

**Lizard ecology along altitudinal  
gradients in Mediterranean mountains**



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Amb molt d'amor, per en Grego i na Laia  
i a n'Ona, en Bru i en Roc.





**Lizard ecology along altitudinal gradients  
in Mediterranean mountains**

**Dissertation presented by Maria del Mar Comas Manresa to obtain  
the Doctor degree by the University of Granada.**

**Memoria presentada por Maria del Mar Comas Manresa para optar  
al grado de Doctora por la Universidad de Granada.**

**The doctoral student,  
La doctoranda,**

**Maria del Mar Comas Manresa  
Granada, 2020.**





El director, el Dr. Jaime Potti Sánchez, Investigador Científico de la Estación Biológica de Doñana (EBD-CSIC) y la tutora, Carmen Zamora Muñoz, Profesora Titular del Departamento de Zoología de la Universidad de Granada,

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Que los trabajos de investigación realizados en la Memoria de Tesis Doctoral: “Lizard ecology along altitudinal gradients in Mediterranean mountains”, son aptos para ser presentados por la Lda. Maria del Mar Comas Manresa ante el tribunal que en su día se designe, para aspirar al Grado de Doctora en Ciencias Biológicas por la Universidad de Granada.

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VºBº Director

VºBº Tutora

**Dr. Jaime Potti Sánchez**

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Un ofegabous *Pleurodeles waltl* mostrejat amb la companyia dels meus pares.

De veritat, algú pensa que es lleig?





La Padrina, Maria Comas i Gordi, es la persona, junt amb en Grego, que més m'ha animat i recolzat per a seguir amb la Ciència. La Padrineta es de les poques persones que em deia que em centrés en treballar a la Universitat (sense cobrar) i no perdés el temps amb altres treballs no científics, tot i que estaven relacionats amb la Biologia i inclús cobrava. No se que hagués fet sense tu durant el màster i els anys següents que vaig estar treballant a la Universitat de Barcelona sense beca, ni massa optimisme. La veritat es que vas ser un gran recolzament, em vas ajudar molt mentre feia el màster amb dos treballs de recerca alhora i mentre treballava. Tenir el dinar fet i inclús els entrepans per endur-me a la universitat van ser la diferència de dormir una hora més per dia quan no tenia temps per dormir-ne més de sis. I segueixes sent la millor Padrineta del món.

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N'Ona, amb només dos anys, llegint-li una rondalla d'una geneta *Genetta genetta* que perseguia una titina *Motacilla alba*, mentre pensava en les interaccions entre ambdues em va preguntar què érem nosaltres. Ser científica es un treball que implica aprendre sempre i fer-se bones preguntes i n'Ona sempre té bones preguntes i ja m'ha ensenyat coses: amb dos anys em va ensenyar que era un *Parasaurolophus*, un dels seus dinosaures



preferits llavors, que jo no coneixia. I només amb tres anys, ja em va parlar d'adaptació local. Avui, mentre escric aquestes línies, m'acaba d'explicar que ha atrapat una papallona, diversos escarabats i dos caragols. I a la papallona li ha donat nèctar perquè estava una mica malalta. Em recorda quan jo era nena i em feia preguntes similars mentre també mirava els animals del jardí i al pati de l'escola. Només que tu ets molt més intel·ligent, i si mai vols fer una tesi, tindràs els teus tiets protegint-te, per tenir un camí menys difícil i més curt.

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En Rai en una ocasió em va proposar de fer-me de guardaespalles. I al bosc de Białowieża no em va voler deixar cercar bitxos sola, perquè hi havia molts homes beguts vora el camí. A més, en Rai i n'Èlia aviat en presentaran en Roc, que estic segura que m'agradarà.

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Amb en Toni Aparicio també em sortit a veure ocells i em fet alguna escapada bitxera, i sempre s'aprèn alguna cosa perseguint bitxos arreu. Recordo un *Lacerta viridis* que es va quedar quiet mentre prenia el sol mig endormiscat mentre tots dos li fèiem una pila de fotos. Espero la propera escapada fer-la també amb na Cristina i en Guillem.

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Jamal Chioua ahora parece que estas más lejos, pero ha sido un honor tenerte cerca tanto tiempo cerca y espero que nos volvamos a encontrar, como mínimo cuando vuelva a muestrear geckos en el Atlas.



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I parlant de bons companys, a la EBD, n'he tingut molts. Com n'Arlo Hinckley. Les passejades per cercar amfibis per Sierra Norte han estat increïbles. Recordo una salamandra espectacular creuant un petit riuet sec mentre plovia. Espero que hi poguem tornar! ¡Y con Alicia!



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In the Konrad Lorenz Institute, I passed three months in the middle of a forest, with *Zamenis longissimus* near the door and the lab at less than a minute from home there! It was a great experience, especially because of



people as Dustin Penn. Dustin is one of the brightest scientists that I know, and lovely at the same time. Like Sarah, it was really great having both you there. Moreover, I had the chance to have a very good lab teacher like Steve Smith. In the Konrad Lorenz, I had also very good partners, like Miro, Sonia, and Maria among others. With Miro and Sonia we look for snakes, newts and frogs near the Danube. I remember how, with the help of Miro, we capture a very agile frog! And the photograph of the sand lizard of this thesis was of one of those days looking for animals nearby.

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I am writing this while COVID-19 crisis is at its peak. Because of that, I especially to thank all members of the tribunal its consideration to access and come to this event with this difficult situation.



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Durant el llarg decurs d'aquesta tesis, he tingut l'oportunitat de conèixer Doñana, i el seu interior "protegit". L'he vist ans i després del pas de determinat grup de gent. Ans, ple d'abellerols i després, difícilment se'n podia veure algun. I el cert es que els abellerols també em va acompanyar durant els mostrejos per Doñana, almenys abans del pas d'aquesta gent. Els cavalls i les litrones de cervesa en varen destruir-ne els nius. Si bé és cert que una bona dosis d'alcohol pot fer que hom perdi certes competències, això no treu que només un monstre -borratxo o no- es l'únic que podria destruir quelcom tant bell.



*Abellerol Merops apiaster*



I a tots els que m'han posat entrebancs en aquest camí tant llarg, també els hi haig d'agrair, doncs hi ha persones que el reconeixement només els saben demostrar amb entrebancs.

Finalment, agraeixo especialment als protagonistes d'aquesta tesi haver cedit “voluntàriament” una mostra, biomètrics, i més temps del que haurien desitjat per a la Ciència. Recordo quan empaitant un mascle del gecko de l'Atlas, tot fugint de mi, va entrar en el territori d'un altre. Ambdós van començar a moure el cos fent-se senyals i vocalitzacions. No sabre mai que es van dir, però si que no es van agredir físicament, i que tots dos es van refugiar en una mateixa escletxa. Els dos se'm van escapar mentre els observava. Ells no van donar cap mostra per a la Ciència, però em van proporcionar una experiència molt difícil d'oblidar.

De fet els enyoro, son adorables.



*Un mascle de gecko de l'Atlas *Zuedenfeldtia trachyblepharus**





El sargantaner gros es una de les sargantanes més intel·ligents que conec: sempre acaba escapant sota una argelaga, una planta que segons el seu nom científic *Genista scorpius* hom pot pensar que es verinosa, però el cert es que no sent-ho, punxa més que un escorpí. Córrer sota argelagues no ajuda a proporcionar mostres per a la Ciència, però córrer rere una sargantana es prou divertit i fa valorar més els sargantaners atrapats.

Espero que molts d'ells segueixin corrent sota argelagues per Sierra Nevada.



*El sargantaner gros Psammodromus algirus*





## ABSTRACT

Elevational gradients imply a huge variation in several biotic and abiotic factors that change with altitude. Because of that, organisms may change their phenotype along the altitudinal gradient being adapted to the local conditions. Consequently, elevational gradients constitute a good model to examine geographic variation offering a natural experiment for carrying out research in evolutionary ecology. In this thesis, I carry out different approaches to study two lizard species along two elevational gradients in the Mediterranean region. On the one hand, I study the Atlas day gecko *Quedenfeldtia trachyblepharus* in the High Atlas of Morocco. On the other hand, I study the large Psammodromus *Psammodromus algirus* in Sierra Nevada of Spain.

In the first chapter, I study the Atlas day gecko trophic ecology. It is hypothesized that increased competition should reduce niche breadth. However, there are scarce field tests on this hypothesis. The Atlas day gecko faces fewer competitors as altitude increases, and thereby, we predict that this species should increase niche breadth and relevant fitness parameters with altitude. We tested this prediction by analysing the isotopic signature of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ). Our results reveal that specimens from higher altitudes showed higher values for both carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotopes, had better body condition and a greater isotopic breadth when compared to specimens from lower altitudes. Altitudinal variation in carbon values was not explained by variation in isotopic concentration in the baseline of the trophic chain. Therefore, results support the prediction that relaxed



interspecific competition favours increased trophic niche breadth. These results also suggest that global warming may represent an important threat for this species, as it may provoke the ascent in altitude of competitors, with negative consequences for the conservation of this endemism.

In the second chapter, I study the variation in probability and intensity of infestation of blood-sucking mites parasitizing the Atlas day gecko depending on host body condition, sex, and elevation. Parasitism is one of the main selective forces in nature, strongly affecting host fitness. Still, knowledge is incomplete concerning how variation in probability and intensity of infestation depends on body condition, sex or geographic variables. I found that mite prevalence was 58.75% and the probability of infestation decreased with host body condition. However, parasitism intensity tended to increase with body condition. The parasite load ranged from 0 to 16 mites per individual, with a mean intensity of  $3.0 \pm 0.37$  (SE) in infested geckos. Prevalence was higher in males (2/3 parasitized) than in females (1/2 parasitized), but the intensity did not significantly differ with sex. Neither prevalence nor intensity varied with elevation. In conclusion, geckos in better body condition harboured heavier parasite loads, but animals with the highest body condition were not infested. These findings suggest that animals with good body condition may tolerate heavier mite infestations, but only animals with the highest body condition may resist infestation.

In the third chapter, I analyse how lifespan and other life-history traits of the lizard large *Psammodromus* *Psammodromus algirus* vary along a 2,200 m elevational gradient in Sierra Nevada. 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. Populations at intermediate altitudes, corresponding to the optimal habitat for this species, had the shortest lifespans, while populations inhabiting marginal habitats (at both low and 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. This study also indicated that reproductive investment and body condition increase with elevation, suggesting that alpine populations are locally adapted.

In the fourth chapter, I investigate the role of life-history traits such as age, sex, body size, body condition, and tail autotomy (i.e self-amputation) in shaping telomere length of six populations of the large *Psammodromus* in Sierra Nevada. The timing of organisms’ senescence is developmentally programmed but also shaped by the interaction between environmental inputs and life-history traits. In ectotherms, ageing dynamics are still poorly understood despite their particularities



concerning thermoregulation, regeneration capacity, or growth trajectory. Our cross-sectional study shows that older lizards have longer telomeres, which might be linked to a higher expression of telomerase across their life or caused by the selective disappearance of individuals with shorter telomeres. Variation in telomere across elevation was explained by age structure of lizards, thus altitude did not affect the telomere length of lizards. In contrast to our predictions, telomere length was unaffected by tail autotomy and was sex-independent, but positively correlated with body condition. Regarding the lack of impacts of elevation on telomeres of lizards, our results suggest that life-history traits such as age and body condition can be major drivers of telomere dynamics for this lizard, whereas environmental conditions apparently had scarce or no effects on their telomeres. Our findings emphasize the relevance of understanding species' life histories and habitat characteristics for fully disentangling the causes and consequences of lifespan trajectory in ectotherms.



## RESUMEN

Los gradientes elevacionales implican una gran variación en varios factores bióticos y abióticos que cambian con la altitud. Debido a eso, los organismos pueden cambiar su fenotipo a lo largo del gradiente altitudinal adaptándose a las condiciones locales. En consecuencia, los gradientes elevacionales constituyen un buen modelo para examinar la variación geográfica y ofrecen unas condiciones de experimento natural para llevar a cabo investigaciones en ecología evolutiva. En esta tesis llevo a cabo diferentes enfoques para estudiar dos especies de saurios a lo largo de dos gradientes elevacionales en la región mediterránea. Por un lado estudio el gecko diurno del Atlas *Quedenfeldtia trachyblepharus* en el Alto Atlas, en Marruecos. Por otro, estudio la lagartija colilarga *Psammodromus algirus* en Sierra Nevada, en España.

En el primer capítulo de esta tesis estudio la ecología trófica del gecko diurno del Atlas. Se hipotetiza que una mayor competencia debería reducir la amplitud de nicho. Sin embargo, hay pocas pruebas de campo sobre esta hipótesis. El gecko diurno del Atlas se enfrenta a menos competidores a medida que aumenta la altitud y, por lo tanto, predecimos que esta especie debería aumentar la amplitud de nicho y los parámetros de condición física relevantes con la altitud. Probamos esta predicción analizando la firma isotópica de carbono ( $\delta^{13}\text{C}$ ) y nitrógeno ( $\delta^{15}\text{N}$ ). Nuestros resultados revelan que las muestras de mayor altitud mostraron valores más altos para los isótopos de carbono ( $\delta^{13}\text{C}$ ) y nitrógeno ( $\delta^{15}\text{N}$ ), y los geckos tenían una mejor condición corporal y una mayor amplitud isotópica en comparación con los geckos de altitudes



más bajas. La variación altitudinal en los valores de carbono no se explicó por la variación en la concentración isotópica de base de la cadena trófica. Por lo tanto, los resultados respaldan la predicción de que una competencia interespecífica relajada favorece una mayor amplitud de nicho trófico. Estos resultados también sugieren que el calentamiento global puede representar una amenaza importante para esta especie, ya que puede provocar el ascenso en altitud de los competidores, con consecuencias negativas para la conservación de este endemismo.

En el segundo capítulo, estudio la variación en la probabilidad y la intensidad de la infestación de ácaros hematófagos que parasitan el gecko diurno del Atlas dependiendo de la condición física del huésped, el sexo y la elevación. El parasitismo es una de las principales fuerzas selectivas en la naturaleza, que afecta fuertemente la eficacia biológica del huésped. Aún así, el conocimiento sobre cómo la variación en la probabilidad y la intensidad de la infestación depende de la condición corporal, el sexo o las variables geográficas es incompleto. Encontré que la prevalencia de ácaros era del 58.75% y la probabilidad de infestación disminuyó con la condición física del huésped. Sin embargo, la intensidad del parasitismo tiende a aumentar con la condición física. La carga del parásito varió de 0 a 16 ácaros por individuo, con una intensidad media de  $3.0 \pm 0.37$  (error típico) en geckos infestados. La prevalencia fue mayor en los machos (2/3 parasitados) que en las hembras (1/2 parasitadas), pero la intensidad no difirió significativamente con el sexo. Ni la prevalencia ni la intensidad variaron con la elevación. En conclusión, los geckos en mejor condición corporal albergaban cargas de parásitos mayores, pero los animales con la





condición corporal más alta no estaban infestados. Estos hallazgos sugieren que los animales con buena condición corporal pueden tolerar mayores infestaciones de ácaros, pero solo los animales con la condición corporal más alta pueden resistir la infestación.

En el tercer capítulo analizo cómo la esperanza de vida y otros rasgos de la estrategia vital de la lagartija colilarga *Psammmodromus algirus* varían a lo largo de un gradiente de elevación de 2.200 m en Sierra Nevada. La esperanza de vida es uno de los principales componentes de la estrategia vital. Se pueden esperar vidas más cortas en hábitats marginales. Sin embargo, en el caso de los ectotermos, la esperanza de vida generalmente aumenta con la altitud, aunque la temperatura, uno de los principales factores para determinar la estrategia vital de los ectotermos, disminuye con la elevación. Este patrón puede explicarse por el hecho de que un tiempo de actividad más corto favorece la supervivencia. Las poblaciones a altitudes intermedias, que corresponden al hábitat óptimo para esta especie, tuvieron la esperanza de vida más corta, mientras que las poblaciones que habitaban hábitats marginales (tanto en altitudes bajas como altas) vivieron más tiempo. Por lo tanto, *P. algirus* no siguió el patrón típico de ectotermos, ya que también vivió más tiempo en el límite inferior de su distribución, ni mostró una vida esperanza de vida más larga en áreas con hábitats óptimos. Estos resultados podrían explicarse por una combinación compleja de diferentes gradientes a lo largo de la montaña: el tiempo de actividad disminuye con la altitud, mientras que la disponibilidad de alimentos aumenta. Esto podría explicar por qué la esperanza de vida es máxima tanto en altitudes altas (tiempo de actividad limitado) como



bajas (disponibilidad de alimentos limitada), lo que resulta en una esperanza de vida similar en áreas con condiciones ambientales muy diferentes. Este estudio también indicó que la inversión reproductiva y la condición física aumentan con la elevación, lo que sugiere que las poblaciones alpinas se adaptan localmente.

En el cuarto capítulo, investigo el papel de los rasgos de la estrategia vital, como la edad, el sexo, el tamaño corporal, la condición física y la autotomía de la cola (es decir, la autoamputación) en la longitud de los telómeros de seis poblaciones de la lagartija colilarga en Sierra Nevada. El momento de la senescencia en los organismos está programado durante el desarrollo, pero también está determinado por la interacción entre el ambiente y los rasgos de la historia de vida. En ectotermos, la dinámica de envejecimiento todavía se comprende mal a pesar de sus particularidades con respecto a la termorregulación, la capacidad de regeneración o la trayectoria de crecimiento. Nuestro estudio transversal muestra que los lagartos más viejos tienen telómeros más largos, lo que podría estar relacionado con una mayor expresión de telomerasa a lo largo de su vida o causado por la desaparición selectiva de individuos con telómeros más cortos. La variación en los telómeros a través de la elevación se explicó por la estructura de edad de las lagartijas, por lo tanto, la altitud no tuvo efecto sobre la longitud de los telómeros. En contraste con nuestras predicciones, la longitud de los telómeros no se vio afectada por la autotomía de la cola y fue independiente del sexo, pero se correlacionó positivamente con la condición corporal. Con respecto a la falta de impactos de la elevación en los telómeros de las lagartijas, nuestros resultados sugieren que los



rasgos de la historia de vida, como la edad y la condición corporal, pueden ser los principales impulsores de la dinámica de los telómeros para esta lagartija, mientras que las condiciones ambientales aparentemente tuvieron efectos escasos o nulos en sus telómeros. Nuestros hallazgos enfatizan la relevancia de comprender las estrategias vitales de las especies y las características del hábitat para desenredar por completo las causas y consecuencias de la trayectoria de la vida útil en ectotermos.





## **GENERAL INTRODUCTION**

### **Altitudinal gradients**

Elevational gradients imply a huge variation in several biotic and abiotic factors. Temperature, humidity, radiation, atmospheric pressure, community structure and even human land-use change with altitude. Due to these both biotic and abiotic changes along the elevational gradients, organisms may change their phenotype being adapted to the local conditions, thus offering ideal conditions for exploring evolutionary adaptation over short spatial distances (Badyaev 1997). Besides, as altitude increases, there is a reduction in land area. Moreover, fragmentation of the land area by geological and gravity-related processes reduces the uniform space for any type of habitat creating isolated habitat conditions and thus, climatic mountain islands. However, this habitat diversity and spatial isolation favour population segregation and, consequently enhancing speciation (Körner 2007). Consequently, elevational gradients constitute a good model to examine geographic variation offering a natural experiment for carrying out research in evolutionary ecology (Stevens 1992; Körner 2007; Keller et al. 2013).

Altitudinal gradients follow widespread environmental changes that occur with altitude but also reflect regional peculiarities of each system, such as land use, drought, precipitation and fire, not always associated with altitude in meters above sea level, hereafter, m. a.s.l. (Körner 2007). Then, it should be into account both global and local



changes with altitude before doing generalizations from a determinate mountain system.

### ***Widespread altitudinal gradients***

In addition to a reduction of land area with altitude, there are four main global changes which also depend on altitude: (1) a decreasing total atmospheric pressure and partial pressure of all atmospheric gases; (2) a reduction of temperature, (3) increasing solar radiation and (4) higher UV-B radiation.

1. Atmospheric pressure: For every kilometre gained in altitude, atmospheric pressure declines (about 11%) as well as the partial pressure of all gases. This reduction in pressure and partial pressure has a significant impact on respiration in animals as well as on gas exchange in plants.

2. Temperature: On average, the air temperature drops by 5.5 °C per kilometre of altitude. However, this is not necessarily the reduction of temperature experienced by all species, especially ectotherms that thermoregulate by means of basking.

3. Solar radiation: Considering only clear-sky days, solar radiation increases with altitude. This is because of reduced atmospheric turbidity with altitude and consequently an increased transmissivity. However, for solar radiation in general, depends on clouds and fog, both often



increasing with altitude. Because of that, there is no global altitudinal trend.

4. UV-B radiation: For peak solar radiation, there is a global increasing with altitude, but not for mean daily or seasonal dose. However, more frequent clouds and fog with altitude can reverse this effect.

#### ***Altitudinal gradients depending on local conditions***

Some meteorological parameters show a regional variation with altitude such as precipitation (5), wind velocity (6), and seasonality (7) depending on latitude and orography of the specific mountain system and local conditions.

5. Precipitation: Both precipitation and moisture availability do not follow any trend with altitude globally and precipitation and moisture gradients can go in any direction, depending on regional features of the specific mountain system. Some regions are dry at low altitude and moderately humid at mid-altitude or high altitude, like the Atlas, whereas others show a midaltitude moisture peak (e.g. Mount Kilimanjaro) or are humid lowlands and at higher altitude sharply convert to a mountain desert (for example the Teide).

6. Wind velocity: Mountains offer a topographic shelter for the wind between or within mountain ranges, except for exposed crests, summits and isolated mountains. The windiest climate is in the plains and the



coast but tropical mountains are generally calm. Then, there is no general altitudinal pattern of wind velocity with altitude.

7. Seasonality: Seasons become shorter as altitude increases at high latitudes but there is no pronounced altitudinal change in seasonality in the tropics. In dry low-altitude climate regions, the length of the growing period can become longer with increasing altitude, because of moisture conditions. Interactions of both regional thermal and moisture seasons produce different seasonality trends with altitude. Consequently, season length is driven by temperature and moisture. In humid, temperate mountains, the altitudinal reduction in productivity is mainly explained by the duration of the growing season.

### ***Biotic factors***

As a consequence of environmental gradients with altitude, biotic factors and life history concomitantly varies with elevation. Along elevational gradients, there is a high turnover in the species composition, with changing communities through altitude (Jankowski et al. 2013; Leingärtner et al. 2014; Mena and Vázquez-Domínguez 2005). Despite that it depends on the system and latitude, generally, there is a mid-elevation peak of diversity and a decrease in species richness with altitude, being species richness lower at high elevations (Stevens 1992; Fu et al. 2004; Grytnes and Vetaas 2002; Houss et al. 2012).

Environmental conditions such as temperature, humidity and community composition also affect the prevalence of parasites, its abundance and diversity. Community composition and species richness





affect the biodiversity of parasites because both the number of vectors and hosts decreases with altitude. Because of that, host-parasite dynamics typically vary with altitude (Poisot et al. 2017). Along elevational gradients, parasite intensity generally declines with elevation (Badyaev 1997). As one ascends in elevation, parasites are typically exposed to lower temperatures, reduced daily and annual time available to complete their life cycles and long periods of host hibernation which may increase parasite mortality (Postawa and Nagy, 2016).

Moreover, a shorter growing season at high altitude narrows the temporal window for parasites to reproduce and grow but also narrows the temporal window for the host to reproduce and grow which at the same time affect parasite growth and reproduction. All these factors likely decrease parasite reproduction and growth but also increase parasite mortality (Postawa and Nagy 2016). Although several studies have reported an increased parasite prevalence with elevation (Spoecker 1967; Llanos-Garrido et al. 2017), several studies indeed report reduced parasite prevalence and abundance at higher elevations (Carothers and Jaksic 2001; Álvarez-Ruiz et al. 2018; Carbayo et al. 2019).

Further, slower pace of life at high elevations are attributed to colder temperatures, shorter breeding seasons, lower predation risk, and lower food availability (Badyaev 1997; Sandercock et al. 2005; Turbill et al. 2011; Boyle et al. 2016). Of the previous ecological factors, only is a global trend colder temperature at high altitude. On the one hand, shorter breeding seasons occur only in temperate regions and could impose temporal constraints on reproduction, particularly limiting incubation time and juvenile development. Especially for ectotherms at



temperate regions, the completion of different developmental stages takes longer due to colder temperature at high altitudes slowing down all physiological processes (Keller et al. 2013).

In general, high altitudes tend to harbour lower species diversity. Consequently, in montane populations usually occur fewer species of predators, and thus a lower predation risk (McKinnon et al. 2010; Boyle et al. 2016). However, predation risk may depend on the system and density of predators as well as habitat openness and shelter availability. Furthermore, lower food availability occurs where precipitation gradients do not equalize or invert productivity. In the mountain gradients where there is lower primary productivity at high elevations, it may affect food availability (Conway and Martin 2000; Boyle et al. 2016).

On the other hand, population dynamics are also dependent on environmental factors. For example, colder temperatures may result in increased mortality risk, especially of the juveniles. Moreover, environmental variables also affect reproductive effort. At higher elevations both colder temperatures and lower partial oxygen pressures can increase physiological costs, growth costs, and reproduction costs (Boyle et al. 2016). Indeed, colder temperatures may imply longer incubation times, which could increase the exposure to predation risk. Furthermore, shorter activity periods may impose temporal constraints on the length of the reproductive season.



## Local adaptation

Altitudinal gradients provide a valuable model system to investigate local adaptation (Keller et al. 2013). Altitudinal gradients present a huge variation in both biotic and abiotic conditions, which translates in a huge environmental heterogeneity. To respond to such environmental heterogeneity some organisms show local adaptation, where the fitness of local individuals is higher than that of immigrants from other locations (Kawecki and Ebert 2004). Local adaptation is possible only if populations contain enough genetic variation and if divergent selection between different environments is stronger than gene flow, due to the homogenising effect of gene flow (Kawecki and Ebert 2004; Morjan and Rieseberg 2004). In those cases, local population evolve traits that provide an advantage under its local environmental conditions which make it adapted to its habitat (Kawecki and Ebert 2004).

An example of local adaptation at high altitude is the deer mice (*Peromyscus maniculatus*), where the b-globin variant of high-altitude populations present a higher O<sub>2</sub> affinity in comparison of those from low altitude (Storz et al. 2009).

Further, phenotypic variation in organisms can involve body size clines with altitude, being the general pattern, that body mass increases with elevation (Chown and Klok 2003; Keller et al. 2013; Comas et al. 2014; Comas et al. 2019). This may be due to both that at higher altitude being bigger allows storing larger fat reserves which allow to survive to longer winters (Comas et al. 2014) and implies better thermal adaptation



to colder temperatures following Bergmann's rule (Zamora-Camacho et al. 2014).

Ectotherms, which depend on environmental temperature to do all their physiological processes, at high altitudes with colder temperatures, slow down all these processes to complete different developmental stages during the growing season taking it longer at higher than at lower altitude (Hodkinson 2005; Keller et al. 2013; Zamora-Camacho et al. 2013). However, an adaptation to these conditions implies a compensatory response, with high altitude populations developing faster than low altitude populations (Hodkinson 2005). Such a pattern was observed in different ectotherm species for which development time was correlated with altitude (Keller et al. 2013).

### **Altitudinal vs latitudinal gradients**

Furthermore, biotic and abiotic variables also change similarly in latitudinal gradients (Stevens 1992; Körner 2007; Chown and Klok 2003; Graae et al. 2012), allowing to compare the studies done in both geographical gradients. On one hand, there is a common ecological pattern of a biodiversity decline both in altitude and in latitude as both increases (Stevens 1992). Moreover, phenotypic variation in altitude may match with those reported in latitude (Blackburn and Ruggiero 2001; Ashton and Feldman 2003). On the other hand, not all environmental variables change in the same direction in both geographical gradients. For example, some variables change differently in latitudinal gradients that it does with altitude, such as radiation.



Radiation increases with altitude but decreases with latitude. These differences between the two geographical gradients imply that not all findings were comparable. For example, Zhang and Lu (2012) found that amphibians live longer at higher altitudes but not at higher latitudes. Despite that, some similarities allow to extrapolate the results obtained in elevational gradients to latitudinal gradients. Moreover, elevational gradients present several logistic advantages in front of the study of latitudinal gradients, such as small spatial scales of environmental transitions with the consequent smaller budgets needed to study them and usually, less logistic requirements. Further, the smaller geographical scale of elevational gradients in front of latitudinal gradients implies that confounding effects are less marked, for example, distinct regional evolutionary histories will be likely less divergent along smaller scales (Balanya et al. 2006; Keller et al. 2013).

### **Global change**

Global change implies ascending mean temperatures and a change of the use of the resources as well as land use. This global change is already affecting global biodiversity (Parmesan 2006). Different studies predict high extinction rates for animals as well as plants (Sala et al. 2000; Thomas et al., 2004; Settele et al., 2010). Moreover, some groups show a sharp decline, especially amphibians and reptiles (Gibbons et al. 2000; Whitfield et al. 2007; Sinervo et al. 2010; Moreno-Rueda et al. 2012).



