# Variación altitudinal en las estrategias vitales de la lagartija colilarga, *Psammodromus algirus*, en Sierra Nevada: relaciones entre termorregulación, velocidad de carrera y sistema inmune.

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**TESIS DOCTORAL**

**Departamento de Zoología**

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# UNIVERSIDAD DE GRANADA

## FACULTAD DE CIENCIAS

## Departamento de Zoología

## TESIS DOCTORAL

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PRESENTADA POR

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*Todas las fotografías que figuran en esta tesis son obra del autor, Francisco Javier Zamora Camacho.*

# Variación altitudinal en las estrategias vitales de la lagartija colilarga, *Psammodromus algirus*, en Sierra Nevada: relaciones entre termorregulación, velocidad de carrera y sistema inmune.

Memoria presentada por Francisco Javier Zamora Camacho para optar al Grado de Doctor por la Universidad de Granada.

El Doctorando

Francisco Javier Zamora Camacho
Granada, noviembre de 2014

El Dr. Gregorio Moreno Rueda, Profesor Ayudante Doctor de la Universidad de Granada

CERTIFICA

Que los trabajos de investigación realizados en la Memoria de Tesis Doctoral: "**Variación altitudinal en las estrategias vitales de la lagartija colilarga, *Psammodromus algirus,* en Sierra Nevada: relaciones entre termorregulación, velocidad de carrera y sistema inmune**", son aptos para ser presentados por el Ldo. Francisco Javier Zamora Camacho ante el Tribunal que en su día se designe, para aspirar al Grado de Doctor en Ciencias Biológicas por la Universidad de Granada.

Y para que así conste, en cumplimiento de las disposiciones vigentes, extiendo el presente certificado a 19 de noviembre de 2014 en Granada.

VºBº Director

**Dr. Gregorio Moreno Rueda**

*A mi madre,*

*A quien encuentro en la causa de cada uno de mis logros.*

**Índice**

## Agradecimientos

> "El agradecimiento es la parte principal de un hombre de bien"
>
> Francisco de Quevedo y Villegas

Al azar. Al amor. A la belleza. A las propiedades físico-químicas del carbono. La vida es tan poco probable, particularmente la nuestra, que no hay momento de ella que no deba ser agradecido. Como la humilde amalgama de átomos organizados de manera insólita que somos, deberíamos agradecer cada sencillo instante que vivimos. Al azar, al amor, a la belleza, a esos otros conglomerados de átomos convenientemente estructurados que hacen que seamos mucho más que conglomerados de átomos convenientemente estructurados. Y, si la vivencia más cotidiana es digna de agradecimiento, con más razón, pues, se ha de mostrar gratitud por aquellos momentos que marcan un hito en una vida. Y yo, ahora que me veo ante el abismo de uno de ellos, encuentro que no sé por dónde empezar.

Pero algunos empieces se acometen mejor por el principio. Y en mi caso, el principio empezó a comenzar hace muchos años, o quizás no tantos, en una tierra, mi tierra, a la que añoro, y en una familia, mi familia, en cuyo amor se cimienta mi vida. Y a mi familia les debo tanto que toda mi vida ha de ser un tributo a ella. Si algo bueno hay en mí, si algo bueno he hecho alguna vez, todo se lo debo a mi familia. Todo se lo debo a mi madre, a quien hace ya algunos años porté sobre mi hombro en su último viaje. Aún cargo sobre mi espalda el peso de su marcha. Aún cargo el peso de ese vacío tan grave. Sin embargo, en él pesan también mis recuerdos más felices. Dichosa sea mi carga. Quién no querría cargar con el recuerdo de la felicidad más plena. Quién no querría cargar con el recuerdo de una mirada del amor más absoluto. Ningunos ojos volverán a mirarme tan rebosantes de amor como los suyos. Tuve la fortuna del amor más puro y más cándido. Es imposible tanto amor. Y ahora sólo perdura en mi recuerdo. Por mucho que me pese. Por eso cargo dichoso incluso con los momentos que no puedo recordar: cómo podía imaginar aquella mujer que, preñadísima del que un día habría de ser yo, huía despavorida monte abajo de una serpiente que resultó ser un palo, cómo podría ella imaginar que llevaba dentro un herpetólogo que acabaría llenando su casa de culebras y de escuerzos. Y que ella habría de consentirlo, y hasta fomentarlo. Mientras haya bichos en casa, el niño seguirá viniendo. Ciertamente. Aún sigo yendo en busca de lo que queda fuera de su lápida. Y por fortuna lo que queda no es poco. Tengo la enrome suerte de tener a mi hermana, María Ángeles, con quien tanto comparto, y a quien tanto necesito. Llevar esta carga a medias con ella la hace aún más feliz. Gracias a ella, algunas cosas de mi familia todavía permanecen intactas; gracias a ella, sé que sigue existiendo mi familia, de otra manera muy distinta, pero con la misma esencia. Jamás terminaré de darle las gracias. Gracias también por mi niño, por mi Javi, por la ilusión que nos hizo recordar que existe la magia, esa magia que ha sobrepasado todas las expectativas: uno espera una pequeña porción de tembloroso amor cuando da la bienvenida a un niño, y mi Javi rebosa ese amor hasta cualquier rincón del mundo en que me encuentre. Gracias también a mi cuñado, Manolo, que algo tiene que ver en todo esto. También por su paciencia para acudir a rescatar mi coche atascado en mitad de los pinares una vez… y otra… y otra… No es un cuadro, es una pinacoteca entera, pero aun así, le doy las gracias. Gracias también a mi padre, Paco, quien no solo me ha legado un nombre un tanto corriente, sino que ha intentado aportar su granito de arena a cada disparatada idea de su hijo, aunque no siempre las comprendiera del todo bien. Gracias por el camaronero, gracias por aquellos primeros tritones y gallipatos, por las noches de lluvia conduciendo para mí por esas carreteras llenas de anfibios… y de poco más en kilómetros a la redonda. Gracias por los viajes a la sierra en busca de salamandras. Y por aquella excursión a Portugal que terminamos sepa Dios dónde.

Sin alejarme de aquellos cándidos tiempos cuando el regajo de la curva delimitaba los confines del mundo, también agradezco los felicísimos veranos de mi infancia en el campo de mis tíos Antonio y Felipa. Aunque no llegaba al metro lo que yo alzaba del suelo, recuerdo vívidamente aquella noche en que convencí a mi tía Felipa, que se dejaba gustosa convencer por mí con enorme facilidad, para que me acompañase a un charcón de la cuneta donde abundaban las ranas. Y estoy seguro (porque en no pocas ocasiones lo referimos) de que los dos recordamos cómo, absorto e hipnotizado, me sacó de la pileta asiéndome por el brazo cuando ya el agua me cubría casi hasta el cuello. Después de eso vinieron algunos remojones más (los remojones son parte inherente de este trabajo), pero el primero debo agradecérselo a ella. Agradezco la suave paciencia de mi tío Antonio, ese hombre tan serenamente dulce que convirtió en un gozo el levantarse a las siete de la mañana a pensar a las gallinas. Sigue siendo utilísima su idea de poner botes de tomate frito Fruco enterrados en el montón de patatas en el almacén, con un pedacito de queso dentro, por si caía un ratón, para que yo lo viera. Aunque mi impaciencia infantil me obligaba a revisar las trampas cada dos minutos, y claro, espantaba a los ratones. Aun así, algunos caían. Fue así como me inicio mi tío Antonio en el trampeo de caída, cuando yo contaría con apenas 6 años; a día de hoy, he instalado algunas trampas más. También recuerdo las brevísimas horas acurrucado en aquel pasillo (siempre he gustado yo de espacios retirados) leyendo "Fascinante Naturaleza", el libro sobre flora y fauna de Europa que me regaló mi prima Mari Feli. ¡Qué buen regalo fue aquel, que buena culpa tiene de que esté escribiendo ahora estas líneas! "¿En dónde está el niño?" "Mira a ver si está en el pasillo con el libro" ¿Cuántas veces habrán escuchado aquellas paredes este diálogo? Todavía lo conservo, ajado ya y raído, en alguna estantería. Agradezco el cariño de mi prima Toñi, con quien tener accidentes no es tan malo. Lo digo de buena tinta, que he sobrevivido a varios. Doy las gracias a ambas por mis sobrinos, ya hombres y mujeres (o casi) que tienen el sino de ser siempre nuestros niños. Doy las gracias por las vueltitas en la moto a Álvaro y Carolina. ¡Cuantísimo las echo de menos! Doy las gracias a Dani y a Adán por el entusiasmo ante las noches de lluvia y sapos, aunque no estuviera muy claro de qué iba eso. Y por supuesto no me olvido de las excursiones en busca de ranas y tortugas con Ángel. Los cangrejos de río que soltamos en el pilón donde goteaban los grifos del riego, ¡qué miedo me daban, con esas pinzas casi más grandes que mis manos, tan rojas y tan llenas de protuberancias! Pero de qué manera me fascinaban. Recuerdo igualmente mis primeras crías de lagarto, que se encontró Roldán apropiadamente refugiados bajo el plástico de un líneo. Recuerdo los días buscando y rebuscando en los bidones y los charcos, llenos de pececitos gordos (hoy los llamo renacuajos, al menos cuando hablo en voz alta), pececitos flacos (luego supe que eran larvas de mosquito), y culebritas rojas (larvas de quironómidos, quién me iba a decir que nombrachos como este me acabarían resultando cotidianos). Recuerdo allí tantas cosas que es difícil enumerarlas. Hoy esa finca está en venta. Los avatares de la vida así lo han querido. Pero esos momentos de plena felicidad, esos nos los quedamos.

Agradezco, pues, a toda mi familia su paciencia, su disposición, y sobre todo su inconmensurable amor hacia mí. Agradezco cómo fomentaron la curiosidad, la candidez y el entusiasmo de aquel niño que fui, y que el hombre que hoy soy sigue sacando a buscar bichos en cada ocasión que tengo, como prometo seguir haciendo. Y a mi tierra le agradezco la luz y el agua: las tardes de lluvia (esa manera de caer, recia y operística, que tiene la lluvia en Huelva), las charcas de Malatao, y todas las charcas; el arroyo Tariquejo, y todos los arroyos; los jarales, los majanos, los suaves arenales tapizados de pinos y musgo. Agradezco cada momento de mi infancia, que quisiera (y aún espero) repetir una vez, y otra, y otra…

Pero aquel niño creció, y un día, que recuerdo intensamente teñido de melancolía, tuvo que hacer las maletas y trasponer a Granada. Hasta entonces, habría dicho mudarse, marcharse, irse, trasladarse. Pero en Granada aprendí esa palabra. Y cascar. Y chicolear. Y golismear. Y cuchi. Y bocaná. Y malafollá. Y apañao. Y collejo. Y bonico, y ratico, y fritico (sé que aun después de diez años a muchos allá en mi tierra les chirrían estos diminutivos en –ico). Y es que después de tanto tiempo, tan largamente vivido, llevo también dentro de mí esta tierra seca, adusta, difícil. En Granada aprendí tantas cosas… Aprendí tanto y de tantos maneras, que no puedo sino estar agradecido: al bien que me han hecho, y al mal, parafraseando los versos de la Môme Piaf. En Granada conocí también la soledad, en muchísimos sentidos. Tuve la oportunidad única de dirigir mi vida solo. De resolver mi vida solo. De ser el único culpable, o simplemente causante, de todo lo que me pasara. Pero eso no significa que estuviera siempre solo. Ni mucho menos. En Granada maduré, y eso es una de las principales cosas que debo agradecer a la vida. Y, cómo no, a las personas que me acompañaron en el proceso.

Tengo tanto que agradecer a tantas personas… A Sonia, que ha elevado el concepto de amistad a niveles inauditos. Con quien tantas vivencias he compartido, las más íntimas, las más bonitas, las más dolorosas de los últimos diez años. Esa persona con quien, al quitarme la coraza, no me siento vulnerable, sino más fuerte. Esa persona que convierte mi flaqueza en fortaleza. Esa persona con quien no hacen falta palabras, con quien el entendimiento mutuo es tan profundo que basta una tácita mirada para decirlo todo. Nos entendemos incluso cuando no decimos nada. ¡Amor! Gracias, porque esa palabra ha alcanzado en esta amistad su forma más perfecta. Gracias por tantísimo apoyo y tantísima comprensión, por tantísimo cariño, por tantísimos momentos. Gracias por las charlas entre clase y clase, y durante las clases, porque aunque no sea decoroso decirlo, teníamos discreción para que ningún profesor detuviera nuestra conversación, aun estando en primerísima fila. Doy las gracias por los macarrones con queso, y con champiñones pequeños. Por el “llévame el bolso a mi cuarto”, que es sin duda uno de los descuidos más tiernos que recuerdo. Por diez años de felicitaciones a las 00:00. Por las “otra, otra por si acaso”. Por saber exactamente qué decir y qué hacer en cada momento. Por conocerme mejor que yo mismo, y mira que no es fácil, con lo “especial” que es uno, y lo “hombre” que es a veces…

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## Resumen

Esta tesis estudia el efecto de la altitud sobre la termorregulación, la velocidad de carrera, el sistema inmune y sus relaciones en la lagartija colilarga, *Psammodromus algirus*, en seis poblaciones distribuidas uniformemente entre los 300 y los 2500 metros sobre el nivel del mar en Sierra Nevada (SE de España).

Mediante transectos estandarizados durante todo el período de actividad, encontramos que su fenología fue similar a lo largo del gradiente. No obstante, en contraste con las poblaciones medias y bajas, la actividad en poblaciones altas fue máxima al principio del período de actividad, probablemente por limitaciones en el tiempo para la reproducción.

Estudios sobre la temperatura corporal de los animales en el campo y la seleccionada en un gradiente térmico revelaron el papel termoconservador de esta especie en este sistema. Pese a las diferencias altitudinales en temperatura ambiental, las lagartijas podían termorregular eficientemente seleccionando distintos microhábitats a diferentes horas. No obstante, las temperaturas seleccionadas fueron siempre mayores que las presentadas en el campo, lo que sugiere que otros factores podrían constreñir la capacidad de termorregulación de estas lagartijas. De hecho, la temperatura corporal estuvo limitada por la ambiental en las poblaciones más elevadas. Las hembras posparto seleccionaron temperaturas más altas, lo cual probablemente les permite mejorar su condición corporal tras el esfuerzo reproductor.

Un estudio de variación de tamaño y tasas de intercambio térmico mostró que las lagartijas de poblaciones elevadas fueron de mayor tamaño, acorde con las clinas de Bergmann, que prevén mayor talla corporal en hábitats fríos, reduciéndose la pérdida de calor al disminuir la relación superficie/volumen corporal. En consonancia, los animales de zonas altas, más grandes, tuvieron mayor inercia térmica.

Por otro lado, un desafío inmune sólo disminuyó la temperatura seleccionada por los ejemplares de poblaciones bajas, lo que probablemente les permite ahorrar energía, pero no afectó a las de poblaciones levadas, posiblemente porque los costes de no termorregular en un hábitat térmicamente deficiente podrían ser mayores que los de termorregular.

Por otra parte, la velocidad de carrera estuvo positivamente correlacionada con la longitud de las patas, y negativamente con la masa, por lo que la acumulación de grasas parece comprometida con la capacidad de huir de los depredadores. No obstante, las hembras grávidas no fueron más lentas que las no grávidas. Sin embargo, los machos, más conspicuos a los depredadores, sí fueron más rápidos que las hembras. La altitud no tuvo ningún efecto sobre la velocidad.

En cuanto al efecto de un desafío inmune sobre la velocidad de carrera, sólo afectó a los machos, que disminuyeron su velocidad, probablemente por ser el sexo que más energía invierte en ella.

Por último, la velocidad de carrera fue mayor a temperaturas corporales óptimas que subóptimas para todas las altitudes, lo que sugiere que las preferencias térmicas y la dependencia térmica del desempeño locomotor están coadaptadas. Por otra parte, las hembras grávidas, que a temperatura óptima no eran más lentas que las no grávidas, sí lo fueron a temperaturas subóptimas. Estos resultados señalan que la velocidad de carrera es altamente conservadora en altitud.

## Abstract

This thesis studies the effect of elevation on thermoregulation, sprint speed, immune system, and their relationships, in the large Psammodromus, *Psammodromus algirus*, in six populations uniformly distributed from 300 to 2500 meters above the sea level in Sierra Nevada (SE Spain).

We found that lizard phenology was similar along the gradient, by using standardized transects throughout the whole activity period. However, contrasting with mid and low elevation populations, activity in high populations peaked at the beginning of the activity period, probably due to limitations in the window of time for reproduction.

Studies on field and thermal-gradient selected body temperatures showed that this lizard was thermally conservative in this system. Despite differences in environmental temperature, lizards could thermoregulate effectively selecting different microhabitats at different times of the day. Nonetheless, selected body temperature was always higher than field body temperature, which suggests that other factors could be constraining lizard thermoregulation ability. In fact, field body temperature was limited by environmental temperature in the highest populations. Postpartum females selected higher temperatures, which probably allows them to improve their body condition after the reproductive investment.

A study on variation in body size and thermal exchange rates showed that high-elevation lizards were larger, according to Bergmman’s clines, which predict larger body size in cold habitats, diminishing heat loss by a decrease in the body surface/volume ratio. Moreover, highland lizards, the largest, showed higher thermal inertia.

On the other hand, an immune challenge only diminished selected body temperature in lizards from mid and low populations, which probably allows them to save energy, but did not affect high-elevation lizards, possibly because

the cost of no-thermoregulation in a thermally-limiting habitat outweigh those of thermoregulation.

Also, sprint speed was positively correlated with limb length, and negatively with body mass, so fat accumulation seems to be traded-off with the ability of fleeing from predators. However, gravid females were not slower than non-gravid females. Nevertheless, males, more conspicuous to predators, were faster than females. Elevation had no effect on sprint speed.

As for the effect of an immune challenge on sprint speed, only males were affected, diminishing their speed, probably because they invest more energy than females in sprint speed.

Finally, sprint speed was faster at optimal than at suboptimal body temperatures for lizards from all elevations, suggesting that thermal preferences and thermal dependence of sprint performance are coadapted. On the other hand, gravid females, which were not slower than non-gravid females at optimal body temperatures, were indeed at suboptimal body temperatures. These results confirm that sprint speed is highly conservative in elevation.

## Preámbulo

*Imagínatelo por un momento: ponte en las escamas de una pequeña lagartija. Yaces casi inerte en alguna oquedad que sepultó hace meses una espesa capa de nieve cegadora. Pero hoy, al fin, amanece detrás de tus párpados. Sobre todo pesa el frío. Resulta difícil domeñar los músculos ateridos, pero refulge un haz de luz que se entrevera, como la hoja de un alfanje que hiende un odre de vino, por un resquicio de la roca, maganel de la hura que ha sido dormitorio y fortaleza durante los rigores del invierno. Y eso lo significa todo. Mientras el hielo se desangra, frío y transparente, encharcando la tierra, las extremidades se desentumecen a duras penas, y la roca gélida parece derretirse, y se derrite, al contacto marrón de tus costados. Ya fuera, el sol se despereza desde el otro lado de las cumbres afiladas, que recortan el azul cerúleo como los dientes de un serrucho oxidado. Imagínatelo. No eres más que una mancha parduzca entre las rocas grisáceas y las ramas de un enebro que chorrean, que humean temblorosas, pero sientes un cálido aliento desde las alturas. Y, presas de un calor casi olvidado, las vísceras gritan por dentro: ¡Aire! ¡Hambre! ¡Libido! Unas sombras deslizan sobre las matas, sin segarlas, su silueta de falce, y despiertan a chillidos los temores ancestrales que latían adormecidos. Y un año más, tal vez el último, reanudas la eterna, la inacabable carrera...*

# 1.-Introducción General

## *1.1.-Introducción*

Si pensamos en la biosfera como conjunto y en los distintos niveles de organización que la constituyen, resulta difícil encontrar patrones comunes. Es cierto que todos los seres vivos comparten un adarme de características esenciales: una composición molecular basada en el carbono (Graur y Li, 2000), mecanismos de transmisión de información genética encriptada en secuencias de ácidos nucleicos (Darlington, 1939), funciones vitales similares (Gurney y Nisbet, 1998). Pero sobre todo, en todos los niveles de organización de la vida, la característica común más palmaria es precisamente la diversidad (Wilson, 1994). Baste citar los casi dos millones de especies biológicas descritas hasta la fecha (Mora et al., 2011). Pero la diversidad de la biosfera va más allá de una cifra ingente: a nivel intra o interespecífico, desde las biomoléculas hasta las biocenosis, pasando por genes, tejidos o poblaciones, todos los niveles de organización de la vida son asombrosamente diversos.

Considerada hasta entonces por los fijistas como errores o imperfecciones en la expresión del modelo tipo, la diversidad intraespecífica es objeto de especial atención por parte de los biólogos evolutivos desde que Charles Darwin llamara la atención sobre su importancia como motor de evolución por Selección Natural (Barberousse y Samadi, 2010). Por tanto, comprender los factores que promueven la diversidad intraespecífica es clave para entender el funcionamiento intrínseco de la evolución.

Para que la Selección Natural genere evolución es necesaria una variabilidad heredable correlacionada con la eficacia biológica o *fitness* (Kozlowski, 1996), y el hecho de que un determinado rasgo proporcione o no una ventaja adaptativa depende del ambiente en que se exprese. Dado que el ambiente varía geográficamente (Sunday et al., 2010), los gradientes ambientales proporcionan una herramienta única para el estudio de la generación y mantenimiento de la diversidad intraespecífica, así como para entender los fenómenos de especiación (Doebeli y Dieckmann, 2003).

La temperatura ambiental puede limitar fuertemente el desempeño de la mayoría de funciones vitales de los organismos, así como su supervivencia (White et al., 2006) -especialmente en los ectotermos que dependen estrechamente de ella (Bennett, 1980)-, por lo que el estudio de la variación en la temperatura ambiental es de especial interés. Entre los patrones térmicos de variación geográfica, los más predecibles, y por tanto los más adecuados para los estudios acerca de los efectos de la calidad térmica del hábitat sobre la biología de los organismos, son el latitudinal (Sunday et al., 2010) y el altitudinal (Stevens, 1992): al aumentar ambos disminuye la temperatura media ambiental (Graae, 2012). No obstante, el gradiente altitudinal presenta varias ventajas sobre el latitudinal, y es que al tener lugar en menores distancias no sólo facilita la logística, sino que además permite un mejor control de otros factores tanto bióticos como abióticos (Körner, 2007). Por este motivo, los gradientes altitudinales constituyen una excelente oportunidad para el estudio de los patrones de adaptación local (Keller et al., 2013).

La temperatura puede afectar muchos otros aspectos fisiológicos de los organismos, tales como la velocidad de carrera y la capacidad inmune. Diversos estudios han mostrado que la velocidad de carrera en ectotermos es dependiente de la temperatura, existiendo una temperatura óptima a la que el animal desempeña su velocidad de forma máxima (Chen et al., 2003). Del mismo modo, la respuesta inmune depende de la temperatura, ya que uno de los principales aspectos de la respuesta inmune es la fiebre (Kluger et al., 1998), aunque en ocasiones la respuesta inmune puede provocar hipotermia (Deen y Hutchison, 2001). Tanto velocidad de carrera como capacidad inmune están bajo una fuerte presión selectiva ejercida por depredadores y patógenos (Caro, 2005; Schmid-Hempel, 2011). Por tanto, en los gradientes altitudinales podemos predecir una compleja relación entre temperatura, velocidad de carrera y capacidad inmune, especialmente en ectotermos.

Considerando lo expuesto, esta tesis intenta arrojar luz sobre la variación intraespecífica y los procesos de adaptación local examinando las relaciones entre la temperatura corporal, el desempeño en la velocidad de carrera y en el sistema inmune, a lo largo de un gradiente altitudinal de 2200 metros en Sierra Nevada (España), en un ectotermo, la lagartija colilarga (*Psammodromus algirus*).

### *1.2.-Temperatura corporal*

La temperatura corporal es uno de los principales condicionantes de la fisiología de los animales, ya que los procesos metabólicos que permiten la vida sólo pueden tener lugar dentro de un rango de temperaturas más o menos estrecho (White et al., 2006). Por lo tanto, en el reino animal no faltan las estrategias para mantener la temperatura corporal dentro de los márgenes adecuados. Las diversas estrategias térmicas posibles pueden clasificarse en dos grandes tipos: la endotermia y la ectotermia. La endotermia conlleva la capacidad de generar calor metabólico, lo que proporciona una importante independencia térmica respecto al entorno (Bennett y Ruben, 1979). La ectotermia, por el contrario, implica una estrecha dependencia de fuentes de calor ambientales por la carencia de mecanismos fisiológicos que generen calor en cantidades suficientes (Hertz et al., 1993). La principal ventaja de la ectotermia sobre la endotermia es su bajo coste energético (Pough, 1980; Pough et al., 2001). El ahorro energético que permite la ectotermia debe ser considerable, teniendo en cuenta los elevados costes que lleva asociada (Alford y Lutterschmidt, 2012).

Y es que pocos factores condicionan de una manera tan decisiva la biología de muchos saurios como su ectotermia (Bennett, 1980). En muchos saurios se ha documentado ampliamente la dependencia térmica de procesos como el crecimiento (Brewster et al., 2013), la locomoción (Xiang et al., 1996), la digestión (McConnachie y Alexander, 2004) o la reproducción (Du et al., 2005; Sun et al., 2012). Por ello, la mayoría de saurios posee un conjunto de mecanismos, principalmente comportamentales, que les permiten mantener su temperatura corporal en el rango de temperatura preferido (Hutchinson y Maness, 1979; Hertz et al., 1993), dentro del cual el desempeño metabólico, digestivo y locomotor, entre otros, resulta óptimo (Adolph y Porter, 1993). De hecho, como evidencia de la importancia de la termorregulación en los saurios, un estudio sobre la biología térmica de dos lacértidos mediterráneos concluyó que la lagartija colirroja (*Acanthodactylus erithrurus*) dedica a la adquisición de calor el 40% de su tiempo, porcentaje que en la lagartija colilarga (*Psammodromus algirus*) aumenta hasta superar el 47% (Belliure et al., 1996). Esto sin contar que la termorregulación como tal no sólo implica el asoleamiento, sino el mecanismo opuesto que consiste en evitar activamente el sol para disipar el calor cuando es excesivo.

Ese es precisamente uno de los grandes costes de la ectotermia: el consumo de tiempo. La termorregulación acapara una importante fracción del tiempo de actividad del animal, lo que reduce la proporción disponible para otras funciones vitales, como la alimentación (Verwaijen y Van Damme, 2007), la defensa de un territorio o la búsqueda de parejas sexuales (Huey y Slatkin, 1976), con lo que el reparto del tiempo entre diferentes actividades está sujeto a un importante compromiso, entendiendo como compromiso la competencia por los mismos recursos entre rasgos de la estrategia vital de un organismo (Zera y Harshman, 2001; Roff, 2002).

Por otra parte, para muchos saurios el asoleamiento es la principal estrategia de adquisición de calor (Bauwens et al., 1996). Este comportamiento es más complejo de lo que puede parecer a primera instancia: lejos de consistir en simplemente colocarse bajo el sol, la orientación paralela o perpendicular del cuerpo del animal con respecto a la trayectoria de sus rayos regula la ganancia de calor (Muth, 1977), y de hecho varía de manera circadiana y estacional según los requerimientos térmicos de los animales (Waldschmidt, 1980). No obstante, este comportamiento no está exento de costes, dado que durante la exposición para el asoleamiento los animales son especialmente conspicuos para los depredadores, lo que causa un notable riesgo de depredación (Herczeg et al., 2008).

Dado que los ectotermos dependen en buena medida de fuentes externas de calor, otro factor importante en su termorregulación es la calidad térmica del hábitat (Fei et al., 2012). De hecho, se ha demostrado que las tasas de supervivencia de los reptiles aumentan con la temperatura ambiental (Moreno-Rueda y Pleguezuelos, 2007). Más que un coste en sí, la calidad térmica del hábitat puede significar una limitación a la termorregulación, que incrementa los costes anteriores al requerirse más tiempo para conseguir una temperatura corporal adecuada. En hábitats térmicamente limitantes disminuye por tanto el tiempo disponible para otras funciones y aumenta el riesgo de depredación (Christian, 1998; Blouin-Demers y Weatherhead, 2001).

En consecuencia, la dedicación de un reptil a la termorregulación está sujeta a un balance entre sus costes y sus beneficios, como fue propuesto por Huey y Slatkin (1976). Su modelo de coste-beneficio de la termorregulación predecía que 1) el óptimo fisiológico no siempre ha de coincidir con el ecológico, lo cual es una consecuencia de los costes de la termorregulación; 2) la termorregulación sólo es beneficiosa cuando sus costes son bajos; 3) los especialistas o estenotermos termorregularán con más eficiencia que los generalistas o euritermos, salvo que los costes de la termorregulación sean altos; y 4) la termorregulación será más eficiente en hábitats de mayor productividad o de menor competencia por los recursos térmicos. Según este modelo, pues, la eficiencia de la termorregulación disminuirá en hábitats térmicamente limitantes, desplazándose las estrategias hacia la termoconformación, definida como la ausencia de termorregulación activa (Nicholson et al., 2005). Aunque no faltan los trabajos que apoyan este modelo (Rummery et al., 1994; Herczeg et al., 2006; Herczeg et al., 2008), otros autores han encontrado que varios reptiles de hábitats térmicamente limitantes no sólo no se vuelven termoconformadores, sino que termorregulan con gran precisión (Christian, 1998; Blouin-Demers y Weatherhead, 2001; Blouin-Demers y Nadeau, 2005;

Besson y Cree, 2010). En este contexto, Vickers et al. (2011) propusieron el modelo extendido de coste-beneficio de la termorregulación, que predice que los ectotermos aumentarán su esfuerzo de termorregulación en hábitats de menor calidad térmica, de modo que mantengan los beneficios de una temperatura corporal óptima. Aunque este modelo fue descrito y testado con saurios de climas excesivamente cálidos, también podría explicar casos similares en ambientes fríos, como la eficaz termorregulación de *Zootoca vivipara* en climas subárticos (Herczeg et al., 2003) o el aumento en la eficacia de la termorregulación en ambientes térmicamente limitantes del tuátara, *Sphenodon punctatus* (Besson y Cree, 2010).

Recapitulando, la eficacia de la termorregulación depende del balance entre sus costes y sus beneficios (Vickers et al., 2011), este balance está determinado en buena medida por la calidad térmica del hábitat (Huey, 1991), y esta calidad varía geográficamente (Sunday et al., 2010). El rendimiento y el desempeño de la termorregulación de un ectotermo, por tanto, dependen íntimamente del ambiente térmico en el que se desenvuelve (Shine, 2005), reduciéndose en climas fríos donde la disponibilidad térmica es menor (Hertz et al., 1993).

Los climas fríos, como los hallados en alta montaña, imponen a los ectotermos una serie de importantes limitaciones ecológicas, sobre todo por la menor calidad térmica del hábitat (McCain, 2010). La altitud puede modificar las necesidades térmicas de los reptiles, así como su comportamiento y su fenología (Chettri et al., 2010; Guitérrez et al., 2010). Los reptiles deben ajustar su fenología a diferentes condiciones climáticas (Castilla et al., 1999). De hecho, se ha demostrado en diversas ocasiones que la fenología de los ectotermos está íntimamente relacionada con la temperatura ambiental del hábitat (Smith, 1998; Moreno-Rueda et al., 2009). La *fitness* puede verse dramáticamente afectada si fenómenos tales como la gametogénesis, la cópula, el parto o, en su caso, la puesta de huevos y su eclosión no tienen lugar en el momento apropiado (Sinervo y Adolph, 1989; Mathies y Andrews, 1995).

Además, al disminuir los períodos térmicamente aptos para la actividad circadiana, los reptiles deben dedicar más tiempo a la termorregulación (Nelson y Gregory, 2000), por lo que disponen de menos tiempo para la búsqueda de presas (Díaz y Carrascal, 1993) y por tanto acumulan menos reservas (Van Damme et al., 1989, Carrascal et al., 1992). Para un saurio con menos reservas es más difícil superar el período de hibernación (Naya et al., 2008), y en climas fríos este es más prolongado, dado que el período de actividad circaanual también se acorta (Busack y Jaksic, 1982).

Todo ello podría compensarse por fenómenos de adaptación local (Kawecki, 2008), es decir, si los animales que viven en hábitats más fríos pudieran realizar sus actividades vitales con igual eficiencia a temperaturas más bajas. Dos hipótesis clásicas complementarias intentan explicar la adaptación de los ectotermos al ambiente térmico. Por un lado, la hipótesis lábil predice que estos fenómenos de adaptación local pueden producirse, y las temperaturas óptimas pueden adecuarse a las temperaturas ambientales, de modo que sean más fáciles de alcanzar (Hertz et al., 1983; Rodríguez-Serrano et al., 2009). Por otro lado, la hipótesis estática sostiene que la biología térmica es evolutivamente conservadora, y puede ser bastante constante a pesar de la variación climática (Crowley, 1985; Hertz et al., 1993). Cuál de estas dos hipótesis sobre la adaptación de los ectotermos al ambiente térmico funcione en cada caso particular dependerá de los mecanismos subyacentes y de la capacidad de adaptación de la biología térmica.

Por otra parte, es frecuente que los animales que afrontan climas fríos presenten adaptaciones específicas que facilitan la adquisición y retención de calor. Por ejemplo, las extremidades de algunos animales de climas fríos son de menor tamaño, con lo que se reduce la relación superficie/volumen corporal, de modo que existe menos superficie por unidad de volumen por la que pueda disiparse el calor. Este fenómeno se conoce como regla de Allen (Allen, 1877), y ha sido demostrado en buen número de mamíferos (Yom-Tov y Nix, 1986), aves (Symonds y Tattersall, 2010), reptiles (Tang et al., 2012) y anfibios (Alho et al., 2011). Otra regla macroecológica deriva de la relación entre el tamaño corporal y la temperatura, la llamada regla de Bergmann, predice que los animales en climas más fríos serán más grandes, otra manera de reducir la relación superficie/volumen corporal (Bergmann, 1847). Esta regla ha sido ampliamente validada en endotermos, para los que fue descrita (Meiri y Dayan, 2003; Millien et al., 2006). No obstante, la validez de esta regla para ectotermos es controvertida, ya que se han documentado casos de ectotermos que la siguen (Ashton, 2002; Blanckenhorn & Demont, 2004; Millien *et al.*, 2006), pero no faltan los trabajos que citan resultados opuestos (Mousseau, 1997; Ashton & Feldman, 2003; Olalla-Tárraga *et al.*, 2006; Adams & Church, 2008; Hu *et al.*, 2011). Parece faltar, pues, un marco teórico capaz de encuadrar la variedad de respuestas que el tamaño corporal de los ectotermos puede presentar ante la variación en temperatura ambiental.

### *1.3.-Velocidad de carrera*

La menor disponibilidad de recursos tanto térmicos como tróficos (no ya estimada como abundancia de presas, sino de tiempo que dedicar a su localización y captura) en los hábitats marginales podría tener efectos no sólo en la termorregulación, sino también en otros factores como la velocidad de carrera. De hecho, la velocidad de carrera en ectotermos es fuertemente dependiente de la temperatura corporal: en la mayoría de los casos existe una temperatura óptima para la carrera, por encima y por debajo de la cual el desempeño de la locomoción disminuye (Waldschmidt and Tracy, 1983; Xiang et al., 1996; Pinch and Claussen, 2003). Por lo tanto, dado que la temperatura ambiental puede limitar la temperatura corporal de los ectotermos, las limitaciones en la temperatura ambiental podrían afectar a la capacidad de huida de los saurios.

Dos hipótesis excluyentes modelan las distintas relaciones entre el desempeño de funciones vitales como la locomoción, y la temperatura corporal. La hipótesis de variación contragradiente funciona en los casos en que la variabilidad genética puede compensar la influencia de diferentes ambientes térmicos en los fenotipos de los ectotermos, de manera que, a nivel metabólico, ocurren procesos que permiten a los animales mejorar el desempeño de sus actividades en rangos de temperatura que incluso pueden estar fuera de los que los organismos presentan durante su actividad (Conover and Schultz, 1995; McElroy, 2014). Alternativamente, la hipótesis de coadaptación se aplica cuando el desempeño de las funciones vitales es máximo dentro de los rangos de temperatura que los ectotermos experimentan cuando están activos (Gilchrist, 1995; Angilletta, 2009).

Como otros rasgos, la velocidad de carrera de muchos saurios está sujeta a dimorfismo sexual (Lailvaux, 2007). Este dimorfismo en desempeño puede estar mediado por el dimorfismo subyacente en la talla, cuando uno de los sexos, habitualmente el masculino, alcanza tamaños mayores que el otro (Braña, 1996; Cox et al., 2003), o en la forma del cuerpo, ya que las hembras, como ventaja reproductora que permite albergar más huevos, suelen tener troncos proporcionalmente más largos que los machos, que a su vez suelen tener cabezas más grandes que las hembras (Forsman y Shine, 1995; Braña, 1996; Olsson et al., 2002). Por otra parte, especialmente en los machos, los poros femorales segregan productos de señalización química implicados en la reproducción (Martín et al., 2007), de modo que se seleccionan en ellos extremidades posteriores más largas capaces de albergar más poros y por tanto ser más eficientes en la atracción de hembras (Iraeta et al., 2011), pero también de correr más rápido, ya que la velocidad de carrera en saurios presenta una elevada correlación con la longitud de la extremidad posterior (Bauwens et al., 1995; Bonine y Garland, 1999).

La masa corporal, por su parte, constituye una carga que disminuye la velocidad en muchos animales (Witter y Cuthill, 1993), lo que explicaría los resultados de un trabajo de Pérez-Tris et al. (2004), que encontraron que la lagartija colilarga, *P. algirus*, disminuye su masa en presencia de depredadores, probablemente optimizando así su capacidad de huida. Un caso especial de aumento de masa corporal es la gravidez de las hembras. De hecho, se ha encontrado que las hembras grávidas de varias especies de lacértidos tienen menor velocidad de carrera (Shine, 1980), por lo que parece haber un compromiso entre la velocidad de carrera y la eficiencia reproductora de las hembras de lacértidos (Miles et al., 2000).

Por otra parte, la velocidad de carrera es un rasgo costoso que consume una buena parte de los recursos de los saurios (Christian et al., 1997). De hecho, la velocidad de carrera puede verse afectada negativamente por un estado energético deteriorado (Hertz et al., 1983; Sinervo y Losos, 1991). La velocidad de carrera es una de las principales estrategias de defensa de los saurios frente a sus depredadores (López y Martín, 2002; Perry et al., 2004; Husak y Fox, 2006), y por lo tanto está sujeta a selección natural (Calsbeek e Irschick, 2007). Además, una elevada velocidad de carrera facilita la captura de presas (Irschick y Garland, 2001; Lailvaux e Irschick, 2006). Por todo ello, la velocidad de carrera se correlaciona positivamente con el éxito reproductor (Husak y Fox, 2006; Peterson y Husak, 2006) y la supervivencia (Miles et al., 2000; Warner y Andrews, 2002; Le Galliard et al., 2004) en muchas especies. No en vano, se considera un elemento clave de la *fitness* de los saurios (Bonine y Garland, 1999).

### ***1.4.-Sistema Inmune***

Otro factor con un gran impacto en la *fitness* de los saurios, como de la mayoría de los animales, es el sistema inmune, y es que si la velocidad de carrera era su principal defensa contra los depredadores, el sistema inmune lo es frente a los patógenos (Schmid-Hempel, 2011). De hecho, una mayor eficacia del sistema inmune mejora la supervivencia (Møller y Saino, 2004). El sistema inmune de los vertebrados está en general bastante conservado: incluye una serie de glóbulos blancos (serie celular) que reconocen y atacan a los elementos patógenos mediante fagocitosis, anticuerpos (serie humoral), agentes oxidantes o lisozimas (Wakelin y Apanius, 1997), aparte de fiebre en muchas ocasiones

(Kluger et al., 1975; Kluger et al., 1998). Sin embargo, la puesta en marcha del sistema inmune es costosa. Por un lado, una respuesta inmune excesiva puede desencadenar enfermedades autoinmunes o estrés oxidativo (Sorci y Faivre, 2009), entendido el estrés oxidativo como el desequilibrio entre la producción de sustancias oxidantes y las defensas antioxidantes (Halliwell, 2007). Por otro lado, la actividad inmune es metabólicamente costosa en términos de energía y de consumo de aminoácidos y proteínas (Lochmiller y Deerenberg, 2000; Demas, 2004; Schmid-Hempel, 2011). Entre los componentes del sistema inmune, la fiebre es particularmente costosa, no obstante su eficacia demostrada (Kluger et al., 1975), y su efecto positivo sobre la supervivencia tanto de endotermos como de ectotermos (Kluger et al., 1998). Para los endotermos, que generan calor internamente, supone un aumento del gasto energético (Marais et al., 2011). Para los ectotermos supone un incremento de la magnitud de los costes propios de la termorregulación discutidos anteriormente (principalmente exposición a depredadores; Herczeg et al., 2008), así como un aumento del gasto energético, dado que el metabolismo se incrementa con la temperatura corporal (Sherman and Stephens, 1998).

Por lo tanto, cabe esperar un compromiso entre el sistema inmune y otros rasgos energéticamente costosos. De hecho, se ha demostrado que el éxito reproductor (Råberg et al., 2000; French et al., 2007; Meylan et al., 2013), la capacidad de atracción sexual (López et al., 2009), la capacidad de aprendizaje (Grindstaff et al., 2012), el crecimiento (Uller et al., 2006), e incluso la supervivencia (Moret and Schmid-Hempel, 2000; Hanssen et al., 2004; Eraud et al., 2009) pueden competir energéticamente con el sistema inmune. Por ello, cabe esperar también un compromiso entre el sistema inmune y la termorregulación, así como la velocidad de carrera. La velocidad de carrera puede verse comprometida por el sistema inmunitario, no sólo porque ambos consumen una considerable cantidad de energía (Lochmiller y Deerenberg, 2000; Hancock y Gleeson, 2002; Schmid-Hempel, 2011), sino porque ambos compiten por recursos proteicos (Christian et al., 1997; Lochmiller y

Deerenberg, 2000). Y no sólo eso: la acción del sistema inmunitario puede aumentar el estrés oxidativo, perjudicando el desempeño de la velocidad de carrera, ya que la carrera también produce estrés oxidativo (Fisher-Wellman and Bloomer 2009).

La relación entre el sistema inmune y la termorregulación es especialmente compleja, dado que uno de los medios de actuación del sistema inmune es en muchas ocasiones la fiebre, un aumento de la temperatura corporal con efectos beneficiosos para otros componentes del sistema inmune, o con efectos perjudiciales directos para los patógenos (Kluger et al., 1998). La fiebre no está exenta de costes, como se dijo anteriormente. En determinadas circunstancias, los costes de la fiebre para los ectotermos pueden superar a sus beneficios, lo cual dependerá en buena medida del balance entre costes y beneficios que la adquisición de calor suponga en unas circunstancias ambientales dadas. De hecho, aunque existe constancia de ectotermos que responden a las infecciones con fiebre (Kluger et al., 1998), en otras ocasiones está documentada una respuesta hipotérmica (do Amaral et al., 2002; Merchant et al., 2008). Dado que los costes de la termorregulación dependen de la temperatura ambiental, y esta varía geográficamente (Sunday et al., 2011), cabe esperar variación geográfica en la respuesta térmica de los ectotermos a un desafío inmune.

## 2.-La especie de estudio: La lagartija colilarga

La lagartija colilarga, *Psammodromus algirus* (Linnaeus, 1758), es un lacértido de tamaño medio, distribuido en los extremos Sudoeste de Europa y Noroeste de África, donde aparece en hábitats arbustivos de carácter mediterráneo, en los que se comporta como un depredador generalista, siendo a su vez presa de numerosos depredadores tanto aéreos como terrestres, y hospedador de múltiples ecto y endoparásitos (Salvador, 2011).

## *2.1.-Filogenia y biogeografía*

Junto con el género *Gallotia*, constituido por los lagartos gigantes de las Canarias, el género *Psammodromus* (del griego *psammé*, “arena”, y *dromein*, “correr”) integra la subfamilia Gallotiinae, taxón monofilético que se considera grupo hermano de la subfamilia Lacertinae. Ambos grupos conforman la familia Lacertidae (Fu, 2000; Arnold et al., 2007; Mayer y Pavlicev, 2007). Aunque el origen biogeográfico del género *Psammodromus* es discutido, dada la distribución de sus especies, el grupo (así como la subfamilia Gallotiinae en la que se clasifica) debió de surgir en el noroeste de África o el sudoeste de Europa. Tradicionalmente, se han considerado cuatro especies integrantes de dicho género: *P. blanci* en Túnez, Argelia y Marruecos, *P. microdactylus* en Marruecos, *P. algirus* en el noroeste de África, la Península Ibérica y el sudoeste de Francia, y *P. hispanicus* en la Península Ibérica y el sudoeste de Francia. Sin embargo, recientes estudios de Fitze et al. (2011) sobre características ambientales, geográficas, fenotípicas y filogenéticas de *P. hispanicus* pusieron de manifiesto la existencia de tres clados en dicha especie. Posteriormente, un estudio taxonómico confirmó la necesidad de elevar dichos linajes a la categoría específica, añadiéndose así al género las especies *P. edwardsianus*, correspondiente al grupo oriental del complejo *P. hispanicus*, y *P. occidentalis*, en el oeste de la distribución del grupo, permaneciendo como *P. hispanicus* las restantes poblaciones (Fitze et al., 2012).

Del mismo modo, la filogeografía de *P. algirus* se ha visto sometida a revisión recientemente. Por un lado, basándose en estudios de alozimas y ADN mitocondrial, Busack y Lawson (2006) propusieron un origen africano de la especie, dado que las poblaciones marroquíes presentaban mayor grado de diferenciación que las ibéricas, y estimaron la escisión de las poblaciones a ambos lados del Estrecho de Gibraltar en 2,98-3,23 millones de años, mientras que para la Península Ibérica propusieron una separación de las poblaciones centrales y meridionales hace 1,40-1,54 millones de años. A la luz de estos resultados, Busack et al. (2006) propusieron considerar dichos clados ibéricos como especies separadas, situando a *P. manuelae* en el centro de la Península, y a *P. jeanneae* en la mitad meridional, mientras que se entendían como *P. algirus* las poblaciones del Magreb.

Sin embargo, paralelamente Carranza et al. (2006) propusieron para esta especie un origen ibérico, al hallar evidencias de la separación de dos linajes, uno occidental y otro oriental, unos 3,6 millones de años atrás. Según sus resultados, las poblaciones norteafricanas, emparentadas con el linaje occidental ibérico, se habrían diferenciado de las europeas hace unos 1,9 millones de años, coincidiendo con la reapertura del Estrecho de Gibraltar tras la crisis del Mesiniense. Asimismo, las poblaciones occidentales constan de cuatro subclados, uno norteafricano, otro en las provincias de Cádiz y Málaga, otro en el suroeste y un último subclado en el noroeste (Carranza et al., 2006; Verdú-Ricoy et al., 2010). Las especies *P. manuelae* y *P. jeanneae* corresponderían respectivamente a los subclados norte y sur del linaje occidental, según Verdú-Ricoy et al. (2010). Además, parece haber cierto grado de flujo génico entre los clados occidental y oriental de la Península Ibérica (Verdú-Ricoy, 2013). Posteriormente, Pina Martins (2007) validó el origen ibérico de la especie basándose en análisis de ADN mitocondrial; no obstante, según su propuesta, el clado oriental ibérico habría colonizado en primer lugar el norte de África, y desde allí se habría producido una recolonización de la Península Ibérica, que habría dado origen a las poblaciones occidentales que en ella se hallan. Siguiendo esta línea de evidencia, Verdú-Ricoy et al. (2010) y Carretero et al. (2011) pusieron en duda la validez de las especies descritas por Busack et al. (2006), considerando nuevamente *P. algirus* como una sola especie con dos linajes, uno oriental y otro occidental, sin validez taxonómica.

