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Age structure of a lizard along an elevational gradient reveals non-linear lifespan patterns with altitude

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Abstract

Lifespan is one of the main components of life history. Shorter lifespans can be expected in marginal habitats. However, in the case of ectotherms, lifespan typically increases with altitude, even though temperature –one of the main factors to determine ectotherms' life history– declines with elevation. This pattern can be explained by the fact that a shorter activity time favours survival. In the present study, we analysed how lifespan and other life-history traits of the lizard *Psammodromus algirus* vary along a 2,200 m elevational gradient in Sierra Nevada (SE Spain). Populations at intermediate altitudes (1,200–1,700 m), corresponding to the optimal habitat for this species, had the shortest lifespans, while populations inhabiting marginal habitats (at both low and at high altitudes) lived longest. Therefore, this lizard did not follow the typical pattern of ectotherms, as it also lived longer at the lower limit of its distribution, nor did it show a longer lifespan in areas with optimal habitats. These results might be explained by a complex combination of different gradients along the mountain, namely that activity time decreases with altitude whereas food availability increases. This could explain why lifespan was maximum at both high (limited activity time) and low (limited food availability) altitudes, resulting in similar lifespans in areas with contrasting environmental conditions. Our findings also indicated that reproductive investment and body condition increase with elevation, suggesting that alpine populations are locally adapted.

Key words: age structure, elevation, life history, longevity, marginal habitats, relative clutch mass

The habitat of a particular species may be defined as a set of resources and conditions needed for survival and reproduction of individuals of that species (Chase and Leibold 2003). Accordingly, the central-marginal hypothesis states that zones with optimal or near-optimal conditions can be referred to as core habitats. Nevertheless, as one moves away from the core habitat areas, the environment usually becomes progressively less suitable for the species, implying lower survival probability and/or reproductive success and hence decreased fitness (Pironon et al. 2017). These habitats have border conditions that the species can tolerate for survival and reproduction and are therefore considered suboptimal or marginal (Kawecki 2008).

Core and marginal habitats for a given species can be found along altitudinal gradients. Mountain environments harbour a high level of ecological heterogeneity because several abiotic factors change with altitude; mainly, temperature and the partial pressure of oxygen decrease with altitude, while solar radiation increases (Barry 2008). These abiotic factors exert selective pressures on animals and plants, causing communities to vary along the elevational gradient (e.g., Carothers et al. 2001; Navas 2002; Fu et al. 2007). Hence, a species inhabiting an elevational gradient may occupy core habitats as well as marginal ones in a relatively small geographical area. As such, elevational gradients provide researchers with a natural experimental setting to study how life-history varies according to habitat quality.

One of the main life-history traits is lifespan, which is both influenced by and influences other life-history traits, as it has a direct effect on several ecological and evolutionary outcomes (Metcalf and Pavard 2007). Lifespan primarily depends on extrinsic mortality (Cichoń 1997), but life history theory suggests that it could also be shortened by selection for greater reproductive investment (Araya-Ajoy et al. 2018). Although lifespan show a marked geographical variation (Valcu et al. 2016), we only have a limited understanding of how it varies with elevation in species distributed across a large range of altitudes and the causes of this variation. The available studies generally assume lifespan varies linearly with altitude and provide contradictory results where lifespan lengthens, shortens or remains relatively unchanged along the altitudinal gradient (e.g., review for birds in Boyle et al. 2016).

Furthermore, the elevational pattern in lifespan could differ between ectotherms and endotherms, since temperature, which is the main abiotic factor to vary with elevation (Körner 2007), has a more significant effect on the physiology of ectotherms than that of endotherms (Angilleta 2009). Environments become harsher at high elevations, where storms, strong winds and snow are frequent, resulting in low thermal quality and long hibernation periods. This scenario may bring about an early death and therefore reduce lifespan (Sears 2005). Most studies on ectotherms, however, report that lifespan increases with altitude (Zhang and Lu 2012). This pattern is attributed to several concomitant factors: (1) shorter activity seasons at high elevations, which reduces metabolic damage and time exposed to predators; (2) reduced predator pressure with altitude; and (3) changes in life history, as populations at high elevations often lead a slower pace of life and invest less in reproduction and more in self-preservation (review in Cabezas-Cartes et al. 2018).

In the present study, we evaluate how lizard lifespan varies across a wide elevational gradient by studying the lizard *Psammodromus algirus* in the Sierra Nevada mountain (SE Spain). We assumed that habitat quality for this species in Sierra Nevada is maximum at mid elevations (1,200–1,700 m) and diminishes as the species ascends or descends the elevational gradient. This assumption is based on two lines of evidence. First, thermal quality is one of the main factors affecting habitat quality in ectotherms (Angiletta 2009), and for this species in Sierra Nevada it becomes maximal at intermediate elevations (Zamora-Camacho et al. 2016). Second, population density is typically lower in marginal habitats than in core ones (Sagarin et al. 2006) and the density of this species in Sierra Nevada is highest at mid elevations (Zamora-Camacho et al. 2013). Different environmental conditions can lead to a suboptimal habitat and hence, depending on the trait or traits affecting lizard fitness and how they vary with elevation, lifespan may present different elevational patterns. Accordingly, in the present study, we tested competitive hypotheses developed to explain how lifespan varies with elevation in *P. algirus* (Table 1).

The central-marginal hypothesis: This hypothesis affirms that fitness is lower in low-quality habitats because adult survival decreases with habitat quality. As such, we expect the maximum lifespan in the core habitat (mid elevations), with lifespans being shorter in marginal habitats situated at both high and low elevations. Adult survival may be shorter at high elevations due to harsh and extreme climate conditions (Sears 2005). Meanwhile, at the lower limit, interspecific competition is often more intense (Comas et al. 2014), which can have negative consequences for adult survival (Dunham 1980). Moreover, at low elevations, predation risk may increase due to the presence of more and a greater diversity of predators (Fox et al. 1994), while at high elevations it can increase due to greater conspicuousness since more time is devoted to thermoregulation (Alford and Lutterschmidt 2012).

The resource-allocation hypothesis: This hypothesis claims that fitness is higher in better quality habitats because they favour successful reproduction (this is also predicted by the central-marginal hypothesis). So, animals could be selected for greater investment in reproduction in core habitats, hence allocating fewer resources to self-preservation and thereby reducing lifespan (Kirkwood and Rose 1991). For example, *Crinia pseudinsignifera* frogs invest more in reproduction when living in more favourable areas, this reducing their lifespan with respect to harsher areas (Reniers et al. 2015). In such cases, lifespan would be longer in marginal habitats than in core habitats. The assumptions made in this hypothesis are supported by studies in other populations of *P. algirus*, which suggest that reproductive success is impaired at both high and low elevations. In highlands, low soil temperatures delay hatching and harm hatchlings' body condition (Monasterio et al. 2011), whereas at lower altitudes, hatchling survival is lower as a consequence of scarcer food availability (Iraeta et al. 2006). Moreover, predators are typically more abundant in lowland areas and tend to have a greater impact on juveniles than on adults (Ballinger 1979). In addition, juvenile mortality is very high during hibernation (Civantos and Forman 2000), which is longer at high elevations.

The rate-of-living hypothesis and the activity-exposition hypothesis: The relevance of temperature for ectotherms may prevail, as it is the main determinant of lifespan across the gradient. Indeed, ectotherms frequently show an increased lifespan with altitude and when inhabiting cold environments in general (Morrison and Hero 2003; Munch and Salinas 2009; Zhang and Lu 2012; Scharf et al. 2015; Cabezas-Cartes et al. 2018; Stark et al. 2018). This generalised pattern can be explained by two different hypotheses. According to the rate-of-living hypothesis, in cold environments, ectotherms live through shorter activity seasons, which reduces metabolic damage and consequently increases survival and lifespan (Speakman 2005). Supporting this contention, Bestion et al. (2015) experimentally showed that increased temperatures in the lizard *Zootoca vivipara* enhances growth and reproductive investment, resulting in reduced longevity. Furthermore, according to the activity-exposition hypothesis, reduced activity would also reduce exposure to predators, and therefore increase survival at high altitudes (Adolph and Porter 1993). Several studies with ectotherms show that longevity increases for shorter activity seasons (Cvetković et al. 2008; Liao et al. 2016; Cabezas-Cartes et al. 2018), although the exact mechanism behind this pattern is still not completely known. In our study population, the length of the activity season decreases with elevation (Zamora-Camacho et al. 2013), and, as an evidence of reduced metabolic damage, oxidative stress also decreases with elevation (Reguera et al. 2014a, 2015). In other *P. algirus* populations, lizard survival during the activity period was lower at low altitudes, presumably due to longer activity time (Iraeta et al. 2015).

