OXFORD

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Loss of largest and oldest individuals of the Montpellier snake correlates with recent warming in the southeastern Iberian Peninsula

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Abstract

The effects of climate change on organisms are now being extensively studied in many different taxa. However, the variation in body size, usually shrinkage in response to increasing temperature, has received little attention regarding to reptiles. During past periods of global warming, many organisms shrank in size, and current evidence and experiments manipulating temperature have shown a biomass decrease in some organisms with increasing temperatures. Here we test whether the body size of the Montpellier snake *Malpolon monspessulanus* from the southeastern Iberian Peninsula is changing and correlated with the increasing temperature in this region during a 39-year period (1976–2014). We measured the snout–vent length (SVL) of vouchers in scientific collections to check for trends in adult body size at the population level in relation with temperature, while controlling for the age of the individuals (estimated by skeletochronology, $n = 141$). Given the great ontogenetic variation in body size of the study species, we categorized age in 3 classes: "young adults" (under 5 years old), "intermediate adults" (from 5 to 7 years old), and "old adults" (from 8 to 14 years old). By means of linear mixed models, we found a negative relationship between SVL of "old adults" and average annual temperature in the region during the lifetime of each individual. Our results indicate that largest and oldest individuals of the Montpellier Snake, that is, males because of strong sexual size dimorphism in this species, disappeared from the study population, and suggest that it occurred in response to rising environmental temperature.

Key words: body shrinkage, climate warming, differential mortality, Malpolon monspessulanus, skeletochronology, snakes, Spain.

Our planet has always been changing and, therefore, varying its biodiversity (Smith et al. 2012). The problem of the recent climate change led by human activities lies in its intensity and speed, as the global average surface temperature has increased 0.74 ± 0.18 °C during the period 1906–2005 (IPCC 2007), mostly during the last 50 years (0.13 ± 0.03 °C per decade). The forecast expects an increase of the global average surface temperature for the decade 2090–2099 relative to the period 1980–1999 of 1.8 °C (1.1–2.9 °C) in the best possible scenario (B1), whereas 4.0 °C (2.4–6.4 °C) in the worst (A1F1), as well as an intensification of the extreme meteorological events (IPCC 2007). In this scenario, the study of the effects of climate change on organisms is now being extensively studied in different taxa. These effects can be classified as: (1) shifts in the distribution range, (2) local plasticity or adaptation through phenotypical and/or phenological changes to remain in the place of origin, and finally (3) extinction on a local or larger scale (Millien et al. 2006). Most studies in this field deal with range shifts and phenological changes in organisms (Walther et al. 2002; Parmesan 2006), with body-size variation being one of the responses that has received the least attention (review in Gardner et al. 2011). During

past periods of global warming, marine and terrestrial organisms shrank in size (Smith et al. 2009), and experiments manipulating temperature have shown a decline in the size or biomass of some organisms with rising temperature; for example, in phytoplankton (Sommer et al. 2007), fish (Desai and Singh 2009), and beetles (Stillwell and Fox 2009). Body size is the most evident morphologic trait of a given organism, it is easy to quantify, and large sample sizes can be obtained with little effort. In addition, many life-history traits depend on this parameter: clutch (or litter) size and weight, brood time or gestation time, water and energy requirements, metabolic rate, position in the trophic chain, maturation time, intrinsic rate of population growth, lifespan, and extinction risk, among others (Blueweiss et al. 1978; Millien et al. 2006; Meiri 2008; Gardner et al. 2011).

Within the animal kingdom, the most widely studied groups for body-size shrinkage in response to climate change have been the endothermic ones, whereas the terrestrial ectotherms have received little attention (reviews in Gardner et al. 2011; Sheridan and Bickford 2011). For instance, body shrinkage was correlated to recent warming in *Bufo bufo* females from the UK over a 22-year period (Reading 2007); and also, body shrinkage at the population level correlated to recent warming in 6 species of plethodontid salamanders from the USA during a 55-year period (Caruso et al. 2014). However, no proof of this trend is available in the literature for reptiles. The opposite reaction, increasing body size related to climate warming, has been detected in a few cases, generally restricted to organisms of high latitudes or elevations, as in the lizard *Zootoca vivipara* in France (Chamaillé-Jammes et al. 2006). Particularly within snakes, there are few studies on responses to climate change (Weatherhead and Madsen 2009), and to our concern, none available on body-size shift as a response to gradual long-term increase in environmental temperature.

