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1 *Running head: Dehydration and stress in lizards*

2 **Chronic water restriction triggers sex-specific oxidative stress and telomere**
3 **shortening in lizards**

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13 **Abstract**

14 Animals use a variety of strategies to avoid acute dehydration and death. Yet, how chronic
15 exposure to sub-lethal dehydration may entail physiological and fitness costs remains elusive.
16 In this study, we experimentally tested if water restriction causes increased oxidative stress
17 (OS) and telomere length (TL) shortening, two well-described mediators of environment-
18 fitness relationships. We exposed 100 yearling female and male common lizards (*Zootoca*
19 *vivipara*) either to a 51-day period of water restriction or to water *ad libitum*, followed by 45
20 days in common garden outdoor conditions. We measured the kinetic changes in OS and TL
21 and found that water restricted males enhanced antioxidant defences and decreased oxidative
22 damage at day 36, whereas females did not immediately respond. A month and half after
23 water restriction, both sexes experienced a drop in antioxidant capacity but only males
24 exhibited significant TL shortening. In the following 3 years, we found that lizards with
25 longer initial TL and those who maintained stronger antioxidant defences experienced higher
26 longevity, irrespective of sex and water restriction. Together, these results unravelled sex-
27 specific responses to water restriction, with potential applications in better understanding the
28 physiological costs of increasing summer droughts as a result of global climate change.

29

30 **Keywords.** Aging, antioxidant, longevity, oxidative damage, sex, water

31 **Introduction**

32 Water is becoming alarmingly scarce due to increasingly frequent droughts in several areas,
33 with dramatic consequences on terrestrial wildlife [1]. How animals are going to cope with
34 newly warmer and drier conditions yet remains greatly overlooked [2]. Dehydration risk is
35 often considered when regarding the extreme death risk caused by climate severity both in
36 humans and wild species [3–5]. Comparatively, less is known about the impacts of chronic
37 and sub-lethal water restriction periods on organismal life history trajectories [6].

38 Dehydration may incur diverse physiological stresses including oxidative stress (OS),
39 the unbalance between pro-oxidant molecules and antioxidant defences [7,8]. Moreover, OS
40 may affect the telomere length (TL), the DNA sequences capping chromosomes that are
41 involved in genome integrity and cellular replication [9–11]. At the whole organism level,
42 both OS and short TL may be associated to long-lasting fitness costs, which is why those are
43 often considered as reliable mediators of life history trade-offs [12,13]. Nevertheless, the
44 covariation between environmental stress, OS, TL shortening, and long-lasting fitness costs
45 remain greatly elusive [11].

46 Here, we tested whether chronic water restriction may trigger OS and shorten TL early
47 in life of the common lizard (*Zootoca vivipara*), and if this may further impact individual
48 longevity over the following 3 years. In squamate reptiles, correlative evidences imply that
49 drought conditions in lowland climates may trigger OS and TL shortening [14,15]. We
50 focused on sexually immature yearling individuals to standardise chronological age (one year
51 old) and reproductive state (non-reproductive), and given that i) TL is more likely to vary in
52 early life stages [9], and ii) yearling TL integrates stressful climate conditions and correlates
53 with population decline in this species [15]. We hypothesized that dehydration should trigger

54 immediate physiological changes and delayed fitness costs. We therefore expected water
55 restricted individuals to face higher OS, shortened TL, and thereafter lower longevity.

56 **Material & Methods**

57 *Studied species and experiments*

58 In May 2016, we captured 100 yearling lizards (57 females and 43 males) from 10 outdoor
59 enclosures (100 m²) at the CEREEP-Ecotron IleDeFrance (48°17'N, 2°41'E). Lizards were
60 identified with their unique toe clip codes and randomly affiliated to a water treatment, and
61 individualised in terraria maintained under standardised conditions as described previously
62 [8]. Most lizards were unrelated but 23 individuals were siblings from a total 82 families,
63 which we characterised henceforth by a factor called “Mother ID”. We initially provided
64 lizards with water *ad libitum* in water bowl (~100 mL) and with three mists (~50 mL each)
65 per day (09:00, 13:00, 17:00). At the onset of the experiment (see extensive details about
66 experiment timing in [6]), water restriction consisted in reducing the water access to one
67 water spray in the morning (09:00) to mimic the water accessible with morning dew in natural
68 populations. Treatments lasted for 51 days to mimic summer droughts [6]. Females and males
69 from both treatments were then released evenly amongst 10 identical outdoor enclosures
70 under common garden outdoor conditions (Table S1). After an additional 45-day period under
71 relatively warm and dry summer climate [16], we recaptured all lizards we found within a day
72 and obtained a representative sample size (n = 74) to determine the long-lasting consequences
73 on physiology [6].

