



Variation of preferred body temperatures along an altitudinal gradient: A multi-species study



Audrey Trochet^{a,*}, Andréaz Dupoué^a, Jérémie Souchet^a, Romain Bertrand^{a,b}, Marine Deluen^{a,c}, Sophie Murarasu^{a,e}, Olivier Calvez^a, Albert Martinez-Silvestre^d, Isabel Verdaguer-Foz^d, Elodie Darnet^a, Hugo Le Chevalier^a, Marc Mossoll-Torres^{f,g}, Olivier Guillaume^a, Fabien Aubret^a

^a CNRS, Station d'Ecologie Théorique et Expérimentale, UMR 5321 CNRS – Université Paul Sabatier, 09200 Moulis, France

^b CNRS, Centre for Biodiversity Theory and Modelling, Station d'Ecologie Théorique et Expérimentale, UMR 5321 CNRS – Université Paul Sabatier, 09200 Moulis, France

^c Université de Perpignan, Via Domitia, 66100 Perpignan, France

^d Catalonia Reptile and Amphibian Rescue Center (CRARC), 08783 Masquefa, Barcelona, Spain

^e Université Pierre et Marie CURIE, 4 place Jussieu, 75252 Paris, France

^f Pirenalía, c/ de la rectoria, 2 Casa Cíntet, AD200 Encamp, Andorra

^g Bomosa, Pl. Parc de la Mola, 10 Torre Caldea 7, AD700 Les Escaldes, Andorra

ARTICLE INFO

Keywords:

Body preferred temperatures

Lizards

Amphibians

Altitude

Countergradient hypothesis

Thermal strategies

ABSTRACT

Temperature affects the physiological functions of ectotherms. To maintain optimal body temperature and ensure physiological performance, these organisms can use behavioral adjustments to keep the body temperature in their specific temperature range, so-called preferred temperature (T_{pref}). It is therefore crucial to describe and understand how T_{pref} vary within and amongst populations to predict the effects of climate change of altitudinal range shifts in organisms. We aimed at determining the altitudinal variations in T_{pref} in three ectothermic species (the Pyrenean brook salamander – a semi-aquatic and thigmothermic amphibian – the European common lizard and the wall lizard – both heliothermic species). Using an experimental approach where T_{pref} were measured along a temperature gradient in laboratory conditions, we used a cross-sectional approach to compare the variation of T_{pref} measured in populations sampled along the altitudinal gradient in the Pyrenees. We hypothesized a complex and highly variable intra-specific response of T_{pref} along geographical clines, with a positive relationship between T_{pref} and altitude (as predicted by the countergradient variation), the reverse pattern (referring to the adaptation of local optima hypothesis), or no relationship at all. Our results corroborated partially the countergradient hypothesis in the salamander (middle to high elevation part). At high altitude level, individuals may compensate for lower opportunities of favorable conditions by choosing a high temperature which maximizes their activities. However, populations from low elevation level hence better supported the adaptation of local optima hypothesis, such as both lizard species, for which T_{pref} tended instead to decrease with altitude. Lizards from cold climates may be physiologically adapted to low temperature, with the ability to reach optimal functioning at lower temperature than individuals from low altitude. Our findings suggest that predicting future niche models should therefore take into account the variability of T_{pref} , where species might be differently affected by global warming.

1. Introduction

Environmental (operative) temperature varies seasonally and geographically along latitudinal and altitudinal clines (Gvozdík, 2002; Sunday et al., 2014). For several decades, the variation of mean global air temperature has been associated with anthropogenic activities resulting notably in a global increase in temperature over the world (+ 0.12 °C per decade [0.08–0.14 °C] in average since 1951; IPCC, 2014).

The current climate change generates important and heterogeneous spatial redistribution of historical climate conditions (Loarie et al., 2009), with various consequences at every level of biodiversity complexity (e.g. Peñuelas et al., 2013). Indeed, global warming may force organisms to adapt, migrate (by driving upslope and poleward range shifts of many populations and/or species towards new sustainable climate conditions; Botts et al., 2015) or, when these are not possible, go extinct (Jump and Peñuelas, 2005; Parmesan, 2006; Aitken et al.,

* Corresponding author.

E-mail address: audrey.trochet@sete.cnrs.fr (A. Trochet).

<https://doi.org/10.1016/j.jtherbio.2018.08.002>

Received 24 April 2018; Received in revised form 24 July 2018; Accepted 6 August 2018

Available online 08 August 2018

0306-4565/ © 2018 Elsevier Ltd. All rights reserved.

2008; Sinervo et al., 2010). A major driver of biological responses to climate change is the physiological sensitivity of organisms in the face of climatic conditions of their natural environment (Bernardo et al., 2007; Calosi et al., 2007). Evaluating the impacts of operative temperature on such functional responses is therefore essential to parameterize predictive models and to clarify the organisms' response to climate changes (Buckley et al., 2010; Li et al., 2013).

Ectotherms (more than 98% of animal species) are likely to be highly vulnerable in the face of climate change because their physiological performance is affected by body temperature, for which variation depends on the availability of environmental (operative) temperatures (Angilletta, 2009). The relationship between physiological performance and body temperature in ectotherms is a nonlinear asymmetric curve, bounded by extreme low and high temperatures (i.e. lower and upper critical temperatures; Paaijmans et al., 2013). The thermal zone between both critical limits shapes the thermal tolerance range, where the given species can tolerate the environmental temperatures (Stevenson et al., 1985; Sinclair et al., 2016) with a maximum performance reached at an optimal temperature, usually higher than the preferred body temperature (T_{pref} is the body temperature maintained by an ectotherm; Hertz et al., 1993). To maintain optimal body temperature and ensure physiological performance, ectotherms mainly rely on behavioral thermoregulation (Angilletta, 2009). Behavioral thermoregulation is characterized by a set of measures including T_{pref} (Porter et al., 1973; Stevenson, 1985; Hertz et al., 1993; Kearney et al., 2009). One important point is that T_{pref} is not necessarily homogenous within populations and may notably vary along environmental clines (Artacho et al., 2017). But T_{pref} can also stay fairly stable across ecogeographical clines (see an example in the large *Psammotromus* lizard; Zamora-Camacho et al., 2013). Thus, to investigate the current and future effects of climate change of altitudinal range shifts in organisms, it is crucial to first describe and understand how T_{pref} varies within populations that typically span several hundred or even thousand meters in altitudinal range.

