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1 **INTENSE NOCTURNAL WARMING ALTERS GROWTH STRATEGIES,**
2 **COLORATION, AND PARASITE LOAD IN A DIURNAL LIZARD**

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25 **ABSTRACT**

26 1. In the past decades, nocturnal temperatures have been playing a disproportionate role in the
27 global warming of the planet. Yet, they remain a neglected factor in studies assessing the impact
28 of global warming on natural populations. In ectotherms, physiological performance is
29 influenced by thermal conditions and an increase in body temperature by only 2°C is sufficient
30 to induce a disproportionate increase in metabolic expenditure.

31 2. Here, we question whether an intense augmentation of nocturnal temperatures is beneficial
32 or deleterious to ectotherms. Warmer nights may expand a species thermal niche and open new
33 opportunities for prolonged activities and improve foraging efficiency. However, increased
34 activity may also have deleterious effects on energy balance if exposure to warmer nights
35 reduces resting periods and elevates resting metabolic rate.

36 3. We assessed whether warmer nights affected an individual's growth, dorsal skin colouration,
37 thermoregulation behaviour, oxidative stress status and parasite load by exposing yearling
38 common lizards (*Zootoca vivipara*) from four populations to either ambient or high nocturnal
39 temperatures for approximately five weeks.

40 4. Warmer nocturnal temperatures increased the prevalence of ectoparasitic infestation and
41 altered allocation of resources toward structural growth rather than storage. We found no
42 change in markers for oxidative stress. The thermal treatment did not influence thermal
43 preferences, but influenced dorsal skin brightness and luminance, in line with a predicted
44 acclimation response in colder environments to enhance heat gain from solar radiation.

45 5. Altogether, our results highlight the importance of considering nocturnal warming as an
46 independent factor affecting ectotherms life-history in the context of global climate change.

47

48 **Keywords:** coloration change; ectotherms; energetic balance; nocturnal temperatures;
49 oxidative stress.

50 INTRODUCTION

51 Mean air temperature has been increasing in the past decades and is expected to continue
52 increasing through 2100 (Allen et al., 2018). Yet, while most studies accentuated the role of
53 diurnal temperatures (T_D), historical records suggest that climate warming is driven by a faster
54 increase of nocturnal temperatures (T_N) (Vose, Easterling, & Gleason, 2005). Reasons
55 underlying this asymmetry remain under debate, but include changes in global cloudiness,
56 precipitation, soil moisture or in the thickness of the planetary boundary layer (Davy *et al.*,
57 2017 and references therein). The escalation of T_N influences multiple aspects of terrestrial
58 ecosystems' functioning (Gaston, 2019) by affecting vegetation maturation (Mu et al., 2015)
59 and structure (Alward, Detling, & Milchunas, 1999), microbial activity (Palareti et al., 2016)
60 community composition and food web dynamics (Barton & Schmitz, 2018; Ma, Le Lann, van
61 Baaren, & Ma, 2020; Miller et al., 2017).

62 At present, little is known about the ecophysiological consequences of nocturnal warming
63 on terrestrial animals. Most studies exploring the impacts of global warming are daytime-biased
64 and focus on average warming, ignoring consideration of nocturnal ecology (Gaston, 2019). As
65 a consequence, the daily partitioning of global warming effects between diurnal and nocturnal
66 components has received scant attention, with the very notable exception of several studies
67 emphasizing insects. For example, Kingsolver et al. (2015) demonstrated that an experimental
68 increase in ambient temperature of 5°C during the night or day results in differential growth
69 effects for tobacco hornworms (*Manduca sexta*). Other studies have suggested that a change in
70 nocturnal temperatures should alter multiple traits in insects including metabolic expenditure,
71 growth rates and developmental time (Colinet, Sinclair, Vernon, & Renault, 2015; Zhao, Zhang,
72 Hoffmann, & Ma, 2014).

73 Physiological processes and behavioural activity of ectothermic species are directly
74 influenced by the temperature of their environment (Seebacher & Franklin, 2005). For such

75 species, the relationship between metabolism, performance, or behavioural activity and body
76 temperature often follows a right-skewed bell-shaped curve (Fig.1.a): the trait value gradually
77 increases from the critical thermal minimum (CT_{\min}) up to the maximal performance at the
78 thermal optimum (T_{opt}), before experiencing a swift decrease toward the critical thermal
79 maximum (CT_{\max}). Due to the shape of this curve, it is commonly assumed that increasing
80 temperatures near CT_{\min} may have a lower impact on metabolism, performance, or behavioural
81 activity than increasing temperatures beyond T_{opt} , where the slope towards CT_{\max} is steeper
82 (Sinclair et al., 2016; Sunday et al., 2014). Yet, in critical traits involved in energy balance such
83 as metabolism, temperature increments ranging between 0.5°C and 2°C have been
84 demonstrated to cause a disproportionate increase in metabolic expenditure (respectively about
85 +3% to about + 17%) (Dillon, Wang, & Huey, 2010). In diurnal species, the energy balance
86 during daytime depends on individual's activity level and energy intake from foraging. At night,
87 metabolic expenditure declines steadily and depends on ambient temperatures, because
88 nocturnal thermoregulatory behaviour is often limited for diurnal species (Colinet et al., 2015).
89 In this context, a warmer T_N that shifts the thermal conditions away from CT_{\min} may thus
90 substantially raise metabolic expenditure (Fig.1.b), with different consequences for an
91 individual's energy balance.

92 Current assessments about the impacts of rising T_N on ectothermic species in the literature
93 are rare but differ regarding the potential costs and benefits (Barton & Schmitz, 2018; Speights,
94 Harmon, & Barton, 2017). Some studies demonstrate that higher T_N may open new temporal
95 niches by, extending an individual's daily activity period (Clarke & Zani, 2012; Ziska, 2014),
96 increasing the number of frost-free nights (Andrade, Krespi, Bonnardot, van Baaren, &
97 Outreman, 2016), or altering activity towards night-time in environments where daily
98 temperatures exceed CT_{\max} (Lara Resendiz, 2019; Nordberg & Schwarzkopf, 2019). With
99 increasing T_N , individuals may also benefit from an acceleration of development, that can lead

100 to earlier onset of reproduction (Speights et al., 2017) or enhanced immediate survival by
101 improving the ability to escape predators (Burraco, Díaz-Paniagua, & Gomez-Mestre, 2017).
102 However, if exposure to higher T_N is severe and long-lasting, one might also expect several
103 negative effects. First, the short-term advantage provided by an extended period of activity can
104 have long term costs as the supplementary energy expended overnight could disrupt an
105 individual's energetic (e.g. fat reserve) and physiological (e.g. telomere lengths, oxidative
106 stress) balance, which could decrease life expectancy (Alonso-Alvarez et al., 2006; Burraco,
107 Orizaola, Monaghan, & Metcalfe, 2020; Metcalfe & Monaghan, 2001). Second, high T_N may
108 have an instantaneous negative effect by affecting an individual's rest and recovery time (Bai,
109 Ma, Cai, & Ma, 2019; Kayser, Yue, & Sehgal, 2014; Ma, Hoffmann, & Ma, 2015). For many
110 species, low T_N constitutes a thermal refuge, where individuals recover from diurnal activity
111 (Speights et al., 2017; Zhao et al., 2014). If this period of quiescence is compromised,
112 individuals may not be able to recuperate from their diurnal activity, which may in turn have
113 negative effects on their physiology, alter their daily behaviour and, if nocturnal warming is
114 long enough, have negative repercussions on their stress levels and life cycle.