Despite that, in order to adapt to those changes, several animal species are changing their distributions (Hickling et al. 2006; Enriquez-Urzelai et al. 2019), ascending in altitude or latitude (Perry et al. 2005; Wilson et al. 2005; Lenoir et al. 2005; Hickling et al. 2006; Moreno-Rueda et al. 2012). Some species may be able to change their distribution in order to adapt to climate warming. However, others, because of their physiological needs or because of geographical barriers and/or a lack of corridors, maybe will not. For instance, species that inhabit mountains, in the case of rising temperatures, may ascend in altitude (e.g. Wilson et al. 2005). However, this change in their distribution is only possible until reaching the mountain peak. Moreover, valleys around the mountain peaks maybe not a suitable habitat to allow dispersion to all species, for example, high mountain species may be more restricted to a mountain-specific habitat. Further, competing species adapted to a lowland's climate, with rising temperatures may ascend in altitude, and this may affect the long-term survival of mountain species due to interspecific competition (Comas et al. 2014). This may add a survival risk to alpine species in a global change scenario (Raxworthy et al. 2008; Dubey and Shine 2011).

Moreover, species restricted to alpine habitat may suffer a habitat loss, due to a reduction in the land area towards the top of the mountains and upward range shifts in animals' distributions because of climate warming (Franzen and Molander 2012; Freeman and Freeman 2014).

Mountain habitats, because of its isolation and particularities, are hotspots of endemic species (Myers et al. 2000), being a large number of these species restricted to habitats above the tree line (Essl et al. 2009).



The shift upward of the tree line in response to climate warming may constrict this habitat with the consequent possible extinction of the populations above the tree line, most of those endemic (Theurillat and Guisan 2001; Sekercioglu et al. 2008; Dirnböck et al. 2011), as already it occurs in different mountain ranges as a consequence of climate warming (Harsch et al. 2009; Dirnböck et al. 2011).

Further, with climate warming, thermal environments at high altitudes may become more similar to the current thermal environments at lower altitudes. Because of that, range shifts may not be enough for locally adapted populations to track the environment which were adapted (Parmesan and Yohe 2003; Parmesan 2006) with the consequent possible extinction of the populations. A mechanism to deal with environmental variability is phenotypic plasticity. However, plastic responses are possible within certain limits (Gienapp et al. 2008). Indeed, some studies reflect microevolutionary changes in response to global warming (Bradshaw et al. 2006; Hoffmann and Sgrò 2011; Keller et al. 2013).

For all that, altitudinal gradients are climate gradients and because of that are particularly useful to study the possible responses of populations in front of the climate warming (Reusch and Wood 2007; Keller et al. 2013), providing an opportunity to investigate the current and historical responses to climate warming.

In the specific case of the ectotherms, the temperature is one of the most important environmental variables that affect their physiology. Ectotherms depend on environmental temperature to keep their bodies warm and carry out all their physiological functions (Niehaus et al. 2012). Because of that, a rise of the temperature may affect their



physiology and climate warming may imply a challenge for the survival of some populations, especially those near their range-limits of its distribution (Terborgh 1971).





## **OBJECTIVES**

The main objective of this thesis is to add knowledge to the lizard ecology in mountain systems and know-how a global change can affect the viability of their populations. With this thesis, I intend to know how trophic ecology, age structure and parasitisation in reptiles vary along montane elevation gradients, in order to predict the impact that climate change will have on reptiles. The specific objectives are:

- 1.- Examine the variation of the trophic niche with the altitude, in order to determine if the niche width and trophic position change with altitude.
- 2.- Examine the variation of body condition with the altitude.
- 3.- Examine how the prevalence and abundance of ectoparasites (hematophagous mites) vary along the altitudinal gradient.
- 4.- Examine how the age structure of the population varies along the altitudinal gradient.
- 5.- Examine how telomere lengths change depending on age structure and altitude.





## **HYPOTHESES**

Considering the previous objectives of this thesis, the corresponding hypotheses will be:

1.- Trophic availability will differ with altitude, as a result of a decrease in the number of competitors at high altitude. Therefore, the trophic niche is expected to be wider and the trophic position higher at higher altitude.

2.- Body condition will increase with altitude, as a result of a decrease in the number of competitors and parasites at a higher altitude.

3.- I expect that prevalence and abundance of parasites will decrease with altitude as longer winters may increase parasite mortality.

4.- The age structure will change as altitude increases. Longer life is predicted at higher altitude, since at higher altitude, the winters are longer and a longer hibernation is correlated with greater longevity.

5.- Telomere lengths will change depending on age structure and altitude, because with age usually, telomere length shortens and with altitude a decrease of the oxygen partial pressure may imply less oxidative stress, which may be related to a longer telomere length.





## STUDY AREA

### The High Atlas



The Atlas runs along 2.400 km in northwest Africa. The Atlas is the main mountain range of Morocco, which is formed by the Middle Atlas, the High Atlas and the Anti-Atlas. It rises in the west at the Atlantic Ocean and stretches at East to the Algerian border. At the Atlantic and to the southwest the range drops abruptly and makes a sharp transition to the coast and the Anti-Atlas range. To the north, close to Marrakech, the range descends less abruptly.

Jebel Toubkal is the highest peak of the High Atlas, which reaches 4.167 m a.s.l. and lies in Toubkal National Park. In the High Atlas also stand out the Jebel Ayachi, which reaches 3.747 m. At Oukaimeden the highest peak achieves 3.268 m (Fig. 1). In the Middle



Atlas, there are other considerable peaks, such as the Jebel Bou Naceur which reaches 3.356 m, Jebel Mouâsker of 3.277 m, and Jebel Bou Iblane of 3.192 m, near Immouzer Marmoucha.



**Figure 1.** The High Atlas at Oukaimeden at 2.755 m a.s.l.

The photo was taken by Mar Comas.

The range serves as a weather system barrier in Morocco running east-west and separating the Sahara from the Mediterranean and continental zones to the north and west.

In the High Atlas, snow lasts into late spring mostly on the northern faces of the range, providing water for several year-round rivers, the majority of them which flow to the north. However, several wadis and seasonal rivers terminate in the deserts to the south and plateaux to the east.



The Atlas formation is a consequence of the collision between the Eurasian and the African plate. The Atlas has been formed during the Cenozoic (last 65 Ma) by compression of previous extensive Mesozoic basins, except for the Anti-Atlas, where Paleozoic deformation is reported. The first phase of the Atlas formation took place during the opening of the Atlantic Ocean. Meanwhile, a second phase took place during the Mesozoic Era (65 million years ago) and consisted of a generalized extension of the Earth's crust that dislocated and separated from the continents. This extension was responsible of the formation of thick intracontinental sedimentary basins present in the Atlas. Most of the rocks that form the surface of the current High Atlas were deposited under the ocean at that time. Lastly, in the tertiary period, the Atlas erected meanwhile Europe and Africa plates collided at the southern tip of the Iberian Peninsula.

The geology of the High Atlas is complex. The western High Atlas is the oldest, with Jurassic and Cretaceous formations notched by deep erosion-carved valleys. The central High Atlas is formed by a solid chalk mass morphologically dominated by tabular zones. The eastern part of the High Atlas forms vast plateaux at high altitude which provides the headwaters for the Moulouya River. It extends from the town of Khenifra and includes the solid mass of the Tamlelt whose northern edge is occupied by its higher peaks, such as Jebel Ayachi. The altitude falls towards the east where the mountains join the pre-Saharan zone.

There are two types of alpine climates in the High Atlas depending on altitude and latitude: oceanic climate and continental semi-arid.



Oceanic climate dominates the north and south of the Western High Atlas in the Jebel Toubkal National park, as well as the northern part of Central High Atlas until Imilchil, owing to their exposition to the perturbations coming from the North Atlantic Ocean. The Western High Atlas is relatively humid with an annual rainfall is between 600 and 1.000 mm. The drought in the summer months is usually intense but interrupted by thunderstorms which at the end of summer become storms sometimes torrential. Snow falls between November and April but can persist from September to June in the peaks. The most important rivers in this region flow through the valleys Asif Melloul, Oued n'Fis, and Oued Tessaout, where there are forests of pine, oak and cedars. However, these forests are in decline due to their over-exploitation, the overgrazing of goats and sheep together with the reduced precipitations (Aloui Haroni et al. 2009).

Continental semi-arid climate dominates the southern part of the Central High Atlas, and the whole Oriental High-Atlas, from Toubkal to Imilchil and down of Imilchil. Central and Oriental High Atlas are marked by huge variations in temperatures. This continental climate extends southward from the steppe lands into rocky desert. Forests are rare in these regions, because of the overexploitation, overgrazing, and because forest recovering is more difficult in these regions with lower precipitations.

The Atlas biodiversity is huge and there are numerous endemic species. The most common forests are Atlas cedar *Cedrus atlantica* forests, evergreen oak forests, and semi-evergreen oaks forests, such as the Algerian oak *Quercus canariensis*.





Examples of some animal endemic species or animals that mainly inhabit the Atlas include the Barbary macaque *Macaca sylvanus*, Barbary stag *Cervus elaphus barbarus*, Barbary sheep *Ammotragus lervia*, Cuvier's gazelle *Gazella cuvieri*, Maghreb green woodpecker *Picus vaillantii*, the Atlas mountain viper *Vipera monticola*, the Atlas day gecko *Quedenfeldtia trachyblepharus*, *Q. moerens* and the Andreansky's lizard *Atlantolacerta andreanszkyi*.

In the High Atlas, the reptile community is very rich, particularly at middle and at low altitude. At high altitude there are fewer lizard species, however, there is sharing habitat two alpine specialist, the Atlas day gecko *Quedenfeldtia trachyblepharus* and Andreansky's lizard *Atlantolacerta andreanszkyi* (Fig. 2). The Atlas day gecko is a scansorial species meanwhile the Andreansky's lizard is a ground-dwelling species (Bons & Geniez 1996; Schleich et al. 1996).

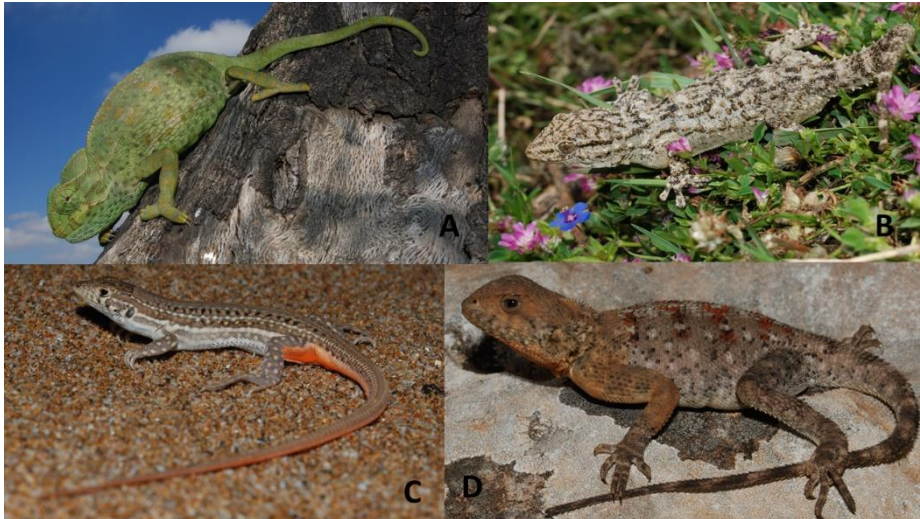


**Figure 2.** Different species which inhabit the High Atlas, mainly at high or mid-altitude: Moroccan rock lizard *Scelarcis perspicillata* (A), Vaucher's wall lizard *Podarcis vaucheri* (B) and Andreansky's lizard *Atlantolacerta andreanszkyi* (C).

Photos were taken by Mar Comas.



Nevertheless, biodiversity increases below 2,500 m.a.s.l. with numerous scansorial species such as the Moroccan rock lizard *Scelarcis perspicillata*, the Vaucher's wall lizard *Podarcis vaucheri* and the Moorish gecko *Tarentola mauritanica* and also ground-dwelling species as the large psammodromus *Psammodromus algirus*, but also species of larger size such as the Moroccan eyed lizard *Timon tangitanus* and the Atlas agama *Agama impalearis* (Bons & Geniez 1996; Schleich et al. 1996; Fig. 3). Also, there is a replacement of predators with altitude. For example, saurophagic snakes such as the Montpellier snake *Malpolon monspessulanus* is common at low altitudes meanwhile the Atlas mountain viper *Vipera monticola* is the most common snake at higher altitudes (Schleich et al. 1996).



**Figure 3.** Different species which inhabit the High Atlas, mainly at low or mid-altitude: Mediterranean chameleon *Chamaleo chamaeleon* (A), Moorish gecko *Tarentola mauritanica* (B), spiny-footed lizard *Acanthodactylus erythrurus* (C) and Atlas agama *Agama impalearis* (D). Photos were taken by Mar Comas.



## Sierra Nevada



Sierra Nevada is a mountain range belonging to the Betic Cordilleras, specifically to the Penibetic System of Southeastern Spain. It rises in the south-east of the Iberian Peninsula. It is located mostly in the province of Granada, although in its easternmost part it extends to the province of Almería. Sierra Nevada is delimited by the Lecrín valley to the west, the Guadalfeo river valley to the south, the Bética depression to the north, the Nacimiento river to the east, and the Andarax valley to the south-east.

Sierra Nevada counts with the highest peak of the Iberian Peninsula, the Mulhacén, with 3.482 m above sea level followed by Pico Veleta, with 3.479 m a.s.l. It is the highest altitude mountain range in all of Western Europe, after the Alps. It runs along almost 90 km and its surface is about 2.000 km<sup>2</sup>.

Sierra Nevada was declared a Biosphere Reserve by UNESCO in 1986 and in 1999 a large part of its territory was declared a National Park for its endemisms and natural values.

The north face of Sierra Nevada is the birthplace of several rivers, located almost all in the Guadalquivir basin: such as the Nacimiento, the Fardes, and the Genil river. The rivers belonging to the Mediterranean side are born on the west and south faces, such as the Dúrcal or Ízbor



river, the Trevélez, the Poqueira, tributaries of the Guadalfeo, which is also born in the mountains, and the Adra and Andarax rivers. Moreover, in this same slope, are about fifty high mountain lagoons as a result of the ancient presence of glaciers.

Sierra Nevada was formed during alpine orogenesis in the Tertiary Era, along with the rest of the Betic mountains, with sediments from the Primary and Secondary era: quartz grains, clays and iron oxides. The accumulation of a thick layer of materials at the bottom of a syncline, covered by the marine waters of the Tethys Sea over 200 million years ago, and subjected to the enormous pressures of the depths (between 3.000 - 4.000 m deep), transformed sedimentary rocks to metamorphic. Later, in the second half of the Tertiary Era, in the Pliocene, the lifting of the materials already folded in ancient orogenesis begins and gradually emerges from the waters, like all the Alpine formation that, starting its pressure in the south, was moving northwards, towards the Castilian plateau, which acted stopping its advance. Consequently, it reached a point where the lifting reached its limit and the materials broke, sliding on top of each other, and forming a series of characteristic failures.

The geology of Sierra Nevada is complex: in the central part, we can find mica slates or shales of low hardness from the Paleozoic era surrounded by gneiss, marbles, amphibolite serpentine, some quite metamorphized volcanic rocks and formations of the Triassic. In the external part there is a limestone zone, it is formed by a belt of dolomites and Triassic limestones, and as a more characteristic example, we have the Trevenque



peak of 2.079 m a.s.l. (Fig. 4). In the surroundings, tertiary lands extend with conglomerate and clayey extensions.



**Figure 4.** Trevenque, a limestone zone in the external part of Sierra Nevada. The photo was taken by Mar Comas.

The climate of Sierra Nevada is Mediterranean, with hot and dry summers, and relative aridity (due to its west-east orientation, and the prevailing winds of the west) and strong insolation (Valle et al. 2004). Annual mean temperatures vary between 0 to 17.6 °C and annual precipitations between 267 to 753 mm (REDIAM 2001), depending on the elevation. During colder months (from December to February), precipitations fall usually as snow, particularly above 2000 m asl. In fact, snow cover lasts about six months per year, from November to May (Fig. 5).





**Figure 5.** Forests of Pyrenean oak and evergreen oak below the high peaks covered with snow. The photo was taken by Mar Comas.

The slope in Sierra Nevada is very pronounced, therefore, both temperature and rainfall vary sharply with altitude; however, the northern slope is colder. Bioclimatic floors also change with altitude, from the Mesomediterranean lowlands to the Oromediterranean highlands.

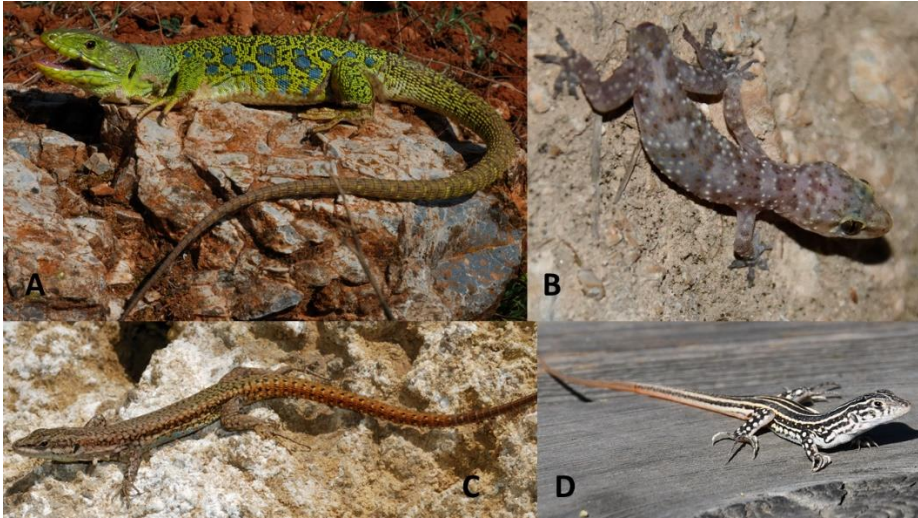
It was estimated that, during the Würm Glaciation, the limit of the perpetual snows of Sierra Nevada was around 2.400 meters. Its relief modelling was the cause of the formation of high mountain lagoons and U-shaped valleys. At the 19th century was reported the remains of a glacier in the area Los Corrales del Veleta, just in the shadow of the peak El Veleta, and recently it was discovered that even today remains certain permafrost areas at various points in the mountain range. The estimated age of the fossil ices present in the subsoil was between 100 and 9.000 years. These remaining permafrost areas are considered reminiscent of the ancient glaciers present in Sierra Nevada.



Because of the isolation and altitude of Sierra Nevada, since the end of the Würm Glaciation, it has remained as a glacial refuge for numerous endemisms, such as 66 endemic vascular plant species and 80 animal species.

A Sierra Nevada characteristic and endangered forest is Sierra Nevada scots pine forest *Pinus sylvestris nevadensis*. Moreover, there some botanical endemism of high altitude such as the Sierra Nevada chamomile *Artemisa granatensis*, the Sierra Nevada daffodil *Narcissus nevadensis* and *Plantago nivalis*. However, few animal species live at high altitude. This is a typical feature of the Sierra Nevada wildlife, compared to other European mountain ranges. The main ones are the snowy vole (*Chionomys nivalis*) among mammals and the alpine accentor (*Prunella collaris*) among birds. Moreover, of some typical animal species as the ibex *Capra pyrenaica hispanica*.

In the Sierra Nevada massif, the reptile community is richer particularly at middle and at low altitude, with few species achieving more than 2.000 m a.s.l. like the large psammodromus *Psammodromus algirus* and the Iberian wall lizard *Podarcis hispanica complex*. Nevertheless, these both species can be found along most of the gradient. At mid-altitude, we can find species such as the Sierra Nevada lizard *Timon nevadensis* and the Edward's psammodromus *Psammodromus edwardsianus* and a low altitude the spiny-footed lizard *Acanthodactylus erythrurus*, the Mediterranean house gecko *Hemidactylus turcicus* and the Moorish gecko *Tarentola mauritanica*, however, the latter is possible to find it also at middle altitude (Fig.6).



**Figure 6.** Different species which inhabit Sierra Nevada, mainly at low and mid-altitude: Sierra Nevada lizard *Timon nevadensis* (A), Mediterranean house gecko *Hemidactylus turcicus* (B), Iberian wall lizard *Podarcis hispanica complex* (C), spiny-footed lizard *Acanthodactylus erythrurus* (D). Photos were taken by Mar Comas.





## MODEL SPECIES

### **The Atlas day gecko *Quedenfeldtia trachyblepharus***

Etymology: Genus dedicated to the German entomologist O. V. Quedenfeldt. From Greek, trachys: rough; blepharon: eyelid.

The Atlas day gecko *Quedenfeldtia trachyblepharus* (Boettger, 1874) is a member of the Sphaerodactylidae family endemic to the Moroccan High Atlas (Fig. 7).



**Figure 7.** A female Atlas day gecko at Oukaïmedene (High Atlas, Morocco).

The photo was taken by Mar Comas.



*Quedenfeldtia* is a genus containing two lizard species *Quedenfeldtia trachyblepharus* and *Quedenfeldtia moerens*, with distinctive pholidosis, features and colour pattern (Arnold 1990). Recent genetic analysis supports these two current species (Barata et al. 2012). Depending on genetic analysis done with mitochondrial DNA there are two lineages of *Quedenfeldtia trachyblepharus*: one that includes the population from Oukaïmeden and Toubkal and a second that includes the population from the Jebel Sirwa. There was low diversity within groups, up to a maximum of approximately 3% (Barata et al. 2012). However, analysis done with nuclear DNA differs from the mitochondrial analysis because individuals from Toubkal were well differentiated from Oukaïmeden and almost identical to the remaining *Q. trachyblepharus* localities (Barata et al. 2012). The nuclear divergence between the lineages of *Q. trachyblepharus* between Oukaïmeden and Jebel Sirwa is of 0.5% (Barata et al. 2012). The small intraspecific variation found suggests that the Atlas day gecko current distribution is the result of rapid expansion from very small populations or a bottleneck effect (Barata et al. 2012).

*Quedenfeldtia moerens* inhabits a wide range of habitats from the sea level to 3.000 m above the sea level whereas *Q. trachyblepharus* inhabits mountain habitats from 1.200 to 4.000 m, but is particularly abundant from 2.500 m on (Arnold 1990; Bons and Geniez 1996; Schleich et al. 1996; Bouazza et al. 2016). *Quedenfeldtia moerens* occurs in flat and dry areas with temperatures below 25 °C during summer and linked to sparse vegetation, meanwhile *Q. trachyblepharus* occurs in sloping and humid areas with high levels of precipitation, especially



during winter (Barata et al. 2012). Although there are some specific environmental conditions where both species could coexist in the High Atlas, to date, no regions of sympatry have been found (Barata et al. 2012).

The Atlas day gecko *Q. trachyblepharus* is the dominant species in lizard communities at alpine levels becoming scarcer at lower elevations. The Atlas day gecko is strictly diurnal and presents variation in colouration: orange, yellow, and white variants were found in approximately equal proportions in both sexes, and in both juveniles and adults (Blouin-Demers et al. 2013). The Atlas day geckos present sexual dimorphism, having males a dark spot on the venter and in the ventral part of posterior legs (Fig. 8). Moreover, males are bigger than females and present larger heads, both longer and wider (Table 1).



**Figure 8.** Female (left) and male (right) of the Atlas day gecko where it is possible to see the lack of dark ventral spots in the female and its presence in the male.

Photos were taken by Mar Comas.



**Table 1.** Biometrics of the Atlas day gecko by sex (mean  $\pm$  SD): snout-vent length (SVL), tail length (Tail.L), head length (HL), head width (HW), linear measurements in mm and weight in g.

Sex	SVL	Tail.L	HL	HW	Weight
Male	45.3 $\pm$ 2.1	48.3 $\pm$ 19.6	11.7 $\pm$ 0.8	8.3 $\pm$ 0.5	2.75 $\pm$ 0.3
Female	43.36 $\pm$ 2.7	40.0 $\pm$ 15.2	10.8 $\pm$ 0.6	7.9 $\pm$ 0.5	2.4 $\pm$ 0.4

Gravid females show an enlargement of the abdomen when are pregnant. Moreover, most females show an enlargement of the endolymphatic glands located at the base of the throat and associated with the calcium carbonate storing (Bauer 1989; Brown et al. 1996; Bouazza et al. 2016; Fig. 9). This calcium storage of the endolymphatic glands is likely used by females as calcium reservoir used in the eggshell deposition. This hypothesis is especially likely for members of the families Gekkonidae and Sphaerodactylidae, which lay calcareous shelled eggs (Bauer 1989).



**Figure 9.** This female Atlas day gecko presents two enlarged endolymphatic glands clearly visible at the base of the throat. The photo was taken by Mar Comas.



In our study area, snout-vent length (SVL) varies from 38.7 to 48.5 mm with a mean of 44.2 mm, and mean tail length 44.7 mm with a maximum tail length of 68.5 mm. These geckos lack eyelids (Fig. 10) but present prominent upper lids to protect the eyes, likely to protect the eyes in a montane zone with strong winds.



**Figure 10.** Most geckos lack eyelids, but they often lick their eyes to keep them clean and moist, as is doing this Atlas day gecko. The photo was taken by Mar Comas.

These geckos are saxicolous and scansorial with no predilection for any type of rock, inhabiting granite, schists and sandstone and looking for refuge in fissures and under rocks. On rock faces fissures can be several meters deep with a microclimate close to the yearly average, offering retreats for hibernation and during the summer heat. Generally, the Atlas day gecko aggregates in rock crevices for shelter and nesting (Bouazza et al. 2016). Hibernation lasts from October to February,



however, in sunny winter days is possible to see geckos basking at least if temperatures are above 6 °C (Schleich et al. 1996). In order to increase radiation absorption, many lizards modify their skin colour while basking (Sherbrooke et al. 1994) and in the Atlas day gecko occur marked colour changes especially when the air temperature is cold, often showing darker colouration (Bouazza et al. 2016).

The Atlas day gecko is a highly social species. The visual communication system includes several ritualized postures: of the head, legs, body and gular pouch; as well as motions: of the head, legs and tail (Schleich et al. 1996). Males are territorial meanwhile females tolerate each other. However, hostilities are ritualized with a rich array of different displays and serious damage seems to be rare with most rival attacks between males directed to their tails.

Autotomy is frequent, in fact, in the study area, only 7.5 % of the geckos show an intact tail (Fig. 11). Nevertheless, tail regeneration occurs in both regenerate and unregenerate tails (Schleich et al. 1996).



**Figure 11.** Here, one of the few geckos found with an intact tail (a male).

The photo was taken by Mar Comas.





The reproductive period for these oviparous geckos is from March to June (Bouazza et al. 2016). There are two or three clutches of a single egg per season. The oviposition is done in rock fissures where eggs are glued to the substrate. Collective egg-laying sites are used by several females in one colony (Schleich et al. 1996).

The Atlas day geckos are alert during most of their activity period, maintaining an observation posture with raised forebodies and elevated heads. They sit on vantage points which allow to survey the surroundings but are active predators (Schleich et al. 1996). They feed mainly on coleoptera, homoptera, hymenoptera, heteroptera, diptera, formicidae, lepidoptera, araneae and opilioni (ordered by occurrence percentage according to Carretero et al. 2006), but also of other arthropods such as small mantids as *Ameles* (Schleich et al. 1996). The main prey of the Atlas day gecko is coleoptera of small size, and are consumed more by males than by females. Moreover, adults consumed more frequently coleopters than immatures (Carretero et al. 2006). This is due to a stronger bite force of males than females as well as a stronger bite force of adults in comparison with immatures. However, despite males having bigger heads than females, both sexes show a similar trophic niche (Comas et al. 2014).

Trophic niche width of the Atlas day gecko is greater as altitude increases. Consequently, it has an expanded trophic niche at higher elevations (Comas et al. 2014), possibly as a consequence of reduced competition at high elevations where fewer species coexists (Comas et al. 2014). Accordingly, the Atlas day gecko shows a higher trophic position at higher altitude (Comas et al. 2014).



Their main predator at highlands is the Atlas viper *Vipera monticola*. However, other snakes such as the Montpellier snake *Malpolon monspessulanus* (Fig. 12) may prey on geckos at lower altitude. Their main antipredator strategy is flight from predators, but when caught they also can emit vocalizations (Schleich et al. 1996). The tail is autotomic, so that the gecko detaches itself mechanically if it receives a physical attack. In this way, the predator can attack the tail meanwhile the gecko flees.



**Figure 12.** A Montpellier snake *Malpolon monspessulanus*, one of the main predators of the Atlas day gecko lowlands. The photo was taken by Mar Comas.





### **The large psammodromus *Psammodromus algirus***

Etymology: from Greek, psammos: sand and dromos: a running. And from Latin, Algius: Argelian.

The large psammodromus *Psammodromus algirus* (Linnaeus, 1758) is a medium-sized lizard of 53–95 mm of snout-vent length (SVL) in our study area, with a tail that can be twice or even three times its SVL. Its dorsal colouration is generally brown, with two lighter dorsolateral lines that run through the body from the back of the head to the hind legs. In the dorsal part, the scales are overlapped being fairing and pointed. Behind the forelimbs usually have two blue ocelli, although their number and size may vary (Fig. 13). The ventral colouration goes from white to grey and frequently with yellow pigmentation in the throat region that varies in extent and intensity (Carretero 2002; Salvador 2011).



**Figure 13.** Large psammodromus *Psammodromus algirus* where it is possible to see its characteristic brown colouration, the two lighter dorsolateral lines and the overlapped scales. The photo was taken by Mar Comas.



This species has sexual dimorphism. Males have more and larger ocelli, larger heads and longer tails, while females have a proportionally longer body, which increases their ability to house eggs (Salvador 2011). There are two Iberian lineages described, one eastern and one western (Carranza et al. 2006; Verdú-Ricoy et al. 2010), which differ according to various morphological and colouring characteristics. Western populations are related to populations of North Africa. During the reproductive season, the males present a spot in the throat that is yellow in the eastern lineage (Fig. 14) and orange or reddish in the western lineage (Díaz 1993).