In general, different demographic and life-history traits are expected near the upper and lower distribution boundaries with respect to the core distribution. Accurate assessments of lifespan are therefore necessary to discern between competing hypotheses concerning the nature of selective forces driving life-history evolution. Indeed, the three hypotheses make different predictions on how lifespan should vary with elevation in our study system (Table 1): peak at mid elevations (central-marginal hypothesis), U-shaped (resource-allocation hypothesis), and linear increase (both the rate-of-living hypothesis and the activity-exposition hypothesis). In this study, we estimate the age structure (by means of skeletochronology) of a population of *P. algirus* across 2,200 m of an elevational gradient. Moreover, to disentangle the causes of elevational variation in lifespan, we present additional data to test the assumption that habitat quality is optimal at mid elevations. Specifically, to differentiate between core and marginal populations, we test for different proxies of habitat quality (following Hoffmann and Blows 1994): the proportion of juveniles (which is expected to be higher in optimal habitats), population density, and a measure of a fitness-related trait such as body condition. Furthermore, given that the resource-allocation hypothesis implies longer lifespans in marginal habitats as a consequence of life-history trade-offs, this hypothesis also predicts greater reproductive investment (estimated as relative clutch mass) in core habitats.

Material and Methods

General procedures

The lizard *Psammodromus algirus* is a medium-large lacertid (53–80 mm snout-vent length, SVL, in our study area) that inhabits shrubby habitats in the Mediterranean region of south-west Europe and north-west Africa (Salvador 2015). The field work was performed in the Sierra Nevada mountain system (SE Spain), where *P. algirus* is found from 200 to 2600 m above sea level (hereafter, m asl) (Fernández-Cardenete et al. 2000). We sampled from six sites, at 300, 700, 1200, 1700, 2200, and 2500 m asl (Figure 1). Lizards were captured by hand during their activity season in Sierra Nevada, which spans from March to September (Zamora-Camacho et al. 2013). We assessed a total of 125 individuals over four years (sample size per year; 2010: 9, 2011: 39, 2012: 72, and 2013: 5 individuals). We tried to assess equal numbers of each sex at each elevation (samples sizes of females/males for each altitude: 300, 12/11; 700, 11/8; 1200, 10/10; 1700, 11/10; 2200, 9/10; 2500, 11/12). Males were distinguished by their wider heads, larger and more numerous femoral pores in the hind limbs, and orange spots in the corners of their mouths (Carretero 2002; Iraeta et al. 2011). Because the lizards were part of a long-term study they were marked by toe clipping. These toe samples were used to estimate lizards' age using phalanx skeletochronology (more details below). Toe clipping is a marking method frequently used in lizards with limited impact on their welfare (Perry et al. 2011).

We measured the lizards' snout-vent length (SVL) to the nearest 1 mm with a metal ruler, and body mass to the nearest 0.01 g with a digital balance (Model Radwag WTB200). With these data, we estimated the body-condition index (BCI) as the residuals of regressing log mass on log SVL. This is a widely used index that represents the relative energy reserves of an animal (Schulte-Hostedde et al. 2005). Lizards often detach their tails as a defensive mechanism. The lack of a complete tail could affect BCI calculation. However, a multiple regression with individuals possessing a full tail showed that most of the body mass was explained by SVL (partial correlation $r = 0.85$, $r^2 = 0.72$, $P < 0.001$), tail length having a non-significant effect on body mass (partial correlation $r = 0.11$, $r^2 = 0.01$, $P = 0.32$). Therefore, the presence of individuals with a partial tail should have a negligible effect on the BCI estimation.

In 2010, to quantify lizard relative abundance, we sampled 500-m transects every two weeks at each sampling site during the annual activity season. Censuses were repeated every two hours from sunrise until sunset. We recorded the number of active adults and juveniles seen in each transect. Juveniles were discriminated from adults based on body size and colouration, especially the tail (redder in juveniles). We assumed lizard detectability to be the same at all sampling stations and that the number of active individuals counted by this procedure correlated positively with the real population density (Blomberg and Shine 1996). From these transects, we estimated relative abundance at each site as the mean number of adults detected. We also estimated the percentage of juveniles and took it as a birth rate indicator for the population.

A subset of gravid females ($n = 102$) not included in previous analyses was used to estimate reproductive investment along the elevational gradient. We recognised gravid females by manual palpation of developing eggs. Gravid females were transported to a lab and placed in individual terrariums (100 × 20 × 40 cm) with water (in form of aqueous nutritious gel) and food (*Tenebrio molitor* larvae) *ad libitum*, indirect access to sunlight, and a heat cable at one end of the cage, switched on three hours/day (11 h – 14 h) to allow thermoregulation. The substrate was bare soil from the study area. When females laid eggs, we recorded clutch mass and estimated relative clutch mass, an indicator of their reproductive investment (Shine 1980), as a percentage of female body mass. Females and their offspring were released at the point where the female had been caught. No lizard died or suffered permanent injury in this study.

Skeletochronology age estimation

The age of the lizards was determined by phalanx skeletochronology (Comas et al. 2016), which is one of the most accurate age estimation techniques in animals (Zhao et al. 2019). Ectotherms with indeterminate growth may present a cyclic growth pattern in hard body structures, corresponding to alternate periods of growth and resting. Therefore, age can be estimated by examining cyclic growth patterns in bones (Figure 2). Phalanx skeletochronology provides an age estimation by counting annual growth rings in the phalanges (Comas et al. 2016). One toe of each lizard was clipped and preserved in ethanol 70%, after which the wound was properly disinfected with chlorohexidine. The toes were decalcified in 3% nitric acid for 3.5 h. Cross-sections (10 µm) were prepared using a freezing microtome (CM1850 Leica) at the Centre of Scientific Instrumentation, University of Granada. Cross-sections were stained with Harris hematoxylin for 20 min, dehydrated through an alcohol chain (70%, 96%, 100%; 5 min each), and washed in xylol for 15 min. They were then fixed with DPX (histology mounting medium), mounted on slides, and examined for the presence of LAGs (line of arrested growth) using a light microscope (Leitz Dialux 20, Leica Microsystems, Wetzlar, Germany) at 400× magnification. We took 10-20 photographs (with a ProgresC3 camera) of several representative cross-sections for each individual, discarding any cuts with unclear LAGs. We selected diaphysea sections where the size of the medullar cavity was at its minimum and that of the periosteal bone at its maximum (Comas et al. 2016). The number of LAGs detected in the periosteal bone was counted on three separate occasions by the same person (MC) while blinded to the specimen identification. Each LAG may approach one year of life, so the number of LAGs indicates the lizard's approximate age with an accuracy of ± 1 year (Figure 2).

We used the skeletochronological data to estimate adult lifespan, that is, the expected average longevity of individuals that had reached maturity, by means of Seber's (1973) formula: Lifespan = 0.5 + 1/(1 – S), where S is the survival rate. Survival rate was calculated according to Robson and Chapman's (1961) formula: S = T/(R + T – 1), where S is the finite annual survival rate estimate, $T = N_1 + 2N_2 + 3N_3 + 4N_4$ and so on to complete age classes, R is $\sum N_i$, and N_i is the number of individuals in the age class i.

