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## *Research article*

### **Short-term change in water availability influences thermoregulation behaviors in a dry-skinned ectotherm**

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## Abstract

1. Mechanistic models of terrestrial ectotherms predict that climate warming will induce activity restriction due to heat stress and loss of shade, leading to the extinction of numerous populations.

Such models rely on the assumption that activity patterns are dictated by simple temperature thresholds independent of changes in water availability. However, changes in water availability may further influence thermoregulation behavior of ectotherms through dehydration risk perception, changes in water balance or changes in microclimatic conditions.

2. Here, we experimentally assess the interactive effects of thermal conditions and water availability on activity patterns, shade selection and thermoregulation efficiency in a model ectothermic species. 3. Thermoregulation behavior of adult common lizards (*Zootoca vivipara*) was monitored in outdoor mesocosms as we manipulated water availability, providing water as mist in the morning and free-standing water during the daytime. We recorded operative temperatures and micro-meteorological conditions to infer thermal constraints and dehydration risk.

4. Activity and shade selection were better predicted by continuous changes in thermal conditions and dehydration risk, respectively, than by threshold functions. In addition, water supplementation increased activity in males and reduced shade selection in both sexes, most probably as a behavioral response to the perception of a stronger dehydration risk. Water supplementation also influenced the thermal quality of the environment, which in turn altered daily activity patterns and thermoregulation statistics.

5. This demonstrates that dual effects of heat and water stress on activity patterns may lead to stronger activity restriction as a result of climate change than currently predicted.

## Introduction

In a warming world, mechanistic models predict that excessive heat and loss of shade should restrain activity periods and compromise the long-term energy balance of terrestrial ectotherms, which could ultimately lead to their population decline (Kearney, 2013; Sinervo et al., 2010).

Temperature thresholds for activity and shade selection behavior are crucial to predict the consequences of climate change on populations of terrestrial ectotherms (Adolph & Porter, 1993; Kearney, 2013; Kearney, Shine, & Porter, 2009; Sinervo et al., 2010). Yet, the thermal dependence of activity patterns of these species is more complex than previously thought and our understanding of it requires more quantitative data (Gunderson & Leal, 2015, 2016; Vickers, Manicom, & Schwarzkopf, 2011). In addition, current mechanistic models neglect the water-dependence of behavioral thermoregulation (Rozen-Rechels, Badiane, Agostini, Meylan, & Le Galliard, 2020a; Rozen-Rechels et al., 2019; Ryan et al., 2016; Sannolo & Carretero, 2019), which becomes critical given predicted changes in drought propensity as the climate is warming worldwide (Field, Barros, Stocker, & Dahe, 2012). In fact, we still lack empirical evidence to understand and parameterize the joint effects of water and heat stress on ectotherms activity for future mechanistic modeling.

Behavioral thermoregulation encompasses all behavioral processes allowing the regulation of body temperature (Angilletta, 2009). It enables animals to regulate their energy budget and buffer the potentially deleterious consequences of heat stress (Huey et al., 2012; Kearney et al., 2009). Behavioral thermoregulation often involves temporal (e.g., Diaz & Cabezas-Diaz, 2004; Vasconcelos, Santos, & Carretero, 2012) and spatial (e.g., shade selection; Blouin-Demers & Weatherhead, 2002; Muri et al., 2015) changes in activity patterns. In addition, behavioral thermoregulation also depends on water availability, and future changes in water availability may cause behavioral and ecological changes in terrestrial ectotherms independently from heat constraints (Cahill et al., 2012; Rozen-Rechels et al., 2019). Understanding behavioral consequences of both heat and water restriction is thus essential to predict activity and growth patterns (Kearney, Munns, Moore, Malishev, & Bull, 2018; Pirtle, Tracy, & Kearney, 2019). For example, water balance regulation, and not thermoregulation, may promote behavioral selection of shady areas during droughts and can cause animals to retreat into a refuge, thus shortening activity periods (e.g., Davis & DeNardo, 2009). In addition, water availability and rainfall patterns can change micro-climatic conditions on the short-term and induce long-term modifications in vegetation development or trophic interactions (e.g., Collins et al., 2014; Grimm-Seyfarth,

Mihoub, Gruber, & Henle, 2018; Pincebourde & Woods, 2012; Ryan et al., 2016). These effects may in turn modify thermoregulation strategies of terrestrial ectotherms, which depend on multiple factors such as microhabitat availability, predation risks and food intake (Grimm-Seyfarth et al., 2018; Huang, Kearley, Hung, & Porter, 2020; Rozen-Rechels et al., 2020a). When trophic interactions are controlled for, behavioral responses of organisms to water availability could thus be driven by direct physiological effects or by indirect microclimatic changes (Rozen-Rechels et al., 2019).

Thermoregulation and hydroregulation behaviors are intimately related (Rozen-Rechels et al., 2019), as heat and water stress conditions are often correlated, making it hard to disentangle one from the other. Yet, mechanistic models of ectotherms populations typically describe behavioral thermoregulation with simple thermal functions that fail to predict how these behaviors should respond to joint changes in temperature and water availability (Gunderson & Leal, 2016; Kearney & Porter, 2009). In terrestrial ectotherms, active thermoregulation (*contra* thermal conformance) allows individuals to maintain body temperatures during activity close to their thermal preference set, defined as the range of body temperatures selected by individuals in cost-free environments (Angilletta, 2009). Opportunities to reach the thermal preference set depend on social parameters and environmental conditions such as “operative” temperatures, referring to the local environmental conditions determining the equilibrium body temperatures of a perfectly thermoconforming animal (Dzialowski, 2005). Optimal thermoregulation is therefore limited when operative temperatures deviate from the thermal preference set, leading to higher thermoregulation costs, shifts in shade selection, lower activity or a temporal shift in activity patterns (Herczeg, Gonda, Saarikivi, & Merilä, 2006; Porter, Mitchell, Beckman, & DeWitt, 1973). However, how environmental temperatures, activity, and shade selection relate to each other is still debated (Gunderson & Leal, 2015). Two models have been proposed regarding activity patterns depending on decision rules for shuttling behaviors associated to thermoregulation: classical threshold models in which activity occurs within a specific “thermal activity window” (Adolph & Porter, 1993; Kearney, 2013; Porter et al., 1973; Sinervo et al., 2010), and continuous models in which activity vary smoothly with operative or body temperatures (Gunderson & Leal, 2015, 2016; Hertz, Huey, & Garland, 1988).

