# Acclimation to Water Restriction Implies Different Paces for Behavioral and Physiological Responses in a Lizard Species

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## ABSTRACT

Chronic changes in climate conditions may select for acclimation responses in terrestrial animals living in fluctuating environments, and beneficial acclimation responses may be key to the resilience of these species to global changes. Despite evidence that climate warming induces changes in water availability, acclimation responses to water restriction are understudied compared with thermal acclimation. In addition, acclimation responses may involve different modes, paces, and trade-offs between physiological and behavioral traits. Here, we tested the dynamical acclimation responses of a dry-skinned terrestrial ectotherm to chronic water restriction. Yearling common lizards (*Zootoca vivipara*) were exposed to sublethal water restriction during 2 mo of the summer season in laboratory conditions, then released in outdoor conditions for 10 additional months. Candidate behavioral (exploration, basking, and thermal preferences) and physiological (metabolism at rest and standard water loss rate) traits potentially involved in the acclimation response were measured repeatedly during and after water restriction. We observed a sequential acclimation response in water-restricted animals (yearlings spent less time basking during the first weeks of water deprivation) that was followed by delayed sex-specific physiological consequences of the water restriction during the following months (thermal depression in males and lower standard evaporative water loss rates in females). Despite short-term negative effects of water restriction on body growth, annual growth, survival, and reproduction were not significantly different between water-restricted and control yearlings. This demonstrates that beneficial acclimation responses to water restriction involve both short-term flexible behavioral responses and delayed changes in thermal and water biology traits.

*Keywords:* body temperature, water availability, activity, evaporative water loss, exploration, metabolism, reptiles.

## Introduction

Adaptive plasticity is essential for organisms to cope with spatiotemporal variability of their environment and is often the leading mechanism by which they can face the negative ecological impacts of ongoing and future global changes (Somero 2010; Kelly et al. 2012; Seebacher et al. 2015; Wong and Candolin 2015). In particular, thermal plasticity refers to the capacity to adjust phenotypes to changes in thermal conditions and is a major component of species' resistance to climate warming (Sinervo et al. 2010; Gunderson and Stillman 2015; Mitchell et al. 2018). Global changes also entail modifications to rainfall regimes or drought events and therefore water availability in the environment (Field et al. 2012). Water is a critical resource for most terrestrial animals, altering, for example, their locomotor performances (Cheuvront and Kenefick 2014; Anderson and Andrade 2017) and life-history strategies (Lorenzon et al. 2001; Marquis et al. 2008). Terrestrial organisms have therefore evolved numerous plastic strategies to cope with spatial and temporal variation in water availability, hereafter referred to as "hydroregulation tactics" (Ostwald et al. 2016; Eto et al. 2017; Pirtle et al. 2019). Hydroregulation tactics play a key role in water balance regulation (the balance between water inputs and water losses), and the plasticity of hydroregulation tactics will be as critical as thermal plasticity to predicting the consequences of global changes on organisms (e.g., Peterman and Semlitsch 2014; Kearney et al. 2018).

Chronic changes in water availability or water losses induced by predictable seasonal fluctuations in rainfall and temperature or by sustained weather events (e.g., warm spells) may lead to

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acclimation responses in terrestrial animals. These acclimation responses may allow organisms to reduce the costs of performance loss associated with dehydration and, thus, be beneficial in their response to environmental changes in water balance regulation (i.e., beneficial acclimation hypothesis; Leroi et al. 1994; Huey et al. 1999; see Anderson and Andrade 2017 for an example). The acclimation response of a given species is usually a multifaceted process that involves a range of reversible morphological and physiological changes to maintain water balance, including modifications of metabolic rates, adjustments in renal function and osmoregulation, modulations of cutaneous and respiratory water loss rates, and adjustments of body temperature (Peterson 1996; McKechnie 2004; Muir et al. 2007). In terrestrial ectothermic vertebrates, three important water-saving strategies are metabolic depression (e.g., Muir et al. 2007), reduction of transcutaneous evaporative water loss (e.g., Anderson et al. 2017), and thermal depression (Ladyman and Bradshaw 2003). A reduction of resting metabolism following chronic water deprivation is expected to reduce respiratory water loss because metabolism scales linearly with ventilation rate (Woods and Smith 2010; Dupoué et al. 2017*a*). Another water conservation mechanism involves decreased permeability of the water barrier of the skin (Lillywhite 2006; McCormick and Bradshaw 2006). Ectotherms may also respond to water restriction by thermal depression, that is, the lowering of their preferred set temperature (Ladyman and Bradshaw 2003; Köhler et al. 2011; Anderson and Andrade 2017). This is because higher body temperatures are associated with stronger respiratory and cutaneous water loss rates during activity (e.g., Oufiero and Van Sant 2018; Senzano and Andrade 2018). Previous studies focused mostly on one of these acclimation mechanisms, and their prevalence or pace has been little investigated so far, especially in dry-skinned ectotherms.

Physiological mechanisms involved in acclimation may be energetically or ecologically costly, and cheaper alternative responses to cope with dehydration may consequently limit the implementation of beneficial acclimation (Marais and Chown 2008; Huey et al. 2012). Relevant examples in terrestrial animals are dispersal responses (i.e., behavioral flight) or changes in behavioral activity and microhabitat choice within the home range (i.e., behavioral fight responses consisting in behavioral and physiological responses to cope with environmental changes while staying in the same home range, inspired from Hertz et al. 1982), which may concur to increase water intake and reduce water loss. In response to drought, individuals could enhance their exploration rate and locomotor activity to find more suitable hydric environments (e.g., Rozen-Rechels et al. 2018) or, on the contrary, decrease activity and exposure to drying conditions (e.g., less basking) to reduce water loss rates (e.g., Lorenzon et al. 1999; Davis and DeNardo 2010; Kearney et al. 2018). Individuals can also select cooler and wetter microhabitats (Guillon et al. 2013; Dupoué et al. 2015*b*; Pintor et al. 2016). Such flight and fight behavioral strategies are not mutually exclusive and might take place relatively quickly, within hours or days after exposure to chronic water stress if environmental conditions are conducive to behavioral plasticity (Huey et al. 2003; Rozen-Rechels et al. 2018). According to the Bogert effect (Bogert 1949; Huey et al. 2003; Marais and Chown 2008), behavioral flexibility buffers environmental variations to which individuals are exposed and should consequently reduce the benefits of physiological acclimation (i.e., "behavioral inertia" evolutionary scenario; see Huey et al. 2003; Muñoz and Losos 2018). Unfortunately, quantification of joint behavioral and physiological acclimation responses to chronic water stress is rare, and the relative importance of each response mechanism is yet to be elucidated.

One intuitive alternative to the Bogert effect is that each trait response follows a distinct pace, whereby some physiological and behavioral responses come first, followed by other physiological and behavioral adjustments. For example, physiological models of chronic stress responses in vertebrates, such as the allostatic model (McEwen and Wingfield 2003) and the reactive scope model (Romero et al. 2009), predict nonlinear dynamics of behavioral, physiological, and life-history traits when individuals are exposed to a chronic stressor. In these models, behavioral adjustments, being less energetically costly, are expected in the short-term and facilitate the activation of an emergency state. Examples of the kinetics of behavioral and physiological responses to chronic stressors support this hypothesis (Timmerman and Chapman 2004; Romero and Wingfield 2015). To our knowledge, no study to date has examined the kinetics of plastic responses to chronic water deprivation in terrestrial animals, including potential long-lasting effects and fitness responses.