115 In this study, we investigated the functional responses of the common lizard (*Zootoca*
116 *vivipara*) to an experimental increase in nocturnal temperatures. In the past decade, this species
117 faced several heatwaves in the southern part of the distribution, sometimes leading to extreme
118 T_N . The common lizard is a cold-adapted specialist and recent studies highlighted that
119 functional traits correlate with geographic differences in nocturnal temperatures across its
120 southern range. For example, the nocturnal temperatures of local populations correlate with
121 individual physiological strategies, including heating rate (Rutschmann et al., 2020), baseline
122 corticosterone level (Dupoué et al., 2016) or water balance strategy (Dupoué, Rutschmann, Le
123 Galliard, Miles, et al., 2017). More concerning results point out that warmer nocturnal
124 temperatures also correlate with faster erosion of telomeres early in life and population decline,

125 and therefore with a higher “stress level” of individuals and populations (Dupoué, Rutschmann,
126 Le Galliard, Clobert, et al., 2017). Yet, a mechanistic understanding of the biological impact of
127 T_N remains lacking. To better assess the consequences of warmer nights, we studied the
128 response of yearling common lizards to an intense increase in nocturnal temperatures. We
129 exposed individuals to two nocturnal thermal treatments over a period of 38 days. A control
130 treatment simulated T_N close to the common lizard’s lower limit of activity, whereas lizards in
131 the treatment group experienced an increase in T_N , matching extreme temperatures recorded
132 overnight in natural populations. Both groups experienced similar ambient temperature
133 conditions during the day. We predicted that warmer T_N should increase metabolic expenditure
134 and individual growth rate, which may trigger a trade-off with other physiological and
135 metabolic functions. To test this prediction, we therefore compared several functional traits
136 before and after treatment.

137 We predicted individuals from the warmer treatment to grow faster as a direct
138 consequence of a higher assimilation rate (Bestion, Teyssier, Richard, Clobert, & Cote, 2015;
139 Dupoué, Rutschmann, Le Galliard, Clobert, et al., 2017). In addition, we expected enhanced
140 growth rates to incur a cost to other morphological traits such as body mass or fat reserve, which
141 may lead to elevated physiological stress (Dupoué, Rutschmann, Le Galliard, Clobert, et al.,
142 2017). To verify this second prediction, we quantified the balance between oxidative damage
143 and antioxidant defences (i.e., oxidative stress) (Costantini, 2014; Metcalfe & Monaghan, 2001;
144 Monaghan, Metcalfe, & Torres, 2009). If exposure to warmer nocturnal temperatures induced
145 a stress response, then we also predicted an increased susceptibility to parasitic infestation due
146 to the immunosuppressive effect of stress (Harvell et al., 2002). Finally, we tested if and how
147 lizards could buffer the physiological costs of increased nocturnal temperatures by selecting
148 cooler body temperatures during the day (Angilletta, Cooper, Schuler, & Boyles, 2002) and by

149 developing a lighter colouration that reduces heat gain during daytime (thermal melanism
150 hypothesis, Clusella Trullas, van Wyk, & Spotila, 2007).

151 **METHODS**

152 *Study species and populations*

153 The common lizard (*Z. vivipara*) is a small-sized species in the family Lacertidae whose
154 distribution encompasses Northern Europe and Asia. In the study populations, lizards emerge
155 in spring from winter hibernation to grow and reproduce over summer. The population structure
156 consists of juveniles (born in the summer), yearlings (non-reproductive one-year old
157 individuals) and adults (two-year-old individuals or older). We focused on yearling males,
158 because they exhibit the highest growth rate during the activity season. We sampled individuals
159 from four populations (ca. 20 lizards per populations, total n=78), located in the Massif Central,
160 France (Table 1) and brought them back to the laboratory. Populations were selected based on
161 their similarity in diurnal and nocturnal thermal characteristics. Yet, significant differences
162 existed between populations, notably when comparing extreme temperatures recorded
163 overnight (97.5% upper quantile of the distribution of nocturnal temperatures; see table 1,
164 Fig.2.a-b and Appendix 1). To control for these differences, and detect patterns related to them,
165 population identity was always included in our models as a fixed effect.

166 *Thermal conditions*

167 Each lizard was housed individually in a plastic terrarium (11x18x11cm) containing a
168 cardboard shelter and a substrate of sterilized soil. Terraria were misted four times a day.
169 Lizards were allowed to bask under a 25W bulb for six hours a day. Every second day, lizards
170 were fed live crickets, maggots or mealworms *ad libitum*. Lizards were randomly divided into
171 two thermal treatments. During the day, all lizards were held in a common room under the same
172 thermal conditions. Every evening between 17:30 and 18:30, lizards in the control group (T_{Nc})
173 were transferred to a room tracking ambient temperature. Lizards in the warm nocturnal
174 temperature treatment (T_{Nw}) were transferred to a room maintained at higher nocturnal

175 temperatures. Every morning between 8:00 and 8:30, lizards were returned to the main room
176 (See Fig.2c and Appendix 1). All terraria were randomly allocated on the shelves every morning
177 and evening. Note that we matched individuals for size and mass between treatment groups to
178 eliminate potential bias toward heavier or longer individuals in one treatment (See Appendix 2
179 and Fig.S4.).

180 In each treatment, two iButtons (Thermochron[®], Maxim Integrated Products, Sunnyvale,
181 CA, USA) were buried in two different terraria to estimate thermal conditions experienced by
182 the lizards. Temperatures were recorded every hour during the experimental period.
183 Temperatures in T_{Nw} were chosen to reflect the extreme nocturnal temperatures recorded in
184 natural populations (Table 1), simulating the potential effect of global warming. Temperatures
185 in T_{Nc} were chosen to be near the lower limit for activity for the common lizard that is around
186 an air temperature of 17-20 °C (average temperature in $T_{Nc} = 17.8 \pm 0.7^{\circ}\text{C}$ and $T_{Nw} = 24.6 \pm$
187 0.9°C ; $t_{1195.7} = 145.65$, $p < 0.005$) (Rozen-Rechels et al., 2020).

188 *Timing of the experiment*

189 All measurements described in the next sections were performed twice, before and after the
190 five-week (38 days) treatment. For each lizard and for each trait i , a differential Δ_i was
191 calculated as the difference between post- and pre-treatment measurements (e.g., $\Delta_{SVL} = SVL$
192 post-treatment – SVL pre-treatment). A period of 38 days was chosen to mirror the typical
193 duration of summer heat waves in the Massif Central region (personal observations). It also
194 covers approximately 25% of the summer activity (May to September) for common lizards and
195 corresponds to a period of rapid growth for young lizards. Thus, this duration was sufficient to
196 detect differences in individual growth rates and observable changes in oxidative balance
197 (Dupoué, Angelier, et al., 2020). Lizards were fasted between the day of capture and the end of
198 the first set of measurements (two days). Measurements were taken in the following order after
199 capture: 1. ectoparasite removal, 2. morphological measurements, 3 digital scanning for color

200 analysis, 3. preferred body temperature, and 4. blood sampling. All lizards were fed after blood
201 sampling. We fasted lizards for two days prior to the second run of measurements. All
202 individuals were released to their site of capture. See Appendix 3 for a timeline of the
203 experimental design.

204 ***Morphological measurements***

205 We measured snout to vent length (SVL, mean \pm SD = 40.2 ± 2.5 mm), body length (= SVL +
206 tail length: BL = 93.6 ± 13.4 mm) and body mass (BM = 1.31 ± 0.25 g). We estimated tail's
207 volume (TV = 331.1 ± 76.7 mm³) as an index of fat reserves (Avery, 1974) and muscular state
208 (see Appendix 4 for more details). We also calculated an index of body condition (BC) as the
209 residuals of a linear regression relating BL and BM (Richard, Massot, Clobert, & Meylan,
210 2012).