Statistical analyses

A Chi-Square test was used to test for any differences in age structure between sexes or elevations. Since there were no 5-year-old males, we used four age categories to avoid creating cells with a value of 0: 1 year, 2 years, 3 years and >3 years (4 and 5 years together). To examine simultaneously the effect of sex and altitude on age, we used two approximations. On one hand, we tested whether the lizards' average age varied with elevation and sex by using an ANOVA, taking altitude (six levels, corresponding to the six sites sampled), sex (two levels) and interaction as factors. We also employed a multinomial model with age (four levels) as the dependent variable, and altitude (six levels), sex (two levels) and their interaction as predictors. To consider possible cohort effects, we repeated the previous analyses including the year of capture (2011 and 2012) as a factor (years 2010 and 2013 were not included in this analysis because of the small sample size). ANOVA were also used to test for elevational variation in relative abundance, percentage of juveniles, BCI, and relative clutch mass. In these analyses, percentage of juveniles and relative clutch mass were arcsine-transformed (Quinn and Keough 2002). Data were checked for outliers, normality and homoscedasticity following Zuur et al. (2010).

Results

The age structure of the lizards did not differ between sexes ($\chi^2_3 = 1.47$, $P = 0.69$; Figure 3A; sample sizes given in the figure). Nevertheless, females had a maximum lifespan of 5 years and males of 4 years. The lizards showed a similar frequency of individuals aged 1, 2 and 3 years, but there was a decrease of almost 50% in the number of lizards reaching the age of 4 years. The age frequency distribution varied significantly in function of altitude ($\chi^2_{15} = 36.58$, $P = 0.001$; Figure 3B; sample sizes given in the figure). The frequency of individuals aged ≥ 4 years was lower at medium elevations than at low and high ones (only 1 of the 41 individuals at mid altitudes > 3 years old, versus 7/42 at low and 6/42 at high elevations). The annual survival rate was about 0.70 at each elevation, except at 1200 m where the rate was 0.60. Similarly, the lifespan was approximately 4 years at each elevation, except at 1200 m where it was only 3 years (Figure 4).

The lizards' mean age varied with elevation ($F_{5,\ 113} = 5.89$, $P < 0.001$; $n = 125$), following a U-shaped pattern (Figure 5). Mean age did not differ with sex ($F_{1,\ 113} = 0.99$, $P = 0.32$). However, the pattern with altitude differed slightly between sexes, males having a higher average age than females at 300 m, while females were older than males at all other elevations (interaction sex*altitude, $F_{5,\ 113} = 3.55$, $P = 0.005$; Figure 5). The multinomial model gave similar results, with a significant effect of elevation ($\chi^2_{15} = 40.12$, p = 0.0004) and the interaction sex*altitude ($\chi^2_9 = 21.03$, $P = 0.01$) on lizard age, but no effect of sex ($\chi^2_3 = 1.81$, $P = 0.61$). When the analyses were repeated including year of capture as a factor, the results were qualitatively the same (data not shown for simplicity), with no significant effect of year or the interactions year*altitude, year*sex, and triple interaction. There were no differences in age structure with elevation between 2011 and 2012 ($\chi^2_5 = 1.18$, $P = 0.95$).

The relative abundance of adult lizards varied significantly with elevation ($F_{5,\ 49} = 5.09$, $P < 0.001$, $n = 55$ samplings), presenting a maximum at mid elevations (1200 and 1700 m; Figure 6). The percentage of juveniles detected in transects ranged between 43.4% at 2500 m and 74.3% at 2200 m, but did not differ significantly between elevations ($F_{5,\ 34} = 0.78$, $P = 0.57$, $n = 40$ samplings in which at least one juvenile was detected; Figure 6). Meanwhile, body condition was minimal at 700 m, and improved with elevation ($F_{5,\ 119} = 3.26$, $P = 0.0085$, $n = 125$; Figure 7). The reproductive investment registered minimal values at low elevations and followed a tendency to increase with elevation ($F_{5,\ 96} = 2.43$, $P = 0.04$, $n = 102$ gravid females; Figure 8).

Discussion

Our findings (summarised in Table 2) show that the age structure of the lizard *Psammodromus algirus* in the Sierra Nevada mountain changes with altitude following a curvilinear pattern: populations at low and high elevations (presumed to be marginal habitats) harbour older individuals in comparison with populations at mid elevations. We discarded a cohort effect because the elevational age structure did not vary with sampling year and the altitudinal effect remained after controlling for year of capture. The findings cannot be easily explained through either nonlinear clines in predators or parasites in our study system. Although we have no accurate data on predator pressure, anecdotal observations during fieldwork suggest that predator abundance is lower at high elevations (see also Fox et al. 1994; Camacho and Avilés 2019). Meanwhile parasites showed a complex pattern: the prevalence of ectoparasites (mites) decreased linearly with ascending elevation, while haemoparasites increased linearly (Álvarez-Ruiz et al. 2018). Consequently, the altitudinal pattern observed in age structure initially appears to support the resource-allocation hypothesis (see Table 1), which postulates that lizards should have a faster pace of life, invest less in self-preservation and therefore present shorter lifespans in core habitats where reproduction is favoured. However, the resource-allocation hypothesis relies on the assumption of higher reproductive investment at mid elevations, but our data did not support this prediction, given that reproductive investment, measured as relative clutch mass, tended to increase with altitude. In fact, the resource-allocation hypothesis is based on the life-history theory, which predicts an inverse relationship (i.e., a trade-off) between lifespan and reproductive investment (Stearns 1992; Roff 2002). Although such a trade-off has been evidenced in reptiles in general (Scharf et al. 2015), it is unclear whether it applies to lacertids (Bauwens and Díaz-Uriarte 1997). Therefore, the observed pattern seems more complex than predicted by the competitive hypotheses presented in the Introduction, and the longer lifespan in highland and lowland lizards could be due to different ecological processes.

An initial question is why *P. algirus* lizards live longer at high elevations than at intermediate elevations. We assumed that alpine zones constitute marginal habitats because lizard density and thermal quality were lower than those at the middle elevations (Zamora-Camacho et al. 2013, 2016). Moreover, *P. algirus* is a lacertid typical of North Africa and Mediterranean environments in the Iberian Peninsula (Carranza et al. 2006), and thus, a priori, it is presumed to be poorly adapted to alpine habitats (see Monasterio et al. 2011). However, while data presented in the current study support that lizard densities are greater at middle rather than high elevations, other proxies of habitat quality show a more complex picture: the proportion of juvenile lizards, as a measurement of population growth, did not differ across elevations, while body condition increased with altitude. This calls into question the assumption that alpine habitats are suboptimal for *P. algirus*. In our study population, alpine lizards show a number of phenotypic traits, well differentiated from lizards at middle and low altitudes, such as a darker colouration and larger body size, which appear to be adaptations to cope with alpine habitats by improving thermoregulation in cold environments (Reguera et al. 2014b; Zamora-Camacho et al. 2014). These adaptations may mean this lizard is locally adapted to alpine zones in Sierra Nevada, thus the alpine zone may not be a suboptimal habitat. Furthermore, food availability is greater at higher elevations in our study system (Moreno-Rueda et al. 2018). So, the increased food availability in the alpine zone (Moreno-Rueda et al. 2018), in combination with low oxidative stress (Reguera et al. 2014a, 2015) and activity time (Zamora-Camacho et al. 2013), implies highland lizards could invest more in reproduction without a cost in the form of reduced lifespan.

By contrast, our findings support the idea that lowlands harbour suboptimal habitat for the lizard *P. algirus*. In lowlands, thermal quality and food availability were the lowest, while oxidative stress was maximal (Table 2). Consequently, lizard density, body condition and reproductive investment were the lowest (Table 2). In fact, in Mediterranean environments, lowlands show low precipitation and high temperatures during summer, which can be very restrictive for lizards. Indeed, several studies in Mediterranean areas report a lower food availability and growth rate in lowland habitats than those at 1200-1800 m, which is the midland range in our study area (Iraeta et al. 2006; Ortega et al. 2015, 2017). However, in spite of the harmful environmental conditions and the long activity time (Zamora-Camacho et al. 2013), lifespan was not the shortest in the lowlands. A possible explanation is that the lower food availability would lead to poor body condition and so to low reproductive investment (see Bronikowski and Arnold 1999), thereby lengthening lifespan and balancing the negative impact derived from high activity time and oxidative damage (Figure 9).

