Bergmann’s Rule predicts larger body sizes in colder habitats, increasing organisms’ ability to conserve heat. Originally formulated for endotherms, it is controversial whether Bergmann’s Rule may be applicable to ectotherms, given that larger ectotherms show diminished capacity for heating up. We predict that Bergmann’s Rule will be applicable to ectotherms when the benefits of a higher conservation of heat due to a larger body size overcompensate for decreased capacity to heating up. We test this hypothesis in the lizard *Psammodromus algirus*, which shows increased body size with elevation in Sierra Nevada (SE Spain). We measured heating and cooling rates of lizards from different elevations (from 300 to 2500 m above sea level) under controlled conditions. We found no significant differences in the heating rate along an elevational gradient. However, the cooling rate diminished with elevation and body size: highland lizards, with larger masses, have a higher thermal inertia for cooling, which allows them to maintain heat for more time and keep a high body temperature despite the lower thermal availability. Consequently, the net gaining of heat increased with elevation and body size. This study highlights that the heat conservation mechanism for explaining Bergmann’s Rule works and is applicable to ectotherms, depending on the thermal benefits and costs associated with larger body sizes.
During the activity period of this lizard, environmental lizards being larger at high elevations (see Results). Bergmann’s clines in a 2200-metre elevational gradient, Nevada (SE Spain). In Sierra Nevada, this lizard follows *Psammodromus algirus* (Yom-Tov & Geffen, 2010; for exceptions to this view see Ashton & Feldman, 2003; Cruz et al., 2005; Olalla-Tárraga et al., 2006; Olalla-Tárraga & Rodríguez, 2007). Despite this point of view, many ectotherm groups show Bergmann’s clines (e.g. aforementioned references; also see Ashton, 2002a; Blanckenhorn & Demont, 2004; Millien et al., 2006). Both the converse of Bergmann’s clines as well as the lack of covariation between body size and environmental temperature have also been found in several ectotherms (Mousseau, 1997; Ashton & Feldman, 2003; Olalla-Tárraga et al., 2006; Adams & Church, 2008; Hu et al., 2011), contributing to the controversy regarding the applicability of Bergmann’s Rule to ectotherms.

Under the view that the heat conservation hypothesis fails to explain why ectotherms comply with Bergmann’s Rule, several alternative hypotheses (not mutually exclusive) have been proposed to explain Bergmann’s clines in ectotherms (Atkinson & Sibly, 1997; Arnett & Gotelli, 2003; Angilletta et al., 2004a; Wilson, 2009; Arendt, 2011). However, we consider that arguments suggesting that the heat conservation hypothesis does not explain Bergmann’s Rule in ectotherms rely on simplistic assumptions. In ectotherms, net heat gain ($N$) is a function of heat gain ($G$) minus heat loss ($L$). Both $G$ and $L$ are functions of body mass ($m$), everything else being equal, $G = −αm$ and $L = −βm$. The parameters $α$ and $β$ indicate the slopes at which heat gain and heat loss decrease with body mass, and they are negative as thermal inertia increases with body mass (Carothers et al., 1997). Therefore, the net gain of heat would be $N = (β − α)m$. Typically, researchers have implicitly assumed that in ectotherms $β < α$, and therefore, net gain of heat decreases with body mass, which would favour smaller body sizes in cold sites (the converse Bergmann’s cline). Nevertheless, the fact is that for some ectotherms, $β < α$ (*Iberolacerta monticola*; Carrascal et al., 1992), but for others, $β > α$ (*Liolaeus altissimus*; Carothers et al., 1997).

We hypothesized that the heat conservation hypothesis may explain the occurrence of Bergmann’s clines in ectotherms. Here, we test this hypothesis using the lizard *Psammodromus algirus* as a model system in Sierra Nevada (SE Spain). In Sierra Nevada, this lizard follows Bergmann’s clines in a 2200-metre elevational gradient, lizards being larger at high elevations (see Results). During the activity period of this lizard, environmental temperature decreases an average of 8 °C from the lowest (300 m asl) to the highest elevation (2500 m asl) in our study area (Zamora-Camacho et al., 2013). We predict that the advantage of increased thermal inertia in larger individuals inhabiting cold habitats should compensate for the disadvantage of reduced heating capabilities, explaining the body size cline with elevation in this population; that is, we expect to find $β > α$.

