



# The peak of thermoregulation effectiveness: Thermal biology of the Pyrenean rock lizard, *Iberolacerta bonnali* (Squamata, Lacertidae)



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## ARTICLE INFO

### Article history:

Received 18 October 2015

Received in revised form

29 December 2015

Accepted 10 January 2016

Available online 13 January 2016

### Keywords:

Thermoregulation

Cold-adapted

Global warming

Mountains

*Iberolacerta bonnali*

Lacertidae

## ABSTRACT

We studied, at 2200 m altitude, the thermal biology of the Pyrenean rock lizard, *Iberolacerta bonnali*, in the glacial cirque of Cotatuero (National Park of Ordesa, Huesca, Spain). The preferred thermal range (PTR) of *I. bonnali* indicates that it is a cold-adapted ectotherm with a narrow PTR (29.20–32.77 °C). However, its PTR (3.57 °C) is twice as wide as other *Iberolacerta* lizards, which may be explained by its broader historical distribution. The studied area is formed by a mosaic of microhabitats which offer different operative temperatures, so that lizards have, throughout their entire daily period of activity, the opportunity to choose the most thermally suitable substrates. *I. bonnali* achieves an effectiveness of thermoregulation of 0.95, which makes it the highest value found to date among the Lacertidae, and one of the highest among lizards. Their relatively wide distribution, their wider PTR, and their excellent ability of thermoregulation, would make *I. bonnali* lizards less vulnerable to climate change than other species of *Iberolacerta*. Thanks to its difficult access, the studied area is not visited by a large number of tourists, as are other areas of the National Park. Thus, it is a key area for the conservation of the Pyrenean rock lizard. By shuttling between suitable microhabitats, lizards achieve suitable body temperatures during all day. However, such thermally suitable microhabitats should vary in other traits than thermal quality, such as prey availability or predation risk. Hence, it seems that these non-thermal traits are not constraining habitat selection and thermoregulation in this population. Therefore, future research in this population may study the causes that would lead lizards to prioritize thermoregulation to such extent in this population.

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## 1. Introduction

Temperature strongly influences the rate of biochemical reactions (e.g. Pörtner, 2002) and body temperature can have a deep influence in the performance of biochemical procedure (Angilletta, 2009). Environmental temperature varies with latitude (e.g. Sunday et al., 2014), altitude (e.g. Gvozdík, 2002; Zamora-Camacho et al., 2013, 2015), weather (e.g. Hammerson, 1989), seasons (e.g. Díaz et al., 2006; Ortega et al., 2014), microhabitat structure (e.g. Sears and Angilletta, 2015), and even is currently varying as a consequence of climate change (e.g. Parmesan, 2006), and this variation determines the availability of temperatures for organisms. This thermal variation influences two dimensions of thermal biology (Angilletta, 2009), which define the strategies of organisms to deal with environmental thermal heterogeneity: thermal sensitivity (e.g. Huey and Kingsolver, 1989) and thermoregulation (e.g. Hertz et al., 1993). Thermal sensitivity describes the extent to

which physiological performance of an organism depends on temperature, from thermal specialists, whose performance is optimal in a narrow temperature range, to thermal generalists, which are able to perform well in a wide range of temperatures. The other dimension of thermal biology is thermoregulation, or the ability to actively regulate body temperature, which ranges from thermoconformers, whose body temperature equals the environment temperatures anytime, to perfect thermoregulators, whose body temperature is practically independent of environmental temperature (see a review in Angilletta, 2009).

Lacertid lizards are effective thermoregulators, which regulate their body temperature mainly shuttling between microhabitats (Arnold, 1987; Castilla et al., 1999). Some studies have addressed thermal biology of lacertid lizards living at high altitudes (e.g. Aguado and Braña, 2014; Martín and Salvador, 1993; Monasterio et al., 2009), although much additional work would be necessary to understand the thermal biology at high altitudes.

There are three factors that make rock lizards one of the most endangered group of reptiles in front of climate change. First, they live at mountaintops, so they do not have a higher and colder place

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to migrate (e.g. Berg et al., 2010). Then, they tend to be cold-adapted, which not only implies that their optimal temperatures would be exceeded sooner than for not cold-adapted ectotherms, but the impact of exceeding the optimal temperatures would be stronger (e.g. Huey et al., 2012). Finally, a faster increase of environmental temperatures and drought is expected throughout the 21st century in the Iberian mountains (Araújo et al., 2006; Maiorano et al., 2013). Moreover, climate warming not only entails hotter habitats for high mountain lacertids, it may provoke the ascension of low-elevation lizards, which may compete with alpine rock lizards (Comas et al., 2014).

