



Adaptive seasonal shifts in the thermal preferences of the lizard *Iberolacerta galani* (Squamata, Lacertidae)



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ABSTRACT

The León rock lizard, *Iberolacerta galani*, lives in isolated mountains of Spain. We studied the seasonal changes in the thermal biology of *I. galani* between spring and summer. We calculated precision, accuracy and effectiveness of thermoregulation and the habitat thermal quality for spring, and compared with the values of summer. In addition, we studied how the shift in the thermal preferences of lizards would contribute to achieve a higher effectiveness of thermoregulation. Thermal preferences of León rock lizards are among the lowest in lacertids, and are also very narrow, maintaining the narrowness among seasons. As for summer (27.90–29.70 °C, mean value =28.76 °C), the thermal preferences of *I. galani* are also low in spring (29.60–31.10 °C, mean value =30.38 °C), supporting the idea that this species is adapted to cold environments. The habitat thermal quality is lower in spring (10.99 °C) than in summer (9.36 °C), while the effectiveness of thermoregulation is higher in spring (0.92) than in summer (0.80). We found that the seasonal shift in thermal preferences contributes significantly to enhance the effectiveness of thermoregulation in both seasons, more in spring (0.45 °C) than in summer (0.16 °C). Because *I. galani* inhabits isolated mountains, where the activity period is reduced from April to October, we hypothesize that the observed adaptation of the thermal preferences, which enhance thermoregulation to a larger extent in spring, may evolved to maximize performance during the reproductive season.

1. Introduction

Environmental temperatures available for organisms vary with latitude, altitude and habitat composition (Graae et al., 2012; Sears et al., 2011; Zamora-Camacho et al., 2015). Moreover, ectotherms must deal with daily and seasonal changes of environmental temperatures (Díaz et al., 2006; Díaz and Cabezas-Díaz, 2004; Ortega et al., 2014), and with the recently added thermal variation of global warming (Gunderson and Stillman, 2015; Huey et al., 2012; Solomon et al., 2007). Seasonal thermal fluctuations may lead ectotherms to adapt their physiology and behaviour, since they are quite predictable (Angilletta, 2009; Christian and Bedford, 1995; Huey, 1982). Through seasonal adaptation of thermal biology, lizards may be able to achieve body temperatures closer to their thermal preferences for each season (Angilletta, 2009; Huey, 1982).

Some studies have addressed the effect of seasonality in thermal ecology Mediterranean lacertids. Seasonality is the main factor affecting body temperature of *Podarcis guadarramae* and environmental constraints are the main forces driving seasonal changes in microhabitat selection, regardless sex, age or body size (Ortega and Pérez-

Mellado, 2016). In addition, seasonal changes of thermoregulation are known to interact with habitat structure in *Podarcis lilfordi* (Ortega et al., 2014). Seasonal patterns of thermoregulation and activity also change with altitude, as found in *Psammodromus algirus*, probably synchronizing reproductive cycles (Zamora-Camacho et al., 2013). Finally, the contribution of different behavioural mechanisms to thermoregulation also vary seasonally (Díaz and Díaz-Cabezas, 2004).

Regarding thermal preferences, seasonal shifts have been reported in several species of temperate areas (e.g. Díaz et al., 2006; Díaz and Cabezas-Díaz, 2004; Patterson and Davies, 1978; Van Damme et al., 1986). Furthermore, the seasonal change in thermal preferences facilitates thermoregulation in some species, so it may involve an adaptive advantage (Díaz et al., 2006; Seebacher, 2005; Truter et al., 2014). For high mountain lizards, seasonal adaptation of thermal biology may be critical in order to survive in such extreme environmental conditions, on which the time with suitable temperatures is daily and annually restricted (Gutiérrez et al., 2010; Zamora-Camacho et al., 2013). We studied the seasonal changes in thermal biology of the León rock lizard, *Iberolacerta galani*. First, we assessed the thermal biology of *I. galani* using the protocol of Hertz et al. (1993) in spring,

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and compared the results with data from summer (Ortega et al., 2016a). In addition, we studied the adaptation of the thermal preferences of the species to the seasonal changes of the habitat thermal quality. Our aim was to assess the effect of seasonal changes in the thermal biology of this species and to study the possible seasonal adaptations.

