Intense nocturnal warming alters growth strategies, colouration and parasite load in a diurnal lizard

Rutschmann Alexis [1, 2, *], Dupoue Andreaz [3], Miles Donald B. [1, 4], Megia-Palma Rodrigo [5, 6], Lauden Clemence [1], Richard Murielle [1], Badiane Arnaud [3], Rozen-Rechels David [3, 7], Brevet Mathieu [1], Blaimont Pauline [8], Meylan Sandrine [3], Clobert Jean [1], Le Galliard Jean-Francois [3, 9]

[1] CNRS, USR5321, Stn Ecol Theor & Expt SETE, Moulis, France.
[2] Univ Auckland, Sch Biol Sci, Auckland, New Zealand.
[3] Sorbonne Univ, Inst Ecol & Sci Environm Paris iEES Paris, CNRS, INRA,IRD, Paris, France.
[4] Ohio Univ, Dept Biol Sci, Athens, OH 45701 USA.
[5] Univ Porto, CIBIO, InBIO Res Network Biodivers & Evolutionary Biol, Vairao, Portugal.
[6] Univ Alcala De Henares, Sch Pharm, Dept Biomed & Biotechnol, Alcala De Henares, Spain.
[7] La Rochelle Univ, CNRS, Ctr Etud Biol Chize, Villiers En Bois, France.
[8] Rider Univ, Dept Biol, Lawrenceville, NJ 08648 USA.
[9] PSL Univ, CNRS, Ctr Rech Ecol Expt & Predict CEREEP Ecotron Ile F, Ecole Normale Super,Dept Biol, St Pierre Les Nemours, France.

* Corresponding author : Alexis Rutschmann, email address : alexis.rutschmann@gmail.com

Abstract **:**

1. In the past decades, nocturnal temperatures have been playing a disproportionate role in the global warming of the planet. Yet, they remain a neglected factor in studies assessing the impact of global warming on natural populations.

2. Here, we question whether an intense augmentation of nocturnal temperatures is beneficial or deleterious to ectotherms. Physiological performance is influenced by thermal conditions in ectotherms and an increase in temperature by only 2 degrees C is sufficient to induce a disproportionate increase in metabolic expenditure. Warmer nights may expand ectotherms' species thermal niche and open new opportunities for prolonged activities and improve foraging efficiency. However, increased activity may also have deleterious effects on energy balance if exposure to warmer nights reduces resting periods and elevates resting metabolic rate.

3. We assessed whether warmer nights affected an individual's growth, dorsal skin colouration, thermoregulation behaviour, oxidative stress status and parasite load by exposing yearling common lizards (Zootoca vivipara) from four populations to either ambient or high nocturnal temperatures for approximately 5 weeks.

4. Warmer nocturnal temperatures increased the prevalence of ectoparasitic infestation and altered allocation of resources towards structural growth rather than storage. We found no change in markers for oxidative stress. The thermal treatment did not influence thermal preferences, but influenced dorsal skin

brightness and luminance, in line with a predicted acclimation response in colder environments to enhance heat gain from solar radiation.

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

Keywords : colouration change, ectotherms, energetic balance, nocturnal temperatures, oxidative stress

INTRODUCTION

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

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

Physiological processes and behavioural activity of ectothermic species are directly 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 ± 0.7°C and T_{Nw} = 24.6 ±

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 $\mathbf{\Delta_i}$ was

191   calculated as the difference between post- and pre-treatment measurements (e.g., $\mathbf{\Delta_{SVL}}$ = 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 ± 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 mm^3) 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 μL 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 Spray^{TM}). 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 ($\mathbf{\Delta_{LUM}}$) 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 ($\mathbf{\Delta_{COL\_1}}$ to

285      $\mathbf{\Delta_{COL\_4}}$). 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 $\mathbf{\Delta_{Tpref}}$ 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 ($\mathbf{\Delta_{ROM}}$)

295   and in OXY ($\mathbf{\Delta_{OXY}}$) 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 ±

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; Brusch, Lourdais, 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 (Metcalfe, 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   (Δ_{ROM} = -0.2 ± 2.2) while the antioxidant capacity of the plasma increased in both treatments

435   (Δ_{OXY} = 10.3 ± 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 (Metcalfe & 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 ($\mathbf{\Delta_{bT}}$ = 2.09 °C ± 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 ±

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.

Conclusions

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

Our results also show minor, but interesting differences in the responses of lizards among different populations. Indeed, lizards from Bouvier were the only individuals to change toward darker colors and exhibit a lower rate of blood parasite infections in the warmer treatment. Both were unexpected results. Despite an initial choice of populations based on their similar climate conditions, a closer examination of the weather data revealed that extreme overnight temperatures during the summer are significantly higher in Bouvier and Barnassac than in Col du Pendu and Vialas. Whether the high temperatures at night in Bouvier, or other environmental differences not quantified such as the risk of predation or grazing, have selected 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

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

REFERENCES

Allen, M. R., Dube, O. P., Solecki, W., Aragón-Durand, F., Cramer, W., Humphreys, S., …
Zickfeld, K. (2018). Framing and Context. In *Global Warming of 1.5°C. An IPCC
Special Report on the impacts of global warming of 1.5°C above pre-industrial levels
and related global greenhouse gas emission pathways, in the context of strengthening
the global response to the threat of climate change,* (p. 49).