Short-term changes in water availability may influence activity patterns in terrestrial ectotherms when these decision rules depend on the perceived risks of dehydration and/or on the hydration state of individuals (Davis & DeNardo, 2009; Lorenzon, Clobert, Oppliger, & John-Alder, 1999;

Rozen-Rechels et al., 2018, 2020a). On the one hand, high body and environmental temperatures both increase water loss rates from evaporation and respiration in terrestrial ectotherms (Davis & DeNardo, 2009; Lourdais et al., 2017). Short-term water restriction may lead to physiological dehydration in warm environments, thus increasing the behavioral sensitivity of terrestrial ectotherms to excessive heat (Kearney et al., 2018). On the other hand, short-term water supplementation, for example due to rainfall events, could also entail local cooling effects from evaporation of excess water in vegetation and soil (Campbell & Norman, 1998). This may lead to changes in operative temperatures and environmental constraints on thermoregulation, especially during heat waves, and concurrent changes in thermoregulation behaviors (Caillon, Suppo, Casas, Arthur Woods, & Pincebourde, 2014; Pincebourde & Woods, 2012). Thus, despite the current lack of empirical evidence, we expect a positive covariation between overheating risks and dehydration risks and both should jointly influence activity patterns and shade selection (Davis & DeNardo, 2009).

Behavioral shifts in response to short-term changes in water availability could further impair the efficiency of thermoregulation. According to prevailing theory, a perfect thermoregulator should be able to maintain its preferred body temperature through flexible thermoregulation efforts when the benefits of reaching thermal preferences exceed the costs of active thermoregulation (Angilletta, 2009; Huey & Slatkin, 1976; Vickers et al., 2011). Yet, only few studies to date tested how non-energetic costs induced by changes in water availability influence thermoregulation efficiency (Grimm-Seyfarth et al., 2018; Rozen-Rechels et al., 2020a; Sannolo & Carretero, 2019). It has been demonstrated that chronic dehydration may lead to thermal depression, that is behavioral selection of lower preferred body temperatures (e.g., Ladyman & Bradshaw, 2003; Rozen-Rechels et al., 2020a), but short-term changes in water availability can also trigger thermal depression in lizards (Sannolo & Carretero, 2019). If dehydration risk is a cost to thermoregulation, this should alter thermoregulation effort and lower thermoregulation efficiency. We thus expect dehydration risk to influence thermoregulation efficiency, in addition to activity and shade selection patterns.

In this study, we manipulated short-term water availability in semi-natural outdoor mesocosms. We compared how different metrics of thermal constraints, based on thresholds or continuous variables, and how water supplementation jointly predict activity patterns, shade selection behavior and thermoregulation statistics in the European common lizard (*Zootoca vivipara*, Lichtenstein, 1823). This cold-adapted species is tightly dependent on moist conditions and

permanent access to water for long-term persistence of natural populations (Dupoué, Rutschmann, Le Galliard, Miles, et al., 2017; Lorenzon, Clobert, & Massot, 2001; Lorenzon et al., 1999; Massot, Clobert, Lorenzon, & Rossi, 2002), and is also an efficient heliothermic thermoregulator sensitive to ambient temperatures (Herczeg et al., 2006; Herczeg, Kovács, Hettyey, & Merilä, 2003). This lizard species displays high evaporative water loss rates compared with other squamate reptile species but relatively similar to those of other mesic habitat species (Cox & Cox, 2015). We tested whether water supplementation modifies the mean of behavioral traits as well as the thermal dependence of behavioral traits. When water supplementation modifies the thermal dependence, we further investigated whether this was driven by indirect effects on microclimate conditions or not, with the prediction that water supplemented habitats should be cooler on average. To do so, we precisely examined the effect operative temperatures have on thermoregulation behavior, quantified environmental constraints on water balance, and tested alternative mechanisms of thermal and water constraints on thermoregulation behavior and statistics.

## **Material and methods**

### ***Study species and sampling***

Common lizards were captured in semi-natural outdoor enclosures located at CEREEP-Ecotron IleDeFrance research center in France (48° 17' N, 2° 41' E). These predation-free enclosures include a natural vegetation with refuges and basking sites made of rocks and logs, and free water in artificial ponds. Natural populations of this species occupy habitats ranging from shady understory in some lowland environments to open grassland and even rocky habitats in the coldest areas. Individuals in this study were acclimatized to open, humid grassland habitats similar to their origin open, heathlands and bogs from Massif Central, France. From May 3<sup>rd</sup> to May 7<sup>th</sup>, 2018, we captured 30 adult pregnant females and measured their snout-vent length (SVL:  $66 \pm 3$  mm) and body mass (BM:  $5.68 \pm 1.13$  g). We then placed them in individual terraria (18×11×12 cm) with a shelter and sterilized peat soil as substratum, located in a temperature-controlled room (23°C from 09:00 to 18:00, 16°C at night). Individuals were fed three times a week with  $300 \pm 10$  mg of live house crickets (*Acheta domestica*). Water was available *ad-libitum* in a petri dish and sprayed three times a day (09:00, 13:00 and 17:00). Females were kept in these laboratory conditions until parturition and were then maintained in the same conditions for two to three weeks in order to standardize their postpartum physiological conditions before the experiment (Le Galliard, Le Bris, & Clobert, 2003). Females were sorted in three trial groups (10 females per group) according to

their laying date (Table S1). Males were captured in outdoor enclosures two days before the experiment (N=30, 10 males per trial: SVL:  $59 \pm 2$  mm; BM:  $4.27 \pm 0.48$  g). They were also placed in individual terraria (18×11×12 cm) with a shelter and sterilized peat soil as substratum for habituation. We note that both sexes were not in the same pre-experimental conditions. The effort of females in reproduction has also consequences on their thermal physiology (Le Galliard et al., 2003). Lizards were not fed during the two days preceding the experiment in order to standardize satiety state.