We expected a complex and highly variable intra-specific response of T_{pref} in the face of climate change along geographical clines (Valladares et al., 2014). Individuals at high altitude (i.e. a cold environment) may select higher temperatures in order to compensate for lower opportunities of finding favorable temperatures than at low altitude (Aubret and Michniewicz, 2010; Marco et al., 2016; Tedeschi et al., 2016), as predicted by the countergradient variation hypothesis (Conover and Schultz, 1995). This strategy may allow individuals to maximize their performance under low frequent favorable conditions. In contrast, individuals may select colder temperatures (i.e. a decrease T_{pref}) as elevation increase in order to be adapted to local climate conditions, following the adaptation of local optima hypothesis (Levinton, 1983). A last hypothesis also supposes that T_{pref} might stay stable across altitudinal gradient, because ectotherms may buffer selection pressure of thermal environment on T_{pref} by behavioral thermoregulation (Bogert, 1949; Stevenson, 1985). The three hypotheses differ in their assumptions on trade-offs but can result from plasticity or genetic local adaptation. As a consequence, climate change is expected to have contrasting effects on species along geographical gradients (Vittoz et al., 2009; Rosbakh et al., 2014) affecting future range shifts depending intraspecific variation in species temperature response. To the best of our knowledge, considering species temperature response as a varying trait throughout the geographical space to explain and predict the impact of climate change on species remain under-investigated. Predicting future niche shift should therefore take into account the variability of T_{pref} , instead of projecting of current suitable temperature range for a given organism into a changing environment (i.e. upwards move of the climate envelope). Considering ecogeographical variation in T_{pref} could challenge actual predictions of the climate change impacts on biodiversity. Indeed, it has been recently demonstrated that local adaptation to current climate conditions could lead to higher decrease in species range under future climate change scenarios (Valladares

et al., 2014).

In the current study, we aimed at determining the altitudinal variations in T_{pref} in three ectothermic species occurring in different thermal conditions. We used a cross-sectional approach to compare the variation of T_{pref} measured in standard conditions in populations sampled along the altitudinal gradient in the Pyrenees mountains. Specifically, we focused on species from middle to high altitude, the Pyrenean brook salamander (*Calotriton asper*, $n = 159$ individuals from 8 populations) and the European common lizard (*Zootoca vivipara*, $n = 264$ individuals from 12 populations), and a species of low elevation level, the wall lizard (*Podarcis muralis*, $n = 90$ individuals from 9 populations). The three species exhibit different behavioral thermoregulation, from thigmothermy (behavioral thermoregulation by contact with substrates or a warm object in the environment) in the brook salamander to heliothermy (behavioral thermoregulation by radiant heat from the sun) in lizards, both mechanisms involved in heat gain to access optimal body temperature in ectotherms (Carrascal et al., 1992; Hutchison and Dupré, 1992; Belliure and Carrascal, 2002). Because the relationship between altitude and temperature is not straightforward, and that thermal conditions can also vary with slope orientation or top-valley, we sampled individuals from an extended altitudinal gradient in order to compensate for change in microclimatic conditions. We tested if T_{pref} co-varies with altitude following the hypotheses exposed above and finally discussed the pattern observed in regards of its potential consequences to understand and predict ectotherm species' response to climate change.

2. Material and methods

2.1. Study species and populations

2.1.1. The Pyrenean brook salamander

The Pyrenean brook salamander (*Calotriton asper*) is a large-bodied urodele species native to the Pyrenees Mountains, at the interface between France, Andorra and Spain. This species is commonly found between 400 and 2500 m above sea level (Clergue-Gazeau and Martínez-Rica, 1978). Individuals grow to about 105–120 mm total length in males and 110–140 mm total length in females. Moreover, this species is considered as a low-disperser animal, with low dispersal rate and weak gene flow between populations (Milá et al., 2010) which could favor local adaptation, with strong morphological variability among populations (Serra-Cobo et al., 2000).

Individuals are active during both day and night, and can be relatively easily found under rocks or walking on the bottom of a body of water. Habitats of the Pyrenean brook salamander are small rivers and lakes (rivers with no or low fish density, width of river and cover vegetation variable) but few low-elevation populations live in caves. Individuals from rivers can move to basins of water or under rocks where water flow is lower and temperature is higher than in the middle of the river (pers. comm.). The brook salamander mainly relies on thigmothermy (behavioral thermoregulation by contact with substrates or a warm object in the environment) and is able to select microhabitats (Spotila, 1972; Heath, 1975; Balogová and Gvoždík, 2015).

Because the relationship between altitude and temperature is not straightforward, and that thermal conditions can also vary with slope orientation or top-valley, we sampled 159 Pyrenean brook salamanders (86 females and 73 males) from eight different populations (North and South slopes) across an elevation gradient from 625 m to 2189 m in altitude (Appendix A). Individuals captured between the 10th and the 25th of June 2017 were housed at the Catalonia Reptile and Amphibian Rescue Center (CRARC, Masquefa, Spain, coordinates: 41°29'52.61"N, 1°48'51.44"E), and those captured between the 15th of June and the 27th of July 2017 were housed at the Station d'Ecologie Théorique et Expérimentale (SETE, Moulis, France; coordinates: 42°57'29.82"N, 1°05'11.27"E). We captured non-gravid females to avoid influence of reproduction on T_{pref} (Gvoždík, 2005). All individuals were PIT-tagged

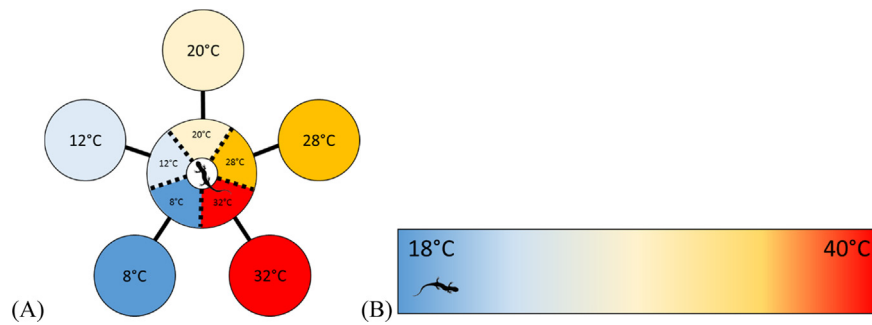


Fig. 1. Schematic representation of the experimental devices for testing the preferred body temperatures (T_{pref}) in (A) *Calotriton asper* and (B) the two lizard species *Zootoca vivipara* and *Podarcis muralis*.