Alonso-Alvarez, C., Bertrand, S., Devevey, G., Prost, J., Faivre, B., Chastel, O., & Sorci, G.
(2006). an Experimental Manipulation of Life-History Trajectories and Resistance To
Oxidative Stress. *Evolution*, *60*(9), 1913. doi: 10.1554/05-644.1

Alward, R. D., Detling, J. K., & Milchunas, D. G. (1999). Grassland vegetation changes and
nocturnal global warming. *Science*, *283*(5399), 229–231. doi:
10.1126/science.283.5399.229

Andrade, T. O., Krespi, L., Bonnardot, V., van Baaren, J., & Outreman, Y. (2016). Impact of
change in winter strategy of one parasitoid species on the diversity and function of a
guild of parasitoids. *Oecologia*, *180*(3), 877–888. doi: 10.1007/s00442-015-3502-4

Angilletta, M. J., Cooper, B. S., Schuler, M. S., & Boyles, J. G. (2002). The Evolution of
Thermal Physiology in Endotherms. *Journal of Thermal Biology*, *2*, 249–268. doi:
10.2741/E148

Avery, R. A. (1974). Storage lipids in the lizard Lacerta vivipara: a quantitative study.
Journal of Zoology, Vol. 173, pp. 419–425. doi: 10.1111/j.1469-7998.1974.tb04124.x

Bai, C. M., Ma, G., Cai, W. Z., & Ma, C. Sen. (2019). Independent and combined effects of
daytime heat stress and night-time recovery determine thermal performance. *Biology
Open*, *8*(3). doi: 10.1242/bio.038141

Barton, B. T. (2017). Beyond global warming: Putting the "climate" back into "climate
change ecology." *Food Webs*, *13*, 51–52. doi: 10.1016/j.fooweb.2017.03.002

571   Barton, B. T., & Schmitz, O. J. (2018). Opposite effects of daytime and nighttime warming on

572   top-down control of plant diversity. *Ecology*, *99*(1), 13–20. doi: 10.1002/ecy.2062

573   Bateman, P. W., & Fleming, P. A. (2009). To cut a long tail short: A review of lizard caudal

574   autotomy studies carried out over the last 20 years. *Journal of Zoology*, *277*(1), 1–14.

575   doi: 10.1111/j.1469-7998.2008.00484.x

576   Bestion, E., Teyssier, A., Richard, M., Clobert, J., & Cote, J. (2015). Live Fast, Die Young:

577   Experimental Evidence of Population Extinction Risk due to Climate Change. *PLoS*

578   *Biology*, *13*(10), 1–19. doi: 10.1371/journal.pbio.1002281

579   Brusch, G. A., Lourdais, O., Kaminsky, B., & DeNardo, D. F. (2018). Muscles provide an

580   internal water reserve for reproduction. *Proceedings of the Royal Society B: Biological*

581   *Sciences*, *285*(1881). doi: 10.1098/rspb.2018.0752

582   Burraco, P., Díaz-Paniagua, C., & Gomez-Mestre, I. (2017). Different effects of accelerated

583   development and enhanced growth on oxidative stress and telomere shortening in

584   amphibian larvae. *Scientific Reports*, *7*(1), 1–11. doi: 10.1038/s41598-017-07201-z

585   Burraco, P., Orizaola, G., Monaghan, P., & Metcalfe, N. B. (2020). Climate change and

586   ageing in ectotherms. *Global Change Biology*, *26*(10), 5371–5381. doi:

587   10.1111/gcb.15305

588   Chamaille-Jammes, S., Massot, M., Aragon, P., & Clobert, J. (2006). Global warming and

589   positive fitness response in mountain populations of common lizards Lacerta vivipara.

590   *Global Change Biology*, *12*(2), 392–402. doi: 10.1111/j.1365-2486.2005.01088.x

591   Clarke, D. N., & Zani, P. A. (2012). Effects of night-time warming on temperate ectotherm

592   reproduction: Potential fitness benefits of climate change for side-blotched lizards.

593   *Journal of Experimental Biology*, *215*(7), 1117–1127. doi: 10.1242/jeb065359

594   Clusella Trullas, S., van Wyk, J. H., & Spotila, J. R. (2007). Thermal melanism in ectotherms.

595   *Journal of Thermal Biology*, *32*(5), 235–245. doi: 10.1016/j.jtherbio.2007.01.013

596   Colinet, H., Sinclair, B. J., Vernon, P., & Renault, D. (2015). Insects in Fluctuating Thermal

597   Environments. *Annual Review of Entomology*, *60*(1), 123–140. doi: 10.1146/annurev-

598   ento-010814-021017

599   Costantini, D. (2014). Oxidative Stress and Hormesis in Evolutionary Ecology and

600   Physiology. In *Oxidative Stress and Hormesis in Evolutionary Ecology and Physiology*.

601   doi: 10.1007/978-3-642-54663-1

602   Costantini, D. (2016). Oxidative stress ecology and the d-ROMs test: facts, misfacts and an

603   appraisal of a decade's work. *Behavioral Ecology and Sociobiology*, *70*(5), 809–820.

