



HAL
open science

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

George Bruschi, Jean-François Le Galliard, Robin Viton, Rodrigo Gavira, Jean Clobert, Olivier Lourdaï

► To cite this version:

George Bruschi, Jean-François Le Galliard, Robin Viton, Rodrigo Gavira, Jean Clobert, et al.. 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. *Oikos*, 2023, 2023 (3), pp.e09536. 10.1111/oik.09536 . hal-03906465

HAL Id: hal-03906465

<https://hal.science/hal-03906465v1>

Submitted on 15 Nov 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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
4 **Authors:** George A. Brusch IV^{1,2*}, Jean-François Le Galliard^{3,4}, Robin Viton¹, Rodrigo Gavira¹,
5 Jean Clobert⁵, and Olivier Lourdais^{1,6}

6 *Author for correspondence: (gbrusch@okstate.edu)

7 1 – Centre d’Etudes Biologiques de Chizé, CNRS, 79360 Villiers en Bois, France

8 2 – Department of Integrative Biology, Oklahoma State University, Stillwater, OK, 74078, USA

9 3 – Sorbonne Université, CNRS, IRD, INRAe, Institut d’écologie et des sciences de
10 l’environnement (IEES), 4 Place Jussieu, 75252 Paris Cedex 5, France

11 4 – Ecole normale supérieure, PSL University, Département de biologie, CNRS, UMS 3194,
12 Centre de recherche en écologie expérimentale et prédictive (CEREEP-Ecotron IleDeFrance), 11
13 chemin de Busseau, 77140 Saint-Pierre-lès-Nemours, France

14 5 – Station d’Ecologie Théorique et Expérimentale de Moulis, CNRS, UMR 5321, Saint Girons,
15 France

16 6 – School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501, USA

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 ± 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 (± 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 (± 3 mOsm kg⁻¹; 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⁻¹) in accordance with factory recommendations. We ran samples in
214 triplicate and used 290 mOsm kg⁻¹ standards to check the osmometer for variation after every
215 sample. If the standard varied more than the limits of the osmometer (± 3 mOsm kg⁻¹), 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 7th 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 (± 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 ± 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 kg^{-1}), but both groups lost the same amount of muscle during pregnancy (both -0.12 ± 0.02 mm).
317 Similarly, females exposed to cold and dry days had higher osmolality (27.91 ± 0.93 mOsm kg^{-1})
318 compared to those with *ad libitum* access to water (4.97 ± 0.62 mOsm kg^{-1}). However, water
319 restricted cold day females lost a higher amount of muscle (-0.18 ± 0.02 mm) when compared to
320 cold day females in wet conditions (-0.04 ± 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 ± 1.08
325 bpm) compared to females in dry conditions (13.20 ± 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 ± 1.61 bpm and
328 9.05 ± 1.28 bpm, respectively) and lower in females exposed to hot days and nights or cold days
329 and nights (8.14 ± 2.35 bpm and 8.25 ± 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 ± 0.05 mm), however, when
332 comparing across water treatments, these increases were driven by females in wet conditions
333 (0.19 ± 0.08 mm) while females in dry conditions tended to lose tail width (-0.02 ± 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 ± 0.10 g), whereas females in hot nights lost less
345 (1.62 ± 0.73 g) compared to females in cold nights (2.00 ± 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 ± 0.05 g) compared to females in cold nights (0.97 ± 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 Guillette 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.