**Figure 14.** A large psammodromus *Psammodromus algirus* from the eastern population, where is possible to see the yellow colouration of the throat and two blue ocelli. The photo was taken by Mar Comas.



Male western lineage lizards extend the orange or reddish colouration to the head meanwhile in the eastern male's orange colouration is reduced to a small spot in the commissures which are visible only when open their mouth. Moreover, the blue ocelli are bigger and more numerous in the western lineage (Salvador 2011; Fig. 15).



**Figure 15.** A large psammmodromus from Morocco -related to the western population from the Iberian Peninsula- where it is possible to see the orange colouration in the upper commissures and four blue ocelli. The photo was taken by Mar Comas.

The subspecies *ketamensis* is considered a monochromatic form of the nominal species (Bons and Geniez 1996) that does not present the characteristic lighter dorsolateral lines (Fig. 16) and inhabit at North Africa.



**Figure 16.** The ketamensis subspecies is a monochromatic form of North Africa that does not present the lighter dorsolateral lines. The photo was taken by Mar Comas.

*Psammmodromus* integrates the Gallotiinae subfamily (which includes the giant Canary island lizards), a monophyletic taxon that is considered a sister group of the Lacertinae subfamily. Both subfamilies make up the family Lacertidae (Fu 2000; Arnold et al. 2007; Mayer and Pavlicev 2007). Several species integrate this genus: *Psammmodromus microdactylus* in Morocco, *P. blanci* in the Maghreb, *P. algirus* in both the Iberian Peninsula and the Maghreb, and *P. hispanicus* complex in the Iberian Peninsula and the southwest of France (Bons and Geniez 1996). *Psammmodromus hispanicus* has been recently split into three different species (Fitze et al. 2011): *P. edwardsianus* correspondent to the eastern group, *P. occidentalis*, at the western part of the Iberian Peninsula, meanwhile the other populations are considered as *P. hispanicus* (Fitze et al. 2012).



The taxonomy of *P. algirus* has been reviewed recently. Busack et al. (2006) proposed split *P. algirus* into different species, considering two different Iberian clades (based on allozymes and morphological features), placing *P. manuelae* in the centre of the Peninsula, and *P. jeanneae* in the southern half, considering an estimated divergence between the two Iberian clades of 1.40-1.54 million years, while the Maghreb populations were still considered *P. algirus*, based on estimates of 2.98-3.23 million years of divergence from the populations of the Iberian Peninsula. However, other authors (Carranza et al. 2006) estimated a separation for the Iberian clades of 3.6 million years. According to their results, the North African populations, related to the Iberian western lineage, would have differed from the European ones about 1.9 million years ago. In addition, Carranza et al. (2006) consider that the western populations consist of four subclades, three Iberian (one in the provinces of Cádiz and Málaga, other in the northwest and another in the southwest) and one North African (Carranza et al. 2006; Verdú-Ricoy et al. 2010). According to Verdú-Ricoy et al. (2010), the species *P. manuelae* and *P. jeanneae* would correspond respectively to the northern and southern subclades of the western lineage. Verdú-Ricoy et al. (2010) questioned the validity of the species described by Busack et al. (2006), again considering *P. algirus* as a single species with two lineages, one western and one eastern, without taxonomic validity.

The distribution of the large psammotriton is Iberian-Maghreb, occupying most of the Iberian Peninsula (except the Cantabrian area), Andorra and the southwest and centre-west of France, in the Eastern Pyrenees, Hérault and Gard. Some introductions on islands have been





described, such as in Mallorca or Isolotto dei Conigli, in Italy. In the Maghreb, it is located in the centre and north of Morocco, and the north of Algeria and Tunisia (Salvador 2011).

Regarding its altitudinal distribution, it can be found from sea level to more than 2.500 m a.s.l. in the Sierra Nevada (SW of Iberian Peninsula) and in the High Atlas, in Morocco. Generally, at higher latitudes it reaches lower altitudes, therefore, while in France they achieve 800 meters high, in the Pyrenees and the Central System they reach 1.500, and in Sierra Nevada and the High Atlas (in Morocco) they reach 2.600 m a.s.l. (Salvador 2011).

The large psammophilus is generally found in bushes and forested areas, such as pine and oak groves, although it can also be found in crop fields, in the edges of roads and highways, in coastal sandbanks, in pastures with bushes and even on burned terrain (Moreno-Rueda et al. 2019). It generally selects the areas with the highest scrubland coverage or herbaceous vegetation (Salvador 2011).

The large psammophilus is a diurnal reptile with a period of activity that extends from the end of February to October, with considerable geographical variation according to the climate and altitude (Díaz et al. 2006). In coastal areas and especially in the south of its range, lizards can be active throughout the year. The activity is more intense in spring, decreasing towards the summer (which is usually bimodal, with activity peaks in the early and late hours of the day), and increasing at the beginning of autumn (Salvador 2011).

The large psammophilus reproduces in spring and early summer, with some geographical variation depending on weather conditions and



altitude. The courtship consists of a chase from the male to the female, who bites in the abdomen or neck, to facilitate the mating (Salvador and Veiga 2001). After this, the male can escort the female for one or even up to three days (Salvador and Veiga 2001). The laying takes place 30 - 40 days after the mating, under stones or in indentations in the ground or holes made by the female, but in any case, the female performs the laying protecting it from the weather. Sometimes communal clutches have been found (Salvador 2011). The laying season goes from March to August, although it varies geographically and according to the altitude (Salvador 2011). The clutch size varies from 1 to 11 eggs (Fig. 17) and increases with the size of the female and altitude (Salvador 2011). Females from fragmented habitats lay fewer and smaller eggs (Díaz et al. 2005).



**Figure 17.** Here we can see a newborn lizard, a lizard being born and an egg already hatched from the same laying of a large psammophilus.

The photo was taken by Mar Comas.



The temperature affects the duration of the incubation: at 27°C the incubation lasts 60-72 days, while at 30°C this period is reduced to 48-55 days (Iraeta et al. 2006). At the northern end of its distribution, however, incubation periods are shorter even at the same temperature, which has been interpreted as a strategy that allows early hatching despite thermal limitations (Díaz et al. 2007). Moreover, at high altitude, large psammophilus are darker, likely as a response to low temperature and high UV radiation at high altitudes (Reguera et al. 2014).

In general, males of greater size and older age are those who develop nuptial colouration (Salvador and Veiga 2000), present higher levels of testosterone and are more aggressive (Martín and López 1999), being more active and spending more time on courtship (Martín and López 1999). On the other hand, they are more susceptible to parasitization and their survival is lower (Belliure et al. 2004; Salvador 2011).

Parasitism in large psammophilus depends on altitude: ectoparasites such as mites, ticks, mosquitoes, and sandflies abundance decreased with altitude but hemoparasite prevalence and intensity increased with altitude (Alvárez-Ruiz et al. 2018).

The large psammophilus is a generalist predator, which reduces the time spent searching for its prey, mostly arthropods. It can hunt waiting (sit and wait) or actively looking for prey. Their diet varies seasonally and geographically depending on the availability of prey. Its spectrum of prey is very wide: it is mainly composed of ants, beetles, homoptera, orthoptera, diptera and spiders, although they are also generally found in a proportion less than 5%, solifugal, scorpionids,





chilopods, odonates, blatoids, mantoids, dermaptera, phasmids, neuroptera, lepidoptera and gastropods (Moreno-Rueda et al. 2018). Large adults may occasionally prey on juveniles of *Acanthodactylus erythrurus* (Salvador 2011).

There is a great variety of predators that prey on the large psammodromus, such as raptors as *Falco tinnunculus*, *Falco naumanni*, *Circaetus gallicus*, *Circus pygargus*, *Aegypius monachus*, *Milvus milvus*, *Accipiter gentilis*, *Elanus caeruleus*, *Buteo buteo*, *Bubo bubo*, *Tyto alba*, *Athene noctua* and *Otus scops* and other groups of birds such as *Bubulcus ibis*, *Ciconia ciconia*, *Sturnus unicolor*, *Burhinus oedicephalus*, *Lanius excubitor* and *Upupa epops* (Salvador 2011). Among their terrestrial predators, there are snakes such as *Coronella girondica* (Fig. 18), *Malpolon monspessulanus*, *Macroprotodon cucullatus* and *Vipera latastei*, and lizards such as *Acanthodactylus erythrurus* and *Timon lepidus*, and mammals such as *Sus scrofa*, *Felis silvestris*, *Genetta genetta*, *Martes foina*, *Vulpes vulpes* and *Lutra lutra* (Salvador 2011).



**Figure 18.** The southern smooth snake *Coronella girondica* is one of the numerous terrestrial predators of the large psammodromus. The photo was taken by Mar Comas.



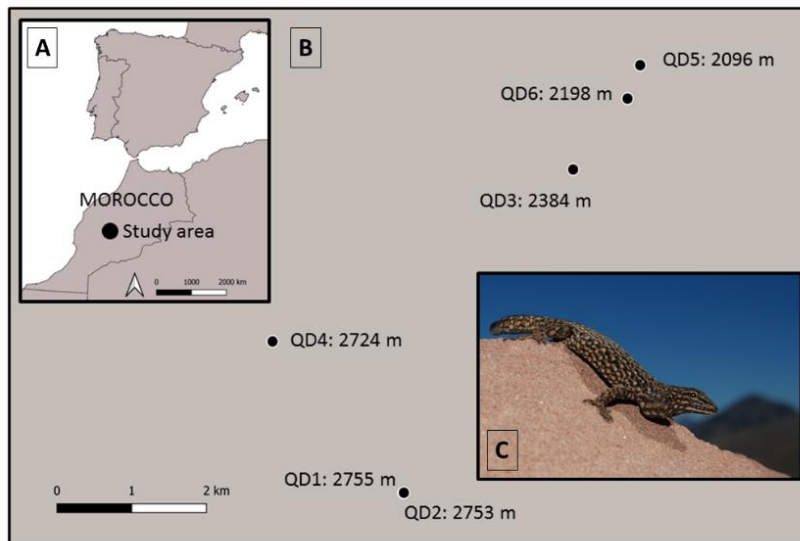
Their main antipredator strategy is flight from predators. Both adults and juveniles take refuge mainly in leaf litter or surrounding shrub vegetation, although some individuals use rocks, or even climb tree trunks up to two meters (Martín and López 1995). In fact, lizards adjust its escape distance according to the predator's attack speed (Cooper 2006). Moreover, the two lighter dorsolateral lines may confuse the predator during the flight, because it makes it difficult to perceive the direction of the race. The tail is autotomic, so that the lizard detaches itself mechanically if it receives a physical attack. In this way, the predator can attack the tail while the lizard flees.



## MATERIAL AND METHODS

### High Atlas Fieldwork

In the High Atlas, fieldwork was performed at Oukaïmedene, in the High Atlas of Morocco (31.21° N, 7.83° W; Fig. 19).



**Figure 19.** (A) Location of the study area in Morocco. (B) Spatial distribution of the six localities sampled, indicating their altitude above the sea level. Notice that QD1 and QD2 were so closed that the point was indistinguishable. (C) Photography of the Atlas day gecko.

The climate at Oukaïmedene is Mediterranean, with precipitation values around 400-500 mm per year. Average temperatures range from 23.5 °C in the warmest month (July) to -2.7 °C in the coldest month (January), with 82 to 139 days of frost per year (Aloui Haroni et al.



2009). Snowfall occurs mainly between November and March. However, sometimes it remains until the end of May (Bouazza et al. 2016). The vegetation consists of grasslands, with richer vegetal communities at lower elevations (Mediterranean shrubs of *Retama spp.* and Atlas Cedars *Cedrus atlantica* plantations).

In the High Atlas the sampling was conducted in September 2010. The specimens of Atlas day gecko were captured by hand and later released at the capture site. No specimen suffered permanent damage as a consequence of this study and sampling was done following animal care protocols. A total of 42 adult males, 38 adult females and 11 juveniles were captured from different elevations (ranging from 2096 to 2755 m).

For every gecko captured, I took the following biometrics: snout-vent length (SVL), measured from the tip of the snout to the posterior border of the vent; head length (HL), from the tip of the snout to the posterior border of the collar; head width (HW), at the broadest point of the head (to the nearest 0.1 mm), tail length (TL), from the snout to the tip of the tail, and we also recorded whether the tail was intact or regenerated. Weight was measured using a precision balance (Denver Instrument Company Model 100A; accuracy 0.1 g). Body condition was estimated as residuals from the logarithm of body weight regressed against the logarithm of SVL (Schulte-Hostedde et al. 2005). Sex was distinguished visually, male geckos having dark spots on their bellies and undersides of their legs (Blouin-Demers et al. 2013).

The captured individuals were assigned to two elevation categories, i.e. under 2500 m ( $n = 21$ ) or above 2500 m ( $n = 59$ ), based

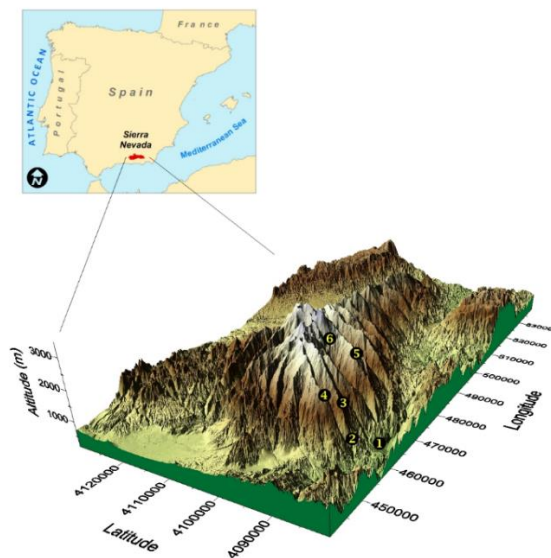


on the preferred habitat of the gecko, more abundant above 2500 m asl. Thus, elevation was considered to depend on two intervals, low elevation (L) from 2096 to 2385 m and high elevation (H) from 2725 to 2755 m.

I obtained tissue samples from individual tails, which were stored immediately in vials containing 1.5 mL of 70% ethanol. After that, the wound was disinfected with chlorhexidine.

### Sierra Nevada Fieldwork

In Sierra Nevada fieldwork was performed in the southern side of the mountain ( $36^{\circ}54'57.8318''\text{N}$ ,  $3^{\circ}37'59.8698''\text{W}$ ) with six sampling points separated each other by approximately 500 m in elevation (at 300 m, 700 m, 1200 m, 1700 m, 2200 m, and 2500 m asl., Figs. 20 and 21).



**Figure 20.** Study localities in Sierra Nevada where the six-sampling points along the elevational gradients were marked as 1 to 6, from the lowest (300 m a.s.l.) to the highest altitude (2500 m a.s.l.).



**Figure 21.** The highest study locality in Sierra Nevada at 2500 m, near Poqueira refuge. The photo was taken by Mar Comas.

In Sierra Nevada the climate of the study area is Mediterranean with air temperature ranging from  $25.0 \pm 5.09$  °C to  $17.2 \pm 4.87$  °C, depending on altitude (Zamora-Camacho et al. 2013). Due to variation in vegetation structure and cover along altitude, vegetation structure was measured in each location. Transects of 50 meters were performed randomly, and both the presence or absence of vegetation and the height and the kind of plants were recorded every meter.

During a long-term study, 492 *Psammodrommus algirus* (255 females and 237 males) were sampled from 2010 to 2013, during their activity season, from March to October. Males were distinguished because they have wider heads, larger and more numerous femoral pores



in the hind limbs and orange spots in the corners of their mouths. Different biometrics were taken, such as snout-vent length (SVL; in mm) and tail length, with a millimetre-marked ruler, and body mass with a digital balance (model CDS-100, precision 0.01 g). We estimated the body condition index (BCI) as the residuals of the regressing log mass on log SVL. This widely used index represents the relative energy reserves of an animal (Schulte-Hostedde et al. 2005). We also recorded whether the tail was intact or regenerated.

Gravid females, recognized by palpation of developing eggs inside the trunk, were translated to a lab and placed in individual terrariums (100×20×40 cm) with a heat cable at one end of the cage, switched on three hours a day (11 h-14 h) to allow thermoregulation, indirect access to sunlight, and water (in form of aqueous nutritious gel) and food (*Tenebrio molitor* larvae) ad libitum. The substrate was bare soil from the study area. We maintained eggs laid in terrariums until hatching. Then, we took a portion of the tail of hatchlings for genetic analyses (see below). In order to avoid pseudoreplication, only one neonate per litter (N = 37) was randomly used for telomere analyses. Females and their neonates were released at the point the female was caught. No lizard died or suffered permanent pain during the study.

We obtained tissue samples from individual tails, which were stored immediately in vials containing 1.5 mL of 70% ethanol. Moreover, we cut one toe per individual. We also stored this toe in vials containing 1.5 mL of 70% ethanol. After that, the wounds were disinfected with chlorhexidine.





## Stable isotopes analysis

We obtained muscle tissue samples from individual tails, which were stored in vials containing 1.5 mL of 70% ethanol for stable isotope determination (Hobson et al. 1997).

To reduce isotopic variability due to differential content of lipids, these were extracted with several rinses of chloroform-methanol (2:1, v/v) (Logan et al. 2008). Once lipids were extracted, each sample of muscle was dried to constant mass powdered and homogenized and aliquots of 0.3 mg were placed into tin capsules and crimped for combustion. Isotope analyses were carried out at the Scientific Services of the University of Barcelona by means of elemental analysis isotope ratio mass spectrometry using a Thermo Finnigan Flash 1112 (CE Elantech, Lakewood, NJ, USA) elemental analyser, coupled to a delta isotope ratio mass spectrometer via a CONFLOIII interface (Thermo Finnigan MAT, Bremen, Germany). Stable-isotope ratios are expressed in  $\delta$  notation as parts per thousand (‰) deviations from the international standards (Pee Dee Belemnite marine limestone for  $\delta^{13}\text{C}$  and atmospheric nitrogen for  $\delta^{15}\text{N}$ ; Fry 2006), according to the equation:  $\delta X = [(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}] \times 1000$ , where X is  $^{13}\text{C}$  or  $^{15}\text{N}$  and R is  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ . Based on several hundred replicates of laboratory standards, we estimated laboratory measurement error to be  $\pm 0.1\%$  for carbon and  $\pm 0.2\%$  for nitrogen.

There were no significant differences in isotopic proportions in geckos with complete or regenerated tail (MANOVA test; Lambda de Wilks = 0.98,  $F_{(2, 88)} = 0.98$ ,  $P = 0.38$ ). Moreover, we collected samples





from the more representative plant species in high and low altitude, which were used to estimate the signature baseline.

### **Parasite fieldwork**

Captured geckos were carefully inspected searching to count mites on their body surface, especially those under scales. Probability of infection was estimated as the presence or absence of mites, and intensity as the number of mites in infested individuals (Margolis et al. 1982; Bush et al. 1997; Rózsa et al. 2000).

### **Telomere length measurement**

Once in the laboratory, we stored tail samples at  $-20\text{ }^{\circ}\text{C}$  until assayed. We extracted DNA from the epidermis using a high-salt DNA extraction protocol (Lahiri and Nurnberger 1991). This method eliminates the use of toxic reagents such as phenol or chloroform, at the same time that yield a high amount of good-quality DNA. We used a Nanodrop (Thermo Scientific) to quantify the quantity and quality of DNA. Since storage conditions, extraction method, or tissue type can affect telomere length measures (Nussey et al. 2014) we used the same conditions for all samples to avoid confounding factors.

We quantified relative telomere length through quantitative polymerase chain reactions (qPCRs), which is one of the most widely used method for estimating telomere length (Nussey et al. 2014). We compared the cycle threshold (Ct) of telomeric sequences with the Ct of



a control sequence that is autosomal and non-variable in copy number (Cawthon 2002; Nussey et al. 2014). We used previously published primer sequences for GAPDH and telomere fragments (Criscuolo et al. 2009). As a reference sequence, we amplified GAPDH sequences using 5'-AACCAGCCAAGTACGATGACAT-3' (GAPDH-F) and 5'-CCATCAGCAGCAGCCTTCA-3' (GAPDH-R) as forward and reverse primers, respectively. The use of GAPDH as a single copy gene is widely spread in telomere studies with vertebrates and has been previously used in other studies in sand lizards (Pauliny et al. 2018). In addition, we confirmed that the among-individual variation was low for this gene (average Cq value 25.37 showing a Standard Error of 0.32). For telomere sequences, we used Tel1b Tel2b as forward and reverse primers, respectively:

5'CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3'  
(Tel1b)

5'-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3'  
(Tel2b)

Conditions of qPCR for GAPDH fragment consisted of 10 min at 95 °C and 40 cycles of 10 sec at 95 °C, 20 secs at 58 °C, and 1 min at 72 °C, and for telomere fragment of 10 min at 95 °C, and 10 secs at 95 °C, 20 secs at 58 °C, and 1 min at 72 °C. We conducted qPCR assays for each gene in separate plates on a LightCycler 480 (Roche) and ran a melting curve from 65 to 95 °C, as a final step in each qPCR to check for specific amplicons. The melting curve showed a normal shape, indicating a high specificity of GAPDH and telomere primers. For each sample, we added 20 ng of genomic DNA and used both sets of primers



at a final concentration of 100nM in a 20  $\mu$ L master mix containing 10  $\mu$ L of Brilliant SYBR Green (QPCR Master Mix, Roche). All samples were run in duplicate. Samples with a coefficient of variation higher than 5 % were measured again. We calculated qPCR-plates efficiency by including five serial diluted standards in triplicate (120, 40, 10, 2.5 and 0.66 ng/uL both for GAPDH and telomere sequences), obtained from a golden standard sample containing a pool of three samples from each elevation. We calculated the relative telomere length by applying the following formula (Pfaffl 2001):  $[(\text{Etelomere})\Delta\text{Ct telomere (control-sample)}]/[(\text{EGAPDH})\Delta\text{Ct GAPDH (control-sample)}]$ ; where Etelomere and EGAPDH are the qPCR efficiency of telomere and GAPDH fragment, respectively;  $\Delta\text{Ct telomere (control-sample)}$  and  $\Delta\text{Ct GAPDH (control-sample)}$  is the deviation of standard – telomere or GAPDH sequences for each sample, respectively. Efficiencies of qPCR were  $1.99 \pm 0.02$  S.E. and  $1.93 \pm 0.02$  S.E. for GAPDH and telomere fragments, respectively. The intra-assay CV% was 4.07% for GAPDH gene and 1.38% for telomere gene. The inter-assay CV% was 11.26% for relative telomere length. All the  $R^2$  of the standard curves were higher 0.985.

### **Estimation of age with skeletochronology**

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 (Comas et al. 2016) which is one of the most accurate age estimation techniques in animals. It has to take into account that toe clipping is a marking method frequently used in



lizards with limited impact on their welfare (Perry et al. 2011). 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). Moreover, I used all bones from fourteen *Psammoudromus algirus* from the scientific collection of the University of Granada, in order to assess the age estimate precision using different long bones.

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. 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 chlorhexidine. The toes were decalcified in 3% nitric acid for 3.5 h. Cross-sections (10  $\mu$ m) were prepared using a freezing microtome (CM1850 Leica) at the Centre of Scientific Instrumentation, University of Granada.

We did several trials to estimate the necessary time for decalcification. Finally, the samples were decalcified in 3% nitric acid for at least 3 hours and 30 minutes. Although we used only one phalanx per lizard, the phalanx number was assigned at random in order to examine whether different phalanges are more or less suitable for estimating age. The basal and middle phalanges of each finger provide



better resolution than does the most distal phalanx (Castanet and Smirina 1990). Decalcified samples were conserved in PBS (phosphate-buffered saline) solution with sucrose (for cryoprotection) for at least 48h at 4°C, until they were sectioned with the freezing microtome.

Glass-slides were treated (before use) with a solution of glycerol (5 gr/L) and chromium (III) potassium sulphate (0.5 gr/L). Glycerol is used to improve the placing of the cross-sections on glass-slides. Chromium (III) potassium sulphate is used to improve sample conservation before applying the staining and fixation protocol. Glass slides were submerged for at least 5 min in glycerol-chromium (III) potassium sulphate solution and then oven-dried for 24 h. Finally, the treated slides were refrigerated until used.

For cross-sections, samples were embedded in gel O.C.T. (optimum cutting temperature) and then sectioned at 10-12  $\mu\text{m}$  for phalanges and 14-30  $\mu\text{m}$  for the longer bones, using a freezing microtome (CM1850 Leica) at the Centre of Scientific Instrumentation of the University of Granada. Cross-sections were stained with Harris hematoxylin for 20 min and then the excess stain was rinsed by washing the slides in tap water for 5 min. Later, stained sections were dehydrated with an alcohol series (70%, 96%, 100%; 5 min each), washed in xylol for 15 min, and were finally fixed with DPX (mounting medium for histology) and mounted on slides.

Thereafter, cross-sections were examined for the presence of LAGs using a light microscope (Leitz Dialux20) at magnifications from 50 to 125X. With a ProgresC3 camera, we took several photographs (a mean of 33.67 per individual) of various representative cross-sections,



discarding those in which cuts were unsuitable for examining the LAGs. We selected diaphysis sections in which the size of the medullar cavity was at its minimum and that of the periosteal bone at its maximum (Castanet and Smirina 1990).

Because inferring age from the number of LAGs requires knowing the annual number of periods of arrested growth for each year, we compared our age estimates with juveniles, whose age is known -less than a year-. Multiple LAGs were found in juveniles in their first period of growth -which was counted as a single year-, while adults usually showed a single additional LAG per year. When various LAGs were found much closed, they were considered as a single LAG in order to avoid overestimation of age. Different LAG pattern depending on age may be explained because juvenile lizards usually are more active and show activity periods more intermittent than adults.

I counted the number of LAGs detected in the periosteal bone three times independently on different occasions, always blindly regarding the specimen identification (Sagor et al. 1998). Lizards were collected in summer. Therefore, LAGs deposited during previous winter hibernation were discernible from the outer edge of the bone. Consequently, the outer edge of the bone was not counted as a LAG.

### **Data analysis**

Data were checked for outliers, normality and homoscedasticity following Zuur et al. (2010). In order to satisfy model assumptions, when was needed, those variables which not followed a normal distribution or



were heteroscedastic were transformed with the Naperian logarithm. When possible, analyses were done with parametric statistics. Means of descriptive statistics are given with the standard deviation (SD). All analyses were performed with R 3.5.1 (R Development Core Team). Specific statistics for each chapter are detailed in statistical analysis or data analysis section.



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**Chapter I: Stable isotope analysis reveals variation in trophic niche depending on altitude in an endemic alpine gecko**

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## **Stable isotope analysis reveals variation in trophic niche depending on altitude in an endemic alpine gecko**

**Mar Comas, Daniel Escoriza, Gregorio Moreno-Rueda**

### **Abstract**

Interspecific competition is considered a major determinant of ecological niche. It is hypothesized that increased competition should reduce niche breadth. However, there are scarce field tests on this hypothesis. Here, we test this central hypothesis in ecology by using the Atlas day gecko *Quedenfeldtia trachyblepharus*. This alpine gecko faces fewer competitors as altitude increases, and thereby, we predict that this species should increase niche breadth and relevant fitness parameters with altitude. We tested this prediction by analyzing the isotopic signature of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ). Our results reveal that specimens from higher altitudes showed higher values for both carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotopes, had better body condition and a greater isotopic breadth when compared to specimens from lower altitudes. Altitudinal variation in carbon values was not explained by variation in isotopic concentration in the baseline of the trophic chain. Therefore, our findings support the prediction that relaxed interspecific competition favours increased trophic niche breadth. These results also suggest that global warming may represent an important threat for this species, as it may provoke the ascent in altitude of competitors, with negative consequences for the conservation of this endemism.



## **Introduction**

The concept of the ecological niche is a cornerstone of Ecology (Chase & Leibold 2004). The ecological niche occupied by one species has important implications, not only for the ecology of that species but also for the ecology of sympatric species (Schoener 1974). This is because the fundamental niche of a given species is predicted to be limited by competition with sympatric species, and species may be forced to occupy a smaller niche (the realized niche; e.g., Pianka 1974). Consequently, interspecific competition is considered a major factor determining the ecological niche breadth of sympatric species (May & MacArthur 1972). Increased competition for food can cause a decrease in trophic niche breadth, growth rate and/or body condition (Jones & Barmuta 1998; Gómez-Mestre & Tejedo 2002; Benard & Middlemis Maher 2011). Therefore, in a gradient of decreasing competition, niche breadth should increase. Because of this, species inhabiting islands or alpine environments may occupy a wider trophic niche due to a lower presence of competitors (MacArthur, Diamond, & Karr 1972).

In mountain habitats, there is a gradual turnover in species composition with altitude (Jankowski, Ciecka, Meyer, & Rabenold 2009). As a general pattern, species richness decreases with altitude or has a peak at middle elevations, which depends mainly on the gradient of temperature and moisture (Van Rensburg, Chown, & Gaston 2002). Those spatial changes in species composition with altitude (e.g. Fig. 1) imply changes in the number of competitors: at higher altitudes, there is an ecological release in competition, and consequently, an expansion of



trophic niche space is expected (Nimmo, James, Kelly, Watson, & Bennett 2011).

High mountains present harsh environmental conditions for life, as low temperatures imply reduced primary productivity, which is moreover concentrated in a small window of time. For this reason, this type of environment presents lower species richness, although it shows elevated rates of endemism (Terborgh 1977). In order to complete their life cycles, endemic alpine species display adaptations to the special environmental conditions under which they live (Arribas & Galán 2005). However, generalist species may occur in mountain communities, where they exploit these environments with lower competition, predation and, consequently, higher resource availability for a short period of time (Luiselli, Filippi, & Di Lena 2007). Nonetheless, the gradual constraints on the distributions of generalist species with altitude imply that alpine specialists have greater access to resources at high elevations, which in turn may increase population density (Díaz 1997).