604   doi: 10.1007/s00265-016-2091-5

605   Costantini, D. (2019). Understanding diversity in oxidative status and oxidative stress: The

606   opportunities and challenges ahead. *Journal of Experimental Biology*, *222*(13). doi:

607   10.1242/jeb.194688

608   Costantini, D., Marasco, V., & Møller, A. P. (2011). A meta-analysis of glucocorticoids as

609   modulators of oxidative stress in vertebrates. *Journal of Comparative Physiology B:*

610   *Biochemical, Systemic, and Environmental Physiology*, *181*(4), 447–456. doi:

611   10.1007/s00360-011-0566-2

612   Davy, R., Esau, I., Chernokulsky, A., Outten, S., & Zilitinkevich, S. (2017). Diurnal

613   asymmetry to the observed global warming. *International Journal of Climatology*, *37*(1),

614   79–93. doi: 10.1002/joc.4688

615   Díaz, J. A., Iraeta, P., & Monasterio, C. (2006). Seasonality provokes a shift of thermal

616   preferences in a temperate lizard, but altitude does not. *Journal of Thermal Biology*,

617   *31*(3), 237–242. doi: 10.1016/j.jtherbio.2005.10.001

618   Dillon, M. E., Wang, G., & Huey, R. B. (2010). Global metabolic impacts of recent climate

619   warming. *Nature*, *467*(7316), 704–706. doi: 10.1038/nature09407

620   Dimitris Rizopoulos. (2020). *Package 'GLMMadaptive.'*

621   Dupoué, A., Angelier, F., Brischoux, F., Denardo, D. F., Trouvé, C., Parenteau, C., &

622   Lourdais, O. (2016). Water deprivation increases maternal corticosterone levels and

623   enhances offspring growth in the snake Vipera aspis. *Journal of Experimental Biology*,

624   *219*(5), 658–667. doi: 10.1242/jeb.132639

625   Dupoué, A., Angelier, F., Ribout, C., Meylan, S., Rozen-Rechels, D., Decencière, B., … Le

626   Galliard, J. F. (2020). Chronic water restriction triggers sex-specific oxidative stress and

627   telomere shortening in lizards. *Biology Letters*, *16*(2). doi: 10.1098/rsbl.2019.0889

628   Dupoué, A., Blaimont, P., Rozen-Rechels, D., Richard, M., Meylan, S., Clobert, J., … Le

629   Galliard, J. F. (2020). Water availability and temperature induce changes in oxidative

630   status during pregnancy in a viviparous lizard. *Functional Ecology*, *34*(2), 475–485. doi:

631   10.1111/1365-2435.13481

632   Dupoué, A., Rutschmann, A., Le Galliard, J. F., Clobert, J., Angelier, F., Marciau, C., …

633   Meylan, S. (2017). Shorter telomeres precede population extinction in wild lizards.

634   *Scientific Reports*, *7*(1), 1–8. doi: 10.1038/s41598-017-17323-z

635   Dupoué, A., Rutschmann, A., Le Galliard, J. F., Miles, D. B., Clobert, J., DeNardo, D. F., …

636   Meylan, S. (2017). Water availability and environmental temperature correlate with

637   geographic variation in water balance in common lizards. *Oecologia*, *185*(4), 561–571.

638   doi: 10.1007/s00442-017-3973-6

639   Gaston, K. J. (2019). Nighttime ecology: The "nocturnal problem" revisited. *American

640   Naturalist*, *193*(4), 481–502. doi: 10.1086/702250

641   Geen, M. R. S., & Johnston, G. R. (2014). Coloration affects heating and cooling in three

642   color morphs of the Australian bluetongue lizard, Tiliqua scincoides. *Journal of Thermal

643   Biology*, *43*(1), 54–60. doi: 10.1016/j.jtherbio.2014.04.004

644   Gilbert, A. L., & Miles, D. B. (2019). Antagonistic responses of exposure to sublethal

645   temperatures: Adaptive phenotypic plasticity coincides with a reduction in organismal

646   performance. *American Naturalist*, *194*(3), 344–355. doi: 10.1086/704208

647   González-Suárez, M., Le Galliard, J.-F., & Claessen, D. (2011). Population and life-history

648   consequences of within-cohort individual variation. *American Naturalist*, *178*(4), 525–

649   537. doi: 10.1086/661906

650   Gvoždík, L. (2002). To heat or to save time? Thermoregulation in the lizard *Zootoca vivipara*

651   (Squamata: Lacertidae) in different thermal environments along an altitudinal gradient.

652   *Canadian Journal of Zoology*, *80*(3), 479–492. doi: 10.1139/z02-015

653   Harvell, C. D., Mitchell, C. E., Ward, J. R., Altizer, S., Dobson, A. P., Ostfeld, R. S., &

654   Samuel, M. D. (2002). Climate Warming and Disease Risks for Terrstrial and Marine

655   Biota. *Science*, *296*, 2158–2162.

656   Hertz, P. E., Huey, R. B., & Stevenson, R. D. (1993). Evaluating Temperature Regulation by

657   Field-Active Ectotherms: The Fallacy of the Inappropriate Question. *The American*

658   *Naturalist*, *142*(5), 796–818. doi: 10.1086/285573

659   Horváthová, T., Cooney, C. R., Fitze, P. S., Oksanen, T. A., Jelić, D., Ghira, I., … Jandzik, D.

660   (2013). Length of activity season drives geographic variation in body size of a widely

661   distributed lizard. *Ecology and Evolution*, *3*(8), 2424–2442. doi: 10.1002/ece3.613

662   Huey, R. B., & Kingsolver, J. G. (2019). Climate warming, resource availability, and the

663   metabolic meltdown of ectotherms. *American Naturalist*, *194*(6), E140–E150. doi:

664   10.1086/705679

665   Huey, R. B., & Slatkin, M. (1976). Cost and Benefits of Lizard Thermoregulation. *The*

666   *Quarterly Review of Biology*, *51*(3), 363–384.

