Environmental drivers of growth rates in Guadarrama wall lizards: a reciprocal transplant experiment

JESÚS ORTEGA*, PILAR LÓPEZ and JOSÉ MARTÍN

Departamento de Ecología Evolutiva, Museo Nacional de Ciencias Naturales, C.S.I.C., José Gutiérrez Abascal 2, E-28006 Madrid, Spain

Growth rates have complex sources and may determine adult body size in organisms with indeterminate growth. Thus, the interpretation of interpopulation differences in body size along geographical gradients requires the examination of growth and to distinguish between the proximal and ultimate causes of it. Several studies support a link between growth rates and habitat production via climatic effects. Environmental constraints, such as food abundance, may be correlated with climatic conditions and could, in turn, limit growth rate because of limited energy availability. We performed a reciprocal transplant experiment to disentangle the effects of environmental and genetic factors on body size interpopulational divergence in the lizard *Podarcis guadarramae* along a 500-m elevation gradient with contrasting environmental conditions. Our results showed that the growing environment determined growth rates of juvenile lizards, independently of the population of origin. Hatchlings experiencing the high-altitude growing environment, which had colder and more humid climatic conditions, grew faster than those growing in the low-altitude environment. However, mother's site of origin did not affect growth. We conclude that the drivers of growth rate differences in *P. guadarramae* lizards are probably related to between-sites differences in water and food availability.

ADDITIONAL KEYWORDS: body size – climatic conditions – food availability – growth – lizards – productivity.

INTRODUCTION

Life histories are a crucial part of the ecology of species and play a central role in evolutionary theory (Stearns, 2000; Du *et al*., 2013). Studies of intraspecific variation in life histories are typically concerned with identifying environmental sources of variation that are correlated with the observed variation (Tinkle & Ballinger, 1972; Ballinger, 1977; Jones, Ballinger & Porter, 1987; Ford & Siegel, 1989). In some cases, variation in life-history traits is environmentally induced by extrinsic factors (phenotypic plasticity) (Losos *et al*., 2000; Via & Lande, 1985), in others it is mediated by intrinsic differences linked to geographically variable conditions (local adaptation) (Travis, 1994) or a combination of both (Sá-sousa Sears & Angilletta, 2003). Hence, the understanding of the ecological and evolutionary significance of intraspecific geographic variation in life-history traits requires teasing apart genetic and environmental sources of variation (Ferguson &

Brockman, 1980; Ballinger, 1983; Berven & Gill, 1983; Jones *et al*., 1987; Ford & Siegel, 1989; Sá-sousa Sears & Angilletta, 2003).

Geographical variation of body size along environmental gradients has been widely studied (Aragón & Fitze, 2014), mostly from a Bergmann's rule perspective (Bergmann, 1847; Rensch, 1938). Nonetheless, there are alternative explanations such as the temperature-size rule, which predicts that larger body sizes are associated with colder climates, since a negative relationship between ontogenetic temperature and size at maturity has been found in many ectotherms (Atkinson, 1994) or the starvation resistance hypothesis, which states that, as energy stores increase with size faster than metabolic rate, larger body sizes are adaptive to long inactivity periods in more seasonal environments (Lindsey, 1996; Ashton, 2001). However, most research has focused on describing trends rather than searching for plausible explanations behind the observations (Watts, Mitchell & Salewski, 2010). In ectotherms, the multiple hypotheses proposed and the lack of a general pattern shows that it is unlikely that a single mechanism for geographic variation in body

*Corresponding author. E-mail: jortega@mncn.csic.es

size exists for this group (Ficetola *et al*., 2010). In ectothermic vertebrates, a large body size may result from a faster growth rate and/or a longer duration of growth (Angilletta *et al*., 2004). This is the consequence of a continuous growth beyond sexual maturity, so all else being equal and according to this formula: body size = f (initial size + growth rate × age), faster growth rates would lead to larger body sizes at maturity (Liao & Lu, 2012). Hence, growth rate defines the relationship between body size and age, and therefore it is clearly an important factor to consider in life-history theory (Arendt, 1997). Geographic variation in growth has complex sources, depending on the interplay among resource availability and the efficiency with which an organism can assimilate energy (Congdon, 1989; Ferguson & Talent, 1993; Arendt, 1997). For example, Niewiarowski & Roosenburg (1993) found a genotype × environment interaction in *Sceloporus undulatus* lizards in which the thermal environment and population of origin shaped lizard growth rates. Thus, the interpretation of interpopulation differences in growth rates along geographical gradients requires distinguishing between the proximal (environmental) and ultimate (genetic) causes of growth (Iraeta *et al*., 2006). Environmental factors, such as temperature, precipitation and food availability, play a key role in ectotherm growth rates (Sinervo & Adolph, 1989; Niewiarowski, 1995; Andrews, Mathies & Warner, 2000); however, temperature and precipitation (a cue of food availability) tend to be negatively correlated in temperate regions (Iraeta *et al*., 2006). This implies that the interaction between resource levels and biophysical constraints may greatly influence interpopulation differences in life histories (Grant & Dunham, 1990). In this context, Mediterranean areas offer an excellent opportunity to examine the effects of environmental variations in temperature, precipitation and food availability on lizard growth rates (Iraeta *et al*., 2006). Mediterranean climates are defined by a cold winter, the coincidence of low summer rainfall and maximum temperatures with the consequent period of drought of at least 2 months, and a highly interannual variability in precipitation, which altogether accentuates the severity of these climates for vegetation and associated arthropod faunas (Nahal, 1981). Most of the studies of ectotherms assume that environmental conditions, such as temperature and humidity, and other factors that determine the activity period, should be more restrictive as altitude increases so that the time available for hatchling growth prior to winter is more limited than at lower altitude (Olsson & Shine, 1997; Sears, 2005). However, this assumption does not hold for Mediterranean climates where drought becomes less restrictive as altitude increases due to milder temperatures and higher precipitation, which increases productivity (Nahal, 1981; Iraeta, Salvador & Díaz, 2012).

Several studies support a link between growth rates and productivity via climate effects (Bauwens, 1985; Bauwens & Verheyen, 1987; Heulin, 1985; Aragón & Fitze, 2014). So, environmental constraints such as thermoregulation opportunities or food abundance may be correlated with humidity and could, in turn, limit growth rate because of limited energy availability (Ballinger, 1977; Dunham, 1978; Ballinger & Congdon, 1980; Stamps & Tanaka, 1981). Hence, the main problem for ectotherms in Mediterranean lowlands during the dry season (i.e. summer) may be food scarcity rather than thermal constraints, where offspring may experience reduced growth due to food shortage (Iraeta *et al*., 2006).