### ***2.2.-Corología***

La lagartija colilarga se extiende por un amplio territorio del sudoeste de Europa y el noroeste de África. Sus poblaciones europeas se distribuyen por

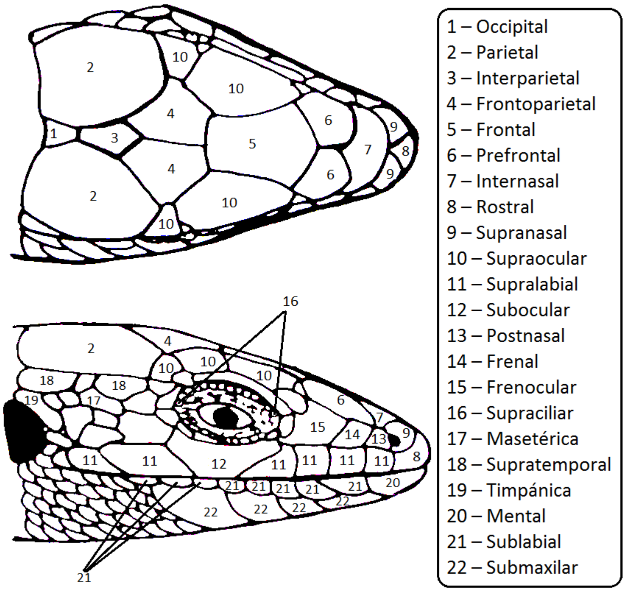
Distribución mundial de la lagartija colilarga.

la mayor parte de la Península Ibérica (salvo el extremo norte, de clima marcadamente eurosiberiano) y el sudoeste y centro-oeste de Francia (Jourde, 2004), en los departamentos de Pirineos Orientales, Hérault y Gard. Ha sido igualmente descrita en Andorra (Schlüter, 2011). Las poblaciones del italiano Isolotto dei Conigli, en las inmediaciones de la Isla de Lampedusa, parecen provenir de Marruecos y no de la más cercana Túnez, lo que apunta a una posible introducción antrópica (Carretero et al., 2009). Además, se han descrito recientes introducciones antrópicas en la isla de Mallorca (Vicens, 2005; Pinya y Carretero, 2011). En el Magreb, se encuentra en el centro y norte de Marruecos, así como en el norte de Argelia y Túnez (Schlueter, 2012). A pesar de que su área de distribución en general no parece estar en regresión (Pleguezuelos et al., 2002), en los últimos años se ha registrado su extinción en la isla gerundense de Meda Gran (Carretero et al., 1993).

### *2.3.-Morfología*

La lagartija colilarga es un saurio de mediana talla, si bien su tamaño está sujeto a variación geográfica. En la Península Ibérica, los ejemplares del noreste parecen ser de menor tamaño (entre 52,1 y 74,5 mm de longitud hocico-cloaca [LHC]; Carretero y Llorente, 1997) que los del centro (67-85 mm de LHC; Salvador y Veiga, 2001) o el sudoeste (59,3-79 mm de LHC; Mellado y Martínez, 1974; Pérez-Quintero, 1996), aunque el patrón latitudinal no es uniforme, lo que indica que otros factores, bióticos o abióticos, deben de estar influyendo en el crecimiento de las lagartijas. Destaca por el tamaño de su cola, cuya longitud puede llegar a ser más de dos veces y media la LHC. Las extremidades aparecen bien desarrolladas, y son responsables del desempeño en la carrera (Bauwens et al., 1995). En los machos, además, las extremidades posteriores están implicadas en la señalización sexual de tipo químico, ya que muslos más largos pueden albergar más y mayores poros femorales, encargados de las secreciones de señalización química en esta especie (Iraeta et al., 2011).

Las escamas cefálicas son grandes y bien diferenciadas, destacando las escamas temporales de gran tamaño. Esta especie se distingue por poseer una escama postnasal, y carecer de gránulos entre las escamas supraoculares y supraciliares. Posee 7 supralabiales, de las cuales la 5ª alcanza el ojo. Las escamas gulares aparecen imbricadas, sin ningún tipo de pigmentación oscura. El pliegue gular es poco patente, y el collar no está presente, apareciendo en su lugar cierto grado de imbricación de las escamas. La pupila es circular y de color negro, siendo el iris anaranjado o cobrizo. Las escamas dorsales son puntiagudas en su parte posterior, están fuertemente carenadas, muy imbricadas entre sí. Las escamas ventrales son cuadrangulares y se disponen en seis hileras, donde aparecen superpuestas unas a otras. Las escamas caudales son también cuadrangulares, estructuradas en círculos concéntricos.



Folidosis cefálica de la lagartija colilarga.

Su coloración dorsal está sujeta a cierta variabilidad, pudiendo aparecer tonalidades pardas, oliváceas o cobrizas (Carretero, 2002). En la cabeza, son características dos líneas supraciliares y otras dos supralabiales de color crema, con una banda más oscura intercalada. Dorsolateralmente, sendas líneas de color claro se destacan sobre el fondo parduzco, desvaneciéndose hacia los cuartos traseros del animal. En algunos individuos aparece además una línea vertebral más oscura. Excepcionalmente, pueden encontrarse individuos sin este diseño, con un color dorsolateral uniformemente parduzco. Lateralmente, tras las extremidades anteriores aparecen ocelos azules, variables en número y tamaño. La coloración ventral es grisácea (Carretero, 2002).

Como en muchos otros saurios, el dimorfismo sexual es muy marcado en esta especie, particularmente durante la época de celo. Los machos presentan cabezas más anchas y largas, así como colas más largas, y los poros femorales aparecen en mayor número y tamaño. Por su parte, el tronco de las hembras es proporcionalmente más largo, lo que incrementa su capacidad de albergar huevos (Olsson et al., 2002). En cuanto a la coloración, los machos lucen más y mayores ocelos que las hembras. Durante la estación reproductora, los machos del linaje occidental muestran una mancha gular de extensión variable, de color rojizo o anaranjado muy intenso (Díaz, 1993; Mellado y Martínez, 1974), que es de color amarillo en machos y hembras del linaje oriental. Las hembras de mayor tamaño pueden presentar también manchas amarillentas en la garganta (Carretero, 2002).

## *2.4.-Variación geográfica*

Los dos clados ibéricos, oriental y occidental, descritos por Carranza et al. (2006), y confirmados como linajes por Verdú-Ricoy et al. (2010), pueden diferenciarse por varias características morfológicas. Así, las poblaciones occidentales, con las que se relacionan las norteafricanas, se caracterizan por un color dorsal uniforme, mientras que en las poblaciones orientales es muy frecuente la aparición de una línea vertebral más oscura. Además, durante la

Diferencias en la coloración gular de machos de los linajes oriental (izquierda) y occidental (derecha) de *P. algirus*.

época de celo, los machos del linaje occidental desarrollan una vívida coloración nupcial roja o anaranjada en la garganta y la cabeza, a la vez que en los costados se pueden alternar escamas amarillas con otras escamas oscuras a modo de bandas verticales; por su parte, en los machos del linaje occidental la coloración nupcial queda reducida a una mancha naranja en la comisura labial que sólo se aprecia cuando el animal abre la boca. Por otro lado, los ocelos azulados postaxilares son más grandes y numerosos en el linaje occidental.

### ***2.5.-Hábitat***

La lagartija colilarga es en general un lacértido de exigencias mesófilas en cuanto a humedad y temperatura ambientales. Como consecuencia, no aparece en el extremo norte de la Península Ibérica, donde las precipitaciones son más abundantes por la influencia atlántica. Igualmente, su presencia en África se relaciona con las zonas donde la humedad es relativamente más alta, desapareciendo en los climas desérticos o subdesérticos. A partir de cierto tamaño (0,5 hectáreas), es la calidad del hábitat, y no su extensión, la que explica la presencia de la especie, prefiriendo coberturas arbóreas caducifolias a las perennes (Díaz et al., 2000; Santos et al., 2008). Se encuentra en general en espacios abiertos, generalmente asociados a vegetación arbustiva, donde se cobija (Díaz y Carrascal, 1991). En áreas boscosas, como pinares y robledales donde es frecuente (Mellado et al., 1975), así como en arenales costeros (Mellado, 1980; Seva, 1982; 1984), y dehesas (Martín y López, 2002; Godinho et al., 2011), selecciona las zonas con mayor cobertura de matorrales, hierbas u hojarasca en su defecto (Carrascal et al., 1989; 1990). De hecho, su abundancia se correlaciona con la presencia de arbustos de más de 20 cm de altura, entre otros factores (Díaz y Carrascal, 1991). La selección de microhábitats puede variar de forma estacional: Martín y López (1998) encontraron que en robledales del centro de España *P. algirus* prefiere cobijarse en rocas y en matorrales perennes, mientras que en verano selecciona matorrales de robledal y herbazales. Tolera cierto grado de alteración (antrópica o no) del medio, por lo que no es infrecuente en campos de cultivo o en los bordes de caminos y carreteras (Ribeiro et al., 2009), en hábitats modificados por vivares de conejos (Gálvez Bravo et al., 2009), o en terrenos incendiados (Santos y Poquet, 2010). No obstante, un excesivo deterioro del hábitat puede inducir un estrés fisiológico capaz de empeorar la condición corporal y aumentar la parasitemia mediante un incremento de la vulnerabilidad a depredadores, especialmente de las hembras (Amo et al., 2007).

Al tratarse de un animal generalista en muchas de sus exigencias relativas al hábitat, puede encontrarse desde el nivel del mar hasta un amplio espectro altitudinal. No obstante, a mayores latitudes alcanza menores altitudes, probablemente por limitaciones térmicas. Así, mientras sus poblaciones en Francia se encuentran hasta los 800 metros de altitud, en los Pirineos y el Sistema Central alcanzan los 1500, y en las Sierras Béticas y el Atlas marroquí llegan a rebasar los 2500 (Carretero et al., 1997; Fernández-Cardenete et al., 2000; Monasterio et al., 2011). Monasterio et al. (2011) han propuesto que la temperatura de incubación de los huevos puede ser un factor limitante para la distribución altitudinal de la especie en la Sierra de Guadarrama, ya que huevos incubados a menores temperaturas de incubación tardaron más en eclosionar y produjeron neonatos de menor tamaño y peor condición corporal.

En cuanto al significado ecológico de la selección de hábitat en esta especie, Díaz et al. (2005) encontraron que las lagartijas evitaban los jarales y espacios abiertos en favor del matorral de encina tanto en primavera como en verano. No obstante, desde el punto de vista térmico, el uso de matorral resultaba más restrictivo en primavera, lo cual indica que la selección de hábitat está más relacionada con factores como la presencia de alimento o la protección frente a depredadores que con la biología térmica.

### *2.6.-Abundancia*

Es una especie común y abundante en toda su área de distribución. No obstante, sus poblaciones se rarifican en los límites de su distribución. Sus densidades poblacionales varían geográficamente, habiéndose estimado unas densidades de 13-18 individuos/hectárea (ha) en Madrid (Cano, 1984), 25 en León (Delibes y Salvador, 1986), 130 en Guadarrama (Salvador y Veiga, 2001), o 178 en la abulense Sierra de Gredos (Gil Costa, 1992). Igualmente, Díaz (1993) encontró cierta variabilidad interanual en las densidades poblacionales de esta especie en Madrid, hallando 30 individuos/ha un año y 22,5 el año siguiente.

Debido a la elevada abundancia de la mayoría de sus poblaciones, Mateo-Miras et al. (2009) la definen como Preocupación Menor (LC) en la Lista Roja de la Unión Internacional por la Conservación de la Naturaleza (IUCN en sus siglas en inglés: International Union for Conservation of Nature).

### *2.7.-Ecología térmica*

Carretero y Llorente (1995) calcularon que la temperatura promedio de los individuos activos era de 31,5ºC, mientras que Díaz (1997) encontró que en gradiente térmico seleccionan una temperatura de 35,6ºC de media. En el campo, mantienen su temperatura corporal entre los 30 y los 33ºC, lo que

Ejemplar de *P. algirus* asoleándose protegido entre las espinas de una aulaga, *Ulex sp*.

consiguen alternando activamente parches de sol y sombra (Carrascal y Díaz, 1989). Generalmente se solea cerca de los arbustos que le sirven de cobijo, incluso sobre ellos hasta un metro de altura, principalmente a primera y última hora del día, actividad a la que dedica el 47,3% de su tiempo, lo que da cuenta de la importancia de la termorregulación para esta especie (Belliure et al., 1996). Se ha constatado que los períodos ininterrumpidos de asoleamiento matutinos suelen prolongarse durante unos 9 minutos de media a primera hora del día, disminuyendo su duración a medida que avanza la mañana (Díaz, 1991). Para solearse, dispone su cuerpo de manera que la insolación sea máxima, lo cual incrementa la eficiencia del calentamiento (Díaz, 1992).

Díaz y Cabezas-Días (2004) estudiaron la variabilidad estacional en la ecología térmica de esta especie. Encontraron que tanto la temperatura que los animales desarrollan en el campo como la que seleccionan en gradiente térmico en laboratorio era menor en primavera que en verano. En primavera, la actividad diaria de los animales contribuyó a la termorregulación más que en verano, ya que la temperatura ambiental es más próxima a la temperatura corporal óptima de la especie. Por el contrario, la selección activa de parches soleados y a la sombra fue mayor en verano que en primavera. Esto se explica porque en verano, cuando la temperatura ambiental excede la temperatura óptima de la especie, los individuos necesitan seleccionar parches sombreados para poder mantenerse activos sin calentarse por encima de sus límites de tolerancia. Díaz et al. (2006) encontraron un efecto similar de la estacionalidad sobre las preferencias térmicas de la especie, pero no detectaron ningún efecto de la altitud.

### *2.8.-Actividad*

La lagartija colilarga es un reptil eminentemente diurno. Su período de actividad se extiende desde finales de febrero hasta octubre. No obstante, este parámetro está sujeto a variación geográfica dependiendo fuertemente del clima. Así, en climas suaves de las costas alicantinas (Seva, 1982) u onubenses (Pérez-Quintero, 2001) pueden verse individuos activos durante todo el año, si bien durante el período invernal la actividad de la especie es más escasa, llevada a cabo principalmente por individuos inmaduros. La actividad es más intensa en primavera, disminuyendo hacia el verano, y aumentando tímidamente al inicio del otoño (Busak y Jaksic, 1982; Carretero y Llorente, 1995). Además, los ciclos circadianos son unimodales en primavera, con un máximo de actividad a mediodía, y bimodales en verano, cuando evitan las horas centrales del día, más calurosas (Pérez-Quintero, 2001).

### *2.9.-Reproducción*

Las hembras que han copulado pueden reconocerse por la marca en la zona por donde el macho las sujetó con sus mandíbulas.

La lagartija colilarga se reproduce en primavera y principios de verano, con cierta variación geográfica dependiendo de las condiciones climáticas. La parada nupcial consiste en una persecución más o menos prolongada por parte del macho, tras la cual este retiene a la hembra mordiéndola firmemente en el abdomen o el cuello, para facilitar el acoplamiento que dura de 3 a 15 minutos (Salvador y Veiga, 2001). Tras la cópula, el macho puede escoltar a la hembra durante 1 a 3 días (Salvador y Veiga, 2001). Los machos grandes suelen copular con hasta 3 hembras por temporada, mientras que las hembras se aparean con 1 o 2 machos (Salvador y Veiga, 2001). La disponibilidad reproductora de las hembras no es sincrónica, lo que implica que los machos pueden aparearse sucesivamente con diferentes hembras a lo largo de una misma estación de celo (Salvador y Veiga, 2001).

Los machos más grandes presentan una ventaja, al emerger más temprano de la hibernación (Veiga y Salvador, 2001). Además, los machos que desarrollan coloración nupcial son más grandes y tienen más edad (Salvador y Veiga, 2000), mayores niveles de testosterona en sangre (Díaz et al., 1994; Puerta et al., 1996), son más agresivos (Martín y Forsman, 1999; Martín y López, 1999), están más activos (Díaz, 1993; Martín y López, 1999), y dedican más tiempo al cortejo de hembras (Salvador et al., 1996; Martín y Forsman, 1999; Martín y López, 1999). Como contrapartida, son más susceptibles a la infección por endoparásitos (Belliure et al., 2004), y sus tasas de supervivencia son más bajas (Díaz, 1993). Los machos más pequeños podrían obtener cierta ventaja al mimetizarse con las hembras y obtener cópulas forzadas (Salvador et al., 1997), si bien los otros machos pueden expulsarlos tras reconocerlos mediante sus secreciones femorales (López et al., 2003). Aparte del color de la garganta, una correlación positiva demostrada por Salvador y Veiga (2008) entre la saturación del color de los ocelos axilares, el éxito de emparejamiento y la supervivencia sugiere que estos ocelos podrían ser una señal honesta de calidad de los machos. Por otra parte, la composición de la secreción de los poros femorales de los machos adultos varía con la edad, por lo que dicha secreción podría informar a otros individuos sobre la edad de su emisor (Martín y López, 2006). Además, la diferente respuesta de las hembras a las secreciones femorales según la carga parasitaria de los machos y su estado de salud sugiere que dichas secreciones informan a las hembras sobre la calidad de los machos (Martín et al., 2007).

La puesta tiene lugar un mes después de la cópula, en orificios practicados en el suelo por la hembra, o bajo piedras, a temperaturas más bajas que las ambientales. En ocasiones se han encontrado puestas comunales (Valladolid y González-Fernández, 2003; Pleguezuelos et al., 2004). En el sudoeste, las puestas tienen lugar desde abril hasta agosto (Pérez-Quintero, 1996), mientras en el sudeste se encuentran de marzo a julio (Escarré y Vericad, 1981; Seva, 1982). Esta amplitud puede deberse a la asincronía en el celo de las hembras, o podría indicar la existencia de varias puestas al año. El tamaño de puesta aumenta con la talla de la hembra, aunque está sujeto a variación geográfica (Escarré y Vericad, 1981; Pollo y Pérez-Mellado, 1990; Pérez-Quintero, 1996; Carretero y Llorente, 1997) y altitudinal (Iraeta et al., 2013), ya que las hembras a mayor altitud y latitud ponen más huevos, pero de menor tamaño (Díaz et al., 2007). Se ha observado también que las hembras procedentes de hábitats fragmentados ponen menos huevos y de menor tamaño (Díaz et al., 2005).

Neonato de *P. algirus*.

La temperatura afecta a la duración de la incubación: a 27ºC la incubación dura 60-72 días, mientras que a 30ºC este período se reduce a 48-55 días (Iraeta et al., 2006). No obstante, las lagartijas prefieren realizar la puesta en sitios más fríos que lo esperable según la temperatura ambiental (Iraeta et al., 2007). El tiempo de incubación se explica fundamentalmente por el linaje, entre otros efectos relacionados con la madre y la población de procedencia, según Iraeta et al. (2012). En el extremo norte de su distribución, no obstante, los períodos de incubación son más cortos incluso a la misma temperatura, lo que se ha interpretado como una estrategia que permite una eclosión temprana a pesar de las limitaciones térmicas (Díaz et al., 2007). Recientemente, Verdú-Ricoy et al. (2014) hicieron experimentos sobre cómo afectan la temperatura y la humedad de incubación a poblaciones de diferentes altitudes de los linajes oriental y occidental. Por una parte, los huevos incubados a mayor humedad produjeron neonatos más grandes y pesados. Por otra parte, las poblaciones occidentales mostraron mayor tasa de supervivencia que las orientales, así como los juveniles incubados a mayor temperatura de ambos linajes. Del mismo modo, la tasa de crecimiento fue más alta en los juveniles incubados a mayor temperatura, de manera que tras un mes de crecimiento alcanzaron el mismo tamaño que los neonatos incubados a menor temperatura, que presentaron mayor tamaño al nacer. Además, Iraeta et al. (2012) encontraron que la pérdida temprana de la cola reduce la tasa de crecimiento de los juveniles.

## *2.10.-Dieta*

La lagartija colilarga es un depredador generalista, lo cual optimiza la adquisición de energía (Carretero y Llorente, 1993a) y reduce el tiempo dedicado a la búsqueda de alimento (Díaz y Carrascal, 1993), en comparación con una estrategia especialista. Se alimenta principalmente de artrópodos (Verwaijen y Van Damme, 2008a). Puede cazar a la espera (estrategia comúnmente conocida con el giro inglés *sit and wait*), o, más comúnmente, buscando activamente a sus presas (Verwaijen y Van Damme, 2008b), que localiza mediante el sistema vomeronasal, la vista o el oído (Bateman, 1995). Su espectro de presas es muy amplio y ha sido profusamente estudiado: principalmente se compone de araneidos, formícidos, coleópteros, homópteros, ortópteros y dípteros, aunque también se encuentran, en general en proporción menor al 5%, solífugos, escorpiónidos, quilópodos, odonatos, blatoideos, mantoideos, dermápteros, fásmidos, neurópteros, lepidópteros, gasterópodos (Valverde, 1967; Mellado et al., 1975; Escarré y Vericad, 1981; Pérez-Mellado, 1982; Seva, 1984; Pollo y Pérez-Mellado, 1988; Díaz y Carrascal, 1990; Ortega-Rubio, 1991; Gil Costa, 1992; Carretero y Llorente, 1993b; Pérez-Quintero y Rubio-García, 1997; Arab y Doumandji, 2003; Baierl, 2011) e incluso los ejemplares de gran tamaño pueden devorar puntualmente pequeños vertebrados como ejemplares juveniles de lagartija colirroja (*Acanthodactylus erythrurus*; Albornà et al., 2004) o incluso de su propia especie (A. Salvador, com. pers.). Dada la amplitud de sus espectro de presas, la dieta varía estacional y geográficamente dependiendo de la disponibilidad de presas (Escarré y Vericad, 1981; Díaz y Carrascal, 1990; Ortega-Rubio, 1991; Carretero y Llorente, 1993; Pérez-Quintero y Rubio-García, 1997).

## *2.11.-Depredadores*

Dados su gran tamaño y su abundancia, su papel como presa es preponderante en los ecosistemas que habita. Así, se han descrito como depredadores aéreos rapaces como *Falco tinnunculus* (Valverde, 1967; Bernis, 1973), *Circaetus gallicus* (Garzón, 1973; Amores y Franco, 1981; Aragón y Sánchez-Fernández, 2013), *Circus pygargus* (Hiraldo et al., 1975a), *Aegypius monachus* (Hiraldo, 1976), *Falco naumanni* (Franco y Andrada, 1977), *Elanus caeruleus* (Amat, 1979), *Milvus milvus* (Blanco et al., 1987), *Accipiter gentilis* (Padial et al., 1998) o *Buteo buteo* (Múgica, 1990), otros grupos de aves, como *Burhinus oedicnemus* (Valverde, 1967), *Lanius excubitor* (Valverde, 1967), *Upupa epops* (Valverde, 1967), *Bubulcus ibis* (Herrera, 1974), *Ciconia ciconia* (Lázaro, 1984), o *Sturnus unicolor* (Pascual, 1992), e incluso rapaces nocturnas como *Bubo bubo* (Hiraldo et al, 1975b), *Tyto alba* (Vargas y Antúnez, 1982), *Athene noctua* (Máñez, 1983), u *Otus scops* (Amores et al., 1984).

Igualmente, entre sus depredadores terrestres han sido descritos reptiles como *Acanthodactylus erythrurus* (Valverde, 1967), *Coronella girondica* (Valverde, 1967), *Malpolon monspessulanus* (Valverde, 1967; Díaz-Paniagua, 1976; Vericad y Escarré, 1976; López-Jurado y Dos Santos, 1979), *Vipera latastei* (Valverde, 1967; Vericad y Escarré, 1976), *Timon lepidus* (Castilla et al., 1991) o *Macroprotodon cucullatus* (Pleguezuelos et al., 1994), así como mamíferos, tales como *Genetta genetta* (Delibes, 1974; Gil-Sánchez, 1998), *Vulpes vulpes* (Amores, 1975; Fedriani, 1996), *Lutra lutra* (Callejo-Rey et al., 1979; Ruiz-Olmo et al., 1989), *Martes foina* (Amores, 1980), *Sus scrofa* (Abaigar, 1993) o *Felis silvestris* (Moleón y Gil-Sánchez, 2003).

### *2.12.-Estrategias antidepredatorias*

Dada la enorme presión de depredación que sufren los saurios en general, y esta especie en particular (ver arriba), tanto por parte de depredadores aéreos como terrestres, su *fitness* depende estrechamente de su capacidad de evitar a los depredadores (Irschick y Garland, 2001). Esta especie basa su estrategia de huida en la velocidad de carrera, y ajusta sus patrones de movimiento y vigilancia en consonancia con la vulnerabilidad frente a los depredadores en cada hábitat (López y Martín, 2013). De hecho, la lagartija colilarga ajusta su distancia de huida acorde a la velocidad del ataque del depredador (Cooper, 2006). Además, su diseño rayado longitudinalmente podría confundir al depredador durante la huida, pues dificulta la percepción del sentido de la carrera (Pleguezuelos et al., 2010).

La velocidad guarda una relación directa con la longitud de las extremidades, y con su simetría (Martín y López, 2001a). Empero, diversos autores han detectado variaciones en el uso del espacio y de la velocidad dependiendo de diversos parámetros. De este modo, se ha constatado que los machos de mayor tamaño recorren mayores distancias en sus huidas, que comienzan a mayor distancia del atacante (Martín y López, 2000). Además, la distancia de huida puede variar estacionalmente, ya que en primavera, cuando la vegetación escasea, las lagartijas inician la huida a mayor distancia del depredador hasta alcanzar un refugio, mientras en verano dicha distancia se reduce al ser más abundante la vegetación que les sirve de cobijo (Martín y López, 1995a). La ausencia de cola también disminuye la distancia recorrida durante la huida, así como la velocidad (Martín y Avery, 1998). En ocasiones, cuando huyen largas distancias, hacen resonar de manera ruidosa la hojarasca reseca. Esto se ha explicado como una señalización de capacidad de escape hacia el depredador (Martín y López, 2001b).

Al igual que los adultos, los juveniles se refugian principalmente en la hojarasca o la vegetación arbustiva circundante, si bien algunos individuos usan rocas, o incluso trepan por los troncos de los árboles hasta los dos metros (Martín y López, 1995b). No obstante, se ha constatado que los patrones de huida de los individuos inmaduros son más erráticos, con frecuentes paradas, lo que hace pensar que los juveniles confían más en su capacidad críptica que los adultos, probablemente debido a que su menor tamaño les facilita pasar desapercibidos (Martín y López, 1996). También se ha comprobado que los individuos juveniles disminuyen su velocidad de huida después de haberse alimentado (Martín, 1996). La presión de depredación ralentiza las tasas de crecimiento de los juveniles de esta especie, aunque no afecta a la condición corporal ni a la capacidad inmunitaria (Civantos et al., 2010).

En trabajos llevados a cabo por Iraeta et al. (2010) en el centro de España, se comprobó que la velocidad de las hembras grávidas es menor, y sus carreras más cortas, que en las hembras no grávidas. No obstante, estos autores encontraron también variación espacial en este patrón, que puede relacionarse con la disponibilidad de refugios: así, en una localidad a menor altitud, con menor disponibilidad de refugios, la distancia de escape fue más corta que en otra localidad a mayor altitud, donde la disponibilidad de refugios era mayor.

Pérez-Tris et al. (2004) demostraron la existencia de un compromiso entre la alimentación y la velocidad de huida causado por la presión de depredación. Dado que los animales con mayor masa sufren un lastre durante la huida, las lagartijas sometidas experimentalmente a presión de depredación redujeron sus tasas de alimentación, y por ende su masa, cuando se les proporcionó abundante alimento. No obstante, los individuos mantenidos con baja densidad de presas mantuvieron su tasa de alimentación, pero igualmente redujeron su masa, lo que indica que el fenómeno puede estar mediado por el estrés fisiológico que supone la amenaza de un depredador.

Al igual que la mayoría de los saurios, la cola es autotómica en esta especie, de manera que la lagartija se desprende de ella de manera mecánica si esta recibe un ataque físico. Como última arma disuasoria, un individuo atrapado propinará con violencia fuertes mordiscos a su depredador con sus poderosas mandíbulas.

### *2.13.-Parásitos*

Como parásitos de esta especie se han citado cestodos (*Diplopylidium acanthotetra*), nematodos (*Parapharyngodon echinatus*; Roca et al., 1986; Roca y Lluch, 1988), hemogregarinas (Veiga et al., 1998), y se ha constatado su papel como reservorio de la espiroqueta *Borrelia burgdorferi*, causante de la enfermedad de Lyme (Dsouli et al., 2006). Igualmente, se han constatado parasitaciones de estadios larvarios de garrapatas (*Ixodes ricinus*), especialmente en invaginaciones cutáneas a cada lado del cuello, de manera que la lagartija evita ser parasitada en las axilas, donde las garrapatas podrían obstaculizar la movilidad, y en el oído, donde podrían empeorar la detección de presas y depredadores (Salvador et al., 1999).

## 3.-La zona de estudio: Sierra Nevada

Fue nombrada por los romanos como Mons Solarium; como Mont Oriens por los visigodos. La media luna la llamó Yabal Sulayr, y otras veces, no contenta con un solo nombre, Yabal-al-Tay: Monte del Sol el primero, Monte de la Nieve el segundo. Bajo insignia cristiana fue bautizada y rebautizada: Monte del Sol, del Aire y del Sol, de la Nieve, Sierra de la Helada… Sierra Nevada es el macizo montañoso más elevado de la Península Ibérica, y el segundo más alto de toda Europa Occidental después de los Alpes. Es además el más meridional de los grandes sistemas montañosos europeos. Su extensión es reducida en comparación con otros macizos, lo cual acentúa su pendiente.

### *3.1.-Su formación en contexto: la Península Ibérica*

La Península Ibérica ostenta una historia biogeográfica particularmente rica y compleja, que le ha valido la calificación de *hotspot* de biodiversidad en la Cuenca Mediterránea (Médail y Quézel, 1999). En primer lugar, por su ubicación geográfica ha servido como corredor entre África y Europa, principalmente al emerger las tierras bajo el Estrecho de Gibraltar durante la desecación responsable de la crisis salina del Messiniense hace unos 7,2 millones de años (García-Castellanos y Villaseñor, 2011). Esta conexión terrestre permitió un flujo bidireccional de organismos entre la Península Ibérica y el Magreb, con los subsecuentes fenómenos de especiación divergente a cada lado del estrecho, favorecidos por el aislamiento tras el hundimiento de dichas tierras. Esto ha sido ampliamente documentado en mamíferos (Palmer y Cambefort, 2000; Agustí et al., 2006), herpetofauna (Harris et al., 2002; Carranza y Arnold, 2003; Carranza et al., 2006; Pleguezuelos et al, 2008), invertebrados (Pinto-Juma et al., 2008; Sousa et al., 2012) y plantas (Lavergne et al., 2013).

Además, por su posición meridional respecto al resto de Europa, y el efecto atenuante que sobre las temperaturas tienen las masas oceánicas que la rodean, ha servido como refugio templado para muchas especies durante las glaciaciones del Cuaternario (Hewitt, 1996; Schmitt, 2007). Igualmente, el Magreb también actuó como refugio de biodiversidad durante las glaciaciones (Husemann et al., 2014), lo cual reforzó el número de especies implicadas en el continuo intercambio entre el Magreb y la Península Ibérica.

Por otra parte, la Península está muy compartimentada por la profusión de sistemas montañosos, la mayoría de los cuales se distribuyen siguiendo un eje de oeste a este, de modo que difícilmente pueden ser flanqueados por muchas especies hacia el norte y hacia el sur, favoreciendo los fenómenos de divergencia por aislamiento a ambos lados (García-París et al., 2001) y dentro de cada sistema montañoso (Hewitt, 1996).

Por último, sus variadas condiciones climáticas, causadas principalmente por las influencias atlántica por el norte y el oeste, mediterránea por el este, y sahariana por el sur, han favorecido una gran variedad de hábitats, que han propiciado a su vez un incremento notable de la biodiversidad tanto vegetal (Cowling et al., 1996; Ferrer-Castán y Vetaas, 2005) como animal (Moreno-Rueda y Pizarro, 2007; Moreno-Rueda y Pizarro, 2009).

### *3.1.1.-Historia Geológica*

En la formación de la Península Ibérica como entidad geológica, cabe distinguir distintas etapas, si bien fueron los fenómenos de orogenia Herciniana, durante la Era Primaria, y Alpina, durante la Era Terciaria, los principales responsables de su actual orografía (Terán y Solé, 1968; Casas-Sainz y de Vicente, 2009).

Durante la Era Arcaica (4000-600 millones de años), los plegamientos precámbricos hicieron emerger del mar una banda de tierra arqueada de noroeste a sudeste, formada principalmente por sustratos de pizarras y gneis, que comprendía la mayor parte de la actual Galicia. Igualmente, surgieron algunos puntos aislados del Sistema Central y los Montes de Toledo. No obstante, este macizo fue arrasado posteriormente por la erosión y cubierto casi por completo por los mares del Paleozoico.

Durante la Era Primaria (600-225 millones de años), por tanto, casi toda la actual Península Ibérica estaba cubierta por el mar. Sin embargo, al final de este período tuvo lugar la orogenia Herciniana, el primer gran proceso geológico de formación de la Península. Estos materiales, principalmente graníticos, pizarrosos y de cuarcita, se elevaron conformando el Macizo Hespérico o Ibérico en el oeste, que fue intensamente erosionado durante la propia Era Primaria. Este cuadrilátero de tierras emergidas se correspondía aproximadamente con la mitad septentrional de la actual Península: la Meseta, Galicia y la mayor parte de Portugal. Igualmente, surgieron el macizo Bético-Rifeño, y los de Aquitania, Catalano-Balear y del Ebro.

Durante la Era Secundaria (225-68 millones de años), predominó cierta calma orogénica, con avances y retrocesos del Mar de Tetis, precursor del actual Mediterráneo, no más allá de Madrid o Segovia. Las corrientes marinas depositaron sedimentos calcáreos, de areniscas o margas, de mayor grosor en las aguas más profundas, en terrenos que hoy están emergidos.

Durante la Era Terciaria (68-1,7 millones de años) tuvo lugar el segundo gran proceso orogénico en la formación de la Península Ibérica, la orogénesis Alpina, a causa del choque entre las placas Africana y Euroasiática, responsable en gran medida de los relieves que conocemos en la actualidad (Vegas y Banda, 1982). El proceso tuvo lugar en dos fases diferenciadas: una primera fase de compresión, en que la aproximación de las placas provocó el plegamiento de las capas de sedimentos, y una segunda fase de distensión, en la que se produjeron los reajustes tectónicos correspondientes a fallas y demás planicies entre montañas. Como resultado, se alzaron las grandes sierras alpinas de la Península y sus depresiones paralelas, como los Pirineos y las Sierras Béticas. Entre estas nuevas sierras y los macizos antiguos quedaron depresiones acentuadas por los movimientos de distensión: la depresión del Ebro y la del Guadalquivir. También se alzaron el Macizo Galaico, la Cornisa Cantábrica, el Sistema Central, los Montes de Toledo o Sierra Morena.

Durante la Era Cuaternaria (1,7 millones de años hasta hoy) tuvo lugar una fase de asentamiento de bloques. La erosión modeló los relieves terciarios, rellenando las depresiones del Ebro y el Guadalquivir. De hecho, las marismas del Guadalquivir pueden interpretarse como el resultado del proceso de sedimentación de depósitos en lo que fue un brazo de mar en su transición hacia río continental. Estos procesos, que continúan en la actualidad (Stich et al., 2006), terminaron de conformar los relieves ibéricos que conocemos en nuestros días.

### *3.1.2.-Litología*

Debido a los diferentes fenómenos causantes de las distintas fases de su formación, en la Península Ibérica predominan tres tipos de sustrato (Hernández-Pacheco, 1934). El sustrato silíceo es preponderante en su mitad occidental, aunque aparece también en otros puntos de manera aislada, como en los Pirineos, el Sistema Ibérico, Sistema Bético o la Sierra Costera Catalana, donde abundan materiales como el granito, la pizarra, el gneis o la cuarcita. Se trata de rocas endógenas, surgidas por metamorfismo causado por las altas presiones y temperaturas a las que se han visto sometidas, así como por una intensa actividad volcánica. Son los más antiguos, pues se originaron en la Era Primaria, o incluso con anterioridad. Posteriormente fueron cubiertos por depósitos sedimentarios, pero volvieron a aflorar a causa de la erosión. Dado que se erosionan con facilidad por la acción del agua, son frecuentes los perfiles suaves y las formas redondeadas.

El sustrato calcáreo traza una especie de Z invertida en la región oriental de la Península. Se extiende por la mayor parte de los Pirineos, y los Sistemas Ibérico y Bético. Son rocas sedimentarias carbonatadas surgidas durante la Era Secundaria, mayormente en medios marinos, que se elevaron durante el plegamiento Alpino. Las rocas calcáreas y conglomeradas son las más frecuentes, aunque también se encuentran piedras arenosas, margas y gres. Las formaciones geológicas de los sustratos calcáreos se caracterizan por fenómenos kársticos, tales como poljes, dolinas o cuevas.

El sustrato arcilloso es minoritario. Se extiende por las llanuras litorales de la mitad oriental y las depresiones del Ebro, del Guadalquivir y de la Meseta. Se trata de materiales sedimentarios y continentales, como margas y arcillas, de las Eras Terciaria y Cuaternaria. Son por tanto de los terrenos más recientes, por lo que no se han visto afectados por procesos orogénicos y se extienden como relieves planos, erosionados de forma tabular o de relieves costeros. Al ser materiales impermeables, el agua tiene un gran efecto erosivo sobre ellos.

### *3.1.3.-Clima*

Por su posición encrucijada entre el Mediterráneo, el Atlántico, el Sáhara y Europa, así como por su compleja orografía, la Península Ibérica posee una gran diversidad climática (Capel Molina, 2000). En su mayoría, el clima de la Península es de tipo mediterráneo, en distintas variedades. Así, en las costas orientales y meridionales, así como en el Valle del Guadalquivir, predomina un clima mediterráneo costero, donde las temperaturas son más suaves y las precipitaciones menos escasas, salvo en verano. Sin embargo, hacia el interior el clima se vuelve de tipo mediterráneo continental, con temperaturas y regímenes de precipitaciones y sequías más extremos. Por otra parte, la mitad occidental recibe más precipitaciones que la oriental. De este modo, el extremo sudeste corresponde con un clima árido o semiárido con menos de 300 mm de precipitaciones anuales. En puntos de las costas orientales de Andalucía también se da un clima subtropical, caracterizado por temperaturas cálidas durante todo el año y precipitaciones moderadas. Por otro lado, los extremos norte y occidental poseen un clima oceánico, donde las precipitaciones son abundantes a lo largo del año, y las temperaturas son frescas. En los sistemas montañosos como la Cordillera Cantábrica, los Montes de León, los Pirineos, la Cordillera Ibérica, el Sistema Central y las Cordilleras Béticas, se da un clima de montaña donde los inviernos son muy fríos y las precipitaciones son frecuentemente en forma de nieve durante los meses invernales.

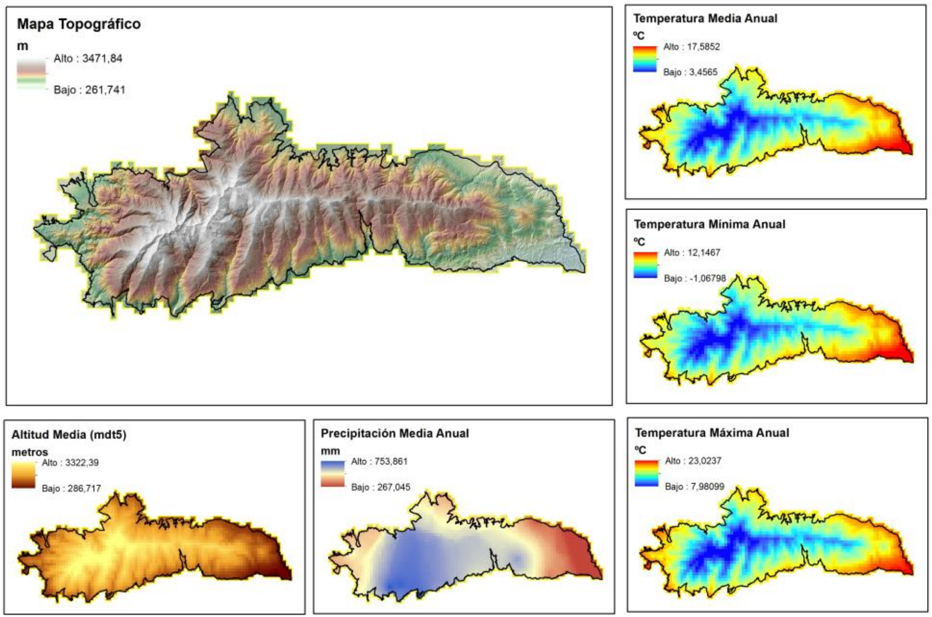
## ***3.2.-Sierra Nevada***

El macizo de Sierra Nevada, en el sudeste de la Península Ibérica (36º56’10”0 - 37º12’58”9 de latitud norte y 2º56’41”2 - 3º38’02”2 de longitud oeste), pertenece a los Sistemas Penibéticos. Se formó al alzarse durante la Era Terciaria los materiales submarinos sedimentados durante las Eras Primaria y Secundaria, por el impacto tectónico entre las placas Euroasiática y Africana. Abarca una extensión de casi 90 km de oeste a este, con un área de unos 20.000

km$^2$. En este sistema montañoso se alza el pico más alto de la Península Ibérica, el Mulhacén, con 3479 metros sobre el nivel del mar (msm).

La parte central de Sierra Nevada, donde se localizan las mayores elevaciones del sistema, está formada por pizarras metamórficas o esquistos. Alrededor, se extiende una aureola de rocas diversas, como mármoles, gneis, serpentinas, anfibolitas o pizarras. La zona exterior está integrada por un cinturón de dolomías y calizas. Por último, se encuentran arcillas y conglomerados de origen terciario.

Aunque enormemente variables dependiendo de la altitud, la orientación, o el perfil meteorológico de cada año, las temperaturas medias anuales oscilan entre los 3.5 y los 17.6ºC (REDIAM, 2001). El rango de precipitaciones anuales oscila entre los 267 y los 753 mm (REDIAM, 2001). Durante los meses más fríos, la precipitación ocurre por lo general en forma de nieve por encima de los 2000 msm, durando la capa de nieve alrededor de 6 meses al año (Noviembre-Mayo). Por debajo de dicha altitud, las precipitaciones suelen acontecer en primavera y otoño, disminuyendo la



Mapa topográfico de elevación, precipitaciones y temperaturas anuales medias, mínimas y máximas de Sierra Nevada.

probabilidad de nevadas gradualmente hacia cotas más bajas. Junto al deshielo vernal y estival, estas precipitaciones mantienen relativamente alta la humedad edáfica. No obstante, como corresponde a un clima típicamente mediterráneo, el verano es seco, con gran escasez de precipitaciones (Valle et al., 2004).

Por sus características bioclimáticas, en Sierra Nevada aparece una variedad de termotipos que se suceden altitudinalmente, variando desde el Mesomediterráneo hasta el Oromediterráneo, mientras que los ombrotipos van de seco a subhúmedo superior. Además, su pronunciada pendiente hace que estas regiones se sucedan en muy poco espacio. En lo tocante a su clasificación biogeográfica, la vegetación del área se puede encuadrar en la provincia Bética, en los sectores Nevadense y Alpujarreño-Gadorense respectivamente, dentro de los distritos Nevadense-Filábrico y Alpujarreño (Valle et al., 2004).

## 4.-Objetivos

Basándose en todo lo descrito anteriormente, esta tesis pretende arrojar luz sobre la forma en que la altitud afecta a las relaciones entre la termorregulación, la velocidad de carrera y el sistema inmune de un ectotermo, la lagartija colilarga, a lo largo de un gradiente altitudinal de 2200 metros en Sierra Nevada. Concretamente, los objetivos planteados son:

-Conocer cómo afecta la altitud a la fenología y a los patrones de actividad circadianos y circaanuales de la lagartija colilarga en Sierra Nevada. Las limitaciones térmicas en altitud deberían constreñir temporalmente ambos patrones. No obstante, si las lagartijas muestran ciclos de actividad similares a lo largo del gradiente de altitud, esto revelará adaptación local o plasticidad fenotípica. Este objetivo se desarrollará en el **Capítulo 1**.

-Explorar la temperatura corporal que experimentan las lagartijas a lo largo de un gradiente de altitud, y sus relaciones con la temperatura ambiental y la del sustrato, para conocer la fuente de calor preferida por estos animales. Del mismo modo, conocer experimentalmente la temperatura corporal que las lagartijas seleccionan en un gradiente térmico. A partir de estos datos, contrastándolos con un modelo nulo de no-termorregulación correspondiente con la temperatura corporal de los animales si su estrategia fuera la termoconformación, calcular la idoneidad térmica del hábitat, la precisión de la temperatura corporal y la efectividad de la termorregulación de ejemplares en distintos estados reproductores. Un cambio en las preferencias térmicas en altitud apoyaría la hipótesis lábil de la adaptación de los ectotermos a su ambiente térmico; si las preferencias se mantienen, los resultados estarían acorde con la hipótesis estática. Este objetivo se desarrollará en el **Capítulo 2**.

-Estudiar las variaciones de tamaño en altitud para comprobar la ocurrencia de clinas de Bergmann, que predicen que en zonas más altas, y por tanto más frías, los animales deberían alcanzar tallas mayores. Seguidamente, analizar cómo varían las tasas de calentamiento y de enfriamiento de individuos en distintos estados reproductores, y corroborar si, como prevé la regla de Bergmann, hay diferencias en la inercia térmica. Esperamos que esta sea mayor en los individuos de mayor tamaño, con menor relación superficie/volumen y, por tanto, con menor pérdida de calor. Este objetivo se desarrollará en el **Capítulo 3**.

-Comprobar el efecto de un desafío inmune sobre las preferencias térmicas de lagartijas de distintas altitudes en un gradiente térmico artificial. Dado que el desafío inmune tiene unos costes asociados similares en todas las poblaciones, la respuesta térmica de los animales dependerá del balance entre costes y beneficios de la termorregulación entre los distintos hábitats de procedencia, pudiendo variar desde la fiebre, si los costes de la termorregulación son bajos, a la hipotermia, si resultan elevados, pasando por la ausencia de cambio. Este objetivo se desarrollará en el **Capítulo 4**.

-Analizar experimentalmente las posibles correlaciones entre la velocidad de carrera y diferentes variables biométricas en un gradiente de altitud: longitud de las extremidades y masa. Previsiblemente, extremidades mayores contribuirán a una mayor velocidad, mientras que la masa será un impedimento al desempeño de la locomoción. Igualmente, comprobar si existen diferencias en la velocidad de carrera desarrollada por machos, hembras no grávidas y hembras grávidas. La predicción de partida es que los machos, más activos y territoriales, alcanzarán velocidades mayores. Este objetivo se desarrollará en el **Capítulo 5**.

-Constatar las posibles diferencias entre machos y hembras en el efecto de un desafío inmune sobre la velocidad de carrera a lo largo de un gradiente de altitud. Dado que tanto el sistema inmune como la locomoción consumen una gran cantidad de energía, y compiten por recursos como las proteínas, puede predecirse que un desafío inmune reducirá la velocidad, al menos en los individuos que destinen a ella más recursos en condiciones normales. Este objetivo se desarrollará en el **Capítulo 6**.

-Investigar el efecto de la temperatura corporal sobre el desempeño en locomoción a lo largo de un gradiente altitudinal. Esperamos que la velocidad sea menor a temperaturas subóptimas. Además, si las lagartijas de alta montaña están localmente adaptadas a los climas fríos, podrán desempeñar mayores velocidades a temperaturas corporales subóptimas que las de otros hábitats, de acuerdo con la hipótesis de contragradiente. Por el contrario, la ausencia de diferencias apuntaría a unos requerimientos térmicos similares a pesar de las diferencias en temperatura ambiental, acorde con la hipótesis de coadaptación. Este objetivo se desarrollará en el **Capítulo 7**.

## 5.-Materiales y Métodos Generales

### *5.1.-¿Por qué la lagartija colilarga?*

Por varias de sus características ya descritas (véase apartado 2), la lagartija colilarga resulta idónea para los objetivos de esta tesis. En primer lugar, su abundancia garantiza un número apropiado de ejemplares para los experimentos. Su amplia valencia ecológica facilita la localización de sus poblaciones. Pero sobre todo, su amplio rango de distribución altitudinal hace de ella una especie muy apropiada para el estudio de este tipo de gradientes.