However, snakes can be model organisms in analyzing body-size shifts in response to climate change, as they have no limbs and most of the growth is expressed in the longitudinal axis (at least when compared with limbed ectotherms). In addition, the Mediterranean Basin has been proposed as one of the 2 most responsive regions to climate change in the world (Giorgi 2006). Furthermore, the Iberian Peninsula is one of the European regions where the effects of global warming to herptiles will be the most dramatic (Araújo et al. 2006; Carvalho et al. 2010). Specifically for the southeastern Iberian Peninsula, a temperature increase of 0.07 °C per year has been previously proved (for the 1983–2004 period; Moreno-Rueda et al. 2009; see also Jones et al. 1999).

The aim of the present study is to determine whether the population of the Montpellier snake *Malpolon monspessulanus* from the southeastern Iberian Peninsula, has reduced in body size over the period 1976–2014 in relation to temperature. We expect that the largest adult size class (i.e., the oldest individuals) will be the most responsive to climate change, and, therefore, we included the age class of individuals in our mixed models, to account for the effect of this important variable. We used the body size measurement of vouchers preserved in scientific collections (i.e., 1 observation per individual). Thus, this is a study conducted at the population level.

Materials and Methods

The study area is the southeastern Iberian Peninsula (39°00'–36°30' N; 4°30'–0°15' E), which occupies approximately 110,000 km^2. Climate is typically Mediterranean, with temperatures [mean ± standard deviation (SD)] in winter (January) = 7.2 ± 1.9 °C, in summer (July) = 25.3 ± 2.4 °C, annual average = 16.4 ± 1.7 °C, and yearly rainfall = 412.1 ± 226.7 mm (data from 98 meteorological stations for the 1980–2004 period, summarized in Moreno-Rueda et al. 2009). The vegetation consists of evergreen oak *Quercus rotundifolia*, scrubs, and some species of pines *Pinus* spp., mixed with cultivated areas of cereals and orchards in the foothills, plains, and valleys.

The snake under study was selected for the amount of data available, as it is the commonest snake of the Iberian Peninsula. This species has the widest diet and furthermore is considered the second more generalist snake in habitat use in the study region (Segura et al. 2007). Within our data set, the study species spread 3-1850 m asl, with a mean elevation value of 781.2 m (SD = 367.7). Specimens came from field sampling conducted from 1979 to 2014, within the framework of several studies (i.e., Feriche et al. 2008). During most of the years, we sampled 2–4 days per month (daily searches lasted ~4 h), throughout all months of the year. Specimens were collected among those killed by traffic or local people. Afterwards, individuals were preserved in ethanol at the collection of the University of Granada, Spain. The review of scientific collections has been proposed as an important tool to understand the effect of climate change on body sizes (Millien et al. 2006).

To check for snake body-size variation during the study period, we used the snout–vent length (SVL) of specimens, measured by a cord along the middorsal line, and afterwards by a flexometer (instrumental error = ±1 mm). The measurement error of snake's SVL has been quantified as being approximately 1% of the body size (Blouin-Demers 2003); therefore, it is possible to detect shrinkage if this is greater than the error value. Reptile vouchers from scientific collections and especially snakes, shrink due to the fixative and preservative, and measurements of fresh specimens are not directly comparable to measurements of preserved ones (Barry 2011). As not all the vouchers in collection were measured for body size when fresh, we needed to estimate this value from some specimens. We regressed fresh (SVL$_1$) against preserved (SVL$_2$) body size from a subset of individuals that offered both measurements ($n = 62$), assuming the shrinking process is not continuous, but rather stops after the first days of preservation in alcohol (Shields and Carlson 1996). This has been tested for green iguanas *Iguana iguana*, finding that shrinking stops after 30 days in preservative (Vervust et al. 2009).