### ***Experimental design***

Behavioral observations were conducted in 20 outdoor mesocosms (diameter = 150 cm; EcoBovin 600L) filled with local soil (i.e. sandy cambisol) up to 40 cm high. Dates when individuals were placed in the mesocosm are indicated in Table S1. The mesocosm habitat was set up in order to test the basic hypotheses of mechanistic models of ectotherms behavioral thermoregulation and thus included a very simplified microhabitat landscape with short grass, full-sun conditions, shady conditions and a burrow (Kearney & Porter, 2009). During the experiment, the surface of the mesocosms was covered with 5-cm high grass that did not generate substantial shade. A shady spot was created on the south side of each mesocosm with a tile maintained with an angle of 30°. The top of the tile served as a basking spot. We also simulated a natural underground burrow that lizards could use to hide deep in the soil on the north side of the mesocosm with corrugated tube (25 cm) closed at the deeper end with a plug and fitted with a smooth tube (25×5 cm), pushed in the soil with a 60° angle. This system allowed us to take the “burrow” out of the soil without damage when we removed the corrugated tube from the smooth tube. A small plastic tank (10×5×3 cm) on the east side of the mesocosms served as free water point.

We implemented two different water supplementation treatments, hereafter called water conditions. In the wet conditions, the entire mesocosm was sprayed homogeneously with water for 7-9 seconds at 7:30 in the morning in order to simulate dew. The water tank was filled the night before. In the dry conditions, the water tank was emptied the night before and the mesocosm was not watered in the morning. All mesocosms were watered after sunset to maintain vegetation. Mesocosms were protected from rain with a tarpaulin every night from 21:30 to 07:30 local time. In the event of rain during daytime, which happened only twice (i.e., a total of one hour of missing data), mesocosms were immediately covered and uncovered as soon as the rain stopped to avoid greenhouse effects.

Each lizard in each trial spent eight days in the same mesocosm; four days in wet conditions and four days in dry conditions. A pseudo-random sequence was generated to get half of the mesocosms in dry conditions and the other half in wet conditions every day. Lizards were fed every day at 10:00 with  $200 \pm 10$  mg of live house crickets. Lizards were placed in wet conditions mesocosms for habituation the day prior to the behavioral measurements at 14:00. Two individuals died in the mesocosm during the experiment. They were excluded from the analysis. The other 58 individuals gained weight during the 8 days in the mesocosm ( $0.18 \pm 0.03$  g).

### ***Behavioral data***

Every day, we recorded the individual's behavior with a focal sampling every 30 minutes from 08:00 to 17:00. Since the common lizard is a typical actively foraging species with a strong percentage of time spent moving (Van Damme, Bauwens, & Verheyen, 1987), we considered that individuals were active when they were visible in the mesocosm or at the entrance of the shelter (when not active, individuals hide deep in their burrows, pers. obs.). Vegetation height was maintained short and constant (ca. 5 cm high) all along the experiment to allow the detection of individuals. When the individual was active, we recorded the substratum on which it was first seen and the shade conditions of this micro-habitat (sunny or shady). We also measured its surface body temperature on a small spot of the back (called  $T_{\text{body}}$ ) at a distance using an infrared thermometer (Raytek, Raynger MX2). This was only possible when the individuals did not hide after being detected. We used data from an independent sample of adults in the laboratory to calibrate the relationship between cloacal body temperatures (core body) and surface infrared measurements (Artacho, Jouanneau, & Le Galliard, 2013;  $N=70$ ,  $T_{\text{IR}}=4.34(\pm 0.35)+0.82(\pm 0.02) \times T_{\text{body}}$ ,  $R^2=0.96$ ).

### ***Thermal and water conditions proxies***

Operative temperatures were measured in each mesocosms under the full sun with two hollow copper tubes physical models fitted with temperature probes and a data logger (HOBO® Pro v2 U23-003, ONSET Cape Cod, Massachusetts). Following standard recommendations (Dzialowski, 2005), the copper tube physical model, which had the same dimension than an adult common lizard (1 cm in diameter, 6-cm long), was closed at one end with an Eppendorf tube® plug, and was painted in a mix of brown and green color and with black and light lines in order to mimic the reflectance properties of a common lizard. The temperature probe was fixed inside the tube without direct contact to the copper using pipette tips cut at the right diameter. Operative temperatures were measured in full sun on the tile and on top of the grass every 5 minutes. We

used previous data (September 2016) from the physical models against core temperature body measurements of dead adult lizards to calibrate operative temperatures data (calibration under full-sun:  $N=3590$  (5 individuals),  $T_{\text{core}}=-0.93 (\pm 0.74)+1.04(\pm 0.01)\times T_{\text{physical model}}$ ,  $R^2=0.95$ ). Since we recorded behavioral and body temperature data every 30 minutes and since biophysical models typically assume an hourly resolution (Kearney, Matzelle, & Helmuth, 2012), we decided to average operative temperature records from all mesocosms for each hour to calculate operative temperature ( $T_e$ ) under full-sun conditions and characterize heat constraints during the study (Camacho, Trefaut Rodrigues, & Navas, 2015).  $T_e$  was averaged from all mesocosms measurements in order to reduce sampling biases due to differences in the exact location of the sensors in each mesocosm and to average out measurement errors. Temperature conditions were highly variable during the study, ranging from cool summer days to heat wave conditions (Figure 1A, Figure S1). This is similar to observations made in natural populations (Rutschmann et al. 2016). Our study design therefore successfully allowed for the comparison of the behavior of the same lizards in two different water conditions and under variable thermal constraints.

We also measured ambient temperature under the tile and in the shelter (Figure S1).

Meteorological conditions were continuously recorded every hour with a weather station (Campbell Scientific, BWS200) equipped with an air temperature and relative humidity sensor (CS215), an anemometer (CS03002) and a pyranometer (CS301). The weather station was placed approximately 120 cm above the mesocosm surface. We used these measurements to calculate the predicted cutaneous evaporative water loss (CEWL) of a lizard at the soil surface using the Niche Mapper model (Kearney & Porter, 2009) wrapped in the R package “NicheMapR” (Kearney & Porter, 2017). This biophysical model predicts the hourly micro-climatic conditions, heat and water balance using data from the weather conditions, data about the soil properties and data about lizard morphology and physiology. Here, we calculated CEWL assuming an inactive individual basking full sun of the average body weight calculated from individuals used in the study right before entering the mesocosm ( $4.14 \pm 0.53$  g – calculations detailed in Supplementary Information 1). We found that CEWL and  $T_e$  were highly correlated ( $R^2=0.93$ , Figure S4), which suggests that CEWL is not a measure of water constraints independent from thermal constraints.

