Universidad Autónoma de Tlaxcala

Posgrado en Ciencias Biológicas

La termorregulación como rasgo complejo ante la variación estacional de rasgos subordinados en un gradiente altitudinal en la lagartija del mezquite

TESIS

QUE PARA OBTENER EL GRADO ACADÉMICO DE

DOCTOR EN CIENCIAS BIOLÓGICAS

Presenta

Juan Carlos González Morales

Codirectores
Dr. Víctor Manuel Fajardo Guadarrama
Dr. Amando Bautista Ortega

Tlaxcala, Tlax.

Julio, 2022

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Este trabajo fue realizado bajo la codirección del Dr. Víctor Manuel Fajardo Guadarrama y el Dr. Amando Bautista Ortega y la asesoría de mi comité tutoral en el Centro Tlaxcala de Biología de la Conducta (CTBC) de la Universidad Autónoma de Tlaxcala, y en el Laboratorio de Ecofisiología de Fauna Silvestre del Instituto para la Conservación de la Cordillera ante el Cambio Climático, Toluca, Estado de México.

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COMITÉ ACADÉMICO
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Sirva este medio para describir el proceso de revisión de la tesis realizada por el estudiante Juan Carlos González Morales titulada “La termorregulación como rasgo complejo ante la variación estacional de rasgos subordinados en un gradiente altitudinal en la lagartija del mezquite” para optar por su grado de **Doctor en Ciencias Biológicas**.

El documento de la tesis de Juan Carlos González Morales fue revisado por mí como directora de tesis antes de presentarse en cada examen tutoral y, posteriormente a los exámenes tutorales, los miembros de su comité tutoral realizaron también sus respectivas observaciones. De manera que el documento, llevó un proceso de revisión por varios profesores expertos en el tema. En el mes de abril, el documento final de la tesis fue procesado con el programa Plagiarism no encontrando similitudes ni coincidencias con otros textos.

Por lo anterior, confirmo que **la estudiante no incurrió en ninguna práctica no deseable** en la escritura de la tesis, ni en ninguno de los artículos derivados de su trabajo de investigación doctoral.

Sin más por el momento, reciban atentos saludos.

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Investigar y Educar para Conservar
“2022 año Internacional de las Ciencias Básicas y el Desarrollo Sostenible”

Dr. Victor Manuel Fajardo Guadarrama
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PRESENTE

Los abajo firmantes, miembros del jurado evaluador del proyecto de tesis que **Juan Carlos González Morales** realiza para la obtención del grado de **Doctor en Ciencias Biológicas**, expresamos que, habiendo revisado la versión final del documento de tesis, damos la aprobación para que ésta sea impresa y defendida en el examen correspondiente. El título que llevará es **"La termorregulación como rasgo complejo ante la variación estacional de rasgos subordinados en un gradiente altitudinal en la lagartija del Mesquite".**

Sin otro particular, aprovechamos para enviarle un cordial saludo.

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Resumen

Los vertebrados ectotermos, como las lagartijas, ante la baja producción interna de calor por vías fisiológicas lo adquieren de fuentes ambientales. Por ejemplo, exponiéndose a la radiación solar o colocándose en sustratos con una temperatura más alta o baja. Es decir, la termorregulación conductual es la forma principal de regulación de la temperatura corporal (*Tc*). La *Tc* tiene un efecto prominente en los procesos biológicos de las lagartijas, por lo que deben de alcanzar y mantener un rango de *Tc* para optimizar procesos como la locomoción, digestión y crecimiento. La dependencia de dicha característica, le da un papel central como rasgo complejo. La regulación de la *Tc* en lacertilios puede ser lábil y modificarse de acuerdo con el tipo de ambiente o puede ser conservada, en la que las preferencias térmicas generalmente son similares entre especies cercanas o en la misma especie. Los rasgos complejos a su vez, están compuestos por rasgos subordinados en niveles fenotípicos inferiores (sistemas, órganos, tejidos o células) a la conducta. A la variación de dichos rasgos se le ha asociado el mantener lo más constante posible a los rasgos complejos. Para esta tesis, se seleccionó a la lagartija *Sceloporus grammicus* como modelo de estudio, ya que presenta una distribución altitudinal que va desde los 500 m y supera los 4000 m, por lo que de forma natural está habituada a distintos ambientes térmicos. Lo anterior, aunado al hecho de que esta especie se ha considerado térmicamente conservativa, la convierte en un modelo adecuado para evaluar si la termorregulación como rasgo complejo se mantiene de esa forma por la variación estacional de los rasgos subordinados. Para comprobar lo anterior, se seleccionaron tres poblaciones (2500, 3400 y 4300 m) que visitamos de forma estacional. De dichas poblaciones obtuvimos la temperatura de selección, la velocidad máxima de carrera o "*sprint*", las tasas térmicas y la reflectancia dorsal.

El objetivo de esta investigación fue comparar los rasgos complejos (temperatura de selección y velocidad de *sprint*) por elevación y estación del año e indagar si los rasgos subordinados (reflectancia dorsal y tasas térmicas) variaban con el fin de mantener estables a los rasgos complejos a pesar de las fluctuaciones térmicas del ambiente. En cada uno de los siguientes capítulos se muestra el trabajo realizado durante la tesis doctoral.

Capítulo 1: En lagartijas que habitan en ambientes térmicamente distintos, como ocurre en gradientes de altitud, puede esperarse que las poblaciones de alta elevación que están expuestas a temperaturas más bajas, presenten costos altos asociados con la termorregulación. Podría darse una disyuntiva entre el tiempo que se invierte en termorregular y el tiempo que se invierte en otras actividades, como el forrajeo o la reproducción. No obstante, algunos mecanismos auxiliares de ganancia de calor podrían mitigar dichos costos. Por ejemplo, según la hipótesis del melanismo térmico, la menor reflectancia dorsal en organismos que habitan en zonas con temperaturas ambientales bajas, puede incrementar la absorción de calor respecto a organismos claros o con alta reflectancia dorsal. Nosotros evaluamos si las tasas de calentamiento y de enfriamiento, la retención de calor y la ganancia neta de calor, difieren entre poblaciones de diferente altitud durante el verano. Evaluamos los potenciales cambios en la reflectancia del dorso inducidos por los cambios en la temperatura corporal, y la relación de esta con las tasas térmicas. Nuestros resultados mostraron que, durante el verano, no existen diferencia en las tasas de calentamiento y de enfriamiento, ganancia neta y retención de calor entre poblaciones de diferente altitud. Por otro lado, las lagartijas de mayor elevación fueron más oscuras que sus conespecíficas de baja y media altitud. Finalmente, a pesar de que las tasas térmicas no diferían significativamente entre altitudes, de forma general e intra poblacional, las lagartijas más oscuras y pequeñas tuvieron las tasas de calentamiento y de enfriamiento más grandes, así como la ganancia neta de calor. Estos resultados nos permiten dilucidar que la hipótesis del melanismo térmico se acepta en *Sceloporus grammicus*.

Capítulo 2: La velocidad de escape máxima o velocidad de "*sprint*" se considera como un rasgo de rendimiento (desempeño o *performance*) que está implicado en el escape a depredadores, obtención de alimento y como señal de estatus social. En lagartijas, dicho rasgo es dependiente de la temperatura corporal, por lo que se optimiza a cierta temperatura. Tomando en cuenta los costos asociados con la termorregulación en ambientes de alta elevación, nosotros predecíamos que a temperaturas corporales bajas (10 y 20 °C), la velocidad de sprint fuera mayor en las lagartijas de alta elevación respecto a aquellas provenientes de la zona baja e intermedia. Por otra parte, la reflectancia dorsal puede funcionar como una forma de evasión en la que las lagartijas pueden pasar desapercibidas ante depredadores mientras están expuestas durante el

proceso de obtención de calor por las mañanas. Por lo que es de esperarse una relación negativa entre la velocidad de "*sprint*" y la reflectancia dorsal. Nosotros evaluamos si la velocidad de sprint y la reflectancia dorsal de las lagartijas diferían a través del gradiente altitudinal (2500, 3400 y 4300 m). Nuestros resultados mostraron que, a mayor temperatura, la velocidad de sprint era más alta en las tres elevaciones. Sin embargo, solo a 30 °C encontramos que las lagartijas de la población alta eran más rápidas, mientras que a 10 y 20 °C no apreciamos diferencia entre elevaciones. Finalmente, solo a 20 °C se relacionó significativamente la velocidad de sprint y la reflectancia dorsal, las lagartijas más claras eran más rápidas.

Capítulo 3: La variación de la temperatura ambiental suele ser más marcada en zonas templadas respecto a zonas tropicales, por lo que la termorregulación en lagartijas podría tener costos diferenciales por estación, más aún en lagartijas que mantienen los mismos requerimientos térmicos a pesar de distribuirse en un rango amplio de elevación. Nosotros evaluamos si algunos rasgos térmicos como la temperatura preferida en laboratorio, temperatura corporal en campo, precisión y eficiencia de la termorregulación permanecen estáticos a lo largo del año en *Sceloporus grammicus* en tres diferentes elevaciones. Obtuvimos las temperaturas operativas colocando modelos biofísicos en cada una de las zonas de estudio, estos modelos tenían en el interior un sensor de temperatura que registró la temperatura cada 30 minutos durante 4 años. Los resultados obtenidos indican que las temperaturas operativas difieren por elevación y entre estaciones, siendo el verano la estación del año con las temperaturas operativas más bajas. Obtuvimos la temperatura corporal de campo y la temperatura preferida en laboratorio, estos fueron afectados por la altitud y la estación del año. Además, los índices de termorregulación variaron a lo largo de las estaciones del año, siendo el otoño la estación donde las lagartijas fueron más precisas y eficientes para alcanzar sus requerimientos térmicos. Concluimos que, al menos en nuestra zona de estudio, no es posible considerar estrictamente conservativa la biología térmica de *Sceloporus grammicus*.