74 *Oxidative stress and telomere assays*

75 We bled lizards from postorbital sinus (whole blood volume: 40 µl) on three occasions
76 (sampling sessions) to measure the kinetics of physiological changes during water restriction:
77 at day 0 (onset of experiment), at day 36 during water restriction period, and at day 96 (Fig.

78 1). We assessed plasma OS using the reactive oxygen metabolites (ROMs) as an index of
79 damage, and the total plasma antioxidant capacity (OXY) as an index of defence, using
80 colorimetric kits (MC003 & MC435, Diacron International, Italy) and a standard method [8].
81 These two measurements provide reliable markers of oxidative status in vertebrates [17,18].
82 We used rabbit plasma measured at least 8 times in each plate to assess coefficients of
83 variation in ROMs (intra-plate: 2.7%; inter-plate: 4.9%) and OXY (intra-plate: 10.9%; inter-
84 plate: 12.9%). Due to small body size, 3 individuals could not be bled at day 0. In addition,
85 and due to bleeding failure, some individuals did not account in OS analyses. Changes in
86 ROMs (Δ ROMs) and OXY (Δ OXY) were successfully determined on 97% of lizards at day
87 36 (ROMs: n = 96; OXY: n = 94) or after the recapture at day 96 (ROMs and OXY: n = 72).

88 We further used a quantitative Polymerase Chain Reaction (qPCR) to determine
89 relative TL from red blood cells following the same methodology (see all details in ESM
90 methods) as described previously [19]. Changes in TL (Δ TL) compared to initial TL (TL day
91 0) are more likely to occur on a longer time scale than OS, so we maximised the time delay to
92 determine Δ TL (96 days) on a subsample group of lizards (n = 48). The subsample was
93 chosen randomly in each treatment group.

94 *Longevity*

95 We recaptured surviving individuals once each year over the next 3 years to determine the
96 long-lasting consequences of water restriction early in life on longevity. In these captive
97 populations, the recapture rate is above 95% and thereby represents a reliable measure of
98 survival [20]. Longevity within 4 years was a relevant estimate of individual total lifespan
99 given the life expectancy of adult females and males averages 4 and 3 years, respectively [21].
100 In 2019, 15 females and 2 males were still alive, and were therefore censored in the following
101 analyses.

102 *Statistical analyses*

103 All analyses were performed on R software [22]. Physiological data were initially centered by
104 subtracting the mean to facilitate model convergence and interpretation of model output. We
105 used linear mixed models (package *lme4* [23]) to check whether the variation in initial values
106 of OS (ROM-Day0 or OXY-Day0) and TL were explained by random effects of Mother ID
107 (family effect), and the fixed effects of sex, treatment affiliation and their interaction (Table
108 S2). We further used linear mixed models to analyse the physiological changes in OS
109 including either Δ ROM (Δ ROM-Day36 = ROM-Day36 - ROM-Day0 and Δ ROM-Day96 =
110 ROM-Day96 - ROM-Day36) or Δ OXY (Δ OXY-Day36 = OXY-Day36 - OXY-Day0 and
111 Δ OXY-Day96 = OXY-Day96 - OXY-Day36) as the dependent variable, their respective
112 initial value as a linear covariate and sex, hydric treatment, time, first and second order
113 interactions as fixed factors (Table S3). We accounted for Mother ID, lizard ID (repeated
114 design) and the outdoor enclosures (common garden conditions) as random terms (Table S3).
115 Δ TL were measured only at the end of experiments at day 96 so models were simpler (Table
116 S3). Eventually, we used a Cox regression model (package *survival* [24]) and performed
117 hazard ratio tests to examine the impacts of hydric treatment and sex on mortality risk. We
118 further tested the influence of initial values of ROM, OXY and TL (Day0) and their changes
119 over the time in the experiments (Δ ROM-Day36, Δ OXY-Day36, Δ ROM-Day96, Δ OXY-
120 Day96 and Δ TL-Day96), which were previously adjusted by extracting the residuals from the
121 linear regression over their respective initial value. We treated each physiological variable
122 separately in 8 models to maximise the sample size and thereby, the statistical power of
123 analyses.