### ***Thermal preferences and thermoregulation statistics inside mesocosms***

In the morning of the day prior to the mesocosm experiments, we measured thermal preferences of all individuals following standard procedures for this species, as detailed in Supplementary Material 2. We obtained individual and time-specific approximations of the average thermal

preferences ( $T_{pref}$ ) and of the set of thermal preferences defined by the 25% and 75% quantile ( $T_{set}$ ). We note that females had higher thermal preferences than males as expected after parturition (Le Galliard et al., 2003). Using body temperatures inside mesocosms and individual statistics on thermal preferences, we calculated thermoregulation inaccuracy  $D_b$  for each  $T_{body}$  at the 30 minutes time scale, defined as  $T_{pref} - T_{body}$  (Hertz et al. 1993). We also calculated different metrics of the thermal constraint based on  $T_{pref}$  following Gunderson & Leal, (2015) and Hertz, Huey, & Stevenson (1993). We first calculated two indices of habitat thermal quality at the hourly scale to fit micro-climatic predictions,  $D_e$ , which is the absolute value of the difference between operative temperatures under the sun and individual  $T_{pref}$  ( $|T_{pref} - T_e|$ ), and  $D_{e2}$ , which is the signed difference of the operative temperatures under the sun to the  $T_{pref}$  ( $T_{pref} - T_e$ ). We further calculated three alternative metrics of thermal constraints on thermoregulation behavior: (1) the “continuous” index of operative temperature  $I_{cont}$  which is 0 when  $T_e$  is within  $T_{set}$  or is equal to the absolute difference between  $T_e$  and the closest  $T_{set}$  limit when  $T_e$  is out of  $T_{set}$  range; (2) the “medium constraint” index  $I_{med}$  which is 1 if  $T_e$  is higher than the upper  $T_{set}$  limit, 0 otherwise; and (3) the “maximum constraint” index  $I_{max}$  which is 0 when  $T_e$  is within  $T_{set}$  range, 1 otherwise. Finally, we also calculated the thermoregulation efficiency index for each  $T_{body}$  measurement (at the 30 minutes / day / individual scale):  $E = D_e - D_b$  which is 0 for a thermoconformer, positive for a thermoregulator, negative for an individual with a less precise thermoregulation than a conformer (Blouin-Demers & Nadeau, 2005). These metrics are summarized in Table 1.

### **Statistical analyses**

Statistical analyses were performed using R version 3.4.4 (R Core Team, 2018). We fixed activity as “1” when the individual was active, “0” if it was not. We acknowledge that the lack of observation could underlie that we missed the individual; this bias should however not affect our results as the whole experiment was conducted with the same trained observer with a constant and low risk of missing an observation due to low vegetation. We also fixed shade selection as “1” when the individual was active in full-sun, “0” when it active in the shade. Activity and shade selection behavior thus followed a Bernoulli distribution. We used model selection and model averaging procedures to determine whether variations of activity and shade selection are better explained by a “thermal activity window” ( $I_{med}$ ,  $I_{max}$ ), by linear or quadratic variations of temperatures ( $D_e$ ,  $D_{e2}$ ,  $I_{low}$ ), by linear or quadratic variations of the dehydration risk (CEWL), or by linear or quadratic variations of the hour of the day. We chose to fit quadratic relationships instead of non-linear functions to capture the monotonic temperature-dependent curves with a

single optimum and allow for robust model comparison procedures. We also tested whether the thermal constraint model could be water-dependent by creating two dummy variables: (1)  $I_{\text{waterMAX}}$  in which thermoregulation follows the  $I_{\text{max}}$  model in wet conditions and the  $I_{\text{med}}$  model in dry conditions, (2)  $I_{\text{waterMED}}$  in which thermoregulation follows the  $I_{\text{med}}$  model in wet conditions and the  $I_{\text{max}}$  model in dry conditions. We fitted generalized linear mixed models (GLMMs) with the behavioral traits as a response variable and one of these explanatory variables as fixed effect using the *glmer* function (“lme4” package, Bates, Mächler, Bolker, & Walker, 2015) with a binomial error. These models had a satisfactory fit to the data based on Pearson’s chi-square goodness-of-fit tests and examination of residuals. To test for effects of water supplementation on activity patterns, we tested whether these models were improved by the two-way interaction of each variable with water treatment in the mesocosm. We also tested the additive and the two-way interaction effects of sex and water treatment. We modeled inter-individual variability by fitting a random individual identity effect and we also tested the additive fixed effect of trial to account for potential differences between trials. The best model was selected based on comparison of Akaike Information Criterion corrected for small sample size (Burnham & Anderson, 2002). We followed a similar procedure to test whether body temperature and thermoregulation inaccuracy were better explained by the hour of the day, meteorological conditions ( $T_e$ ), or individual characteristics (sex and  $T_{\text{pref}}$ ). As operative temperatures  $T_e$  were highly correlated to standard water loss rates CEWL ( $r = 0.93$ , see Supplementary Information 1), we included effects of  $T_e$  instead of CEWL in these models. We compared models fitting linear and quadratic regressions of the hour of the day or  $T_e$ , linear regression of  $T_{\text{pref}}$  or a sex difference using LMMs with the *lme* function (“nlme” package, Pinheiro & Bates, 2006). The thermoregulation effort  $E$  is traditionally compared between populations or seasons since it is usually treated as a population statistic (Angilletta, 2009; Hertz et al., 1993). Here, we compared the  $E$  estimated every 30 minutes between wet and dry conditions using a LMM with the water condition as fixed effect and the individual identity nested in the mesocosm identity as random effects.

We also compared hourly variations of  $T_e$  at the mesocosm level between water conditions using a LMM testing the two-way interaction between a quadratic variation of the hour of the day and the water conditions, adding the mesocosm identity as a random effect. If the thermal quality of the mesocosm differed according to water supplementation, we performed the model selection procedure described above with both operative temperature records averaged over all mesocosms (see above, called  $T_e$ ) and with records of operative temperatures averaged in each water treatment

(hereafter called  $T'_e$ ). By comparing the fit of the models with  $T'_e$  to the ones based on  $T_e$  averaged for all mesocosms, we can test whether water supplementation changes thermoregulation behavior through indirect changes in microclimate conditions only (in that case fitting  $T'_e$  would suppress the effect of water supplementation) or through the perception of dehydration risk by individuals (in that case water supplementation keeps explaining variations of a trait even when fitting  $T'_e$ ).