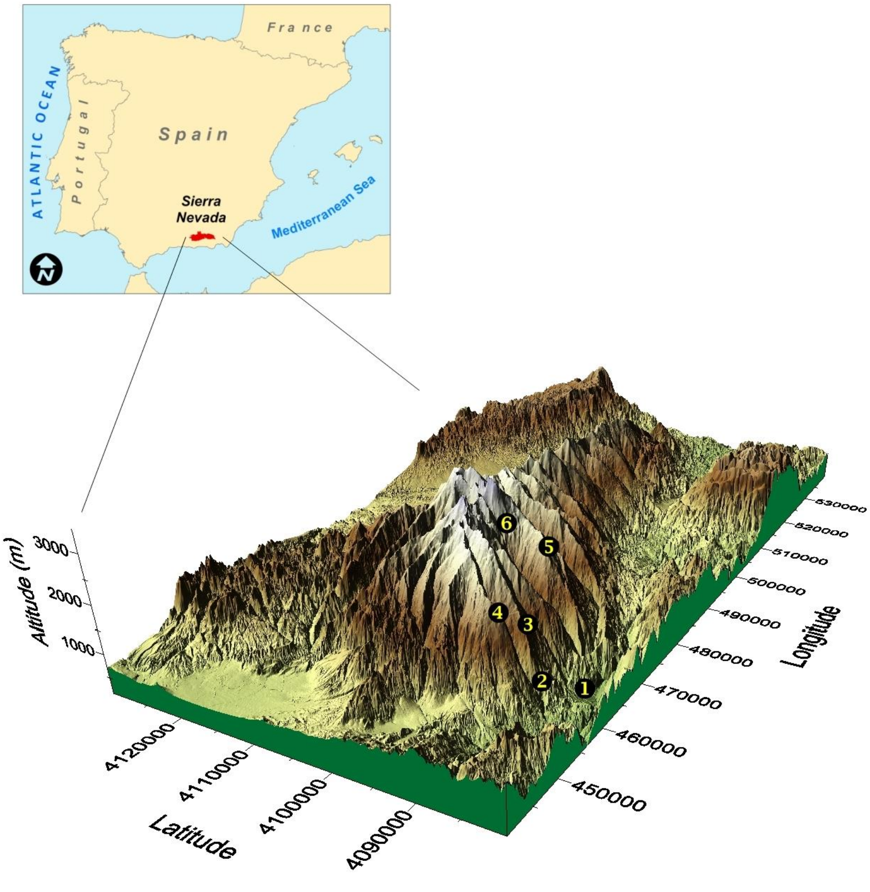
Además, la biología térmica de esta lagartija es suficientemente conocida, por lo que se sabe que es un eficaz termorregulador. El elevado número de depredadores potenciales que la amenazan hace también especialmente apropiado el estudio de su estrategia antidepredatoria más palmaria: la velocidad de carrera. Del mismo modo, se conoce un buen número de parásitos y patógenos que pueden atacarla, lo que convierte a su sistema inmune en un buen modelo de estudio.

### *5.2.-¿Por qué Sierra Nevada?*

Sierra Nevada se muestra como el enclave más apropiado para este estudio por diversas razones (véase apartado 3.2). Su posición meridional dentro de la Península Ibérica garantizaba un entorno suficientemente cálido para una amplia distribución altitudinal de los reptiles, mientras que su notoria elevación aseguraba unas temperaturas suficientemente frías en altitud para proporcionar un gradiente térmico suficiente. Por otra parte, su pronunciada pendiente facilitaba logísticamente disponer de estaciones de muestreo ampliamente separadas en altitud, pero poco distantes geográficamente, a la vez que reducían las posibilidades de sesgos por aislamiento físico entre las poblaciones. Por sus condiciones térmicas más favorables, se seleccionó la ladera sur.

### *5.3.-Poblaciones de muestreo*

El trabajo de campo fue desarrollado en la ladera sur de Sierra Nevada, en seis poblaciones distantes entre sí unos 500 metros de altitud, a 300, 700, 1200, 1700, 2200 y 2500 msm respectivamente. Debido a la pronunciada pendiente de Sierra Nevada, apenas 17,5 kilómetros separan las poblaciones de 300 y 2500 msm desde una perspectiva cenital.



Localización de Sierra Nevada en la Península Ibérica, y de las poblaciones de muestreo en Sierra Nevada, numeradas de menor a mayor altitud.

**Población de muestreo 300**: se encuentra a 300 msm, localizada a 36°53' de latitud norte y 3°24' de longitud oeste. En esta localidad los veranos son calurosos y escasos en precipitaciones, mientras que las heladas son infrecuentes en invierno. La vegetación potencial se clasifica en la serie termomediterránea, bética, algarviense y mauritánica, seca-subhúmeda, basófila de la encina (*Quercus rotundifolia*): *Smilaco mauritanicae-Querceto rotundifoliae, S. faciación típica.* No obstante, la zona está altamente antropizada, de modo que la vegetación actual añade al matorral propio de dicha serie de vegetación (*Rosmarinus officinalis, Retama sphaerocarpa, Genista sp., Artemisia sp. y Thymus sp.*) (Valle et al., 2004), cultivos de *Eucalyptus sp*. y diversos frutales.

**Población de muestreo 700**: se halla a 700 msm, a 36°55' de latitud norte y 3°26' de longitud oeste. El clima es similar al de la localidad anterior, principalmente por la influencia del río Guadalfeo, que propicia las condiciones propias del termotipo termomediterráneo. La vegetación potencial se encuadra en la serie termomediterránea, bética, algarviense y mauritánica, seca-subhúmeda, basófila de la encina (*Quercus rotundifolia*): *Smilaco mauritanicae*-*Querceto rotundifoliae S. faciación típica*. Sin embargo, debido a la alteración antrópica de la zona, en la actualidad la vegetación añade al matorral propio de la serie de vegetación en cuestión (*Cystus sp., Retama sphaerocarpa*, *Rosmarinus officinalis*, *Genista sp.*, etc.) (Valle et al., 2004), plantaciones de *Ficus carica*, *Olea europea* y *Prunus dulcis*.

**Población de muestreo 1200**: se encuentra a 1200 msm, a 36°56' de latitud norte y 3°25' de longitud oeste. Los veranos en esta localidad son aún calurosos, mientras que, por su mayor altitud, las heladas son más frecuentes en invierno. La vegetación potencial se clasifica en la serie supra-mesomediterránea filábrica y nevadense silicícola de la encina (*Quercus rotundifolia*): *Adenocarpo decorticantis*-*Querceto rotundifoliae S. faciación mesomediterránea* con *Retama sphaerocarpa*. El matorral es primordialmente el propio de dicha serie (Valle et al., 2004), si bien aparecen cultivos de *Castanea sativa*, *Ficus carica*, *Morus nigra* u *Olea europaea*.

**Población de muestreo 1700**: se halla a 1700 msm, a 36°57' de latitud norte y 3°26' de longitud oeste. En esta localidad, los veranos son cálidos, mientras que las heladas son frecuentes, así como las nevadas, en invierno. La vegetación potencial se encuadra en la serie supramediterránea nevadense y malacitano-almijarense silicícola del roble melojo (*Quercus pyrenaica*): *Adenocarpo decorticantis-Querceto pyrenaicae S. faciación* típica supramediterránea. En este punto se encuentra el melojar natural que crece a mayor altitud de la Península. Por su parte, en las laderas con mayor incidencia solar aparece la serie supra-mesomediterránea filábrica y nevadense malacitano-almijarense y alpujarreño-gadorense silicícola de la encina (*Quercus rotundifolia*): *Adenocarpo decorticantis-Querceto rotundifoliae* S. Abunda el matorral propio de dichas series de vegetación (*Genista sp.*, *Cystus sp.*, etc.) (Valle et al., 2004), mientras que apenas existe vegetación no autóctona.

**Población de muestreo 2200**: se encuentra a 2200 msm, a 36°58' de latitud norte y 3°19' de longitud oeste. Aunque los veranos son templados en esta localidad, las heladas se vuelven muy frecuentes a esta altitud, con abundantes nevadas en invierno. De hecho, la nieve cubre el suelo durante unos seis meses al año, variando la duración y el espesor de dicha capa según las condiciones meteorológicas de cada año. La vegetación potencial en este punto pertenece a la serie oromediterránea filábrico-nevadense silicícola del enebro rastrero (*Juniperus communis subsp. nana*): *Genisto baeticae-Junipereto nanae* S. El matorral dominante se compone principalmente de *Genista versicolor* en los claros que se abren en los bosques de *Pinus sylvestris* subsp. *nevadensis*, de origen antrópico (Valle et al., 2004).

**Población de muestreo 2500**: se halla a 2500 msm, a 37°01' de latitud norte y 3°19' de longitud oeste. Rodean a este enclave algunos manantiales de deshielo, así como los ríos Veleta y Mulhacén. El clima es similar al de la localidad anteriormente descrita, pero los fríos invernales son aún más extremos. Por ello, no hay vegetación arbórea en esta altitud. La vegetación potencial pertenece a la serie oromediterránea filábrico-nevadense silicícola del enebro rastrero (*Juniperus communis* subsp. *nana*): *Genisto baeticae-Junipereto nanae* S. (Valle et al., 2004).

### *5.4.-Manipulación y mantenimiento*

Los ejemplares de lagartija colilarga usados en esta tesis se capturaron durante sus períodos de actividad de los años 2010 a 2013. En la mayoría de los casos la captura se realizó manualmente, entre dos personas: una acechaba a las lagartijas alrededor de sus refugios (principalmente arbustos) mientras la otra la hacía salir de ellos en la dirección apropiada. Tras la captura, los animales eran transportados al laboratorio en bolsas de algodón que permitían una correcta ventilación. En el laboratorio, eran mantenidos en terrarios de plástico individuales de 20 × 13 × 9 cm. La cercanía de una ventana permitía el ajuste de los ciclos circadianos, mientras que un cable calefactor bajo un extremo del terrario encendido durante las horas centrales del día facilitaba la adquisición de una temperatura corporal apropiada. La comida, larvas de *Tenebrio molitor*, y el agua, en forma de gel acuoso vitaminado, eran proporcionadas *ad libitum*.

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# I - Chapter 1

## Patterns of seasonal activity in a Mediterranean lizard along a 2200 m altitudinal gradient.

This chapter reproduces the published article:

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**I.1.-Abstract** - Reptiles' ectothermy involves strong environmental temperature dependence to maintain body temperature. High mountains are extreme habitats which, because of their marked temperature decrease with altitude, impose difficulties on reptile thermoregulation, as the number of optimum-temperature days diminish in altitude. We studied the activity patterns of the altitudinally wide-distributed lizard *Psammodromus algirus* in six sampling stations that spanned an altitudinal range from 300 to 2500 m asl in Sierra Nevada (SE Iberian Peninsula). We performed censuses of the species during the seasonal and daily activity period, and measured lizard body temperature and environmental temperature in the six sampling plots along the altitude gradient. While environmental temperature decreased around nine degrees in that gradient, lizards' body temperature kept very similar in altitude. Seasonal activity peaked at the beginning of the activity period in highlands, but in the middle of the season in mid and lowlands. However, newborns appeared in the field almost at the same time along the altitude gradient. We suggest that lizards' thermoregulatory strategies must vary along this altitudinal gradient, permitting lizards to keep their body temperature around a very similar optimum and thus, develop very similar circa-annual activity patterns.

## I.2.-Introduction

Animals' activity patterns depend on biotic (ecological interrelationships, vegetation structure, etc.) and abiotic (altitude, environmental temperature, moisture, etc.) conditions in the environment they inhabit. Reptiles, as ectotherms, are strongly dependent on environmental temperature for keeping their physiological processes (Bennet, 1980). They display a set of mainly ethological mechanisms to keep their body temperature as close as possible to their metabolic optimum (Hutchinson and Manness, 1979). Preferred temperature range (PTR; Hertz et al., 1993) is defined as the body temperature range ectotherms select. At PTR, locomotion, digestion and metabolism are supposed to be the most effective for the highest maintenance and production rates (Adolph and Porter, 1993). Thus, reptile dependence on environmental temperature may constrain their life-history patterns, and limit their ability to inhabit in cold habitats, where heat sources are less available (Hertz et al., 1993).

Due to environmental constraints, terrestrial reptiles' PTR varies in latitude both inter and intraspecifically, diminishing the lower thermal limits as latitude increases (Sunday et al., 2010). As environmental temperature follows a similar pattern in latitude and in altitude (Graae et al., 2012), we can also expect a PTR variation in altitude. High mountains encompass extreme low temperature habitats (McCain, 2010), and overall, gradients of environmental parameters such as temperature, solar radiation, and atmospheric pressure from low to highlands (Körner, 2007). Days and hours with suitable temperature during the reptile activity season diminish as altitude increases, which seriously limits reptile thermoregulation capabilities in high elevations. This variation in thermal environment is likely to involve differences in lizards' thermoregulatory needs, behaviors and phenology along altitudinal gradients (Adolph and Porter, 1993; Chettri et al., 2010; Gutiérrez et al., 2010). Nevertheless, there are opposing effects of the adiabatic temperature decrease and the increase in solar radiation in altitude (Angilleta, 2009). As altitude increases, the narrower lay of atmosphere results in a higher transmission of radiation (Gates, 1980). This could have buffering consequences in lizards' operative temperatures in high altitude.

Phenology is a key aspect in an organism's life history: cardinal phenomena such as hibernation, gametogenesis, mating, egg-laying and hatching or parturition, may cause a reproductive failure or death if they do not take place in the appropriate moment (Mathies and Andrews, 1995; Sinervo and Adolph, 1989). This is especially relevant in short-life animals such as many lizards, since they may lose one of their scarce -or even their only- opportunity to reproduce. Because of their ectothermy, reptiles phenology is closely constrained by the thermal cycle in the place they inhabit (Moreno-Rueda et al., 2009; Smith, 1998), so it is expected to vary in altitudinal gradients (Mathies and Andrews, 1995; Navas, 2002). Reptiles must synchronize their activity patterns with the daily and annual thermal cycles, in order to optimize their productivity (Ibargüengoytía, 2005). Therefore, lowland and highland reptiles are expected to have different phenology due to the different climatic conditions they stand (Castilla et al., 1999). Moreover, the lower thermal availability in altitude might cause differences in field body temperature and phenology. Otherwise, reptiles could be locally adapted, in which case phenology and body temperature could not be as different as expectable considering the contrasting climatic conditions along the altitudinal gradient.

In this work, we analyzed circadian and circa-annual activity patterns of the Large Psammodromus lizard (*Psammodromus algirus*) along an altitudinal gradient, in Sierra Nevada (SE Spain). In the region, the species can be found there from the sea level to more than 2500 m asl (Salvador, 2011), being one of the widest altitudinal ranges for a single lizard species in Europe. Therefore, this species is an ideal model for the study of the effects of altitude on reptiles. In lowlands, high environmental temperature might limit lizard’s activity during summer, whilst in highlands, low environmental temperature might constrain activity in spring and autumn. In this altitude gradient, we predict that the circadian activity patterns within the same season and circa-annual activity cycles will be wider in lowlands. On the other hand, if some degree of local adaptation exists, highland lizards will be active at lower temperatures than lowland lizards.

## I.3.-Materials and methods

*Psammodromus algirus* is a medium-sized lacertid distributed in South West Europe and North West Africa, strongly linked to warm Mediterranean areas with shrub formations (Salvador, 2011). It behaves as an eurioic and generalist species for most of its ecological traits. This lizard is diurnal and avoids the coldest time of the year by hibernating for a variable period, from October-November until March-April, depending on the environmental temperature. The taxonomy of this species is controversial (Verdú-Ricoy et al., 2010), but all the populations studied here belong to the same clade.

The field work was performed in 2010, in the southern slope of Sierra Nevada (SE Spain), which shows a pronounced altitudinal range (200-3481 m asl). We established six sampling stations in the study area, separated about 500 meters in altitude from each other, from 300 to 2500 m asl (Appendix A), within the altitudinal range of the species in the study area (200-2600 m asl). All sampling stations met the ecological requirements of *P. algirus*, i.e.: shrubs and bushes 40-100 cm high and rather high vegetation cover (Díaz and Carrascal, 1991; Appendix A). Mean vegetation cover ranged between 53 and 67%, except for 2200 m asl, where it reached 82%. For the six sampling stations, neither vegetation cover nor mean shrub height followed any clear altitudinal pattern (Appendix A).

To quantify lizard activity, we performed 500m transects every two weeks in each sampling station during the annual activity season of the lizard in the study area (ca. April-October), although we began in May in the highest sampling station, because the access was closed by snow. Censuses were replicated every two hours along daytime, since sunrise until sunset, and we recorded the number of active adult and newborn individuals seen in each transect. We assumed lizards' detectability to be the same in all sampling stations. Air temperature was measured one meter above the ground, under a shadow, using a thermometer Hibok 14 (accuracy 0.1 ºC). This census method allowed us to quantify the relative circadian and circa-annual activity of lizards in altitude for comparative purposes in relation with temperature (Blomberg and Shine, 1996). Number of active individuals obtained by this procedure are assumed to be positively related to the real population density (Díaz et al., 2006b).

We captured adult individuals from the study area, but far away (> 100 m) from the transect zone. We measured lizards' body temperature within 30 seconds of capture, with a one-millimeter diameter thermocouple, eight millimeters inside the cloaca (TP (K) INCONEL 600), assembled to the thermometer (Hibok 14). At the same time, air temperature was also measured as during transects (above). This allowed us to compare body temperature of active individuals to environmental temperature. We used 5x1cm hollow copper models in order to obtain which temperature lizards would show if they did not actively thermoregulate (null hypothesis). Copper models heat up like a lizard, and have been largely used for similar purposes in this and other species (Bauwens et al., 1996; Díaz, 1997). Models were almost totally sealed in their both ends with synthetic cork, but for a small hole in one of them to insert the thermocouple of the thermometer. In every sampling plot, we placed 31 hollow copper models separated one meter one from another, with a "ventral" part in contact with the substrate. We measured the temperature of every model five times, once an hour, during the morning, in every sampling plot, recording the environmental temperature in each case as described above. We recorded whether each model were fully exposed to sun (F), partially exposed (P), or under a shadow (S).

All data were analyzed with parametric tests when the residuals accomplished the assumptions of homoscedasticity and normality (Quinn and Keough, 2002). When not, we applied non-parametric tests (Siegel and Castellan, 1988). We tested whether newborns' mean appearance date varied with altitude by ANOVA. For analyzing the circa-annual variation in adults' activity, we used the average of the adults' observations made in all transects of every sampling day by altitude sampling plot. We considered three seasons: Spring (April, May, June), with months of increasing average environmental temperature; Summer (July, August), with months of asymptotical high temperature; and Autumn (September, October), with months of decreasing average environmental temperature. We considered two independent variables: season and altitude (categorical), and conducted a General Linear Model (GLM) of Ordinal Least Squares (OLS). In order to analyze adults' circadian activity patterns, we tested for seasonal and altitudinal differences among three defined hourly intervals: H1 from sunrise to 11:59, H2 from 12:00 to 15:59, H3 from 16:00 to sunset. We performed a three-ways ANOVA, and analyzed the interactions among hour, season and altitude. To know the lizards' operative temperature variation in altitude, we analyzed the temperature of the copper models by an ANOVA, including the altitude, the exposure to sun, and the interaction among them. We repeated the analysis controlling for the environmental temperature at the moment of the measurement, to obtain information about the effects of other heat sources than air temperature, i. e., solar radiation.

## I.4.-Results

### *I.4.1.-Temperature and activity*

Environmental temperature measured in transects ($T_t$) diminished significantly as altitude increased ($F_{5, 63} = 6.10$; $P = 0.0001$), and varied along the activity season, with a maximum in the mid of the season (Kruskal-Wallis $H_{6, 69} = 22.40$, $P = 0.001$; Fig. I.1). When we found active lizards in transects, $T_t$ was higher than when we did not (20.77 ± SE = 0.34 vs. 19.25 ± 0.48, respectively; $F_{1, 351} = 6.64$; $P = 0.01$), suggesting that lizards were active when temperature was higher. Lizards were active at lower $T_t$ as altitude increased ($F_{5, 216} = 14.48$; $P < 0.001$). The temperature of the copper models ($T_m$) showed a general tendency to fall as altitude increased ($F_{5, 882} = 122.15$; $P < 0.0001$; Fig. I.2). Copper model temperature depended on the degree of exposure to sun of the model ($F_{2, 882} = 176.28$; $P < 0.0001$), being highest in totally exposed models, intermediate in partially exposed models, and minimum in totally shadowy models. When controlling for environmental temperature at the moment of the measurement, $T_m$ tended to increase with altitude ($F_{5, 881} = 24.758$; $P < 0.0001$), suggesting that thermal sources other than air temperature contributed to warm copper models.

We found slight differences among sampling station in body temperature ($T_b$) of active lizards ($H_{5, 207} = 13.25$, $P = 0.02$), but with no clear pattern with altitude (Fig. I3). Environmental temperature when active lizards were caught ($T_e$) diminished significantly in altitude ($F_{5,\ 206} = 35.31$; $P < 0.001$; Fig. I.3), suggesting that as altitude increases, lizards may be active at lower environmental temperatures. We also found that the relationship between $T_e$ and $T_b$ differed with altitude (significant interaction, $F_{5,\ 195} = 5.34$; $P < 0.001$). Body temperature of active lizards was not correlated with $T_e$ in 300, 700 and 1700 m asl ($P > 0.50$ in all cases), but it was positively correlated with $T_e$ in 2500 m asl ($r = 0.39$; $P = 0.006$) and 2200 m asl ($r = 0.27$; $P = 0.06$). Strangely, we found a significant negative correlation between $T_b$ and $T_e$ in 1200 m asl ($r = -0.62$; $P = 0.004$).

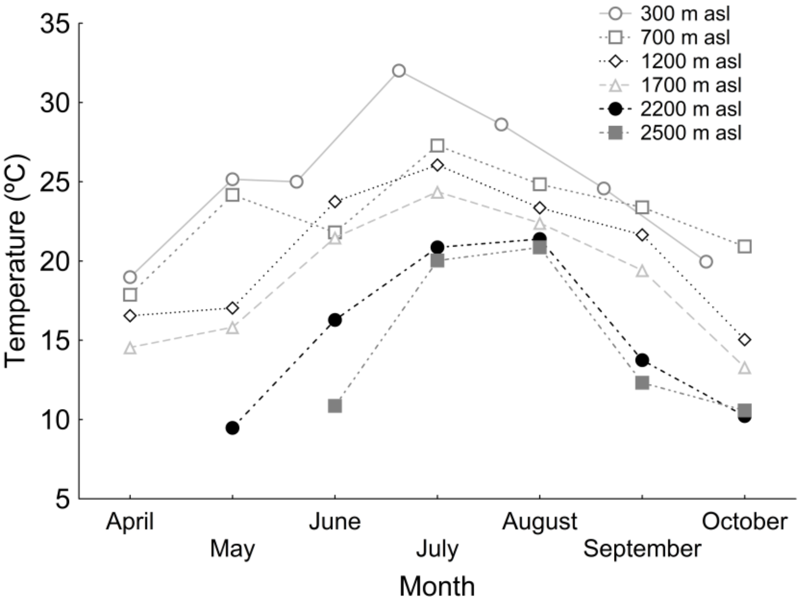

Fig. I.1 - Altitudinal variation of average monthly air temperature (ºC) (measured 1m above the ground) along the activity period of *Psammodromus algirus* in the southern slope of Sierra Nevada (SE Spain).

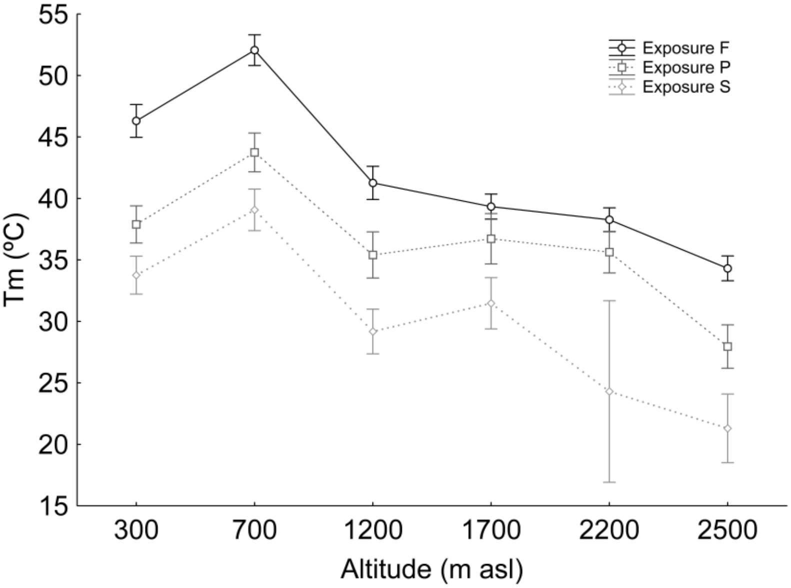

Fig. I.2 - Copper models temperature ($T_m$) diminished as altitude increased, following similar patterns in fully sun-exposed (Exposure F, continuous line), partially exposed (Exposure P, discontinuous line) and shadowy (Exposure S, spotted line) models. Bars indicate 95% Confidence Intervals.

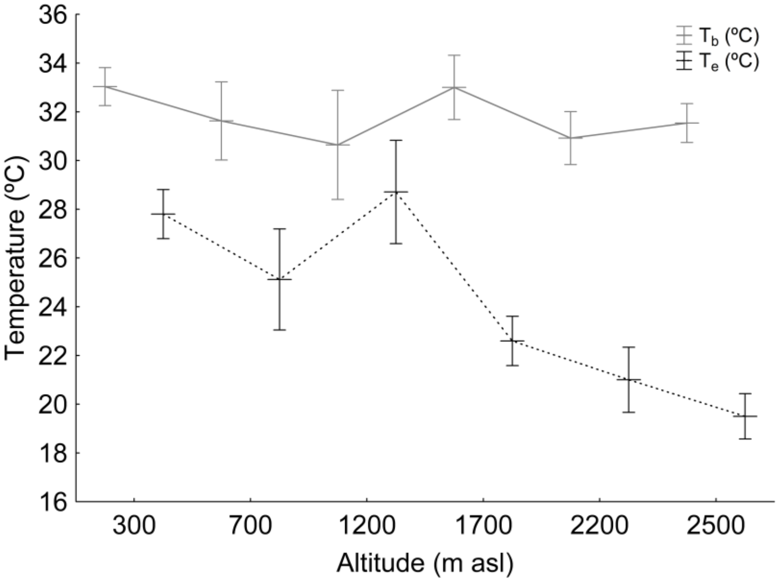

Fig. I.3 - Body temperature ($T_b$) of *Psammodromus algirus* and environmental temperature ($T_e$) in the six sampling plots ranked according altitude in Sierra Nevada (SE Spain). Bars indicate 95% Confidence Intervals.

### *I.4.2.-Circa-annual and circadian activity patterns*

The number of adults observed in transects showed a significant variation in altitude ($F_{5,\ 54} = 5.45$; $P = 0.0004$), being maximum in mid altitudinal ranges, intermediate in highlands and minimum in lowlands (Fig. I.4). The interaction among altitude and season was significant ($F_{10,\ 54} = 2.17$; $P = 0.034$): relative density had a peak in the middle of the activity period, except in the highest altitudes, where it peaked at the beginning of the activity season (Fig. I.4). This implies that the seasonal activity patterns varied in altitude. Newborns' appearance mean date did not show statistical differences in altitude ($F_{5,\ 26} < 0.01$; $P = 0.89$). The variance of newborn appearance did not vary with altitude, either (Levene's test, $P = 0.53$).The number of active individuals detected did not vary with the hourly interval ($F_{2,\ 326} = 1.94$; $P = 0.14$). The statistical interaction among season and hour ($F_{4,\ 326} = 1.48$; $P = 0.21$), among hour and altitude ($F_{10,\ 326} = 1.62$; $P = 0.10$), and the triple interaction among hour, altitude and season, were not significant ($F_{20,\ 326} = 0.96$, $P = 0.51$). Thus, we detected no clear variation in circadian activity pattern.

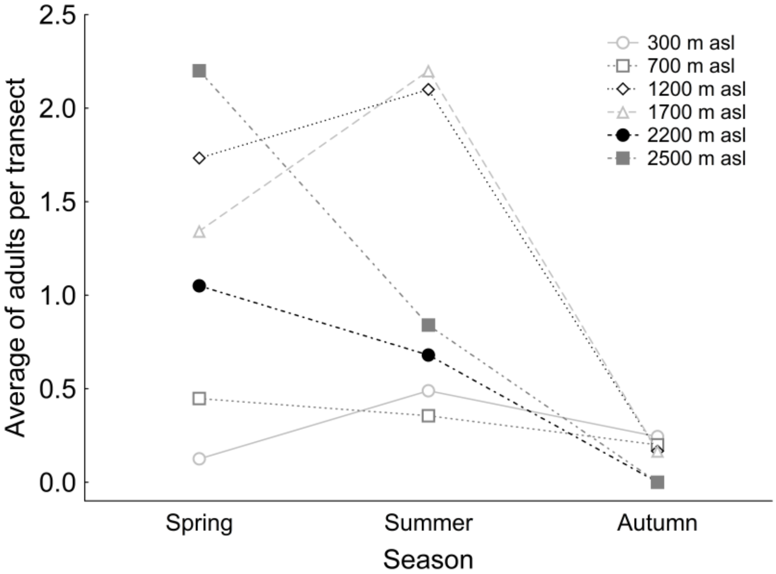

Fig. I.4 - Altitudinal variation in the number of *Psammodromus algirus* individuals censed along the three seasons of the activity period in Sierra Nevada (SE Spain).

## I.5.-Discussion

### *I.5.1.-Activity patterns*

Reptile activity patterns show close connection to many factors (Díaz et al., 2005a), as population density, habitat availability, prey abundance, predation pressure, and mainly environmental temperature (Moreno-Rueda et al., 2009; Nelson and Gregory, 2000; Vidal et al., 2010). As some of these factors vary with altitude, particularly thermal environment, we could expect lizard activity patterns to be dissimilar along an elevational gradient (Grant and Dunham, 1990). We found that environmental temperature diminished in altitude, but kept a very similar seasonal pattern all along the gradient: ascendant temperature during spring, peak in July-August, and descendant temperature from August forward. Lizards' activity patterns linked up these seasonal temperature-stages in mid and low altitudes, reaching a maximum of activity in the middle of the activity period, coinciding with the highest temperatures. The higher heat availability at mid-season could increase their thermoregulation accuracy, augmenting their chance to get their optimum body temperature faster (Huang et al., 2006). But in the two highest altitudes, the activity peak occurred at the beginning of the season, when the environmental temperature is not the highest (compare Fig. I.1 vs Fig. I.4). Lizards' activity period (both, seasonal and diary) should be narrower as altitude increases, as the availability of optimum-temperature days in year, and hours in day, diminishes. Therefore, high altitude lizards would have less time to forage and reproduce. However, they could sort this thermal constrain by an early activity peak; waiting for higher activity at the thermally more favorable summer period would cause a delay in mating that could lead to a reproductive failure in the year. Highland lizards showed to be active at lower environmental temperature, which enabled these lizards to be more active at the beginning of the activity period.

Differences in reproductive phenology are also expected to appear in this thermal gradient (Díaz et al., 2005b; Doughty and Shine, 1998). However, newborns appeared at the end of the activity season, almost synchronal along the gradient. This reproductive synchrony, altogether with similar adult field body temperature along the gradient, may point to altitudinal differences in thermoregulation which are necessary to develop similar reproductive cycles in quite a different set of habitats (Du et al., 2005).

Although environmental temperature changed within daytime, circadian activity showed no clear pattern in altitude, and active lizards were found at any sunny hour in day. This supports the idea that lizards possess capabilities to sort thermal constrains (Bauwens et al., 1996).

### *I.5.2.-Thermal gradient*

The difference of the average environmental temperature ($T_e$) between the highest and the lowest altitudes was about 9 ºC. Despite this thermal gradient, the difference of the average body temperature ($T_b$) of active lizards among sampling stations was quite small, with no clear altitudinal pattern. Thus, *P. algirus* showed to be a thermally conservative lizard in the altitudinal gradient of Sierra Nevada. In fact, we found no significant correlation between $T_b$ and $T_e$ in the medial and lowest altitudes (except at 1200 m asl). However, $T_b$ was positively correlated with $T_e$ at the highest altitudes. This result suggests that thermoregulation in this lizard is not limited by $T_e$ in low and midlands. Nevertheless, thermoregulation seems to be limited in highlands, where $T_e$ is positively correlated with lizard $T_b$, probably because an increase in $T_e$ in highlands allows lizards to reach a $T_b$ closer to optimum. The fact that *P. algirus* reaches similar $T_b$ in spite of strong differences in $T_e$ with altitude suggests that this lizard possess different thermoregulatory capabilities along the gradient. Díaz et al. (2006a) studied *P. algirus* thermal preferences in two 700-altitude-meters separated sampling plots in central Spain, and they also failed to find thermal preferences differences, which supports our findings. The altitudinal pattern found in this lizard is similar to other thermally conservative species, as the wide-distributed lizard *Microlophus atacamensis* which shows no latitudinal differences in field body temperature (Sepúlveda et al., 2008). Similarly, lizards of the genus *Sceloporus* have no significant variation in body temperature in altitudinal gradients, from 45 to 4400 m asl (Andrews, 1998).

Temperature measured in copper models ($T_m$) decreased with altitude in a similar way as $T_e$. However, when we controlled statistically for environmental temperature of the air, $T_m$ increased with altitude, suggesting that solar radiation contributed to increase the temperature of the copper models. Nevertheless, the effect of increased solar radiation with altitude was not enough to counteract the adiabatic cooling in altitude. Considering all models (totally exposed to sun, partially exposed, and totally shadowy), $T_m$ differed 12ºC among the lowest and the highest altitudes. Therefore, copper models reflect that, if lizards were not capable to thermoregulate actively, their $T_b$ would decrease as altitude increases, and altitudinal differences would be similar (12ºC) to $T_e$ altitudinal differences (9ºC). The scarce differences in $T_b$ among sampling stations we found, with no altitudinal pattern, point to the presence of an active thermoregulation strategy (Díaz and Cabezas-Días, 2004).

### *I.5.3.-Thermoregulation mechanisms*

All previous results suggest differences in thermoregulation in altitude that may permit animals under very different thermal conditions to reach a similar optimum temperature and, thus, develop similar circa-annual activity and reproductive patterns (Bauwens et al., 1996). Many factors, such as solar radiation, convective heat flow, longwave radiation, conductive heat flow, metabolic heat gain and respiratory energy loss, are involved in lizards thermoregulation, as can be seen in recent physical models (Fei et al., 2012). The relative importance of some of those factors may vary seasonally (Díaz and Cabezas-Díaz, 2004), so their seasonal availability in altitude may constrain *P. algirus* thermoregulation capabilities in this system.

Several physiological or ethological traits could permit high altitude lizards in our system to reach body temperatures as high as low altitude lizards, despite the lower environmental temperature. A trait implicated in the thermoregulation of an animal is coloration, since darker animals heat faster than pale ones (Clusella-Trullas et al., 2007, 2008). In our study system, dorsal coloration is significantly darker at high altitude (Reguera et al., submitted). Dark coloration could protect highland lizards against the harmful effects of ultraviolet radiation (Porter and Norris, 1969), help them to benefit from the altitudinal increase in radiation to warm up faster (Vences et al., 2002), or both.

The basking behavior may be a key ethological thermoregulatory mechanism in these diurnal lizards (Belliure and Carrascal, 1998). The time a lizard spends on sunbathing has been proved to depend on environmental factors such as light composition, increasing basking time when wavelengths < 540 nm are removed (Tosini and Avery, 1996a), and physiological factors such as dermal light sensibility, which determines its ability to position under a heat source (Tosini and Avery, 1996b). For example, the high altitude lizard *Iberolacerta monticola* spends more time basking when environmental temperature is lower, but sunbathes more times and during shorter periods when environmental temperature is higher (Carrascal et al., 1992). Small lizards have shorter sunbathing periods, as they heat up faster, and there could be an inverse relationship among body size and heat gain (Carrascal et al., 1992). In our study system, lizards are larger at high altitude (unpublished data), so they could reach high temperatures by spending more time on sunbathing. Furthermore, bigger body masses in altitude slows down cooling rates, helping lizards to keep high temperature once they reach it (unpublished data). Differences in thermoregulation according to altitude could be defined by phenotypic plasticity or local adaptation mechanisms. Phenotypic plasticity could mean a higher individual possibility to move among different habitats (Lorenzon et al., 2001), as the same genotype can result in different phenotypes depending on the environmental pressures during the organism development. Then, plastic individuals can survive under different environmental conditions, and some degree of gene flow can be possible among disparate habitats. Otherwise, local adaptation implies a scenario that limits gene flow among populations because of a negative selection on the individuals allocated outside from the habitats they are adapted to (North et al., 2011). Therefore, population differentiation may occur in the second case (Palo et al., 2003). Thereby, local selection has been pointed as a determinant factor in coloration differentiation in Aegean lizard (*Podarcis gaigeae*) populations between the continent and some islets they inhabit (Runemark et al., 2010).

### *I.5.4.-Implications under the Global Change scenario*

Mediterranean animals have evolved in a rather unpredictable climate, they are adapted to survive to short-term environmental variations, so they may be able to successfully face the present Global Warming (Chamaillé-Jammes et al., 2006; Moreno-Rueda and Pleguezuelos, 2007; Zamora-Camacho et al., 2010). In mountains, the Global Warming is expected to make animals to increase their altitudinal upper limit for their thermal optimum; otherwise, some of them could come extinct (Wilson et al., 2005). In the case of *P. algirus*, our results suggest little thermal limitations to climb up in altitude in response to climatic change. Limitations for this species can be related to availability of shrub formations, which in the southern slope of Sierra Nevada now arrive to 2600 m asl. However, if bush responds to Climate Change climbing up in altitude (Randin et al., 2009; Benito et al., 2011), lizards seem able to follow them. If this ability is similar in other species, we expect Mediterranean reptiles from low and mid altitudes to have little trouble to colonize higher altitudes, mainly if their capability to face different environmental conditions is due to phenotypic plasticity mechanisms. But Eurosiberian species which are now isolated in high altitudinal ranges, like the smooth snake, *Coronella austriaca*, in Sierra Nevada (Santos et al., 2009; Caro et al., 2012), may then suffer from the new Mediterranean incomers, some of them acting either as predators or competitors. In that forecasted scenario, Eurosiberian species in Mediterranean mountains could be the losers.

## I.6.-Conclusions

*Psammodromus algirus* showed to be a thermoconservative lizard in Sierra Nevada. Field body temperature kept rather similar along a 2200-meters altitudinal gradient, whilst the average environmental temperature decreased more than 9 ºC within the same altitude range when lizards were caught active. Circa-annual activity peak occurred at the beginning of the season in high altitude, despite the warmest period to be later, probably because the constraint in reproductive time requires full activity immediately after emergence from hibernation. In mid and lowlands, when time for reproduction is wider, the activity peak occurred in the mid of the season, when the temperature is highest, as expected. Nevertheless, newborns appeared at once all along the gradient and almost at the same time of the year, meaning altitudinal similitude in reproductive cycles in spite of strong environmental shift. These findings point to differences in thermoregulatory mechanisms with altitude that permit lizards to exploit dissimilar environmental heat conditions and reach similar body temperature and synchronized reproductive cycles.

## I.7.-Acknowledgements

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# II - Chapter 2

# Thermoregulation in a temperate lizard along a 2200-m elevational gradient.

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This chapter reproduces the submitted article:

Zamora-Camacho, F.J., Reguera, S., Moreno-Rueda, G. 2014. Thermoregulation in a temperate lizard along a 2200-m elevational gradient. Oecologia, submitted.

**II.1.-Abstract** - Body temperature is a key determinant of animal fitness. Since ectotherm thermoregulation depends upon environmental temperature, lizard thermal performance and preferences can be constrained in thermally challenging environments. Moreover, reproductive condition may alter thermal needs through different physiological processes. We tested these hypotheses measuring field and laboratory-selected body temperatures of lizards of different reproductive conditions from a 2200-m elevational gradient, as well as determining thermal quality of the habitat, and accuracy and effectiveness of thermoregulation indexes. We found that both environment and selected body temperature diminished with increasing elevation, while field body temperature showed no elevational trend, suggesting that other factors in addition to thermal availability constrain lizard thermoregulation in the wild. Field body temperature was constrained by environmental temperature only at high elevations. Moreover, while environmental temperature fell more than 7ºC, selected body temperature dropped only 0.6ºC with elevation. Notably, thermal quality of the habitat was more suitable at high than at low elevations, the best being at middle elevations. Low-elevation lizards face environmental temperatures exceeding the optimum. Nonetheless, thermal quality of high-elevation habitats was more suitable in fully sun-exposed microhabitats, which increases thermoregulation costs, and at day central hours, which limits daily activity periods. As for reproductive condition, the accuracy and effectiveness of thermoregulation was better in gravid and non-gravid females than in males, probably because males spend more time defending their territories and searching for mates. Moreover, postpartum females selected higher body temperatures than did gravid and non-gravid females or males, which might thus improve body condition by increasing metabolic rate.

## II.2.-Introduction

Body temperature is a key determinant of animal fitness, since physiological and biochemical processes such as digestion (McConnachie and Alexander 2004), locomotion (Xiang et al. 1996), growth (Brewster et al. 2013), and reproduction (Du et al. 2005; Sun et al. 2012) strongly depend upon temperature. Ectotherms do not generate enough metabolic heat for efficient body-temperature upkeep (Brown and Au 2009), so they depend upon external heat sources to attain body temperatures within their preferred temperature ranges (PTR), at which physiological processes are optimised (Hertz et al. 1993). Most ectotherms heat up ethologically, mainly by means of two complementary mechanisms: heliothermy, which consists of heat gain by solar radiation, and thigmothermy, which consists of heat transmission by direct conduction (Belliure and Carrascal 2002). However, despite the metabolic benefits of heat acquisition and maintenance, these are particularly costly for ectotherms (Alford and Lutterschmidt 2012). Heat acquisition makes ectotherms highly conspicuous to predators, thereby increasing their predation risk (Herczeg et al. 2006). Moreover, they spend much of their time adjusting their body temperature, which reduces the time available for foraging (Verwaijen and Van Damme 2007), mating, or defending a territory (Huey and Slatkin 1976).

Also, the thermal environment can greatly constrain body temperature, especially in thermally challenging habitats (Fei et al. 2012). Environmental thermal quality for ectotherms varies geographically (Sunday et al. 2010). Although global temperature changes are often uneven, some general trends exist: temperature becomes gradually colder as latitude (Sunday et al. 2010) and elevation (Stevens 1992) increase. Classically, two opposite hypotheses address the issue of ectotherm thermal-environment adaptation: the labile and the static hypotheses. The labile hypothesis states that ectotherm thermal preferences can evolve accordingly to thermal environment, so that optimum temperatures are easier to achieve (Hertz et al. 1983; Rodríguez-Serrano et al. 2009). On the contrary, the static hypothesis posits that ectotherm thermal preferences are evolutionarily conservative, and remain fairly constant despite thermal-environment shifts (Hertz et al. 1983; Crowley 1985).

Irrespective of the relationship between thermal environment and ectotherm thermal preferences, ectotherm thermoregulation depends on its cost/benefit balance (Huey and Slatkin 1976). In thermally challenging habitats, the costs of no-thermoregulation often outweigh the costs of thermoregulation, since the reduced chances to thermoregulate make it a priority (Blouin-Demers and Nadeau 2005). Therefore, the extended model of thermoregulation cost/benefit balance predicts that ectotherms will thermoregulate accurately in thermally challenging habitats where environmental temperature exceeds PTR (Vickers et al. 2011). Nevertheless, the same reasoning is valid for ectotherms in cold-climate habitats, as in high latitudes or elevations. In fact, tuataras (*Sphenodon punctatus*) kept at a suboptimal thermal regime maintain higher body temperatures than do individuals kept at optimal thermal regimes (Besson and Cree 2010). Reflecting the importance of thermoregulation in cold habitats, alpine lizards spend more time searching for basking spots (Díaz 1997), and prioritize heat acquisition when traded-off with immune-system deployment (Zamora-Camacho et al. submitted). Accordingly, lower thermal-tolerance limits decrease as latitude increases in many terrestrial ectotherms, while upper thermal-tolerance limits remain unaltered (Sunday et al. 2010). Thus, cold-habitat ectotherms can face lower temperatures, but temperatures as high as those attained by warm-habitat ectotherms are also suitable for them (Valdecantos et al. 2013).

Besides habitat thermal quality, physiological and metabolic conditions can also affect ectotherm body-temperature performance. For instance, lizards can select lower body temperatures in response to starvation (Yu et al. 2008), hypoxia (He et al. 2013), or dehydration (Lorenzon et al. 1999). Similarly, thermal requirements of reproductive processes can shift ectotherm thermal preferences. For example, male adders *Vipera berus* show lower body temperatures imposed by spermiogenesis (Herczeg et al. 2007), and some snakes are geographically constrained by climatic limitations for their spermiogenesis cycles (Saint Girons 1982). Pregnant females of the viviparous lizard *Eulamprus tympanum* exhibit temperatures similar to those of males and non-gravid females, even when they require more basking time (Schwarzkopf and Shine 1991). Nevertheless, female lizards may show a trend to select lower temperatures when gravid than after parturition both in oviparous (Mathies and Andrews 1997) and viviparous species (Le Galliard et al. 2003). In fact, embryos gestated at lower temperatures display better body conditions and escape speed in those species (Mathies and Andrews 1997; Le Galliard et al. 2003). However, in the oviparous lizard *Podarcis muralis* those differences between gravid and non-gravid females appear only in the field, but disappear when lizards are allowed to thermoregulate in a thermal gradient, suggesting that environmental constraints limit thermoregulation accuracy in gravid females (Braña 1993).

In this study, we seek to characterize lizard thermoregulation along an elevational gradient, addressing the following issues:

A) Based on the extended model of thermoregulation cost/benefit balance (Vickers et al. 2011), we expect lizards to thermoregulate accurately in high-elevation, cold-environment habitats (Andrews et al. 1998). Therefore, according to this model, we expect to find support for the static hypothesis of ectotherm thermal-environment adaptation, which states that lizards will not shift their thermal preferences despite thermal-environment constraints. The failure of our predictions would support the labile hypothesis, which predicts that lizards will prefer and attempt to achieve lower body temperatures in high-elevation, low-thermal-quality habitats.

B) We predict that gravid females will choose lower body temperatures for better embryo development (Mathies and Andrews 1997; Le Galliard et al. 2003).

C) We seek to elucidate elevational trends in habitat thermal quality, accuracy of thermoregulation, and effectiveness of thermoregulation.

For these purposes, we used the lizard *Psammodromus algirus* as a model species in a 2200-m elevation gradient, from 300 to 2500 m asl (meters above sea level) in Sierra Nevada (SE Spain). Environmental temperatures when lizards were active dropped by 9ºC along this gradient (Zamora-Camacho et al. 2013), implying an elevational increase in thermal constraint. Firstly, we examined how thermal environment varies with elevation in our study system. Then, we compared field body temperatures of lizards from different elevational thermal environments, regarding their reproductive condition. In addition, we recorded selected body temperature of lizards from different elevations, in different reproductive states, in a free-ranging laboratory thermal gradient. Furthermore, we established a null hypothesis of no-thermoregulation using hollow copper models. With those data, we assessed habitat thermal quality, accuracy of thermoregulation and effectiveness of thermoregulation indexes.

## II.3.-Materials and Methods

### *II.3.1.-Experimental species handling*

*Psammodromus algirus* is an abundant oviparous, generalist, medium-sized Lacertid lizard (53-95 mm of adult snout-vent length [SVL] in our study area) which inhabits shrubby zones in Western Europe and Africa Mediterranean climates, from the sea level to more than 2600 m asl (Salvador 2011). It is an opportunistic arthropod predator, which can either use a “sit-and-wait” strategy or (more often) search for preys actively (Salvador 2011). Lizards heat up mainly heliothermically (Carretero and Llorente 1995; Díaz and Cabezas-Díaz 2004), by sunbathing near the shrubbery that they use as a shelter when they detect a potential predator (Martín and López 2000).

