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# Reproducing in a changing world: combined effects of thermal conditions by day and night and of water constraints during pregnancy in a cold-adapted ectotherm

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1 **Title:** Reproducing in a changing world: Combined effects of thermal conditions by day and  
2 night and of water constraints during pregnancy in a cold-adapted ectotherm

3  
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17  
18 **Key words:** pregnancy; climate change; environmental constraints; hydration; temperature;  
19 reproductive output

20  
21 **Abstract:**

22 Climate change will continue to increase mean global temperatures with daily minima increasing  
23 more than daily maxima temperatures. Altered rainfall patterns due to climate change will also  
24 disrupt water availability for terrestrial organisms already facing climatic warming. To explore

25 how organisms may adjust to changes in multiple, concurrent climate-related environmental  
26 conditions, we manipulated day and night temperatures as well as water availability during  
27 gestation in female common lizards (*Zootoca vivipara*), a cold and wet adapted species facing  
28 climatic changes notably in populations located on the warm margin. We jointly manipulated  
29 temperature (hot or cold) independently during the daytime and nighttime as well as water  
30 availability ( $\pm$  *ad libitum* access to water) throughout pregnancy and quantified the effects on  
31 maternal traits (morphology, physiology, and phenology) and reproductive output. Overall, we  
32 found that higher day or night temperatures decreased gestation length and increased energetic  
33 demands. Higher temperatures during the day, coupled with water restrictions, increased  
34 dehydration, and water restrictions affected ability to allocate resources but had no impact on  
35 reproductive output. While high temperatures during the day were beneficial to current  
36 reproductive output and success, high temperatures during the night had the opposite effect. Our  
37 results suggest that high nighttime temperatures can dramatically increase the burden on  
38 pregnant mothers already constrained by heavy resource and water investment. This could  
39 provide a mechanistic explanation for the long-term declines of warm-margin populations in this  
40 species.

41 **1. Introduction**

42 Organismal attempts to maintain homeostasis during accelerating shifts in climatic conditions  
43 involve both behavioral and physiological mechanisms (Hoffmann et al. 2003; Deutsch et al.  
44 2008; Dillon et al. 2010; New et al. 2011). The capacity or not to maintain a relatively stable  
45 internal environment may constrain the future survival of many species in this context (Kearney  
46 and Porter 2009; Buckley et al. 2010). The physiological resistance to environmental stressors  
47 can vary significantly among populations and species (Stillman 2003; Johansen and Jones 2011;  
48 Riddell et al. 2018) and often involves resource-based trade-offs (Zera and Harshman 2001).  
49 Understanding how multiple climatic stressors shape plastic responses and influence life-history  
50 strategies is therefore critical to predicting climate change effects (Huey et al. 2012). Yet, to a  
51 large degree, current studies provide little information on how concurrent environmental  
52 constraints will challenge organismal physiology and life history trade-offs.

53 Most of the previous research on animals' response to global climate change has  
54 understandably focused on the effects of changing temperatures (Harvell et al. 2002; Portner and  
55 Farrell 2008; Helmuth et al. 2010), especially increases in mean daily temperature (Thomas et al.  
56 2004; Parmesan 2006; Musolin 2007; Huey and Tewksbury 2009). This appears relevant for the  
57 majority of species, which are ectotherms, as their locomotion, growth, and reproduction are  
58 temperature-sensitive (Tewksbury et al. 2008). However, climate warming involves a  
59 characteristic set of changes in diurnal and geographic patterns of temperature, precipitation, and  
60 other atmospheric conditions (Kingsolver 2002; Helmuth et al. 2005). For example, nocturnal  
61 warming is greater than diurnal warming in most regions, leading to reduced variation in daily  
62 temperatures (Dai et al. 1999; Davy et al. 2017). In fact, nearly all the climatic warming seen in  
63 the northern hemisphere over the past century is due to increased daily minima (Vose et al.

64 2005). Shifts in rainfall patterns and water availability are equally important to consider in  
65 terrestrial ecosystems as both are similarly affected by climate change (Fung et al. 2011;  
66 Sanderson et al. 2011). Current and predicted climatic conditions thus include shifts in diurnal  
67 and nocturnal temperatures, precipitation patterns, and interactions between temperature and  
68 precipitation change (Vose et al. 2005; Adler et al. 2008; Zhou et al. 2009).

69 While it remains unclear how organisms will be influenced by these compounded  
70 changes, the effects are likely to be significant (Bonebrake and Mastrandrea 2010). Many  
71 terrestrial ectotherms are likely to be at risk from climate change as their body temperature  
72 depends on behavioral thermoregulation (Huey and Tewksbury 2009; Kearney and Porter 2009).  
73 However, most previous studies have primarily focused on changing average thermal conditions  
74 (Deutsch et al. 2008; Herrando-Pérez et al. 2019; Verheyen and Stoks 2019), and it remains  
75 unclear how ectotherms will be affected by independent shifts in daytime and nighttime  
76 temperatures. For example, rising temperatures during the day may force ectotherms above their  
77 optimal temperature, leading to heat stress and reduced performance (Niehaus et al. 2012; Stoks  
78 et al. 2017), while rising temperatures at night may either result in enhanced performance  
79 (Speights et al. 2017) or increased energetic costs and parasite loads (Rutschmann et al. 2021).  
80 Recent studies have begun to document physiological and behavioral responses to changes in  
81 temperature and water availability (Rozen-Rechels et al. 2019; Dupoué et al. 2020a) or to shifts  
82 in day and night temperatures (Barton and Schmitz 2018). Reproduction is a particularly  
83 sensitive period that requires a considerable amount of energy and water investment as well as  
84 appropriate temperatures for the developing embryos. Reproductive mode may drive  
85 vulnerability to climate changes and viviparous species may be more susceptible because of  
86 substantial maternal investment during pregnancy (Pincheira-Donoso et al. 2013). In addition,

87 viviparity generates parent-offspring conflicts for energetic resources (Calow 1973; Doughty and  
88 Shine 1997; Reznick et al. 2000; Kotiaho 2001) and hydric resources (Dupoue et al. 2015;  
89 Bruschi et al. 2017; Bruschi et al. 2018; Dupoué et al. 2018; Bruschi and DeNardo 2019; Bruschi et  
90 al. 2020), which may be altered by climate change.

91 To address these questions, we manipulated day and night temperatures as well as water  
92 availability during gestation in female common lizards (*Zootoca vivipara*), a small cold- and  
93 wet-adapted and widespread viviparous lizard with a strictly diurnal activity. We applied  
94 ecologically relevant, daily thermal cycles mimicking behavioral thermoregulation during  
95 daytime and thermoconformity at night. Common lizards exhibit characteristics of mixed income  
96 and capital breeding (Bleu et al. 2013) and must make multiple physiological adjustments in  
97 response to climatic conditions during periods of heavy investment such as reproduction. We  
98 made the following three general hypotheses. First, warmer days would have a positive effect on  
99 reproductive output because of extended access to preferred temperature but would result in  
100 increased food intake to match the higher body-reserve mobilization, provided food availability  
101 is not limiting. Second, conversely, warmer nights would have a net negative impact on  
102 reproduction by increasing metabolic demands without providing thermal benefits for foraging  
103 (Rutschmann et al. 2021). Third, water constraints would overall negate potential thermal  
104 benefits by negatively impacting maternal physiology (Bruschi et al. 2020).

