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

Zaida Ortega⁎, Abraham Mencía, Valentín Pérez-Mellado

Department of Animal Biology, University of Salamanca, Campus Miguel de Unamuno, 37007 Salamanca, Spain

**ARTICLE INFO**

*Keywords:* Thermoregulation
Mountains
*Iberolacerta*
Seasonal variation
Thermal preferences
Thermal biology
Lacertidae

**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 (Diaz and Diaz-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 (Diaz 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,
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; Mencia 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 (Tb) immediately after capture, as to 1800 GMT, during their daily activity period. For every lizard, we from 2011 to 2013. Adult lizards were captured by noosing from 0800 during spring (May) of 2012 and summer (August) from 2011 to 2013. We obtained 2311 measures of Tb 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 Tsel as the selected body temperatures in general, and to PTR as the 50% central values of the Tsel. 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 db): the mean of absolute values of the deviations between each Tb from the preferred temperature range. The values of the index of accuracy of thermoregulation are opposite to their meaning: higher values of d db indicate lower accuracy of thermoregulation, and vice-versa. The second is the index of habitat thermal quality (mean d sq): the mean of absolute values of the deviations of each Tb 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 sq 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.
versa. The third is the index of effectiveness of thermoregulation (E), that is calculated as \( E = 1 - \frac{d_{p}}{d_{a}} \). 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: \( T_{b} \), \( T_{a} \) and \( T_{s} \), and was programmed to perform bootstraps of 100 iterations, building pseudo-distributions of three kinds of output values: \( d_{p} \), \( d_{a} \), 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 (\( d_{p-spring} \)) with the potential habitat thermal quality of spring (\( d_{p-spring} \)). The \( d_{p-spring} \) is the mean of absolute values of the deviations of each \( T_{b} \) of spring from the PTR of spring. While the \( d_{p-spring} \) is the mean of absolute values of the deviations of each \( T_{b} \) of spring from the PTR of summer (that is, the potential \( d_{p} \) that lizards would show it the PTR of spring was the one that they have in summer). Then, we compared the real (\( d_{p-spring} \)) 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, the potential \( d_{p} \) that lizards would show it the PTR of spring was the one that they have in summer). Then, we compared the real (\( d_{p-spring} \)) 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, the potential \( d_{p} \) that lizards would show it the PTR of spring was the one that they have in summer). Then, we compared the real (\( d_{p-spring} \)) 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, the potential \( d_{p} \) that lizards would show it the PTR of spring was the one that they have in summer). Then, we compared the real (\( d_{p-spring} \)) 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, the potential \( d_{p} \) that lizards would show it the PTR of spring was the one that they have in summer). Then, we compared the real (\( d_{p-spring} \)) 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, the potential \( d_{p} \) that lizards would show it the PTR of spring was the one that they have in summer). Then, we compared the real (\( d_{p-spring} \)) 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, the potential \( d_{p} \) that lizards would show it the PTR of spring was the one that they have in summer).

### 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).

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Spring</th>
<th>Summer</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T_{b} )</td>
<td>29.45 ± 0.45 (26)</td>
<td>30.89 ± 0.27 (79)</td>
</tr>
<tr>
<td>( T_{a} )</td>
<td>26.33 ± 0.64 (26)</td>
<td>28.22 ± 0.39 (79)</td>
</tr>
<tr>
<td>( T_{s} )</td>
<td>28.65 ± 0.69 (26)</td>
<td>30.55 ± 0.42 (79)</td>
</tr>
<tr>
<td>( T_{sel} )</td>
<td>30.38 ± 0.49 (24)</td>
<td>28.76 ± 0.54 (24)</td>
</tr>
<tr>
<td>( PTR )</td>
<td>29.60–31.10 (24)</td>
<td>27.90–29.70 (24)</td>
</tr>
<tr>
<td>( T_{c} )</td>
<td>36.64 ± 0.27 (15)</td>
<td>32.94 ± 0.16 (15)</td>
</tr>
</tbody>
</table>

Mean values are reported with standard errors (SE). 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 ± 0.61 mm (mean ± SE; N=97) and mean weight was 6.99 ± 0.19 g (N=97). There were no differences regarding reproduction condition for selected temperatures of females in spring (pregnant: mean \( T_{sel} = 30.33 ± 0.18 °C \), N=36; non-pregnant: mean \( T_{sel} = 30.33 ± 0.18 °C \), 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 \( T_{sel} = 30.33 ± 0.09 °C \), N=29; females: mean \( T_{sel} = 30.42 ± 0.07 °C \), N=44; ANOVA, \( F_{1,72} = 0.432 \), p=0.623) and summer (males: mean \( T_{sel} = 28.70 ± 0.07 °C \), N=43; females: mean \( T_{sel} = 28.82 ± 0.10 °C \), N=37; ANOVA, \( F_{1,72} = 0.340 \), p=0.576). 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 \( T_{b} = 29.04 ± 0.67 °C \), N=11; females: mean \( T_{b} = 29.76 ± 0.60 °C \), N=15; ANOVA, \( F_{1,22} = 0.633 \), p=0.434) and summer (males: mean \( T_{b} = 30.53 ± 0.38 °C \), N=38; females: mean \( T_{b} = 31.23 ± 0.38 °C \), N=41; ANOVA, \( F_{1,78} = 1.642 \), 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 \( T_{b} \) and \( T_{s} \) in both seasons (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: \( T_{b} = 23.26+0.24*T_{s} \), \( R^{2}=0.113 \); summer: \( T_{b} = 21.26+0.34*T_{s} \), \( R^{2}=0.250 \); test of slopes of ANCOVA, \( F_{1,75} = 0.474 \), p=0.666; Fig. 3). In addition, there was also a positive correlation between \( T_{b} \) and \( T_{s} \) 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: \( T_{b} = 16.05+0.47*T_{s} \), \( R^{2}=0.730 \); summer: \( T_{b} = 17.20+0.45*T_{s} \), \( R^{2}=0.487 \); test of slopes of ANCOVA, \( F_{1,102} = 0.033 \), p=0.856; Fig. 3).

The values of the index of habitat thermal quality of habitat (\( d_{p} \)) were significantly higher in spring than in summer (spring: mean
de = 10.99 ± 0.05 °C; summer: mean de = 9.36 ± 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 (db) during spring than in summer, which means a higher accuracy of thermoregulation in spring (spring: mean db = 0.93 ± 0.03 °C; summer: mean db = 1.88 ± 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 ± 0.002; summer: mean E=0.80 ± 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 (de-spring < dp-spring), while the PTR of summer is significantly closer to T_e of summer than to T_e of spring (de-summer < dp-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 Psammobromus algirus, the other lacertid lizard studied with the same protocol (Díaz...
and AM by predoctoral grants from the University of Salamanca. This work was also supported by the research projects CGL2009-12926-C02-02 and CGL2012-39850-C02-02 from the Spanish Ministry of Science and Innovation. All research was conducted in compliance with ethical standards and procedures of the University of Salamanca.

References


