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

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Running title: Geographic variation in thermo-hydroregulation biology

Abstract

Thermo-hydroregulation strategies involve concurrent changes in functional traits related to energy and water balance as well as 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 geographic 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 lousiantzi*) across a multidimensional environmental gradient involving independent variation in air temperature, rainfall **and differences in habitat features (access to free standing water and forest cover)**. We sampled adult males for morphology, resting metabolic rate, total evaporative water loss and cutaneous evaporative water loss (EWL) and thermal preferences in 15 populations from the rear to the leading edge of the distribution across an altitudinal gradient ranging from sea level to **1750 m**. Besides a decrease in adult body size with **increasing** environmental temperatures, we found little effects of thermal conditions on thermo-hydroregulation strategies. **In particular, relict, lowland populations from the warm rear edge showed no specific eco-physiological 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 through the season further influenced EWL. **Our study thus emphasizes the importance of rainfall and habitat features rather than thermal conditions on geographic variation in lizard morphology and physiology.**

Keywords: aridity - evaporative water loss – lizards – metabolism – morphology - thermal preferences - reptiles

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 louislantzi*, 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 louislantzi* (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 μ 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 μ l) were immediately frozen at -28°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°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 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°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_{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_{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 ± 0.01 , $p < 0.0001$,
234 **R² > 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 CO₂ and O₂ 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 CO₂ and O₂
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 VO₂ (volume of O₂ consumed in ml per hour) and the respiratory quotient as the ratio of CO₂ produced
251 by O₂ 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 VO₂ 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 m² 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⁻².h⁻¹ 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 (mm²), thermal preferences (T_{pref} , °C), total water loss
280 (TEWL, mg per hour), cutaneous water loss (CEWL, g.m⁻².h⁻¹) and resting metabolic rate (VO₂, 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 VO₂. 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_{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⁻¹.h⁻¹; *Z. vivipara louislantzi*: TEWL = 1.387 mg. g⁻¹.h⁻¹; *Z.*
331 *vivipara vivipara*: TEWL = 1.489 mg. g⁻¹.h⁻¹; ANCOVA: Taxa: F_{2,140} = 10.9, p < 0.0001; Body mass:
332 F_{1,140} = 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_{2,652} = 66.3, p < 0.0001). VO₂ rates corrected for body mass, were 26% lower
334 in *P. muralis* than in *Z. vivipara* (ANCOVA: Body mass: F_{1,160} = 336.6, p < 0.0001, Taxa: F_{2,160} = 9.63,
335 p = 0.0001). T_{pref} were significantly lower in *P. muralis* compared to *Z. vivipara* subspecies (ANOVA:
336 F_{2,1640} = 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_{14,127} = 4.7, p < 0.0001 and R_{interpop} = 0.38 for SVL and
341 ANOVA: Population effect: F_{14,126} = 7.0, p < 0.0001 and R_{interpop} = 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 ± 0.021 mm, $p = 0.03$; for 10th scale : slope = 0.074 ± 0.015 mm per mm, $p < 0.0001$), the altitude (for
360 max width : slope = 0.0006 ± 0.0002 mm per m, $p = 0.0009$; for 10th scale : slope = 0.0005 ± 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 ± 0.04 mm per °C, $p = 0.018$; for 10th scale : slope = -0.09 ± 0.03 mm per °C, p
363 = 0.006) and the mean precipitation during activity period (for max width : slope = 0.001 ± 0.0007 mm
364 per mm, $p = 0.046$; for 10th scale : slope = 0.001 ± 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 ± 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 ± 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 ± 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 ± 18 mOsm kg⁻¹ and was different among populations (ANOVA:
386 Population effect: $F_{14,119} = 3.8$, $p < 0.0001$ and $R_{interpop} = 0.35$), 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°C, range = 19.80-39.40°C,
390 Figure 3B) compared to previous studies with the same species (e.g., $T_{pref} = 31.5$; Gvoždík & Castilla,
391 2001; Trochet et al., 2018). T_{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 $R_{interpop} = 0.18$, 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_{pref} than lizards from forest habitats.