Capítulo 4: La reflectancia dorsal es considerada como una característica fenotípica que puede funcionar como un mecanismo auxiliar en la obtención de calor mediante el incremento de la tasa de calentamiento, conocido como "hipótesis de melanismo térmico". Dicha hipótesis ha recibido apoyo mixto: en algunas especies se aprecia el efecto benéfico de ser obscuro durante el proceso de obtención de calor, pero en otras especies el efecto es indirecto o ausente. Por otro lado, son pocos los estudios que han abordado si la reflectancia dorsal permanece estática o es variable a lo largo del año en lagartijas, incluso, no hay trabajos disponibles en los que se relacione la reflectancia dorsal y las tasas de calentamiento y enfriamiento, y la retención de calor en función de la altitud y la estación. La variación en las temperaturas disponibles para la termorregulación en *Sceloporus grammicus* en las mismas elevaciones utilizadas en los capítulos anteriores, nos proporcionó una excelente oportunidad para evaluar dicha relación. Nuestros resultados mostraron que: 1) los cambios en la reflectancia dorsal inducidos por la temperatura corporal de las lagartijas no fueron constantes ni homogéneos entre altitudes y estaciones del año, 2) durante la estación del año con las temperaturas ambientales más bajas, la reflectancia dorsal fue menor (fueron más oscuras), 3) las tasas térmicas fueron más altas durante el verano y el otoño, incluso la retención de calor fue de más del doble en otoño respecto a la primavera y 4) durante todas las estaciones del año, las lagartijas con menor reflectancia dorsal presentaron tasas de calentamiento y ganancia neta de calor superiores a sus conespecíficas con mayor reflectancia dorsal. Nuestros resultados abren la interrogante acerca de los costos energéticos asociados con los cambios a corto y largo plazo de la reflectancia dorsal (cambios por temperatura corporal y estaciones, respectivamente).

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1. INTRODUCCIÓN

Los vertebrados pueden ser clasificados de acuerdo con la forma en que obtienen y mantienen su temperatura corporal (*Tc*) en dos grandes grupos: ectotermos y endotermos. Ambos grupos presentan diferencias entre sí: 1) los ectotermos adquieren temperatura corporal principalmente por medio de la exposición a fuentes de calor en el ambiente; los endotermos adquieren temperatura corporal principalmente a través de la producción de calor corporal de forma autónoma e independiente de la temperatura ambiental; 2) cuando la temperatura ambiental disminuye, la temperatura corporal de los ectotermos también y con ello su metabolismo y la demanda de energía. En el caso de los endotermos la disminución de la temperatura ambiental juega un papel inverso debido a que están obligados a mantener su temperatura corporal constante, por lo que se ven en la necesidad de aumentar la producción de calor; 3) manteniendo la temperatura corporal constante induce a que un endotermo pueda invertir más tiempo forrajeando que los ectotermos (Pough 1980, Pough y cols. 2001).

La morfofisiología de los organismos ectotermos les permite usar poca energía y producir de manera eficiente biomasa respecto a los organismos endotermos. En endotermos, la mayor parte de la energía proviene del metabolismo aerobio, por lo que el uso de energía es alto, constante y por periodos prolongados (Bennet y Dawson 1972, Angilletta y cols. 2010). En ectotermos, entre el 50 y el 98% de la energía que requieren para sus actividades diarias se obtiene del metabolismo anaerobio, por lo que solo usan altas tasas de energía en periodos específicos como al escapar de un depredador o capturar una presa, aunque después de realizar dichas actividades, deben de mantenerse en reposo por periodos prolongados hasta recuperar sus niveles basales de ácido láctico (Bennet y Dawson 1972; ver Pough 1980). La mayor parte de los ectotermos siguen este patrón, en el cual se puede generar una gran cantidad de energía sin el costo energético de mantener la tasa metabólica basal alta característica de los sistemas aeróbicos. Aún con los beneficios de la regulación ectotérmica existen importantes límites asociados, el más prominente está en la restricción impuesta por las bajas temperaturas ambientales a la posibilidad de aumentar su distribución espacial (altitud y/o latitud), contrario a lo que sucede con los individuos endotérmicos quienes se distribuyen casi ilimitadamente en el espacio, lo cual explica la presencia de estos organismos en sitios térmicamente adversos como las zonas polares (Pough 1980). Sin embargo, existen especies ectotérmicas en hábitats considerados fríos, como es el caso del género *Sceloporus* y *Liolaemus* en América del Norte y del Sur, respectivamente (Lemos-Espinal y Ballinger 1995; Rodríguez-Serrano y cols. 2011), por lo que Hock (1967) sugirió que las bajas temperaturas ambientales no eran limitantes para la distribución de ectotermos. A partir de estas observaciones se ha tratado de entender cómo es que estos organismos sobreviven en dichos ambientes (Snyder y Wheaters 1997).

Aunado a lo anterior y debido a que los ambientes de gran altitud se consideran como térmicamente adversos para ectotermos, es posible realizar estudios de potenciales adaptaciones morfológicas y fisiológicas que dichos organismos presentan para contrarrestar las bajas temperaturas (Pincheira-Donoso y cols. 2008; Díaz de la Vega-Pérez y cols. 2019). En ambientes de alta elevación, la disminución de la temperatura ambiental representa un factor de estrés que generalmente va acompañado de baja disponibilidad de oxígeno y alta radiación UV respecto a entornos de bajas altitudes en latitudes similares (Korner 2007; Storz y cols. 2010). Partiendo del hecho de que la temperatura es esencial para mantener la vida de los vertebrados, tanto ectotermos como endotermos, estos presentan una serie de mecanismos que les permite aclimatarse o adaptarse a los ambientes con bajas temperaturas ambientales como los de alta montaña. En ectotermos estas adaptaciones incluyen ajustes en la termorregulación, por ejemplo, varias especies del género *Liolaemus* presentan una menor temperatura corporal en zonas de alta elevación (Rodríguez-Serrano y cols. 2009) o *Psammodromus algirus* que presenta una menor velocidad de enfriamiento en zonas de alta elevación (Zamora-Camacho y cols. 2014a).

2. ANTECEDENTES

La selección natural actúa con mayor fuerza sobre la conducta y el desempeño (definido como la capacidad de un organismo de realizar una actividad ecológicamente relevante como la locomoción o la fuerza de la mordida; ver Irschick y cols. 2010; Irschick y Garland 2001), ya que están fuertemente relacionados con la supervivencia y la reproducción. Estos rasgos son conocidos como “rasgos biológicos altos” o “rasgos complejos” y están compuestos por rasgos subordinados en niveles de organización biológica menores como sistemas, órganos, tejidos y células. Los rasgos subordinados pueden modificarse mediante vías fisiológicas, bioquímicas y morfológicas de los organismos (Sinervo y Calsbeek 2003) y tener efecto sobre los rasgos complejos, por ejemplo, la afinidad del oxígeno por la hemoglobina puede influir en la tasa cardiaca máxima o el desempeño muscular (Orr y Garland 2017).

Los organismos pueden adaptarse al ambiente gracias a la plasticidad fenotípica. La plasticidad fenotípica se define como la capacidad de un genotipo de producir varios fenotipos en respuesta a cambios ambientales o como la habilidad de un organismo para modificar su fenotipo ante algún cambio ambiental (Futuyma 1998; Pigliucci 2005). Esta plasticidad puede o no ser reversible (flexibilidad o regresión fenotípica: Piersma y Drent 2003) dependiendo de a) el tiempo de vida de los organismos, si este es menor a la duración del cambio que ha propiciado la modificación en el fenotipo, este no será reversible (i.e. los eventos reproductivos únicos de algunas lagartijas semelparas, en la que no existe la fase de regresión en las gónadas; Rodríguez-Romero y cols. 2011) y b) la duración del cambio ambiental: los eventos selectivos pueden ser instantáneos (i.e. depredación) o prolongados (i.e. la estacionalidad o el calentamiento global), la exposición a estos eventos puede producir cambios en el fenotipo con consecuencias adaptativas y por ende evolutivas, ya que al otorgar un mayor rendimiento a un rasgo, este puede favorecer ciertas aptitudes darwinianas como la supervivencia mediante selección direccional (Garland y Adolph 1991; Gabriel 2005). Sin embargo, la plasticidad fenotípica puede tener consecuencias negativas o ser costosa en términos de energía, ya que producir estructuras o mantenerlas puede reducir la energía disponible para otros rasgos como la reproducción. En crustáceos del género *Daphnia* la presencia de depredadores induce a que produzcan espinas como defensa y esto se ha relacionado con la disminución del éxito reproductivo en comparación con especies de *Daphnia* sin espinas (Black y Dodson 1990).

En ambientes estacionales (donde las variables abióticas no permanecen constantes a lo largo del año), los diferentes eventos asociados con la reproducción y la supervivencia tienden a ocurrir en espacios predecibles de tiempo, acompañados de cambios en el fenotipo adulto (i.e. desarrollo de gónadas y muda de plumas), dicha correspondencia permite a los organismos desempeñarse de manera óptima en varias condiciones ambientales (Nityananda y cols. 2016). Un ejemplo de estos cambios (que además es cíclico) es el plumaje de *Lagopus mutus* el cual es blanco durante la mayor parte del año lo cual les permite mimetizarse con la nieve, cuando la nieve desaparece durante el verano, los organismos mudan la pluma a color café, como resultado de esto, continúan mimetizándose en el ambiente (Montgomerie y cols. 2001). El grado de cambio en los factores abióticos de una estación del año a otra varía por regiones, siendo más marcada en zonas alejadas del ecuador. La variación en factores ecológicos como la temperatura ambiental, humedad, lluvias, concentración de oxígeno o salinidad del agua causan patrones estacionales en las poblaciones e inducen cambios en rasgos como la reproducción, función inmunitaria y la supervivencia (Huyghe y cols. 2010; Nityananda y cols. 2010).

2.1. La temperatura de selección y la velocidad de *sprint*

El rango de temperatura que buscan mantener los ectotermos durante su periodo de actividad es conocido como temperatura de selección (*Tsel*), en este rango suele encontrarse la temperatura corporal (*Tc*) en el cual se maximiza la eficiencia de la digestión y de la locomoción entre otros rasgos ([temperatura óptima]; Hertz y cols. 1993; Adolph y Porter 1993). Existen dos vertientes que intentan explicar la termorregulación en reptiles: 1) la hipótesis lábil: la biología térmica (*Tsel* y *Tc*) puede ser plástica y modificarse de acuerdo con el tipo de ambiente térmico, de modo que las "temperaturas óptimas" son más factibles de alcanzar (Adolph 1990). Es el caso de *Iberalocertha galani*, en la que la *Tsel* es menor en primavera que en verano, lo cual reduce los costos de la termorregulación (Ortega y cols. 2016); 2) la hipótesis estática: la biología térmica (*Tsel* y *Tc*) es evolutivamente conservativa y permanece constante a pesar de los cambios térmicos en el ambiente, por lo que no se aprecian diferencias entre individuos de la misma especie en diferentes ambientes o entre especies estrechamente relacionadas. Generalmente, cambios conductuales en la adquisición de calor ambiental son suficientes para mitigar cambios en el ambiente, por ejemplo, aumentar el tiempo que se invierte expuesto a la radiación solar. Este es el caso de la mayoría de lagartijas del género *Sceloporus*, en la que no se aprecian diferencias en la *Tc* y *Tsel* (Crowley 1985, Andrews 1998; Lara-Reséndiz y cols. 2014) No obstante, la falta de estudios estacionales impide observar potenciales cambios en algunos rasgos de termorregulación, tomando en cuenta que estas especies llegan a habitar en zonas fluctuantes de temperatura ambiental como son los gradientes altitudinales (Garland y Adolph 1991). Este es el caso de la lagartija *Sceloporus torquatus* en la que recientemente se han descrito cambios en la *Tsel* y temperaturas críticas máximas y mínimas a lo largo del año (Domínguez-Guerrero 2019).