For estimating the age of snake vouchers, we used a skeletochronology approach. This was based on the dense lines in bones of ectotherms from temperate regions, which corresponded to active osteogenesis periods (growth marks, thereafter GM), followed by no visible marks, meaning a non-osteogenesis period (Peabody 1961). In temperate latitudes GM generally matches the seasons of the year, bone growth stopping in winter, but also during the reproductive or the warmest periods, which can lead to more than 1 GM per year (Pérez et al. 1979). Even so, given the infrequent feeding of snakes, 1 large prey could imply the appearance of a small GM, and these possibilities were taken into account in estimating the age of the specimens. We removed from the snake skull the ectopterygoid, one of the most flattened bones in the snake skeleton. We cleaned the ectopterygoid with acetone to eliminate the non-bone tissues and afterwards we made the bone transparent by brief immersion in xylol (dimethyl benzene); finally, we counted the GM by light microscopy, under 100× magnification. Because of the absence of limb bones in snakes, the ectopterygoid is more reliable for determining the age of these reptiles than are other bones, such as caudal vertebrae (Reed and Tucker 2011), and reliable results have been achieved with ophidians by the external examination of flattened bones (Castanet et al. 1993). To prevent biased interpretations

influenced by snake body size, we counted GM in each bone without knowing the body size of the given voucher. For better accuracy, GM counting and age estimation interpreted by one of us (M.F.) was assessed by two of us (C.L.C. and J.M.P.), who counted the GM in ectopterygoids from 30 vouchers taken at random. The repeatability of GM measures from different observers was then estimated by means of Generalized Linear Mixed Model (GLMM)-based repeatability using Penalized Quasi-Likelihood estimation (PQL) estimation for count data (Nakagawa and Schielzeth 2010). We utilized *log* as the link function for transformation into a Gaussian distribution of errors, and we modeled overdispersion as an additional parameter to the distribution from which the original responses are assumed to be drawn (*multiplicative overdispersion model*).

The ontogenetic variation in body size of snakes is huge (SVL from 252 to 1574 mm; this study), and previous studies have found body-size shrinkages in snakes only for the largest individuals; that is, since they require more energy and resources, they are more vulnerable to environmental changes (Madsen and Stille 1988; Winne et al. 2010). To test different responses to warming in relation to age class of individuals, we categorized age as a factor with 3 levels: "young adults" (under 5 years old, $n = 45$), "intermediate adults" (from 5 to 7 years old, $n = 59$), and "old adults" (from 8 to 14 years old, $n = 37$). Since only 2 females were categorized as "old adults", we eliminated both vouchers from subsequent analysis.

The climatic trend in temperature over the years was checked from 14 meteorological stations that provided climatic data within the study area for the period 1976–2014 (www.aemet.es; see Supplementary Table 1), a period that includes the estimated lifespan of snake vouchers. Since we expect that environmental temperature in the study area is increasing over time, we also can expect a negative trend in body size of Montpellier snake during the study period. Thus, with exploratory purpose, we represented a scatterplot of raw adult body size versus years of the study period.

To detect potential relationships between body size and climate change, we used linear mixed models, where body size (fresh SVL, in mm) was the response variable, whereas sex, age class, elevation and temperature were the input variables. Also, we included the 2-way interaction term of sex with temperature as an explanatory variable, testing potential sexual differences in the effect of climate on body size. In the same line, we included as an explanatory variable the interaction term of age class with temperature, testing different effects of climate on body size in relation to age class. The year when a given individual died was included in the model as the random effect, thus controlling for interannual variation in the response variable. We also included elevation as a covariate because a body-size shift with increasing elevation has been found in a lizard from the southeastern Iberian Peninsula (Zamora-Camacho et al. 2014). A given individual of *M. monspessulanus* can live up to 14 years in the wild (this study), and therefore, its growth and body size does not depend on the climatic conditions of the year it is recorded in the scientific collection. Consequently, we used as a climatic parameter the average of annual temperature in the study area over the entire life of each specimen.

We evaluated all possible candidate models that could be built with our initial global models following the procedures described by Grueber et al. (2011). We first standardized the input variables entering the global model scaling them by dividing means by 2 SDs, which allowed comparison on the same scale of coefficients of binary factors (e.g., sex), and covariates. Therefore, parameter estimates were of standardized effect sizes and were on a comparable scale (Gelman 2008; Grueber et al. 2011). The most parsimonious of all possible candidate models was determined using Akaike

information criterion (AICc) corrected for small sample size. We calculated Akaike weight (w) for each candidate model (i) that can be interpreted as the probability that (2) is the best model, given the data and set of candidate models (Burnham and Anderson 2002). The final model was built by averaging the parameter estimates from top models at a cutoff criterion of ΔAICc < 6.0 (Richards 2008). Candidate models to perform the model averaging are presented in Supplementary Table 2.