Alternatively, the altitudinal pattern found for age structure could be a consequence of elevational variation in intraspecific competition. Intraspecific competition may be an important selective agent (Calsbeek and Cox 2010), and strong intraspecific competition may reduce survival (Balbontín and Møller 2015), at least under certain circumstances. In fact, *P. algirus* is a strongly territorial lizard in which aggressions are frequent (Civantos 2000). Effectively, when density is high, aggressions between lizards may affect their survival (Le Galliard et al. 2005). Consistent with this idea, *P. algirus* lifespan and survival were the lowest at mid elevations, where abundance was the highest.

Our results also reveal that the lizards had similar survival rates until they were 3 years old and then survival declined sharply. That is, few lizards reached 4 years old and only three females attained the maximum lifespan of 5 years in our study area. The peak of mortality after 3 years may be a consequence of senescence. Senescence implies a deterioration of physiological conditions in older individuals, resulting in greater mortality (Massot et al. 2011). In fact, senescence often implies a deterioration of the immune system (Zamora-Camacho and Comas 2018), which also leads to a higher mortality due to pathogens and parasites. However, senescence is strongly determined by telomere length (Haussmann and Marchetto 2010) and, in our study population, telomeres lengthen up to 4 years old, and then shorten (Burraco et al. 2019). Therefore, it is still unclear why mortality sharply increases when lizards are 4 years old.

In conclusion, our findings contrast with most of those published to date on lizards (and ectotherms in general), which typically report greater longevity at higher altitudes. As summarised in the Figure 9, several factors may affect lifespan in complex ways. Consequently, a pattern of enhanced longevity with altitude is not universal and our study highlights some causes that could be responsible for exceptions to the rule.

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References

Adolph SC, Porter WP, 1993. Temperature, activity, and lizard life histories. *Am Nat* **142**:273–295.

Alford JG, Lutterschmidt WI, 2012. Modeling energetic and theoretical costs of thermoregulatory strategy. *J Biol Dyn* **6**:63–79.

Álvarez-Ruiz L, Megía-Palma R, Reguera S, Ruiz S, Zamora-Camacho FJ, Figuerola J, Moreno-Rueda G, 2018. Opposed elevational variation in prevalence and intensity of endoparasites and their vectors in a lizard. *Curr Zool* **64**:197–204.

Angilletta MJ, 2009. *Thermal adaptation: a theoretical and empirical synthesis*. Oxford: Oxford University Press.

Araya-Ajoy YG, Bolstad GH, Brommer J, Careau V, Dingemanse NJ, Wright J, 2018. Demographic measures of an individual's "pace of life": fecundity rate, lifespan, generation time, or a composite variable? *Behav Ecol Sociobiol* **72**:20132645.

Balbontín J, Møller AP, 2015. Environmental conditions during early life accelerate the rate of senescence in a short-lived passerine bird. *Ecology* **96**:948–959.

Ballinger RE, 1979. Intraspecific variation in demography and life history of the lizard, *Sceloporus Jarrovi*, along an altitudinal gradient in Southeastern Arizona. *Ecology* **60**:901–909.

Barry RG, 2008. *Mountain weather and climate*. New York: Cambridge University Press.

Bauwens D, Díaz-Uriarte R, 1997. Covariation of life-history traits in lacertid lizards: A comparative study. *Am Nat* **149**:91–111.

Bestion E, Teyssier A, Richard M, Clobert J, Cote J, 2015. Live fast, die young: Experimental evidence of population extinction risk due to climate change. *PLoS Biol* **13**:e1002281.

Blomberg S, Shine R, 1996. Reptiles. In: Sutherland WJ, editor. *Ecological census techniques, a handbook*. Cambridge: Cambridge University Press, 218–226.

Boyle WA, Sandercock BK, Martin K, 2016. Patterns and drivers of intraspecific variation in avian life history along elevational gradients: a meta-analysis. *Biol Rev* **91**:469–482.

Bronikowski AM, Arnold SJ, 1999. The evolutionary ecology of life histories variation in the garter snake *Thammophis elegans*. *Ecology* **80**:2314–2325.

Burraco P, Comas M, Reguera S, Zamora-Camacho FJ, Moreno-Rueda G. 2019. Telomere length covaries with age across an elevational gradient in a Mediterranean lizard. *bioRxiv:* 732727. doi: https://doi.org/10.1101/732727

Cabezas-Cartes F, Boretto JM, Ibargüengoytía NR, 2018. Effects of climate and latitude on age at maturity and longevity of lizards studied by skeletochronology. *Integr Comp Biol* **58**:1086–1097.

Calsbeek R, Cox RM, 2010. Experimentally assessing the relative importance of predation and competition as agents of selection. *Nature* **465**:613–616.

Camacho L, Avilés L, 2019. Decreasing predator density and activity explain declining predation of insect prey along elevational gradients. *Am Nat* **194**:334–343.

Carothers JH, Jaksić FM, Marquet PA, 2001. Altitudinal zonation among lizards of the genus *Liolaemus*: questions answered and unanswered questions. *Rev Chil Hist Nat* **74**:313–316.

Carranza S, Harris DJ, Arnold EN, Batista V, Gonzalez De La Vega JP, 2006. Phylogeography of the lacertid lizard, *Psammodromus algirus*, in Iberia and across the Strait of Gibraltar. *J Biogeogr* **33**:1279–1288.

Carretero MA, 2002. Sources of colour pattern variation in Mediterranean *Psammodromus algirus*. *Neth J Zool* **52**:43–60.

Chase JM, Leibold MA, 2003. *Ecological niches: Linking classical and contemporary approaches*. Chicago: University of Chigado Press.

Cichoń M, 1997. Evolution of longevity through optimal resource allocation. *Proc R Soc B* **264**:1383–1388.

Civantos E, 2000. Home-range ecology, aggressive behaviour, and survival in juvenile lizards, *Psammodromus algirus*. *Can J Zool* **78**:1681–1685.

Civantos E, Forsman A, 2000. Determinants of survival in juvenile *Psammodromus algirus* lizards. *Oecologia* **124**:64–72.

Comas M, Escoriza D, Moreno-Rueda G, 2014. Stable isotope analysis reveals variation in trophic niche depending on altitude in an endemic alpine gecko. *Basic Appl Ecol* **15**:362–369.

Comas M, Reguera S, Zamora-Camacho FJ, Salvadó H, Moreno-Rueda G, 2016. Comparison of the effectiveness of phalanges vs. humeri and femurs to estimate lizard age with skeletochronology. *Anim Biodiv Conserv* **39**:237–240.

Cvetković D, Tomašević N, Ficetola GF, Crnobrnja-Isailović J, Miaud C, 2009. Bergmann's rule in amphibians: combining demographic and ecological parameters to explain body size variation among populations in the common toad *Bufo bufo*. *J Zool Syst Evol Res* **47**:171–180.

Dunham AE, 1980. An experimental study of interspecific competition between the Iguanid lizards *Sceloporus merriami* and *Urosaurus ornatus*. *Ecol Monogr* **50**:309–330.

Fernández-Cardenete JR, Luzón-Ortega JM, Pérez-Contreras J, Tierno de Figueroa JM, 2000. Revisión de la distribución y conservación de los anfibios y reptiles en la provincia de Granada (España). *Zool Baet* **11**:77–104.

Fox SF, Perea-Fox S, Castro Franco R, 1994. Development of the tail autotomy adaptation in lizards under disparate levels of predation at high and low elevations in Mexico. *Southwest Nat* **39**:311–322.

Fu C, Wang J, Pu Z, Zhang S, Chen H, Zhao B, Chen J, Wu J, 2007. Elevational gradients of diversity for lizards and snakes in the Hengduan Mountains, China. *Biodivers Conserv* **16**:707–726.

Haussmann MF, Marchetto NM, 2010. Telomeres: Linking stress and survival, ecology and evolution. *Curr Zool* **56**:714–727.

Hoffmann AA, Blows MW, 1994. Species borders: ecological and evolutionary perspectives. *Trends Ecol Evol* **9**:223–227.

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

Iraeta P, Monasterio C, Salvador A, Díaz JA, 2011. Sexual dimorphism and interpopulation differences in lizard hind limb length:Locomotor performance or chemical signalling? *Biol J Linn Soc* **104**:318–329.