(BIOLOG-ID, France) following the same procedure before the experiments, allowing unambiguous identification of each individual. Animals were all housed in same-species groups of 10 individuals in aquaria of $80 \times 35 \times 40$ cm (sex-ratios per group: mean \pm SD: 0.91 ± 0.20 ; range: from 0.40 to 1.86). They were kept at a water temperature of 15 °C (Raffaelli, 2007; Silvestre, 2011). Animals were kept under controlled air temperature conditions (mean \pm SD: $20^\circ\text{C} \pm 1^\circ\text{C}$) and exposed to a photoperiod (12 h of light and 12 h of darkness) provided by artificial lighting (28 W heating bulb). Because digestion may influence T_{pref} in newts (Gvoždík, 2003), salamanders were fed with mealworms and *Tubifex* worms 24 h before experiments and ad libitum outside test periods.

2.1.2. The common lizard

The common lizard (*Zootoca vivipara*) is a small (adult snout-vent length \sim 50–75 mm), widespread species in the family Lacertidae that inhabits peat bogs and heathlands across northern Eurasia. In the Pyrenees Mountain range, this species is at the southern limit of its distribution area and common between 300 and 2500 m (Salvador et al., 2014). The common lizard inhabits a wide variety of habitats although it is mostly found in great density in humid and cold habitats. This species mainly heat up in basking in the sun while alternating with shade area (Gvoždík, 2002).

We sampled 264 individuals from 12 populations distributed from 488 to 2001 m above sea level (Appendix A). Individuals (males and non-reproductive females) were captured between 15th July and 8th August 2017. The day of capture, lizards were brought back to the SETE and individualized in terrarium ($26 \times 38 \times 23$ cm). All terraria were equipped with sterilized soil, a shelter and a basking point under a 25 W light bulb to provide a thermal gradient (20 – 35 °C) for 6 h per day (09:00–12:00 and 14:00–17:00). Individuals were kept two days before thermal measurements and were not fed to avoid the effect of digestion on T_{pref} (Qu et al., 2011). Water was sprayed three times per day (09:00, 13:00, and 17:00). The day following the measurement, all individuals were released at the population locality.

2.1.3. The wall Lizard

The wall lizard (*Podarcis muralis*) is a small (adult snout-vent length \sim 48–68 mm) species in the family Lacertidae. This species is widely distributed in Europe around the Mediterranean area, from sea level up to 2500 m above sea level. The wall lizard inhabits a wide variety of habitats, including rocky and scree areas, scrubland, deciduous and coniferous woodland, orchards, vineyards, fields, stone walls, and on buildings. This species mainly heat up in basking in the sun while alternating with shade area. A complete description of this species is given by Pérez-Mellado (1998) (see also Diego-Rasilla, 2015).

We sampled 90 individuals from 9 populations distributed from 474 to 1400 m above sea level (Appendix A). Individuals were captured between 19th April and 11th May. The day of capture, individuals were brought back to the SETE, marked with low temperature cautery (Bovie®) by the technique of heat branding (Winne et al., 2006).

Individuals from the same population were housed by two in one terrarium of $26 \times 38 \times 23$ cm. All terrarium was equipped with sterilized substrate, a shelter and ad libitum water and food (mealworms). Individuals was exposed to a photoperiod (9 h of light and 15 h of darkness) provided by artificial lighting (28 W heating bulb). Individuals were kept few days and were not fed one day before thermal measurements to avoid the effect of digestion on preferred body temperatures (Qu et al., 2011).

2.2. Measurements of T_{pref}

2.2.1. Tests on *Calotriton asper*

In both places (SETE in France and CRARC in Spain), water preferred body temperatures were recorded on 159 individuals (73 males and 86 females) using standard device and protocol under controlled-temperature conditions (mean \pm SD: $15^\circ\text{C} \pm 1^\circ\text{C}$). The experimental device (Fig. 1) featured six basins, with a central test basin connected to five peripheral basins with different water temperatures (from 8 °C to 32 °C). The “cold” basins were cooled using coolers (Chiller 200 BLUE MARINE) which maintained the water temperature at $8^\circ\text{C} \pm 2^\circ\text{C}$ and $12^\circ\text{C} \pm 2^\circ\text{C}$. The “temperate” basin was filled with tap water where the temperature was $20^\circ\text{C} \pm 2^\circ\text{C}$. The “warm” basins were heated using thermostatic heaters (thermocontrol 100 W EHEIM JÄGER) which maintained the water temperature at $28^\circ\text{C} \pm 2^\circ\text{C}$ and $32^\circ\text{C} \pm 2^\circ\text{C}$ respectively. The central basin was connected with each peripheral basin by a darkened tube about 5 cm in diameter and 40 cm long. In the central basin, a pipe allowed the evacuation of water to an auxiliary tank. Then, a pump redistributed the water from the tank to the peripheral basins, creating a continuous water flow in a closed circuit. All the tests (habituation and survey) were filmed using a video camera.

Each individual was placed into the central test basin (Fig. 1A) and maintained with a little transparent box for 2 min of habituation. Then, we removed the transparent box and left for individual temperature choice during 30 min. During the experiment (between 10:00 and 18:00) individuals were free to move into the test basin and could also enter into the darkened tubes between the test and the peripheral basins. To take into account temperature variation ($21.11 \pm 0.85^\circ\text{C}$), we also recorded the water temperature of each basin between each experiment. From videos we recorded for each individual the time spent in each zone (i.e. 8 °C, 12 °C, 20 °C, 28 °C or 32 °C; Fig. 1A) and the number of zone changes during the 30 min of experiment. In newt and salamander species, body temperatures closely approach those of the surrounding water (Gvoždík, 2005). We therefore considered temperature of each zone as a reliable indirect estimate of body temperatures in Pyrenean brook salamanders. We then calculated a water preferred temperature (T_{pref}) (with T_{pref} variance) using the weighted average of the time spent in each zone (correcting by the temperature recorded in each basin between tests).

2.2.2. Tests on *Zootoca vivipara* and *Podarcis muralis*

The tests were performed into an air conditioned room at 18 °C and under constant room light. The experimental design comprised 15 heat gradient tracks each measuring 100 × 20 × 30 cm. A heating bulb (28 W) was placed at one end of each track and generates a thermal gradient on air of track from 18 °C to 40 °C (Fig. 1B).

Each individual was placed two days post-prandial on a track and let habituated from one hour. We then measured skin surface temperature with multiple sampling every 15 min (12 measures in *Z. vivipara*; 21 measures in *P. muralis*) between 9:00 and 17:00 (Fig. 1B). The temperature readings were taken on the dorsal surface of each individual using an infrared thermometer (Trotec® BP21). This technique has the advantage of avoiding any manipulation of the lizards and thus to avoid a measurement bias related to stress (Hare et al., 2007) and has been shown to strongly correlate with body core temperature in lizards (Carretero, 2012). For each individual, we calculated a mean preferred temperature (T_{pref}) (with T_{pref} variance) by averaging the body temperature recorded. After the measurements, individuals were housed in the terrarium one day before releasing at the exact capture locality.