We study the thermal biology of the Pyrenean rock lizard, *Iberolacerta bonnali*, which was thought to be a case of strange non thermoregulatory lacertid (Martínez-Rica, 1977). Following the protocol of Hertz et al. (1993), we assess precision of thermoregulation, thermal quality of the habitat, as well as accuracy and effectiveness of thermoregulation. Thus, we will test the hypothesis of thermoregulation (Hertz et al., 1993) for this species. Furthermore, we assess the spatial and thermal heterogeneity of the habitat of *I. bonnali* in order to describe it for future comparisons between other high mountain lizards. Rock lizards live in high mountain isolated habitats (Pérez-Mellado et al., 2009), where they were located after the last glaciation, as other species of *Iberolacerta* (Crochet et al., 2004; Mouret et al., 2011). Given the vulnerability of Pyrenean rock lizards to global warming (see above), together with the fact that their populations are fragmented, small and isolated, *I. bonnali* lizards might have little chances to survive the current climate change. In this framework, this study could be important because it provides useful information in order to ascertain the capacity of these endemic lizards to survive climate warming.

## 2. Material and methods

### 2.1. Studied species and studied area

The Pyrenean rock lizard, *I. bonnali* (Lantz, 1927), is endemic to the Pyrenees mountains, with a fragmented range between 1550 m and 3062 m of altitude (Arribas, 2004; Arribas, 2009). This species belongs to the "Pyrenean group" of *Iberolacerta*, with a different evolutionary history from other species of the genus (Mayer and Arribas, 2003). Mean snout-vent length of the studied lizards is  $56.07 \pm 0.64$  mm and mean weight is  $4.24 \pm 0.15$  g, lacking sexual dimorphism for body size (unpub. data,  $N=38$ ). They are insectivorous lizards, whose morphology and coloration is similar to the other two species of the "Pyrenean group" (Arribas, 1994). Like other species of the genus, *I. bonnali* is subjected to a short annual period of activity due to snow coverage most of the year (Arribas, 2009, 2010; Martínez-Rica, 1977).

We studied the thermal biology of *I. bonnali* at the glacial cirque of Cotatuero (Huesca, Spain), in the National Park of "Ordesa y Monte Perdido", at Central Pyrenees. The study area is at 2200 m of altitude, and consists mainly of rocky crops and moist meadows, crossed by several streams. It is a well-preserved and undisturbed area, probably because the access is restricted through a difficult mountain pass that prevents the racking of tourists that is common in the rest of the National Park.

### 2.2. Field sampling

Body temperatures ( $T_b$ ) were sampled between the 9th and the 14th of August of 2013. From 07.00 to 16.00 h GMT, we captured 46 *I. bonnali* adult lizards (23 males and 23 females) by noosing. For each lizard, we measured cloacal temperature ( $T_b$ ) immediately after capture with a Testo<sup>®</sup> digital thermometer ( $\pm 0.1$  °C

precision), introducing the thermal probe 0.5 mm into the vent of lizards. We also measured air temperature ( $T_a$ ) 1 cm above the capture point, and substrate temperature ( $T_s$ ) at the capture point. All temperatures were measured shading the thermal probe, in order to avoid a temperature bias due to sun radiation.

We recorded operative temperatures ( $T_e$ ) in the same area of study, simultaneously to  $T_b$ , sampling in order to assure the same weather conditions. We used copper models as null  $T_e$  models (Bakken and Angilletta, 2014). For small ectotherms, hollow copper models of the same length and diameter of lizards match appropriately the temperature of non-thermoregulating individuals, in order to be used as a null hypothesis of thermoregulation (Shine and Kearney, 2001). One thermocouple probe was placed into each hollow model and connected to a data logger HOBO H8<sup>®</sup> (Onset Computer Corporation), programmed to take a temperature recording every five minutes. We used 12 data loggers and models, which were randomly placed in different microhabitats: (1) *under rock* ( $N=661$ ), (2) *flat rock in shade* ( $N=660$ ), (3) *grass in shade* ( $N=663$ ), (4) *soil in shade* ( $N=659$ ), (5) *grass in filtered sun* ( $N=665$ ), (6) *flat rock in full sun* ( $N=664$ ), (7) *rock facing North in full sun* ( $N=662$ ), (8) *rock facing South in full sun* ( $N=661$ ), (9) *rock facing East in full sun* ( $N=526$ ), (10) *rock facing West in full sun* ( $N=659$ ), (11) *grass in full sun* ( $N=663$ ), and (12) *soil in full sun* ( $N=665$ ).