To test this hypothesis, we examined whether elevational differences in body size are related to different thermoregulatory capabilities, by measuring lizards’ heating and cooling rates under controlled conditions (Yom-Tov & Geffen, 2011). The main novelty of this study is that, for the first time, we check whether the mechanism of the heat conservation hypothesis works in an animal showing a Bergmann cline.

### Materials and methods

*Psammodromus algirus* is a medium-sized (53–95 mm snout-vent length (SVL) in our study area) generalist Lacertid inhabiting mid-moisture shrubby areas in south-western Europe and north-western Africa, from sea level to 2600 m asl (Salvador, 2011). Several linages appear to comprise *P. algirus* (Verdú-Rico et al., 2010), but all the populations in our study belong to the same lineage, thus avoiding phylogenetic divergence (Cruz et al., 2005; Meiri & Thomas, 2007).

During June and July 2012, we caught lizards from sampling plots at 300, 700, 1200, 1700, 2200 and 2500 m asl in the southern slope of Sierra Nevada (SE Spain), in habitats chosen as similar as possible based on habitat structure (Zamora-Camacho et al., 2013). All specimens caught were taken to the laboratory. We used males, nongravid females and gravid females. We distinguished males because they have wider heads, larger and more numerous femoral pores in the hind limbs and orange spots in the corners of their mouths. We recognized pregnant females by manual palpation of developing eggs inside the trunk. During their stay in the laboratory, we maintained each lizard in an individual terrarium, where water (in the form of nutritive aqueous gel) and food (mealworm larvae, *Tenebrio molitor*) were provided *ad libitum*, and a natural light source was provided for the circadian physiological processes. A heat source (heat cable) was put in one corner of the terrarium to permit natural thermoregulation behaviour of the animals.

Prior to the experiments, we measured biometric parameters likely involved in heat exchange, including body mass with a balance (model CDS-100, precision 0.01 g), snout-vent length (SVL; in mm) with a millimetre-marked ruler and back lightness with a SCM-2600d/2500d spectrophotometer (Clusella-Trullas et al., 2007). Colour data provided by this device were composed of three coordinates ($L^*, a^*, b^*$) from the Commission Internationale d’Eclairage chromatic value colour.
space. Darkness is the inverse of lightness (L, measured in % of reflectance; Montgomerie, 2006). We measured
the colour of the central point of every lizard’s dorsum. The spectrophotometer measured three times and pro-
vided the average value, which we used for the analyses. As body mass, more than SVL, is the physical
attribute most involved in heat exchange (Carothers et al., 1997; Fei et al., 2012), we focused our analyses
on body mass, avoiding the expectable collinearity of these biometrically related factors ($r = 0.85; P < 0.001;
n = 61$).

To measure heat transfer rates, we immobilized liz-
ards individually on a thermally stable synthetic cork
plaque, using a soft lace knot around lizards’ neck, and
another one around lizards’ waist, fastened firmly but
loosely enough so that the lizards could not either
escape or suffer any pain. We placed the lizards 13 cm
below a 60-W blue-light bulb. In a laboratory at 22 °C
room temperature, we started the trials when lizards
attained 27 °C body temperature and switched off the
bulb when their body temperature reached 35 °C. We
chose a bulb over the animals as heat source because
these lizards are heliothermic, and heat up mainly by
sunbathing (Díaz & Cabezas-Díaz, 2004). The ranges
of body temperature are below lethal temperature, being
their optimum temperature range of 33–35 °C (Díaz
et al., 2006). Actually, the ranges of body temperature
chosen are within the body-temperature range that ani-
mals show in the field when active (Zamora-Camacho
et al., 2013). Once the bulb was switched off, the exper-
iment continued until body temperature diminished to
32 °C. Body temperature during the experiment was
measured with a small catheter inserted 8 mm inside
the cloaca and connected to a thermometer (Hybok
18). The body temperature of each animal was regist-
ered every 30 s during the whole process. No animal
suffered damage during this study. Once the exper-
iment ended, lizards were returned to the place where
they were captured.

Graphing body temperature over time, the standard
heat transfer curve of a lizard consisted of a linear tem-
perature increase while the bulb was switched on, a
variable-duration transition lapse after switching off the
bulb, while body temperature remained almost unal-
tered, and a linear temperature decrease after the iner-
tia lapse (Fig. 1). From those three phases, we obtained
for each individual: (i) the heating rate, calculated as
the regression slope of body temperature increase over
time during the heating period, in °C min$^{-1}$; (ii) the
transition lapse, as the time (in min) lapse since we
switched off the bulb until lizard body temperature
started to diminish; and (iii) the cooling rate, calculated
as the regression slope of body temperature decrease
over time during the cooling period, in °C min$^{-1}$.