2. Materials and methods

2.1. Species under study

The León rock lizard, *Iberolacerta galani*, is a recently described lacertid lizard endemic to Northwest Spain that inhabits rocky substrates above the tree line, >1400 m (Arribas et al., 2006; Mencía et al., 2016). The study took place at the Natural Monument “Lago de la Baña” (León, Spain), in the surroundings of a glacial lake at 1400 m. The habitat is formed by rocks, meadows and shrubs and circled by mountain peaks of more than 2000 m. Thermal biology and microhabitat selection of *I. galani* during summer has been reported by Ortega et al. (2016a) in a comparative study with the syntopic lizard *Podarcis bocagei* for the same study area. In summer, the lake partially dries and wide shores arise, where both species of lizards coexist, selecting different microhabitats (Ortega et al., 2016a). In spring the lake is full and *I. galani* lizards inhabits a shrubby area, mainly formed by heathers, while *P. bocagei* inhabits a rocky area more than 100 m away (Fig. 1).

2.2. Field temperatures

Although some data from summer have been published in a different comparative study (Ortega et al., 2016a), we used here again these raw data for different analyses. We studied thermal biology of *Iberolacerta galani* during spring (May) of 2012 and summer (August) from 2011 to 2013. Adult lizards were captured by noosing from 0800 to 1800 GMT, during their daily activity period. For every lizard, we measured cloacal body temperature (T_b) immediately after capture, as well as air temperature (T_a), 1 cm above the capture point, and substrate temperature (T_s) of the capture point, with a Testo® 925 digital thermometer (± 0.1 °C precision).

We registered operative temperatures (T_e) as estimates of temperatures of non-thermoregulating lizards, that is, as a null hypothesis of thermoregulation. The study of T_e and T_b was simultaneous and in the same area, in order to avoid climatic variation and assure that the differences between both variables were attributable to lizards' thermo-

regulation. We employed copper models as null T_e models (Bakken and Angilletta, 2014). One thermocouple probe was placed into each hollow model and connected to a data logger HOB0 H8 (® Onset Computer Corporation) programmed to take a temperature record every five minutes. We randomly placed the data loggers with the copper models in different microhabitats during May of 2012 and August from 2011 to 2013. We obtained 2311 measures of T_e in spring and 6082 in summer.

2.3. Preferred temperature range (PTR)

Selected body temperatures of *I. galani* were measured in a laboratory thermal gradient during May of 2012, for the spring sample, and during August of 2011, for the summer sample. For both experiments, lizards were captured from Lago de la Baña (León, Spain). All conditions, laboratory protocols and methodology were identical. Lizards were housed on individual terraria and fed with mealworms and crickets. Water was provided ad libitum. The thermal gradient was built in a glass terrarium (100×60×60 cm³) with a 150 W infrared lamp over one side, obtaining a gradient between 20–60 °C. A data of a selected temperature of a lizard was measured with a digital thermometer each hour from 0800 to 1800 GMT. We used 24 adult lizards (12 males, 12 females) for each season, obtaining 144 selected temperatures. We considered the 50% of central values of selected body temperatures as the preferred temperature range to assess thermoregulation (Blouin-Demers and Nadeau, 2005; Hertz et al., 1993). Thus, we refer to T_{sel} as the selected body temperatures in general, and to PTR as the 50% central values of the T_{sel} . After both experiments, lizards were released in the same places of capture.

2.4. Data analysis

2.4.1. Thermoregulation indexes

To test the null hypothesis of thermoregulation we followed the protocol developed by Hertz et al. (1993) and calculated the three indexes of thermoregulation. The first is the index of accuracy of thermoregulation (mean d_b): the mean of absolute values of the deviations between each T_b from the preferred temperature range. The values of the index of accuracy of thermoregulation are opposite to their meaning: higher values of d_b indicate lower accuracy of thermoregulation, and vice-versa. The second is the index of habitat thermal quality (mean d_e): the mean of absolute values of the deviations of each T_e from the preferred temperature range. Accordingly, the values of the index of habitat thermal quality are also opposite to their meaning: higher values of d_e indicate a lower habitat thermal quality, and vice-