397 3.5. Covariation between thermo-hydroregulation traits

398 We found no significant correlation between TEWL, CEWL, VO_2 , the body surface area of the animal
399 and the T_{pref} (Figure 4). First, TEWL, T_{pref} and 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 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 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 ± 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)
596 [10.5281/zenodo.6366222](https://dx.doi.org/10.5281/zenodo.6366222)

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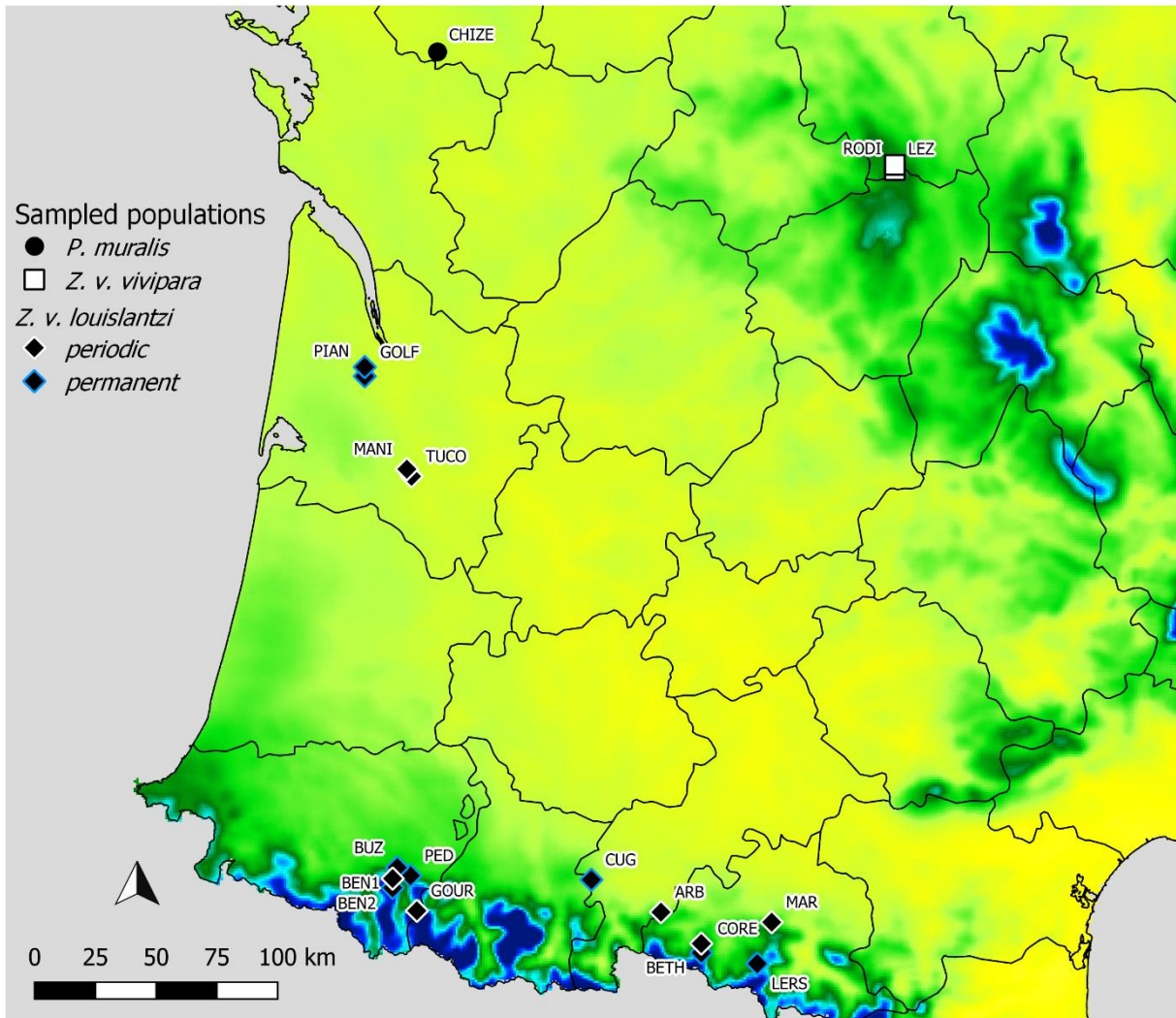
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905 **Figures and Legends**