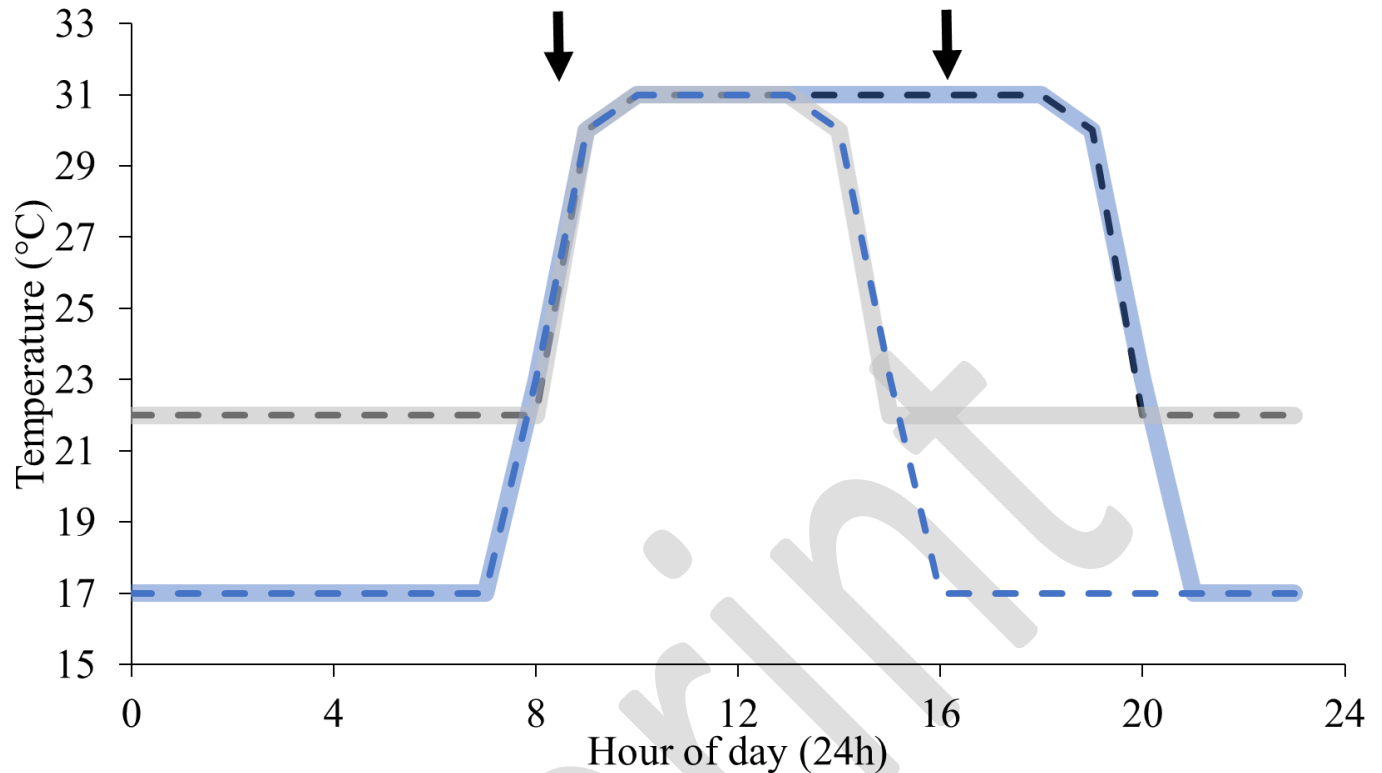
528

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

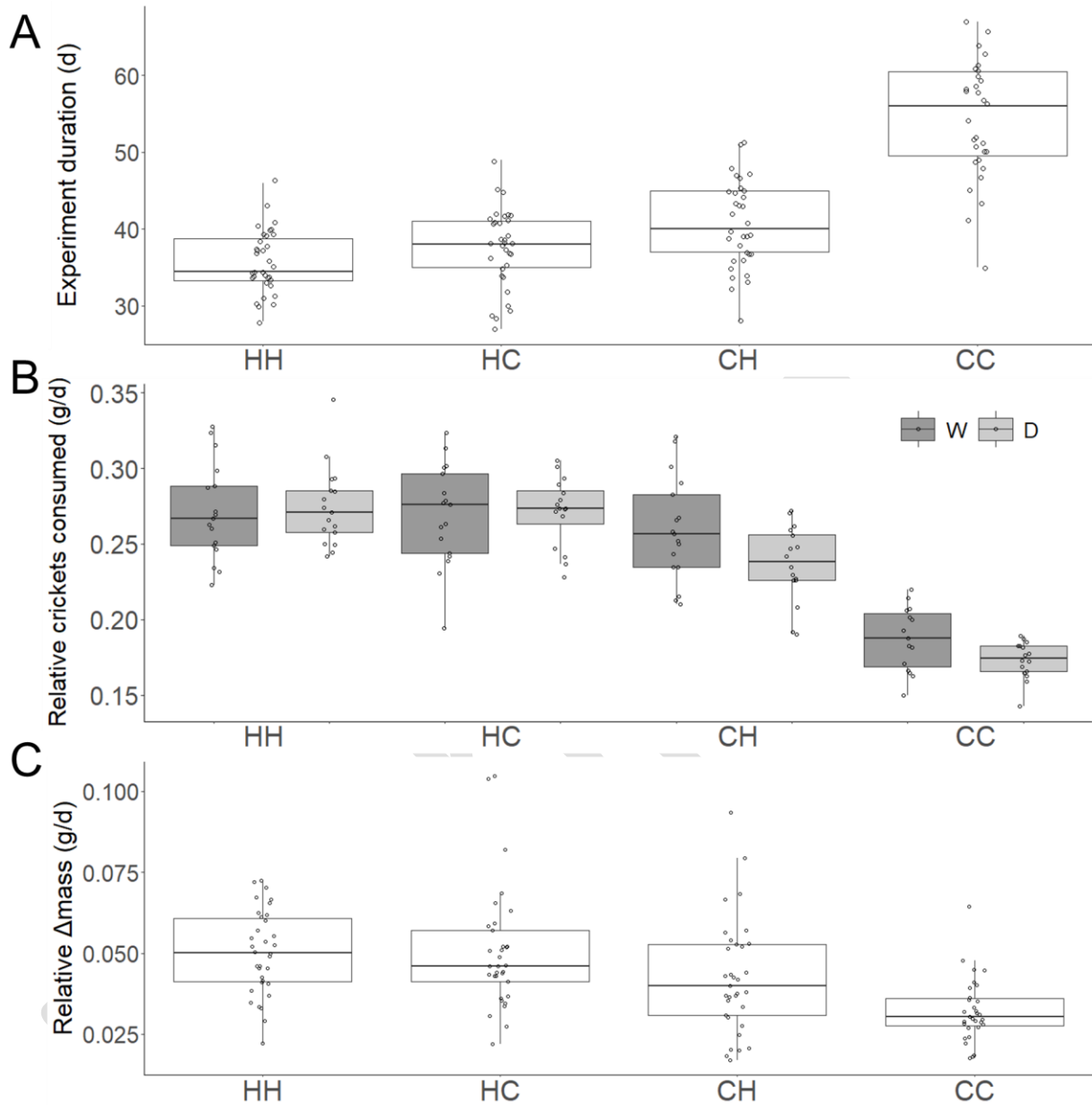
530 **Data availability:** The datasets supporting this article can be accessed at

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).