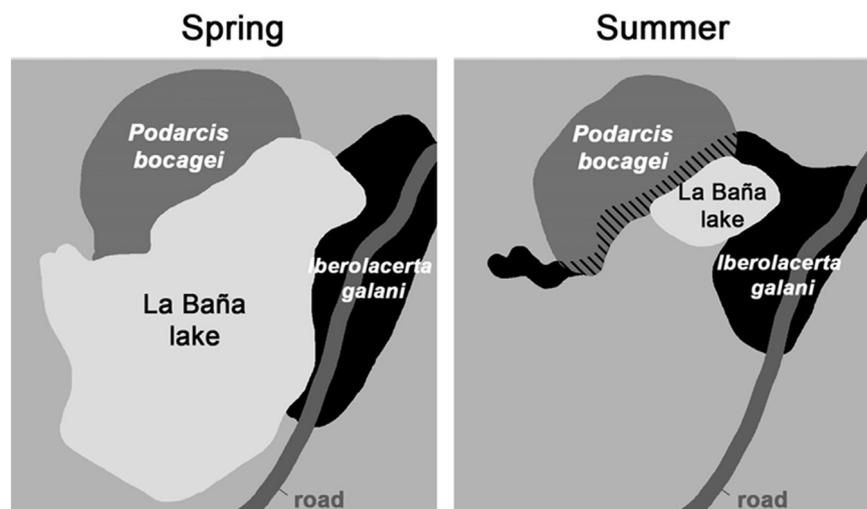


Fig. 1. Observed distribution of *Iberolacerta galani* at La Baña lake during spring (left) and summer (right). Their interaction with *Podarcis bocagei* at the area of syntopy was studied in Ortega et al. (2016a). Here we assess the changes in thermal ecology of *I. galani* between both seasons.

Table 1

Mean body temperatures (T_b), air temperatures (T_a), substrate temperatures (T_s), selected temperatures in a laboratory thermal gradient (T_{sel}) and preferred temperature range (PTR; the 50% central of T_{sel} values) of *Iberolacerta galani*, and mean operative temperatures (T_e) of their habitat during spring and summer. Mean \pm SE (N). All temperatures are reported in $^{\circ}\text{C}$.

	Spring	Summer
T_b	29.45 \pm 0.45 (26)	30.89 \pm 0.27 (79)
T_a	26.33 \pm 0.64 (26)	28.22 \pm 0.39 (79)
T_s	28.65 \pm 0.69 (26)	30.55 \pm 0.42 (79)
T_{sel}	30.38 \pm 0.49 (24)	28.76 \pm 0.54 (24)
PTR	29.60–31.10 (24)	27.90–29.70 (24)
T_e	36.64 \pm 0.27 (15)	32.94 \pm 0.16 (15)

versa. The third is the index of effectiveness of thermoregulation (E), that is calculated as $E = 1 - (d_b/d_e)$. Values of E range from 0 to 1, where a higher effectiveness of thermoregulation translates into a higher value of E (see Hertz et al., 1993). Effectiveness of thermoregulation was calculated with THERMO, a Minitab module that has been used in previous studies of thermal biology (e.g. Ortega et al., 2014) and uses three kinds of input data: T_b , T_e and T_{sel} of the PTR, and was programmed to perform bootstraps of 100 iterations, building pseudo-distributions of three kinds of output values: d_b , d_e , and E .

2.4.2. Contribution of seasonal change in the PTR to thermoregulation

Following the methodology described in Díaz et al. (2006), we studied the contribution of the seasonal changes in the PTR of *I. galani* to the effectiveness of thermoregulation. The hypothesis is that a seasonal change of thermal preferences would improve thermoregulatory efficiency. In order to test the hypothesis, we studied the contribution of the change of the PTR to the effectiveness of thermoregulation for both seasons.