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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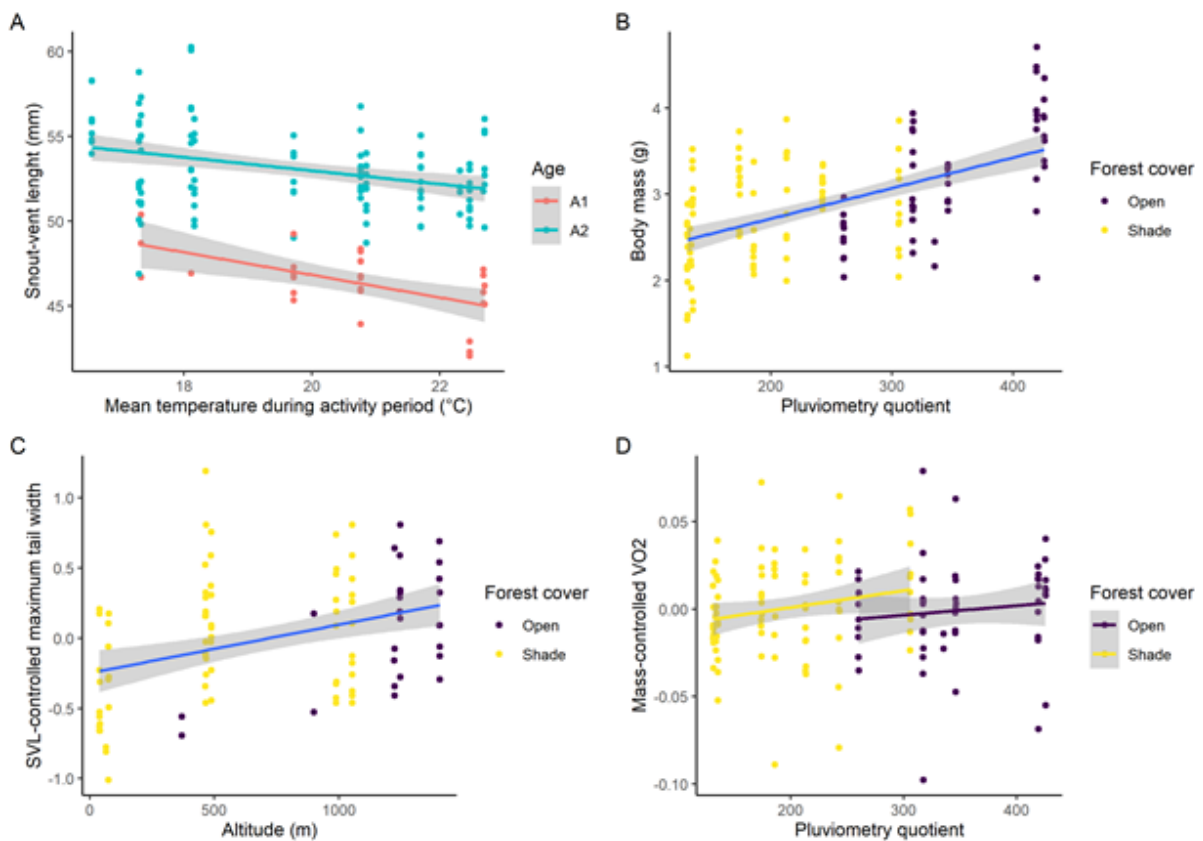
	<i>Z. vivipara louislantzi</i>	<i>Z. vivipara vivipara</i>	<i>P. muralis</i>
BM (g)	2.90 \pm 0.66	3.15 \pm 0.75	5.26 \pm 1.60
TEWL (mg/h)	3.63 \pm 0.78	3.97 \pm 0.68	4.08 \pm 1.70
TEWL per mass (mg/h/g)	1.42 \pm 0.29	1.54 \pm 0.27	0.87 \pm 0.24
CEWL (g/m²/h)	16.92 \pm 4.35	17.86 \pm 3.11	10.59 \pm 2.23
VO₂ (ml/h)	0.186 \pm 0.03	0.203 \pm 0.03	0.254 \pm 0.06
VO₂ per mass (ml/h/g)	0.075 \pm 0.013	0.073 \pm 0.012	0.058 \pm 0.008
<i>T</i>_{pref} (°C)	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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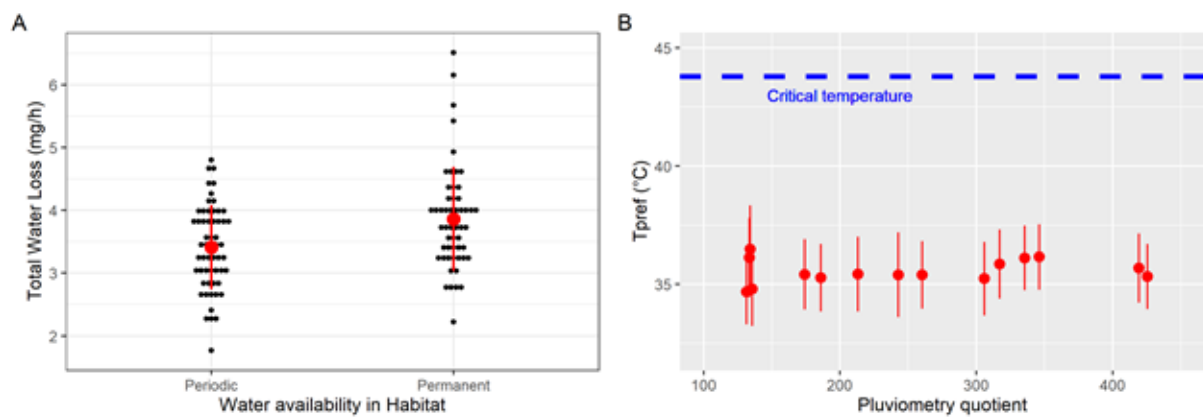
