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## Climate aridity and habitat drive geographical variation in morphology and thermo-hydroregulation strategies of a widespread lizard species

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### Abstract :

Thermo-hydroregulation strategies involve concurrent changes in functional traits related to energy, water balance and thermoregulation and play a key role in determining life-history traits and population demography of terrestrial ectotherms. Local thermal and hydric conditions should be important drivers of the geographical variation of thermo-hydroregulation strategies, but we lack studies that examine these changes across climatic gradients in different habitat types. Here, we investigated intraspecific variation of morphology and thermo-hydroregulation traits in the widespread European common lizard (*Zootoca vivipara loulisantzii*) across a multidimensional environmental gradient involving independent variation in air temperature and rainfall and differences in habitat features (access to free-standing water and forest cover). We sampled adult males for morphology, resting metabolic rate, total and cutaneous evaporative water loss and thermal preferences in 15 populations from the rear to the leading edge of the distribution across an elevational gradient ranging from sea level to 1750 m. Besides a decrease in adult body size with increasing environmental temperatures, we found little effect of thermal conditions on thermo-hydroregulation strategies. In particular, relict lowland populations from the warm rear edge showed no specific ecophysiological adaptations. Instead, body mass, body condition and resting metabolic rate were positively associated with a rainfall gradient, while forest cover and water access in the habitat throughout the season also influenced cutaneous evaporative water loss. Our study emphasizes the importance of rainfall and habitat features rather than thermal conditions for geographical variation in lizard morphology and physiology.

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**Keywords** : aridity, evaporative water loss, lizards, metabolism, morphology, reptiles, thermal preferences

## 1 **1. Introduction**

2 Ongoing climate change dramatically impacts biodiversity and increasing our ability to predict future  
3 ecological effects of climate changes is one of the main challenges facing research in ecology today  
4 (Bellard et al., 2012). In widely distributed organisms, gradient analyses of intraspecific variation along  
5 contrasted climate conditions can inform us on **the environmental sensitivity** and capacity of different  
6 species to adapt to future changes (Blois et al., 2013). Terrestrial ectotherms are sensitive to  
7 environmental temperatures and highly vulnerable to global warming, especially to heat and drought  
8 stress that impairs activity, causes physiological stress and can reduce fitness and population growth  
9 (Huey et al., 2009, 2012; Sinervo et al., 2010; Kubisch, Fernández, & Ibarguengoytía, 2016).

10 Terrestrial ectotherms can cope **with heat and drought risks** through changes in thermoregulation (i.e.,  
11 heat exchange and body temperature regulation) and hydoregulation traits (i.e., water balance  
12 regulation), collectively referred to as their “thermo-hydoregulation” strategies (Rozen- Rechels et al.,  
13 2019). Yet, large uncertainties remain about the respective roles of physiological and behavioral  
14 response and the geographic variability of body temperature and water balance regulation. Behavioral  
15 and physiological maintenance of an optimal body temperature and hydration state depends to a large  
16 extent on spatio-temporal variability in operative temperatures, water availability and water vapor deficit  
17 **(Sears et al., 2019; Rozen-Rechels et al., 2019).**

18 Historically, **comparative physiology** research has however focused on the thermal biology of  
19 terrestrial ectotherms (Huey et al., 2012; Sunday et al., 2014; Seebacher, White, & Franklin, 2015;  
20 Artacho et al., 2017; Caldwell, While, & Wapstra, 2017; Rutschmann et al., 2020). For example,  
21 populations of the same species of lizards can display **different** critical thermal limits depending on  
22 local thermal conditions, particularly those characterizing the cold end of the thermal performance  
23 curves (Pontes-da-Silva et al., 2018; Herrando- Pérez et al., 2020; Bodensteiner et al., 2021). Another  
24 general finding is the metabolic cold adaptation in energy expenditure where organisms from cooler  
25 climates have higher basal metabolic rates, probably to partly compensate for the slow development and  
26 pace-of-life associated with low environmental temperatures in ectotherms (Seebacher, 2005; Pettersen,  
27 2020; Dupoué et al 2017a). **Instead, intraspecific studies on thermal preferences (i.e., preferred**  
28 **body temperatures in the absence of thermoregulation costs) have led to inconclusive results, with**  
29 **some authors demonstrating geographic differences in thermal preferences (Trochet et al., 2018;**  
30 **Rozen-Rechels et al., 2021) whereas others suggest that thermal preferences are more**  
31 **evolutionarily rigid than other thermal biology traits (Clusella-Trullas & Chown, 2014). In**  
32 **addition, we lack knowledge on how intraspecific variations correspond with the observed**  
33 **variation between species.**

34 Heretofore, most studies of geographic variation in reptile thermal traits have focused on thermal clines  
35 such as broad altitudinal or latitudinal gradients in air temperatures (Gvoždík & Castilla, 2001; Zamora-

36 Camacho, 2013; Osojnik et al., 2013; Clusella-Trullas & Chown, 2014; Trochet et al., 2018; Plasman et  
37 al., 2020). Such broad thermal clines usually confound with variation in precipitation, making the two  
38 factors difficult to distinguish. Yet, habitat **characteristics** can amplify or buffer thermal variability and  
39 recent works have highlighted the importance of water availability and microclimatic conditions when  
40 studying responses **of terrestrial ectotherms to environmental modifications** (Wegener, Gartner, &  
41 Losos, 2014; Miller & Lutterschmidt, 2014; Chiacchio et al., 2020). In squamate reptiles, availability of  
42 water and suitable microhabitats are essential for optimal thermoregulation and hydroregulation, as  
43 behavioral exploitation of microhabitats (such as wet shelters from the vegetation) can buffer these  
44 species from the deleterious effects of a temperature increase or a rainfall reduction (Sears et al., 2016;  
45 Rozen- Rechels et al., 2020). In addition, the costs of maintaining high body temperature in heliothermic  
46 species usually increase when those species face a reduction of water availability in the environment,  
47 leading to the selection of suboptimal body temperature (Rozen- Rechels et al., 2020). Variation in  
48 morphological traits can also be strongly affected by water availability and microhabitat features  
49 (Ashton, 2001; Olalla-Tárraga et al., 2009, Roitberg et al., 2020). Teasing apart the effects of thermal  
50 gradients, water availability and local habitats on thermo-hydroregulation strategies and morphology  
51 requires comparative analyses along independent clines of ambient temperature and rainfall **in sites with**  
52 **contrasted microhabitat features.**

53 Another limitation is that comparative studies of ectotherm hydroregulation strategies are still lagging  
54 behind those of thermoregulation (Pirtle, Tracy, & Kearney, 2019). Hydroregulation involves both  
55 behavioral and physiological mechanisms such as behavioral activity and shelter use, behavioral  
56 microhabitat selection, or physiological mechanisms of evaporative water loss through the skin (Pirtle  
57 et al., 2019). Skin resistance to evaporative water loss is primarily determined by the presence of a lipid  
58 layer in the epidermis of lizards and snakes, which acts as a physical barrier to water loss and determines  
59 total evaporative water loss rates (TEWL, Roberts & Lillywhite, 1980). Plastic changes in TEWL rates  
60 over a few weeks or months have been demonstrated in some lizards and snakes when individuals are  
61 exposed to different air moisture or water availability conditions (Kobayashi, Mautz, & Nagy, 1983;  
62 Moen, Winne, & Reed, 2005). Phylogenetic analyses of TEWL in Lacertid lizards also indicate that this  
63 trait is more evolutionarily labile and more variable than thermal preferences, and evolves towards lower  
64 values in warmer environments (Garcia-Porta et al., 2019). Yet, studies of co-variation in TEWL and  
65 thermoregulation traits are rare, and those suggest that the two sets of functional traits may often vary  
66 independently from each other (Sannolo et al., 2020; S'khifa et al., 2020).

67 Here, we performed such a comparative study in the Lacertid *Zootoca vivipara louislantzi* (Arribas,  
68 2009), which is a ground-dwelling lizard with an oviparous reproductive mode. We examined variation  
69 in thermoregulation and hydroregulation **traits** across a geographic gradient **in South-Western France**  
70 from relict populations located at the hot distribution margin to populations located at the colonization  
71 front in cold, highland habitats (**Dupoué et al., 2021**). Using an integrative approach, we quantified

72 geographic variation in (a) **thermal preferences** ( $T_{pref}$ ), (b) hydroregulation physiology (total  
73 evaporative water loss TEWL and its cutaneous sub-component CEWL), (c) energy metabolism (resting  
74 metabolic rate) and (d) morphology (body size, body surface, body condition and caudal reserve) of  
75 adult male lizards from fifteen populations. **We also evaluated physiological dehydration through**  
76 **osmolality to investigate its correlation with climate conditions and habitat. To avoid confounding**  
77 **effects of inter-individual differences due to age, sex or seasonality, we sampled only sexually**  
78 **mature males during the reproductive season.** We further compared our studied populations with  
79 those of two taxonomic outgroups sampled with the same technique, including two nearby French  
80 populations of the closely related viviparous reproductive mode *Z. vivipara vivipara* and one population  
81 of the distantly related wall lizard *Podarcis muralis* from semi-mesic, saxicolous environments (Garcia-  
82 Porta *et al.*, 2019). This sampling design allowed us to **test** if hydroregulation physiology and energy  
83 metabolism traits are more flexible than thermal preferences, if those traits covary or vary independently  
84 from each other, and if intraspecific variability is more constrained than interspecific variability. We  
85 hypothesized that geographic variation is best explained by considering both local (habitat) and large-  
86 scale (climate) features.

87 First, populations with a restricted access to water should have lower TEWL rates and thermal  
88 preferences than those with permanent access after controlling for effects of the macrohabitat climate  
89 (Dupoué *et al.*, 2017b; Rozen-Rechels *et al.*, 2021).

90 Second, populations from **more** arid environments should have lower TEWL rates as seen in  
91 interspecific comparisons for reptiles (Cox & Cox, 2015; Garcia-Porta *et al.*, 2019), but it is unclear  
92 whether thermal preferences should increase or not with macrohabitat temperature and aridity.  
93 Metabolism at rest should be lower in warmer and more arid environments, as seen in recent broad scale  
94 comparisons (Dupoué, Brischoux, & Lourdais, 2017a).

95 Third, we expect inter-individual covariation between functionally related traits. For example, total  
96 evaporative water loss (TEWL) being the sum of respiratory (REWL) and cutaneous evaporative water  
97 losses (CEWL), we expect a positive correlation between TEWL and CEWL measured in similar  
98 conditions in the same animals and also a correlation between TEWL and oxygen consumption ( $VO_2$ ),  
99 since REWL scales linearly with  $VO_2$  (Pirtle *et al.*, 2019).

100

## 101 **2. Material and methods**

### 102 2.1. Study species

103 The common lizard *Zootoca vivipara* is a small lacertid lizard (Reptilia: Lacertidae) with a wide  
104 Eurosiberian distribution ranging from Southern France and Central Europe to Japan on Hokkaido Island  
105 (Surget- Groba *et al.*, 2006). It occupies cold and wet habitats including open heat lands, humid  
106 grasslands and peat bogs as well as clearances and clear-cuts surrounded by forests. Most European

107 populations are viviparous except for some oviparous lineages distributed at the Southwestern margin  
108 of the range in France and in Southern Central Europe (Surget-Groba et al., 2001). We studied the  
109 oviparous South-West European common lizard, *Z. vivipara lousilantzi*, which consists of four major  
110 subclades and inhabits a range of habitats from sea levels to highland (Milá et al., 2013). We focused  
111 our field sampling on clade B2 in France to avoid inter-population differences due to genetic  
112 differentiation between clades with potential introgression (Milá et al., 2013; Dupoué et al., 2021).

113

## 114 2.2. Sampling design and sampling site

115 **In a recent study of the range distribution of this sub-species in South-Western France, we found**  
116 **that temperature, rainfall and forest cover shape demographic and genetic variability from the**  
117 **rear to the leading edges (Dupoué et al., 2021). We sub-sampled sites within this geographic range**  
118 **to select fifteen populations along a sharp climate gradient for both temperature and rainfall, and**  
119 **contrasted habitat features including differences in access to free-standing water and in forest**  
120 **cover. These variables adequately quantify aspects of the thermal and hydric gradients that**  
121 **should be relevant considering previous findings showing interactive effects of hydric and thermal**  
122 **conditions on thermoregulation behavior (Rozen-Rechels et al., 2021). In addition, geographic**  
123 **variation of some morphological and reproductive traits correlates with habitat humidity and**  
124 **rainfall in this species (Lorenzon, Clobert, & Massot, 2001; Marquis, Massot, & Le Galliard, 2008;**  
125 **Dupoué et al., 2017b).**

126 **To select our focal populations,** we gathered altitude, habitat data and climate information for ca. 130  
127 known occurrence sites in South-Western France (Cistude Nature and Nature en Occitanie, unpub. data).  
128 For each site, we calculated **the average temperature and the cumulative precipitation during the**  
129 **activity season (from April to September)** using 30 Arcsec resolution climatic data derived from  
130 AURELHY climatic model outputs developed by Meteo-France (averages from the 1971-2000 period  
131 of weather survey, (Canellas et al., 2014)). We also extracted the minimum temperature of the coldest  
132 month (Tcold) and the maximum of the hottest month (Thot) to calculate the annual pluviometry  
133 quotient  $Q=(100 \times P) / (T_{hot}^2 - T_{cold}^2)$ , also called the Emberger index (Emberger, 1955). This  
134 parameter allows to discriminate mesic (high Q index) from arid (low Q index) climates.

