



HAL
open science

Influence of landscape connectivity on newt's response to a warmer climate

Laurane Winandy, Félix Pellerin, Lucie Di Gesu, Delphine Legrand, Julien
Cote

► **To cite this version:**

Laurane Winandy, Félix Pellerin, Lucie Di Gesu, Delphine Legrand, Julien Cote. Influence of landscape connectivity on newt's response to a warmer climate. *Landscape Ecology*, In press, 10.1007/s10980-023-01685-z . hal-04135879

HAL Id: hal-04135879

<https://hal.science/hal-04135879v1>

Submitted on 21 Jun 2023

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

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

1 **Influence of landscape connectivity on newt's response to a warmer climate**

2 Laurane Winandy^{1,2}, Félix Pellerin², Lucie Di Gesu², Delphine Legrand³, Julien Cote².

3 ¹ Laboratory of Ecology and Conservation of Amphibians (LECA), Freshwater and Oceanic Science Unit of
4 Research (FOCUS), University of Liège, 4020 Liege, Belgium

5
6 ² Laboratoire Évolution and Diversité Biologique (EDB), UMR5174, CNRS, IRD, Université Toulouse III Paul
7 Sabatier, 118 Route de Narbonne, 31062 Toulouse, France

8
9 ³ Station d'Écologie Théorique et Expérimentale (SETE), UAR2029, CNRS, 2 route du CNRS, 09200 Moulis,
10 France

11

12 **Landscape Ecology, 2023, <https://doi.org/10.1007/s10980-023-01685-z>**

13 Corresponding author:

14 Laurane Winandy, laurane.winandy@uliege.be (0000-0001-6255-2503)
15 0032479486161

16 Laboratory of Ecology and Conservation of Amphibians (LECA), Freshwater and Oceanic Science Unit of
17 Research (FOCUS), University of Liège, 4020 Liege, Belgium

18

19 Co-author contacts:

20 Felix Pellerin, felix.pellerin23@gmail.com (0000-0002-7062-4957)

21 Lucie Di Gesu, l.digesu@ecomail.fr

22 Delphine Legrand, Delphine.LEGRAND@sete.cnrs.fr

23 Julien Cote, julien.cote@univ-tlse3.fr (0000-0002-4453-5969)

24

25 **Acknowledgements:**

26 We thank the editor and the two anonymous reviewers for their constructive comments and suggestions,
27 Dominic Zugai for editing and reviewing this manuscript for English language, Olivier Guillaume, Thomas
28 Deruelles, Guillaume Toumi, Elvire Bestion and Audrey Trochet for their help or technical support in the
29 achievement of the project.

30 **Author contributions:**

31 JC, LW and DL designed the study. LW, FP and LDG performed the experiment. JC analyzed the pictures on
32 ImageJ. LW performed the statistical analysis and wrote the first version of the manuscript. All authors
33 contributed substantially to manuscript revisions and gave final approval for publication.

34 **Funding:**

35 This work was supported by funding from the European Research Council (ERC) under the European Union's
36 Horizon 2020 research and innovation program (grant agreement no. 817779 to JC), by the French Laboratory of
37 Excellence project 'TULIP' (grant nos. ANR-10-LABX-41 and ANR-11-IDEX-0002-02) and by an
38 'Investissements d'avenir' program from the Agence Nationale de la Recherche (grant no. ANR-11-INBS-
39 0001AnaEE-Services). LW was supported by the Fyssen Foundation Post-Doctoral Fellowship and is currently a
40 postdoctoral researcher at the Fonds de la recherche scientifique (F.R.S.-FNRS).

41

42 **Conflict of interest:**

43 The authors declare no conflict of interest.

44 **Data availability:**

45 The datasets analyzed during the current study are available from the corresponding author on reasonable
46 request.

47 **Abstract**

48 Context

49 Climate change and habitat fragmentation exert considerable pressures on biodiversity. The spatial distribution
50 of microclimatic refuges in the landscape can influence species responses to warming climates.

51 Objectives

52 Using a semi-natural experiment, we investigated the potential synergetic effects of climate warming and habitat
53 connectivity on a single amphibian species.

54 Methods

55 We monitored populations of the palmate newt, *Lissotriton helveticus*, under two climate treatments, a warmer
56 climate (+~2°C) or a present-day climate, in mesocosms either isolated or connected to the other climatic
57 conditions. We assessed the abundance and phenotype (snout-vent length, body condition and skin coloration:
58 darkness and redness) of juvenile and adult newts, and the dispersal propensity of juveniles.

59 Results

60 Over the 4 years of climatic manipulation, populations tended to increase more in the present-day than in the
61 warmer climate. Warmer climates decreased the abundance of adult newts and altered the phenotypic
62 composition of populations with darker and less red newts. However, connectivity between the two climates
63 cancelled out the effect of a warmer climate on abundance and reversed the effects on phenotype composition.
64 We further found that juvenile newts from the present-day climate treatment tended to emigrate more from
65 warmer conditions during our laboratory dispersal assay and that population isolation disrupted a common
66 covariation between emigration propensity and body size, leg length and skin darkness.

67 Conclusions

68 Our results point to a synergetic effect of climate warming and fragmentation on the demography of newt
69 populations and emigration decisions of juveniles, suggesting that microclimate refuges and their accessibility
70 play a key role in buffering the impacts of climate warming, with potential implications for amphibian diversity
71 at a regional scale.

72

73 Keywords: Amphibian, Global warming, Dispersal syndrome, Habitat fragmentation, Microclimate

74

75

76 **Introduction**

77 Global warming is one of the largest human-induced disturbances of natural ecosystems affecting biodiversity
78 worldwide (Díaz et al. 2019) and could lead to the extinction of 5 to 37% of all species (Urban 2015). Direct
79 evidence of warming-induced population extinction is scarce (Román-Palacios and Wiens 2020) because
80 organisms may respond to climate change through phenotypic plasticity, selection or relocation (McGaughan et
81 al. 2021). Climate change is indeed already changing the phenotypic composition and life history traits of
82 populations (Merilä and Hendry 2014), with phenological shifts occurring in many species (Parmesan and Yohe
83 2003) and an accelerated pace of life in ectotherm species (Bestion et al. 2015b; Burraco et al. 2020), for
84 example. These changes can alter population dynamics and increase the risk of population extinction, but they
85 can also facilitate species persistence under new conditions (Pellerin et al. 2022). Phenotypic plasticity and
86 genetic selection can result in behavioral, physiological and/or morphological adaptations to prevent extinction
87 (Huey et al. 2012). A notable and widespread example of phenotypic plasticity is thermal acclimation, which
88 allows species to extend their tolerance to higher temperatures (Seebacher et al. 2014).

89 Species can also shift their geographic range through dispersal towards suitable climatic conditions at
90 higher latitudes and/or elevations (Lenoir and Svenning 2015). Highly mobile organisms, such as birds and
91 butterflies, can travel long distances to locate suitable climatic conditions, while other taxa, such as amphibians
92 and reptiles, are more limited in their movement. These less mobile species need much more time to disperse to
93 distant cooler habitats (Sillero 2021), and may instead use locally variable habitats (*i.e.*, microclimate refugia,
94 Suggitt et al. 2018). The survival of these organisms could therefore be more dependent on successful short-
95 distance movements across climatically heterogeneous environments. Heterogeneous landscapes can offer a high
96 variety of microclimates (Milling et al. 2018), some of which are similar to conditions at higher latitude or
97 elevation, and these conditions can buffer the consequences of extreme temperatures (*i.e.*, extirpation, Scheffers
98 et al. 2013; Suggitt et al. 2018). Dispersal therefore plays a central role in species' ability to locate habitats that
99 meet their thermal requirements at regional or local scales (Bestion et al. 2015a). Yet dispersal among
100 microclimates may vary by phenotype, and particularly by thermal requirements. Individuals with lower thermal
101 requirements might disperse towards cooler microhabitats, while more thermally tolerant individuals tend to
102 remain in or search for warmer environments (Bestion et al. 2015a; Pellerin et al. 2019). So-called adaptive
103 dispersal syndrome (Clobert et al. 2009) can lead to climate-induced shifts in phenotypic distributions, with
104 consequences for population persistence.

105 Climate-driven dispersal is structured by the spatial distribution of microclimate habitats, which are
106 becoming increasingly fragmented due to human activities (Opdam and Wascher 2004). By reducing landscape
107 connectivity, fragmentation may impede individual movements from temporarily or permanently local
108 detrimental conditions to refuge areas, and may force some species to move longer distances. We therefore
109 expect climate warming and habitat fragmentation to act synergistically to modify population responses to
110 climate change (Opdam and Wascher 2004). However, empirical evidence of this phenomenon is scarce (Urban
111 et al. 2014), necessitating new experimental approaches to test for the combined effects of habitat fragmentation
112 and spatial variability in climate on dispersal.

113 As ectotherms, amphibians are particularly sensitive to climate change because their body temperature,
114 and hence their physiological functions, strongly depend on environmental temperatures (Urban 2015).
115 Moreover, the limited dispersal abilities of many amphibians restrict their options to relocate to optimal climates
116 at regional scales, making them even more threatened by climate change (Urban et al. 2014). Yet thermal
117 acclimation through phenotypic plasticity can potentially reduce the impact of climate change. Previous studies
118 show that newts' locomotor activity, heat tolerance and metabolism rate can be acclimated to higher
119 temperatures (Gvoždík et al. 2007; Winterová & Gvoždík 2022). At a morphological level, changing body size
120 has emerged in many organisms as a response to climate warming but remains underexplored in amphibians (Li
121 et al. 2013). Melanin-based coloration plays an important role in thermoregulation in many animals, with
122 individuals of varying darkness presenting different levels of sensitivity to climate change: while dark skin may
123 provide protection against UV-radiation, it can also increase the risk of overheating (Roulin 2014). In
124 amphibians, skin coloration also results from carotenoid pigments (Mochida et al. 2013), which are acquired
125 through diet and are associated with improved individual fitness and may prevent pro-oxidative effects of
126 enhanced UV (Ogilvy and Preziosi 2012; Mochida et al. 2013). However, predicting the impact of climate
127 change in accordance with color traits depends mostly on the adaptive function of the coloration and how it
128 covaries with physiology and behavior in the studied species (Roulin 2014). For example, darkness is positively
129 correlated to boldness, exploration and dispersal in some species (Maflí et al. 2011; Mateos-Gonzalez and Senar
130 2012; Roulin, 2014; Saino et al., 2014) and would be expected to influence their responses to climate change,
131 particularly in fragmented landscapes.