## Results

Temperatures in the different microhabitats varied across the study period (Figure S1). Full-sun operative temperatures during the coolest days usually reached  $T_{set}$  in the afternoon (Figure 1A). During the hottest days, operative temperatures in full-sun were above the maximal critical threshold  $CT_{max}$  in the afternoon (based on Gvoždík & Castilla, 2001). During an average day, temperatures in the afternoon were mostly above  $T_{set}$  but below the maximal critical threshold (Figure 1A). During the coldest days,  $T_{set}$  temperatures were available in full-sun from 09:30 until the end of the day. During the morning, operative temperatures in full-sun were mostly below or within the  $T_{set}$ , while they were mostly above  $T_{set}$  or above the maximal critical threshold in the afternoon (Figure 1B). On average, temperatures under the tile and in the burrow displayed less variability than full-sun temperatures (Figure S1).

In addition, microhabitats differed for dehydration risks as indicated by predicted vapor pressure deficit for a lizard, which had the highest amplitude under full-sun, being 5 times more desiccating during the day than in the shade (Figure S2B). Conditions were constantly humid with small predicted water loss rates for lizards inside the burrows. We further found that operative temperatures  $T_e$  were also slightly cooler in wet than in dry mesocosms, especially during the hottest hours of the day (Figure S5). Thus, water supplementation had some effects on microclimatic conditions inside the mesocosms. Consequently, we compared the fit of the models with  $T'_e$  to the ones based on  $T_e$  averaged for all mesocosms when relevant (Table S2).

The results of model selection procedures are summarized in Table S2. Activity rate increased up to maximum of 50% at 11:00 and then decreased smoothly during the afternoon (Figure 1C). Males were less active on average in dry conditions than in wet conditions (sex  $\times$  water conditions:  $\chi^2_1 = 7.50$ ,  $p = 0.006$ ). The best model for activity probability also included a two-way interaction of the quadratic effect of  $D_{e2}$  with water conditions ( $D_{e2} \times$  water conditions:  $\chi^2_1 = 10.7$ ,  $p = 0.001$ ;  $D_{e2}^2 \times$  water conditions:  $\chi^2_1 = 2.02$ ,  $p = 0.15$ ). Activity in wet conditions reached an optimum when  $-D_{e2}$  approximately equaled  $-0.9$  °C. This optimum shifted to slightly

cooler temperatures ( $-1.9^{\circ}\text{C}$ ) and activity rate dropped faster with increasing temperatures in dry conditions than in wet conditions (Figure 1D). When we reran the same models with  $D'_{e2}$  calculated based on  $T'_e$  averaged in each water treatment, the best selected model showed that males were less active in dry conditions than in wet conditions as previously found. However, this model indicated that the thermal dependence of activity patterns was the same in both treatments (maximum activity at  $D'_{e2} = -1.4^{\circ}\text{C}$  in both conditions; Table S2, Supplementary Information 3, Figure S6).

Full-sun micro-habitats were preferentially selected early in the day, and individuals gradually shifted to shady micro-habitats and had a full-sun micro-habitat selection rate comprised between 50 and 60% during the afternoon (Figure 1E). According to the best model, full-sun micro-habitat selection was almost at 100% at low CEWL and then decreased with increasing CEWL (Figure 1F; CEWL:  $\chi^2_1 = 47.37$ ,  $p < 0.0001$ , CEWL<sup>2</sup>:  $\chi^2_1 = 5.07$ ,  $p = 0.02$ ). Full-sun micro-habitat selection was slightly lower on average in dry habitats (contrast =  $-0.20 \pm 0.09$  SE,  $\chi^2_1 = 4.32$ ,  $p = 0.04$ ).

Intra-individual variations of  $T_{\text{body}}$  were significantly explained by linear change in  $T_e$  ( $T_e$ :  $F_{1,544} = 301.7$ ,  $p < 0.0001$ ,  $T_e^2$ :  $F_{1,543} = 2.49$ ,  $p = 0.12$ ) and water supplementation inside the mesocosm ( $F_{1,544} = 4.7$ ,  $p = 0.03$ ), but not by dual changes in both variables ( $T_e \times$  water conditions:  $F_{1,543} = 2.0$ ,  $p = 0.16$ ).  $T_{\text{body}}$  increased with  $T_e$  and were on average slightly higher in dry conditions (Figure 2A, contrast =  $0.44 \pm 0.20$  SE). On average,  $T_{\text{body}}$  reached  $T_{\text{set}}$  when full-sun operative temperatures were slightly above  $T_{\text{set}}$ . Expectedly, variations of  $D_b$  were also significantly explained by linear variations of  $T_e$  ( $T_e$ :  $F_{1,544} = 296.3$ ,  $p < 0.0001$ ,  $T_e^2$ :  $F_{1,543} = 2.56$ ,  $p = 0.11$ ) and water conditions in the mesocosm ( $F_{1,544} = 4.6$ ,  $p = 0.03$ ), but not by dual changes in both variables ( $T_e \times$  water conditions:  $F_{1,543} = 1.5$ ,  $p = 0.23$ ).  $D_b$  decreased with  $T_e$  and was lower in dry conditions (Figure 2B, contrast =  $-0.45 \pm 0.21$  SE). In general,  $T_{\text{body}}$  reached and exceeded  $T_{\text{pref}}$  at lower temperatures in dry conditions, suggesting lower thermoregulation accuracy (Figure 2B). Indeed, thermoregulation efficiency  $E$  was slightly lower in dry than in wet conditions (contrast =  $-0.63 \pm 0.32$ ;  $F_{1,545} = 3.92$ ,  $p = 0.05$ ; Figure 2C). When we reran the same models with records of operative temperatures averaged in each treatment ( $T'_e$ ), we did not find significant effect of the water conditions (Table S2;  $T_{\text{body}}$ :  $F_{1,544} = 0.66$ ,  $p = 0.42$ ;  $D_b$ :  $F_{1,544} = 0.67$ ,  $p = 0.42$ ;  $E$ :  $F_{1,545} = 1.49$ ,  $p = 0.22$ ; Figure S7).