135 We then selected 15 representative populations of *Z. vivipara lousilantzi* (clade B2) distributed along  
136 the altitudinal gradient with different temperature (maximum temperatures during activity period  
137 ranging from 20°C to 26°C), rainfall levels (total precipitation during activity period ranging from  
138 360mm to 850mm) and access to free standing water at the same altitude (see Supplementary Table S1  
139 and Figure 1). These populations were also associated to an altitudinal gradient (40-1750 m) and to a  
140 variety of habitats including (1) sites with permanent access to free standing water (**presence of water**  
141 **sources available to the lizards throughout the year such as lake, streams, peat bogs)** versus  
142 temporary access to free standing water (**water body only present after a rain or during the wet**

143 **season, see Dupoué et al., 2017b)** and (2) sites with forest cover versus open habitats, **calculated with**  
144 **a forest cover index detailed in Rutschmann et al. (2016). We chose to sample populations with as**  
145 **much contrasted access to water and forest cover along the altitude and aridity gradients as**  
146 **possible, and then there was no significant correlation between mean temperature or mean**  
147 **rainfall and water access, but a small and significant correlation (Kruskal-Wallis test = 5, df = 1,**  
148 **p = 0.02) between temperature or rainfall and forest cover.** For comparative purposes, we further  
149 sampled 2 populations of the viviparous *Z. vivipara vivipara* clade E1 from the nearest locations in the  
150 Limousin area at ca. 800m asl (Figure 1). We also sampled wall lizards (*Podarcis muralis*) from a  
151 lowland population in the same area (Chizé population, 46°08'51.5"N 0°25'36.4"W, ca. 64 m asl), **which**  
152 **fell within the climatic range of the common lizard populations in order to limit geographic effects.**  
153 This widespread species is adapted to more arid environments and served us as an outgroup to compare  
154 with the common lizard, which is adapted to mesic and cold environments (Figure 1). We attempted to  
155 capture ca. 10 adult males per population (see Table S1, Supp.Info).

156 Oviparous populations from South-Western France are characterized by a strong seasonal activity  
157 pattern, a sexual maturation at the age of one to two years old, facultative multiple clutches per year,  
158 and with an adult snout-vent length (SVL) ranging from 45 to 75 mm in males (Heulin, 1987; Heulin,  
159 Osenegg-Leconte, & Michel', 1997). **Sexually mature males were told apart by body coloration,**  
160 **presence of femoral pores, and hemipenis shape. Since sexually mature males most likely emerge**  
161 **earlier during the season in warmer environments, lowland populations were sampled first to**  
162 **reduce differences in reproductive phenology among populations (from 17th of April 2019 in the**  
163 **lowland populations until the 7th of May 2019 in the highland).**

### 164 2.3. Field measurements and housing

165 We captured individuals by hand and brought them back to a laboratory (CEBC, CNRS, Villiers-en-  
166 bois, France) within two to four days following capture. **Age class of sexually mature males was**  
167 **unambiguously scored using external morphology to separate young adults (aged less than one**  
168 **calendar year and born in 2018) from older adults (aged two or more than two calendar years).**  
169 One person (JFLG) then measured SVL and total length to the nearest mm with a plastic ruler and body  
170 weight to the nearest mg with an electronic balance. **The ventral and dorsal surface of all males were**  
171 **scanned at 600 dots per inch using a flatbed scanner (Hewlett-Packard Co., ScanJet 3670, see**  
172 **Brusch et al., 2020) with animals evenly laid flat on the scanner on their belly and back using a**  
173 **blue foam, to measure body surface and tail width, since body area is a critical determinant of**  
174 **total water loss. To do so, we processed the digital images with the software ImageJ (Schneider,**  
175 **Rasband, & Eliceiri, 2012) using the Analyze Particles function to calculate the body area. We**  
176 **first determined a threshold in terms of color of pixels to tell the lizard apart from the background,**  
177 **and then the software automatically counted the number of pixels corresponding to the lizard**  
178 **body area. We repeated the analysis with ventral and dorsal scans of each lizard and summed the**

179 **two areas.** Tail width was further measured at the 7th, 10th and 12th subcaudal scales, and at maximum  
180 width (Brusch et al., 2020). Scans were not always exploitable for this measurement with 124 usable  
181 values out of 142 for the 10th and 12th subcaudal scales, 117 for the 7th subcaudal and 94 for the  
182 maximum width. The day of capture, we further took a blood sample from the post-orbital sinus using  
183 1 to 2 micro-capillary tubes (ca. 20–30  $\mu$ l whole blood) and kept samples at  $\sim 4^{\circ}\text{C}$  in a cooler. **We used**  
184 **blood sample to assess how plasma osmolality (one of the best proxies for physiological**  
185 **dehydration in lizards, see Peterson, 1996) vary among populations and whether lizards could**  
186 **maintain normosmolality or not in the face of strong variation in environmental conditions**  
187 (Dupoué *et al.*, 2017). In the laboratory, samples were centrifuged at 11,000 rpm for 5 min to separate  
188 plasma from red blood cells. Plasma samples (ca. 5–15  $\mu$ l) were immediately frozen at  $-28^{\circ}\text{C}$  until  
189 osmolality assays. Later, plasma osmolality was determined using a vapor pressure osmometer (model  
190 Vapro 5600, ELITechGroup) with the protocol described in Wright, Jackson, & DeNardo (2013) and  
191 adjusted to small plasma volumes (Dupoué et al., 2017b). Before analyses, plasma was diluted (1:3) in  
192 standard saline solution (Osmolarity =  $280 \text{ mOsm}\cdot\text{l}^{-1}$ ) to obtain 2 duplicates per sample. Thus, we were  
193 able to estimate an intra-assay coefficient of variation ( $\text{CV} < 3\%$ ).

194 Animals were kept separated in individual terraria ( $35 \times 25 \times 12.5 \text{ cm}$ ) with peat soil, a PVC tube for shelter  
195 and a water cup with permanent access to free standing water. During captivity, we provided all lizards  
196 with the same thermal gradient from  $20$  to  $34^{\circ}\text{C}$  for 8h per day (09:00-17:00) using heating wires placed  
197 under one end of each terrarium. Lizards were all fed with live crickets (*Acheta domesticus*) every two  
198 days and had water sprayed 3 times a day in addition to free water available *ad libitum*. Physiological  
199 assays and behavioral trials started after a one-week acclimation period (see below). Once all  
200 measurements were completed, all males were released at capture location.

#### 201 2.4. Ecophysiological measurements

202 We separated animals from different populations randomly in 12 lots of 15 lizards. Then, each lot went  
203 through the same succession of laboratory measurements as summarized in Figure S1 (Supp.Info).  
204 Individuals were maintained without food for 4 days and weighed with a high precision scale ( $\text{BM} \pm$   
205  $0.01 \text{ mg}$ ) at 8 pm at the end of Day 3. They were then placed overnight in an open-top plastic box with  
206 no water nor food inside a climatic chamber set at  $20^{\circ}\text{C}$  and 60% relative humidity. All lizards were re-  
207 weighted at 8 am the following day (Day 4) and moved back to their individual terrarium. We assessed  
208 total evaporative water loss (TEWL) from the loss of mass in mg per hour after removing individuals  
209 having defecated during the night ( $\text{N} = 27$ ). In squamate reptiles, body mass loss reflects TEWL (i.e., the  
210 sum of ventilatory and cutaneous evaporative water losses) in resting animals because variation in body  
211 mass is mostly due to water loss (Dupoué et al., 2015; Moen et al., 2005). We further measured body  
212 mass loss at rest during metabolic rate assays in Day 6 (Figure S1) and found a strong correlation  
213 between these two measurements of TEWL, showing that this protocol was highly repeatable



214 **(Repeatability estimation using the lmm method including animals that did not defecate during**  
215 **the tests, N = 59, R = 0.64 ± 0.08, p<0.0001).**

216

217 The next day (Day 5), we measured preferred body temperatures  $T_{\text{pref}}$  of fasted lizards in a photothermal  
218 gradient inside a neutral arena where water was available *ad libitum* and lizards were safe from predators  
219 and competitors (Artacho, Jouanneau, & Le Galliard, 2013). **Around noon, lizards were placed**  
220 **individually in a 60 cm-long plastic box with a 40W light bulb at one end and a water cup (Trochet**  
221 **et al., 2018).** By maintaining the air of the room at 18°C, we could simulate a thermal gradient ranging  
222 from 18°C to 43°C below the light bulb. Experiments started around 1pm and we collected data after at  
223 least a 30 min long acclimation period. Every 20 min during 3h30, we measured the surface temperature  
224 of the lizard on the back with a high precision infrared thermometer (Raytek, Raynger MX2). We  
225 collected 11 repeated measurements for each individual to assess thermal preference ( $T_{\text{pref}}$ ) values.  
226 Differences in thermal preferences between populations were then analyzed using all observations  
227 except those of animals buried in the soil, which we considered irrelevant because lizards were  
228 “inactive”. **At the end of the day, we also measured the core body temperature of 120 individuals**  
229 **(randomly chosen among types and populations) with the tip of a K-type temperature probe**  
230 **(Hanna HI 935002, Hanna Instruments) inserted in the cloaca.** This allowed us to calculate a  
231 calibration curve between surface temperature and core body temperature. We found a highly  
232 significant, close to 1:1 relationship between core body temperature and surface infrared measurements  
233 for the 3 taxonomic groups of lizards (major axis regression forced to 0, slope =  $0.96 \pm 0.01$ ,  $p < 0.0001$ ,  
234 **R<sup>2</sup> > 0.99**). We used this regression to convert all surface temperatures to core body temperatures for  
235 further analyzes.

236 At Day 6, we used the indirect calorimetric method with a closed respirometer system described in  
237 (Foucart et al., 2014) to measure resting metabolic rate from the CO<sub>2</sub> and O<sub>2</sub> gas exchanges between  
238 animals and the atmosphere. Lizards fasted for 6 days were weighted with high precision scale and then  
239 placed in opaque test chambers (1L) covered with an air-tight glass lid. Chambers were installed in a  
240 temperature-controlled environment (set at 20°C, a temperature that does not elicit significant locomotor  
241 activity) 2 hours before beginning of trial. Outside air was drawn into each test chamber using a Bioblock  
242 Scientific 55 L air pump for 5 min ensuring +99% air turnover. Baseline samples of outside air were  
243 collected at the onset of each trial. Trials were performed during the night (12h) so that lizards were  
244 inactive most of the time. At the end of each trial, an air sample was collected using two 150 ml syringes  
245 and we noted if the lizard defecated during the night (**N=41**). The baseline and final CO<sub>2</sub> and O<sub>2</sub>  
246 concentrations were determined using high precision gas analyzers (CA10 & FC10A, Sable Systems,  
247 Las Vegas, NV). Air was pushed from the syringes using an infusion pump (KDS210; KD Scientific,  
248 Inc., Holliston, MA, USA), passed through Drierite to remove water and then sent at a controlled rate  
249 to the analyzer calibrated before each trial. With baseline and final concentrations, we calculated the

250 VO<sub>2</sub> (volume of O<sub>2</sub> consumed in ml per hour) and the respiratory quotient as the ratio of CO<sub>2</sub> produced  
251 by O<sub>2</sub> consumed. The respiratory quotient varied around 0.75 (mean = 0.77 ± 0.032, range = 0.695-  
252 0.911), which corresponds to the situation of a fasting animal that catabolizes lipids (Schmidt-Nielsen,  
253 1997). **We therefore subsequently analyzed resting metabolic rate using VO<sub>2</sub> values.**

254 At Day 7, fast was ended and at Day 8 we used an evaporimeter (Aquaflux BIOX AF200, Biox Systems  
255 Ltd, UK) that quantified the trans-epidermal water vapor flux from measurements of a humidity gradient  
256 within a closed chamber (Imhof et al., 2009; Guillon et al., 2013). We measured independently  
257 cutaneous evaporative water loss (CEWL rate, g of water per m<sup>2</sup> per hour) from 4 locations on the back  
258 of each lizard in a laboratory room maintained at 23°C. We used an in vivo nail cap with rubber O-ring  
259 (diameter 2.6 mm) to ensure a complete seal between the measurement chamber and the lizard's skin.  
260 The evaporimeter was calibrated at the beginning of each trial following manufacturer procedures. We  
261 then gently pressed the probe against the skin of the lizard for a few minutes to perform measurements.  
262 Each measurement trial ended once real time CEWL reading, monitored on a graphic interface, was  
263 stabilized (± 0.01 g.m<sup>-2</sup>.h<sup>-1</sup> for 10 s). If any movement caused a leak in the seal between the measurement  
264 chamber and the animal's skin, we repeated the trial. In total, we performed 4 measurements for each  
265 animal and evaluated a posteriori their quality according to the shape of the curve as recommended by  
266 the manufacturer. We kept only good quality measurements for subsequent analyzes (range = 2-4 per  
267 lizard, mean = 3.66). **Repeatability for measurements on the same individual was estimated using  
268 the lmm method and was significant (R = 0.56 ± 0.04, p<0.0001).**

## 269 2.5. Statistical analyses

270 All statistical analyses were performed in R software version 3.5.3 (2019-03-11) "Great Truth" (RC  
271 Team 2019). **In our results, we provide the standard deviation of the mean as the dispersion  
272 measure (mean ± SD).** In a first set of analyses, we compared measurements obtained in the two clades  
273 of common lizards and the wall lizards (*Z. vivipara louislantzi*, *Z. vivipara vivipara* and *P. muralis*) with  
274 an analysis of variance. For each measurement, we performed an **ANCOVA** test controlling for inter-  
275 individual differences in body mass. In a second set of analyses, we studied geographic variation among  
276 oviparous populations of *Z. vivipara louislantzi*. To test for potential correlations between  
277 morphological, behavioral and physiological traits on one hand and the population characteristics on the  
278 other hand, we used a two-step model selection procedure. We analyzed independently variation of body  
279 size (SVL, mm), body mass (W, g), body surface (mm<sup>2</sup>), thermal preferences ( $T_{pref}$ , °C), total water loss  
280 (TEWL, mg per hour), cutaneous water loss (CEWL, g.m<sup>-2</sup>.h<sup>-1</sup>) and resting metabolic rate (VO<sub>2</sub>, ml per  
281 hour) using linear mixed models with the function lme from the package nlme (Pinheiro et al., 2006).  
282 For each variable, we followed the same model selection procedure (as shown in Supplementary tables  
283 S3 to S11).