211 ***Colour measurements***

212 Pre- and post-treatment dorsal images of yearlings were obtained with a high-resolution digital
213 scanner (Canon[®], CanoScan Lide 110; image size: 2550x2600 pixels). Each scan was cropped
214 to the area of interest (i.e., dorsal section, see Fig.S6) and the background was removed. We
215 analysed two aspects of colour variation with the *colordistance* R package (Weller, 2019), as
216 described in Appendix 5. Briefly, we focused first on the luminance of each dorsal section and
217 calculated the distance between the luminance before and after treatment (Δ LUM). Second, we
218 estimated the change in colouration by quantifying the variation in colour composition by
219 extracting the predominant colours in all pictures (pre-and post-treatment pictures grouped
220 together; four colours were selected: COL_1 to COL_4; Fig.4.a; Fig.S8). Then, we evaluated
221 their relative abundance for each individual, before and after the treatment, to calculate four
222 colouration distances (Δ COL_1 to Δ COL_4). Colours can be classified from the darker to the lighter
223 one (position on the spectral range) as follows: COL_1 (HEX Code: #41362E), COL_2
224 (#604B40), COL_3 (#6E5F4C) and COL_4 (#7E7167).

225 ***Thermoregulatory behaviour***

226 The preferred temperature T_{pref} is defined as the range of body temperatures selected by an
227 ectotherm in a cost-free environment (Angilletta et al., 2002; Hertz, Huey, & Stevenson, 1993).
228 We measured T_{pref} using ten plastic tracks (120 x 25 x 20 cm) lined with a substrate of sterile
229 soil. In each track, we created a thermal gradient by heating one side with a 60W incandescent
230 bulb (39.4 ± 4.02 °C) while the cool side was maintained at ambient temperature (23.7 ± 2.7
231 °C). Two UVB 30W fluorescent lamps positioned over each track provided uniform
232 illumination with white light to avoid a potential effect of a luminance gradient. Thermal
233 gradient tracks were heated at least 45 minutes prior to each experiment. We assigned a lizard
234 to each track at random and allowed them to thermoregulate for 80 minutes after 10 minutes of
235 acclimation. We recorded the back-surface temperature of each lizard as a proxy of body
236 temperature (Rutschmann et al., 2020). Measurements were repeated every 10 minutes (9
237 values per individual), with an infrared thermometer (Raytek, Raynger MX2; distance-to-spot
238 ratio is 60:1) and with minimal disturbance. The distance of detection (300mm) was calibrated
239 between all measures according to the manufacturer recommendations for a spot diameter of 6
240 mm.

241 ***Oxidative status***

242 Oxidative status was assessed using two blood markers. Lizards were bled (60 μ L whole blood)
243 from the post-orbital sinus at days 0 and 39. Samples were immediately centrifuged for 5
244 minutes at 13,500 rpm to separate plasma and blood cells and kept frozen at -28°C in airtight
245 tubes until analyses. Plasma concentration of reactive oxygen metabolites (ROM) and non-
246 enzymatic antioxidant capacity (OXY) were used as indices of oxidative damage and defences,
247 respectively. We followed a protocol developed for this species (Josserand et al., 2020). ROM
248 concentration was measured with the d-ROMs test (MC003, Diacron International, Italy). This
249 test measures the oxidative capacity of a plasma sample in relation to the activity of organic

250 hydroperoxides (biomolecules damaged by free radicals, Costantini, 2016). The antioxidant
251 capacity of the plasma was assessed with the OXY-absorbent kit (MC435, Diacron
252 International, Italy). This test measures the ability of plasma to buffer an oxidant attack of
253 hypochlorous acid (Costantini, Marasco, & Møller, 2011). Samples for d-ROMs were read at
254 540nm after 75min of incubation at 37°C. Samples for OXY were read at 540nm after 10
255 minutes of incubation at 37°C. Plates were randomised by population, treatment and timing
256 (pre- vs post-). We measured ROM and OXY using two plates, so we repeated 14 samples to
257 estimate the coefficient of variation both within (intraplate; ROM: 8.86%, OXY: 1.02%) and
258 between plates (inter-plate; ROM: 9.19%, OXY: 2.05%).

259 ***Parasite load***

260 Ecto- and endo-parasite load was estimated for each individual. The day of capture, all
261 individuals were examined for mites (genus *Ophionyssus*). All parasites were hand removed
262 with tweezers and the lizard treated with an antiparasitic solution of 0.29% fipronil (Frontline
263 SprayTM). Twenty days later, we checked for the presence of mites to avoid infestation in the
264 laboratory. We took advantage of this opportunity to once again categorize lizards for presence
265 or absence of ectoparasites (EctoP = 0 or 1). We also took advantage of blood sampling to test
266 for the presence of blood parasites (BloodP, *Karyolysus spp.*, RM-P, personal observation)
267 before and after treatment. When individuals were bled, a drop of blood was smeared on a slide
268 whenever blood flow was sufficient. Blood was fixed with 100% methanol for 5 minutes and
269 stained for 40 minutes with a 10% solution of Giemsa stain (azur-eosin methylene). BloodP
270 were counted under a Leica Microsystems DMLB microscope at 1000x magnification. A total
271 of 2,000 blood cells per blood smear were screened following Megía-Palma *et al.* (2018).

272 **Statistics**

273 All statistics were performed using R software (R core Team, 2020, v. 3.2.0). All differences
274 between post- and pre-treatment measures (Δ) were analysed using linear models, with the

275 exception of ecto- and blood-parasite loads that were analysed using Generalised Linear Model
276 and Generalised Linear Mixed Model respectively (see dedicated section).

277 *Morphology* - The difference (Δ_i) for each morphological trait i , was analysed with a Gaussian
278 linear model. We included temperature treatment and population of origin as fixed effects. The
279 initial value of the morphological trait ($i_{initial}$) was entered as a covariate. We also included the
280 interaction between treatment and population.

281 *Colouration* - The luminance differential (Δ_{LUM}) was analysed with a Gaussian linear model,
282 including population of origin, temperature treatment, initial luminance and the interaction
283 between treatment and population as fixed effects. We used four linear models to test whether
284 the four most represented dorsal colours changed during the experimental treatment (Δ_{COL_1} to
285 Δ_{COL_4}). Two lizards were discarded from the analyses, because their images revealed moult
286 residuals, affecting their colouration.

287 *Thermal preferences* – We used a linear mixed-effects model to analyse T_{pref} . For each
288 individual, we estimated $\Delta_{T_{pref}}$ as the difference between the mean body temperature calculated
289 over the post-experimental run and the mean body temperature calculated over the pre-
290 experimental run. In the fixed part of the model, we included initial T_{pref} , population of origin
291 and experimental treatment. We also included the interaction between the population of origin
292 and treatment. The identity of the experimental arena was added as a random effect to capture
293 the variance between the ten thermal arenas used repeatedly in the experimental setup.

294 *Oxidative status* – We used a Gaussian linear model to test whether changes in ROMs (Δ_{ROM})
295 and in OXY (Δ_{OXY}) were impacted by the experimental design, the population of origin, the
296 initial values of ROMs (or OXY) and the interaction between the treatment and the population
297 of origin.

298 *Parasite load* – Ectoparasite presence (EctoP) was examined using a generalized linear model
299 (GLM) with a binomial distribution. Initial state of parasitism (presence/absence), population

300 of origin and treatment (and their interaction) were included as predictor variables.
301 Unfortunately, some individuals were more difficult to bleed than others before or after the
302 treatment notably because of small body size, but also because of individuals' level of
303 hydration, body temperature and person in charge. Yet, despite standardized before blood
304 sampling to avoid any bias, we were not able to collect blood to assess *BloodP* for all individuals
305 (N pre-treatment warm= 25, control= 25; N post-treatment warm= 35, control= 34).
306 Consequently, it was not possible to analyse the difference in *BloodP* load, since some
307 individuals in the post-treatment sample were not included in the in the pre-treatment sample,
308 and *vice versa*. *BloodP* exhibited an excess number of zeros, so we used a generalized linear
309 mixed model, with a zero-inflated Poisson distribution (GLMMadaptive package; Dimitris
310 Rizopoulos, 2020). Population, timing (pre-vs post-treatment) and treatment, as well as the
311 interactions between timing and treatment and population and treatment were included as fixed
312 terms in the model. We included individual identity as a random effect.