Partiendo del hecho de que la termorregulación en reptiles es principalmente conductual, esta puede considerarse como un rasgo complejo debido a su implicación en la supervivencia, ya que, para adquirir calor, los organismos tienen que exponerse no solo al calor del ambiente, sino también a depredadores, por lo que la velocidad de *sprint* (o velocidad de arranque) suele asociarse tanto a la capacidad de escapar de depredadores como al éxito de captura de presas (Scales y cols. 2009). Ciertas características en el fenotipo pueden determinar el desempeño de la velocidad de sprint, por ejemplo, la longitud de las patas traseras o el porcentaje de fibras musculares blancas y rojas en los músculos asociados con la locomoción (Bonine y cols. 2001, Quintana y cols. 2014).

Por otra parte, la coloración dorsal es considerada como un rasgo potencialmente auxiliar que puede ayudar a minimizar los costos de termorregulación en zonas frías como altas montañas o grandes latitudes. Por consiguiente, la coloración dorsal puede tener gran relevancia en la sobrevivencia de los organismos, ya que el porcentaje de reflectancia en el dorso (como una forma de cuantificar la coloración dorsal) de ciertos vertebrados (y algunos insectos) está relacionada con la capacidad de adquirir calor corporal en menos tiempo. Los organismos con menor reflectancia (más oscuros) pueden absorber una mayor cantidad de luz, lo que se refleja en una mayor tasa de calentamiento. Esta relación es conocida como "hipótesis de melanismo térmico" (Norris 1967; Clusella-Trullas y cols. 2007). Mientras que en mamíferos se ha demostrado que los organismos de altas elevaciones mantienen una mejor eficiencia térmica producto de su baja reflectancia, en reptiles esta hipótesis ha recibido apoyo mixto. En algunas especies como *Tiliqua sincoides* y *Cordylus niger* se ha mostrado que los organismos más oscuros tienen una tasa de calentamiento superior a la que presentan sus conespecíficos claros (Clusella-Trullas y cols. 2009; Geen y Johnston 2014), por otra parte, en otras especies como *Psammodromus algirus,* a pesar de que la reflectancia dorsal disminuye gradualmente con la altitud, no hay una relación directa entre la reflectancia dorsal y la tasa de calentamiento (Reguera y cols. 2014; Zamora-Camacho y cols. 2014).

La variación estacional en algunos rasgos subordinados como la reflectancia dorsal podría influir en la conservación de las preferencias térmicas y la velocidad de sprint en el género *Sceloporus,* por lo que es indispensable evaluar rasgos complejos y rasgos subordinados en un mismo sitio de estudio.

Sceloporus grammicus es una especie de lagartija de talla pequeña (longitud hocico-cloaca [LHC] = 52 mm y peso = 4.6 g en promedio) que presenta una distribución desde el sur de Texas hasta el estado de Oaxaca, México, llegando a distribuirse por arriba de los 4000 m de altitud (Lemos-Espinal 1992; Bastiaans y cols. 2014). Al estar presente en distintos tipos de ambientes y pertenecer a un género considerado térmicamente conservativo, representa un modelo de estudio interesante para describir la posible variación de rasgos complejos y subordinados asociada con la altitud y la estacionalidad. En esta tesis presentamos una serie de comparaciones que involucran tanto a rasgos complejos (*Tsel* y velocidad de sprint) como rasgos subordinados (reflectancia dorsal y tasas térmicas) de *S. grammicus* en diferentes altitudes y por estación del año.

3. HIPÓTESIS

Si los rasgos complejos como la *Tsel* y la velocidad de *sprint* son conservados en el género *Sceloporus,* entonces *S. grammicus* no presentará diferencias en estos rasgos asociados a variaciones en altitud y estación del año. Por otra parte, los rasgos subordinados como la reflectancia dorsal y las tasas térmicas presentaran variacion a lo largo de las estaciones del año para mantener constantes a los rasgos complejos.

4. OBJETIVOS

4.1. Objetivo general

Evaluar la temperatura de selección (*Tsel*) y la velocidad de *sprint* como rasgos complejos y su relación con los rasgos subordinados (reflectancia dorsal y tasas térmicas) en tres elevaciones y por estación del año.

4.2. Objetivos particulares

Comparar por estaciones del año: la temperatura de selección, la velocidad de sprint, las tasas térmicas y la reflectancia dorsal en organismos de *Sceloporus grammicus* en tres elevaciones diferentes.

5. METODOLOGÍA

Se utilizaron tres zonas de estudio en el Estado de México: Texcalyacac a 2500 m (elevación baja [EB]) (19º 07’ 37’’ N, 99º 29’ 41’’ W), Las Lágrimas a 3400 m (elevación media [EM]) (19º 06’ 82’’ N, 99º 49’ 48’’ W) y Nevado de Toluca a 4100 m (elevación alta [EA]) (19º 06’ 06’’ N, 99º 46’ 03’’ W). Se realizaron cuatro periodos de capturas correspondientes a cada una de las estaciones ambientales, durante mayo (primavera), julio (verano), octubre (otoño) y enero-febrero (invierno), obteniendo 193 lagartijas en total (primavera: 65, verano: 64, otoño: 64 e invierno: 0). Los meses para la captura de los organismos se seleccionaron de modo que coincidiera con la mitad de cada estación del año y evitar efectos de confusión por el empalme entre estaciones. El procedimiento descrito a continuación fue empleado de forma idéntica durante todos los muestreos.

Las lagartijas fueron capturadas a mano o con caña de pescar. Inmediatamente después de la captura, se registró su temperatura corporal (*Tc*) introduciendo en la cloaca un termopar conectado a un termómetro digital; también se registró la temperatura del sustrato (*Ts*) y del aire (*Ta*) (a 5 cm del sustrato) de la zona en donde se observó a la lagartija por primera vez. Todas las lagartijas fueron transportadas en bolsas de algodón al Laboratorio de Ecofisiología de Fauna Silvestre del Instituto para la Conservación de la Cordillera Neovolcánica ante el Cambio Climático A.C. (ICCoNeCC A.C.) ubicado en Toluca, Estado de México. Posteriormente, de cada lagartija se registró el peso corporal con una balanza digital (precisión: 0.01 g), longitud hocico-cloaca (LHC) y longitud de la pata trasera derecha con un vernier digital (precisión: 1 mm) y el grosor del fémur con un espesímetro (Mitutoyo, precisión: 0.01 mm). Los residuos de la regresión entre el logaritmo de la masa corporal y el logaritmo de la LHC se consideraron como el valor de la condición corporal de cada individuo (Schulte-Hostedde y cols. 2005). Cada lagartija se mantuvo en un terrario individual con focos de calor y placas térmicas. Fueron alimentadas *ad libitum* con larvas de *Tenebrio molitor* he hidratadas con agua, únicamente se les privó del alimento durante las pruebas de calentamiento y enfriamiento.

5.1. Temperatura de selección e índices de termorregulación

Se utilizó la metodología propuesta por Hertz y cols. (1993), la cual ha sido utilizada ampliamente en estudios de termorregulación de otros lacertilios (Fierro-Estrada y cols. 2019, Díaz de la Vega-Pérez y cols. 2019, entre otros). Esta consiste en lo siguiente: para evaluar las temperaturas operativas (*Top*), se colocó en cada una de las zonas de estudio un sensor de temperatura conectado a dos modelos biofísicos que simulan en forma y tamaño a las lagartijas, adicionalmente, en todas las zonas de estudio el sensor fue movido a varios sitios de cada una de las zonas de estudio con la finalidad de registrar de forma aleatoria las condiciones térmicas. Los modelos se construyeron con PVC y las temperaturas que registran están altamente correlacionadas con las *Tc* de las lagartijas (r= 0.82, p < 0.001, n= 59). Los sensores registraron cada 30 minutos la temperatura ambiental que experimentarían las lagartijas en caso de que no termorregularán de forma activa (*Top*). Los sensores se mantuvieron durante los años 2016, 2018, 2019 y 2020. Solo se consideran las *Top* durante el periodo de actividad circadiano de las lagartijas, la cual depende de la elevación y consiste en los siguientes periodos: EB: 08:00-18:30; EM: 09:00-17:30 y EA: 10:00-16:30.

El primer rasgo evaluado en el laboratorio fue la temperatura de selección (*Tsel*), para la cual se colocaron a las lagartijas en una caja de policarbonato de 150×100×80 cm (largo × ancho × alto) con 4 focos de calor (80 W cada foco) agrupados en un extremo de tal manera que daban como resultado un gradiente térmico dentro de la caja (de 20 a 50 °C), adicionalmente, se colocaron rocas para permitir la conducta natural de termorregulación. Cada 90 minutos las lagartijas eran capturadas para registrar su *Tc*, teniendo 5 datos por cada lagartija de cada población. Se consideró el rango de *Tsel* como los cuartiles 1 y 3, que representan el 25% y 75% de los datos, respectivamente.

A partir de los datos obtenidos de la *Tc* de campo, *Tsel* y *Top,* se calcularon los índices de termorregulación propuestos por Hertz y cols. (1993) y Blouin-Demmers y Weatherhead (2001):

1. Calidad térmica del hábitat (*de*): se calcula como la media de valores absolutos de cada *Top* respecto al rango *Tsel*, cuanto mayor es el valor *de* peor es la calidad térmica del ambiente y viceversa.

2. Precisión de la termorregulación (*db*): se calcula como la media de valores absolutos de las desviaciones de las *Tc* de campo respecto al rango *Tsel* en laboratorio. Cuanto mayor es el valor *db*, menor es la precisión de la termorregulación y viceversa.

Efectividad de la termorregulación:

A) Hertz y cols. (1993): se calcula como 1 - (*db*/*de*), valores cercanos a 0 indican termoconformidad, valores cercanos a 1 indican termorregulación activa y valores por debajo de 0 indican que evitan la *Tsel*

B) Blouin-Demers y Weatherhead (2001): definida como el grado de esfuerzo en °C que requiere una lagartija para alcanzar su *Tsel* en campo, calculada como *de*-*db*. Valores mayores indican un mayor esfuerzo para alcanzar la *Tsel*.