In this study, we test the prediction that alpine species should increase their trophic niche breadth with altitude, as a consequence of reduced competition. We also expect that species inhabiting communities with fewer competitors will present better body condition. We analysed these predictions by exploring variations with altitude in the trophic niche breadth of an endemic alpine gecko, the Atlas day gecko *Quedenfeldtia trachyblepharus*, using a stable isotope approach. It is endemic to the mountain range of the High Atlas and occurs from 1,400 m altitude, reaching up to 4,000 m (Bons & Geniez 1996; Schleich, Kästle, & Kabish 1996; Fig. 1); it is the only alpine gecko of the



Mediterranean region. The Atlas day gecko is the dominant species in lizard communities from the alpine stage in our study area, although it becomes scarcer as altitude decreases, coinciding with an increase in the number of competitors (Fig. 1). When different species coexist, competition may result in reduced abundance, fecundity and growth (e.g., Eccard & Ylönen 2002). Therefore, if competition is higher at low altitude, we predict that this gecko's trophic niche should be narrower and that its body condition should worsen with decreasing altitude.

We investigated the trophic niche of the Atlas day gecko by using stable isotope analysis. Given that the frequency of isotopes varies throughout the trophic levels, the isotopic composition of animal tissues reflects dietary signatures of the consumed resources, as well as its position in the trophic chain (Fry 2006). This approach provides a more stable signal over time than those obtained from stomach contents or faeces (Seminoff, Jones, Eguchi, Jones, & Dutton 2006; Warne, Gilman, & Wolf 2010; Rosenblatt & Heithaus 2013). Then, stable isotope analysis became a powerful tool to overcome some of the limitations of conventional dietary studies, such as differential digestibility of prey items or their inability to reflect longer-term diet. Therefore, stable isotope analysis allows the evaluation of the trophic niche breadth of a species (Bearhop, Adams, Waldron, Fuller, & Macleod 2004; Newsome, Del Rio, Bearhop, & Phillips 2007). For our study, we used stable carbon ( $^{13}\text{C}/^{12}\text{C}$ ,  $\delta^{13}\text{C}$ ) and nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ,  $\delta^{15}\text{N}$ ) isotopes, the most frequently used isotopes for studying trophic relationships (Kelly 2000). The large dietary diversity within reptiles suggests that stable isotope analysis (SIA) can be broadly applied for studying ecological questions in this



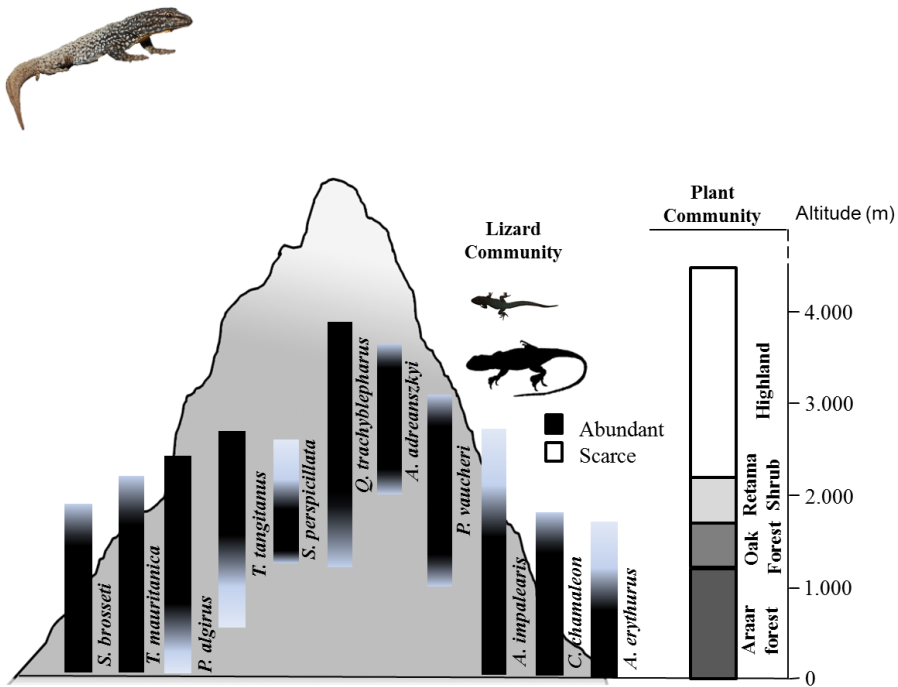
group (for example, Willson, Winne, Pilgrim, Romanek, & Gibbons 2010; Brischoux, Bonnet, Cherel, & Shine 2011; Murray & Wolf 2013).

## **Material and methods**

### **Study area**

The study zone is Oukaïmedene Plateau in the High Atlas of Morocco (31.21° N, 7.83° W). The climate is Mediterranean, with precipitation values around 400-500 mm per year. Average temperatures range from 23.5 °C in the warmest month to -2.7 °C in the coldest month, with 82 to 139 days of frost per year (Aloui Haroni, Alifriqui, & Simonneaux 2009). The vegetation consists of dry and wet grasslands at the plateau of Oukaïmedene with richer vegetal communities at lower altitudes (Mediterranean shrubs of *Retama spp.* and Atlas Cedars *Cedrus atlantica* plantations).

In this region, endemic alpine reptile species co-occur with other species with broader ranges, including species from Eurasia together with species of Mediterranean and Ibero-Maghrebian range (Bons & Geniez 1996; Schleich et al. 1996; Fig. 1).



**Figure 1.** Altitudinal distribution of lizard community inhabiting Oukaimeden, High Atlas (Bons & Geniez 1996; Schleich et al. 1996).

Above 2,500 m.a.s.l., the Atlas day gecko shares its habitat mainly with the lizard *Atlantolacerta andreanszkyi* (Bons & Geniez 1996). Nevertheless, populations below 2,500 m.a.s.l. must share the habitat with an increasing diversity of other species of scansorial lizards with similar or slightly higher mass (*Podarcis vaucheri*, *Scelarcis perspicillata*, *Tarentola mauritanica*) and ground-dwelling species as *Psammmodromus algirus*, but also species of larger size such as *Timon tangitanus* and *Agama impalearis* (Bons & Geniez 1996; Schleich et al. 1996). Also, there is a replacement of saurophagic snakes with altitude, with *Malpolon monspessulanus* at low altitudes and *Vipera monticola* at higher altitudes (Schleich et al. 1996).





## Sampling

Sampling was carried out in September 2010. The specimens of Atlas day gecko were captured by hand and after manipulation were released at the capture site. No specimen suffered permanent damage as a consequence of this study. A total of 42 adult males, 38 females and 11 immatures were captured from different altitudes (ranging from 2,096 m a.s.l. to 2,755 m a.s.l.) at six different sample points. We obtained muscle tissue samples from individual tails, which were stored immediately in vials containing 1.5 mL of 70% ethanol for posterior stable isotope determination (Hobson, Gibbs, & Gloutney 1997). There were no significant differences in isotopic proportions in geckos with complete or regenerated tail (MANOVA test; Lambda de Wilks = 0.98,  $F_{(2, 88)} = 0.98$ ,  $P = 0.38$ ). In addition, we took the following biometrics: snout-vent length (SVL), measured from the tip of the snout to the posterior border of the vent; head length (HL), from the tip of the snout to the posterior border of the collar; and head width (HW), at the broadest point of the head (to the nearest 0.1 mm). Weight was measured using a precision balance (Denver Instrument Company Model 100A; accuracy 0.1 g). Body condition was estimated as residuals from the body length-body weight linear regression (Green 2001). Moreover, we collected samples from the more representative plant species in high and low altitude, which were used to estimate the signature baseline.



## Stable isotopic analyses

To reduce isotopic variability due to differential content of lipids, these were extracted with several rinses of chloroform-methanol (2:1 v/v) (Logan, Jardine, Miller, Bunn, Cunjak et al. 2008). Once lipids were extracted, each sample of muscle was dried to constant mass powdered and homogenized and aliquots of 0.3 mg were placed into tin capsules and crimped for combustion. Isotope analyses were carried out at the Scientific Services of the Barcelona University by means of elemental analysis-isotope ratio mass spectrometry using a Thermo Finnigan Flash 1112 (CE Elantech, Lakewood, NJ, USA) elemental analyser, coupled to a delta isotope ratio mass spectrometer via a CONFLOIII interface (Thermo Finnigan MAT, Bremen, Germany). Stable-isotope ratios are expressed in  $\delta$  notation as parts per thousand (‰) deviations from the international standards (Pee Dee Belemnite marine limestone for  $\delta^{13}\text{C}$  and atmospheric nitrogen for  $\delta^{15}\text{N}$ ; Fry 2006), according to the equation:  $\delta X = [(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}] \times 1,000$ , where X is  $^{13}\text{C}$  or  $^{15}\text{N}$  and R is  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ . Based on several hundred replicates of laboratory standards, we estimated laboratory measurement error to be  $\pm 0.1\%$  for carbon and  $\pm 0.2\%$  for nitrogen.

## Data analysis

The covariation between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values and body condition, with altitude was analysed through Linear Models (LM) of Ordinal Least Squares (OLS) with sum of squares type III. Statistical



models were performed using the packages "vegan" (Oksanen, Kindt, Legendre, O'Hara, Stevens et al. 2013) and "car" (Fox & Weisberg 2009) in R (R Development Core Team 2012). Specimens were classified by sex (immature, which not present adult colouration [Blouin-Demers, Lourdais, Bouazza, Verreault, El Mouden et al. 2013], were not included in the analyses) and assigned to two altitude intervals (above and below 2,500 m). This altitudinal threshold coincides approximately with the described altitudinal limits for generalist species such as *P. vaucheri* and *S. perspicillata* (Bons & Geniez 1996). Sexual dimorphism was tested by including sex as a factor in the models. Differences in head size (width and length) were tested with ANCOVA, with altitude and sex as categorical factors and SVL as continuous variable to control for allometric relationships. The assumptions of normality and homoscedasticity of the residuals were checked with the Shapiro-Wilk's test and Levene's tests, respectively.

In order to analyse differences in isotopic niche breadth with altitude and considering sample sizes differed between altitudes ( $n = 59$  specimens for high altitude and  $n = 21$  specimens for low altitude), we used a randomly extracted sub-sample of high altitude specimens, thus comparing similar sample sizes ( $n = 21$  specimens for each altitude range). Differences in isotopic niche breadth between high and low lands were statistically tested by using  $F$ -ratio tests (Bearhop et al. 2004). Isotopic niche breadth was measured as the variance of carbon and nitrogen isotopic ratios. Spearman correlations were performed to test correlations among average isotopic variables and body condition with values of altitude of each sampling point. Pearson correlations were used



to examine correlations between morphological variables and isotopic niche. Mann Whitney *U*-test was performed for testing differences in isotopic ratios in plants between altitudes, and *F*-ratio test was done for testing differences in the variance of isotopic ratios with altitude in plants.

## Results

The concentrations of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were significantly higher in high altitude populations (Table 1; Fig. 2).

At the same time, body condition also increased with altitude (Table 1). These variables did not show significant differences between sexes or for the sex\*altitude interaction (Table 1), which implies that males and females overlap in their trophic niche (Fig. 2). Similarly, Spearman correlations showed an increase of average isotopic ratios with altitude for both nitrogen ( $r_s = 0.94$ ,  $P = 0.0048$ ) and carbon ( $r_s = 1$ ,  $n = 6$  sampling points), while average body condition trended to increase with altitude ( $r_s = 0.77$ ,  $P = 0.072$ ).

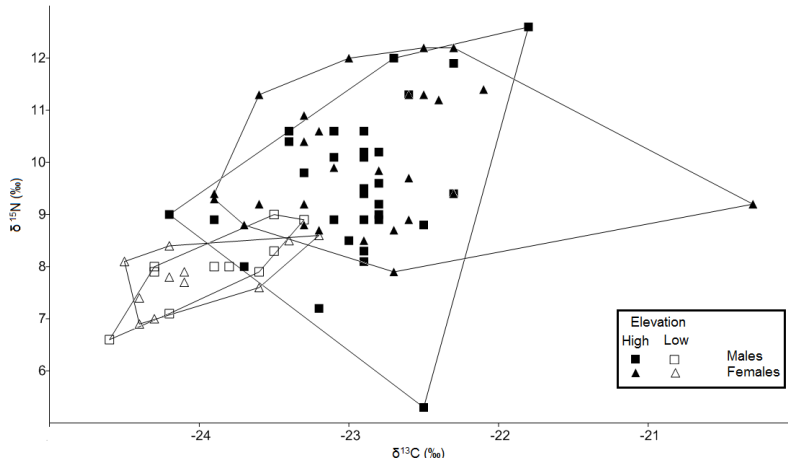
Isotopic variability of the Atlas day gecko increased with altitude for both  $\delta^{13}\text{C}$  (*F*-ratio = 2.96,  $P = 0.02$ ) and  $\delta^{15}\text{N}$  (*F*-ratio = 3.22,  $P = 0.01$ ; Fig. 2). We found a higher isotopic niche breadth in  $\delta^{13}\text{C}$  for females than for males at high altitude (*F*-ratio = 2.17,  $P = 0.04$ ; variance for females: 0.70, for males: 0.48). Females and males showed similar isotopic niche breadth for  $\delta^{15}\text{N}$  at high altitude (*F*-ratio = 1.62,  $P = 0.21$ ; variance for females: 1.18, for males: 1.50). At low altitude, females and males did not differ in their niche breadth for both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$



(respective variances: 0.59 vs. 0.71 and 0.43 vs. 0.45,  $F$ -ratios = 1.42 and 1.07,  $P = 0.59$  and 0.91).

**Table 1.** Stable-carbon ( $\delta^{13}\text{C}$ ), nitrogen isotope ( $\delta^{15}\text{N}$ ) concentrations (mean ‰) of lipid-extracted tails muscle tissues, body condition (residuals), SVL (mm), body mass (g), head length and head width (mm) in the Atlas day gecko at two altitudes by sex (raw data, mean  $\pm$  SE). Sample size is indicated ( $n$ ). The effect of altitude and sex ( $F$ -values,  $df = 1, 77$ ) is shown. Note that for head length and head width, SVL was included as covariate, and therefore  $df = 1, 76$  for these variables. Non-significant effects are indicated by "ns", §  $0.10 > P > 0.05$ , \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ . The interactions altitude\*sex interaction was non-significant in every case and were not included in final models.

Variable	Lowland (< 2500 m)		Highland (> 2500 m)		Altitude	Sex
	Females	Males	Females	Males		
	(n = 11)	(n = 10)	(n = 27)	(n = 32)	$F_{1, 77}$	$F_{1, 77}$
$\delta^{13}\text{C}$ (‰)	$-24.1 \pm 0.17$	$-23.9 \pm 0.18$	$-22.9 \pm 0.11$	$-22.9 \pm 0.10$	57.50***	0.00 <sup>ns</sup>
$\delta^{15}\text{N}$ (‰)	$7.80 \pm 0.37$	$7.96 \pm 0.39$	$9.94 \pm 0.24$	$9.62 \pm 0.22$	37.33***	0.51 <sup>ns</sup>
Condition	$-0.05 \pm 0.03$	$-0.05 \pm 0.03$	$-0.01 \pm 0.02$	$0.04 \pm 0.02$	9.11**	3.19 <sup>§</sup>
SVL	$42.6 \pm 0.71$	$46.2 \pm 0.74$	$43.0 \pm 0.45$	$45.1 \pm 0.42$	0.30 <sup>ns</sup>	21.93***
Mass	$2.20 \pm 0.11$	$2.69 \pm 0.12$	$2.37 \pm 0.07$	$2.78 \pm 0.07$	1.82 <sup>ns</sup>	25.00***
Head length	$10.79 \pm 0.21$	$11.91 \pm 0.23$	$10.78 \pm 0.14$	$11.63 \pm 0.13$	0.48 <sup>ns</sup>	18.49***
Head width	$7.54 \pm 0.15$	$8.29 \pm 0.16$	$7.86 \pm 0.10$	$8.33 \pm 0.09$	3.35 <sup>§</sup>	7.27**



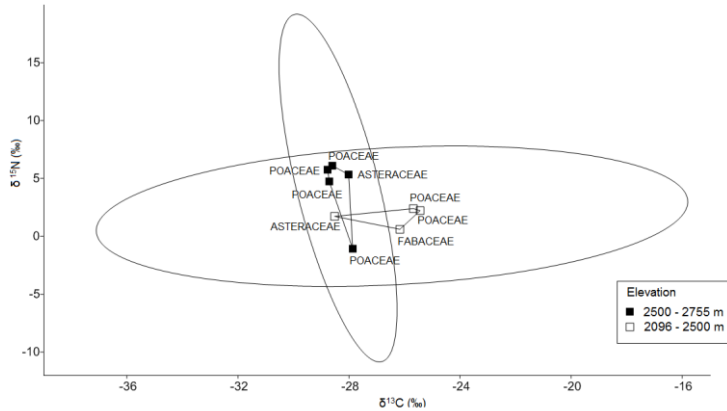
**Figure 2.** Bivariate plot of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for the Atlas day gecko (*Quedenfeldtia trachyblepharus*). Lines represent the “Convex Hull Polygon” proposed by Layman, Arrington, Montaña, & Post (2007). Black figures indicate high altitude and empty figures represent low altitude data. Squares are males, triangles are females.

We examined whether differences found in trophic niche and niche breadth may be a consequence of differences in morphology. Despite niche overlap between sexes, males showed larger snout-vent length, mass and head size (length and width, controlled for body size) than females (Table 1). In contrast, altitudinal variation in trophic niche and trophic niche breadth could not be explained by differences in morphology, as we found no differences in SVL, body mass or head morphology between altitudes (Table 1). Nevertheless, head width tended to increase with altitude ( $P = 0.071$ ; Table 1). We found no significant correlation among morphological variables and isotopic niche of geckos (always  $P > 0.10$ ).

The isotopic signature of plants in the environment did not differ significantly between altitudes, neither for nitrogen (mean  $\pm$  SD at high



altitude:  $4.93 \pm 2.99$  ‰, at low altitude  $1.86 \pm 0.81$  ‰;  $z = 1.47$ ,  $P = 0.142$ ), nor for carbon (high altitude:  $-28.78 \pm 0.42$  ‰; low altitude:  $-26.82 \pm 1.46$  ‰;  $z = -1.72$ ,  $P = 0.086$ ; Fig. 3).



**Figure 3.** Bivariate plot of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for the representative families of plants of the Oukaïmeden, High Atlas. Black lines represent the “Convex Hull Polygon” proposed by Layman, Arrington, Montaña, & Post (2007), and in grey are represented Gaussian bivariate ellipses corresponding to the 95% probability region. Full squares represent high altitude and empty squares represent low altitude.

Plant nitrogen variance tended to increase with altitude (Fig. 3), but not significantly (high altitude: 8.942, low altitude: 0.662;  $F$ -ratio = 13.52,  $P = 0.058$ ). However, plant carbon variance significantly decreased with altitude (high altitude: 0.174, low altitude: 2.118;  $F$ -ratio = 12.14,  $P = 0.0035$ ).



## Discussion

### Niche width

The Atlas day gecko showed significant isotopic variability depending on altitude. This isotopic variability was greater in populations located above 2,500 m, for both carbon and nitrogen isotopes. Greater isotopic variability at higher altitude suggests that niche breadth of the Atlas day gecko is greater as altitude increases (Bearhop et al. 2004). Consequently, the Atlas day gecko, as predicted, presented an expanded trophic niche where fewer competitor species co-occur.

Increased amplitude in gecko's nitrogen ratios with altitude could be explained by increased variance in baseline nitrogen isotopes with altitude, given that we found that  $\delta^{15}\text{N}$  variance in vegetation trend to increase with altitude. This explanation, nonetheless, may be discarded for the increase in carbon isotopic variance. That is, the increase in carbon isotope amplitude with altitude detected in the geckos cannot be explained by an increased variance in carbon isotopes in plants with altitude, as the variance in carbon isotopes in plants decreased with altitude. Moreover, variation in trophic niche amplitude with altitude cannot be explained by differences in body size or head morphology, although we found a non-significant trend for geckos at high altitude to have wider heads.

Therefore, our findings suggest that the Atlas day gecko modifies its trophic breadth as altitude increases, possibly as a consequence of





reduced competition. At high altitudes, the only abundant lizard species that appears in syntopy with Atlas day gecko (a scansorial lizard) is *A. andreanszkyi*, a ground-dwelling lacertid species. Below 2,500 m, the Atlas day gecko shares trophic resources with a larger number of competitors with similar scansorial habitats such as *P. vaucheri*, *S. perspicillata* and *T. mauritanica* (Carretero, Perera, Harris, Batista, & Pinho 2006). The presence of competing species is expected to restrict niche breadth (Bolnick, Svanbäck, Araújo, & Persson 2007).

### **Trophic position**

We found greater isotopic values for both carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) at higher altitude in the Atlas day gecko. This implies that at high altitude, gecko tissues contain a higher proportion of the heavy isotope for both C and N (Fry 2006). Stable carbon and nitrogen isotopes values are correlated with the trophic position in the trophic chain (Bearhop, Waldron, Votier, & Furness 2002; Fry 2006; Newsome et al. 2007). This is especially relevant for the nitrogen stable isotope, given that  $\delta^{15}\text{N}$  values in the predator tissues tend to be between 2.5‰ and 5‰ greater than those of their prey (Post 2002). Therefore, greater values suggest a higher trophic position for populations living at higher altitudes. Carbon stable isotope ratio also increases with trophic level, but to a much lesser degree than nitrogen (about 1‰; Inger & Bearhop 2008).

The fact that isotopic niche is different at higher altitude could reflect different isotopic concentration with altitude in the available prey



(Matthews & Mazumder 2004). For example, changes found in  $\delta^{13}\text{C}$  with altitude might reflect changes in C isotope in the primary producers (Warne, Pershall, & Wolf 2010). Regarding  $\delta^{15}\text{N}$ , previous studies found that this element is relatively constant with altitude (Graves, Romanek, & Navarro 2002; Hobson, Wassenaar, Milá, Lovette, Dingle et al. 2003), or even that  $\delta^{15}\text{N}$  decreases with altitude (Bai, Boutton, Liu, Wu, Archer, et al. 2009). Nevertheless, our findings suggest that differences in trophic niche with altitude cannot be explained by isotopic variation in the baseline, given that average isotopic signature in representative plants of the ecosystem did not vary with altitude.  $\delta^{15}\text{N}$  tended to increase with altitude (not significantly), but  $\delta^{13}\text{C}$ , in fact, tended to decrease with altitude, a pattern opposite to that found in geckos. Therefore, our findings suggest that the Atlas day gecko also modifies its diet as altitude increases, seeming to change to a higher trophic position as consequence of decreased presence of competitors.

The analysis of the isotopic signal revealed an overlap in the trophic niche occupied by both sexes. Trophic overlap between sexes has been also observed by analysing the diet by conventional methods (Carretero et al. 2006). Differences have been found in trophic niches occupied by males and females in other species, especially if the sexual dimorphism is marked (Perry 1996). In the Atlas day gecko, sexual dimorphism for body size and head morphology is pronounced (males have larger body size and head size), but this sexual dimorphism did not translate into sexual differences in trophic niche, which is consistent with other studies (Costa, Vitt, Pianka, Mesquita, & Colli 2008). In the Atlas day gecko, morphological differences between sexes could be



explained by different sexual roles, such as greater aggressiveness of males, as it occurs in other species of lizards (Lailvaux & Irschick 2007).

### **Body condition**

The Atlas day gecko showed a significant loss of physical condition as the number of competitors increased -at low altitude. This loss of body condition may be due to competition for food at lower altitudes, where trophic resources have to be partitioned within a richer community. This fact could also explain the decrease in density of the populations at lower altitudes (see Eccard & Ylönen 2002). However, we cannot rule out that better body condition in highland geckos is a consequence of increased fat-storing for hibernation (Warne, Gilman, Garcia & Wolf 2012).

### **Potential effects of global change**

Global change is causing changes in species distribution and abundance (review in Parmesan 2006; for reptiles see Sinervo, Méndez-de-la-Cruz, Miles, Heulin, Bastiaans et al. 2010; Moreno-Rueda, Pleguezuelos, Pizarro, & Montori 2012). Moreover, organisms restricted to alpine habitat suffer particularly from global warming (Raxworthy, Pearson, Rabibisoa, Rakotondrazafy, Ramanamanjato et al. 2008; Dubey & Shine 2011), and because the distribution of the Atlas day gecko is strictly limited to alpine areas, it may be particularly vulnerable (Thomas, Franco, & Hill 2006). Our study shows how niche width



changes with altitude and suggests that the Atlas day gecko is affected by interspecific competition. Therefore, we conclude that the Atlas day gecko might be potentially vulnerable to climate warming by upslope displacement of distributions of competing species. Species with narrower niches are at greater risk of extinction (Williams, Williams, VanDerWal, Isaac, Shoo et al. 2009). Because the Atlas day gecko shows a narrower niche where there are more competitors, upslope displacement of generalist species may form an important potential extinction threat for this species.

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**Chapter II: Body condition, sex, and elevation in relation to mite parasitism in a high-mountain gecko**

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## **Body condition, sex, and elevation in relation to mite parasitism in a high-mountain gecko**

**Mar Comas**

### **Abstract**

Parasitism is one of the main selective forces in nature, strongly affecting host fitness. Still, knowledge is incomplete concerning how variation in probability and intensity of infestation depends on body condition, sex or geographic variables. Here, I study the variation in probability and intensity of infestation of blood-sucking mites parasitizing the Atlas day gecko (*Quedenfeldtia trachyblepharus*) depending on host body condition, sex, and elevation, in the High Atlas (Morocco). Parasite prevalence was 58.75% and the probability of infection decreased with host body condition. However, parasitism intensity tended to increase with body condition. The parasite load ranged from 0 to 16 mites per individual, with a mean intensity of  $3.0 \pm 0.37$  (SE) in infested geckos. Prevalence was higher in males (2/3 parasitized) than in females (1/2 parasitized), but intensity did not significantly differ with sex. Neither prevalence nor intensity varied with elevation. In conclusion, geckos in better body condition harboured heavier parasite loads, but animals with the highest body condition were not infested. These findings suggest that animals with good body condition may tolerate heavier mite infestations, but only animals with the highest body condition may resist infestation.



## **Introduction**

Parasites, by taking resources from hosts, reduce host fitness (Schmid-Hempel, 2011). The deleterious effects of parasitism on host fitness include a decreased reproductive success as well as increased mortality (Rätti, Dufva & Alatalo, 1993; Hakkarainen et al., 2007). Moreover, parasites consume energy and hence often reduce host energy stores, harming body condition (Hakkarainen et al., 2007; Mougeot et al., 2009; Sánchez et al., 2018). Therefore, parasitism has consequences in most aspects of host's life history (e.g. Combes, 2001; Marzal et al., 2005). Altogether, parasites constitute a strong selective pressure, affecting host population structure and ultimately ecosystem functioning (Hudson et al., 2002). Consequently, the immune system of hosts has evolved different defence mechanisms against parasites, such as resistance and tolerance (Dawkins, 1990; Schmid-Hempel, 2011; Owen & Hawley, 2014). Resistance is the host's ability to reduce parasite establishment. Meanwhile, tolerance is the host's ability to withstand a given parasite load and maintain fitness in the presence of infestation (Råberg, Sim & Read, 2007; Råberg, Graham & Read, 2009; Ayres & Schneider, 2008; Medzhitov, Schneider & Soares, 2012). Then, to combat the parasite, a host may bolster its resistance to infection by reducing pathogen fitness or, alternatively, may increase its tolerance by reducing the damage caused by the pathogen (Ayres & Schneider, 2008). Nevertheless, the development of mechanisms against parasites, either resistance or tolerance, is costly in terms of energy, structural resources such as amino-acids, or the generation of free radicals (Toft & Karter,





1990; Schmid-Hempel, 2011; Demas & Nelson, 2012). Thus, there is often a trade-off between the immune response and other physiological demands such as self-maintenance, growth, and reproduction (Demas & Nelson, 2012).

Body condition typically reflects an animal's overall health, energetic state, and survival capabilities (Schulte-Hostedde et al., 2005; Beldomenico et al., 2008; Budischak et al., 2018). Examining the relationship between body condition and infestation may be a good proxy to measure parasite impact on the host (Sánchez et al., 2018). However, the relationship between host body condition and parasitism is complex (e.g. Amo, López & Martín, 2004; Knapp et al., 2019). Immune function is condition-dependent (Møller et al., 1998), and hosts in good condition may use more resources to resist infestation or to tolerate high intensities of parasitism (Budischak et al., 2018; Sánchez et al., 2018; Carbayo, Martín & Civantos, 2019). If hosts use resources to fight parasites, hosts in poor condition could suffer impaired immune defence that would lead to the greater probability of infection and heavier parasite loads, and ultimately higher mortality (Merino et al., 2000; Botzler & Brown, 2014). However, if the hosts with better body condition use more resources to tolerate heavier parasitic loads, then a positive relationship may be expected between host body condition and parasite intensity (Budischak et al., 2018). Moreover, other aspects may affect the relationship between body condition and parasite intensity. Parasites are expected to diminish the host body condition (Hatchwell et al., 2001; Garvin, Szell & Moore, 2006) and several studies reported negative associations between host body condition and infestation (Dawson &



Bortolotti, 2000; Mougeot et al., 2009; Cook et al 2013). Nevertheless, if heavily parasitized individuals in bad body condition suffer an increase in mortality, parasitized individuals may show a higher body condition compared to non-infested ones (Amo, López & Martín, 2005).