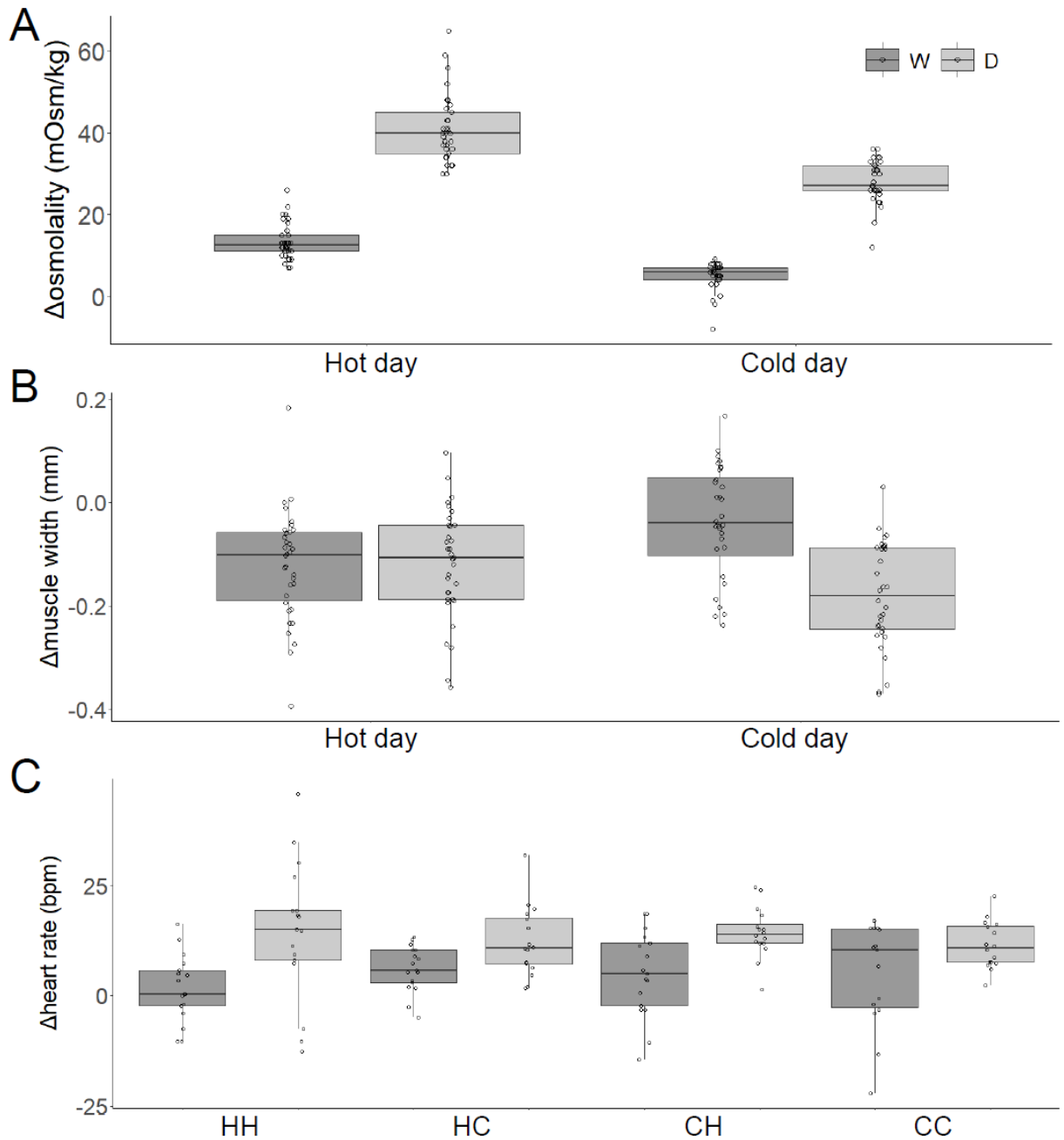


535
 536 Figure 1 – Hourly temperature treatments of reproductive *Zootoca vivipara* throughout the
 537 majority of pregnancy. Females were exposed to either hot day and hot night (dashed grey line; n
 538 = 34), hot day and cold night (solid blue line; n = 33), cold day and hot night (solid grey line; 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).