284 As our variables of interest are **influenced by both individual-level population-level factors**, we  
285 proceeded in two steps. First, we fitted a full model including all individual and experimental covariates  
286 as fixed effects and then selected a first minimum adequate model using backward model selection based  
287 on AIC. We also assessed homoscedasticity and normality of residuals using the full model.  
288 Experimental covariates included measurement conditions (time of the day, temperature and humidity  
289 in the room when the experiment was not performed in climatic chambers), and presence of feces for  
290 TEWL and VO<sub>2</sub>. Individual covariates included age class, SVL, body surface and/or body mass  
291 depending on **traits** (see details below and in Supplementary tables). Full models included additive  
292 linear effects of all covariates. For thermal preferences data, we analyzed all body temperatures recorded  
293 for each individual rather than a composite variable (e.g., mean or variance). Population identity was  
294 always included as a random effect to control for non-independence among individuals from the same  
295 population. Individual identity nested in the population identity was also included as a random effect to  
296 account for repeated measurements on the same individual for  $T_{\text{pref}}$  and CEWL data. In a second step,  
297 we tested if traits differed significantly among populations due to differences in climate or habitat. We  
298 used a model averaging procedure using the Akaike information-based criterion (AIC) comparison  
299 procedure (Burnham & Anderson, 2004) to compare the relative importance (sum of AICc corrected for  
300 small sample size over all models in which the variable appears) of environmental variables related to  
301 elevation, climate conditions (mean temperature and rainfall during activity season, pluviometry  
302 quotient), and habitat features (water presence and forest cover). The model averaging procedure was  
303 performed with maximum likelihood estimates of model parameters using the MuMIn package  
304 (Burnham & Anderson, 2002; Barton & Barton, 2015). Models were constructed from the best model  
305 selected during the first step described above in which we added the environmental variables alone as a  
306 fixed effect or the additive effects of one variable describing climatic conditions and one variable  
307 describing habitat features. Correlations between the environmental variables and the habitat variables  
308 were low since we chose to sample populations with as much contrasted access to water and forest cover  
309 along the altitude and aridity gradients as possible. For the purpose of model averaging calculations, all  
310 continuous covariates were centered and scaled and categorical covariates (age, water presence and  
311 forest cover) had their contrasts summed to zero. We selected the main effects with significant  
312 contributions to the variability of the traits based on the AICc difference between models, the relative  
313 weight of the models and the importance as well as conditional averages from model averaging  
314 procedure (Burnham & Anderson, 2004). If the minimal model was included in the set of best models,  
315 this implies uncertainty in the importance of environmental variables. If numerous concurrent best  
316 models were observed, this implies uncertainty about which environmental variable explains the most  
317 geographic variation in the trait. All model averaging tables and conditional averages of the important  
318 environmental variables are reported in the supplementary materials (Tables S3 to S11).

319 To complement these independent analyses of each trait, we further performed a path analysis  
320 with Piecewise Structural Equation modeling using the R package PiecewiseSEM (Lefcheck,  
321 2016). This procedure allowed us to test causal paths identified in our model selection approach  
322 in a single model accounting for the multivariate relationships between our interrelated traits (see  
323 Supplementary information S13 for more details).

### 324 3. Results

#### 325 3.1. Differences between taxa

326 We found significant differences for all thermo-hydroregulation traits between the wall lizards and the  
327 two groups of common lizards, but not between oviparous and viviparous populations of the common  
328 lizard, even after controlling for allometric relationship (Table 1, see details in Supplementary Table  
329 S2). TEWL was 40% lower in *P. muralis* than in the two clades of *Z. vivipara* after controlling for body  
330 mass (*P. muralis*: TEWL = 0.853 mg.g<sup>-1</sup>.h<sup>-1</sup>; *Z. vivipara louislantzi*: TEWL = 1.387 mg. g<sup>-1</sup>.h<sup>-1</sup>; *Z.*  
331 *vivipara vivipara*: TEWL = 1.489 mg. g<sup>-1</sup>.h<sup>-1</sup>; ANCOVA: Taxa: F<sub>2,140</sub> = 10.9, p < 0.0001; Body mass:  
332 F<sub>1,140</sub> = 77.2, p < 0.0001). Similarly, CEWL was 40% smaller in *P. muralis* than in the two clades of *Z.*  
333 *vivipara* (ANOVA: Taxa: F<sub>2,652</sub> = 66.3, p < 0.0001). VO<sub>2</sub> rates corrected for body mass, were 26% lower  
334 in *P. muralis* than in *Z. vivipara* (ANCOVA: Body mass: F<sub>1,160</sub> = 336.6, p < 0.0001, Taxa: F<sub>2,160</sub> = 9.63,  
335 p = 0.0001). T<sub>pref</sub> were significantly lower in *P. muralis* compared to *Z. vivipara* subspecies (ANOVA:  
336 F<sub>2,1640</sub> = 47.19, p < 0.001). None of the post-hoc tests revealed any difference between *Z. vivipara*  
337 *louislantzi* and *Z. vivipara vivipara* (Table 1).

#### 338 3.2. Morphological variation among oviparous populations of *Z. vivipara*

339 The SVL and body condition (body mass corrected for SVL with a linear regression) varied among  
340 populations (ANOVA: Population effect: F<sub>14,127</sub> = 4.7, p < 0.0001 and R<sub>interpop</sub> = 0.38 for SVL and  
341 ANOVA: Population effect: F<sub>14,126</sub> = 7.0, p < 0.0001 and R<sub>interpop</sub> = 0.42 for BM) and these differences  
342 were partly explained by climatic conditions. The most important environmental and individual  
343 variables explaining variation of SVL included the age class (contrast between age 1 and older males =  
344 -3.2 ± 0.25 mm, p < 0.0001), mean temperature during activity period (slope = -0.5 ± 0.16 mm per °C, p  
345 = 0.01) and the pluviometry quotient (slope = 0.01 ± 0.005 mm per Q unit, p = 0.04, see Table S3).  
346 Thus, lizards had a larger average body size in colder climates (Figure 2A). The most important  
347 environmental variables explaining geographic variation of body condition were the pluviometry  
348 quotient (slope = 0.002 ± 0.001 g per Q unit, p = 0.03), the altitude (estimate = 0.0003 ± 0.0001 g per  
349 m, p = 0.03) and the mean temperature during activity period (estimate = -0.07 ± 0.03 g per °C, p =  
350 0.04). According to the best supported model, lizards had a higher body condition in highland  
351 populations with a high pluviometry quotient and in open habitats than under dense forest cover (see  
352 variations of body mass in Figure 2B, Table S4). Body surface was as expected strongly correlated with

353 body mass ( $r = 0.9$ ,  $p < 0.0001$ ) and followed a similar pattern of geographic variation than body  
354 condition (see Table S5).

355 Tail width at the three recorded positions was correlated with SVL and body mass and the different  
356 measurements of tail width were strongly correlated with each other (all  $r > 0.75$ ,  $p < 0.0001$ ). We  
357 present only the analyses on maximum tail width and tail width at 10th subcaudal scale. For both, the  
358 most important environmental and individual variables included the SVL (for max width : slope =  $0.046$   
359  $\pm 0.021$  mm,  $p = 0.03$  ; for 10th scale : slope =  $0.074 \pm 0.015$  mm per mm,  $p < 0.0001$ ), the altitude (for  
360 max width : slope =  $0.0006 \pm 0.0002$  mm per m,  $p = 0.0009$  ; for 10th scale : slope =  $0.0005 \pm 0.0001$   
361 mm per m,  $p = 0.0003$ , see Figure 2C, Tables S6), the mean temperature during activity period (for max  
362 width : slope =  $-0.11 \pm 0.04$  mm per °C,  $p = 0.018$  ; for 10th scale : slope =  $-0.09 \pm 0.03$  mm per °C,  $p$   
363 =  $0.006$ ) and the mean precipitation during activity period (for max width : slope =  $0.001 \pm 0.0007$  mm  
364 per mm,  $p = 0.046$  ; for 10th scale : slope =  $0.001 \pm 0.0005$  mm per mm,  $p = 0.03$ ). The best supported  
365 models retained the effect of age class and SVL as individual covariates and of altitude and forest cover,  
366 indicating a joint effect of climate conditions and local habitat (see Figure 2C, Tables S6).

### 367 3.3. Physiological variation among oviparous populations of *Z. vivipara*

368 The TEWL at rest increased with body mass (estimate =  $0.0008 \pm 0.0001$  mg/h per g,  $p < 0.0001$ ). There  
369 was some degree of variation in TEWL among populations (ANOVA: Population effect:  $F_{14,94} = 3.5$ ,  $p$   
370 =  $0.0001$  and  $R_{interpop} = 0.37$ ) and our model comparison procedure uncovered that the permanent access  
371 to free water in the habitat was a significant variable explaining this geographic variation (Figure 3A,  
372 Table S7). Lizards living in habitats with no permanent access to water had lower TEWL (contrast = -  
373  $0.26 \pm 0.07$ ,  $p = 0.001$ ). The effects of other variables were more uncertain with models suggesting that  
374 TEWL decreased weakly with altitude and in open habitats compared to forest habitats (Table S7). The  
375 CEWL values showed a much greater variability among individuals within a population than among  
376 populations ( $R_{interpop} = 0.22$ ;  $R_{interind} = 0.47$ ). The model that best explained CEWL variation included  
377 only experimental conditions effects with a positive correlation of the absolute humidity of the room at  
378 the time of measurement (estimator =  $0.70 \pm 0.04$ ,  $p = 0.0001$ , Table S8).

379 Apart from the significant positive effect of body mass on  $VO_2$  at rest (estimate =  $6.14 \cdot 10^{-5} \pm 5.10 \cdot 10^{-6}$   
380 ml/h per mg,  $p < 0.0001$ ), there was some uncertainty about the effects of environmental variables on  
381  $VO_2$  despite variation among populations (ANOVA: Population effect:  $F_{17,144} = 1.91$ ,  $p = 0.02$  and  
382  $R_{interpop} = 0.25$ ). According to the best supported model, lizards tended to have higher  $VO_2$  in populations  
383 with a high pluviometry quotient, and, in addition, in forest habitats than in open areas (Figure 2D, Table  
384 S9).

385 Mean plasma osmolality was  $306 \pm 18$  mOsm  $\text{kg}^{-1}$  and was different among populations (ANOVA:  
386 Population effect:  $F_{14,119} = 3.8$ ,  $p < 0.0001$  and  $R_{\text{interpop}} = 0.35$ ), but we found no significant effect of  
387 environmental variables on plasma osmolality (Table S11).

### 388 3.4. Variation of thermal preferences in oviparous populations of *Z. vivipara*

389 Thermal body preferences were relatively high in our sample (mean =  $34.94^{\circ}\text{C}$ , range =  $19.80$ - $39.40^{\circ}\text{C}$ ,  
390 Figure 3B) compared to previous studies with the same species (e.g.,  $T_{\text{pref}} = 31.5$ ; Gvoždík & Castilla,  
391 2001; Trochet et al., 2018).  $T_{\text{pref}}$  showed a great intra-population variability but varied little across  
392 populations (ANOVA: Population effect:  $F_{14,1420} = 5.11$ ,  $p < 0.001$  and  $R_{\text{interpop}} = 0.18$ , see Figure 3B)  
393 and this geographic variation was not significantly explained by environmental variables. Our model  
394 comparison procedure only revealed the effect of experimental covariates and suggested some potential  
395 but weak effects of forest cover (not significant in conditional averages, Table S10). Lizards living in  
396 open habitats tended to have higher  $T_{\text{pref}}$  than lizards from forest habitats.

### 397 3.5. Covariation between thermo-hydroregulation traits

398 We found no significant correlation between TEWL, CEWL,  $\text{VO}_2$ , the body surface area of the animal  
399 and the  $T_{\text{pref}}$  (Figure 4). First, TEWL,  $T_{\text{pref}}$  and  $\text{VO}_2$ , which provide three potentially related information  
400 about the thermo-hydroregulation strategy of lizards, were not strongly correlated (Pearson product  
401 moment correlation, **all  $r < 0.3$** , all  $p < 0.05$ ). Second, we explored the relationship between TEWL and  
402 the potential subcomponents of evaporative water losses, such as CEWL, body surface and ventilation  
403 rate (which is linearly related to  $\text{VO}_2$ ), but we found no correlation between them (**all  $r < 0.2$** , all  $p >$   
404  $0.05$ ). **In addition, in the SEM result CEWL and  $\text{VO}_2$  were weakly positively correlated ( $r = 0.26$ ,**  
405  **$p = 0.018$ , see Supp. Info S13).**

## 406 **4. Discussion**

407 Studies of intraspecific variation in functional traits across environmental gradients have tended to focus  
408 on the thermal biology of ectotherms instead of the joint variation and integration of their thermo-  
409 hydroregulation strategies (Artacho et al., 2017; Domínguez- Guerrero et al., 2021). Here, we compared  
410 ecophysiological traits linked to both water balance and body temperature regulation in populations of  
411 *Zootoca vivipara louislantzi* ranging from rear edge nearby sea level to the leading edge of their  
412 distribution in highland habitats. In addition, we compared these populations with two outgroups  
413 including a different species and another subspecies, with which we expected substantial  
414 ecophysiological differences.

415 Strong ecophysiological variation was indeed observed between wall lizards *Podarcis muralis* and  
416 common lizards *Zootoca vivipara*, as expected given the broad ecological and habitat differences  
417 between these two species. The wall lizard is found on average in warmer and drier areas than the  
418 common lizard and this species also exhibits distinct preferences for dry saxicolous rather vegetated

419 habitats where common lizards thrive, even though these two species can occur sympatrically (Mole,  
420 2010). On the one hand, *Podarcis muralis* had lower water loss rates (both TEWL and CEWL) and basal  
421 metabolic rates ( $VO_2$ ) than *Zootoca vivipara*. Comparative studies of squamate reptiles and other  
422 vertebrates have uncovered similar findings, with higher resistance to evaporative water loss and lower  
423 basal energy expenditure in species from hot and dry environments (Dupoué et al. 2017, Le Galliard et  
424 al., 2021a, Cox & Cox, 2015). On the other hand, *P. muralis* displayed lower preferred temperatures  
425 ( $T_{pref}$ ) than our populations of *Z. vivipara* instead of the predicted higher  $T_{pref}$  expected for organisms  
426 from warmer habitats (Garcia-Porta et al., 2019). Previous studies of *P. muralis* and *Z. vivipara* have  
427 found that the  $T_{pref}$  of adult males can vary seasonally with high  $T_{pref}$  during the early mating season,  
428 lower  $T_{pref}$  during the spring season and then higher  $T_{pref}$  during the summer season (Osojnik et al., 2013).  
429 Thus, one possibility is that wall lizards, who tend to have an earlier phenology than common lizards,  
430 had lower  $T_{pref}$  because they were sampled slightly later during their mating season than common lizards.  
431 Another possibility is that the relatively smaller  $T_{pref}$  of wall lizards reflects adjustments to water  
432 limitation and habitat aridity in their native population, as shown in laboratory experiments (Sannolo &  
433 Carretero, 2019; Le Galliard et al., 2021b).

