce offspring; 1 = pairs that produced offspring; CM: control individuals in Control Moisture (moisture 80%), LM females: stressed individuals in Loss of Moisture (moisture 50%))*

2. Individual physiological traits

2.A. Immune cells viability

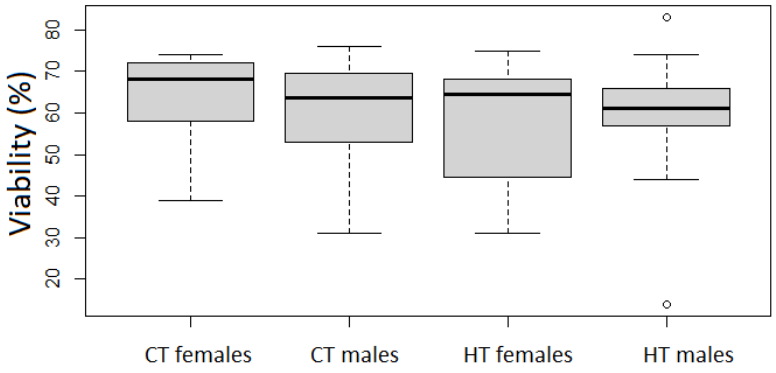


Figure 2.A.1.: Effect of thermal stress on immune cell viability (% of live cells)
CT females: control females in Control Temperature (20°C), HT females: stressed females in High Temperature (28°C), CT males: control males in Control Temperature (20°C), HT males: stressed males in High Temperature (28°C)

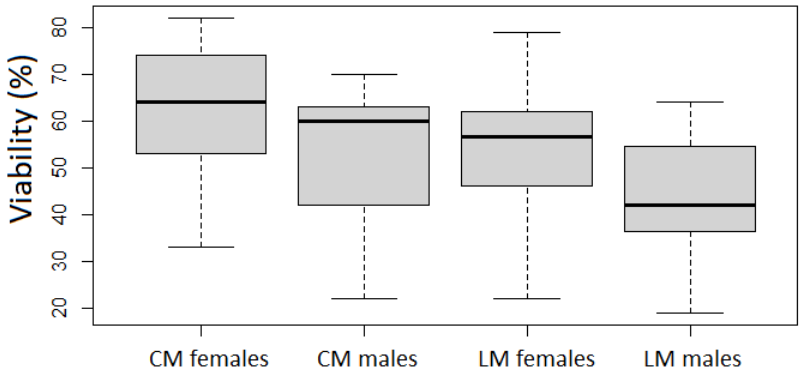


Figure 2.A.2.: Effect of water stress on immune cell viability (% of live cells)
CM females: control females in Control Moisture (moisture 80%), LM females: stressed females in Loss of Moisture (moisture 50%), CM males: control males in Control Moisture (moisture 80%), LM males: stressed males in Loss of Moisture (moisture 50%)

2.B. Immune cells size

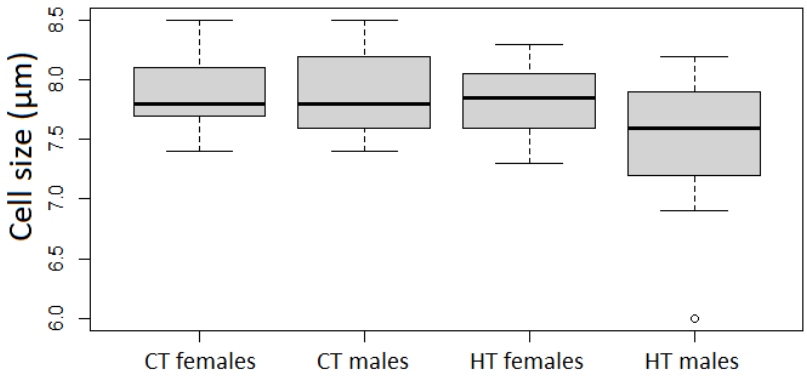


Figure 2.B.1.: Effect of thermal stress on immune cells size (in µm)

CT females: control females in Control Temperature (20°C), HT females: stressed females in High Temperature (28°C), CT males: control males in Control Temperature (20°C), HT males: stressed males in High Temperature (28°C)