105

## 106 **2. Materials and methods**

### 107 *2.1 Study species*

108 *Zootoca vivipara* are strictly diurnal, heliothermic lizards of small body size (adult snout-vent  
109 length, SVL 50-75 mm) with a very efficient thermoregulation behavior. Individuals are

110 typically found in cool, mesic habitats such as bogs, heathland or wet meadows across northern  
111 Eurasia (Dely and Böhme 1984). This species occurs in both viviparous and oviparous forms  
112 across Europe, but we considered a viviparous mode inhabiting populations from the Plateau de  
113 Millevache (Limousin) in France. Here, males copulate with females shortly after emerging in  
114 the spring with fertilization occurring in mid-late May. Pregnancy lasts 2–3 months, with  
115 parturition of 1-12 offspring occurring between mid-July and early August, depending on female  
116 body size and climatic conditions (Foucart et al. 2014; Dupoué et al. 2018). After parturition,  
117 females replenish their reserves throughout the summer before entering over-wintering dormancy  
118 in late September (Avery 1974; Bauwens and Verheyen 1985).

119

## 120 *2.2 Experimental design*

121 Females used for this study were captured between 22-26 May (n = 115) or on 8 June (n = 20)  
122 2019 from two different sites separated by less than 7 km. Pregnant females were captured by  
123 hand and reproductive status was inferred by the presence of recent male bite marks on the  
124 abdomen. We confirmed that all females captured were in the end stages of vitellogenesis or  
125 early pregnancy using ultrasonography (Sonosite MicroMaxx, Bothell, WA) and previously  
126 described methods (i.e., presence of spherical, echolucent follicles or uterine ova ~1 cm long;  
127 Gilman and Wolf 2007). Non-reproductive females were released at the site of capture within 24  
128 h. All reproductive females were transported to the Centre d'Etudes Biologiques de Chizé,  
129 France. We determined body mass by placing each lizard into a plastic box on a tared balance ( $\pm$   
130 0.01 g) and SVL using digital calipers ( $\pm$  0.1 mm), after which all ectoparasites were removed  
131 with forceps and females were treated with Frontline (Merial Inc. Duluth, GA, USA) to avoid a  
132 high burden of ectoparasites. Females were then housed individually with a 12L:12D light

133 regimen in opaque containers (30 × 18 × 10 cm) with mesh tops securely fastened with elastic  
134 bands, filled with approximately 2 cm of moist peat, two different shelters, and *ad libitum* access  
135 to food (crickets – *Acheta domestica*) and water. During the 12 h light cycle, a subsurface  
136 heating element was provided below one end of each cage to create a thermal gradient (20° –  
137 40°C) and light was provided using LED rope lights (Ever Lighting, Chalu, China). Females  
138 were held in these conditions for 6-9 d prior to the start of the experiment after which we  
139 collected (in order) a blood sample, mass, scanned image, muscle measurement, and heart rate  
140 (see below) of all females. Three of the females failed to ovulate and one female needed  
141 veterinary assistance during parturition. These females were subsequently returned to their site of  
142 capture and not used for any analyses.

143 Experimental manipulations started on 3-5 or 12 June and lasted until 48 h post-  
144 parturition for all females. We used a body condition index, based on standardized residuals from  
145 a linear regression using mass and SVL, to equally distribute lizards of similar size, condition,  
146 and date and location of capture and assign them to one of two treatments: with permanent  
147 access to water or water restricted (hereafter referred to “wet” or “dry”, respectively) following  
148 previously described protocols (Lorenzon et al. 1999; Dupoué et al. 2018). Females in the wet  
149 treatment (n = 66) were provided with water *ad libitum* and water was sprayed (~20 mL) twice  
150 per day (morning [0800 - 0900 h] and afternoon [1600 – 1700 h]) on one wall of each container  
151 so that drops could be used for drinking up to 45 min after spraying. Females assigned to the dry  
152 group (n = 65) were only provided with water once per day (morning) by similarly spraying one  
153 wall of each container.

154 Lizards were further distributed into one of four temperature treatments based on body  
155 condition with a factorial manipulation of day temperature (hereafter “hot” or “cold day”) and



156 night temperature (hereafter “hot” or “cold night”, see Figure 1 for details). During a portion of  
157 the day, females were either held at their preferred body temperature (31°C) from 10:00 to 18:59  
158 (~9 hours, hot day) or from 10:00 to 13:59 (~4 hours, cold day). Overnight, females were either  
159 held at 22°C (~13-18 hours, hot night) or at 17°C (~11-16 hours, cold night). In between daytime  
160 and nighttime conditions, lizards spent 2 to 4 hours where temperatures were either gradually  
161 increasing or decreasing to avoid abrupt changes. Light regimens were similarly adjusted so that  
162 lights were on or off during day and night, respectively. Stable temperatures were maintained by  
163 housing females of similar treatment groups in climatic chambers (Vötsch VP 600, Balingen,  
164 Germany). The relative humidity in each chamber was adjusted depending on the temperature so  
165 that the water vapor deficit remained constant overnight (0.7 kPa at 17°C and 22°C) or for  
166 portions of the day (1.6 kPa at 31°C).

167         In the wild, pregnant females maintain relatively stable body temperatures during the day  
168 (29–33°C) by thermoregulating and are inactive during the night when their body temperatures  
169 decrease (15–20°C; Le Galliard et al. 2003; Dupoué et al. 2017a). We chose environmental  
170 conditions that are within the range of variation seen between cold and some warm habitats or  
171 years excluding extreme situations (in the most endangered populations or the warmest years)  
172 when environmental temperatures exceed the preferred temperatures or maximum critical  
173 thermal limit. The daytime conditions include the range of variation between highland, cold  
174 habitats where females have restricted access to their preferred body temperatures during the day  
175 and lowland, warm habitats where females reach a maximum access to their preferred body  
176 temperatures (Rutschmann et al. 2016). The nighttime conditions also reflect the range of  
177 variation observed for soil surface temperatures during summer season in populations across an  
178 altitudinal gradient with a mean variation ranging between 8 and 15°C (Dupoué et al. 2017b) and

179 a variation of upper quartiles ranging between 19 and 23°C (Dupoué and Rutschmann,  
180 unpublished data). Furthermore, the manipulation of water availability mirrors the geographic  
181 variation in water availability across populations, where some populations have permanent  
182 access to free standing water throughout the year (similar to our wet treatment group) whereas  
183 others have only temporary access to free standing water (dry treatment group). In the latter,  
184 water access is often restricted to morning mist and rainfall during the summer, dry season  
185 (Dupoué et al. 2017b). Additionally, the duration of our selected water restrictions was designed  
186 to fit with the entire length of the gestation period.

187 During the study, each female was provided with live crickets ( $0.80 \pm 0.01$  g) three times  
188 per week between 07:30 and 09:30, which were dusted with vitamin powder containing calcium  
189 and vitamin D3 (ReptiVite, Zoo Med Laboratories Inc, USA). During subsequent feedings any  
190 remaining crickets were removed and counted in order to estimate food consumption in the  
191 previous 48-72 hrs. Throughout the study, females were weighed twice per week, always before  
192 feedings between 07:00 and 08:00, and each cage was visually inspected twice per day for  
193 parturition. On the day of parturition, offspring were moved to separate containers while the  
194 same maternal variables from the start of the experiment were collected, after which females  
195 were returned to their respective treatment regimens for an additional 48 hrs in case of delayed  
196 parturition. Only one female in this study exhibited delayed parturition, which required  
197 veterinary assistance, and was removed from all analyses. Concomitantly, offspring from each  
198 female were quantified and weighed ( $\pm 1$  mg) in the following categories: living, stillborn, or  
199 unfertilized ova. The ratio of living offspring to total litter size was used as a measure of  
200 reproductive success. We also recorded total litter size and litter mass (number and mass of  
201 living, stillborn, and unfertilized ova). Forty-eight hours after parturition, females were removed

202 from the study, provided a standard thermal gradient (20° – 40°C) and *ad libitum* access to food  
203 and water, before being released at their site of capture.