Finally, we also tested a correlation between raw age of the subset of "old adults" and averaged temperature during their lifespan. The result from this correlation indicates if older individuals, not only the largest ones, are disappearing from the population in correlation with recent warming.

All the statistical analyses were run out in R, version 3.0.2, and α was set at 0.05. We employed the libraries *MuMIn* (Bartón 2015), *lme4* (Bates et al. 2016), *AICcmodavg* (Mazerolle 2015), *rptR* (Nakagawa and Schielzeth 2010), and *ggplot2* (Wickham and Chang 2016) using R version 3.0.2 (R Development Core Team 2013).

Results

The linear regression among measures of body size for fresh (SVL_1) and preserved individuals (SVL_2) proved highly significant ($R^2 = 0.987$; $P < 0.001$; $n = 62$), the mean shrinkage rate of vouchers being 5.55% of the initial body size. Thus, we estimated the fresh body-size measurement (SVL_1) for the vouchers without this value using the following equation:

$$SVL_2 = 0.93 * SVL_1 + 10.35.$$

The linear character of this equation shows that the shrinkage process depends neither on body size nor on sex.

In the southeastern Iberian Peninsula, during the study period (1976–2014), adult body size of *M. monspessulanus* averaged 883.7 mm SVL (SD = 208.6; $n = 141$), ranging between 555 and 1574 mm. There was marked sexual dimorphism in body size ($P < 0.001$; Kruskal–Wallis $\chi^2 = 32.16$; df = 1); mean body size of the adult males was 950.0 mm SVL (SD = 224.8; $n = 93$), whereas mean body size of the adult females was 755.1 mm SVL (SD = 71.7; $n = 48$). This sexual dimorphism in body size was driven by the age of individuals: males can live more years (up to 14 years) than females (up to 8 years), so they can attain larger body size (Figure 1). Raw data utilized in this study is showed in Supplementary Table 3.

The estimated repeatability of the GM counting on ectopterygoid bones was high and significant [original scale repeatability = 0.923; SE = 0.098; CI = (0.373, 0.748); $P = 0.001$]. Furthermore, the linear regression between age as an independent variable and fresh SVL as a dependent variable was significant (intercept = 445.72; slope = 69.538; $R^2 = 0.777$; $P < 0.001$; $n = 141$; Figure 1). The intercept of this linear regression is a reliable body size before the first GM appears on the bone (i.e., the body size that a given individual can attain during the first year of life; see Feriche et al. 2008). Thus, we concluded that our approach for estimating the age of the individuals is reliable.

We found a significant linear increase in annual temperature over the study period 1976–2014 for the southeastern Iberian Peninsula ($R^2 = 0.40$; $P < 0.001$; $n = 39$; Figure 2). Furthermore, as a preliminary result, we observed that maximum body size of *M. monspessulanus* declined in the study population in recent years (Figure 3). The effects of sex and age class on SVL were significant in the linear mixed model, indicating respectively, the sexual dimorphism
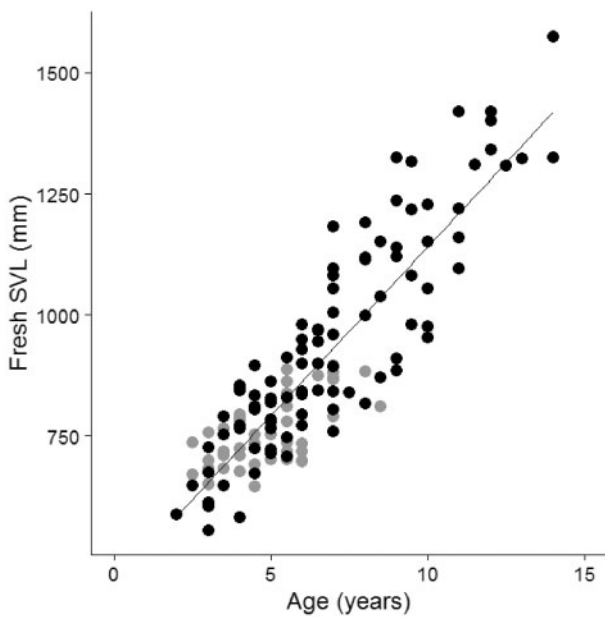
Figure 1. Linear regression between body size and estimated age of the Montpellier snake, *Malpolon monspessulanus*, in the southeastern Iberian Peninsula during the period 1986–2014. Sample size is 141 vouchers: 93 males (black points) and 48 females (gray points).
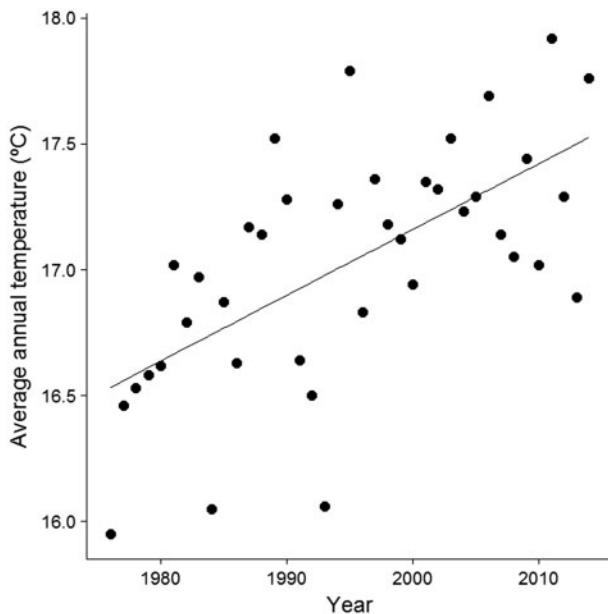