In this study, we investigated temporal patterns of behavioral and physiological adjustments during and after chronic water restriction in the European common lizard, *Zootoca vivipara* (Lacertidae, Lichtenstein, 1823). This species is widespread in Eurasia and depends on cold wetlands and permanent access to free-standing water for demographic persistence (Lorenzon et al. 2001; Dupoué et al. 2017*b*). Acute or chronic exposure to sublethal water deprivation and reductions in water availability in the habitat are associated with dehydration and physiological stress (Dupoué et al. 2017*c*, 2018*a*), a reduction in behavioral activity and body growth (Lorenzon et al. 1999), and some behavioral flight responses (Rozen-Rechels et al. 2018). In addition, the potential reversal of population growth to population decline is associated with increased habitat dryness and thermal stress (Lorenzon et al. 2001; Dupoué et al. 2017*b*). Here, we compared morning activity patterns, standard exploration behavior, thermal preferences, resting metabolic rates, and standard water loss rates from yearling lizards exposed to a 2-mo water restriction period with those of yearlings that had access to water ad lib. During water restriction in the laboratory and after release in outdoor enclosures, lizards were given opportunities to adjust their behavior. We predicted that chronic water restriction in this age class, characterized by limited dispersal behavior and fast growth linked with sexual maturation, would induce immediate water conservation behavioral responses (shifts in activity patterns and fewer exploratory behaviors) along with delayed physiological responses, including thermal and metabolic depression. We further predicted that delayed but potentially long-lasting reduction of standard water loss rates, especially from the skin, would be a consequence of water restriction.

## Material and Methods

### *Study Species, Sampling, and Rearing Conditions*

All common lizards were captured from captive populations maintained in 100-$m^2$ outdoor enclosures at the Centre de Recherche en Écologie Expérimentale et Prédictive (CEREEP-Ecotron IleDeFrance), France (48°17′N, 2°41′E). Enclosures include a natural vegetation layer with permanent access to free water and piles of rocks and logs for basking and sheltering. Ground and avian predation are avoided with plastic walls and nets. The density of the populations are similar (23 ± 5 SD individuals, approximately 67% of yearlings). In these enclosures, hibernation usually lasts from October to February or March, and mating season starts upon emergence of female individuals around March or April and lasts around 2–4 wk. In our study populations, age at maturation ranges from 1 yr (yearlings) to 2 yr old.

In May 2016, 100 sexually immature yearlings (57 females and 43 males) were captured by hand in 10 enclosures, identified by their unique toe clip, and measured for snout-vent length (SVL ± 1 mm) and body mass ($M_b$ ± 1 mg). Lizards were then housed in individual terraria (18 cm × 11 cm × 12 cm) with a shelter and sterilized peat soil as substratum. A basking site (around 35°C), created using a 25-W bulb above the shelter and light from a UVB 30-W neon (ReptiSun 10.0, white light), was available for each terrarium 9 h/d (from 0900 to 1800 hours). Terraria were located in a temperature-controlled room (23°C from 0900 to 1800 hours, 16°C at night). In this setup with a thermal gradient and some microhabitat heterogeneity, lizards express a range of typical field behaviors such as locomotor activity, basking, and hiding in the shelter or in the soil. We therefore expected that these conditions allowed for behavioral responses such as changes in daily activity profiles, propensity of basking and hiding behaviors, and differences in body temperature. Individuals were fed three times a week with 300 ± 10 mg of live house crickets (*Acheta domestica*). Under normal housing conditions, water was available ad lib. in a petri dish and sprayed on one of the walls of the terrarium three times a day (0900, 1300, and 1700 hours). Individuals were released in enclosures on August 1, 2016. Individuals shared the enclosure with adults and newly born juveniles (same density conditions in all enclosures: 41 ± 3 SD, 72% of juveniles). From September 12 to September 16, 2016, corresponding to the late-summer activity season, we recaptured as many individuals as we could (33 males and 40 females). We then recaptured all survivors (recapture rate close to 100%; Le Galliard et al. 2004) during the next reproductive season from May 15 to May 27, 2017 (32 males and 34 females). Nonrecaptured individuals are supposed to have not survived.

### *Water Restriction Manipulation*

After acclimation of all individuals to standard housing conditions in late May 2016, we randomly assigned lizards to two experimental conditions of water availability (Lorenzon et al. 1999; Dupoué et al. 2018*a*; Rozen-Rechels et al. 2018), with a balanced sample of 29 females and 21 males in the water-restricted treatment and 28 females and 22 males in the control treatment. In the water-restricted treatment, individuals were sprayed only once a day at 0900 hours, and the petri dish providing water ad lib. was removed. These conditions mimic habitats in which water is available only with the morning dew during summer (Lorenzon et al. 1999, 2001; Dupoué et al. 2017*b*). This experimental water restriction has already been implemented in past studies over shorter periods from a few days to 2 wk. This protocol is sublethal but is known to enhance physiological responses, although this is relatively less clear in yearlings compared with adults (Dupoué et al. 2018*a*). It also enhances transgenerational and delayed effects in reproductive females (Dupoué et al. 2018*a*; Rozen-Rechels et al. 2018).

In the control treatment, water conditions remained similar to the normal ones described above. These conditions mimic habitats with permanent access to water such as peat bogs and streams. Water restriction treatment lasted from June 10 to July 31, 2016, which is equivalent to a chronic early-summer drought in natural populations (Dupoué et al. 2017*b*, 2018*b*). After that, lizards were released in enclosures corresponding to common-garden conditions. This would allow us to test whether effects of water restriction under laboratory conditions last over time. In case we did not find any effects of water restriction, this setup would allow us to test whether individuals have different life-history trajectories depending on the treatment they experienced in laboratory conditions. Individuals were split randomly into five groups of 20 individuals each (10 control individuals and 10 water-restricted individuals) so that each group was measured on one day (5 d total for one measurement session). We defined five standard measurement sessions of behavioral and physiological data: June 4–8, 2016 (week 0, before water restriction), July 2–6, 2016 (week 4, 1 mo later), July 25–29, 2016 (week 7, 2 mo later and before release in outdoor enclosures), and in September 2016 and May 2017. The chronology of the measurements is summarized in figure 1.

### *Body Mass and Size Measurements*

$M_b$ provides a good indicator of hydration state in reptiles (Lillywhite et al. 2012; Dupoué et al. 2015*a*, 2018*a*). Change in $M_b$ ($\Delta M_b$) was calculated as the difference between initial $M_b$ at the onset of the experiment (week 0) and $M_b$ focally measured throughout the water restriction (weeks 1, 2, 4, and 6–8). In order to standardize measurements, $M_b$ was always measured 2 d after a feeding event. To assess growth of structural size, SVL was also measured in weeks 0 and 4, as well as in September 2016 and in May 2017. Structural growth rate was then defined as the difference in SVL between two measurement sessions divided by the number of days between those sessions. We calculated early growth during the laboratory experiment (growth between weeks 0 and 4; $\Delta \mathrm{SVL}_{\mathrm{experiment}}$), summer growth (growth between week 4 and September 2016; $\Delta \mathrm{SVL}_{\mathrm{summer}}$), and annual growth (growth between September 2016 and May 2017; $\Delta \mathrm{SVL}_{\mathrm{annual}}$).

### *Behavioral Tests*

We measured individual behavior at all standard sessions when lizards were in a postabsorptive state.