We studied two populations of the Guadarrama wall lizard, *Podarcis guadarramae guadarramae* (formerly named as *P. hispanicus* type 1, altitudinal range 650–2100 m), located at each end of a 500-m elevational gradient in the Guadarrama Mountains (central Spain) without geographical barriers but with contrasting environmental conditions. We selected these two populations because they differ in adult body size, morphology, coloration and chemical sexual signals, in spite of being genetically closely related (Martín & López, 2006; Gabirot, López & Martín, 2012, 2013). Results from a common garden experiment provided evidence that the divergent adult body sizes of these two populations of *P. g. guadarramae* lizards are not driven by size at hatching nor intrinsic post-hatching growth rates (Ortega, López & Martín, 2015). The work presented here constitutes the next step and aims to determine which factors drive phenotypic plasticity along elevational gradients in this system. Thus, we performed a reciprocal transplant experiment of hatchling lizards and compared hatchling growth rates to determine the relative contribution of phenotypic plasticity and local adaptation to different climatic conditions. Specifically, we hypothesized that if environmental differences were responsible for growth rate differences of juveniles, then the source population would not determine growth rates and, hence, we would find the same phenotypes in the same environment. Conversely, and considering that previous research showed an absence of intrinsic differences (Ortega *et al*., 2015), if phenotypic divergence between populations is maintained across different environments, maternal effects could be responsible for different juvenile growth rates due to differential allocation of resources to the eggs. We also measured food availability in both populations to test the hypothesis that productivity (i.e. food availability) has a direct effect on lizard growth rates. We predicted that, if extrinsic (environmental) factors are more important than intrinsic (genetic) ones, lizards

would grow faster, attaining a larger body size, in the site with higher humidity, milder temperatures and, ultimately, higher ecosystem productivity.

MATERIAL AND METHODS

STUDY SITES

During April–May 2013, gravid female *P. g. guadarramae* lizards ($n = 12$, per population) were captured by noosing at two nearby populations in the Guadarrama Mountains (Central Spain). The lower elevation site was 'La Dehesa de la Golondrina', an oak forest near Cercedilla village (40°44′N, 04°02′W; 1250 m altitude). The higher elevation population was found in the upper part of a mountain valley ('Fuenfría') (40°47′N, 04°03′W; 1750 m altitude) occupying granite rock walls and rock piles at the edge of a pine forest. These populations are located at different elevational ranges with contrasting climatic conditions separated 6 km by air (Ortega *et al.*, 2015).

CLIMATIC CONDITIONS

We summarized the available environmental temperatures and precipitations in the study areas by using long-term (from 1988 to 2002, as data from 2002 to 2014 were not available) daily data from two nearby meteorological stations: 'Embalse de Navacerrada' (40°43′51″N, 04°00′49″; elevation 1207 m; Madrid province) and 'Puerto de Navacerrada' (40°46′50″N, 04°00′37″W; elevation 1894 m; Madrid province) for the lower- and higher-elevation populations, respectively (data available from the Spanish Meteorological Agency, 'Agencia Española de Metereología, AEMET'; http://www.aemet.es). Monthly temperatures were measured as means of daily mean temperatures, and we also calculated means of daily maximum air temperatures, as advised for ecophysiological studies of reptiles (Huey, 1982). We also included total precipitation per month in our analyses.

ADULT HUSBANDRY

Captured lizards were immediately transported to 'El Ventorrillo' field station facilities about 5 km away from the capture localities. Female lizards were kept in individual plastic terraria (40 × 30 × 25 cm; length × height × width) filled with a moistened coconut fibre substrate and provided with a shallow water bowl and a brick (24 × 8 × 11 cm; length × height × width) with six longitudinal holes (3 cm of diameter and 24 cm long) that allowed shelter and climbing opportunities. A 50 W halogen lamp (hotspot) was suspended over one end of the terrarium providing a diurnal temperature gradient (21–45°C) during the photoperiod

and allowed lizard thermoregulation (preferred temperature: 34.4°C; Bauwens *et al.*, 1995). In addition, fluorescent bulbs over the terraria provided ambient lighting mimicking the natural photoperiod, and mercury vapour bulbs (Exoterra Solar Glow 125 W) provided ultraviolet radiation during 1.5 h a day (from 14.00 to 15.30). Terraria were placed inside a climatic chamber (Ibercex V-450-D walk-in chamber; ASL S.A., Madrid, Spain) where temperature (diurnal = 21 °C; nocturnal = 15 °C) and photoperiod (12 h: 12 h, light: dark) were easily controlled automatically. Water was sprayed over the terraria and water bowls were filled with fresh water every day. Lizards were fed crickets (*Acheta domesticus*) and mealworms (*Tenebrio mollitor*) *ad libitum*, dusted with a commercial vitamin and calcium supplement. Adult lizards were returned to their capture sites in late June.

EGGS AND HATCHLINGS HUSBANDRY

Females laid egg within 9.3 ± 0.4 days (mean ± SE) after being captured. Cages were carefully checked for the presence of eggs twice daily. We focused our analysis on first clutches as they potentially reflect the field conditions (e.g. food availability) experienced by females in the wild before being captured, minimizing the effects of captivity. Eggs were individually placed in 60-mL closed plastic cups filled with 10 g of moistened perlite (perlite: water ratio = 1: 1) and transferred to an incubator at 27.5 °C (94 × 60 × 60 cm; IRE-160; Raypa, Barcelona) where they were randomly distributed in the shelves, and the shelves were rotated inside the incubator every week to control for possible position effects (Telemeco *et al.*, 2010). The incubator was checked for lizard hatching daily. Immediately after hatching, we measured body size using a ruler [snout-vent length (SVL) to the nearest 1 mm]. We measured 'body mass' with a digital scale (to the nearest 0.01 g).

Preliminary studies revealed that in the field, under natural conditions, there is a high hatchling mortality during the winter (Ortega J, unpublished results). Thus, we decided to keep all hatchlings, which hatched in July and August, in the laboratory until the next spring under standardized environmental conditions, similar to those experienced in a previous common garden experiment (see Ortega *et al.*, 2015). A 50-W halogen lamp was suspended over one end of the terrarium providing a diurnal temperature gradient (21–45 °C) allowing thermoregulation of lizards. A fluorescent bulb on each shelf provided ambient lighting mimicking the natural photoperiod, and mercury vapour bulbs (Exoterra Solar Glow 125 W) provided ultraviolet radiation during 1.5 h per day (from 1400 to 1530 h). From December to February, we simulated winter conditions decreasing ambient temperature to 5 °C and the availability of a hotspot

to 1.5 h a day. As this species is active year-round and does not have a strict hibernation (Ortega J, unpublished data), the former procedure was suspended for 3 days each month and ambient temperature was raised to 15 °C and the hotspot made available for 5 h. Crickets (*A. domesticus*) and fruit flies (*Drosophila hydei*) of an appropriate size were offered *ad libitum* the first day of each of these winter breaks. The presence of snow prevented access to the high-elevation site and thus, releasing hatchlings before May was not possible.