313 **Initial values** – We screened for potential differences between experimental groups at the onset
314 of the experiment for all measurements. Except for ROM (1.27 ± 0.41 , $p = 0.003$), we found no
315 difference between treatment for initial values (all $p > 0.07$). The difference in ROM was driven
316 by five individuals, all assigned in T_{Nw}, exhibiting extreme high values. These high initial
317 values of ROM were uncorrelated with any of other traits studied here and therefore individuals
318 were retained in our analyses (See Appendix 6 for more information).

319

320 **RESULTS**

321 Complete summary tables for all traits are available in Appendix 7.

322 **Morphology** - Δ_{SVL} was positively influenced by the warmer treatment (i.e., higher growth; Fig.
323 3a; 0.59 ± 0.25 , $t = 2.38$, $p = 0.02$). In contrast, Δ_{MASS} was not impacted by the experimental
324 treatment (Fig. 3b; p -value= 0.84). Change in body condition Δ_{BC} was negatively influenced by

325 warmer nocturnal temperatures (Fig. 3c; -0.07 ± 0.02 , $t = -3.01$, $p = 0.003$). Finally, Δ_{TAIL} was
326 negatively influenced by T_{Nw} (Fig. 3d; -26.6 ± 9.48 ; $t = -2.8$; $p = 0.006$). All morphological
327 differences were negatively impacted by the initial measurement (all p-values <0.007). Neither
328 the population of origin (all p-value > 0.12), nor the interaction between treatment and
329 population (all p-values > 0.06) had an effect on morphological differences.

330 **Colouration** Δ_{LUM} was significantly higher in T_{Nw} (3.43 ± 1.64 ; $t = 2.1$; $p = 0.04$) in all but one
331 population (Bouvier), as indicated by the significant interaction between population and
332 treatment (Fig. 4b; -5.53 ± 2.25 ; $t = -2.259$; $p = 0.02$; all other $p > 0.58$). Initial luminance had
333 a negative correlation with Δ_{LUM} (-0.79 ± 0.12 ; $t = -6.46$; $p < 0.005$). Regarding colouration
334 (Δ_{COL} ; Fig.4c), three out of the four dominant colours were significantly affected by T_{Nw} . The
335 proportion of the darkest colour (Δ_{COL_1} ; #41362E) declined in all populations (-0.14 ± 0.06 ; t
336 $= -2.34$; $p < 0.02$), except in Bouvier where an opposite and significant difference was observed
337 (0.19 ± 0.03 ; $t = -2.48$; $p < 0.015$; all other $p > 0.45$). In all populations, the proportion of
338 COL_2 (Δ_{COL_2} ; #604B40) increased in T_{Nw} (0.02 ± 0.01 ; $t = 2.07$; $p = 0.04$), and particularly
339 in Col du Pendu (0.03 ± 0.01 ; $t = 2.1$; $p = 0.03$, all other $p > 0.6$). Δ_{COL_3} (#6E5F4C) was
340 unaffected by the treatment (-0.05 ± 0.02 , $t = 1.63$, $p = 0.11$) except for Bouvier, where it
341 increased significantly in T_{Nc} (-0.05 ± 0.02 , $t = -2.15$, $p = 0.03$). The proportion of COL_4
342 (#7E7167) was positively affected by T_{Nw} for all populations (0.04 ± 0.02 ; $t = 2.24$; $p = 0.03$).
343 All Δ_{COL} were negatively affected by the initial proportion of colouration (all $p < 0.005$).

344 **Thermal preferences** - We found no effect of nocturnal temperatures on ΔT_{pref} (-0.001 ± 0.19 ,
345 $t=0.32$, $p = 0.99$; Fig.S.10). Yet, on average, individuals favoured warmer temperatures during
346 the second measurement after controlling for a negative correlation with initial T_{pref} ($-0.81 \pm$
347 0.07 , $t=-10.88$, $p < 0.005$). We found no influence of population (all p-value > 0.07).

348 **Oxidative status** - Both Δ_{ROM} and Δ_{OXY} were negatively influenced by the initial levels of
349 ROMs and OXY (ROMs: -1.02 ± 0.07 , $t = -14.26$, $p < 0.005$; OXY: -1.00 ± 0.02 , $t = -59.01$, p

350 < 0.005). We did not detect any effect of treatment on Δ_{ROM} and Δ_{OXY} (See Fig.S.11; $p > 0.20$),
351 of the population of origin (all $p > 0.14$) nor of the interaction between the population of origin
352 and the treatment (all $p > 0.11$).

353 **Parasitic Load** - Ectoparasitic infestation (**EctoP**) was influenced by the experimental treatment
354 (Fig. 5a; 1.63 ± 0.55 , $z = 2.97$, $p = 0.003$) but not by the initial presence of ectoparasites (p -
355 value = 0.19). **EctoP** did not differ among populations (all $p > 0.30$). Blood parasite load
356 (**BloodP**) did not change due to the treatment (zero-inflation model, interaction between
357 treatment and time: p -value = 0.72). **BloodP** also did not differ between treatments (Fig. 5b;
358 count model, interaction between treatment and time: p -value = 0.84). Yet, individuals from
359 Bouvier showed a lower rate of infection in T_{Nw} (-1.61 ± 0.68 , z -value = -2.35 , p -value = 0.02).

360

361 **DISCUSSION**

362 After five weeks of exposure to manipulated, high nocturnal temperatures, without changes in
363 diurnal conditions, we found significant alterations in morphology, colouration and rate of
364 infestation by ectoparasites in yearlings of the common lizard, *Z. vivipara*. We did not detect
365 an effect of treatment on thermal preferences, infection rate by blood parasites, or oxidative
366 stress status.

367 ***Intense nocturnal warming drives allocation of energy toward structural growth rather than***
368 ***storage.***

369 In all populations, exposure to high warm nocturnal temperatures had a positive influence on
370 the growth in body length (SVL), with lizards from T_{Nw} growing 20% faster on average
371 compared to controls. These results corroborate similar patterns from previous studies on the
372 same species in which juvenile common lizards exhibited enhanced growth rates in body size
373 when exposed to elevated daily temperatures (Bestion et al., 2015; Chamaille-Jammes, Massot,
374 Aragon, & Clobert, 2006). Comparable effects of T_{N} have also been described for other
375 ectothermic species, with warmer T_{N} accelerating egg, larval or pupal development in several

376 insect species (Speights & Barton, 2019; Whitney-Johnson, Thompson, & Hon, 2005; Wu, Hao,
377 Sun, & Kang, 2012). However, to the best of our knowledge, this study is the first to show that
378 warmer T_N can have a direct, positive effect on a lizard growth rate.

379 Faster body growth may be attributable to higher energy acquisition or higher allocation
380 towards structural growth during warmer nights or both (Speights et al., 2017). Yet, if not
381 compensated by higher caloric intake, higher energetic expenditure during warmer nights may
382 require rapid functional adjustments, including depletion of energetic reserves (Dillon et al.,
383 2010; Huey & Kingsolver, 2019). Despite lizards having unrestricted access to food and water
384 in captivity, our results indicate that faster growth rate triggered a potential trade-off with other
385 key morphological traits. First, body mass did not differ between treatments. Consequently,
386 yearlings grew faster in T_{Nw} but showed lower post-treatment body condition. In addition,
387 lizards in T_{Nw} had a reduced tail volume compared to controls. Tail volume in lizards is a
388 reliable proxy of reserves available to respond to energetic or hydric stress, by metabolizing fat
389 or proteins, respectively (Bateman & Fleming, 2009; Bruschi, Lourda, Kaminsky, & DeNardo,
390 2018). We therefore hypothesize that the nocturnal treatment initiated two distinct strategies
391 resulting from a plastic allocation of energy toward structural growth versus storage.
392 Individuals from T_{Nw} expended more available energy to compensate for increased metabolic
393 activity, whereas individuals from T_{Nc} built-up reserves for future needs.