434 We found no significant ecophysiological variation between the two clades of *Zootoca vivipara* despite  
435 their ancient evolutionary divergence and a major difference in reproduction mode between the two  
436 clades. Phylogenetic studies revealed six major genetic clades for *Zootoca vivipara* in Eurasia with four  
437 widespread viviparous clades present in most of the distribution range and two oviparous clades  
438 restricted to the southern margin of the range distribution in Western or Eastern Europe (Surget- Groba  
439 et al., 2006). The most parsimonious evolutionary scenario proposes that viviparity evolved only once  
440 but then a reversal back to oviparity occurred in the Western populations from our study area (Recknagel  
441 et al., 2018; Horreo et al., 2018). This transition back to oviparity is associated with climate warming,  
442 since oviparous forms are generally favored over viviparous forms in warmer habitats (Shine, 1985).  
443 The two reproductive modes are probably separated given the limited gene flow and reproductive  
444 isolation between them (e.g., Cornetti et al., 2015). Viviparity is considered as a key evolutionary  
445 transition to life under cold climatic conditions (Horreo, Jiménez-Valverde, & Fitze, 2021), with  
446 oviparous lizards being adapted to warmer and more variable environment during the reproductive  
447 season (as suggested by Horreo et al., 2021). This scenario led us to expect major differences in thermo-  
448 hydroregulation strategies between clades B (oviparous) and E (viviparous). However, we found no  
449 physiological or behavioral adaptation as the two clades were very similar in terms of TEWL,  $T_{pref}$  and  
450  $VO_2$ . In our experiment, interindividual and interpopulation differences on these traits were stronger  
451 than the divergence due to clades. However, we encourage further studies of the ecophysiological  
452 strategies of adult females, especially during gestation when phenotypic differences between the two  
453 reproductive modes are likely to be stronger (Recknagel & Elmer, 2019).

454 Morphological differences among oviparous populations were explained by climatic conditions  
455 including temperature and the pluviometry quotient Q. First, **body** condition of males was explained by  
456 both environmental temperature and humidity: sexually mature males were on average heavier in the  
457 more mesic habitats (sensu Q index), whereas relative tail width increased with altitude (and cold  
458 temperatures) and with rainfall. In this species, caudal width represents a good proxy of tail reserves,  
459 which are an important body component for fat and protein storage whose variation can reflect changes  
460 in food availability, energy intake and also water availability (Bateman & Fleming, 2009; Bruschi et al.,  
461 2020). Body condition might increase with humidity because of positive effects of rainfall and water  
462 availability on habitat quality, including food availability, but this should be accompanied by an increase  
463 of body size with humidity. Alternatively, low environmental temperatures and high water availability  
464 might reduce energy expenditure and the reliance of lizards on fat or protein catabolism to restore energy  
465 and water balance (e.g., Bruschi et al., 2020). Hence, we can hypothesize that males from rear edge, drier  
466 and hotter populations were more prompt to catabolize tail reserves, probably to fuel some higher  
467 metabolic demand (in warmer climates) and to support water demands (in drier climates). Further  
468 examinations of physiological traits in standard conditions tend to confirm the later hypothesis.

469 Second, size was influenced mostly by environmental temperature: sexually mature males were on  
470 average longer in the colder habitats. This geographic variability for body size is globally consistent  
471 with previous data collected across the altitudinal range of other ectothermic vertebrates (Peterman,  
472 Crawford, & Hocking, 2016; Trochet et al., 2019). These two aspects suggest a positive effect of cold  
473 temperature and humidity on body size and body conditions. If the effect on size has been documented  
474 previously (Lu et al., 2018; Roitberg et al., 2020), the effect of aridity is rarely tested. A recent thorough  
475 analysis of the body size variation across the range distribution of *Z. vivipara* suggested that body size  
476 varies non-monotonously with the length of the activity season in this species because of the underlying  
477 thermal plasticity of growth and sexual maturation (Roitberg et al., 2020). This scenario of thermal  
478 plasticity predicts a shift towards a larger mean body size of adult individuals in colder climates because  
479 age at first reproduction is delayed by a year or more in a seasonal environment (Adolph and Porter,  
480 1996). Thus, adult males are bigger in colder climates because they mature older and therefore at a larger  
481 body size on average than adults growing at higher temperatures but maturing earlier in life (Atkinson,  
482 1994; **Angilletta, 2009**). However, under this scenario, we would not expect the size of young males of  
483 the year (age class A1) to be smaller in warmer habitats if those yearlings are born earlier and grow  
484 faster than those of colder habitats (Sorci et al., 1996; Roitberg et al., 2020), which is what we found  
485 here. Our sample of 1-year-old individuals was however likely biased as we only captured sexually  
486 mature young males, whereas most young are not mature at this age, especially in cold climates. We  
487 therefore performed a complementary analysis of a larger dataset including many more *Z. vivipara*  
488 populations and both sexually mature and immature young males of the year. In this supplementary  
489 database, we had a much stronger variability in the body size of young males of the year. This updated



490 analysis confirmed a positive thermal cline for the body size of 1 year-old males with SVL increasing  
491 by  $1.03 \pm 0.29$  mm per °C mean activity temperature ( $p = 0.0006$ , see supplementary Table S12). This  
492 reverse thermal clines for body size in young males of the year and older males conforms exactly with  
493 the “saw-tooth” relationship between body size and seasonality proposed by Adolph and Porter (1996)  
494 and seen across the broad geographic distribution of *Z. vivipara* across Europe (Roitberg et al., 2020).  
495 Altogether, these results indicate that broad scale climate gradients instead of local habitat features were  
496 the main determinants of geographic variation in morphology.

497 Lizards living in habitats with temporary access to water had lower total evaporative water loss rates  
498 than those from habitats with permanent access and the plasma osmolality was remarkably consistent  
499 across habitat types and climate conditions, similar to a previous finding in adult males and females of  
500 the viviparous clade E in Massif Central, France (Dupoué et al., 2017b). This confirms that common  
501 lizards can adjust or adapt their total water loss to cope with seasonal habitat dryness and that water  
502 availability in the environment is more consistently related to this functional trait of the water budget  
503 than rainfall or thermal conditions. We also examined traits involved in TEWL variation including body  
504 surface (which relates to the total exchange area for cutaneous evaporative water loss), standard  
505 cutaneous water loss rates (which relates to skin permeability to water loss), and  $VO_2$  at rest (which  
506 relates to basal energy expenditure and also correlates with respiratory water loss). Quite surprisingly,  
507 we found no effect of water availability in the habitat on these three traits and little inter-individual  
508 correlation between these traits and TEWL, which does not allow us to point a specific avenue by which  
509 lizards down-regulated standard TEWL rates in habitats with temporary access to water. One possibility  
510 is that some behavioral mechanisms were driving the observed pattern. For example, ocular water loss  
511 can represent a significant avenue for TEWL in small lizard species (Pirtle et al., 2019) and the time  
512 spent with eyes open could be variable during inactivity periods.

513 Contrary to our expectations, the  $VO_2$  of adult males were poorly influenced by climate conditions,  
514 especially environmental temperatures, except for a trend towards higher  $VO_2$  in more mesic conditions  
515 that would require further testing with a larger sample size. Note that this weak pattern of variation of  
516  $VO_2$  with climate aridity is the same than the one seen in some desert species of birds and rodents  
517 (McNab & Morrison, 1963; Tieleman, Williams, & Bloomer, 2003). We also found a small positive  
518 effect of forest cover on  $VO_2$  but this effect remains difficult to interpret given that closed habitats tended  
519 to be more frequent at a lower elevation. Given the strong and consistent differences in thermal and  
520 rainfall conditions between the relict lowland populations and those in highland mountains, we expected  
521 that the  $VO_2$  of adult males would be significantly lower in low altitude populations (i.e. metabolic cold  
522 adaptation or Krogh’s rule described in Krogh & Lindhard, 1914) as it has been shown in other species  
523 of lizards (e.g. Plasman et al., 2020). However, a recent review by Pettersen (2020) on reptiles  
524 demonstrates little support for counter gradient variation in metabolic rate (which would reflect an  
525 adaptive response to geographic gradient), whereas it did reveal such an adaptive response on

526 development time. Here, we do not find a difference in  $VO_2$  at adult stage across a 2000 m elevation  
527 gradient but cannot exclude that geographic variation may be more substantial at earlier stages of life **or**  
528 **in females**, but also at a different season, for example at the end of the summer following seasonal  
529 acclimatization of resting metabolic rate.

530 **Regarding thermoregulation behavior, we studied thermal preferences to quantify**  
531 **thermoregulation strategies under controlled laboratory conditions.** Variation in  $T_{pref}$  was often  
532 higher among individuals within a population than among populations and fell 6-7°C below the critical  
533 thermal maximum (Gvoždík & Castilla, 2001; Trochet et al., 2018). **Individual differences in  $T_{pref}$**   
534 **were independent from variation in water loss rates or basal metabolism.** The  $T_{pref}$  also remained  
535 similar on average among populations with contrasted climatic and habitat conditions confirming the  
536 results of several previous comparative studies of thermal preferences in this species in other geographic  
537 areas (Van Damme, Bauwens, & Verheyen, 1990; Gvoždík & Castilla, 2001; Rozen-Rechels et al.,  
538 2021; Carretero, Roig, & Llorente, 2005). Note that we cannot exclude stronger geographic differences  
539 of  $T_{pref}$  later in the season due to different acclimatization conditions across our geographic gradients, **as**  
540 **a strong seasonality effect was found in a meta-analysis on thermoregulation efficiency of lacertid**  
541 **lizards (Ortega & Martín-Vallejo, 2019).** On the same species, Trochet et al. (2018) found a slight  
542 decrease of the  $T_{pref}$  of adult males and females from ca. 31-32°C at 500 m elevation to ca. 29-30°C at  
543 2000 m elevation during the late spring season. Irrespective of this possibility, the fact that  $T_{pref}$  varied  
544 little across a temperature gradient of 6°C for mean temperature during the activity season suggests that  
545 behavioral thermoregulation is optimized at grossly similar body temperatures over the geographic  
546 gradient, perhaps because the species is a thermal generalist (Angilletta et al. 2002).

547 Assuming a strong selection for optimization of thermal performances, behavioral adjustments may  
548 explain the absence of geographic variability in  $T_{pref}$ . First, we know that lizards can use microhabitats  
549 (e.g., rocks, shaded vegetation or burrows) to maintain an optimal body temperature and buffer the  
550 negative effects of cold or hot environmental temperatures on their performance (Gaudenti et al., 2021;  
551 Taylor et al., 2021). Hence, macroclimatic conditions, here quantified by average air temperatures,  
552 might be less relevant than microclimatic conditions since common lizards can shift microhabitat  
553 selection to keep an optimal temperature even in the extreme parts of our geographic gradient (Rozen-  
554 Rechels et al., 2021). This supposes a great spatial variation in operative temperature to allow efficient  
555 thermoregulation even in cold and warm environments (Logan, van Berkel, & Clusella-Trullas, 2019).  
556 **Ultimately, some components of the ecological niche of the species could also change along the**  
557 **climatic gradients. For example, there might be a shift toward sunny, low vegetation habitats in**  
558 **high altitude populations compared to more shaded forest habitats in lowland populations.**  
559 **Complementary studies of the spatial distribution of contemporary populations are required to**  
560 **confirm this hypothesis.** However, our thermal cline was quite substantial and we found strong  
561 morphological differences along the gradient, which were likely caused by differences in the length of

562 the activity season. This suggests that body temperature regulation was to some extent constrained by  
563 cold climate conditions early and late in the activity season. Another compensatory mechanism could  
564 be the possibility of geographic changes in basking behavior and effort (Stevenson, 1985): here, animals  
565 from cold populations could spend more time basking to keep an optimal temperature even at the cost  
566 of spending less time foraging (Buckley, Ehrenberger, & Angilletta, 2015). Yet, a previous study  
567 examined behavioral thermoregulation between lowland and highland populations of *Z. vivipara* and  
568 found no compensatory behavior of this kind in high altitude (Gvoždík, 2002). In any case, our results  
569 suggest that some aspects of **the thermal preferences** or the ecological niche did compensate at least  
570 partly for the strong macroclimatic differences in environmental temperatures. The behavioral  
571 mechanisms allowing common lizards to maintain optimal body temperature and performance across  
572 gradients of environmental temperatures are important to study in the future since they can shield  
573 organisms from environmental variation and cause evolutionary inertia of physiological traits (i.e.,  
574 Bogert effect, Muñoz, 2021).

575 **Overall, our study of intraspecific variation of morphology and thermo-hydroregulation traits in**  
576 ***Z. vivipara louislantzi* indicate that macroclimate gradients were the main determinants of**  
577 **geographic variation in morphology but not for thermo-hydroregulation traits. Relict populations**  
578 **from the warm rear edge of the range distribution displayed no particular ecophysiological**  
579 **adaptations. Habitat features such as water access and forest cover had a stronger influence on**  
580 **EWL than thermal conditions. We suggest that habitat features should be included in future**  
581 **comparative studies of terrestrial ectotherms' physiology in order to better understand their role**  
582 **relative to climate conditions.**

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## 594 **6. Data Availability**

595 The data underlying this article are available in *Zenodo* at [https://dx.doi.org/](https://dx.doi.org/10.5281/zenodo.6366222)  
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598 **7. References**

599

600 ADOLPH, S.C. & PORTER, W.P. (1996) Growth, Seasonality, and Lizard Life Histories: Age and Size  
601 at Maturity. *Oikos* **77**, 267.602 ANGILLETTA JR, M.J. & ANGILLETTA, M.J. (2009) Thermal adaptation: a theoretical and  
603 empirical synthesis. Oxford University Press.604 ANGILLETTA, M.J., NIEWIAROWSKI, P.H. & NAVAS, C.A. (2002) The evolution of thermal  
605 physiology in ectotherms. *Journal of Thermal Biology* **27**, 249–268.606 ARRIBAS, O.J. (2009) Morphological variability of the Cantabro-Pyrenean populations of *Zootoca*  
607 *vivipara* (JACQUIN, 1787) with description of a new subspecies. *Herpetozoa* **21**, 123–146.608 ARTACHO, P., JOUANNEAU, I. & LE GALLIARD, J.-F. (2013) Interindividual Variation in  
609 Thermal Sensitivity of Maximal Sprint Speed, Thermal Behavior, and Resting Metabolic Rate in a  
610 Lizard. *Physiological and Biochemical Zoology* **86**, 458–469.611 ARTACHO, P., SARAVIA, J., PERRET, S., BARTHELD, J.L. & LE GALLIARD, J.-F. (2017)  
612 Geographic variation and acclimation effects on thermoregulation behavior in the widespread lizard  
613 *Liolaemus pictus*. *Journal of Thermal Biology* **63**, 78–87.614 ASHTON, K.G. (2001) Body Size Variation Among Mainland Populations of the Western Rattlesnake  
615 (*Crotalus Viridis*). *Evolution* **55**, 2523–2533.616 ATKINSON, D. (1994) Temperature and organism size: a biological law for ectotherms? *Advances in*  
617 *ecological research* **25**, 1–58.