2.3. Statistical analyses

To investigate the relationship between T_{pref} and altitude, we fitted mixed-effects linear models with Gaussian errors including the T_{pref} as the response variable, “population” as random variable and altitude as explanatory variable with both simple and quadratic terms. Because preferred body temperatures can depend on sex in ectotherms (Van Damme et al., 1986; Rodríguez-Díaz and Braña, 2011), we also added sex as a covariate. Mean effects and confidence intervals were assessed by bootstrapped models ($n = 5000$) as the average and the range of parameter estimates containing 95% of the bootstrap distributions respectively. First, we identified significant variables and terms from the complete model as coefficient values having at least 95% of their bootstrap distribution less or more than 0 (that is, a threshold $\alpha = 0.05$). Second, we fitted a model based on significant variables only. The “lme4” (Bates et al., 2015) R-package were used to build mixed-effects models. All statistical analyses were performed using R 3.1.0 (R Development Core Team, 2014).

3. Results

In *C. asper*, T_{pref} varied from 11.10 °C to 32 °C (mean \pm SD: 21.08 °C \pm 4.60 °C). In both lizard species, T_{pref} varied from 18.87 °C to 37.23 °C and 28.36–36.25 °C respectively for *Z. vivipara* and *P. muralis* (mean \pm SD: 29.24 °C \pm 3.22 °C and 32.89 °C \pm 1.53 °C respectively). In all species, differences between marginal and conditional R^2 showed that T_{pref} variability was higher within population than between populations (Appendix B). We showed that T_{pref} was significantly explained by altitude in all species and sex (Table 1). The variation of T_{pref}

along the elevation differed among species. In the Pyrenean brook salamander, we found that both the simple and quadratic terms of altitude were significant (Table 1), materializing a non-linear relationship where T_{pref} was maximum at both low (451 m a.s.l.) and high (2158 m a.s.l.) altitudes (Fig. 2A; $R^2 = 0.1895$). Preferred body temperature was minimum at medium altitude (~1250 m a.s.l.). In both lizard species, T_{pref} showed a negative and linear relationship with altitude (Fig. 2C–D). Both a higher decrease in T_{pref} along the altitudinal gradient and a higher part of variation in T_{pref} explained by the altitude were observed in the wall lizard compared (mean slope = -0.4599 [-0.7183 ; -0.1925]; $R^2 = 0.2628$) to the common lizard (mean slope = -0.2196 [-0.4068 ; -0.0324]; $R^2 = 0.1494$). Males tended to prefer warmer temperatures compared to females, but such differences were not significant whatever the species (Table 1; no significant differences between sexes in *C. asper* $P = 0.1774$; in *P. muralis* $P = 0.2186$; in *Z. vivipara* $P = 0.1302$).

4. Discussion

Despite a strong individual variability of T_{pref} within populations, our results showed significant relationships between T_{pref} and altitude in three ectothermic species along an altitudinal gradient in the Pyrenean mountains (e.g. Navas, 2002). These relationships highlighted complex species-specific behavioral responses to altitude. That is, the brook salamander showed non-linear relationship between T_{pref} and altitude while lizards from low altitude populations tended to select higher temperatures than those from higher altitude populations (i.e. adaptation of local optima hypothesis). Below, we discuss these species-specific variation in T_{pref} along altitude and the further implications of these patterns in regard with current theories and future climate change.

4.1. Species-specific behavioral responses along altitudinal gradient

We found two types of relationships between T_{pref} and altitude, non-linear in the brook salamander and linear in two lizard species. It is worth noting that such species-specific pattern is consistent with previous investigations showing variable tendencies amongst (Huey et al., 1989; Andrews, 1998; Feder et al., 2000; Melville and Schulte, 2001; Winne and Keck, 2005) and within species (Mathies and Andrews, 1997; Le Galliard et al., 2003; Xu and Ji, 2006; Lin et al., 2008). The studied species mainly differ in habitat use, with aquatic (i.e., brook salamander) versus terrestrial (i.e., lizards) lifestyle during the active season, and therefore specific mode of thermoregulation. Therefore, different behavioral strategies (i.e. thigmothermy in *C. asper* and heliothermy in both lizard species) leading to species-specific pattern of T_{pref} variation with altitude could emerge from these differences in lifestyle, thermoregulation and temperature variation exposure.

Specifically, in the brook salamander, some divergences in the life-

Table 1

Effects of altitude and sex on species temperature preferences estimated through Bayesian bootstrapped mixed-effects linear models ($n = 5000$). Significant effects are displayed in bold ($P < 0.05$). Response and explanatory variables have been scaled for comparison purposes. N = number of populations. R^2 = marginal R^2 (i.e. the part of T_{pref} variance explained by fixed effects only).

Variable	<i>Calotriton asper</i> $R^2 = 0.1895$ [0.0586;0.3385]; N = 8			<i>Zootoca vivipara</i> $R^2 = 0.1494$ [0.0504;0.2742]; N = 12			<i>Podarcis muralis</i> $R^2 = 0.2628$ [0.0986;0.4433]; N = 9		
	Mean estimate	CI95%	P-value	Mean estimate	CI95%	P-value	Mean estimate	CI95%	P-value
Altitude (simple term)	-0.193	[-0.4473; 0.058]	0.0714	-0.2196	[-0.4068; -0.0324]	0.011	-0.4599	[-0.7183; -0.1925]	0
Altitude (quadratic term)	0.4042	[0.1467; 0.6597]	0.0004	-0.0904	[-0.0993; 0.2822]	0.1718	-0.0656	[-0.3297; 0.2056]	0.3126
Sex (female)	-0.061	[-0.3004; 0.1839]	0.3096	-0.0494	[-0.2589; 0.1551]	0.3176	-0.0761	[-0.3928; 0.2315]	0.31
Sex (male)	0.069	[-0.1891; 0.325]	0.3004	0.0713	[-0.1545; 0.2928]	0.2686	0.1228	[-0.2574; 0.3868]	0.342