Statistical analyses were performed with parametric
statistics (Quinn & Keough, 2002). Data distributions
matched the assumptions of parametric statistics
(homoscedasticity and normality of residuals). The vari-
bles cooling rate and body mass required log-transfor-
mation. We performed an ANOVA to test for elevational
differences in back lightness, SVL and body mass.
Another ANOVA tested whether there were statistical dif-
fferences in heat transfer processes among males, gravid
females and nongravid females (a factor with three lev-
ecls). Finally, we used ordinary least squares linear mod-
els to test for differences in heating rates, inertia lapses
and cooling rates according to elevation and body mass.
We did not include elevation and body mass in the
same model because models with the two variables
showed problems of collinearity (in an ANOVA, elevation
explained body mass with a multiple $R = 0.68$). There-
fore, to avoid multicollinearity, for each dependent vari-
able, we performed two models, one with elevation
and other with body mass. Given that sex and female
reproductive status, as well as dorsal lightness, could
affect thermoregulation (heating rate in the case of dor-
sal lightness), we controlled for these variables in the
models. We performed those analyses with software
Statistica 7.1 (StatSoft, Inc., Tulsa, OK, USA).

## Results

### Morphological variation in elevation

Body mass ($F_{3,53} = 9.07; P < 0.001$; Fig. 2) and SVL
($F_{3,57} = 7.44; P < 0.001$) were significantly larger
in highland than in mid- and lowland lizards, which is
consistent with a Bergmann’s cline. There were no dif-
ferences in body mass ($F_{2,53} = 1.00; P = 0.376$) or SVL
($F_{2,57} = 0.99; P = 0.376$) among males, gravid and
nongravid females. Dorsal lightness ($L^*$), inverse indicator of darkness, significantly decreased with elevation ($F_{5,56} = 5.92; P < 0.001$), so individuals’ dorsum was significantly darker as elevation increased. There were no differences in lightness with sex and female reproductive status ($F_{2,56} = 1.973; P = 0.149$). Lizards’ average body mass at every sampling elevation was negatively correlated with average environmental temperature during the activity period ($r = -0.836; P = 0.038, n = 6$ sampling points).

**Heating rates**

On average, lizards heated at a rate of 1.08 °C min$^{-1}$. Heating rates did not significantly differ with elevation ($F_{5,52} = 2.016; P = 0.092$), sex or female reproductive status ($F_{2,52} = 2.323; P = 0.108$), or dorsal lightness ($F_{1,53} = 1.656; P = 0.204$). When body mass was introduced in the model in the place of elevation, we found a concave downward relationship between body mass and heating rate (linear effect: $F_{1,52} = 4.454; P = 0.040$; quadratic effect: $F_{1,52} = 5.521; P = 0.023$; Fig. 3); the effect of sex and female reproductive status, and dorsal lightness remained nonsignificant (data not shown). Lizards from the highest elevation (where lizards are largest) showed a significant, negative relationship between body mass and heating rate ($r = -0.626; P = 0.009$), whereas no significant relationship between body mass and heating rate was found when considering lizards of populations below 2500 m asl ($r = 0.100, P = 0.513$).

**Transition lapses**

After switching off the bulb, lizards’ body temperature remained stable for 1.27 min on average. Transition lapses tended to increase with elevation ($F_{5,57} = 12.554; P < 0.001$; Fig. 4). In this model, there were almost significant differences in transition lapses among males, nongravid females and gravid females ($F_{2,57} = 3.065; P = 0.054$; Fig. 5a), gravid females tending to have larger inertia lapses than males. When body mass was included in the model in the place of elevation, transition lapses significantly increased with body mass (effect of body mass: $\beta = 0.562; F_{1,57} = 30.153; P = 0.001$; effect of sex and female reproductive status: $F_{2,57} = 2.211; P = 0.119$). Neither lizards from the highest elevation ($r = 0.240$;
nor from populations below \((r = 0.238; P = 0.115)\) showed a significant relationship between body mass and transition lapses.