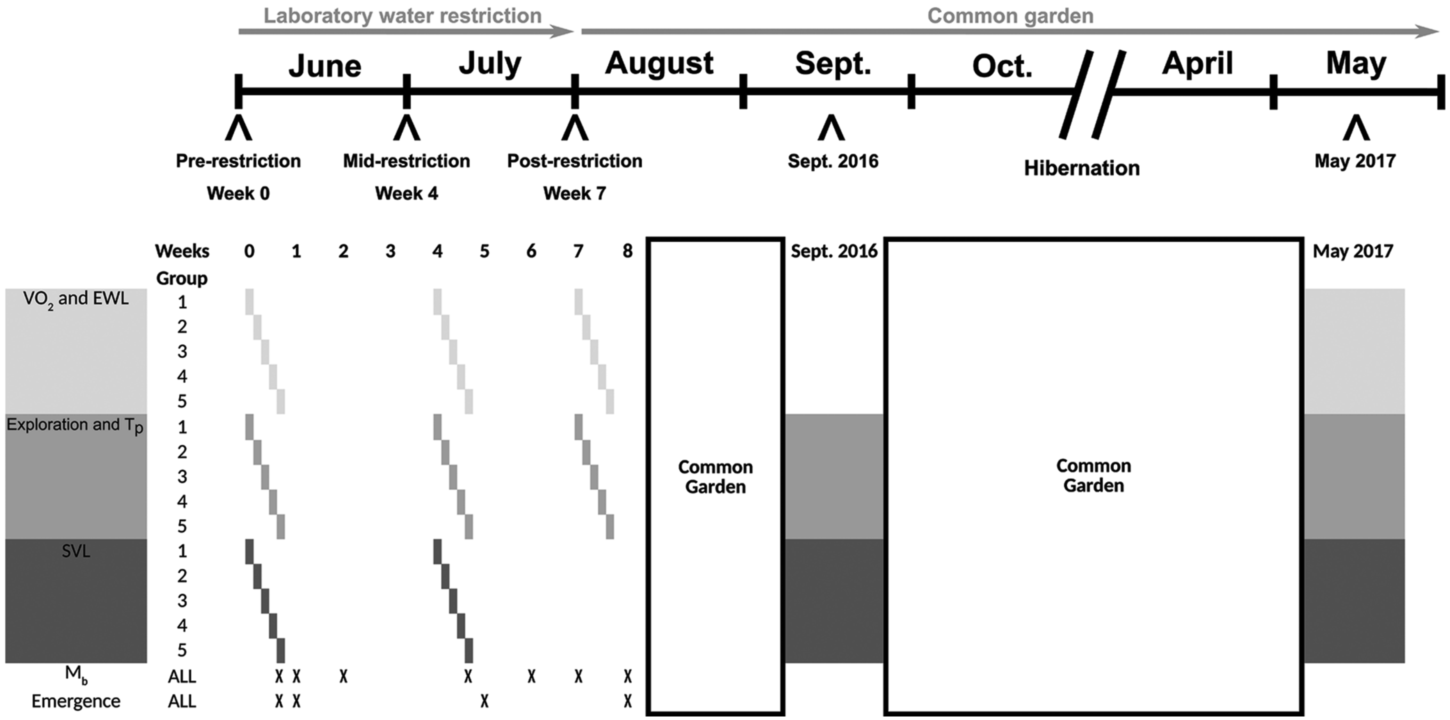

Figure 1. Summarized chronology of the experiment across months. $\dot{V}O_2$ = oxygen consumption; EWL = evaporative water loss; $T_p$ = thermal preferences; SVL = snout-vent length; $M_b$ = body mass. A color version of this figure is available online.

*Emergence Time and Basking Effort in the Home Cage.* We scored lizards' behavior in their home cage during weeks 0, 1, 4, and 7. These measurements were made for all 100 individuals in the same day. From 0830 to 1200 hours, an observer recorded the lizards' behaviors in their cage every 15 min (15 records per trial) with minimum disturbance. Behavioral items included the following: lizard hidden and inactive, lizard basking under the heat source, and any other active behaviors (e.g., moving, scratching the soil). From this, we calculated emergence time (first time the individual was seen active) and basking effort (proportion of observations seen basking).

*Exploration Behavior in a Neutral Arena.* In a temperature-controlled room at 25°C, individuals were tested for their exploration behavior in 30-min tests between 0900 and 1130 hours in a neutral arena. Neutral arenas (44.5 cm × 24 cm × 26 cm) contained a layer of white sand as substrate and were warmed with a 40-W light bulb at a heated basking point. Two UVB 30-W neon tubes were suspended above the arena to homogenize light conditions and avoid phototropism. During the first 10 min, individuals were allowed to habituate to the arena and recover from handling stress (Rozen-Rechels et al. 2018). We then recorded their behaviors using webcams set at five images per second for 20 min. Before each trial, the sand layer was dried and sterilized at 150°C for 2 h in a stove, and arenas were washed between each trial in order to suppress conspecifics' odors in the arena. We extracted 12 behavioral units related to exploration (detailed in app. A) from the videos using the same protocol of image analysis as previously described (Rozen-Rechels et al. 2018). We then used a multivariate analysis to calculate a composite exploration score for each individual within each session (see app. A, table A1, and Rozen-Rechels et al. 2018 for the complete analytical procedure).

*Thermal Preferences in a Neutral Arena.* We measured thermal preferences in a neutral thermal gradient (80 cm × 15 cm × 20 cm) filled with a substratum of dried and sterilized peat soil. We placed a plate of wood on the ground (for basking and hiding) and installed a 40-W heat bulb 15 cm above the ground at the warm side of the gradient (49.1° ± 6.7°C). The cold side of the gradient was maintained at ambient temperature (25.5° ± 1.9°C). A UVB 30-W neon provided white, natural light conditions above each thermal gradient. Heat bulbs were turned on at 1200 hours for 1 h before individuals were randomly placed in one of 20 thermal gradients for habituation for 30 min. Every 20 min until 1710 hours (12 measurements), skin surface temperature ($T_\mathrm{p}$) of lizards was measured at the same focal distance (ca. 30 cm; i.e., measurement in a 6-mm-diameter circle that we were able to assess thanks to three lasers indicating the center of the circle and the diameter of the spot when measuring the temperature) using an infrared thermometer (Raytek Rayynger MX2). This method allows temperature measurements without handling lizards, which are strongly correlated with core temperatures, and provides an accurate measure of preferred body temperature (Bucklin et al. 2010; Artacho et al. 2013). We analyzed preferred temperature in comparison with initial preferred temperatures at the onset of the experiment. To do so, we calculated the difference between each skin surface temperature measurement and the measurement done on the same individual at the same time of the day at week 0, hereafter referred to as $\Delta T_\mathrm{p}$.

### Metabolic Rate and Water Loss

Resting metabolic rate (RMR) and total evaporative water loss (TEWL) were measured overnight at 25°C, when individuals are normally inactive. We measured oxygen consumption ($\dot{V}\mathrm{O_2}$) using closed-system respirometry methods as previously described (Foucart et al. 2014), and we measured TEWL concomitantly. We weighed the lizards ($M_{\mathrm{b}_1}$) and let them acclimate at 25°C for 3 h in late afternoon. After collecting a baseline air sample with adapted locked syringes at the onset of trial (two 140-mL syringes, Medtronic Monoject Luer Lock), we closed the jar and placed the lizards for 12 h in a controlled climatic chamber (Aqualytic TC255). We collected a final air sample from each container and weighed the lizards a second time ($M_{\mathrm{b}_2}$) to estimate body mass loss ($M_{\mathrm{b}_2} - M_{\mathrm{b}_1}$). Oxygen proportion in air samples was determined using an $\mathrm{O_2}$ analyzer (FOXBOX, Sable Systems, Las Vegas, NV). Using an infusion pump (KDS 200, KD Scientific, Holliston, MA), air was sent at a constant flow (60 mL $\mathrm{min^{-1}}$), dried in a column of Drierite, and sent in the $\mathrm{O_2}$ analyzer, which was calibrated before each trial using outdoor air. Oxygen consumption (mL $\mathrm{h^{-1}}$) was calculated as the difference between final $\mathrm{O_2}$ and baseline $\mathrm{O_2}$ multiplied by the exact chamber volume (mL; calculated by measuring the mass of water it can contain) divided by trial duration (h). We also measured $\mathrm{CO_2}$ production (mL $\mathrm{h^{-1}}$), which was highly correlated to oxygen consumption ($R^2 = 0.98$). We calculated the respiratory exchange ratio, defined as the ratio between $\mathrm{CO_2}$ produced and $\mathrm{O_2}$ consumed, which was not affected by the water restriction ($F_{1,98} = 0.04$, $P = 0.83$; ~0.7 in yearlings, ~0.8 in adults).