124 **Results**

125 At day 0, all lizards exhibited similar levels of ROMs, OXY or TL irrespective of sex,
126 treatment and their interaction (Table S2). At day 36, females did not respond to hydric
127 treatment (Fig. 1a,b, Table S3), while water restricted males showed a decrease in ROMs
128 (Fig. 1d, Table S3) compared to control males paralleled with a slight increase in OXY, albeit
129 not different between treatments (Fig. 1e, Table S3). At day 96, water restricted males faced
130 stronger increase in ROMs than controls (Fig. 1d, Table S3), and both sexes previously
131 exposed to water restriction had a drop in OXY (Fig. 1, Table S3). Water restriction had no
132 impact on female TL (Fig. 1c), whereas water restricted males experienced stronger TL
133 shortening than controls (Fig. 1f). TL was not correlated with ROMs markers, but TL tended
134 to shorten in lizards which displayed greater OXY (Fig. S1).

135 During the following 3 years, lizard longevity was higher in females than males ($z =$
136 2.0 , $p = 0.043$, Fig. 2) and similar between hydric treatments ($z = -0.5$, $p = 0.647$, Fig. 2). In
137 addition, longevity was positively explained by variation in OXY at day 96 ($z = -2.4$, $p =$
138 0.018) and by initial TL ($z = -2.0$, $p = 0.042$, Fig. 2), while other physiological traits had no
139 effect (all $p > 0.268$, Fig. 2). Differences between lizards with long and short TL occurred
140 over the next two years following the experimentation irrespectively of lizard sex (Fig. S2),
141 without any effect of hydric treatment on lizard mortality rate (controls: $48 \pm 7\%$; water-
142 restricted: $50 \pm 7\%$).

143 **Discussion**

144 Here, during the first month of water restriction, male lizards faced a drop in ROM paralleled
145 with a slight increase in OXY, but two months later they endured stronger increase in ROM
146 and TL shortening compared to controls. Females were less sensitive to water restriction on
147 the short term, and experienced reduced OXY at the end of the experiment. In a concomitant
148 study, chronic dehydration triggered rapid mass loss, interruption of growth and activity

149 depression irrespective of lizard sex and besides, water restriction had no impact on resting
150 metabolic rate and evaporative water loss [6]. Yet, at the end of experiment, males exhibited a
151 delayed depression of activity and thermoregulation behaviour compared to females,
152 illustrating some other sex-specific tendencies, high activity levels being one of the strongest
153 determinant of telomere erosion in non-human vertebrates [11]. This echoes previous studies
154 showing that males of this species are more active and generally more sensitive to water
155 availability both in early life and during adulthood [16,25,26]. Sex-specific responses to
156 environmental constraints such as the ones described here are more rarely documented [27],
157 despite being ultimately critical for understanding the evolution of reproductive strategies
158 [13].

159 In animals, OS and TL often represent key physiological determinants of life history
160 trajectories, in particular longevity [28,29]. Mechanistically, when ROM exceeds OXY,
161 oxidative imbalance may lead to dysregulated cellular functioning, inflammation and tissue
162 damage, as precursors of injuries, TL shortening and higher mortality risk [10,12]. However,
163 in this study, lizard longevity was neither affected by water restriction, nor by physiological
164 changes. Instead, we found that initial TL as well as the capacity to enhance antioxidant
165 shielding during the following 3 months predicted longevity over the next 3 years.
166 Surprisingly, we also found evidences suggesting that greater Δ OXY negatively correlated
167 with TL erosion during experimentation. Altogether, these findings suggested that individual
168 quality rather than physiological impacts of dehydration *per se* may determine TL in early life
169 and predict long-lasting mortality costs [30].