Iraeta P, Salvador A, Díaz JA, 2015. A reciprocal transplant study of activity, body size, and winter survivorship in juvenile lizards from two sites at different altitude. *Écoscience* **15**:298–304.

Kawecki TJ, 2008. Adaptation to marginal habitats. *Annu Rev Ecol Evol Syst* **39**:321–342.

Kirkwood TBL, Rose MR, 1991. Evolution of senescence: late survival sacrificed for reproduction. *Phil Trans R Soc B* **332**:15–24.

Körner C, 2007. The use of "altitude" in ecological research. *Trends Ecol Evol* **22**:569–574.

Le Galliard J-F, Fitze PS, Ferrière R, Clobert J, 2005. Sex ratio bias, male aggression, and population collapse in lizards. *Proc Natl Acad Sci U S A* **102**:18231–18236.

Liao WB, Luo Y, Lou SL, Di Lu, Jehle R, 2016. Geographic variation in life-history traits: Growth season affects age structure, egg size and clutch size in Andrew's toad (*Bufo andrewsi*). *Front Zool* **13**:6.

Massot M, Clobert J, Montes-Poloni L, Haussy C, Cubo J, Meylan S, 2011. An integrative study of ageing in a wild population of common lizards. *Funct Ecol* **25**:848–858.

Metcalf CJE, Pavard S, 2007. Why evolutionary biologists should be demographers. *Trends Ecol Evol* **22**:205–212.

Monasterio C, Shoo LP, Salvador A, Siliceo I, Díaz JA, 2011. Thermal constraints on embryonic development as a proximate cause for elevational range limits in two Mediterranean lacertid lizards. *Ecography* **34**:1030–1039.

Moreno-Rueda G, Melero E, Reguera S, Zamora-Camacho FJ, Álvarez-Benito I, 2018. Prey availability, prey selection, and trophic niche width in the lizard *Psammodromus algirus* along an elevational gradient. *Curr Zool* **64**:603–613.

Morrison C, Hero J-M, 2003. Geographic variation in life-history characteristics of amphibians: a review. *J Anim Ecol* **72**:270–279.

Munch SB, Salinas S, 2009. Latitudinal variation in lifespan within species is explained by the metabolic theory of ecology. *Proc Natl Acad Sci USA* **106**:13860–13864.

Navas CA, 2002. Herpetological diversity along Andean elevational gradients: Links with physiological ecology and evolutionary physiology. *Comp Biochem Physiol A* **133**:469–485.

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

Ortega J, López P, Martín J, 2017. Environmental drivers of growth rates in Guadarrama wall lizards: A reciprocal transplant experiment. *Biol J Linn Soc* **122**:340–350.

Perry G, Wallace MC, Perry D, Curzer H, Muhlberger P, 2011. Toe clipping of amphibians and reptiles: Science, ethics, and the law. *J Herpetol* **45**:547–555.

Pironon S, Papuga G, Villellas J, Angert AL, García MB, Thompson JD, 2017. Geographic variation in genetic and demographic performance: New insights from an old biogeographical paradigm. *Biol Rev* **92**:1877–1909.

Quinn GP, Keough MJ, 2002. Experimental design and data analysis for biologists. Cambridge: Cambridge University Press.

Reguera S, Zamora-Camacho FJ, Trenzado CE, Sanz A, Moreno-Rueda G, 2014a. Oxidative stress decreases with elevation in the lizard *Psammodromus algirus*. *Comp Biochem Physiol A* **172**:52–56.

Reguera S, Zamora-Camacho FJ, Moreno-Rueda G, 2014b. The lizard *Psammodromus algirus* (Squamata: Lacertidae) is darker at high altitudes. *Biol J Linn Soc* **112**:132–141.

Reguera S, Zamora-Camacho FJ, Melero E, García-Mesa S, Trenzado CE, Cabrerizo MJ, Sanz A, Moreno-Rueda G, 2015. Ultraviolet radiation does not increase oxidative stress in the lizard *Psammodromus algirus* along an elevational gradient. *Comp Biochem Physiol A* **183**:20–26.

Reniers J, Brendonck L, Roberts JD, Verlinden W, Vanschoenwinkel B, 2015. Environmental harshness shapes life-history variation in an Australian temporary pool breeding frog: A skeletochronological approach. *Oecologia* **178**:931–941.

Robson DS, Chapman DG, 1961. Catch curves and mortality rates. *Trans Am Fish Soc* **90**:181–189.

Roff DA, 2002. *Life history evolution*. Sunderland: Sinauer Associates.

Sagarin RD, Gaines SD, Gaylord B, 2006. Moving beyond assumptions to understand abundance distributions across the ranges of species. *Trends Ecol Evol* **21**:524–530.

Salvador A, 2015. Lagartija colilarga – *Psammodromus algirus* (Linnaeus, 1758). In: Salvador A, Marco A, editors. *Enciclopedia Virtual de los Vertebrados Españoles*. Madrid: Museo Nacional de Ciencias Naturales.

Scharf I, Feldman A, Novosolov M, Pincheira-Donoso D, Das I, Böhm M, Uetz P, Torres-Carvajal O, Bauer AM, Roll U, Meiri S, 2015. Late bloomers and baby boomers: Ecological drivers of longevity in squamates and the tuatara. *Global Ecol Biogeogr* **24**:396–405.

Schulte-Hostedde AI, Zinner B, Millar JS, Hickling GJ, 2005. Restitution on mass-size residuals: Validating body condition indices. *Ecology* **86**:155–163.

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

Seber GAF, 1973. *The estimation of animal abundance and related parameters*. London: Griffin.

Shine R, 1980. "Costs" of reproduction in reptiles. *Oecologia* **46**:92–100.

Speakman JR, 2005. Body size, energy metabolism and lifespan. *J Exp Biol* **208**:1717–1730.

Stark G, Tamar K, Itescu Y, Feldman A, Meiri S, 2018. Cold and isolated ectotherms: drivers of reptilian longevity. *Biol J Linn Soc* **125**:730–740.

Stearns SC, 1992. The evolution of life-histories. Oxford: Oxford University Press.

Valcu M, Dale J, Griesser M, Nakagawa S, Kempenaers B, 2014. Global gradients of avian longevity support the classic evolutionary theory of ageing. *Ecography* **37**:930–938.

Zamora-Camacho FJ, Comas M, 2018. Early swelling response to phytohemagglutinin is lower in older toads. *PeerJ* **6**:e6104.

Zamora-Camacho FJ, Reguera S, Moreno-Rueda G, Pleguezuelos JM, 2013. Patterns of seasonal activity in a Mediterranean lizard along a 2200m altitudinal gradient. *J Thermal Biol* **38**:64–69.

Zamora-Camacho FJ, Reguera S, Moreno-Rueda G, 2014. Bergmann's Rule rules body size in an ectotherm: heat conservation in a lizard along a 2200-metre elevational gradient. *J Evol Biol* **27**:2820–2828.

Zamora-Camacho FJ, Reguera S, Moreno-Rueda G, 2016. Thermoregulation in the lizard *Psammodromus algirus* along a 2200-m elevational gradient in Sierra Nevada (Spain). *Int J Biometeorol* **60**:687–697.

Zhang L, Lu X, 2012. Amphibians live longer at higher altitudes but not at higher latitudes. *Biol J Linn Soc* **106**:623–632.

Zhao M, Klaassen CAJ, Lisovski S, Klaassen M, 2019. The adequacy of aging techniques in vertebrates for rapid estimation of population mortality rates from age distributions. *Ecol Evol* **9**:1394–1402.

Zuur AF, Ieno EN, Elphick CS, 2010. A protocol for data exploration to avoid common statistical problems. *Methods Ecol Evol* **1**:3–14.

Table 1. Summary of the hypotheses used to explain altitudinal lifespan patterns in lizards with an explanation of each hypothesis and its prediction for our study system.