5.2. Velocidad de escape

Para la prueba de velocidad de sprint, primero se aclimató a las lagartijas al menos durante una hora a cada una de las temperaturas utilizadas durante cada prueba. Con cada lagartija se realizó la prueba a 10, 20 y 30 °C dos veces consecutivas y a una temperatura por día. La prueba consistió en colocar a cada lagartija de forma individual en un carril de 2 m, elaborado de policarbonato y una base de lija para permitir una adecuada tracción. El carril estaba marcado cada 10 cm con la finalidad de poder observar con detalle el momento preciso cuando la lagartija cruzará las marcas. Las carreras se grabaron con una cámara digital (Motorola) a una resolución de 30 cuadros por segundo y los archivos fueron transferidos a la computadora (HP Pavilión) para analizarse con el programa Adobe Premier Pro CC (versión 12.1.1), que permite descomponer los videos en cuadros, obteniendo así el tiempo preciso que tomaba recorrer cada sección del carril. La velocidad de *sprint* se calculó sobre cada 50 cm y solo se consideró la velocidad máxima obtenida en cada carrera (modificado de Lowie y cols. 2019). El valor más alto entre la primera y segunda carrera se correlacionaron altamente ($r= 0.96$, $p < 0.001$, $n= 118$), por lo que los datos son replicables. El valor más alto se usó para el resto de los análisis.

5.3. Reflectancia dorsal

Se utilizaron los mismos tratamientos de temperatura corporal usados en la velocidad de sprint, la coloración dorsal se obtuvo como el porcentaje de reflectancia dorsal promedio de tres mediciones de la zona dorsal, estas fueron tomadas en la misma zona y por la misma persona bajo las mismas condiciones de luminosidad. Para cerciorarnos de que la medida se tomara en la misma zona en cada uno de los tratamientos, se colocó previa a las mediciones una marca con tinta no tóxica a un costado de la zona de medición.

Para obtener los valores de reflectancia dorsal se utilizó un espectrofotómetro portátil (Jazz Ocean Optics) calibrado con un blanco estándar. Se midió la reflectancia cada 20 nm sobre un rango de 300 a 700 nm y se consideró el promedio como la reflectancia dorsal (Matthews y cols. 2006).

5.4. Tasas térmicas y lapso térmico

En esta prueba, cada lagartija se sujetó con cinta adhesiva a un trozo de plástico para evitar el movimiento y ajustes conductuales en las pruebas. Las lagartijas fueron enfriadas hasta los 20 °C de *Tc* en un refrigerador. Se les insertó en la cloaca (4 mm) un termopar conectado a un termómetro de alta precisión para monitorear la *Tc* durante toda la prueba. Cada lagartija fue colocada bajo una lámpara de 100 W a una distancia de 15 cm, cuando la *Tc* alcanzó los 21 °C se inició el registro del calentamiento, la *Tc* se registró cada 30 segundos (s) hasta alcanzar los 33 °C (elegido por ser la media de la *Tsel* reportada por Lara-Reséndiz y Díaz de la Vega-Pérez 2013). Posteriormente, la lámpara fue apagada y la lagartija fue trasladada a un lugar donde no hubiera irradiado el calor de la lámpara, se registró el tiempo que la *Tc* tardó en empezar a disminuir, esta medición se considerado como lapso térmico. Cuando la *Tc* empezó a disminuir se registró nuevamente cada 30 s hasta que alcanzó 27 °C (esta temperatura está por abajo del rango de *Tsel*). Con el valor de la pendiente de la regresión entre el aumento o la disminución de la *Tc* y el tiempo se obtuvieron las tasas de calentamiento y de enfriamiento, respectivamente. Finalmente, el valor de la tasa de enfriamiento fue restado de la tasa de calentamiento para calcular la ganancia neta de calor.

Todas las pruebas fueron realizadas en un periodo de 08:00 a 10:00 h en un cuarto con temperatura ambiente controlada a 20 °C (modificado de Zamora-Camacho y cols. 2014). Después de concluir las pruebas, las lagartijas fueron regresadas al lugar de captura.

6. Capítulo I

To be small and dark is advantageous for gaining heat in mesquite lizard, *Sceloporus grammicus* (Squamata: Phrynosomatidae).

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El trabajo que a continuación se presenta, contiene datos de la reflectancia dorsal, tamaño corporal, tasas térmicas (tasas de calentamiento y de enfriamiento, ganancia neta de calor y retención de calor) en *Sceloporus grammicus* provenientes de tres elevaciones durante el verano. La literatura disponible muestra que, en ciertos organismos, la reflectancia dorsal y el tamaño corporal pueden influir en la adquisición y mantenimiento del calor corporal en lacertilios. Los resultados mostraron que, las tasas térmicas no fueron estadísticamente diferentes entre las tres elevaciones. Las lagartijas tienen una menor reflectancia dorsal (son más oscuras) y son más pequeñas a mayor elevación. Además, se encontró que la reflectancia dorsal y el tamaño corporal influyen en la tasa de calentamiento y ganancia neta de calor, es decir, entre más oscuras y pequeñas eran las lagartijas, mayor era la tasa de calentamiento y ganancia neta de calor.

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To be small and dark is advantageous for gaining heat in mezquite lizards, *Sceloporus grammicus* (Squamata: Phrynosomatidae)

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Body temperature is important in determining individual performance in ectotherms such as lizards. Environmental temperature decreases with increasing altitude, but nevertheless many lizards inhabit high-altitude environments. The 'thermal melanism hypothesis' proposes that a dark dorsal coloration enables darker individuals to heat up faster because they absorb more solar radiation and thus being darker may be advantageous in cold habitats. The aim of the present study is to evaluate how heating rate, cooling rate and net heat gain vary with body size and dorsal skin coloration in *Sceloporus grammicus* lizards along an altitudinal gradient. We measured these traits multiple times in the same individuals with a radiation heat source and spectrophotometry under laboratory conditions. Our results showed that *S. grammicus* lizards are smaller and darker at high elevations than at low elevations. In addition, the smallest and darkest lizards showed the greatest heating rate and net heat gain. Therefore, in *S. grammicus*, we suggest that small body size and dark dorsal coloration provide thermoregulatory benefits in high-altitude environments. Hence, this study supports the thermal melanism hypothesis in a lizard species under varied thermal environments.

ADDITIONAL KEYWORDS: altitude – body size – reflectance – *Sceloporus grammicus* – thermoregulation

INTRODUCTION

Body temperature affects physiological and behavioural performance (Huey & Kingsolver, 1989; Angilleta,

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2009). For example, it is necessary for an organism to remain within a range of body temperatures to optimize blood flow (Snyder, 1971), digestive efficiency (Xiang *et al.*, 1996) and sprint velocity (Hertz *et al.*, 1983; Zajitschek *et al.*, 2012). Although the body temperature of endotherms relies mainly on metabolic heat, ectotherms depend on environmental heat to maintain optimal body temperature (Pough, 1980). This makes ectotherms very sensitive to their thermal environment, often precluding them from living in habitats where environmental temperatures are low (Pough, 1980).

Environmental temperature generally decreases with altitude (Körner, 2007). However, many lizards do inhabit high-altitude environments where temperatures are too low for them to be active most of the time (Zamora-Camacho *et al.*, 2013). High-altitude lizards have thus become a model system for understanding how ectotherms adapt to extreme thermal conditions (Hertz & Huey, 1981; Gvozdík, 2002; Reguera *et al.*, 2014; Zamora-Camacho *et al.*, 2014; González-Morales *et al.*, 2015; Ortega *et al.*, 2016; Gilbert & Miles, 2019). High-altitude environments may be considered marginal habitats for lizards (Zamora-Camacho *et al.*, 2013), so when lizards successfully inhabit such habitats, local adaptation processes may be expected (Kawecki, 2008). Indeed, genetic and phenotypic changes in traits such as body size have been frequently described along altitudinal gradients (review in Keller *et al.*, 2013).

One way by which lizards may adapt to colder environments is by changes in their dorsal coloration. The thermal melanism hypothesis predicts that dark coloured organisms should heat faster than equivalent light-coloured ones (Clusella-Trullas *et al.*, 2007). Darker individuals may have an advantage in cold environments as they could heat faster; however, they would be disadvantaged in hot environments due to overheating. This has been supported by mathematical models (Stevenson, 1985; Walton & Bennet, 1993); however, these models do not account for the many traits of living animals such as body size, body shape or physiological and behavioural adjustments (Dzialowski & O´Connor, 2004; Clusella-Trullas *et al.*, 2007). Empirically, several studies have shown that melanic organisms warm up faster than non-melanic conspecifics (e.g. Clusella-Trullas *et al.*, 2009; Geen & Johnston, 2014; Moreno-Azócar *et al.*, 2016). Therefore, it is predicted that high-altitude animals should be darker than their close relatives at lower altitudes. This pattern has been widely tested in butterflies and flies (Ellers & Boggs, 2004; Munjal *et al.*, 1997); however, for reptiles the information is scarce and inconsistent. For example, *Sceloporus graciosus* and *Sceloporus jarrovi* do not show any relationship between colour and altitude (Norris, 1967); however, the lizards *Podarcis hispanica*, *Psammodromus algirus* and *Iberolacerta monticola* are darker at higher altitudes (Gabirot *et al.*, 2013; Reguera *et al.*, 2014; Aguado de la Paz, 2015).

In endotherms, body size has an important role in thermoregulation; Bergmann's rule suggests that large body size favours heat conservation, selecting for larger body size in colder environments (review in Blackburn *et al.*, 1999). For ectotherms, it has been shown that larger body size has a favourable effect on corporal heat retention (Gabirot *et al.*, 2013; Zamora-Camacho *et al.*, 2014; Moreno-Azócar *et al.*, 2016). Turtles, some lizards, and insects follow Bergmann's rule, but other ectotherms appear to follow the reverse pattern, including snakes, some lizards and frogs (Ashton & Feldman, 2003; Sears & Angilletta, 2004; Olalla-Tárraga & Rodríguez, 2007; Adams & Church, 2008; Pincheira-Donoso *et al.*, 2008; Ma *et al.*, 2009; González-Morales *et al.*, 2017). Smaller lizards heat up faster than larger ones; however, the cooling rate is also higher (Carothers *et al.*, 1997; Gabirot *et al.*, 2013; Zamora-Camacho *et al.*, 2014). The net effect of body size on heat gained (Net Heat Gain, hereafter, NHG) depends on the relative speed of heating and cooling rates according to body size. For example, in *Psammodromus algirus*, larger lizards exhibit a greater net increase in body temperature (Zamora-Camacho *et al.*, 2014); however, in *I. monticola*, smaller body size allows for greater net increase in body temperature (Carrascal *et al.*, 1992).