667   Josserand, R., Haussy, C., Agostini, S., Decencière, B., Le Galliard, J. F., & Meylan, S.

668   (2020). Chronic elevation of glucorticoids late in life generates long lasting changes in

669   physiological state without a life history switch. *General and Comparative*

670   *Endocrinology*, *285*(August 2019), 113288. doi: 10.1016/j.ygcen.2019.113288

671   Kayser, M. S., Yue, Z., & Sehgal, A. (2014). A critical period of sleep for development of

672   courtship circuitry and behavior in Drosophila. *Science*, *344*(6181), 269–274. doi:

673   10.1126/science.1250553

674   Kingsolver, J. G., Higgins, J. K., & Augustine, K. E. (2015). Fluctuating temperatures and

675   ectotherm growth: Distinguishing non-linear and time-dependent effects. *Journal of*

676   *Experimental Biology*, *218*(14), 2218–2225. doi: 10.1242/jeb.120733

677   Lara Resendiz, A. R. (2019). ¿ QUÉ IMPLICACIONES ECOFISIOLÓGICAS TIENE LA

678   ACTIVIDAD NOCTURNA EN REPTILES " DIURNOS "? UNA REVISIÓN What are

679   the ecophysiological implications of nocturnal activity in " diurnal " reptiles ? A review.

680   *ACTA BIOLÓGICA COLOMBIANA*, *25*(2), 314–326.

681   Le Galliard, J.-F., Clobert, J., & Ferrière, R. (2004). Physical performance and darwinian

682   fitness in lizards. *Nature*, *432*(November), 1–4.

683   Ma, G., Hoffmann, A. A., & Ma, C. Sen. (2015). Daily temperature extremes play an

684   important role in predicting thermal effects. *Journal of Experimental Biology*, *218*(14),

685   2289–2296. doi: 10.1242/jeb.122127

686   Ma, G., Le Lann, C., van Baaren, J., & Ma, C.-S. (2020). Night Warming Affecting

687   Interspecific Interactions: Implications for Biological Control. In *Integrative Biological*

688   *Control* (pp. 231–248).

689   Megía-Palma, R., Martínez, J., Cuervo, J. J., Belliure, J., Jiménez-Robles, O., Gomes, V., …

690   Merino, S. (2018). Molecular evidence for host–parasite co-speciation between lizards

691   and Schellackia parasites. *International Journal for Parasitology*, *48*(9–10), 709–718.

692   doi: 10.1016/j.ijpara.2018.03.003

693   Megía Palma, R., Jiménez-Robles, O., Hernández-Agüero, J. A., & de la Riva, I. (2020).

694   Plasticity of haemoglobin concentration and thermoregulation in a mountain lizard.

695   *Journal of Thermal Biology*, *in Press*.

696   Metcalfe, N. B., & Monaghan, P. (2001). Compensation for a bad start: Grow now, pay later?

697   *Trends in Ecology and Evolution*, *16*(5), 254–260. doi: 10.1016/S0169-5347(01)02124-3

698   Miller, C. R., Barton, B. T., Zhu, L., Radeloff, V. C., Oliver, K. M., Harmon, J. P., & Ives, A.

699   R. (2017). Combined effects of night warming and light pollution on predator - Prey

700   interactions. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1864). doi:

701   10.1098/rspb.2017.1195

702   Monaghan, P., Metcalfe, N. B., & Torres, R. (2009). Oxidative stress as a mediator of life

703   history trade-offs: Mechanisms, measurements and interpretation. *Ecology Letters*, *12*(1),

704   75–92. doi: 10.1111/j.1461-0248.2008.01258.x

705   Monasterio, C., Shoo, L. P., Salvador, A., Iraeta, P., & Díaz, J. A. (2013). High temperature

706   constrains reproductive success in a temperate lizard: Implications for distribution range

707   limits and the impacts of climate change. *Journal of Zoology*, *291*(2), 136–145. doi:

708   10.1111/jzo.12057

709   Moore, G., Penniket, S., & Cree, A. (2020). Greater basking opportunity and warmer nights

710   during late pregnancy advance modal birth season in a live-bearing gecko, lowering the

711   risk of reduced embryonic condition. *Biological Journal of the Linnean Society*, *130*(1),

712   128–141. doi: 10.1093/biolinnean/blaa017

713   Mu, J., Peng, Y., Xi, X., Wu, X., Li, G., Niklas, K. J., & Sun, S. (2015). Artificial asymmetric

714   warming reduces nectar yield in a Tibetan alpine species of Asteraceae. *Annals of*

715   *Botany*, *116*(6), 899–906. doi: 10.1093/aob/mcv042

716   Nordberg, E. J., & Schwarzkopf, L. (2019). Heat seekers: A tropical nocturnal lizard uses

717   behavioral thermoregulation to exploit rare microclimates at night. *Journal of Thermal*

718   *Biology*, *82*(January), 107–114. doi: 10.1016/j.jtherbio.2019.03.018

719   Palareti, G., Legnani, C., Cosmi, B., Antonucci, E., Erba, N., Poli, D., … Tosetto, A. (2016).

720   Comparison between different D-Dimer cutoff values to assess the individual risk of

721   recurrent venous thromboembolism: Analysis of results obtained in the DULCIS study.

722   *International Journal of Laboratory Hematology*, *38*(1), 42–49. doi: 10.1111/ijlh.12426

723 Pintor, A. F. V., Schwarzkopf, L., & Krockenberger, A. K. (2016). Extensive acclimation in

724   ectotherms conceals interspecific variation in thermal tolerance limits. *PLoS ONE*, *11*(3),

725   1–15. doi: 10.1371/journal.pone.0150408

726 R core Team. (2020). *R: A language and environment for statistical computing. R Foundation*

727   *for Statistical Computing.* Vienna, Austria.

