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### ► **To cite this version:**

Andréaz Dupoué, Frédéric Angelier, Cecile Ribout, Sandrine Meylan, David Rozen-Rechels, et al.. Chronic water restriction triggers sex-specific oxidative stress and telomere shortening in lizards. *Biology Letters*, 2020, 16 (2), pp.20190889. 10.1098/rsbl.2019.0889 . hal-02487162

**HAL Id: hal-02487162**

**<https://hal.science/hal-02487162>**

Submitted on 27 Nov 2020

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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 m<sup>2</sup>) 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 ( $\Delta$ ROMs) and OXY ( $\Delta$ 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 ( $\Delta$ 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  $\Delta$ 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  $\Delta\text{ROM}$  ( $\Delta\text{ROM-Day36} = \text{ROM-Day36} - \text{ROM-Day0}$  and  $\Delta\text{ROM-Day96} =$   
110  $\text{ROM-Day96} - \text{ROM-Day36}$ ) or  $\Delta\text{OXY}$  ( $\Delta\text{OXY-Day36} = \text{OXY-Day36} - \text{OXY-Day0}$  and  
111  $\Delta\text{OXY-Day96} = \text{OXY-Day96} - \text{OXY-Day36}$ ) 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  $\Delta\text{TL}$  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 ( $\Delta\text{ROM-Day36}$ ,  $\Delta\text{OXY-Day36}$ ,  $\Delta\text{ROM-Day96}$ ,  $\Delta\text{OXY-}$   
120  $\text{Day96}$  and  $\Delta\text{TL-Day96}$ ), 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  $\Delta$ 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].

### 177 **Acknowledgments**

178 We thank all people who helped during experiments. We are particularly grateful to Dr.  
179 Darryl McLennan and Dr. Hans Recknagel for sharing with us the qPCR methodology for  
180 telomere measurements and to Dr. Pauline Blaimont for English proofing. We thank Dr.  
181 Gregorio Moreno-Rueda and Dr. Pablo Burraco for providing constructive comments on early  
182 draft of this manuscript.

### 183 **Competing interest**

184 The authors declare no conflicts of interest.

### 185 **Funding**

186 This study received the financial and technical support from the Centre National de la  
187 Recherche Scientifique (CNRS), and was funded by the Agence Nationale de la Recherche  
188 under DESTRESS project (ANR-13-JSV7-0011-01 to SM), the AQUATHERM project  
189 (ANR-17-CE02-0013 to JFLG), and the CPER ECONAT. The work also benefited from  
190 financial support of the Regional Council of Ile-de-France (I-05-098/R) and the French  
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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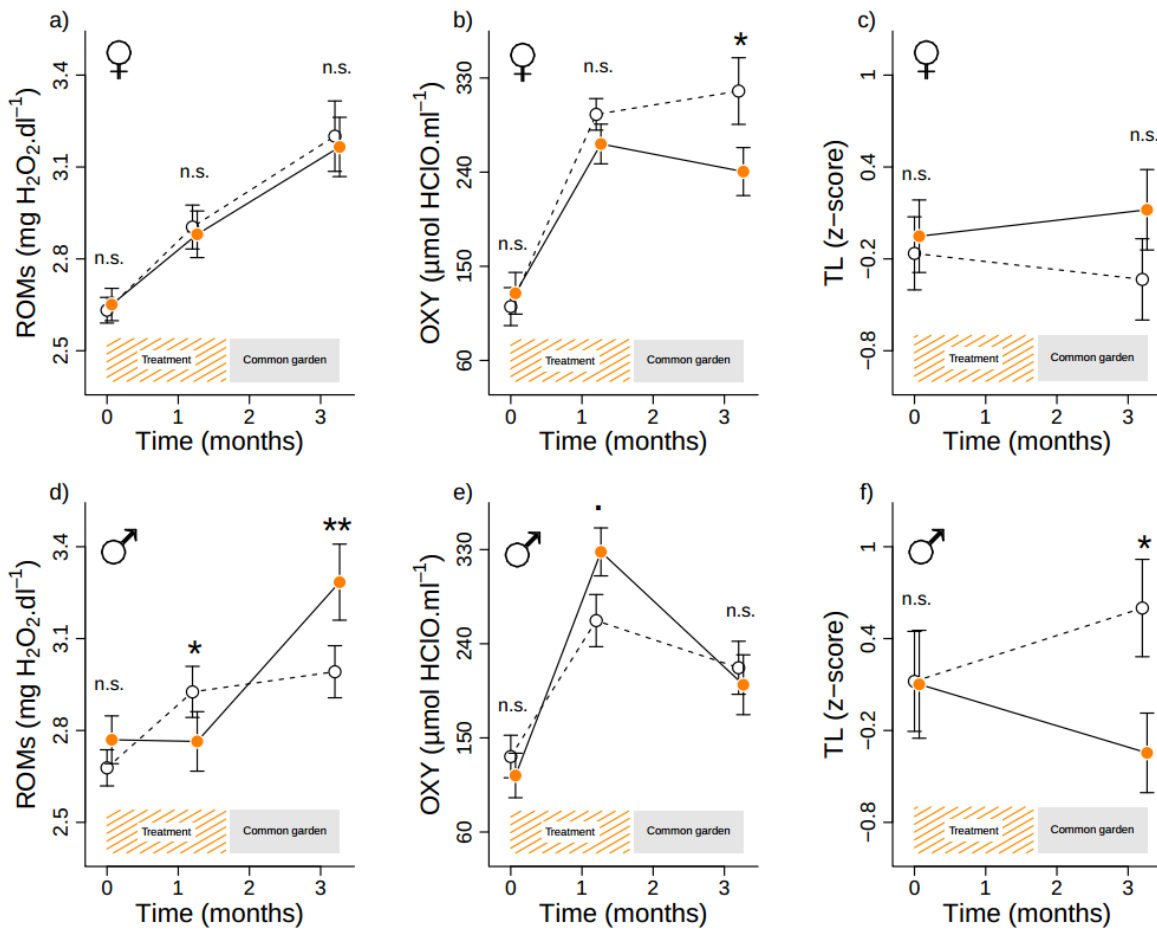
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300 oxidative stress and telomere shortening in lizards. *Zenodo*. See  
301 <https://doi.org/10.5281/zenodo.3606405>.
- 302
- 303