To study the contribution of the PTR of spring to the effectiveness of thermoregulation, we compared the real habitat thermal quality of spring ($d_{e-spring}$) with the potential habitat thermal quality of spring ($d_{p-spring}$). The $d_{e-spring}$ is the mean of absolute values of the deviations of each T_e of spring from the PTR of spring, while the $d_{p-spring}$ is the mean of absolute values of the deviations of each T_e of spring from the PTR of summer (that is, the potential d_e that lizards would show if the PTR of spring was the one that they have in summer). Then, we compared the real ($d_{e-spring}$) and the potential ($d_{p-spring}$) thermal quality of habitat: if operative temperatures of spring deviate less from the PTR of spring than from the PTR of summer (that is, if $d_{e-spring} < d_{p-spring}$), then the seasonal change in the PTR would result in a higher thermoregulatory efficiency in spring. Thus, the contribution of the seasonal shift of the PTR to thermoregulation in spring is calculated as ($d_{e-spring} - d_{p-spring}$).

Respectively, we calculated the real value of the index of the habitat thermal quality in summer ($d_{e-summer}$) and the potential value of the index of habitat thermal quality in summer if the PTR of lizards would be the PTR of spring ($d_{p-summer}$). If summer operative temperatures deviate less from the PTR of summer than from the PTR of spring, then the PTR of summer would contribute to achieve a higher thermoregulatory efficiency. Thus, the contribution of the seasonal shift of the PTR to thermoregulation in summer is calculated as ($d_{e-summer} - d_{p-summer}$) (see Bauwens et al., 1996; Díaz et al., 2006; Díaz and Cabezas-Díaz, 2004). The potential indexes were also calculated with THERMO, as the real thermoregulation indexes.

2.4.3. Statistical analyses

Parametric statistics were performed when data followed the assumptions of normality and variance homogeneity. If these assumptions were not fulfilled, even after log-transformation, non-parametric equivalents were carried out (Crawley, 2012; Sokal and Rohlf, 1995).

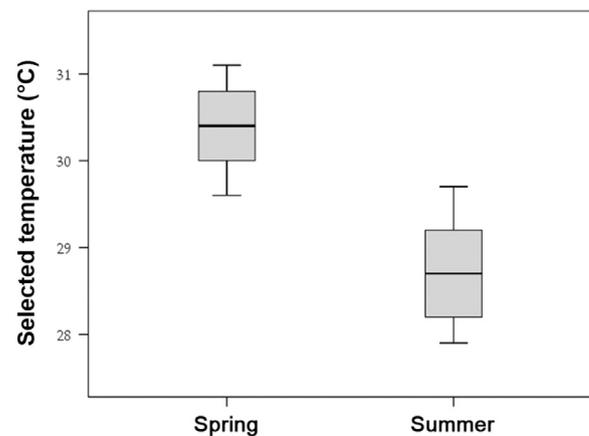


Fig. 2. The preferred temperature range (PTR) of *Iberolacerta galani* is significantly higher in spring (29.60–31.10 $^{\circ}\text{C}$) than in summer (27.90–29.70 $^{\circ}\text{C}$) while its narrowness is statistically similar for both seasons. Boxes depict the PTR (that is, the 50% central values, or the interquartile range, of the selected temperatures) and whiskers comprise the 90 percentile values.

Analyses were conducted on R, version 3.1.3 (R Core Team, 2015). Mean values are reported with standard errors (SE).

3. Results

Mean SVL of studied lizards was 67.05 \pm 0.61 mm (mean \pm SE; N=97) and mean weight was 6.99 \pm 0.19 g (N=97). There were no differences regarding reproduction condition for selected temperatures of females in spring (pregnant: mean T_{sel} =30.13 \pm 0.19 $^{\circ}\text{C}$, N=37; non-pregnant: mean T_{sel} =30.33 \pm 0.18 $^{\circ}\text{C}$, N=36; ANOVA, $F_{1, 72}$ =0.581, p =0.449), so we pooled T_{sel} of females of spring for subsequent analysis. Selected body temperatures were similar for males and females, both during spring (males: mean T_{sel} =30.33 \pm 0.09 $^{\circ}\text{C}$, N=29; females: mean T_{sel} =30.42 \pm 0.07 $^{\circ}\text{C}$, N=44; ANOVA, $F_{1, 71}$ =0.623, p =0.432) and summer (males: mean T_{sel} =28.70 \pm 0.07 $^{\circ}\text{C}$, N=43; females: mean T_{sel} =28.82 \pm 0.10 $^{\circ}\text{C}$, N=37; ANOVA, $F_{1, 78}$ =0.920, p =0.340). However, the T_{sel} were significantly higher in spring than in summer (ANOVA, $F_{1, 151}$ =374.283, p <0.0001; Table 1). Thus, the PTR of *I. galani* must be considered separately for each season (Table 1). The breadth of the PTR is similar for both seasons (Levene test, F =1.127, p =0.290; Fig. 2).