394 It is interesting to note here that lizards were fed *ad libitum* and provided with a constant
395 supply of water. We did not record potential differences in food consumption between
396 treatments, however, recent unpublished data (Brusch et al., pers. comm.) suggest that warmer
397 conditions over night increase food consumption rates in adult female common lizards. This
398 suggests that yearling common lizards were probably eating more in the warm nocturnal
399 treatment group because food was not a limiting factor. All else being equal, the potential food
400 limitation occurring in a natural population should therefore exacerbate the ‘trade-off’ between

401 structural and reserve growth effect mediated by T_N observed in our experiment. In other words,
402 the trade-off should be stronger when food availability becomes a limiting factor and lizards
403 cannot increase their food intake as they could in the laboratory conditions. Ultimately, this
404 mechanism could alter food webs, as already observed in different studies (Speights & Barton,
405 2019). The potential for warmer temperatures to alter patterns of energy allocation challenges
406 assumptions about the optimal energy budget for common lizards prior to sexual maturation, as
407 modelled in González-Suárez, Le Galliard and Claessen (2011). Their model compared a net
408 allocation scenario and a net production one. In the net allocation model, yearlings first assigned
409 a fixed proportion of energy to reproduction, the remainder being paid to maintenance and
410 growth, respectively. Alternatively, in the net production model, maintenance is paid first, and
411 the remainder is assigned to growth and reproduction, proportionally. In contrast, our results
412 suggest fluctuating allocation rules towards growth, reserves and future reproduction that vary
413 with environmental temperatures, hence a potential a change in the assimilation and allocation
414 rules as well. Further work, also including females, would help to disentangle the effect of T_N
415 on male versus female' reproduction allocation strategies.

416 Assessing which pace of life strategy (faster growth with a lower condition vs slower
417 growth with a higher condition) yields a higher life-time fitness advantage is difficult, as
418 differential growth early in life may have important long-term effects (Metcalf, Monaghan and
419 Metcalfe, 2001). There are multiple fitness advantages for early growth and warmer
420 temperatures on current and future reproduction (Horváthová et al., 2013; Sears, 2005). For
421 example, experimental acceleration of growth in juvenile common lizards has been
422 demonstrated to favour rapid sexual maturation and earlier reproduction (Bestion et al., 2015).
423 Furthermore, reproduction is known to be size-dependent in reptiles, with larger females often
424 producing larger clutches or litters (Le Galliard, Clobert, & Ferrière, 2004). However, rapid
425 growth early in life may compromise the accumulation of energy reserves (this study), and lead

426 to lower survival of individuals with reduced energy reserves, as well as the ability invest into
427 reproduction later in their life (Bestion et al., 2015; Dupoué, Rutschmann, Le Galliard, Clobert,
428 et al., 2017).

429 *Impacts of a faster growth on oxidative balance and parasitic susceptibility*

430 One option to assess whether effects of accelerated growth are deleterious is to measure
431 individual stress levels, by quantifying their oxidative balance (Costantini, 2014; Salin et al.,
432 2015). Contrary to our prediction, we did not find any changes in oxidative status attributable
433 to the thermal treatment. In fact, oxidative damages showed a slight decrease in both treatments
434 ($\Delta_{\text{ROM}} = -0.2 \pm 2.2$) while the antioxidant capacity of the plasma increased in both treatments
435 ($\Delta_{\text{OXY}} = 10.3 \pm 40.7$). One potential explanation is that despite the difference in T_N , general
436 laboratory conditions were benign with respect to the risks of oxidative stress. Common lizard
437 populations characterized by higher T_N exhibit lower levels of oxidative stress in the field,
438 which suggests that warmer environmental conditions may not necessarily challenge oxidative
439 balance regulation in this species (Dupoué, Blaimont, et al., 2020). Similar reduction of
440 oxidative damage has been observed in the corn snake (*Pantherophis guttatus*) after a simulated
441 heat wave (Stahlschmidt, French, Ahn, Webb, & Butler, 2017). Yet, faster growth rate is
442 correlated with increased oxidative stress across many species (Metcalf & Monaghan, 2001;
443 Monaghan et al., 2009; Salin et al., 2015). Here, we found no correlation between individual
444 growth rate and Δ_{ROM} ($r = 0.003$; $t_{71} = 0.03$, $p = 0.97$) or Δ_{OXY} ($r = 0.07$; $t_{71} = 0.62$, $p = 0.53$). A full
445 assessment of whether or not warmer nights induce an increase in cellular stress might require
446 further analyses including multiple markers of oxidative stress (Stier et al., 2017). Moreover,
447 frequent sampling for the signature of oxidative stress and over a longer duration would enhance
448 the ability to characterize the dynamics of oxidative damage over time (Dupoué, Angelier, et
449 al., 2020).

450 Our results also showed that increased T_N can have immediate effects on susceptibility to
451 parasite infestation. We did not detect any statistical difference between treatments regarding
452 blood parasites. However, we found clear evidence that individuals from T_{Nw} were more
453 vulnerable to an ectoparasitic infestation. The provenance of mites re-infesting individuals is
454 questionable. Even if we hand removed and applied an antiparasitic treatment, it is possible that
455 some mites survived and re-infected individuals during captivity. It is also possible that mites
456 survived the sterilisation of the soil used as substrate in each terrarium. In our study, we did not
457 detect an influence of mites on SVL or body mass (Appendix 7), but we removed parasites from
458 individuals before deleterious effects could become obvious. In wild populations,
459 hematophagous mites are known to influence common lizards' mass and/or survival (Sorci &
460 Clobert, 1995) and could represent a serious burden for infected yearlings. It is difficult,
461 however, to determine whether the increased parasitic infestation in the warmer nocturnal
462 treatment is the result of a weakened immune response, a consequence of an extended period
463 of activity of lizards, or the by-product of a favourable environment for ectoparasite
464 reproduction.

465 *Behavioural and colour adjustment to warmer nocturnal temperatures*

466 Behavioural adjustments during the daytime may alleviate physiological costs of higher T_N . For
467 example, lizards acclimated to warmer T_N may adopt diurnal thermoregulation strategies to
468 reduce body temperatures and minimize levels of activity. Yet, contrary to our expectations,
469 diurnal T_{pref} was not altered by the treatment. Instead, average T_{pref} was higher at the end of the
470 experiment in both treatments ($\Delta_{bT} = 2.09 \text{ }^\circ\text{C} \pm 2.45$), suggesting acclimation of thermal
471 preference by lizards to general laboratory conditions (Pintor, Schwarzkopf and Krockenberger,
472 2016) or to seasonality (post-hibernation vs mid-summer conditions) (Díaz, Iraeta and
473 Monasterio, 2006). It is also possible that the differences in elevation between capture sites and
474 laboratory facilities (585m a.s.l.) contributed to this pattern (mean elevation difference = $814 \pm$

475 146 m) (Megía Palma et al., 2020). The absence of differences between treatments is consistent
476 with previous comparative studies that demonstrated a relative insensitivity of T_{pref} to variation
477 in thermal conditions in the common lizard (Gvoždík, 2002). Yet, we cannot disregard that
478 other behavioural strategies may be used by lizards during the activity period to buffer effects
479 of warmer T_N . For example, lizards may change their basking effort, shift the timing of their
480 daily activity (Rozen-Rechels et al., 2020) or alter the exploitation of different micro-habitats
481 (Gvoždík, 2002) to compensate for physiological effects of changes in T_N .