TEWL (mg $\mathrm{h^{-1}}$) was calculated from body mass loss divided by the time between $M_{\mathrm{b}_1}$ and $M_{\mathrm{b}_2}$ measures, a method previously validated for this species (Dupoué et al. 2017*c*). RMR and TEWL were measured in weeks 0, 4, and 7 during the laboratory experiment (the night before behavioral measurements), as well as in May 2017 at the end of the study (the night after behavioral measurements in order to ensure the same postprandial digestive state of individuals 2 d after recapture). For each individual, because we expected physiological traits to change with time and within individuals, we calculated the changes in RMR (ΔRMR) and TEWL (ΔTEWL) as the difference between individual record of the measurement session and the one at week 0.

### Statistical Analyses

Statistical analyses were performed with R version 3.4.1 (R Core Team 2018). In most analyses, trait variation was analyzed with ANCOVAs using linear models and backward model selection from a full model including the three-way interaction between treatment, sex, and time (measurement session). In the cases of $\Delta M_\mathrm{b}$, ΔSVL, ΔRMR, and ΔTEWL, the initial value at the onset of the experiment was included as fixed effect. For ΔRMR and ΔTEWL, we included individual $M_b$ as a fixed effect in the ANCOVA to account for body mass scaling. Metabolism is indeed positively related to body mass (Gillooly et al. 2001), and water loss rates depend on the whole-body area and respiratory exchanges, both scaling with body mass. For ΔTEWL, we also considered the presence of feces as a fixed, categorical effect to account for corresponding mass loss due to defecation. We separately analyzed treatment effects during the laboratory experiment and delayed effects after recapture in outdoor enclosures, given the substantial changes in sample sizes and individual contribution between sessions. In the first three measurement sessions, we used linear mixed models (lme function from nlme package; Pinheiro et al. 2007) and set individual identity as a random factor to account for repeated measurements between sessions. Sessions in September 2016 and in May 2017 were investigated separately (one observation per individual in each). We included enclosure identity as a random factor to account for among-enclosures variability. In all cases, the best model fit was determined using log-likelihood ratio tests and elimination of nonsignificant factors at 5% critical rate.

We analyzed variation in $\Delta T_p$ in a different way because body temperature was recorded sequentially during the day, and we wanted to account for potential daytime trends in thermal preferences (Artacho et al. 2013). For laboratory sessions, the full mixed effect model thus included a two-way interaction between treatment and sex and a three-way interaction between treatment, measurement session, and time of day. September 2016 and May 2017 thermal preferences were analyzed in separate similar models.

We estimated whether the treatment impacted emergence time using the Cox survival model from the package coxme (Therneau 2018). By analogy to a survival analysis, at each observation, an individual would emerge with a probability $P$ ("die"). If we did not have any observation of the individual at the end of the experiment, we consider that it never went out ("survived"). The model estimates the mean time of emergence. We analyzed the proportion of time spent basking by testing the influence of all possible two-way interactions between treatment, session, and sex on the number of basking events using generalized linear mixed models from the package lme4, considering that the number of basking events is the result of a binomial probability distribution.

We controlled the family-wise error rate due to multiple comparisons by calculating the corrected threshold of significance with the Holm-Bonferroni method based on the number of tests, $m$, realized at each step of the study (laboratory experiment: $m = 8$; after 1 mo in common gardens: $m = 3$; after winter in common gardens: $m = 5$). In each case, $P$ values of significance of the treatment effect were ranked from the lowest to the highest, and the new threshold was calculated as $\alpha_{\text{Holm}} = 0.05/(m + 1 - \text{rank})$ (Holm 1979).

## Results

Initial $M_b$, SVL, $T_b$, RMR, and TEWL values (week 0, before the beginning of the water restriction) are shown in table 1.

### *Effects of Water Restriction on Body Mass and Growth*

Body mass increased on average during the laboratory manipulation ($F_{5,490} = 288.9, P < 0.0001$) but with different temporal trends between treatments (treatment: $F_{1,96} = 28.5, P < 0.0001$; treatment × time: $F_{5,490} = 10.6$, $P < 0.0001$, $\alpha_{\text{Holm}} = 0.006$). Water-restricted yearlings had a decreased $\Delta M_b$ 1 and 2 wk after the beginning of the experiment, but this difference vanished in subsequent sessions (fig. 2*A*). In addition, males had a lower $\Delta M_b$ than females ($F_{1,96} = 4.47$, $P = 0.037$), and $\Delta M_b$ was negatively correlated to initial body mass ($F_{1,96} = 8.2$, $P = 0.005$). During the first month, water-restricted individuals also had a marginally lower $\Delta \text{SVL}_{\text{experiment}}$ compared with control individuals ($F_{1,96} = 4.235, P = 0.042, \alpha_{\text{Holm}} = 0.008$; see fig. 2*B*). The treatment did not affect $\Delta \text{SVL}_{\text{summer}}$ and $\Delta \text{SVL}_{\text{annual}}$ (all $P > 0.59$ , all $\alpha_{\text{Holm}} < 0.05$; see fig. 2*B*). Males had consistently lower ΔSVL than females (all $P < 0.007$), and ΔSVL was negatively correlated to initial body size (all $P < 0.002$).

### *Emergence and Basking Effort in Home Cage*

Time since the beginning of the experiment significantly explained variation in emergence behavior ($\chi^2_3 = 107.7$, $P < 0.0001$), but treatment had no effect ($\chi^2_1 = 1.01$, $P = 0.31$, $\alpha_{\text{Holm}} = 0.01$;

Table 1: Physiological and behavioral parameters measured at week 0 (before the start of the water restriction treatment)

| | Control individuals | | Water-restricted individuals | |
|---|---|---|---|---|
| Parameter | Females | Males | Females | Males |
| $M_b$ (g) | 1.68 ± .04 | 1.57 ± .04 | 1.64 ± .04 | 1.57 ± .03 |
| SVL (mm) | 49.6 ± .4 | 46.5 ± .5 | 50.0 ± .5 | 46.3 ± .5 |
| $T_p$ (°C) | 33.4 ± .1 | 33.2 ± .1 | 32.9 ± .1 | 33.9 ± .1 |
| RMR (mL $h^{-1}$) | .32 ± .01 | .30 ± .01 | .31 ± .01 | .32 ± .01 |
| TEWL (mg $h^{-1}$) | 3.6 ± .2 | 4.0 ± .2 | 3.8 ± .2 | 4.2 ± .3 |

Note. Values are average ± SE. See text for abbreviation definitions.