Hypotheses	*Assumptions and Predictions*	*Altitudinal pattern predicted*
The central-marginal hypothesis	Adult survival decreases with decreased habitat quality	∩-shaped pattern
The resource-allocation hypothesis	Animals are selected for greater investment in reproduction in core habitats, allocating fewer resources to self-preservation and thereby reducing lifespan	U-shaped pattern
The rate-of-living hypothesis	In cold environments, the length of activity seasons for ectotherms is shorter and reduced activity implies lower metabolic damage, thereby increasing survival and lifespan	Linear increase
The activity-exposition hypothesis	In cold environments, the length of activity seasons for ectotherms is shorter and reduced activity implies lower exposure to predators, which increases survival	Linear increase

Table 2. Summary of results describing altitudinal pattern in the present study and in previous studies performed in the same study system.

Variable	Altitudinal pattern	Reference
This study		
Mean age	U-shaped pattern	
Annual survival	U-shaped pattern	
Lifespan	U-shaped pattern	
Lizard abundance	∩-shaped pattern	
% of juveniles	No altitudinal pattern	
Body condition	Increased with altitude	
Reproductive investment	Increased with altitude	
Previous studies		
Thermal quality	∩-shaped pattern	Zamora-Camacho et al. 2016
Lizard abundance	∩-shaped pattern	Zamora-Camacho et al. 2013
Activity season length	Decreases with altitude	Zamora-Camacho et al. 2013
Oxidative stress	Decreases with altitude	Reguera et al. 2014a, 2015
Ectoparasites (mites)	Decreases with altitude	Álvarez-Ruiz et al. 2018
Haemoparasites	Increases with altitude	Álvarez-Ruiz et al. 2018
Dorsal coloration	Darker with altitude	Reguera et al. 2014b
Body size	Increases with altitude	Zamora-Camacho et al. 2014
Food availability	Increases with altitude	Moreno-Rueda et al. 2018

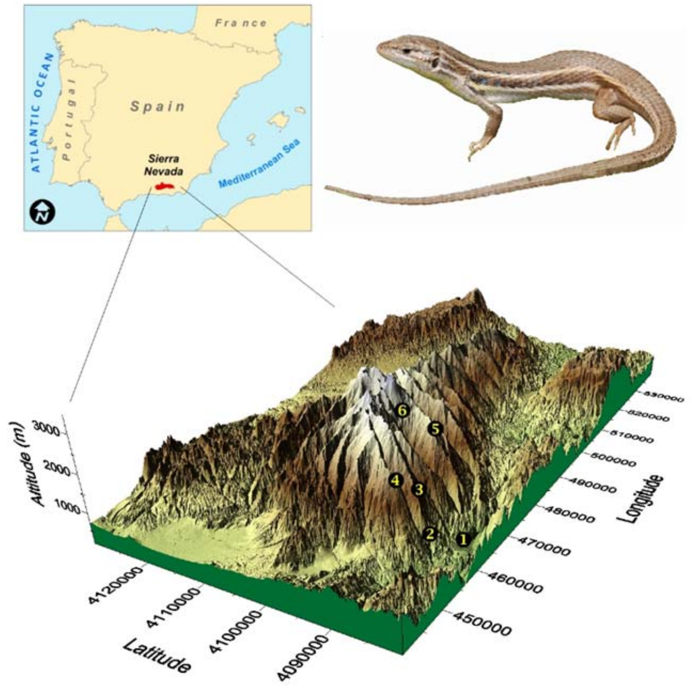


Figure 1. Location of the Sierra Nevada mountain range in the Iberian Peninsula (top, left panel) and a three-dimensional map of Sierra Nevada (lower panel), showing the location of the sampling sites (1 to 6 correspond to the sites at 300, 700, 1200, 1700, 2200, and 2500 m asl, respectively). An image of the lizard *Psammodromus algirus* appears in the top right panel.

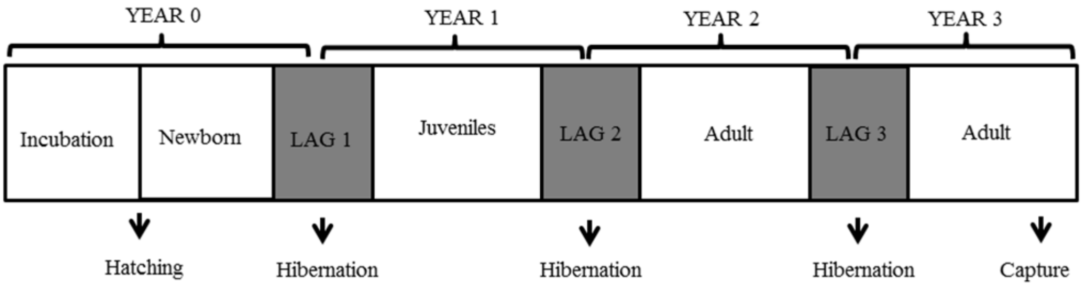
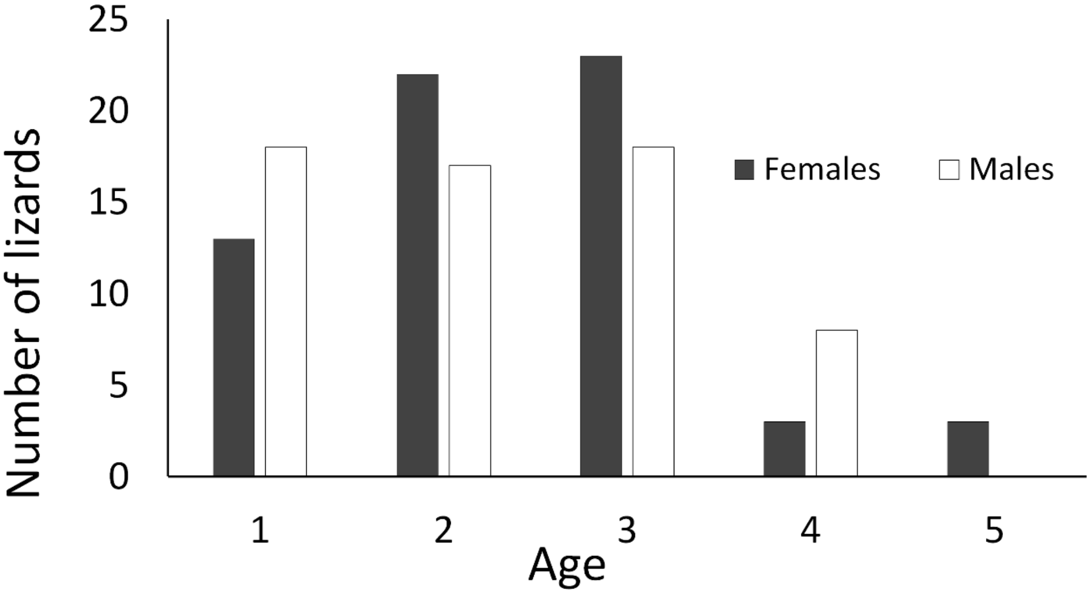


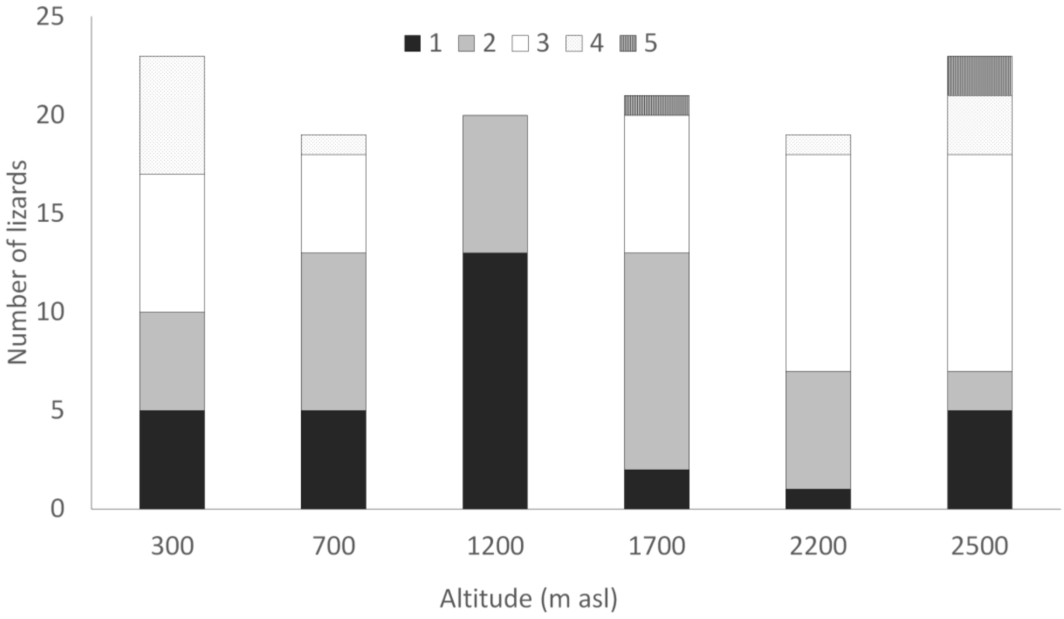
Figure 2. Life cycle of the lizard *Psammodromus algirus* with the example of a lizard estimated to be 3 years old.