FIELD PROCEDURES

Four outdoor enclosures (5 × 5 m²) were built in the typical habitats of the two localities where lizards were captured, without modifying the natural vegetation cover and the surroundings. Galvanized metal walls of 85 cm height, partly buried in the ground (25 cm), demarcated each enclosure. A net covered the enclosures from above to avoid bird predation. Five artificial boulders per enclosure, each one composed of four concrete bricks (50 × 25 × 25 cm; length × height × width) and covered with granite rocks, were added to mimic the saxicolous habitat of the species. Under each boulder, we excavated a pit of 25 cm of depth to fit a clay brick (24 × 8 × 11 cm; length × height × width) with six longitudinal holes (3 cm of diameter and 24 cm long) to provide additional underground shelter.

In each locality, we placed two enclosures, one with lower elevation hatchlings (n = 13) and other with higher elevation hatchlings (n = 13). Thus, hatchling lizards from mothers from the higher and lower altitude experienced the two growing environments. Specifically, we followed a split clutch design where half of the hatchlings from a given clutch (clutch mates) were randomly assigned to a lower-elevation enclosure and the other half to a higher-elevation one, so that at least one hatchling per female was raised in each treatment (lower elevation vs. higher elevation).

Hatchlings (n = 52 in total) were toe-clipped and photographed for further identification, measured and weighed prior to release in the outdoor enclosures in early spring (1 May 2014). In late spring (6–8 June) and late summer (9–11 September) we searched for juveniles during 3 days below bricks and rocks around 9:00 am, before lizards became active. Juveniles were measured again in each recapture to estimate changes in body size, body mass, size-specific and mass-specific growth rates between recapture periods.

FOOD AVAILABILITY AND DIVERSITY

Potential prey availability and diversity in each population were estimated in three periods (at hatchling release, early spring and late summer). We walked arbitrary transects within the study area, and then tossed a 20 × 20 cm² wooden frame every 40 steps from a distance of about 1.5 m; we counted arthropods contained within it (or escaping from it, such as grasshoppers) during a 1-min interval (Díaz & Carrascal, 1990). As *P. guadarramae* is a saxicolous species (Salvador & Carretero, 2014), we concentrated our sampling on rock boulders, rock cliffs and their surroundings. All prey items were identified to order except ants which were considered separately from Hymenoptera due to its high abundance. Also, because *P. guadarramae* is a generalist species (Salvador & Carretero, 2014), the availability of arthropods mirrors the potential prey availability. Diversity of arthropods was calculated by means of the Shannon–Weaver index for the taxonomic categories identified (Magurran, 1988): $H' = -\Sigma pi \ln pi$ where pi is the proportion of the species i.

STATISTICAL ANALYSIS

The size-specific (SVL) and mass-specific growth were expressed as the proportionate increase in size or mass, measured in days⁻¹ units (Iraeta *et al*., 2006, 2012) according to the equation: $G = [(\ln (s_t/s_i)/(d)]$, where s_t is the body length or body mass at time t, s_i is the initial body length or body mass and d is the time elapsed in days. To control for possible familiar effects, we also compared the size-specific growth rates of clutch mates released at different sites using repeated-measures general linear model (GLM). Body condition was estimated using the residuals of the regression of log–body mass on log-SVL. We calculated growth rates and body condition for two periods: late spring and late summer. Except for the third recapture period, where we performed Mann–Whitney's *U*-tests due to the low number of recaptured juveniles, all the statistical analyses were performed with GLMs with mother origin, release site and their interactions as fixed factors and were conducted in Statistica 8.0 (StatSoft Inc., Tulsa, OK, USA). We compared arthropod diversity and abundance between populations and seasons with GLMs with season, population and their interactions as fixed factors. Differences in mean and maximum air temperatures, and total precipitation between populations and months, were examined with GLMs with season, population and their interactions as fixed factors. Before the data analyses, normality and homoscedasticity were systematically checked for each variable using the Kolmogorov–Smirnov and Hartley tests, respectively. We used Tukey's honestly significant difference tests in pairwise comparisons.

RESULTS

CLIMATIC CONDITIONS

The mean air temperatures increased significantly within a given year from January to August in all populations and reached the greatest values in July and August but temperatures were significantly greater in the lower-elevation population in all these months (GLM: month: $F_{11,336} = 445.04$, $P < 0.001$; locality: $F_{1,336} = 552.41$, $P < 0.001$; month × locality: $F_{11,336} = 1.65$, $P = 0.083$) (Fig. 1a). A similar result was observed for the means of daily maximum air temperatures, but the significant interaction indicated that the magnitude of the differences between sites differed among months so the discrepancy was greater during summer than during winter (GLM: month: $F_{11,336} = 271.52$, $P < 0.001$; locality: $F_{1,336} = 450.97$, $P < 0.001$; month × locality: $F_{11,336} = 2.57$, $P = 0.004$) (Fig. 1b). Total precipitation varied greatly between months, decreased significantly from May to August in both populations and was significantly greater in the higher-elevation site (GLM: month: $F_{11,336} = 10.34$, $P < 0.001$; locality: $F_{1,336} = 21.95$, $P < 0.001$; month × locality: $F_{11,336} = 0.44$, $P = 0.937$) (Fig. 1c). Tukey's *post hoc* tests revealed significant differences in mean and maximum temperatures between localities from May to September (all $Ps > 0.004$), while although precipitations tended to be higher in high elevation from May to September, the differences were not significant (all $Ps > 0.100$). Overall, the higher-elevation population has a climate that is colder and more humid than in the lower-elevation population.

HATCHLING MORPHOLOGY

Body size, body mass and body condition of hatchlings from low- and high-altitude populations were similar at hatching in the laboratory (GLMs: SVL: $F_{1,50} = 0.62$, $P = 0.433$; body mass: $F_{1,50} = 0.77$, $P = 0.385$; body condition: $F_{1,50} = 0.06$, $P = 0.808$) (Fig. 3). Body size, body mass and body condition were still similar at the moment of release in the enclosures in early spring (May) (GLMs: SVL: $F_{1,50} = 1.56$, $P = 0.218$; body mass: $F_{1,50} = 2.59$, $P = 0.114$; body condition: $F_{1,50} = 0.01$, $P = 0.99$) (Fig. 2).