Thermal heterogeneity of the habitat was also quantified as the standard deviation of the mean operative temperatures of the data loggers, following Logan et al. (2015). Furthermore, spatial heterogeneity was described by mean values of the proportion of coverage and the frequency of each microhabitat type of 15 lineal transects, 25 m long each.

### 2.3. Preferred temperature range (PTR)

We studied the PTR of *I. bonnali* on the 15th and 16th of August 2013, immediately after the field work to assure similar weather conditions. We captured 24 Pyrenean rock lizards (12 males and 12 females) during the field work days, which were housed on individual terraria, fed daily with mealworms and crickets, and provided with water ad libitum. The thermal gradient was built in a glass terrarium ( $100 \times 60 \times 60$  cm<sup>3</sup>) with a 150 W infrared lamp over one of the sides, obtaining a gradient between 20 and 60 °C. A value of a selected temperature ( $T_{set}$ ) of a lizard was recorded with a digital thermometer each hour from 08.00 to 18.00 h (GMT), obtaining 143 selected temperature values. The 50% of central values of selected body temperatures was considered as the PTR to assess thermoregulation (Hertz et al., 1993; Blouin-Demers and Nadeau, 2005). The 80% of central values of selected body temperatures or 80% PTR was only used in plots, not in analysis. After the experiment, lizards were released in the same places of capture.

### 2.4. Data analysis

We followed the protocol developed by Hertz et al. (1993) in order to test the null hypothesis of thermoregulation in *I. bonnali*. Thus, we calculated the indexes of accuracy of thermoregulation ( $d_b$ ), thermal quality of habitat ( $d_e$ ) and effectiveness of thermoregulation ( $E$ ). The index of accuracy of thermoregulation ( $d_b$ ) is the average of absolute values of each deviation between  $T_b$  and the PTR. Thus, the higher is the value the lower is the accuracy of thermoregulation. The index of thermal quality of the habitat ( $d_e$ ) is the average of absolute values of each deviation between  $T_e$  and the PTR. Thus, the higher is the value the lower is the thermal quality of the habitat. Hence, if we compare the deviation of temperatures that lizards would reach without behavioral thermoregulation (i.e.  $T_e$ ) and the actual temperatures of active lizards

in relation with the PTR, we are able to quantify the degree of behavioral thermoregulation. To do this, we use the index of effectiveness of thermoregulation ( $E$ ) developed by Hertz et al. (1993). The index of effectiveness of thermoregulation ( $E$ ) is calculated as:  $E = 1 - d_b / d_e$ , and ranges between 0 (for a full thermoconformer) to 1 (for a perfect thermoregulator; see more details in Hertz et al. (1993)). In our study, effectiveness of thermoregulation was obtained performing a bootstrap of 500 iterations to build pseudo-distributions of three kinds of output values: mean accuracy (mean  $d_b$ ), mean thermal quality of the habitat (mean  $d_e$ ), and mean effectiveness of thermoregulation (mean  $E$ ).

Mean values are accompanied by standard errors (SE). Parametric statistics were performed when data followed the assumptions of normality and homogeneity of variances. If, even after log-transformation, these assumptions were not fulfilled, non-parametric equivalents were carried out (Crawley, 2012; Sokal and Rohlf, 1995). Analyses were conducted on R, version 3.1.3 (R Core Team, 2015). Post-hoc comparisons of Kruskal-Wallis tests were computed with Nemenyi test with the package PMCMR Pohlert (2014).

### 3. Results

The preferred temperatures range (PTR) of *I. bonnali* was 29.20–32.77 °C, with no differences between males and females (males: mean  $T_{set} = 31.26 \pm 0.20$  °C,  $N=36$ ; females: mean  $T_{set} = 31.02 \pm 0.16$  °C,  $N=37$ ; One-way ANOVA,  $F_{1,71} = 0.926$ ,  $p=0.367$ ; Fig. 1). Body temperatures were marginally higher for males than females, and air temperatures ( $T_a$ ) and substrate temperatures ( $T_s$ ) where male and female lizards were captured were similar between sexes (Table 1).

Correlation between  $T_b$  and  $T_a$  was statistically significant ( $r=0.306$ ,  $p=0.019$ ,  $N=46$ ). However, the regression fit was very poor ( $R^2=0.094$ , Fig. 2). Correlation between  $T_b$  and  $T_s$  was also significant ( $r=0.440$ ,  $p=0.001$ ,  $N=46$ ), although the regression fit was also poor ( $R^2=0.193$ , Fig. 2). The studied habitat offered

**Table 1**

Mean  $\pm$  SE body temperatures ( $T_b$ ), air temperatures ( $T_a$ ) and substrate temperatures ( $T_s$ ) of *Iberolacerta bonnali* lizards regarding sex, and results of paired comparisons (One-way ANOVA). All temperatures are in °C.