132 Here, we experimentally assessed the interaction between warmer climate and landscape connectivity
133 on the abundance, phenotypic distribution and movement strategy of the palmate newt (*Lissotriton helveticus*).
134 We monitored populations of palmate newts inhabiting 24 semi-natural mesocosms undergoing either a warmer
135 ($+2\text{C}^\circ$) or a present-day climatic treatment across multiple years. Two mesocosms, one for each set of climatic
136 conditions, were paired and either isolated or connected with corridors. We assessed the impact of these
137 conditions on juvenile and adult population abundance and phenotypic traits. We predicted newt abundance
138 would be lower in warm and isolated populations, where newts could not evade the warming temperatures. We
139 also measured morphological traits (body size, body condition), coloration (darkness and redness) and dispersal
140 propensity and their covariation with temperature and phenotype. We predicted phenotypic differences between
141 newts experiencing different climatic conditions, particularly in isolated populations, where the lack of
142 connectivity prevents them from evading warming temperatures. More specifically, we predicted a reduction in
143 body size and body condition at warmer temperatures, as has been shown in previous studies (Reading 2007).
144 We expected that thermal stress would decrease the red coloration, while the expected effects on skin melanism
145 were less clear since darkness of color can have either positive (UV protection) or negative (overheating risk)
146 effects on thermoregulation.

147 Finally, we measured the emigration propensity of juvenile newts at two temperatures in a laboratory
148 setting and its covariation with phenotypic traits (*i.e.*, dispersal syndrome). We expected newts coming from
149 present-day climate populations to emigrate more from warmer temperatures than newts that were exposed
150 initially to warmer conditions (*i.e.*, from warmer climates), suggesting that newts coming from warmer climates
151 would be acclimated to warmer temperatures. While emigrants are expected to have greater body and leg length
152 and to be darker (*e.g.*, Winandy et al. 2019), we expected challenging environmental conditions experienced in

153 warm and/or isolated populations to disrupt dispersal syndrome (e.g., Cote et al. 2017, 2022), possibly inciting
154 all individuals to emigrate regardless of phenotype (Legrand et al. 2015).

155 **Material and methods**

156 *Model species*

157 The palmate newt (*Lissotriton helveticus*) is a small salamandridae (i.e., tailed amphibian) occurring in a wide
158 range of habitats in Europe, from wooded to more open areas such as meadows, field edges and gardens. The
159 palmate newt has an aquatic larval stage before undergoing metamorphosis into terrestrial juveniles. Juveniles
160 usually stay on land for two to three years before reaching sexual maturity and returning to the water to breed.
161 Adult newts have strictly aquatic reproduction and remain in the water during the mating period which lasts
162 several months. As home ranges of newts are quite small (< 50.000 m² (Verell 1987)), aquatic breeding sites
163 must be close to the terrestrial habitat (Joly and Miaud 1989). Breeding sites range from small puddles to larger
164 ponds or even lakes and are usually stagnant, permanent or filled with water for at least three months covering
165 the period of reproduction and larval development. On land, newts are usually found sheltering under logs or
166 stones or in small burrows.

167 *Study site and experimental design*

168 The study took place in the Station d'Ecologie Théorique et Expérimentale in Moulis (France). The Station has a
169 national agreement for use of animals in the laboratory (number B09583), and our experiments were conducted
170 in accordance with French ethics regulations (Ethics permit number APAFIS#30486-2021031815341692v4).
171 The newts were caught in the Metatron under license number 09-2016- 02. At the end of the experiment, they
172 were released exactly where they were caught, following recommendations of the permit.

173 For this study, we used populations of palmate newts naturally occurring in the Metatron, which is a
174 system of 48 semi-natural and interconnected mesocosms in which climatic conditions can be manipulated
175 (Legrand et al. 2012). Mesocosms are large (10 x 10 m), delimited by tarpaulins buried 30 cm into the ground,
176 and fully enclosed with a net allowing the isolation of each enclosure. This system prevents both the escape of
177 the study species and predation from birds and snakes present outside of the Metatron. Each mesocosm acts as a
178 mini-ecosystem of a wet meadow with dense natural vegetation, two permanent pools (30 x 50 x 15cm), shelters
179 (logs and rocks) and large invertebrate communities. They therefore offer all the ecological features required for
180 the different life stages of palmate newts. Temperature, light intensity and hygrometry in each enclosure are
181 monitored every 30 minutes and can be manipulated using motor-driven shutters and a sprinkler system. In
182 addition, mesocosms can be connected by 19 m-long corridors allowing species to move between mesocosms.
183 Newts usually exhibit strong breeding site fidelity, with individuals frequently returning to their natal site (Joly
184 and Miaud 1989) and thus movements between mesocosms and breeding patches could be considered as
185 dispersal (Clobert et al. 2009). While breeding adult newts usually disperse between twenty to a few hundred
186 meters (Cayuela et al. 2018; Denoël et al. 2018), natal dispersal distances are unknown and likely higher as
187 juvenile newts wander on land for several years (Pittman et al. 2014). While the corridor length in the
188 mesocosms does not allow for distinguishing dispersal from migration movements, the harsh conditions in
189 corridors (i.e, a narrow width devoid of shelter and/or puddles) make the crossing challenging for newts and
190 ensures that movements between mesocosm are not routine foraging movements (Fronhofer et al. 2018).

191 In this study, we used 24 mesocosms in which we applied the climatic treatment for four years (2013-
192 2017) and the connectivity treatments for two years (2015-2017). The climatic treatment was created with
193 shutters that automatically closed above certain temperature thresholds: 28°C for the present-day climate and
194 38°C for the warm climate. Mesocosms were intrinsically warmer than outside because of the greenhouse effect
195 associated with the nets. The present-day climate treatment created temperatures similar to the mean temperature
196 outside of the Metatron (Bestion et al. 2015a). Since climatic treatments depended on outdoor climatic
197 conditions, they were efficient only during daytime in summer (from mid-June to October), with variation within
198 and between days. This resulted in a 1-2°C difference in mean summer daily temperatures between the present-
199 day and warm climates from June 2013 to July 2017 ($\chi^2_1 = 77.12$, $P < 0.001$, the mean \pm SE are $26.49 \pm 0.10^\circ\text{C}$
200 and $27.76 \pm 0.11^\circ\text{C}$ for present-day and warm climate treatments respectively). There was also a 2-3°C
201 difference of the summer maximum temperature ($\chi^2_1 = 135.62$, $P < 0.001$, the mean \pm SE are $29.43 \pm 0.13^\circ\text{C}$ and
202 $31.88 \pm 0.20^\circ\text{C}$ for present-day and warm climate treatments respectively). The warm climate treatment matched
203 the scenario SSP5-8.5 for 2041–2060 and SSP2-4.5, SSP3-7.0 and SSP5-8.5 for 2081–2100 (Masson-Delmotte
204 et al., 2021). While most global warming studies focus on the effects of a strict increase in mean temperatures,
205 an increase in the magnitude of daily temperature fluctuation (*i.e.*, difference between the maximum and the
206 minimum day temperature) is also predicted (Thompson et al. 2013). Accordingly, in our study, there was also
207 an effect of treatments on the difference between the maximum and the minimum temperature during summer
208 ($\chi^2_1 = 124.96$, $P < 0.001$, the mean \pm SE are $15.39 \pm 0.15^\circ\text{C}$ and $17.94 \pm 0.20^\circ\text{C}$ for present-day and warm
209 climate treatment respectively). During the climate treatment, all mesocosm were watered with soft water every
210 day in the evening to avoid a drying effect of the treatment.

211 We used 12 pairs of present-day and warm climate mesocosms that were either connected by opening
212 the corridor between them (*i.e.*, for connected treatment) or not to simulate habitat fragmentation (*i.e.*, isolated
213 treatment). The six pairs of each connectivity treatment were randomly distributed across the system.

214 *Population monitoring in the Metatron*

215 After the construction of the Metatron in 2009, all mesocosms remained open for an entire year and, between
216 2010 and 2012, for 2-3 weeks a year to allow colonization by local species (*e.g.*, plants, insects, amphibians,
217 mollusks) present on the site. The palmate newts within habitats around the Metatron rapidly colonized and
218 established themselves inside the mesocosms. In 2012, the 24 mesocosms were closed for a long-term
219 experiment on common lizards (see Bestion et al. 2015b; Pellerin et al. 2022) and migration to and from the
220 mesocosms was thus impossible. The climatic treatment began in 2013 while the connectivity treatment began in
221 2015. Newt abundance in each mesocosm was estimated annually beginning in 2013 with a standardized
222 sampling effort. Our climate treatments were only applied in the summer (from the end of June to the beginning
223 of October) and during the warmest times of the day, while our sampling took place in the early mornings in
224 June, prior to climate treatments. Consequently, we assumed our probability of detection did not differ across
225 climate treatments. Between 2013 and 2016, the sampling consisted of two observers searching for newts in the
226 vegetation during 10 min on the four sides of each mesocosm, where newts were localized due to the presence of
227 moss providing moist conditions.