482 Behaviour is the main strategy that ectotherms use for short-term regulation of body
483 temperature, and the common lizard is often described as an active thermoregulator (Rozen-
484 Rechels et al., 2020). Yet, other mechanisms may also mitigate the effects of extreme thermal
485 environments such as the production of heat shock proteins, changes in critical thermal limits
486 and basal metabolic rate or the modulation of body shape and coloration (Gilbert & Miles, 2019;
487 Rutschmann et al., 2020; Seebacher & Franklin, 2005). In our study system, lizards experienced
488 changes in dorsal colour depending on the treatment: yearlings shifted toward lower luminance
489 and darker colours in T_{Nc} in all populations with the exception of Bouvier. In contrast, lizards
490 in T_{Nw} exhibited a higher luminance and lighter colours on their dorsum. According to the
491 thermal melanism hypothesis, lighter-coloured individuals should be favoured in environments
492 where the risk of overheating risks is high since they will not absorb solar radiation and heat as
493 fast as darker individuals (Clusella Trullas et al., 2007; Geen & Johnston, 2014). Heat gain due
494 to radiation is close to null during over-night, but warmer T_N could increase sensitivity to
495 diurnal heat stress (Speights et al., 2017) and be a criterion of developmental plasticity affecting
496 skin darkness. That is, lizards from the warmer treatment may adjust colouration toward a
497 lighter dorsal colouration in order to diminish skin solar absorbance and mitigate the
498 consequences of heat stress in a warmer environment. In any case, our results illustrate that T_N
499 may represent a relevant driver triggering a plastic adjustment of body colouration.

500 **Conclusions**

501 Recent evidence suggests that warmer nocturnal temperatures can advance the timing of
502 reproduction and increase the quality of the offspring as demonstrated in the long-lived,
503 viviparous Otago gecko *Woodworthia sp.* (Moore, Penniket, & Cree, 2020). Other studies have
504 shown an augmentation of T_N of 6°C increased the probability of a second clutch in the common
505 side-blotched lizard *Uta stansburiana* (Clarke & Zani, 2012). These studies corroborate our
506 results and support the current paradigm: ectotherms can rapidly acclimate to warmer T_N and
507 use them to improve energy intake and expand their thermal niche over part of the night or in
508 different seasons (Lara Resendiz, 2019; Ziska, 2014). Yet, our work, as well as previous
509 findings (Speights et al., 2017; Zhao et al., 2014) also suggests that one cannot ignore the
510 potential costs of nocturnal warming, especially when warming is intense (our results) and when
511 diurnal temperatures are already high (Monasterio, Shoo, Salvador, Iraeta, & Díaz, 2013; Zhao
512 et al., 2014). Long-term experimental studies, monitoring changes in an individuals' life history
513 traits are necessary to resolve under which conditions warmer nights are detrimental to
514 ectotherms. The extent to which T_N will affect common lizard populations and other ectotherms
515 species therefore remains an open question.

516 Our results also show minor, but interesting differences in the responses of lizards
517 among different populations. Indeed, lizards from Bouvier were the only individuals to change
518 toward darker colors and exhibit a lower rate of blood parasite infections in the warmer
519 treatment. Both were unexpected results. Despite an initial choice of populations based on their
520 similar climate conditions, a closer examination of the weather data revealed that extreme
521 overnight temperatures during the summer are significantly higher in Bouvier and Barnassac
522 than in Col du Pendu and Vialas. Whether the high temperatures at night in Bouvier, or other
523 environmental differences not quantified such as the risk of predation or grazing, have selected
524 for lizards with contrasted responses to extreme T_N is difficult to assess without complementary

525 analyses. We encourage future studies to incorporate the effects of nocturnal temperatures in
526 experimental designs (Speights, Wolff, Barton, & Barton, 2018), but we also stress the
527 importance of using multi-population comparison approaches to obtain a comprehensive
528 perspective on the responses of species to climate change (Barton, 2017).

529

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537 **Authors contribution** - AR, AD, SM and JFLG designed the study. AR, CL, MB, DBM and
538 JC conducted field work. AR and CL performed the experiments. DRR and PB designed the
539 thermal preference experiment. MR and RMP, respectively analysed plasma samples for
540 oxidative status and blood parasites prevalence. AR analysed all data with the help of DRR
541 (thermal data) and AB (coloration data) and drafted the manuscript with AD. All authors
542 interpreted the results, provided editorial advices and gave final approval for publication.

543 **Data availability statement** – Data will be archived online if the manuscript is accepted for
544 publication.

545

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792

793 FIGURES CAPTIONS

794 **Fig.1. Hypothetical impact of an increase in diurnal and nocturnal temperature on**
795 **individual performance.** For ectotherms, the relationship between performance and
796 temperature follows a right-skewed bell-shaped curve (Huey & Slatkin, 1976). **(a)** Increasing
797 diurnal temperatures are considered a potential threat for ectotherms as they may push
798 individuals from temperatures near T_{opt} (yellow box) towards CT_{max} (red box), therefore
799 reducing performance (red arrow). **(b)** Nocturnal temperatures are generally low (blue
800 rectangle) and associated with a resting metabolic expenditure. An increase in nocturnal
801 temperatures (yellow rectangle) may increase individual performance (red arrow) by moving
802 body temperatures towards activity levels, reducing optimal resting time and raising energetic
803 costs of rest.

804 **Fig.2. Environmental conditions in source populations and daily cycle of temperature in**
805 **laboratory conditions.** Average (a.) and extreme (b.) nocturnal temperatures recorded
806 overnight in the four sampled populations. Letters indicate significant differences among
807 populations, according to a post-hoc Tuckey test. Extreme high temperatures are defined as the
808 97.5% upper quantile of the distribution of nocturnal temperatures. Panel (c.) represents the
809 differences between the warm (yellow) and the control (blue) nocturnal temperature treatments
810 over a 24 hours day-night cycle. Over the day, lizards from the two thermal treatments were
811 maintained in similar conditions and were allowed to bask under a 25W bulb for six hours a
812 day, from 9.00 to 12.00 and from 14.00 to 17.00. The absence of heat during the mid-day time
813 (12.00 to 14.00) explains the pattern of diurnal temperatures.

814 **Fig.3. Morphology** - *a.* Yearling growth rate in body size (in mm), *b.* body mass difference (in
815 g), *c.* body condition (in g) and *d.* difference in tail volume (in mm^3) according to temperature
816 treatment (T_{Nc} : pale-blue; T_{Nw} : pale-yellow). To ease the graphical representation, Δ_{SVL} , Δ_{Mass}
817 and Δ_{Tail} are all corrected for their initial values. As a residual, Δ_{BC} is not corrected.

818 **Fig.4. Colouration** - (a) The four most dominant colours in individual dorsal patterns and their
819 associated HEX code. (b) Dorsal luminance (in cd/m²) depending on the treatment. (c) Change
820 of the proportion of colours in the dorsal pattern depending on the treatment. To ease the
821 graphical representation, Δ_{COL} and Δ_{LUM} are both corrected for initial values of **Col** and **Lum**.

822 **Fig.5. - Parasite load.** (a) Yearlings infestation rate by mites depending on the treatment. (b)
823 Proportion of yearlings infested by blood parasites at the end of the treatment.

824

825 **Table 1: Habitat characteristics.** Elevation (in m), latitude and longitude of each population
826 sites and average nocturnal (T_N) and diurnal (T_D) temperatures (in °C). Extreme temperatures
827 ($T_{N(EX)}$ and $T_{D(EX)}$) correspond to the 97.5% upper quantile of the temperature distribution
828 recorded in each location.