The aim of the present study was to evaluate how heating rate, cooling rate and net heat gain vary with body size and dorsal coloration in the lizard *Sceloporus grammicus* from three populations from different altitudes. Moreover, we also test whether dorsal coloration is phenotypically plastic, varying with environmental temperatures. *S. grammicus* is a small lizard averaging 52 mm snout-vent length (SVL) and 4.6 g body mass. It occurs from southern Texas (USA) to the Mexican state of Oaxaca and has a wide altitudinal distribution, including populations above 4600 m (Lemos-Espinal & Ballinger, 1995; Bastiaans *et al.*, 2014). The dorsal coloration of *S. grammicus* generally shows small transverse black bars (Sites, 1982; Fig. 1). Regarding the dorsal coloration, we predicted that high-altitude lizards would exhibit darker dorsal coloration than lizards from lower altitudes, and darker lizards would exhibit greater net heat gain than lighter lizards. Within individuals, we predicted that dorsal coloration would be darker at cool temperatures than at warm temperatures. With respect to body size in ectotherms, it is difficult to predict *a priori* how body size will vary with altitude; however, we predict that, if body size decreases with altitude, then smaller lizards would exhibit greater net heat gain than larger lizards.

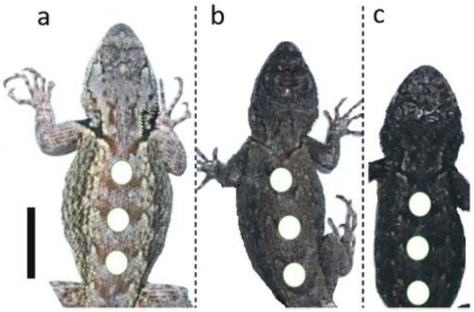

Figure 1. Representative images of the dorsal skin of *S. grammicus* from different altitudes: (a) low altitude, (b) middle altitude and (c) high altitude. The solid black line represents 1 cm in length. White circles show sample points for DSR measurements.

MATERIAL AND METHODS

ETHICS STATEMENT

All experimental procedures were carried out following the guidelines of the Universidad Autónoma del Estado de México (UAEM), as well as the Mexican Federal Regulation for Animal Experimentation and Care (NOM-062-ZOO-2001; governmental approval SGPA/DGVS/02407/13).

FIELD SITES AND SAMPLING

During June and July of 2018, we captured animals from three populations as previously described in González-Morales *et al.* (2017): Texcalyacac, at 2500 m (19º 07' 37" N, 99º 29' 41" W) (*N* = 12), Las Lágrimas, at 3400 m (19º 06' 82" N, 99º 49' 48" W) (n = 23) and Nevado de Toluca, at 4300 m (19º 06' 06" N, 99º 46' 03" W) (n = 28). We will refer to these sites as our low, middle and high-altitude sites, respectively. Texcalyacac has a temperate subhumid climate with rain in summer; the habitat of the lizards is characterized by igneous extrusive rocks, in addition to pine forest with a range of annual average temperatures between 12–14 °C (INEGI, 2009). Las Lágrimas has a subtropical climate with rain in summer, very cold autumns and winters, and very hot springs which is reflected in a range of annual average temperatures between 10–20 °C and high humidity. The lizards' habitat is characterized by fallen pine trunks and living pine trees (INEGI, 2009). Nevado de Toluca presents a cold climate with a range of annual average temperatures of -5 to 5 °C, with rainfall throughout the year, but mainly in summer. The characteristic habitat of this area is known as paramo and consists of igneous exposed rocks, with little or no vegetation (CONANP, 2016).

We captured the animals by hand or lasso. We only used adult animals, identified by having a SVL > 40 mm and secondary sexual ventral coloration (Zúñiga-Vega *et al.*, 2008; Bastiaans *et al.*, 2014). No pregnant females were used in the study. Lizards were transported (1.5 h drive) in cloth bags to the Laboratorio de Ecofisiología de Fauna Silvestre at the Instituto para la Conservación de la Cordillera Neovolcánica ante el Cambio Climático, where each lizard was maintained in an individual terrarium (40 cm length × 20 cm width × 24 cm height). Water and food (larvae of *Tenebrio molitor*) were provided ad libitum, a natural light source was provided for circadian physiology processes, and a heat source (heat bulb) and rocks were put in one corner of each terrarium to allow normal thermoregulatory behaviour. Prior to the experiment, we measured body mass with a balance (g; precision: 0.01 g) and SVL with a digital calliper (precision: 1 mm). The same individuals were used for all tests described below.

MEASUREMENT OF DORSAL SKIN REFLECTANCE

Each lizard was placed in a thermal chamber set at 10, 20 or 30 °C for 2 h, on three different days. These temperatures were used because the lizards face this range of environmental temperatures naturally (Texcalyacac: 11–24.5 °C, Las Lagrimas: 10–25.9 °C, Nevado de Toluca: 5–28.2 °C) (González-Morales *et al.*, unpublished data). We randomized the order in which each lizard experienced these three temperatures. After 2 h, we measured dorsal skin reflectance at three points in the dorsal region. A small ink mark was placed next to the measurement site to ensure that the same site was used for all reflectance measurements. We used the average of measurements taken from the upper, medial and lower dorsal regions of each lizard. Reflectance was measured within the 300–700 nm range, which includes most of the spectrum reflected by dorsal skin, using a spectrophotometer (Jaz Ocean Optics) with a Pulsed Xenon Light Source (Jaz-PX) connected to an optical fibre. The probe was mounted within a holder that ensured readings were taken from 1 mm in diameter at a constant distance of 3 mm from the skin surface with a 45 ° angle (Megía-Palma *et al.*, 2016). All measurement was relative to a 99% WS-1 with standard reflectance (all components from Ocean Optics Inc., Dunedin, FL, USA). Raw spectra were imported into the software CLR, smoothed using a running average computed over a 20 nm interval, and the average reflectance was considered as dorsal skin reflectance. For each lizard, all reflectance measurements were made in less than 1 min.

THERMAL RATES AND TRANSITIONAL LAPSES

To measure thermal rates and transitional lapses, we immobilized lizards individually on a thermally stable synthetic plaque, using a soft lace knot around each lizard's chest, and another around each lizard's limbs, fastened firmly enough that the lizard could not escape but loosely enough to avoid injury. Next, we placed the lizards 13 cm below a 60 W bulb in a laboratory at 20 °C room temperature. All measurements were performed between 08:00 and 10:00, local time. We started the trials when the lizards attained a body temperature of 21 °C and switched off the bulb when their body temperature reached 33 °C. This body temperature range is within the preferred temperature range reported for this species (31.1 ± 3.7 °C) and does not approach the critical thermal maximum (40 °C) or minimum (9.2 °C) values (Díaz de la Vega-Pérez *et al.*, 2019). We observed how long it took the body temperature to begin to decrease from 33 °C, and that value was considered as the transitional lapse. Once the bulb was switched off, the experiment continued until body temperature diminished to 27 °C, because this temperature is below the preferred temperature range (Lara-Reséndiz & Díaz de la Vega-Pérez, 2013; Díaz de la Vega-Pérez *et al.*, 2019). Body temperature of each lizard was monitored with a small T-type thermocouple inserted 4 mm inside the cloaca and connected to a thermometer (Fluke 52-II). Body temperature was recorded every 30 s during the whole process. To make sure that the lizards were not harmed, they recuperated in the laboratory for 3 days before we released them at their capture site.

The heating rate was calculated as the linear regression slope of body temperature increase over time during the heating period. The transitional lapse was calculated as the time (min) between switching off the bulb until the lizard's body temperature started to diminish. The cooling rate was calculated as the linear regression slope of body temperature decrease over time during the cooling period. The net gain of heat (NGH) was obtained by subtracting the cooling rate from the heating rate; this relationship expresses the total heat gain (Zamora-Camacho *et al.*, 2014).

STATISTICAL ANALYSES

Normality and homogeneity of the data (in addition to the sphericity for repeated measures ANOVA) were examined prior to data analyses (Zuur *et al.*, 2010). We used two-way ANOVA (factors sex and altitude) to compare body mass and SVL among populations. When we detected significant effects, we performed post hoc comparisons to detect pairwise significant differences using the Bonferroni test. We used a three-way repeated measures ANOVA to compare the dorsal skin reflectance using sex, altitude and the temperature at which lizards were acclimated, as factors. Because the most common field body temperatures of these lizards are between 20 and 30 °C and dorsal skin reflectances at these temperatures were highly correlated ($r = 0.53$, $P < 0.001$), we combined these variables using a Principal Components Analysis (PCA). PC1 explained 79.40% of the variance in dorsal skin reflectance (eigenvalues = 1.50, loading = 0.70), and thus was considered as an indicator of "dorsal reflectance".

In our first analysis, we performed an ANOVA to test for differences in heating rate, cooling rate, transitional lapses and NGH by altitude. Analyses with sex as a co-factor provided qualitatively similar results, and so they are not shown. The interaction altitude*sex was never significant and so it was not included in the analyses reported here. Similarly, we used Pearson product-moment correlations to estimate the association between each thermal rate and transitional lapse with reflectance and body mass. Finally, to ascertain the independent effect of each predictive variable on thermal rates, controlling by the effect of the each other variables, we performed linear models with each of the thermal rates as dependent variables, altitude and sex as factors, and body mass and dorsal reflectance as covariates. We did not use SVL as a predictor of thermal rates because it is strongly correlated with body mass ($r = 0.61$, $P < 0.01$). We performed all analyses with the program jamovi (The jamovi Project 2019, v.1.2.2).

RESULTS

MORPHOLOGICAL PARAMETERS

Body mass and SVL differed by altitude ($F_{2,60} = 5.86$, $P < 0.05$; $F_{2,60} = 8.91$, $P < 0.001$; respectively). Lizards from low altitudes were heavier and larger (higher SVL) than those from middle and high altitudes (Table 1). Although the altitude*sex interaction was not significant (body mass: $F_{1,53} = 2.28$, $P = 0.11$; SVL: $F_{1,53} = 2.69$, $P = 0.07$), we detected sexual dimorphism in body mass in the high-elevation population (post hoc Tukey test = 3.85, $P < 0.001$).