228 In June 2017, we estimated newt abundance with a more thorough sampling. To estimate juvenile newt
229 abundance, we did three sessions (on different days) of standardized sampling early in the morning, when there

230 was still morning dew and juvenile newts were more active. Each capture session was conducted by the same
231 two people searching for newts for 10 minutes in each mesocosm. After each session, all newts captured were
232 brought back to the lab. As described above, the climate treatment was not applied during early June and dawn
233 was coolest and wettest part of the day, thus we assumed that there was no difference in detection probability
234 between treatments during sampling. Yet the large size of the mesocosm and the dense vegetation cover likely
235 prevented detection and capture of all juvenile newts. Therefore, the number of captured juveniles in this study
236 represents an estimate of abundance that is comparable between treatments due to equal sampling effort.

237 With respect to adult newts, we ran two capture sessions on different days by dipnetting for 5 minutes in
238 each of the two pools available to newts in each mesocosm. In June, most adults inhabit water for reproduction
239 purposes. Given the small size of the pools (30 x 50 x 15 cm), we were confident of capturing all adults present
240 in the water at that time. Therefore, we feel that our abundance estimates are likely to match the actual number
241 of adults in each mesocosm.

242 *Housing conditions and phenotype assessment*

243 From the sampling above, we kept 67 randomly selected juvenile newts from 16 mesocosms in the lab: 31 newts
244 were from seven mesocosms in warm climate (n = 15 in isolated and n = 16 in connected treatments); 36 newts
245 were from nine mesocosms in present-day climate (n = 18 in isolated and n = 18 in connected treatments). The
246 remaining juveniles were released back to the mesocosms after a maximum of three days spent in the lab. The 67
247 juveniles were identified using dorsal and ventral pictures (see below) and housed in individual terraria (25 x
248 15.5 x 15 cm) filled with natural moss, an egg carton as a shelter, and a small pool (3 cm diameter 1 cm depth).
249 Newts were fed five micro-cricket (2-3 mm, *Acheta domestica*) daily. A natural neon light provided light from
250 9am to 7pm and the temperature of the room was set at 18°C, which is in the range of preferential temperatures
251 for European newts (Balogová & Gvoždík 2015; Gvoždík 2015). All terraria were sprayed with water twice a
252 day to maintain a high humidity level. Juvenile newts were housed in common environmental conditions for 2
253 weeks prior the dispersal test (see below) to reduce the effect of stressful new condition on their emigration rate.

254 We also randomly selected 86 adult newts: 32 from nine mesocosms with the warm climate (n = 6 in
255 isolated and n = 26 in connected treatments); 54 from 10 mesocosms with the present-day climate (n = 28 in
256 isolated and n = 26 in connected treatments). Adult newts were kept in the lab to measure morphological traits
257 for several days before being released back in the mesocosms. Newts were housed in aquaria (35 cm L × 18 cm l
258 × 20 cm water deep) with a maximum of four individuals from the same mesocosm per aquarium. We provided a
259 shelter and an egg laying substrate (strips of filter paper) and fed individuals 50 mg of thawed *Chironomus*
260 larvae per newt daily to create optimal conditions for palmate newts during aquatic reproduction.

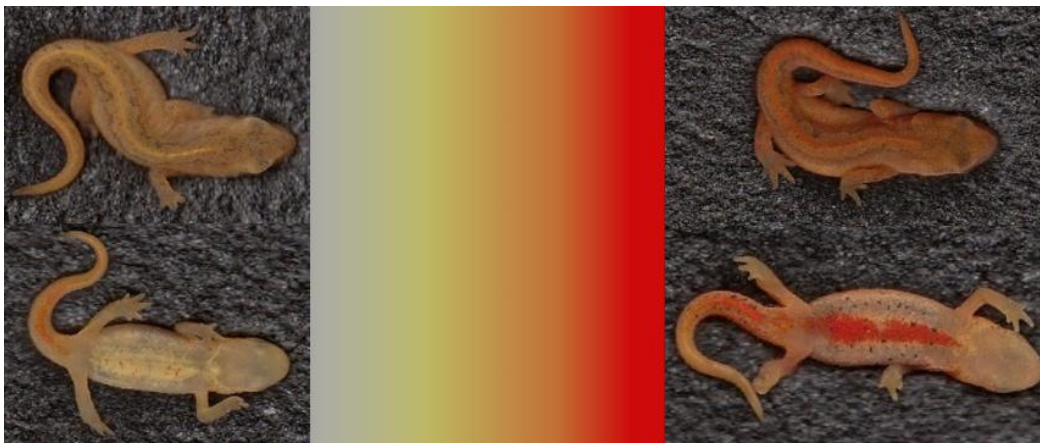
261 *Morphological traits*

262 We measured snout-vent length and total length of newts using a ruler (measurement to the mm) and weighed (to
263 0.001 g) the selected newts (n = 67 juveniles and n = 86 adults) on the day of their capture. The body mass of
264 newts highly depends on their degree of hydration, so we made sure to weigh fully hydrated newts. Adult newts
265 in their aquatic phase, are obviously fully hydrated. For the juveniles, just after capture, they were maintained in
266 boxes filled with soaked paper towels, ensuring their proper hydration. We calculated the body condition of
267 newts using the mass/snout-vent length residuals. We took ventral and dorsal photographs to assess skin

268 coloration of the 67 juveniles. However, due to a technical issue we were only able to photograph 27 adult newts
269 [warm climate: 12 newts from 7 mesocosms (2 in isolated and 10 in connected treatments), present-day climate:
270 15 newts from 7 mesocosms (9 in isolated and 6 in connected treatments)]. For photographs, each newt was
271 carefully positioned on a computer scanner (Canon CadoscanLide110) lined with high density foam to avoid
272 injuries. Photographs were taken at a resolution of 400 dpi and analyzed using ImageJ software. We delimited a
273 zone for the dorsal (from the top of the neck to the posterior legs) and ventral areas (from the throat to the
274 posterior legs). The photographs were transformed in shades of grey and then in black and white according to a
275 fixed grey threshold of 60 chosen to maximize variance in darkness among individuals. A mean grey value and a
276 percentage of black pixels in each zone were computed. We further extracted HSL values (Hue Saturation
277 Lightness, Endler 1990). Hue, often referred to as ‘color’ (*i.e.*, the difference between green, red or blue),
278 corresponds to the angular position on a color circle where 0° corresponds to red. Saturation refers to the
279 intensity of a hue, from grey tone (scored as 0) to pure and vivid color (scored as 1). Lightness is the luminance
280 (a photometric measure of the luminous intensity per unit area of light travelling in a given direction) ranging
281 from dark (scored as 0) to light (scored as 1). On the ventral images we also measured hindlimb length (to the
282 nearest mm) of juvenile newts, as leg length is a locomotor trait potentially linked to emigration propensity
283 (Denoël et al. 2018; Winandy et al. 2019).

284 Using the FactoMinR package (Lê et al. 2008), adult and juvenile darkness was scored with Principal
285 Component Analyses (PCA) on the mean grey, the percentage of black pixels, and the lightness values of both
286 dorsal and ventral photographs (mean and variance scaled). In adults, the PC1 axis explained 52.6% of variance,
287 had an eigenvalue of 3.16, and was positively correlated to dorsal and ventral lightness and mean grey, and
288 negatively correlated to the percentage of black pixels (Table S1). In juveniles, the PC1 axis explained 57.3% of
289 variance, had an eigenvalue of 3.43, and was positively correlated to dorsal and ventral lightness and mean grey,
290 and negatively correlated to the percentage of black (Table S1). To obtain a darkness score ranging from pale to
291 dark, we multiplied scores by -1.

292 We scored the red coloration of juvenile newts with PCA using the dorsal and ventral hue and saturation
293 values (variance and mean scaled). The PC1 axis explained 54.4% of variance, had an eigenvalue of 2.17, and
294 was positively correlated to dorsal and ventral hue, and negatively correlated to ventral and dorsal saturation
295 (Table S1). To obtain a redness score (Fig. 1), from grey tone yellow to pure red, we multiplied scores by -1. We
296 did not measure redness in adult newts as they have a beige-brown coloration.



297

298 **Fig. 1** Coloration of juvenile newts. Redness scores scale from high hue and low saturation (grey-tone yellow
299 coloration) to low hue and high saturation (pure red), photography by L. Winandy.

300

301 *Emigration propensity*

302 Emigration propensity was assessed only in juvenile newts two weeks after their capture, allowing a period of
303 acclimation to common garden conditions in the laboratory. We used eight experimental systems placed in a
304 greenhouse with controlled temperature and natural light for this experiment. Each system was made of two 130
305 L plastic containers 78 x 56 x 43 cm) connected by a circuitous plastic pipe (diameter: 10 cm, total length: 4.4
306 m). The departure container was filled with soil litter, with several terrestrial shelters and a pool (25 cm
307 diameter, filled with water and natural moss). The corridor (plastic pipe) was directly connected to the departure
308 container and filled with a thin layer of moist soil. To go from the departure to the arrival containers, juvenile
309 newts had to enter the plastic pipe (*i.e.*, the corridor) and fall into the arrival container which was filled with
310 natural moss. Once in the arrival container, newts could not re-enter the corridor. This system has been used in
311 previous studies to assess palmate newt's emigration decisions (Fronhofer et al. 2018; Cote et al. 2022). Here we
312 measured the emigration probability of each individual at two different temperature regimes: a warm
313 temperature (with a daytime temperature of $30.7 \pm 0.27^\circ\text{C}$ and a nighttime temperature of $24.6 \pm 0.07^\circ\text{C}$), and a
314 cooler temperature (with a daytime temperature of $22.83 \pm 0.18^\circ\text{C}$ and a nighttime temperature of $20.6 \pm 0.3^\circ\text{C}$).
315 These temperatures corresponded to local natural temperatures at this time of the year (*i.e.*, in July).