A GLM with body size and body mass of lizards recaptured in late spring (i.e. June; 38 ± 1 days after they had been released) showed that lizards were significantly larger and heavier in the higher-elevation enclosures independent of the population of origin (release site: $N = 20$, higher elevation; $N = 19$, lower elevation; population of origin: $N = 21$, higher elevation; $N = 18$, lower elevation; SVL: GLM, release site: $F_{1,35} = 28.70$, $P < 0.001$; site of origin: $F_{1,35} = 1.83$, $P = 0.185$; release site × site of origin: $F_{1,35} = 0.42$, $P = 0.523$; body mass: GLM, release site: $F_{1,35} = 18.33$, $P < 0.001$;
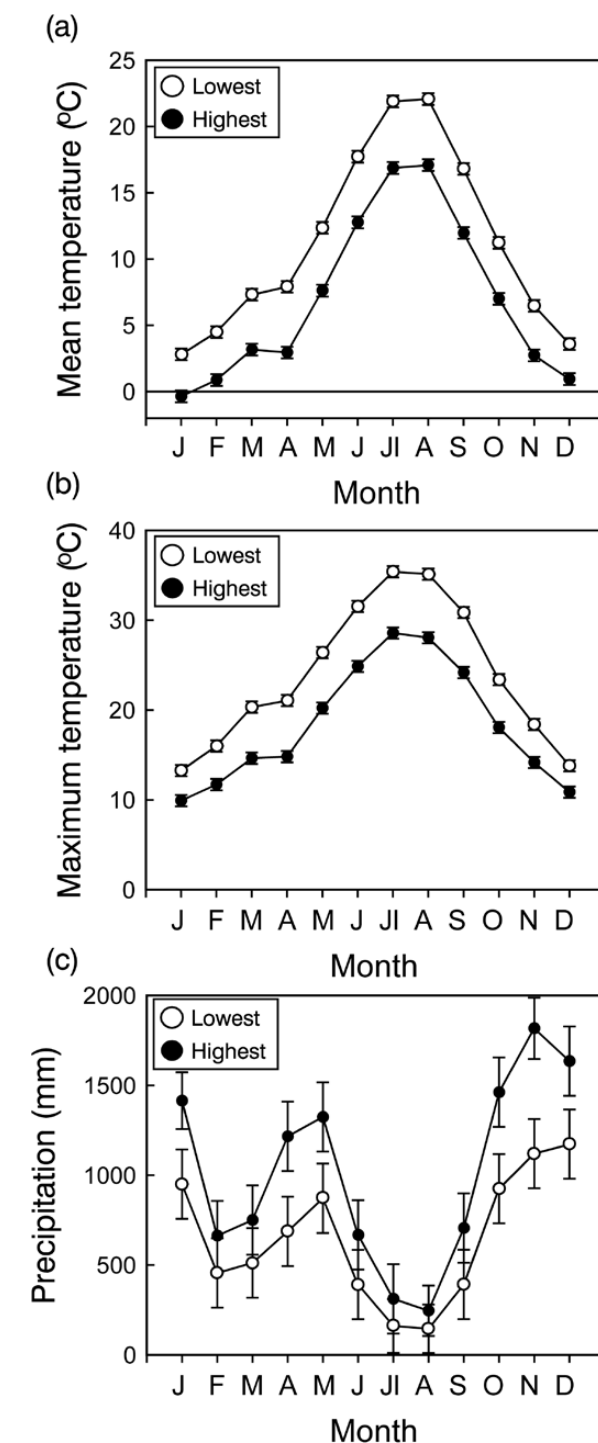
Figure 1. Variation in (a) daily mean and (b) daily maximum air temperatures, and (c) total monthly precipitation in the lower- and higher-elevation localities. Data show average (±SE) monthly values for a 15-year period (1988–2002).

site of origin: $F_{1,35} = 2.71$, $P = 0.109$; release site × site of origin: $F_{1,35} = 0.07$, $P = 0.794$) (Fig. 2a, b). However, body condition was similar between release sites and
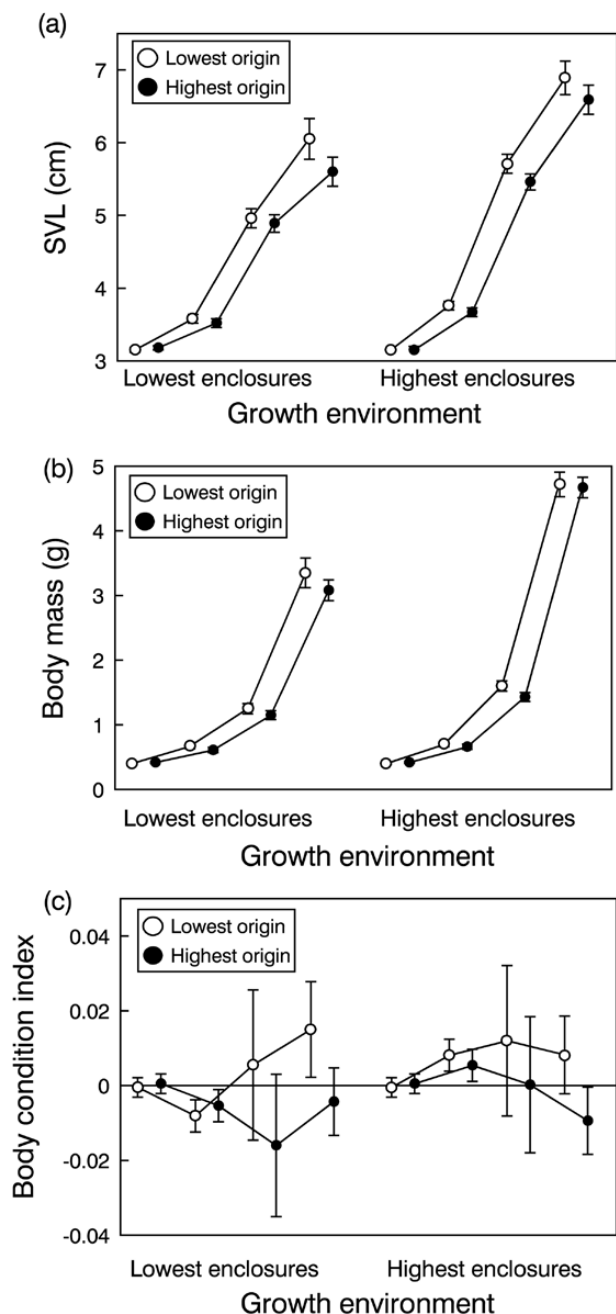
Figure 2. Changes in mean (±SE) (a) body length [snout-to-vent length (SVL) in cm], (b) body mass (g) and (c) body condition indexes of juvenile *Podarcis guadarramae* lizards at hatching, when they were released (early spring, 1 May 2014) and at two recapture periods (late spring and late summer, 6–8 June and 9–11 September, respectively), depending on mother origin and the growth environment (i.e. release site or localization of enclosures). Error bars depict, from left to right: release, early spring, late spring and late summer.

population of origin (GLM: release site: $F_{1,35} = 0.34$, $P = 0.560$; site of origin: $F_{1,35} = 0.74$, $P = 0.394$; release site × site of origin: $F_{1,35} = 0.06$, $P = 0.804$) (Fig. 2c).

Lizards recaptured in late summer (i.e. September; 96 ± 1 days after they had been released) were also larger ($N = 7$, higher elevation; $N = 6$, lower elevation; Mann–Whitney's U-test: $U = 2.00$, $Z = -2.71$, $P = 0.007$) and heavier in the higher-elevation enclosures (Mann–Whitney's U-test: $U = 0.00$, $Z = -3.00$, $P = 0.003$) (Fig. 2a, b). The population of origin did not significantly affect body size ($N = 8$, higher elevation; $N = 5$; lower elevation; Mann–Whitney's U-test: $U = 13.50$, $Z = -0.95$, $P = 0.341$) or body mass (Mann–Whitney's U-test: $U = 20.00$, $Z = 0.01$, $P = 0.99$) (Fig. 1a, b). However, body condition did not significantly differ between release sites (Mann–Whitney's U-test: $U = 21.00$, $Z = 0.01$, $P = 0.99$) or between populations of origin (Mann–Whitney's U-test: $U = 8.00$, $Z = -1.76$ $P = 0.079$) (Fig. 2c).