Body temperatures of active lizards (T_b) were also similar for males and females, both in spring (males: mean T_b =29.04 \pm 0.67 $^{\circ}\text{C}$, N=11; females: mean T_b =29.76 \pm 0.60 $^{\circ}\text{C}$, N=15; ANOVA, $F_{1, 24}$ =0.633, p =0.434) and summer (males: mean T_b =30.53 \pm 0.38 $^{\circ}\text{C}$, N=38; females: mean T_b =31.23 \pm 0.38 $^{\circ}\text{C}$, N=41; ANOVA, $F_{1, 77}$ =1.642, p =0.204). Thus, males and females were grouped for further analyses. Body temperatures (T_b ; ANOVA, $F_{1, 103}$ =1.779, p =0.185) and substrate temperatures (T_s ; ANOVA, $F_{1, 103}$ =0.073, p =0.787) were similar in spring and summer, while air temperatures (T_a ; Mann–Whitney U test, U =696.0, p =0.014) were significantly lower in spring than in summer (Table 1).

We found a positive correlation between T_a and T_b during spring (r =0.336, p =0.047, N=26) and summer (r =0.500, p <0.0001, N=79). Nonetheless, slopes of linear regression are similar in both seasons (spring: T_b =23.26+0.24 $\cdot T_a$, R^2 =0.113; summer: T_b =21.26+0.34 $\cdot T_a$, R^2 =0.250; test of slopes of ANCOVA, $F_{1, 103}$ =0.516, p =0.474; Fig. 3). In addition, there was also a positive correlation between T_s and T_b in spring (r =0.730, p <0.0001, N=26) and summer (r =0.698, p <0.0001, N=79), and slopes of linear regression are also similar for both seasons (spring: T_b =16.05+0.47 $\cdot T_s$, R^2 =0.730; summer: T_b =17.20+0.45 $\cdot T_s$, R^2 =0.487; test of slopes of ANCOVA, $F_{1, 103}$ =0.033, p =0.856; Fig. 3).

The values of the index of habitat thermal quality of habitat (d_e) were significantly higher in spring than in summer (spring: mean

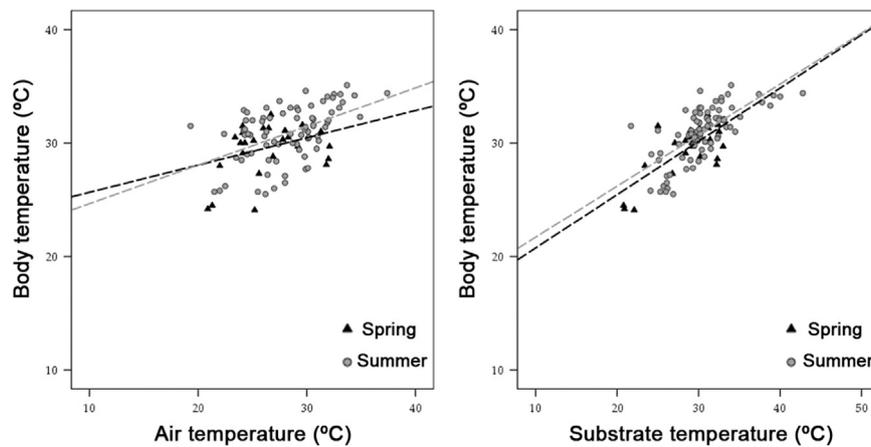


Fig. 3. Linear regression models between body and air temperatures and between substrate and body temperatures of *Iberolacerta galani* show statistically similar slopes for both spring and summer.