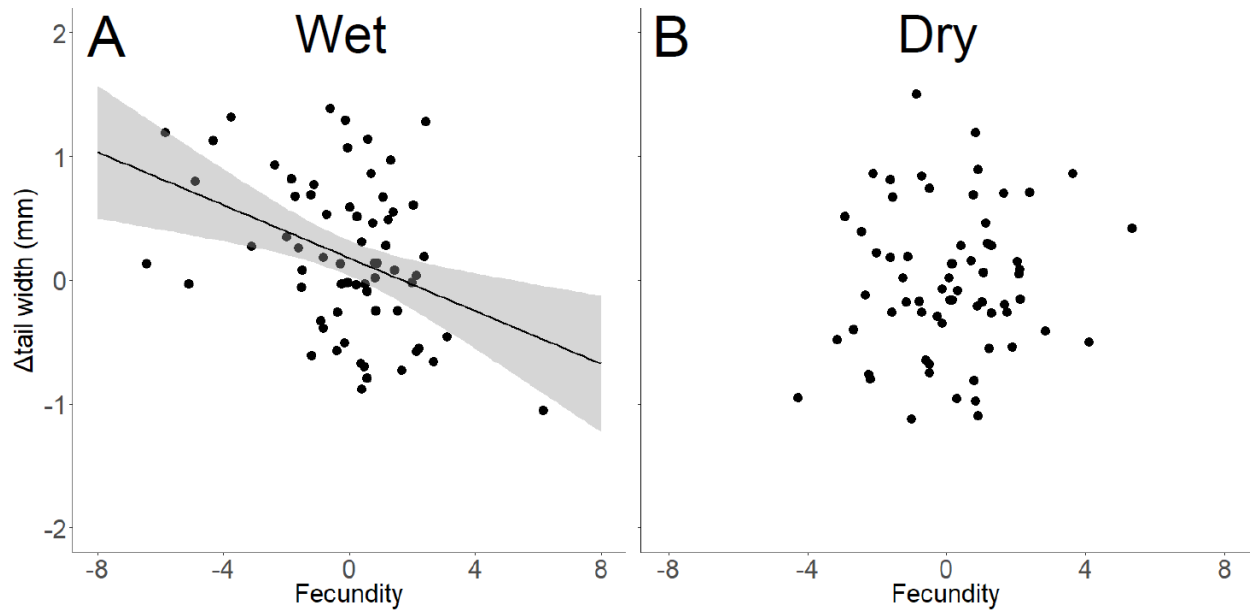
547

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.

567 **References**

- 568 Adler, R.F., Gu, G., Wang, J.J., Huffman, G.J., Curtis, S. & Bolvin, D. 2008. Relationships
569 between global precipitation and surface temperature on interannual and longer
570 timescales (1979–2006). – J. Geophys. Res. Atmos. 113: D22104.
- 571 Anderson, M. and Cribble, N. 1998. Partitioning the variation among spatial, temporal and
572 environmental components in a multivariate data set. – Austral. J. Ecol. 23: 158-167.
- 573 Angilletta Jr, M. J. 2001. Thermal and physiological constraints on energy assimilation in a
574 widespread lizard (*Sceloporus undulatus*). – Ecology 82: 3044-3056.
- 575 Arnold, T. W. 2010. Uninformative parameters and model selection using Akaike's Information
576 Criterion. – J. Wildl. Manage. 74: 1175-1178.
- 577 Avery, R. 1974. Storage lipids in the lizard *Lacerta vivipara*: a quantitative study. – J. Zool. 173:
578 419-425.
- 579 Avery, R. 1975. Clutch size and reproductive effort in the lizard *Lacerta vivipara* Jacquin. –
580 Oecologia 19: 165-170.
- 581 Bartholomew, G. A. 1982. Physiological control of body temperature. – Biol. Reptilia 12: 167-
582 211.
- 583 Barton, B. T. and Schmitz, O. J. 2018. Opposite effects of daytime and nighttime warming on
584 top-down control of plant diversity. – Ecology 99: 13-20.
- 585 Bateman, P. and Fleming, P. 2009. To cut a long tail short: a review of lizard caudal autotomy
586 studies carried out over the last 20 years. – J. Zool. 277: 1-14.
- 587 Bauwens, D. and Verheyen, R. 1985. The timing of reproduction in the lizard *Lacerata vivipara*:
588 differences between individual females. – J. Herpetol. 19: 353-364.
- 589 Beaupre, S. J. 1996. Field metabolic rate, water flux, and energy budgets of mottled rock
590 rattlesnakes, *Crotalus lepidus*, from two populations. – Copeia 1996: 319-329.
- 591 Blackburn, D. G. 2015. Viviparous placentotrophy in reptiles and the parent–offspring conflict. –
592 J. Exp. Zool. B: Mol.Dev. Evol. 324: 532-548.
- 593 Bleu, J., Le Galliard, J.-F., Fitze, P.S., Meylan, S., Clobert, J. & Massot, M. 2013. Reproductive
594 allocation strategies: a long-term study on proximate factors and temporal adjustments in
595 a viviparous lizard. – Oecologia 171: 141-151.
- 596 Bonebrake, T. C. and Mastrandrea, M. D. 2010. Tolerance adaptation and precipitation changes
597 complicate latitudinal patterns of climate change impacts. – Proc. Natl Acad. Sci. USA
598 107: 12581-12586.
- 599 Brusch IV, G.A., Billy, G., Blattman, J.N. & DeNardo, D.F. 2017. Reproduction alters hydration
600 state but does not impact the positive effects of dehydration on innate immune function in
601 children's pythons (*Antaresia childreni*). – Physiol. Biochem. Zool. 90: 646-654.
- 602 Brusch IV, G. A. and DeNardo, D. F. 2019. Egg desiccation leads to dehydration and enhanced
603 innate immunity in python embryos. – Dev. Comp. Immunol. 90: 147-151.
- 604 Brusch IV, G.A., Gavira, R.S., Viton, R., Dupoué, A., Leroux-Coyau, M., Meylan, S., Le
605 Galliard, J.-F. & Lourdais, O. 2020. Additive effects of temperature and water
606 availability on pregnancy in a viviparous lizard. – J. Exp. Biol. 223: jeb228064.
- 607 Brusch IV, G.A., Heulin, B. & DeNardo, D.F. 2019. Dehydration during egg production alters
608 egg composition and yolk immune function. – Comp. Biochem. Physiol. A Mol. Integr.
609 Physiol. 227: 68-74.
- 610 Brusch IV, G.A., Lourdais, O., Kaminsky, B. & DeNardo, D.F. 2018. Muscles provide an
611 internal water reserve for reproduction. – Proc. Royal Soc. B, 285: 20180752.