### Cooling rates

On average, lizards cooled by \(0.58 \, ^\circ \text{C min}^{-1}\). Similar to inertia lapses, cooling rates significantly decreased with elevation \((F_{5,52} = 11.068, P < 0.001; \text{Fig. 6})\). Consistent with our results for the inertia lapse, gravid females tended to lose heat more slowly than males \((F_{2,52} = 4.112; P = 0.022; \text{Fig. 5b})\). When body mass was introduced in the model in the place of elevation, cooling rates significantly decreased with body mass \((\text{effect of body mass: } \beta = -0.568; F_{1,56} = 37.582, P = 0.001; \text{Fig. 3})\). That is, consistently with the heat conservation hypothesis, larger lizards lost heat more slowly than small lizards. Lizards from the highest elevation showed a significant, positive relationship between body mass and cooling rate \((r = 0.775, P < 0.001)\), whereas no significant relationship between body mass and cooling rate was found when considering lizards of populations below 2500 m asl \((r = 0.241, P = 0.116)\).

### Net heating rate

We estimated the net heating rate as the heating rate minus the cooling rate. Net heating rate significantly increased with elevation \((F_{5,52} = 4.061, P = 0.003; \text{Fig. 7a})\). Similarly, net heating rate increased with body mass, suggesting a trend towards a concave down function \((\text{linear effect: } F_{1,53} = 5.965, P = 0.018; \text{quadratic effect: } F_{1,53} = 3.979, P = 0.051; \text{Fig. 7b})\). Lizards from the highest elevation showed no significant relationship between body size and net heating rate \((r = 0.243, P = 0.365)\), whereas a positive linear relationship between body size and net heating rate was found when considering lizards from populations below 2500 m asl \((r = 0.326, P = 0.035; \text{Fig. 7b})\).

### Discussion

Bergmann’s Rule, larger size in colder areas, has been validated in some endotherms \((\text{Ashton et al., 2000; Ashton, 2002b; Meiri & Dayan, 2003; Millien et al., 2006; Rodríguez et al., 2006, 2008; Ramírez et al., 2008})\). Nevertheless, its applicability for ectotherms remains controversial, since some of them follow Bergmann’s cline, while numerous ectotherms do not, or even follow the converse to Bergmann’s cline relationship between body mass and cooling rate \((r = 0.775, P < 0.001)\), whereas no significant relationship between body mass and cooling rate was found when considering lizards of populations below 2500 m asl \((r = 0.241, P = 0.116)\).
Bergmann’s Rule in elevation in a lizard

A set of hypotheses have been proposed to explain Bergmann’s clines based on physiological by-products of temperature-dependent growth and development, or temperature-dependent life-history traits (mortality or fecundity; Angilletta et al., 2004b). There is evidence that other ecological traits, besides thermoregulation, may cause body size divergence at interspecific or interpopulation levels, such as differences in longevity (Liao & Lu, 2011), habitat use (Collar et al., 2011), predation pressure (de Barros et al., 2010), feeding habits (Ochociriska & Taylor, 2003), food availability (Yom-Tov & Geffen, 2006), starvation resistance (Arnett & Gotelli, 2003) and life-history traits (Angilletta et al., 2004b), among others. These factors are not mutually excluding, may act in contrasting directions and complicate enormously the interpretation of the causes of Bergmann’s clines (Jones et al., 2005).

However, the basis of the heat conservation hypothesis (higher inertia in larger animals in conjunction with a cline of increasing body size with decreasing environmental temperature) has not been properly tested until now (Partridge & Coyne, 1997). Evidence about body-size-dependent differences in heat transfer rates are needed to support this hypothesis (Watt et al., 2010). In this sense, our study is novel because we showed that net heat gaining increased as lizards were larger. Nonetheless, the relationship between body size and net heating rate seemed to reach a limit, lizards at the highest elevation (the largest lizards) showing no relationship between body size and net heating rate. According to our results, larger lizards in cold habitats have an adaptive advantage over smaller lizards, which evolutionarily would favour larger sizes in alpine habitats, explaining the elevational pattern of lizard’s body size found in our study. Our findings strongly support the heat conservation hypothesis and demonstrate that this hypothesis may operate in ectotherms, provided that the slope of cooling rate with body mass (β) is higher than the slope of heating rate with body mass (α; Figs 3 and 7b). In turn, we predict that when β < α, ectotherms should tend to show the converse Bergmann’s cline, which would explain the contrasting patterns found in several studies (Ashton & Feldman, 2003; Olalla-Tárraga et al., 2006; Olalla-Tárraga & Rodríguez, 2007). In fact, the origin of a tendency to achieve larger body sizes in colder habitats is ancestral for tetrapods, before endothermy evolved (De Queiroz & Ashton, 2004); thus, we expect to find Bergmann’s rule both in endotherms and ectotherms.