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933 Figure 3: Variation in oviparous populations of *Z. vivipara* A. Rates of total evaporative water loss
934 (TEWL) according to the availability of water in the habitat; red dot is mean value and red lines are
935 standard deviations. B. Mean and 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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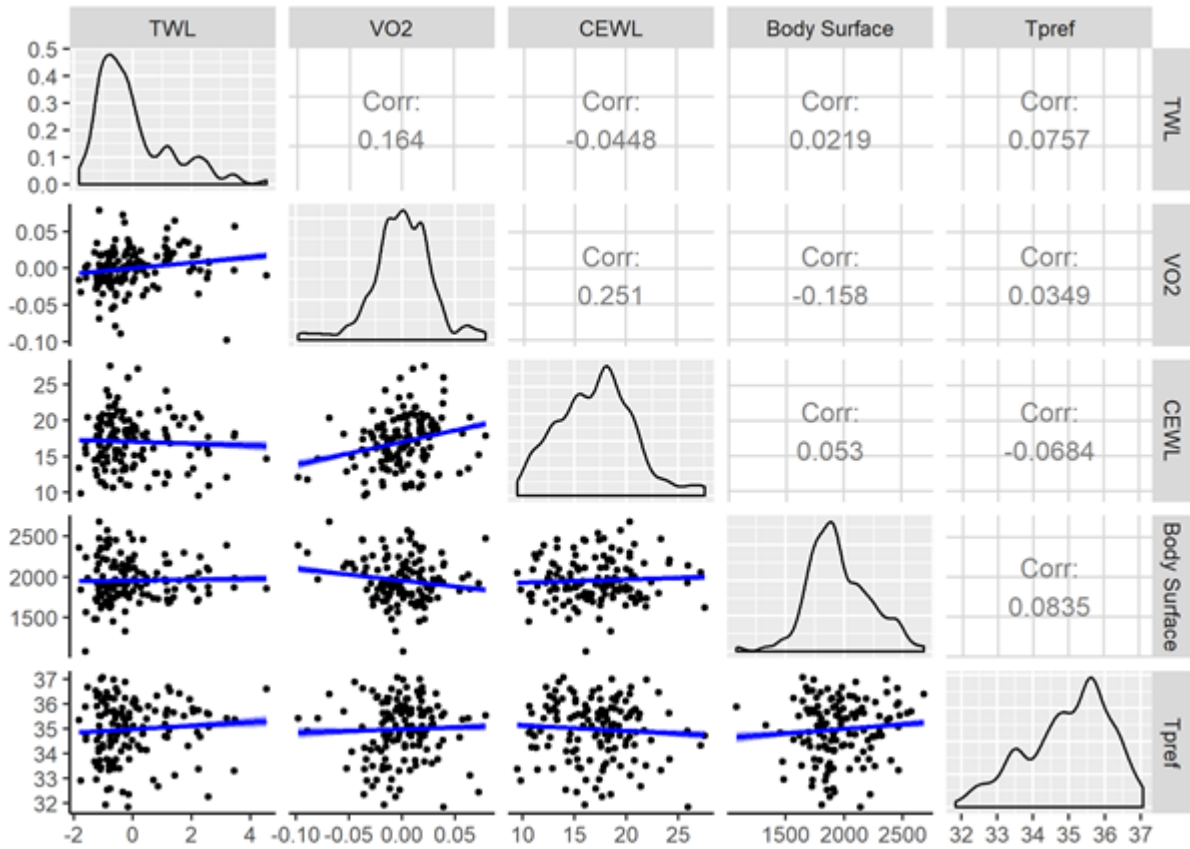
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942 Figure 4: Scatterplot matrix of total and cutaneous water losses (TEWL, $\text{mg}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ and CEWL, $\text{g}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$),
 943 metabolic rate (VO_2 , $\text{ml}\cdot\text{h}^{-1}$), total body surface (mm^2) and preferred body temperature (T_{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