204

### 205 *2.3 Blood sample collection and determination of plasma osmolality*

206 We used 20 µL hematocrit tubes to collect a ~50 µL blood sample from the postorbital sinus of  
207 all reproductive females. Total time for capture, restraint, and blood collection did not exceed 4  
208 min. We immediately centrifuged the blood samples at 4,000 g for 5 min at 20°C to separate  
209 plasma from blood cells, and then aliquoted plasma (~40 µL) into separate vials and froze at -  
210 30°C until we used them to measure plasma osmolality. We determined plasma osmolality in  
211 triplicate using a vapor pressure osmometer ( $\pm 3$  mOsm kg<sup>-1</sup>; model 5100C; Wescor Inc., Logan,  
212 Utah, USA). Prior to use, we calibrated the osmometer using sealed osmolality standards (100,  
213 290, and 1000 mOsm kg<sup>-1</sup>) in accordance with factory recommendations. We ran samples in  
214 triplicate and used 290 mOsm kg<sup>-1</sup> standards to check the osmometer for variation after every  
215 sample. If the standard varied more than the limits of the osmometer ( $\pm 3$  mOsm kg<sup>-1</sup>), the  
216 osmometer was recalibrated. If it continued to vary more than the limits, the osmometer head  
217 was cleaned and the machine was recalibrated before continuing duplicate analysis beginning  
218 with the last sample prior to calibration/cleaning to verify correct measurement of the sample.

219

### 220 *2.4 Tail width, muscle width, and heart rate.*

221 On the day experimental manipulations began and at the end of parturition, we collected the  
222 following measurements between 07:30 and 08:30. First, ventral surfaces of all females were  
223 scanned at 600 dots per inch using a flatbed scanner (Canon Inc., Canoscan LiDE 400) to  
224 measure tail width at the 7<sup>th</sup> sub-caudal scale using ImageJ2 (Rueden et al. 2017). Next, we

225 measured transverse muscle thickness of the right hind leg at the mid region of the upper limb  
226 segment (i.e., midpoint between joint and body on thigh). We used digital calipers ( $\pm 0.1$  mm;  
227 Mitutoyo Corp., model 547-301) and took measurements in triplicate. Finally, females were  
228 allowed to acclimate undisturbed at 20°C for 1 hour. After which, we used ultrasonography  
229 (Sonosite MicroMaxx, Bothell, WA) to measure heart rate within 75 seconds of removing them  
230 from their cages and before any noticeable increase in heart rates due to handling.

231

### 232 *2.5 Statistical analyses*

233 We analyzed treatment effects on each morphological, physiological and reproductive output  
234 traits independently using model selection procedures in R software (R Development Core  
235 Team, version 3.5.2) with the packages ‘MASS’ (Venables and Ripley 2013), ‘multcomp’  
236 (Hothorn et al. 2008), ‘car’ (Fox and Weisberg 2018), and ‘lmerTest’ (Zeileis and Hothorn 2002).  
237 All initial models included additive and interactive effects of the three experimental treatments  
238 (categorical variables). We also controlled for inter-individual differences in size and  
239 reproductive investment among females because size indicates differences in condition,  
240 reproductive phenology, and/or age and because treatments may have stronger effects in females  
241 with a higher reproductive investment (Dupoué et al. 2020b). Water treatment started at or just  
242 prior to ovulation where final litter size is set in this lecithotrophic lizard (Blackburn 2015).  
243 Therefore, we did not expect any effect of experimental manipulations on total litter size. We  
244 first used relative body size (individual SVL – mean SVL of all females) as a linear covariate  
245 instead of mass, especially because the body mass fluctuated considerably over the experimental  
246 period. To avoid collinearity issues, we further included in our initial models the effect of size-  
247 controlled fecundity or developed fecundity (standardized residuals from a linear regression of

248 total litter size or developed litter size [i.e., number of living and stillborn offspring not including  
249 unfertilized ova] against SVL). Thus, our full models included fixed effects of day and night  
250 temperatures, water availability, and all interactions, additive effects of relative body size (RBS)  
251 and fecundity, and two-way interactions between water availability and fecundity and between  
252 water availability and RBS. These interactions were included because allocation trade-offs  
253 between mothers and embryos may change with differences in water availability (Dezetter et al.  
254 2021). Starting with these initial models, we used stepwise removal of insignificant coefficients  
255 as recommended for null hypothesis testing in manipulative studies (Arnold 2010; Zuur et al.  
256 2010). For models comparing change in a variable (muscle width, heart rate, etc.), we also  
257 included initial values as a covariate. To model post-parturition body condition (residuals from a  
258 linear regression using post-parturition mass and SVL), we used initial body condition as a  
259 covariate to control for initial differences in reserves between females. To compare differences in  
260 litter size and litter mass, we only included female RBS as a covariate. Most traits were fitted  
261 with linear models except reproductive success (proportion of number of living offspring/total  
262 offspring [i.e., number of living and stillborn offspring]) for which we used general linear  
263 models with a binomial distribution with overdispersion with maternal body size as a covariate  
264 (see Tables S1-11 for details of full and final models).

265         After comparing the combined effects of water availability, day, and night temperatures,  
266 we further used variance partitioning methods described by Anderson and Cribble (Anderson and  
267 Cribble 1998) to decompose our full response into orthogonal subsets. Using these subsets, we  
268 performed separate linear models to examine how day temperatures (hot or cold) influenced the  
269 relationship between plasma osmolality and heart rate with initial heart rate as a covariate. We  
270 also used orthogonal subsets and separate linear models to examine how water availability ( $\pm ad$

271 *libitum* access to water) influenced the relationships between dynamics of energetic reserves  
272 (change in tail width or change in muscle width) and parturition mass change and litter mass  
273 using maternal size as a covariate.

274 A Tukey's HSD *post hoc* test was used to compare treatment groups. We checked all  
275 initial models to ensure the data met the assumptions for parametric testing and used  
276 transformations where necessary. Data are presented as mean  $\pm$  SEM and differences were  
277 accepted as significant at the level of  $p < 0.05$ . Females at the start of the experiment had similar  
278 body mass ( $F_{7,123} = 0.53$ ,  $p = 0.813$ ), plasma osmolality ( $F_{7,123} = 1.11$ ,  $p = 0.363$ ), muscle width  
279 ( $F_{7,123} = 1.53$ ,  $p = 0.164$ ), heart rate ( $F_{7,123} = 0.98$ ,  $p = 0.449$ ), and tail width ( $F_{7,123} = 0.43$ ,  $p =$   
280  $0.883$ ; Table S13) in the 8 treatment groups.

281

### 282 **3. Results**

#### 283 *3.1 Reproductive phenology, food consumption, and mass change*

284 The length of gestation was negatively related to female size ( $F_{1,126} = 5.54$ ,  $p = 0.020$ ) and was  
285 further influenced by an interaction between day and night temperatures ( $F_{1,126} = 33.86$ ,  $p <$   
286  $0.001$ ), where hot days or hot nights drastically reduced the duration of gestation but a  
287 combination of the two had a much smaller effect (Fig. 2A). After accounting for a positive  
288 effect of female size ( $F_{1,123} = 14.41$ ,  $p < 0.001$ ), the average mass of crickets consumed per day  
289 during gestation was influenced by an interaction between day and night temperatures ( $F_{1,123} =$   
290  $50.37$ ,  $p < 0.001$ ) and by an interaction between day temperature and water availability ( $F_{1,123} =$   
291  $6.34$ ,  $p = 0.013$ ). Overall, females exposed to hot days consumed high amounts of crickets per  
292 day (mean consumption of  $0.27 \pm 0.01$ g) regardless of night temperatures and water availability.