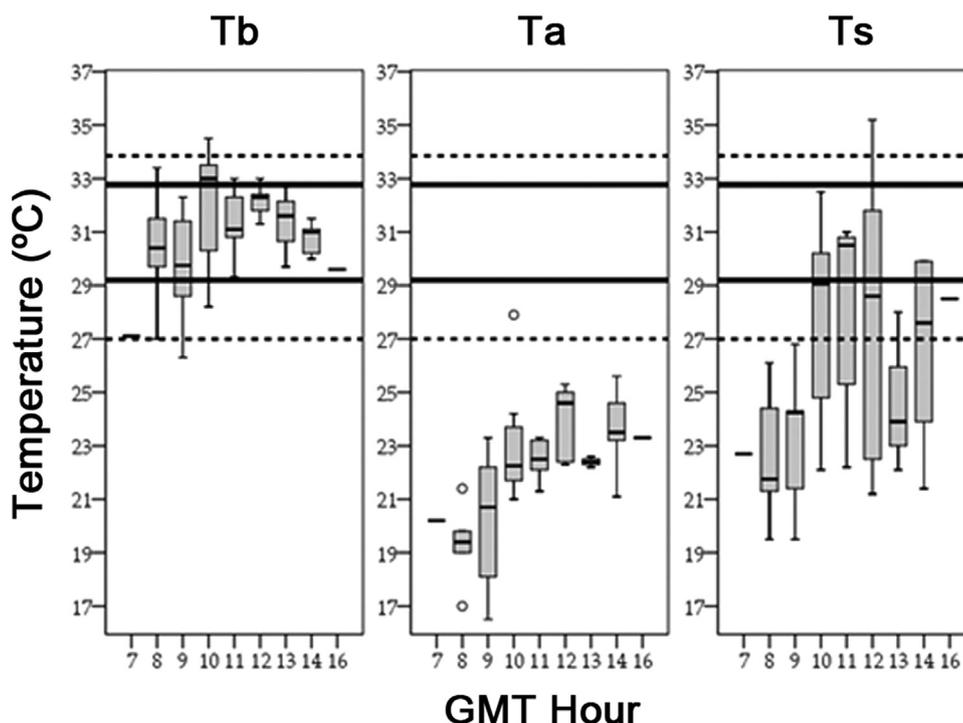
	Males (N=23)	Females (N=23)	Total	$F_{1,44}$	$p$
$T_b$	31.44 $\pm$ 0.34	30.56 $\pm$ 0.43	30.90 $\pm$ 0.28	3.922	0.054
$T_a$	22.25 $\pm$ 0.52	21.51 $\pm$ 0.45	21.88 $\pm$ 0.34	1.166	0.286
$T_s$	26.82 $\pm$ 0.92	24.75 $\pm$ 0.68	25.78 $\pm$ 0.59	3.271	0.077

significantly different thermal microhabitats for the thermoregulation of *I. bonnali* (Kruskal-Wallis test,  $N=7808$ ,  $gl=11$ ,  $H=2735.08$ ,  $p < 0.0001$ , Fig. 3). Shaded microhabitats showed similar  $T_e$ , which did not reach the PTR of *I. bonnali* at any moment of the day, being clearly unsuitable microhabitats for thermoregulation (Fig. 3). Grass in filtered sun offered  $T_e$  between shaded and sunny microhabitats, which reached the PTR between 10.00 and 16.00 GMT (Fig. 3). Microhabitats in full sun were divided in four different thermal types: (1) rock facing South and rock facing West, with similar  $T_e$  that were lower than the rest of sunny places, and (2) rock facing North and rock facing East, grass and soil, that offered the higher  $T_e$  available, which exceeded the PTR in the central hours of the day (Fig. 3).

Thermal heterogeneity of the habitat was 5.52 °C ( $N=12$ ). Spatial heterogeneity is described in Table 2, showing that dominant microhabitats are mixed meadows and big rocks. The index of thermal quality of the habitat was  $7.70 \pm 0.02$  °C, that is, the mean deviation between  $T_e$  and the PTR. The index of accuracy of thermoregulation of *I. bonnali* was  $0.38 \pm 0.01$  °C, that is, the mean deviation between  $T_b$  and the PTR. Finally, the index of effectiveness of thermoregulation was  $0.95 \pm 0.001$  (Fig. 4).