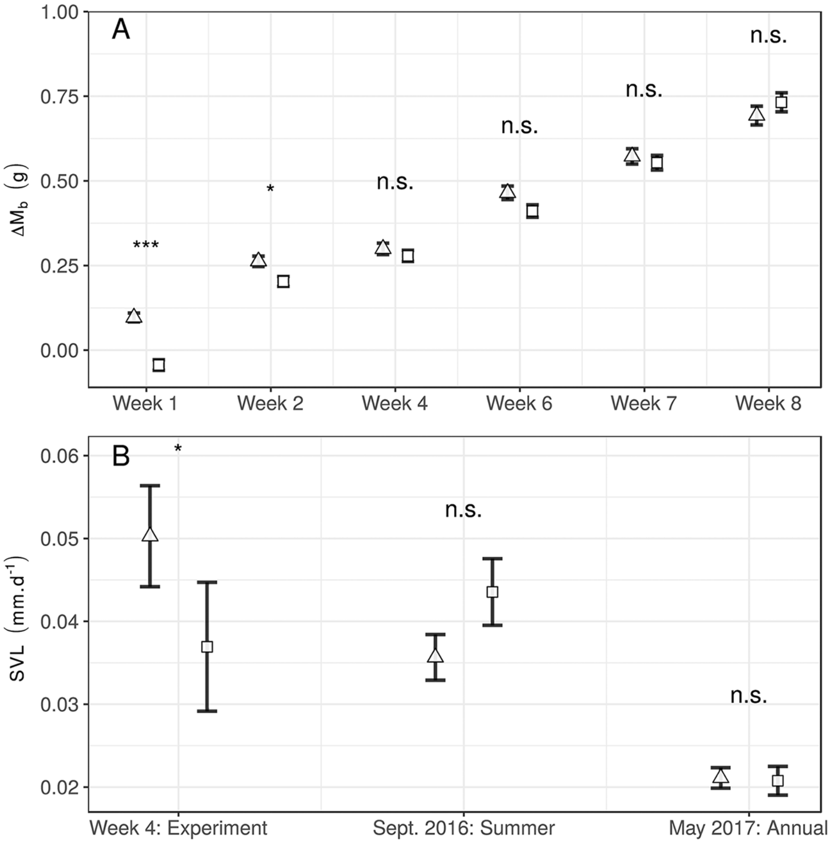

Figure 2. *A*, Average change in body mass ($\Delta M_b$; g ± SE) during the laboratory experiment. *B*, Change in body size growth (ΔSVL; mm $d^{-1}$ ± SE) during and after the laboratory experiment. Change was calculated since the beginning of the experiment. Control individuals are represented by a triangle; water-restricted individuals are represented by a square. Symbols indicate the significance of the difference between treatments at each measurement: n.s. = nonsignificant; $^*P < 0.05$; $^{***}P < 0.001$.

see fig. 3*A*). Basking effort was also influenced by time since the beginning of the experiment but in interaction with water restriction treatment (treatment × time: $\chi^2_3 = 14.2$, $P = 0.003$, $\alpha_{\text{Holm}} = 0.007$; fig. 3*B*) and in interaction with sex (time × sex: $\chi^2_3 = 10.3$, $P = 0.02$). One week after the beginning of the experiment, control yearlings spent significantly more time basking than water-restricted individuals (fig. 3*B*), whereas differences between treatments later disappeared (all $P > 0.25$). In addition, no sex differences existed during the first month (all $P > 0.17$), but males basked significantly less than females at the end of the experiment ($z = -3.18$, $P = 0.001$).

### *Exploration Score in Neutral Arenas*

Exploration score was lower on average 1 mo and 2 mo after the start of the experiment than at the beginning of the study ($F_{2,196} = 10.84$, $P < 0.0001$), but there was no effect of water restriction on exploration ($F_{1,98} = 0.13$, $P = 0.72$; treatment × time: $F_{4,194} = 0.91$, $P = 0.40$, $\alpha_{\text{Holm}} = 0.016$; see fig. 4*A*). One month after the end of the experiment (September 2016), exploration score was influenced by a two-way interaction between treatment and sex ($F_{1,69} = 5.7$, $P = 0.02$, $\alpha_{\text{Holm}} = 0.017$–$0.025$). Control females and males did not differ in their exploration rate ($t_{69} = 1.57$, $P = 0.12$) nor did control and water-restricted females ($t_{69} = 1.78$, $P = 0.08$). However, water-restricted males explored their environment significantly less than control males ($t_{69} = -2.38$, $P = 0.02$). One year later, water restriction treatment had no effect on exploration score ($F_{1,64} = 0.02$, $P = 0.9$, $\alpha_{\text{Holm}} = 0.05$).

### *Thermal Preferences*

Preferred body temperature did not change significantly during the laboratory study ($F_{1,2,273} = 1.73$, $P = 0.19$) or between treatments ($F_{1,98} = 0.26$, $P = 0.61$; treatment × time: $F_{1,2,273} = 0.23$, $P = 0.63$, $\alpha_{\text{Holm}} = 0.05$; see fig. 4*B*). One month after the end of the laboratory study, intraindividual $\Delta T_p$ was influenced by a two-way interaction between sex and treatment ($F_{1,69} = 5.74$, $P = 0.02$, $\alpha_{\text{Holm}} = 0.017$–$0.025$; see fig. 4*B*), as well as by time of day ($F_{1,802} = 7.94$, $P = 0.005$). At this stage, water restriction did not change thermal preferences of females ($t_{69} = 0.38$, $P = 0.47$) but decreased the thermal preferences of

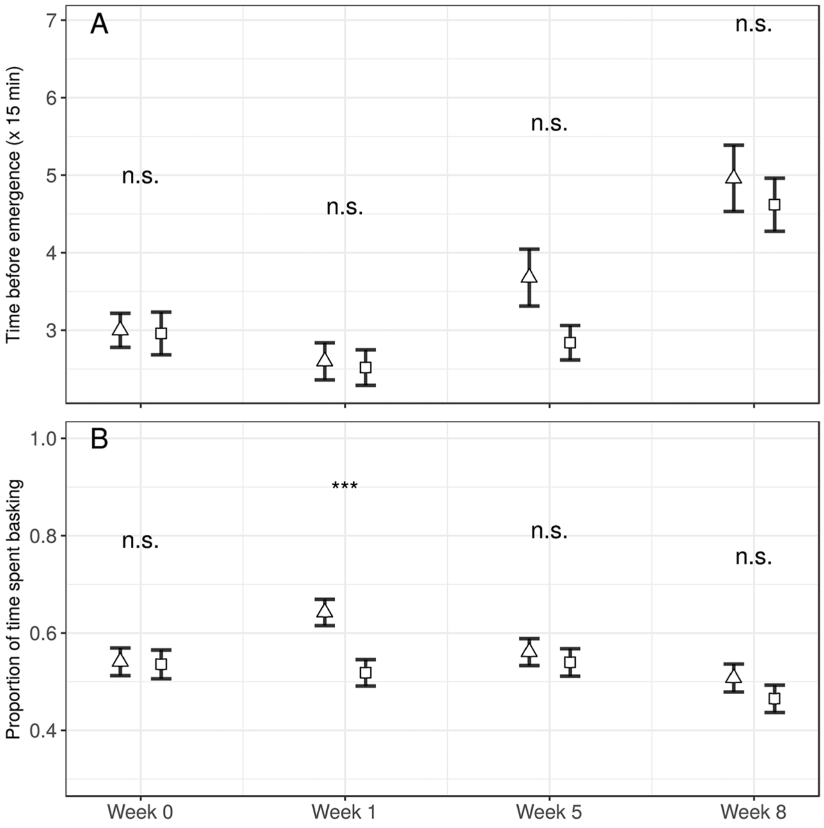

Figure 3. Time spent before emergence (in 15-min steps ±SE; *A*) and proportion of time (±SE) spent basking (*B*) by lizards in their home cages during morning (laboratory experiment). Control individuals are represented by a triangle; water-restricted individuals are represented by a square. Symbols indicate the significance of the difference between treatments at each measurement: n.s. = nonsignificant; $^{***}P < 0.001$.

males ($t_{69} = -2.39, P = 0.02$). One year later, we found no effect of treatment conditions ($F_{1,64} = 0.26, P = 0.61, \alpha_{\text{Holm}} = 0.017$).