293 Conversely, females exposed to cold days consumed more if nights were hot compared to cold,  
294 independently from water availability ( $0.26 \pm 0.01\text{g}$  versus  $0.19 \pm 0.01\text{g}$ , see Fig. 2B).  
295 Dry conditions slightly reduced food intake in females exposed to cold days but had no  
296 detectable effect in females exposed to hot days (Fig. 2B).

297 Females, regardless of treatment, gained mass ( $1.78 \pm 0.05\text{ g}$ ) up until parturition. Mass  
298 increase per unit time was positively influenced by body size ( $F_{1,122} = 54.19$ ,  $p < 0.001$ ) and by  
299 an interaction between water availability and litter size ( $F_{1,122} = 4.61$ ,  $p = 0.034$ ). Litter size did  
300 not covary with relative mass gain in females in wet conditions ( $\beta = 0.001$ ,  $SE < 0.01$ ,  $p = 0.066$ ,  
301  $R^2_{\text{adj}} = 0.037$ ) but positively covaried in females in dry conditions ( $\beta = 0.003$ ,  $SE < 0.01$ ,  $p <$   
302  $0.001$ ,  $R^2_{\text{adj}} = 0.165$ ). We further found an interaction between day and night temperatures ( $F_{1,122}$   
303  $= 4.66$ ,  $p = 0.033$ ) that mirrored results on the length of gestation: hot days or nights led to  
304 increased daily mass gains relative to the cold controls, while a combination of the two did not  
305 have an additive effect (Fig. 2C; Table S12). Post-parturition body condition was positively  
306 influenced by initial body condition at the start of the experiment ( $F_{1,125} = 32.57$ ,  $p < 0.001$ ). In  
307 addition, females either exposed to hot nights or held in dry conditions had lower post-parturition  
308 body conditions ( $F_{1,125} = 5.87$ ,  $p = 0.017$ ;  $F_{1,125} = 3.95$ ,  $p = 0.049$ , respectively; Table S11).

### 309 310 *3.2 Maternal physiology*

311 Significant day temperature by water availability interactions influenced plasma osmolality  
312 ( $F_{1,126} = 4.47$ ,  $p = 0.036$ ; initial osmolality:  $F_{1,126} = 9.65$ ,  $p = 0.002$ ) and muscle width ( $F_{1,125} =$   
313  $11.46$ ,  $p = 0.001$ ; initial muscle width values:  $F_{1,125} = 61.84$ ,  $p < 0.001$ ) changes throughout the  
314 experiment. Females exposed to hot days had larger increases in osmolality when conditions  
315 were dry ( $40.88 \pm 1.44\text{ mOsm kg}^{-1}$ ) compared to when conditions were wet ( $13.41 \pm 0.76\text{ mOsm}$

316  $\text{kg}^{-1}$ ), but both groups lost the same amount of muscle during pregnancy (both  $-0.12 \pm 0.02$  mm).  
317 Similarly, females exposed to cold and dry days had higher osmolality ( $27.91 \pm 0.93$  mOsm  $\text{kg}^{-1}$ )  
318 compared to those with *ad libitum* access to water ( $4.97 \pm 0.62$  mOsm  $\text{kg}^{-1}$ ). However, water  
319 restricted cold day females lost a higher amount of muscle ( $-0.18 \pm 0.02$  mm) when compared to  
320 cold day females in wet conditions ( $-0.04 \pm 0.02$  mm; Fig. 3A, B). We found no main effects or  
321 interactions (all  $p > 0.05$ ) when comparing night temperatures between the treatment groups  
322 (Table S4).

323 When comparing changes in heart rate and accounting for initial heart rate ( $F_{1,125} = 63.22$ ,  
324  $p < 0.001$ ), females in wet conditions had relatively small increases in heart rate ( $4.31 \pm 1.08$   
325 bpm) compared to females in dry conditions ( $13.20 \pm 1.19$  bpm; water availability:  $F_{1,125} =$   
326  $44.87$ ,  $p < 0.001$ ). Irrespective of water treatment, heart rate increases were slightly higher in  
327 females exposed to cold days and hot nights or hot nights and cold days ( $9.43 \pm 1.61$  bpm and  
328  $9.05 \pm 1.28$  bpm, respectively) and lower in females exposed to hot days and nights or cold days  
329 and nights ( $8.14 \pm 2.35$  bpm and  $8.25 \pm 1.72$  bpm, respectively; day  $\times$  night temperature  
330 interaction:  $F_{1,125} = 4.01$ ,  $p = 0.047$ ; see Fig. 3C).

331 Overall, females gained tail width throughout gestation ( $0.09 \pm 0.05$  mm), however, when  
332 comparing across water treatments, these increases were driven by females in wet conditions  
333 ( $0.19 \pm 0.08$  mm) while females in dry conditions tended to lose tail width ( $-0.02 \pm 0.07$  mm).  
334 After including the effects of initial tail width ( $F_{1,126} = 59.88$ ,  $p < 0.001$ ), a significant interaction  
335 between water availability and fecundity ( $F_{1,126} = 4.54$ ,  $p = 0.035$ ) influenced changes in tail  
336 width. In females in wet conditions, fecundity negatively covaried with tail width change ( $\beta = -$   
337  $0.057$ ,  $\text{SE} = 0.03$ ,  $p = 0.041$ ,  $R^2_{\text{adj}} = 0.438$ ), but not in females in dry conditions ( $\beta = 0.031$ ,  $\text{SE} =$   
338  $0.03$ ,  $p = 0.368$ ,  $R^2_{\text{adj}} = 0.269$ ; Fig. 4).



339

### 340 *3.3 Reproductive output*

341 Mass loss at parturition increased with female size ( $F_{1,127} = 111.05$ ,  $p < 0.001$ ) and was  
342 influenced by day ( $F_{1,127} = 4.17$ ,  $p = 0.043$ ) and night temperatures ( $F_{1,127} = 14.46$ ,  $p < 0.001$ ) but  
343 not by their interaction ( $p > 0.05$ ). Females exposed to hot days lost slightly more mass ( $1.89 \pm$   
344  $0.09$  g) compared to females in cold days ( $1.71 \pm 0.10$  g), whereas females in hot nights lost less  
345 ( $1.62 \pm 0.73$  g) compared to females in cold nights ( $2.00 \pm 0.81$  g). Similarly, after correcting for  
346 female size ( $F_{1,128} = 99.44$ ,  $p < 0.001$ ), females exposed to hot nights had a smaller litter mass  
347 ( $0.86 \pm 0.05$  g) compared to females in cold nights ( $0.97 \pm 0.05$  g;  $F_{1,128} = 99.44$ ,  $p < 0.001$ ). We  
348 also found a significant effect of nighttime temperatures ( $F_{1,130} = 5.70$ ,  $p = 0.020$ ) on  
349 reproductive success, where females exposed to hot nights had a 26% higher chance of  
350 producing stillborn or unfertilized ova at parturition. We found no significant effect of daytime  
351 temperatures or water availability on reproductive success or litter mass (both  $p > 0.05$ ; Table  
352 S9, S14). Total litter size was not influenced by day- or nighttime temperatures, or water  
353 availability (all  $p > 0.05$ ; Table S14).