### 4. Discussion

The studied population of *I. bonnali* achieves an effectiveness of thermoregulation of 0.95. Thus, *I. bonnali* is the best



**Fig. 1.** Daily evolution of body temperatures ( $T_b$ ) of the Pyrenean rock lizards, and air temperatures ( $T_a$ ) and substrate temperatures ( $T_s$ ) in the capture sites. Continuous lines comprise the 50% PTR and the dotted lines comprise the 80% PTR. Sample sizes of each GMT hour from 07.00 to 16.00 h are: 1, 6, 10, 10, 5, 3, 5, 0, and 1.

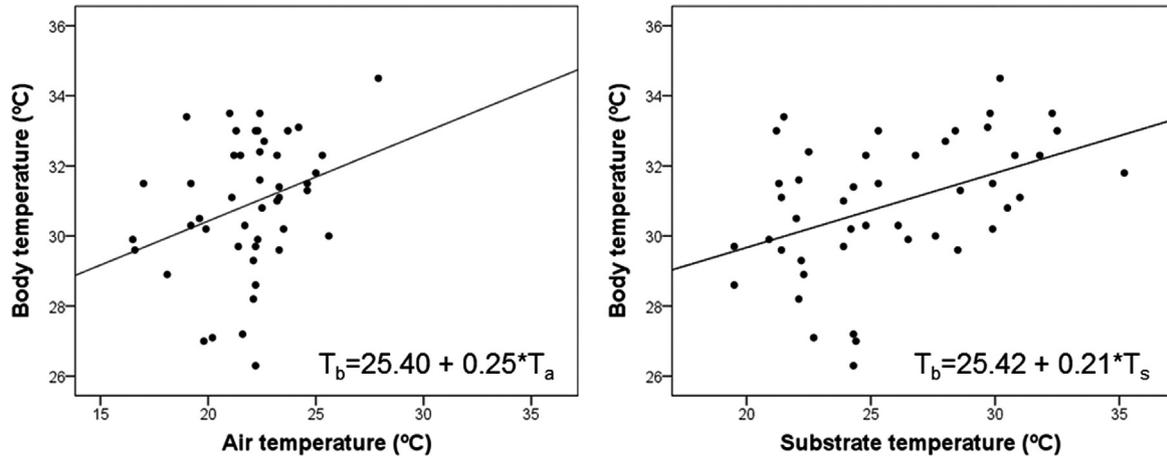


Fig. 2. Lineal regressions between body temperatures ( $T_b$ ) and air temperatures ( $T_a$ ), and between  $T_b$  and substrate temperatures ( $T_s$ ) are provided for *Iberolacerta bonnali*.