Figure 3. (A) Frequency (number of lizards) of female (black bars) and male (white bars) lizards according to estimated age. Age structure is similar between sexes, but only females reached the age of 5. (B) Frequency of lizards at each age class (black: 1 year; grey: 2 years; white: 3 years; dotted: 4 years; hatched: 5 years) according to elevation.

(A)



(B)



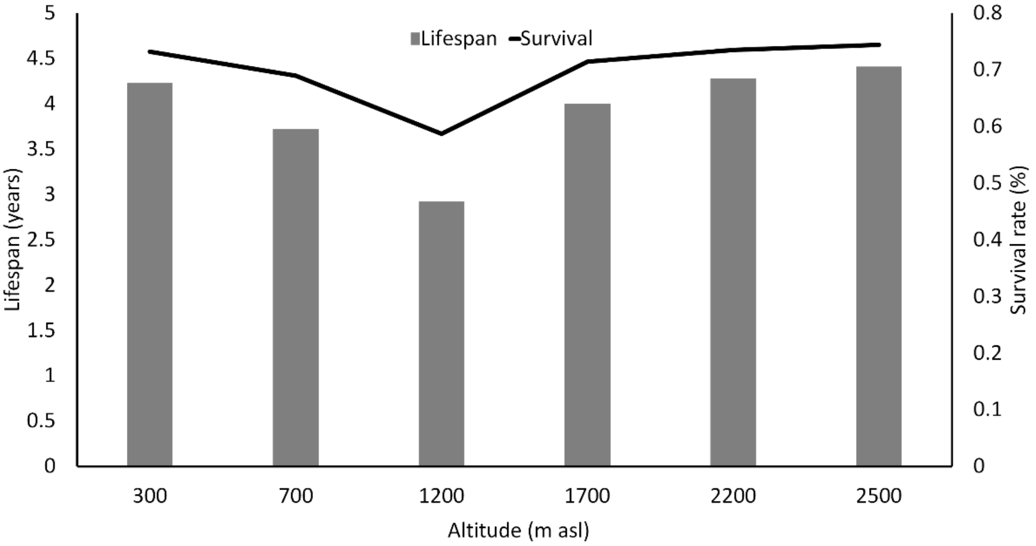


Figure 4. Estimated lifespan (grey bars) and survival rate (black line) for *Psammodromus algirus* lizards according to elevation (m asl).

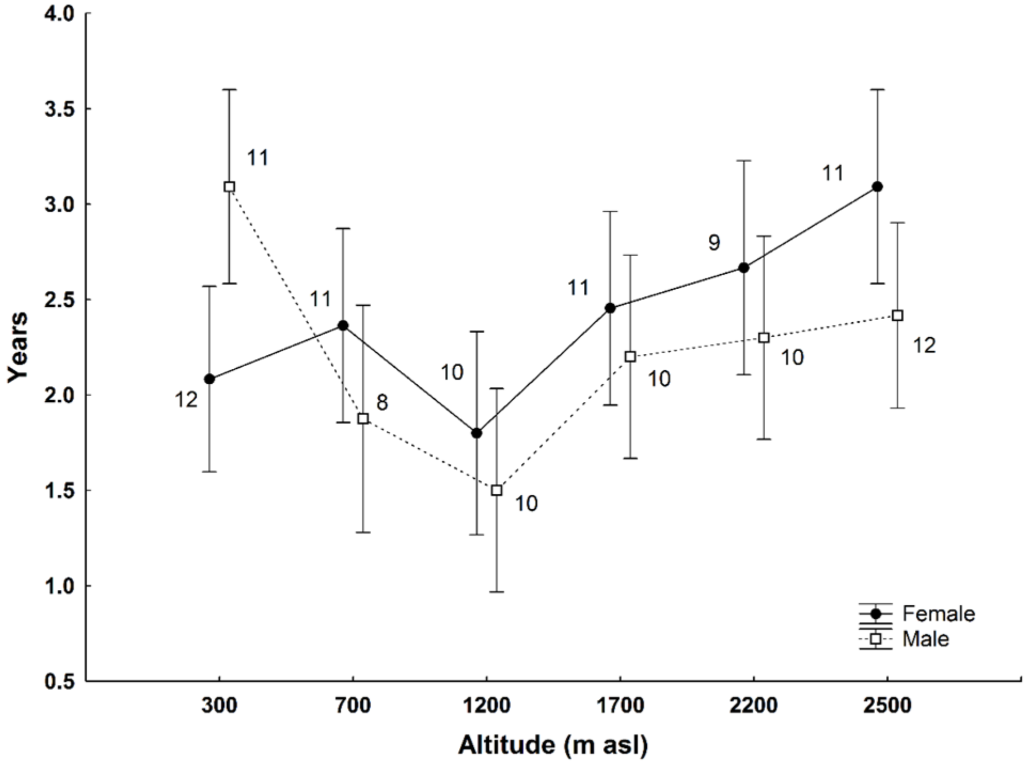


Figure 5. Average age (with 95% CI, vertical bars) of female (black dots, solid line) and male (white squares, dashed line) lizards depending on altitude. Sample size for each category is indicated on the graph, close to the corresponding data point.

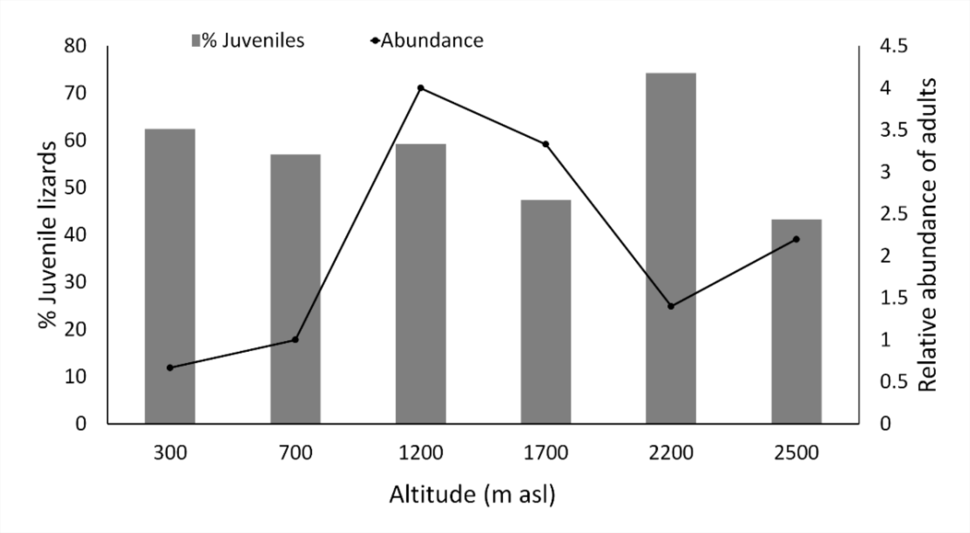


Figure 6. Maximal abundance values registered in transects for adult lizards (black line) and percentage of juvenile lizards (grey bars) depending on altitude.