## Discussion

Our mesocosm experiment was conducted under ambient thermal constraints ranging from cool to warm summer conditions with contrasted operative temperature, thus exposing individuals to severe heat constraints only at specific times of the day and during some of the hottest days of the study. This climate variability, to which each individual was confronted at least four times in each treatment, is usually considered as the leading cause of variability in behavioral thermoregulation in terrestrial ectotherms (Angilletta, 2009; Sunday et al., 2014). Indeed, behavioral activity was strongly predicted by a continuous variable describing the availability of operative temperatures close to the thermal preference set. Full-sun micro-habitat selection was instead predicted by a continuous variable describing the dehydration risk, a variable strongly positively correlated to the operative temperature in the habitat. Thermoregulation statistics correlated strongly with thermal conditions during the day, a potential proxy of heat stress and dehydration risks especially in the full sun as shown by our microclimatic data. Body temperatures increased linearly with operative temperature until reaching the thermal preferences range, which was almost never exceeded, showing that lizards avoided overheating in general. In addition, as hinted by recent models of thermo-hydroregulation behavior (Rozen-Rechels et al., 2019), a short-term restriction of water availability caused significant changes in behavioral thermoregulation because lizard activity was lower on average in water-restricted environments independently from microclimatic conditions. Full-sun micro-habitat selection was also slightly reduced in dry conditions independently from weather conditions. Finally, short-term water supplementation changed local thermal constraints on activity patterns and thermoregulation, most likely through changes in microclimate conditions. Wet mesocosms were slightly cooler on average during the afternoon and lower operative temperatures inside these mesocosms led to slightly more activity, lower body temperatures and less accurate thermoregulation.

Most mechanistic models assume that activity patterns are characterized by thermal thresholds defining when an individual is active or not, but different threshold models have been hypothesized so far (Adolph & Porter, 1993; Kearney, 2013; Porter et al., 1973; Sinervo et al., 2010). Here, we calculated five metrics of the thermal environment from individual data on the thermal preference set and independent data on habitat thermal quality (Adolph & Porter, 1993; Gunderson & Leal, 2015). Some metrics were based on threshold functions and thus assumed thermal boundaries above or below which activity should shift. Our model comparison procedure however supported Gunderson & Leal (2016) suggestion that continuous functions better capture temporal changes in activity according to environmental temperatures than threshold functions.

For example, behavioral activity of *Anolis* lizards, a typical sit-and-wait taxonomic group, is strongly thermal-dependent with a peak around the preferred body temperature and a steep decrease below and above this optimum (Gunderson & Leal, 2015). We found qualitatively similar results in the common lizard, which is an actively foraging, thermal generalist and heliothermic species (Herczeg et al., 2008). Altogether, our results thus suggest that continuous functions might provide a better fit to quantitative patterns of behavioral thermoregulation than threshold functions for a wide range of lizard species (Gunderson & Leal, 2015, 2016).

More importantly, we found significant effects of short-term water supplementation on activity patterns even though our experimental conditions were less likely to induce substantial changes in the water balance of lizards than a chronic water restriction (Dupoué et al., 2018). We found a lower average activity in dry conditions in males but not in females. Even though pre-experimental conditions were not similar between both sexes, these results are consistent with those of past studies that showed a higher sensitivity to hydric stress in males than in females in this species (Dupoué et al., 2019; Rozen-Rechels et al., 2020b). The observed change in average activity is consistent with a water conservation strategy aiming at reducing water loss rates under high thermal constraints, as seen in wild populations of the Gila monster *Heloderma suspectum* (Davis & DeNardo, 2009). This finding is also consistent with field observations of an increased activity vigor in response to increased availability of free-standing water or moisture in a range of lizard species (Davis & DeNardo, 2009; Kearney et al., 2018; Pirtle et al., 2019). Activity changes could be explained by underground shelters selection patterns (burrows). In our study, conditions in burrows were cooler and wetter than at soil surface based on micro-climatic predictions. Burrows are commonly used by reptiles to reduce water losses (Beck & Jennings, 2003), and our study confirms the critical role of shelters, especially burrows, in explaining activity decision rules.

We further observed water-dependent changes in activity patterns under heat constraint. More specifically, individuals shifted from selecting preferred temperatures in wet conditions to a stronger heat avoidance in dry conditions, with heat stress shifting activity to 1°C lower in water restricted mesocosms. Our complementary analyses further suggest that short-term water supplementation influenced the thermal dependence of activity patterns by cooling the environment rather than through direct effects of water supplementation on the water biology of the lizards or through some behavioral perception of the combined risks of heat and dehydration. First, operative temperatures were slightly lower, especially during the hottest time of the day, in

water supplemented than in water restricted mesocosms. This difference is most likely a consequence of evaporative cooling from water supplemented as mist in the morning and/or from free standing water stored in the tank. Second, the thermal dependence of activity patterns was not different between treatments when we accounted for these treatment-specific differences in microclimate conditions instead of using microclimate conditions averaged over both treatments. The reported shift from full-sun to shadier micro-habitats selection during water restriction confirms that access to shade is critical to maintain activity under high heat and water constraints. Dehydration risk was indeed strongly correlated with high operative temperatures  $T_e$  and was thus a good proxy of "hot and dry" weather conditions. Similar changes in shade selection have been reported in whiptail lizards, *Apidoscelis exsanguis*, exposed to natural variation in rainfall patterns (Ryan et al., 2016). Shade selection patterns are relevant to hydroregulation behavior and a preference for shady micro-habitats at high temperatures is expected when thermoregulation is influenced by water loss risks (Grimm-Seyfarth, Mihoub, & Henle, 2017; Huang et al., 2020). The importance of shade selection for water budget and hydroregulation is well supported in this study by our calculation that shady environments were almost 5 times less drying than full-sun environments. One should note that the effect of water availability on shade selection behavior was however relatively small and most pronounced under low desiccation conditions whereas full-sun selection was rare and overall similar between the two treatments in the most desiccating conditions. We also caution against naive extrapolation from our data on shade selection behavior because our mesocosm set-up might not have provided enough variability in shade availability due to our choice of a standardized and homogeneous short vegetation layer. Future studies should examine a more natural and wider range of microhabitats.

Thermoregulation statistics during activity, including body temperatures and the accuracy of thermoregulation, changed linearly with operative temperatures  $T_e$  as expected from models of optimal thermoregulation (Herczeg et al., 2006, 2003; Vickers et al., 2011). On average, most individuals reached their thermal preferences when operative temperatures in full-sun conditions were slightly above the thermal preference set,  $T_{set}$ . Body temperatures were closer to  $T_{set}$  than  $T_e$  in the coolest conditions, a pattern indicative of active thermoregulation strategy irrespective of the environmental costs of thermoregulation (Huey & Slatkin, 1976). As shown in analyses of thermoregulation statistics after controlling for the relationship between water supplementation and microclimate conditions, water supplementation had no effects on thermoregulation statistics. This result runs against those of previous laboratory studies that found thermal depression (i.e.,

lower body temperatures) of animals maintained in water restricted environments (Ladyman & Bradshaw, 2003; Lourdais et al., 2017; Rozen-Rechels et al., 2020a). Thermal depression is usually considered as an adaptive acclimation response that helps reduce water loss rates and allows to maintain water balance more efficiently in water restricted habitats. The change in water availability and dehydration risk was likely too short-term in this study to induce such acclimation responses.