829

Figure 1

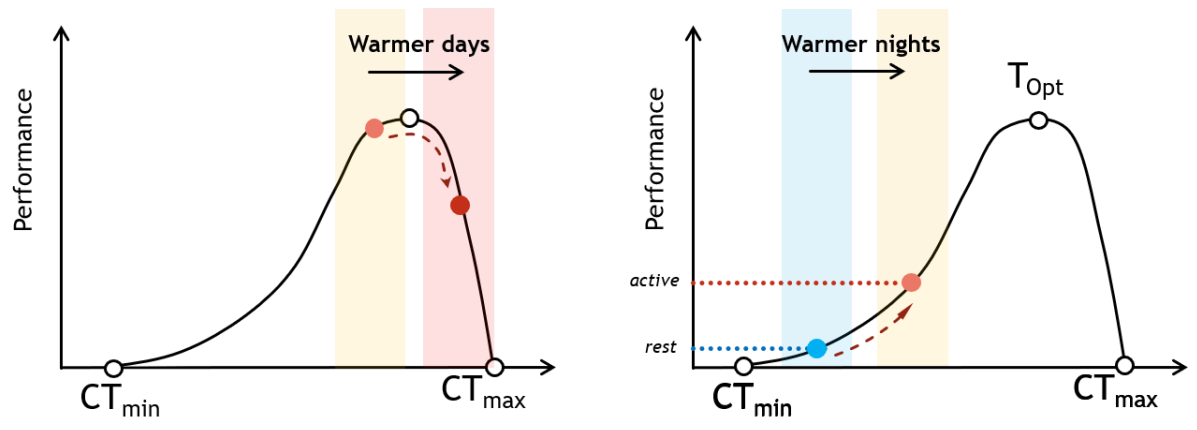


Figure 2

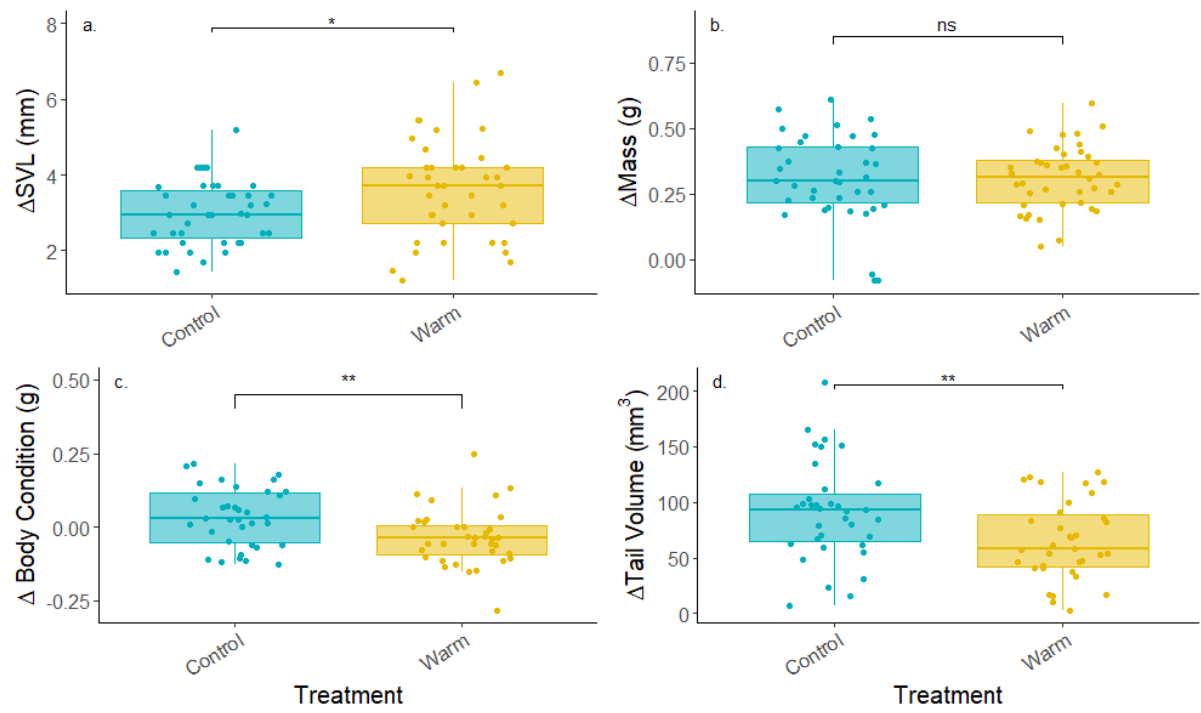


Figure 3

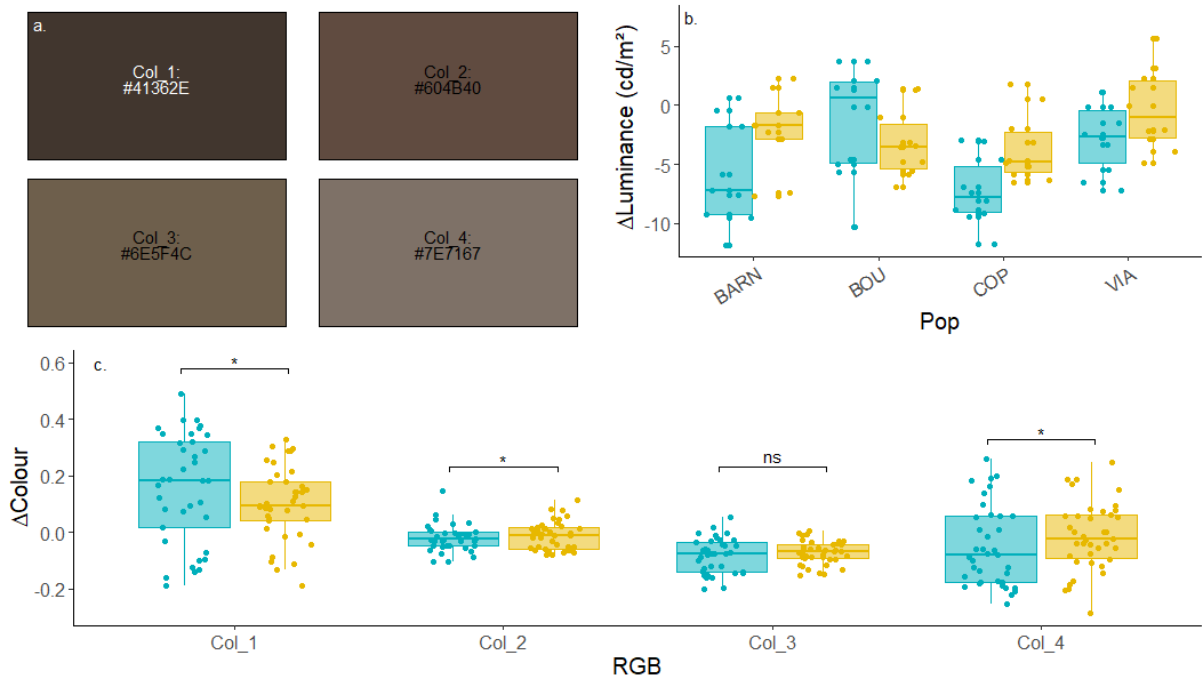


Figure 4

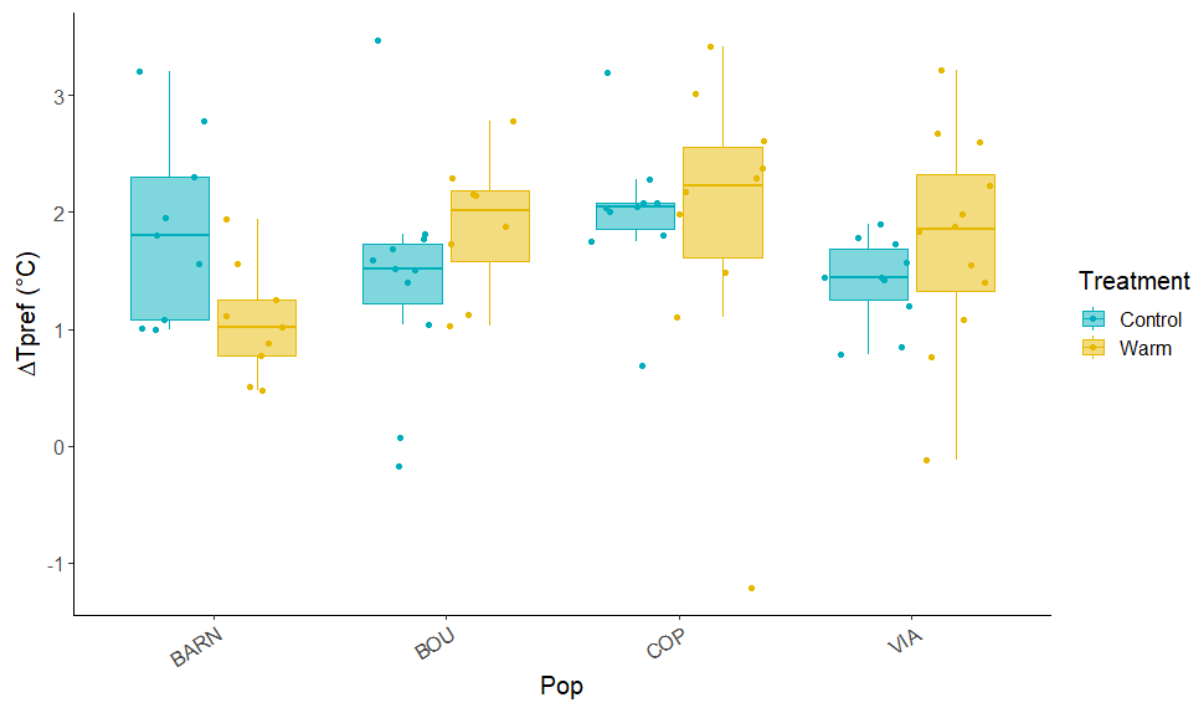


Figure 5

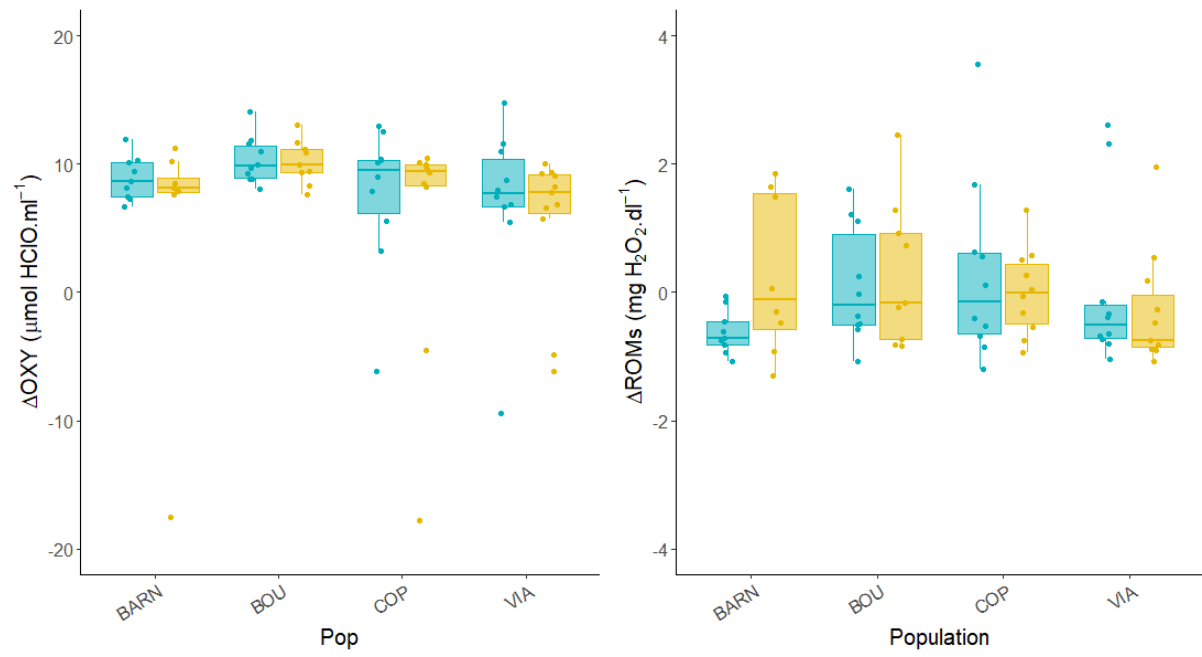


Figure 6

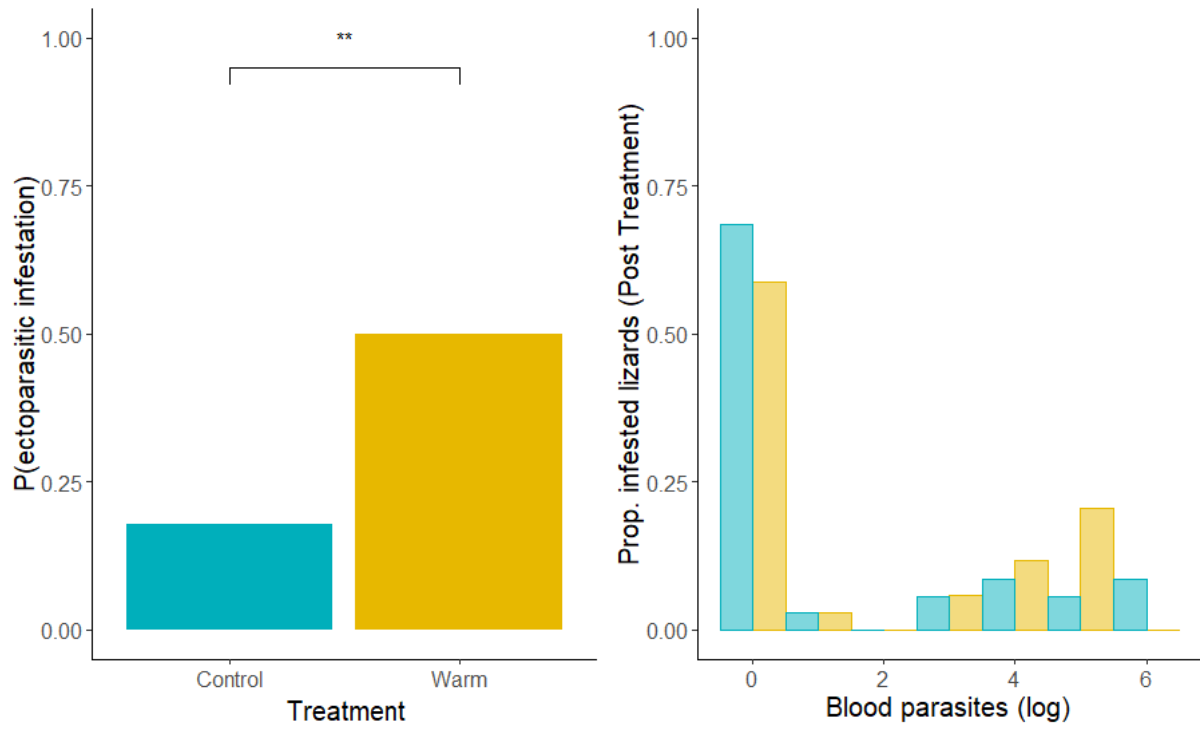


Table 1

Population	Elevation	T_N	$T_{N(Ex)}$	T_D	$T_{D(Ex)}$	Lat.	Long.
Barnassac	1527	13.6 ± 4.2	23.1	18.6 ± 7.3	33.6	44°25'40.4"N	3°45'50.1"E
Vialas	1190	13.1 ± 3.7	23.0	16.9 ± 6.1	30.5	44°20'17.4"N	3°46'4.7"E
Col du Pendu	1420	12.6 ± 3.1	19.5	17.1 ± 6.1	31.5	44°39'18.3"N	4°01'49.7"E
Bouvier	1461	12.4 ± 3.7	21.0	18.8 ± 8.7	37.5	44°45'25.6"N	3°31'05.7"E