*Standard Metabolism and Water Loss*

During the laboratory experiment, ΔRMR was negatively related to initial values ($F_{1,97} = 21.3, P < 0.0001$) but independent of treatment and session groups (treatment: $F_{1,97} = 2.23, P = 0.14$; treatment × time: $F_{1,97} = 0.25, P = 0.62, \alpha_{\text{Holm}} = 0.025$) and sex (treatment × sex: $F_{1,95} = 1.15, P = 0.29$). ΔRMR measured from metabolic rate at recapture during the next reproductive season was marginally lower in water-restricted yearlings ($F_{1,59} = 3.66, P = 0.061, \alpha_{\text{Holm}} = 0.0125$; see fig. 5*A*). In addition, ΔTEWL decreased during the laboratory experiment ($F_{1,96} = 28.5, P < 0.0001$; see fig. 5*B*) independently from the treatment (treatment: $F_{1,97} = 1.64, P = 0.20$; treatment × time: $F_{1,96} = 0.75, P = 0.39, \alpha_{\text{Holm}} = 0.01$). ΔTEWL at recapture during the next reproductive season was significantly explained by a two-way interaction between sex and treatment ($F_{1,48} = 4.35, P = 0.04, \alpha_{\text{Holm}} = 0.01$; see fig. 5*B*). Water-restricted females had lower ΔTEWL than control females ($t_{48} = -2.69, P = 0.01$), whereas no treatment effect was detected in males ($t_{48} = 0.33, P = 0.75$). In all cases, ΔTEWL was negatively correlated to the initial TEWL value (all $P < 0.0001$). ΔTEWL during the laboratory experiment was positively correlated to body mass ($P = 0.0001$).

## Discussion

We exposed yearling lizards to a sublethal, chronic water restriction episode lasting almost 2 mo, during which lizards could substantially buffer dehydration with behavioral shifts. Our data revealed acclimation kinetics of the behavioral and physiological responses to water deprivation. The kinetics of these behavioral and physiological responses were generally in agreement with our initial predictions, since flexible behavioral changes came first, followed by delayed sex-specific acclimation responses in thermal physiology (thermal depression) and standard evaporative water loss. However, in contrast to our expectations, we did not find any short-term thermoregulation adjustments in our laboratory setting. Despite significant short-term negative effects of water deprivation on body mass change and marginal negative effects on body size growth during the first weeks of manipulation, which could suggest short-term physiological adjustments that we did not measure, the annual life-history strategy of subadult lizards

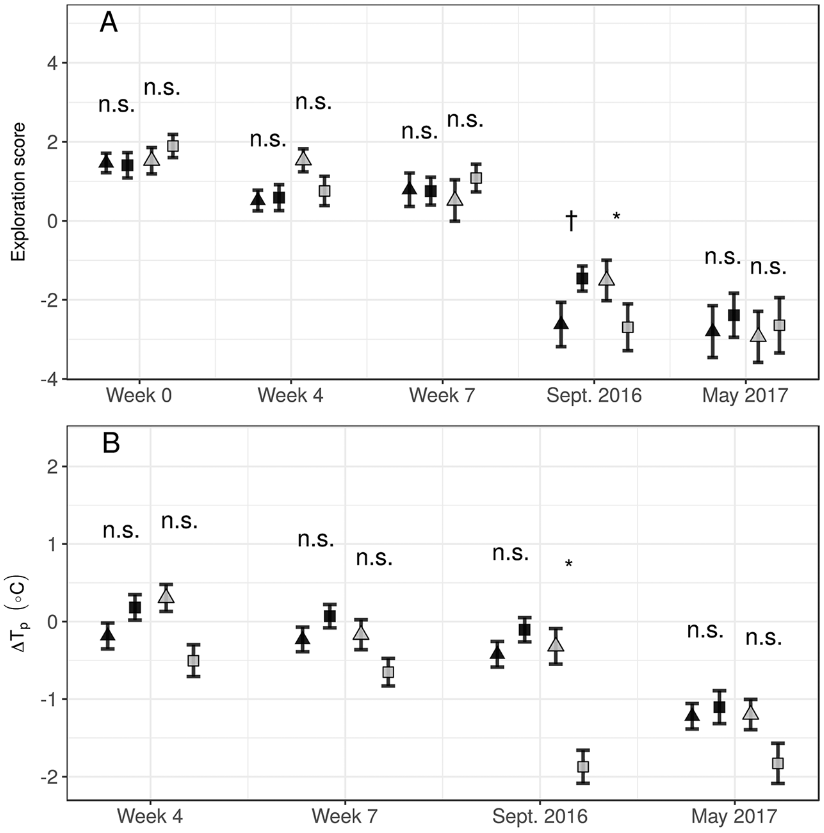

Figure 4. *A*, Mean exploration score (±SE) calculated from behavioral measurements in a neutral arena at each session. *B*, Intraindividual change in thermal preferences ($\Delta T_p$; °C ± SE) since the beginning of the experiment. Black and gray symbols represent females and males, respectively. Control individuals are represented by a triangle; water-restricted individuals are represented by a square. Symbols indicate the significance of the difference between treatments at each measurement: n.s. = nonsignificant; †$P < 0.10$; *$P < 0.05$.

from the two treatments did not differ for total annual size growth or for annual survival (see app. B) and future reproduction of females (see app. C). Differences in body mass change might be the consequence of differences in muscle volume (storing water and sources of metabolic water) or differences in hydration state. We thus conclude that phenotypic plasticity in *Zootoca vivipara* compensates to some degree for the homeostatic load imposed by dehydration risk. This supports the beneficial acclimation hypothesis, at least in our laboratory setting (Huey et al. 1999; Kelly et al. 2012), and the kinetics are consistent with previous studies on acclimation to other stressors. In the sailfin molly *Poecilia latipinna*, fishes exposed to extreme hypoxia display an immediate physiological response (increased ventilation) and an immediate behavioral response (increased aquatic surface respiration) that decrease over time as gradual acclimation processes take place (e.g., changes in hemoglobin concentration; Timmerman and Chapman 2004). Testing whether observed adjustments give significant advantages in the water-restricted environment would however be necessary to strictly conclude that we observe beneficial acclimation.