Figure 3. Scatterplot plot of raw adult body size of the Montpellier snake, *Malpolon monspessulanus*, versus years of the study period in the southeastern Iberian Peninsula. Sample size is 290 vouchers: 206 males (black points) and 84 females (gray points).

Figure 2. Climate-warming trend in the southeastern Iberian Peninsula for the period 1976–2014. Values for each year represent the mean of 14 weather stations (see Supplementary Table 1). Sample size is 39 years.

Table 1. Summary results after model averaging of the effects of environmental temperature on snake body size (fresh SVL in mm)

Parameter	Estimate	SE	95% CI
Intercept	700.26	23.90	(653.0, 747.5)
Age class (intermediate)	102.32	23.28	(56.3, 148.4)
Age class (old)	377.84	28.55	(321.3, 434.3)
Sex	59.89	22.46	(15.4, 104.3)
Altitude	−7.55	19.97	(−47.1, 32.0)
Temperature	−24.44	46.62	(−116.7, 67.8)
Age class (intermediate) * temperature	**−16.72**	**45.08**	**(−105.9, 72.5)**
Age class (old) * temperature	−138.83	54.98	(−247.6, −30.1)
Sex * temperature	−16.61	47.20	(−110, 76.8)

Notes: We used as climatic variable, the average annual temperature during the lifetime of each individual. Parameters estimated for predictors with confidence intervals (95% CI) that do not include zero are highlighted in bold (i.e., significant effects). The reference levels of the fixed factors sex and age class were, respectively, "female" and "young adult." We considered the year when a given individual died as the random effect. Sample size is 139 adult individuals.

and the increasing body size with age class. The linear mixed model also revealed a significant effect of the interaction term between age and temperature, indicating a negative linear relationship between body size of "old adults" and average annual temperature during their lifespan (Table 1; Figure 4). The effect of elevation on SVL was nonsignificant in the linear mixed model, indicating that individuals do not differ in body size according to elevation. The interaction term between sex and temperature was nonsignificant in the linear
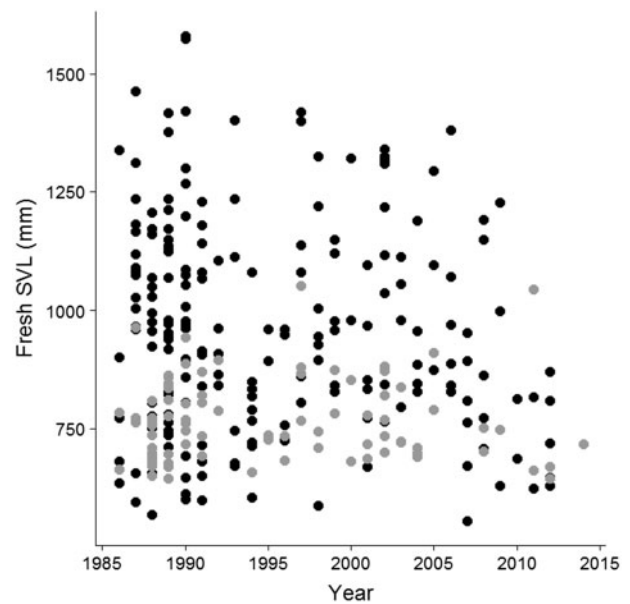
mixed model, indicating that there is no difference across sexes in the correlation between body size and temperature. Nevertheless, we noticed that all individuals categorized as "old adults" were males, thus the relationship we found between body size and temperature was only operating in males; since they lived longer than females, they could attain longer body sizes (Figure 1). Top models set selected for averaging are presented in Supplementary Table 2.