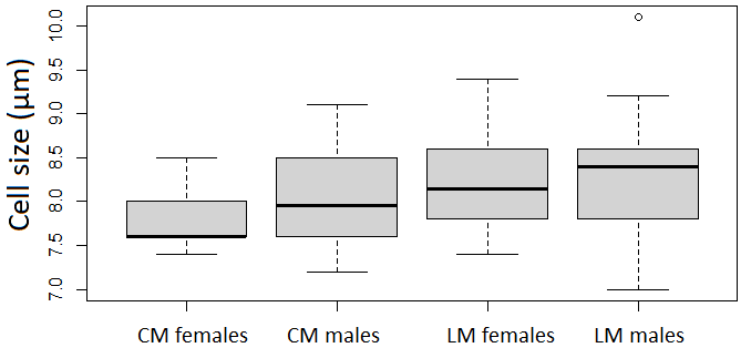


Figure 2.B.2.: Effect of water stress on immune cells size (in µm)

CM females: control females in Control Moisture (moisture 80%), LM females: stressed females in Loss of Moisture (moisture 50%), CM males: control males in Control Moisture (moisture 80%), LM males: stressed males in Loss of Moisture (moisture 50%)

2.C. Immune cells density

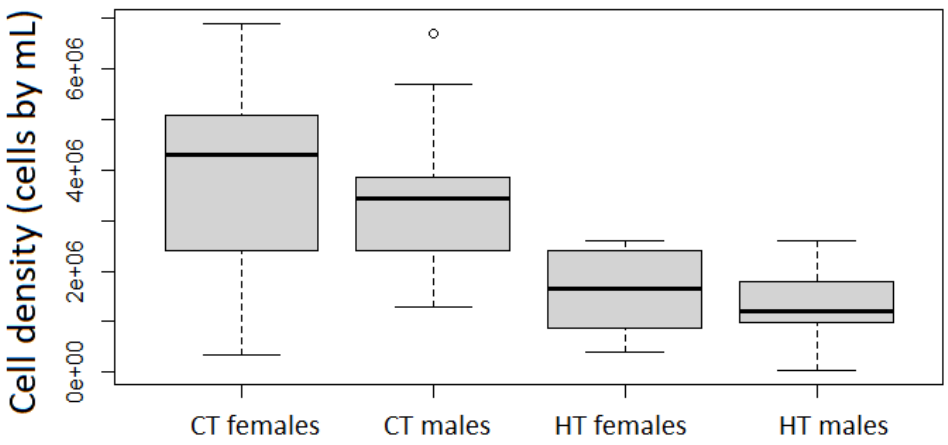


Figure 2.C.1.: Effect of thermal stress on immune cells density (number of cells per mL of haemolymph)
CT females: control females in Control Temperature (20°C), HT females: stressed females in High Temperature (28°C), CT males: control males in Control Temperature (20°C), HT males: stressed males in High Temperature (28°C)

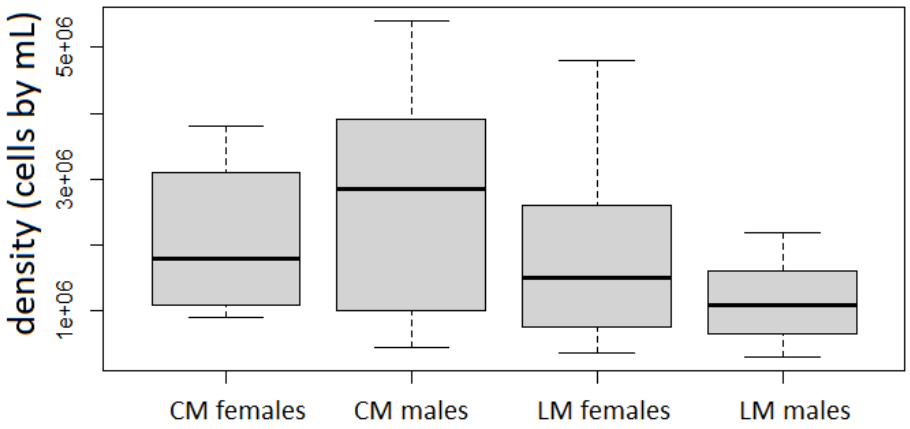
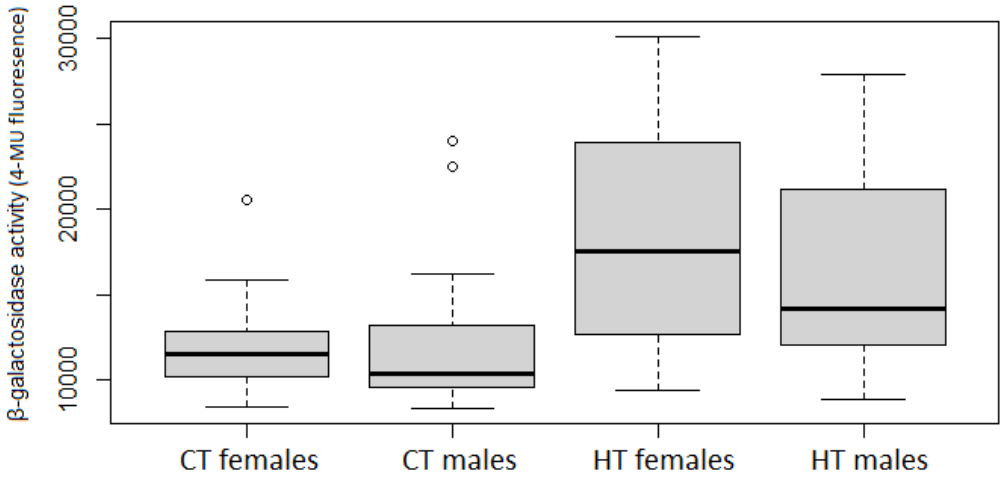