Lizards were captured within their reproductive periods in 2010-2013. We assigned each lizard caught an individual identification (ID), and considered three reproductive conditions: males, non-gravid females, and gravid females. The lizards were sexed based on the fact that males have proportionally wider heads, orange spots in the corners of their mouths, and more numerous and conspicuous femoral pores in their hind limbs. Gravid females were recognized by manual palpation of developing eggs. Moreover, we kept gravid females in the laboratory until they laid eggs, thereby obtaining postpartum females as the fourth reproductive condition. While captured lizards were not being used in an experiment, they were kept individually in plastic terrariums of 20×13×9 cm. Throughout the process, all lizards had water (in form of aqueous nutritious gel) and food (*Tenebrio molitor* larvae) available *ad libitum*, and a window let in natural daylight, allowing them to adjust circadian rhythms. Lizards suffered no pain as a consequence of this study, and they were set free at their provenance population as soon as the experiment was finished.

### *II.3.2.-Field measurements*

Lizards were captured on the southern face of Sierra Nevada (SE Spain), in six populations at 300, 700, 1200, 1700, 2200, and 2500 m asl. We chose places as similar as possible regarding habitat structure (see Appendix A in Zamora-Camacho et al. 2013). We measured field body temperature ($T_b$) of all lizards caught within 30 s after capture, putting a 1-mm diameter thermocouple, assembled to a thermometer (model Hibok 14, accuracy 0.1 ºC), 8 mm into the cloaca. Next, we recorded environmental temperature ($T_e$) with the same setup, one meter above the ground, under shade. Also, when available, substrate temperature ($T_s$) was measured at the exact point where the lizard was first detected, every time we caught an active lizard.

Furthermore, on a clear day, halfway through the reproductive season of 2012, we ascertained the body temperature that lizards would attain in the field if they did not thermoregulate actively, thus providing a null hypothesis of no thermoregulation, by using hollow copper models (5×1 cm), which heat up like lizards (Hertz 1992; Bauwens et al. 1996; Díaz 1997). Both ends of copper models were sealed with artificial cork, but we perforated a small hole in one end to insert the thermocouple. In each sampling plot, 31 copper models were distributed one meter from each other, putting a "ventral" part in contact with the substrate. We measured copper model internal temperature ($T_m$) once per hour throughout the morning, from 10:00 to 14:00 (local time). Also, we considered three microhabitats regarding the exposure of the copper models to the sun: fully exposed to sunlight, partially exposed, or under shade.

### *II.3.3.-Laboratory measurements*

Moreover, lizards captured in 2012 and 2013 were taken to the laboratory, and placed one day for acclimation in individual terrariums (100×20×40 cm) built in 5mm-thick methacrylate, with a 150W red-light bulb at one end, 15 cm over the pine-cork substrate. The bulb was switched on during daytime, generating a thermal gradient of approximately 20-55ºC. The following day, we recorded lizards' body temperature five times, once an hour, from 10:00 to 14:00 (local time). This procedure was repeated for postpartum females the day after egg laying. Then we calculated each lizard's selected body temperature in the laboratory ($T_{sel}$) as the mean of those five measurements. As we kept gravid females in the laboratory until egg laying, we could record clutch size (number of eggs), egg burden (female investment in clutch), and days from each measurement (days when we performed $T_b$ and $T_{sel}$ measurements, respectively) until oviposition. For gravid females, we calculated egg burden as the difference in body mass between the moment of temperature measurement ($T_b$ in the field and $T_{sel}$ in the laboratory) and right after egg laying. For postpartum females, we considered egg burden as the difference in body mass just before and just after egg laying.

### ***II.3.4.-Indexes***

The information collected on $T_b$, $T_{sel}$, and $T_m$ allowed us to calculate some thermoregulation indexes widely used in ectotherm thermal ecology (Hertz et al. 1993; Díaz 1997; Blouin-Demers and Nadeau 2005). Nevertheless, we first needed to calculate the set-point ranges or preferred body temperature ranges ($T_{set}$), as the central distribution of $T_{sel}$ values. Some debate exists on the use of 50% or 80% central boundaries of these values (Blouin-Demers and Nadeau 2005), so we calculated both. Since the results were rather similar, and 50% is mostly preferred (Blouin-Demers and Nadeau 2005), we opted for showing the findings with this value. However, results calculated with the central 80% are available in the Appendix B. We calculated the following indexes:

A) Thermal quality of the habitat ($d_e$): defined as the average of the absolute values of deviations of $T_m$ relative to the nearest $T_{set}$ boundary. Thermal quality of the habitat is higher as $d_e$ index approaches 0. This index was calculated for every microhabitat in each elevation, as well as hourly.

B) Accuracy of body temperature ($d_b$): defined as the average of the absolute values of deviations of $T_b$ relative to the nearest $T_{set}$ boundary. Accuracy of body temperature is higher as $d_b$ index approaches 0.

C) Effectiveness of thermoregulation: is calculated from the previous indexes, by means of several formulae:

C.1) E (Hertz et al. 1993): calculated as $1-(d_b/d_e)$. Values close to 0 indicate thermoconformity, values close to 1 indicate thermoregulation, and values below 0 indicate avoidance of preferred temperatures. The validity of this index is controversial (Blouin-Demers and Weatherhead 2001), so we show these results in the Appendix B.

C.2) $d_e$-$d_b$ (Blouin-Demers and Weatherhead 2001): as the case before, values close to 0 indicate thermoconformity, values close to 1 indicate thermoregulation, and values below 0 indicate avoidance of preferred temperatures. We opted for showing findings reached with this index in the present work.

### *II.3.5.-Statistical analyses*

Since all models fulfilled the criteria of residual normality and homoscedasticity, we conducted parametrical statistics (Quinn and Keough 2002). We analysed most of the data with Linear Models by Ordinary Least Squares. We first performed a two-way ANOVA in order to check $T_b$ differences depending on elevation and reproductive condition. We used a one-way ANOVA to test elevational differences in $T_e$ and $T_s$. A three-way ANOVA was performed to ascertain differences in $d_e$, among elevations, microhabitats, and hours. A two-way ANOVA was used to test differences in $d_b$ among elevations and reproductive conditions. Furthermore, Spearman’s correlations were performed to check elevational trends in $T_e$, $T_s$, $T_b$, $T_{sel}$, $d_b$, $d_e$-$d_b$, and E, using the average values in each of the 6 populations. In addition, multiple regressions were used to test the effect of $T_s$ and $T_e$ on $T_b$. The effect of reproductive condition on $T_{sel}$ was analysed with a Linear Mixed Model of

Restricted Maximum Likelihood (REML-LMM), including ID as a random factor (Zuur et al. 2009), since gravid and postpartum females were the same individuals at different points of their reproductive cycle (except for a few cases in which we missed one or other measurement). Moreover, for gravid and postpartum females, we searched for correlations of both $T_b$ and $T_{sel}$ with clutch size, egg burden, and days until oviposition, by using Pearson's product-moment correlations. We also tested correlations of postpartum female $T_{sel}$ with clutch size and egg burden. Postpartum females were excluded from every other analysis. Finally, we checked differences between $T_b$ and $T_{sel}$ with a t-test for dependent samples, and then we used a two-way ANOVA to detect possible effects of elevation and reproductive condition on the difference between $T_{sel}$ and $T_b$. We performed most tests with the software Statistica 8 (StatSoft Inc. 2007), except the mixed model, which was performed with the software R version 3.1.1 (R Development Team 2014).

## II.4.-Results

### *II.4.1.-Field body temperature ($T_b$)*

As expected, we found elevational differences in $T_e$ ($F_{5,\ 311}$; $P < 0.001$; Fig. II.1), and Spearman's correlation showed a significant trend of $T_e$ to diminish with rising elevation ($n = 6$ populations; $r_s = -0.83$; $P = 0.042$). In turn, although $T_s$ when lizards were active varied among populations ($F_{5,\ 201}$; $P < 0.001$), it did not show a pattern with elevation (Fig. II.1), so Spearman's correlation was not significant ($r_s = -0.66$; $P = 0.16$). Similarly, although lizards from different elevations had different $T_b$ ($F_{5,\ 307} = 3.91$; $P = 0.002$), we detected no clear elevational pattern (Fig. II.1). Accordingly, Spearman's correlation between average $T_b$ and elevation was not significant ($r_s = -0.49$; $P = 0.33$).

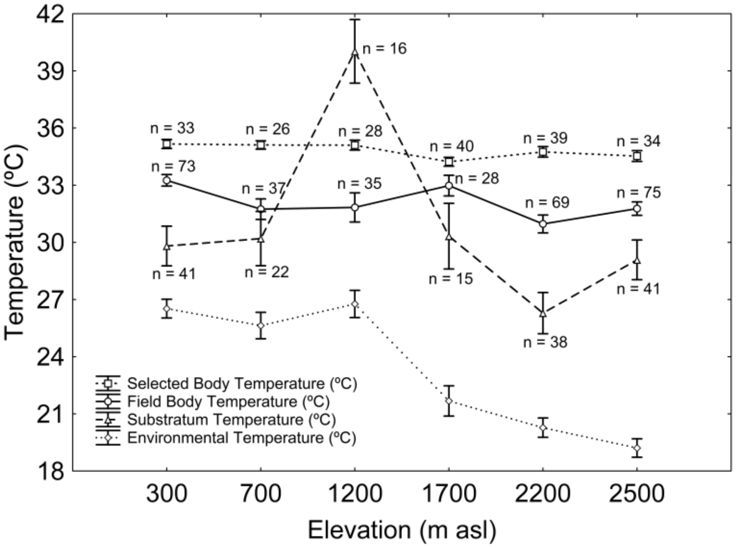

Fig. II.1 - Lizard selected ($T_{sel}$) and field body temperatures ($T_b$), as well as substratum ($T_s$) and environmental temperature ($T_e$) in each sampling plot. Although we detected population differences in all the four variables, only $T_{sel}$ and $T_e$ showed a significant elevational trend. Sample size of environmental and field body temperatures were the same at each elevation, since both measurements were made at once. Vertical bars represent standard errors.

Consequently, the difference between $T_b$ and $T_e$ ($\Delta T_{be}$) tended to decrease with increasing elevation ($F_{5, 350} = 18.36$, $P < 0.001$). As a whole, $\Delta T_{be}$ followed a non-linear relationship with $T_e$ (linear effect: $F_{1, 353} = 12.38$, $P < 0.001$; quadratic effect: $F_{1, 353} = 69.68$, $P < 0.001$; $R^2 = 0.69$; Fig. II.2a). For high $T_e$, $T_b$ was smaller than $T_e$ (negative $\Delta T_{be}$). As $T_e$ decreased, $T_b$ was relatively higher following a linear trend. When $T_e$ was below ~30ºC, $T_b$ was higher than $T_e$ (positive $\Delta T_{be}$). However, for low $T_e$ (between 20 and 25ºC), when the difference $\Delta T_{be}$ was 9.07ºC (breakpoint), the slope strongly decreased, from -0.82 to -0.46 (Piecewise linear regression; $R^2 = 0.73$), the relationship between $T_e$ and $\Delta T_{be}$ approaching to an asymptote (Fig. II.2a). The non-linear relationship between $T_e$ and $\Delta T_{be}$ was general across populations (Table II.1 – note that $\beta$ values were consistently negative for the quadratic effect). As a consequence of this non-linear relationship, $T_b$ was not correlated with $T_e$ in the three lowest elevations ($P > 0.16$ in all cases), but it showed a positive correlation in populations at 1700 ($r = 0.52$; $P = 0.005$), 2200 ($r = 0.45$; $P < 0.001$) and 2500 m asl ($r = 0.31$; $P = 0.007$).

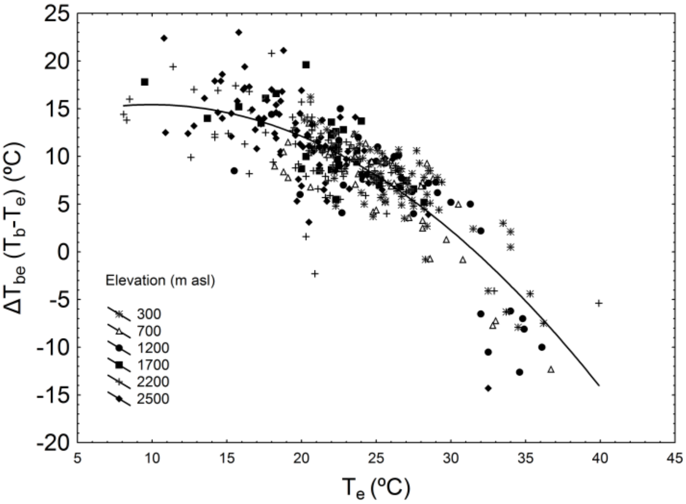

Fig. II.2a

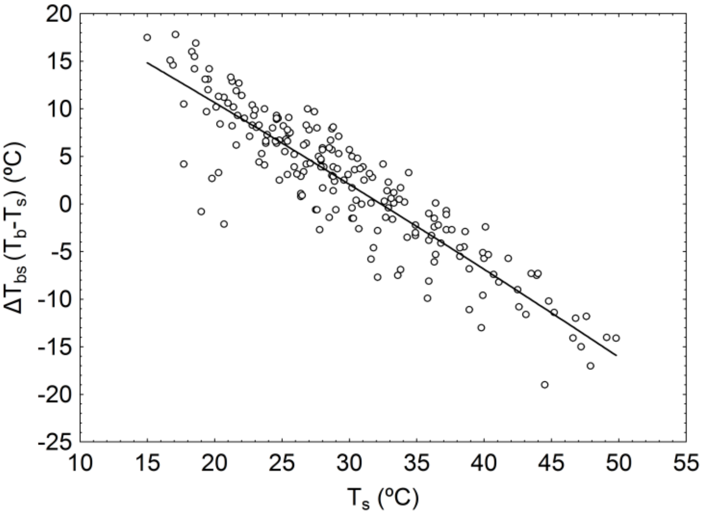

Fig. II.2b

Fig. II.2 - $\Delta T_{be}$, calculated as body temperature ($T_b$) minus environmental temperature ($T_e$) followed a non-linear relationship with $T_e$ (Fig. II.2a). The asymptote at low $T_e$ suggests that $T_b$ is constrained by $T_e$ at low $T_e$. Meanwhile, $\Delta T_{bs}$, calculated as body temperature ($T_b$) minus substratum temperature ($T_s$) followed a linear relationship with $T_s$ (Fig. II.2b). The linear relationship suggests no constraint of $T_s$ on $T_b$.

| **Elevation** | ***D.f.*** | **Linear effect** | | **Quadratic Effect** | |
|---|---|---|---|---|---|
| | | ***F*** | ***β*** | ***F*** | ***β*** |
| **300** | 1, 78 | 1.76 | 0.93 | **6.23*** | **-1.76** |
| **700** | 1, 36 | **19.55***** | **3.05** | **31.49***** | **-3.87** |
| **1200** | 1, 35 | **20.15***** | **2.81** | **33.96***** | **-3.65** |
| **1700** | 1, 27 | 0.04 | 0.16 | 1.24 | -0.91 |
| **2200** | 1, 81 | 0.12 | 0.11 | **6.78*** | **-0.80** |
| **2500** | 1, 81 | **6.20*** | **1.13** | **16.99***** | **-1.86** |
| **Total** | 1, 353 | **12.38***** | **0.59** | **69.68***** | **-1.41** |

Table II.1 - Linear and quadratic relationships between $T_e$ and $\Delta T_{be}$ in all populations. *F* statistics and *β* values are shown. In bold, significant results. Symbols indicate: * for $P < 0.05$; *** for $P < 0.001$.

Regarding the relationship between $T_b$ and $T_s$, the difference between $T_b$ and $T_s$ ($\Delta T_{bs}$) followed a linear relationship with $T_s$ (Fig. II.2b). That is, $T_b$ did not seem to be constrained by $T_s$: when $T_s$ was relatively high, $\Delta T_{bs}$ was negative, and when $T_s$ was relatively low, $\Delta T_{bs}$ was positive. Note that this finding contrasts with that found for $T_e$, given that for low values of $T_e$, lizards showed difficulties to thermoregulate adequately (see Fig. II.2a). The relationships between $T_s$ and $T_b$ were not consistent across elevations. $T_s$ showed a positive correlation with $T_b$ at 700 and 2200 populations ($r > 0.4$; $P < 0.05$ in both cases), while no correlation was found in the remaining elevations ($P > 0.05$). We searched for the effect of $T_s$ and $T_e$ on $T_b$ by using multiple regressions for each elevation. In general, $T_e$ influenced $T_b$ more than $T_s$, except at 2200 population (Table II.2).

Reproductive condition had no effect on $T_b$ ($F_{2, 338} = 0.36$; $P = 0.70$; Fig. II.3). Neither clutch size ($r = -0.013$; $P = 0.92$) nor egg burden ($r = -0.078$; $P = 0.56$), nor days until oviposition, correlated significantly with gravid female $T_b$ ($r = -0.094$; $P = 0.70$; Fig. II.4).

| **Elevation** | ***D.f.*** | **$T_s$** | **$T_e$** | **$T_e^2$** |
|---|---|---|---|---|
| **300** | 1, 41 | 3.12 | **5.15*** | **5.28*** |
| **700** | 1, 20 | **12.89**** | **47.10***** | **50.37***** |
| **1200** | 1, 14 | 0.05 | **1.17***** | **19.79***** |
| **1700** | 1, 11 | 0.26 | 0.26 | 0.27 |
| **2200** | 1, 46 | **8.86**** | 1.52 | 0.93 |
| **2500** | 1, 45 | 2.53 | **6.36*** | **8.83**** |

Table II.2 - Multiple regressions testing the effects of $T_s$, $T_e$, and $T_e^2$ on $T_b$ for each elevation. In bold, significant results. Symbols indicate: * for *P* < 0.05; ** *P* < 0.01; *** for *P* < 0.001.

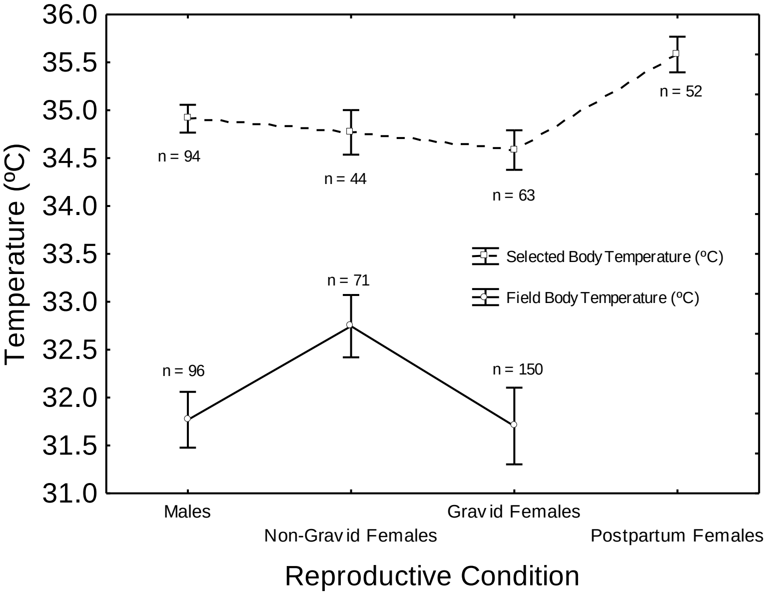

Fig. II.3 - Lizard selected ($T_{sel}$) and field body temperature ($T_b$) regarding reproductive condition. Note that for postpartum females we only had records of $T_{sel}$. Differences in $T_b$ among reproductive conditions were not significant. Postpartum females chose a $T_{sel}$ higher than that of the other reproductive conditions. Vertical bars represent standard errors.

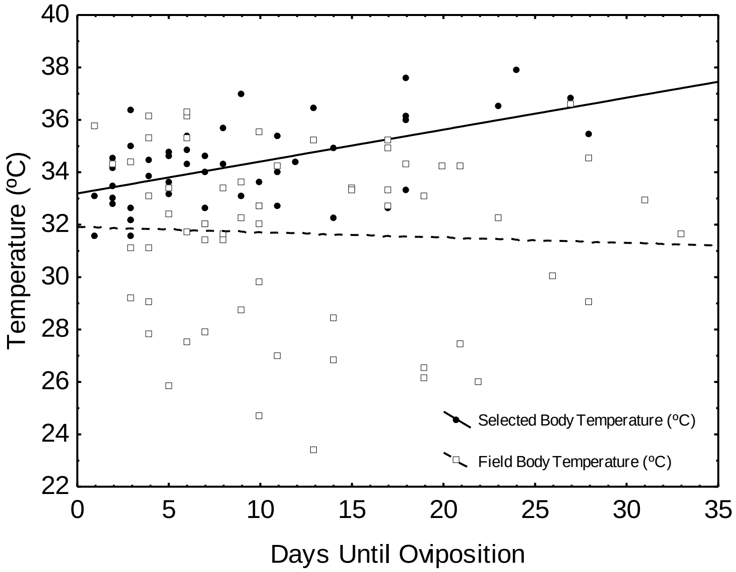

Fig. II.4 - Correlations between both gravid female selected ($T_{sel}$) and field body temperature ($T_b$) and days until oviposition. Gravid females preferred lower $T_{sel}$ as oviposition approached. However, we found no significant correlation between $T_b$ and days until oviposition. Sample sizes were 46 gravid females for $T_{sel}$ and 61 for $T_b$.

### *II.4.2.-Selected body temperature ($T_{sel}$)*

Elevational differences in $T_{sel}$ were marginally non-significant, with a trend to diminish with rising elevation ($F_{5,\ 190}$ = 2.17; $P$ = 0.059; Fig. II.1). Hence, Spearman's correlation between average $T_{sel}$ and elevation was significant ($r_s$ = -0.83; $P$ = 0.042), confirming that lizards tended to prefer lower $T_{sel}$ as their provenance elevation rose. However, the difference in $T_{sel}$ between the lowest and the highest populations was only of 0.6ºC.

Reproductive condition had a significant effect on $T_{sel}$, and postpartum females exhibited higher $T_{sel}$ than males as well as gravid and non-gravid females, even when controlling for elevation ($F_{3,\ 244}$ = 13.5; $P$ = 0.002; Fig. II.3). Neither clutch size ($r$ = -0.18; $P$ = 0.21) nor egg burden ($r$ = -0.24; $P$ = 0.12) were correlated with gravid female $T_{sel}$. Nevertheless, we found a positive correlation between days until oviposition and gravid female $T_{sel}$ ($r$ = 0.55; $P$ <

0.001; Fig. II.4). For postpartum females, neither clutch size ($r$ = -0.008; $P$ = 0.96) nor egg burden ($r$ = 0.095; $P$ = 0.52) were correlated with $T_{sel}$.

Lizard $T_{sel}$ was significantly higher than field $T_b$ (paired t-test, $t_{78}$ = 6.65; $P$ < 0.001; Figs. II.1, II.3 and II.4). Nevertheless, neither elevation ($F_{5, 71}$ = 0.58; $P$ = 0.72) nor reproductive condition ($F_{2, 71}$ = 0.38; $P$ = 0.69) significantly affected the difference between $T_b$ and $T_{sel}$.

### *II.4.3.-Thermal quality of the habitat ($d_e$)*

Thermal quality of the habitat varied with elevation, microhabitat, and hour in a complex way, as reflected by the multiple interactions between the three factors (Table II.3; Fig. II.5). In general, thermal quality was worse for lizards in exposed microhabitats than in the shade —except at high elevations—, and, in general, at the low elevations than in middle and high elevations. In any case, lizards from all elevations could select habitats of adequate thermal quality by shifting between shaded or sun-exposed microhabitats (Fig. II.5a). Nevertheless, at low elevations, thermal quality of the habitat was maximal under shade, but at high elevations only fully sun-exposed microhabitats were of maximal quality (Fig. II.5a). Moreover, the thermal quality of the different microhabitats varied with the hour. At low elevations, habitat thermal quality was maximal at the beginning of the day, but at high elevations it was maximal in the central hours (Fig. II.5b). Furthermore, maximal thermal quality of the habitat occurred in fully sun-exposed microhabitats early in the day, shifting gradually to partially exposed, and eventually to shaded microhabitats (Fig. II.5c). We found similar results when using 80% of the values of $T_{sel}$ to estimate habitat thermal quality (Table B.S1; Fig. B.S1 in Appendix B).

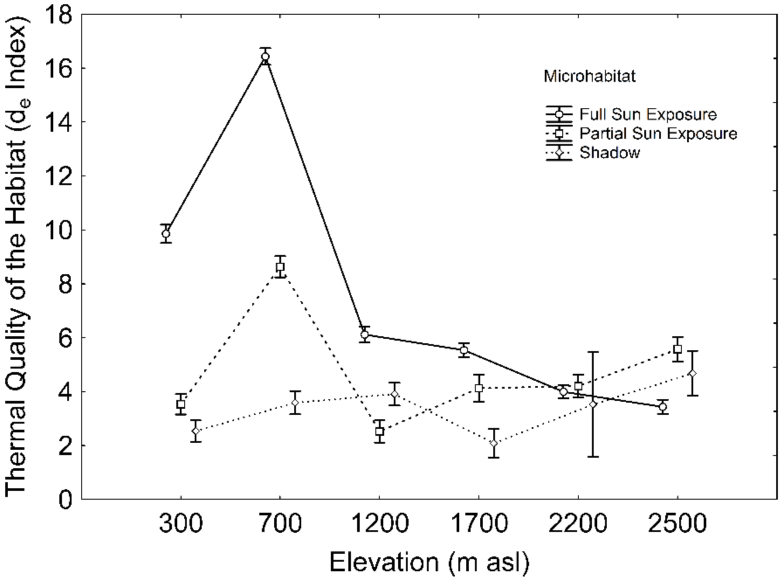

Fig. II.5a

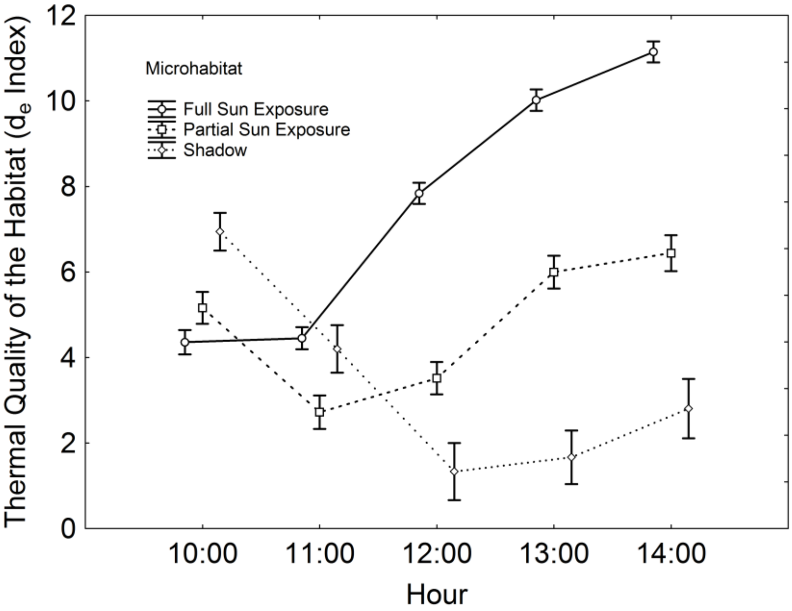

Fig. II.5b

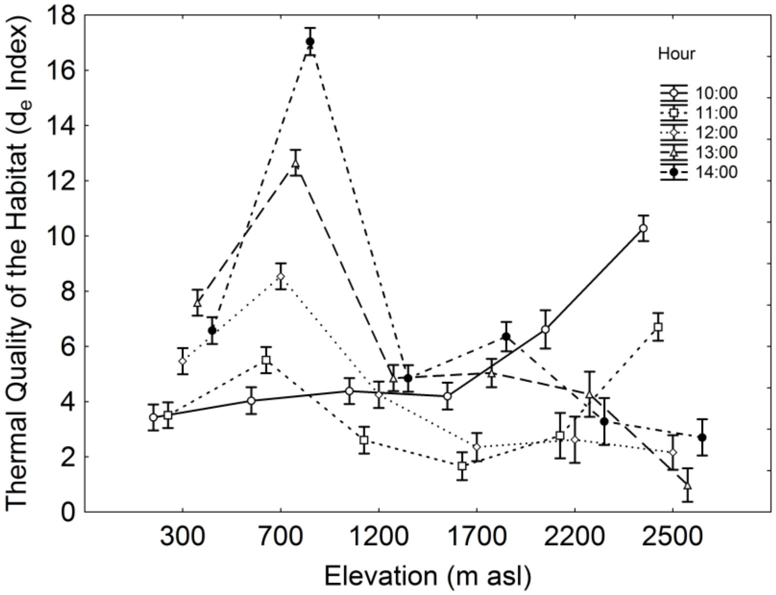

Fig. II.5c

Fig. II.5 - Thermal quality of the habitat (measured as $d_e$ index) increases as $d_e$ value approaches 0. In low elevations, thermal quality of the habitat is maximal under shade, but at high elevations full sun exposure is more suitable (Fig. II.5a). At low elevations, habitat thermal quality is maximal at the beginning of the day, while at high elevations the central day hours are best (Fig. II.5b). Maximal thermal quality of the habitat occurred in fully sun-exposed microhabitats at the beginning of the day, shifting gradually to partially exposed and finally to shaded microhabitats (Fig. II.5c). Vertical bars represent standard errors. These results were calculated from $T_{set}$ considered as 50% central values of $T_{sel}$. Results from $T_{set}$ considered as 80% central values of $T_{sel}$ were very similar, and are available in Appendix B (Fig. B.S1).

| | *D.f.* | *F* |
|---|---|---|
| **Elevation** | 5, 881 | 82.51*** |
| **Hour** | 4, 881 | 28.40*** |
| **Microhabitat** | 2, 881 | 118.32*** |
| **Elevation*Hour** | 20, 881 | 33.86*** |
| **Elevation*Microhabitat** | 10, 881 | 48.38*** |
| **Hour*Microhabitat** | 8, 881 | 41.13*** |

Table II.3 - Results of three-way factorial ANOVA testing the effect of elevation, hour, microhabitat, and their interactions on thermal quality of the habitat ($d_e$). Symbols indicate: *** for $P < 0.001$. Results of de from $T_{set}$ calculated as 80% central values of $T_{sel}$ were very similar, and are available in Table B.S1 in Appendix B.

### *II.4.4.-Accuracy of thermoregulation ($d_b$) and effectiveness of thermoregulation ($d_e$-$d_b$)*

Although we found differences among populations ($F_{5,\ 292} = 2.83$; $P = 0.02$), $d_b$ showed no clear elevational pattern (Fig. II.6). In fact, Spearman's correlation between $d_b$ and elevation was not significant ($r_s = 0.26$; $P = 0.62$). However, females thermoregulated more accurately than did males ($F_{2,\ 290} = 3.72$; $P = 0.025$; Fig. II.7). Results proved similar after using 80% of the $T_{sel}$ values to estimate the accuracy of body temperature (Figs. B.S2 and B.S3 in Appendix B, respectively).

Regarding effectiveness of thermoregulation, lizards from low elevations (300 and 700 m asl) thermoregulated more effectively than those from mid (1200 and 1700 m asl) and high elevation (2200 and 2500 m asl) populations (Tables B.S2-B.S5 in Appendix B). Spearmans' correlations were negative for lizards in all reproductive conditions, although they were marginally non-significant only for gravid ($r_s = -0.77$; $P = 0.07$) and non-gravid females ($r_s = -0.77$; $P = 0.07$), but not for males ($r_s = -0.49$; $P = 0.33$). This result agrees with female having higher thermoregulation accuracy.

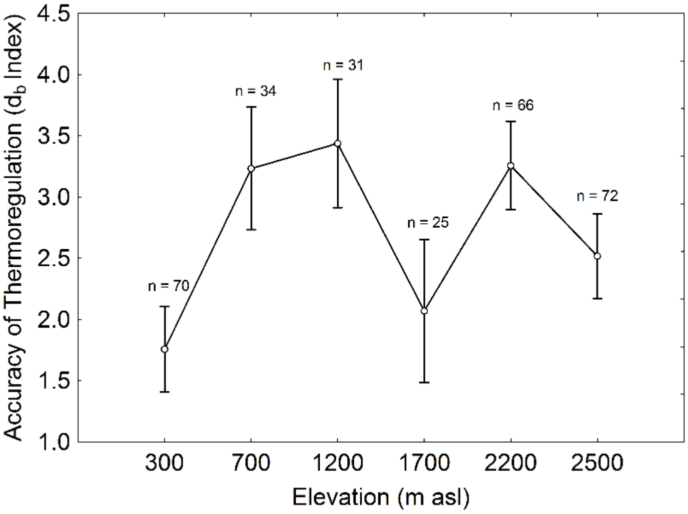

Fig II.6 - Accuracy of thermoregulation (measured as $d_b$ index) showed no clear elevational pattern. Accuracy of thermoregulation increases as $d_b$ value approaches 0. Vertical bars represent standard errors. These results were calculated from $T_{set}$ considered as 50% central values of $T_{sel}$. Results from $T_{set}$ considered as 80% central values of $T_{sel}$ were very similar, and are available in Appendix B (Fig. BS2).

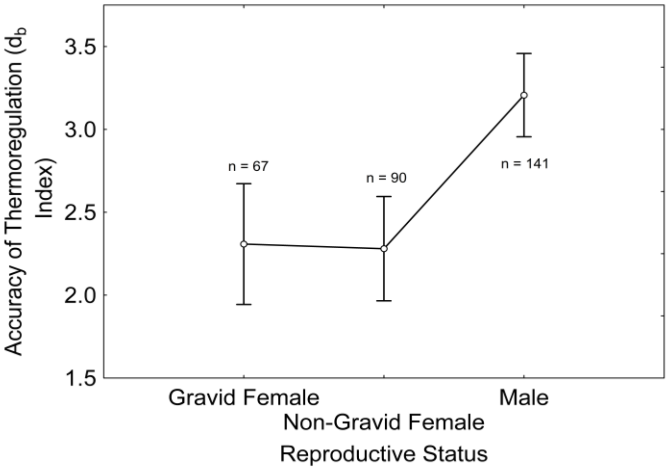

Fig. II.7 - Accuracy of thermoregulation (measured as $d_b$ index) according to reproductive condition. Accuracy of thermoregulation increases as $d_b$ value approaches 0. Both gravid and non-gravid females thermoregulated more accurately than did males. Vertical bars represent standard errors. These results were calculated from $T_{set}$ considered as 50% central values of $T_{sel}$. Results from $T_{set}$ considered as 80% central values of $T_{sel}$ are available in Appendix B (Fig. B.S3).

## II.5.-Discussion

### *II.5.1.-Elevation*

Ectotherm thermal ecology is closely related to environmental temperature (Angilletta 2009), which decreases with elevation as well as with latitude (Hertz and Huey 1981). Thus, lizard thermal performance could be gradually constrained in this 2200-meter elevational gradient, where we detected a trend of $T_e$ to diminish with rising elevation. Nonetheless, elevational differences in $T_b$ did not follow any detectable elevational gradient, as we have reported in a previous study with a subsample of data used here (Zamora-Camacho et al. 2013). For instance, the $T_b$ of lizards at 2500 m asl (31.6ºC in average), where $T_e$ was the lowest, was very similar to that of lizards at high $T_e$ populations such as 700 and 1200 m asl (31.5 and 32.0ºC, respectively). Accordingly, Díaz et al. (2006) found no differences in $T_{sel}$ in two populations of *P. algirus* separated 700 m in elevation. We found that $T_{sel}$, which is attained without environmental constraints, diminished gradually as elevation rose for 0.6ºC. Nevertheless, $T_e$ fell more than 7.3ºC with rising elevation.

The fact that $T_{sel}$ was consistently higher than $T_b$ suggests that thermoregulation capability in the wild is constrained, which may determine the temperature that lizards can actually attain in the field. Consistent with that result, Aguado and Braña (2014) found that $T_{sel}$ was higher than $T_b$ in the high-elevation lizard *Iberolacerta cyreni*. Evidently, one of the main constraints on ectotherm thermoregulation is environmental temperature (Grant and Dunham 1990; Adolph and Porter 1993; Medina et al. 2009). We found a correlation between $T_e$ and $T_b$ in the highest sampling populations, suggesting that environmental temperature is limiting only at high elevations. Our findings suggest that lizard thermoregulation is constrained below 20-25ºC. When $T_e$ is below this temperature, the relationship between the difference in $T_b$-$T_e$ and $T_e$ seems to approach an asymptote. A number of abiotic factors, with predictable geographical patterns, such as solar radiation, long-wave radiation, or conductive and convective heat flow, can affect lizard thermoregulation (Fei et al. 2012). In addition, other biotic factors may be constraining lizard ability to thermoregulate in the field, such as predation pressure (Carter et al. 2010), food availability (Yu et al. 2008), or reproduction opportunities (Shine et al. 2000). In our study, environmental temperature explained around 70% of variation in $T_b$. The relationship between $T_s$ and $T_b$, and thus the influence of thigmothermy in this species remains obscure, but it does not seem to be preponderant.

These data suggest that $T_e$ is limiting in high elevations. Surprisingly, although lizards in all elevations can attain appropriate $T_b$ by shifting among microhabitats, thermal quality of the habitat improved at high elevations with respect to low elevations, the best being at mid-elevation populations. This result indicates that lizards at low elevations face excess $T_e$. Accordingly, the thermal quality of the habitat at low elevations was more suitable for lizards at the beginning of the day, and in shaded microhabitats. The excessively hot thermal environment lizards face in low elevation populations might explain why these populations harbour the lowest densities (Zamora-Camacho et al. 2013). Moreover, this finding has important consequences in a scenario of climate warming, as it suggests that these populations would be in risk of extinction if thermal environment worsens.

On the contrary, thermal environment in high elevations was more appropriate in the central hours of the day, and in sunny microhabitats. This finding implies that, although overall habitat thermal quality is better at high elevations than at low elevations, lizards still have to sunbathe actively in order to reach an appropriate $T_b$, which increases thermoregulation costs, especially predation risk (Alford and Lutterschmidt 2012). Moreover, daily optimal temperature at high elevations appeared to be constrained to the central hours, when thermal quality of the habitat is more suitable. This temporal constraint is also a major limitation to lizard circadian activity. In fact, thermoregulation was more effective at low than at high elevations, suggesting that lizards can more easily attain appropriate $T_b$ by avoiding heat when it is excessive than by acquiring it when temperature is a bit below of the adequate one. Furthermore, habitat thermal quality was measured halfway through the reproductive season, when lizard abundance was maximal in most populations (Zamora-Camacho et al. 2013). Thus, habitat thermal quality could be worse at high and better at low elevations in spring or autumn, when environmental temperature is lower (Zamora-Camacho et al. 2013).

Hence, our results tally with the static hypothesis of ectotherm thermal-environment adaptation (Crowley 1985; Gilchrist 1995), which posits that ectotherm capability to modify thermal physiology, and thus thermal preferences (Hertz et al. 1983), can be evolutionarily constrained. However, Labra et al. (2009) found little effect of phylogeny on adaptation to thermal environment in 32 species of *Liolaemus* lizards inhabiting very different thermal environments. Therefore, phylogenetic relationships could not be limiting lizard thermal ecology evolution (Rodríguez-Serrano et al. 2009). Nevertheless, Moreno Azócar et al. (2013) found that critical thermal minimum in relation to average air temperature are mainly shaped by air temperature in the *Liolaemus goetschi* group, while critical thermal maximum and preferred body temperature are phylogenetically more conservative. Therefore, the extent to which phylogeny can constraint different aspects of lizard thermal biology seems variable.

On the other hand, our results match the extended model of thermoregulation cost/benefit balance (Vickers et al. 2011), which predicts that ectotherms will thermoregulate appropriately in thermally challenging habitats because the costs of no-thermoregulation outweigh those of thermoregulation. In fact, high-elevation lizards managed to attain $T_b$ values as high as those of mid-elevation lizards, despite the ecological and thermal constraints. Similarly, several species of *Liolaemus* and *Phymaturus* lizards thermoregulate suitably at high elevations (Ibargüengoytía et al. 2008; Valdecantos et al. 2013). Lizards can display several mechanisms for accurate thermoregulation in thermally challenging habitats. For instance, montane *Iberolacerta monticola* uses long basking periods to accumulate heat (Carrascal et al. 1992), and *Zootoca* *vivipara* spends more time basking at 1450 than at 250 m asl (Gvoždík 2002). High elevation *Iberolacerta cyreni* lizards spend 80% of their time in thermoregulation when radiation intensity is low (Aguado and Braña 2014). Furthermore, *P. algirus* is darker at higher elevations in our study system (Reguera et al. 2014), which can help them heat up faster, according to the thermal melanism hypothesis (Clusella-Trullas et al. 2007). Moreover, *P. algirus* cools down more slowly at higher elevations as a consequence of their size increasing with elevation (Zamora-Camacho et al. 2014a). In addition, although copper-model temperature decreased at higher elevations (Fig. 3 in Zamora-Camacho et al. 2013), this trend reversed when controlling for environmental temperature, suggesting that heat sources other than environmental temperature can boost high-elevation lizard thermoregulation (Zamora-Camacho et al. 2013). In conclusion, *P. algirus* seems to have developed adaptations in order to maintain thermoregulation performance in high elevations in Sierra Nevada (darker dorsal colour and larger body size).

### ***II.5.2.-Reproductive condition***

Lizard $T_b$ was similar irrespective of reproductive condition. However, male accuracy and effectiveness of thermoregulation were lower than those of gravid and non-gravid females. This result may be the result of males spending more time searching for mates, as observed in the snake *Thamnophis sirtalis scalaris* (Shine et al. 2000), or defending their territories, as in the lizard *Sceloporus jarrovi* (Marler et al. 1995), to the detriment of thermoregulation.

Regarding gravid females, they might be facing a trade-off between their own PTR and optimum embryo-development temperature, which has proven to be lower in several oviparous (Mathies and Andrews 1997) and viviparous (Beuchat 1986; Shine and Harlow 1993) lizards. Actually, we found that gravid female $T_{sel}$ was lower as egg laying approached. This finding suggests that the embryo need for lower temperatures increases at more advanced developmental stages. Although other lizards show impaired escape speed when gravid (Shine 2003), and thus avoid exposure to predators by reducing basking time, leading to reduced body temperatures (Lin et al. 2008), we can discard this explanation since gravid *P. algirus* in this study system do not suffer impaired escape speed (Zamora-Camacho et al. 2014b).

However, gravid female $T_{sel}$ was similar to that of non-gravid females and males. Only postpartum females showed a significantly higher $T_{sel}$ than for the rest of reproductive conditions. This finding is surprising considering findings by other authors, which have shown that gravid females prefer lower $T_{sel}$ (Mathies and Andrews 1997; Le Galliard et al. 2003). Although we still found lower $T_{sel}$ in gravid than in postpartum females, in our case this result was not due to an active selection of lower $T_{sel}$ by gravid females, but to preferences of higher $T_{sel}$ by postpartum females. A possible explanation for this result is that females pay a cost for reproduction (Shine 1980), including postpartum diminished body condition (Olsson et al. 2000; Itonaga et al. 2012). Higher body temperatures could improve their predatory ability (Avery et al. 1982) as well as their metabolic rate, and thus, efficacy of digestive processes such as higher assimilation efficiency and lower gut-passage times (McConnachie and Alexander 2004), allowing them to restore body condition before hibernation.

## II.6.-Conclusions

In short, $T_e$ and $T_{sel}$ tended to diminish with rising elevation, while $T_b$ differences among populations showed no elevational tendency. This result suggests that, in the field, factors in addition to thermal availability constrain lizard thermoregulation capacity. In fact, $T_b$ seems to be constrained by $T_e$ at the highest elevations. Nevertheless, thermal quality of the habitat is more suitable at high elevations, suggesting that low-elevation lizards face excess environmental temperature. However, thermal quality of the habitat at high elevations is better in fully sun-exposed microhabitats and in the central hours of the day, indicating that high-elevation lizards still have to sunbathe actively to attain appropriate $T_b$, thus increasing thermoregulation costs, and their optimal daily activity period is limited to the central hours of the day.

For reproductive status, the accuracy and effectiveness of thermoregulation was better in gravid and non-gravid females than in males, probably because males spend more time defending their territories and searching for mates. Postpartum females showed higher $T_{sel}$ than did males or gravid and non-gravid females, probably improving their body condition by increasing their metabolic rate. Furthermore, gravid females chose lower $T_{sel}$ as egg laying approached, this being consistent with lower optimum embryo-development temperatures.

## II.7.-Acknowledgements

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# III - Chapter 3

# Bergmann’s Rule rules body size in an ectotherm: heat conservation in a lizard along a 2200-meter elevational gradient.

This chapter reproduces the published article:

Zamora-Camacho, F.J., Reguera, S., Moreno-Rueda, G. 2014. Bergmann’s Rule rules body size in an ectotherm: heat conservation in a lizard along a 2200-meter elevational gradient. Journal of Evolutionary Biology, DOI: 10.1111/jeb.12546.

**III.1.-Abstract** - Bergmann’s Rule predicts larger body sizes in colder habitats, increasing organisms’ ability to conserve heat. Originally formulated for endotherms, it is controversial whether Bergmann's Rule may be applicable to ectotherms, given that larger ectotherms show diminished capacity for heating up. We predict that Bergmann's Rule will be applicable to ectotherms when the benefits of a higher conservation of heat due to a larger body size overcompensate for decreased capacity to heating up. We test this hypothesis in the lizard *Psammodromus algirus*, which shows increased body size with elevation in Sierra Nevada (SE Spain). We measured heating and cooling rates of lizards from different elevations (from 300 to 2500 meters above sea level) under controlled conditions. We found no significant differences in the heating rate along an elevational gradient. However, the cooling rate diminished with elevation and body size: highland lizards, with larger masses, have a higher thermal inertia for cooling, which allows them to maintain heat for more time and keep a high body temperature despite the lower thermal availability. Consequently, the net gaining of heat increased with elevation and body size. This study highlights that the heat conservation mechanism for explaining Bergmann's Rule works and is applicable to ectotherms, depending on the thermal benefits and costs associated with larger body sizes.

## III.2.-Introduction

Physiological processes are possible only at certain temperature ranges (Xiang et al., 1996; Clark et al., 2005; Sun et al., 2012). Nonetheless, environmental temperature is generally variable, frequently shifting away from organisms´ physiological optima. Thus, organisms show a set of adaptations for keeping their internal temperature compatible with physiological processes in spite of the unsettled environmental temperature. Animals show two main thermal strategies according to the relative importance of metabolic and environmental heat in body temperature upkeep: ectothermy and endothermy. Ectotherms resort mainly to external environmental heat sources to maintain their internal body temperature inside the preferred temperature range (Hertz et al., 1993), which endotherms achieve using internal metabolic heat sources (Bennett and Ruben, 1979).

Several macroecological rules arose from the influence of the thermal environment on animals' fitness, showing different applicability in ectotherms and endotherms. Bergmann's Rule establishes a general tendency of endotherms to achieve larger body sizes in higher latitudes, and thus, in colder environments (Bergmann, 1847). This increase in body size with decreasing environmental temperature, known as Bergmann's clines, could be explained by the positive relationship between body mass and thermal inertia (heat conservation hypothesis): heat is dissipated more slowly in larger animals as the surface-area to volume ratio diminishes, so it would be a thermal advantage in colder habitats (Meiri, 2011). Bergmann's Rule has been widely proven in endotherms (Meiri and Dayan, 2003; Millien et al., 2006), although the mechanism causing Bergmann's Rule is still under discussion (Blackburn et al., 1999; Rodríguez et al., 2006; Yom-Tov and Geffen, 2006; Meiri et al., 2007; McNab, 2010; Watt et al., 2010).

For ectotherms, the application of Bergmann's Rule is even less clear: although a larger ectotherm would retain body heat for a longer time, a big body mass could decrease its capacity to warm up from an external heat source, mainly in cold habitats (Stevenson, 1985). For this reason, most scientists consider that the traditional explanation for Bergmann's clines (the heat conservation hypothesis) is not applicable to ectotherms (Watt et al., 2010; for exceptions to this view see Ashton and Feldman, 2003; Cruz et al., 2005; Olalla-Tárraga et al., 2006; Olalla-Tárraga and Rodríguez, 2007). Despite this point of view, many ectotherm groups show Bergmann's clines (e.g., aforementioned references; also see Ashton, 2002a; Blanckenhorn and Demont, 2004; Millien et al., 2006). Both the converse of Bergmann's clines as well as the lack of covariation between body size and environmental temperature have also been found in several ectotherms (Mousseau, 1997; Ashton and Feldman, 2003; Olalla-Tárraga et al., 2006; Adams and Church, 2008; Hu et al., 2011), contributing to the controversy regarding the applicability of Bergmann's Rule to ectotherms.