316 Assays were run on small groups (4-5 newts per system) to prevent high density in departure
317 populations. We carried out two temporal replicates of eight experimental groups each. Juvenile newts from each
318 climatic treatment and mesocosm were homogeneously distributed between the temporal replicates. Half of the
319 newts were tested first at the cooler temperature and the other half were tested first at the warmer temperature.
320 Replicates were done several days apart, and the moss and water pools were changed to limit the risk of scent
321 remaining inside the system. Ten days after the first assays, we repeated the assay under the other temperature
322 treatment. For each assay, groups of newts were released in the departure tank in the late afternoon for a 24-h
323 acclimation period to the system and thermal conditions. Food (micro-crickets) was available *ad libitum* in the
324 departure tank. During the acclimation period, the dispersal pipes were closed to prevent any stress-induced
325 movement from the departure tank. We then opened the pipes for one night as juvenile newts usually move from
326 dusk to dawn. The next morning, we closed all dispersal pipes, captured residents and emigrants (*i.e.*, newts
327 found in departure and arrival tanks, respectively) and identified individuals using the ventral and dorsal
328 photographs. No newts were found inside the dispersal pipes.

329 *Statistical analysis*

330 *Abundance*

331 Firstly, we ran a generalized linear mixed model (GLMM) using a poisson family (with a log link function) to
332 assess the effect of climatic treatments (warm *vs.* present-day) on the evolution of newt abundance across years
333 (from 2013 to 2017). The mesocosm identity (Mesocosm_ID) was included as a random intercept. Since the
334 connectivity treatment only began in summer 2015, we checked that there was no prior effect of this treatment
335 on newt abundance using a generalized linear model (GLM), using a quasipoisson family to correct for

336 overdispersion, and testing the effect of connectivity treatment (isolated vs. connected) and its interaction with
337 climatic treatment on newt abundance every year from 2013 to 2015.

338 Secondly, we analyzed abundance in 2017, in which sampling was more extensive than previous
339 sampling and took place after two years of connectivity treatment. We ran a GLM using a quasipoisson family
340 for adult and juvenile abundance, testing the effect of the climate treatments, the connectivity treatment, and
341 their interaction as fixed effects. Since mesocosms were not seeded with the same number of individuals prior to
342 the start of the experiment, we added the abundance of newts in 2013 as a covariate. For juvenile abundance, we
343 also added the adult abundance as a covariate to prevent multiple testing issues as juvenile and adult abundances
344 should be positively correlated.

345 *Phenotype*

346 We ran linear mixed models (LMM) for morphological traits [body size (*i.e.*, snout-vent length), body condition
347 (*i.e.*, mass/size residuals) and darkness for adults and juveniles plus leg length (using leg length/body size
348 residuals) and red coloration for juveniles]. All models included the climate treatments, the connectivity
349 treatment, and their interaction as fixed effects. The mesocosm identity (Mesocosm_ID) was included as a
350 random intercept. For adult body size and body condition, the sex and its interaction with treatments were
351 included. Redness and darkness were positively correlated ($F_{1,65} = 18.91$; $P < 0.001$), so for both variables we
352 used the residuals of the regression models (residuals of redness on darkness for the redness variable and
353 darkness/redness residuals for the darkness variable) to obtain an independent analysis of color parameters. For
354 coloration traits, we also added body size (*i.e.*, snout-vent length) as a covariate in the models.

355 *Emigration probability*

356 We used a generalized linear mixed model (GLMM) with a binomial error distribution and a logit link function
357 for the emigration status of juveniles (*i.e.*, 0 = resident, 1 = emigrant), with climate, connectivity, and
358 temperature treatments during the emigration assay and all interactions between these factors as fixed effects.
359 Individual identity (Newt_ID), experimental group (Group_ID) and the mesocosm identity (Mesocosm_ID) were
360 added as random intercepts. The order of passage (warm or cooler temperature first) as well as the temporal
361 replicate (*i.e.*, first or second block of eight experimental groups) did not affect the emigration rate ($\chi^2_1 = 0.355$,
362 $P = 0.552$; $\chi^2_1 = 0.624$, $P = 0.43$, respectively) and were therefore removed from the model to avoid over
363 parameterization.

364 *Dispersal syndromes*

365 We performed a multivariate analysis of variance (MANOVA) to assess the effects of treatments and emigration
366 status on newt phenotypes. The dependent variables were body size, leg length (using leg length/body size
367 residuals), darkness (using darkness/redness residuals), and redness (using redness/darkness residuals). These
368 variables were centered and scaled. Fixed effects included climate treatment, connectivity treatment, and their
369 interactions with the emigration status. In a second step, we ran univariate analysis on the four traits separately.

370 *General procedure*

371 Analyses were performed in R 3.5.1 (www.r-project.org). We checked model assumptions by visually screening
372 the residuals for normality and homoscedasticity. Body size for adult newts and body condition for juvenile
373 newts were log-transformed to achieve normality. We used lme4 (Bates et al. 2008) and sjstats (Lüdecke and
374 Lüdecke 2019) packages for all models and estimated the significance of factors with Wald χ^2 and likelihood
375 ratio tests. We used a backward selection procedure (with *a priori* level of significance of 0.05) to remove non-
376 significant interactions and covariates, while keeping the main factors of interest (*i.e.* the climatic and
377 connectivity treatments) in the models. After the model selection, we used the performance package (Lüdecke et
378 al. 2021) to calculate adjusted R^2 for GLMs and conditional R^2 (R^2_c , effect of the fixed and random effects) and
379 marginal R^2 (R^2_m , effect of the fixed effect) for LMMs and GLMMs. For mixed models, when the variance of a
380 random effect was equal to 0 (*i.e.*, $R^2_c = NA$), the random variable of concern was removed from the model and
381 the adjusted R^2 was calculated if there were no more random variables included in the model. When we found a
382 significant interaction between climate and connectivity treatments, we investigated the influence of climate in
383 each connectivity treatment by separately running the models for each connectivity treatment.

384 Results

385 Abundance

386 During the 4 years of study, we found no effect on abundance from the climate treatment ($\chi^2_1 = 0.201$, $P = 0.65$).
387 However, we found a strong effect for the specific year ($\chi^2_4 = 330.331$, $P < 0.001$), showing an increase of newt
388 abundance over time and a marginal interaction between year and climate treatment ($\chi^2_4 = 8.834$, $P = 0.065$),
389 with newt abundance increasing slightly more in the present-day climate than in the warm climate (Fig. 2A). The
390 conditional and marginal R^2 of the model were $R^2_c = 0.889$ and $R^2_m = 0.36$, respectively, and the variance of the
391 mesocosm identity was $1.532 \pm 1.238SD$. Before 2017, there was no effect of connectivity treatment or any
392 interaction with climate treatment on newt abundance ($P > 0.2$ for all years from 2013 to 2015, see Table S2 for
393 details).

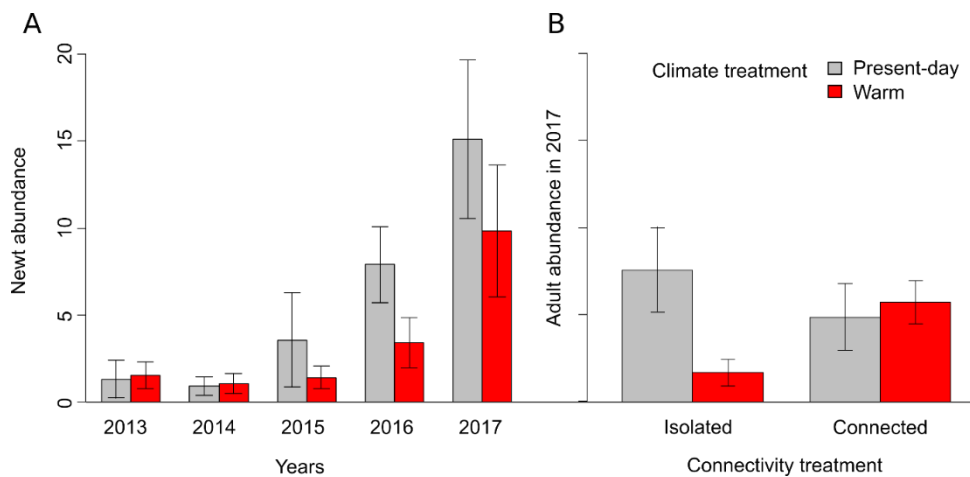
394 In 2017, over all mesocosms, we captured 187 juvenile newts and 118 adults (52 males and 66 females,
395 see Table S3 for details on the number of newts captured in each mesocosm). We captured 80% of adults in the
396 water during the first session and 13% during the second session. Only 7% of adults were captured on land
397 during the 3 sessions of juvenile sampling (*i.e.* $n = 8$; 4 newts in each climate treatment), confirming that finding
398 adult newts on land was rare at this time of the year. We found an interaction between the effects of climate and
399 connectivity treatments on the abundance of adult newts (Table 1). In isolated populations, newt abundance was
400 much lower in the warm climate treatment than in the present-day treatment (Fig. 2B; $\chi^2_1 = 7.182$, $P = 0.007$),
401 while there was no difference in the connected populations (Fig. 2B; $\chi^2_1 = 0.13$, $P = 0.718$). We found no effect
402 of treatment on juvenile abundance, but there was a positive relationship between juvenile and adult abundance
403 (Table 1). The abundance of adult newts in 2013 and 2017 were positively correlated, but the abundance of
404 adults in 2013 and juvenile abundance in 2017 were not related (Table 1).

405

406 **Table 1:** Results of GLM (after backward selection procedure) testing the effect of climate treatment,
407 connectivity treatment, and their interaction on the abundance of adult ($R^2_{adj} = 0.903$) and juvenile newts ($R^2_{adj} =$
408 0.227) in 2017. Estimates for climate and connectivity are given for the present-day climate and the connected
409 population, respectively.