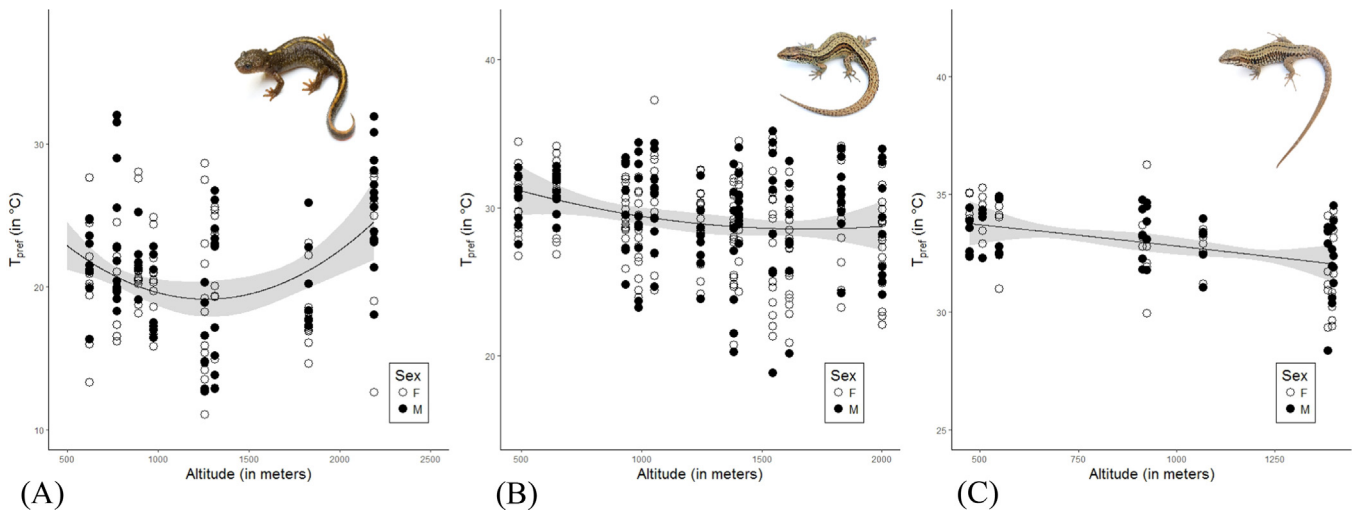


Fig. 2. Variation of mean individual preferred body temperatures (T_{pref}) along an altitudinal gradient in the 3 ectothermic species studied in our study. The relationship between the T_{pref} and altitude was quadratic in A) the *Calotriton asper*, and negative in B) *Zootoca vivipara* and in C) *Podarcis muralis*. Black lines and grey areas show the relationships fitted on significant variables and terms only through mixed-effects models (see [Table 1](#)) and their 95% confidence intervals respectively.

cycle (i.e. mating season) or in phenotypic differences depending on the elevation site have been reported (Martínez-Rica, 1980; Gasser and Clergue-Gazeau, 1981; Serra-Cobo et al., 2000; Miaud and Guillaume, 2005; Oromi et al., 2014). Here, the quadratic relationship between preferred body temperatures and altitude may illustrate two behavioral strategies in this species. Notably, populations living at low elevation level showed activity periods from May–June to September. Moreover, low-level sites were more forestry, with a continuous forest vegetation over the river, which can buffer the influence of air temperature on water temperature. As a result, individuals from low altitude might have no opportunities to find microhabitats with higher temperature for thermoregulation (passive to thermal heterogeneity; see Piasecna et al., 2015). In this context, T_{pref} follows the decrease in temperature with altitude, which could make individuals adapted to cold temperature conditions (following the adaptation of local optima hypothesis). On the contrary, from 1300 m high onwards, the snow duration linearly increases with elevation (Appendix C). For these populations from middle and high elevation levels, we suggested that Pyrenean brook salamanders may have a restricted time to accomplish their life cycle (at 2400 m of altitude, the mean snow duration is up to 150 days, which induce a reduction in the activity season -from July to September- of this species; Appendix C). This might force animals to select relatively higher temperatures in order to compensate for lower opportunities of favorable temperature (short breeding season or short time to prepare for winter; i.e. the countergradient hypothesis). Furthermore, we observed in the field at high elevation levels (rivers with surrounded by meadow with no vegetation cover over) that individuals can move to basins of water or under rocks where water flow was lower and temperature was higher than in the middle of the river. As a consequence, individuals from high altitude might be able to thermoregulate by selecting microhabitats (Spotila, 1972; Heath, 1975; Balogová and Gvoždík, 2015).

In both lizard species, we found convergent tendencies that follow the prediction of the adaptation of local optima hypothesis (Levinton, 1983). That is, individuals from lower altitude and warmer conditions have higher T_{pref} than those from cooler habitats allowing them to be adapted to local temperature conditions, even if we cannot exclude that low temperature lizards were more prompt for inactivity.

4.2. Evolutionary implications of T_{pref} variation

Following the countergradient theory (Conover and Schultz, 1995), we would have expected higher T_{pref} in individuals from high altitude

habitats that associate lower temperature and thereby less frequent opportunities to perform crucial physiological functions to complete their life cycle (Freidenburg and Skelly, 2004; Artacho et al., 2017). Indeed, lower access to favorable temperature should then compromise the individuals to perform critical activities (locomotion, digestion, reproduction, etc.). Therefore we hypothesized that T_{pref} , one component of behavioral thermoregulation should be related to altitude as individuals from high elevation and “cold” populations maximize the intensity in basking.

Our results corroborated partially this hypothesis in the Pyrenean brook salamander (middle to high elevation part), as reported in other studies (Fanguie et al., 2009). At high altitude level, individuals may compensate for lower opportunities of favorable conditions by choosing a high temperature which maximize their activities. However, populations from low elevation level hence better supported the adaptation of local optima hypothesis, such as both lizard species, for which T_{pref} tended instead to decrease with altitude. In our study, lizards from cold climates (i.e. high altitude) may be physiologically adapted to low temperature, with the ability to perform their activities (breeding, feeding, dispersal, etc...) at lower temperature than individuals from low altitude (Laugen et al., 2003; Muir et al., 2014). Behavioral adjustments of thermoregulation could be viewed as the proximate regulator within species physiological limits (Huey and Slatkin, 1976; Buckley et al., 2013).

4.3. Perspectives and conservation applications

Plasticity and adaptation of thermoregulation behaviors can interact and influence the vulnerability of organisms in the face to climate change (Huey et al., 2012). We showed here that populations from different elevation levels displayed differences in preferred body temperatures, which could reveal that they might be differently affected by global warming. In general, models predicting the responses of species in face to climate change do not consider any inter- and intraspecific and spatially structured variability in temperature preferences. That is, niche models (largely used in this context) do not consider either of the adaptation of local optima hypothesis or the countergradient hypothesis. Based on our results, we stress that this modelling approach has missed one important point: preferred body temperatures are not necessarily homogenous within populations and may notably vary along the altitudinal gradient, therefore affecting future range shifts depending on the shape, slope and intensity of the T_{pref} gradient (which is highly related to the optimal body temperature). Some recent

simulations confirmed the fears that this parameter omission could have strong consequences on the determination of future range shifts (Valladares et al., 2014). More generally, predicting the responses of species face to climate change requires an understanding of the factors that could constrain their cycle life (such as physiological, behavioral, ecological, and evolutionary factors), it becomes crucial to increase our understanding on the influence of temperature on those aspects. Notably, determining the patterns leading to thermal physiology is essential to understand the evolutionary responses of species to climatic variations and correctly predict the future of species.