618 BARTON, K. &amp; BARTON, M.K. (2015) Package ‘mumin’. Version 1, 18.

619 BATEMAN, P.W. & FLEMING, P.A. (2009) To cut a long tail short: a review of lizard caudal  
620 autotomy studies carried out over the last 20 years. *Journal of Zoology* **277**, 1–14.621 BELLARD, C., BERTELSMEIER, C., LEADLEY, P., THUILLER, W. & COURCHAMP, F. (2012)  
622 Impacts of climate change on the future of biodiversity. *Ecology Letters* **15**, 365–377.623 BLOIS, J.L., WILLIAMS, J.W., FITZPATRICK, M.C., JACKSON, S.T. & FERRIER, S. (2013)  
624 Space can substitute for time in predicting climate-change effects on biodiversity. *Proceedings of the*  
625 *National Academy of Sciences* **110**, 9374–9379.626 BODENSTEINER, B.L., AGUDELO- CANTERO, G.A., ARIETTA, A.Z.A., GUNDERSON, A.R.,  
627 MUÑOZ, M.M., REFSNIDER, J.M. & GANGLOFF, E.J. (2021) Thermal adaptation revisited: How  
628 conserved are thermal traits of reptiles and amphibians? *Journal of Experimental Zoology Part A:*  
629 *Ecological and Integrative Physiology* **335**, 173–194.630 BRUSCH, G.A., GAVIRA, R.S.B., VITON, R., DUPOUÉ, A., LEROUX-COYAU, M., MEYLAN,  
631 S., LE GALLIARD, J.-F. & LOURDAIS, O. (2020) Additive effects of temperature and water  
632 availability on pregnancy in a viviparous lizard. *The Journal of Experimental Biology* **223**, jeb228064.633 BUCKLEY, L.B., EHRENBERGER, J.C. & ANGILLETTA, M.J. (2015) Thermoregulatory  
634 behaviour limits local adaptation of thermal niches and confers sensitivity to climate change.  
635 *Functional Ecology* **29**, 1038–1047.636 BURNHAM, K.P. & ANDERSON, D.R. (2002) A practical information-theoretic approach. *Model*  
637 *selection and multimodel inference 2*. Springer New York.

- 638 BURNHAM, K.P. & ANDERSON, D.R. (2004) Multimodel Inference: Understanding AIC and BIC  
639 in Model Selection. *Sociological Methods & Research* 33, 261–304.
- 640 CALDWELL, A.J., WHILE, G.M. & WAPSTRA, E. (2017) Plasticity of thermoregulatory behaviour  
641 in response to the thermal environment by widespread and alpine reptile species. *Animal Behaviour*  
642 132, 217–227.
- 643 CANELLAS, C., GIBELIN, A.-L., LASSÈGUES, P., KERDONCUFF, M., DANDIN, P. & SIMON,  
644 P. (2014) The 1981-2010 Aurelhy gridded climate normals. <https://doi.org/10.4267/2042/53750>.  
645
- 646 CARRETERO, M.A., ROIG, J.M. & LLORENTE, G.A. (2005) Variation in Preferred Body Temperature in  
647 an Oviparous Population of *Lacerta (Zootoca) Vivipara*. *The Herpetological Journal* 15, 51–55.
- 648 CHIACCHIO, M., GRIMM- SEYFARTH, A., HENLE, K. & MIHOUB, J. (2020) Water availability  
649 as a major climatic driver of taxonomic and functional diversity in a desert reptile community.  
650 *Ecosphere* 11.
- 651 CLUSELLA-TRULLAS, S. & CHOWN, S.L. (2014) Lizard thermal trait variation at multiple scales:  
652 a review. *Journal of Comparative Physiology B* 184, 5–21.
- 653 CORNETTI, L., BELLUARDO, F., GHIELMI, S., GIOVINE, G., FICETOLA, G.F., BERTORELLE,  
654 G., VERNESI, C. & HAUFFE, H.C. (2015) Reproductive isolation between oviparous and viviparous  
655 lineages of the Eurasian common lizard *Zootoca vivipara* in a contact zone: Speciation in the  
656 Common Lizard. *Biological Journal of the Linnean Society* 114, 566–573.
- 657 COX, C.L. & COX, R.M. (2015) Evolutionary shifts in habitat aridity predict evaporative water loss  
658 across squamate reptiles: BRIEF COMMUNICATION. *Evolution* 69, 2507–2516.
- 659 DOMÍNGUEZ- GUERRERO, S.F., BODENSTEINER, B.L., PARDO- RAMÍREZ, A.,  
660 AGUILLÓN- GUTIERREZ, D.R., CRUZ, F.R.M. LA & MUÑOZ, M.M. (2021) Thermal physiology  
661 responds to interannual temperature shifts in a montane horned lizard, *Phrynosoma orbiculare*. *Journal*  
662 *of Experimental Zoology Part A: Ecological and Integrative Physiology* 335, 136–145.
- 663 DUPOUÉ, A., BRISCHOUX, F., ANGELIER, F., DENARDO, D.F., WRIGHT, C.D. & LOURDAIS,  
664 O. (2015) Intergenerational trade-off for water may induce a mother–offspring conflict in favour of  
665 embryos in a viviparous snake. *Functional Ecology* 29, 414–422.
- 666 DUPOUÉ, A., BRISCHOUX, F. & LOURDAIS, O. (2017a) Climate and foraging mode explain  
667 interspecific variation in snake metabolic rates. *Proceedings of the Royal Society B: Biological*  
668 *Sciences* 284, 20172108.
- 669 DUPOUÉ, A., RUTSCHMANN, A., LE GALLIARD, J.F., MILES, D.B., CLOBERT, J.,  
670 DENARDO, D.F., BRUSCH, G.A. & MEYLAN, S. (2017b) Water availability and environmental  
671 temperature correlate with geographic variation in water balance in common lizards. *Oecologia* 185,  
672 561–571.
- 673 DUPOUÉ, A., TROCHET, A., RICHARD, M., SORLIN, M., GUILLON, M., TEULIERES-  
674 QUILLET, J., VALLÉ, C., RAULT, C., BERRONEAU, M., BERRONEAU, M., LOURDAIS, O.,  
675 BLAIMONT, P., BERTRAND, R., POTTIER, G., CALVEZ, O., ET AL. (2021) Genetic and  
676 demographic trends from rear to leading edge are explained by climate and forest cover in a cold-  
677 adapted ectotherm. *Diversity and Distributions* 27, 267–281.
- 678 EMBERGER, L. (1955) Une classification biogéographique des climats.
- 679 FOUCART, T., LOURDAIS, O., DENARDO, D.F. & HEULIN, B. (2014) Influence of reproductive  
680 mode on metabolic costs of reproduction: insight from the bimodal lizard *Zootoca vivipara*. *Journal of*  
681 *Experimental Biology* 217, 4049–4056.

- 682 GARCÍA-MUÑOZ, E. & CARRETERO, M.A. (2013) Comparative ecophysiology of two sympatric  
683 lizards. Laying the groundwork for mechanistic distribution models. *Acta Herpetologica* 8, 123-128–  
684 128.
- 685 GARCIA-PORTA, J., IRISARRI, I., KIRCHNER, M., RODRÍGUEZ, A., KIRCHHOF, S., BROWN,  
686 J.L., MACLEOD, A., TURNER, A.P., AHMADZADEH, F., ALBALADEJO, G., CRNOBRNJA-  
687 ISAILOVIC, J., DE LA RIVA, I., FAWZI, A., GALÁN, P., GÖÇMEN, B., ET AL. (2019)  
688 Environmental temperatures shape thermal physiology as well as diversification and genome-wide  
689 substitution rates in lizards. *Nature Communications* 10, 4077.
- 690 GAUDENTI, N., NIX, E., MAIER, P., WESTPHAL, M.F. & TAYLOR, E.N. (2021) Habitat  
691 heterogeneity affects the thermal ecology of an endangered lizard. *Ecology and Evolution* 11, 14843–  
692 14856.
- 693 GUILLON, M., GUILLER, G., DENARDO, D.F. & LOURDAIS, O. (2013) Microclimate preferences  
694 correlate with contrasted evaporative water loss in parapatric vipers at their contact zone. *Canadian*  
695 *Journal of Zoology* 92, 81–86. NRC Research Press.  
696
- 697 GVOŽDÍK, L. (2002) To heat or to save time? Thermoregulation in the lizard *Zootoca vivipara*  
698 (Squamata: Lacertidae) in different thermal environments along an altitudinal gradient. *Canadian*  
699 *Journal of Zoology* **80**, 479–492.
- 700 GVOŽDÍK, L. & CASTILLA, A.M. (2001) A Comparative Study of Preferred Body Temperatures  
701 and Critical Thermal Tolerance Limits among Populations of *Zootoca vivipara* (Squamata: Lacertidae)  
702 along an Altitudinal Gradient. *Journal of Herpetology* 35, 486–492. Society for the Study of  
703 Amphibians and Reptiles.
- 704 HERRANDO- PÉREZ, S., MONASTERIO, C., BEUKEMA, W., GOMES, V., FERRI- YAÑEZ, F.,  
705 VIEITES, D.R., BUCKLEY, L.B. & ARAÚJO, M.B. (2020) Heat tolerance is more variable than cold  
706 tolerance across species of Iberian lizards after controlling for intraspecific variation. *Functional*  
707 *Ecology* 34, 631–645.
- 708 HEULIN, B. (1987) Temperature diurne d'activité des males et des femelles de *Lacerta vivipara*.  
709 *Amphibia-Reptilia* 8, 393–400. Brill.
- 710 HEULIN, B., OSENEGG-LECONTE, K. & MICHEL', D. (1997) Demography of a Bimodal  
711 Reproductive Species of Lizard (*Lacerta vivipara*): Survival and Density Characteristics of Oviparous  
712 Populations. *Herpetologica*, 14.
- 713 HORREO, J.L., JIMÉNEZ-VALVERDE, A. & FITZE, P.S. (2021) Climatic niche differences among  
714 *Zootoca vivipara* clades with different parity modes: implications for the evolution and maintenance of  
715 viviparity. *Frontiers in Zoology* 18, 32.  
716
- 717 HORREO, J.L., PELAEZ, M.L., SUÁREZ, T., BREEDVELD, M.C., HEULIN, B., SURGET- GROBA, Y.,  
718 OKSANEN, T.A. & FITZE, P.S. (2018) Phylogeography, evolutionary history and effects of glaciations  
719 in a species (*Zootoca vivipara*) inhabiting multiple biogeographic regions. *Journal of Biogeography*  
720 **45**, 1616–1627.
- 721 HUEY, R.B., DEUTSCH, C.A., TEWKSBURY, J.J., VITT, L.J., HERTZ, P.E., ÁLVAREZ PÉREZ,  
722 H.J. & GARLAND, T. (2009) Why tropical forest lizards are vulnerable to climate warming.  
723 *Proceedings of the Royal Society B: Biological Sciences* 276, 1939–1948.
- 724 HUEY, R.B., KEARNEY, M.R., KROCKENBERGER, A., HOLTUM, J.A.M., JESS, M. &  
725 WILLIAMS, S.E. (2012) Predicting organismal vulnerability to climate warming: roles of behaviour,  
726 physiology and adaptation. *Philosophical Transactions of the Royal Society B: Biological Sciences*  
727 367, 1665–1679.

728 IMHOF, R.E., JESUS, M.E.P.D., XIAO, P., CIORTEA, L.I. & BERG, E.P. (2009) Closed-chamber  
729 transepidermal water loss measurement: microclimate, calibration and performance. *International*  
730 *Journal of Cosmetic Science* 31, 97–118.

731 KOBAYASHI, D., MAUTZ, W.J. & NAGY, K.A. (1983) Evaporative Water Loss: Humidity  
732 Acclimation in *Anolis carolinensis* Lizards. *Copeia* 1983, 701–704. [American Society of  
733 Ichthyologists and Herpetologists (ASIH), Allen Press].

734 KROGH, A. & LINDHARD, J. (1914) On the average composition of the alveolar air and its  
735 variations during the respiratory cycle. *The Journal of physiology* 47, 431. Wiley-Blackwell.

736 KUBISCH, E.L., FERNÁNDEZ, J.B. & IBARGÜENGOYTÍA, N.R. (2016) Vulnerability to climate warming  
737 of *Liolaemus pictus* (Squamata, Liolaemidae), a lizard from the cold temperate climate in Patagonia,  
738 Argentina. *Journal of Comparative Physiology B* **186**, 243–253.

739

740 LEFCHECK, J.S. (2016) piecewiseSEM: Piecewise structural equation modelling in r for  
741 ecology, evolution, and systematics. *Methods in Ecology and Evolution* **7**, 573–579.

742 LE GALLIARD, J.-F., CHABAUD, C., DE ANDRADE, D.O.V., BRISCHOUX, F., CARRETERO,  
743 M.A., DUPOUÉ, A., GAVIRA, R.S.B., LOURDAIS, O., SANNOLO, M. & VAN DOOREN, T.J.M.  
744 (2021a) A worldwide and annotated database of evaporative water loss rates in squamate reptiles.  
745 *Global Ecology and Biogeography* 30, 1938–1950.