Finally, we also found a significant correlation between raw age of "old adults" and average temperature during their lifespan ($r_s = -0.40$; $P = 0.014$). Therefore, the result we found for the body size of *M. monspessulanus* is related to a differential mortality of older individuals in correlation to recent warming, rather than a
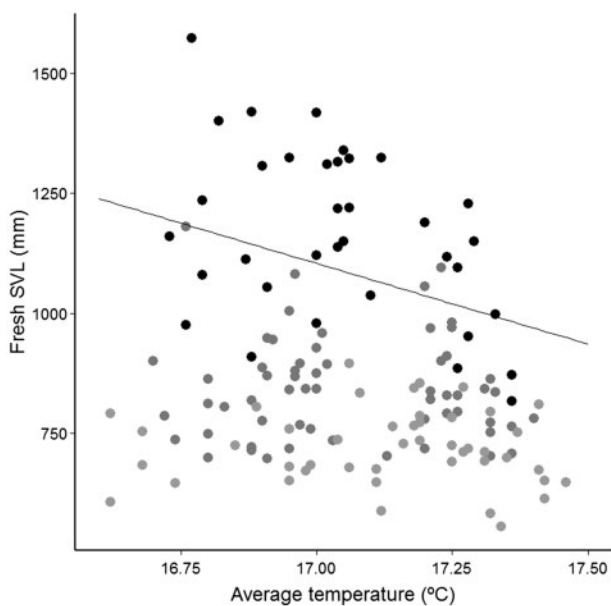
Figure 4. Relationship between adult body size of the Montpellier snake, *Malpolon monspessulanus*, and average annual temperature in the southeastern Iberian Peninsula during the lifespan of each individual. Points represent observed values of body size in response to the average temperature that a given voucher experienced. Straight line represents the body size values predicted by the linear mixed model for old adults (Table 1). Sample size is 139 vouchers: 35 old adults (black points), 59 intermediate adults (dark gray points), and 45 young adults (gray points).

body-size shrinkage. In other words, we found a loss of larger and older individuals from the study population in recent years, but not a reduction in body size for individuals of same age.

Discussion

Based on GM counting on ectopterygoid bones, we estimated the age of 141 adults of *M. monspessulanus*, which allowed us to include this variable in the mixed models to test relationships between body size and temperature. The longevity of both sexes was quite different but fits within the range of this trait for late-maturing temperate colubrids (Parker and Plummer 1987). Climate warming in the southeastern Iberian Peninsula for the 1976–2014 period was confirmed by the meteorological data considered in this study, in agreement with previous and more general studies (Castro et al. 2005; Moreno-Rueda et al. 2009). Finally, we found a negative linear correlation between the body size of old individuals from our study population and the average annual temperature they experienced during their lifespan. However, our results proved that body shrinkage at the population level is not occurring, but larger and older individuals are disappearing; they are dying in correlation with the increasing temperature, rather than reducing their body size. Previous herpetological studies have found, in correlation with environmental changes, a body-size shift only for the largest size class (Madsen and Stille 1988; Jessop et al. 2006; Winne et al. 2010). However, these studies did not measure age of individuals and thus our study highlights the importance of ageing when testing body size shifts in long-lived organisms, such as large snakes. Other studies on snakes also found a differential mortality according to age (Parker and Plummer 1987), but to our knowledge, no study has

found a gradual loss of the oldest and largest individuals according to a gradual environmental change. We hypothesize that larger individuals are more vulnerable to environmental changes because they require more energy and resources for maintenance.