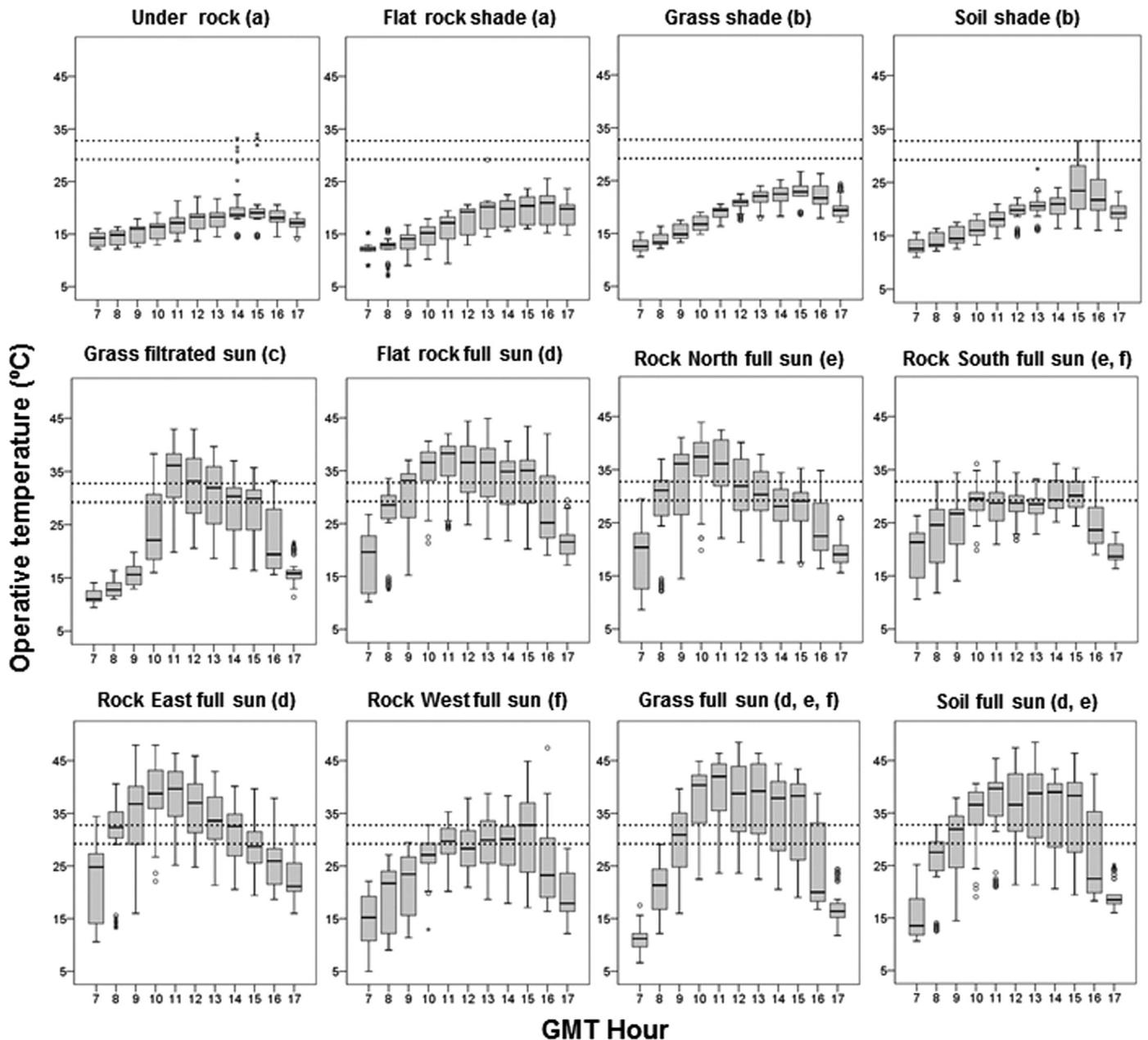


Fig. 3. Daily evolution of operative temperatures ( $T_e$ ) available for the thermoregulation of *Iberolacerta bonnali*. Microhabitats with similar  $T_e$  in the Nemenyi paired tests ( $p > 0.05$ ) are marked with the same letter between brackets. The dotted lines comprise the 50% PTR. Empty circles are outlier data, which exceed the 90 percentiles.