Newt stage	Factors	Estimates	χ^2_1	CI (95%)	P
Adults	Climate	4.4527	0.948	(1.721, 10.719)	0.330
	Connectivity	3.093	1.382	(0.238, 8.397)	0.190
	Climate * Connectivity	-6.667	4.262	(-13.377, -0.385)	0.039
	Abundance in 2013	1.092	7.629	(0.04, 0.24)	0.006
Juveniles	Climate	-0.657	0.815	(-12.823, 9.129)	0.367
	Connectivity	-0.834	1.839	(-20.371, 4.624)	0.175
	Adult abundance	0.156	7.279	(0.114, 0.173)	0.007
	Abundance in 2013	0.063	0.574	(-0.10, 0.25)	0.449

410



411

412 **Fig. 2** Effect of climate treatments (mean \pm SE) on newt abundance across years (A), and of climate and
413 connectivity treatments on adult newt abundance in 2017 (B).

414

415 *Phenotypic differences*

416 The climate and connectivity treatments alone or in interaction did not influence body size, leg length or body
417 condition of adult or juvenile newts (Table S4). Adult newts were significantly darker in warm than in present-
418 day climates (Table 2, Fig. 3A), while connectivity treatments had no effect (Table 2). There was an interaction
419 between climate and connectivity treatments on juvenile darkness (Table 2, Fig. 3B): in connected populations,
420 newts were darker in the present-day climate than in the warm climate ($\chi^2_1 = 4.564$, $P = 0.033$). However this
421 was not the case for isolated populations ($\chi^2_1 = 0.740$, $P = 0.39$). There was an interaction between climate and
422 connectivity treatments on juveniles' redness (Table 2, Fig. 3C): in isolated populations, newts were redder in
423 the present-day climate than in the warm climate ($F_{1,31} = 6.462$, $P = 0.016$), while in connected populations newts
424 tended to be redder in the warm climate than in the present-day climate ($F_{1,32} = 2.764$, $P = 0.106$). Body size had
425 no effect on coloration traits in adults or in juveniles ($P > 0.1$ in all models) and was therefore removed during
426 model selection.

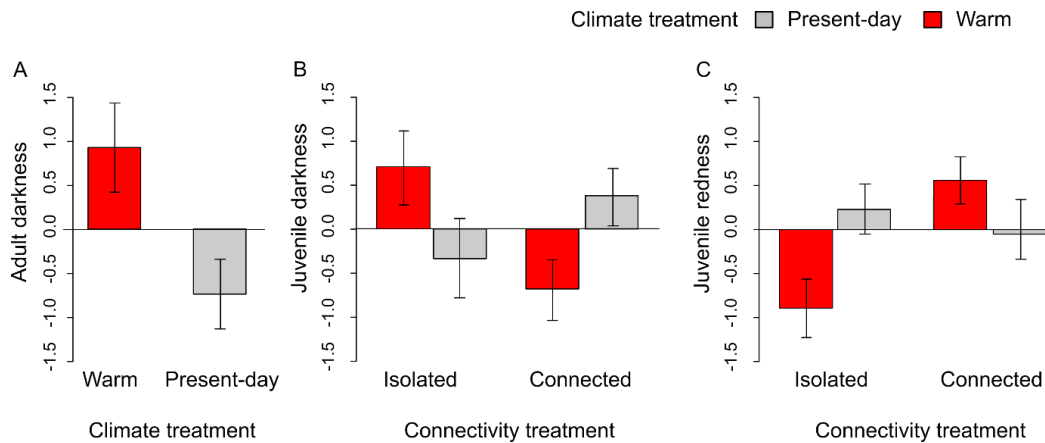
427

428 **Table 2:** Results of LMM (after backward selection procedure) testing the effect of climate treatment,
429 connectivity treatment, and their interaction on the darkness of adults ($n = 27$, $R^2_c = 0.350$, $R^2_m = 0.206$) and on
430 darkness and redness of juveniles ($n = 67$, $R^2_c = 0.238$, $R^2_m = 0.083$ and $R^2_{adj} = 0.12$). Estimates for climate and
431 connectivity are given for the present-day climate and for the connected population, respectively.

Newt coloration	Factors	Estimates	SE	Test value	CI (95%)	P
Adult					(-3.07, -0.15)	
Darkness	Climate	-1.609	0.781	$\chi^2_1 = 4.247$	(-1.3, 1.66)	0.039
	Connectivity	0.180	0.790	$\chi^2_1 = 0.052$		0.820
	Mesocosm_ID		Variance = 0.512±0.716SD			
Juvenile						
Darkness	Climate	-0.928	0.742	$\chi^2_1 = 0.0004$	(-2.23, 0.36)	0.985
	Connectivity	-1.31	0.782	$\chi^2_1 = 0.269$	(-2.68, 0.05)	0.604
	Climate* Connectivity	1.847	1.042	$\chi^2_1 = 3.968$	(0.04, 3.71)	0.046
	Mesocosm_ID		Variance = 1.35±1.16SD			
Juvenile Redness	Climate	1.125	0.430	$F_{1,63} = 0.739$	(0.27, 1.98)	0.393
	Connectivity	1.445	0.442	$F_{1,63} = 3.105$	(0.56, 2.33)	0.083
	Climate* Connectivity	-1.703	0.602	$F_{1,63} = 8.002$	(-2.91, -0.50)	0.006
	Mesocosm_ID		Variance = 0*			

432 *Since the variance of the mesocosm identity (Mesocosm_ID) was equal to 0 it was removed from the final
433 model.

434



435 Climate treatment Present-day Warm
436 **Fig. 3** Effect of treatments on newt coloration (mean ± SE): Effect of climate treatment (red bar for warm
437 climate and grey bar for present-day climate) on adult newt darkness (A), and of climate and connectivity
438 treatments on juvenile newt darkness (B) and on juvenile newt redness (C).

439

440 *Emigration probability and dispersal syndromes*

441 While connectivity treatments had no effect on emigration probability and dispersal syndromes, we found a
442 marginal interaction between climate and assay temperature treatments (Table 3, Fig. 4), showing that newts
443 coming from the present-day climates tended to emigrate more often in warm conditions than in cooler
444 conditions, but that the difference was not significant ($\chi^2_1 = 2.300$, $P = 0.129$). Newts coming from the warm
445 climate emigrated similarly in warm and cooler conditions ($\chi^2_1 = 0.512$, $P = 0.474$).

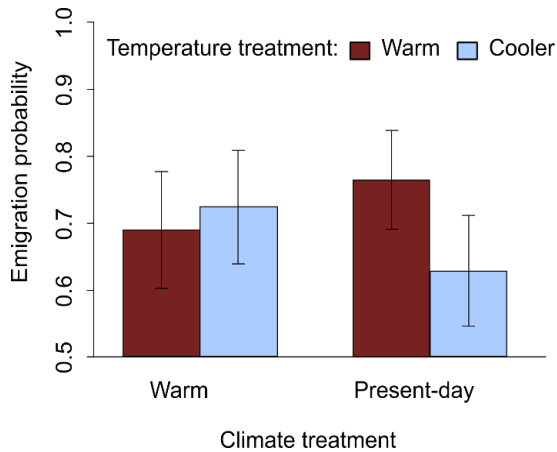
446

447 **Table 3:** Results of GLMM (after backward selection procedure) testing the effect climate treatment,
448 connectivity treatment, temperature treatment, and their interaction on the emigration probability of juvenile
449 newts ($n = 67$, $R^2_c = 0.990$, $R^2_m = 0.013$). Estimates for climate, connectivity and temperature are given for the
450 present-day climate, for the connected population, and for cooler temperatures, respectively.

Response	Factors	Estimates	SE	χ^2_1	CI (95%)	P
Emigration probability	Climate	-0.2	1.681	0.014	(-3.5, 3.1)	0.991
	Connectivity	-0.018	1.679	0.0001	(-3.31, 3.27)	0.905
	Temperature	-1.298	1.003	1.675	(-3.26, 0.67)	0.196
	Climate*Temperature	-6.342	3.456	3.368	(-13.11, 0.43)	0.066
	1 newt_ID	Variance = 311.3±17.64SD				
	1 Group_ID	Variance = 0*				
	1 Mesocosm_ID	Variance = 0*				

451 *Since the variance of the experimental group (Group_ID) and the mesocosm identity (Mesocosm_ID) were
452 equal to 0, these were removed from the final model.

453



454

455 **Fig. 4** Interactive effect of climate treatment and temperature treatment (*i.e.*, applied during the movement assay)
456 on newt emigration probability (mean ± SE).