728 Richard, M., Massot, M., Clobert, J., & Meylan, S. (2012). Litter quality and inflammatory

729   response are dependent on mating strategy in a reptile. *Oecologia*, *170*(1), 39–46. doi:

730   10.1007/s00442-012-2282-3

731 Rozen-Rechels, D., Dupoué, A., Meylan, S., Qitout, K., Decencière, B., Agostini, S., &

732   Galliard, J.-F. Le. (2020). Acclimation to water restriction implies different paces for

733   behavioral and physiological responses in a lizard species. *Physiological and*

734   *Biochemical Zoology*, *93*(2).

735 Rutschmann, A., Rozen-rechels, D., Dupoué, A., Blaimont, P., Villemereuil, P. De, Miles, D.

736   B., … Clobert, J. (2020). *Climate dependent heating efficiency in the common lizard*.

737   (February), 1–11. doi: 10.1002/ece3.6241

738 Salin, K., Auer, S. K., Rudolf, A. M., Anderson, G. J., Cairns, A. G., Mullen, W., …

739   Metcalfe, N. B. (2015). Individuals with higher metabolic rates have lower levels of

740   reactive oxygen species in vivo. *Biology Letters*, *11*(9), 4–7. doi: 10.1098/rsbl.2015.0538

741 Sears, M. W. (2005). Geographic variation in the life history of the sagebrush lizard: The role

742   of thermal constraints on activity. *Oecologia*, *143*(1), 25–36. doi: 10.1007/s00442-004-

743   1767-0

744 Seebacher, F., & Franklin, C. E. (2005). Physiological mechanisms of thermoregulation in

745   reptiles: A review. *Journal of Comparative Physiology B: Biochemical, Systemic, and*

746 *Environmental Physiology*, *175*(8), 533–541. doi: 10.1007/s00360-005-0007-1

747 Sinclair, B. J., Marshall, K. E., Sewell, M. A., Levesque, D. L., Willett, C. S., Slotsbo, S., …

748 Huey, R. B. (2016). Can we predict ectotherm responses to climate change using thermal

749 performance curves and body temperatures? *Ecology Letters*, *19*(11), 1372–1385. doi:

750 10.1111/ele.12686

751 Sorci, G., & Clobert, J. (1995). Effects of maternal parasite load on offspring life-history traits

752 in the common lizard (Lacerta uivipara). *Journal of Evolutionary Biology*, *723*(8), 711–

753 723.

754 Speights, C. J., & Barton, B. T. (2019). Timing is everything: Effects of day and night

755 warming on predator functional traits. *Food Webs*, *21*, e00130. doi:

756 10.1016/j.fooweb.2019.e00130

757 Speights, C. J., Harmon, J. P., & Barton, B. T. (2017). Contrasting the potential effects of

758 daytime versus nighttime warming on insects. *Current Opinion in Insect Science*, *23*, 1–

759 6. doi: 10.1016/j.cois.2017.06.005

760 Speights, C. J., Wolff, C. L., Barton, M. E., & Barton, B. T. (2018). Why and how to create

761 nighttime warming treatments for ecological field experiments. *Yale Journal of Biology*

762 *and Medicine*, *91*(4), 471–480.

763 Stahlschmidt, Z. R., French, S. S., Ahn, A., Webb, A., & Butler, M. W. (2017). A simulated

764 heat wave has diverse effects on immune function and oxidative physiology in the corn

765 snake (Pantherophis guttatus). *Physiological and Biochemical Zoology*, *90*(4), 434–444.

766 doi: 10.1086/691315

767 Stier, A., Dupoue, A., Picard, D., Angelier, F., Brischoux, F., & Lourdais, O. (2017).

768 *Oxidative stress in a capital breeder (Vipera aspis) facing pregnancy and water*

769 *constraints*. 1792–1796. doi: 10.1242/jeb.156752

770 Sunday, J. M., Bates, A. E., Kearney, M. R., Colwell, R. K., Dulvy, N. K., Longino, J. T., &

771   Huey, R. B. (2014). Thermal-safety margins and the necessity of thermoregulatory

772   behavior across latitude and elevation. *Proceedings of the National Academy of Sciences*

773   *of the United States of America*, *111*(15), 5610–5615. doi: 10.1073/pnas.1316145111

774   Vose, R. S., Easterling, D. R., & Gleason, B. (2005). Maximum and minimum temperature

775   trends for the globe: An update through 2004. *Geophysical Research Letters*, *32*(23), 1–

776   5. doi: 10.1029/2005GL024379

777   Weller, H. I. (2019). *colordistance: Distance Metrics for Image Color Similarity. R package*

778   *version 1.1.0.*

779   Whitney-Johnson, A., Thompson, M., & Hon, E. (2005). Responses to Predicted Global

780   Warming in Pieris rapae L. (Lepidoptera): Consequences of Nocturnal Versus Diurnal

781   Temperature Change on Fitness Components . *Environmental Entomology*, *34*(3), 535–

782   540. doi: 10.1603/0046-225x-34.3.535

783   Wu, T., Hao, S., Sun, O. J., & Kang, L. (2012). Specificity responses of grasshoppers in

784   temperate grasslands to diel asymmetric warming. *PLoS ONE*, *7*(7), 1–8. doi:

785   10.1371/journal.pone.0041764

786   Zhao, F., Zhang, W., Hoffmann, A. A., & Ma, C. Sen. (2014). Night warming on hot days

787   produces novel impacts on development, survival and reproduction in a small arthropod.