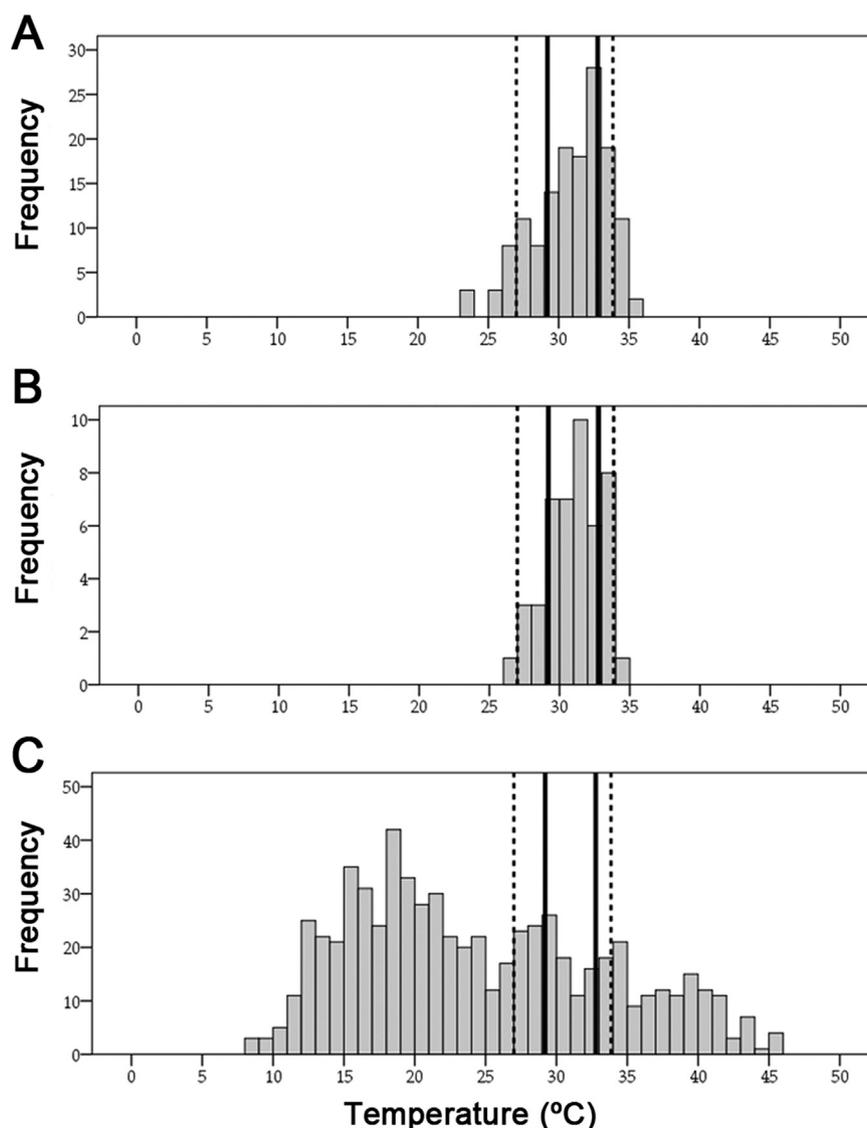
**Table 2**

Spatial heterogeneity of the glacial cirque of Cotatuero was assessed by measuring the available microhabitats in lineal transects. Mean (SE) percentages of microhabitat cover and frequency are provided for the 15 transects. Slab means a big flat rock that does not provide shelter for lizards.

	Slab	Rocks > 50 cm	Rocks < 50 cm	Loose stones	Soil	Grass > 15 cm	Grass < 15 cm	Shrubs
<b>Cover</b>	12.51% (1.81)	21.84% (4.05)	8.56% (2.09)	6.05% (2.71)	1.92% (0.52)	33.07% (5.21)	14.99% (3.20)	1.07% (0.52)
<b>Frequency</b>	16.13% (2.26)	19.16% (2.29)	11.23% (1.97)	6.64% (2.27)	5.90% (1.42)	20.37% (2.49)	19.74% (3.45)	0.83% (0.39)

thermoregulator found to date among lacertid lizards (Table 3). Our results do not support the results of Martínez-Rica (1977), which suggested that the Pyrenean rock lizard would lack the mechanisms to regulate its body temperature. It is probable that Martínez-Rica (1977) reached that conclusion because his measurements of body temperatures of lizards were unsuitable (see Arribas, 2009). Our results are consistent with those obtained by Arribas (2009) for body, air and substrate temperatures. Arribas (2009) suggests that *I. bonnali* is a good thermoregulator, but he was unable to confirm it without a test of the null hypothesis of thermoregulation (Hertz et al., 1993). Here we confirm the great capacity of the Pyrenean lizards to regulate their body temperatures and we found their astounding effectiveness for thermoregulation.

Another interesting result is a lesser degree of thermal specialization of *I. bonnali* in comparison with other species of the genus *Iberolacerta*. In the absence of the thermal reaction norm of performance for the Pyrenean rock lizard, as sprint speed, we can assume that the breadth of the PTR would approximately resemble the breadth of the thermal physiological performance curve (e.g. Angilletta, 2009; Bauwens et al., 1995). The precision of thermoregulation of other species ranges between 1.25 °C and 1.80 °C (in preparation). However, the Pyrenean rock lizard has a precision of 3.57 °C (PTR of 29.20–32.77 °C), doubling the other species of the genus. The result could be related with the evolutionary history of *I. bonnali*: its wider PTR could allow lizards to occupy a larger distributional range than other species of lizards from the same genus. The seasonality hypothesis of Janzen (Gaston and Chown, 1999; Janzen, 1967) predicts



**Fig. 4.** Histograms of: (A) selected temperatures, (B) body temperatures, and (C) operative temperatures for *Iberolacerta bonnali*. Continuous lines comprise the 50% PTR and the dotted lines comprise the 80% PTR.

**Table 3**Many available data of effectiveness of thermoregulation (*E*) of Lacertid lizards found to date.

Species	Study	Season	Altitude (m)	<i>E</i>
<i>Lacerta trilineata</i> (mainland)	Sagonas et al. (2013b)	spring	< 600	0.64
<i>Lacerta trilineata</i> (island)	Sagonas et al. (2013b)	spring	unknown	0.72
<i>Podarcis gaigeae</i> (Diavates)	Sagonas et al. (2013a)	unknown	unknown	0.72
<i>Dalmatolacerta oxycephala</i>	Scheers & Van Damme (2002)	summer	250	0.78
<i>Zootoca vivipara</i>	Gvozdík (2002)	summer	800	0.79
<i>Podarcis muralis</i>	Monasterio et al. (2009)	spring and summer	1770	0.79
<i>Podarcis muralis</i>	Grbac & Bauwens (2001)	autumn	10	0.81
<i>Podarcis melisellensis</i>	Scheers & Van Damme (2002)	summer	250	0.83
<i>Zootoca vivipara</i>	Gvozdík (2002)	summer	1450	0.85
<i>Podarcis gaigeae</i> (Skyros)	Sagonas et al. (2013a)	unknown	unknown	0.87
<i>Podarcis lilfordi</i>	Ortega et al. (2014)	spring and summer	10	0.88
<i>Psammodromus algirus</i>	Díaz (1997)	summer	1450	0.90
<i>Psammodromus algirus</i>	Díaz (1997)	summer	675	0.90
<i>Podarcis hispanica</i>	Díaz et al. (1996)	unknown	675	0.91
<i>Psammodromus algirus</i>	Díaz and Cabezas-Díaz (2004), Díaz et al. (2006)	spring	675	0.91
<i>Psammodromus algirus</i>	Díaz and Cabezas-Díaz (2004), Díaz et al. (2006)	summer	675	0.92
<i>Podarcis muralis</i>	Monasterio et al. (2009)	spring and summer	1770	0.92