170 To conclude, this study illustrates sex-dependent physiological responses to chronic
171 water restriction and provides evidences supporting the hypothesis that lifespan correlates
172 with inter-individual differences in OS and TL. We predict that increasingly frequent and
173 intense water deprivation during heat waves and summer droughts should cause higher

174 cellular OS and faster TL shortening in ectotherms. In addition with other known detrimental
175 effects of dehydration (e.g., limited field activity, oxidative costs of reproduction, [8,31,32]),
176 this may therefore partly explain the rapid decline observed in this lizard species [15].

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182 draft of this manuscript.

183 **Competing interest**

184 The authors declare no conflicts of interest.

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191 government (ANR-11-INBS-0001 and ANR-10-IDEX-0001-02).

192 **Data accessibility**

193 The data supporting this article are available at the Zenodo Repository [33].

194 **Authors' contribution**

195 AD conceived the study, led the experiments, performed the oxidative stress assays, ran the
196 analyses and drafted the manuscript. FA and CR completed the telomere assays. SM

197 conceived the study and contributed to the experiments. DRR, BD and SA contributed to the
198 experiments. JFLG supervised and coordinated this study including its conception and long-
199 term data collection. All authors co-wrote the manuscript and agreed to be held accountable
200 for the work performed therein.

201 **Ethics**

202 This study was approved by an independent ethical committee (Apafis 2016040811314849).

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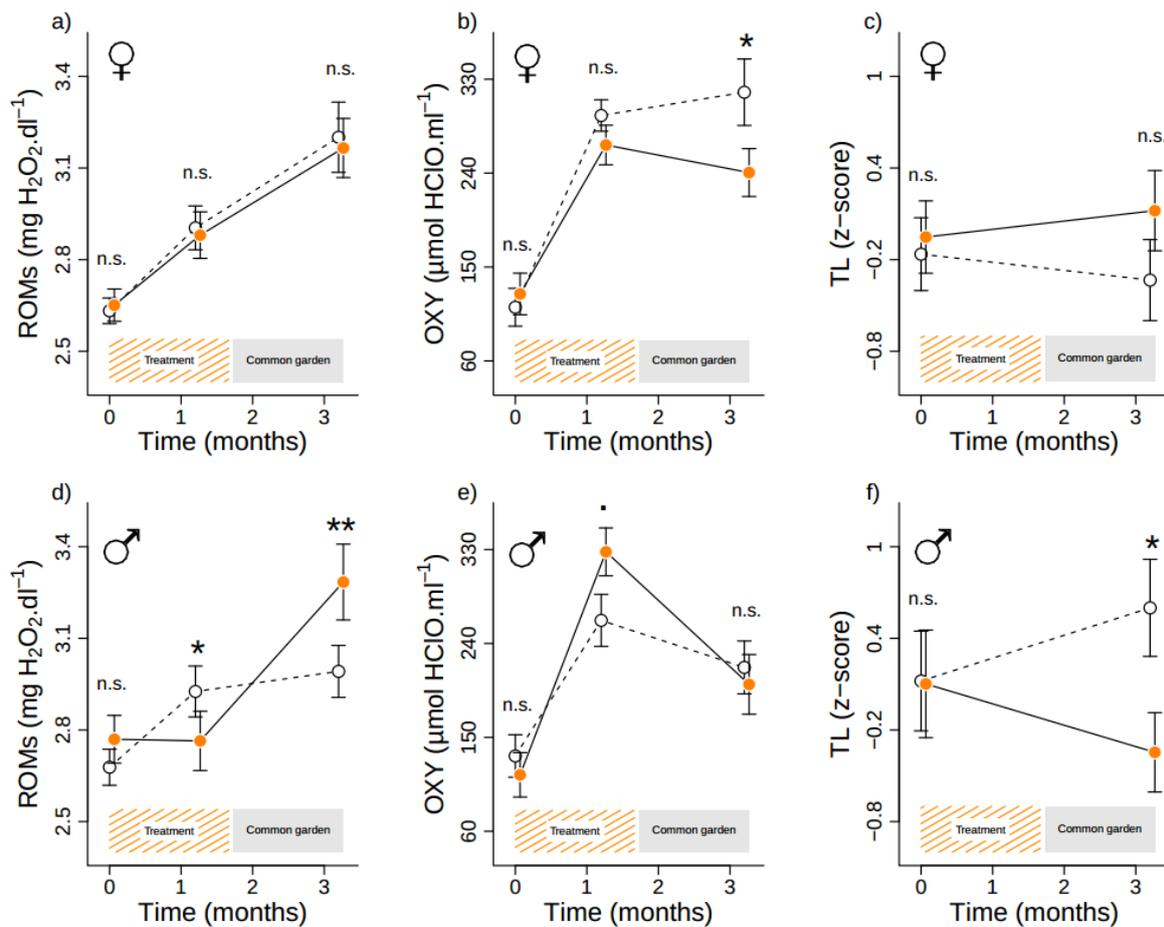
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- 302
- 303