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

We hypothesized that the heat conservation hypothesis may explain the occurrence of Bergmann's clines in ectotherms. Here, we test this hypothesis using the lizard *Psammodromus algirus* as a model system in Sierra Nevada (SE Spain). In Sierra Nevada, this lizard follows Bergmann’s clines in a 2200-meter elevational gradient, lizards being larger at high elevations (see Results). During the activity period of this lizard, environmental temperature decreases an average of 8 ºC from the lowest (300 m asl) to the highest elevation (2500 m asl) in our study area (Zamora-Camacho et al., 2013). We predict that the advantage of increased thermal inertia in larger individuals inhabiting cold habitats should compensate for the disadvantage of reduced heating capabilities, explaining the body size cline with elevation in this population; i. e., we expect to find $\beta > \alpha$. To test this hypothesis, we examined whether elevational differences in body size are related to different thermoregulatory capabilities, by measuring lizards' heating and cooling rates under controlled conditions (Yom-Tov and Geffen, 2011). The main novelty of this study is that, for the first time, we check whether the mechanism of the heat conservation hypothesis works in an animal showing a Bergmann cline.

## III.3.-Materials and methods

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

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

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

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

Graphing body temperature over time, the standard heat transfer curve of a lizard consisted of a linear temperature increase while the bulb was switched on, a variable-duration transition lapse after switching off the bulb, while body temperature remained almost unaltered, and a linear temperature decrease after the inertia lapse (Fig. III.1). From those three phases we obtained for each individual: (1) the heating rate, calculated as the regression slope of body temperature increase over time during the heating period, in ºC/min; (2) the transition lapse, as the time (in min) lapse since we switched off the bulb until lizard body temperature started to diminish; and (3) the cooling rate, calculated as the regression slope of body temperature decrease over time during the cooling period, in ºC/min.

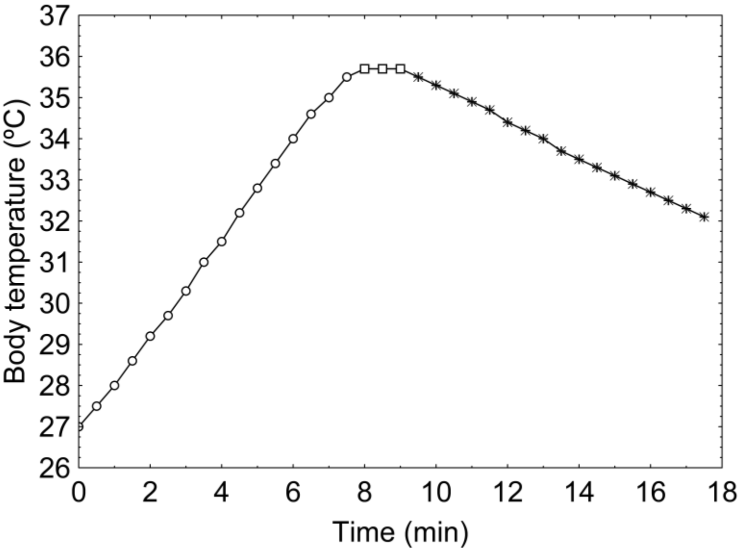

Fig. III.1 - Changes in body temperature during the main stages of the thermal experiment in a standard, hypothetical lizard. All lizards followed a similar pattern of temperatures during the trials: linear heating phase when the bulb is on (circle-dotted line), thermally-stable transition lapse after switching the bulb off (square-dotted line), and linear cooling phase after the transition lapse (asterisk-dotted line).

Statistical analyses were performed with parametric statistics (Quinn and Keough, 2002). Data distributions matched the assumptions of parametric statistics (homoscedasticity and normality of residuals). The variables cooling rate and body mass required log-transformation. We performed an ANOVA to test for elevational differences in back lightness, SVL and body mass. Another ANOVA tested whether there were statistical differences in heat transfer processes among males, gravid females, and non-gravid females (a factor with three levels). Finally, we used Ordinary Least Squares Linear Models to test for differences in heating rates, inertia lapses and cooling rates according to elevation and body mass. We did not include elevation and body mass in the same model because models with the two variables showed problems of collinearity (in an ANOVA, elevation explained body mass with a multiple $R$ = 0.68). Therefore, in order to avoid multicollinearity, for each dependent variable, we performed two models, one with elevation and other with body mass. Given that sex and female reproductive status, as well as dorsal lightness, could affect thermoregulation (heating rate in the case of dorsal lightness), we controlled for these variables in the models. We performed those analyses with software Statistica 7.1.

## III.4.-Results

### *III.4.1.-Morphological variation in elevation*

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

### *III.4.2.-Heating rates*

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

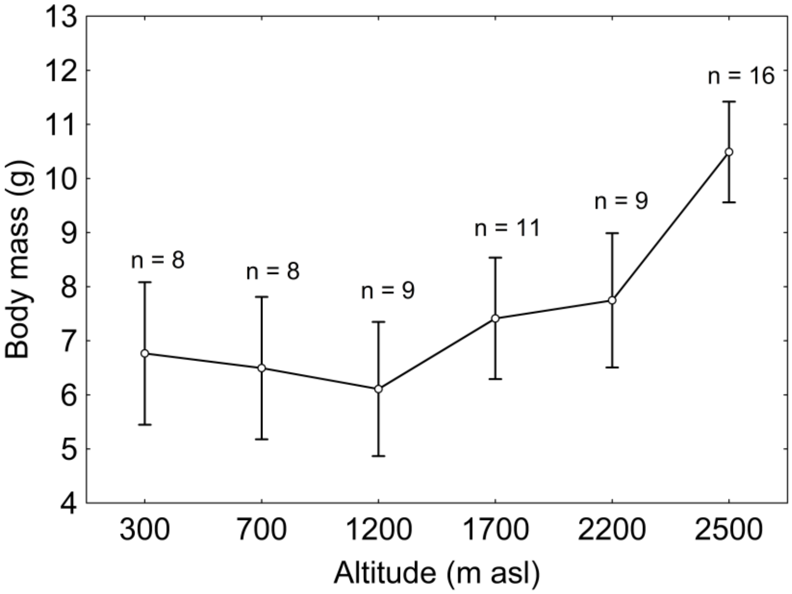

Fig. III.2 - Body mass was significantly bigger in the highlands, according to predictions of Bergmann's Rule. Vertical bars denote 0.95 confidence intervals. Sample size is indicated on bars.

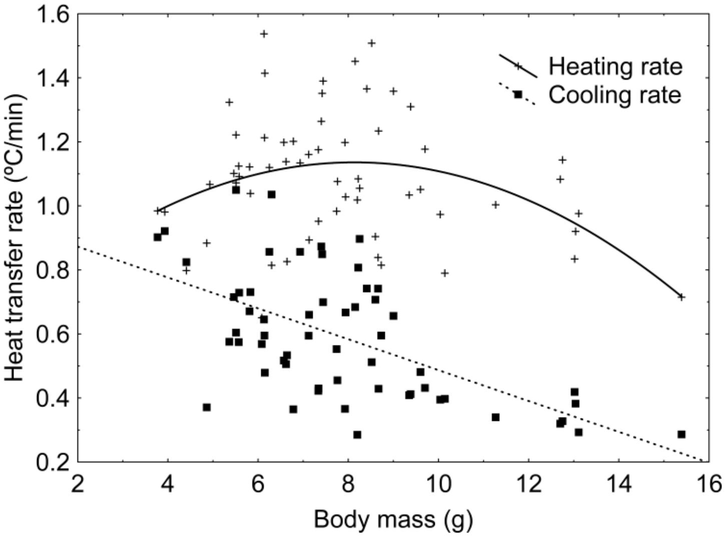

Fig. III.3 - Ordinary Least Squares Regression of heating rate (crosses) and cooling rate (squares) over body mass. While cooling rate had a significant, higher-sloped, negative relationship with body mass, heating rate showed a significant concave down relationship with body mass.

### *III.4.3.-Transition lapses*

After switching off the bulb, lizards' body temperature remained stable for 1.27 min on average. Transition lapses tended to increase with elevation ($F_{5, 57}$ = 12.554; $P < 0.001$; Fig. III.4). In this model there were almost significant differences in transition lapses among males, non-gravid females and gravid females ($F_{2, 57}$ = 3.065; $P$ = 0.054; Fig. III.5a), gravid females tending to have larger inertia lapses than males. When body mass was included in the model in the place of elevation, transition lapses significantly increased with body mass (effect of body mass: $\beta$ = 0.562; $F_{1, 57}$ = 30.153; $P$ = 0.001; effect of sex and female reproductive status: $F_{2, 57}$ = 2.211; $P$ = 0.119). Neither lizards from the highest elevation ($r$ = 0.240; $P$ = 0.370) or from populations below ($r$ = 0.238; $P$ = 0.115) showed a significant relationship between body mass and transition lapses.

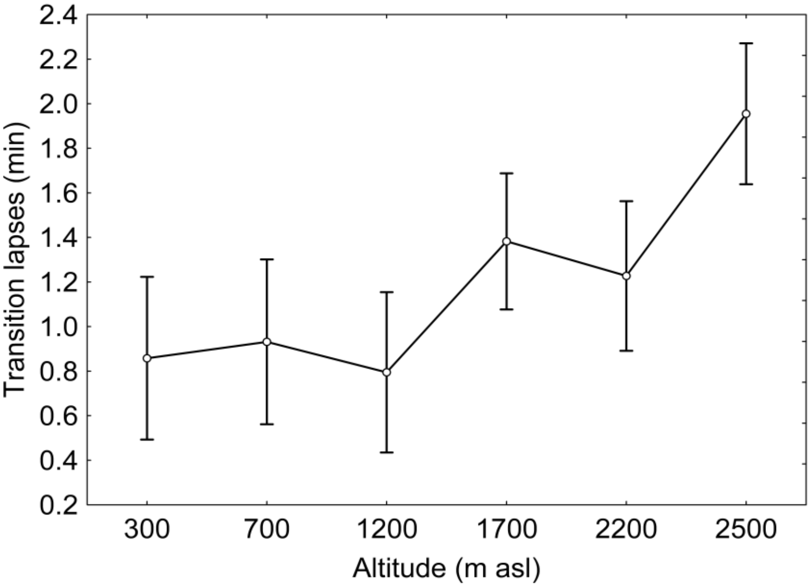

Fig. III.4 - Transition lapses, i. e., time elapsed from the moment we switched off the bulb until lizard started cooling down, were bigger in high elevations. Thus, larger-size high-elevation lizards showed increased thermal inertia, following the predictions of Bergmann's rule. Vertical bars denote 0.95 confidence intervals. Sample size was shown in Fig. III.2.

### *III.4.4.-Cooling rates*

On average, lizards cooled by 0.58ºC/min. Similar to inertia lapses, cooling rates significantly decreased with elevation ($F_{5,\ 52} = 11.068$, $P < 0.001$; Fig. III.6). Consistent with our results for the inertia lapse, gravid-females tended to lose heat more slowly than males ($F_{2,\ 52} = 4.112$; $P = 0.022$; Fig. III.5b). When body mass was introduced in the model in the place of elevation, cooling rates significantly decreased with body mass (effect of body mass: $\beta = -0.568$; $F_{1,\ 56} = 37.582$, $P = 0.001$; Fig. III.3; effect of sex and female reproductive status: $F_{2,\ 56} = 2.801$, $P = 0.069$). That is, consistently with the heat conservation hypothesis, larger lizards lost heat more slowly than small lizards. Lizards from the highest elevation showed a significant, positive relationship between body mass and cooling rate ($r = 0.775$, $P < 0.001$), while no significant relationship between body mass and cooling rate was found when considering lizards of populations below 2500 m asl ($r = 0.241$, $P = 0.116$).

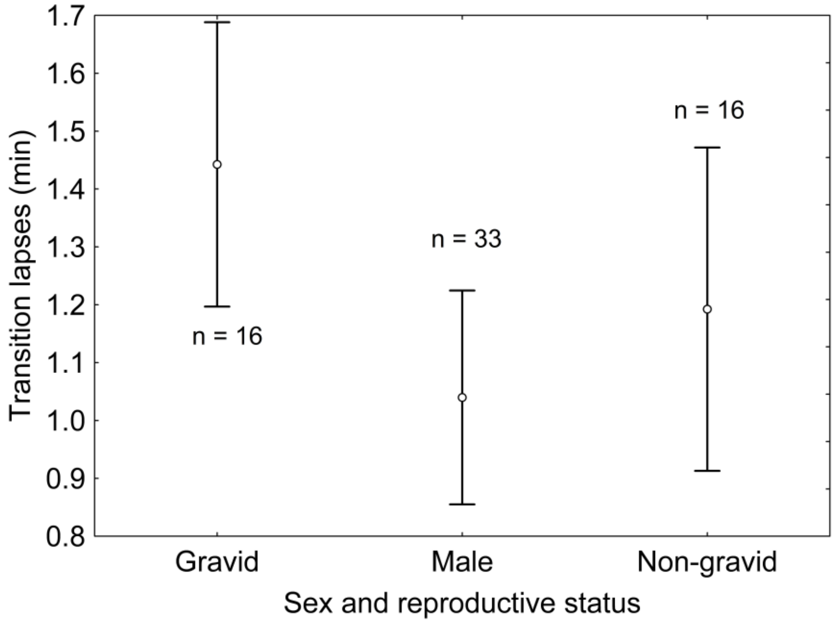

Fig. III.5a

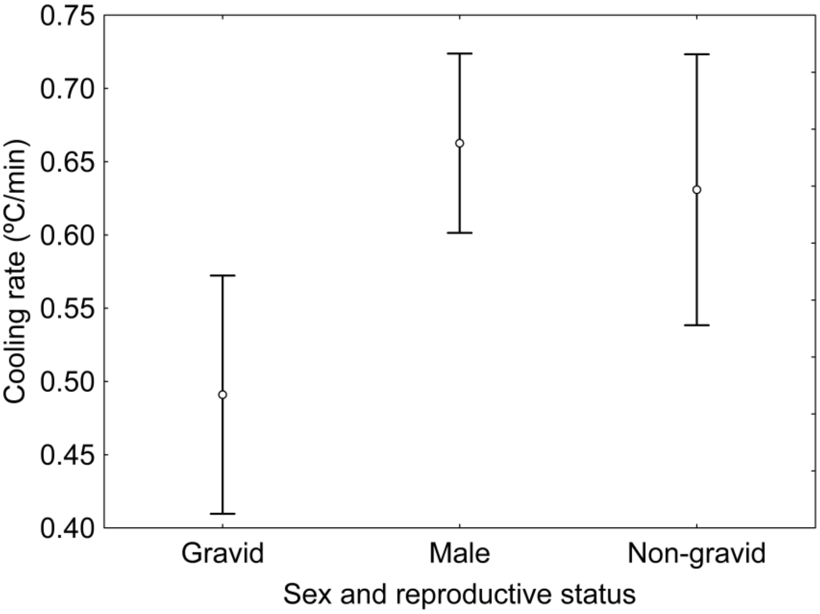

Fig. III.5b

Fig. III.5 - Gravid females had higher transition lapses (Fig. III.5a) and lower cooling rates (Fig. III.5b) than males and non-gravid females. Vertical bars denote 0.95 confidence intervals. Sample sizes on bars in Fig. III.5a.

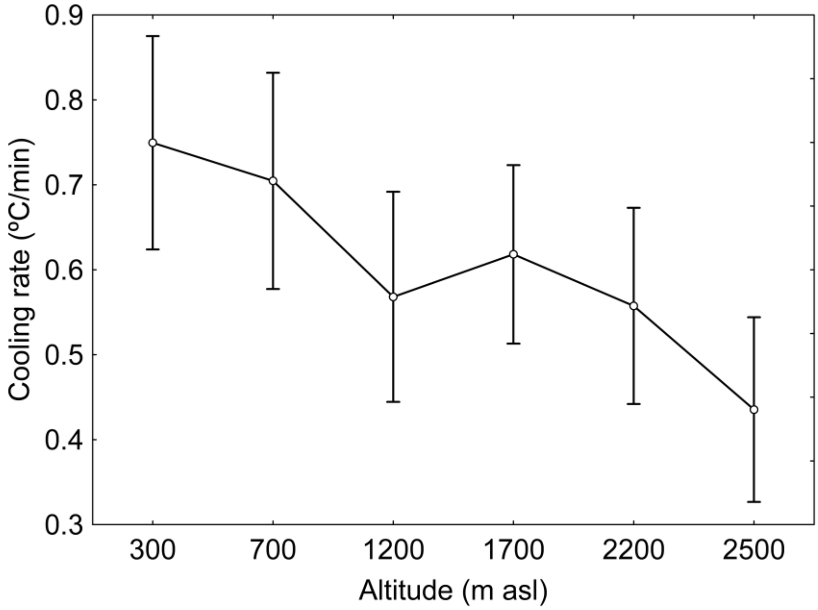

Fig. III.6 - Cooling rate diminished in elevation. Larger-sized highland lizards showed to retain heat for longer, supporting both the morphological and the thermal predictions of Bergmann's Rule. Vertical bars denote 0.95 confidence intervals. Sample sizes as in Fig. III.2.

### *III.4.5.-Net heating rate*

We estimated the net heating rate as the heating rate minus the cooling rate. Net heating rate significantly increased with elevation ($F_{5,\ 52}$ = 4.061, $P$ = 0.003; Fig. III.7a; sex and female reproductive status, $F_{2,\ 52}$ = 0.183, $P$ = 0.833). Similarly, net heating rate increased with body mass, suggesting a trend toward a concave down function (linear effect: $F_{1,\ 53}$ = 5.965, $P$ = 0.018; quadratic effect: $F_{1,\ 53}$ = 3.979, $P$ = 0.051; Fig. III.7b; effect of sex and female reproductive status, $F_{2,\ 53}$ = 1.453, $P$ = 0.243). Lizards from the highest elevation showed no significant relationship between body size and net heating rate ($r$ = -0.243, $P$ = 0.365), while a positive linear relationship between body size and net heating rate was found when considering lizards from populations below 2500 m asl ($r$ = 0.326, $P$ = 0.035; Fig. III.7b).

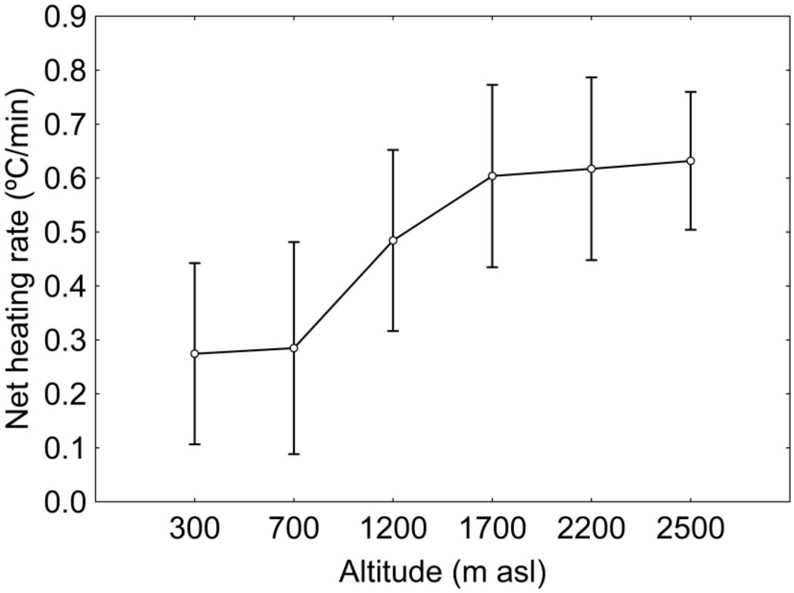

Fig. III.7a

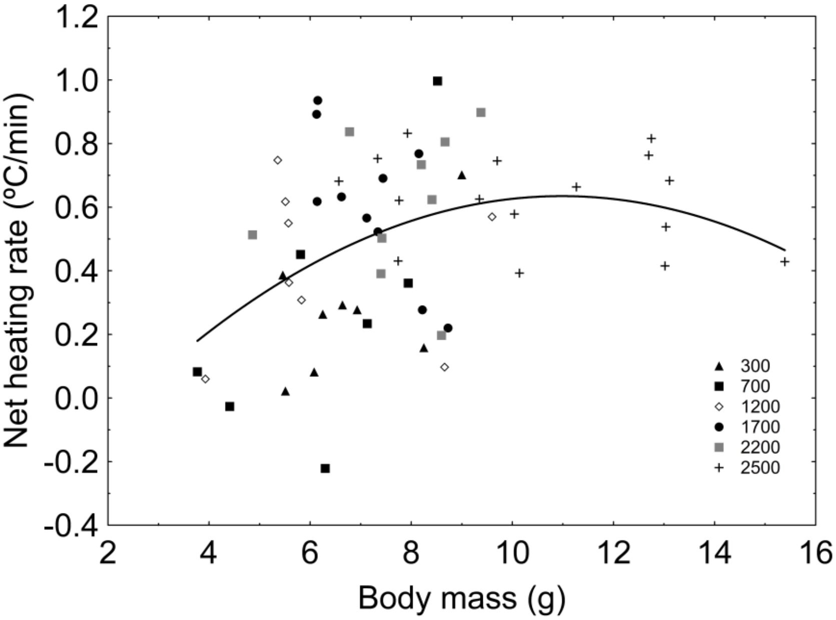

Fig. III.7b

Fig. III.7 - Net heating rate (ºC/min) tended to increase with elevation (Fig. III.7a), and follow a concave down relationship with body mass (Fig. III.7b). Sample sizes for Fig. III.7a as in Fig. III.2. Vertical bars denote 0.95 confidence intervals.

## III.5.-Discussion

Bergmann’s Rule, larger size in colder areas, has been validated in some endotherms (Ashton et al., 2000; Ashton, 2002b; Meiri and Dayan, 2003; Millien et al., 2006; Rodríguez et al., 2006; Ramírez et al., 2008; Rodríguez et al., 2008). Nevertheless, its applicability for ectotherms remains controversial, since some of them follow Bergmann's cline, whilst numerous ectotherms do not, or even follow the converse to Bergmann’s cline (Mousseau, 1997; Ashton and Feldman, 2003; Blackenhorn and Demont, 2004; Olalla-Tárraga et al., 2006; Olalla-Tárraga and Rodríguez, 2007; Adams and Church, 2008; Hu et al., 2011; Oufiero et al., 2011). The results of our study support Bergmann’s Rule. In an elevational gradient of 2200 m, *P. algirus* populations showed larger size in high than in mid and low elevations, and population average body size negatively correlated with temperature. The fact that the increase in body size is much more marked in high elevations matches with other studies showing that the increase in body size with decreasing temperature is stronger in cold habitats (Rodríguez et al., 2008).

Nonetheless, the mechanism underlying Bergmann's clines is still under debate. Even for endotherms, many researchers have argued that the heat conservation hypothesis does not completely explain Bergmann's clines (McNab, 2010). In ectotherms, in which the heat conservation hypothesis has been generally (and prematurely) rejected, a set of hypotheses have been proposed to explain Bergmann's clines based on physiological by-products of temperature-dependent growth and development, or temperature-dependent life-history traits (mortality or fecundity; Angilletta et al., 2004b). There is evidence that other ecological traits, besides thermoregulation, may cause body size divergence at interspecific or interpopulation levels, such as differences in longevity (Liao and Lu, 2011), habitat use (Collar et al., 2011), predation pressure (De Barros et al., 2010), feeding habits (Ochocińska and Taylor, 2003), food availability (Yom-Tov and Geffen, 2006), starvation resistance (Arnett and Gotelli, 2003), and life-history traits (Angilletta et al., 2004b), among others.

These factors are not mutually excluding, may act in contrasting directions, and complicate enormously the interpretation of the causes of Bergmann's clines (Jones et al., 2005).

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

We found that heating rate showed a concave down relationship with body mass. This contrasts with previous studies showing that small lizards heat up faster than larger lizards (Carothers et al., 1997). In our study system, highland lizards are darker; darker back colors could help these lizards to warm up faster (Clusella-Trullas et al., 2007), counteracting the negative effect of larger body mass on heating capacity, and smoothing out any elevational differences in heating rates (Cruz et al., 2005; Harris et al., 2012). Consequently, larger body mass would not limit heating rates in our study system. In fact, highland lizards reach field body temperatures similar to lowland lizards, despite lower environmental temperatures, which suggests an efficient exploitation of poorer heat sources in high elevations (Zamora-Camacho et al., 2013). However, we failed to find convincing evidence of an effect of darker dorsal color on heating rate.

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

## III.6.-Conclusions

In conclusion, we show that *P. algirus* complies with Bergmann's Rule in an elevational system. We also demonstrate that larger lizards have a thermal advantage in colder habitats, as they retain accumulated heat for longer, but they heat up as fast as smaller lizards, providing the strongest empirical evidence that the heat conservation hypothesis may apply to ectotherm body size trends.

According to the relative thermal costs and benefits of a larger body size, ectotherms may follow Bergmann's Rule, if the thermal costs of a larger body size exceed the benefits.

## III.7.-Acknowledgements

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# IV - Chapter 4

# Elevational variation in body-temperature response to immune challenge in a lizard.

This chapter reproduces the submitted article:

Zamora-Camacho, F.J., Reguera, S., Moreno-Rueda, G. 2014. Elevational variation in body-temperature response to immune challenge in a lizard. Journal of Experimental Biology, submitted.

**IV.1.-Abstract -** Immunocompetence determines animal fitness by combating pathogens, but entails a number of costs. One of its main components is fever, which in ectotherms involves two main types of costs: energy expenditure and predation risk. Whenever those costs of fever outweigh the benefits, ectotherms are expected not to develop fever, or even to show hypothermia, reducing costs of thermoregulation and diverting the energy saved to other components of the immune system. Environmental thermal quality, and therefore the thermoregulation cost/benefit balance, varies geographically. Hence, we hypothesize that, in temperate environments, immune-challenged ectotherms might show either a hypothermic or a febrile response, depending on thermoregulation cost/benefit balance. However, in alpine habitats, immune-challenged ectotherms are more probable to show no thermal response, given that (1) hypothermia would be very costly, as the available temporal window for reproduction is extremely small, and (2) fever would also have a prohibitive cost, as heat acquisition is limited in that habitat. We tested this hypothesis in *Psammodromus algirus* lizards from Sierra Nevada (SE Spain), by testing which body temperature is preferred by alpine and non-alpine lizards in a thermal gradient, before and after activating their immune system with a typical innocuous pyrogen. Non-alpine lizards responded to immune challenge by decreasing preferential body-temperature, presumably allowing them to save energy. On the contrary, immune-challenged alpine lizards maintained their body-temperature preferences. These results agree with our predictions, matching with increased costs of no thermoregulation with elevation, due to the reduced window of time for reproduction in their alpine environment.

## IV.2.-Introduction

Immunocompetence greatly determines animal fitness by combating pathogens (Schmid-Hempel, 2011). Nevertheless, deploying an efficient immune response entails a number of costs (Lochmiller and Deerenberg, 2000; Demas, 2004; Schmid-Hempel, 2011), which force animals to trade off immune defence with other costly traits (Sheldon and Verhulst, 1996; Norris and Evans, 2000; Zuk and Stoehr, 2002). These costs imply that other life-history traits may be impaired by the activation of the immune system, such as breeding success (Råberg et al., 2000), learning capacity (Grindstaff et al., 2012), growth (Uller et al., 2006), sexual attractiveness (López et al., 2009), and even survival (Moret and Schmid-Hempel, 2000; Hanssen et al., 2004; Eraud et al., 2009).

In addition to humoral and cellular responses, one of the main components of immune response in most animals -endotherms as well as ectotherms- is fever, a rise of body temperature with beneficial effects on other components of the immune system and/or direct negative effects on pathogens (Kluger et al., 1998). The efficacy of fever against pathogens has long been documented (Kluger et al., 1975), and survival following infections is well known to increase with temperature in both endotherms and ectotherms (Kluger et al., 1998). Nevertheless, the costs of producing fever and the optimal response considering these costs have received little attention (Romanovsky and Szekely, 1998). Endotherms generate heat endogenously, and thus fever implies increased energy expenditure (Marais et al., 2011). In turn, ectotherms regulate body temperature mainly behaviourally, by exposing themselves to environmental heat sources (Belliure and Carrascal, 1998). This leads to two types of costs: (1) energy expenditure, as metabolism increases with body temperature (Sherman and Stephens, 1998), and (2) predation risk, as ectotherms' mechanisms to gain heat, such as basking, usually imply an elevated exposure to predators (Herczeg et al., 2008). Therefore, under certain circumstances, the costs of raising body temperature might outweigh the benefits of fever. In such cases, no thermal response to pathogens may occur. Animals may even show hypothermia in order to save energy.

Maintaining optimal temperature is energetically costly (Bennett and Ruben, 1979; Alford and Lutterschmidt, 2012; Brewster et al., 2013). Moreover, mounting an immune response is also energetically costly, even without fever. For example, in toads *Rhinella marinus* maintained at the same temperature (thus, precluding febrile response), those injected with an antigen showed increased metabolic costs as a consequence of immune response (Sherman and Stephens, 1998). Therefore, simultaneously eliciting an immune response and maintaining body temperature might be under an energy trade-off. In this scenario, on the one hand, a trade-off might be expected between immune response and thermoregulation (given that both functions are energetically costly), and thus body temperature decreases in immune-challenged ectotherms (hypothermia). On the other hand, a body-temperature increase might also be expected in response to an immune challenge, i.e. an increase in body temperature in immune-challenged ectotherms (fever). The result would depend of the costs/benefits balance of eliciting fever.

Indeed, although fever is a common response to pathogens, some endotherm as well as ectotherm vertebrates respond to antigens with hypothermia (Owen-Ashley et al., 2006; Merchant et al., 2008; King and Swanson, 2013). Given that survival following infections usually decreases at lower temperatures (Kluger et al., 1998, and references therein), these findings suggest the existence of a trade-off between thermoregulation and eliciting an immune response. Therefore, fever, the absence of thermal response, or hypothermia in response to an immune challenge will depend on the cost/benefit balance of thermoregulation. Animals in a scenario of high thermoregulation cost/benefit balance might respond to an immune challenge with hypothermia, while animals in which costs of hyperthermia are low, or benefits are high, might respond to immune challenge with fever. Supporting this hypothesis, febrile or hypothermic response of iguana (*Iguana iguana*) juveniles to an activation of the immune system depends on the energetic state of each individual, only those individuals in the best energetic condition developing fever (Deen and Hutchison, 2001).

Given that ectotherms depend mainly on environmental temperature to thermoregulate, and environment thermal quality varies geographically (Sunday et al., 2010), the cost/benefit balance of producing fever should also vary geographically, especially in an elevational gradient. On the one hand, thermal quality for ectotherms typically decreases with elevation (Hertz and Huey, 1981). Consequently, ectotherms spend more time searching for basking sites in alpine zones (Díaz, 1997); therefore, the cost of thermoregulation increases with elevation. Accordingly, we predict that ectotherms in alpine zones should not develop fever in response to an immune challenge. On the other hand, in an elevational gradient, the window for reproduction declines with rising elevation (Zamora-Camacho et al., 2013). For alpine ectotherms, the costs of no thermoregulation may be high, as it would imply a loss of valuable time for reproduction. Therefore, we hypothesize that, everything being equal, in an elevational gradient, alpine immune-challenged ectotherms should prioritize heat acquisition, and thus their body temperature should be maintained in comparison with non-alpine immune-challenged ectotherms. Regarding non-alpine ectotherms challenged with an antigen, we predict that they might show either a hypothermic or a febrile response, depending on the cost/benefit balance in their circumstances, as mentioned above. In sum, alpine ectotherms should avoid hypothermia when immune challenged, considering the small window for reproduction, but it is highly improbable that they develop fever, considering the low thermal quality of the alpine environment.

In this work, we test this hypothesis by comparing the effect of an immune challenge on the temperature selected by a lizard, the large Psammodromus (*Psammodromus algirus*; Linnaeus, 1758), from alpine (2200-2500 m asl [metres above sea level]) and non-alpine (300-1700 m asl) zones in a mountain. In our study system, alpine lizards face a colder environment than non-alpine lizards do, but despite the differences in thermal environment, keep field body temperatures similar to those of non-alpine lizards (Zamora-Camacho et al., 2013). Alpine lizards in our study area are, indeed, active at lower temperatures than non-alpine lizards, which allows them to complete their life cycle in the small window of time available in alpine areas (Zamora-Camacho et al., 2013). According to the above reasoning, we predict that the thermal response (hypothermia or fever) to a typical pyrogen would be less evident in alpine lizards, as a consequence of their elevated costs of thermoregulation (low-thermal-quality environment) and of no-thermoregulation (small window of time for reproduction).

## IV.3.-Materials and Methods

*Psammodromus algirus* is a medium-sized (53-95 mm of snout-vent length [SVL] in our study zone) lacertid lizard that inhabits shrubby areas in the Western Mediterranean, from the sea level to more than 2600 m asl (Salvador, 2011). We caught lizards during their reproductive season (April-July) in 2012, in Sierra Nevada mountain (SE Spain). The study area was divided into six sampling plots distributed approximately every 500 m of elevation, four of them placed in non-alpine zones (300, 700, 1200, and 1700 m asl), the remaining two in alpine zones (2200 and 2500 m asl, respectively). In alpine zones, winter precipitation takes the form of snow, which usually covers the soil during six months a year on average (November-May); summers are moderately warm. Alpine vegetation is well adapted to those harsh climatic conditions: forests are open and composed of conifers, the timberline appears at roughly 2300 m asl, and shrubs, where trees lack, are dense and small. By contrast, in non-alpine zones, precipitation typically takes the form of rain, winter snowfalls being occasional or absent, and summers are hot. Predominant vegetation in non-alpine zones consists of Mediterranean sclerophyllous forests and associated shrubbery (more details in Appendix A in Zamora-Camacho et al., 2013).

We took lizards to the laboratory, and measured SVL with a ruler (accuracy 1 mm) and body mass with a balance model CDS-100 (accuracy 0.01 g). The first day in the lab (day 1), we placed lizards into individual terrariums (100×20×40 cm), built using 0.5mm-thick methacrylate, with a 150W red-light bulb on one side, 15 cm above the pine-bark substrate. Bulbs were lit during daytime, generating a 20-55ºC temperature gradient, which covers the temperatures usually preferred by this lizard (Díaz, 1997). A window provided natural light-darkness cycles, so that lizards could adjust their circadian rhythms. Being left undisturbed during the entire day, all the lizards spent the same time in the same environment before trials, independently of their origin, thereby avoiding differences that could be ascribed to thermal conditions (Deen and Hutchinson, 2001).

On day 2, lizards were allowed to thermoregulate during the entire day, and we registered their preferred temperatures ($T_{pref}$) five times, once per hour, from 10:00 to 14:00 h (local time). Body temperature was measured with a small catheter connected to a thermometer (Hybok 18, accuracy 0.1ºC) and inserted 8 mm inside the cloaca. Each time we handled a lizard, it was returned to the centre of the terrarium with the body aligned perpendicularly to the thermal gradient. We did so in order to avoid an effect of lizard position on body-temperature preferences (Llewellyn et al., 2013).

On day 3, lizards were randomly assigned to experimental or control groups. At 9:45 h, lizards in the experimental group were inoculated subcutaneously in the hind sole pad with 0.1 mg of LPS (serotype 055:B5, L-2880, Sigma Aldrich), diluted in 0.01 ml of phosphate buffer. Meanwhile, control lizards were inoculated with 0.01 ml of isotonic PBS, which has no physiological effects. LPS is an innocuous antigen that stimulates the immune system, without other effects on the organism, provoking an immune response that peaks four hours after inoculation (Parmentier et al., 1998). LPS is probably the most frequent pyrogen used in studies on fever (Kluger, 1991). To evaluate the response to the antigen, we measured the thickness of the inoculated sole pad using a pressure-sensitive micrometer (Mitutoyo; accuracy 0.01 mm) immediately before and four hours after injecting the substance. The degree of inflammation of the region injected is directly related to the strength of the immune response (Parmentier et al., 1998). We took three sole-pad thickness measurements each time, and calculated the average value, the difference being the immune-response magnitude. Immediately after the inoculations, lizards were individually reallocated in the temperature-gradient terrariums (as described above), and we recorded their body temperatures five times, once an hour, from 10:00 to 14:00 h (local time). Thus, $T_{pref}$ was registered while lizards were developing the immune response. Then, we calculated the average values of the five $T_{pref}$ measurements on day 2 (before inoculation) and day 3 (after inoculation), as well as the difference between the two measurements.

Since immune challenge reduces female lizard reproductive output (Uller et al., 2006), we used only adult males in their reproductive season for this experiment, in order to avoid interfering with ovogenesis and gestation. We identified males because they have proportionally larger heads, orange mouth commissures, and more prominent femoral pores. During the entire process, lizards were provided *ad libitum* with mealworms (*Tenebrio molitor* larvae) and water (in form of a nutritious aqueous gel). No lizard suffered any damage as a consequence of this experiment. Once the experiment ended, lizards were returned to their place of capture.

Since the data fulfilled the criteria of residual normality and homoscedasticity (Quinn and Keough, 2002), we used parametric statistics. We conducted Linear Models of Ordinary Least Squares, performing factorial ANOVAs to test for an effect of treatment, elevation (non-alpine vs. alpine origin of the lizards) and their interaction on body mass and SVL. We used repeated-measures ANOVAs to test for the effect of treatment, elevation and their interaction on immune-response magnitude (sole-pad thickness before and after trials), and $T_{pref}$ before and after the experimental inoculations. We predicted an effect of treatment on the change in $T_{pref}$ beteween day 2 and 3, as well as a significant treatment×elevation interaction. We performed those analyses with the software Statistica 7.1 (StatSoft, 2007).

## IV.4.-Results

Since lizards were randomly assigned to each treatment, there were no significant differences between treatments in morphology or preferred body-temperature ($T_{pref}$) prior to inoculations (Fig. IV.1, Table IV.1). Alpine lizards were larger than non-alpine lizards, but showed similar $T_{pref}$ before the treatment (Fig. IV.2, Table IV.1). A repeated-measures ANOVA showed that treatment was effective in stimulating an immune response, as evidenced by a significant swelling of the sole pad in lizards inoculated with lipopolysaccharide (LPS) of bacterial wall of *Scherichia coli*, while lizards inoculated with phosphate buffer solution (PBS) showed no change in the thickness of their sole pad (interaction treatment×measurement, $F_{1,\ 29} = 11.074$, $P = 0.002$, Fig. IV.3). In turn, elevation ($F_{1,\ 29} = 0.303$, $P = 0.586$) and its interaction with treatment ($F_{1,\ 29} = 0.083$, $P = 0.775$) had no significant effect on sole pad swelling. However, although $T_{pref}$ did not differ between LPS- and PBS-inoculated lizards or alpine and non-alpine lizards, a repeated measurement ANOVA showed that non-alpine LPS-inoculated lizards decreased their $T_{pref}$ significantly more than non-alpine PBS-inoculated lizards (Fig. IV.1, Table IV.2). However, immune-challenged alpine lizards did not show lower $T_{pref}$ than control (see threefold interaction in Table IV.2, Fig. IV.2). The elevational difference in $T_{pref}$ in LPS-inoculated lizards cannot be ascribed to differences in immune response, as the magnitude of immune response to LPS of alpine and non-alpine lizards was similar (Table IV.1).

| **Effect** | ***D.f.*** | ***F*** | ***P*** |
|---|---|---|---|
| **Between subjects component** | | | |
| **Treatment** | 1, 29 | 9.87 | 0.004 |
| **Elevation** | 1, 29 | 9.55 | 0.004 |
| **Treatment×Elevation** | 1, 29 | 0.40 | 0.53 |
| **Within subjects component** | | | |
| **Repeated measurement** | 1, 29 | 16770.40 | < 0.001 |
| **Measurement×Treatment** | 1, 29 | 0.53 | 0.47 |
| **Measurement×Elevation** | 1, 29 | 0.42 | 0.52 |
| **Measurement×Treatment×Elevation** | 1, 29 | 7.73 | 0.009 |

Table IV.2 - Repeated measurements ANOVA testing the effect of treatment (LPS vs. PBS) and elevation (alpine vs. non-alpine) on preferential body temperature ($T_{pref}$) before and after the inoculations (repeated measurements).

| Variable | LPS group (n = 18) | PBS group (n = 15) | Alpine (n = 15) | Non-alpine (n = 18) | Effect of treatment | Effect of elevation | Treatment * Elevation |
|---|---|---|---|---|---|---|---|
| **Body mass (g)** | 7.41 ± 0.64 | 6.88 ± 0.70 | 8.95 ± 0.55 | 5.68 ± 0.50 | $F_{1, 29}$ = 0.51 | **$F_{1, 29}$ = 19.71$^{***}$** | $F_{1, 29}$ = 0.84 |
| **Snout-vent length (mm)** | 66.06 ± 1.79 | 64.07 ± 1.96 | 69.87 ± 1.61 | 61.22 ± 1.47 | $F_{1, 29}$ = 0.78 | **$F_{1, 29}$ = 17.79$^{***}$** | $F_{1, 29}$ = 2.65 |
| **Pre-inoculation $T_{pref}$ (ºC)** | 35.50 ± 0.31 | 34.99 ± 0.34 | 35.39 ± 0.35 | 35.16 ± 0.32 | $F_{1, 29}$ = 1.06 | $F_{1, 29}$ = 0.17 | $F_{1, 29}$ = 1.06 |
| **Post-inoculation $T_{pref}$ (ºC)** | 34.62 ± 0.32 | 35.48 ± 0.26 | 35.49 ± 0.31 | 34.61 ± 0.29 | $F_{1, 29}$ = 4.16$^{§}$ | **$F_{1, 29}$ = 4.27$^{*}$** | **$F_{1, 29}$ = 4.93$^{*}$** |
| **$T_{pref}$ difference (ºC)** | -0.88 ± 0.34 | 0.49 ± 0.37 | 0.10 ± 0.34 | -0.55 ± 0.41 | **$F_{1, 29}$ = 6.88$^{*}$** | $F_{1, 29}$ = 1.34 | $F_{1, 29}$ = 0.50 |
| **Immune response (mm)** | 0.08 ± 0.02 | -0.02 ± 0.02 | 0.02 ± 0.02 | 0.05 ± 0.03 | **$F_{1, 29}$ =11.07$^{**}$** | $F_{1, 29}$ = 0.30 | $F_{1, 29}$ = 0.08 |

Table IV.1 - Average values ± standard errors of body mass, snout-vent length (SVL), preferential body temperature ($T_{pref}$) before and after the inoculations, differences in $T_{pref}$ (after minus before inoculation), and immune response (sole-pad swelling in mm), for both groups (LPS and PBS), as well as for alpine (2200-2500 m asl) and non-alpine (300-1700 m asl) lizards. Sample size is shown in parentheses. Differences between treatments and elevations were tested with a factorial ANOVA. In bold significant results ($^{§}$: marginally non-significant with $P$ = 0.051, *: $P<0.05$; ***: $P<0.001$).

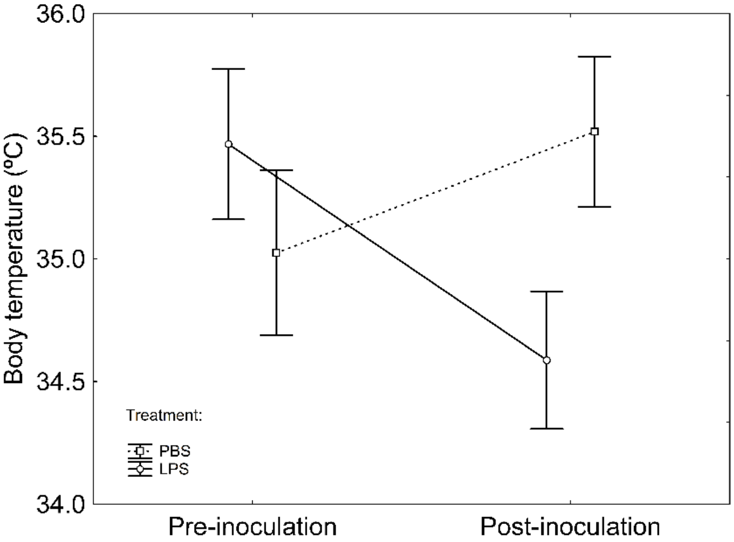

Fig. IV.1 - Lizard $T_{pref}$ before and after inoculations regarding treatment. All lizards showed similar preferred body-temperature ($T_{pref}$) before inoculations. Then, LPS-inoculated lizards decreased their $T_{pref}$, while PBS-inoculated lizards did not significantly vary their $T_{pref}$. Sample sizes are shown in Table IV.1. Vertical bars represent standard errors.

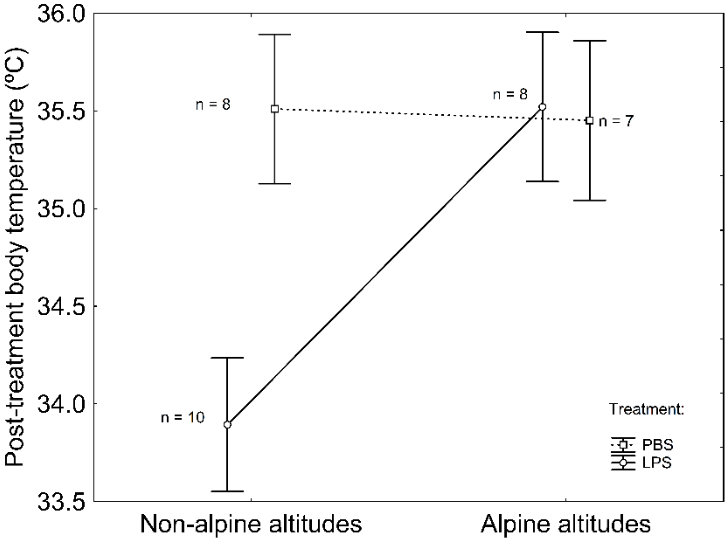

Fig. IV.2 - Lizard $T_{pref}$ regarding elevation and treatment. A trade-off between preferred body temperature ($T_{pref}$) and immune system appeared in non-alpine lizards, .Nevertheless, this trade-off did not affect alpine lizards, which selected similarly high temperatures regardless of the substance inoculated. Vertical bars represent standard errors.

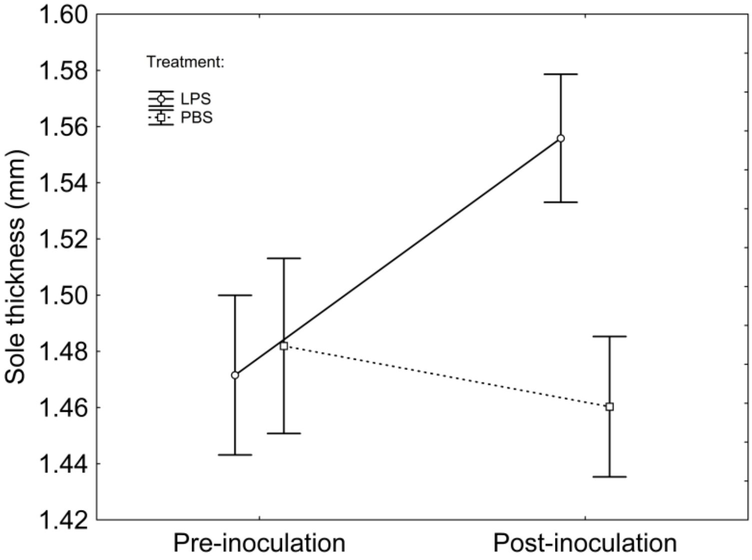

Fig. IV.3 - Sole-pad thickness before and after inoculations. Sole-pad thickness was similar in both LPS and PBS-inoculated lizards. Four hours after the inoculation, sole-pad thickness of PBS-inoculated lizards did not change significantly, while an inflammation occurred in LPS-inoculated lizards, pointing to an actual physiological effect of LPS. Sample sizes are shown in Table IV.1. Vertical bars represent standard errors.

## IV.5.-Discussion

In this study, we found that the thermal response of a lizard to an immune challenge varied along an elevational gradient. Non-alpine immune-challenged lizards showed evidence of a trade-off between thermoregulation and immune response, $T_{pref}$ decreasing when immune challenged. Meanwhile, alpine lizards maintained $T_{pref}$ when immune-challenged, probably because the cost of non-thermoregulation in the environment where they have evolved (reduced time available for reproduction) was so high that they could not afford to suppress thermoregulation.