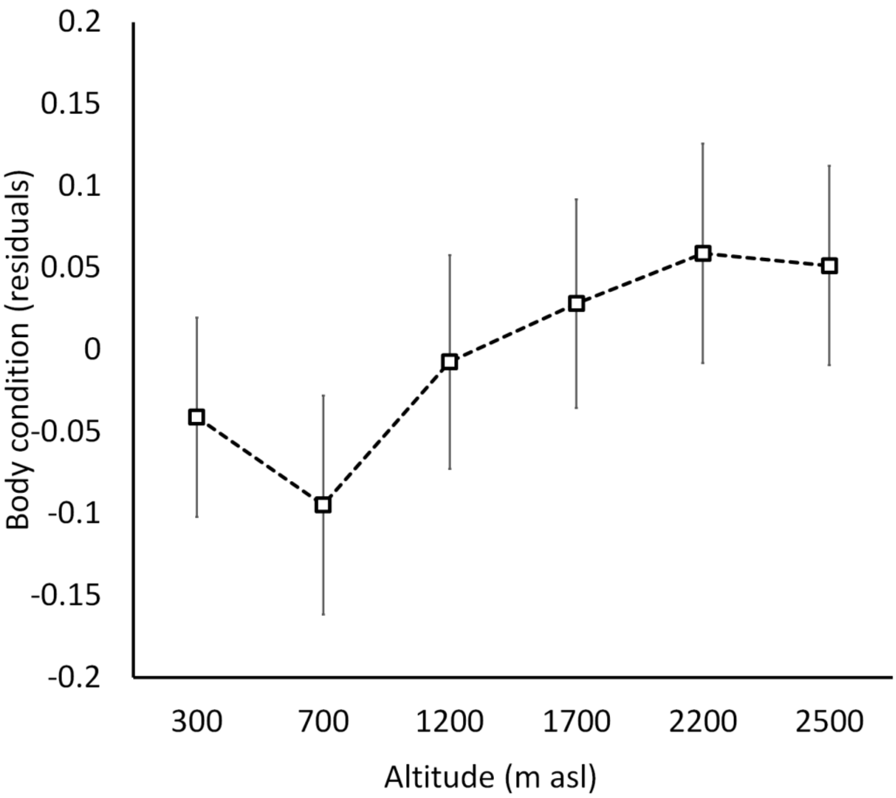


Fig 7. Average body condition (residuals of the body mass regarding the SVL, both log-transformed) with 95% CI (bars) in function of altitude.

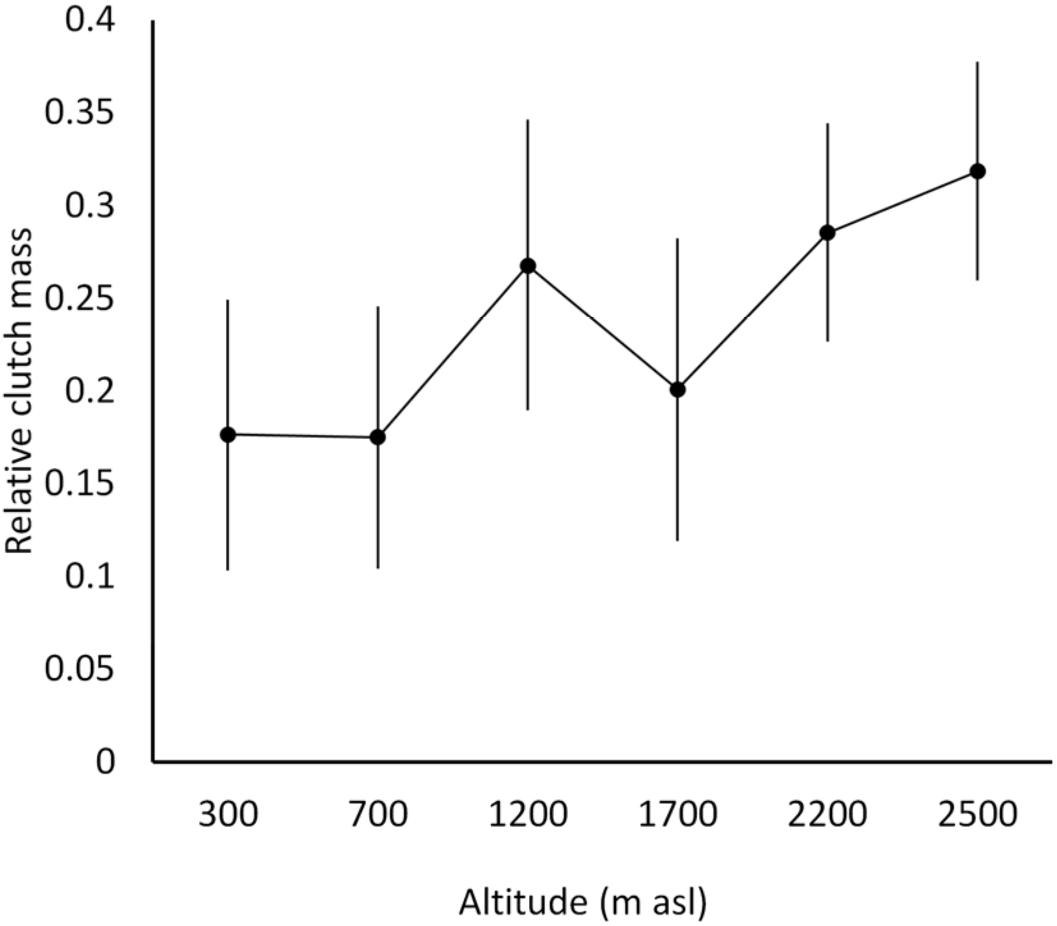


Figure 8. Average reproductive investment with 95% CI (estimated as relative investment in the clutch) depending on altitude.

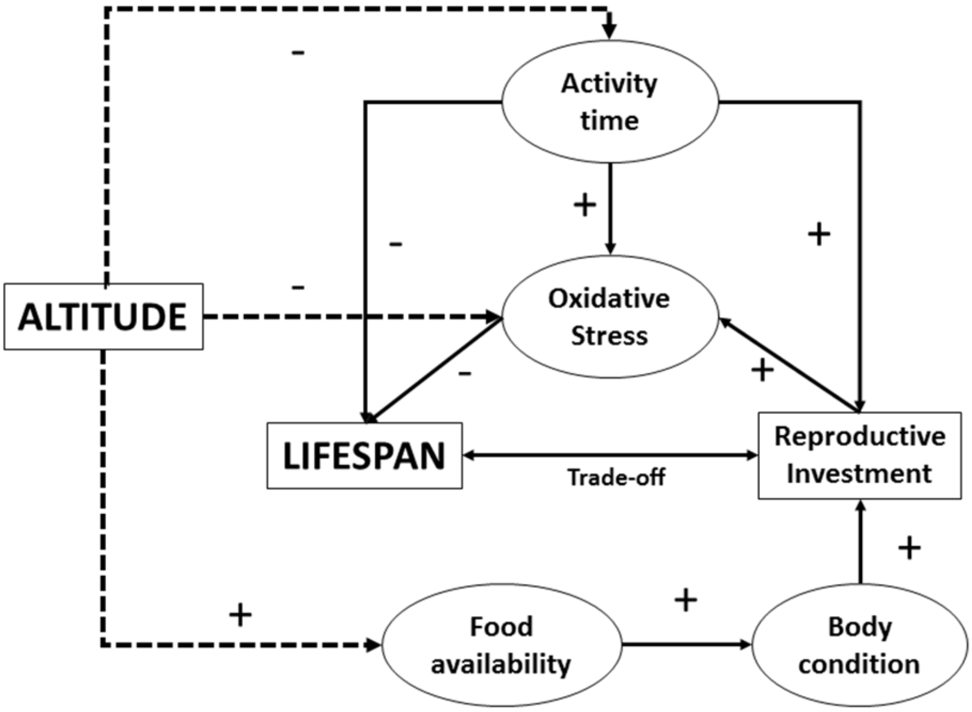


Figure 9. Flow chart of the interactions that could explain the elevational variation in lifespan of the lizard *P. algirus*. There is a trade-off between lifespan and reproductive investment. Activity time and oxidative stress decrease with altitude, whereas food availability increases. Both activity time and oxidative stress have negative effects on lifespan; activity time increases reproductive investment, which, in turn increases oxidative stress. Meanwhile, food availability improves body condition, which at the same time boosts reproductive investment.