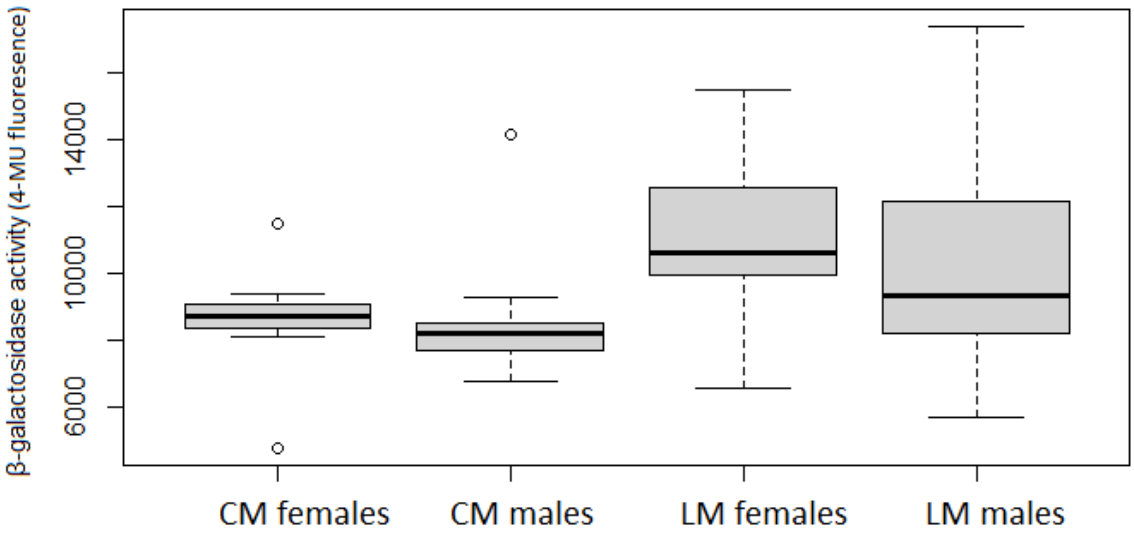
Figure 2.C.2.: Effect of water stress on immune cells density (number of cells per mL of haemolymph)
CM females: control females in Control Moisture (moisture 80%), LM females: stressed females in Loss of Moisture (moisture 50%), CM males: control males in Control Moisture (moisture 80%), LM males: stressed males in Loss of Moisture (moisture 50%)

820 **2.D**. β-galactosidase activity



821

822 ***Figure 2.D.1.: Effect of thermal stress on β-galactosidase activity***
823 *CT females: control females in Control Temperature (20°C), HT females: stressed females in High Temperature (28°C), CT*
824 *males: control males in Control Temperature (20°C), HT males: stressed males in High Temperature (28°C)*
825



826

827 ***Figure 2.D.2.: Effect of water stress on β-galactosidase activity***
828 *CM females: control females in Control Moisture (moisture 80%), LM females: stressed females in Loss of Moisture (moisture*
829 *50%), CM males: control males in Control Moisture (moisture 80%), LM males: stressed males in Loss of Moisture (moisture*
830 *50%)*
831

832