HATCHLING GROWTH

Between early and late spring (May to June), we found that hatchlings released at the higher-elevation site increased body size and body mass faster than those released at the lower-elevation site, independently of the origin of their mothers (Tables 1 and 2).

A repeated-measures GLM comparing the (average) growth rates of clutch mates reared at both sites showed that size-specific (SVL) growth rates were consistently higher at the higher elevation site (repeated-measures GLM: release site: $F_{1,14} = 64.32$, $P < 0.001$; site of origin: $F_{1,14} = 0.26$, $P = 0.620$; release site × site of origin: $F_{1,14} = 1.09$, $P = 0.315$). We found a similar result for mass-specific growth (repeated-measures GLM: release site: $F_{1,14} = 22.52$, $P = 0.003$; site of origin: $F_{1,14} = 0.31$, $P = 0.587$; release site × site of origin: $F_{1,14} = 0.01$, $P = 0.909$).

Lizards recaptured in late summer (September) showed a significantly higher size-specific growth rate and a significantly higher mass-specific growth rate in the higher-elevation than in the lower-elevation enclosures. However, we did not find significant differences due to the population of origin in size-specific growth or mass-specific growth (Tables 1 and 2).

FOOD AVAILABILITY AND DIVERSITY

The number of available potential prey items was significantly higher in the higher-elevation site in all seasons and there were significant differences among seasons in both populations (GLM: season: $F_{2,138} = 13.16$, $P < 0.001$; population $F_{1,138} = 90.15$, $P < 0.001$; season × population: $F_{2,138} = 1.53$, $P = 0.220$) (Fig. 3a). Tukey's *post hoc* tests revealed that overall prey availability was lower in late summer than in early spring ($P = 0.007$) and in late spring ($P < 0.001$), while there were no significant differences between the two spring sampling periods ($P = 0.095$).

Table 1. Mean (±1 SE) values for growth rates of juvenile *Podarcis g. guadarramae* lizards in two recapture periods depending on the population of origin of their mothers and the growth environment (i.e. release site or location of enclosures)

	Late spring		Late summer	
	Size-specific growth rate (days^{-1})	Mass-specific growth rate (days^{-1})	Size-specific growth rate (days^{-1})	Mass-specific growth rate (days^{-1})
Population of origin				
Lower elevation	0.07298 ± 0.00199	−0.01253 ± 0.00246	0.03457 ± 0.00121	0.01356 ± 0.00152
Higher elevation	0.07298 ± 0.00184	−0.01105 ± 0.00228	0.03361 ± 0.00096	0.01301 ± 0.00120
Release site				
Lower elevation	0.06972 ± 0.00071	−0.01790 ± 0.00194	0.03160 ± 0.00056	0.00996 ± 0.00038
Higher elevation	0.07768 ± 0.00167	−0.00587 ± 0.00189	0.03603 ± 0.00052	0.01601 ± 0.00035

Size refers to snout-vent length (cm).

Table 2. Effects of release site, site of origin and release site × site of origin on size-specific and mass-specific growth of hatchlings from two populations of *P. g. guadarramae* lizards in two recapture periods

|	Release site	Site of origin	Release site × site of origin
Late spring			
Size-specific growth	$F_{1,35} = 10.84$ $P = 0.002$	$F_{1,35} = 0.64$ $P = 0.428$	$F_{1,35} = 0.02$ $P = 0.902$
Mass-specific growth	$F_{1,35} = 18.93$ $P < 0.001$	$F_{1,35} = 0.19$ $P = 0.663$	$F_{1,35} = 0.17$ $P = 0.682$
Late summer			
Size-specific growth	$U = 0.01, Z = -3.00$ $P = 0.003$	$U = 16.50, Z = -0.52$ $P = 0.608$	
Mass-specific growth	$U = 0.01, Z = -3.00$ $P = 0.003$	$U = 20.00, Z = 0.01$ $P = 0.999$	

The diversity of potential prey differed significantly among seasons in both populations and tended, although non-significantly, to be higher in the higher-elevation site (GLM: season: $F_{2,138} = 3.59$, $P = 0.030$; population $F_{1,138} = 3.20$, $P = 0.076$; season × population: $F_{2,138} = 0.34$, $P = 0.713$) (Fig. 3b). Tukey's *post hoc* tests revealed significant differences in prey diversity between late spring and late summer ($P = 0.035$), while early spring did not significantly differ from late spring ($P = 0.129$) or late summer ($P = 0.888$).

DISCUSSION

Our results showed that lizards grew faster in the higher-altitude environment, independent of their population of origin. Thus, this study confirms our previous results from a common garden experiment suggesting that differences in growth and adult body size are a plastic response and not a product of intrinsic genetic differences between populations (Ortega *et al*., 2015). We can also rule out population-specific maternal effects as the interactions between maternal origin and release site were not significant in any case. Hence, all these evidences point out that body size differences are solely the result of environmental elements acting directly on hatchlings.

Lizards growing at the higher altitude, independent of their origin, have a larger body size than lizards growing at the lower altitude, following Bergmann's rule (i.e. larger body size in colder environments) (Ortega *et al*., 2015). This is in sharp contrast with the vast majority of squamates which exhibit reversed Bergmann's clines (Ashton & Feldman, 2003; Angilletta *et al*., 2004). One of the reasons that could explain the deviation from the reverse Bergmann's cline in these *P. g. guadarramae* lizard populations is that resource limitation impairs somatic growth in warmer environments (Congdon, 1989; Dunham *et al*., 1989). Several studies support a link between growth rates and productivity via climate effects that may be responsible for differential body size and life histories (Bauwens, 1985; Bauwens & Verheyen, 1987; Heulin,

(a)