Our results complement those of recent tests of the role of water availability on activity patterns, micro-habitat selection and thermoregulation statistics in diverse species of squamates in both natural and laboratory conditions (Grimm-Seyfarth et al., 2018; Kearney et al., 2018; Pirtle et al., 2019; Rozen-Rechels et al., 2020a, 2020b; Sannolo & Carretero, 2019). Currently, species investigated for their hydroregulation behaviors live in a wide range of climates ranging from arid to mesic environments and in habitats ranging from open semi-desertic areas to shady forest understory. The behavioral responses to hydric stress observed in these species include a lower activity vigor, some micro-habitat selection shifts, and sometimes a less accurate thermoregulation. Similar to conclusions about the thermal biology of ectotherms (Huey et al., 2012), this suggests that the vulnerability of many terrestrial ectotherms to climate warming will depend on their behavioral plastic responses in addition to their physiological resistance.

Squamate reptiles from more arid environments generally have evolved a higher resistance to water loss, but there is a high residual variability in resistance to water loss among species even when controlling for climate adaptations (Cox & Cox, 2015). Within a given community of ectotherms, we predict that the species least resistant to water loss should rely more on behavioral flexibility to adjust their water balance when conditions deteriorate (Davis & DeNardo, 2009; Rozen-Rechels et al., 2020b). In addition, vegetation cover and the spatial heterogeneity of shade will also play an important role because behavioral flexibility is most efficient in the most heterogeneous habitats that provide sufficient opportunities of thermoregulation (Grimm-Seyfarth et al., 2017). In a warming world where vegetation cover and habitat heterogeneity are expected to decrease with aridity, behavioral thermo-hydroregulation will be further challenged and terrestrial ectotherms will face higher risk of extinction. Future studies should try to examine whether behavioral responses of terrestrial ectotherms relate to their physiological resistance to drought events and should test how behavioral responses are influenced by vegetation cover and heterogeneity.

Given the increasing probabilities and severity of drought events during the hottest seasons in numerous regions of the world (Field et al., 2012), our data emphasize that proximate mechanisms of activity restriction will involve a dual effect of warming and drying. These results call for a better consideration of joint water and thermal availability changes in future climate change studies of ectotherms (Rozen-Rechels et al., 2019). Models ignoring this dual effect might overestimate or underestimate activity restriction amplitude in terrestrial ectotherms depending on future climate trends and interactive effects of water and heat on behavioral thermoregulation. For example, we hypothesize that future climate conditions of heat and drought stress should restrain activity in natural populations of the common lizard. Instead, classical mechanistic models predict that these lizards are more likely to extend their activity time during warmer years, without consideration of water restriction, and emphasize that loss of shade is the critical determinant of activity restriction (Kearney, 2013). In line with our behavioral data, decades of field observations in a range of common lizard populations across France and climate change experiments suggest that activity restriction and summer spells are critical determinants of population extinction in this species (Bestion, Clobert, & Cote, 2015; Dupoué, Rutschmann, Le Galliard, Clobert, et al., 2017; Sinervo et al., 2010). Loss of shade does not seem to occur in these populations; instead, habitats tend to close and become more diverse in vegetation height because of slow changes in land use and human disturbance in the recent years (Clobert et al., pers. obs). Altogether, our results along with previous evidence support the idea that a dual increase in heat constraints and water restriction during summer, and their impact on activity patterns, is a potential driver of local extinction in natural populations of the common lizard (Chamaillé-Jammes, Massot, Aragon, & Clobert, 2006; Rutschmann, Miles, Clobert, & Richard, 2016).

Our experiment demonstrates unequivocally that even a small reduction in water availability and the concomitant changes in water balance can drive flexible changes in patterns of behavioral thermoregulation under thermal constraints without modifying thermoregulation strategies. The described interaction between water conditions and thermal constraints appears also to be tightly dependent on how thermal constraints are measured. Indeed, water supplementation influenced local thermal constraints, which in turn influenced activity patterns at the population level. This questions the methodology of most studies on thermoregulation in which the effect of temperature on the biology of species is never unequivocally demonstrated independently from other biotic or abiotic parameters.

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#### **Authors' contributions**

DRR, JFLG and SM conceived the ideas and designed methodology. All authors participated to lizard captures and breeding. PF and DRR collected the data. DRR, PF and JFLG analyzed the data. DRR led the writing of the manuscript with JFLG. All authors participated to the interpretation of results and the writing of the manuscripts and approved the manuscript.

#### **Data availability statement**

Analyses reported in this article can be reproduced using the data provided in Zenodo (Rozen-Rechels et al. 2020c, 10.5281/zenodo.3775431).

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**Table 1.** Summary of thermal indices used in the study.

<b>Index</b>	<b>Abbreviation</b>	<b>Calculation</b>
Operative temperatures	$T_e$	/
Body temperature	$T_{\text{body}}$	/
Body temperature in the neutral thermal gradient	$T_p$	/
Average thermal preferences	$T_{\text{pref}}$	$T_p$
Thermal preferences set	$T_{\text{set}}$	Range from the 25% quantile of $T_p$ to the 75% quantile
Thermoregulation inaccuracy	$D_b$	$T_{\text{pref}} - T_{\text{body}}$
Habitat thermal quality	$D_e$	$ T_{\text{pref}} - T_e $
Habitat thermal quality 2	$D_{e2}$	$T_{\text{pref}} - T_e$
“Continuous” index of operative temperature	$I_{\text{cont}}$	0 when $T_e$ is in $T_{\text{set}}$ minimal $ T_{\text{set}} - T_e $ when out of $T_{\text{set}}$
“Medium constraint” index of operative temperature	$I_{\text{med}}$	1 when $T_e > T_{\text{set}}$ 0 otherwise
“Maximum constraint” index of operative temperature	$I_{\text{max}}$	0 when $T_e$ is in $T_{\text{set}}$ 1 otherwise
Thermoregulation efficiency	$E$	$D_b - D_e$