457

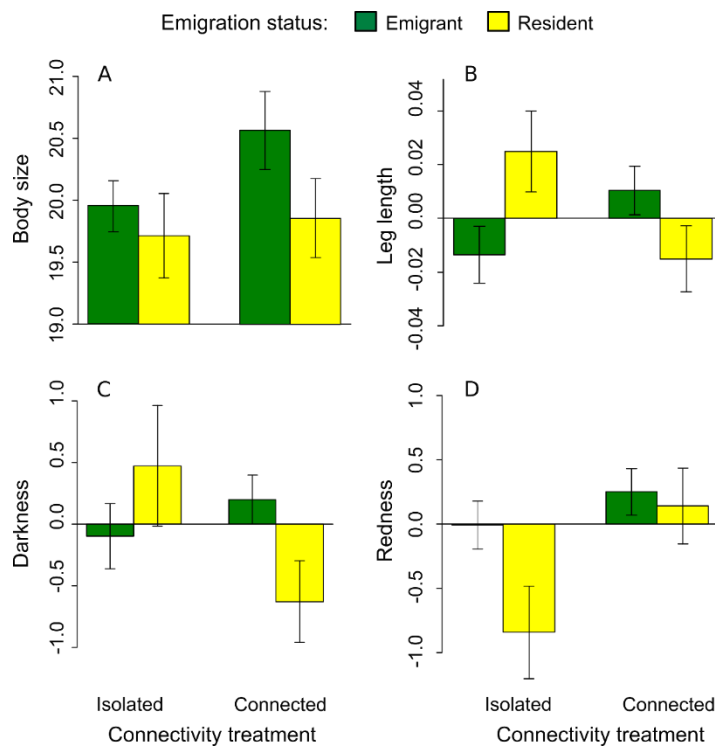
458 The multivariate analysis revealed a significant interaction between emigration status and connectivity
459 treatment (Table 4, Fig. 5). Compared to residents in connected populations, emigrants were bigger (Fig. 5A),
460 had longer legs (Fig. 5B), and were darker (Fig. 5C), while in isolated populations they had shorter legs (Fig.
461 5B) and were redder (Fig. 5D). The univariate analyses showed that this interaction was particularly significant
462 for darkness and leg length (Table 4). Emigrants were darker than residents in connected populations ($F_{(1,65)} =$
463 4.39, $P = 0.04$, Fig. 5A) but not in isolated populations ($F_{(1,58)} = 0.90$, $P = 0.347$, Fig. 5A). Emigrants had slightly
464 but non-significantly longer legs than residents in connected populations ($F_{(1,65)} = 2.44$, $P = 0.123$), Fig. 5B),
465 while in isolated populations emigrants had slightly shorter legs than residents ($F_{(1,55)} = 3.80$, $P = 0.056$, Fig. 5B).
466 The univariate analysis also revealed that emigrants tended to be redder than residents (mean ± SE 0.21 ± 0.14
467 and -0.44 ± 0.29 , respectively, Table 4). Finally, the univariate analysis also revealed a marginal interaction
468 between climate treatment and emigration status on leg length (Table 4). In the warm climate, residents tended to
469 have longer legs than emigrants (mean ± SE 0.45 ± 0.010 and 0.42 ± 0.010 , respectively, $F_{(1,56)} = 3.21$, $P =$
470 0.079), while residents and emigrants had similar leg length in the present-day climate (mean ± SE 0.40 ± 0.01
471 and 0.43 ± 0.01 , respectively; $F_{(1,67)} = 0.41$, $P = 0.526$).

471

472 **Table 4:** Results of the multivariate analysis (MANOVA) and univariate analysis (ANOVAs) testing the effect
473 of emigration status and its interaction with the climate and the connectivity treatment on newt traits. Significant
474 and marginal effects are respectively highlighted in bold and underlined.

	Emigration status		Emigration status *		Emigration status *		Emigration status *	
	F-test	P	F-test	P	F-test	P	F-test	P
Manova								
All traits	$F_{(4,119)} = 1.72$	0.149	$F_{(4,119)} = 3.63$	0.008	$F_{(4,119)} = 1.62$	0.175	$F_{(4,119)} = 0.44$	0.780
Anovas								
Body size	$F_{(1,119)} = 2.32$	0.130	$F_{(1,119)} = 0.52$	0.473	$F_{(1,119)} = 0.24$	0.624	$F_{(1,119)} = 0.07$	0.797
Leg length	$F_{(1,119)} = 0.10$	0.747	$F_{(1,119)} = 6.62$	0.011	$F_{(1,119)} = 3.74$	<u>0.055</u>	$F_{(1,119)} = 1.05$	0.308
Darkness	$F_{(1,119)} = 0.40$	0.529	$F_{(1,119)} = 5.68$	0.019	$F_{(1,119)} = 0.21$	0.651	$F_{(1,119)} = 0.52$	0.472
Redness	$F_{(1,119)} = 3.64$	<u>0.059</u>	$F_{(1,119)} = 2.35$	0.128	$F_{(1,119)} = 1.83$	0.179	$F_{(1,119)} = 0.27$	0.604

475



476

477 **Fig. 5** Interactive effects of connectivity treatment (isolated *versus* connected) and emigration status (resident
478 *versus* disperser) on newt phenotypes (mean \pm SE): body size (A), leg length (B), darkness (C) and redness (D).

479

480 Discussion

481 Using experimental mesocosms to simulate natural habitats, we investigated whether population and phenotypic
482 responses of palmate newts to a $\sim 2^\circ\text{C}$ warmer climate depended on the connectivity between warmer and cooler
483 habitats. We found that warmer climates negatively influenced the abundance of newts, altered the phenotypic
484 composition of populations, and influenced the emigration propensity in juvenile newts. As predicted,
485 connectivity between thermal habitats negated or reversed these main effects and added a distinct effect on
486 dispersal syndromes. Our results highlight the importance of considering habitat connectivity and microclimates
487 in predicting species responses to warming climates.

488

489 *Population abundance and phenotypic composition*

490 Newt abundance decreased over four years in mesocosms with warmer climates as has been demonstrated in
491 previous studies where climate warming reduced newt survival and increased the risk of population extinction
492 (Li et al. 2013; Weinbach et al. 2018). The change in newt abundance differed by life-stage, as only adult
493 abundance was reduced in warmer climates. However, our results must be interpreted cautiously as our initial
494 abundance estimates were based on natural colonization of newts rather than establishing standardized
495 population sizes across all mesocosms. Nonetheless this initial abundance was not different across the two
496 climate treatments and our results provide evidence suggesting a negative impact of warm climate on population
497 growing over time. Warmer climates could have impacted adults more than juveniles because adults may not be
498 able to compensate for the energy expenditures induced by warmer conditions. Indeed, unlike juveniles, adult
499 newts may spend part of the summer in the aquatic habitat to avoid hot and dry conditions in the terrestrial
500 habitat (Denoël 2006). However, such habitat shifts might include a cost in depletion of caloric reserves as the
501 metabolic rate of newts is higher in water than on land (Kristín and Gvoždík 2014). Adults may therefore enter
502 hibernation with a lower body condition, in turn impacting their survival. Alternatively, warm climates could
503 provoke an ageing effect that could impact adult survival as observed in other ectotherms (reviewed in Burraco
504 et al. 2020).

505 Previous studies have reported deleterious effects of hot summers on juvenile newt abundance, due
506 mostly to desiccation of breeding sites. Desiccation of breeding sites drastically reduced larval amphibian
507 survival, which had a direct impact on the abundance of post-metamorphic juveniles (Cayuela et al. 2016;
508 Weinbach et al. 2018). In our study, we added water to mesocosms daily, so the aquatic habitats did not dry out
509 even in the warm climate, which could explain the absence of any effect of a warm climate on juvenile
510 abundance. In some species, early life stages are also less impacted by warmer climates (Bestion et al. 2015b)
511 because accelerated energy acquisition in warmer climates are useful for growth. While warmer temperature can
512 accelerate larval development and the timing of metamorphic emergence in amphibians (Gvoždík 2022), it can
513 also result in a smaller size at metamorphosis, which can reduce post metamorphic survival (Urban et al. 2014).
514 At early life stages, amphibians can also show greater thermal tolerance and thermoregulatory behavior, favoring
515 acclimation to warmer temperatures (Fan et al. 2021). Finally, previous studies have showed that compared to
516 cold winters, warm winters more frequently lead to lower survival because they result in depletion of energy
517 reserves in juveniles unable to look for food (Weinbach et al. 2018). We applied climate treatments only during
518 summer, which could again explain the lack of impact on juvenile abundance. However, unlike adult abundance,
519 which we believe was estimated precisely, estimates for juveniles provide relative abundances among
520 mesocosms due to a lower detection probability of juveniles in dense vegetation than adults in pools.

521
522 Climate change can induce multiple phenotypic changes in species. Decreased body size has emerged as
523 a universal response in many organisms to climate warming but seems less pronounced in amphibians (Li et al.
524 2013) and was not observed in our study. In accordance with other taxa, Reading (2007) found that, compared to
525 cold winters, mild winters reduced body size and condition in common toads by depleting more energy reserves
526 during hibernation. Alternatively, in some frog species, body size was increased in warmer habitats (Tryjanowski
527 et al. 2006), which could be explained by either a positive effect of insect prey availability on growth rate (i.e.
528 developmental plasticity), or by an adaptive role of larger body size in desiccation resistance (i.e. selection). In a
529 previous study using the same experimental system, we found that after 3 years of treatment, common lizards

530 inhabiting warm mesocosms were bigger than in the present-day climate, mainly because of accelerated growth
531 rate at the juvenile stage (Pellerin et al. 2022). In our study, the absence of a climate effect on body size may
532 result from the summertime climatic manipulation or from opposite effects of energetic saving and prey
533 availability.

534 Adult newts and, to a smaller extent, juvenile newts were darker in warm climates. Climate warming is
535 predicted to induce a darker skin for UV protection or a paler skin to avoid overheating (Roulin 2014), with the
536 resulting effect depending on the cost-benefit balance between UV protection and overheating. In our
537 experiment, the climatic treatment was created with shutters that automatically closed above certain temperature
538 thresholds, so the warmer treatment implied more exposure to the sun and UV radiation. Amphibian skin is
539 particularly thin and UV radiation can induce strong damage that can directly affect survival (reviewed by
540 Blaustein et al. 2010). Coloration traits have been shown to be highly plastic in amphibians (Nilsson Sköld et al.
541 2013) and the development of darker skin under UV exposure has been observed in several amphibian species
542 (Garcia et al. 2004; Blaustein et al. 2010).