612 Buckley, L.B., Urban, M.C., Angilletta, M.J., Crozier, L.G., Rissler, L.J. & Sears, M.W. 2010.
613 Can mechanism inform species' distribution models? – *Ecol. Lett.* 13: 1041-1054.

614 Burg, M. B. and Ferraris, J. D. 2008. Intracellular organic osmolytes: function and regulation. –
615 *J. Biol. Chem.* 283: 7309-7313.

616 Calow, P. 1973. The relationship between fecundity, phenology, and longevity: a systems
617 approach. – *Am. Nat.* 107: 559-574.

618 Chamaillé-Jammes, S., Massot, M., Aragon, P. & Clobert, J. 2006. Global warming and positive
619 fitness response in mountain populations of common lizards *Lacerta vivipara*. – *Glob.*
620 *Chang. Biol.* 12: 392-402.

621 Dai, A., Trenberth, K.E. & Karl, T.R. 1999. Effects of clouds, soil moisture, precipitation, and
622 water vapor on diurnal temperature range. – *J. Clim.* 12: 2451-2473.

623 Davy, R., Esau, I., Chernokulsky, A., Outten, S. & Zilitinkevich, S. 2017. Diurnal asymmetry to
624 the observed global warming. – *Int. J. Climatol.* 37: 79-93.

625 Dawson, W. R. and Templeton, J. R. 1963. Physiological responses to temperature in the lizard
626 *Crotaphytus collaris*. – *Physiol. Zool.* 36: 219-236.

627 Dely, O. G. and Böhme, W. 1984. *Lacerta vivipara* Jacquin 1787–Waldeidechse. – *Handbuch*
628 *der Reptilien und Amphibien Europas 2*: 362-393.

629 DeMarco, V. 1993. Metabolic rates of female viviparous lizards (*Sceloporus jarrovi*) throughout
630 the reproductive cycle: do pregnant lizards adhere to standard allometry? – *Physiol. Zool.*
631 66: 166-180.

632 Demarco, V. and Guillette Jr, L. J. 1992. Physiological cost of pregnancy in a viviparous lizard
633 (*Sceloporus jarrovi*). – *J. Exp Zool.* 262: 383-390.

634 Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak, D.C. &
635 Martin, P.R. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. –
636 *Proc. Natl Acad. Sci. USA* 105: 6668-6672.

637 Dezetter, M., Le Galliard, J.F., Guiller, G., Guillon, M., Leroux-Coyau, M., Meylan, S.,
638 Brischoux, F., Angelier, F. & Lourdais, O. 2021. Water deprivation compromises
639 maternal physiology and reproductive success in a cold and wet adapted snake *Vipera*
640 *berus*. – *Conser. Physiol.* 9: coab071.

641 Dillon, M.E., Wang, G. & Huey, R.B. 2010. Global metabolic impacts of recent climate
642 warming. – *Nature* 467: 704-706.

643 Doughty, P. and Shine, R. 1997. Detecting life history trade-offs: measuring energy stores in
644 “capital” breeders reveals costs of reproduction. – *Oecologia* 110: 508-513.

645 Dupoué, A., Brischoux, F., Angelier, F., DeNardo, D.F., Wright, C.D. & Lourdais, O. 2015.
646 Intergenerational trade-off for water may induce a mother–offspring conflict in favour of
647 embryos in a viviparous snake. – *Funct. Ecol.* 29: 414-422.

648 Dupoué, A., Rutschmann, A., Le Galliard, J.F., Clobert, J., Angelier, F., Marciau, C., Ruault, S.,
649 Miles, D. & Meylan, S. 2017a. Shorter telomeres precede population extinction in wild
650 lizards. – *Sci. Rep.* 7: 1–8.

651 Dupoué, A., Rutschmann, A., Le Galliard, J.F., Miles, D.B., Clobert, J., DeNardo, D.F., Bruschi,
652 G.A. & Meylan, S. 2017b. Water availability and environmental temperature correlate
653 with geographic variation in water balance in common lizards. – *Oecologia* 185: 561-571.

654 Dupoué, A., Le Galliard, J.F., Josserand, R., DeNardo, D.F., Decencièrè, B., Agostini, S.,
655 Haussy, C. & Meylan, S. 2018. Water restriction causes an intergenerational trade-off
656 and delayed mother–offspring conflict in a viviparous lizard. – *Funct. Ecol.* 32: 676-686.

657 Dupoué, A., Blaimont, P., Rozen-Rechels, D., Richard, M., Meylan, S., Clobert, J., Miles, D.B.,
658 Martin, R., Decencièrre, B. & Agostini, S. 2020a. Water availability and temperature
659 induce changes in oxidative status during pregnancy in a viviparous lizard. – *Funct. Ecol.*
660 34: 475-485.