354

### 355 *3.4 Orthogonal subset analyses*

356 We used orthogonal subset analyses to independently assess the effect of hydric state on heart  
357 rate and explore the relationships between maternal dynamics of energetic reserves and their  
358 reproductive output. Plasma osmolality changes positively covaried with heart rate changes in  
359 females exposed to hot days ( $\beta = 0.325$ ,  $SE = 0.07$ ,  $p < 0.001$ ,  $R^2_{adj} = 0.416$ ) as well as in females  
360 exposed to cold days ( $\beta = 0.293$ ,  $SE = 0.71$ ,  $p < 0.001$ ,  $R^2_{adj} = 0.441$ ). Furthermore, muscle width  
361 changes negatively covaried with parturition mass loss ( $\beta = -2.634$ ,  $SE = 0.55$ ,  $p < 0.001$ ,  $R^2_{adj} =$

362 0.616; Fig. S1A) and with litter mass ( $\beta = -1.106$ ,  $SE = 0.37$ ,  $p = 0.004$ ,  $R^2_{adj} = 0.471$ ; Fig. S1C)  
363 in females provided in wet conditions but not in those in dry conditions (both  $p > 0.05$ ; Fig. S1B,  
364 D). Similarly, tail width changes also negatively covaried with both parturition mass loss ( $\beta = -$   
365  $0.244$ ,  $SE = 0.11$ ,  $p = 0.031$ ,  $R^2_{adj} = 0.514$ ; Fig. S2A) and litter mass ( $\beta = -0.205$ ,  $SE = 0.07$ ,  $p =$   
366  $0.003$ ,  $R^2_{adj} = 0.476$ ; Fig. S2C) in females in wet conditions but not in those in dry conditions  
367 (both  $p > 0.05$ ; Fig. S2B, D).

368

#### 369 **4. Discussion**

370 Our study independently manipulated day and nighttime temperatures as well as water  
371 availability to examine how concurrent environmental conditions impact an organism's ability to  
372 maintain homeostasis when faced with multiple abiotic constraints during reproduction. Overall,  
373 (1) hot day or night treatments put reproductive females in the "fast lane", decreasing gestation  
374 length and increasing resource demands per unit time; (2) hot day treatments, coupled with water  
375 restrictions, increased dehydration during reproduction; (3) water restrictions affected females'  
376 ability to allocate resources but had no impact on reproductive output; and (4) longer access to  
377 preferred temperature (hot days) was beneficial to current reproductive output and success  
378 whereas hot nights had the opposite effect.

379

##### 380 *4.1 High temperatures put females in the "fast lane"*

381 As expected, hot days or hot nights drastically decreased pregnancy duration, but the effects of  
382 higher temperatures were not additive because the combination of hot days and hot nights did not  
383 significantly shorten pregnancy durations even though mean daily temperature was the highest in  
384 this treatment group (Fig. 1 and 2A). This suggests that females exposed to hot days and nights

385 were at or near a phenological limit (~28 days in our study) for embryonic development (Olsson  
386 and Shine 1997a; Olsson and Shine 1997b; Lourdais et al. 2004). Reductions in gestation length  
387 can have important implications on offspring fitness and survival (Chamaillé-Jammes et al.  
388 2006; Warner and Shine 2007; While et al. 2009), and, as suggested by our study, require  
389 tremendous increases in resource acquisition per unit time. Daytime and nighttime temperatures  
390 both influenced the mass of crickets consumed per unit time and contrasting temperature regimes  
391 had distinct effects on energetic intake, possibly due to temperature-dependent changes in  
392 foraging and digestive efficiencies as well as the temporal dissociation of these two mechanisms  
393 influencing net energy gain. Body temperatures in ectotherms impact feeding behaviors and  
394 digestive physiology, where lizards exposed to higher temperatures drastically increase the  
395 quantity of food consumed. However, gut passage time ultimately limits the ability to  
396 continuously consume food (Angilletta 2001; Levy et al. 2017). In juxtaposition, colder  
397 temperatures decrease feeding behaviors while also reducing gut-throughput rates (Van Damme  
398 et al. 1991). Irrespective of nighttime temperatures, females in our study exposed to hot days  
399 were potentially consuming the physiological “maximum” amount of food. However, females  
400 exposed to cold days may have been behaviorally limited in their food consumption and were  
401 further reliant on nighttime temperatures to influence their digestive efficiencies (Fig. 2B).

402 Food consumption was also influenced by daytime temperatures and water availability,  
403 where animals with limited access to preferred temperatures did not consume as much relative  
404 crickets especially when water was restricted. After a meal is ingested, a significant amount of  
405 fluid is initially secreted into the lumen of the gastro-intestinal tract which is essential for  
406 digestion, absorption, and motility (Skoczylas 1978). While the vast majority of those fluids are  
407 eventually reabsorbed in the large intestine, the initial hydric cost of ingesting a meal may have

408 forced pregnant females in dry conditions to forgo meal consumption. Previous studies in other  
409 species of reptile have similarly found that animals can experience negative water balance after  
410 consuming prey (Beaupre 1996; Wright et al. 2013; Murphy and DeNardo 2019). In addition, it  
411 is not clear if lower temperatures during daytime decreased drinking behaviors as is seen with  
412 feeding in this species (Van Damme et al. 1991). Interestingly, within temperature treatments,  
413 females in wet or dry conditions gained similar relative mass per day, suggesting that females in  
414 wet conditions either drank less or females in dry conditions were able to reduce evaporative  
415 water loss. Temperatures were imposed by climatic chambers to eliminate the possibility of  
416 behavioral thermoregulation, but differences in transcutaneous or ocular water loss rates may  
417 have occurred due to changes in behavioral hydroregulation or plasticity of the skin barrier to  
418 transepidermal water loss (Dupoué et al. 2015; Refsnider et al. 2019; Dezetter et al. 2021).

419

#### 420 *4.2 High temperatures and water restrictions increase physiological costs of reproduction*

421 Limited hydric resources for pregnant females should result in higher maternal-offspring  
422 conflicts for water (Speakman and Garratt 2014; Dupoué et al. 2015) and burden mothers with  
423 increased plasma osmolality (Dupoué et al. 2018) and higher muscle catabolism (Brusch et al.  
424 2018). Our results confirm this and further suggest that daytime temperatures can exacerbate  
425 these conflicts and increase some of the physiological costs of reproduction. First, females  
426 exposed to high temperatures during the day had greater increases in plasma osmolality during  
427 gestation compared to those exposed to colder daytime temperatures. When considering water  
428 restrictions, these increases in plasma osmolality were further pronounced and highest in hot day  
429 females in dry conditions. Second, all females experienced muscle loss during the experiment  
430 and losses were highest in females in dry conditions, especially if they had limited access to

431 preferred temperatures during the daytime. Muscle wasting during pregnancy is common in  
432 reptiles (Lourdais et al. 2004; Lourdais et al. 2013; Lorigou et al. 2016) and skeletal muscle has  
433 been shown to be an important substrate for the reallocation of hydric resources when demands  
434 are high and intake is limited (Brusch et al. 2018; Brusch et al. 2020; Dezetter et al. 2021).  
435 Because proteins are a much lower energetic source compared to lipids and their oxidation  
436 provides five times more water (Jenni and Jenni-Eiermann 1998), increased muscle catabolism  
437 likely supported the hydric costs of reproduction in our study. Third, females in wet conditions  
438 throughout gestation had better post-parturition body conditions compared to those in dry  
439 conditions. Maternal condition after giving birth can have major impacts on their future  
440 reproduction (Bleu et al. 2013), and a female with a lower body condition and smaller muscles  
441 might not have the ability to capture prey, avoid predators, and survive until the next year.