788   *Journal of Animal Ecology*, *83*(4), 769–778. doi: 10.1111/1365-2656.12196

789   Ziska, L. H. (2014). Increasing minimum daily temperatures are associated with enhanced

790   pesticide use in cultivated soybean along a latitudinal gradient in the mid-western United

791   States. *PLoS ONE*, *9*(6). doi: 10.1371/journal.pone.0098516

792

FIGURES CAPTIONS

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

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

Fig.3. Morphology - *a.* Yearling growth rate in body size (in mm), *b.* body mass difference (in g), *c.* body condition (in g) and *d.* difference in tail volume (in mm^3) according to temperature treatment (T_{Nc}: pale-blue; T_{Nw}: pale-yellow). To ease the graphical representation, Δ_{SVL}, Δ_{Mass} 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
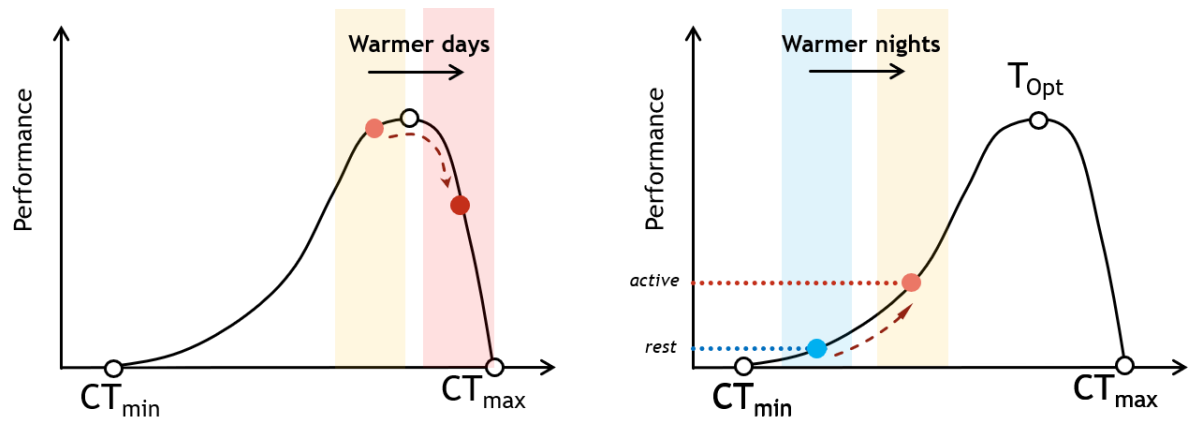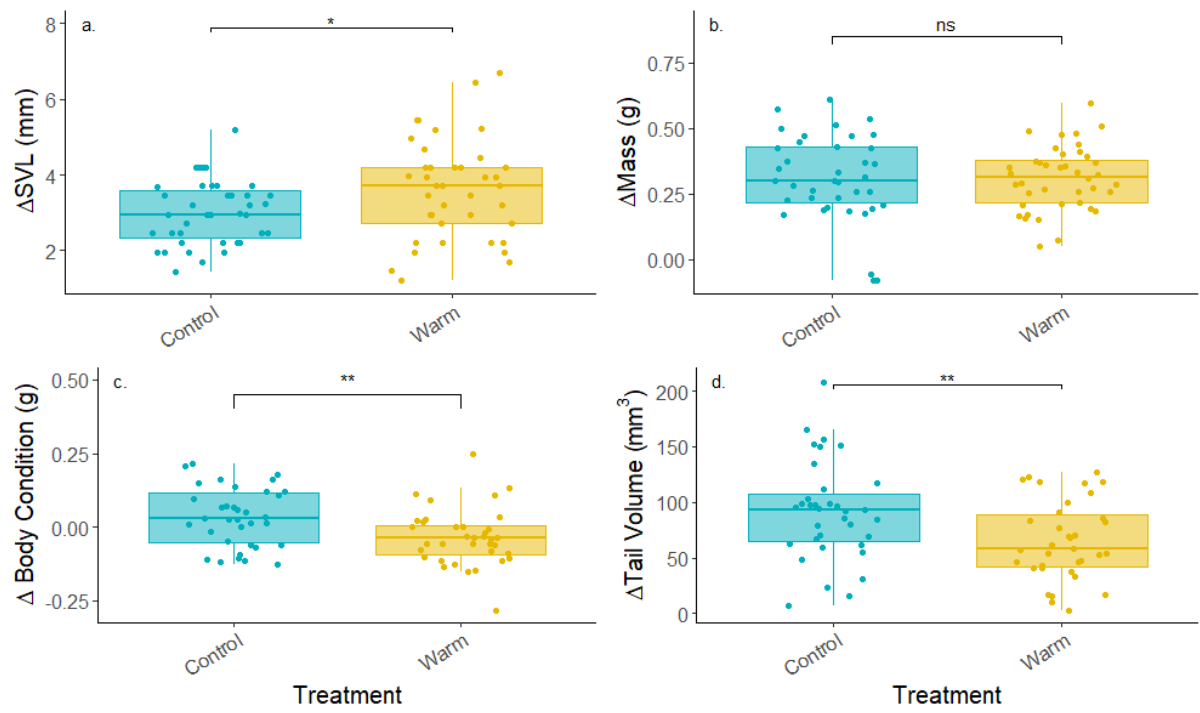
828 recorded in each location.
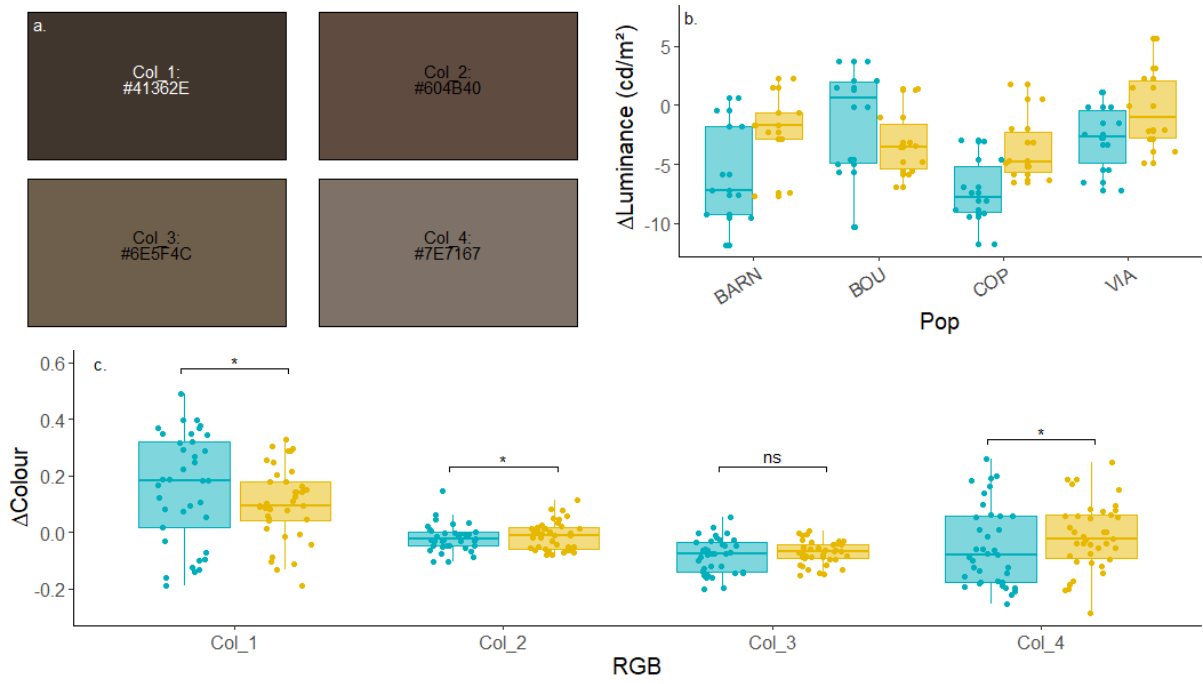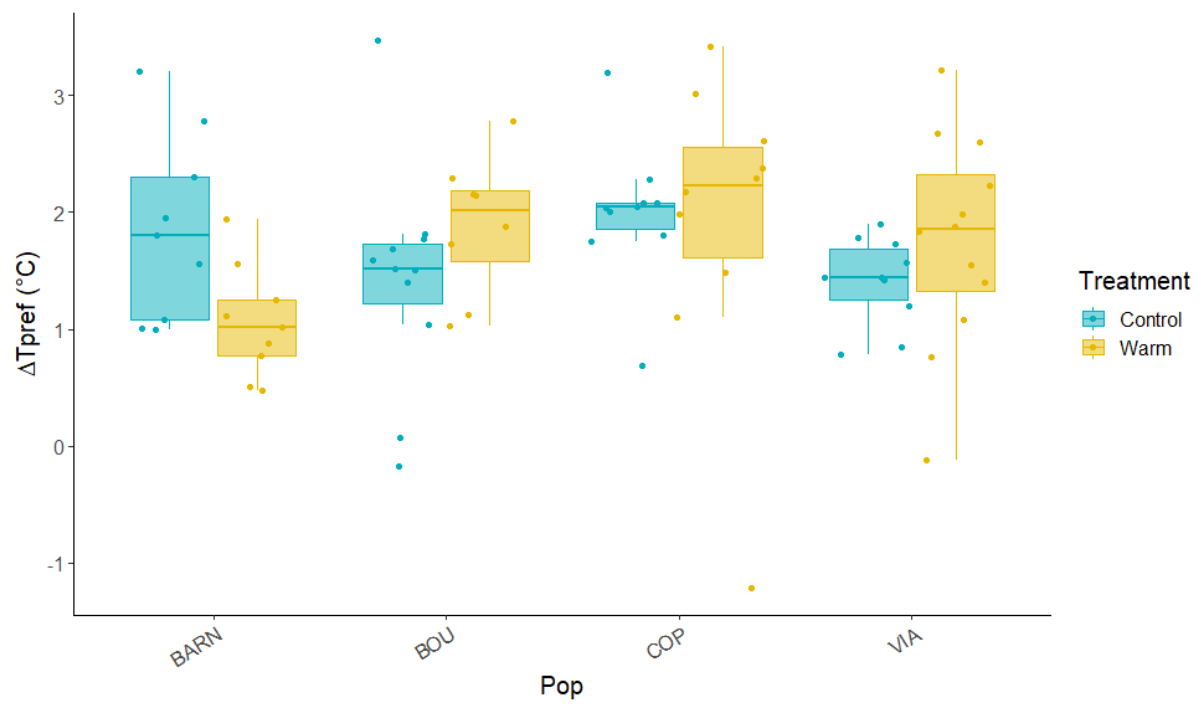
829

