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Physiology

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

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

1. Introduction

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

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

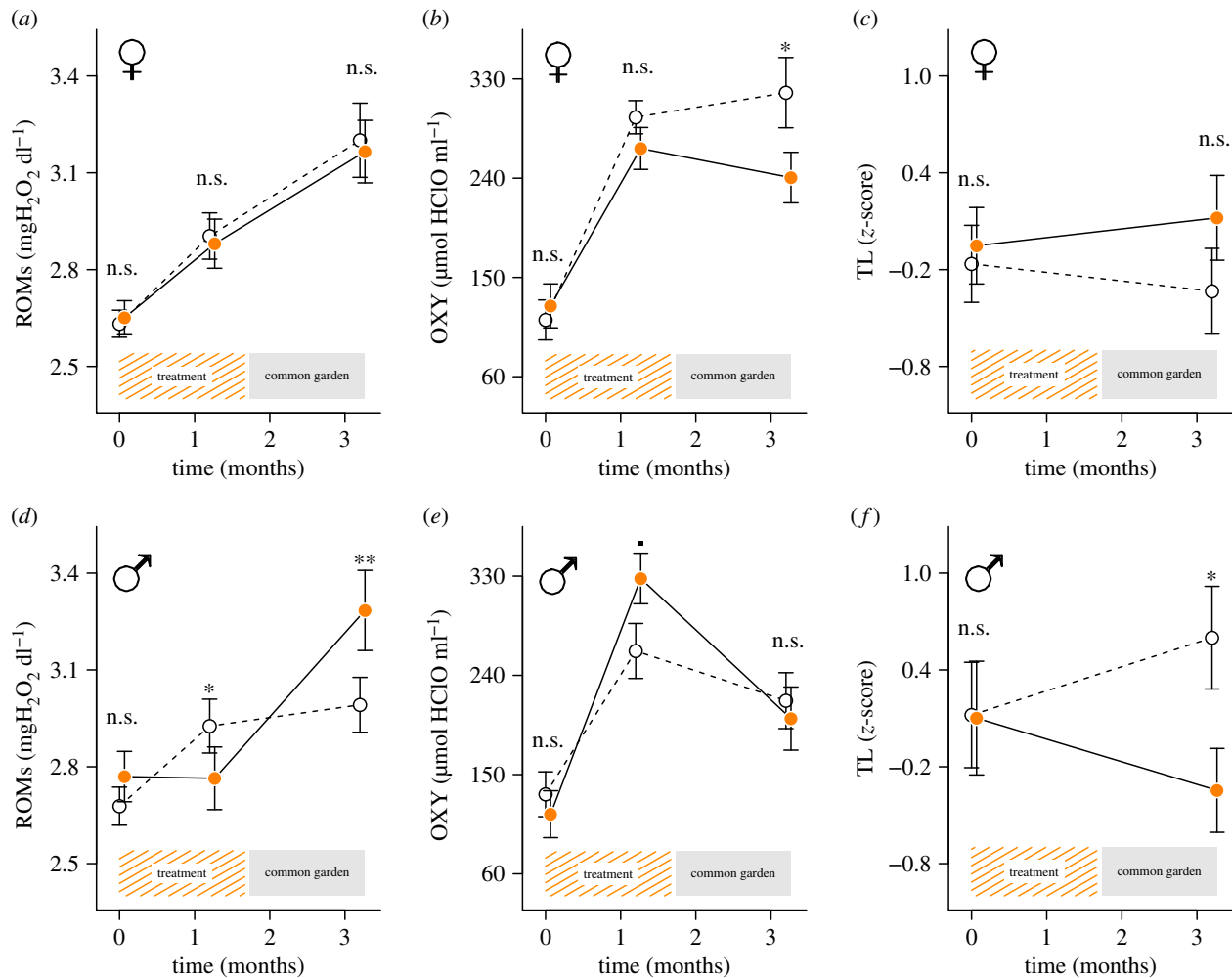


Figure 1. Impacts of dehydration on OS and TL over a 36-day period of water restriction (water-restricted individuals: orange symbols and solid lines, controls: white symbols and dashed lines) followed by 15 additional days under treatment and 45 days in common outdoor conditions. (a–c) Female and (d–f) male kinetic responses to water restriction (means \pm s.e.) differed in oxidative damage (ROMs, left panels), antioxidant defences (OXY, middle panels) and telomere length (TL, right panels). Differences between treatments: ** $p < 0.01$; * $p < 0.05$; ■, $p < 0.06$; and n.s., non-significant.

Here, we tested whether chronic water restriction may trigger OS and shorten TL early in life of the common lizard (*Zootoca vivipara*), and if this may further impact individual longevity over the following 3 years. In squamate reptiles, correlative evidences imply that drought conditions in lowland climates may trigger OS and TL shortening [14,15]. We focused on sexually immature yearling individuals to standardize chronological age (1 year old) and reproductive state (non-reproductive), and given that (i) TL is more likely to vary in early life stages [9] and (ii) yearling TL integrates stressful climate conditions and correlates with population decline in this species [15]. We hypothesized that dehydration should trigger immediate physiological changes and delayed fitness costs. We therefore expected water-restricted individuals to face higher OS, shortened TL and thereafter lower longevity.