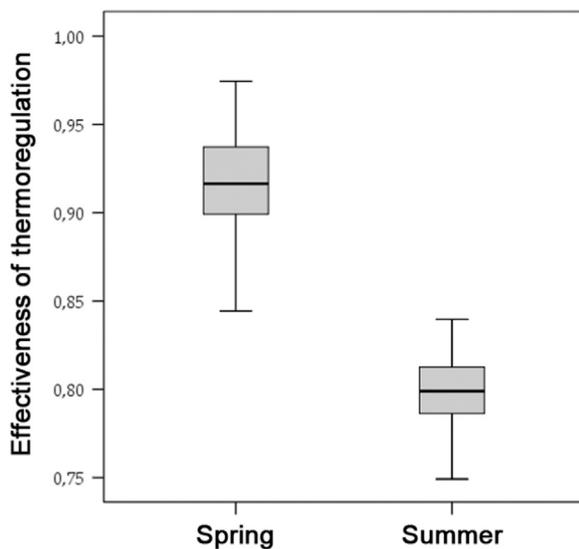


Fig. 4. *Iberolacerta galani* achieves a significantly higher effectiveness of thermoregulation in spring than in summer. Boxes depict the interquartile range of the effectiveness of thermoregulation obtained by bootstrapping, and whiskers comprise the 90 percentile values.

Table 2

Proportion (%) of the body temperatures of *Iberolacerta galani* and the operative temperatures at their habitat that felt below and above the PTR for spring and summer.

	Body temperatures		Operative temperatures	
	< PTR	> PTR	< PTR	> PTR
Spring	60.0%	40.0%	34.9%	65.1%
Summer	15.7%	84.3%	49.4%	50.6%

$d_e=10.99 \pm 0.05$ °C; summer: mean $d_e=9.36 \pm 0.03$ °C; Mann-Whitney *U* test, $U=10.0$, $p < 0.0001$), which means a lower habitat thermal quality in spring. However, *I. galani* achieves a significantly lower value of the index of accuracy of thermoregulation (d_b) during spring than in summer, which means a higher accuracy of thermoregulation in spring (spring: mean $d_b=0.93 \pm 0.03$ °C; summer: mean $d_b=1.88 \pm 0.02$ °C; test *U* de Mann-Whitney, $U=45.0$, $p < 0.0001$). Thus, effectiveness of thermoregulation (*E*) is significantly higher in spring than in summer (spring: mean $E=0.92 \pm 0.002$; summer: mean $E=0.80 \pm 0.002$; test *U* de Mann-Whitney, $U=5.0$, $p < 0.0001$; Fig. 4). Finally, the PTR of spring is significantly closer to T_e of spring than to those of summer ($d_{e-spring} < d_{p-spring}$), while the PTR of summer is

Table 3

Contribution of the seasonal shift in the thermal preferences of *Iberolacerta galani* to enhance thermoregulation of each season. Mean values of the real habitat thermal quality (d_e) are compared with mean values of the potential quality of the habitat (d_p) for both seasons.

	$d_e \pm SE$	$d_p \pm SE$	$F_{1, 198}$	<i>p</i>	$(d_e - d_p)$
Spring	10.99 ± 0.05 °C	11.44 ± 0.05 °C	38.595	< 0.0001	0.45 °C
Summer	9.36 ± 0.03 °C	9.52 ± 0.03 °C	11.949	0.001	0.16 °C

significantly closer to T_e of summer than to T_e of spring ($d_{e-summer} < d_{p-summer}$; Tables 2, 3).

4. Discussion

Although PTR values are sometimes lower than the actual optimal thermal range of lizards, it can still be used as a proxy for performance and fitness (Hertz et al., 1993; Martin and Huey, 2008). The PTR of spring was similar for pregnant and non-pregnant females, a situation that has been also reported for other lacertids (Braña, 1993; Ortega et al., 2016c). The PTR of *I. galani* is similar for males and females in both seasons, and markedly narrow and low: 29.60–31.10 °C in spring and 27.90–29.70 °C in summer, being, to our knowledge, the lowest among lacertid lizards. Thermal preferences of other Lacertidae species range from approximately 30 °C in *Zootoca vivipara* and *Iberolacerta cyreni* (Aguado and Braña, 2014; Gvoždík, 2002; Ortega et al., 2016b) to more than 37 °C in *Acanthodactylus erythrurus* (Bauwens et al., 1995; Gil et al., 1993). The low breadth of the PTR of *I. galani* was similar between seasons and would be reflecting a consistent precision of thermoregulation, despite seasonal changes. Therefore, *I. galani* would be a cold-adapted lizard, as the other studied species of *Iberolacerta* (Aguado and Braña, 2014; Martín and Salvador, 1993; Žagar et al., 2015; Ortega et al., 2016d), with cold preferences both in spring and summer. The conservation of the PTR breadth between seasons is an interesting finding that should be more deeply studied in relation to thermal specialization.