Acknowledgments

We especially thank all people involved in the capture and field work. Namely, we thank Jules Teulière Quillet, Clément Vallé, Adoué Adotevi. We also greatly thank the members of the ECTOPYR project. This work was supported by the French Laboratory of Excellence project "TULIP" (ANR-10-LABX-41; ANR-11-IDEX-0002-02) and by the Interreg Poctefa Ectopyr (no. EFA031/15). We are grateful to the 'Office Nationale des Forêts', the 'Parc National des Pyrénées', and the region Occitanie for allowing us to sample animals.

Author contributions

This study was designed by AT, AD, JS and FA. Field data were obtained by all authors. AT, AD, RB and JS led the data analyses and the manuscript writing, which was corrected by all authors.

Competing interest

We declare no competing financial interest.

Ethics

The Pyrenean brook salamander (*Calotriton asper*) is protected by French national legislation (Order of 19 November 2007, Article 2), Spanish national legislation ("Real Decreto" of 1980 and 1986) and is listed on Appendix II of the Bern Convention and on Annex IV of the EU Habitats Directive. The Pyrenean brook salamander is also listed as "near threatened" in the IUCN Redlist, because is considered likely to be vulnerable to climate change. The wall lizard (*Podarcis muralis*) is protected by French national legislation (Order of 19 November 2007, Article 2), and is listed on Appendix II of the Bern Convention, and on Annex IV of the EU Habitats Directive. Our work complies with the international animal care guidelines of the Association for the Study of Animal Behaviour, and all required French (permit no. 2017-s-02) and Spanish (Catalan government "Departament de Medi Ambient i Habitatge de la Generalitat de Catalunya"; permit no. SF/966) permits relating to an authorization of capture, marking, transport, detention, use and release of protected amphibian species; and animal experimentation accreditation n°A09-1) were been obtained. The project was approved by the "Conseil Scientifique Régional du Patrimoine Naturel (CSRPN)" of the region Occitanie the 30th of March 2017. Some authors (JS, AT) have also been trained in the use of animals in wildlife not hosted in scientific purposes (Decree No. 2013-118 and approval of the Ministry of Agriculture under No. I-75-MNHN-F-15).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jtherbio.2018.08.002.

References

Aitken, S.N., Yeaman, S., Holliday, J.A., Wang, T., Curtis-McLane, S., 2008. Adaptation, migration or extirpation: climate change outcomes for tree populations. *Evol. Appl.* 1,

95–111.

Andrews, R.M., 1998. Geographic variation in field body temperature of *Sceloporus* lizards. *J. Therm. Biol.* 23, 329–334.

Angilletta, M.J., 2009. *Thermal Adaptation: a Theoretical and Empirical Synthesis*. Oxford University Press, Oxford.

Artacho, P., Saravia, J., Perret, S., Bartheld, J.L., Le Galliard, J.F., 2017. Geographic variation and acclimation effects on thermoregulation behavior in the widespread lizard *Liolaemus pictus*. *J. Therm. Biol.* 63, 78–87.

Aubret, F., Michniewicz, R.J., 2010. Warming up for cold water: influence of habitat type on thermoregulatory tactics in a semi-aquatic snake. *Amphib.-Reptil.* 31, 525–531.

Balogová, M., Gvoždík, L., 2015. Can newts cope with the heat? Disparate thermoregulatory strategies of two sympatric species in water. *PLoS One* 10, e0128155.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1–48.

Belliure, J., Carrascal, L.M., 2002. Influence of heat transmission mode on heating rates and on the selection of patches for heating in a Mediterranean lizard. *Physiol. Biochem. Zool.* 75, 369–376.

Bernardo, J., Ossola, R.J., Spotilla, J.R., Crandall, K.A., 2007. Interspecies physiological variation as a tool for cross-species assessments of global warming-induced endangerment: validation of an intrinsic determinant of macroecological and phylogeographic structure. *Biol. Lett.* 3, 695–698.

Botts, E.A., Erasmus, B.F.N., Alexander, G.J., 2015. Observed range dynamics of South African amphibians under conditions of global change. *Aust. Ecol.* 4, 309–317.

Bogert, C.M., 1949. Thermoregulation in reptiles; a factor in evolution. *Evolution* 3, 195–211.

Buckley, L.B., Nufio, C.R., Kingsolver, J.G., 2013. Phenotypic clines, energy balances, and ecological responses to climate change. *J. Anim. Ecol.* 83, 41–50.

Buckley, L.B., Urban, M.C., Angilletta, M.J., Crozier, L.G., Rissler, L.J., Sears, M.W., 2010. Can mechanism in form species' distribution models? *Ecol. Lett.* 13, 1041–1054.

Calosi, P., Bilton, D.T., Spicer, J.I., 2007. Thermal tolerance, acclimatory capacity and vulnerability to global climate change. *Biol. Lett.* 4, 99–102.

Carrascal, L.M., Lopez, P., Martin, J., Salvador, A., 1992. Basking and antipredator behaviour in high altitude lizard: implications of heat-exchange rate. *Ethology* 92, 143–154.

Carretero, M.A., 2012. Measuring body temperatures in small lacertids: infrared vs. contact thermometers. *Basic. Appl. Herpetol.* 26, 99–105.

Clergue-Gazeau, M., Martínez-Rica, J.P., 1978. Les différents biotopes de l'urodèle pyrénéen: *Euproctus asper*. *Bull. Soc. Hist. Nat. Toulouse* 114, 461–471.

Conover, D.O., Schultz, E.T., 1995. Phenotypic similarity and the evolutionary significance of countergradient variation. *Trends Ecol. Evol.* 10, 248–252.

Diego-Rasilla, F.J., 2015. In: Salvador, A., Marco, A. (Eds.), *Lagartija roquera - Podarcis muralis*. En: *Enciclopedia Virtual de los Vertebrados Españoles*. Museo Nacional de Ciencias Naturales, Madrid. <http://www.vertebradosibericos.org/>.

Fangue, N.A., Podrabsky, J.E., Crawshaw, L.I., Schulte, P.M., 2009. Countergradient variation in temperature preference in populations of killifish, *Fundulus heteroclitus*. *Physiol. Biochem. Zool.* 82, 776–786.

Feder, M.E., Bennett, A.F., Huey, R.B., 2000. Evolutionary physiology. *Ann. Rev. Ecol. Syst.* 31, 62–71.