543 Juvenile newts in our study were also less red in warmer than in present-day climates. In amphibians,
544 the red pigments, mostly carotenoids, are acquired through dietary sources (Mochida et al. 2013). In red-eyed
545 tree frogs (*Agalychnis callidryas*), a diet rich in carotenoids leads to redder skin and has a positive effect on
546 juvenile development and female fecundity (Ogilvy et al. 2012). Warm climates may reduce newt's prey
547 availability and diversity, and hence access to dietary pigments. Carotenoids may also provide protection against
548 the pro-oxidative effects of UV induced by our warming treatment (Ogilvy and Preziosi 2012) and making
549 carotenoids less available for skin color. Regardless of the exact determinant, redder individuals are often
550 considered healthier, advantaged against predators (*i.e.*, toxicity signal, (Mochida et al. 2013), and have higher
551 reproductive success (Ogilvy and Preziosi 2012). Overall, the decrease of red pigments in juveniles from our
552 experiment suggests the potential for negative effects from warmer conditions.

553

554 *Connectivity mitigates the effects of climate warming*

555 Interestingly, access to microclimatic refuges negated the effect of warming on newt abundance and suggests a
556 mitigating effect of these refuges for population persistence (Scheffers et al. 2013). Newts in our study may have
557 migrated to the cooler climate habitats during the warmer season to prevent overheating, as found in other
558 ectotherms (Chukwuka et al. 2021). However,, we could not monitor movements between microclimates because
559 newts were not individually marked. Movements could also have allowed a reorganization of individuals
560 according to their thermal optimum, with the phenotypes less adapted to warm climate settling in cooler habitats
561 and vice-versa (Bestion et al. 2015a). Such adaptive dispersal could reduce the negative impacts of warming
562 climate on (meta)population persistence (Pellerin et al. 2019), and is a likely hypothesis given the changes in
563 phenotypic composition in connected populations.

564 Contrary to patterns observed in isolated habitats, newts were paler in the warm climate than in the
565 present-day climate in connected habitats. Darker newts may have moved from the warm climate to the present-
566 day climate to prevent overheating (Roulin 2014), and paler individuals may have moved to the warmer climate
567 to meet their thermal needs. In amphibians, smaller sized juveniles are at greater risk of desiccation and their
568 strictly terrestrial lifestyle may lead them to search for terrestrial microclimatic refuges (Enriquez-Urzelai et al.
569 2019). Darker individuals may therefore seek microclimatic refuges to prevent overheating. In contrast to

570 juveniles, darker adults could avoid risk of overheating by remaining in the water during summer, while still
571 remaining more protected against UV radiation.

572 Connectivity between microclimates further mitigated the effect of warm climate on red coloration.
573 This suggests that the possibility to reach a cooler microclimate can decrease oxidative stress and may leave
574 more carotenoids available for the skin (Ogilvy and Preziosi 2012) or increase the availability of prey that
575 provide the pigments. Information on food availability and diet preference of newts in the two climatic
576 treatments would allow a better understanding of the underlying mechanisms.

577

578 *Emigration propensity and dispersal syndrome*

579 We found that newts from populations with present-day climate temperatures emigrated slightly more often from
580 warmer than from cooler conditions, while newts from populations with warmer climates emigrated equally
581 under both thermal conditions. This might suggest an acclimation to warmer temperatures by newts from warm
582 climates induced by developmental plasticity. Acclimation to warm temperatures has previously been shown in
583 several newt species through locomotor activity, metabolic rate and critical thermal maximum, and these plastic
584 responses can mitigate the impacts of climate change to some extent (Gvoždík et al. 2007; Winterová & Gvoždík
585 2022). Acclimation to warmer temperatures could also result from natural selection, but given the short duration
586 of this study this explanation is unlikely.

587 While dispersal can facilitate colonization of areas with suitable climatic conditions, this depends on the
588 phenotypic specialization of the dispersers, which modulates their success in travelling or adapting to novel
589 conditions (Clobert et al. 2009). In this study we assessed dispersal syndrome by measuring traits that enhance
590 movement (body size and leg length) on the one hand, and traits related to local adaptation/acclimation
591 (coloration) and movement propensity on the other. In connected populations, we found evidence of dispersal
592 syndrome among almost all the traits measured. Emigrant newts were bigger, had longer legs and were darker
593 than residents. Body size and leg length are the main common enhancing traits of dispersers, and this
594 phenomenon has been well-documented in other amphibians (Denoël et al. 2018; Winandy et al. 2019). The
595 positive association between darkness in coloration and dispersal has been mostly described in birds (Roulin,
596 2014; Saino et al., 2014), and may arise as a by-product of correlation with other physiological, morphological
597 and behavioral traits. For example, darkness is positively correlated to boldness and exploration (Mafli et al.
598 2011; Mateos-Gonzalez and Senar 2012), two behavioral traits often associated to a higher dispersal propensity.
599 Yet habitat isolation largely altered these syndromes in our study. Emigrants had shorter legs than residents but
600 did not differ in body size and darkness. As found in other species (Cote et al. 2022), specific environmental
601 conditions can disrupt the relationships between phenotypic traits and dispersal and may result in additional
602 dispersal costs (Winandy et al. 2019). While the higher abundance of newts in connected populations in our
603 study could suggest adaptive movements, a longer-term follow-up of the fitness of individuals of different
604 phenotypes and emigration decisions is needed to validate this hypothesis. The interaction between the
605 phenotype and environmental conditions is complex, and challenging environments such as warmer and more
606 fragmented habitats can modify dispersal syndrome. This could have consequences for the success of range
607 shifts and, to a greater extent, on phenotypic composition and/or survival of populations (Pellerin et al. 2019).

608

609 *Conclusion*

610 The spatial configuration of habitats in the landscape can influence the response of organisms to warming
611 climates (Opdam and Wascher 2004). In this study, we found interactive effects of these factors on newt
612 populations: when newts do not have the opportunity to escape warmer temperature, their abundance declines
613 and the phenotypic composition of populations is altered, while microclimate connectivity seems to alleviate
614 these effects. We cannot tease apart the effects of genetic selection and phenotypic plasticity on the observed
615 phenotypic changes. Yet coloration traits have been shown to be highly plastic in amphibians (Nilsson Sköld et
616 al. 2013). By buffering the adverse effects of high temperatures, phenotypic plasticity can be a first step of
617 population persistence (Seebacher et al. 2014). Indeed, in amphibians, thermal acclimation through behavioral
618 thermoregulation or physiological heat tolerance can act as efficient compensatory mechanisms (Gvoždík et al.
619 2007). We did not measure these thermal traits in our study, so we do not know if newts developed thermal
620 compensation in warmer climates. Yet even the most plastic traits may prove insufficient to fully compensate for
621 the consequences of overheating in ectotherms (Gunderson and Stillman 2015). In this case, emigration of less
622 adapted phenotypes to a habitat that better matches their phenotype could be a way of ensuring population
623 persistence, a hypothesis which needs to be validated through long term monitoring of the survival and
624 reproduction of individuals (Merilä and Hendry 2014).

625 Landscape connectivity to microclimatic refuges seems to be a key factor that could buffer species
626 against regional extirpation (Suggitt et al. 2018). Access to microclimatic refuges can be particularly important
627 in the case of amphibians such as newts that have relatively limited dispersal capacity. Conservation of
628 microclimatic areas like forests and wetlands as well as maintaining landscape connectivity should be a primary
629 consideration to reduce the impact of climate warming on amphibian diversity (Scalercio et al. 2009; Frey et al.
630 2016).

631

632 **References**

- 633 Balogová M, Gvoždík L (2015) Can Newts Cope with the Heat? Disparate Thermoregulatory Strategies of Two
634 Sympatric Species in Water. *PLoS ONE* 10(5): e0128155.
- 635 Bates D, Mächler M, Bolker B, Walker S (2008) Fitting mixed-effects models using the lme4 package in R. In:
636 International Meeting of the Psychometric Society Bestion E, Clobert J, Cote J (2015a) Dispersal
637 response to climate change: scaling down to intraspecific variation. *Ecology Letters* 18:1226–1233
- 638 Bestion E, Teyssier A, Richard M, et al (2015b) Live fast, die young: experimental evidence of population
639 extinction risk due to climate change. *PLoS biology* 13:e1002281
- 640 Blaustein AR, Walls SC, Bancroft BA, et al (2010) Direct and indirect effects of climate change on amphibian
641 populations. *Diversity* 2:281–313
- 642 Burraco P, Orizaola G, Monaghan P, Metcalfe NB (2020) Climate change and ageing in ectotherms. *Global
643 Change Biology* 26:5371–5381
- 644 Cayuela H, Arsovski D, Bonnaire E, et al (2016) The impact of severe drought on survival, fecundity, and
645 population persistence in an endangered amphibian. *Ecosphere* 7:e01246
- 646 Cayuela H, Grolet O, Joly P (2018) Context-dependent dispersal, public information, and heterospecific
647 attraction in newts. *Oecologia* 188:1069–1080
- 648 Chukwuka CO, Mello RS, Cree A, Monks JM (2021) Thermal heterogeneity of selected retreats in cool-
649 temperate viviparous lizards suggests a potential benefit of future climate warming. *Journal of Thermal
650 Biology* 97:102869