304 **Figure captions.**

305 **Figure 1.** Impacts of dehydration on OS and TL over a 36-day period of water restriction
306 (water restricted individuals: orange symbols – solid lines, controls: white symbols – dashed
307 lines) followed by 15 additional days under water restriction and 45 days in common outdoor
308 conditions. a,b,c) Female and d,e,f) male kinetic responses to water restriction (means \pm SE)
309 differed in oxidative damages (ROM, left panels), antioxidant defences (OXY, middle panels)
310 and telomere length (TL, right panels). Differences between treatments are symbolised: ** p
311 < 0.01, * p < 0.05, • p < 0.06, and n.s. non-significant.

312

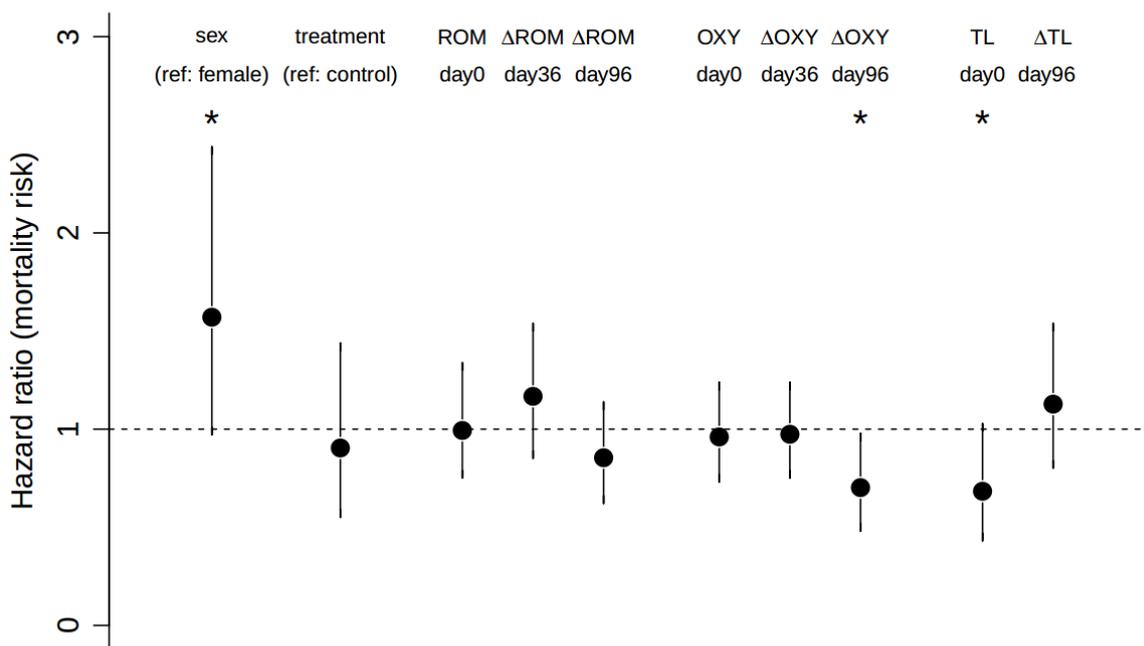
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315

316 **Figure 2.** Effects of sex (n = 57 females and 43 males), hydric treatment (n = 50 control and
 317 50 water restricted), and physiological variables on mortality risk over 3 years. The effect of
 318 each physiological covariate was analysed separately on all lizards of both sexes to maximise
 319 sample size and statistical power. Physiological changes were previously adjusted to initial
 320 value (residuals of linear relationships). For each factor and covariate, hazard ratio (HR) tests
 321 were performed and associated p-values are symbolised: * p < 0.05 when HR was different or
 322 similar to 1 (dashed line). HR > 1 when mortality risk is increased and inversely for HR < 1.
 323



324

325