Parasitism often proves decisive in sexual-selection processes, females usually choosing less parasitized males (Hamilton & Zuk, 1982; Andersson, 1994; Able, 1996, Kelly et al., 2018). Overall, across species, females usually have stronger immune responses to parasite infestation than males (Klein, 2000; Klein, 2004; Roberts, Buchanan & Evans, 2004; Foo et al., 2017). Especially in reptiles, prevalence and intensity of ectoparasite infestations tend to be lower in females (e.g. Václav, Prokop & Fekiač, 2007; Dudek et al., 2016; Llanos-Garrido et al., 2017). Males have higher testosterone levels than females do, and testosterone (or certain behavioural and physiological processes associated with testosterone) may have an immunosuppressive effect (Roberts et al., 2004; Foo et al., 2017). Furthermore, males with higher testosterone levels typically show greater mobility, which may increase the exposure to parasites from infested conspecifics during encounters with females as well as during fights with other males (Olsson et al., 2000; Amo et al., 2005).

Environmental conditions such as community composition, temperature, and humidity affect parasite prevalence and load, and consequently host-parasite dynamics typically vary geographically (Poisot et al., 2017). In this sense, elevational gradients, which imply a huge variation in several biotic and abiotic factors, constitute a good model to examine geographic variation in host-parasite interactions. It is



generally thought that parasite intensity declines with elevation (Badyaev, 1997). As one ascends in elevation, ectoparasites are typically exposed to lower temperatures, reduced daily and annual time available to complete their life cycles and long periods of host hibernation. Altogether, these factors likely increase ectoparasite mortality (Postawa & Nagy, 2016). For lizard-mites systems, although increased mite prevalence with elevation has been reported (Spoecker, 1967; Llanos-Garrido et al., 2017), several studies indeed report reduced mite prevalence and load at higher elevations (e.g. Carothers & Jaksic, 2001; Álvarez-Ruiz et al., 2018; Carbayo et al., 2019).

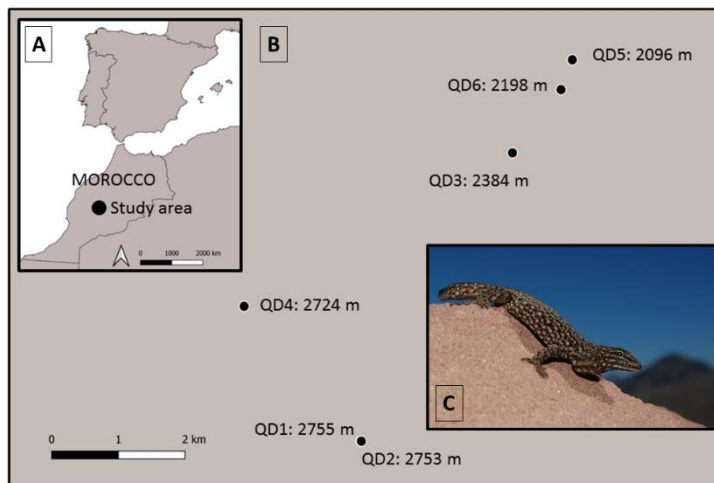
In this study, I examine the interaction between a reptile endemic to the Moroccan High Atlas, the Atlas day gecko *Quedenfeldtia trachyblepharus* (Boettger, 1874), and blood-sucking mites along an elevational gradient. Specifically, I test: 1) The relationship between the probability of infection and intensity of mites with gecko body condition. If geckos in better body condition are more resistant to parasites, then I predict a negative relationship between body condition and mite probability of infection and intensity; but if enhanced body condition improves tolerance to parasitism, then a positive relationship between body condition and parasite intensity is expected. 2) Differences in the probability of infection and intensity of mites depending on sex. I expect males to harbour more parasites than females, given their higher testosterone levels. 3) Differences in prevalence and intensity depending on elevation. Specifically, I expect reduced mite parasitism at higher elevations, given that harsh conditions at high elevations are expected to negatively affect parasite survival and life cycle.



## Material and Methods

### Study system

The study was performed along an elevational gradient at six localities in Oukaïmedene, in the High Atlas of Morocco (31.21°N, 7.83°W; Fig. 1).



**Figure 1** (A) Location of the study area in Morocco. (B) Spatial distribution of the six localities sampled, indicating their altitude above the sea level. Notice that QD1 and QD2 were so closed that the point was indistinguishable. (C) Photography of the Atlas day gecko.

The study area has a Mediterranean climate, with average annual precipitation of approximately 400–500 mm. Average temperatures range from 23.5°C in the warmest month (July) to  $-2.7^{\circ}\text{C}$  in the coldest month (January), with 82 to 139 days of frost per year (Alaoui Haroni,



Alifriqui & Simonneaux, 2009). Snowfall occurs mainly between November and March (corresponding to the hibernation period of geckos). However, sometimes snow cover remains until the end of May (Bouazza et al. 2016). The vegetation consists of grasslands, with richer vegetal communities at lower elevations (Mediterranean shrubs of *Retama spp.* and Atlas Cedars *Cedrus atlantica* plantations).

The Atlas day gecko (*Quedenfeldtia trachyblepharus*) is a member of the Sphaerodactylidae family endemic to the Moroccan High Atlas that inhabits from 1200 to 4000 m above the sea level but is particularly abundant from 2500 m on (Arnold, 1990; Bons & Geniez, 1996; Schleich, Kästle & Kabisch, 1996; Bouazza et al. 2016). This gecko is strictly diurnal (Blouin-Demers et al. 2013) and the dominant species in lizard communities at alpine levels becoming scarcer at lower elevations. Above 2500 m, the Atlas day gecko shares its habitat with the lizard *Atlantolacerta andreanszkyi* (Bons & Geniez, 1996). Nevertheless, populations below 2500 m must share the habitat with an increasing diversity of other lizard species such as *Podarcis vaucheri*, *Scelarcis perspicillata*, *Tarentola mauritanica*, *Psammodromus algirus*, *Timon tangitanus*, and *Agama impalearis* (Bons & Geniez, 1996; Schleich et al., 1996). The reproductive period for the geckos is from March to June (Bouazza et al. 2016).



## Sampling

Sampling was conducted in September 2010. The specimens of Atlas day gecko were captured by hand and later released at the capture site. No specimen suffered permanent damage as a consequence of this study and sampling was done following animal care protocols. A total of 42 adult males and 38 adult females were captured from different elevations (ranging from 2096 to 2755 m). The captured individuals were assigned to two elevation categories, i.e. under 2500 m ( $n = 21$ ) or above 2500 m ( $n = 59$ ), based on the preferred habitat of the gecko, more abundant above 2500 m asl. Thus, elevation was considered to depend on two intervals, low elevation (L) from 2096 to 2385 m and high elevation (H) from 2725 to 2755 m. Sex was distinguished visually, male geckos having dark spots on their bellies and undersides of their legs (Blouin-Demers et al. 2013). The snout-vent length (SVL) was measured from the tip of the snout to the posterior border of the vent with digital callipers (Gyros Digi-science Accumatic Pro, Gyros Precisions Tools, Inc, Monsey, NY, USA; accuracy 0.01 mm), and weight was recorded using a precision balance (Denver Instrument Company Model 100A; Denver Instrument, Bohemia, NY, USA; accuracy 0.1 g). Body condition was estimated as residuals from the logarithm of body weight regressed against the logarithm of SVL (Schulte-Hostedde et al., 2005). Captured geckos were carefully inspected searching to count mites on their body surface, especially those under scales. Probability of infection was estimated as the presence or absence of mites, and intensity as the



number of mites in infested individuals (Margolis et al. 1982; Bush et al. 1997; Rózsa, Reicsigel & Majoros, 2000).

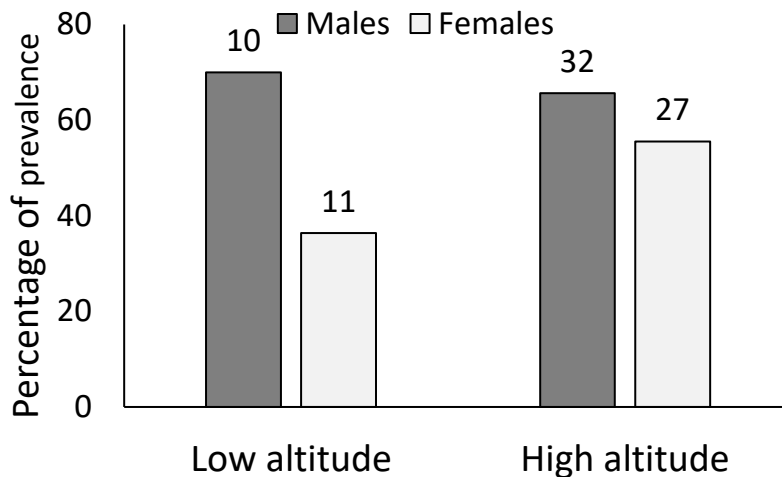
### **Statistical analysis**

To test for the variation in the probability of infection I used Generalized Linear Models (GLM) with binomial distribution, linked to a logit function, with body condition (continuous), elevation (two levels), and sex (two levels), as independent variables. Also, to test for variation in intensity, I performed several linear models (LM) with intensity (log-transformed) as a dependent variable, and body condition, elevation, and sex as predictors. No interaction between independent variables proved significant (results not shown), and hence interactions were not included in the final models. To select the best models I used Akaike Information Criterion (AIC) and I chose those with the smaller value of AIC (Quinn & Keough, 2002). Normality and homoscedasticity of variables and model residuals were checked following Zuur, Ieno & Elphick (2010). Some variables (such as intensity) were transformed with the Naperian logarithm to satisfy model assumptions. Basic statistics are given as mean  $\pm$  SE (standard error). All analyses were performed with R 3.5.1 (R Development Core Team, 2017).



## Results

Mite prevalence was 58.75% (47/80; Fig. 2). The best model depending on AIC was which included the three predictor variables, being this model indistinguishable of that which only included sex and BCI (Table 1).



**Figure 2** Prevalence (percentage of Atlas day geckos infested with mites) depending on elevation and sex (dark: males, white: females). The sample size is indicated over the bars.

The GLM showed a negative relationship between probability of mite infection and body condition (Estimate = -8.34,  $z = 2.66$ ,  $P = 0.008$ ; Fig. 3), as well as a significant variation with sex: higher probability of mite infection in males than in females (Males = 67%,  $n = 42$ ; Females = 50%,  $n = 38$ ;  $z = -2.03$ ,  $P = 0.042$ ), but no variation in probability of mite infection with elevation was found ( $z = -1.46$ ,  $P = 0.145$ ). When the





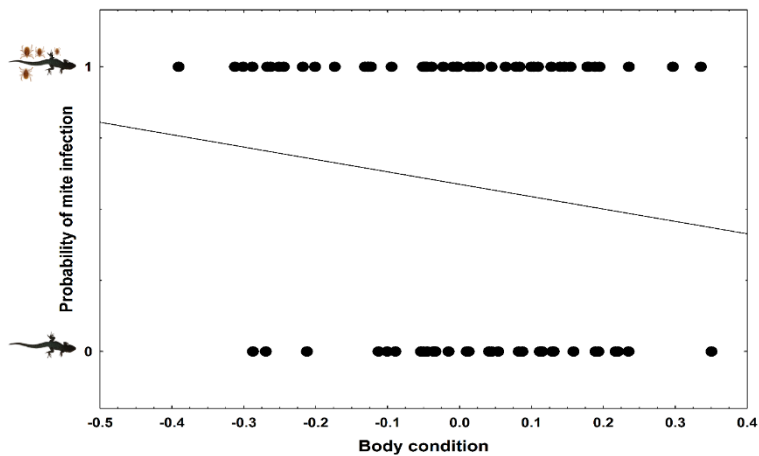
analyses were repeated without the two more extreme values, the effects of body condition and sex on probability of mite infection remained significant (data not shown for simplicity).

**Table 1:** AIC values and AIC increment of the models done for the probability of infection and intensity with the variables included in the models indicated, in bold those that were significant at  $P < 0.05$ .

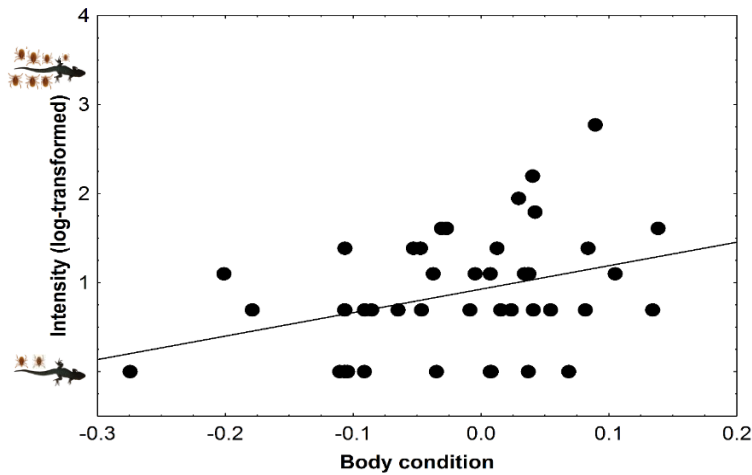
Variable	AIC	$\Delta$ AIC
<i>Probability of infection</i>		
<b>BCI, sex, altitude</b>	105.65	0.00
<b>BCI, sex</b>	105.82	0.17
<b>BCI, altitude</b>	108.01	2.36
<b>BCI</b>	108.16	2.51
Sex, altitude	110.15	4.50
Sex, altitude	111.78	6.13
Altitude	111.97	6.32
<i>Intensity</i>		
<b>BCI</b>	89.36	0.00
BCI, altitude	90.53	1.17
<b>BCI, sex</b>	91.33	1.97
Altitude	92.22	2.86
BCI, sex, altitude	92.46	3.10
Altitude, sex	93.88	4.52
Sex	95.28	5.92



The number of mites per host ranged from 0 to 16 mites, and in infested geckos, mean intensity was  $3.0 \pm 0.37$  mites per host ( $n = 47$ ). The best model depending on AIC was which included only BCI (Table 1). The LM analysing the relationship between parasite intensity and body condition was significant ( $t = 2.51$ ,  $P = 0.016$ , Fig. 4), but no significant differences in intensity were found depending on sex (Males =  $3.14 \pm 0.57$ ,  $n = 28$ ; Females =  $2.79 \pm 0.42$ ,  $n = 19$ ;  $t = -0.45$ ,  $P = 0.65$ ) or elevation (Low elevation =  $2.00 \pm 0.30$ ,  $n = 11$ ; High elevation =  $3.31 \pm 0.47$ ;  $n = 36$ ;  $t = -1.80$ ,  $P = 0.079$ ).



**Figure 3** Relationship between the probability of mite infection and body condition: Atlas day geckos with lower values of body condition were more likely to be infested with mites.



**Figure 4** Relationship between the intensity of mite infestation and Atlas day gecko's body condition.

## Discussion

The results in this study show complex relationships between gecko body condition and mite parasitism, probability of infection being lower in geckos having better body condition, but infestation tending to intensify with body condition. These apparently contradictory results suggest that the better the body condition of an individual, the lower the likelihood of being parasitized, but a good body condition also implied more tolerance to parasites, allowing the individual to deal with a greater parasitic load after the initial infestation (Amo et al., 2005). Anti-parasitic defences are costly (Hakkarainen et al., 2007), requiring the allocation of resources to the immune system. Therefore, individuals with a better body condition may invest more resources to their immune system to fight parasites, but also may invest more resources to deal with



parasites and to be more tolerant. Additionally, different mite species may specialize in geckos depending of its tolerance or resistance. Nevertheless, if the infestation reduces survival, only individuals in good body condition could survive and, consequently, individuals with the poorest body condition might have died before the sampling (Amo et al., 2005). This might explain the greater infestation in individuals with greater body condition.

As expected, prevalence proved greater in males than females, with two-thirds of the males but only half of the females parasitized. However, the intensity of infestation did not change according to sex, in agreement with another study conducted in the same area with the same species (Blouin-Demers et al. 2013). High testosterone levels may imply immunosuppressive effects that, particularly in reptiles, boosts ectoparasite intensity (Salvador et al., 1996; Olsson et al., 2000; Klukowski & Nelson, 2001). These factors may explain greater prevalence in males. However, males registered higher values of body condition than females (Comas, Escoriza & Moreno-Rueda, 2014). Better body condition implies more resources to fight parasites, allowing more resistance to parasites (Arriero et al. 2018), perhaps explaining why males do not show a higher intensity of infestation than females do, although the absence of significant interaction sex\*body condition does not support this contention. Still, males suffered more prevalence of mites. Higher mobility and more frequent social interactions of males may facilitate parasite transmission during contacts with females and in fights with other males (Olsson et al., 2000; Amo et al., 2005). If higher



male mobility applies to Atlas day geckos, increased mobility could explain their higher prevalence.

Selective pressures may vary with elevation as a consequence of the changing biotic and abiotic conditions (Körner, 2007). For example, at higher elevations, hibernation lasts longer, resulting in a narrow temporal window for both host and parasites to reproduce and grow. Moreover, at higher elevations, ectoparasites are exposed longer to lower temperatures which may increase ectoparasite mortality during hibernation (Postawa & Nagy, 2016). In fact, other studies show differences in parasitism with elevation, lizards typically harbouring more parasites at low elevations (e.g. Álvarez-Ruiz et al., 2018). However, this contention was not supported by the results, given that both prevalence and intensity did not differ with altitude. This result could be explained in several ways. The reptile community composition and gecko's population density change with elevation. The Atlas day gecko is alpine, being the most common reptile above 2500 m, with lower densities below this elevation (Schleich et al., 1996). The high density of individuals intensifies the risk of parasite transmission (Altizer et al., 2004; Hakkarainen et al., 2007). However, as elevation decreases, gecko densities also decrease but many other lizard species occur, with the reptile community being richer in the lowlands, which could act as a mite reservoir. Moreover, other factors could intervene, such as the fact that body condition is better in highland populations (Comas et al., 2014) and geckos in better body condition may divert more resources to fight parasites. Consequently, different conflicting effects could be acting: environmental conditions change with elevation, as well as gecko's body



condition, population densities, and reptile communities. The contradictory results concerning those reported in the literature suggest that elevational patterns in the lizard-mite interaction may be complex and specific for each system.

In conclusion, the findings suggest that the patterns of probability and intensity of infestation of mites parasitizing the Atlas day gecko did not vary with elevation, but are complex: factors encouraging a higher probability of infection not necessarily promoting higher intensity. Geckos in better body condition were less likely to be parasitized, probably as a consequence of a higher investment in resistance against mites. However, on being parasitized, geckos in better body condition tended to harbour more mites, either as a consequence of higher mortality of geckos in worse body condition, or because of a greater capacity in geckos in better condition to invest in tolerance to mites. Males were more likely to be parasitized than females. However, once infested, males and females did not differ in the intensity of the infestation.

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### **Chapter III: Age structure of a lizard along an elevational gradient reveals non-linear lifespan patterns with altitude**

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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 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.

## **Introduction**

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 shows 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 (Angilletta 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 *Psammmodromus 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 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).



**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

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, thus reducing their lifespan with respect to harsher areas (Reniers et al. 2015). In such cases, the 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). Besides, 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 (using 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.

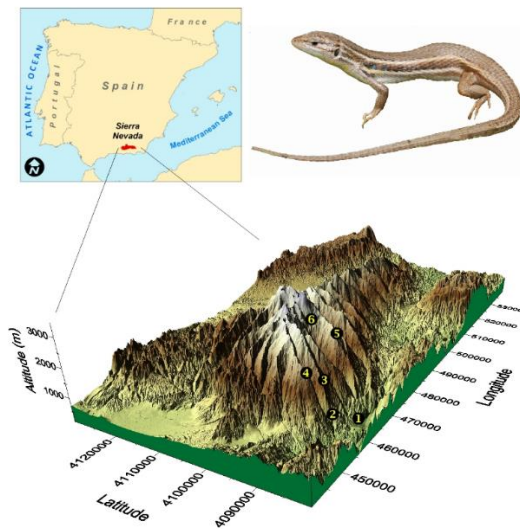




## Material and Methods

### 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 fieldwork 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).



**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.



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



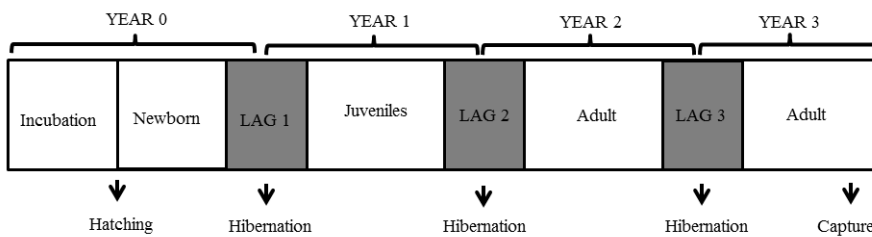
reproductive investment (Shine 1980), as a percentage of female body mass. Females and their offspring were released at the point where the female had been caught. No lizard died or suffered permanent injury in this study.

### **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 chlorhexidine. The toes were decalcified in 3% nitric acid for 3.5 h. Cross-sections (10  $\mu$ m) 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 $\times$  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 diaphyseal 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  $\pm 1$  year (Figure 2).



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

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:

$$\text{Lifespan} = 0.5 + 1/(1 - S), \text{ where } S \text{ is the survival rate.}$$

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  $i$ .

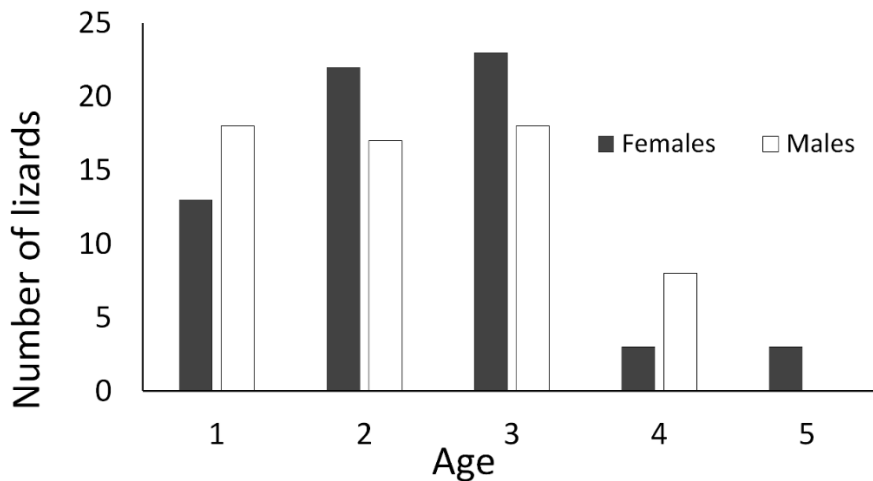
### **Statistical analyses**

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, the 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).



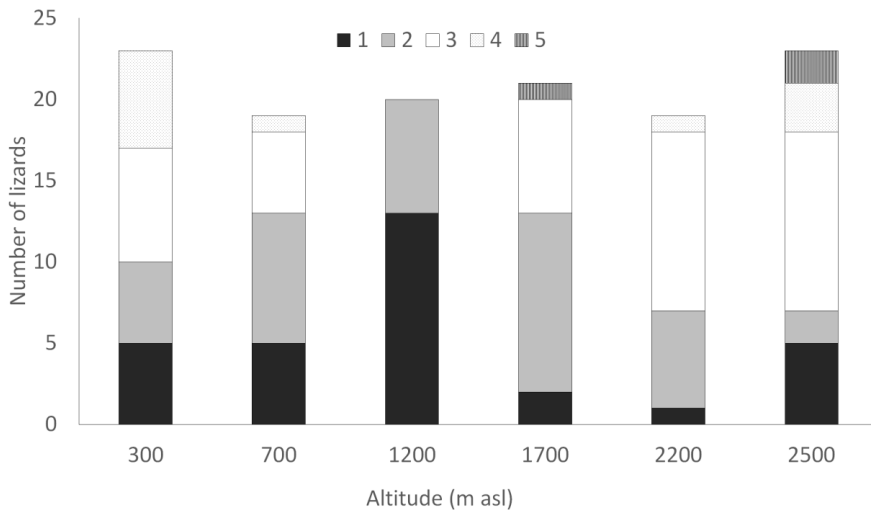
## Results

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).



**Figure 3A.** 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.

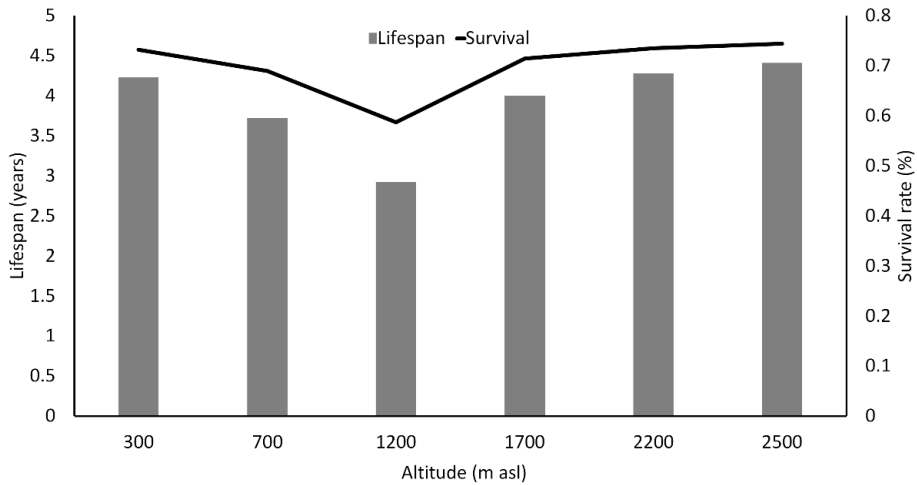
Nevertheless, females had a maximum lifespan of 5 years and males of 4 years. The lizards showed a similar frequency of individuals aged 1, 2 and 3 years, but there was a decrease of almost 50% in the number of lizards reaching the age of 4 years. The age frequency distribution varied significantly in function of altitude ( $\chi^2_{15} = 36.58, P = 0.001$ ; Figure 3B; sample sizes given in the figure).



**Figure 3B.** 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.

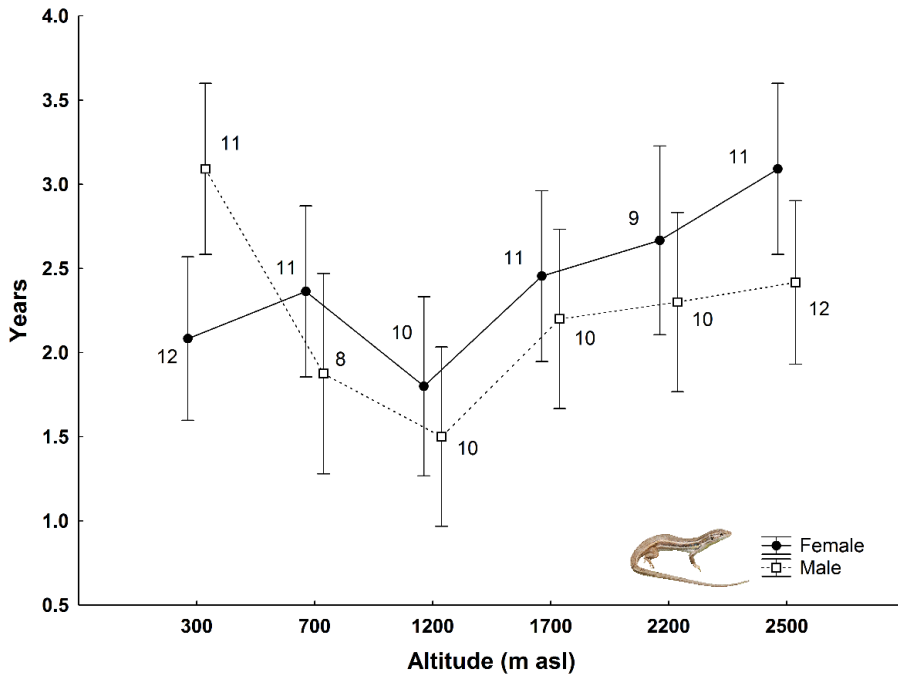
The frequency of individuals aged  $\geq 4$  years was lower at medium elevations than at low and high ones (only 1 of the 41 individuals at mid altitudes  $> 3$  years old, versus 7/42 at low and 6/42 at high elevations). The annual survival rate was about 0.70 at each elevation, except at 1200 m where 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).





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

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).

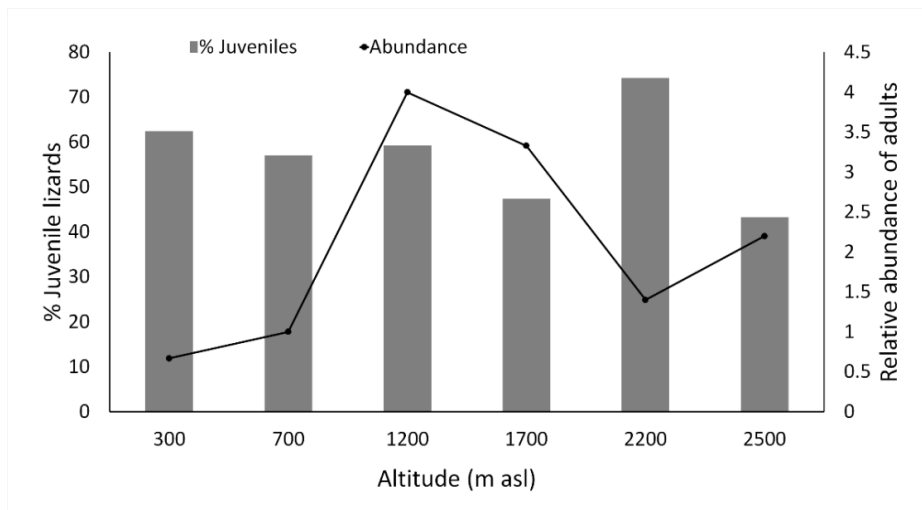


**Figure 5.** Average age (with 95% CI, vertical bars) of female (black dots, solid line) and male (white squares, dashed line) lizards depending on altitude. The sample size for each category is indicated on the graph, close to the corresponding data point.