These findings cannot be ascribed to differences in immune response between alpine and non-alpine lizards, as the magnitude of immune response to LPS was similar in both. Nor can they be attributed to acclimation to different thermal conditions, which may affect the response to pyrogens (Deen and Hutchison, 2001), given that lizards were acclimated to the same conditions in the laboratory. In fact, control lizards showed the same $T_{pref}$ regardless of their elevational origin. In the field, body temperature does not differ with elevation, either (Zamora-Camacho et al., 2013). Therefore, the findings in this study suggest that, as we predicted, lizards use different thermal response to pathogens according to elevation.

Why did immune-challenged non-alpine lizards decrease their $T_{pref}$ rather than showing fever? The immune system is energetically costly (review in Schmid-Hempel, 2011), and high body temperature implies raised metabolism, with the concomitant expenditure of resources (Gillooly et al., 2001). In fact, inoculation of LPS in ectotherms increases metabolic expenditure (Sherman and Stephens, 1998). Therefore, we propose that LPS-inoculated lizards that selected lower body temperature diminished the energetic costs associated with metabolism, diverting the energy saved to immune system. In fact, hypothermia is not an infrequent response to immune challenges (Deen and Hutchison, 2001; do Amaral et al., 2002; Owen-Ashley et al., 2006; Merchant et al., 2008; King and Swanson, 2013; and references therein), although its evolutionary importance has received little attention in comparison with fever. Consequently, in consideration of the costs and benefits involved in a thermal response to pathogens, fever may not always be the best option.

Hypothermia may also occur in immune-challenged non-alpine lizards because it helps them to combat parasites. Although the benefits of fever against pathogens are well established, and improved survival associated with fever in infected lizards has been demonstrated (Kluger et al., 1975), some parasites, such as haemogreagerines, undergo reduced multiplication rates at lower temperatures (Oppliger et al., 1996). Therefore, decreased body temperature could be an adaptive response against some pathogens.

A third possibility would be that immune-challenged lizards had problems to thermoregulate due to sickness behaviour (Llewellyn et al., 2013).

Sickness behaviour is usually elicited when the immune system is activated, and it implies diminished motility (Adelman and Martin, 2009). Therefore, sick lizards could have problems to select the right position to thermoregulate. Nonetheless, in such case -no thermoregulation-, we would expect higher variance in body temperature in LPS-inoculated lizards. We tested the differences in variance in post-inoculation $T_{pref}$ between LPS and PBS lizards from the non-alpine zone, and found no significant differences (Levene test, $F_{1, 16} = 2.61$, $P = 0.13$). Consequently, our findings cannot be ascribed to differences in sickness behaviour.

In any case, lower body temperature in immune-challenged lizards would have a number of detrimental effects on fitness derived either from lower motility, such as reduced mating opportunities, territory loss or reduced food intake, or from physiological constraints, such as longer food-passage time (Van Damme et al., 1991; Chen et al., 2003; Llewellyn et al., 2013). Benefits of hypothermia (which remain poorly known) should be sufficiently high to compensate for these costs.

Then, why did immune-challenged alpine lizards not decrease their $T_{pref}$? We propose that alpine lizards, which inhabit an environment with a small window of time for reproduction, should prioritize heat acquisition, because costs of non-thermoregulation are higher for alpine than for non-alpine lizards (Blouin-Demers and Nadeau, 2005). Additionally, alpine lizards in our study area are adjusted to their cold environment with a number of adaptations that improve their thermoregulatory efficiency. They are larger, which improves heat retention, and thus maintain more easily high body temperatures (Atkinson and Sibly, 1997). Moreover, they are darker than non-alpine lizards (Reguera et al., 2014), which may improve heat gain (Clusella-Trullas et al., 2008). Thus, everything being equal, the costs of thermoregulation would be lower in alpine lizards (adapted to cold environments), a feature that might have contributed to the findings in this study.

## IV.6.-Conclusions

In conclusion, our results suggest that lizards adjust their thermal response to an immune challenge according to the costs/benefits balance, leading to an elevational variation in the thermal response to the activation of the immune system. Non-alpine lizards showed decreased $T_{pref}$ in response to immune challenge, presumably in order to save energy. However, alpine lizards prioritized heat gain, and maintained their $T_{pref}$. This matches with increased costs of non-thermoregulation in alpine lizards, due to the reduced window for reproduction in the environment where they have evolved.

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## V - Chapter 5

# Effects of limb length, body mass, gender, gravidity, and elevation on escape speed in the lizard *Psammodromus algirus*.

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This chapter reproduces the published article:

Zamora-Camacho, F.J., Reguera, S., Rubiño-Hispán, M.V., Moreno-Rueda, G. 2014. Effects of limb length, body mass, gender, gravidity, and elevation on escape speed in the lizard *Psammodromus algirus*. Evolutionary Biology, 41:509-517.

**V.1.-Abstract -** Most animals rely on their escape speed to flee from predators. Here, we test several hypotheses on the evolution of escape speed in the lizard *Psammodromus algirus*. We test that: (1) Longer limbs should improve speed sprint. (2) Heavier lizards should be impaired regarding their sprint speed ability, suggesting a trade-off between fat storage and escape capability. (3) Males should achieve faster speeds due to their higher exposure to predators. (4) Gravid females, with increased body mass, should perform lower speed than non-gravid females. And (5) there are inter-population differences in sprint speed across an elevational gradient. We measured lizards sprint speed in a lineal raceway in the laboratory, filming races in standardized conditions and then calculating their maximal speed. We found that hind limb length greatly determined maximal sprint speed, lizards with longer limbs being faster. In parallel, higher body masses reduced maximal speed, which points to a trade-off between fat storage and escaping capability. Sexual differences also arose, as males were faster than females, as a consequence of males having longer limbs. Regarding females, gravidity did not impair maximal sprint speed, suggesting adaptations which compensate for the increased body mass. Finally, we found no elevational trend in both limbs length and sprint speed. In any case, this study suggests that selection on escape capacity may cast morphological evolution, and affect other life-history traits, such as fat storage and reproduction.

## V.2.-Introduction

Predation is one of the main selective forces in nature (Abrams, 2000), strongly influencing the evolution of physiology, morphology, behaviour, and life-history traits in animals (Caro, 2005). The first way to minimize the risk of predation may be to remain within a shelter. Although, eventually, individuals should leave their shelter in order to search for mates, food, or other resources. In this situation, when prey is detected by a predator, their first option is usually to escape (Lima and Dill, 1990). Morphology strongly influences the success of escape behaviour. For example, birds with longer wings show improvement in their escape capability, probably because longer wings allow to fly faster (Moreno-Rueda, 2003). Consequently, predators shape the evolution of wing morphology in birds (Møller and Nielsen, 1997). Similarly, limb morphology has an effect on lizard escape behaviour (Schulte et al., 2004), and, as a consequence, limb length is also under selection by predation in reptiles (Losos et al., 2004).

On the other hand, heavier birds show reduced escape capability (Witter and Cuthill, 1993). Animals frequently need to accumulate energetic reserves in order to survive periods of inactivity (mainly hibernation or aestivation). Nevertheless, the heavier individuals may have lower locomotor capacity. Likewise, for gravid females, the increase in body mass involves a reduction in escape capability (Shine, 1980; Lee et al., 1996; Shaffer and Formanowicz, 1996). In fact, gravid females are more likely to be predated than males (review in Magnhagen, 1991). In such cases, escape ability cannot be maximized, and there should be a trade-off with other life-history traits, such as resistance against starvation (MacLeod et al., 2005) or reproductive output (Miles et al., 2000). In fact, lizard body weight diminishes under predation risk (Pérez-Tris et al., 2004), which might improve their ability to flee from predators.

Consequently, escape capability is costly, and hence natural selection should favour lower escape capacity in populations with lower predation risk. Supporting this idea, the lizard *Podarcis sicula* has lower sprint speeds when inhabiting predator-free islands (Vervust et al., 2007). Furthermore, reptiles run faster in open habitats, where they are more exposed to predators (Melville and Swain, 2000; Goodman, 2009). In the same way, if one gender is more exposed to predators, it should show improved escape capacity. Males are usually more exposed to predators (Promislow et al., 1992), as they spend more time out of shelter, searching for mates and defending their territories (Magnhagen, 1991). Accordingly, males show higher sprint speed than females in some territorial lizard species (reviewed in Lailvaux, 2007).

In the present study, we examine intra- and inter-population variability in escape capacity in the lizard *Psammodromus algirus*, in a 2200-meter elevational gradient in SE Spain. *Psammodromus algirus* is a medium-sized (53-95 mm snout-vent length (SVL) in our study area) Lacertid lizard that inhabits shrubby areas (Díaz and Carrascal, 1991) in the Western Mediterranean, from the sea level to more than 2600 meters above the sea level (m asl) (Salvador, 2011). In our study system, some lowland lizards can be active in warm winter days (mainly juveniles), while highland lizards hibernate during the snowy season, from November to March approximately (Zamora-Camacho et al., 2013). Average environmental temperature when lizards were active diminished 9ºC in elevation, while average lizard field temperature diminished only 1ºC from the lowest to the highest elevation studied, suggesting that lizards are well adapted to local environmental conditions along the altitudinal gradient (Zamora-Camacho et al., 2013).

This lizard is found mainly foraging or basking on the ground around bushes, where it spends 20.7% of its time walking about (Verwaijen and Van Damme, 2008), and seeks shelter under shrubs and only occasionally within rock crevices (Salvador, 2011; personal observations). Male home ranges are 312 $m^2$ on average (46 to 864 $m^2$ range), females averaging 74 $m^2$ (3 to 276 $m^2$ range) (Salvador and Veiga, 2001). In females, carrying eggs involves an additional burden, diminishing gravid females' escape speed (Iraeta et al., 2010).

When this lizard detects a potential predator, individuals escape by means of short and fast races directly to the nearest bush, where they hide (Martín and López, 2000). Maximal sprint speed, therefore, may be vital, as slow lizards are more likely to be preyed on (Husak, 2006). In our study, we estimated sprint speed of *P. algirus* in order to examine:

(1) The relationship between limb length and maximal sprint speed. We predict that, all else being equal, *P. algirus* with longer limbs should be faster, which has been frequently found in lizards (Bauwens et al., 1995).

(2) The effect of body mass on sprint speed. As shown for birds, we predict that heavier lizards should be slower, all else being equal. This would imply a trade-off between fat storage and capacity to escape from predators.

Nevertheless, the effect of body mass on lizard locomotor performance has not received much attention (Pérez-Tris et al., 2004).

(3) Sexual differences in sprint speed. As discussed above, male lizards are often more exposed to predators (Martín and López, 1999). Therefore, we predict that, all else being equal, males should show higher sprint speed than females.

(4) Sprint speed in gravid and non-gravid females. We predict that, all else being equal, gravid females should be slower than non-gravid females, as a consequence of increased body mass during gravidity. Reduced locomotion of gravid females is well-reported in reptiles (Lin et al., [2008] and references therein), including our target species (Iraeta et al., 2010).

(5) Population variation in sprint speed. We compared sprint speed among three populations across an elevational gradient in order to test whether there is geographic variation in sprint speed. Given that factors such as predation pressure or habitat structure may affect the risk of predation, and these factors probably vary in an altitudinal gradient (e.g. Schemske et al., 2009), we predict that there should be interpopulation variation in sprint speed.

## V.3.-Materials and Methods

We captured individuals of *P. algirus* from Sierra Nevada (SE Spain), during the activity seasons (April-September) in 2011 and 2012. Six sampling plots were placed at 300, 700, 1200, 1700, 2200 and 2500 m asl, which almost spanned the full range of elevation of this species. For a better detection of elevational trends, we grouped those sampling plots in three elevation tracks: elevation track 1 (including 300 and 700 m asl plots), elevation track 2 (including 1200 and 1700 m asl plots) and elevation track 3 (including 2200 and 2500 m asl plots). This grouping was justified by similarity between tracks in different population traits, such as activity patterns, morphology, and even genetic proximity (Zamora-Camacho et al., 2013; unpublished data). Since habitat structure influences morphology and sprint speed (Melville and Swain, 2000; Herrel et al., 2002; Goodman, 2009), even in our study species (Martín and López, 1995), which usually walks around the bushes they use as shelters (Salvador, 2011), we measured vegetation structure in each sampling station. We randomly set a 50-meter-long string in five times, and recorded the absence or presence of vegetation every meter along the string. We estimated the height of each plant found at every one-meter mark of the string as < 25 cm, 25-50 cm, and > 50 cm. Vegetation cover and vegetation height did not statistically differ with elevation (vegetation cover: $\chi^2_5 = 0.09$, $P = 1.00$; frequency of plants of 25-50 cm: $\chi^2_5 = 0.16$, $P = 1.00$). Moreover, vegetation cover and height showed no clear pattern with elevation, and they were not related to sprint speed ($r_s = 0.58$ for males, $r_s = 0.12$ for females, $P > 0.20$ in both cases).

Captured individuals were taken to laboratory, where we recorded gender, female reproductive status (gravidity), occiput-vent length (OVL), fore and hind limbs length (with a ruler, accuracy 0.5 mm), and body mass (with a balance model WTB200, accuracy 0.01 g). We used OVL instead of SVL in order to control for the distance between fore and hind limbs, since head length differed almost significantly between genders ($F_{1,\ 80} = 3.92$; $P = 0.051$). We distinguished genders because males have proportionally larger heads, more abundant and conspicuous femoral pores, and orange spots in the corners of the mouth. Females were classified as gravid if a manual palpation revealed the presence of developing eggs inside the trunk, and as non-gravid otherwise. We did not use tailless lizards in this study, because tail loss diminishes speed in this species (Martín and Avery, 1998). During their captivity, as brief as possible, lizards were kept in individual plastic terrariums (20 × 13 × 9 cm), and provided with food (mealworms, *Tenebrio molitor* larvae) and water (nutritious aqueous gel) *ad libitum*. Lizards were marked by toe clipping (excluding the longest toes) as part of a longstanding ongoing study. However, toe clipping has been proved not to alter Lacertids' locomotor capacities (Huey et al., 1990; Dodd, 1993; Husak, 2006).

Two days after capture, sprint speed was measured on a wooden linear raceway (3.2 × 0.2 × 0.4 m), with a cork base to provide an adequate traction (Bauwens et al., 1995). A dark background was placed at the end of the raceway to make lizards take it for a shelter and stimulate them to run forward. We released lizards in the other end of the raceway, and chased them to encourage the race. The raceway was divided into five stretches 50 cm long with a contrasting-coloured strip, because this is the approximate distance lizards run in the wild to their shelters (Martín and López, 2000). Each lizard ran only one race, and only one lizard ran at a time. All lizards ran at 32ºC of body temperature, in order to avoid confounding effects of body temperature on escape speed (see Pérez-Tris et al., 2004; Iraeta et al., 2010). We selected the same temperature for all lizards of every population given that in our study zone lizards select the same temperature independently of their elevation (Zamora-Camacho et al., 2013; see also Díaz et al., 2006). It is well established that the temperature lizards select corresponds with the temperature at which sprint speed is maximal (Hertz et al., 1983; Van Damme et al., 1989; Bonino et al., 2011), and temperature at which lizards achieve maximal sprint speed varies little or nothing among lizard populations (Garland and Adolph, 1991). The temperature selected for the study (32ºC) is inside of the optimal temperature of this species (Díaz and Cabezas-Días, 2004). We ascertained body temperature by putting a thermocouple (1 mm diameter) connected to a thermometer (Hibok 18, accuracy 0.1ºC) 8 mm into every lizard's cloaca.

Races were recorded with a video camera Canon EOS 550D, at 25 frames per second. Then, films were analyzed using the software Movavi v. 11, which allows to measure time intervals in milliseconds (Chen et al., 2003). In each race, we measured the time interval for every stretch, counting every stretch strip as reached when the snout of the lizard arrived to it (Martín and López, 2001). As long as we needed to know maximal sprint speed, we performed our analyses with the speed value of the fastest stretch for every lizard. Furthermore, lizards frequently stopped while running, so the average speed values of all the stretches would not be an accurate indicator of sprint speed. Nonetheless, we used both maximal and average speed to compare gravid and non-gravid females, since lower average speeds in gravid females could indicate that they are more prone to fatigue than non-gravid females due to the egg burden. After the races, gravid females were kept in captivity until oviposition, so we could estimate the time to oviposition remaining in the moment of the race was carried out. The longer that time, the less is the egg load. Moreover, this allowed us to know clutch sizes and estimate egg burdens, as the difference between gravid females weigh in the moment of the race and postpartum females weigh.

All data were analysed with parametric tests, checking that they accomplished the criteria of residual normality and homoscedasticity (Quinn and Keough, 2002). Sexual, elevational, and gravidity-dependent differences for each variable were tested with one-way ANOVA. The relationship between sprint speed and morphological variables were examined with regression techniques. Nonetheless, note that for fore and hind limbs, we controlled either for OVL or body mass in all the analyses, but not for both altogether due to the collinearity caused by a high correlation between them ($r = 0.868$; $P < 0.001$). Once we performed exploratory ANOVAs and regressions, we performed Ordinary Least Squares (OLS) Linear Models (LM) in which relevant independent variables were included in order to control for indirect relationships among variables (Quinn and Keough, 2002). Thereby, linear models informed us about direct relationships between sprint speed and independent variables, unaffected by the remaining independent variables included in the models, and revealed effects of variables which remained masked by the interrelations with other variables.

## V.4.-Results

### *V.4.1.-Morphology, gender and sprint speed*

Sprint speed was higher in males than in females (Table V.1). Sprint speed covaried positively (almost significantly) with fore limbs length (Table V.2) and (significantly) with hind limbs length (Table V.2), but not with OVL (Table V.2) or body mass (Table V.2). These results imply that lizards with longer hind limbs sprinted faster. Given that males had significantly larger fore and hind limbs than females (Table V.1), we analysed sexual differences in sprint speed controlling for morphology. We performed two separate models for fore and for hind limbs due to collinearity derived from high correlation between them ($r = 0.689$; $P < 0.001$). In the two models, we controlled for body mass. Sprint speed showed a positive correlation with fore limb length (Table V.3a), negative with body mass (Table V.3a), and sex differences became non-significant (Table V.3a). Regarding hind limbs, sprint speed showed again a positive relation with hind limb length (Table V.3b), negative with body mass (Table V.3b), sex differences becoming non-significant (Table V.3b). Therefore, males were faster than females simply because they have longer limbs.

| **Variable** | **Males (n = 45)** | **Females (n = 37)** | $F_{1,80}{}^{\dagger}$ |
|---|---|---|---|
| **Sprint speed (cm/s)** | 158.17 ± 8.45 | 126.28 ± 9.32 | 6.425* |
| **Occiput-vent length (mm)** | 50.05 ± 1.08 | 53.60 ± 1.19 | 4.903* |
| **Body mass (g)** | 7.99 ± 0.37 | 7.25 ± 0.41 | $1.861^{ns}$ |
| **Fore limb length (mm)** | 22.91 ± 0.19 | 21.54 ± 0.21 | 39.381*** |
| **Hind limb length (mm)** | 37.20 ± 0.37 | 33.65 ± 0.41 | 52.253*** |

Table V.1 - Average values ± SE for males and females of measured variables. The signification of the *F*-statistic between sexes appears as a superscript on each value ($^{ns}$ = non significant; * = $P < 0.05$; *** = $P < 0.001$).

$^{\dagger}$Since fore and hind limbs were analysed controlling for OVL (introduced in the model as covariate), the degrees of freedom of both were of 1 and 79.

| Variable | $\beta$-value | $F_{1, 80}$[†] |
|---|---|---|
| **Fore limb length** | 0.239 | $3.706^{\S}$ |
| **Hind limb length** | 0.296 | 7.189** |
| **Occiput-vent length** | -0.010 | $0.008^{ns}$ |
| **Body mass** | -0.066 | $0.349^{ns}$ |

Table V.2 - Relationship between escape speed and several morphological variables. The signification of the *F*-statistic appears as a superscript on each value ($^{ns}$ = non significant; $^{\S}$ = marginally non-significant; * = $P < 0.05$; ** = $P < 0.01$).

[†] Since fore and hind limbs were analyzed controlling for OVL (introduced in the model as covariate), the degrees of freedom of both were of 1 and 79.

| Variable | $\beta$-value | $F_{1, 78}$ |
|---|---|---|
| **Fore limb length** | 0.320 | 4.199* |
| **Body mass** | -0.313 | 4.609* |
| **Sex** | | $2.883^{ns}$ |

Table V.3a

| Variable | $\beta$-value | $F_{1, 78}$ |
|---|---|---|
| **Hind limb length** | 0.340 | 5.511* |
| **Body mass** | -0.257 | 4.353* |
| **Sex** | | $1.010^{ns}$ |

Table V.3b

Table V.3 - Models testing the relationships of fore limb length (Table V.3a), hind limb length (Table V.3b), body mass, and sex with escape speed. The signification of the *F*-statistic appears as a superscript on each value ($^{ns}$ = non significant; * = $P < 0.05$).

### *V.4.2.-Elevational differences in sprint speed*

Lizard sprint speed did not differ significantly among elevation tracks (Table V.4; Fig. V.1a). Lizards showed increased body mass (Table V.4; Fig. V.1b) and OVL (Table V.4; Fig. V.1c) in the highest elevation track, while fore (Table V.4; Fig. V.1d) and hind limb length (Table V.4; Fig. V.1e) did not differ in elevation when controlling for allometry by using OVL as covariate. In all the previous models, sex was introduced as factor, in order to control for sexual differences in the measured variables (for sexual differences in the variables, see the previous paragraph). The interaction between sex and elevation was significant for sprint speed ($F_{2,\ 75}$ = 4.169; $P$ = 0.019). For females, sprint speed was similar across elevation tracks ($F_{2,\ 34}$ = 1.618; $P$ = 0.213), while males at mid-elevation lands tended to show lower sprint speed than at low- or high-elevation ($F_{2,\ 42}$ = 2.827; $P$ = 0.070; Fig. V.1a). Finally, a full model including elevation track, sex, body mass, and hind limbs length as predictors of sprint speed showed no significant elevational variation ($F_{2,\ 73}$ = 0.416; $P$ = 0.661). In this model, the interaction elevation*sex was again significant ($F_{2,\ 73}$ = 3.878; $P$ = 0.026).

| Variable | Elevation<br>$F_{2,76}^{\dagger}$ | Gender<br>$F_{1,76}^{\dagger}$ | Elevation*Gender<br>$F_{2,76}^{\dagger}$ |
|---|---|---|---|
| **Escape speed** | $0.457^{ns}$ | 4.321* | 3.602* |
| **Body mass** | 24.526*** | 5.121* | $0.756^{ns}$ |
| **Occiput-vent length** | 18.429*** | $3.631^{\S}$ | $1.069^{ns}$ |
| **Fore limb length** | $0.689^{ns}$ | 32.481*** | $1.481^{ns}$ |
| **Hind limb length** | $0.835^{ns}$ | 44.876*** | $1.735^{ns}$ |

Table V.4 - Models testing the relationships of elevation, gender, and their interaction with escape speed and several morphological variables. The signification of the *F*-statistic appears as a superscript on each value ($^{ns}$ = non significant; $^{\S}$ = marginally non-significant; * = $P < 0.05$: *** = $P < 0.001$).

$^{\dagger}$Since fore and hind limbs were analyzed controlling for OVL (introduced in the model as covariate), the degrees of freedom of both were of 2 and 75.

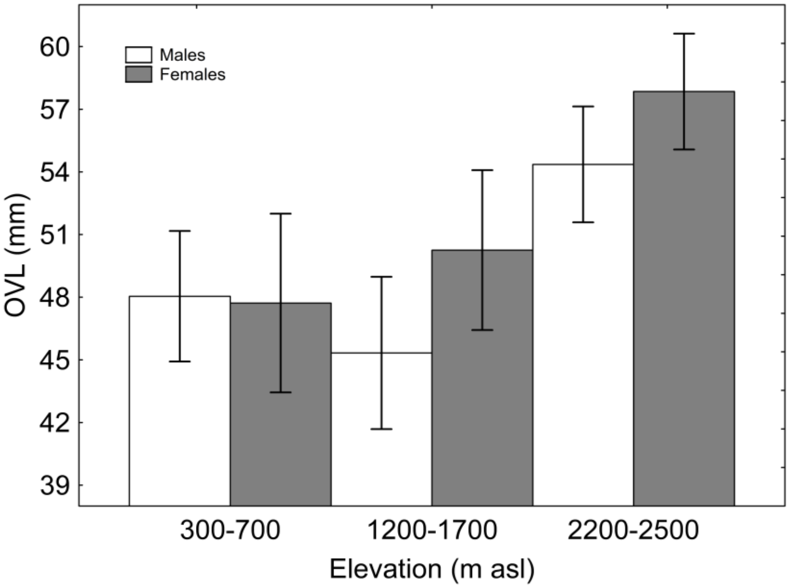

Fig V.1a

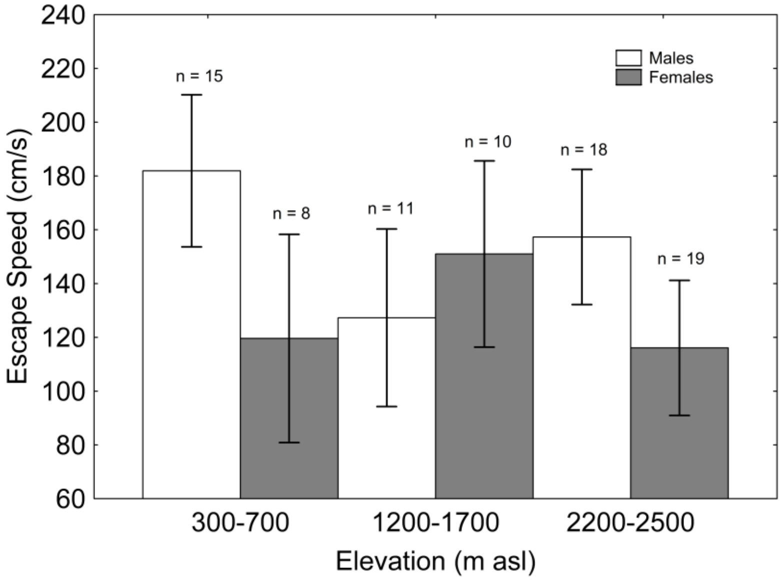

Fig. V.1b

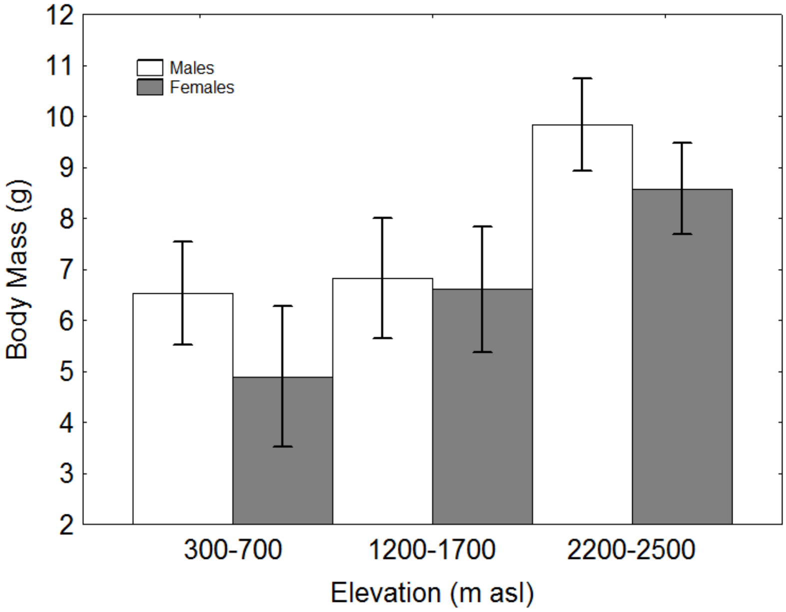

Fig. V.1c

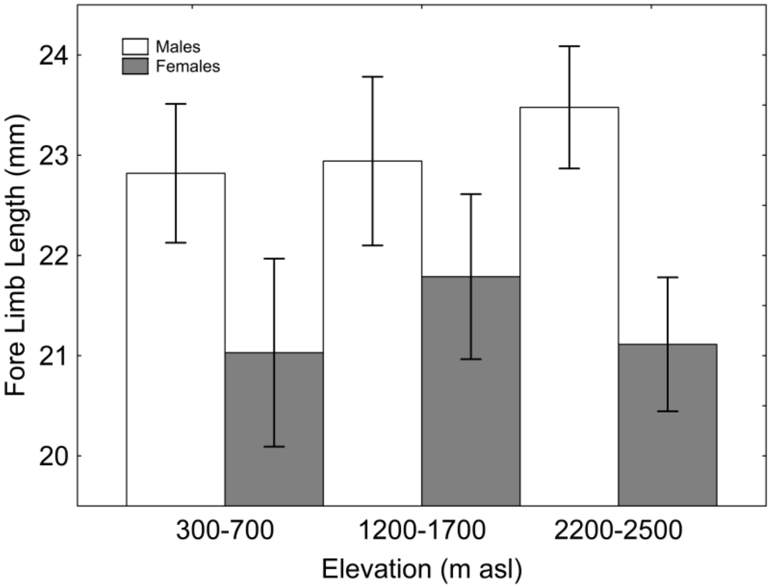

Fig. V.1d

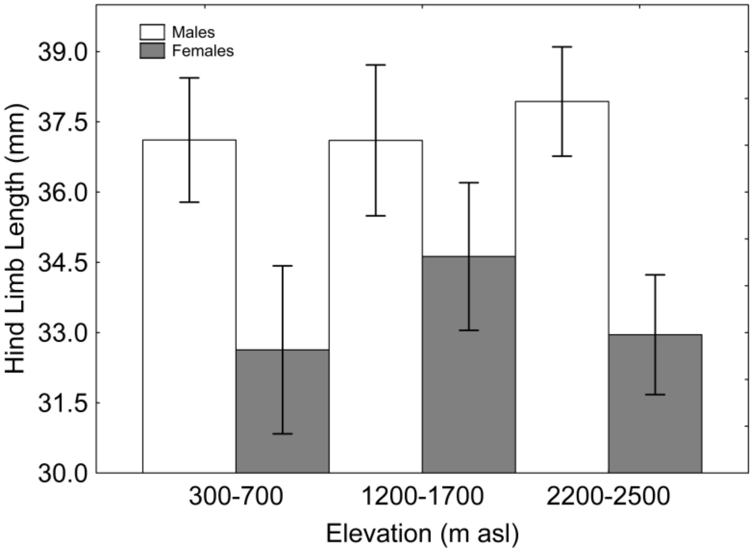

Fig. V.1e

Fig. V.1 - Elevation had no effect on escape speed (Fig. V.1a), which matches the lack of altitudinal trend in both fore (Fig. V.1d) and hind limbs (Fig. V.1e). Nevertheless, both OVL (Fig. V.1b) and body mass (Fig. V.1c) increased in the highest elevations, both in males (white bars) and females (grey bars). Bars indicate the 95% Confidence Intervals. Sample size is indicated in Fig. V.1a.

### *V.4.3.-Sprint speed in gravid females*

We found no significant differences in maximal or average sprint speed among gravid and non-gravid females (Table V.5). Actually, gravid females showed a tendency to run faster than non-gravid females (Table V.5), even closer to signification when controlling for body mass ($F_{1,\ 48}$ = 3.579; $P$ = 0.065). Body mass, which reduces sprint speed (see above) tended to be higher in gravid than in non-gravid females, but differences did not reach significance (Table V.5). Nevertheless, when controlling for elevation, these differences turned significant, gravid females being heavier than non-gravid ($F_{1,\ 45}$ = 7.102; $P$ = 0.011). Gravid lizards laid 3 to 12 eggs 0 to 27 days after the races; no correlation appeared between speed and days until oviposition ($r$ = 0.223; $P$ = 0.36), clutch size ($r$ = 0.125; $P$ = 0.62), or egg burden ($r$ = 0.20; $P$ = 0.46).

| **Variable** | **Non Gravid (n = 32)** | **Gravid (n = 19)** | $F_{1,\ 49}$[†] |
|---|---|---|---|
| **Maximal speed (cm/s)** | 123.95 ± 9.95 | 151.48 ± 12.91 | $2.852^{ns}$ |
| **Average speed (cm/s)** | 86.35 ± 7.48 | 86.78 ± 9.71 | $0.001^{ns}$ |
| **Occiput-vent length (mm)** | 54.27 ± 1.27 | 55.14 ± 1.64 | $0.117^{ns}$ |
| **Body mass (g)** | 7.26 ± 0.47 | 8.76 ± 0.61 | $3.840^{\S}$ |
| **Fore limb length (mm)** | 21.56 ± 0.19 | 21.58 ± 0.25 | $0.081^{ns}$ |
| **Hind limb length (mm)** | 33.38 ± 0.30 | 33.95 ± 0.39 | $1.063^{ns}$ |

Table V.5 - Average values ± SE for non-gravid and gravid females of measured variables. The signification of the *F*-statistic between sexes appears as a superscript on each value ($^{ns}$ = non significant; $^{\S}$ = marginally non-significant).

[†] Since fore and hind limbs were analyzed controlling for OVL, the degrees of freedom of both were of 1 and 48.

## V.5.-Discussion

Locomotor performance is a relevant ecological and evolutionary trait in reptiles, which does not only affect escaping from predators, but also foraging, dominance, and mating (review in Irschick and Garland, 2001). In fact, locomotor musculature consumes a large proportion of lizards' resource intake (Christian et al., 1997). Thus, it is not remarkable that locomotor capacity is strongly under selection in lizards (Miles et al., 2000; Le Galliard et al., 2004; Losos et al., 2004; Husak et al., 2006; Calsbeek and Irschick, 2007; Irschick and Meyers, 2007; Calsbeek and Smith, 2008; Calsbeek and Cox, 2010). Herein, we examined different aspects related to maximal escape speed in the lizard *P. algirus*: limbs length, body mass, gender, gravidity stage and population along an elevational gradient. Maximal escape speed measured in laboratory is an upper limit of escape speed in the field, but it is not necessarily correlated with escape speed in the field (Irschick and Garland, 2001). However, a number of studies have shown that locomotor performance measured in laboratory is a good indicator of field survival in lizards (Miles et al., 2000; Warner and Andrews, 2002; Le Galliard et al., 2004). Therefore, considering the escape behaviour of *P. algirus*, we are confident that laboratory escape speed is a good indicator of escape capacity.

We found a positive relation between limbs length and escape speed. This is an unsurprising finding, as it is well documented that hind limb length is the main responsible for motor impulse during race in lizards (Bauwens et al., 1995; Bonine and Garland, 1999). Interestingly, previous studies with other populations of our target species found contradictory results, one study finding a positive relation between hind limbs length and maximal escape speed (Bauwens et al., 1995), while other two studies found no relation (Martín and López, 2001; Iraeta et al., 2011).

As expected, body mass restricted lizards' speed. The negative effect of body mass on escape capacity has been well studied in birds (review in Witter and Cuthill, 1993). This relationship leads to trade-offs between fat storage and escape-from-predators capacity in birds (Gosler et al., 1995). Our findings suggest that reptiles should solve similar trade-offs. However, despite the abundant literature supporting the idea that gravid females or recently fed lizards show reduced speed (Shine, 2003), previous studies have failed to find a relationship between mass or body condition indexes (indicators of fat storage) and escape speed (Pérez-Tris et al., 2004; Amo et al., 2006; Vervust et al., 2008). Lizards need to accumulate fat in order to survive hibernation (Díaz et al., 1994). Therefore, if fat storage reduces escape capacity from predators, reptiles -as birds- should trade fat storage with escape capacity.

Males ran faster than females, as a consequence of having longer limbs, which is consistent with a higher exposure to predators due to a more active life and a brighter colouration (Díaz, 1993). Thereby, natural selection would favour longer limbs in males in order to achieve higher speed. However, hind limbs are involved in sexual signalling by means of secretions from femoral pores present along them (Martín et al., 2007), and longer hind limbs can contain more pores which may also improve mating success (Iraeta et al., 2011). On the other hand, sexual selection may favour faster males, as different studies show that faster males are dominant, maintain larger territories, and sire more offspring (Husak et al., 2006). Consequently, the selective force that shapes hind limb length in *P. algirus* males remains unknown, since males could be faster as a consequence of either natural or sexual selection, or both.

An interesting result in our study is that *P. algirus* females did not show decreased escape speed as a cost of reproduction. Gravid and non-gravid females ran at similar maximal and average speeds. Gravidity obviously increases body mass, and moreover depresses the physiological condition of females; therefore, it has been shown to reduce escape capacity in a number of lizards (Shine, 1980, 2003; Sinervo et al., 1991; Miles et al., 2000; Lin et al., 2008). In fact, Iraeta et al. (2010) found in other population of *P. algirus* that females are slower when gravid than after oviposition. This fact implies geographic differences in the cost of reproduction for *P. algirus*. Interpopulation differences in the cost of reproduction have already been found in other species (Qualls and Shine, 1997). Our findings suggest that gravid females in our population develop adaptations in order to compensate for the disadvantage of increased body mass, resulting in unimpaired escape capacity. Gravidity does not only impair escape speed by physical reasons, physiological deterioration contributing to this impairing (Olsson et al., 2000). The fact that female *P. algirus* feed while gravid probably contributed to avoid physical deterioration, keeping an optimal escape capacity. Although the underlying mechanism remains unexplained, faster speed in gravid females has important advantages for offspring development. Gravid females forced to be sheltered in response to predation show reduced body condition (Amo et al., 2007), so an increment in escape speed would favour them, allowing more basking time and reducing the costs of sheltering.

Finally, we found that lizards’ escape speed did not vary in elevation. Iraeta et al. (2011) neither found elevational differences in maximal escape speed in other *P. algirus* population. Other studies, however, have found inter-population differences in lizards' escape speed: *Podarcis tiliguerta* runs slower at high elevations than at sea level (Van Damme et al., 1989). Nonetheless, limb length and escape speed did not vary with elevation in our system, which suggests that limb length is under similar selection pressure along the elevational gradient. Interestingly, males tended to run faster than females in low and high lands, but they both ran at similar maximum speed in mid-elevation lands. This pattern might suggest that selection on escape speed of males and females varies non-linearly with elevation. The causes of such as pattern remain unknown. However, the mid-elevation track corresponds with the zone with maximal population density in our study system (see Fig. 4 in Zamora-Camacho et al., 2013), implying higher competence in territorial males. Given that faster males are more successful maintaining their territories (Husak et al., 2006), probably selection for speed was the highest in mid-elevation populations, the most dense.

## V.6.-Conclusions

In sum, in *P. algirus* maximal escape speed was determined mainly by limbs length, lizards with longer limbs being faster. In return, heavier lizards were slower. This involves a trade-off between fat storage and escape-from-predators capacity in lizards. We found sexual differences, as males were faster than females, due to their longer limbs. Selection favouring longer limbs in males might be a consequence of their greater exposure to predators. Regarding females, gravidity did not impair maximal escape speed, suggesting that gravid females develop adaptations which offset the increased body mass. Finally, we found no inter-population differences in both limbs length and escape speed along an elevational gradient.

## V.7.-Acknowledgements

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# VI - Chapter 6

## Eliciting an immune response reduces sprint speed in a lizard.

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This chapter reproduces the published article:

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**VI.1.-Abstract** - Predators and pathogens can diminish organisms' fitness, and consequently natural selection favors anti-predatory and anti-parasitic strategies. Nevertheless, since both kinds of strategies consume resources, animals cannot maximize investment in both strategies simultaneously, which should lead to trade-offs between the two. Accordingly, we hypothesize that there is a trade-off between sprint capacity and immune response, which are among the most important anti-predatory and anti-parasitic strategies, respectively, of many animals. Consequently, we predict that animals eliciting an immune response should reduce their sprint capacity. We experimentally tested this prediction in the lizard *Psammodromus algirus*. A group of lizards was inoculated with the antigen LPS (lipopolysaccharide of the cell wall of *Escherichia coli*), which activates the immune system, while the other group (control) was inoculated with phosphate buffer, which has no physiological effect. Before and after the inoculations, we filmed sprint capacity of lizards, estimating the maximal sprint speed. Males were faster than females before the activation of their immune system. Nonetheless, males' sprint speed diminished after the inoculation of LPS, while females' did not. Therefore, our results support the hypothesis of a trade-off between sprint speed and immune response in males. Consequently, male lizards combating a pathogen may suffer either higher risk of predation, or reduced fitness if they avoid this risk by spending more time in shelter.

## VI.2.-Introduction

Predators and pathogens can exert a major impact on the fitness of organisms (Caro, 2005; Schmid-Hempel, 2011). Predators may affect fitness directly by means of predation, reducing the future fitness of the prey to zero. Predators also affect prey fitness indirectly, by altering prey behavior and physiology in order to adopt anti-predatory strategies, which may be costly in terms of reduced growth, foraging success, or breeding success (review in Lima, 1998). Pathogens and parasites take resources from the hosts, reducing their fitness, and ultimately their survival. Consequently, hosts show a set of anti-parasite strategies, of which the immune system is the most sophisticated (Wakelin and Apanius, 1997). In sum, there is a strong selection favoring the evolution of anti-predatory and anti-parasitic strategies.

Quick fleeing when a predator is detected is one of the main anti-predatory strategies in lizards (e.g. Martín and López, 2000), and consequently, survival increases the higher the sprint speed (Irschick and Meyers, 2007). In fact, locomotor capacity is under strong natural selection in lizards (Calsbeek and Irschick, 2007). As an example of the importance of predators as a selective pressure, lizards show higher sprint speed in populations where they are more exposed to predators (Vervust et al., 2007). High-speed sprint is energetically costly (Christian et al., 1997). The fact that lizards reduce their sprint speed when they evolve in an environment without predators (Vervust et al., 2007) suggests that morphological and physiological adaptations necessary to maintain a high sprint speed are costly to develop or maintain. Moreover, the muscular exertion during running increases the production of pro-oxidant substances, with the consequent risk of increased oxidative stress (Fisher-Wellman and Bloomer, 2009). Oxidative stress is the excess production of pro-oxidant substances relative to antioxidant defenses, with negative effects on organism health (review in Halliwell and Gutteridge, 2007).

Regarding pathogens and parasites, the immune system is one of the most important defenses. The immune system includes a number of white corpuscles that recognize and attack alien elements (antigens) that have invaded the organism (Wakelin and Apanius, 1997). Immune response implies a number of mechanisms to kill pathogens, such as antibodies, oxidant agents ($NO_2$), lysozymes, and phagocytosis. Survival increases with immunocompetence (Møller and Saino, 2004), but heavy immune responses may lower fitness by generating auto-immune diseases or increased oxidative stress (Sorci and Faivre, 2009). Moreover, the activity of the immune system is costly, in terms of using energy (e.g., to generate fever) as well as specific resources such as proteins and amino acids (review in Schmid-Hempel, 2011).

Therefore, both sprint speed and mounting an immune response appear to be costly and consume a huge quantity of resources. Consequently, it is improbable that sprint speed and immunocompetence could be maximized simultaneously. When organisms cannot simultaneously maximize different life-history functions, optimal trade-offs emerge among life-history traits in favor of fitness (Roff, 2002). The genetic architecture of different life-history traits can make them co-vary positively despite their costs (Lailvaux et al., 2010). Nonetheless, even with positive genetic covariation, phenotypic trade-offs may emerge when life-history traits compete for the same resources (Zera and Harshman, 2001), experimental manipulation being necessary to ascertain the existence of such phenotypic trade-offs (Garland, 2014).

In the case we are dealing here, several facts make us expect a trade-off between immune response and sprint speed. First, both traits need proteins (Christian et al., 1997; Lochmiller and Deerenberg, 2000), and thus the re-allocation of proteins to the immune system when an antigen should be combated, could weaken muscles used in sprint activity. Alternatively, the re-allocation of amino acids to the immune system could depress brain activity (which also needs amino acids), thus increasing torpor (see Stevenson et al., 2010; Otti et al., 2012). Second, both fleeing and an immune response are energetically costly (Christian et al., 1997; Hancok and Gleeson, 2002; Schmid-Hempel, 2011), therefore immune-activated animals probably re-allocate energy to the immune system, and energy demands of musculature involved in sprint capacity could not be fulfilled. Third, mounting an immune response together with locomotion augment the burst of reactive molecules and thus increase oxidative stress (Fisher-Wellman and Bloomer, 2009; Sorci and Faivre, 2009). Indeed, immunocompetence is traded with several other traits, such as sexual signals, reproduction, growth rate, or even survival (examples from reptiles in Uller et al., 2006; French et al., 2007; López et al., 2009; Meylan et al., 2013).

In sum, given the competition for resources and the fact that both sprint and immune responses increase oxidative stress, here we propose that there is a trade-off between immune response and sprint capacity. On the one hand, this hypothesis predicts that animals under increased predation risk should reduce their immune response in order to increase their sprint capacity. This part of the hypothesis is well supported by studies showing that animals usually reduce their immune response when exposed to predators (Rigby and Jokela, 2000; Zhang et al., 2003; Navarro et al., 2004; Hõrak et al., 2006; Stoks et al., 2006; Mikolajewski et al., 2008). On the other hand, the hypothesis also predicts that immune-challenged animals should reduce their escape capacity, an implication which has received little attention. There is some evidence that eliciting an immune response increases the probability of predation. In the damselfly *Calopteryx splendens*, immune-challenged males lose their territories and are more susceptible to bird predation (Rantala et al., 2010). In field crickets (*Gryllus campestris*), immune-challenged males spend more time outside their shelters and show less reaction to predators, but its top crawling speed is not affected by the immune challenge (Otti et al., 2012).

In the present study, following the aforementioned reasoning, we test the prediction that an immune challenge should diminish the sprint capacity of animals. We used the lizard *Psammodromus algirus* as model system. In a group of lizards, we stimulated the immune system by the inoculation of an innocuous antigen (lipopolysaccharide of the cell wall of *Escherichia coli*, LPS), while another group of lizards served as a control. Afterwards, we analyzed the change in sprint speed as a consequence of the immune challenge, under the prediction that lizards inoculated with LPS would reduce their sprint speed, while control lizards would show no change.

In addition, we also tested gender-based differences in this trade-off between immune response and sprint speed. On the one hand, testosterone has an immunosuppressant effect that provokes males to have lower immune response than females (Møller et al., 1998), which may also occur in the lizard studied here (Belliure et al., 2004). Therefore, we would expect a higher immune response in females, and thus the trade-off will be more evident in females. On the other hand, male lizards sprint faster than females in different species (Lailvaux et al., 2003) such as *P. algirus* (Zamora-Camacho et al., 2014). Accordingly, diverting resources for immune response might be more appellant in males than in females, conducing to a more evident trade-off in males than in females.

## VI.3.-Materials and Methods

*Psammodromus algirus* is a medium-size lacertid lizard (53-95 mm of snout-vent length [SVL] in our study zone) that inhabits shrubby areas in western Mediterranean climates, from the sea level to more than 2600 m a.s.l. (Salvador, 2011). We caught lizards on the southern slope of Sierra Nevada (SE Spain), in six plots placed at 300, 700, 1200, 1700, 2200, and 2500 m a.s.l. (Fig. S1, Appendix C), during the lizards' activity season (April-September), in 2011 and 2012. The escape strategy varies in this species with habitat characteristics (Martín and López, 1995; Iraeta et al., 2010), so we chose locations as similar as possible in vegetation and habitat structure. In fact, elevation provenance did not affect the findings in this study (Tables C.S1 and C.S2 in Appendix C).

We brought all captured individuals to the laboratory and registered biometrical measures involved in sprint speed, such as body mass (with a balance model WTB200, accuracy 0.01 g), SVL, and fore- and hind-leg length (with a millimeter ruler). In order to differentiate sexes, we distinguished males for having proportionally larger heads, more abundant and conspicuous femoral pores, and orange spots at the corners of the mouth. We did not include gravid females in the study to avoid confounding effects on sprint speed (Iraeta et al., 2010); gravid females were detected by palpation for the presence of eggs. Also, tailless lizards were not used, because tail loss diminishes sprint speed in this species (Martín and Avery, 1998). During their stay in captivity, lizards were kept in individual terrariums (20×13×9 cm), and provided *ad libitum* access to mealworms (*Tenebrio molitor* larvae) and water (nutritious aqueous gel). Lizards were marked by toe clipping (excluding the longest toes) as part of a longstanding ongoing study, two days before the trials. This marking method has been demonstrated not to alter lacertids' locomotive capacities (Huey et al., 1990; Dodd, 1993).