661 Dupoué, A., Sorlin, M., Richard, M., Le Galliard, J.F., Lourdais, O., Clobert, J. & Aubret, F.
662 2020b. Mother-offspring conflict for water and its mitigation in the oviparous form of the
663 reproductively bimodal lizard, *Zootoca vivipara*. – *Biol. J. Linn. Soc. Lond.* 129: 888-
664 900.

665 Foucart, T., Lourdais, O., DeNardo, D.F. & Heulin, B. 2014. Influence of reproductive mode on
666 metabolic costs of reproduction: insight from the bimodal lizard *Zootoca vivipara*. – *J.*
667 *Exp. Biol.* 217: 4049-4056.

668 Fox, J. and Weisberg, S. 2018. *An R companion to applied regression*. Sage publications.

669 Fung, F., Lopez, A. & New, M. 2011. Water availability in + 2 C and + 4 C worlds. – *Philos.*
670 *Trans. R. Soc. A* 369: 99-116.

671 Gilman, C. A. and Wolf, B. O. 2007. Use of portable ultrasonography as a nondestructive
672 method for estimating reproductive effort in lizards. – *J. Exp. Biol.* 210: 1859-1867.

673 Harvell, C.D., Mitchell, C.E., Ward, J.R., Altizer, S., Dobson, A.P., Ostfeld, R.S. & Samuel,
674 M.D. 2002. Climate warming and disease risks for terrestrial and marine biota. – *Science*
675 296: 2158-2162.

676 Helmuth, B., Broitman, B.R., Yamane, L., Gilman, S.E., Mach, K., Mislán, K.A. & Denny,
677 M.W. 2010. Organismal climatology: analyzing environmental variability at scales
678 relevant to physiological stress. – *J. Exp. Biol.* 213: 995-1003.

679 Helmuth, B., Kingsolver, J.G. & Carrington, E. 2005. Biophysics, physiological ecology, and
680 climate change: does mechanism matter? – *Annu. Rev. Physiol.* 67: 177-201.

681 Herrando-Pérez, S., Ferri-Yáñez, F., Monasterio, C., Beukema, W., Gomes, V., Belliure, J.,
682 Chown, S.L., Vieites, D.R. & Araújo, M.B. 2019. Intraspecific variation in lizard heat
683 tolerance alters estimates of climate impact. – *J. Anim. Ecol.* 88: 247-257.

684 Hoffmann, A.A., Hallas, R.J., Dean, J.A. & Schiffer, M. 2003. Low potential for climatic stress
685 adaptation in a rainforest *Drosophila* species. – *Science* 301: 100-102.

686 Hoffmann, A.A., Hallas, R.J., Dean, J.A. & Schiffer, M. 2008. Simultaneous inference in general
687 parametric models. – *Biom. J.* 50: 346-363.

688 Huey, R.B., Kearney, M.R., Krockenberger, A., Holtum, J.A., Jess, M. & Williams, S.E. 2012.
689 Predicting organismal vulnerability to climate warming: roles of behaviour, physiology
690 and adaptation. – *Philos. Trans. R. Soc. Lond., B, Biol. Sci.*, 367: 1665-1679.

691 Huey, R. B. and Tewksbury, J. J. 2009. Can behavior douse the fire of climate warming? – *Proc.*
692 *Natl Acad. Sci. USA* 106: 3647-3648.

693 Jenni, L. and Jenni-Eiermann, S. 1998. Fuel supply and metabolic constraints in migrating birds.
694 – *J. Avian Biol.* 29: 521-528.

695 Johansen, J. L. and Jones, G. P. 2011. Increasing ocean temperature reduces the metabolic
696 performance and swimming ability of coral reef damselfishes. – *Glob. Chang. Biol.* 17:
697 2971-2979.

698 Kearney, M. and Porter, W. 2009. Mechanistic niche modelling: combining physiological and
699 spatial data to predict species' ranges. – *Ecol. Lett.* 12: 334-350.

700 Kingsolver, J. G. 2002. Impacts of global environmental change on animals. *The Earth System:*
701 *Biological and Ecological Dimensions of Global Environmental*, pp. 55-66. Wiley