## Figure legends

**Figure 1.** Variation of operative temperatures  $T_e$  as a function of time (A) for the hottest, the coolest and a median day. Points are raw data averaged for all mesocosms every 5 minutes. Loess smoothers are fitted to these data to visualize the non-linear patterns (plain lines). The  $T_{set}$  range is plotted with red dashed lines, and the mean maximal critical threshold,  $CT_{max}$  (Gvoždík & Castilla, 2001), is plotted with an orange line. In B, we combined all  $T_e$  data to calculate the proportions of measurements that are below, in or above  $T_{set}$ , and above  $CT_{max}$  at each hour. Hourly variations of activity rate (C) and full-sun micro-habitat selection rate (E) are plotted according to the water conditions in the mesocosm. Points are for hourly averaged behaviors. Loess smoothers are fitted to these data to visualize the non-linear patterns (plain lines). The variations of activity rate as a function of  $D_{e2}$  are shown in D, points are calculated average activity rates in  $1^\circ\text{C}$   $D_{e2}$  bins according to the water conditions and the sex (circle: females; triangles: males). Note that the  $x$  axis was inverted for in order to show increasing temperatures relatively to  $T_{pref}$ . Lines represent the predictions from the best model (females: dashed lines; males: dotted lines). The variations of full-sun selection rate with CEWL are shown in F where CEWL was scaled for modeling purposes. Squares represent averaged full-sun selection rate in each water conditions in 0.1 bins of scaled CEWL. Best model predictions for full-sun selection according to water conditions are plotted as dotted lines. Sex did not predict variations in full-sun selection rate. Error bars show the standard errors.

**Figure 2.** Thermoregulation statistics. Variations of body temperatures  $T_{body}$  (A), and accuracy of thermoregulation  $D_b$  (B, high values indicate inaccurate thermoregulation) as a function of operative temperature  $T_e$  and water conditions in the mesocosm. Points represent raw data collected during the experiment. Plain lines stand for predicted value from a GLMM fitting a two-way interaction of the quadratic variations of  $T_e$  and the water conditions in the mesocosm to visualize group specific trends. Black and gray dashed lines show the predicted standard errors. Red dashed lines in A stand for the  $T_{set}$  range on each axis. In B, red dashed lines stand for the  $T_{set}$  limits on the  $T_e$  axis and when  $D_b = 0$ , i.e., when  $T_{body}$  equals perfectly thermal preferences  $T_{pref}$ , on the  $D_b$  axis. C, variations of thermoregulation efficiency  $E$  according to water conditions (values close to zero indicate inefficient thermoregulation). Jittered points show raw individual values. Large points show predicted values from the model presented in the Results section. Error bars show the predicted standard errors.

Figure 1

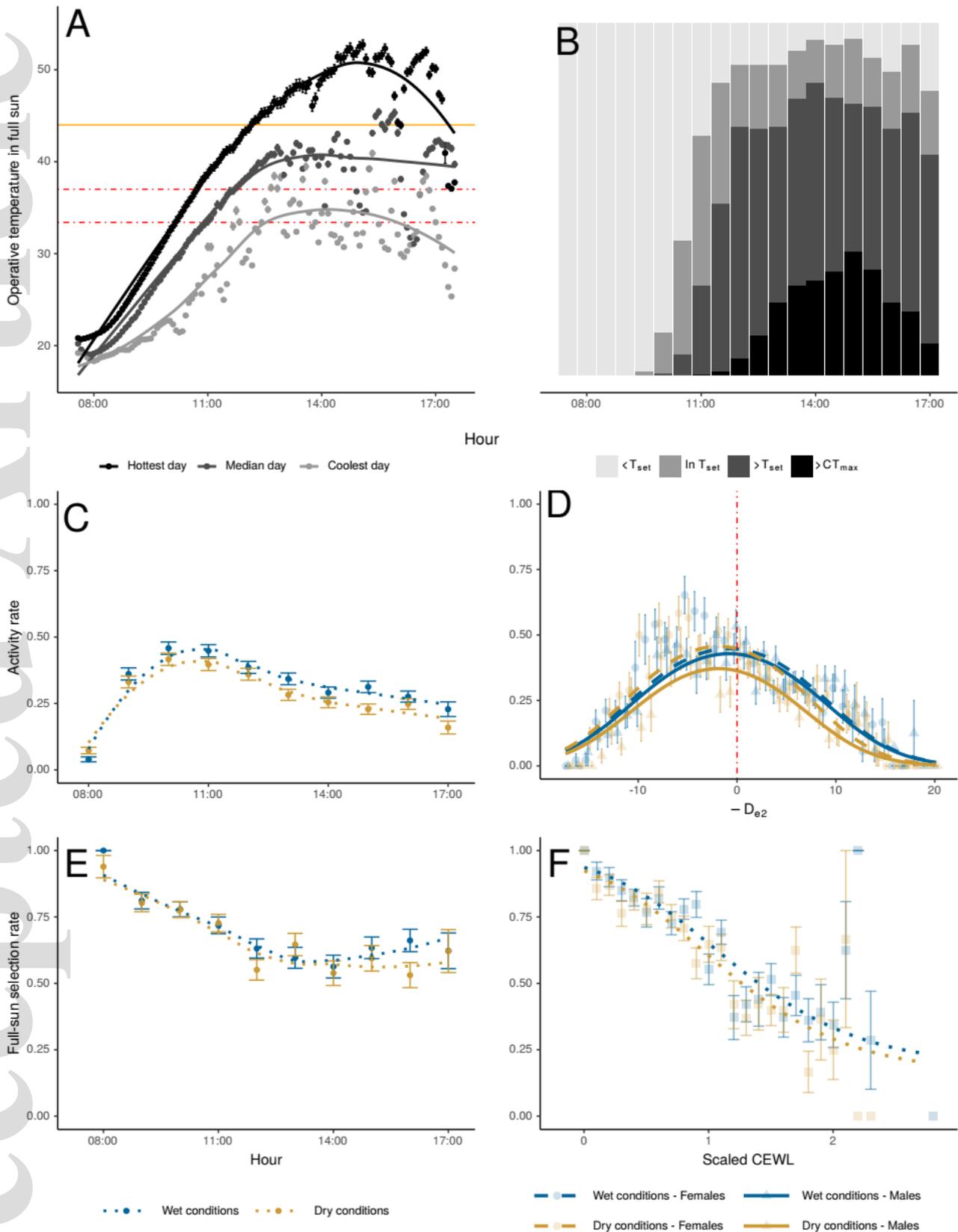


Figure 2