2. Material and methods

(a) Studied species and experiments

In May 2016, we captured 100 yearling lizards (57 females and 43 males) from 10 outdoor enclosures (100 m²) at the CEREEP-Ecotron IleDeFrance (48°17'11" N, 2°41'46" E). Lizards were identified, randomly affiliated to a water treatment and individualized in terraria maintained under standardized conditions as described previously [8]. Most lizards were unrelated but 23 individuals were siblings from a total 82 families, which we

characterized henceforth by a factor called 'mother ID'. We provided lizards with water ad libitum in a water bowl (approx. 100 ml) and with three mists (approx. 50 ml each) per day (at 09.00, 13.00, 17.00). At the onset of the experiment (see extensive details about experiment timing in [6]), water restriction consisted in reducing the water access to one water spray in the morning (09.00) to mimic the water accessible with morning dew in natural populations. Treatments lasted for 51 days to mimic summer droughts. Water-restricted lizards experienced a slight mass loss the first week ($-2.36 \pm 0.97\%$), followed by lower mass gain than controls [6]. They were then released evenly among 10 identical outdoor enclosures under common garden outdoor conditions (electronic supplementary material, table S1). After an additional 45-day period under relatively warm and dry summer climate [16], we recaptured all lizards we found within a day and obtained a representative sample size ($n = 74$) to determine the long-lasting consequences on physiology [6].

(b) Oxidative stress and telomere assays

We bled lizards from postorbital sinus (whole blood volume: 40 μ l) on three occasions (sampling sessions) to measure the kinetics of physiological changes during water restriction: at day 0 (onset of experiment), at day 36 during the water restriction period and at day 96 (figure 1). We assessed plasma OS using the reactive oxygen metabolites (ROMs) as an index of damage, and the total plasma antioxidant capacity (OXY) as an index of defence, using colorimetric kits (MC003 and MC435, Diacron International, Italy) and a standard method [8]. These two measurements

provide reliable markers of oxidative status in vertebrates [17,18]. We used rabbit plasma measured at least eight times in each plate to assess coefficients of variation in ROMs (intra-plate: 2.7%; inter-plate: 4.9%) and OXY (intra-plate: 10.9%; inter-plate: 12.9%). Owing to small body size and bleeding failure, some individuals did not account in OS analyses. Overall, changes in ROMs (Δ ROMs) and OXY (Δ OXY) were successfully determined for 97% of lizards at day 36 (ROMs: $n = 96$; OXY: $n = 94$) or after the recapture at day 96 (ROMs and OXY: $n = 72$).

We further used a quantitative polymerase chain reaction (qPCR) to determine relative TL from red blood cells following the same methodology (see all details in electronic supplementary material, Methods) as described previously [19]. Changes in TL (Δ TL) compared with initial TL (TL day 0) are more likely to occur on a longer time scale than OS, so we maximized the time delay to determine Δ TL (96 days) on a subsample group of lizards ($n = 48$). The subsample was chosen randomly in each treatment group.

(c) Longevity

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

(d) Statistical analyses

All analyses were performed with R software [22]. Physiological data were initially centred by subtracting the mean to facilitate model convergence and interpretation of model output. We used linear mixed models (package *lme4* [23]) to check whether the variation in initial values of OS (ROM-Day0 or OXY-Day0) and TL were explained by random effects of mother ID (family effect), and the fixed effects of sex, treatment affiliation and their interaction (electronic supplementary material, table S2). We further used linear mixed models to analyse the physiological changes in OS, including either Δ ROM (Δ ROM-Day36 = ROM-Day36 – ROM-Day0 and Δ ROM-Day96 = ROM-Day96 – ROM-Day36) or Δ OXY (Δ OXY-Day36 = OXY-Day36 – OXY-Day0 and Δ OXY-Day96 = OXY-Day96 – OXY-Day36) as the dependent variable, their respective initial value as a linear covariate, and sex, treatment, time, first- and second-order interactions as fixed factors (electronic supplementary material, table S3). We accounted for mother ID, lizard ID (repeated design) and the outdoor enclosures (common garden conditions) as random terms (electronic supplementary material, table S3). Δ TL was measured only at the end of experiments at day 96 so that models were simpler (electronic supplementary material, table S3). Eventually, we used a Cox regression model (package 'survival' [24]) and performed hazard ratio tests to examine the impacts of treatment and sex on mortality risk. We further tested the influence of initial values of ROM, OXY and TL (Day0) and their changes over the time in the experiments (Δ ROM-Day36, Δ OXY-Day36, Δ ROM-Day96, Δ OXY-Day96 and Δ TL-Day96), which were previously adjusted by extracting the residuals from the linear regression over their respective initial value. We treated each physiological variable separately in eight models to maximize the sample size and thereby, the statistical power of analyses.