442         In reptiles, cardiac function is maximized when individuals are maintained in their  
443 preferred temperature ranges allowing the animal to optimize oxygen consumption to meet  
444 metabolic demands (Lillywhite et al. 1999). In viviparous lizards, oxygen consumption  
445 drastically increases during later stages of pregnancy to support the increased metabolic demands  
446 of developing offspring (DeMarco and Guillet Jr 1992; DeMarco 1993; Foucart et al. 2014).  
447 Here, heart rate increases were largest in females held in dry conditions and the difference was  
448 more extreme in females exposed to hot days and hot nights (Fig. 3C). Furthermore, our  
449 exploration of increased hydric demands using orthogonal subsets revealed that changes in heart  
450 rate were positively related to changes in plasma osmolality. Increases in plasma osmolality may  
451 be due to decreased water volume, increased solutes (mainly sodium and chloride, Burg and  
452 Ferraris 2008), or both, and we cannot tell if our results were due to hypovolemia or  
453 hypernatremia. Regardless, mild levels of dehydration and decreased blood volume typically

454 increase heart rate, while clinical levels of dehydration elicit decreased heart rates in reptiles  
455 (Mitchell 2006). Many species of reptile can tolerate wide ranges of plasma osmolality (Minnich  
456 1982) and even the most dehydrated female in our study was within the range of previously  
457 reported osmolality from wild populations (Dupoué et al. 2020a). This suggests that females in  
458 our study were at sub-clinical levels of dehydration as the result of water restrictions, which led  
459 to higher heart rates. Lizards are able to increase heart rates to meet metabolic demands (Dawson  
460 and Templeton 1963; Licht 1965) and to rapidly gain heat during periods of intermittent basking  
461 (Bartholomew 1982; Seebacher 2005). While females exposed to hot days and hot nights  
462 expectedly had larger increases in heart rates, ours is the first study to similarly link dehydration  
463 with increased heart rate.

464

#### 465 *4.3 Water restrictions affect allocation of resources but not reproductive investment*

466 The tail is an essential site for protein and lipid storage in many species of lizards (Bateman and  
467 Fleming 2009), including *Z. vivipara* where tail volume and energy content fluctuate seasonally  
468 throughout the year (Avery 1974). As intermediates on the capital-income breeding spectrum, *Z.*  
469 *vivipara* can accumulate vast lipid reserves after parturition, prior to hibernation (Avery 1975),  
470 but are also able to assimilate nutrients during pregnancy (Bleu et al. 2013). Previous studies  
471 have found that higher temperatures, compounded by dehydration, reduce tail reserves in  
472 gestating lizards (Brusch et al. 2020). Our results align with this and further suggest a potential  
473 trade-off between tail reserves and fecundity, where larger female with small litter size are able  
474 to assimilate and invest nutrients into tail reserves but only if water is readily available (Fig. 4A).  
475 In contrast, water restricted females may not have had this option, and were required to use all  
476 available resources to immediately fuel their reproductive efforts, regardless of fecundity (Fig.

477 4B). Similarly, females in wet conditions were able to use both stored lipid (in the tail) and  
478 protein (in the leg muscle) resources to fuel reproductive investment, while those in dry  
479 conditions could not (Fig. S1 and S2). Such dynamical changes in tail and leg muscles may be  
480 relevant for future survival and reproduction. Together, these findings suggest that water  
481 restrictions during pregnancy may negatively affect a female's ability to allocate resources  
482 between current and future reproduction unless post-partum females are able to rapidly make up  
483 the deficit.

484

#### 485 *4.4 High nighttime temperatures negatively impact reproductive output*

486 Total litter size was not influenced by climatic conditions during gestation, which is not entirely  
487 surprising given that our treatments started at the tail-end of vitellogenesis or start of pregnancy,  
488 after the number of ova is typically determined in lizards (see Price 2017 for review). However,  
489 females held in hot nights had lower mass loss during parturition, significantly lighter litters, and  
490 reduced number of live neonates per litter when compared to females held in cold nights. While  
491 females exposed to hot days had higher parturition mass loss compared to females exposed to  
492 cold days, daytime temperatures had no effect on litter mass. These results suggest that  
493 pronounced downstream effects of nighttime temperatures on reproductive effort. During non-  
494 reproductive periods of the year, *Z. vivipara* experimentally exposed to warmer night conditions  
495 direct more resources towards growth at the expense of body condition (Rutschmann et al. 2021).  
496 Females in our experiment who were exposed to hot nights may have adjusted their allocation  
497 strategies as has been previously documented in viviparous lizards facing resource constraints  
498 (Shine and Downes 1999; Wapstra 2000; Bleu et al. 2013). Interestingly, water deprivation had  
499 no effect on reproductive output and the number of live neonates. Water availability in the field

500 has been shown to influence growth, reproduction, and reproductive output (Lorenzon et al.  
501 1999; Le Galliard et al. 2006; Marquis et al. 2008), but our study supports other laboratory  
502 findings that suggest that mothers primarily pay the cost during maternal-offspring conflicts for  
503 hydric resources (Dupoue et al. 2015; Bruschi et al. 2018; Dupoué et al. 2018). However, the  
504 exploration of more cryptic traits beyond litter mass and number of offspring has also found  
505 evidence of a water-based mother-offspring intergenerational trade-off, where both mothers and  
506 offspring are similarly impacted by water restrictions (Bruschi and DeNardo 2019; Bruschi et al.  
507 2019).

508

#### 509 *4.5 Conclusion*

510 Recent work examining how invertebrate (Speights and Barton, 2019; Higashi et al. 2020) and  
511 vertebrate (Clarke and Zani, 2012; Rutschmann et al. 2021) ectotherms will be impacted by  
512 increasing nocturnal temperatures has generally found that abnormally warmer nights may have  
513 a net negative effect on components of lifetime reproductive success. Climate change will also  
514 continue to alter rainfall patterns and disrupt water availability for a plethora of organisms  
515 around the globe (Fung et al. 2011; Sanderson et al. 2011). Ours is the first study to examine the  
516 combined effects of day and nighttime temperatures, and water restrictions in a vertebrate. By  
517 using pregnant females experiencing a crucial life-history stage marked by high physiological  
518 demands, we were able to broaden our understanding of the ways in which organisms may or  
519 may not be able to physiologically adjust to abiotic shifts including future climate conditions.  
520 Overall, our results suggest that high nighttime temperatures and, to some extent, reduced water  
521 availability further increase the burden on individuals already constrained by heavy resource  
522 investment during pregnancy. Negative effects of higher nighttime temperatures may provide a



523 mechanistic explanation of recent observations that warm-edge populations are vulnerable to hot  
524 and dry summer conditions and that these vulnerabilities may trigger demographic declines  
525 (Dupoué et al. 2017a, 2018). Future research should examine potential inter-generational  
526 impacts of these same climatic variables and explore how offspring might be similarly affected  
527 by maternal physiological condition during pregnancy.