The degree to which the natural history of organisms is affected by temperature is diverse, and global warming has already led to significant shifts in the distribution, phenology, body size, and behavior of plants and animals (Brodie and Russell 1999; Parmesan and Yohe 2003; Sheridan and Bickford 2011). For the species considered here, in the same study area and over approximately the same period, detectability in the field was positively correlated to annual temperature (Moreno-Rueda and Pleguezuelos 2007), as well as the span of the seasonal activity period (Moreno-Rueda et al. 2009). The selective advantage for a Mediterranean snake with higher activity and a longer seasonal activity period could be more opportunity to feed, reproduce, increase population size, move, and perhaps to grow (Moreno-Rueda et al. 2009; Weatherhead and Madsen 2009). Apparently, the thermophylous *M. monspessulanus* responded to the temperature increase in the southeastern Iberian Peninsula by increasing population size in the field (Moreno-Rueda and Pleguezuelos 2007), which led the species to shift its relative importance in the snake community, from 27% to 52% of the total individuals found in field sampling during the last decades (Segura et al. 2007). However, the Montpellier snake was one of the few Iberian reptile species that did not shift its range northwards during the recent period of climate warming (Moreno-Rueda et al. 2012). Moreover, according to the current study, the species did not respond to climate warming by increasing body size within the population, but rather the contrary. Results from previous works and the present study suggest that Montpellier snake is responding to climate warming by raising its dominance within the snake community of the southeastern Iberian Peninsula (Segura et al. 2007), but with increasingly younger and smaller sized individuals.

In snakes and other taxonomic groups, a large body size tends to increase the probability of extinction (Gaston and Blackburn 1995; Webb et al. 2002). Our results agree with this statement, because larger and older specimens are disappearing in recent decades but the population under study exhibits a good conservation status (Santos et al. 2007). The sexual dimorphism in body size of the study species (Feriche et al. 2008) and the important sexual difference in lifespan unveiled in this study could be also behind these results. We observed in this study that both sexes grow approximately at the same rate until 8 years old and to a body size of approximately 890 mm SVL, an age and a body size that females do not overpass. Although in snakes and particularly in the study species, there is a positive correlation between maternal body size and clutch or offspring size (Shine 2003; Feriche et al. 2008), the disappearance of the largest specimens in the studied population is not affecting the recruitment of the population, at least from the maternal contribution. This is because the disappearing subset of the population corresponds to oldest and largest individuals, which excludes females. With respect to males, they exhibit a vernal spermatogenesis cycle (Feriche et al. 2008) of African origin that is highly demanding of warm temperatures during spring, shared in Europe by only *Hemorrhois hippocrepis* (Saint Girons 1982). The recent environmental warming should be favoring snakes with this thermophilous spermatogenetic cycle (Feriche et al. 2008) what in turn, it should explain the good conservation status of these species in the study area (Santos et al. 2007) and also the quick success of their recent invasion events in northern Mediterranean areas (Silva-Rocha et al. 2015).

Because this is a correlational study, we cannot suggest a causal relationship between climate warming and loss of the oldest and largest specimens of *M. monspessulanus* without considering other alternative hypotheses, as is the rule for most studies on ecological effects of climate change (Parmesan and Yohe 2003). For instance, a shift may occur in the mortality according to sex and size due to diverse threatening factors, such as road-killing (see results for *Zamenis longissimus* and *Hierophis viridiflavus* in Bonnet et al. 1999). However, testing this alternative hypothesis requires long-term population studies, a difficult task for scarce and secretive animals like snakes (Parker and Plummer 1987; Reading et al. 2010). In addition, global survival rate could be decreasing over time due to any parameter not accounted in this study; anyway, it would be unlikely that a given specimen of the Montpellier snake reach the largest size class in recent times.

According to previous studies and to the current one, *M. monspessulanus* can be interpreted as having the capacity to resist environmental changes and even to take advantage of climate warming within its European range, in spite of the disappearance of the oldest and largest individuals. Snakes may be disappearing worldwide (Mullin and Seigel 2009) because of multi-faceted causes, but with the global climate change at their root (Reading et al. 2010). We acknowledge that the Montpellier snake is not a species of conservation concern; nevertheless, our findings in this research could be of relevance for other snakes considered to be threatened.

Authors' Contributions

J.M.P. conceived the idea, C.L.-C. analyzed the data, and all authors contributed to collecting the data and writing the manuscript.

Supplementary Material

Supplementary material can be found at http://www.cz.oxfordjournals.org/.

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