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

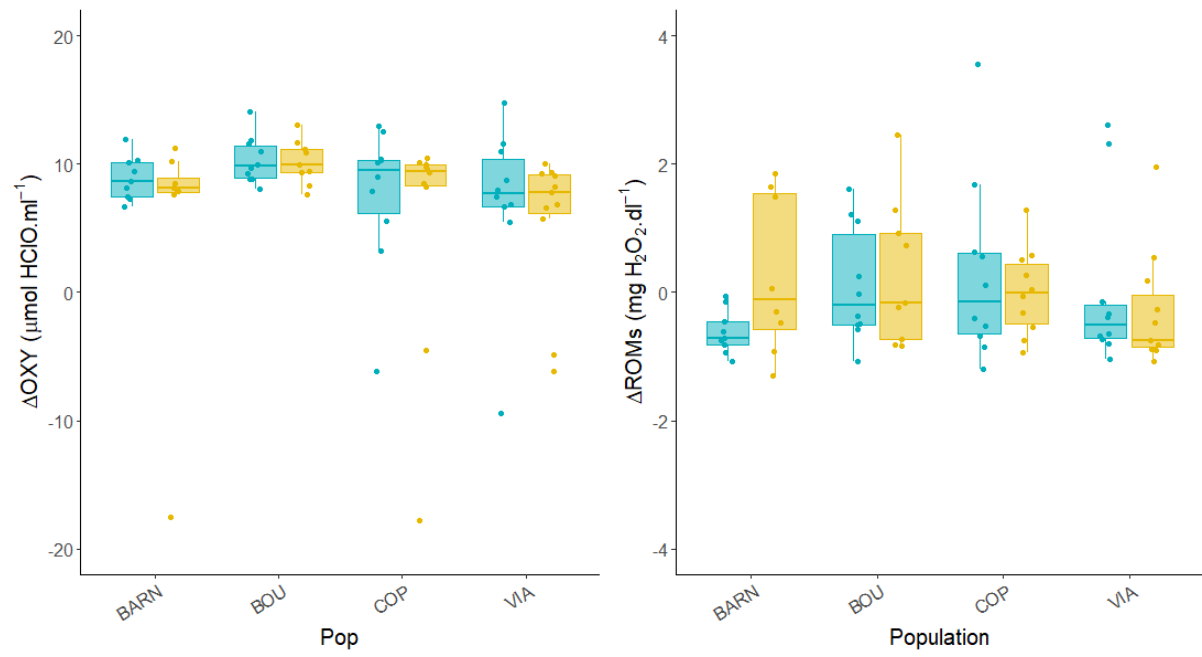Figure 6

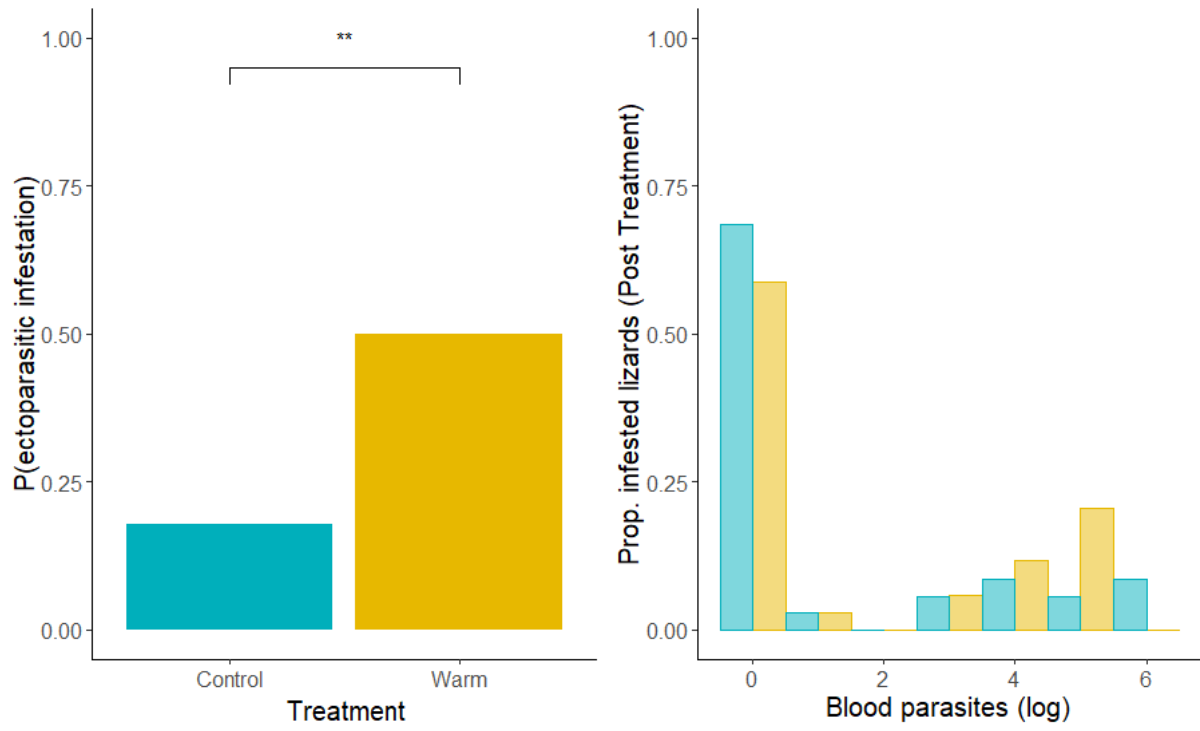Table 1

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