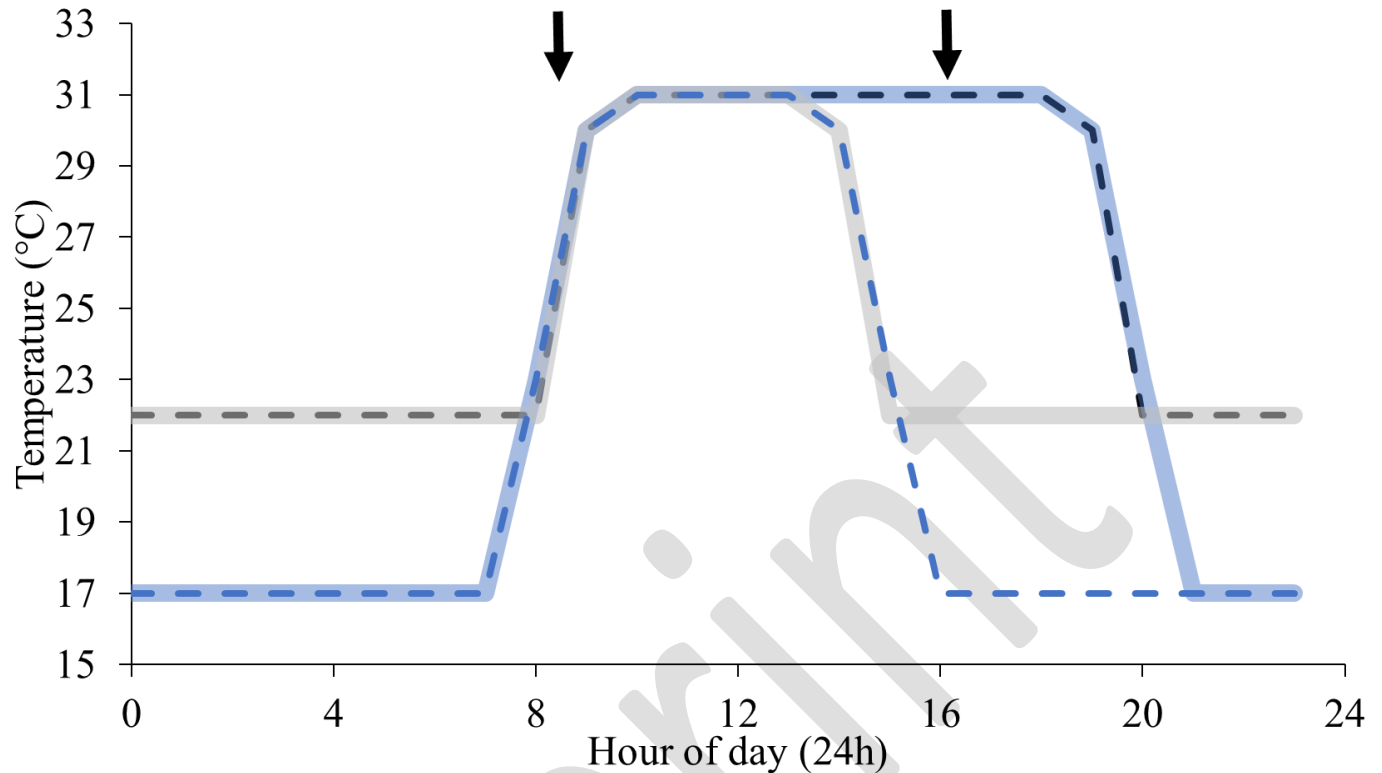
528

529 **Conflict of Interest:** No competing interests declared.

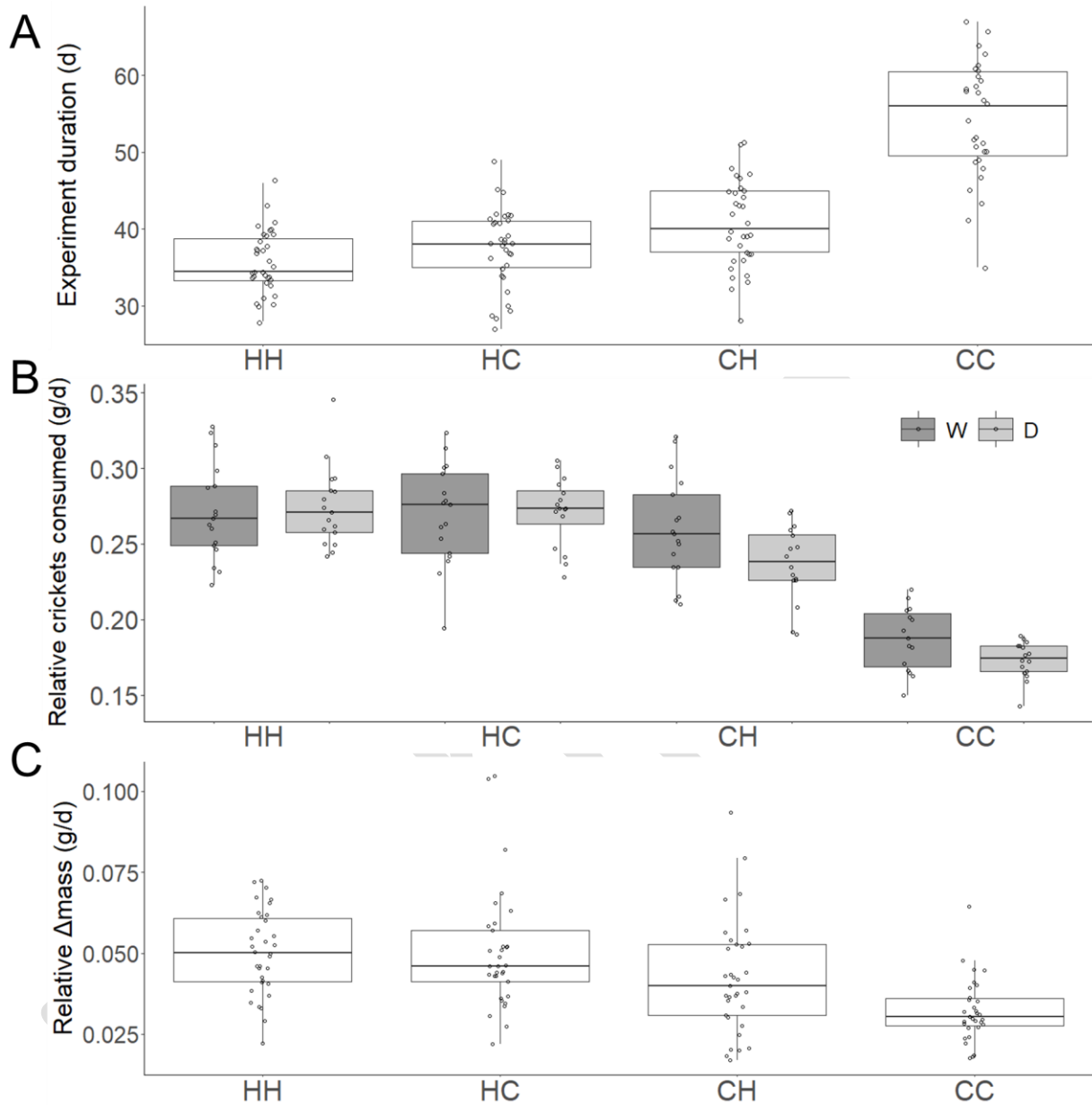
530 **Data availability:** The datasets supporting this article can be accessed at

531 <https://doi.org/10.5281/zenodo.6394479>

532 **Ethics:** This study was performed in accordance with laws relative to capture, transport, and  
533 experiments on *Zootoca vivipara* (DREAL permit #13016\_19042018) and all procedures were  
534 approved by an independent ethical committee (Apafis #2018060111033048\_v5).