746 LE GALLIARD, J.-F.L., ROZEN-RECHELS, D., LECOMTE, A., DEMAY, C., DUPOUÉ, A. &  
747 MEYLAN, S. (2021b) Short-term changes in air humidity and water availability weakly constrain  
748 thermoregulation in a dry-skinned ectotherm. *PLOS ONE* 16, e0247514. Public Library of Science.

749 LOGAN, M.L., VAN BERKEL, J. & CLUSELLA-TRULLAS, S. (2019) The Bogert Effect and  
750 environmental heterogeneity. *Oecologia* 191, 817–827.

751 LORENZON, P., CLOBERT, J. & MASSOT, M. (2001) The Contribution of Phenotypic Plasticity to  
752 Adaptation in *Lacerta Vivipara*. *Evolution* 55, 392–404.

753

754 LU, H.-L., XU, C.-X., JIN, Y.-T., HERO, J.-M. & DU, W.-G. (2018) Proximate causes of altitudinal  
755 differences in body size in an agamid lizard. *Ecology and Evolution* **8**, 645–654.

756 MARQUIS, O., MASSOT, M. & LE GALLIARD, J.F. (2008) Intergenerational effects of climate  
757 generate cohort variation in lizard reproductive performance. *Ecology* 89, 2575–2583.

758 MCNAB, B.K. & MORRISON, P. (1963) Body Temperature and Metabolism in Subspecies of  
759 *Peromyscus* from Arid and Mesic Environments. *Ecological Monographs* 33, 63–82.

760 MILÁ, B., SURGET-GROBA, Y., HEULIN, B., GOSÁ, A. & FITZE, P.S. (2013) Multilocus  
761 phylogeography of the common lizard *Zootoca vivipara* at the Ibero-Pyrenean suture zone reveals  
762 lowland barriers and high-elevation introgression. *BMC Evolutionary Biology* 13, 192.

763 MILLER, M.A. & LUTTERSCHMIDT, W.I. (2014) Cutaneous Water Loss and Epidermal Lipids in  
764 Two Sympatric and Congeneric Pitvipers. *Journal of Herpetology* 48, 577–583.

765

766 MOEN, D.S., WINNE, \* CHRISTOPHER T. & REED‡, R.N. (2005) Habitat-mediated shifts and plasticity  
767 in the evaporative water loss rates of two congeneric pit vipers (Squamata, Viperidae, *Agkistrodon*).  
768 *Evolutionary Ecology Research* **7**, 759–766. Evolutionary Ecology, Ltd.

769 MOLE, S.R.C. (2010) Changes in relative abundance of the western green lizard *Lacerta bilineata* and  
770 the common wall lizard *Podarcis muralis* introduced onto Boscombe Cliffs, Dorset, UK, 6.

- 771 MUÑOZ, M.M. (2021) The Bogert effect, a factor in evolution. *Evolution*, evo.14388.
- 772 OLALLA- TÁRRAGA, M.Á., DINIZ- FILHO, J.A.F., BASTOS, R.P. & RODRÍGUEZ, M.Á. (2009)  
773 Geographic body size gradients in tropical regions: water deficit and anuran body size in the Brazilian  
774 Cerrado. *Ecography* 32, 581–590.
- 775 OSOJNIK, N., ŽAGAR, A., CARRETERO, M.A., GARCÍA-MUÑOZ, E. & VREZEC, A. (2013)  
776 Ecophysiological Dissimilarities of Two Sympatric Lizards. *Herpetologica* 69, 445–454.
- 777 PETERMAN, W.E., CRAWFORD, J.A. & HOCKING, D.J. (2016) Effects of Elevation on  
778 Plethodontid Salamander Body Size. *Copeia* 104, 202–208.
- 779 PETERSON, C.C. (1996) Anhomeostasis: Seasonal Water and Solute Relations in Two Populations of  
780 the Desert Tortoise (*Gopherus agassizii*) during Chronic Drought. *Physiological Zoology* 69, 1324–  
781 1358. The University of Chicago Press.
- 782 PETTERSEN, A.K. (2020) Countergradient Variation in Reptiles: Thermal Sensitivity of  
783 Developmental and Metabolic Rates Across Locally Adapted Populations. *Frontiers in Physiology* 11,  
784 547.
- 785 PINHEIRO, J., BATES, D., DEBROY, S. & SARKAR, D. (2006) nlme: an R package for fitting and  
786 comparing Gaussian linear and nonlinear mixed-effects models. See <http://www.stats.bris.ac.uk/R>.
- 787 PIRTLE, E.I., TRACY, C.R. & KEARNEY, M.R. (2019) Hydroregulation. A neglected behavioral  
788 response of lizards to climate change. *Behavior of lizards: Evolutionary and mechanistic perspectives*,  
789 343–374. CRC Press Boca Raton, FL.
- 790 PLASMAN, M., BAUTISTA, A., MCCUE, M.D. & VEGA- PÉREZ, A.H.D.D.L. (2020) Resting  
791 metabolic rates increase with elevation in a mountain-dwelling lizard. *Integrative Zoology* 15, 363–  
792 374.
- 793 PONTES-DA-SILVA, E., MAGNUSSON, W.E., SINERVO, B., CAETANO, G.H., MILES, D.B.,  
794 COLLI, G.R., DIELE-VIEGAS, L.M., FENKER, J., SANTOS, J.C. & WERNECK, F.P. (2018)  
795 Extinction risks forced by climatic change and intraspecific variation in the thermal physiology of a  
796 tropical lizard. *Journal of Thermal Biology* 73, 50–60.
- 797 RECKNAGEL, H. & ELMER, K.R. (2019) Differential reproductive investment in co-occurring  
798 oviparous and viviparous common lizards (*Zootoca vivipara*) and implications for life-history trade-  
799 offs with viviparity. *Oecologia* 190, 85–98.
- 800
- 801 RECKNAGEL, H., KAMENOS, N.A. & ELMER, K.R. (2018) Common lizards break Dollo’s law of  
802 irreversibility: Genome-wide phylogenomics support a single origin of viviparity and re-evolution of  
803 oviparity. *Molecular Phylogenetics and Evolution* 127, 579–588.
- 804 ROBERTS, J.B. & LILLYWHITE, H.B. (1980) Lipid Barrier to Water Exchange in Reptile  
805 Epidermis. *Science* 207, 1077–1079.
- 806 ROITBERG, E.S., ORLOVA, V.F., BULAKHOVA, N.A., KURANOVA, V.N., EPLANOVA, G.V.,  
807 ZINENKO, O.I., ARRIBAS, O., KRATOCHVÍL, L., LJUBISAVLJEVIĆ, K., STARIKOV, V.P.,  
808 STRIJBOSCH, H., HOFMANN, S., LEONTYEVA, O.A. & BÖHME, W. (2020) Variation in body  
809 size and sexual size dimorphism in the most widely ranging lizard: testing the effects of reproductive  
810 mode and climate. *Ecology and Evolution* 10, 4531–4561.
- 811 ROZEN- RECHELS, D., BADIANE, A., AGOSTINI, S., MEYLAN, S. & GALLIARD, J.-F.L.  
812 (2020) Water restriction induces behavioral fight but impairs thermoregulation in a dry-skinned  
813 ectotherm. *Oikos* n/a.
- 814 ROZEN- RECHELS, D., DUPOUÉ, A., LOURDAIS, O., CHAMAILLÉ- JAMMES, S., MEYLAN,  
815 S., CLOBERT, J. & GALLIARD, J.-F.L. (2019) When water interacts with temperature: Ecological



816 and evolutionary implications of thermo-hydroregulation in terrestrial ectotherms. *Ecology and*  
817 *Evolution* 9, 10029–10043.

818 ROZEN-RECHELS, D., RUTSCHMANN, A., DUPOUÉ, A., BLAIMONT, P., CHAUVEAU, V.,  
819 MILES, D.B., GUILLON, M., RICHARD, M., BADIANE, A., MEYLAN, S., CLOBERT, J. & LE  
820 GALLIARD, J.-F. (2021) Interaction of hydric and thermal conditions drive geographic variation in  
821 thermoregulation in a widespread lizard. *Ecological Monographs* 91, e01440.

822 RUTSCHMANN, A., MILES, D.B., GALLIARD, J.-F.L., RICHARD, M., MOULHERAT, S.,  
823 SINERVO, B. & CLOBERT, J. (2016) Climate and habitat interact to shape the thermal reaction  
824 norms of breeding phenology across lizard populations. *Journal of Animal Ecology* 85, 457–466.

825 RUTSCHMANN, A., ROZEN- RECHELS, D., DUPOUÉ, A., BLAIMONT, P., VILLEMEREUIL,  
826 P. DE, MILES, D.B., RICHARD, M. & CLOBERT, J. (2020) Climate dependent heating efficiency in  
827 the common lizard. *Ecology and Evolution* 10, 8007–8017.

828 SANNOLO, M. & CARRETERO, M.A. (2019) Dehydration constrains thermoregulation and space  
829 use in lizards. *PLOS ONE* 14, e0220384. Public Library of Science.

830 SANNOLO, M., CIVANTOS, E., MARTÍN, J. & CARRETERO, M.A. (2020) Variation in field body  
831 temperature and total evaporative water loss along an environmental gradient in a diurnal ectotherm.  
832 *Journal of Zoology* 310, 221–231.

833  
834 SINERVO, B., MENDEZ-DE-LA-CRUZ, F., MILES, D.B., HEULIN, B., BASTIAANS, E., VILLAGRAN-SANTA  
835 CRUZ, M., LARA-RESENDIZ, R., MARTINEZ-MENDEZ, N., CALDERON-ESPINOSA, M.L., MEZA-  
836 LAZARO, R.N., GADSDEN, H., AVILA, L.J., MORANDO, M., DE LA RIVA, I.J., SEPULVEDA, P.V., ET AL.  
837 (2010) Erosion of Lizard Diversity by Climate Change and Altered Thermal Niches. *Science* **328**,  
838 894–899.

839 SCHMIDT-NIELSEN, K. (1997) *Animal Physiology: Adaptation and Environment*. Cambridge  
840 University Press.

841 SCHNEIDER, C.A., RASBAND, W.S. & ELICEIRI, K.W. (2012) NIH Image to ImageJ: 25 years of  
842 image analysis. *Nature Methods* 9, 671–675.

843 SEARS, M.W., ANGILLETTA, M.J., SCHULER, M.S., BORCHERT, J., DILLIPLANE, K.F.,  
844 STEGMAN, M., RUSCH, T.W. & MITCHELL, W.A. (2016) Configuration of the thermal landscape  
845 determines thermoregulatory performance of ectotherms. *Proceedings of the National Academy of*  
846 *Sciences* 113, 10595–10600.

847  
848 SEARS, M.W., RIDDELL, E.A., RUSCH, T.W. & ANGILLETTA, M.J., JR (2019) The World Still  
849 Is Not Flat: Lessons Learned from Organismal Interactions with Environmental Heterogeneity  
850 in Terrestrial Environments. *Integrative and Comparative Biology* **59**, 1049–1058.

851 SEEBACHER, F. (2005) A review of thermoregulation and physiological performance in reptiles:  
852 what is the role of phenotypic flexibility? *Journal of Comparative Physiology B* 175, 453–461.

853 SEEBACHER, F., WHITE, C.R. & FRANKLIN, C.E. (2015) Physiological plasticity increases  
854 resilience of ectothermic animals to climate change. *Nature Climate Change* 5, 61–66.

855 SHINE, R. (1985) The evolution of viviparity in reptiles: an ecological analysis. *Biology of the*  
856 *Reptilia* 15, 605–694.

857 S’KHIFA, A., KOZIEL, G., VENCES, M., CARRETERO, M.A. & SLIMANI, T. (2020)  
858 Ecophysiology of a lacertid community in the high Moroccan mountains suggests conservation  
859 guidelines. *Journal of Thermal Biology* 94, 102743.

860  
861 SORCI, G., CLOBERT, J. & BELICHON, S. (1996) Phenotypic Plasticity of Growth and Survival in the  
862 Common Lizard *Lacerta vivipara*. *Journal of Animal Ecology* **65**, 781–790. [Wiley, British Ecological  
863 Society].

864 STEVENSON, R.D. (1985) The Relative Importance of Behavioral and Physiological Adjustments  
865 Controlling Body Temperature in Terrestrial Ectotherms. *The American Naturalist* **126**, 362–386.

866 SUNDAY, J.M., BATES, A.E., KEARNEY, M.R., COLWELL, R.K., DULVY, N.K., LONGINO,  
867 J.T. & HUEY, R.B. (2014) Thermal-safety margins and the necessity of thermoregulatory behavior  
868 across latitude and elevation. *Proceedings of the National Academy of Sciences* **111**, 5610–5615.

869 SURGET- GROBA, Y., HEULIN, B., GUILLAUME, C.-P., PUKY, M., SEMENOV, D., ORLOVA,  
870 V., KUPRIYANOVA, L., GHIRA, I. & SMAJDA, B. (2006) Multiple origins of viviparity, or reversal  
871 from viviparity to oviparity? The European common lizard (*Zootoca vivipara*, Lacertidae) and the  
872 evolution of parity. *Biological Journal of the Linnean Society* **87**, 1–11.

873 SURGET-GROBA, Y., HEULIN, B., GUILLAUME, C.-P., THORPE, R.S., KUPRIYANOVA, L.,  
874 VOGRIN, N., MASLAK, R., MAZZOTTI, S., VENCZEL, M., GHIRA, I., ODIERNA, G.,  
875 LEONTYEVA, O., MONNEY, J.C. & SMITH, N. (2001) Intraspecific Phylogeography of *Lacerta*  
876 *vivipara* and the Evolution of Viviparity. *Molecular Phylogenetics and Evolution* **18**, 449–459.

877 TAYLOR, E.N., DIELE- VIEGAS, L.M., GANGLOFF, E.J., HALL, J.M., HALPERN, B.,  
878 MASSEY, M.D., RÖDDER, D., ROLLINSON, N., SPEARS, S., SUN, B. & TELEMECO, R.S.  
879 (2021) The thermal ecology and physiology of reptiles and amphibians: A user’s guide. *Journal of*  
880 *Experimental Zoology Part A: Ecological and Integrative Physiology* **335**, 13–44.