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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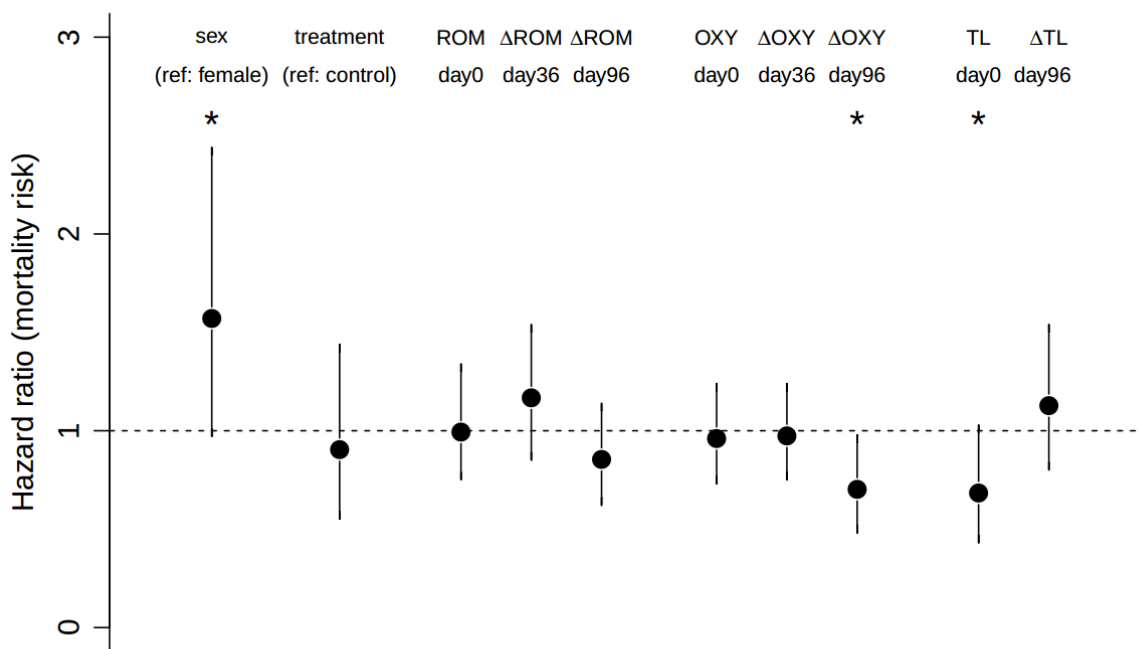


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315



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