- 651 Clobert J, Le Galliard J-F, Cote J, et al (2009) Informed dispersal, heterogeneity in animal dispersal syndromes
652 and the dynamics of spatially structured populations. *Ecology letters* 12:197–209
- 653 Cote J, Bestion E, Jacob S, Travis J, Legrand D, Baguette M (2017). Evolution of dispersal strategies and
654 dispersal syndromes in fragmented landscapes *Ecography*, 40: 56-73
- 655 Cote J, Dahirel M, Altermatt F, et al (2022) Dispersal syndromes in challenging environments: a cross-species
656 experiment. *Ecology Letters* 25: 2675-2687
- 657 Denoël M (2006) Seasonal variation of morph ratio in facultatively paedomorphic populations of the palmate
658 newt *Triturus helveticus*. *Acta oecologica* 29:165–170
- 659 Denoël M, Dalleur S, Langrand E, et al (2018) Dispersal and alternative breeding site fidelity strategies in an
660 amphibian. *Ecography* 41:1543–1555
- 661 Díaz SM, Settele J, Brondízio E, et al (2019) The global assessment report on biodiversity and ecosystem
662 services: Summary for policy makers
- 663 Enriquez-Urzelai U, Bernardo N, Moreno-Rueda G, et al (2019) Are amphibians tracking their climatic niches in
664 response to climate warming? A test with Iberian amphibians. *Climatic Change* 154:289–301
- 665 Fan XL, Lin ZH, Scheffers BR (2021) Physiological, developmental, and behavioral plasticity in response to
666 thermal acclimation. *Journal of Thermal Biology* 97:102866
- 667 Frey SJ, Hadley AS, Johnson SL, et al (2016) Spatial models reveal the microclimatic buffering capacity of old-
668 growth forests. *Science advances* 2:e1501392
- 669 Fronhofer EA, Legrand D, Altermatt F, et al (2018) Bottom-up and top-down control of dispersal across major
670 organismal groups. *Nature Ecology & Evolution* 2:1859–1863
- 671 Garcia TS, Stacy J, Sih A (2004) Larval salamander response to UV radiation and predation risk: color change
672 and microhabitat use. *Ecological Applications* 14:1055–1064
- 673 Gunderson AR, Stillman JH (2015) Plasticity in thermal tolerance has limited potential to buffer ectotherms
674 from global warming. *Proceedings of the Royal Society B: Biological Sciences* 282:20150401
- 675 Gvoždík, L. (2015) Mismatch Between Ectotherm Thermal Preference and Optima for Swimming: A Test of the
676 Evolutionary Pace Hypothesis. *Evolutionary Biology* 42: 137–145
- 677 Gvoždík L, Puky M, ŠUGERKOVÁ M (2007) Acclimation is beneficial at extreme test temperatures in the
678 Danube crested newt, *Triturus dobrogicus* (Caudata, Salamandridae). *Biological Journal of the Linnean
679 Society* 90:627–636
- 680 Huey RB, Kearney MR, Krockenberger A, et al (2012) Predicting organismal vulnerability to climate warming:
681 roles of behaviour, physiology and adaptation. *Philosophical Transactions of the Royal Society B:
682 Biological Sciences* 367:1665–1679
- 683 Joly P, Miaud C (1989) Fidelity to the breeding site in the alpine newt *Triturus alpestris*. *Behavioural Processes*
684 19:47–56
- 685 Kristín P, Gvoždík L (2014) Aquatic-to-terrestrial habitat shift reduces energy expenditure in newts. *Journal of*
686 *Experimental Zoology Part A: Ecological Genetics and Physiology* 321:183–188
- 687 Lê S, Josse J, Husson F (2008) FactoMineR: an R package for multivariate analysis. *Journal of statistical*
688 *software* 25:1–18
- 689 Legrand D, Guillaume O, Baguette M, et al (2012) The Metatron: an experimental system to study dispersal and
690 metaecosystems for terrestrial organisms. *Nature methods* 9:828–833

- 691 Legrand D, Trochet A, Moulherat S, et al (2015) Ranking the ecological causes of dispersal in a butterfly.
692 *Ecography* 38:822–831
- 693 Lenoir J, Svenning J-C (2015) Climate-related range shifts—a global multidimensional synthesis and new
694 research directions. *Ecography* 38:15–28
- 695 Li Y, Cohen JM, Rohr JR (2013) Review and synthesis of the effects of climate change on amphibians.
696 *Integrative Zoology* 8:145–161
- 697 Lüdecke D, Ben-Shachar MS, Patil I, et al (2021) performance: An R package for assessment, comparison and
698 testing of statistical models. *Journal of Open Source Software* 6:
- 699 Lüdecke D, Lüdecke MD (2019) Package ‘sjstats.’ Statistical functions for Regression Models, Version 017 3:
- 700 Mafli A, Wakamatsu K, Roulin A (2011) Melanin-based coloration predicts aggressiveness and boldness in
701 captive eastern Hermann’s tortoises. *Animal Behaviour* 81:859–863
- 702 Masson-Delmotte V, Zhai P, Pirani A, Connors SL, Péan C, Berger S et al. (2021). Climate change 2021: the
703 physical science basis. Contribution of working group I to the sixth assessment report of the
704 intergovernmental panel on climate change, 2.
- 705 Mateos-Gonzalez F, Senar JC (2012) Melanin-based trait predicts individual exploratory behaviour in siskins,
706 *Carduelis spinus*. *Animal Behaviour* 83:229–232
- 707 McGaughran A, Laver R, Fraser C (2021) Evolutionary responses to warming. *Trends in Ecology & Evolution*
708 36:591–600
- 709 Merilä J, Hendry AP (2014) Climate change, adaptation, and phenotypic plasticity: the problem and the
710 evidence. *Evolutionary applications* 7:1–14
- 711 Milling CR, Rachlow JL, Olsoy PJ, et al (2018) Habitat structure modifies microclimate: An approach for
712 mapping fine-scale thermal refuge. *Methods in Ecology and Evolution*, 9: 1648-1657
- 713 Mochida K, Kitada M, Ikeda K, et al (2013) Spatial and temporal instability of local biotic community mediate a
714 form of aposematic defense in newts, consisting of carotenoid-based coloration and tetrodotoxin.
715 *Journal of chemical ecology* 39:1186–1192
- 716 Nilsson Sköld H, Aspengren S, Wallin M (2013) Rapid color change in fish and amphibians—function,
717 regulation, and emerging applications. *Pigment cell & melanoma research* 26:29–38
- 718 Ogilvy V, Preziosi RF (2012) Can carotenoids mediate the potentially harmful effects of ultraviolet light in
719 *Silurana (Xenopus) tropicalis* larvae? *Journal of animal physiology and animal nutrition* 96:693–699
- 720 Ogilvy V, Preziosi RF, Fidgett AL (2012) A brighter future for frogs? The influence of carotenoids on the health,
721 development and reproductive success of the red-eye tree frog. *Animal Conservation* 15:480–488
- 722 Opdam P, Wascher D (2004) Climate change meets habitat fragmentation: linking landscape and
723 biogeographical scale levels in research and conservation. *Biological conservation* 117:285–297
- 724 Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems.
725 *nature* 421:37–42
- 726 Pellerin F, Bestion E, Winandy L, et al (2022) Connectivity among thermal habitats buffers the effects of warm
727 climate on life-history traits and population dynamics. *Journal of Animal Ecology* 91: 2301–2313
- 728 Pellerin F, Cote J, Bestion E, Aguilée R (2019) Matching habitat choice promotes species persistence under
729 climate change. *Oikos* 128:221–234
- 730 Pittman SE, Osbourn MS, Semlitsch RD. (2014). Movement ecology of amphibians: a missing component for
731 understanding population declines. *Biological Conservation*, 169, 44-53.

- 732 Reading CJ (2007) Linking global warming to amphibian declines through its effects on female body condition
733 and survivorship. *Oecologia* 151:125–131
- 734 Román-Palacios C, Wiens JJ (2020) Recent responses to climate change reveal the drivers of species extinction
735 and survival. *Proceedings of the National Academy of Sciences* 117:4211–4217
- 736 Roulin A (2014) Melanin-based colour polymorphism responding to climate change. *Global Change Biology*
737 20:3344–3350
- 738 Saino N, Romano M, Scandolara C, et al (2014) Brownish, small and lousy barn swallows have greater natal
739 dispersal propensity. *Animal Behaviour* 87:137–146
- 740 Scalercio S, Russo M, Dapporto L (2009) Wetlands are refuge areas that delay global warming-induced range
741 shift of Lepidoptera. *Handbook of nature conservation: Global, Environmental and Economic Issues*,
742 Nova Publishers, New York 393–406
- 743 Scheffers BR, Phillips BL, Laurance WF, et al (2013) Increasing arboreality with altitude: a novel biogeographic
744 dimension. *Proceedings of the Royal Society B: Biological Sciences* 280:20131581
- 745 Seebacher F, Beaman J, Little AG (2014) Regulation of thermal acclimation varies between generations of the
746 short-lived mosquitofish that developed in different environmental conditions. *Functional Ecology*
747 28:137–148
- 748 Sillero N (2021) Climate change in action: local elevational shifts on Iberian amphibians and reptiles. *Regional
749 Environmental Change* 21:1–13
- 750 Suggitt AJ, Wilson RJ, Isaac NJ, et al (2018) Extinction risk from climate change is reduced by microclimatic
751 buffering. *Nature Climate Change* 8:713–717
- 752 Thompson RM, Beardall J, Beringer J, et al (2013) Means and extremes: building variability into community-
753 level climate change experiments. *Ecology Letters* 16:799–806
- 754 Tryjanowski P, Sparks T, Rybacki M, Berger L (2006) Is body size of the water frog *Rana esculenta* complex
755 responding to climate change? *Naturwissenschaften* 93:110–113
- 756 Urban MC (2015) Accelerating extinction risk from climate change. *Science* 348:571–573
- 757 Urban MC, Richardson JL, Freidenfelds NA (2014) Plasticity and genetic adaptation mediate amphibian and
758 reptile responses to climate change. *Evolutionary Applications* 7:88–103
- 759 van den Brink V, Dreiss AN, Roulin A (2012) Melanin-based coloration predicts natal dispersal in the barn owl,
760 *Tyto alba*. *Animal Behaviour* 84:805–812
- 761 Verell PA (1987). The directionality of migrations of amphibians to and from a pond in southern England, with
762 particular reference to the smooth newt, *Triturus vulgaris*. *Amphibia-Reptilia* 8: 93-100
- 763 Weinbach A, Cayuela H, Grolet O, et al (2018) Resilience to climate variation in a spatially structured
764 amphibian population. *Scientific Reports* 8:1–9
- 765 Winandy L, Cote J, Di Gesu L, et al (2019) Local predation risk and matrix permeability interact to shape
766 movement strategy. *Oikos* 128:1402–1412