881 TIELEMAN, B.I., WILLIAMS, J.B. & BLOOMER, P. (2003) Adaptation of metabolism and  
882 evaporative water loss along an aridity gradient. *Proceedings of the Royal Society of London. Series*  
883 *B: Biological Sciences* **270**, 207–214.

884 TROCHET, A., DELUEN, M., BERTRAND, R., CALVEZ, O., MARTÍNEZ-SILVESTRE, A.,  
885 VERDAGUER-FOZ, I., MOSSOLL-TORRES, M., SOUCHET, J., DARNET, E., LE CHEVALIER,  
886 H., GUILLAUME, O. & AUBRET, F. (2019) Body Size Increases with Elevation in Pyrenean Newts  
887 (*Calotriton asper*). *Herpetologica* **75**, 30–37.

888 TROCHET, A., DUPOUÉ, A., SOUCHET, J., BERTRAND, R., DELUEN, M., MURARASU, S.,  
889 CALVEZ, O., MARTINEZ-SILVESTRE, A., VERDAGUER-FOZ, I., DARNET, E., CHEVALIER,  
890 H.L., MOSSOLL-TORRES, M., GUILLAUME, O. & AUBRET, F. (2018) Variation of preferred  
891 body temperatures along an altitudinal gradient: A multi-species study. *Journal of Thermal Biology*  
892 **77**, 38–44.

893 VAN DAMME, R., BAUWENS, D. & VERHEYEN, R.F. (1990) Evolutionary rigidity of thermal  
894 physiology: the case of the cool temperate lizard *Lacerta vivipara*. *Oikos*, 61–67. JSTOR.

895 WEGENER, J.E., GARTNER, G.E.A. & LOSOS, J.B. (2014) Lizard scales in an adaptive radiation:  
896 variation in scale number follows climatic and structural habitat diversity in *Anolis* lizards. *Biological*  
897 *Journal of the Linnean Society* **113**, 570–579.

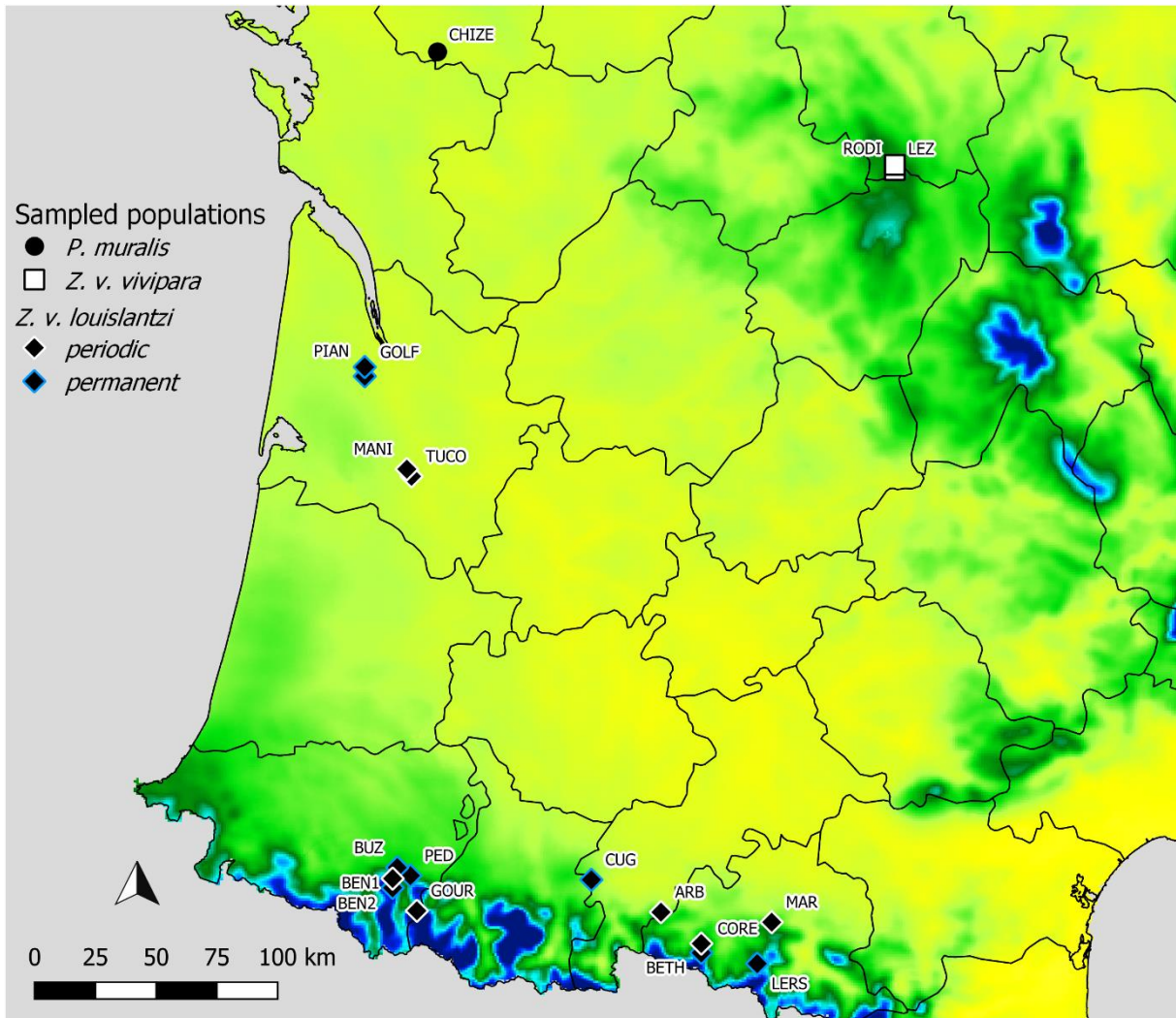
898 WRIGHT, C.D., JACKSON, M.L. & DENARDO, D.F. (2013) Meal consumption is ineffective at  
899 maintaining or correcting water balance in a desert lizard, *Heloderma suspectum*. *Journal of*  
900 *Experimental Biology* **216**, 1439–1447.

901 ZAMORA-CAMACHO, F.J. (2013) Patterns of seasonal activity in a Mediterranean lizard along a  
902 2200m altitudinal gradient. *Journal of Thermal Biology*, **6**.  
903

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905 **Figures and Legends**

906 Figure 1: Map of populations sampled for *Z. vivipara louislantzi* (clade B2) in South-Western France at  
907 different pluviometry quotient levels (background colors, **yellow to blue gradient**: from low to high  
908 pluviometry) and with permanent (diamond with blue outline) or periodic (diamond with white outline)  
909 access to water. Two outgroup populations of *Z. vivipara vivipara* (LEZ and RODI) and one of *P.*  
910 *muralis* (CHIZE) were included for comparative analyses.



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914 Table 1. Mean body mass, water loss rates, oxygen consumption rates and thermal preferences in the  
 915 two sub-species of *Z. vivipara* and in *P. muralis*. Values are the mean values for respectively 142 *Z.*  
 916 *vivipara louislantzi*, 21 *Z. vivipara vivipara* and 17 *P. muralis* adult males ( $\pm$  standard deviation).  
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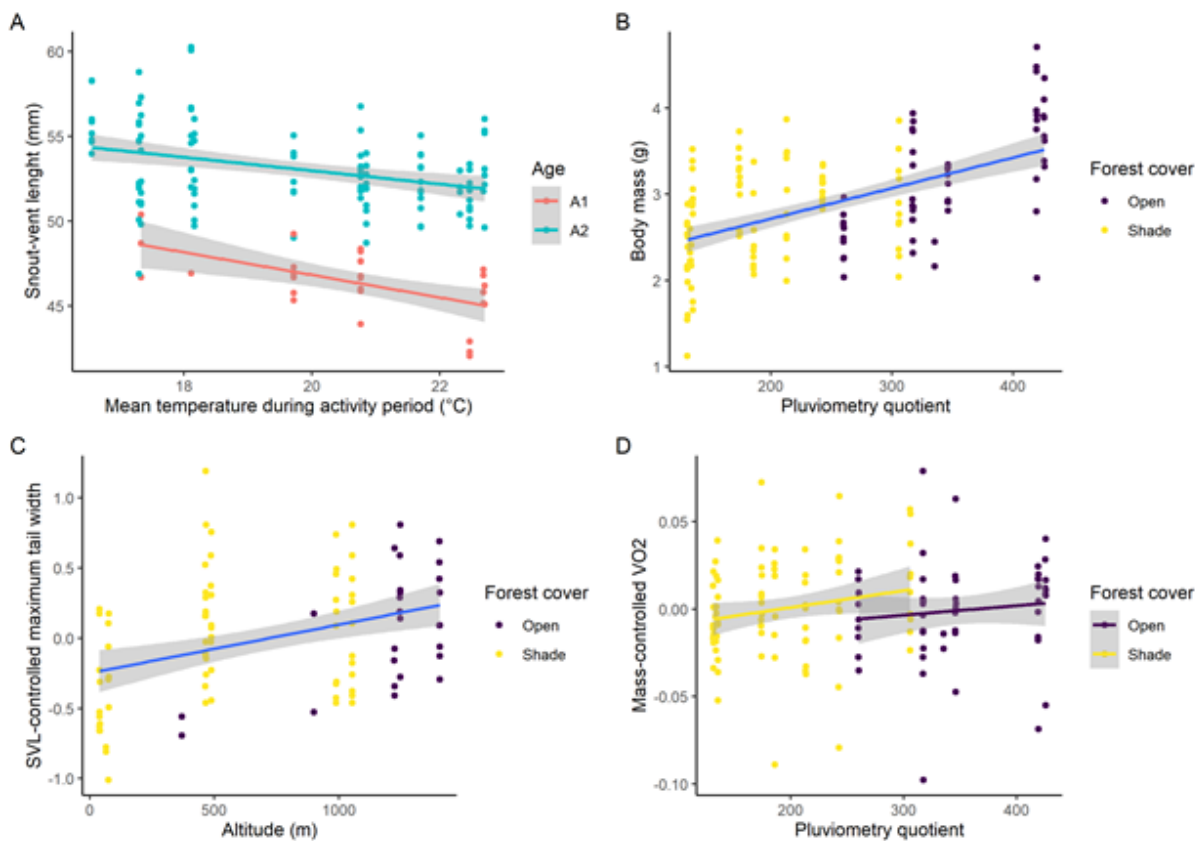
	<i>Z. vivipara louislantzi</i>	<i>Z. vivipara vivipara</i>	<i>P. muralis</i>
<b>BM (g)</b>	2.90 $\pm$ 0.66	3.15 $\pm$ 0.75	5.26 $\pm$ 1.60
<b>TEWL (mg/h)</b>	3.63 $\pm$ 0.78	3.97 $\pm$ 0.68	4.08 $\pm$ 1.70
<b>TEWL per mass (mg/h/g)</b>	1.42 $\pm$ 0.29	1.54 $\pm$ 0.27	0.87 $\pm$ 0.24
<b>CEWL (g/m<sup>2</sup>/h)</b>	16.92 $\pm$ 4.35	17.86 $\pm$ 3.11	10.59 $\pm$ 2.23
<b>VO<sub>2</sub> (ml/h)</b>	0.186 $\pm$ 0.03	0.203 $\pm$ 0.03	0.254 $\pm$ 0.06
<b>VO<sub>2</sub> per mass (ml/h/g)</b>	0.075 $\pm$ 0.013	0.073 $\pm$ 0.012	0.058 $\pm$ 0.008
<b><i>T</i><sub>pref</sub> (°C)</b>	34.94 $\pm$ 2.44	34.67 $\pm$ 2.75	32.08 $\pm$ 3.51

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919 Figure 2: Spatial variation of morphological and physiological traits in an environmental gradient. A:  
 920 Snout to vent length variation along a gradient of mean environmental temperature during activity  
 921 period; color code represents the age class of the lizards (A1=1-year-old, A2=over-1-year-old). B: Body  
 922 mass variation along a gradient of aridity of the environment (low values indicate less mesic  
 923 environments); color separates forest covered habitats (yellow) from open habitats (purple). C:  
 924 Maximum tail width (mm) relative to SVL (residuals of a linear regression) along a gradient of altitude;  
 925 color separates forest covered habitats (yellow) from open habitats (purple). N=94 due to missing values.  
 926 D: Volume of oxygen consumed (ml per hour) relative to the body mass (residuals of a linear regression)  
 927 according to the aridity of the environment; color separates forest covered environments (yellow) from  
 928 open environments (purple).

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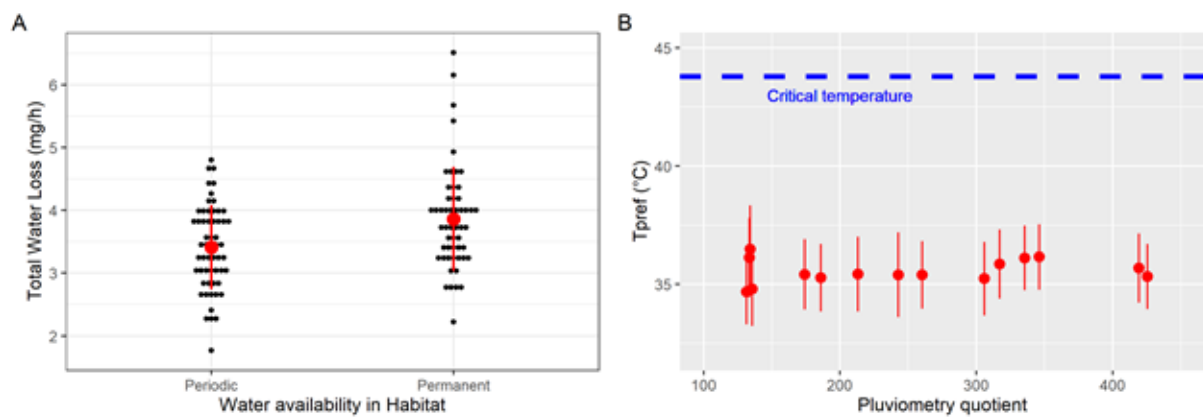
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933 Figure 3: Variation in oviparous populations of *Z. vivipara* A. Rates of total evaporative water loss  
934 (TEWL) according to the availability of water in the habitat; red dot is mean value and red lines are  
935 standard deviations. B. Mean and standard deviation of preferred body temperatures ( $T_{pref}$ ) across  
936 populations along a gradient of aridity of the environment (low values indicate less mesic environments).  
937 The blue dashed line is the critical thermal maximum of *Z. vivipara* (Gvoždík & Castilla, 2001).