- 702 Kotiaho, J. S. 2001. Costs of sexual traits: a mismatch between theoretical considerations and
703 empirical evidence. – *Biol. Rev.* 76: 365-376.
- 704 Le Galliard, J.-F., Le Bris, M. & Clobert, J. 2003. Timing of locomotor impairment and shift in
705 thermal preferences during gravidity in a viviparous lizard. – *Funct. Ecol.* 17: 877-885.
- 706 Le Galliard, J.F., Massot, M., Landys, M., Meylan, S. & Clobert, J. 2006. Ontogenetic sources of
707 variation in sexual size dimorphism in a viviparous lizard. – *J. Evo. Biol.* 19: 690-704.
- 708 Levy, O., Borchert, J.D., Rusch, T.W., Buckley, L.B. & Angilletta Jr, M.J. 2017. Diminishing
709 returns limit energetic costs of climate change. – *Ecology* 98: 1217-1228.
- 710 Licht, P. 1965. Effects of temperature on heart rates of lizards during rest and activity. – *Physiol.*
711 *Zool.* 38: 129-137.
- 712 Lillywhite, H.B., Zippel, K.C. & Farrell, A.P. 1999. Resting and maximal heart rates in
713 ectothermic vertebrates. – *Comp. Biochem. Physiol A Mol Integr Physiol.* 124: 369-382.
- 714 Lorenzon, P., Clobert, J., Oppliger, A. & John-Alder, H. 1999. Effect of water constraint on
715 growth rate, activity and body temperature of yearling common lizard (*Lacerta vivipara*).
716 – *Oecologia* 118: 423-430.
- 717 Lorioux, S., Angelier, F. & Lourdais, O. 2016. Are glucocorticoids good indicators of pregnancy
718 constraints in a capital breeder? – *Gen. Comp. Endo.* 232: 125-133.
- 719 Lourdais, O., Brischoux, F., Denardo, D. & Shine, R. 2004. Protein catabolism in pregnant
720 snakes (*Epicrates cenchria maurus* Boidae) compromises musculature and performance
721 after reproduction. – *J. Comp. Physiol. B* 174: 383-391.
- 722 Lourdais, O., Lorioux, S. & DeNardo, D.F. 2013. Structural and performance costs of
723 reproduction in a pure capital breeder, the Children's python *Antaresia childreni*. –
724 *Physiol. Chem. Zool.* 86: 176-183.
- 725 Marquis, O., Massot, M. & Le Galliard, J.F. 2008. Intergenerational effects of climate generate
726 cohort variation in lizard reproductive performance. – *Ecology* 89: 2575-2583.
- 727 Minnich, J. 1982. The use of water. – *Biol. Reptilia* 12: 325-395.
- 728 Mitchell, M. A. 2006. Therapeutics. *Reptile medicine and surgery*, pp. 631-664. Elsevier Inc.
- 729 Murphy, M. S. and DeNardo, D. F. 2019. Rattlesnakes must drink: meal consumption does not
730 improve hydration state. – *Physiol. Biochem. Zool.* 92: 381-385.
- 731 Musolin, D. L. 2007. Insects in a warmer world: ecological, physiological and life-history
732 responses of true bugs (Heteroptera) to climate change. – *Glob. Chang. Biol.* 13: 1565-
733 1585.
- 734 New, M., Liverman, D., Schroeder, H. & Anderson, K. 2011. Four degrees and beyond: the
735 potential for a global temperature increase of four degrees and its implications. – *Philos.*
736 *Trans. R. Soc. A* 369: 6-19.
- 737 Niehaus, A.C., Angilletta Jr, M.J., Sears, M.W., Franklin, C.E. & Wilson, R.S. 2012. Predicting
738 the physiological performance of ectotherms in fluctuating thermal environments. – *J.*
739 *Exp. Biol.* 215: 694-701.
- 740 Olsson, M. and Shine, R. 1997a. The limits to reproductive output: offspring size versus number
741 in the sand lizard (*Lacerta agilis*). – *Am. Nat.* 149: 179-188.
- 742 Olsson, M. and Shine, R. 1997b. The seasonal timing of oviposition in sand lizards (*Lacerta*
743 *agilis*): why early clutches are better. – *J. Exp. Biol.* 10: 369-381.
- 744 Parmesan, C. 2006. Ecological and Evolutionary Responses to Recent Climate Change. – *Annu.*
745 *Rev. Ecol. Evol. Syst.* 37: 637-669.

746 Pincheira-Donoso, D., Tregenza, T., Witt, M.J. & Hodgson, D.J. 2013. The evolution of
747 viviparity opens opportunities for lizard radiation but drives it into a climatic cul-de-sac.
748 – *Glob. Ecol. Biogeogr.* 22: 857-867.

749 Portner, H. O. and Farrell, A. P. 2008. Physiology and climate change. – *Science* 322: 690-692.

750 Price, E. R. (2017) The physiology of lipid storage and use in reptiles. – *Biol. Rev.* 92: 1406-
751 1426.

752 Refsnider, J.M., Clifton, I.T. & Vazquez, T.K. 2019. Developmental plasticity of thermal
753 ecology traits in reptiles: Trends, potential benefits, and research needs. – *J. Therm. Biol.*
754 84: 74-82.

755 Reznick, D., Nunney, L. & Tessier, A. 2000 Big houses, big cars, superfleas and the costs of
756 reproduction. – *Trends Ecol. Evo.* 15: 421-425.

757 Riddell, E.A., Odom, J.P., Damm, J.D. & Sears, M.W. 2018. Plasticity reveals hidden resistance
758 to extinction under climate change in the global hotspot of salamander diversity. – *Sci.*
759 *Adv.* 4: eaar5471.

760 Rozen-Rechels, D., Dupoué, A., Lourdais, O., Chamaillé-Jammes, S., Meylan, S., Clobert, J. &
761 Le Galliard, J.F. 2019. When water interacts with temperature: Ecological and
762 evolutionary implications of thermo-hydroregulation in terrestrial ectotherms. – *Ecol.*
763 *Evo.* 9: 10029-10043.

764 Rueden, C.T., Schindelin, J., Hiner, M.C., DeZonia, B.E., Walter, A.E., Arena, E.T. & Eliceiri,
765 K.W. 2017. ImageJ2: ImageJ for the next generation of scientific image data. – *BMC*
766 *Bioinformatics*, 18: 1-26.