DORSAL SKIN REFLECTANCE

In an among-population comparison, lizards from the high-altitude site had the lowest (darkest) dorsal skin reflectance (DSR), while lizards from the low- and middle-altitude sites had similar DSR (i.e., lightest dorsal skin; $F_{2,60} = 15.44$, $P < 0.001$; Fig. 2). Females were darker than males ($F_{1,5} = 33.79$, $P = 0.002$); however, the interaction altitude*temperature*sex

Table 1. Morphological parameters of *S. grammicus* lizards. Values are mean ± SE

	Low altitude (Texcalyacac 2500 m)		Middle altitude (Las Lagrimas 3400 m)		High altitude (Nevado de Toluca 4300 m)	
Sex (*N*)	F (6)	M (7)	F (10)	M (13)	F (8)	M (17)
Body mass (g)	$5.60 \pm 0.44^{A\dagger}$	6.13 ± 1.09^{A}	4.65 ± 0.34^{AB}	5.04 ± 0.43^{AB}	3.42 ± 0.18^{B}	$5.15 \pm 0.24^{B,*}$
SVL (cm)	5.82 ± 0.25^{A}	5.97 ± 0.35^{A}	5.34 ± 0.21^{B}	4.95 ± 0.24^{B}	4.78 ± 0.13^{B}	5.23 ± 0.07^{B}

[†] capital letters show differences by altitude.
*shows differences by sex ($P < 0.05$).

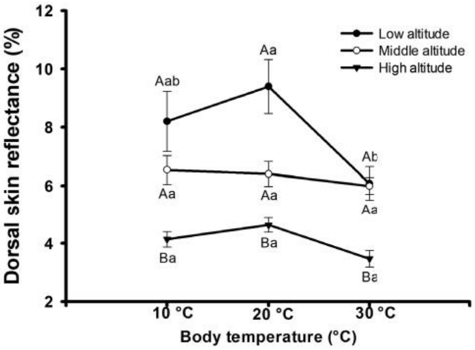

Figure 2. DSR of *S. grammicus* from different altitudes. Numbers represent differences by altitude and letters represent differences by temperature, according to a Tukey post hoc test. Values are represented as mean ± SE.

was not significant ($F_{4,20} = 1.44$, $P = 0.25$). Within individual lizards, DSR also varied with body temperature in the low-altitude population ($F_{2,60} = 17.21$, $P < 0.001$). At 10 and 30 °C, the lizards from low altitudes had lower DSR values than they did at 20 °C ($F_{2,9} = 17.12$, $P < 0.01$; Fig. 2). However, temperature did not affect DSR in middle- and high-altitude lizards (Fig. 2).

THERMAL RATES

Heating rate

Heating rate did not significantly vary with altitude (one-way ANOVA, $F_{2,\ 27} = 1.58$, $P = 0.225$; Table 2). However, univariate analyses showed that heating rate decreased with body mass ($r = -0.51$, $P < 0.003$; Fig. 3a) and with dorsal reflectance ($r = -0.44$, $P < 0.001$; Fig. 4a). That is, smaller lizards and darker lizards warmed up faster. A full model including body mass, dorsal reflectance, altitude and sex explained 65% of variance in heating rate ($F_{5,24} = 8.81$, $P < 0.001$; Table 3). This model showed that the two variables, body mass and dorsal reflectance, negatively and significantly covaried with heating rate in an independent way (respectively: $\beta = -0.465$, $\beta = -0.545$). After controlling for body mass and dorsal reflectance, heating rate varied significantly with altitude, but following a non-linear pattern, with heating rate being the lowest at mid-altitude.

Cooling rate

Cooling rate did not significantly vary with altitude (one-way ANOVA, $F_{2,\ 27} = 1.25$, $P = 0.304$; Table 2). Univariate analyses showed that cooling rate decreased with body mass ($r = -0.46$, $P < 0.006$; Fig. 3b), but did not covary significantly with dorsal reflectance ($r = -0.25$, $P = 0.177$; Fig. 4b). Hence, smaller lizards lost temperature faster, but dorsal reflectance did not affect heat loss. A full model including body mass, dorsal reflectance, altitude and sex was marginally non-significant ($F_{5,\ 24} = 2.59$, $P = 0.052$; Table 3). However, body mass significantly covaried with cooling rate after controlling for the other variables ($\beta = -0.553$; Table 3).

Transitional lapse

Transitional lapse did not significantly vary with altitude (one way ANOVA, $F_{2,\ 27} = 1.27$, $P = 0.296$; Table 2). We did not find a significant correlation between body mass or dorsal reflectance and transitional lapse ($r = 0.223$, $P = 0.236$ and $r = 0.285$, $P = 0.127$, respectively). A full model including body mass, dorsal reflectance, altitude and sex was not significant ($F_{5,\ 24} = 0.88$, $P = 0.51$) and no predictor significantly covaried with transitional lapse (Table 3).

Net gain of heat

NGH did not significantly vary with altitude (one-way ANOVA, $F_{2,\ 27} = 1.27$, $P = 0.296$; Table 2). Pearson correlations showed that NGH decreased with body

Table 2. Thermal rates and transitional lapses in *S. grammicus* lizards. No differences were found between sexes and the interaction altitude*sex was not significant, so data for the two sexes have been pooled. Values are mean ± SE

	Low altitude (Texcalyacac 2500 m)	Middle Altitude (Las Lagrimas 3400 m)	High altitude (Nevado de Toluca 4300 m)
	(N = 10)	(N = 10)	(N = 10)
Heat rate (°C s^{-1})	0.059 ± 0.003	0.0579 ± 0.004	0.0663 ± 0.003
Cooling rate (°C s^{-1})	0.003 ± 0.0003	0.004 ± 0.0009	0.014 ± 0.0006
Net gain of heat (°C s^{-1})	0.045 ± 0.003	0.044 ± 0.003	0.051 ± 0.002
Lapses (s)	60.2 ± 2.96	60.2 ± 6.24	60.2 ± 4.27

mass (r = -0.46, P < 0.01; Fig. 3c) and with dorsal reflectance (r = -0.42, P < 0.01; Fig. 4c). That is, smaller lizards and darker lizards gained more heat from heliothermy than larger and lighter lizards. A full model including body mass, dorsal skin reflectance, altitude and sex explained 64% of variance in NGH ($F_{5,24}$ = 8.59, P < 0.001; Table 3). This model showed that the two variables, body mass and dorsal reflectance, negatively and significantly covaried with NGH in an independent way (Table 3), the effect size of dorsal reflectance (β = -0.657) being almost twice that of body mass (β = -0.388). After controlling for body mass and dorsal reflectance, NGH significantly varied with altitude, but following a non-linear pattern, with NGH being the lowest at mid-altitude.

DISCUSSION

Our results show that *S. grammicus* lizards are smaller and darker at high elevations. Smaller and darker lizards showed the greatest heating rate and net gain of heat and consequently were more efficient in heliothermic heating. Therefore, in *S. grammicus*, it seems that to be small and dark is advantageous in high-altitude environments. However, we failed to find greater heating rate or neat gain of heat in lizards from the high altitude. It is likely that other traits affect heating efficiency, including adjustments in thermoregulation, behavioural postures during basking or physiological characteristics such as blood flow or an increase in the metabolic rate (Carrascal *et al.*, 1992; Gvozdík, 2002; Díaz de la Vega-Pérez *et al.*, 2019; Plasman *et al.*, 2020).

Tests of the thermal melanism hypothesis have yielded mixed support. Zamora-Camacho *et al.* (2014) did not find a direct relationship between dorsal coloration and heat gain in *Psammodromus algirus*, despite this lizard being darker at higher altitudes. Similarly, Gvozdík (1999) failed to find a positive effect of melanism on thermal properties of *Zootoca vivipara*, and even suggested that melanism is not an advantage in animals of relatively small size. Similar results were described by Tosini *et al.* (1992) in *Podarcis muralis*. However, other research has found that lower dorsal skin reflectance in cold environments, such as at high altitudes, can allow individuals to gain heat faster (Clusella-Trullas *et al.*, 2009; Geen & Johnston, 2014). For example, *Cordylus niger* at relative high altitude had lower DSR and a higher heating rate than *Cordylus cordylus* at low altitude (Clusella-Trullas *et al.*, 2009). In addition, the thermal melanism hypothesis is supported in polymorphic lizards such as *Tiliqua scincoides,* in which a dark morph had faster heating rate than lighter-coloured morphs (Geen & Johnston, 2014).

The role of Bergmann's rule in lizards is controversial (Vinarski, 2014). For example, in the lizards *Psammodromus algirus* and *Podarcis hispanica,* body mass and SVL increase with altitude (Zamora-Camacho *et al.*, 2014; Ortega *et al.*, 2015), and it has been shown that larger lizards gain more heat from heliothermic thermoregulation in *Podarcis hispanica* (Gabirot *et al.*, 2013), *Psammodromus algirus* (Zamora-Camacho *et al.*, 2014) and the *Liolaemus goetschi* group (Moreno-Azócar *et al.*, 2016). However, other studies show that lizards follow the inverse of Bergmann's rule (Ashton & Feldman, 2003). In the genus *Sceloporus*, Oufiero *et al.* (2011) reported that most species follow the inverse of Bergmann's rule, although a few species show an increase in size with altitude [e.g. *Sceloporus undulatus* (Sears & Angilletta, 2004)] and others exhibit no relationship between altitude and body size [*Sceloporus graciosus* (Sears & Angilletta, 2004); *Sceloporus torquatus* (González-Morales *et al.*, 2015)]. The relationship between body size and altitude has received mixed support in the genus *Liolaemus,* which inhabits montane environments in the Southern Hemisphere. Pincheira-Donoso *et al.* (2008) did not find a relationship between body size and climate in this genus; however, Cruz *et al.* (2005) reported a positive relationship between body size and latitude in the same genus, suggesting that lizards in this genus follow Bergmann's rule. In our study, body size of *S. grammicus* decreased

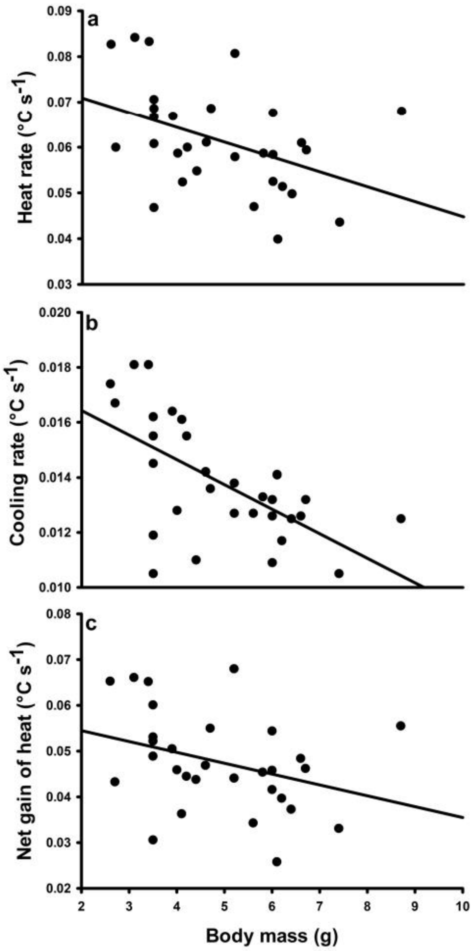

Figure 3. Relationship between body mass and thermal rates in *S. grammicus*, note that as body mass increases the values of thermal trait decrease: (a) heating rate, (b) cooling rate and (c) net heating rate.