Sprint speed was measured in a wooden runway (3.2×0.2×0.4m), with a cork base to provide suitable traction (Bauwens et al., 1995). This runway was divided into 10 stretches every 25 cm with a contrasting color strip, considering that this is approximately the distance that lizards run in nature to reach their refuges (Martín and López, 2000). Finally, a dark background was placed at the end of the runway to resemble a refuge and thus encourage the lizards to run forward. As an internal control, we recorded sprint speed for all lizards prior to treatment. We released lizards at the start of the runway, and chased them by hand to encourage escape behavior. After 24 h, we randomly assigned lizards to one of two treatments. Lizards of one treatment were inoculated subcutaneously in the dorsum with 0.1 mg of LPS of bacterial wall of *Escherichia coli* (serotype 055:B5, L-2880, Sigma Aldrich) diluted in 0.01 ml of isotonic phosphate buffer. This substance acts as an antigen mimicking an infection and thus provoking an immune reaction. The remaining lizards were used as manipulation controls, inoculated with the same volume of phosphate buffer (PBS), which has no physiological effects as it is similar to physiological serum. Trials with treated lizards were conducted 4 h after the inoculation of the respective substance, when the immune reaction to LPS is maximal (Parmentier et al., 1998). All trials were repeated three times for every lizard (Losos et al., 2002; Adolph and Pickering, 2008). No sign of fatigue was observed between trials. All lizards ran at 32ºC of body temperature, in order to avoid confounding effects of body temperature on sprint speed (see Pérez-Tris et al., 2004; Iraeta et al., 2010). This temperature was achieved by placing lizards into an incubator at the trial temperature for ten minutes prior to measurements. Before conducting the trials, lizard body temperature was assessed by inserting a 1-mm diameter thermocouple connected to a thermometer (model Hybok 14, accuracy 0.1ºC) 8 mm inside the cloaca. We used the same temperature for all lizards in each population, given that lizards in our study zone select the same temperature regardless of elevation (Zamora-Camacho et al., 2013). It is well established that the temperature selected by lizards corresponds to the temperature at which sprint speed is maximal (Bonino et al., 2011), and the temperature at which lizards achieve maximal sprint speed varies little or not at all among lizard populations (Garland and Adolph, 1991). Once the experiment was ended, lizards were returned to the same place where they were captured. No lizard died or suffered any damage as a consequence of this study.

All the runs were recorded with a photo and video camera Canon EOS 550D, at 25 frames per second. Then, the films were analyzed with the software Movavi v. 11, which enables time to be measured in milliseconds (ms) (Chen et al., 2003). For each run, we measured the time interval for every stretch, counting a stretch as completed when the lizard's snout reached the dividing strip (Martín and López, 2001). We analyzed speed data of the fastest stretch out of the three runs of each lizard in order to determine the maximal sprint speed of the lizards. Laboratory measurements of maximal sprint speed represent an upper limit of field sprint speed, not necessarily correlated with the sprint speed in the field (Irschick and Garland, 2001). However, several studies have demonstrated that laboratory measures of locomotive performance are good indicators of field survival in lizards (e.g. Le Galliard et al., 2004). Thus, considering the field behavior of this lizard when exposed to a predator (consisting of short runs to the nearest shelter; Martín and López, 2000), we consider the maximal sprint speed measured in the laboratory to be a reliable indicator of sprint capacity in this species.

All data were analyzed with parametric statistics, as they fulfilled the criteria of residual normality and homoscedasticity (Quinn and Keough, 2002). We first checked differences between experimental groups in morphology and previous run with a two-way factorial ANOVA, including treatment and sex as categorical predictors. Then we conducted a similar two-way factorial ANOVA to check the effect of the treatment on post-treatment speed and difference in sprint speed between the two trials, calculated as sprint speed after treatment minus prior sprint speed.

## VI.4.-Results

Since the lizards were randomly assigned to each treatment, there were not significant differences between treatments in body mass, snout-vent length, foreleg length, hind-leg length, or sprint speed prior to the treatment (Tables VI.1 and VI.2). There was sexual dimorphism for limb length, and males ran faster than females (Tables VI.1 and VI.2). The inoculation of LPS had a significant effect on maximal sprint speed, lizards inoculated with LPS showing significantly lower sprint speed than control lizards, inoculated with PBS (Tables VI.1 and VI.2; Fig. VI.1). The effect of the treatment remained significant when the interactions were removed from the model ($F_{1, 69} = 6.39$, $P = 0.014$). Lizards inoculated with LPS significantly decreased their sprint speed in the second trial compared to the first trial, in average -18.68 cm/s, with 95% confidence intervals not including zero (-36.84 and -0.52 cm/s; Nakagawa and Cuthill, 2007). There was an almost significant interaction between sex and treatment explaining sprint speed in the second trial ($p = 0.055$; Table VI.1). When considering only males, post-treatment maximal sprint speed was significantly slower in LPS- than in PBS-inoculated lizards ($F_{1, 33} = 12.09$, $P = 0.001$; Fig. VI.2). However, when only females were considered, the sprint speed did not differ between LPS- and PBS-inoculated lizards ($F_{1, 27} = 0.009$, $P = 0.92$; Fig. VI.2).

| | **Sex** | **Treatment** | **Sex*Treatment** |
|---|---|---|---|
| ***D.f.*** | 1, 68 | 1, 68 | 1, 68 |
| **Mass** | 0.49 | 0.1 | 0.11 |
| **SVL** | 1.86 | 0.01 | 0.003 |
| **FLL** | 12.09*** | 0.12 | 0.17 |
| **HLL** | 29.86*** | 1.35 | 0.18 |
| **Previous speed** | 7.88** | 0.002 | 3.31§ |
| **Post-treatment speed** | 4.24* | 5.68* | 3.81§ |
| **Difference in speed** | 0.55 | 6.33* | 0.03 |

Table VI.1 - Results of the two-way factorial Anova examining variation in body mass, snout-vent length (SVL), foreleg length (FLL), hind-leg length (HLL), maximal sprint speed prior the treatment (previous speed), maximal sprint speed post-treatment (post-treatment speed), and the difference in maximal sprint speed, according to treatment, and sex. *F*-values are shown. Symbols indicate: § for 0.10 > $P$ > 0.05, * for $P$ < 0.05, ** for $P$ < 0.01, and *** for $P$ < 0.001. In bold, significant results. Note that for body mass and SVL, data of one lizard were lost, and therefore the *d.f.* error is 67 for these variables.

| **Variable** | **LPS (n = 38)** | **PBS (n = 34)** | **Males (n = 39)** | **Females (n = 33)** |
|---|---|---|---|---|
| **Body mass (g)** | 7.09 ± 0.34 | 7.22 ± 0.39 | 7.64 ± 0.33 | 6.67 ± 0.40 |
| **SVL (mm)** | 65.98 ± 1.07 | 65.77 ± 1.21 | 65.46 ± 1.04 | 66.29 ± 1.23 |
| **Foreleg length (mm)** | 22.02 ± 0.26 | 21.83 ± 0.29 | 22.65 ± 0.25 | 21.20 ± 0.30 |
| **Hind-leg length (mm)** | 34.89 ± 0.46 | 35.52 ± 0.53 | 37.01 ± 0.45 | 33.40 ± 0.54 |
| **Initial speed (cm/s)** | 143.45 ± 9.19 | 141.61 ± 10.48 | 155.79 ± 8.99 | 129.27 ± 10.65 |
| **Post-treatment speed (cm/s)** | 124.77 ± 9.54 | 154.48 ± 10.88 | 151.99 ± 9.33 | 127.26 ± 11.06 |
| **Difference in speed (cm/s)** | -18.68 ± 9.08 | 12.87 ± 10.35 | -3.80 ± 8.88 | -2.01 ± 10.52 |

Table VI.2 - Average values ± SE of measured variables for both treatments (LPS and PBS), and for both sexes.

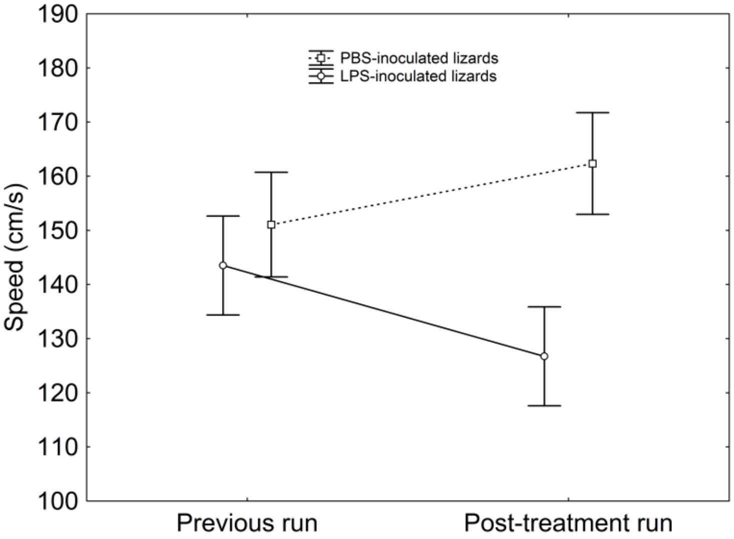

Fig. VI.1 - Lizard sprint speeds were similar between groups in the previous run without antigen treatment, as a result of the random assignation of lizards to one or another group. Then, PBS-inoculated lizards showed a trend to increase their speed, while LPS-inoculated, immune-system-activated lizards ran more slowly, showing a trade-off between immune system and sprint performance. Error bars represent standard error. A total of 39 lizards were injected with LPS, and 34 with PBS.

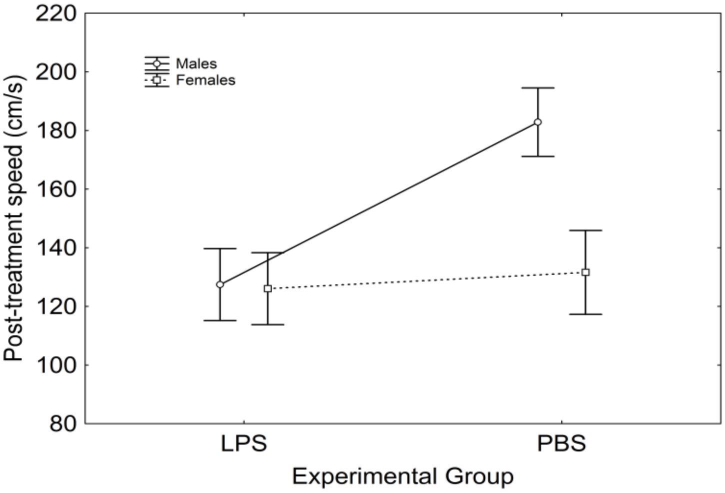

Fig. VI.2 - PBS-inoculated males ran faster than females, repeating the trend in the previous run. In turn, LPS had no effect on female running speed, while male speed diminished. Male sprint speed underwent a trade-off with the immune system, but female sprint speed did not. Error bars represent standard error. Sample sizes were: 19 males and 19 females in the LPS treatment, and 20 males, and 14 females in the PBS treatment.

## VI.5.-Discussion

Results in this study show that when a lizard elicits an immune response against an antigen, its sprint capacity decreases. This finding has an immediate implication: lizards exposed to pathogens, which are eliciting an immune response, have reduced capacity to flee from predators. This result is consistent with previous studies showing that insects challenged with an antigen show higher predation risk (Rantala et al., 2010; Otti et al., 2012). Nevertheless, the fact that Otti et al. (2012) did not find an effect of immune challenge on maximum speed in field crickets suggests that the trade-off between escape capacity and immune capacity is not general in the animal kingdom. In fact, we found such as trade-off only in male lizards.

Our study suggests that unhealthy animals could suffer more risk of predation, not only as a consequence of pathogen virulence, but also as a collateral effect of immune response. Our findings, therefore, help explain the mechanisms by which unhealthy animals are more frequently depredated (Temple, 1987; Hudson et al., 1992; Murray et al., 1997; Møller and Nielsen, 2007). In fact, consistent with our findings, previous studies found that parasitized lizards show reduced take-off capacity (Schall et al., 1992; Oppliger et al., 1996; Main and Bull, 2000), which has also been found in amphibians (Chatfield et al., 2013), birds (Lindström et al., 2003) and mammals (Alzaga et al., 2008). Therefore, animals appear to make a trade-off between avoiding predators and combating pathogens.

Several studies have shown that immunity is a costly trait which requires trade-offs with other life-history traits (see Introduction). Those costs of immunity have been confirmed in reptiles (Zimmerman et al., 2010), in which growth rates (Uller et al., 2006), male and female reproduction success (French et al., 2007; López et al., 2009) or even thermoregulation accuracy (Merchant et al., 2007) are negatively affected by immune challenges. The trade-off between immune competence and sprint speed may be achieved by various non-mutually exclusive ways, as explained in the Introduction.

Therefore, in our trials, lizards obligated to respond to an antigen may have reduced their sprint speed as a consequence of proteins and/or energy being reallocated to immune response, or in order to avoid excessive oxidative imbalance.

On the other hand, it is noteworthy that the trade-off between sprint speed and immune-competence occurred only in males. We can discard an effect of testosterone, as testosterone, being immunosuppressant (Belliure et al., 2004), would relax the trade-off in males, the reverse that we have found. The reason may be that locomotion has a higher impact on male than on female fitness (e.g. Husak et al., 2006). Males usually spend more time than females defending a territory or searching for mates (for our study species, see Díaz, 1993), and consequently they are more exposed to predators (general review in Lima and Dill, 1990). Perhaps for this reason, males have longer limbs than females, and consequently run faster (Zamora-Camacho et al., 2014). Actually, the fact that gender differences in sprint speed in this species disappeared when controlling for body mass suggests that they are a consequence of males having longer limbs (Zamora-Camacho et al., 2014). Given that males have longer limbs, they presumably could need more energy during runs (a larger limb needs more energy to be moved, all else being equal), and thus energy depletion by the immune system could have a stronger effect on males than on females.

It should be noted that the trade-off found does not necessarily imply higher actual predation in lizards eliciting an immune response, if they avoid predators by some means or other. Ill lizards could diminish predation risk by other means, e.g. spending less time outside their shelters. Reduced mobility is characteristic of unhealthy animals (Adelman and Martin, 2009), and it may be adaptive in order to reduce predation risk (Caro, 2005). Thus, if unhealthy lizards avoid predation by remaining sheltered, lizards mounting an immune response would perhaps not pay a cost in increased mortality by predation, but they would pay a cost in reduced fitness as a consequence of reduced vigilance of their territories, foraging or mating (e.g. Civantos et al., 2010). Matching with these predictions, cane toads (*Rhinella marina*) inoculated with LPS showed diminished feeding and activity rates (Llewellyn et al., 2013).

## VI.6.-Conclusions

In short, male lizards were faster than females before activating their immune system, but, while females did not change their sprint speed despite the injection of LPS, male sprint speed diminished when the immune system was challenged by the antigen. This result shows that sprint speed is under a trade-off with immune system in males, since the activation of the latter reduces the performance of the former, but not so in females. These findings imply that male lizards combating a pathogen suffer a higher predation risk, or undergo reduced fitness if they avoid this higher predation risk by staying more time in their shelter, thus suffering a cost in reduced vigilance of their territories, foraging or mating. In addition, according to the trade-off shown in this study, we predict that populations in which predation is reduced, animals should show a stronger immune response, and vice versa in populations with high predation risk. At the same time, in populations with high parasite and pathogen prevalence, animals should show slower sprint speeds.

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## VII - Chapter 7

# Thermal dependence of sprint performance in the lizard *Psammodromus algirus* throughout a 2200-meter elevational gradient: high elevation lizards do not perform better at low temperatures.

This chapter reproduces the submitted article:

Zamora-Camacho, F.J., Rubiño-Hispán, M.V., Reguera, S., Moreno-Rueda, G. 2014. Thermal dependence of sprint performance in the lizard *Psammodromus algirus* throughout a 2200-meter elevational gradient: high elevation lizards do not perform better at low temperatures. Contributions to Zoology, submitted.

**VII.1.-Abstract** - Sprint speed has a capital relevance in most animals' fitness, since flying from predators, foraging, social dominance, or even reproduction are often strongly dependent upon this performance. Sprint performance is maximal within a certain range of body temperatures in ectotherms, whose thermal upkeep relies on exogenous thermal sources. Ectotherms can respond to diverse thermal environments either by shifting their thermal preferences, according to the labile hypothesis of ectotherm thermal-environment adaptation, or maintaining them through different adaptive mechanisms, according to the static hypothesis. Therefore, ectotherms maintaining their thermal preferences should also keep their sprint-speed thermal optima, if coadaptation among these two parameters exists, regardless of environmental thermal quality. If not, cold-habitat ectotherms could improve speed performance at low body temperatures. We tested these hypotheses by recording maximum sprint speed of a lizard that shows static thermal ecology along a 2200-meter elevational gradient, at optimum and suboptimum body temperatures. Lizards ran faster at optimum than at suboptimum body temperature. Notably, high-elevation lizards at suboptimum body temperature were not faster, despite their low-quality thermal environment. This result supports the contention that coadaptation between field body-temperature and thermal dependence of speed performance exists in all elevations. High-elevation lizards display a number of thermoregulatory strategies that allow them to achieve high optimum body temperatures in a low thermal-quality habitat and thus maximize speed performance. Strikingly, gravid females were significantly slower than males and non-gravid females at suboptimum temperature, but performed similarly well at optimal temperature.

## VII.2.-Introduction

Sprint speed is a trait of paramount importance for many animals' fitness, as it is involved in a number of life-history traits. In fact, many species avoid predation by quickly fleeing (Lima and Dill, 1990). In turn, many predator strategies to capture preys are based on speed (Lima, 2002). However, speed does not affect animal fitness exclusively through predator-prey interactions: faster individuals also show social dominance (Garland et al., 1990), or improved mating success (Byers et al., 2010). Regarding the effect of speed on animal fitness, it has been demonstrated that faster lizards have higher survival rates (Husak, 2006a), and produce more offspring (Husak et al., 2006).

Performance of most metabolic and physiologic processes is strongly reliant on body temperature, due to thermal kinetics (Angilletta, 2009; Angilletta et al., 2010). Speed performance also depends on body temperature, since it is closely linked to muscular activity (Taylor et al., 1980). For example, the sprint speed of lizards of the genus *Takydromus* peaks at 30-32ºC of body temperature, diminishing gradually towards warmer or cooler temperatures (Xiang et al., 1997; Chen et al., 2003). Similar results have been found in ants (Hurlbert et al., 2008), fish (Wilson, 2005), a number of other ectotherms (Bennett, 1990), and even endotherms (Rojas et al., 2012; Carr and Lima, 2013).

Speed performance, thus, depends on body temperature in both endotherms and ectotherms. However, body temperature is rather constant in most endotherms (Bennett and Ruben, 1979), whereas in ectotherms, body temperature depends strongly upon environmental temperature (Hertz et al., 1993). Therefore, geographic shifts in environmental temperature can affect ectotherm thermal biology (Van Damme et al., 1989; Andrews, 1998; Sunday et al., 2010). Ectotherms can face different thermal environments by shifting their thermal preferences accordingly (Hertz et al., 1993), as described by the "labile hypothesis" of ectotherm thermal-environment adaptation (Hertz et al., 1983; Rodríguez-Serrano et al., 2009). For instance, crocodiles (Glanville and Seebacher, 2006) and lizards (Yang et al., 2008) experimentally acclimated to, or naturally occurring in (Ibargüengoytía, 2005), low-temperature environments prefer lower body temperatures.

Contrarily, according to the "static hypothesis" of ectotherm thermal-environment adaptation (Hertz et al., 1983; Crowley, 1985), ectotherms may not be able to shift thermal preferences despite environment thermal limitations, due to evolutionary constraints. Support for this hypothesis has also been found both in natural (Gvoždík, 2002; Valdecantos et al., 2013) and experimental scenarios (Besson and Cree, 2010). Consequently, thermal biology is often evolutionarily conserved throughout wide regions, even across continents (Youssef et al., 2008). Several behavioral and physiological mechanisms allow ectotherms to fulfill their thermal preferences in different thermal environments (Stevenson, 1985): alterations in frequency and duration of basking periods (Carrascal et al., 1992; Hertz, 1992; Gvoždík, 2002), in skin color (in order to absorb a wider or narrower spectrum of solar radiation; Clusella-Trullas et al., 2007), or even in metabolic-heat generation (Dickson, 1995) have been reported.

Irrespective of the strategy that ectotherms use to cope with different thermal environments, the relationship between performance and body temperature may differ geographically as environmental temperature varies. Two complementary hypotheses seek to model geographic variation in thermal dependence of ectotherm performance. On the one hand, the hypothesis of countergradient variation predicts that genetic variability can outweigh the influence of different thermal environments on ectotherm phenotypes (Conover and Schultz, 1995; McElroy, 2014). According to this mechanism, compensations at the metabolic level can allow ectotherms to increase performance at a range of temperatures wider than, or even different from, the temperatures that ectotherms actually experience in the wild (Conover and Schultz, 1995). In fact, some lizards show maximum sprint speed outside their field body-temperature ranges (Bennett, 1980; Huey and Bennett, 1987; Huey et al., 1989; McElroy, 2014). For example, in the lizard *Sceloporus merriami*, individuals from high elevations sprint faster than individuals from low elevations when exposed to the same body temperature in the laboratory (Huey et al., 1990).

On the other hand, the hypothesis of coadaptation posits that performance is optimized at body temperatures that match those that ectotherms show in the field (Gilchrist, 1995; Angilletta, 2009). Cases of lizards that optimize sprint-speed performance at their field body temperatures are common as well (Hertz et al., 1983; Bauwens et al., 1995; Bonino et al., 2011; Kubisch et al., 2011). This hypothesis implies that ectotherms that shift their thermal preferences according to environmental temperature adjust their physiology, showing similar performance in different temperature regimes (Glanville and Seebacher, 2006).

Therefore, if thermoregulatory preferences and thermal dependence of speed performance are co-adapted, ectotherms that maintain their thermal preferences despite environmental temperature constraints should also keep their thermal optimum for speed performance. Otherwise, if countergradient variation works, ectotherms inhabiting cold habitats, where heat acquisition is constrained, should show better performance over a wide range of body temperatures than do ectotherms from hotter environments.

We tested these exclusive hypotheses in the lizard *Psammodromus algirus* within a 2200-meter elevational gradient. In our study system, this lizard follows the "static hypothesis" of thermal-environment adaptation: environmental temperature when lizards are active diminishes in elevation 8ºC on average, while the lizard’s field body temperature decreases less than 2ºC from the lowest to the highest elevation (Zamora-Camacho et al., 2013). In fact, lizards from this system selected similar body temperatures in a laboratory thermal gradient, regardless of their elevational provenance (Zamora-Camacho et al., submitted). Moreover, lizards from different elevations showed the same maximal sprint speed in experiments under controlled body temperature (32ºC; Zamora-Camacho et al., 2014a). Therefore, given that lizards face lower environmental temperatures in high elevations when active, if the countergradient hypothesis works in this system, we expect high-elevation lizards to be faster than low-elevation lizards when tested at lower temperatures. However, if the coadaptation hypothesis works here, given that body temperature did not vary with elevation, we expect the relationship between body temperature and sprint speed not to vary with elevation.

## VII.3.-Materials and Methods

### *VII.3.1.-Study species and field area*

*Psammodromus algirus* is a medium-sized (53-95 mm of snout-vent length [SVL] in our study area), generalist lacertid lizard that occupies Mediterranean areas of southwestern Europe and northwestern Africa. It can be found from the sea level to around 2600 m asl (Salvador, 2011). Lizards inhabit shrubby zones, where they crawl around bushes that they use as refuges (Díaz and Carrascal, 1991). They feed on a variety of invertebrates, and their foraging strategy is flexible: they can either use a sit-and-wait strategy, or search actively for prey (Verwaijen and Van Damme, 2008). These lizards select different body temperature seasonally: 30.9–34.7ºC in spring and 32.8–36.9ºC in summer (Díaz and Cabezas-Días, 2004).

We caught 118 lizards by hand during their reproductive season (April-June) in 2013, on the southern face of Sierra Nevada (SE Spain). Sampling was performed in six plots located at 300, 700, 1200, 1700, 2200, and 2500 m asl (see Appendix A in Zamora-Camacho et al., 2013). For accurate detection of geographical trends, we grouped those sampling plots into three elevational belts: low elevations (plots at 300 and 700 m asl), middle elevations (plots at 1200 and 1700 m asl), and high elevations (plots at 2200 and 2500 m asl). Grouping in elevational belts was justified on the basis of similarities in population traits within belts, such as activity patterns, demography, morphology, and even genetic structure (Zamora-Camacho et al., 2013, 2014a, b; Reguera et al., 2014; unpublished data). Since habitat landscape can affect the escape strategy of this species (Martín and López, 1995; Iraeta et al., 2010), we chose locations as similar as possible regarding vegetation structure (see Appendix A in Zamora-Camacho et al., 2013).

### *VII.3.2.-Laboratory procedures*

Captured lizards were transported to the laboratory, where we considered three reproductive conditions: males, non-gravid females, and gravid females. Males were identified by their proportionally larger heads, orange patches in the corners of their mouths, and more numerous and conspicuous femoral pores in their hind limbs. We recognized gravid females by manual abdomen palpation of the developing eggs. Also, we measured biometrical variables involved in sprint speed: hind-limb length (HLL), since it is particularly involved in this species sprint speed (Bauwens et al., 1995; Zamora-Camacho et al., 2014a), and occiput-vent length (OVL), with a millimeter-marked ruler. We used OVL instead of SVL in order to control for distance between fore and hind limbs, because males had significantly longer heads than females ($F_{1, 116} = 13.18$; $P = 0.0004$). Tailless lizards were not used in this study, since tail loss negatively affects sprint speed in this species (Martín and Avery, 1998). We marked lizards by toe-clipping, as a part of a long-term ongoing project. Toe-clipping has been found not to alter Lacertid motility (Huey et al., 1990; Dodd, 1993; Husak, 2006b) and, in any case, we did not clip the longest toes. During their captivity, lizards were kept in individual plastic terrariums (20 × 13 × 9 cm), with pine cork as the substrate, a heat cable underneath to allow thermoregulation, a window as a source of natural light for circadian rhythms, and water (in the form of nutritious aqueous gel) and food (mealworm, *Tenebrio molitor* larvae) provided *ad libitum*.

Two days after capturing the lizards, we performed sprint-speed trials in a wooden, straight raceway (320 × 20 × 40 cm), lined with cork to provide an appropriate traction (Bauwens et al., 1995), to measure the sprint speed. The raceway was divided into twelve 25-cm stretches, delimited with contrasting-color transversal strips, since lizards in the wild run short distances to their shelters (Martín and López, 2000). We put a dark background at one end of the raceway, to simulate a refuge and thereby encourage the lizards to run forward. In each trial, a lizard was released at the opposite end of the raceway, and a researcher chased it to make it run. Lizards ran individually. Each lizard ran three consecutive times in each trial, as they exhibited no sign of fatigue. All lizards performed the first trial at 27ºC of body temperature. Four hours afterwards, they performed the second trial similarly, but randomly assigned to one of two treatments of body temperatures: 27ºC (suboptimum) or 32ºC (optimum). Values of optimal and suboptimal temperature were established according to preferred temperatures in this lizard (e.g., Díaz and Cabezas-Días, 2004). In all cases, the target temperature was achieved by putting lizards into an incubator at the selected temperature for the adequate amount of time (c. 10 min). Body temperature was ascertained by inserting a thermocouple (1mm diameter) assembled to a thermometer (Hybok 18, accuracy 0.1ºC) 8 millimeters inside each lizard's cloaca.

All trials were videotaped with a photo and video camera Canon EOS 550D, at 25 frames per second. Then, videos were analyzed using the software Movavi v.11, with which we could measure millisecond intervals (Chen et al., 2003). Thus, we recorded the time interval for each stretch, considering a stretch as passed when the snout of the lizard reached the strip that delimited it (Martín and López, 2001). Afterwards, we calculated the difference in speed as the second run speed value minus the first. Since our aim was to know how body temperature affected lizard sprint speed to escape predators, we performed our analyses with the fastest stretch of each lizard. Furthermore, lizards often stopped during the trials, so that the average speed of all stretches would be an inaccurate measurement of lizard actual speed. In our study system, gravidity stage, clutch size, or egg burden have no effect on gravid female *P. algirus* sprint speed (Zamora-Camacho et al., 2014a).

### *VII.3.3.-Statistical analyses*

We performed parametric statistics, provided that data accomplished the criteria of residual normality and homoscedasticity (Quinn and Keough, 2002). We conducted Ordinary Least Squares Linear Models, specifically three-way factorial ANOVAs, to test for the effect of treatment, elevation, reproductive condition, and their interactions on OVL, HLL, first run speed, second run speed, and difference in speed. In order to detect relative differences in HLL, we controlled for OVL, introduced into the model as a covariate.

## VII.4.-Results

### *VII.4.1.-Treatment*

Since lizards were assigned randomly to the treatments, there were no significant differences between groups in OVL, HLL, or first run speed (Tables VII.1 and VII.2; Fig. VII.1). In the second run, lizards at 32ºC ran faster than those at 27ºC (Tables VII.1 and VII.2, Fig. VII.1). Consequently, speed differences between trials was significantly higher in lizards running at 32ºC in the second run. Actually, their speed increased significantly, on average 21.75 cm/s, with 95% confidence intervals excluding zero (8.42 and 35.07 cm/s; Nakagawa and Cuthill, 2007), while lizards running at 27ºC in the second run did not shift their speed in the second trial, as the difference in speed was -6.45 cm/s, but 95% confidence intervals did include zero (-22.38 and 9.48 cm/s).

| | *d.f.* | **OVL** | **HLL** | **First Run Speed** | **Second Run Speed** | **Difference in speed** |
|---|---|---|---|---|---|---|
| **Treatment** | 1 | 0.43 | 1.65 | 2.36 | **13.00***** | **7.26**** |
| **Elevation** | 2 | **21.33***** | 0.07 | 0.00 | 0.69 | 0.90 |
| **Reproductive Condition** | 2 | **11.34***** | **18.06***** | **3.74*** | 0.55 | 2.32 |
| **Reproductive Condition*Elevation** | 4 | 0.23 | 0.75 | 1.40 | 1.24 | 1.21 |
| **Reproductive Condition*Treatment** | 2 | 1.55 | 0.02 | 1.49 | 2.15 | 0.97 |
| **Elevation*Treatment** | 2 | 0.60 | 0.22 | 0.94 | 0.04 | 1.32 |
| **Reproductive Condition*Elevation*Treatment** | 4 | 0.24 | 1.18 | 2.38 | 0.16 | 1.71 |
| **Error** | 100 | | | | | |

Table VII.1 - ANOVAs testing the effects of treatment, elevation, reproductive condition, and their interactions on occiput-vent length (OVL), hind limb length (HLL), first run speed, second run speed, and difference in speed of lizards. *F* statistics are shown. Symbols indicate: * for $P < 0.05$, ** for $P < 0.01$, and *** for $P < 0.001$. In bold, significant results. Note that analyses for HLL were conducted controlling for OVL, so error *d.f.* are 99.

| | | n | OVL (mm) | HLL (mm) | First Race Speed (cm/s) | Second Race Speed (cm/s) | Difference in Speed (cm/s) |
|---|---|---|---|---|---|---|---|
| **Treatment** | **27ºC** | 56 | 52.19 ± 0.85 | 35.58 ± 0.41 | 159.38 ± 7.69 | 152.93 ± 9.27 | -6.45 ± 8.03 |
| | **32ºC** | 62 | 51.87 ± 0.81 | 34.90 ± 0.39 | 174.78 ± 6.43 | 196.52 ± 7.76 | 21.75 ± 6.71 |
| **Elevation** | **Low Elevation** | 33 | 46.90 ± 0.85 | 34.64 ± 0.61 | 167.11 ± 10.30 | 185.64 ± 12.42 | 18.53 ± 10.5 |
| | **Mid Elevation** | 43 | 51.09 ± 0.74 | 34.98 ± 0.47 | 166.72 ± 7.35 | 168.70 ± 8.86 | 1.97 ± 7.67 |
| | **High Elevation** | 42 | 57.00 ± 0.75 | 35.92 ± 0.56 | 167.40 ± 8.12 | 169.84 ± 9.80 | 2.44 ± 8.48 |
| **Reproductive Condition** | **Male** | 58 | 49.91 ± 0.71 | 37.18 ± 0.34 | 182.45 ± 5.78 | 181.07 ± 6.97 | -1.38 ± 6.03 |
| | **Non-Gravid Female** | 24 | 49.52 ± 1.10 | 34.08 ± 0.52 | 166.61 ± 9.66 | 166.91 ± 11.66 | 0.30 ± 10.09 |
| | **Gravid Female** | 36 | 57.10 ± 0.90 | 32.81 ± 0.47 | 152.18 ± 9.97 | 176.21 ± 12.02 | 24.03 ± 10.41 |

Table VII.2 - Average ± standard error values of occiput-vent length (OVL), hind-limb length (HLL), first race speed, second race speed, and difference in speed regarding treatment, elevation, and reproductive condition of lizards. Sample sizes (*n*) are indicated for each group.

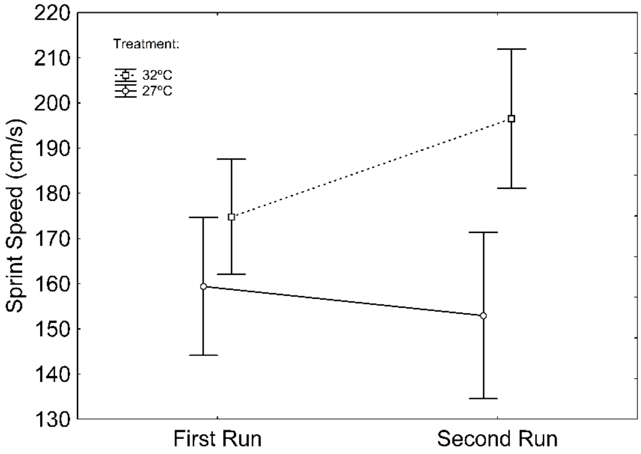

Fig. VII.1 - First run was performed at 27ºC of body temperature for all lizards. Differences in first run sprint speed were not significant regarding treatment. In the second run, the lizards at 27ºC did not change their speed, while lizards at 32ºC ran significantly faster than them, and also than themselves in their first run. Vertical bars represent 95% Confidence Intervals. Sample sizes are indicated in Table VII.2.

### *VII.4.2.-Elevation*

High-elevation lizards showed the largest OVL, but HLL did not vary with elevation (Tables VII.1 and VII.2). Elevation and its interactions had no effect on either the first or second run speed, or on difference in speed (Tables VII.1 and VII.2). This result suggests that body temperature had a similar effect on lizards from different elevations, despite environmental temperature differences that the lizards had to face in their habitats.

### *VII.4.3.-Reproductive Condition*

Gravid females showed larger OVL, but males had relatively longer hind limbs (Tables VII.1 and VII.2). Despite morphological differences, neither reproductive condition nor its interactions had any effect on second run speed or difference in speed (Tables VII.1 and VII.2). Nevertheless, gravid females were significantly slower than males in the first run, at suboptimum temperature (Tables VII.1 and VII.2; Fig. VII.2).

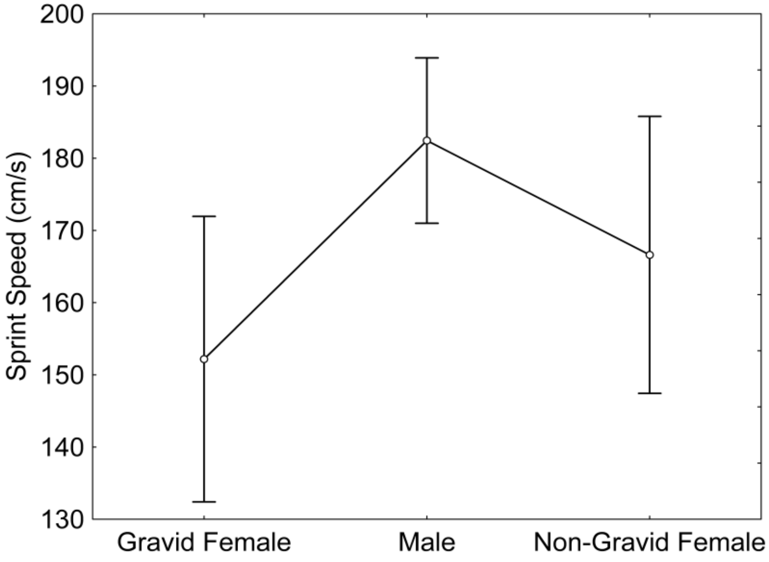

Fig. VII.2 - In the first run, at suboptimum body temperature, gravid females were significantly slower. Vertical bars represent 95% Confidence Intervals. Sample sizes are indicated in Table VII.2.

## VII.5.-Discussion

Sprint speed has a heightened impact on lizard fitness (Irschick and Garland, 2001), since it enhances escape (Lima and Dill, 1990), foraging (Lima, 2002), social dominance (Garland et al., 1990), and reproduction capabilities (Husak et al., 2006). Consequently, it is strongly favored by selection in lizards (Le Galliard et al., 2004; Husak et al., 2006; Irschick and Meyers, 2007; Irschick et al., 2008; Calsbeek and Cox, 2010). Among other life-history traits, sprint speed depends upon body temperature in lizards (Waldschmidt and Tracy, 1983; Pinch and Claussen, 2003; Artacho et al., 2013). Albeit thermoregulation is also costly, especially in terms of conspicuousness to predators (Herczeg et al., 2006), lizards need to thermoregulate in order to keep body temperature within a range at which physiological performance is optimized (Hertz et al., 1993). The costs of achieving and keeping a certain temperature increase with the difference from environmental temperature

(Alford and Lutterschmidt, 2012), since environment is the main heat source for ectotherms (Hertz et al., 1993).

Our results show that lizards run faster at optimum than at suboptimum body temperature. This result is not surprising, since it has been well established that body temperature strongly influences ectotherm overall activity (Grant and Dunham, 1988) and performance (Huey and Kingsolver, 1989), also in the case of sprint speed (e.g., Bonino et al., 2011; Gaby et al., 2011; Herrel & Bonneaud, 2012). Notably, high-elevation lizards did not perform better than middle and low-elevation lizards at suboptimum body temperatures, despite inhabiting a thermally low-quality habitat. Therefore, we failed to find evidence of countergradient variation in sprint speed in our study system. By contrast, our findings support the hypothesis of coadaptation. *Psammodromus algirus* follows the static thermoregulation strategy along the elevational gradient. That is, although environmental temperatures strongly decline with elevation, body temperature of active lizards remains similar along an elevation gradient (Zamora-Camacho et al., 2013). Consequently, consistent with coadaptation between optimal temperature and maximal sprint speed, temperature similarly impacted lizards' speed regardless of the elevation. The hypothesis of coadaptation has also been supported in many other lizards, in which sprint speeds are maximum at the body temperature they seek to establish (Hertz et al., 1983; Bonino et al., 2011; Kubisch et al., 2011), in elevational (Crowley, 1985) and latitudinal thermal gradients (van Berkum, 1988). Sprint speed of several *Anolis* and *Liolaemus* lizard species that differ in field body temperature peaked at each species' field body temperature (van Berkum, 1986; Bonino et al., 2011). In our study system, body temperature did not vary with elevation, so that thermal dependence of sprint speed remained constant along the elevational gradient.

However, other studies have supported the hypothesis of countergradient variation, reporting a number of species for which maximum-performance temperature did not match the body temperature they showed in the field (Bennett, 1980; Huey and Bennett, 1987; Cooper, 2003). For instance, in eastern fence lizards (*Sceloporus undulatus*), sprint speed and stamina are maximum and similar at a range of temperatures even wider than their field-temperature range (Angilletta et al., 2002b). Maximum sprint performance of the cold-habitat skinks *Emoia impar* is higher than that of its sympatric warm-habitat congeneric, *Emoia cyanura,* for any given temperature (McElroy, 2014). Also, several species of nocturnal geckos reach maximum sprint speed at high body temperatures that are not reached when they are active (Huey et al., 1989).

Other studies on elevational gradients report that lizards from colder habitats (high elevations) usually show lower body temperatures in the field, but similar (or even slower) sprint speed for a given temperature than do lizards from warmer environments (Crowley, 1985; Van Damme et al., 1989; Gabirot et al., 2013). As a result, these lizards, when below their optimum body temperature, may not defend themselves from predators by fleeing, but by aggressive attacks, which do not involve sprint speed (Hertz et al., 1982). Also, many lizards flee from predators over greater distances when they are below their optimum temperature, increasing their possibilities to escape successfully in a situation of impaired sprint speed (Rocha and Bergallo, 1990; Braun et al., 2010). In contrast to these studies, in our study system, *P. algirus* showed almost the same body temperature (Zamora-Camacho et al., 2013) and similar sprint speed (Zamora-Camacho et al., 2014a) along the entire elevational gradient. Sprint performance is at least one of the reasons why lizards thermoregulate, when the body temperature that optimizes sprint speed performance matches the body temperature that ectotherms seek (Huey and Kingsolver, 1989; Bennett, 1990). Therefore, the fact that thermal ecology and optimum speed-performance temperature are co-adapted in *P. algirus* has an important consequence: high-elevation *P. algirus* need to be as warm as middle and low-elevation lizards in a lower thermal-quality habitat in order to maximize speed performance. Several thermoregulatory strategies can allow high-elevation lizards to efficiently exploit a low-quality thermal environment.

High-elevation lizards in this system are larger, and exhibit lower cooling rates, enabling them to retain heat for longer time periods (Zamora-Camacho et al., 2014b). Furthermore, they are darker (Reguera et al., 2014), this presumably helping them to warm up faster (Clusella-Trullas et al., 2007). Moreover, high-elevation *P. algirus* probably bask for longer periods, as reported for other alpine lizards (Carrascal et al., 1992; Hertz, 1992; Gvoždík, 2002).

Hence, lizards in this system show conservative thermal ecology and optimum speed-performance temperature along the entire elevational gradient. However, ectotherm thermal physiology seems to be evolutionarily constrained to some extent (Angilletta and Werner, 1998; Angilletta et al., 2002a; Gaby et al., 2011), so that thermal ecology could not be an easily evolvable trait (Van Damme et al., 1990; Angilletta et al., 2002a; Gaston et al., 2009). Evolutionary limitations for thermal ecology to evolve could thus be the mechanism underlying coadaptation (Gilchrist, 1995). Otherwise, when thermal ecology can evolve at the physiological level, the hypothesis of countergradient variation could work instead (Conover and Schultz, 1995). Thus, different degrees of evolvability of thermal ecology among taxa could be the basis of divergence of thermal dependence of sprint-speed performance in different thermal environments (Clusella-Trullas and Chown, 2014).

Consequently, lizards seem to show three different evolutionary strategies in order to inhabit environments with different thermal quality. (1) Some lizards seem to evolve physiological changes that allow them to achieve appropriate speed in colder environments (e.g., McElroy, 2014). (2) Others, such as the lizard in our study, show adaptations in order to achieve similar body temperature regardless of environmental temperature along the elevational gradient, and in this way, maintain the optimal body temperature for its sprint speed. (3) Finally, still others do not seem to raise their body temperature or sprint speed when in colder habitats. In this case, they develop behavioral adaptations, showing alternative strategies against predators (Hertz et al., 1982).

Lastly, at all elevations, males and gravid as well as non-gravid females showed impaired sprint speed at suboptimum body temperatures. Thus, lizards in all reproductive conditions must achieve optimum body temperatures in order to maximize sprint speed. The finding that individuals in all reproductive conditions performed better at optimum than at suboptimum body temperatures suggests that thermal physiology of locomotion does not differ among them (Lailvaux, 2007). As in our study, no sexual differences in sprint speed are frequently found in lizards (Ling-Jun and Wei-Guo, 2007; Tang et al., 2013).

Nevertheless, it bears mentioning that gravid females were significantly slower in the first run, performed at suboptimum body temperature. This result is surprising, since a previous work in this system showed that gravid females at optimum body temperature (32ºC) did not differ from males or non-gravid females in sprint speed (Zamora-Camacho et al., 2014a). Therefore, gravid females under optimum body temperatures may suffer increased predation risk (Sinervo et al., 1991; Miles et al., 2000). This result suggests that the physiologic mechanism that prevents gravid females from impaired sprint speed is temperature-dependent, so they must be at optimum temperatures in order to reduce susceptibility to predators. However, gravid females whose sprint speed is impaired can shift their strategy towards crypsis in order to avoid predators (Schwarzkopf and Shine, 1992). Similarly, Iraeta et al. (2010) found that females of *P. algirus* from two populations in central Spain ran slower, and performed shorter runs, when gravid than after oviposition.

## VII.6.-Conclusions

In conclusion, lizards ran faster at optimum than at suboptimum body temperature. Strikingly, high-elevation lizards at suboptimum temperature were not faster than middle or low-elevation conspecifics, despite the low-quality thermal environment. Taking into account that these lizards kept a quite constant body temperature along this elevational gradient, we found that coadaptation between thermoregulatory preferences and thermal dependence of speed performance exists at all elevations, at the same time as lizard thermal ecology and optimum speed-performance temperature are conservative over the entire elevational gradient. High-elevation *P. algirus* display a number of thermoregulatory strategies that allow them to achieve warm optimum body temperatures in a habitat of low thermal quality and thus maximize speed performance. At suboptimum body temperatures, gravid females were slower than males and non-gravid females, while at optimum temperatures they were not, suggesting that the physiological mechanisms that allow gravid females to offset their egg burden are strongly temperature-dependent. Also, gravid females particularly need to thermoregulate accurately in order to flee effectively from predators.

## VII.7.-Acknowledgements

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# Appendix A

## A.1.-Geographic location

The study area is located in Sierra Nevada, SE Spain (36ºN, 03ºW; Fig. S1). This mountain system presents the highest altitudinal range in the Iberian Peninsula (200-3481 m asl), and rather stepped slopes.

In this mountain system, mean temperature decreases 0.46ºC every 100 m of elevation (Fig. A.S1). Above 2000 m asl, precipitation falls usually in form of snow during the cold season, and the snow cover lasts for around 6 months of the year (November-May). Below 2000 m asl, precipitation in spring and autumn, and thaw, in summer, keep the atmosphere relatively humid during a part of the year; nevertheless, summer is hot and dry, typical of a Mediterranean climate.

Vegetation in Sierra Nevada is strongly influenced by this climatic gradient. In lowlands, *Eucalyptus* sp. (exotic), *Populus alba*, small patches of *Quercus ilex* and cropping *Oliva europaea* and *Prunus dulcis* represent the tree layer; *Rosmarinus officinalis*, *Retama sphaerocarpa* and thorny *Genista* sp. are the most abundant shrubs. As altitude increases, trees are replaced first by *Castanea sativa*, then by *Quercus pyrenaicus* and *Q. ilex* again; *Genista* sp. shrubs are abundant also at middle altitudes. At higher altitudes, there are plantations of *Pinus sylvestris* up to 2400 m asl; over the timberline, meadows with *Juniperus oxycedrus*, *Juniperus sabina* and *Genista florida* appear. Above 3000 m asl, only grassy vegetation appears in summer, when the absence of snow permits.