Freidenburg, L.K., Skelly, D.K., 2004. Microgeographical variation in thermal preference by an amphibian. *Ecol. Lett.* 7, 369–373.

Gasser, F., Clergue-Gazeau, M., 1981. Les protéines sériques de l'urodèle *Euproctus asper* (Dugès). *Éléments de différenciation génétique dans les Prépyrénées espagnols*. *Vie et Milieu* 31, 297–302.

Gvoždík, L., 2002. To heat or to save time? Thermoregulation in the lizard *Zootoca vivipara* (Squamata: lacertidae) in different thermal environments along an altitudinal gradient. *Can. J. Zool.* 80, 479–492.

Gvoždík, L., 2003. Postprandial thermophily in the Danube crested newt, *Triturus dobrovicus*. *J. Therm. Biol.* 28, 545–550.

Gvoždík, L., 2005. Does reproduction influence temperature preferences in newts? *Can. J. Zool.* 83, 1038–1044.

Hare, J.R., Whitworth, E., Cree, A., 2007. Correct orientation of a handheld infrared thermometer is important for accurate measurement of body temperatures in small lizards and tuatara. *Herpetol. Rev.* 38, 311–315.

Heath, A.G., 1975. Behavioral thermoregulation in high altitude tiger salamanders, *Ambystoma tigrinum*. *Herpetologica* 31, 84–93.

Hertz, P.E., Huey, R.B., Stevenson, R.D., 1993. Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. *Am. Nat.* 142, 796–818.

Huey, R.B., Kearney, M.R., Krockenberger, A., Holtum, J.A., Jess, M., Williams, S.E., 2012. Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. *Philos. Trans. R. Soc. B* 367, 1665–1679.

Huey, R.B., Niewiarowski, P.H., Kaufmann, J., Herron, J.C., 1989. Thermal biology of nocturnal ectotherms: is sprint performance of geckos maximal at low body temperatures? *Physiol. Zool.* 62, 488–504.

Huey, R.B., Slatkin, M., 1976. Cost and benefits of lizard thermoregulation. *Q. Rev. Biol.* 51, 363–384.

Hutchison, V.H., Dupré, R.K., 1992. Thermoregulation. In: Feder, M.E., Burggren, W.W. (Eds.), *Environmental Physiology of the Amphibians*. University of Chicago Press, Chicago, pp. 206–249.

IPCC, 2014. *Climate change 2014: synthesis report*. In: Core Writing Team, Pachauri, R.K., Meyer, L.A. (Eds.), *Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. IPCC, Geneva, Switzerland.

Jump, A.S., Peñuelas, J., 2005. Running to stand still: adaptation and the response of plants to rapid climate change. *Ecol. Lett.* 8, 1010–1020.

Kearney, M., Shine, R., Porter, W.P., 2009. The potential for behavioral thermoregulation