We found that heating rate showed a concave down relationship with body mass. This contrasts with previous studies showing that small lizards heat up faster than larger lizards (Carothers et al., 1997). In our study system, highland lizards are darker; darker back colours could help these lizards to warm up faster (Clusella-Trullas et al., 2007), counteracting the negative effect of larger body mass on heating capacity and smoothing

(Mousseau, 1997; Ashton & Feldman, 2003; Blanckenhorn & Demont, 2004; Olalla-Tárraga et al., 2006; Olalla-Tárraga & Rodríguez, 2007; Adams & Church, 2008; Hu et al., 2011; Oufiero et al., 2011). The results of our study support Bergmann’s Rule. In an elevational gradient of 2200 m, P. algirus populations showed larger size in high than in mid- and low elevations, and population average body size negatively correlated with temperature. The fact that the increase in body size is much more marked in high elevations matches with other studies showing that the increase in body size with decreasing temperature is stronger in cold habitats (Rodríguez et al., 2008).

Nonetheless, the mechanism underlying Bergmann’s clines is still under debate. Even for endotherms, many researchers have argued that the heat conservation hypothesis does not completely explain Bergmann’s clines (McNab, 2010). In ectotherms, in which the heat conservation hypothesis has been generally (and prematurely) rejected, a set of hypotheses have been
out any elevational differences in heating rates (Cruz et al., 2005; Harris et al., 2012). Consequently, larger body mass would not limit heating rates in our study system. In fact, highland lizards reach field body temperatures similar to lowland lizards, despite lower environmental temperatures, which suggests an efficient exploitation of poorer heat sources in high elevations (Zamora-Camacho et al., 2013). However, we failed to find convincing evidence of an effect of darker dorsal colour on heating rate.

We also found that gravid females had higher thermal inertia for cooling than males, nongravid females showing an intermediate inertia. Higher body mass is considered as a cause of higher thermal inertia (O’Connor, 1999), but no body mass difference arose among males and females in different reproductive status. This result implies that gravid females would be less dependent on external heat sources for maintaining body temperature during gestation. Pregnant females’ ability to retain body heat could permit them to keep the optimal temperature for eggs or embryos when high predation risk makes them hide (Downes, 2001; Martin & López, 2005). Pregnant females in shelters during prolonged time to avoid predators show lower body temperatures than females in shelters for smaller periods, which provokes deterioration of body condition and immune response (Amo et al., 2007). Therefore, slower cooling rate could help high elevation P. alginus females to prevent this worsening.

Conclusions

We show that P. alginus complies with Bergmann’s Rule in an elevational system. We also demonstrate that larger lizards have a thermal advantage in colder habitats, as they retain accumulated heat for longer, but they heat up as fast as smaller lizards, providing the strongest empirical evidence that the heat conservation hypothesis may apply to ectotherm body size trends. According to the relative thermal costs and benefits of a larger body size, ectotherms may follow Bergmann’s Rule, if the thermal benefits of a larger body size exceed the costs.

Acknowledgments

We thank the personnel from the Espacio Natural de Sierra Nevada for their constant support. This work was economically supported by the Ministerio de Ciencia e Innovación (project CGL2009-13185). FJZC and SR were supported by two predoctoral grants from the Ministerio de Ciencia e Innovación (FPU program). Animals capture was conducted in accordance with Junta de Andalucía research permits issued to the authors FJZC, SR and GMR. María Virtudes Rubiño Hispán, MariCruz Tuset Arcas, Miguel Leandro López Gracia, and Susana Silva González helped us during field work. Comments by Juan Manuel Pleguezuelos, Xavier Santos, Miguel Ángel Carretero, José Martín and José Antonio Hódar improved the manuscript. Jean Mattos-Reaño revised the English. The authors have no conflict of interests to declare.

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Data deposited at Dryad: doi:10.5061/dryad.969q5

Received 12 August 2014; accepted 31 October 2014