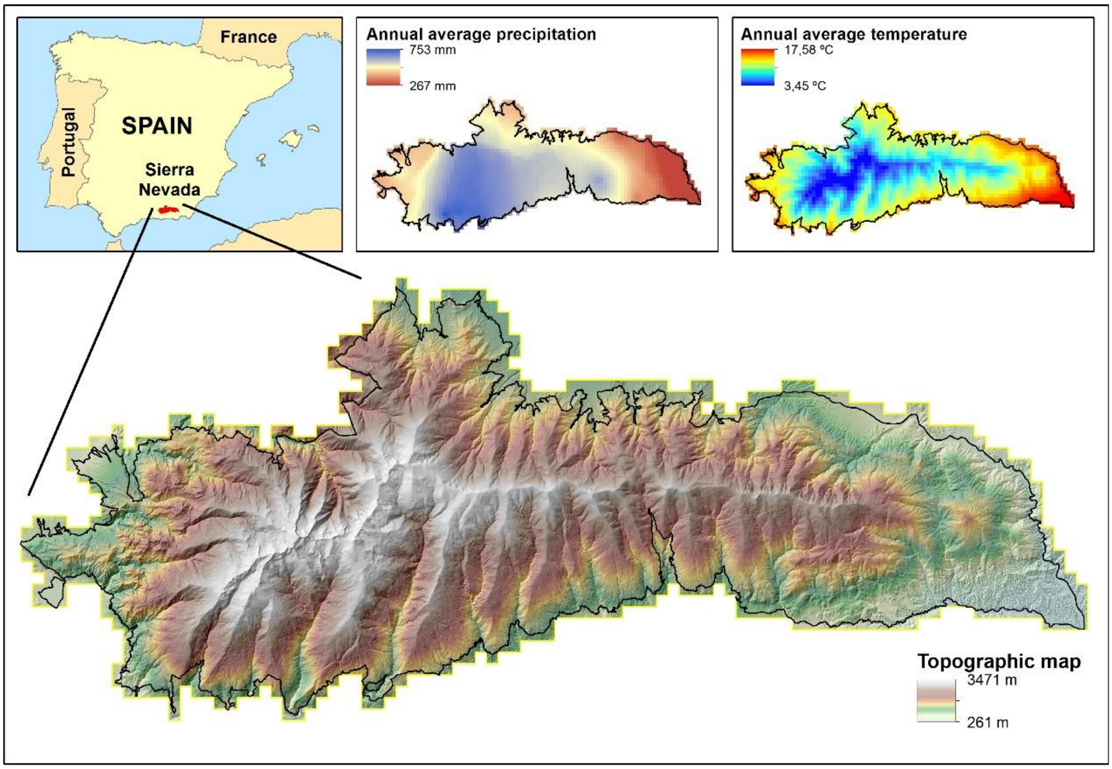

Fig. A.S1 - Altitude and mean values of precipitation and temperature in Sierra Nevada.

## A.2.-Sampling Stations

We conducted our work on the southern slope of Sierra Nevada, where we chose six sampling stations separated from each other by about 500 meters in altitude, at 300, 700, 1200, 1700, 2200 and 2500 m asl (Fig. AS2), all stations having a south orientation. Only 17.33 km over the ground separate the lowest from the highest sampling station.

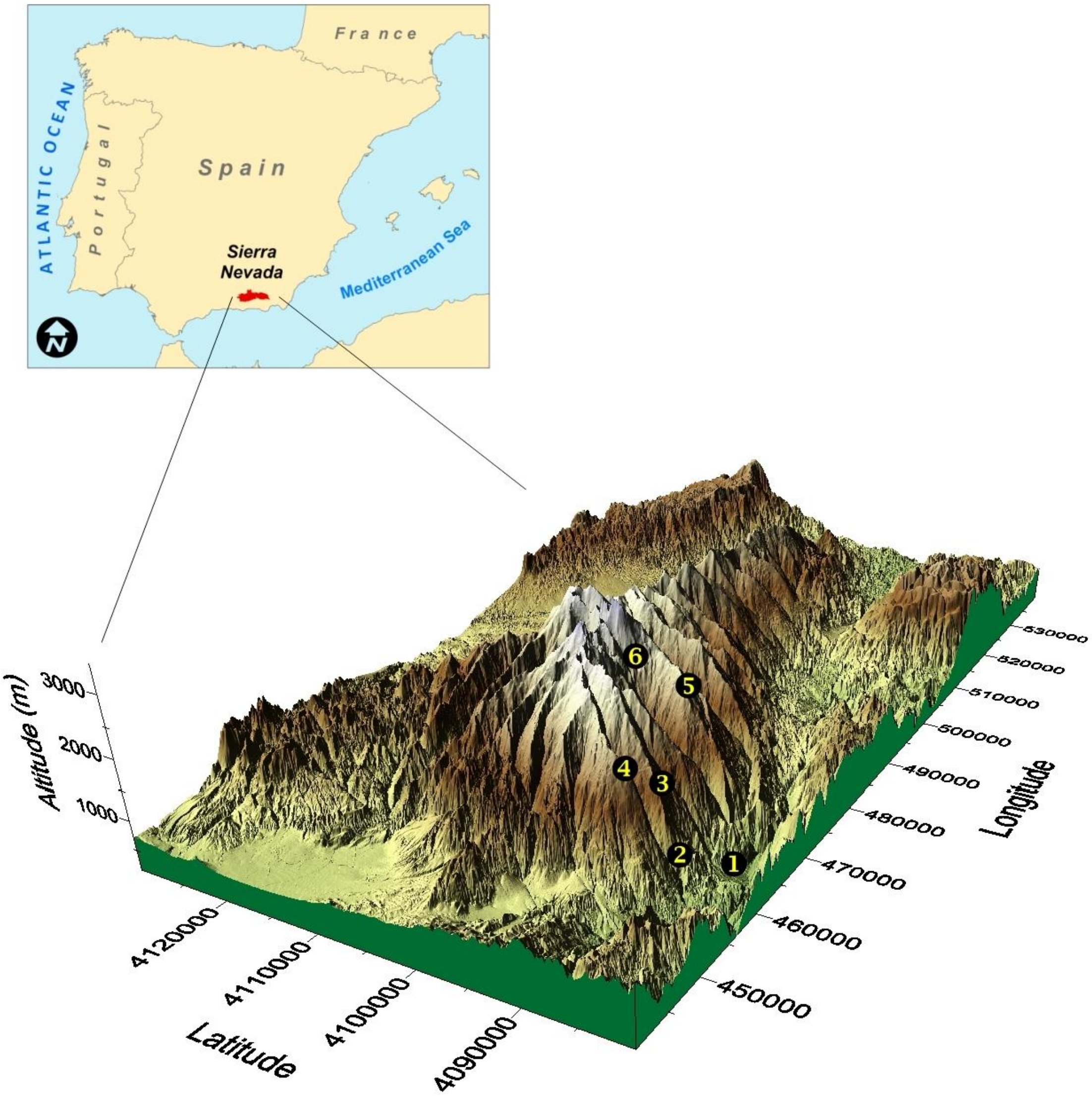

Fig. A.S2 - Three-dimensional map of Sierra Nevada, showing the location of the sampling plots (1 to 6 correspond with S300 to S2500). The localization of Sierra Nevada in the Iberian Peninsula is also indicated.

S300: this is located at 300 m asl (36º53’N, 3º24’W), beside the River Guadalfeo. The climate is characterized by hot summers and generally frost-free winters. The vegetation is composed of an allochthonous *Eucalyptus* sp. forest, a *Populus alba* forest, and several shrubby areas dominated by *Rosmarinus officinalis* and *Retama sphaerocarpa*, with *Genista* sp., *Artemisia* sp. and *Thymus* sp.

S700: this is located at 700 m asl (36º55’N , 3º26’W), beside a deep ravine carved by a stream. The climate is similar to that in S300. Trees are not abundant, although there are some extensive orchards of *Olea europaea* and solitary *Prunus dulcis* and *Ficus carica*. Shrubby vegetation consists mainly of *Cystus* sp., *Retama sphaerocarpa*, *Rosmarinus officinalis* and *Genista* sp.; *Foeniculum vulgaris*, *Capparis spinosa* and *Ruta graveolens* are also present.

S1200: this is located at 1200 m asl (36º56’N, 3º25’W). Some irrigation canals cross the ground. The climate consists of hot summers with infrequent frosts in winter. Dispersed *Castanea sativa* individuals represent the dominant tree vegetation, with some *Morus nigra* and *Ficus carica*. Shrub formations consist mainly of *Genista* sp., although *Cystus* sp. also appears.

S1700: this is located at 1700 m asl (36º57’N, 3º26’W), on steeply sloped terrain where the climate is characterized by warm summers and frequent frosts with snowfall in winter. The dominant trees species are *Quercus ilex* and *Q. pyrenaica*, while *Genista* sp. and *Cystus* sp. are abundant shrubs.

S2200: this is located at 2200 m asl (36º58’N, 3º19’W) around a thaw spring. The climate is characterized by warm summers and very frequent freezes with abundant snowfall in winter, covering the ground with a snow layer of variable thickness . Dense plantations of *Pinus sylvestris* reach this altitude; both in forest clearings and in meadows, the dominant shrub is *Genista florida*.

S2500: this is located at 2500 m asl (37º01’N, 3º19’W) around some thaw springs and not far from the Mulhacén river. The climate is similar to that in S2200, but harsher in winter. No trees appear, being above the timberline.

Creeper *Juniperus communis* and *Genista florida* sub-alpine meadows are the main shrubby formations here.

## A.3.-Habitat structure

We tried to choose places with landscapes as similar as possible, particularly with respect to vegetation cover and height. Nevertheless, flora varied all along the gradient. To measure the vegetation structure in each sampling station, we randomly set a 50-meter-long string, marked every meter, five times over the land, and recorded the absence or presence of vegetation, and the height and the kind of plant found at every one-meter mark of the string; plant height was classified as < 25 cm, 25 - 50 cm, and > 50 cm, because those plant-height values determine major habitat differences for the shrub-linked lizard *Psammodromus algirus*. Differences in vegetation cover and structure unavoidably appeared in this altitudinal gradient, as expected. However, two important components of the landscape, vegetation cover (Fig. A.S3) and vegetation height (Fig. A.S4), showed no clear pattern in altitude. These results suggest that all of our sampling stations comprised the shrubby vegetation physiognomy where *P. algirus* lives (Salvador, 2011).

## A.4.-Aknowledgements

The authors want to thank Manuel Pizarro for the maps (Fig. A.S1 and Fig. A.S2) for this Appendix.

### A.5.-References

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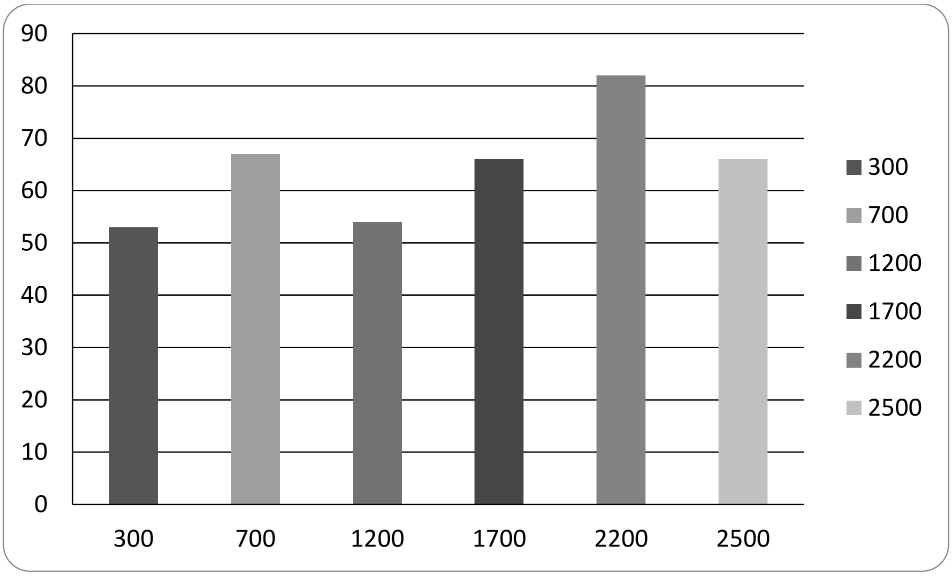

Fig. A.S3 - Percentage of ground surface covered by vegetation in each altitude (n = 253 measures in every altitude).

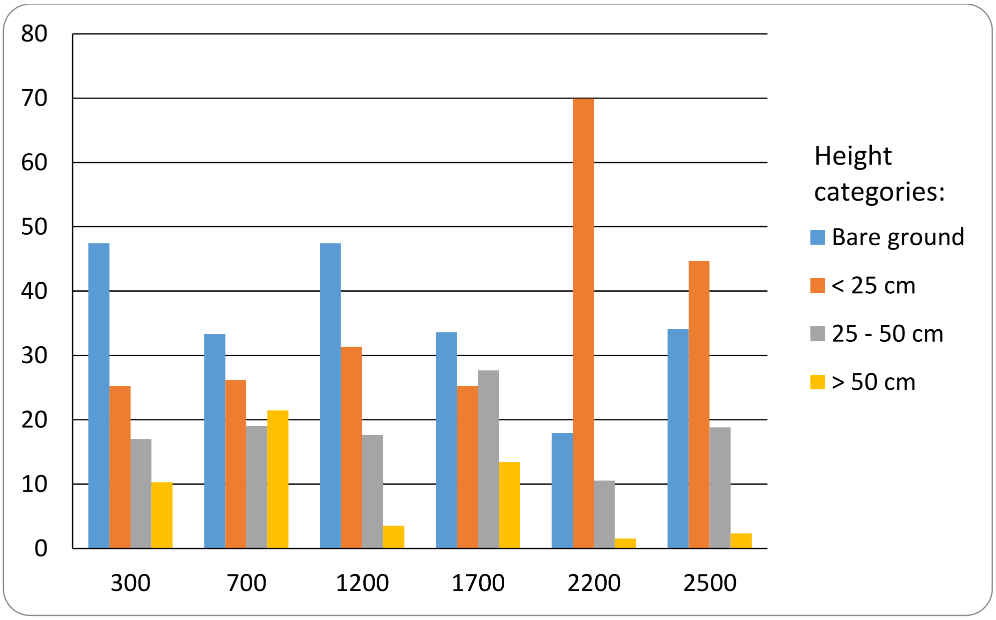

Fig. A.S4 - Percentage of ground surface covered for each vegetation-height category in altitude (n = 253 measures in every altitude).

# Appendix B

## B.1.-Thermal quality of the habitat ($d_e$)

Calculations of $d_e$ index from $T_{set}$ reckoned as 80% central values of $T_{sel}$ were similar to those reckoned as 50% central values. When $T_{set}$ was calculated from 80% central values of $T_{sel}$, $d_e$ followed very similar trends in elevation, microhabitat, and hour (Table B.S1; Fig. B.S1).

## B.2.-Accuracy of thermoregulation ($d_b$)

When $T_{set}$ was calculated from 80% central values of $T_{sel}$, $d_b$ followed very similar results in elevation, with no clear elevational pattern ($F_{5, 292} = 3.09$; $P = 0.01$; Fig. B.S2). Consequently, Spearman's correlation between $d_b$ and elevation was not significant (n = 6 populations; $r_s = -0.14$; $P = 0.79$). Nevertheless, differences in $d_b$ among reproductive status were not significant ($F_{2, 295} = 1.21$; $P = 0.30$; Fig. B.S3).

## B.3.-Effectiveness of thermoregulation

The index $d_e$-$d_b$ calculated from $T_{set}$ as 80% central values of $T_{sel}$ were similar to those based on 50% central values of $T_{sel}$ (Tables B.S2 and B.S3). We also calculated Spearman's correlations between elevation and $d_e$-$d_b$ for non-gravid females ($r_s = -0.77$; $P = 0.07$), gravid females ($r_s = -0.54$; $P = 0.27$), and males ($r_s = -0.61$; $P = 0.11$).

Moreover, results were similar for E [$1-(d_e/d_b)$] reckoned from $T_{set}$ as 50% (Table S4) and as 80% central values of $T_{sel}$ (Table B.S5). We also calculated Spearman's correlations between elevation and E calculated from $T_{set}$ as 50% central values of $T_{sel}$ for non-gravid females ($r_s = -0.77$; $P = 0.07$), gravid females ($r_s = -0.77$; $P = 0.07$) and males ($r_s = -0.49$; $P = 0.33$), and between elevation and E calculated from $T_{set}$ as 80% central values of $T_{sel}$ for non-gravid females ($r_s = -0.77$; $P = 0.07$), gravid females ($r_s = -0.54$; $P = 0.27$) and males ($r_s = -0.71$; $P = 0.11$).

|  | *D.f.* | *F* |
|---|---|---|
| **Elevation** | 5, 881 | 96.09*** |
| **Hour** | 4, 881 | 29.27*** |
| **Microhabitat** | 2, 881 | 128.58*** |
| **Elevation*Hour** | 20, 881 | 33.56*** |
| **Elevation*Microhabitat** | 10, 881 | 48.54*** |
| **Hour*Microhabitat** | 8, 881 | 42.74*** |

Table B.S1 - Results of three-way ANOVA testing the effect of elevation, hour, microhabitat, and their interactions on thermal quality of the habitat ($d_e$). Symbols indicate: *** for $P < 0.001$. These $d_e$ data were obtained from $T_{set}$ calculated as 80% central values of $T_{sel}$.

| Elevation (m asl) | Reproductive status | n | 10:00 | 11:00 | 12:00 | 13:00 | 14:00 | Full Sun | Partial Sun | Shadow |
|---|---|---|---|---|---|---|---|---|---|---|
| **300** | **Non-Gravid Female** | 22 | 0.742 | 0.809 | 0.719 | 0.728 | 0.853 | 0.817 | 0.766 | 0.742 |
| | **Gravid Female** | 14 | 0.531 | 0.640 | 0.482 | 0.481 | 0.697 | 0.650 | 0.547 | 0.509 |
| | **Male** | 34 | 0.488 | 0.638 | 0.525 | 0.552 | 0.731 | 0.668 | 0.581 | 0.529 |
| **700** | **Non-Gravid Female** | 10 | 0.569 | 0.814 | 0.626 | 0.563 | 0.619 | 0.723 | 0.646 | 0.573 |
| | **Gravid Female** | 12 | 0.656 | 0.845 | 0.691 | 0.632 | 0.624 | 0.766 | 0.704 | 0.623 |
| | **Male** | 12 | 0.300 | 0.716 | 0.485 | 0.430 | 0.440 | 0.601 | 0.476 | 0.354 |
| **1200** | **Non-Gravid Female** | 9 | 0.488 | 0.775 | -0.010 | -0.074 | 0.564 | 0.513 | 0.502 | 0.562 |
| | **Gravid Female** | 10 | 0.520 | 0.774 | -0.233 | -0.041 | 0.593 | 0.515 | 0.512 | 0.571 |
| | **Male** | 12 | 0.769 | 0.910 | 0.600 | 0.622 | 0.801 | 0.800 | 0.790 | 0.818 |
| **1700** | **Non-Gravid Female** | 10 | 0.623 | 0.801 | -0.704 | 0.425 | 0.078 | 0.561 | 0.429 | 0.598 |
| | **Gravid Female** | 5 | 0.531 | 0.736 | -1.654 | 0.290 | -0.509 | 0.415 | 0.235 | 0.479 |
| | **Male** | 10 | 0.579 | 0.805 | -0.479 | 0.452 | 0.090 | 0.565 | 0.413 | 0.590 |
| **2200** | **Non-Gravid Female** | 19 | 0.707 | 0.562 | 0.384 | 0.642 | 0.057 | 0.563 | 0.524 | 0.459 |
| | **Gravid Female** | 11 | 0.565 | 0.274 | 0.013 | 0.472 | -0.772 | 0.319 | 0.251 | 0.144 |
| | **Male** | 36 | 0.699 | 0.625 | 0.494 | 0.675 | 0.092 | 0.600 | 0.560 | 0.492 |
| **2500** | **Non-Gravid Female** | 20 | 0.146 | -0.129 | 0.345 | 0.177 | -0.040 | 0.235 | -0.199 | -0.334 |
| | **Gravid Female** | 15 | 0.417 | 0.211 | 0.555 | 0.470 | 0.263 | 0.481 | 0.181 | 0.071 |
| | **Male** | 37 | 0.343 | 0.266 | 0.556 | 0.403 | 0.168 | 0.448 | 0.145 | 0.049 |

Table B.S2 - Effectiveness of thermoregulation ($d_e$-$d_b$) calculated for each hour and each microclimate for each reproductive condition at each elevation. These results were obtained from $T_{set}$ considered as 50% central values of $T_{sel}$.

| **Elevation (m asl)** | **Reproductive status** | **n** | **10:00** | **11:00** | **12:00** | **13:00** | **14:00** | **Full Sun** | **Partial Sun** | **Shadow** |
|---|---|---|---|---|---|---|---|---|---|---|
| **300** | **Non-Gravid Female** | 22 | 0.828 | 0.885 | 0.838 | 0.833 | 0.914 | 0.891 | 0.860 | 0.839 |
| | **Gravid Female** | 10 | 0.556 | 0.830 | 0.660 | 0.560 | 0.641 | 0.744 | 0.659 | 0.576 |
| | **Male** | 9 | 0.457 | 0.780 | -0.633 | -0.251 | 0.539 | 0.483 | 0.458 | 0.539 |
| **700** | **Non-Gravid Female** | 10 | 0.635 | 0.822 | -1.351 | 0.417 | 0.104 | 0.584 | 0.427 | 0.614 |
| | **Gravid Female** | 19 | 0.743 | 0.631 | 0.442 | 0.675 | 0.171 | 0.618 | 0.567 | 0.492 |
| | **Male** | 20 | 0.133 | -0.070 | 0.372 | 0.147 | 0.040 | 0.260 | -0.201 | -0.398 |
| **1200** | **Non-Gravid Female** | 14 | 0.548 | 0.663 | 0.467 | 0.490 | 0.726 | 0.669 | 0.574 | 0.521 |
| | **Gravid Female** | 12 | 0.664 | 0.858 | 0.686 | 0.614 | 0.647 | 0.779 | 0.708 | 0.625 |
| | **Male** | 10 | 0.517 | 0.791 | -0.477 | -0.239 | 0.590 | 0.519 | 0.502 | 0.574 |
| **1700** | **Non-Gravid Female** | 5 | 0.574 | 0.778 | -2.095 | 0.269 | -0.502 | 0.476 | 0.257 | 0.524 |
| | **Gravid Female** | 11 | 0.599 | 0.347 | 0.031 | 0.463 | -1.063 | 0.344 | 0.266 | 0.173 |
| | **Male** | 15 | 0.458 | 0.217 | 0.594 | 0.454 | 0.131 | 0.490 | 0.166 | 0.074 |
| **2200** | **Non-Gravid Female** | 34 | 0.529 | 0.680 | 0.589 | 0.558 | 0.731 | 0.691 | 0.606 | 0.557 |
| | **Gravid Female** | 12 | 0.248 | 0.711 | 0.481 | 0.381 | 0.265 | 0.582 | 0.438 | 0.251 |
| | **Male** | 12 | 0.853 | 0.947 | 0.680 | 0.755 | 0.873 | 0.873 | 0.867 | 0.887 |
| **2500** | **Non-Gravid Female** | 10 | 0.677 | 0.860 | -0.704 | 0.595 | 0.083 | 0.664 | 0.540 | 0.690 |
| | **Gravid Female** | 36 | 0.726 | 0.664 | 0.458 | 0.718 | -0.078 | 0.625 | 0.585 | 0.548 |
| | **Male** | 37 | 0.573 | 0.528 | 0.686 | 0.610 | 0.420 | 0.630 | 0.421 | 0.377 |

Table B.S3 - Effectiveness of thermoregulation calculated as $d_e$-$d_b$ for each hour and each microclimate for each reproductive condition at each population. These results were obtained from $T_{set}$ considered as 80% central values of $T_{sel}$.

| **Elevation (m asl)** | **Reproductive status** | **n** | **10:00** | **11:00** | **12:00** | **13:00** | **14:00** | **Full Sun** | **Partial Sun** | **Shadow** |
|---|---|---|---|---|---|---|---|---|---|---|
| **300** | **Non-Gravid Female** | 22 | 4.070 | 5.998 | 3.615 | 3.788 | 8.173 | 6.319 | 4.636 | 4.065 |
| | **Gravid Female** | 14 | 2.954 | 4.624 | 2.426 | 2.417 | 5.986 | 4.836 | 3.153 | 2.700 |
| | **Male** | 34 | 2.467 | 4.559 | 2.852 | 3.183 | 7.018 | 5.196 | 3.581 | 2.904 |
| **700** | **Non-Gravid Female** | 10 | 2.754 | 9.140 | 3.481 | 2.686 | 3.390 | 5.448 | 3.806 | 2.789 |
| | **Gravid Female** | 12 | 3.241 | 9.264 | 3.815 | 2.929 | 2.827 | 5.583 | 4.054 | 2.808 |
| | **Male** | 12 | 1.315 | 7.738 | 2.892 | 2.313 | 2.410 | 4.617 | 2.784 | 1.679 |
| **1200** | **Non-Gravid Female** | 9 | 3.213 | 11.582 | -0.035 | -0.232 | 4.350 | 3.542 | 3.391 | 4.324 |
| | **Gravid Female** | 10 | 3.594 | 11.375 | -0.626 | -0.129 | 4.817 | 3.522 | 3.478 | 4.403 |
| | **Male** | 12 | 4.359 | 13.224 | 1.966 | 2.151 | 5.254 | 5.238 | 4.935 | 5.870 |
| **1700** | **Non-Gravid Female** | 10 | 4.925 | 12.022 | -1.231 | 2.205 | 0.253 | 3.804 | 2.240 | 4.426 |
| | **Gravid Female** | 5 | 4.420 | 10.869 | -2.430 | 1.592 | -1.314 | 2.770 | 1.199 | 3.585 |
| | **Male** | 10 | 3.912 | 11.737 | -0.920 | 2.341 | 0.281 | 3.693 | 1.996 | 4.087 |
| **2200** | **Non-Gravid Female** | 19 | 5.810 | 3.084 | 1.499 | 4.309 | 0.145 | 3.099 | 2.649 | 2.042 |
| | **Gravid Female** | 11 | 4.858 | 1.407 | 0.048 | 3.339 | -1.627 | 1.754 | 1.250 | 0.630 |
| | **Male** | 36 | 4.999 | 3.584 | 2.100 | 4.471 | 0.217 | 3.219 | 2.738 | 2.082 |
| **2500** | **Non-Gravid Female** | 20 | 0.780 | -0.522 | 2.399 | 0.981 | -0.173 | 1.399 | -0.755 | -1.141 |
| | **Gravid Female** | 15 | 2.180 | 0.813 | 3.807 | 2.699 | 1.089 | 2.826 | 0.675 | 0.235 |
| | **Male** | 37 | 1.706 | 1.181 | 4.083 | 2.207 | 0.659 | 2.653 | 0.554 | 0.169 |

Table B.S4 - Effectiveness of thermoregulation calculated as E [1-($d_e/d_b$)] for each hour and each microclimate for each reproductive condition at each population. These results were obtained from $T_{set}$ considered as 50% central values of $T_{sel}$.

| Elevation (m asl) | Reproductive status | n | 10:00 | 11:00 | 12:00 | 13:00 | 14:00 | Full Sun | Partial Sun | Shadow |
|---|---|---|---|---|---|---|---|---|---|---|
| **300** | **Non-Gravid Female** | 22 | 3.723 | 5.964 | 4.000 | 3.858 | 8.243 | 6.350 | 4.740 | 4.013 |
| | **Gravid Female** | 14 | 2.724 | 4.428 | 1.972 | 2.162 | 5.968 | 4.538 | 3.030 | 2.448 |
| | **Male** | 34 | 2.454 | 4.633 | 3.128 | 2.752 | 5.930 | 4.889 | 3.365 | 2.741 |
| **700** | **Non-Gravid Female** | 10 | 2.219 | 8.625 | 3.437 | 2.249 | 3.158 | 5.127 | 3.423 | 2.403 |
| | **Gravid Female** | 12 | 2.898 | 8.839 | 3.204 | 2.336 | 2.694 | 5.163 | 3.561 | 2.447 |
| | **Male** | 12 | 0.987 | 7.367 | 2.772 | 1.841 | 1.078 | 4.162 | 2.336 | 1.001 |
| **1200** | **Non-Gravid Female** | 9 | 2.612 | 11.027 | -1.204 | -0.623 | 3.638 | 2.903 | 2.625 | 3.631 |
| | **Gravid Female** | 10 | 3.139 | 11.106 | -0.948 | -0.566 | 4.227 | 3.175 | 2.964 | 3.952 |
| | **Male** | 12 | 4.375 | 13.337 | 1.595 | 2.316 | 5.178 | 5.183 | 4.880 | 5.915 |
| **1700** | **Non-Gravid Female** | 10 | 4.401 | 11.662 | -1.455 | 1.808 | 0.295 | 3.550 | 1.890 | 4.022 |
| | **Gravid Female** | 5 | 4.220 | 10.989 | -2.121 | 1.154 | -1.047 | 2.844 | 1.085 | 3.456 |
| | **Male** | 10 | 4.139 | 12.179 | -0.817 | 2.901 | 0.180 | 3.904 | 2.323 | 4.393 |
| **2200** | **Non-Gravid Female** | 19 | 5.406 | 3.202 | 1.484 | 3.889 | 0.387 | 3.033 | 2.453 | 1.816 |
| | **Gravid Female** | 11 | 4.593 | 1.637 | 0.097 | 2.648 | -1.584 | 1.615 | 1.112 | 0.644 |
| | **Male** | 36 | 4.773 | 3.554 | 1.519 | 4.576 | -0.131 | 3.002 | 2.538 | 2.180 |
| **2500** | **Non-Gravid Female** | 20 | 0.616 | -0.261 | 2.376 | 0.691 | 0.166 | 1.410 | -0.670 | -1.141 |
| | **Gravid Female** | 15 | 2.122 | 0.696 | 3.663 | 2.085 | 0.377 | 2.405 | 0.499 | 0.200 |
| | **Male** | 37 | 2.672 | 2.224 | 4.354 | 3.106 | 1.443 | 3.380 | 1.447 | 1.205 |

Table B.S5 - Effectiveness of thermoregulation calculated as E [1-($d_e$/$d_b$)] for each hour and each microclimate for each reproductive condition at each population. These results were obtained from $T_{set}$ considered as 80% central values of $T_{sel}$.

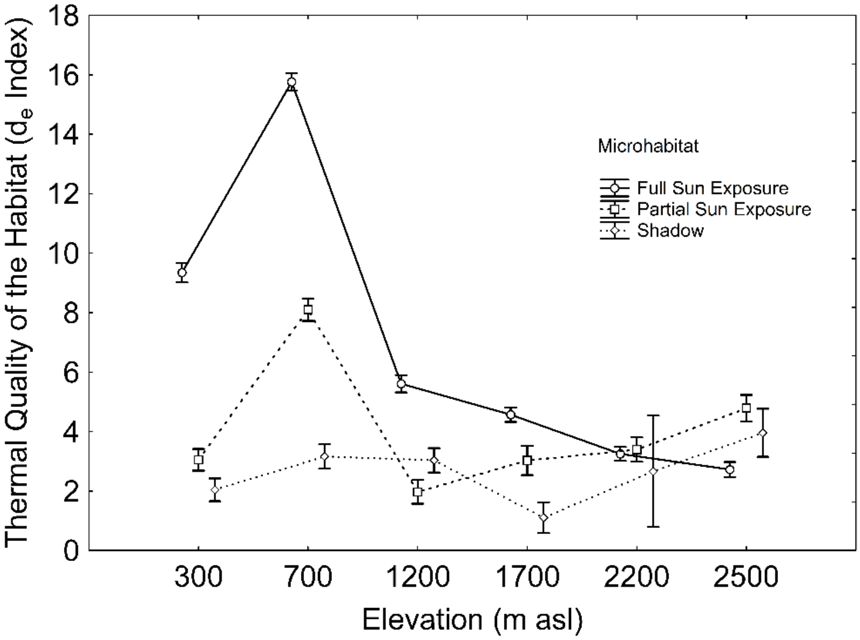

Fig. B.S1a

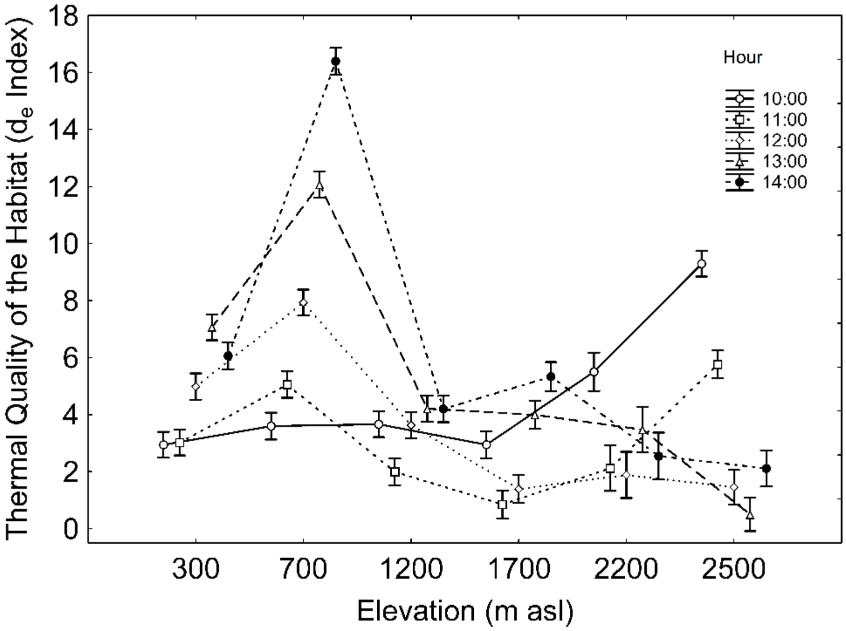

Fig. B.S1b

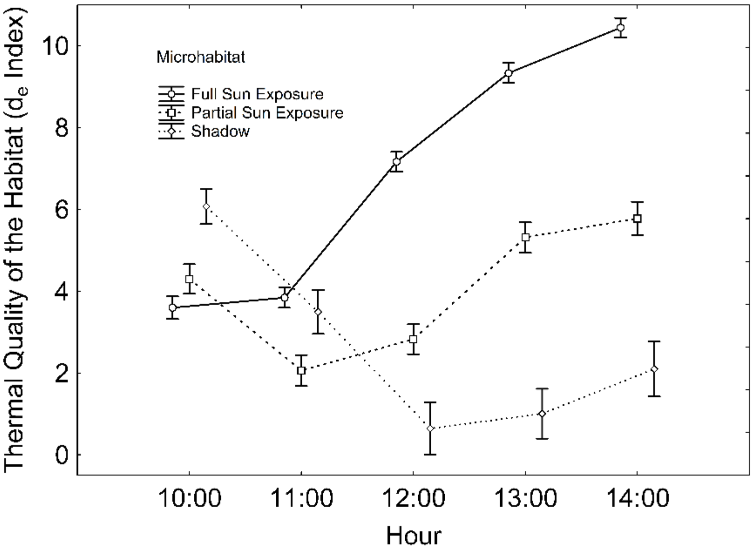

Fig. B.S1c

Fig. B.S1 - Thermal quality of the habitat (measured as $d_e$ index) increases as $d_e$ value approaches 0. These results were calculated from $T_{set}$ considered as 80% central values of $T_{sel}$, and were very similar to those calculated from $T_{set}$ considered as 50% central values of $T_{sel}$. In low elevations, thermal quality of the habitat is maximal under a shadow, but in high elevations full exposition to sun is more suitable (Fig.B. S1a). In low elevations, habitat thermal quality is maximal at the beginning of the day, while in high elevation it increases at central day hours (Fig. B.S1b). Maximal thermal quality of the habitat occurred in fully sun-exposed microhabitats at the beginning of the day, shifting gradually to partially exposed and finally to shadowy microhabitats (Fig. B.S1c). Vertical bars represent standard errors.

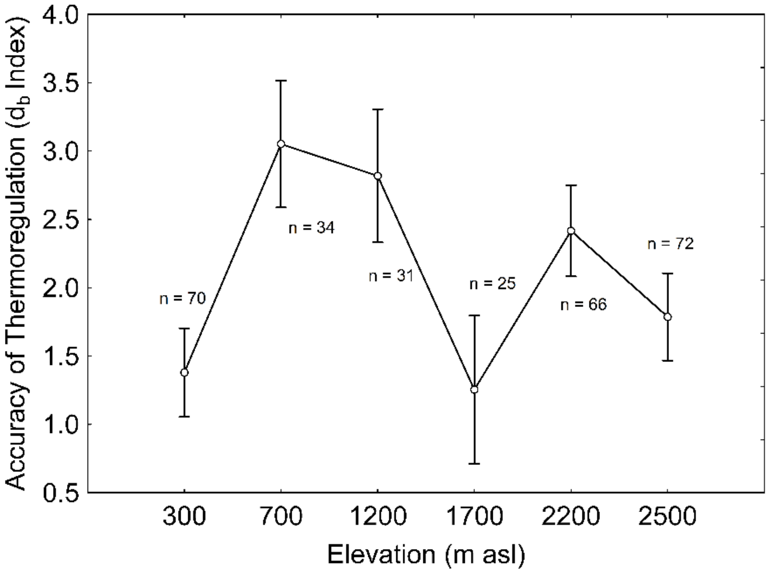

Fig. B.S2 - Accuracy of thermoregulation (measured as $d_b$ index) increased as $d_b$ value approached 0, and showed no clear elevational pattern. These results were calculated from $T_{set}$ considered as 80% central values of $T_{sel}$. Vertical bars represent standard errors.

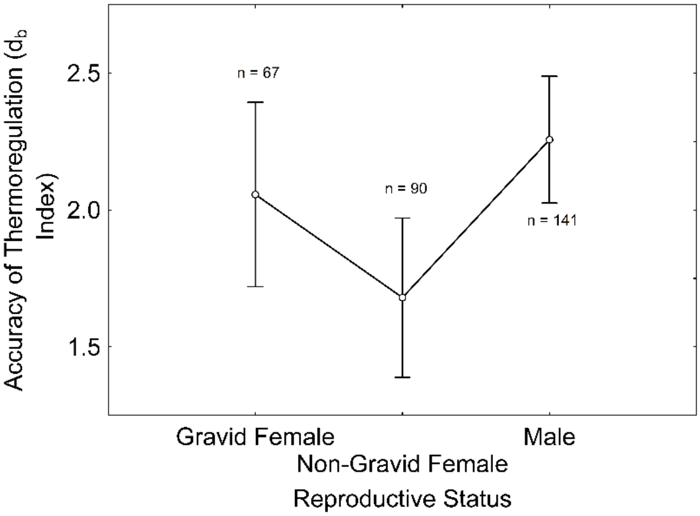

Fig. B.S3 - Accuracy of thermoregulation (measured as $d_b$ index) increased as $d_b$ value approached 0. These results were calculated from $T_{set}$ considered as 80% central values of $T_{sel}$. Contrasting with the results calculated from $T_{set}$ considered as 50% central values of $T_{sel}$, differences among individuals in different reproductive status were not significant. Vertical bars represent standard errors.

## Appendix C

For a better detection of elevational trends, we grouped sampling plots into three elevational belts: low elevations (plots at 300-700 m a.s.l.), mid elevations (plots at 1200-1700 m a.s.l.), and high elevations (plots at 2200-2500 m a.s.l.). This grouping was justified by the similarity between belts in different population traits, such as activity patterns and morphology (see Table SA). Nevertheless, elevation had no significant effect on lizard sprint speed or immune response, even when body mass, SVL, FLL, and HLL were larger in high-elevation lizards (Table SB). A) Average values ± SE of measured variables for low, mid and high elevation lizards. B) Results of a three-way factorial Anova examining variation in body mass, snout-vent length (SVL), foreleg length (FLL), hind-leg length (HLL), maximal sprint speed prior the treatment (previous speed), maximal sprint speed post-treatment (post-treatment speed), and the difference in maximal sprint speed, according to treatment, sex, and elevation. *F*-values are shown. Symbols indicate: § for 0.10 > $P$ > 0.05, * for $P$ < 0.05, and *** for $P$ < 0.001. In bold, significant results. Note that for body mass and SVL, data of one lizard were lost, and therefore the *d.f.* error is 60 for these variables.

| **Variable** | **Low elevations (*n* = 21)** | **Mid elevations (*n* = 17)** | **High elevations (*n* = 34)** |
|---|---|---|---|
| **Body mass (g)** | 5.33 ± 0.50 | 6.81 ± 0.48 | 9.33 ± 0.34 |
| **SVL (mm)** | 60.93 ± 1.56 | 64.51 ± 1.51 | 72.18 ± 1.07 |
| **Foreleg length (mm)** | 21.18 ± 0.38 | 21.65 ± 0.37 | 22.94 ± 0.26 |
| **Hind-leg length (mm)** | 34.15 ± 0.68 | 35.06 ± 0.66 | 36.40 ± 0.47 |
| **Previous speed (cm/s)** | 146.66 ± 13.46 | 139.30 ± 13.04 | 141.64 ± 9.26 |

Table C.S1

| | *D.f.* | **Mass** | **SVL** | **FLL** | **HLL** | **Previous speed** | **Post-treatment speed** | **Difference in speed** |
|---|---|---|---|---|---|---|---|---|
| **Sex** | 1 | 3.53§ | 0.26 | **13.57***** | **26.20***** | 3.62§ | 2.92§ | 0.02 |
| **Elevation** | 2 | **24.19***** | **20.34***** | **8.81***** | **4.06*** | 0.08 | 1.08 | 0.86 |
| **Treatment** | 1 | 0.06 | 0.02 | 0.25 | 0.80 | 0.02 | **4.22*** | **5.25*** |
| **Sex*Elevation** | 2 | 1.12 | 1.36 | 1.36 | 2.30 | 2.59§ | 0.14 | 1.58 |
| **Sex*Treatment** | 1 | 0.34 | 0.35 | 0.01 | 0.55 | 2.88 | 3.57§ | 0.07 |
| **Elevation*Treatment** | 2 | 0.50 | 0.05 | 0.20 | 0.40 | 0.19 | 0.05 | 0.18 |
| **Sex*Elevation*Treatment** | 2 | 1.19 | 1.07 | 0.90 | 0.20 | 0.24 | 0.46 | 0.18 |
| **Error** | 61 | | | | | | | |

Table C.S2

## Conclusiones

La lagartija colilarga, *P. algirus*, se mostró como un termorregulador eficiente en este gradiente altitudinal de 2200 metros en Sierra Nevada. Ha desarrollado estrategias, como la mayor inercia térmica, que le permitieron explotar suficientemente el ambiente térmico subóptimo de las cotas altas. De este modo, consiguió completar su ciclo vital en sincronía a lo largo de todo el gradiente, pese a las limitaciones térmicas. Además, las lagartijas de zonas altas priorizaron la adquisición de calor cuando sufrieron un desafío inmunitario, lo que pone de manifiesto la importancia de la termorregulación en esa zona térmicamente deficiente. Por otra parte, la altitud no afectó a la velocidad de carrera, ni al óptimo térmico de su *performance*. De la misma manera, el sistema inmunitario merma la velocidad de carrera de los machos por igual en todo el gradiente de altitud.

1.- La lagartija colilarga mostró patrones de actividad similares a lo largo de todo el gradiente altitudinal de 2200 metros en Sierra Nevada. No obstante, la actividad en poblaciones altas fue máxima al principio de la estación a pesar de que la temperatura fue mayor en los meses posteriores. Esto contrasta con lo encontrado en poblaciones medias y bajas, donde el pico de actividad se dio a mitad de la estación, coincidiendo con el máximo térmico. Probablemente las lagartijas de poblaciones altas mostraron mayor actividad al comienzo de la estación por las limitaciones en el tiempo para la reproducción. De hecho, los neonatos aparecieron de forma prácticamente sincrónica en todo el gradiente.

2.- Esta especie se ha revelado como un animal termoconservador en este sistema, dado que su temperatura corporal en campo no siguió ningún patrón en altitud, con diferencias de apenas 1ºC entre la población a menor y a mayor altitud. En gradiente térmico, la temperatura seleccionada mostró una tendencia a disminuir en altitud, si bien la diferencia entre la población a menor y a mayor altitud apenas superó los 0,5ºC, mientras que la temperatura ambiental disminuyó significativamente más de 7ºC. No obstante, los resultados muestran que las lagartijas podían termorregular correctamente simplemente por seleccionar el microhábitat adecuado a la hora adecuada del día, siendo las zonas de baja montaña las que presentaron el hábitat de peor calidad térmica para esta especie. A pesar de esto, el hecho de que las temperaturas seleccionadas fueran siempre mayores que las presentadas en el campo sugiere que otros factores aparte de la disponibilidad térmica podrían constreñir la capacidad de termorregulación de estas lagartijas. De hecho, la temperatura corporal sólo está limitada por la ambiental en las poblaciones más elevadas. En cuanto al estado reproductor, sólo las hembras posparto mostraron diferencias con respecto a los otros grupos: seleccionaron temperaturas más altas, lo cual probablemente les permite mejorar su condición corporal tras el esfuerzo reproductor. Igualmente, las hembras grávidas seleccionaron temperaturas más bajas conforme se aproximaba el momento de la puesta. Estos resultados sugieren que este sistema está regido por la hipótesis estática de la adaptación de los ectotermos al ambiente térmico, ya que no existen variaciones sustanciales en la biología térmica de las lagartijas a pesar de que sí las hay en las constricciones térmicas ambientales.

3.- Las escasas diferencias entre la temperatura corporal durante la actividad en el campo a lo largo del gradiente de altitud, así como en la temperatura seleccionada en gradiente térmico, a pesar de la evidente disminución en la calidad térmica del hábitat, sugieren que esta lagartija desarrolla diferentes estrategias de termorregulación que le permiten optimizar el aprovechamiento de los recursos térmicos. Las lagartijas de poblaciones elevadas fueron de mayor tamaño, lo que se corresponde con las clinas de Bergmann, que prevén mayor talla corporal en hábitats fríos, de manera que disminuye la relación superficie/volumen corporal y se reduce la pérdida de calor. Paralelamente, quedó demostrado, por primera vez, que el mecanismo subyacente a estas clinas es efectivamente la mayor inercia térmica, ya que los ejemplares de poblaciones elevadas, de mayor tamaño, presentaron también tasas de enfriamiento más bajas. Mientras tanto, las de calentamiento se mantuvieron constantes, probablemente debido a que los ejemplares de zonas altas son más oscuros y compensan de este modo el retraso en el calentamiento causado por una masa mayor.

4.- Por su parte, un desafío inmune sólo afectó a la temperatura seleccionada por los ejemplares de poblaciones medias y bajas, que respondieron al reto inmune con hipotermia, lo que probablemente les permite ahorrar energía, al no sumar a los costes propios de la termorregulación los de la respuesta inmune. Sin embargo, las lagartijas de poblaciones elevadas no modificaron sus preferencias térmicas a pesar del desafío inmune. Los costes en *fitness* de no termorregular en un hábitat térmicamente deficiente, donde el tiempo para la reproducción es limitado y por tanto se perderían valiosas ocasiones para la reproducción, podrían ser mayores que los de termorregular, a pesar de los costes añadidos del desafío inmune. Este hallazgo sugiere que las lagartijas ajustan su respuesta térmica a un desafío inmune según el balance costes/beneficios de la termorregulación. Igualmente, señalan que la termorregulación es prioritaria en hábitats donde está limitada.

5.- La velocidad de carrera se mostró correlacionada de forma positiva con la longitud de las patas, especialmente las traseras, y de forma negativa con la masa. Este hallazgo sugiere que la acumulación de grasas, necesaria para la supervivencia a la hibernación, está comprometida con la capacidad de huir de los depredadores. No obstante, las hembras grávidas no fueron más lentas que las no grávidas, lo cual apunta a posibles adaptaciones fisiológicas que les permiten compensar el exceso de masa corporal debido a los huevos. Sin embargo, los machos sí fueron más rápidos que las hembras -gracias a que poseen patas más largas-, probablemente a causa de la mayor visibilidad de los machos para los depredadores, ya que la defensa del territorio o la búsqueda de pareja requieren una gran exposición a estos, aparte de verse favorecidas por una mayor velocidad. La altitud no tuvo ningún efecto sobre la velocidad.

6.- En cuanto al efecto de un desafío inmune sobre la velocidad de carrera, sólo afectó a los machos, que disminuyeron su velocidad. Este resultado muestra que la velocidad de carrera y el desafío inmune están sujetos a un compromiso en los machos, que es el sexo que más energía invierte en la primera, ya que ambos conllevan un gran consumo metabólico y proteínico. Por lo tanto, una infección incrementa el riesgo de depredación de los machos, o disminuye su *fitness* si evitan este riesgo permaneciendo refugiados, en lugar de defender su territorio, alimentarse o aparearse. Este efecto no mostró ningún patrón altitudinal.

7.- Por último, la velocidad de carrera fue mayor a temperaturas corporales óptimas que subóptimas. Los ejemplares de poblaciones elevadas no mostraron mejor desempeño que los de poblaciones de altitudes medias y bajas a pesar de la menor disponibilidad de temperatura, lo cual indica que las preferencias térmicas y la dependencia térmica del desempeño locomotor están coadaptadas. Por otra parte, las hembras grávidas, que a temperatura óptima no eran más lentas que las no grávidas, sí lo son a temperaturas subóptimas. Este hecho indica que los mecanismos fisiológicos que permiten a las hembras compensar la carga de los huevos dependen estrechamente de la temperatura corporal. Estos resultados confirman que la velocidad de carrera es altamente conservadora en altitud.