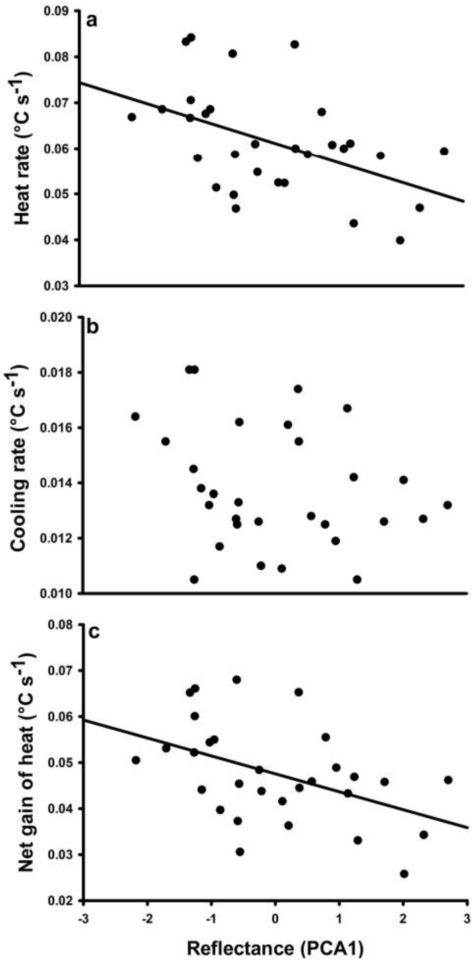

Figure 4. Relationship between "reflectance" and thermal rates in *S. grammicus*, note as dorsal reflectance decreases the values of thermal trait increase: (a) heating rate, (b) cooling rate and (c) net heating rate.

with altitude, following the inverse of Bergmann's rule. Zamora-Camacho *et al.* (2014) suggested that whether or not lizards follow Bergmann's rule may depend on how both heating and cooling rates vary with body size. According to these authors, when the heating rate increases with body size faster than the cooling rate, then the net gain of heat increases with body size, and the lizard is expected to follow Bergmann's rule. This can be seen in *Podarcis hispanicus* and in *Psammodromus algirus* (Gabirot *et al.*, 2013; Zamora-Camacho *et al.*, 2014). However, when the heating rate increases slower than the cooling rate, then the net heat gain decreases with greater body size, and the lizard is expected to follow the inverse of Bergmann's rule. This is the case with *S. grammicus*. Therefore, the fact that *S. grammicus* body size follows the inverse of Bergmann's rule is

Table 3. Full models explaining variation in thermal rates

Variable	d.f.	F-value	*P*-value
Heating rate ($F_{5,\,24} = 8.81, P < 0.001, R^2 = 0.65$)			
Body mass	1, 24	6.27	0.02
Dorsal reflectance	1, 24	8.45	0.008
Altitude	2, 24	4.07	0.03
Sex	1, 24	1.44	0.242
Cooling rate ($F_{5,\,24} = 2.59, P = 0.052, R^2 = 0.35$)			
Body mass	1, 24	4.83	0.038
Dorsal reflectance	1, 24	1.93	0.177
Altitude	2, 24	1.71	0.202
Sex	1, 24	0.60	0.444
Transitional lapse ($F_{5,\,24} = 0.88, P = 0.511, R^2 = 0.15$)			
Body mass	1, 24	0.25	0.622
Dorsal reflectance	1, 24	0.63	0.434
Altitude	2, 24	0.41	0.672
Sex	1, 24	0.71	0.408
Net gain of heat ($F_{5,\,24} = 8.59, P < 0.001, R^2 = 0.65$)			
Body mass	1, 24	4.31	0.049
Dorsal reflectance	1, 24	12.10	0.002
Altitude	2, 24	4.08	0.03
Sex	1, 24	1.15	0.295

consistent with small body size being selectively favoured by cold temperatures at high altitudes.

Body size can be related to the length of the activity period in lizards because shorter activity periods reduce the energy available that individuals could obtain. For example, *Sceloporus undulatus* lizards from New Jersey have 8% less active time than the closely related species *Sceloporus consobrinus* [previously also considered *Sceloporus undulatus* (see Leaché & Reeder, 2002)] from Nebraska, and are also 7% smaller, suggesting that the length of the activity period may be a non-adaptive explanation of why lizards and other ectotherms exhibit small bodies at high altitudes or latitudes (Sears & Angilletta, 2004; also see Adolph & Porter, 1993, 1996). Therefore, the altitude pattern of *S. grammicus* body size could also be a consequence of lizards having reduced growth opportunities at high elevations since they have to bask for more time, reducing time for other activities such as foraging (Gvozdík, 2002; Sears & Angilletta, 2004).

Our findings also indicate that at least part of the altitudinal variation in dorsal skin reflectance may be due to phenotypic plasticity, given that lizards, at least at low altitude, varied their dorsal reflectance when exposed to different temperatures. Lizards from high-altitude populations exhibited the lowest reflectance, which also might be due to a protective role against solar radiation. In fact, lizards at low altitude, when exposed to 30 °C, decreased their dorsal skin reflectance, becoming darker, which may be interpreted as a protective response against solar radiation (Porter & Norris, 1969; Reguera *et al.*, 2014).

Although we observed differences between populations in dorsal coloration that may result from genetic adaptation, our findings also suggest that dorsal skin reflectance is phenotypically plastic in response to changes in temperature, at least in lizards from our low-altitude populations (Cadena *et al.*, 2018). Moreover, at high elevation, females were darker than males at 30 °C. Darker coloration in active females could be adaptive due to differences in habitat use; females move between male territories and could be more exposed to predators than males are [see Quintana *et al.* (2014) and Marshall *et al.* (2015)]. However, we cannot rule out the possibility that dorsal skin reflectance in *S. grammicus* is lower at high altitude as a consequence of other selective pressures, such as crypsis or protection against solar radiation. UV radiation in areas of high elevations is very high as a result of a thinner atmosphere (Blumthaler *et al.*, 1997); hence the dorsal coloration could also have the function of protecting the body from oxidative damage by UV radiation (Porter & Norris, 1969). Nonetheless, evidence that dorsal melanism improves protection against UV radiation is limited (Reguera *et al.*, 2015). On the other hand, lizards from the middle-altitude population did not show differences in the dorsal skin reflectance due to temperature, this zone is characterized by high precipitation, and it has been shown that the dorsal skin reflectance has a negative relationship with the environmental precipitation (see Delhey, 2018). Future studies measuring an increased range of wavelength reflectance, including 700–2100 nm, could be necessary for discrimination between thermal traits and crypsis (Smith *et al.*, 2016; Stuart-Fox *et al.*, 2017).

The lizards we studied are from higher elevations than those used in most previous works in this area (Clusella-Trullas *et al.*, 2009; Zamora-Camacho *et al.*, 2014), and this is the first study that combines data on heat gain, dorsal skin coloration and body size in ectotherms from such an extreme altitudinal gradient. Taken together, our findings suggest that small body sizes and dark dorsal coloration allow for faster heat gain in *Sceloporus grammicus*. Therefore, our results are consistent with the thermal melanism hypothesis, and although we did not find differences in heating rate or NGH by altitude, it is probable that being smaller and darker provides thermoregulatory benefits at high altitudes.

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The authors declare that they have no conflict of interest.

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7. Capítulo II

Fast and dark: the case of Mezquite lizards at extreme altitude.

González-Morales JC, Rivera-Rea J, Moreno-Rueda G, Bastiaans E, Castro-López M, Fajardo V. Journal of Thermal Biology 102: 103-115.

El trabajo que a continuación se presenta, contiene datos de la longitud hocico-cloaca, longitud de las patas traseras, reflectancia dorsal y máxima velocidad de *sprint* en *Sceloporus grammicus* durante el verano en tres poblaciones de diferente elevación. La literatura disponible indica que, en varias especies de lagartijas, la velocidad de *sprint* es dependiente de la temperatura corporal, por lo que nos preguntamos si a bajas temperaturas corporales los organismos de alta elevación podrían mostrar un mejor desempeño respecto a las lagartijas de elevaciones bajas. Por otra parte, la reflectancia dorsal puede tener un papel relevante en evitar la depredación mediante la cripsis, por lo que esperábamos que las lagartijas fueran más oscuras cuando la velocidad de *sprint* era baja. Se obtuvo la máxima velocidad de sprint y la reflectancia dorsal a tres temperaturas corporales (10, 20 y 30 °C). Los resultados muestran que a 30 °C la velocidad de sprint fue mayor en las tres poblaciones respecto a 10 y 20 °C, por otra parte, no encontramos relación entre la velocidad de sprint y la longitud hocico-cloaca y la longitud de las patas inferiores. La velocidad de sprint no es diferente a 20 y 30 °C entre las tres altitudes, sin embargo, a 30 °C, la velocidad de sprint fue mayor en la población de alta elevación. La reflectancia dorsal se relacionó significativamente con la velocidad de sprint a 20 °C, las lagartijas más oscuras eran más lentas.

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Fast and dark: The case of Mezquite lizards at extreme altitude

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ABSTRACT

Sprint speed is a major performance trait in animal fitness involved in escaping from predators, obtaining food, and defending territory. Biotic and abiotic factors may influence sprint speed in lizards. Temperature decreases at higher altitude. Therefore, lizards at high elevations may require longer basking times to reach optimal body temperatures, increasing their vulnerability to predation and decreasing their time for other activities such as foraging or reproduction. Here, we tested whether the maximum sprint speed of a lizard that shows conservative thermal ecology varied along an altitudinal gradient comprising low (2500 m), middle (3400 m) and high-altitude (4300 m) populations. We also tested whether sprint speed was related to dorsal reflectance at different ecologically relevant temperatures. Given that the lizard *Sceloporus grammicus* shows conservative thermal ecology with altitude, we expected that overall average sprint speed would not vary with altitude. However, given that darker lizards heat up quicker, we expected that darker lizards would be faster than lighter lizards. Our results suggest that *S. grammicus* at high altitude are faster and darker at 30 °C, while lizards from low and middle altitude are faster and lighter in color at 20 °C than high altitude lizards. Also, our results suggest a positive relationship between sprint speed and dorsal skin reflectance at 10 and 20 °C. Sprint speed was also affected by snout-vent length, leg length, and leg thickness at 10 °C. These results suggest that, even though predation pressure is lower at extreme altitudes, other factors such as vegetation cover or foraging mode have influenced sprint speed.