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 ( $\chi^2_5 = 1.18$ ,  $P = 0.95$ ).

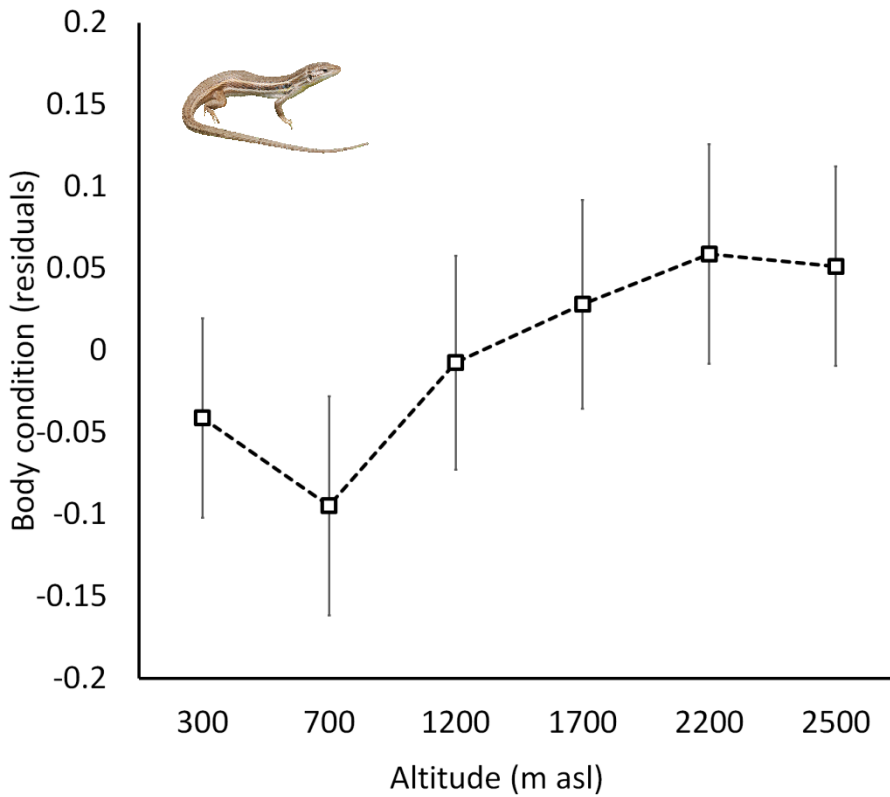


The relative abundance of adult lizards varied significantly with elevation ( $F_{5, 49} = 5.09$ ,  $P < 0.001$ ,  $n = 55$  samplings), presenting a maximum at mid elevations (1200 and 1700 m; Figure 6).



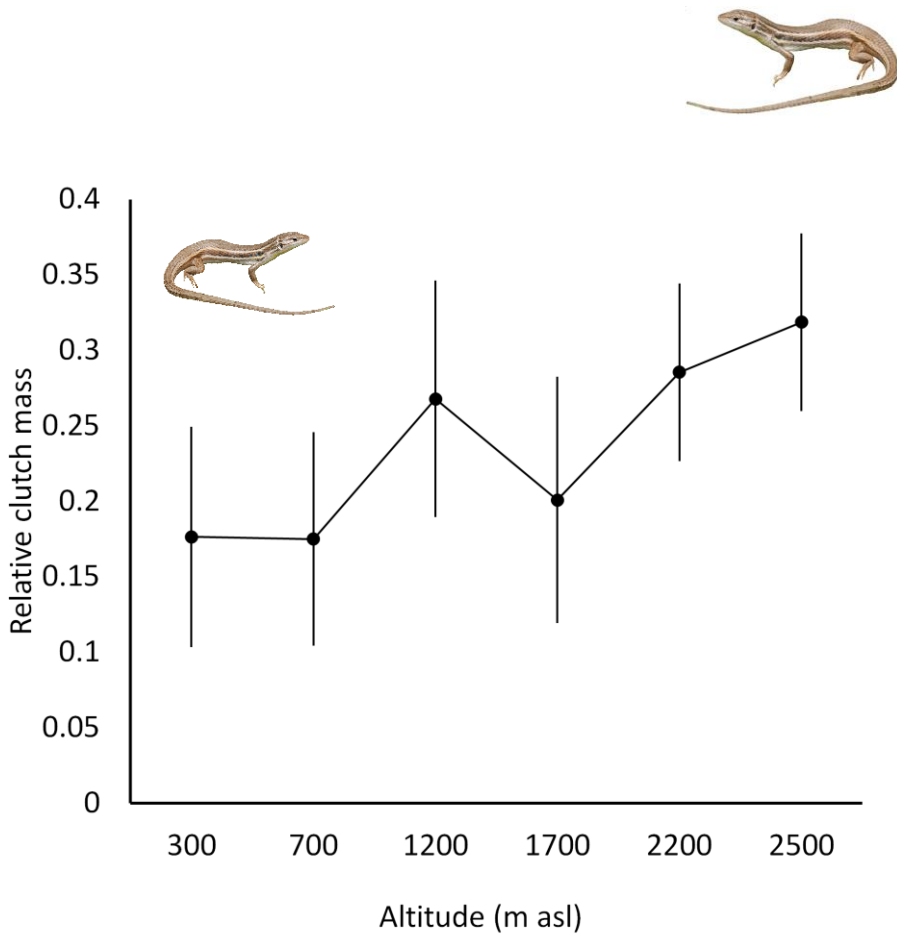
**Figure 6.** Maximal abundance values registered in transects for adult lizards (black line) and percentage of juvenile lizards (grey bars) depending on altitude.

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).



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

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).



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

## 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.

**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
<b>This study</b>		
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	
<b>Previous studies</b>		
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



The findings cannot be easily explained through either nonlinear cline 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.



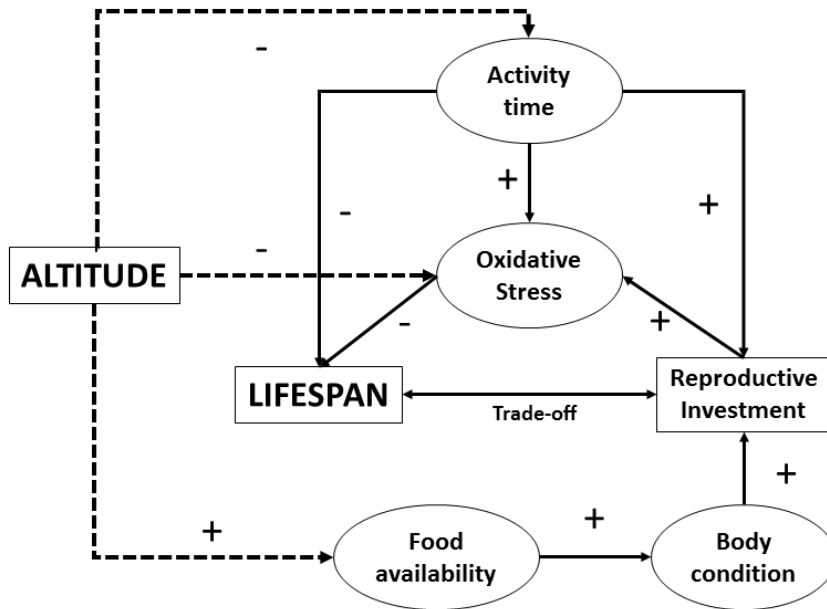
An initial question is why *P. algirus* lizards live longer at high elevations than at intermediate elevations. We assumed that alpine zones constitute marginal habitats because lizard density and thermal quality were lower than those at the middle elevations (Zamora-Camacho et al. 2013, 2016). Moreover, *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 several 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 and references therein). 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, despite 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).



**Fig 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.



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 higher mortality due to pathogens and parasites. However, senescence is strongly determined by telomere length (Hausmann 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 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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#### **Chapter IV: Variation in telomere length in a Mediterranean lizard along a 2200-m altitudinal gradient**

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## **Variation in telomere length in a Mediterranean lizard along a 2200-m altitudinal gradient**

Pablo Burraco, Mar Comas, Senda Reguera, Francisco Javier Zamora-Camacho, Gregorio Moreno-Rueda

### **Abstract**

The timing of organisms' senescence is developmentally programmed but also shaped by the interaction between environmental inputs and life-history traits. In ectotherms, ageing dynamics are still poorly understood despite their particularities concerning thermoregulation, regeneration capacity, or growth trajectory. Here, we investigate the role of life-history traits such as age, sex, body size, body condition, and tail autotomy (i.e self-amputation) in shaping telomere length of six populations of the Large Psammodromus (*Psammodromus algirus*) distributed across an elevational gradient from 300 to 2500 meters above the sea level. Additionally, we show in a review table the available information on reptiles' telomere length. We found longer telomeres in older lizards. We also observed that telomere length and body condition showed a positive relationship, which might be linked to a higher quality of somatic investment. Variation in telomere across elevation was explained by the age structure of lizards, thus altitude did not affect the telomere length of lizards. In contrast to our predictions, telomere length was unaffected by tail autotomy and was sex-independent, but positively correlated with body condition. Our cross-



sectional study shows that older lizards have longer telomeres, which might be linked to a higher expression of telomerase across their life or caused by the selective disappearance of individuals with shorter telomeres. Regarding the lack of impacts of elevation on telomeres of lizards, our results suggest that life-history traits such as age and body condition can be major drivers of telomere dynamics for this sand lizard, whereas environmental conditions apparently had scarce or no effects on their telomeres. Our findings emphasize the relevance of understanding species' life histories and habitat characteristics for fully disentangling the causes and consequences of lifespan trajectory in ectotherms.

## **Introduction**

Environmental conditions can modulate the physiology of individuals altering their rate of ageing (Monaghan 2007; Marasco et al. 2017; Ratikainen and Kokko 2019). The study of the evolutionary underpinnings of ageing has been a long-standing topic both in ecological and medical research. Despite several studies have shown the correlation between mitochondrial metabolism and variation in lifespan across taxa (Selman et al. 2012; Ziegler et al. 2015; Vágási et al. 2019), the machinery governing ageing still remains unclear. Most studies on ageing in vertebrates have been conducted in endotherms. In contrast, ectothermic vertebrates have received scarce attention despite their body temperature, metabolism, or growth trajectory are very sensitive to environmental changes, which might alter their ageing rate (Olsson et al. 2018; Monaghan et al. 2018; but see studies in fish, e.g. Reznick et al.



2002). This contrasts with the extensive literature on senescence of invertebrate ectotherms either in laboratory conditions or more recently in the wild (e.g. Rodriguez-Muñoz et al. 2019a, Rodriguez-Muñoz et al. 2019b). Understanding the link between environmental conditions, life-histories, and senescence in wild ectotherms will increase the current knowledge about their evolutionary and ecological dynamics, then allowing effective conservation actions.

Telomeres are non-coding repeated sequences (TTAGGG<sub>n</sub> in vertebrates) located at the termini of chromosomes, essential for maintaining genomic stability and for protecting cells from chromosome degradation and fusion (O’Sullivan and Karlseder 2010). Telomeric sequences shorten after each cell division due to the *end replication problem*, which occurs once the last piece of RNA primer is removed during replication and DNA cannot be extended (Allsopp et al., 1995). Likely linked to the replication problem, telomere length often decreases with age in endotherms, as observed in several mammals (e.g. Heidinger et al. 2012) and birds (e.g. Hall et al. 2004) although this pattern might not be universal, as discussed below. When telomeres become very short, apoptosis is induced (Aubert and Lansdrop 2008), however, the expression of telomerase (a reverse transcriptase enzyme that adds new telomere repeats to the 3’ end) can alleviate telomere erosion. Telomerase expression is often detected in the germline and in embryonic tissues both in endotherms and ectotherms (Ingles and Deakin 2016). Particularly in ectothermic vertebrates, telomerase is not only active early in life, but also in adult somatic tissues, like in the fish medaka (*Oryzias latipes*; Klapper et al. 1998) or in the African water



frog (*Xenopus laevis*; Bousman et al. 2003). In this line, longer telomeres have been found throughout larval development of the Atlantic salmon (*Salmo salar*, McLennan et al. 2016) and the common frog (*Rana temporaria*; Burraco et al. 2019), as well as during the first years of life in some reptiles (e.g. Olsson et al. 2010; Ujvari et al. 2017). In endotherms, longer telomeres after birth are not widespread and take place under very particular conditions, as during the active season of the edible dormouse (*Glis glis*; Hoelzl et al. 2016), or in some long-lived birds (“elongation hypothesis”, see Haussmann and Mauck 2007).

Such differences in telomere dynamics between ectothermic and endothermic vertebrates might be linked to organisms’ thermoregulation capacity and growth trajectories (typically, indeterminate growth in ectotherms versus determinate growth in endotherms), and explain lifespan across species (Jones et al. 2014). In reptiles, a paraphyletic group, 12 studies have investigated the variation in telomere length across individuals’ lifetime (Table 1).

Five studies found that telomeres shorten with age, whereas in three cases telomere length increased with age in at least one of the two sexes (Table 1). A quadratic sex-dependent relationship between telomere length and age was observed in three cases, i.e. telomeres increase their length until a certain age, and then shorten (Table 1). Meanwhile, three studies found no effect of age on telomere length in reptiles (Table 1). The high inter-species variation regarding the relation between telomere length across reptiles’ lifetime highlights the need of further research to unravel it.



**Table 1.** Summary of the studies describing the relationship between telomere length (TL) with age and/or other traits in reptiles.

Reference	Measured traits	Relationship between TL and age	Relationship between TL and trait(s)	Species
Girondot & Garcia 1998	Age	n.s. (embryos vs. adults)		<i>Emys orbicularis</i>
Scott et al. 2006	body size, sex		body length (-), sex (ns)	<i>Alligator mississippiensis</i>
Bronikowski 2008	Age	(-) (1-13 y/o)		<i>Thamnophis elegans</i>
Hatase et al. 2008	Age	n.s. (0-36 y/o)		<i>Caretta caretta</i>
Ujvari & Madsen 2009	age, survival	(+) for both sexes if including hatchlings (0-20 y/o), the same in longitudinal study (N=8)	longer TL females than in males, hatchling sexes (ns), recaptured hatchlings (ns), recaptured old pythons (+)	<i>Liasis fuscus</i>
Xu et al. 2009	age, sex	(-) for both sexes (3-10 y/o)		<i>Alligator sinensis</i>
Hatase et al. 2010	foraging behaviour		foraging behaviour (ns)	<i>Caretta caretta</i>
Olsson et al. 2010	length, age, activity, ticks, tail regeneration	(+) for females, (-) trend for males (2-8 y/o)	females: length (ns), activity (ns), ticks (ns), tail regeneration (ns); males: ticks (ns), badge size (ns), activity (+), length (-), tail regeneration (-)	<i>Lacerta agilis</i>
Olsson et al. 2011a	heritability, paternal age, offspring survival, malformations	(-) for sires, n.s. for sons (3-7 y/o)	capture probability of sires (+), offspring sex-ratio (+), TL of sons and paternal age at conception (-)	<i>Lacerta agilis</i>
Olsson et al. 2011b	Sex		longer TL in females than in males; females: lifespan (+), lifetime reproductive success (+), males: lifespan (ns), lifetime reproductive success (ns)	<i>Lacerta agilis</i>
Ballen et al. 2012	maternal and offspring TL, body mass, superoxide		offspring TL with maternal TL (+), maternal reproductive investment (+), offspring mass (-), offspring superoxide (-)	<i>Ctenophorus pictus</i>
Plot et al. 2012	sex, age, reproduction	n.s. (hatchlings vs. adults)	reproductive output (+), time to first breeding (+)	<i>Dermochelys coriacea</i>
Giraudeau et al. 2016	colour fading		colour fading (-)	<i>Ctenophorus pictus</i>
Rollings et al. 2017a	head colour, bib presence		competition ability (-), bib presence (-)	<i>Ctenophorus pictus</i>
Dupoué et al. 2017	body size, sex, altitude, extinction risk, Tmin		body size (ns), sex (ns), extinction risk (-), altitude(+), Tmin (+)	<i>Zootoca vivipara</i>



Rollings et al. 2017b	age, sex	quadratic for males, n.s. for females (2-6 y/o)	shorter TL in males, body length (ns), growth (ns), body condition (+)	<i>Thamnophis sirtalis parietalis</i>
Ujvari et al. 2017	age, survival	quadratic (1-8 y/o)	survival (ns)	<i>Chlamydosaurus kingii</i>
Pauliny et al. 2018	paternity probability		probability of siring offspring (+)	<i>Lacerta agilis</i>
Zhang et al. 2018	Temperature		temperature (-)	<i>Phrynocephalus przewalskii</i>
Rollings et al. 2019	sex, tissue		TL varies between sexes and among tissues	<i>Ctenophorus pictus</i>
McLennan et al. 2019	reproductive mode		longer TL in viviparous mothers and offspring than in oviparous	<i>Zootoca vivipara</i>
Singchat et al. 2019	age, sex	(-) for males, quadratic for females	shorter in females up to 5 years of age, older in females older than 5 years	<i>Naja kaouthia</i>
Fitzpatrick et al. 2019	Temperature		hot basking: increased TL. Cold basking treatment: maintained TL	<i>Niveoscincus ocellatus</i>
Burraco et al. (this study)	age, sex, altitude, autotomy, body mass, body condition	(+)	altitude (ns), sex (ns), autotomy (ns), body mass (+), body condition (+)	<i>Psammotromus algirus</i>

Telomere length, at a given ontogenetic point, is not only a function of cell replication but also of the organisms' ability to cope with stress across their life (Dugdale and Richardson 2018). This explains that, although telomere length at birth is highly heritable (Dugdale and Richardson 2018), telomere length early in life and its variation across life can be predictors of lifespan (Monaghan and Haussmann, 2006; Heidinger et al. 2012; Angelier et al. 2019). In vertebrates, harmful conditions often enhance glucocorticoids secretion, which can enhance metabolic processes. Among these processes, the induction of an oxidative state in cells can damage essential biomolecules like lipids, proteins or DNA (Isaksson 2015; Luceri et al. 2018), including telomeres (Haussman and Marchetto 2010; Monaghan 2014; Angelier et al. 2018); however, it has been mainly shown *in vitro* (e.g. von Zglinicki 2002)



whereas remains elusive *in vivo* (Boonekamp et al. 2017). As a consequence of the sensitiveness of telomeric sequences to environmental inputs, telomere length can be used as an indicator of the amount of stress accumulated by organisms across time (Young 2018).

Positive relationships between telomere length and organisms' life expectancy (e.g. Barret et al. 2013; Wilbourn et al. 2018), reproductive outcome (Eastwood et al. 2019), or immunocompetence (Alder et al. 2018) have been found in different taxa. In ectothermic vertebrates, telomere shortening is commonly associated with increased growth rate, bold personality, or predator exposure (reviewed in Olsson et al. 2018). Regarding the available information on telomere dynamics in reptiles, some studies show positive relationships between telomere length and fitness-related traits such as lifetime reproductive success or body conditions. In contrast, no correlation or unexpected relationships between telomere length and other organisms' traits have been found in other studies in reptiles (Table 1). For instance, one might expect telomeres to shorten as body size increases across lifetime since it implies more cellular replications. However, only a few studies on reptiles have observed a significant effect of body size or growth rate on telomere length (Table 1), unlike in fish (McLennan et al. 2016) or amphibians (Burraco et al. 2017a). Therefore, we should not make generalisations -so far- when discussing telomere dynamics in reptiles, being further studies still needed.

Mountains cover *circa* a quarter of the Earth's surface (Körner 2007) and impose wild populations to deep variations in biotic and physical conditions such as predators' abundance, temperature, or



ultraviolet radiation (Barry 2008). Consequently, several physiological adaptations to divergent habitats across altitude have been reported in different taxa (Bozinovic et al. 2011; Keller et al. 2013; Boyle et al. 2016). Such physiological adjustments often imply elevational variation in the relative allocation of energy expenditure to reproduction and somatic maintenance, which potentially can affect telomere dynamics (Stier et al. 2016). Typically, in fact, ectotherms live more time at high elevations (Morrison and Hero 2003; Munch and Salinas 2009; Zhang and Lu 2012; Scharf et al. 2015; Cabezas-Cartes et al. 2018). This generalized pattern could be a consequence of higher investment in somatic maintenance at high elevations, thereby telomere length is expected to lengthen with altitude. However, as far as we know, no study has analysed elevational variation in telomere dynamics in an ectotherm.

Here, we aim to understand the role of elevation on telomere length of the large *Psammodromus* (*Psammodromus algirus*). For this, we studied a substantial altitudinal gradient from 300 to 2500 above the sea level (m.a.s.l. thereafter) in Sierra Nevada mountain system (Spain). In this altitudinal gradient, as a consequence of environmental temperature decrease with altitude, these lizards decrease their activity while hibernation time increases with ascending elevation (Zamora-Camacho et al. 2013). Hibernation is known to speed down telomere attrition in some species (Hoelz et al. 2016; Kirby et al. 2019), therefore telomere dynamics of lizards at higher altitudes might benefit from longer hibernation. On the other hand, the Large *Psammodromus* is a heliothermic species, meaning that they spend a notable amount of time exposed to UV radiation. In this study system, lizards might devote more





time basking with increasing elevation where UV radiation is higher (Reguera et al. 2014a), thereby compensating the dwindling environmental temperature (Zamora et al. 2013; Zamora et al. 2016). The exposure to UV radiation is known to damage DNA (Cadet et al. 2014), thus lizards inhabiting at higher elevations might be more susceptible to telomere shortening. However, lizards at high elevations are darker, which probably protect them from UV radiation (Reguera et al. 2014a). Several studies have related parasites with telomere attrition (Hoelzl et al. 2016; Kirby et al. 2019). In our system, however, the load of ectoparasites and hemoparasites increases and decreases with elevation, respectively (Álvarez-Ruiz et al. 2018), precluding clear predictions of how parasites may affect lizard telomere dynamics across altitude. Indeed, probably as a consequence of reduced activity time and elongated hibernation, oxidative stress is lower in high-elevation lizards (Reguera et al. 2014b; Reguera et al. 2015). Overall, according to the information gathered on this species along the elevational gradient, we predict longer telomeres at high elevations.

The main goal of our study is to analyse how telomere dynamics vary with altitude. However, other factors may potentially affect telomere dynamics. We also considered sexual differences in telomere dynamics. *Psammotriton algirus* lizards do not show a noticeable sexual dimorphism, as both sexes often have similar body sizes, although males can show orange or blue colouration (Carretero 2002). Therefore, we did not expect particular differences in telomere length between sexes beyond the expected higher costs of reproduction in females. Given that telomere dynamics are strongly affected by age, we also estimated the

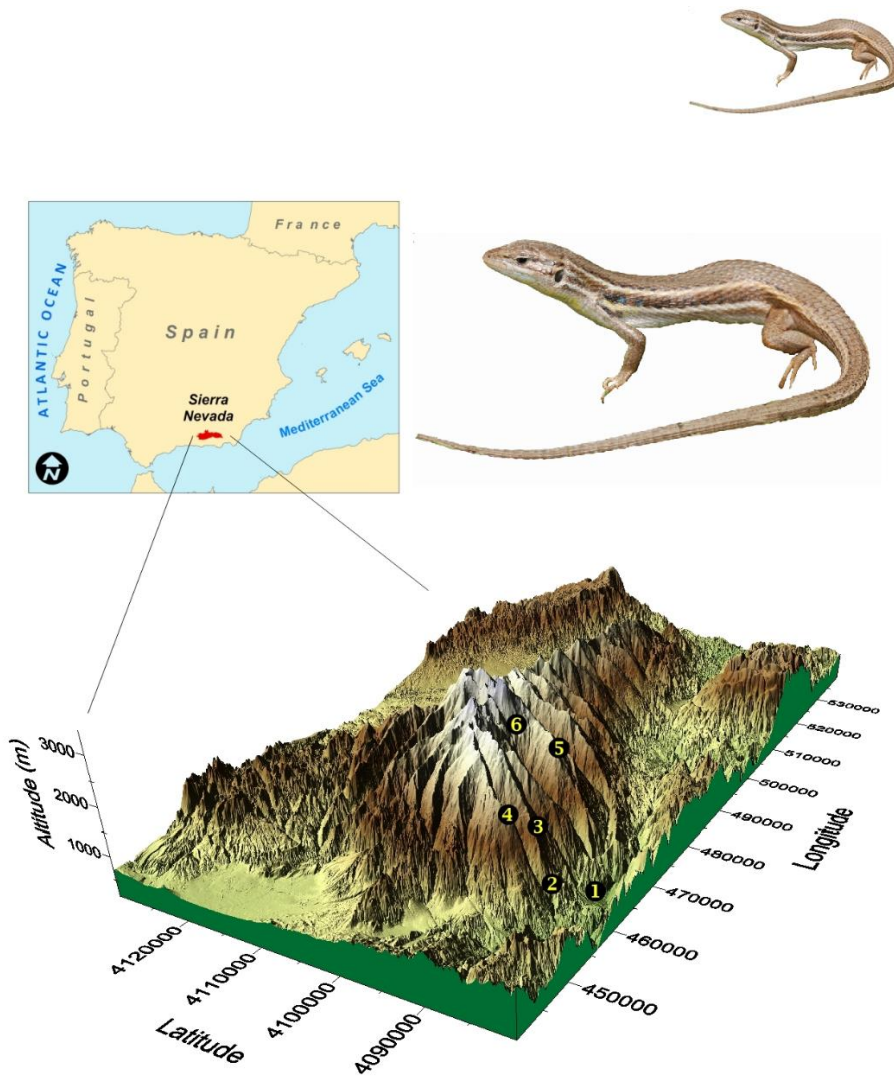


age of lizards sampled. Regarding the available information on telomere dynamics with age in reptiles, we might expect either a positive, negative or quadratic relationship between both factors (Table 1). Finally, we expected that tail loss, which reduces home range size in this species (Salvador and López 1995) and involves metabolic costs in lizards (REF), to enhance telomere shortening in this lizard species. Overall, we aimed to increase the current available information on telomere dynamics in a wild lizard, then gradually filling the gap of knowledge on telomere dynamics in ectothermic vertebrates.

## **Material and methods**

### **General procedures**

The lizard *P. 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 from south-western Europe and north-western Africa (Salvador 2015). In the Sierra Nevada mountain system (SE Spain), we sampled individuals from six populations, which inhabit at 300 (N=18), 700 (N=16), 1200 (N=18), 1700 (N=19), 2200 (N=15), and 2500 (N=20) m.a.s.l. (Fig. 1).



**Figure 1.** Sampling locations used in this study across an altitudinal mountain gradient (Sierra Nevada mountain system, SE Spain). Numbers from one to six correspond with each location, i.e. 300, 700, 1200, 1700, 2200, and 2500 m.a.s.l. respectively.

In total, we caught 106 individuals (50 males and 56 females): 7 in 2010, 28 in 2011, 65 in 2012 and 6 in 2013. Because lizards were part of a long-term study, we marked individuals by toe clipping, a marking method frequently used in lizards, and that have limited impact on their fitness (Perry et al. 2011). We conserved toe samples in ethanol and used them for age class determination using phalanx skeletochronology (more



details below). We collected a portion of the terminal region of lizards' tail (~ 1 cm) in the field -which should have had no effects on lizard welfare- and immersed it in an Eppendorf tube filled with 1.5 mL of absolute ethanol for genetic analyses. Many species of lizards can regenerate lost tails, a trait that has evolved with ecology and with the evolutionary history of lizard lineages (Higham et al. 2013). We took special care to disinfect the wounds caused by both toe clipping and tail sampling with chlorohexidine, closing the wounds with a tissue adhesive glue (Dermabond®).

We measured lizard body mass with a digital balance (Model Radwag WTB200; to the nearest 0.01 g) and SVL with a metal ruler (to the nearest 1 mm). We estimated the body condition index (BCI) as the residuals of the regressing log mass on log SVL. This widely used index represents the relative energy reserves of an animal (Schulte-Hostedde et al. 2005). We also recorded whether the tail was intact or regenerated. Males were distinguished from females mainly because they have more femoral pores in their hind limbs (Iraeta et al. 2011) and an orange spot in the corners of their mouths (Carretero 2002). Gravid females, recognized by palpation of developing eggs inside the trunk, were translated to a lab and placed in individual terrariums (100×20×40 cm) with a heat cable at one end of the cage to allow thermoregulation, indirect access to sunlight, and water (in form of aqueous nutritious gel) and food (*Tenebrio molitor* larvae) *ad libitum*. The substrate was bare soil from the study area. We maintained eggs laid in terrariums until hatching. Then, we took a portion of tail of hatchlings for genetic analyses (see below). In order to avoid pseudoreplication, only one



neonate per litter ( $N = 37$ ) was randomly used for telomere analyses. Females and their neonates were released at the point the female was caught. No lizard died or suffered permanent pain during the study.