(b)
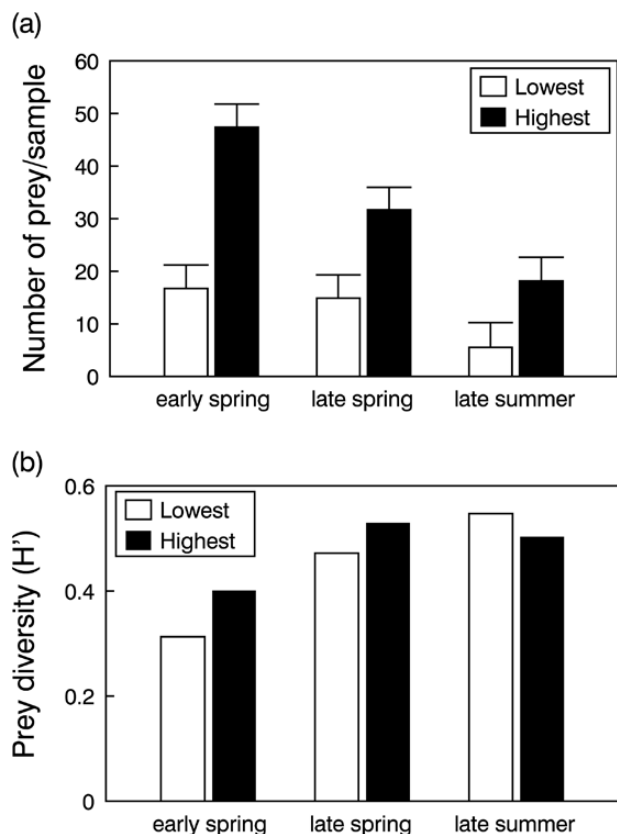
Figure 3. (a) Number of available prey (mean ± SE) and (b) diversity of prey types (H′) in the lower- and higher-elevation localities at three periods of the year (early and late spring and late summer).

1985; Buckley, Irschick & Adolph, 2007; Díaz *et al*., 2011).

In our study system, the main drivers of body size differences between the higher- and lower-altitude populations seem to be food and water availability, which differs both between sites and between seasons and, very likely, directly affect growth rates. In line with this, we also found that precipitation was higher at the higher elevation. We also found that, conversely, mean and maximum temperatures were higher in the lower-elevation site which may also reduce water availability and ambient humidity. Environmental constraints such as food abundance may be correlated with humidity and could, in turn, limit growth rate because of limited energy availability (Stamps & Tanaka, 1981). The abundance of potential prey, size-specific and mass-specific growth rates were higher at the higher-altitude site. In keeping with these results, prey availability fluctuated seasonally and matched lizard growth; size-specific growth rates, mass-specific growth rates and prey availability were maximal in late spring. Moreover, prey diversity was also higher in spring than in late summer, which might also impact

growth as a higher diversity of invertebrates is considered beneficial for a generalist lizard species (Salvador & Carretero, 2014).

In desert habitats, low primary productivity is primarily caused by low water availability (Noy-Meir, 1973, 1974; Webb *et al*., 1978; Cunningham *et al*., 1979; Hadley & Szarek, 1981) which indirectly limits secondary production (Dunham, 1981). Similar environmental restrictions are common in Mediterranean lowlands where drought becomes less restrictive as altitude increases due to milder temperatures and higher precipitation, which increase productivity (Nahal, 1981; Iraeta *et al*., 2012). Thus, the resource acquisition constraints imposed in the lower-elevation site, which are more severe in summer, may be responsible for the observed slower growth rates, as has been demonstrated in other insectivorous lizard species (Dunham, 1978; Ballinger & Congdon, 1980; Iraeta *et al*., 2006). However, we cannot dismiss the possible additional role of limitations in resource harvest rates. For example, in *Sceloporus merriami* lizards, the thermal environment during the summer (i.e. high temperatures) restricts lizard activity to a brief period each day, constraining foraging and, hence, limiting the access to food (Grant & Dunham, 1987). We did not examine whether activity patterns of lizards vary seasonally or between sites. Nonetheless, the effects of summer drought and higher temperatures are more evident in the lower-elevation site, where few lizards are observed active in summer (Ortega *et al*., 2015). Lizards from the lower altitude might also reduce activity to avoid dehydration (Jones *et al*., 1987; Lorenzon *et al*., 1999). Indeed, water availability itself may have a primary limiting effect on growth, even when food supplies are abundant (Stamps & Tanaka, 1981). Thus, the resource limitation and the harvest rate limitation hypotheses are not mutually exclusive and might shape a landscape where the lower-elevation site is a poor environment for food acquisition, has low water availability during summer and, hence, lead to slower growth rates.

Nonetheless, we should acknowledge other potential sources of variation in body size that we did not control in our experimental design. First, as eggs were incubated under common garden conditions, the contrasting temperatures and precipitations between sites might affect incubation conditions so that eggs from higher elevation could experience lower temperatures and increased moisture availability during development which may affect offspring growth rates and body size (Packard *et al*., 1987; Overall, 1994; Telemeco *et al*., 2010) and could increase the magnitude of the phenotypic differences found here. However, female microhabitat selection during egg-laying may buffer potential differences in incubation conditions between high and low elevation. Second, reproductive

phenology (e.g. timing of laying) could differ between high- and low-elevation environments. Nonetheless, we consider this possibility unlikely as our previous study showed that neither laying date nor incubation period differed between sites (Ortega *et al.*, 2015). Last, some ectotherms in colder climates have high rates of growth and development, which would tend to counteract the proximate effects of lower temperatures, such as shorter annual periods for activity and growth (Conover & Schultz, 1995; Oufieroi & Angilletta, 2006). But this is not the case of our system as hatchlings released in the higher-elevation site grew faster and, hence, attained larger body sizes independent of their population of origin, confirming that intrinsic differences are not responsible for this pattern.

It is also worth noting that to generalize our results to Mediterranean lizards in the Iberian Peninsula, we would need more replicates of both higher and lower elevation sites to know if this pattern is general and widespread not just in *P. g. guadarramae* but also in other lizard species with Mediterranean distribution. Although our lower altitude site is located at a medium altitude (1250 m), it experiences typical Mediterranean climatic conditions and, among them, low precipitation levels and high temperatures during the summer are very pronounced due to the continental climatic influence in the centre of the Iberian Peninsula. Our results are in accordance with most research on Mediterranean lizards where primary productivity is an important predictor of body size and/or growth (Aragón & Fitze, 2014; Iraeta *et al.*, 2006).

Our findings agree with previous research in the *P. hispanicus* species complex which emphasizes the high morphological variability both within and between mitochondrial lineages (Kaliontzopoulou, Carretero & Llorente, 2012) and highlight the difficulty of identifying the ecological or evolutionary causes of variation in body size. Thus, phenotypic plasticity shapes a complex evolutionary scenario where this pattern of high morphological variability between populations may be, at least partially, induced by the proximate effects of local climatic conditions. We confirm the previous interpretation that the *P. hispanicus* species complex constitutes a promising model organism for the study of phenotypic diversification within emerging species (Pinho, Harris & Ferrand, 2007). The present study emphasizes how geographical variation in body size can be produced by mechanisms not necessarily related to the Bergmann's rule (Palkovacs, 2003; Aragón & Fitze, 2014). We reveal that hatchlings released in the higher-elevation site grew faster and, hence, attained larger body independent of population of origin, which may be mediated by the milder temperatures and higher humidity that result in a higher availability of food and water at the higher-elevation

site. Thus, the great influence of temperature and precipitation on development can lead to interpopulation differences in life histories that may become genetically fixed over time, giving rise to highly adapted local forms (Du *et al.*, 2010; Iraeta *et al.*, 2012). Hence, our results highlight the importance of environmental factors such as climatic conditions and ecosystem productivity as drivers of phenotypic diversification which is in line with recent research in the family Lacertidae (Hipsley & Müller, 2017).