951 Table_supplementaryS1.xlsx

952 Table_supplementaryS2.xlsx

953 Table_supplementary_S3toS11.xlsx

954 Table_supplementaryS12.xlsx

955 **Supp.InfoS13.docx**

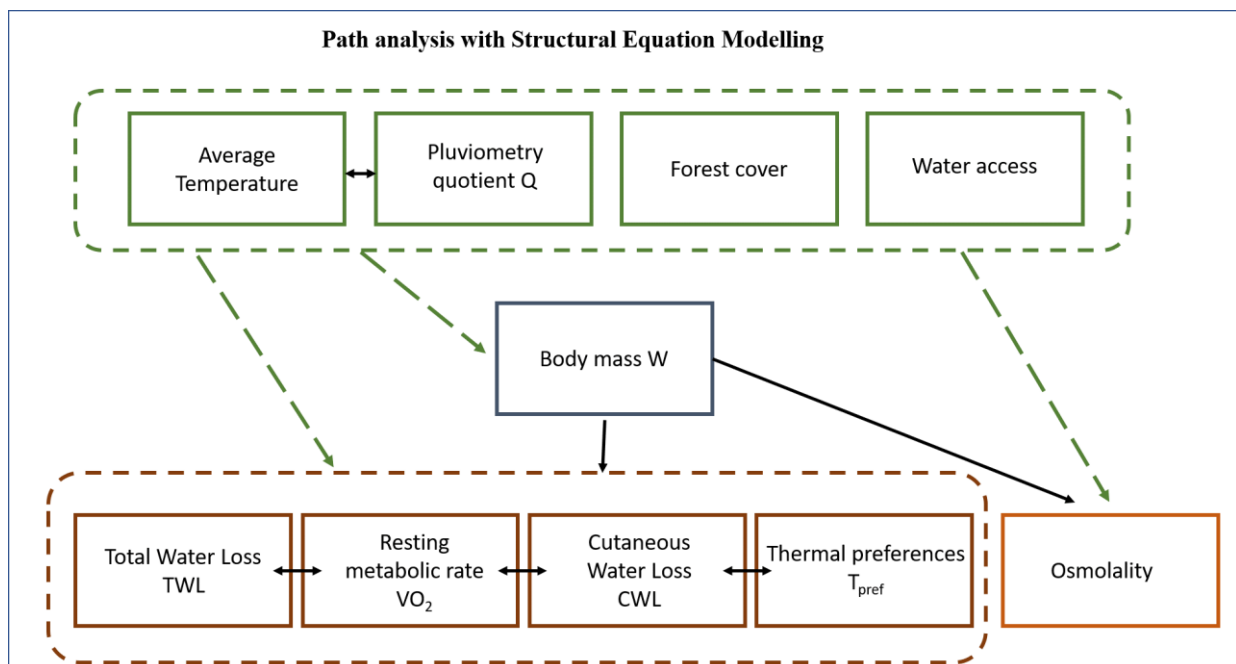
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957 **Appendix S1: Complementary analysis of the multivariate data set using Structural Equation**