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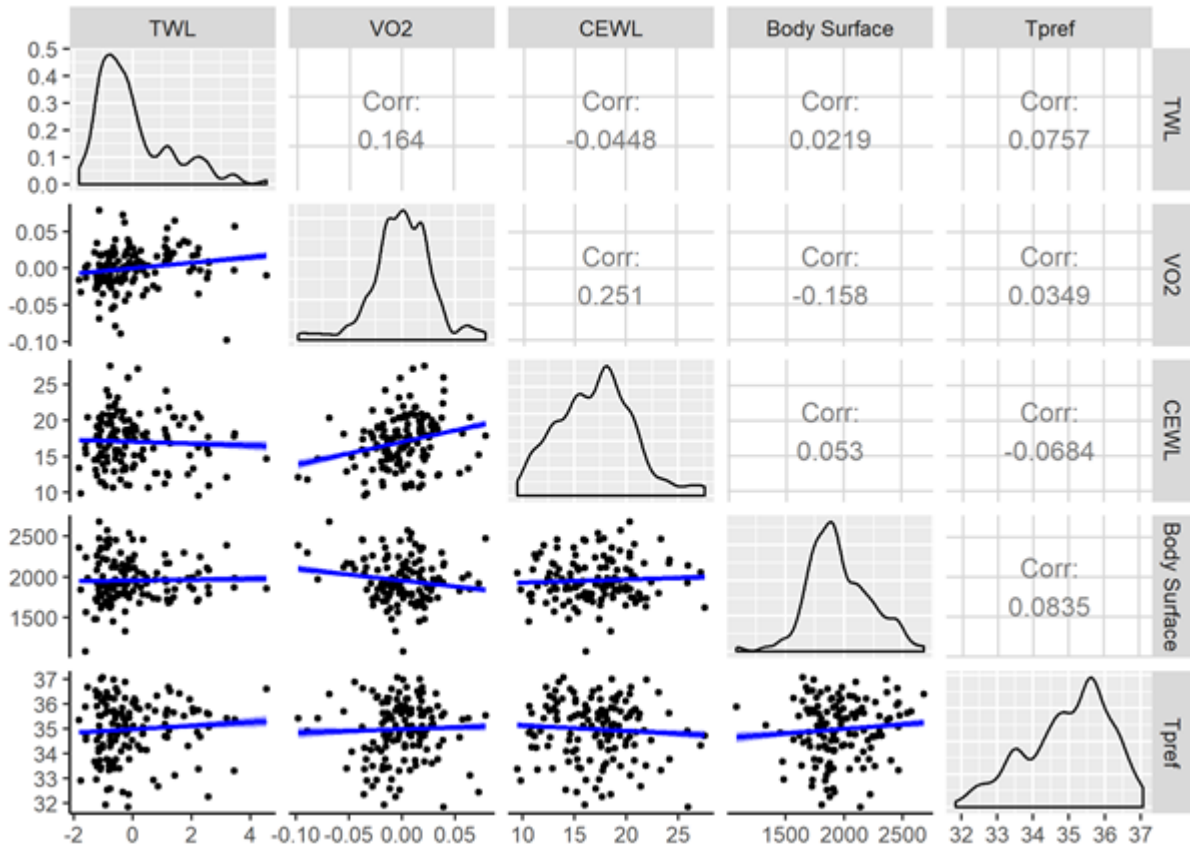
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942 Figure 4: Scatterplot matrix of total and cutaneous water losses (TEWL,  $\text{mg}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$  and CEWL,  $\text{g}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ ),  
 943 metabolic rate ( $\text{VO}_2$ ,  $\text{ml}\cdot\text{h}^{-1}$ ), total body surface ( $\text{mm}^2$ ) and preferred body temperature ( $T_{\text{pref}}$ ,  $^{\circ}\text{C}$ ). The  
 944 diagonal represents the distribution of each variable, the upper triangle provides the Pearson correlation  
 945 coefficients between pairs of variables, and the lower triangle provides scatter plots of each pair of  
 946 variables with the best linear regression line.



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949 SUPPORTING INFORMATION (SEE BELOW)

950 Supp.InfoFigureS1.docx

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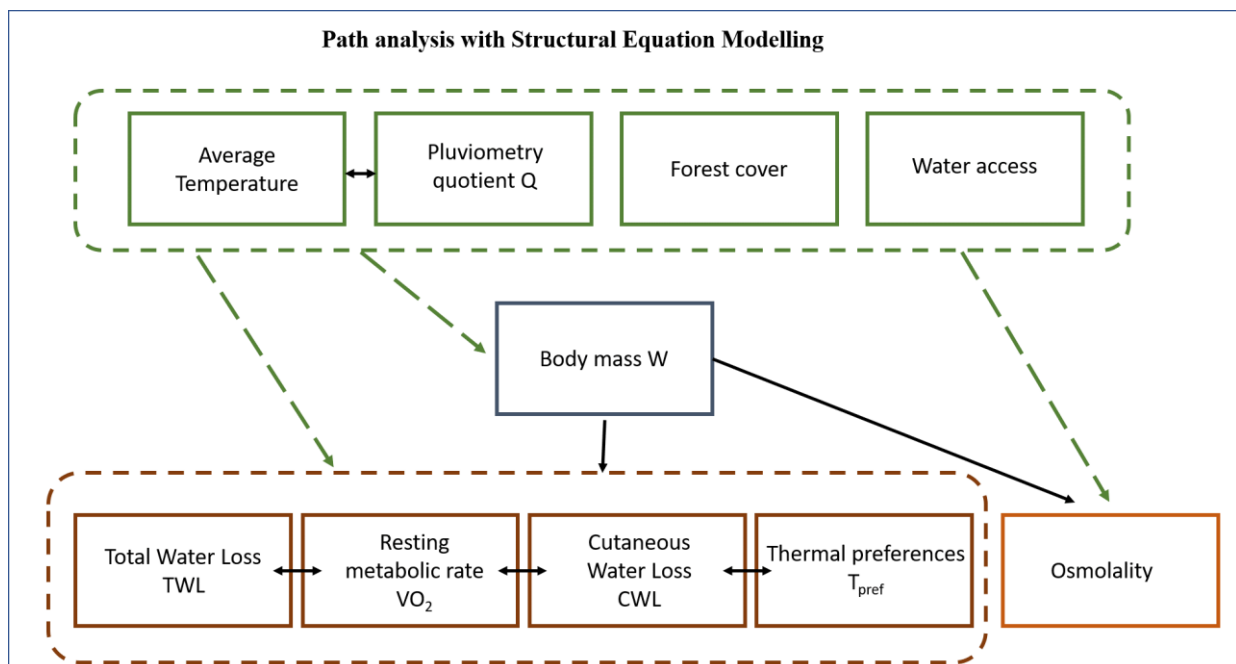


957 **Appendix S1: Complementary analysis of the multivariate data set using Structural Equation**

958 **Modelling**

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960 To further explore the relationships between ecophysiological traits and environmental conditions, we  
961 used piecewise structural equation modelling (SEM) with the *piecewiseSEM* v2.1 package in R  
962 (Lefcheck, 2016) in combination with linear mixed models using the R packages *nlme* (Pinheiro et al.,  
963 2019). We designed our full model based on the same philosophy than the univariate model selection  
964 approach described in the main text (see path diagram below). In our full model, average temperature,  
965 pluviometry quotient Q and the two habitat features (water access and forest cover) have direct,  
966 causal effects on body mass W. In addition, these 4 environmental variables and body mass have direct,  
967 causal effects on plasma osmolality (our index of physiological dehydration) on one side, and on each  
968 of the thermo-hydroregulation traits (TWL,  $VO_2$ , CWL and  $T_{pref}$ ) on the other side. Furthermore, we  
969 assumed correlated errors between average temperatures and pluviometry quotient and between the  
970 four ecophysiological traits. Based on an earlier analysis of this model with tests of direct separation  
971 (Lefcheck, 2016), osmolality and  $VO_2$  appeared to be correlated, so we added correlated errors  
972 between these two variables.



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975 The summary of the outputs of this full model is presented below. The assessment of the goodness-  
976 of-fit showed that it fitted well the data (Fisher's C = 9.372 with P-value = 0.154). In this full model, we  
977 found that significant paths are the same than the ones uncovered in our other model selection  
978 approach in the main text. Indeed, except for body mass which is positively influenced by pluviometry  
979 quotient Q in addition to habitat features, the SEM showed that mostly habitat features and not

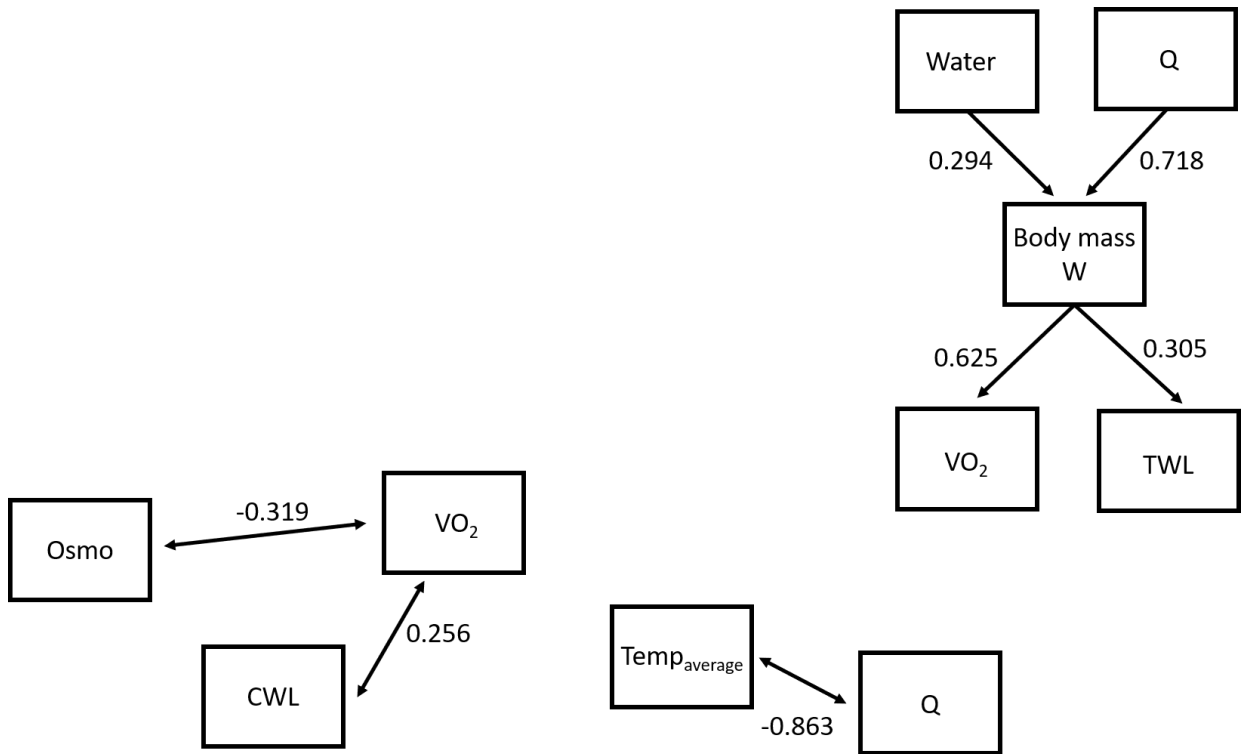
980 thermal conditions influenced our ecophysiological traits (see coefficients of regression and critical  
 981 test values below, significant causal paths are bolded).

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Model	Estimate	SE	P-Value	R <sup>2</sup> <sub>marginal</sub>	R <sup>2</sup> <sub>conditional</sub>
W ~ Taverage	-0.0091	0.077	0.908	0.33	0.42
Q	0.0049	0.002	<b>0.038*</b>		
Forest	0.3467	0.25	0.196		
Water	-0.0466	0.148	0.759		
Osmo ~ W	-2.6415	3.045	0.387	0.05	0.21
Taverage	3.369	2.734	0.246		
Q	0.0749	0.074	0.339		
Forest	5.3381	8.957	0.564		
Water	-5.3288	5.238	0.333		
TWL ~ W	0.6049	0.194	<b>0.002**</b>	0.22	0.28
Taverage	-0.2855	0.138	0.066		
Q	-0.0037	0.004	0.353		
Forest	0.5216	0.456	0.279		
Water	0.7528	0.271	<b>0.019*</b>		
CWL ~ W	-0.5686	0.587	0.335	0.09	0.26
Taverage	0.8019	0.551	0.176		
Q	0.03	0.015	0.073		
Forest	1.6902	1.805	0.371		
Water	-0.7595	1.053	0.487		
VO <sub>2</sub> ~ W	0.0462	0.005	<b>&lt;0.0001***</b>	0.58	0.58
Taverage	-0.0015	0.003	0.609		
Q	0.0001	0.0001	0.189		
Forest	0.0099	0.0096	0.326		
Water	0.0068	0.0058	0.268		
T <sub>pref</sub> ~ W	0.0561	0.195	0.773	0.04	0.04
Taverage	-0.0652	0.107	0.555		
Q	-0.0015	0.003	0.624		
Forest	-0.499	0.3545	0.1896		
Water	0.107	0.2153	0.6301		
Taverage ~~ Q	-0.8626		<b>&lt;0.0001***</b>		
TWL ~~ CWL	0.0218		0.404		
TWL ~~ VO <sub>2</sub>	0.1201		0.088		
TWL ~~ T <sub>pref</sub>	0.123		0.083		
VO <sub>2</sub> ~~ CWL	0.256		<b>0.0018**</b>		
VO <sub>2</sub> ~~ T <sub>pref</sub>	0.0588		0.255		
CWL ~~ T <sub>pref</sub>	-0.061		0.247		
Osmo ~~ VO <sub>2</sub>	-0.319		<b>0.0001***</b>		

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989 Final path diagram with only significant paths showed:  
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