REFERENCES

Andrews RM, Mathies T, Warner DA. 2000. Effect of incubation temperature on morphology, growth, and survival juvenile *Sceloporus undulatus*. *Herpetological Monographs* **14:** 420–431.

Angilletta MJ, Niewiarowski PH, Dunham AE, Leache AD, Porter WP. 2004. Bergmann's clines in ectotherms: illustrating a life-history perspective with sceloporine lizards. *American Naturalist* **164:** E168–E183.

Aragón P, Fitze PS. 2014. Geographical and temporal body size variation in a reptile: roles of sex, ecology, phylogeny and ecology structured in phylogeny. *PLoS ONE* **9:** e104026.

Arendt JD. 1997. Adaptive intrinsic growth rates: an integration across taxa. *The Quarterly Review of Biology* **72:** 149–177.

Ashton KG. 2001. Body size variation among mainland populations of the western rattlesnake (*Crotalus viridis*). *Evolution* **55:** 2523–2533.

Ashton KG, Feldman CR. 2003. Bergmann's rule in nonavian reptiles: turtles follow it, lizards and snakes reverse it. *Evolution* **57:** 1151–1163.

Atkinson D. 1994. Temperature and organism size— a biological law for ectotherms? *Advances in Ecological Resources* **25:** 1–58.

Ballinger RE. 1977. Reproductive strategies: food availability as a source of proximate variation in a lizard. *Ecology* **58:** 628–635.

Ballinger RE. 1983. Life-history variations. In: Huey RB, Pianka ER, Schoener TW, eds. *Lizard ecology: studies of a model organism*. Cambridge: Harvard University Press, 241–260.

Ballinger RE, Congdon JD. 1980. Food resource limitation of body growth rates in *Sceloporus scalaris* (Sauria, Iguanidae). *Copeia* **1980:** 921–923.

Bauwens D. 1985. *Demografische kenmerken en aantalsdynamiek in cen populatie van de Levendbarende Hagedis* (Laceta vivipara). PhD thesis, University of Antwerp, Belgium.

Bauwens D, Verheyen RF. 1987. Variation of reproductive traits in a population of the lizard *Lacerta vivipara*. *Ecography* **10:** 120–127.

Bauwens D, Garland T, Castilla AM, Van Damme R. 1995. Evolution of sprint speed in lacertid lizards: morphological, physiological, and behavioural covariation. *Evolution* **49:** 848–863.

Bergmann C. 1847. Uber die verhaltnisse der warmeokonomie der thiere zu ihrer grosse. *Göttinger Studien* **3:** 595–708.

Berven KA, Gill DE. 1983. Interpreting geographic variations in life–history traits. *American Zoologist* **23:** 85–97.

Buckley CR, Irschick DJ, Adolph SC. 2007. Testing the persistence of phenotypic plasticity after incubation in the western fence lizard, *Sceloporus occidentalis*. *Evolutionary Ecology Research* **9:** 169–183.

Congdon JD. 1989. Proximate and evolutionary constraints on energy relations of reptiles. *Physiological Zoology* **62:** 356–373.

Conover DO, Schultz ET. 1995. Phenotypic similarity and the evolutionary significance of countergradient variation. *Trends in Ecology & Evolution* **10:** 248–252.

Cunningham GL, Syvertsen JP, Reynolds JF, Willson JM. 1979. Some effects of soil-moisture availability on aboveground production and reproductive allocation in *Larrea tridentata* (DC) Cov. *Oecologia* **40:** 113–123.

Díaz JA, Carrascal LM. 1990. Prey size and food selection of *Psammodromus algirus* (Lacertidae) in central Spain. *Journal of Herpetology* **24:** 342–347.

Díaz JA, Iraeta P, Verdú-Ricoy J, Siliceo I, Salvador A. 2011. Intraspecific variation of reproductive traits in a Mediterranean lizard: clutch, population, and lineage effects. *Evolutionary Biology* **39:** 106–115.

Du WG, Ji X, Zhang YP, Lin ZH, Xu XF. 2010. Geographic variation in offspring size of a widespread lizard (*Takydromus septentrionalis*): importance of maternal investment. *Biological Journal of the Linnean Society* **101:** 59–67.

Du WG, Robbins TR, Warner DA, Langkilde T, Shine R. 2013. Latitudinal and seasonal variation in reproductive effort of the eastern fence lizard (*Sceloporus undulatus*). *Integrative Zoology* **9:** 360–371.

Dunham AE. 1978. Food availability as a proximate factor influencing individual growth rates in the iguanid lizard *Sceloporus merriami*. *Ecology* **59:** 770–778.

Dunham AE. 1981. Populations in a fluctuating environment: the comparative population ecology of the iguanid lizards Sceloporus merriami and Urosaurus ornatus. *Miscellaneous Publications Museum of Zoology, University of Michigan* **158:** 1–62.

Dunham AE, Grant BW, Overall KL. 1989. Interfaces between biophysical and physiological ecology of terrestrial vertebrate ectotherms. *Physiological Zoology*. **62:** 335–355.

Ferguson GW, Brockman T. 1980. Geographic differences of growth rate potential of *Sceloporus* lizards (Sauria: Iguanidae). *Copeia* **1980:** 259–264.

Ferguson GW, Talent LG. 1993. Life-history traits of the lizard *Sceloporus undulatus* from two populations raised in a common laboratory environment. *Oecologia* **93:** 88–94.

Ficetola GF, Scali S, Denoël M, Montinaro G, Vukov TD, Zuffi MAL, Padoa-Schioppa E. 2010. Ecogeographical variation of body size in the newt *Triturus carnifex*: comparing the hypotheses using an information-theoretic approach. *Global Ecology and Biogeography* **19:** 485–495.

Ford NB, Siegel RA. 1989. Phenotypic plasticity in reproductive traits: evidence from a viviparous snake. *Ecology* **70:** 1768–1774.

Gabirot M, López P, Martín J. 2012. Differences in chemical sexual signals may promote reproductive isolation and cryptic speciation between Iberian wall lizard populations. *International Journal of Evolutionary Biology* **2012:** 698520.

Gabirot M, López P, Martín J. 2013. Female mate choice based on pheromone content may inhibit reproductive isolation between distinct populations of Iberian wall lizards. *Current Zoology* **59:** 210–220.

Grant BW, Dunham AE. 1987. Thermally imposed time constraints on the activity of the desert lizard *Sceloporus merriami*. *Ecology* **69:** 167–176.

Grant BW, Dunham AE. 1990. Elevational covariation in environmental constraints and life histories of the desert lizard *Sceloporus merriami*. *Ecology* **71:** 1765–1776.

Hadley NF, Szarek SR. 1981. Productivity of desert ecosystems. *Biological Sciences* **31:** 747–753.