3. Results

At day 0, all lizards had similar levels of ROMs, OXY or TL irrespective of sex, treatment and their interaction (electronic supplementary material, table S2). At day 36, females did not

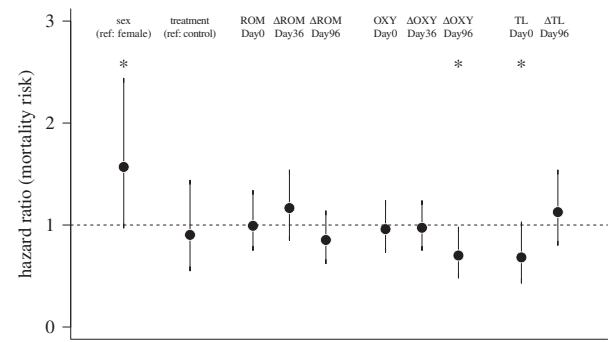


Figure 2. Effects of sex ($n = 57$ females and 43 males), treatment ($n = 50$ control and 50 water-restricted) and physiological variables on mortality risk over 3 years. The effect of each physiological covariate was analysed separately on all lizards of both sexes to maximize sample size and statistical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

respond to treatment (figure 1*a,b*; electronic supplementary material, table S3), while water-restricted males showed a decrease in ROMs compared with control males, paralleled with a marginal increase in OXY (figure 1*d,e*; electronic supplementary material, table S3). At day 96, water-restricted males faced stronger increase in ROMs than controls (figure 1*d*; electronic supplementary material, table S3), and both sexes previously exposed to water restriction exhibited a drop in OXY (figure 1; electronic supplementary material, table S3). Water restriction had no impact on female TL (figure 1*c*), whereas water-restricted males experienced stronger TL shortening than controls (figure 1*f*). TL was not correlated with ROM markers, but TL tended to shorten more in lizards that displayed greater OXY (electronic supplementary material, figure S1).

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

4. Discussion

Here, during the first month of water restriction, male lizards faced a drop in ROMs paralleled with a slight increase in OXY, but two months later they endured a stronger increase in ROMs and TL shortening compared with controls. Females were less sensitive to water restriction in the short term and experienced reduced OXY at the end of the experiment. In a concomitant study, chronic dehydration triggered rapid mass loss, interruption of growth and activity depression irrespective of lizard sex, and besides, water restriction had no impact on resting metabolic rate and evaporative water loss [6]. Yet, at the end of experiment, males exhibited a delayed decrease in activity and thermoregulation behaviour compared with

females, illustrating some other sex-specific tendencies, high activity levels being one of the strongest determinants of telomere erosion in non-human vertebrates [11]. This further echoes previous studies showing that males of this species are more active and generally more sensitive to water availability both in early life and during adulthood [16,25,26]. Sex-specific responses to environmental constraints such as the ones described here are more rarely documented [27], despite being ultimately critical for understanding the evolution of reproductive strategies [13].

In animals, OS and TL often represent key physiological determinants of life-history trajectories, in particular longevity [28,29]. Mechanistically, when ROM exceeds OXY, oxidative imbalance may lead to dysregulated cellular functioning, inflammation and tissue damage, as precursors of injuries, TL shortening and mortality risk [10,12]. However, in this study, lizard longevity was affected neither by water restriction, nor by physiological changes. Instead, we found that initial TL as well as the capacity to enhance antioxidant shielding during the following three months predicted longevity over the next 3 years. Once adults, the majority of lizards reproduce, but in this study we could not test if the relationship between TL and longevity was shaped by the cost of reproduction. Altogether, these findings suggested that individual quality rather than the physiological impacts of dehydration *per se* may determine TL in early life and predict long-lasting mortality costs [30].

To conclude, this study illustrates sex-dependent physiological responses to chronic water restriction and provides evidence supporting the hypothesis that lifespan correlates with inter-individual differences in OS and TL. We predict that increasingly frequent and intense water deprivation

during heatwaves and summer droughts should cause higher cellular OS and faster TL shortening in ectotherms. In addition to other known detrimental effects of dehydration (e.g. limited field activity, oxidative costs of reproduction, [8,31,32]), this may therefore partly explain the rapid decline observed in this lizard species [15].

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

Data accessibility. The data supporting this article are available at the Zenodo repository [33].

Author contributions. A.D. conceived the study, led the experiments, performed the oxidative stress assays, ran the analyses and drafted the manuscript. F.A. and C.R. completed the telomere assays. S.M. conceived the study and contributed to the experiments. D.R.-R., B.D. and S.A. contributed to the experiments. J.-F.L.G. supervised and coordinated this study including its conception and long-term data collection. All authors co-wrote the manuscript, agreed to be held accountable for the work performed therein and approved the final version of the manuscript.

Competing interests. The authors declare no conflicts of interest.

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