Immediate behavioral responses to water restriction included a sharp reduction of basking activity, which was paralleled by a reduction of body size growth. In ectothermic species, an increase of thermoregulation effort and high body temperatures closed to thermal preferences would increase water loss rates compared with resting conditions. A reduction of basking effort can therefore be interpreted as a water conservation strategy (Lourdais et al. 2017). This reduction to conserve water can however conflict with energy intake and allocation to structural growth or reserves (Adolph and Porter 1993; Niewiarowski 2001). Similar results were observed in a previous comparative study with wild-caught yearlings from dry and humid natural habitats: lizards were also less active after a 1-mo-long water scarcity and grew slower in the laboratory, and the spatial variation in growth rates observed between wet and dry natural habitats paralleled the results of the laboratory study (Lorenzon et al. 1999, 2001). During the same time period of our laboratory manipulation, we found no plastic changes for thermal preferences, resting metabolism, and standard water loss rates, which were expected, considering recent studies on other ectotherms (Muir et al. 2007; Anderson and Andrade 2017; Anderson et al. 2017). This may suggest a Bogert effect, as short-term changes in a key behavioral trait, basking activity, compensated for the environmental changes induced by water restriction and protected individuals

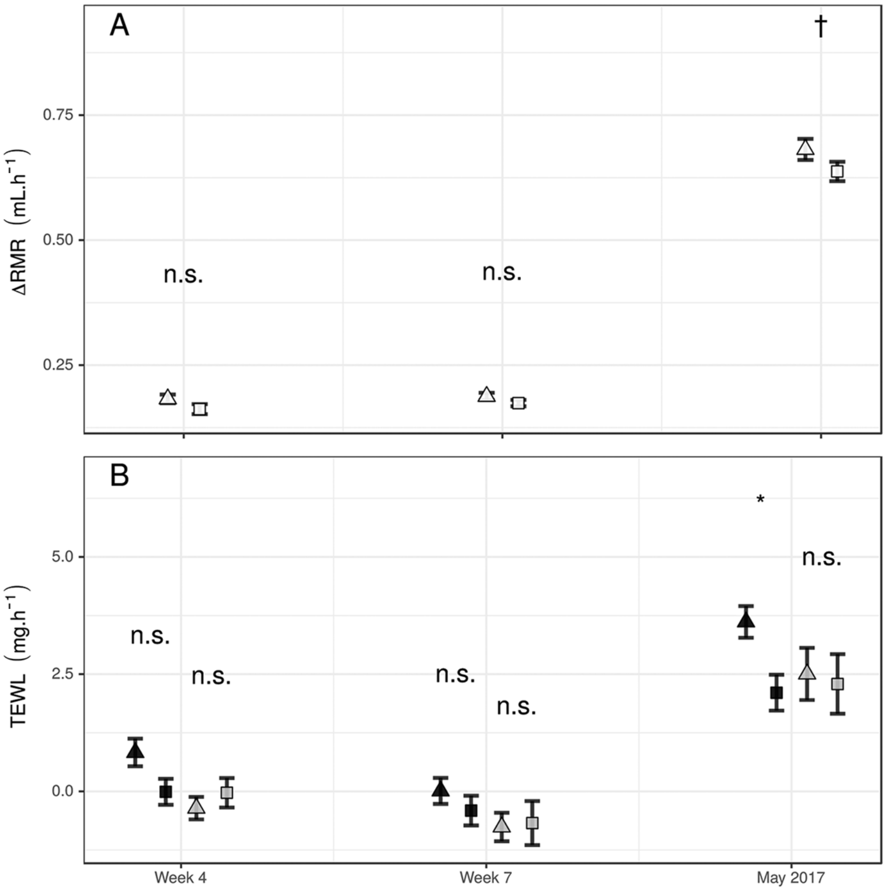

Figure 5. Intraindividual change in basal metabolic rate (ΔRMR ± SE) variation calculated from $O_2$ consumption (*A*) and standard evaporative water loss rates (ΔTEWL ± SE; *B*) during and after the laboratory experiment. Change was calculated since the beginning of the experiment. In *B*, black and gray symbols represent females and males, respectively. Control individuals are represented by a triangle; water-restricted individuals are represented by a square. Symbols indicate the significance of the difference between treatments at each measurement: n.s. = nonsignificant; †$P < 0.10$; *$P < 0.05$.

from its deleterious effects. We, however, cannot exclude that other physiological responses that we did not measure in our study were stimulated by water deprivation (e.g., shift from carbohydrates to protein catabolism to increase metabolic water production [see Brusch et al. 2016] or hormone-induced changes in renal function and osmoregulation to save water lost in urine and feces [see McCormick and Bradshaw 2006]). In addition, some traits may not respond because yearling lizards generally have lower water requirements than adults and are therefore less sensitive to water restriction than older lizards (Dupoué et al. 2018*a*). In support of this hypothesis, no difference in basking activity was detected after the first month of water deprivation and therefore no Bogert effect was expected. Yet we found no acclimation response for other behavioral or physiological traits during the second half of the laboratory experiment, and water-restricted individuals caught up their growth delay. This suggests that fast and reversible physiological adjustments took place.

We predicted delayed responses for some physiological traits but did not expect specifically delayed sex-specific effects of water restriction on exploration behavior and thermal preferences at the end of summer, as observed in male yearlings in this study. Based on a previous study of water-dependent exploration behavior (Rozen-Rechels et al. 2018) and outcomes of thermal acclimation experiments (Clusella-Trullas and Chown 2014), we instead assumed that exploration and, to some extent, thermal preferences would be more flexible and would respond earlier following water deprivation (e.g., Rozen-Rechels et al. 2018). The delayed responses found in males are concordant with a water conservation strategy (i.e., fight response, aiming at buffering the effects of the environmental constraints on the physiology), since lower exploration scores and thermal depression imply lower rates of water loss from locomotor activities and from transcutaneous evaporation (Lourdais et al. 2017). The high sensitivity of male common lizards to weather conditions has already been demonstrated in previous studies (Le Galliard et al. 2006; Dupoué et al. 2018*a*) and is also consistent with recent results showing lower growth rate in juvenile males exposed to abnormally hot summers (Dupoué et al. 2019). Interestingly, during the time period when yearlings were maintained in enclosures in late summer 2016, the study site experienced an abnormal heat wave characterized by very low precipitation in August and a warmer and drier September than average (Meteo France: +0.9°C for August average temperature and +2.4°C for September, with rain deficits of 60% in August and >30% in September compared with previous years). Thus, one possibility is that the delayed acclimation responses were caused by a facilitation process, whereby a new environmental stressor (here, a hot and dry summer) causes a higher than expected stress response in animals that were exposed to past stressful conditions (here, the laboratory water deprivation; see Trompeter and Langkilde 2011 for an example of facilitation response to predation stress in a lizard). To test this hypothesis, future studies that independently manipulate the environmental conditions experienced by male yearlings in two successive later stages are needed.

Another negative delayed effect of water restriction was found for standard water loss rates in female lizards the year after the laboratory experiment, when females had reached sexual maturity and were all gravid. Variations of TEWL were positive, which can be explained by the higher mass of individuals at the adult stage. Water-restricted females, however, had lower change of TEWL than control ones. This effect is weak and needs further confirmation and should therefore be cautiously interpreted, but it is also consistent with a water conservation strategy. Since metabolic rates and body size were not different between water-restricted and control females during this time period, we assume that these differences might reflect changes in cutaneous water loss rates and therefore probably cutaneous resistance to water loss between water-restricted and control females. This explanation is also supported by the robustness of the result of our statistical analyses of TEWL with inclusion of ΔRMR (changes in metabolism, hence, changes in ventilation rates) as a covariate, indicating that water restriction changes total water loss rates even after corrections for differences in respiratory activity (see table B1). This slight change in standard water loss rates supports our initial prediction of a water conservation strategy. Other studies that have tested for plastic changes in cutaneous water loss rates generally found that lizards or snakes acclimated to drier environments or to water-restricted environments can adjust cutaneous water loss rates within days or weeks of exposure (Kobayashi et al. 1983; Kattan and Lillywhite 1989; Moen et al. 2005 but see Neilson 2002; Gunderson et al. 2011). Reduction of cutaneous water loss rates is also well documented in anurans exposed to chronic water deprivation (Anderson et al. 2017; Senzano and Andrade 2018). It is generally accepted that the skin's resistance to water loss depends mainly on the intracellular lipid content and the ultrastructure of the mesos layer of the epidermis (reviewed by Lillywhite 2006). Further investigations of skin properties would be necessary to know the mechanisms underlying delayed acclimation patterns in the common lizard. This female-specific acclimation response of skin properties may be explained by the stronger reliance of females on water during gestation and embryonic development in this viviparous species (Dupoué et al. 2015*a*, 2018*a*).