There was a seasonal shift in thermal preferences of *I. galani*: the PTR was approximately 1.5 °C higher in spring than in summer. Seasonal variation of the thermal preferences is common in reptiles, and may adapt physiology to reduce costs of thermoregulation of each season (Díaz and Cabezas-Díaz, 2004; Seebacher, 2005; Truter et al., 2014). Our results support this hypothesis, since the seasonal shift in the PTR contributed significantly to achieve a more effective thermoregulation on each season. This contribution was less than the 5% of the deviates of operative temperatures form the PTR. However, this small contribution is higher than the shift found in *Psammotromus algirus*, the other lacertid lizard studied with the same protocol (Díaz

et al., 2006). The adaptation of the thermal preferences of *I. galani*, with a higher PTR in spring than in summer would improve thermoregulation and could be related with the enhancement of performance during the mating season. As these montane lizards have short annual activity periods, since their habitat is covered by snow from October to April, they would need to maximize performance in order to reproduce. When they emerge in April (Arribas et al., 2006; pers. obs.) trophic resources are still limited, so lizards may take advantage of a careful thermoregulation that maximizes performance to search for food, look for mates or avoid predators. All studied thermal traits were similar for males and females. Thus, if the physiological seasonal adaptation of thermal biology is related to an increase of reproductive success, it would be equal in both sexes.

If the seasonal shift of thermal preferences contributed to the effectiveness of thermoregulation in less than the 5%, the rest of the thermal regulation would be behavioural, through careful selection of suitable microhabitats (Bauwens et al., 1996; Díaz and Cabezas-Díaz, 2004). *Iberolacerta galani* showed similar body temperatures in spring and summer, being substrate temperatures of the captured places also similar between seasons, and air temperatures of the capture places lower in spring. The relation between body and air temperatures, and between body and substrate temperatures was similar among seasons, suggesting that other physiological adaptations, such as seasonal changes in heating rates, would be unlikely.

Operative temperatures were above the PTR of *I. galani* for both seasons. A previous study in the related lizard *Iberolacerta cyreni* found that the environmental temperatures of the capture places of lizards increased more than 4 °C in the last 25 years, while body temperature of lizards increased less than 2 °C in the same period, being lizards able to buffer the impact of global warming behaviourally (Ortega et al., 2016b). We lack data about the extent of the warming of the habitat of *I. galani* and about thermal ecology of lizards in the past, but we know that the more thermophilic species *P. bocagei* has recently colonized the habitat and is currently thermoregulating more effectively than *I. galani* during summer (Ortega et al., 2016a). There is not information about changes in thermal preferences of lizards due to climate change. Thus, we cannot discard that the observed adaptive seasonal shifts in the PTR of *I. galani* are a recent phenomenon, or that fixed physiological traits are adapted to new environmental circumstances by chance. It is even possible that the PTR of *I. galani* in summer would change in order to avoid competition with *P. bocagei*, since their PTR when they coexist in summer do not overlap.

Previous research found that lizards thermoregulate more effectively in thermally challenging habitats (Besson and Cree, 2010; Blouin-Demers and Weatherhead, 2001, 2002; Monasterio et al., 2009; Sagonas et al., 2013). Environmental temperatures were sub-optimal in spring, but lizards managed to get closer body temperatures to the PTR in spring than in summer. We showed that this is achieved thanks to their behavioural flexibility and a significant contribution of the PTR of each season to facilitate thermoregulation. A higher effort of thermoregulation in spring than in summer will entail more energetic costs, so lizards maintain it more likely because the benefits overcome the costs of thermoregulation (Sears and Angilletta, 2015). Further research is needed to integrate the knowledge about the thermal biology of the Lacertidae and understand how the seasonal changes evolve and affect the biology of lizards, as well as to improve our knowledge of mountain specialists in Mediterranean areas.

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