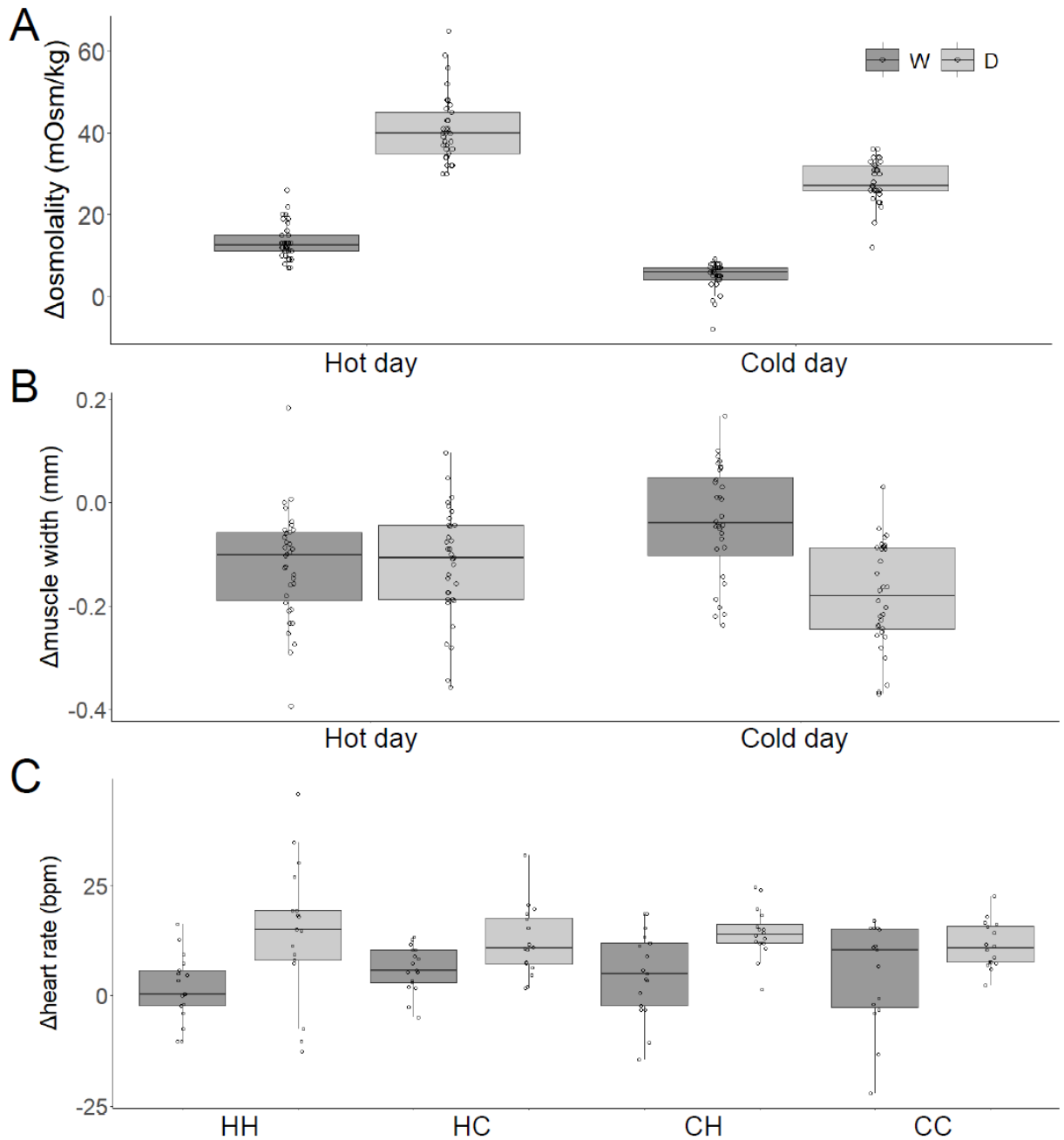


535  
 536 Figure 1 – Hourly temperature treatments of reproductive *Zootoca vivipara* throughout the  
 537 majority of pregnancy. Females were exposed to either hot day and hot night (dashed grey line; n  
 538 = 34), hot day and cold night (solid blue line; n = 33), cold day and hot night (solid grey line; n =  
 539 33), or cold day and cold night (dashed blue line; n = 31). Average daily temperatures were  
 540 highest in the hot day and night treatment (26°C), intermediate and similar in the hot day cold  
 541 night and cold day hot night treatments (23.8°C and 24.2°C), and lowest in the cold day cold  
 542 night treatment (20.9°C). Arrows indicate when water was sprayed on the sides of individual  
 543 cages each morning (wet and dry treatments) and afternoon (wet treatment only). See text for full  
 544 details. Final treatment groups were: hot day and hot night wet (n = 17) or dry (n = 17), hot-day  
 545 and cold-night wet (n = 17) or dry (n = 16), cold-day and hot-night wet (n = 17) or dry (n = 16),  
 546 and cold day and cold night wet (n = 15) or dry (n = 16).



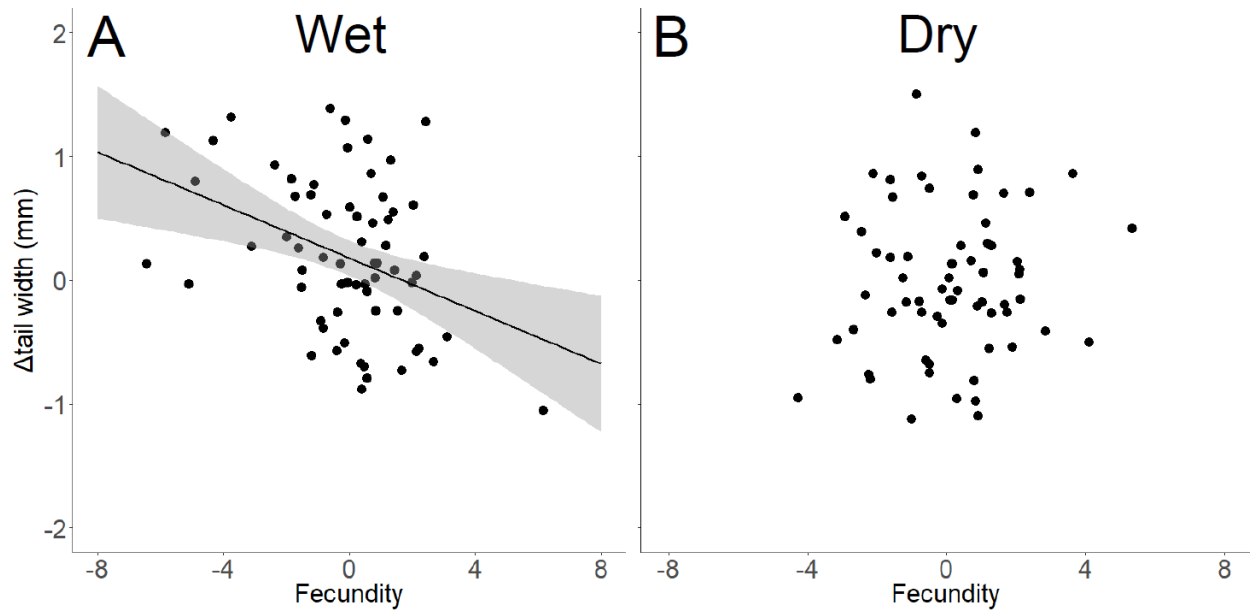
547

548 Figure 2 – Median and quartile distribution of (A) the length of gestation (duration from start of  
 549 the experiment to parturition), (B) the mass of crickets consumed per day and (C) the mass  
 550 increase per day of female *Zootoca vivipara* in wet (W; n = 66) or dry (D; n = 65) conditions and  
 551 exposed to hot day and night (HH; n = 34), hot day and cold night (HC; n = 33), cold day and hot  
 552 night (CH; n = 33), or cold day and night (CC; n = 31) throughout the majority of pregnancy.  
 553 Boxplots of the distribution of data are displayed whereas circles represent individual females.



554

555 Figure 3 – Median and quartile distribution of change from start of experiment until parturition  
 556 for (A) plasma osmolality, (B) muscle width and (C) heart rate of female *Zootoca vivipara* held  
 557 in wet (W; n = 66) or dry (D; n = 65) conditions and exposed to hot day and night (HH; n = 34),  
 558 hot day and cold night (HC; n = 33), cold day and hot night (CH; n = 33), or cold day and night  
 559 (CC; n = 31) throughout the majority of pregnancy. Boxplots of the distribution of data are  
 560 displayed whereas circles represent individual females.



561

562 Figure 4 – Relationships between change in tail width and relative fecundity (residual of litter  
563 size against female body size) in female *Zootoca vivipara* held in wet (n = 66) or dry (n = 65)  
564 conditions throughout the majority of pregnancy. A line of best fit with gray-shaded confidence  
565 interval is included for significant relationships ( $p < 0.05$ ; see text for details). Closed circles  
566 represent individual females.

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