767 Rutschmann, A., Dupoué, A., Miles, D.B., Megía-Palma, R., Laudén, C., Richard, M., Badiane,
768 A., Rozen-Rechels, D., Brevet, M. & Blaimont, P. 2021. Intense nocturnal warming alters
769 growth strategies, colouration and parasite load in a diurnal lizard. – *J. Anim. Ecol.* 90:
770 1864-1877.

771 Rutschmann, A., Miles, D.B., Le Galliard, J.F., Richard, M., Moulherat, S., Sinervo, B. &
772 Clobert, J. 2016. Climate and habitat interact to shape the thermal reaction norms of
773 breeding phenology across lizard populations. – *J. Anim. Ecol.* 85: 457-466.

774 Sanderson, M.G., Hemming, D.L. & Betts, R.A. 2011. Regional temperature and precipitation
775 changes under high-end (≥ 4 C) global warming. – *Philos. Trans. Royal Soc. A* 369: 85-
776 98.

777 Seebacher, F. 2005. A review of thermoregulation and physiological performance in reptiles:
778 what is the role of phenotypic flexibility? – *J. Comp. Physiol. B* 175: 453-461.

779 Shine, R. and Downes, S. J. 1999. Can pregnant lizards adjust their offspring phenotypes to
780 environmental conditions? – *Oecologia* 119: 1-8.

781 Skoczylas, R. 1978. Physiology of the digestive tract. *Biology of the Reptilia* 8, pp 589-717.
782 Academic Press.

783 Speakman, J. R. and Garratt, M. 2014. Oxidative stress as a cost of reproduction: Beyond the
784 simplistic trade-off model. – *BioEssays*, 36: 93-106.

785 Speights, C.J., Harmon, J.P. & Barton, B.T. 2017. Contrasting the potential effects of daytime
786 versus nighttime warming on insects. – *Curr. Opin. Insect. Sci.* 23: 1-6.

787 Stillman, J. H. 2003. Acclimation capacity underlies susceptibility to climate change. – *Science*
788 301: 65.

789 Stoks, R., Verheyen, J., Van Dievel, M. & Tüzün, N. 2017. Daily temperature variation and
790 extreme high temperatures drive performance and biotic interactions in a warming world.
791 – *Curr. Opin. Insect. Sci.* 23: 35-42.

792 Tewksbury, J.J., Huey, R.B. & Deutsch, C.A. 2008. Putting the heat on tropical animals. –
793 Science 320: 1296-1297.

794 Thomas, C.D., Cameron, A., Green, R.E., Bakkenes, M., Beaumont, L.J., Collingham, Y.C.,
795 Erasmus, B.F., De Siqueira, M.F., Grainger, A., Hannah, L., Hughes, L., Huntley, B.,
796 Van Jaarsveld, A.S., Midgley, G.F., Miles, L., Ortega-Huerta, M.A., Peterson, A.T.,
797 Phillips, O.L. & Williams, S.E. 2004. Extinction risk from climate change. – Nature 427:
798 145-148.

799 Van Damme, R., Bauwens, D. & Verheyen, R. 1991. The thermal dependence of feeding
800 behaviour, food consumption and gut-passage time in the lizard *Lacerta vivipara* Jacquin.
801 – Funct. Ecol. 5: 507-517.

802 Venables, W. N. and Ripley, B. D. 2013. *Modern applied statistics with S-PLUS*. Springer
803 Science and Business Media.

804 Verheyen, J. and Stoks, R. 2019. Temperature variation makes an ectotherm more sensitive to
805 global warming unless thermal evolution occurs. – J. Anim. Ecol. 88: 624-636.

806 Vose, R.S., Easterling, D.R. & Gleason, B. 2005. Maximum and minimum temperature trends
807 for the globe: An update through 2004. – Geophys. Res. Lett. 32: L23822.

808 Wapstra, E. 2000. Maternal basking opportunity affects juvenile phenotype in a viviparous
809 lizard. – Funct. Ecol. 14: 345-352.

810 Warner, D. A. and Shine, R. 2007. Fitness of juvenile lizards depends on seasonal timing of
811 hatching, not offspring body size. – Oecologia 154: 65-73.

812 While, G.M., Uller, T., McEvoy, J. & Wapstra, E. 2009. Long-lasting effects of among-but not
813 within-litter timing of birth in a viviparous lizard. – Evol. Ecol. Res. 11: 1259-1270.

814 Wright, C.D., Jackson, M.L. & DeNardo, D.F. 2013. Meal consumption is ineffective at
815 maintaining or correcting water balance in a desert lizard, *Heloderma suspectum*. – J.
816 Exp. Biol. 216: 1439-1447.

817 Zeileis, A. and Hothorn, T. 2002. Diagnostic checking in regression relationships. – R News 2:
818 7-10.

819 Zera, A. J. and Harshman, L. G. 2001. The physiology of life history trade-offs in animals. –
820 Annu. Rev. Ecol. Evol. Syst. 32: 95-126.

821 Zhou, L., Dickinson, R.E., Dirmeyer, P., Dai, A. & Min, S.K. 2009. Spatiotemporal patterns of
822 changes in maximum and minimum temperatures in multi-model simulations. – Geophys.
823 Res. Lett. 36: L02702.

824 Zuur, A.F., Ieno, E.N. & Elphick, C.S. 2010. A protocol for data exploration to avoid common
825 statistical problems. – Methods Ecol. Evol. 1: 3-14.