Heulin B. 1985. Reproduction and morphology of the common lizard (*Zootoca vivipara*) from montane populations in Slovakia. *Zoological Science* **30:** 92–98.

Hipsley CA, Müller J. 2017. Developmental dynamics of ecomorphological convergence in a transcontinental lizard radiation. *Evolution* **71:** 1–13.

Huey RB. 1982. Temperature, physiology and the ecology of reptiles. In: Gans C, Pough FH, eds. *Biology of the Reptilia. Physiology C. Physiological ecology*. London: Academic Press, 25–67.

Iraeta P, Monasterio C, Salvador A, Díaz JA. 2006. Mediterranean hatchling lizards grow faster at higher altitude: a reciprocal transplant experiment. *Functional Ecology* **20:** 865–872.

Iraeta P, Salvador A, Díaz JA. 2012. Life-history traits of two Mediterranean lizard populations: a possible example of countergradient covariation. *Oecologia* **172:** 167–176.

Jones SM, Ballinger RE, Porter WP. 1987. Physiological and environmental sources of variation in reproduction: prairie lizards in a food rich environment. *Oikos* **48:** 325–335.

Kaliontzopoulou A, Carretero MA, Llorente GA. 2012. Morphology of the *Podarcis* wall lizards (Squamata: lacertidae) from the Iberian Peninsula and North Africa: patterns of variation in a putative cryptic species complex. *Zoological Journal of the Linnean Society* **164:** 173–193.

Liao W, Lu X. 2012. Adult body size = f (initial size + growth rate x age): explaining the proximate cause of Bergman's cline in a toad along altitudinal gradients. *Evolutionary Ecology* **26:** 579–590.

Lindsey CC. 1996. Body sizes of poikilotherm vertebrates at different latitudes. *Evolution* **20:** 283–291.

Lorenzon P, Clobert J, Oppliger A, John-Alder H. 1999. Effect of water constraint on growth rate, activity and body temperature of yearling common lizard (*Lacerta vivipara*). *Oecologia* **118:** 423–430.

Losos JB, Creer DA, Glossip D, Goellner R, Hampton A, Roberts G, Haskell N, Taylor P, Ettling J. 2000. Evolutionary implications of phenotypic plasticity in the hindlimb of the lizard *Anolis sagrei*. *Evolution* **54:** 301–305.

Magurran AE. 1988. *Ecological diversity and its measurement.* London: Croom Helm.

Nahal I. 1981. The Mediterranean climate from a biological viewpoint. In: di Castri F, Goodall DW, Specht RL, eds. *Mediterranean-type shrublands*. Amsterdam: Elsevier, 63–86.

Niewiarowski PH. 1995. Effects of supplemental feeding and thermal environment on growth rates of eastern fence lizards, Sceloporus undulatus. *Herpetologica* **51:** 487–496.

Niewiarowski PH, Roosenburg W. 1993. Reciprocal transplant reveals sources of variation in growth rates of the lizard *Sceloporus undulatus*. *Ecology* **74:** 1992–2002.

Noy-Meir I. 1973. Desert ecosystems: environment and producers. *Annual Review of Ecology, Evolution and Systematics* **4:** 25–51.

Noy-Meir I. 1974. Desert ecosystems: higher trophic levels. *Annual Review Ecology and Systematics* **5:** 195–214.

Olsson M, Shine R. 1997. The seasonal timing of oviposition in sand lizards (*Lacerta agilis*): why early clutches are better. *Journal of Evolutionary Biology* **10:** 369–381.

Ortega J, López P, Martín J. 2015. Altitudinally divergent adult phenotypes in Iberian wall lizards are not driven by egg differences or hatchling growth rates. *Oecologia* **177:** 357–366.

Oufieroi CE, Angilletta MJ Jr. 2006. Convergent evolution of embryonic growth and development in the eastern fence lizard (*Sceloporus undulatus*). *Evolution* **60:** 1066–1075.

Overall KL. 1994. Lizard egg environments. In: Vitt LJ, Pianka ER, eds. *Lizard ecology: historical and experimental perspectives*. Princeton: Princeton University Press, 51–72.

Palkovacs EP. 2003. Explaining adaptive shifts in body size on islands: a life history approach. *Oikos* **103:** 37–44.

Packard GC, Packard MJ, Miller K, Boardman TJ. 1987. Influence of moisture, temperature, and substrate on snapping turtle eggs and embryos. *Ecology* **68:** 983–993.

Pinho C, Harris DJ, Ferrand N. 2007. Comparing patterns of nuclear and mitochondrial divergence in a cryptic species complex: the case of Iberian and North African wall lizards (*Podarcis*, Lacertidae). *Biological Journal of the Linnean Society* **92:** 121–133.

Rensch B. 1938. Some problems of geographical variation and species formation. *Proceedings of the Linnean Society of London* **150:** 275–285.

Salvador A, Carretero MA. 2014. *Podarcis hispanicus* (Steindachner, 1870). In: Salvador A (coord.), ed. *Fauna Iberica*, **Vol. 10**. Reptiles, 2ª ed. Madrid: Museo Nacional de Ciencias Naturales, C.S.I.C., 537–556.

Sá-sousa Sears MW, Angilletta MJ Jr. 2003. Life history variation in the sagebrush lizard: phenotypic plasticity or local adaptation? *Ecology* **84:** 1624–1634.

Sears MW. 2005. Geographic variation in the life history of the sagebrush lizard: the role of thermal constraints on activity. *Oecologia* **143:** 25–36.

Sinervo B, Adolph SC. 1989. Thermal sensitivity of growth rate in hatchling Sceloporus lizards: environmental, behavioural and genetic aspects. *Oecologia*. **78:** 411– 419.

Stamps JA, Tanaka S. 1981. The influence of food and water on growth rates in a tropical lizard (*Anolis aeneus*). *Ecology* **62:** 33–40.

Stearns SC. 2000. Life history evolution: successes, limitations, and prospects. *Die Naturwissenschaften* **87:** 476–486.

Telemeco R, Radder R, Baird T, Shine R. 2010. Thermal effects on reptile reproduction: adaptation and phenotypic plasticity in a montane lizard. *Biological Journal of the Linnean Society* **100:** 642–655.

Tinkle DW, Ballinger RE. 1972. *Sceloporus undulatus*: a study of the intraspecific comparative demography of a lizard. *Ecology* **53:** 570–584.

Travis J. 1994. Ecological genetics of life-history traits: variation and its evolutionary significance. In: Real LA, eds. *Ecological genetics*. Princeton: Princeton University Press, 171–204.

Via S, Lande R. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution* **39:** 505–522.

Watts C, Mitchell S, Salewski V. 2010. Bergmann's rule; a concept cluster? *Oikos* **119:** 89–100.

Webb W, Szarek S, Lauenroth W, Kinerson R, Smith M. 1978. Primary productivity and water use in native forests, grasslands, and desert ecosystems. *Ecology* **59:** 1239–1247.