that species that experience a greater annual variation of temperatures, as it happens in high mountain habitats, would tend to be more thermal generalists and have wider ranges (Gaston and Chown, 1999; Sheldon and Tewksbury, 2014). Lizards of the genus *Iberolacerta* experience large annual temperature variations even though they are, among lacertid lizards, thermal specialists, as glacial relict species (Carranza et al., 2004; Crochet et al., 2004; Mouret et al., 2011). Although data about thermal tolerance of *I. bonnali* is needed to test this hypothesis, maybe this species would be more generalist than other species of the Pyrenean *Iberolacerta*, as indicated by its wider altitudinal and geographical range. However, other Iberian species of the genus, as *Iberolacerta cyreni*, have a broader distributional range and a narrow PTR (Aguado and Braña, 2014). A comparative analysis, taking into account the phylogeny, is needed to assess if the seasonality hypothesis is followed here (Sheldon et al., 2015).

The last issue about thermal biology of the Pyrenean rock lizard that needs consideration is its vulnerability to climate change (Gunderson and Leal, 2012; Huey et al., 2012; Pacifici et al., 2015; Young et al., 2012). High mountain cold-specialists are one of the most vulnerable groups in the world because, as temperature decreases from the optimal, its survival decreases slowly, but then rapidly when temperature surpasses the optimal. This is due to the Jensen's inequality property of their thermal reaction norms (Huey et al., 2012; Martin and Huey, 2008). Furthermore, Mediterranean mountain lizards would be especially threatened due to the predictions of warming and drought for this area (Araújo et al., 2006; Carvalho et al., 2010; Maiorano et al., 2011). Because *I. bonnali* lizards have a broader PTR than the other species of *Iberolacerta* (Aguado and Braña, 2014), the expectations for the species survival against global warming would be, somehow, better compared to other species (Huey et al., 2012; Martin and Huey, 2008). In addition, the studied population of Cotatuero seems to inhabit a privileged area that allows lizards to achieve the highest effectiveness of thermoregulation among lacertids. Therefore, we encourage to maintain the excellent conservation work being done in this protected area and to search for other areas that may serve as a climate refuge for the persistence of high mountain lizards (Groves et al., 2012; Shoo et al., 2011). Nonetheless, a future study about the vulnerability of the species under climate change that includes, among other traits, dispersion rates, thermal dependence of physiological performance, or the thermal tolerance limits, will throw more light on the possibilities of persistence of the Pyrenean lizard and the conservation measures necessary to avoid its extinction.

By shuttling between suitable microhabitats, *I. bonnali* lizards might maintain their optimal (or preferred) body temperatures

during most time of the day, at least during summer. However, such thermally suitable microhabitats should vary in other traits than thermal quality, such as prey availability, predation risk, or even risk of being infected by parasites. Hence, it seems that these not-thermal traits are not constraining habitat selection and thermoregulation in this population. Therefore, our results raise the question about the causes that would lead lizards to prioritize thermoregulation to such extent in this population. Thus, the population at Cotatuero offers a good opportunity to further research the costs and benefits of thermoregulation.

### Acknowledgments

We thank Gregorio Moreno-Rueda and two anonymous referees for useful comments that helped us to improve the manuscript. We thank Pepe Galindo for his help climbing to Cotatuero. Lizards were studied under licenses of the Govern of Aragón, with document INAGA 500201/24/2013/4063. Financial support was provided to Zaida Ortega and Abraham Mencía by predoctoral grants of the University of Salamanca (FPI program). This work was also supported by the research project CGL2012-39850-CO2-02 from the Spanish Ministry of Science and Innovation and FEDER funds of EU. All research was conducted in compliance with ethical standards and procedures of the University of Salamanca.

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