### **Telomere length measurement**

Once in the laboratory, we stored tail samples at  $-20\text{ }^{\circ}\text{C}$  until assayed. We extracted DNA from the epidermis using a high-salt DNA extraction protocol (Lahiri and Nurnberger 1991). This method eliminates the use of toxic reagents such as phenol or chloroform, at the same that yield high amount of good-quality DNA. We used a Nanodrop (Thermo Scientific) to quantify the quantity and quality of DNA. Since storage conditions, extraction method, or tissue type can affect telomere length measures (Nussey et al. 2014) we used the same conditions for all samples to avoid confounding factors.

We quantified relative telomere length through quantitative polymerase chain reactions (qPCRs), which is one of the most widely used method for estimating telomere length (Nussey et al. 2014). We compared the cycle threshold ( $C_t$ ) of telomeric sequences with the  $C_t$  of a control sequence that is autosomal and non-variable in copy number (Cawthon 2002; Nussey et al. 2014). We used previously published primer sequences for GAPDH and telomere fragments (Criscuolo et al. 2009). As a reference sequence, we amplified GAPDH sequences using 5'-AACCAGCCAAGTACGATGACAT-3' (GAPDH-F) and 5'-CCATCAGCAGCAGCCTTCA-3' (GAPDH-R) as forward and reverse primers, respectively. The use of GAPDH as a single copy gene is widely



spread in telomere studies with vertebrates and has been previously used in other studies in lizards (Pauliny et al. 2018). In addition, we confirmed that the among-individual variation was low for this gene (average Cq value 25.37 showing a Standard Error of 0.32). For telomere sequences, we used:

5'CGGTTTGGTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3'  
(Tel1b)

5'-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3'  
(Tel2b)

as forward and reverse primers, respectively. Conditions of qPCR for GAPDH fragment consisted of 10 min at 95 °C and 40 cycles of 10 sec at 95 °C, 20 secs at 58 °C, and 1 min at 72 °C, and for telomere fragment of 10 min at 95 °C, and 10 secs at 95 °C, 20 secs at 58 °C, and 1 min at 72 °C. We conducted qPCR assays for each gene in separate plates on a LightCycler 480 (Roche) and ran a melting curve from 65 to 95 °C, as a final step in each qPCR to check for specific amplicons. The melting curve showed a normal shape, indicating a high specificity of GAPDH and telomere primers (Supplementary Material S3). For each sample, we added 20 ng of genomic DNA and used both set of primers at a final concentration of 100nM in a 20 µL master mix containing 10 µL of Brilliant SYBR Green (QPCR Master Mix, Roche). All samples were run in duplicate. Samples with a coefficient of variation higher than 5 % were measured again. We calculated qPCR-plates efficiency by including five serial diluted standards in triplicate (120, 40, 10, 2.5 and 0.66 ng/uL both for GAPDH and telomere sequences), obtained from a *golden standard sample* containing a pool of three samples from each



elevation. We calculated the relative telomere length by applying the following formula (Pfaffl, 2001):  $[(E_{\text{telomere}})^{\Delta C t_{\text{telomere (control-sample)}}}] / [(E_{\text{GAPDH}})^{\Delta C t_{\text{GAPDH (control-sample)}}}]$ ; where  $E_{\text{telomere}}$  and  $E_{\text{GAPDH}}$  are the qPCR efficiency of telomere and GAPDH fragment, respectively;  $\Delta C t_{\text{telomere (control-sample)}}$  and  $\Delta C t_{\text{GAPDH (control-sample)}}$  is the deviation of standard – telomere or GAPDH sequences for each sample, respectively. Efficiencies of qPCR were  $1.99 \pm 0.02$  S.E. and  $1.93 \pm 0.02$  S.E. for GAPDH and telomere fragments, respectively. The intra-assay CV% was 4.07% for GAPDH gene and 1.38% for telomere gene. The inter-assay CV% was 11.26% for relative telomere length. All the  $R^2$  of the standard curves were higher 0.985.

### **Estimation of age class with skeletochronology**

We estimated individual age class by phalanx skeletochronology (Comas et al. 2016), one of the most accurate techniques to estimate age in many vertebrates, including reptiles (Zhao et al. 2019). Vertebrate ectotherms show indeterminate growth, and consequently present a cyclic growth pattern in hard body structures such as bones, corresponding to alternate periods of growth and resting. This pattern is particularly marked in temperate climates, where age can be fairly estimated by counting annual growth rings in the phalanges (Comas et al. 2016). Growth rings are called lines of arrested growth (LAGs). Toes sampled were decalcified in 3% nitric acid for 3 h and 30 min. Cross-sections (10  $\mu\text{m}$ ) were prepared using a freezing microtome (CM1850 Leica), stained with Harris hematoxylin for 20 min and dehydrated



through an alcohol chain (more details in Comas et al. 2016). Next, cross-sections were fixed with DPX (mounting medium for histology), mounted on slides, and examined for the presence of LAGs using a light microscope (Leitz Dialux20) at magnifications from 50 to 125x. We took several photographs (with a ProgresC3 camera, at the University of Barcelona UB) of various representative cross-sections, discarding those photographs in which cuts were unsuitable for observing LAGs. The number of LAGs detected in the periosteal bone was independently and blindly counted three times by a single observer (MC) on three independent occasions.

### **Statistical analysis**

In order not to violate the assumptions of parametric statistics, we log-transformed relative telomere length, body mass, and body condition data. We examined the presence of outliers through a Cleveland plot (Zuur et al. 2010), which revealed that an individual had an extremely abnormal low value (almost zero) of relative telomere length, so we decided to omit this data from all the analyses. Cleveland plots also showed a possible outlier for body condition. Given that we are not sure whether this datum was an outlier, we followed recommendations by Quinn and Keough (2002) and, for analyses implying the variable body condition, we performed the test with and without the datum, and reported the two statistical results.

For the analyses, given that not all lizards had data for each variable (e.g., neonates always have complete tails) and several variables





presented collinearity (e.g. SVL and age), we first did several separate analyses in order to test whether variables potentially affecting telomere dynamics covaried with both relative telomere length or altitude. We performed linear models to check for sexual differences in body mass, age or relative telomere length. A chi-squared test was used to test whether the frequency of males and females differed with elevation. A linear model was also used to test relative telomere length according to the year of capture in order to evaluate possible cohort effects. Since we sampled lizards with intact tails ( $n = 44$ ) and regenerated tails ( $n = 58$ ), and tail regeneration could affect the length of telomeres in tail tissue (Anchelin et al. 2011; Tan et al. 2012; Alibardi 2016), we tested whether there were differences in relative telomere length between lizards with intact or regenerated tail through linear models. A chi-squared test was used to test whether the frequency of individuals with intact or regenerated tails differed with elevation.

We also performed linear models to test how relative telomere length varied with age class. A Spearman correlation was used to test if relative telomere length covaried with average age. We tested for the relationship between the two age and relative telomere length with both body mass and SVL by using Pearson correlations. The relationship between body condition and relative telomere length was also tested with Pearson correlations. A linear model tested for variation in body condition with altitude. We did not test for variation in body mass or SVL with altitude as we have already reported that these variables increase with altitude in our study population (Zamora-Camacho et al. 2014).

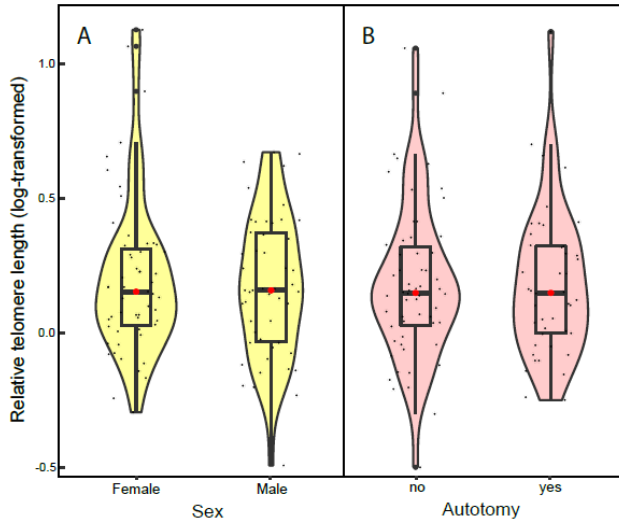


Finally, we tested the altitudinal variation in relative telomere length in lizards. Given that both altitude and age class were significantly related to relative telomere length, we tested the effect of the two variables as predictors on relative telomere length, as dependent variable. We also tested for the altitudinal variation in telomere length only for neonates, in order to know for the existence of genetically-determined variation in telomere length with altitude.

For all linear models carried out, we confirmed that the residuals of the models met parametric assumptions by visual inspection of plots (following Zuur et al. 2010). All statistical analyses were conducted in Statistica software (version 8.0).

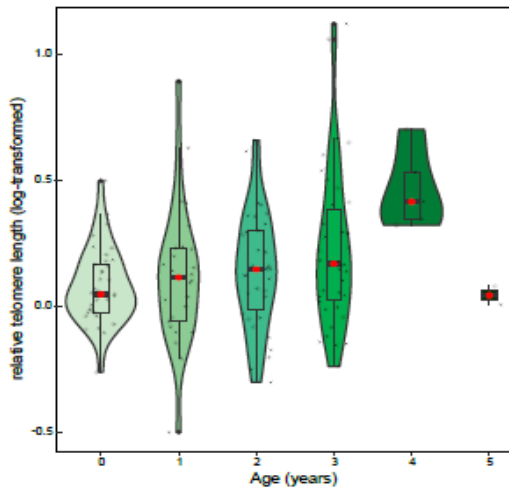
## Results

Lizards did not show sexual dimorphism in body mass ( $F_{1, 102} = 0.11$ ,  $P = 0.74$ ) and average age was similar for both sexes ( $F_{1, 103} = 1.70$ ,  $P = 0.20$ ). The frequency of male and female lizards did not differ across lizard populations ( $\chi^2_5 = 0.66$ ,  $P = 0.98$ ). Relative telomere length did not differ between sexes ( $F_{1, 103} = 0.30$ ,  $P = 0.59$ ; Figure 2A), being average values ( $\pm$  S.E.)  $2.06 \pm 0.31$  for females and  $1.74 \pm 0.15$  for males. The year of capture neither affected telomere length ( $F_{3, 101} = 0.45$ ,  $P = 0.72$ ). The frequency of lizards with autotomized tails did not vary among lizard populations ( $\chi^2_5 = 1.36$ ,  $P = 0.93$ ), and tail autotomy did not affect lizard telomere length ( $F_{1, 100} < 0.01$ ,  $P = 0.99$ ; Figure 2B).



**Figure 2.** Variation in relative telomere length between sexes (A) or in response to autotomy (B) in individuals of the Large Psammotriton (*Psammotriton algirus*). The red point shows the mean value at each age and the boxplot the interquartile range. The kernel density plot shows the probability density of data at different values.

The average telomere lengths ( $\pm$  S.E.) for lizards showing complete tail or tail autotomy were  $1.93 \pm 0.24$  and  $1.89 \pm 0.30$ , respectively. Relative telomere length increased with age class, at least until the fourth year ( $F_{5, 136} = 3.21$ ,  $P = 0.009$ , Fig. 3).

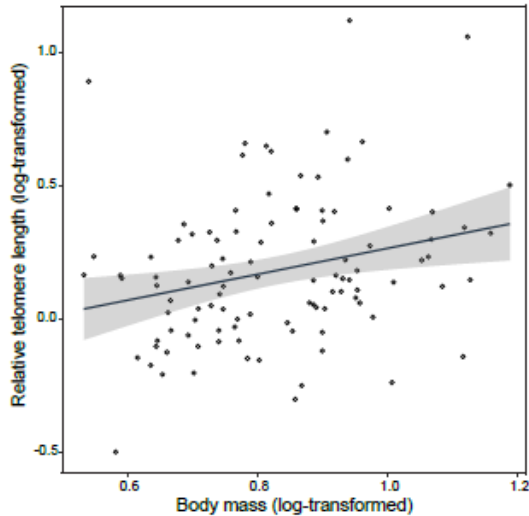


**Figure 3.** Variation in relative telomere length across lifetime of individuals of the Large Psammodromus (*Psammodromus algirus*). The red point shows the mean value at each age and the boxplot the interquartile range. The kernel density plot shows the probability density of data at different values.

In individuals with five years, telomeres tended to shorten, but we only sampled two lizards with this age class. Spearman correlations between telomere length and age showed similar results ( $r = 0.24$ ,  $P = 0.003$ , including neonates,  $N = 147$ ;  $r = 0.19$ ,  $P = 0.047$ , without neonates,  $N = 105$ ). The average telomere lengths from neonate to the fifth year of life of lizards were  $1.27 \pm 0.08$ ,  $1.59 \pm 0.29$ ,  $1.59 \pm 0.16$ ,  $2.32 \pm 0.42$ ,  $3.06 \pm 0.54$ , and  $1.10 \pm 0.09$ , respectively. Age correlated positively with body mass ( $r = 0.57$ ,  $P < 0.001$ ), which is common in organisms with indeterminate growth like lizards. Average values of body mass for adults were  $5.07 \pm 0.28$ ,  $6.86 \pm 0.33$ ,  $8.23 \pm 0.44$ ,  $10.12 \pm 1.50$ ,  $9.20 \pm 0.28$ , respectively for each age class. Likewise, larger



individuals had longer telomeres ( $r = 0.26$ ,  $P = 0.007$ ; Fig. 4), which might indicate that cell replication did not involve a reduction in telomere length in those individuals.

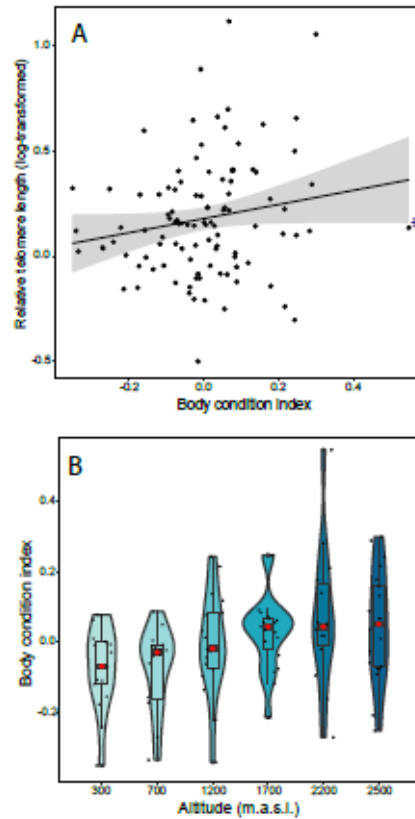


**Figure 4.** Regression between body mass and relative telomere length ( $r = 0.26$ ,  $P = 0.007$ ). Regression line shows the correlation between both parameters in all individuals of the Large Psammmodromus (*Psammmodromus algirus*) sampled in this study, and indicates that telomeres did not shorten, but elongated with cell replication, as observed in larger (and older) individuals. The grey region indicates the 95% confidence intervals.

Average values of body length (SVL) from neonate to five years old lizards were  $58.67 \pm 0.99$ ,  $65.18 \pm 0.99$ ,  $68.66 \pm 1.11$ ,  $74.40 \pm 5.40$ ,  $76.00 \pm 4.00$ , respectively. Relative telomere length tended to increase with body condition ( $r = 0.18$ ,  $P = 0.067$ ). This relationship became significant ( $r = 0.20$ ,  $P = 0.043$ ) when a possible outlier –an individual



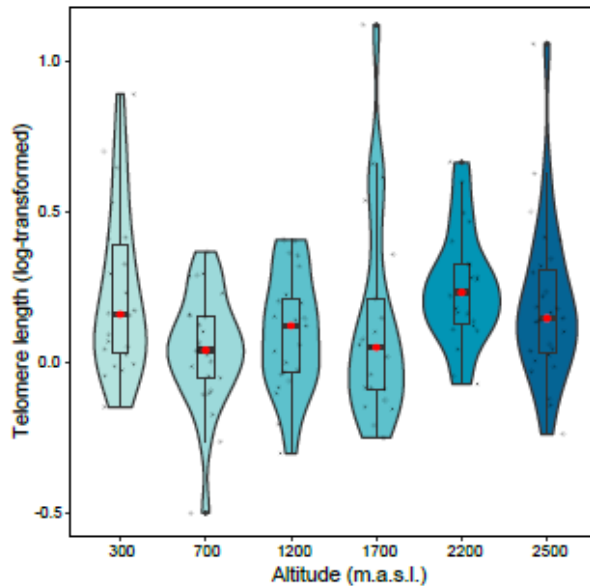
with very high body condition, indicated by the Cleveland plot– was removed (Fig. 5A). Body condition increased with elevation ( $F_{5, 98} = 3.03$ ,  $P = 0.014$ ; Fig. 5B).



**Figure 5.** (A) Regression between body condition and relative telomere length ( $r = 0.20$ ,  $P = 0.043$ ). The asterisk indicates a possible outlier (B) Variation in lizards' body condition across altitude ( $F_{5, 98} = 3.03$ ,  $P = 0.014$ ) in individuals of the Large Psammodromus (*Psammodromus algirus*). The red point shows the mean value at each elevation and the boxplot the interquartile range. The kernel density plot shows the probability density of data at different values.

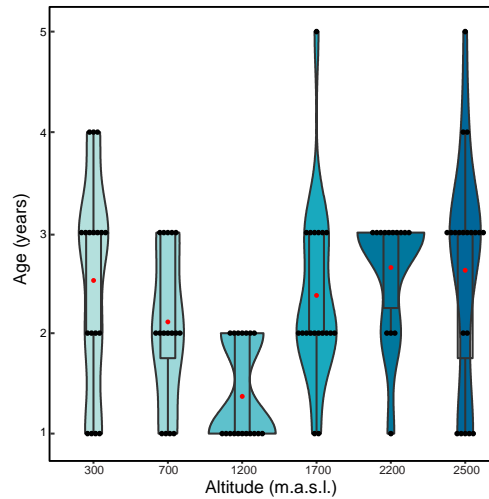


Lizard telomere length varied among lizard populations inhabiting across an elevational gradient, but following a non-linear pattern ( $F_{5,136} = 2.52$ ;  $P = 0.03$  for all individuals, and  $F_{5,99} = 2.07$ ;  $P = 0.070$  when excluding neonates; Fig. 6).



**Figure 6.** Variation in relative telomere length across altitude in individuals of the Large Psammotrypa (*Psammotrypa algirus*). The red point shows the mean value at each age and the boxplot the interquartile range. The kernel density plot shows the probability density of data at different values.

Individuals at 300 and at 2200 m.a.s.l. had the longest telomeres. The average telomere length in lizards at 300, 700, 1200, 1700, 2200, and 2500 m.a.s.l. were  $1.19 \pm 0.09$ ,  $2.01 \pm 0.36$ ,  $1.38 \pm 0.13$ ,  $2.05 \pm 0.61$ ,  $1.94 \pm 0.20$ ,  $1.96 \pm 0.40$ , respectively. However, average age varied with elevation in a similar way ( $F_{5,131} = 5.44$ ;  $P < 0.001$ ; Figure S1).

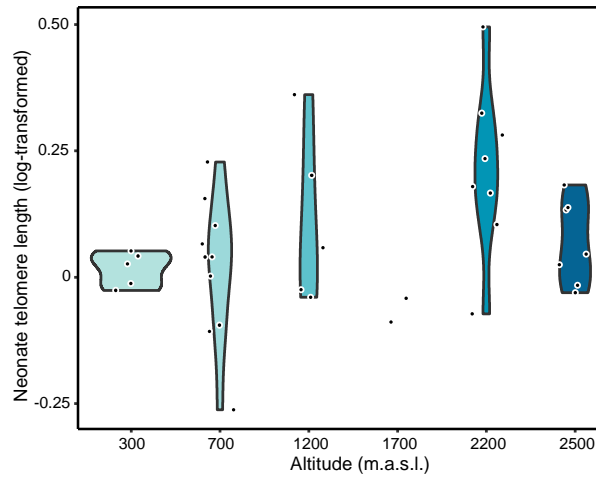


**Figure S1.** Variation in age across altitude of Large *Psammodromus* (*Psammodromus algirus*). The red point shows the mean value at each age and the boxplot the interquartile range. The kernel density plot shows the probability density of data at different values.

When we tested the combined effect of age and elevation on telomere length, the effect of age remained significant ( $F_{5,131} = 2.32$ ;  $P = 0.047$ ), but the effect of elevation was no longer significant ( $F_{5,131} = 1.67$ ;  $P = 0.15$ ).

Neonate telomere length, an indicator of the baseline telomere length at birth, varied among populations ( $F_{5,31} = 2.91$ ;  $P = 0.03$ ), but with no clear pattern; lizard neonates showed the longest telomeres at 2200 m.a.s.l., but the shortest at 1700 m.a.s.l. (Fig. S2).





**Figure S2.** Variation in relative telomere length across altitude of neonates of the large Psammodromus (*Psammodromus algirus*). The red line shows the mean value at each age, whereas the kernel density plot shows the probability density of data at different values.

## Discussion

Life-history trade-offs and environmental conditions can shape ageing across taxa (Wilbourn et al. 2018; Eastwood et al. 2019). Our cross-sectional study suggests that, in the large Psammodromus, telomeres are longer with age until their fourth year, although selective disappearance might have a relevant role here, as discussed below. Likewise, larger (and older) lizards had longer telomeres. Intriguingly, although lizard populations across this substantial elevational gradient are exposed to very different environmental conditions across their life, elevation did not affect lizard telomeres. Differences in telomere length



were sex-independent, unlike found in adults of other sand lizard species (*Lacerta agilis*, Olsson et al. 2011). Sex differences in telomere length may result from sex differences in growth rate, body size, and/or age (Olsson et al. 2018), however, in our study system, lizards did not show sexual dimorphism in size or age structure. Autotomy did not affect telomere length although differences in the regulation of telomere length may be driven by evolutionary pressures such as predation (Olsson et al. 2010), and also by enhanced cell replication during tissue regeneration. Moreover, no cohort effect was detected, as telomere length did not differ with year of sampling.

Our study suggests that lizards have longer telomeres across their first four years of life. This result agrees with previous studies in snakes and lizards (Ujvari and Madsen 2009 and Ujvari et al. 2017, respectively). In individuals with five years old, telomeres tended to be shorter, although this should be interpreted carefully because we only collected two five-year-old individuals. We also found that telomere length showed a positive relationship with body size. Although telomere length and survival had no any association in other lizards such as in the frillneck lizard (Ujvari et al. 2017), larger body size can include lower mortality risk in ectotherms with indeterminate growth (Angilletta et al. 2004). If extrinsic conditions selectively remove individuals in poor condition -with expected shorter telomeres-, then the fact that older lizards have longer telomeres might be indicating a prolonged survival of those individuals (van de Pol and Wright 2009; Salmón et al. 2017). Despite the putative role of selective disappearance in explaining among-differences in telomeres, the positive relationship between telomere



length and body size might suggest that increases in body size -and subsequent higher cell replication- do not imply shorter telomeres by itself. Previous studies have shown that ectotherms, unlike endotherms, can show longer telomeres along their lifetime (Olsson et al. 2018). Such contrasting patterns of telomere dynamics may be related to a higher telomerase expression after birth in somatic cells in ectotherms than in endotherms (Gomes et al. 2010). Hence, telomerase may be relevant for buffering downstream effects of cellular damage in organisms with indeterminate growth such as lizards (Jones et al. 2014). However, telomerase expression may not be enough to protect from telomere shortening in ectothermic vertebrates. For instance, telomerase is expressed in tissues of adult medaka fish (Klapper et al. 1998) but telomeres shorten with age (Hatakeyama et al. 2008). Furthermore, the maintenance of telomerase expression in species with indeterminate growth can imply a trade-off suggested by a higher cancer occurrence in ectotherms (Gomes et al. 2010; Olsson et al. 2018), however, the knowledge about cancer in wildlife is still meagre. In our study system, the use of a longitudinal approach will disentangle the possible role of selective disappearance or telomere elongation (and telomerase activity) in explaining differences in telomere length in older lizards.

In our study, we expected to find longer telomeres in lizard populations at higher elevation, as we know, for instances, that higher-altitude lizards undergo reduced activity time and oxidative damage (Zamora-Camacho et al. 2013; Reguera et al. 2014, 2015). However, we found that elevation did not shape telomeres in these lizard populations. Contrary to our results, Dupoué et al. (2017) found that populations of



the common lizard (*Zootoca vivipara*) inhabiting at low elevations have shorter telomeres and higher extinction risk. In our study system, lowland populations suffer poor habitat quality since they face low thermal quality (risk of overheating, Zamora-Camacho et al. 2016), high ectoparasitism (Álvarez et al. 2018), low food availability (Moreno-Rueda et al. 2018), high oxidative damage (Reguera et al. 2014, 2015), and even high risk of wildfire (Moreno-Rueda et al. 2019). Additionally, at low elevations, lizards increase their activity time while hibernation time decreases (Zamora-Camacho et al. 2013). Despite all this, lizard populations at lowland did not have shorter telomeres than populations at high elevations.

Lizard body condition, temperature, and telomerase expression might explain the lack of variation in telomere length in lizards inhabiting at different elevations. In this study, body condition of lizards was higher in populations at higher elevation and correlated positively with telomere length. It is known that telomere length can show a positive correlation with body condition in other reptiles (*Thamnophis sirtalis*; Rollings et al. 2017), suggesting that body condition is an indirect measure of somatic investment. In addition, it is likely a temperature-mediated regulation of telomerase expression, thus at low elevation telomerase might show a higher expression, then compensating for telomere erosion (Olsson et al. 2018). At the high elevations, the reduction in metabolic rate due to cold conditions may have also favoured a reduction in the rate of telomere erosion due to a reduced production of ROS (Reguera et al. 2014, 2015). Indeed, increases in lifespan are often orchestrated by reductions in metabolic rate



(Speakman, 2005), as suggested by the straightforward influence of latitude on the lifespan of *Rana temporaria* frogs across the Swedish latitudinal gradient (Hjernquist et al. 2012). Furthermore, the variation in the pace-of-life as a consequence of facing particular environmental conditions is also known to alter telomeres, then resulting in complex or unexpected patterns (Giraudeau et al. 2019). For example, shorter telomeres are associated with higher survival in migratory Atlantic salmon (McLennan et al. 2017), which may indicate a trade-off between investment in migration and investment in telomere maintenance. Likewise, amphibian larvae surviving predators, which have larger bodies and larger fat reserves, experience telomere shortening as a consequence of growing faster due to relaxed intraspecific competition (Burraco et al. 2017a). In our system, other factors like diseases or intraspecific interactions might have also modulated ageing in lizards at each elevation. A cross-fostering approach will help to fully clarify the evolutionary impact of both environment and life-history traits on telomeres of this lizard metapopulation.

## **Conclusions**

Our results suggest that telomeres can be longer throughout the first four years of lizards' lifetime. This result would stress the role of telomerase in maintaining ectothermic telomeres, and, likely, in extending lifespan in organisms with indeterminate growth, however, it would be confirmed through a longitudinal approach. This study also highlights that habitat features and repair mechanisms at different



habitats may be relevant for understanding telomere dynamics in ectothermic vertebrates, for example in response to a substantial elevational gradient. Our results emphasize the relevance of understanding species' life histories (e.g. age and body condition) and habitat characteristics for disentangling the causes and consequences of lifespan trajectory.

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## Appendix

### **Comparison of the effectiveness of phalanges vs. humeri and femurs to estimate lizard age with skeletochronology**

This appendix reproduces the published article:

Comas, M., Reguera, R., Zamora-Camacho, F.J., Salvadó, H and Moreno-Rueda, G (2016) Comparison of the effectiveness of phalanges vs. humeri and femurs to estimate lizard age with skeletochronology. *Animal Biodiversity and Conservation* 39: 237-240.

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## **Comparison of the effectiveness of phalanges vs. humeri and femurs to estimate lizard age with skeletochronology**

**M. Comas, S. Reguera, F. J. Zamora-Camacho, H. Salvadó, G. Moreno-Rueda**

### **Abstract**

Skeletochronology allows estimate lizard age with a single capture (from a bone), being unnecessary long-term monitoring. Nevertheless, skeletochronology frequently involve the death of the animal to obtain the bone. We test the reliability of skeletochronology of phalanges (which may be obtained without killing), comparing the estimated age from femurs and humeri) with the age estimated from phalanges. Our results show skeletochronology of phalanges to be a reliable method for estimating age in lizards, as cross-section readings from all bones studied presented a high correlation and repeatability, regardless of the phalanx chosen. Therefore, the killing of lizards for skeletochronology studies is no longer justified.

### **Introduction**

Demography studies -which require knowing the age of the animals studied- are fundamental in population ecology, as well as in conservation biology and wildlife management. However, knowing the age of animals usually requires longitudinal studies, in which animals



are captured and marked for long-term monitoring (Sutherland, 1997). Mark-recapture is a useful and precise method; however, it is highly time-consuming, may be difficult in elusive species or those with high rates of movement, and marks may have negative consequences on individuals (Murray and Fuller, 2000). Mark-recapture has few alternative methods. Nevertheless, some ectotherms with indeterminate growth may present a cyclic growth pattern in hard body structures, corresponding to alternate periods of growth and resting. In this way, age may be estimated by examining cyclic growth patterns in bones (Castanet, 1994).

Femur and humerus are the most commonly used bones in reptile skeletochronology studies (Castanet, 1994). However, the use of the humerus and femur has the disadvantage that individual must be dead or even killed specifically to obtain the bones, which, besides ethical concerns, precludes future studies or experiments with these specimens for which age has been estimated. Alternatively, researchers could use phalanges (easily obtained by toe clipping) to estimate age (e.g. Dubey et al., 2013). Clipping of one or two toes does not significantly reduce survival (Perry et al., 2011), and have no significant effects on key traits of animal behaviour, such as sprint speed (Husak, 2006). Therefore, estimating individual age with skeletochronology of phalanges would allow experimentation or future studies with animals of known age.