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, VO₂, 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 VO₂ 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

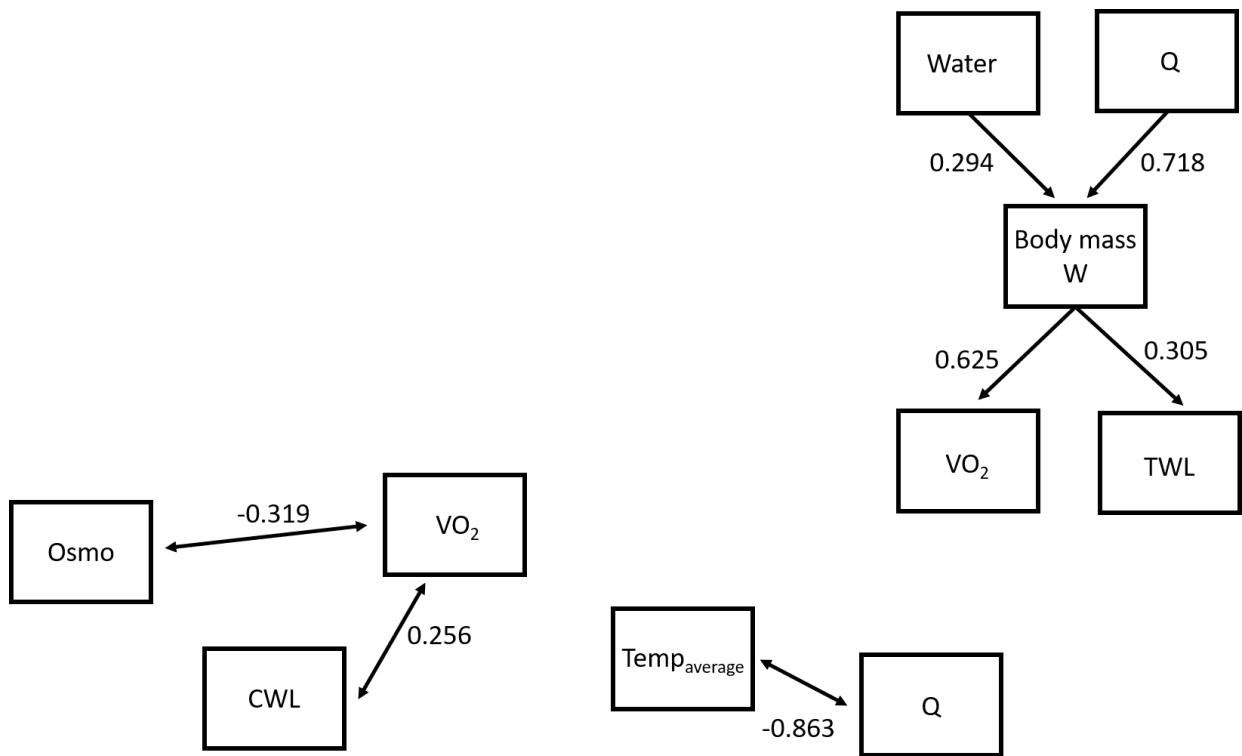
980 thermal conditions influenced our ecophysiological traits (see coefficients of regression and critical
 981 test values below, significant causal paths are bolded).

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Model	Estimate	SE	P-Value	R ² _{marginal}	R ² _{conditional}
W ~ Taverage	-0.0091	0.077	0.908	0.33	0.42
Q	0.0049	0.002	0.038*		
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	0.002**	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	0.019*		
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 ₂ ~ W	0.0462	0.005	<0.0001***	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 _{pref} ~ 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		<0.0001***		
TWL ~~ CWL	0.0218		0.404		
TWL ~~ VO ₂	0.1201		0.088		
TWL ~~ T _{pref}	0.123		0.083		
VO ₂ ~~ CWL	0.256		0.0018**		
VO ₂ ~~ T _{pref}	0.0588		0.255		
CWL ~~ T _{pref}	-0.061		0.247		
Osmo ~~ VO ₂	-0.319		0.0001***		

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989 Final path diagram with only significant paths showed:
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