- to buffer "cold-blooded" animals against climate warming. *PNAS* 106, 3835–3840.
- Laugen, A.T., Laurila, A., Rasanen, K., Merila, J., 2003. Latitudinal countergradient variation in the common frog (*Rana temporaria*) development rates – evidence for local adaptation. *J. Evol. Biol.* 16, 996–1005.
- Le Galliard, J.F., Le Bris, M., Clobert, J., 2003. Timing of locomotor impairment and shift in thermal preferences during gravidity in a viviparous lizard. *Funct. Ecol.* 17, 877–885.
- Levinton, J.S., 1983. The latitudinal compensation hypothesis: growth data and a model of latitudinal growth differentiation based upon energy budgets. I. Interspecific comparison of Ophryotrocha (Polychaeta: dorvilleidae). *Biol. Bull.* 165, 686–698.
- Li, Y., Cohen, J.M., Rohr, J.R., 2013. Review and synthesis of the effects of climate change on amphibians. *Integr. Zool.* 8, 145–161.
- Lin, C.X., Zhang, L., Ji, X., 2008. Influence of pregnancy on locomotor and feeding performances of the skink *Mabuya multifasciata*: why do females shift thermal preferences when pregnant? *Zoology* 111, 188–195.
- Loarie, S.R., Duffy, P.B., Hamilton, H., Asner, G.P., Field, C.B., Ackerly, D.D., 2009. The velocity of climate change. *Nature* 462, 1052.
- Marco, A., Abella, E., Martins, S., Hawkes, L., 2016. Resilience of sea turtles to the impact of global warming on nesting. XIV. Congreso Luso Español Herpetología, pp. 55–56.
- Martínez-Rica, J.P., 1980. Algunos datos sobre las poblaciones meridionales de tritón pirenaico, *Euproctus asper* Dugès. *Stud. Oecol.* 2, 135–154.
- Mathies, T., Andrews, R.M., 1997. Influence of pregnancy on the thermal biology of the lizard *Sceloporus jarrovi*: why do pregnant females exhibit low body temperatures? *Funct. Ecol.* 11, 498–507.
- Melville, J., Schulte, J.A., 2001. Correlates of active body temperatures and microhabitat occupation in nine species of central Australian agamid lizards. *Aust. Ecol.* 26, 660–669.
- Miaud, C., Guillaume, O., 2005. Variation in age, body size and growth among surface and cave-dwelling populations of the Pyrenean newt, *Euproctus asper* (Amphibia; Urodela). *Herpetologica* 61, 241–249.
- Milá, B., Carranza, S., Guillaume, O., Clobert, J., 2010. Marked genetic structuring and extreme dispersal limitation in the Pyrenean brook newt *Calotriton asper* (Amphibia: salamandridae) revealed by genome-wide AFLP but not mtDNA. *Mol. Ecol.* 19, 108–120.
- Muir, A.P., Biek, R., Mable, B.K., 2014. Behavioral and physiological adaptations to low-temperature environments in the common frog, *Rana temporaria*. *BMC Evol. Biol.* 14, 110.
- Navas, C.A., 2002. Herpetological diversity along Andean elevational gradients: links with physiological ecology and evolutionary physiology. *Comp. Biochem. Physiol. A* 133, 469–485.
- Oromi, N., Amat, F., Sanuy, D., Carranza, S., 2014. Life history trait differences between a lake and a stream-dwelling population of the Pyrenean brook newt (*Calotriton asper*). *Amphib.-Reptil.* 35, 53–62.
- Paaijmans, K.P., Heinig, R.L., Seliga, R.A., Blanford, J.I., Blanford, S., Murdock, C.C., Thomas, M.B., 2013. Temperature variation makes ectotherms more sensitive to climate change. *Glob. Chang. Biol.* 19, 2373–2380.
- Parmesan, C., 2006. Ecological and evolutionary responses to recent climate change. *Annu. Rev. Ecol. Evol.* 37, 637–669.
- Peñuelas, J., Sardans, J., Estiarte, M., Ogaya, R., Carnicer, J., Coll, M., Barbeta, A., Rivas-Ubach, A., Llusà, J., Garbulsy, M., Filella, I., Jump, A.S., 2013. Evidence of current impact of climate change on life: a walk from genes to the biosphere. *Glob. Chang. Biol.* 19, 2303–2338.
- Pérez-Mellado, V., 1998. *Podarcis muralis* (Laurenti, 1768). In: Salvador, A., Ramos, M.A. (Eds.), *Reptiles 10. Fauna Ibérica*. Museo Nacional de Ciencias Naturales, CSIC, Madrid, pp. 283–294.
- Piasecna, et al., 2015.
- Porter, W.P., Mitchell, J.W., Beckman, W.A., DeWitt, C.B., 1973. Behavioral implications of mechanistic ecology-thermal and behavioral modeling of desert ectotherms and their microenvironment. *Oecologia* 13, 1–54.
- Qu, Y., Li, H., Gao, J., Xu, X., Ji, X., 2011. Thermal preference, thermal tolerance and the thermal dependence of digestive performance in two coexisting *Phrynocephalus* lizards (Agamidae), with a review of species studied. *Curr. Zool.* 57, 684–700.
- R Development Core Team, 2014. R: A language and environment for statistical computing R Foundation for Statistical Computing, Vienna.
- Raffaëlli, J., 2007. *Les Urodèles du monde*. Pencil edition.
- Rodríguez-Díaz, T., Braña, F., 2011. Shift in thermal preferences of female oviparous common lizards during egg retention: insights into the evolution of reptilian viviparity. *Evol. Biol.* 38, 352–359.
- Rosbakh, S., Bernhardt-Römermann, M., Poschlod, P., 2014. Elevation matters: contrasting effects of climate change on the vegetation development at different elevations in the Bavarian Alps. *Alp. Bot.* 124, 143–154.
- Salvador, A., Roig, J.M., Carretero, M.A., 2014. In: *Vertebrados Españoles*. Salvador, A., Marco, A. (Eds.), *Lagartija de turbera – Zoootoca vivipara*. En: *Enciclopedia Virtual de los. Museo Nacional de Ciencias Naturales*, Madrid. <<http://www.vertebradosibericos.org/>>.
- Serra-Cobo, J., Uiblein, F., Martínez-Rica, J.P., 2000. Variation in sexual dimorphism between two populations of the Pyrenean salamander *Euproctus asper* from ecologically different mountain sites. *Belg. J. Zool.* 130, 39–45.
- Silvestre, M., 2011. L'Élevage des Urodèles : Etude de cinq espèces menaces : *Euproctus platycephalus*, *Calotriton asper*, *Triturus cristatus*, *Salamandra salamandra*, *Speleomantes strinatii*. PhD thesis, Veterinary Scholl of Alfort, France.
- Sinclair, B.J., Marshall, K.E., Sewell, M.A., Levesque, D.L., Willet, C.S., Slotsbo, S., Dong, Y., Harley, C.D.G., Marshall, D.J., Helmuth, B.S., Huey, R.B., 2016. Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? *Ecol. Lett.* 19, 1372–1385.
- Sinervo, B., Méndez-de-la-Cruz, F., Miles, D.B., Heulin, B., Bastiaans, E., Villagrán-Santa Cruz, M., Lara-Resendiz, R., Martínez-Méndez, N., Calderón-Espinosa, M.L., Meza-Lázaro, R.N., Gadsden, H., Avila, L.J., Morando, M., De la Riva, I.J., Sepulveda, P.V., Duarte Rocha, C.F., Ibarquiogoytia, N., Puntriano, C.A., Massot, M., Lepetz, V., Oksanen, T.A., Chapple, D.G., Bauer, A.M., Branch, W.R., Clobert, J., Sites Jr., J.W., 2010. Erosion of lizard diversity by climate change and altered thermal niches. *Science* 328, 894–899.
- Spotila, J.R., 1972. Role of temperature and water in the ecology of lungless salamanders. *Ecol. Monogr.* 42, 95–125.
- Stevenson, R.D., 1985. The relative importance of behavioral and physiological adjustments controlling body temperature in terrestrial ectotherms. *Am. Nat.* 126, 362–386.
- Sunday, J.M., Bates, A.E., Kearney, M.R., Colwell, R.K., Dulvy, N.K., Longino, J.T., Huey, R.B., 2014. Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. *PNAS* 111, 5610–5615.
- Tedeschi, J.N., Kennington, W.J., Berry, O., Whiting, S., Meekan, M.G., Mitchell, N.J., 2016. Heritable variation in heat shock gene expression: a potential mechanism for adaptation to thermal stress in embryos of sea turtles. *Proceedings R. Soc. B.* 283, 20152320.
- Valladares, F., Matesanz, S., Guilhaumon, F., Araújo, M.B., Balaguer, L., Benito-Garzon, M., Cornwell, W., Gianoli, E., van Kleunen, M., Naya, D.E., Nicotra, A.B., Poorter, H., Zavala, M.A., 2014. The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. *Ecol. Lett.* 17, 1351–1364.
- Van Damme, R., Bauwens, D., Verheyen, R.F., 1986. Selected body temperatures in the lizard *Lacerta vivipara* variation within and between populations. *J. Therm. Biol.* 11, 219–222.
- Vittoz, P., Randin, C., Dutoit, A., Bonnet, F., Hegg, O., 2009. Low impact of climate change on subalpine grasslands in the Swiss northern Alps. *Glob. Chang. Biol.* 15, 209–220.
- Winne, C.T., Keck, M.B., 2005. Intraspecific differences in thermal tolerance of the diamondback watersnake (*Nerodia rhombifer*): effects of ontogeny, latitude, and sex. *Comp. Biochem. Physiol.* 140, 141–149.
- Winne, C.T., Willson, J.D., Andrews, K.M., Reed, R.N., 2006. Efficacy of marking snakes with disposable medical cautery units. *Herpetol. Rev.* 37, 52–54.
- Xu, X.F., Ji, X., 2006. Ontogenetic shifts in thermal tolerance, selected body temperature and thermal dependence of food assimilation and locomotor performance in a lacertid lizard *Eremias brenchleyi*. *Comp. Biochem. Physiol.* 143, 118–124.
- Zamora-Camacho, F.J., Reguera, S., Moreno-Rueda, G., Pleguezuelos, J.M., 2013. Patterns of seasonal activity in a Mediterranean lizard along a 2200 m altitudinal gradient. *J. Therm. Biol.* 38, 64–69.