In conclusion, the results of our multivariate analysis of the physiological and behavioral responses to chronic water deprivation in the common lizard are, broadly speaking, consistent with general predictions about the kinetics of a long-term acclimation process and stress response (Huey et al. 1999; Romero and Wingfield 2015). According to this framework, less costly and more flexible adjustments of daily behavioral routines should occur first, followed by delayed responses in thermal preferences, metabolism, and eventually, cutaneous water loss rates (e.g., Timmerman and Chapman 2004). Such dynamical changes may lead to an "emergency life history stage" (sensu Wingfield et al. 1998) that promotes survival in the face of a challenging environment, as seen in this study in which water-restricted lizards had annual growth, survival, and reproduction similar to control lizards. Traits involved in acclimation responses were those most strongly linked to thermoregulation (basking and thermal preferences), but we found no evidence of metabolic depression, although this is a widespread response to water restriction and energy intake reduction in endotherms (Ruf and Geiser 2015) and dry-skinned ectotherms (Christian et al. 1996*a*, 1996*b*). Our results are partly consistent with biophysical models predicting that hydroregulation responses would rely mostly on evaporative water loss changes but also on activity pattern changes and not on metabolic depression (Pirtle et al. 2019). Surprisingly, we found sex-specific acclimation responses, in line with previous findings (e.g., Dupoué et al. 2018*a*), that could be explained by ecological and physiological differences between males and females. Interestingly, males seem more susceptible to water restriction than females, as previous findings might suggest (Le Galliard et al. 2006; Dupoué et al. 2019). This study illustrates the complexity of acclimation responses to water restriction in dry-skinned terrestrial ectotherms.

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## APPENDIX A

### Estimation Methods of Exploration Rate Following Rozen-Rechels et al. (2018)

Behavioral statistics were measured from videos, transformed into image sequences, using the image analysis protocol of Rozen-Rechels et al. (2018). We measured the relative time spent active ($T$), the total distance walked by the lizard ($D$), the number of activity bouts (i.e., number of sequences of consecutive images with positive step length, $N_{\text{bouts}}$), the average distance walked during a bout ($D_{\text{bouts}}$), the average distance walked between two images in a bout ($M_{\text{bouts}}$), the mean standard deviation of distances walked between two images in a bout (homogeneity of displacements; $\text{SD}_{\text{bouts}}$), the time spent by the lizard in the buffer zone along the walls ($T_{\text{walls}}$), the total distance walked out of the buffer zone ($D_{1\text{walls}}$), the mean distance to the walls ($D_{2\text{walls}}$), and the standard deviation of the distance to the walls ($\text{SD}_{\text{walls}}$). We further subdivided the neutral arena into 24 equal squares to estimate the total area visited by the lizards, including the proportion of squares visited at least once by the individual ($\tau_{\text{explo}}$) and the standard deviation of the number of locations per square or heterogeneity of the exploration ($\text{SD}_{\text{explo}}$).

In order to calculate the exploration rate, we transformed each behavioral trait using a Box-Cox power function to meet gaussian requirements. We used principal component analysis (PCA; R package ade4; Dray and Dufour 2007) to identify correlated behaviors (i.e., behavioral syndromes). The first component of the PCA (PC1) corresponded to the one identified by Rozen-Rechels et al. (2018) as an exploration syndrome (same correlations between variables; see table A1). PC1 scores, positively correlated to the time spent walking or the distance walked, were then extracted, and individuals were ranked according to them.

Table A1: Estimation methods of exploration rate following Rozen-Rechels et al. (2018)

| Behavioral measurements | Contribution to PC1 | Contribution to PC2 |
|---|---|---|
| $D$ | **.14** | .002 |
| $T$ | **.12** | .0001 |
| $N_{\text{bouts}}$ | .06 | .003 |
| $M_{\text{bouts}}$ | **.13** | .003 |
| $\text{SD}_{\text{bouts}}$ | **.12** | .0001 |
| $D_{\text{bouts}}$ | **.12** | .00009 |
| $T_{\text{walls}}$ | .02 | **.24** |
| $D_{1\text{walls}}$ | **.14** | .015 |
| $D_{2\text{walls}}$ | .02 | **.27** |
| $\text{SD}_{\text{walls}}$ | .0001 | **.31** |
| $\tau_{\text{explo}}$ | .06 | **.11** |
| $\text{SD}_{\text{explo}}$ | .07 | .05 |

Note. Contributions of each measured behavior to the axes of the principal component analysis. Boldface type indicates contributions that are higher than a random contribution (i.e., $100/12 = 8.33\%$). Axis 1 explained 55% and axis 2 explained 23% of the variance in the data. See appendix A for definitions of behavioral measurements. PC = principal component.

## APPENDIX B

### Effects of Water Restriction on Survival from the End of the Experiment to the Next Reproductive Season

We estimated survival from recapture data collected in May 2017. It was then possible to estimate lizard survival with a high degree of reliability thanks to a recapture probability close to 1. The recapture effort in September 2016 was not high enough to ensure sufficient confidence in the survival data. Still, we captured individuals from the experiment in May 2018 that had not been captured in 2017 (three individuals). We then analyzed survival from August 2016 to May 2017, considering these individuals to be alive even though not captured. We excluded individuals that had been paralyzed because of decalcification at the end of July.

Survival has been analyzed using a mixed effect logistic regression model including a logit link and a binomial error term (package lme4) with a two-way interaction of the water restriction treatment, with the sex of the individual as fixed effect and the enclosure identity as a random effect.

Treatment had no significant effect on survival, whatever the sex (treatment × sex: $\chi^2_1 = 2.1, P = 0.15$; treatment: $\chi^2_1 = 0.07$, $P = 0.79$). Males had significantly higher survival than females ($\chi^2_1 = 4.77, P = 0.03$).

Table B1: Statistics of model explaining variation of ΔTEWL when adding ΔRMR as a fixed additive variable

| Sessions, variable | Numerator df | Denominator df | $F$ | $P$ |
|---|---|---|---|---|
| Weeks 4 and 7: | | | | |
| ΔRMR | 1 | 95 | 3.38 | .06 |
| Time | 1 | 95 | 22.3 | <.0001 |
| Treatment | 1 | 97 | 1.11 | .30 |
| Time × treatment | 1 | 95 | .61 | .44 |
| May 2017: | | | | |
| ΔRMR | 1 | 47 | 1.19 | .28 |
| Sex × treatment | 1 | 47 | 4.51 | .0 |

Note. Effect of the water restriction treatment on ΔTEWL (change in total evaporative water loss) when adding ΔRMR (change in resting metabolic rate) as a fixed additive effect to the previously selected model. Results are unchanged (see “Results”). df = degrees of freedom.

## APPENDIX C

### Effects of Water Restriction on Reproductive Effort

We estimated the reproductive effort of females recaptured in May 2017 by calculating total litter size and mass (i.e., the number and summed mass of all nonfecundated eggs, aborted embryos, dead juveniles, and live juveniles in the litter) and calculating litter size and mass (i.e., only in live juveniles). One female was not pregnant and has been excluded from the analysis.

(Total) litter size was analyzed using a mixed effect logistic regression model including a logit link and a Poisson error term (package lme4; Bates et al. 2015) with the water restriction treatment as fixed effect and the enclosure identity as a random effect. (Total) litter mass was analyzed using a mixed effect linear model (package nlme) with the water restriction treatment as fixed effect and the enclosure identity as a random effect.

Neither (total) litter size nor mass were affected by the water restriction treatment (all $P > 0.19$).

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