In the present study, we examine how well the use of phalanges works to estimate age in reptiles in comparison with the use of the femurs and humeri. For this, we used a collection of preserved individuals of the lizard *Psammodromus algirus* at the University of Granada (Spain). We



estimated the age of these lizards by using phalanges, humeri, and femurs, and compared the estimates made by the three types of bones.

## **Materials and methods**

Fourteen *Psammmodromus algirus* from the scientific collection of the University of Granada was used for the skeletochronological analysis. No lizard was killed for this study. These lizards had died from natural causes while in captivity or by accident while handling during a longstanding study on this species (less than 1% of the lizards handled during the study died). Bodies were preserved in 70% ethanol. Later, long bones (femurs, humeri, and phalanges) were removed and evaluated for age estimation by means skeletochronology (Castanet and Smirina, 1990).

We have done several trials to estimate the necessary time for decalcification. Finally, the samples were decalcified in 3% nitric acid for at least 3 hours and 30 minutes. Although we used only one phalanx per lizard, the phalanx number was assigned at random in order to examine whether different phalanges are more or less suitable for estimating age. The basal and middle phalanges of each finger provide better resolution than does the most distal phalanx (Castanet and Smirina, 1990). Decalcified samples were conserved in PBS (phosphate-buffered saline) solution with sucrose (for cryoprotection) for at least 48h at 4°C, until they were sectioned with the freezing microtome.

Glass-slides were treated (before use) with a solution of glycerol (5 gr/L) and chromium (III) potassium sulphate (0.5 gr/L). Glycerol is



used to improve the placing of the cross-sections on glass-slides. Chromium (III) potassium sulphate is used to improve sample conservation before applying the staining and fixation protocol. Glass slides were submerged for at least 5 min in glycerol-chromium (III) potassium sulphate solution and then oven-dried for 24 h. Finally, the treated slides were refrigerated until used.

For cross-sections, samples were embedded in gel O.C.T. (optimum cutting temperature) and then sectioned at 10-12  $\mu\text{m}$  for phalanges and 14-30  $\mu\text{m}$  for the longer bones, using a freezing microtome (CM1850 Leica) at the Centre of Scientific Instrumentation of the University of Granada. Cross-sections were stained with Harris hematoxylin for 20 min and then the excess stain was rinsed by washing the slides in tap water for 5 min. Later, stained sections were dehydrated with an alcohol series (70%, 96%, 100%; 5 min each), washed in xylol for 15 min, and were finally fixed with DPX (mounting medium for histology) and mounted on slides.

Thereafter, cross-sections were examined for the presence of LAGs using a light microscope (Leitz Dialux20) at magnifications from 50 to 125X. With a ProgresC3 camera, we took several photographs (a mean of 33.67 per individual) of various representative cross-sections, discarding those in which cuts were unsuitable for examining the LAGs. We selected diaphysis sections in which the size of the medullar cavity was at its minimum and that of the periosteal bone at its maximum (Castanet and Smirina, 1990).

Because inferring age from the number of LAGs requires knowing the annual number of periods of arrested growth for each year,



we compared our age estimates with juveniles, whose age is known -less than a year-. Multiple LAGs were found in juveniles in their first period of growth -which were counted as a single year-, while adults usually showed a single additional LAG per year. When various LAGs were found much closed, they were considered as a single LAG in order to avoid overestimation of age. Different LAG pattern depending on age may be explained because juvenile lizards usually are more active and show activity periods more intermittent than adults (Carretero and Llorente, 1995).

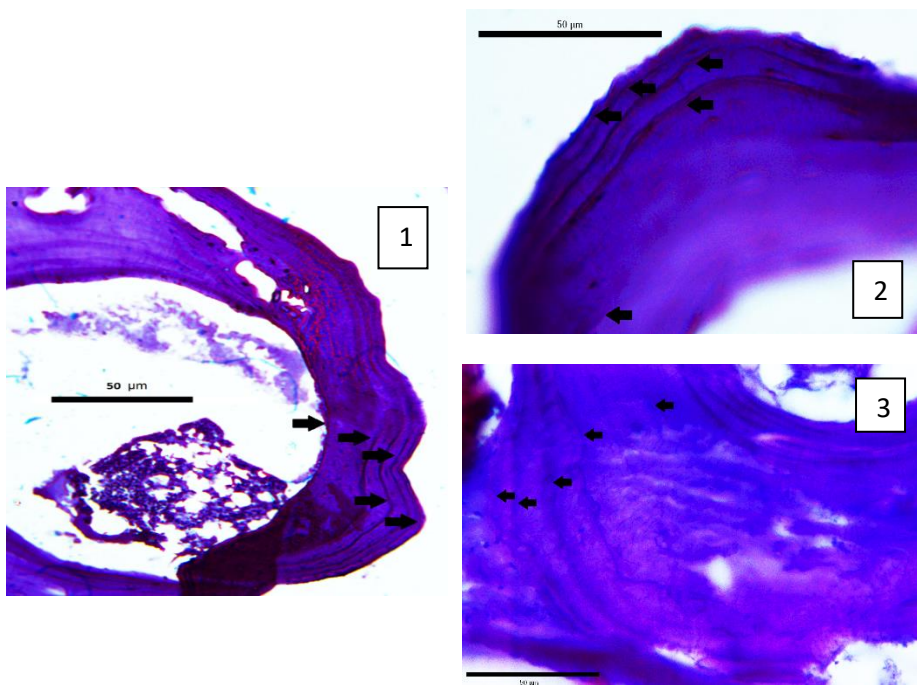
The number of LAGs detected in the periosteal bone was independently counted three times by the same person but on different occasions, always blindly regarding the specimen identification (Sagor et al., 1998). Lizards were collected in summer. Therefore, LAGs deposited during previous winter hibernation were discernible from the outer edge of the bone. Consequently, the outer edge of the bone was not counted as a LAG.

A Pearson's correlation matrix was applied for the three age estimates and for each bone type. Repeatability ( $r_i$ ) was estimated with the formula  $r_i = B/(B+W)$ , where B is the variance between individuals and W is the variance within individuals, estimated from a one-way ANOVA (Senar, 1999).



## Results

In all lizards the number of LAGs remained almost identical for all limb bones analysed and among the three independent readings of the sections, independently of the phalanx number used (for phalanx:  $r_i = 0.982$ ,  $F_{13, 28} = 112.8$ ,  $P < 0.001$ ; humerus:  $r_i = 0.982$ ,  $F_{13, 27} = 108.7$ ,  $P < 0.001$ ; femur:  $r_i = 0.984$ ,  $F_{9, 18} = 123.1$ ,  $P < 0.001$ ; all Pearson's  $r > 0.93$ ; Table 1). In 12 lizards, age estimations were identical for all three readings and all bones studied (Table 1; Fig. 1).



**Figure 1.** The figures show cross-sections of different long bones of the same individual (femur [1], humerus [2], and phalanx [3]), where 5 LAGs can be observed (ID number 10055). Firsts LAGs near marrow cavity correspond to first year of growing. Photo credit: Mar Comas.





**Table 1.** Number of LAGs (age estimates) recorded from three readings of different limb bones: phalanx, femur, and humerus, of 14 individuals of *Psammodromus algirus* (ID number is the identification code of each lizard).

ID number	Phalanx			Femur			Humerus		
	1st	2nd	3th	1st	2nd	3th	1st	2nd	3th
10041	4	4	4	4	4	4	4	4	4
10032	3	3	3	3	3	3	3	3	3
10112	4	4	3	4	4	3	4	3	4
10113	3	3	4	3	3	3	3	3	4
10144	3	3	3	3	3	3	3	3	3
10055	5	5	5	5	5	5	5	5	5
10051	5	5	5	5	5	5	5	5	5
13104	5	5	5	-	-	-	5	5	5
13151	3	3	3	-	-	-	3	3	3
13155	1	1	1	1	1	1	1	1	1
13156	1	1	1	-	-	-	1	1	1
13158	2	2	2	2	2	2	2	2	2
13119	2	2	2	-	-	-	2	2	2
12132	3	3	3	-	-	-	3	3	3



## Discussion

Age estimated from the number of LAGs in all bones was identical in the 85.7% of the lizards, section readings from different bones presenting high correlation and repeatability, similar to that found in a previous study in *Lacerta schreiberi* (Luís et al., 2003), confirming that skeletochronology of phalanges is a reliable method to estimate age in reptiles. Sections from humeri and phalanges were better than those from femurs, and even in some individuals, it was not possible to obtain good sections from femurs because were more difficult to cut. The fact that age was equally well estimated with any phalanx implies that the toe used is irrelevant. Nonetheless, we suggest avoiding clipping toes with special importance for animal movements, such as the longest toe. These results imply that killing lizards to do skeletochronology is no longer justified, and skeletochronology of phalanges should be used instead of skeletochronology with other bones that require the death of the animal, especially in the case of endangered species.

Moreover, the applications of (non-lethal) skeletochronology of phalanges in ecology and conservation biology are numerous and exceed those from skeletochronology implying the death of the specimen. For example, it allows demographic studies with only one visit to the study area, making long-term studies unnecessary. This may fuel research programmes in areas of difficult access, where the mark-recapture method would be ineffective. In this way, skeletochronology of phalanges allows an easy, economic, and ethical way to monitor herpetofauna. In addition, the application of skeletochronology of



phalanges could aid studies on age-related physiology, reproduction or survival in reptiles, with a reduction in costs and disturbance to animals, thereby providing an efficient and cheap alternative to the mark-recapture approach, and having less impact on animals (Langkilde and Shine, 2006).

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## GENERAL DISCUSSION

The results of the first chapter -done with the Atlas day gecko *Quedenfeldtia trachyblepharus*- show significant isotopic variability depending on altitude, being greater in populations located at high altitude, for both carbon and nitrogen isotopes. Greater isotopic variability at higher altitude suggests that niche breadth of the Atlas day gecko is greater as altitude increases (Bearhop et al. 2004). Consequently, the Atlas day gecko presented an expanded trophic niche at high altitude, where fewer competitor species co-occur.

The analysis of the isotopic signal revealed an overlap in the trophic niche occupied by both sexes. In the Atlas day gecko, sexual dimorphism for body size and head morphology is pronounced (males have larger body size and head size), but this sexual dimorphism did not translate into sexual differences in trophic niche. In the Atlas day gecko, morphological differences between sexes could be explained by different sexual roles, such as greater aggressiveness of males, as it occurs in other species of lizards (Lailvaux and Irschick 2007).

Moreover, the Atlas day gecko shows a significant loss of body condition as the number of competitors increased, at low altitude. This loss of body condition may be due to competition for food at lower altitudes, where trophic resources have to be partitioned within a richer community.

Global change is causing changes in species distribution and abundance (review in Parmesan 2006; for reptiles see Sinervo et al. 2010; Moreno-Rueda et al. 2012). Moreover, organisms restricted to alpine



habitat suffer particularly from global warming (Raxworthy et al. 2008; Dubey and Shine 2011), and because the distribution of the Atlas day gecko is strictly limited to alpine areas, it may be particularly vulnerable (Thomas et al. 2006). In the Atlas day gecko niche width changes with altitude and this suggests that it is affected by interspecific competition. Therefore, the Atlas day gecko might be potentially vulnerable to climate warming by upslope displacement of distributions of competing species. Species with narrower niches are at greater risk of extinction (Williams et al. 2009). Because the Atlas day gecko shows a narrower niche where there are more competitors, upslope displacement of generalist species may form an important potential extinction threat for this species.

The results of the second chapter -done with the Atlas day gecko- show complex relationships between gecko body' condition and mite parasitism: the probability of infection being lower in geckos having better body condition, but infestation tending to intensify with body condition. These apparently contradictory results suggest that the better the body condition of an individual, the lower the likelihood of being parasitized, but a good body condition also implied more tolerance to parasites, allowing the individual to deal with a greater parasitic load after the initial infestation (Amo et al. 2005). Anti-parasitic defences are costly (Hakkarainen et al. 2007), requiring the allocation of resources to the immune system. Therefore, individuals with a better body condition may invest more resources to their immune system to fight parasites, but also may invest more resources to deal with parasites and to be more tolerant. Moreover, if the infestation reduces survival, only individuals





in good body condition could survive and, consequently, individuals with the poorest body condition might have died before the sampling (Amo et al. 2005). This might explain the greater infestation in individuals with greater body condition.

As expected, prevalence proved greater in males than females, with two-thirds of the males but only half of the females parasitized. However, the intensity of infestation did not change according to sex, in agreement with another study with the same species (Blouin-Demers et al. 2013). High testosterone levels may imply immunosuppressive effects that, particularly in reptiles, boosts ectoparasite intensity (Salvador et al. 1996; Olsson et al. 2000; Klukowski and Nelson 2001; Fig. 22). These factors may explain greater prevalence in males. However, males registered higher values of body condition than females (Comas et al. 2014). Better body condition implies more resources to fight parasites, allowing more resistance to parasites (Arriero et al. 2018), perhaps explaining why males do not show a higher intensity of infestation than females do. Higher mobility and more frequent social interactions of males may facilitate parasite transmission during contacts with females and in fights with other males (Olsson et al. 2000; Amo et al. 2005). If higher male mobility applies to Atlas day geckos, increased mobility could explain their higher prevalence.



**Figure 22.** Male sand lizard *Lacerta agilis* parasitized with numerous ticks, marked and amplified. Photos were taken by Mar Comas.

Selective pressures may vary with elevation as a consequence of the changing biotic and abiotic conditions (Körner 2007). For example, at higher elevations, hibernation lasts longer, resulting in a narrow temporal window for both host and parasites to reproduce and grow. Moreover, at higher elevations, parasites are exposed longer to lower temperatures which may increase parasite mortality during hibernation (Comas and Ribas 2015; Postawa and Nagy 2016). In fact, other studies show differences in parasitism with elevation, lizards typically harbouring more parasites at low elevations (e.g. Álvarez-Ruiz et al. 2018). However, this contention was not supported by the results, given that both prevalence and intensity did not differ with altitude. This result could be explained in several ways. The reptile community composition and gecko's population density change with elevation. The Atlas day



gecko is alpine, being the most common reptile above 2500 m, with lower densities below this elevation (Schleich et al. 1996). The high density of individuals intensifies the risk of parasite transmission (Altizer et al. 2004; Hakkarainen et al. 2007). However, as elevation decreases, gecko densities also decrease but many other lizard species occur, with the reptile community being richer in the lowlands, which could act as a mite reservoir. Moreover, other factors could intervene, such as the fact that body condition is better in highland populations (Comas et al. 2014) and geckos in better body condition may divert more resources to fight parasites. Consequently, different conflicting effects could be acting: gecko's body condition and population densities which both increase with altitude, meanwhile reptile communities are richer at lower elevation. Therefore, the results obtained in this second chapter respect to those reported in the literature suggest that elevational patterns in the lizard-mite interaction may be complex and specific for each system.

The results of the third chapter -done with the large psammodromus *Psammodromus algirus*- show that its age structure 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 cline in predators or parasites in Sierra Nevada. Although we have no



accurate data on predator pressure, anecdotal observations during fieldwork suggest that predator abundance is lower at high elevations (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 hypothesis 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 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 (Monasterio et al. 2011). However, while data presented in the third chapter 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 Sierra Nevada, alpine lizards show several 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, the results of the third chapter 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. Consequently, lizard density, body condition and reproductive investment were the lowest. 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, despite 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.

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 (Baltontín 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.

Overall, the results of the third chapter 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 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, it should be taken into account that senescence is strongly determined by telomere length (Hausmann and Marchetto 2010; Burraco et al. 2019).



In the fourth chapter, results show that telomeres are longer with age until their fourth year in the large psammmodromus. Likewise, larger (and older) lizards had longer telomeres. Intriguingly, although lizard populations across Sierra Nevada are exposed to very different environmental conditions across their life, elevation did not affect lizard telomeres. Differences in telomere length were sex-independent, unlike found in adults of other lizard species (*Lacerta agilis*, Olsson et al. 2011). Sex differences in telomere length may result from sex differences in growth rate, body size, and/or age (Olsson et al. 2018), however, in our study system, lizards did not show sexual dimorphism in size or age structure.

Our study suggests that lizards have longer telomeres across their first four years of life. This result agrees with previous studies in snakes and lizards (Ujvari and Madsen 2009; Ujvari et al. 2017, respectively). In individuals with five years old, telomeres tended to be shorter, although this should be interpreted carefully because we only collected two five-year-old individuals. We also found that telomere length showed a positive relationship with body size. Although telomere length and survival had no any association in other lizards such as in the frillneck lizard (Ujvari et al. 2017), larger body size can include lower mortality risk in ectotherms with indeterminate growth (Angilletta et al. 2004). If extrinsic conditions selectively remove individuals in poor condition -with expected shorter telomeres-, then the fact that older lizards have longer telomeres might be indicating a prolonged survival of those individuals (van de Pol and Wright 2009; Salmón et al. 2017). Previous studies have shown that ectotherms, unlike endotherms, can



show longer telomeres along their lifetime (Olsson et al. 2018). Such contrasting patterns of telomere dynamics may be related to a higher telomerase expression after birth in somatic cells in ectotherms than in endotherms (Gomes et al. 2010). Hence, telomerase may be relevant for buffering downstream effects of cellular damage in organisms with indeterminate growth such as lizards (Jones et al. 2014). However, telomerase expression may not be enough to protect from telomere shortening in ectothermic vertebrates.

In Sierra Nevada, we expected to find longer telomeres in large psammodromus populations at higher elevation, as we know, for instances, that higher-altitude lizards undergo reduced activity time and oxidative damage (Zamora-Camacho et al. 2013; Reguera et al. 2014a, 2015). Nevertheless, we found that elevation did not shape telomeres in these lizard populations. However, Dupoué et al. (2017) found that populations of the common lizard (*Zootoca vivipara*) inhabiting at low elevations have shorter telomeres and higher extinction risk. In Sierra Nevada, lowland populations suffer poor habitat quality since they face low thermal quality (risk of overheating, Zamora-Camacho et al. 2016), high ectoparasitism (Álvarez et al. 2018), low food availability (Moreno-Rueda et al. 2018), high oxidative damage (Reguera et al. 2014a, 2015), and even high risk of wildfire (Moreno-Rueda et al. 2019). Additionally, at low elevations, lizards increase their activity time while hibernation time decreases (Zamora-Camacho et al. 2013). Despite all this, lizard populations at lowland did not have shorter telomeres than populations at high elevations.





Lizard body condition, temperature, and telomerase expression might explain the lack of variation in telomere length in lizards inhabiting at different elevations. In Sierra Nevada, body condition of large psammmodromus was higher in populations at higher elevation and correlated positively with telomere length. It is known that telomere length can show a positive correlation with body condition in other reptiles (*Thamnophis sirtalis*; Rollings et al. 2017), suggesting that body condition is an indirect measure of somatic investment. In addition, it is likely a temperature-mediated regulation of telomerase expression, thus at low elevation telomerase might show a higher expression, then compensating for telomere erosion (Olsson et al. 2018). At the high elevations, the reduction in metabolic rate due to cold conditions may have also favoured a reduction in the rate of telomere erosion due to a reduced production of ROS (Reguera et al. 2014a, 2015).

Furthermore, the variation in the pace-of-life as a consequence of facing particular environmental conditions is also known to alter telomeres, then resulting in complex or unexpected patterns (Giraudeau et al. 2019). In Sierra Nevada, other factors like diseases or intraspecific interactions might have also modulated ageing in lizards at each elevation. A cross-fostering approach will help to fully clarify the evolutionary impact of both environment and life-history traits on telomeres of large psammmodromus metapopulation along Sierra Nevada.



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## CONCLUSIONS

1- The Atlas day gecko *Quedenfeldtia trachyblepharus* shows greater isotopic variability at high altitude populations, for both carbon and nitrogen isotopes, which implies that niche breadth is greater as altitude increases. This pattern matches with a reduced interspecific competition as altitude increases, which suggest that *Q. trachyblepharus* presents an expanded trophic niche where it has fewer competitors' species.

2- In *Q. trachyblepharus* the analysis of the isotopic signal reveals overlap in the trophic niche occupied by both sexes. Despite the sexual dimorphism of *Q. trachyblepharus* for body size and head morphology, it does not translate into sexual differences in trophic niche.

3- *Quedenfeldtia trachyblepharus* shows better body condition at higher altitude. At lower altitudes, as the number of competitors increased, *Q. trachyblepharus* shows a loss of body condition, where trophic resources have to be partitioned within a richer community.

4- *Quedenfeldtia trachyblepharus* is affected by interspecific competition and it might be vulnerable to climate warming by upslope displacement of distributions of competing species. Because *Q. trachyblepharus* shows a narrower trophic niche where there are more competitors, upslope displacement of generalist species may form a potential extinction threat for this species. Organisms restricted to alpine habitat suffer particularly from global warming, and because the



distribution of *Q. trachyblepharus* is strictly limited to alpine areas, it may be particularly vulnerable.

5- The probability of infection by mites in *Q. trachyblepharus* is lower in geckos with better body condition, but infestation tends to intensify with body condition. This implies that the better the body condition of an individual, the lower the likelihood of being parasitized, but once parasitized, a good body condition also implied more tolerance to parasites, allowing the individual to deal with a greater parasitic load.

6- In *Q. trachyblepharus*, mite prevalence is greater in males than in females. High testosterone levels may imply immunosuppressive effects that, particularly in reptiles, boosts ectoparasite intensity. These factors may explain greater prevalence in males. However, the intensity of infestation did not change depending on sex.

7- In *Q. trachyblepharus*, mite prevalence and intensity did not differ with altitude. This result may be a consequence of different selective pressures which depend on altitude: environmental conditions change with altitude, as well as gecko's body condition and population densities which both increases with altitude, meanwhile reptile communities are richer at lower elevation.

8- In the large psammodromus *Psammodromus algirus* the lifespan is between 3 and 4 years old, depending on the population. Although there



are no differences in age structure between sexes, only females achieve a maximum lifespan of 5 years.

9- *Psammodromus algirus* shows a decrease of almost 50% in the number of lizards reaching the age of 4 years, suggesting that senescence is stronger after 3 years old.

10- Body condition of *P. algirus* improved with elevation. A better body condition with elevation may be due to greater food availability at higher elevations and lower oxidative stress.

11- In *P. algirus* the reproductive investment registered minimal values at low elevations and followed a tendency to increase with elevation. Food availability is greater at higher elevations and, in combination with low oxidative stress and activity time implies highland lizards could invest more in reproduction without a cost in the form of reduced lifespan.

12- The *P. algirus*' mean age follows a U-shaped pattern with altitude: populations at low and high elevations harbour older individuals in comparison with populations at mid-elevations. Similarly, the lifespan was approximately 4 years at each elevation, except at mid-altitude where it was only 3 years. This pattern does not coincide with those found in the bibliography in ectotherms which typically assumes higher longevity at higher altitude. This may be due to the fact that, in Sierra Nevada, there is higher food availability in highlands, which may imply



higher reproduction rate at a lower cost in the way of survival, meanwhile a lower body condition at lower altitude may imply a lower inversion in reproduction which translates into a longer lifespan.

13- The relative abundance of adult *P. algirus* varied significantly with elevation with a maximum at mid-elevations which coincides with its minimal mean age. This suggests that intraspecific competition may reduce lifespan.

14- In the *P. algirus* older individuals show longer telomeres than younger ones. This may be because telomeres lengthen with age, as described in other lizards. Alternatively, this pattern may mirror the selective mortality of individuals with shorter telomeres.

15- In *P. algirus* larger individuals have longer telomeres, which might indicate that cell replication does not involve a reduction in telomere length in these individuals. This result would stress the role of telomerase in maintaining ectothermic telomeres, and, likely, in extending lifespan in organisms with indeterminate growth.

16- In *P. algirus* relative telomere length tended to increase with body condition, which might indicate that a good body condition may involve elongation of telomere length.



## CONCLUSIONES

1- El gecko diurno del Atlas *Quedenfeldtia trachyblepharus* muestra una mayor variabilidad isotópica en las poblaciones de mayor altitud, tanto para isótopos del carbono como del nitrógeno, lo que implica que la amplitud de nicho es mayor a medida que aumenta la altitud. Este patrón coincide con una menor competencia interespecífica a medida que aumenta la altitud, lo que sugiere que *Q. trachyblepharus* presenta un nicho trófico más amplio donde hay menos especies competidoras.

2- En *Q. trachyblepharus* el análisis de la señal isotópica revela una superposición en el nicho trófico ocupado por ambos sexos. A pesar del dimorfismo sexual de *Q. trachyblepharus* para el tamaño del cuerpo y la morfología de la cabeza, este no se traduce en diferencias sexuales en el nicho trófico.

3- El gecónido *Q. trachyblepharus* muestra mejor condición física a mayor altitud. A altitudes más bajas, a medida que aumenta el número de competidores, *Q. trachyblepharus* muestra una pérdida de la condición física, donde los recursos tróficos se particionan entre una comunidad más rica.

4- *Quedenfeldtia trachyblepharus* se ve afectado por la competencia interespecífica y podría ser vulnerable al calentamiento climático por el desplazamiento ascendente de especies competidoras. Debido a que *Q. trachyblepharus* muestra un nicho trófico más estrecho donde hay más



competidores, el desplazamiento ascendente de las especies generalistas puede constituir una potencial amenaza de extinción para esta especie. Los organismos restringidos al hábitat alpino sufren particularmente el calentamiento global, y debido a que la distribución de *Q. trachyblepharus* está estrictamente limitada a las áreas alpinas, puede ser particularmente vulnerable.

5- La probabilidad de infección por ácaros en *Q. trachyblepharus* es menor en geckos con mejor condición física, pero la infestación tiende a intensificarse con la condición corporal. Esto implica que cuanto mejor es la condición corporal de un individuo, menor es la probabilidad de ser parasitado, pero una vez que es parasitado, una buena condición corporal también implica más tolerancia a los parásitos, lo que le permite al individuo lidiar con una mayor carga parasitaria.

6- En *Q. trachyblepharus*, la prevalencia de ácaros es mayor en los machos que en las hembras. Los niveles altos de testosterona pueden implicar efectos inmunosupresores que, particularmente en reptiles, aumentan la intensidad en ectoparásitos. Estos factores pueden explicar una mayor prevalencia en machos. Sin embargo, la intensidad de la infestación no cambió según el sexo.

7- En *Q. trachyblepharus*, la prevalencia e intensidad de los ácaros no difieren con la altitud. Este resultado puede ser consecuencia de diferentes presiones selectivas que dependen de la altitud: la condición





física del gecko y la densidad de sus poblaciones aumentan con la altitud, mientras las comunidades de reptiles son más diversas a menor altitud.

8- En la lagartija colilarga *Psammotriton algirus* la esperanza de vida es de entre 3 y 4 años, dependiendo de la población. Aunque no hay diferencias en la estructura de edad entre sexos, solo las hembras alcanzan una esperanza de vida máxima de 5 años.

9- Las *P. algirus* muestran una disminución de casi el 50% en el número de lagartijas que alcanzan la edad de 4 años, lo que sugiere que la senescencia es más fuerte después de los 3 años.

10- Los valores de condición física de *P. algirus* aumentan con la elevación. Una mejor condición física con la altitud puede deberse a una mayor disponibilidad de alimentos a mayor altitud y a un menor estrés oxidativo.

11- En *P. algirus* la inversión reproductiva muestra valores mínimos en elevaciones bajas y sigue una tendencia a aumentar con la elevación. La disponibilidad de alimentos es mayor en elevaciones más altas y, en combinación con un bajo estrés oxidativo y tiempo de actividad, implica que las lagartijas de alta altitud podrían invertir más en reproducción sin un coste en forma de una menor esperanza de vida.

12- La edad media de *P. algirus* sigue un patrón en forma de U con la altitud: las poblaciones de baja y alta altitud albergan individuos más



viejos en comparación con las poblaciones en elevaciones medias. Del mismo modo, la esperanza de vida fue de aproximadamente 4 años en cada altitud, excepto media altitud, donde es de 3 años. Este patrón no coincide con los que se encuentran en la bibliografía en ectotermos, que generalmente suponen una mayor longevidad a mayor altitud. Esto puede deberse al hecho de que, en Sierra Nevada, a alta altitud hay una mayor disponibilidad de alimento, lo que puede implicar una mayor tasa de reproducción a un menor coste en cuanto a la supervivencia, mientras que una peor condición física a menor altitud puede implicar una menor inversión en reproducción, lo que se traduce en una mayor esperanza de vida.

13- La abundancia relativa de las *P. algirus* adultas varía significativamente con la elevación con un máximo a media altitud que coincide con su mínima edad media. Esto sugiere que la competencia intraespecífica puede reducir la esperanza de vida.

14- En *P. algirus* los individuos de más edad muestran telómeros más largos que los más jóvenes. Esto puede deberse al hecho de que los telómeros se alargan con la edad, como se describe en otras lagartijas. Alternativamente, este patrón puede reflejar la mortalidad selectiva de individuos con telómeros más cortos.

15- En *P. algirus*, los individuos más grandes tienen telómeros más largos, lo que podría indicar que la replicación celular no implica una reducción en la longitud de los telómeros en estos individuos. Este



resultado enfatizaría el papel de la telomerasa en el mantenimiento de los telómeros en ectotermos y, probablemente, en la prolongación de la esperanza de vida en organismos con un crecimiento indeterminado.

16- En *P. algirus*, la longitud relativa de los telómeros tiende a aumentar con la condición física, lo que podría indicar que una buena condición física puede implicar el alargamiento de la longitud de los telómeros.



*The Atlas day gecko *Zuedenfeldtia trachyblepharus**



*The large psammodromus *Psammodromus algirus**