1. Introduction

Natural selection acts more directly on traits related to whole-organism performance (Arnold, 1983; Bonine and Garland, 1999), such as sprint speed (Bauwens et al., 1995; Husak et al., 2006), endurance (Scales et al., 2009; Quintana et al., 2014) or bite force (Verwaijen et al., 2002; Husak and Fox, 2006), than on narrower aspects of morphology or physiology. Organism performance has been implicated as a crucial link in our understanding of how natural selection might mold a phenotype to the environment (Arnold, 1983; Huey et al., 1984; Sinervo and Losos, 1991). Sprint speed is a performance trait of considerable importance to an organism's life history (Bennett, 1990; Sinervo and Losos, 1991; Zamora-Camacho et al., 2015a, 2015b), as it is involved in a number of fitness-related processes, such as escaping predators (Hertz et al., 1982; Lima and Dill, 1990) and obtaining food (Vanhooydonck et al., 2001). In some species, sprint speed may also be a sign of social status (Peterson and Husak, 2006; Zajitschek et al., 2012). In the case of ectothermic organisms such as lizards, sprint speed and other metabolic or physiological traits are strongly related to body temperature. In many cases, performance is optimal within a certain range of body temperatures, but above or below that range it decreases (Angilletta, 2009; Angilletta et al., 2010).

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Geographic variation in environmental temperatures can affect ectotherm thermal biology (Ragland and Kingsolver, 2008; Sunday et al., 2014; Muñoz and Bodensteiner, 2019). Lizards respond to geographic changes in temperature in two manners. In some taxa, the body temperature (*Tb*) at which performance traits such as sprint speed are optimized is very similar among populations or closely-related species living in different thermal environments (*i.e.* altitude or latitude), so individuals from different geographic areas compensate behaviorally for environmental differences to obtain an optimal *Tb* (Crowley, 1985; Hertz et al., 1983; Andrews, 1998). For example, lizards may bask for different periods of time according to environmental temperature. This has been described in *Sceloporus grammicus* from different altitudes (Lemos-Espinal and Ballinger, 1995; Díaz de la Vega-Pérez et al., 2019). Other species' thermal requirements are more plastic and can be modified according to the type of environment. In these taxa, a strong relationship between body and environmental temperatures is expected (Hertz et al., 1983). For example, species of the genus *Liolaemus* exhibit a lower field body temperature in higher altitude areas (Rodríguez-Serrano et al., 2009). Similarly, the lizard *Iberolacerta galani* modifies its body temperature according to seasonal variation in the thermal environmental (Ortega et al., 2016).

Variation in morphology and physiology may imply individual variation in fitness. Sprint speed performance is directly and positively related to body size and hind limb length (Bonine and Garland, 1999; Husak and Fox, 2006; Scales et al., 2009). Muscles involved in lizard locomotion are composed of fibers, which are classified according to their metabolic and contractile properties as slow-twitch, fast-twitch and intermediate (Close, 1972; Peter et al., 1972; Quintana et al., 2014). These muscle fibers can influence whether a lizard is slow, as in horned lizards (*Phrynosoma* spp.), with a high proportion of slow twitch muscle fibers, or fast as in sand lizards (*Uma*, *Callisaurus*, *Cophosaurus* and *Holbrookia*), with a high proportion of fast-twitch muscle fibers (Bonine and Garland, 1999).

In addition to sprint speed, dorsal reflectance is a key predator-avoidance trait in lizards (Bond, 2007; Orton and McBrayer, 2019; Moreno-Rueda et al., 2019). Recent work found that lizards exhibit a negative relationship between dorsal brightness and predation rate (Orton and McBrayer, 2019). This means that in environments with greater predation pressure, lizards are expected to be darker. In polychromatic species, dark morphs flee less often and attack predators more than light morphs, which are more likely to flee from predators (Bruinjé et al., 2019). Dorsal reflectance can function as auxiliar mechanism of thermoregulation. In a previous study with *S. grammicus*, lower reflectance (darkness) increased heating rate in lizards from a high-altitude environment, reducing potential basking time (González-Morales et al., 2021).

In this study, we tested whether *S. grammicus* lizards from different altitudes differ in maximum sprint speed at ecologically relevant body temperatures (*Tb*). In addition, we analyzed whether sprint speed is related to dorsal coloration measured as reflectance. We predicted that high-elevation lizards would perform better at low *Tb* than middle- or low elevation lizards and that sprint speed would be negatively related to dorsal reflectance.

2. Materials and methods

2.1. Study species and study area

Sceloporus grammicus is a small lizard averaging 52 mm snout-vent length (SVL) and 4.6 g body mass. It occurs from southern Texas (USA) to the Mexican state of Oaxaca and has a wide altitudinal distribution, including populations above 4000 m (Lemos-Espinal and Ballinger, 1994; Bastiaans et al., 2014; González-Morales et al., 2017), occupying a wide variety of habitats including rocky areas and trees. We used three populations at different altitudes in central Mexico during June–July of 2019: 1) low altitude population (LAP): Texcalyacac at 2500 m (19° 07′37″ N, 99° 29′41″ W, n = 15), 2) Middle altitude population (MAP): Las Lágrimas at 3200 m (19°06′06″ N, 99°49′48″ W, n = 23) and 3) High altitude population (HAP): Nevado de Toluca at 4300 m (19°06′06″ N, 99°46′03″ W, n = 25).

The LAP has a temperate sub-humid climate with a temperature range of 12–14 °C (INEGI, 2009 [Geostatistical key 15098]). Here, the lizards' habitat is characterized by igneous extrusive rocks. The MAP has a sub-tropical climate with a temperature range of 10–20 °C and a habitat characterized by fallen pine trunks and living pine trees (INEGI, 2009 [Geostatistical key 15004]). The HAP has a cold climate with an annual temperature range of −5 to 5 °C and a habitat consisting of igneous exposed rocks with little or no vegetation (Conanp, 2016).

2.2. Field work and animal maintenance

We captured lizards by hand or lasso during the summer of 2019. We only used adult animals, identified by having a SVL >35 mm and secondary sexual coloration (Zuñiga- Vega et al., 2008). We did not include tailless or pregnant lizards, because these characteristics diminished sprint speed in some lizards (Martin and Avery, 1998). Lizards were transported (1.5 h drive) in cloth bags to the Instituto para la Conservación de la Cordillera Neovolcánica ante el Cambio Climático, where each lizard was maintained in an individual plastic terrarium (23 × 41 × 21 cm; width × length × height, respectively) with substrate of peat moss. Water and food (larvae of *Tenebrio molitor*) were provided *ad libitum*. Lizards were exposed to the natural photoperiod (approximately 12:12). During the day, heat was provided by a 60 W light bulb (20–35 °C range of temperature in each individual terrarium) and rocks were set in one corner of each terrarium to allow normal thermoregulatory behavior. Prior to the experiment, we measured body mass with a balance (precision: 0.01 g) and SVL and hind limb length with a digital caliper (precision: 1 mm). Finally, leg thickness was measured with a pressure-sensitive micrometer at the bisector of the thigh (Mitutoyo, accuracy; 0.01 mm). We estimated the residual values of a linear regression of log-mass vs. log-SVL, in order to calculate body condition index (BCI). The same individuals were used for all tests described below.

We started behavioral trials one day after capture. Sprint speed trials were performed on three different consecutive days with at least 15 h of rest between races. Prior to measurement of sprint speed and dorsal reflectance, lizards were placed in a thermal chamber set to 10, 20, or 30 °C. These temperatures were used because the lizards encounter them under field conditions (Texcalyacac: 11–24.5 °C, Las Lagrimas: 10–25.9 °C, Nevado de Toluca: 5–28.2 °C; González-Morales et al., unpublished data obtained with a biophysical thermoregulation-null model). We randomized the order in which each lizard experienced these three temperatures. After 1 h, we measured dorsal reflectance and sprint speed for each lizard.

2.3. Sprint speed measurement

We performed sprint speed trials in a straight, wooden raceway (200 × 10 × 30 cm) with an inclination of 45° because this is the most frequent angle that the lizards usually run when we approach them in natural conditions in the field. The raceway was lined with artificial cork to provide appropriate traction (Bauwens et al., 1995). The raceway was divided into twenty 10 cm stretches, delimited with contrasting color transversal strips. We set a dark background at the elevated end of the raceway to simulate a refuge and encourage the lizards to run forward and up. Lizards ran individually. Each lizard ran three consecutive times in each trial, as they exhibited no sign of fatigue.

All trials were recorded with a Motorola G5 video camera at 30 frames per second. Then, videos were analyzed using the Adobe Premier CC software, as it is able to analyze each video frame by frame, which allowed us to be especially precise in obtaining the speed values for each video. We recorded the time interval for each stretch, considering a stretch as crossed when the snout of the lizard reached the strip delimiting it. Because our videos were recorded at 30 frames/s, each frame represented 1/30 s, or 0.033 s. Therefore, the time a lizard spent in each segment was obtained by subtracting the initial frame value from the final frame value and dividing it by 0.033. Finally, we divided the distance by time to obtain the running speed for each of the raceway segments. We performed our analyses using the fastest five stretches for each lizard at each body temperature (10, 20 and 30 °C), since lizards in the wild run short distances to their refuges when escaping from potential predators (personal observation). The fastest and second-fastest performances of each individual at the same temperature were highly correlated, indicating high repeatability for sprint speed within lizards ($r = 0.96$, $p < 0.001$).

2.4. Dorsal skin reflectance

Following the protocol of González-Morales et al. (2021), we measured dorsal reflectance at three points on the dorsal region distributed along the body of lizards (lower, middle and upper regions [Fig. 1]). A small ink mark was placed next to the measurement spot to ensure that the same location was used for all reflectance measurements (see González-Morales et al., 2021). For each lizard, and after speed trials, all reflectance measurements were made in less than 1 min and never more than five days after their capture. We used the average of measurement of each lizard. Reflectance was measured using a spectrophotometer (Jaz Ocean Optics) with a Pulsed Xenon Light Source (Jaz-PX) connected to an optical fiber within the 300–700 nm range, which includes most of the spectrum reflected by lizards' dorsa. The probe was mounted within a holder that ensured readings were taken from areas 1 mm in diameter at a constant distance of 3 mm from the skin surface with a 45° angle (Megía-Palma et al., 2016; González-Morales et al., 2021). All measurements were relative to a 99% WS-1 white reflectance standard (all components from Ocean Optics Inc. Dunedin. FL, USA). Raw spectra were imported into the software CLR, smoothed using a running average computed over a 20 nm interval, and the average reflectance was considered as dorsal reflectance. All lizards were released at their exact point of capture after all trials were completed.

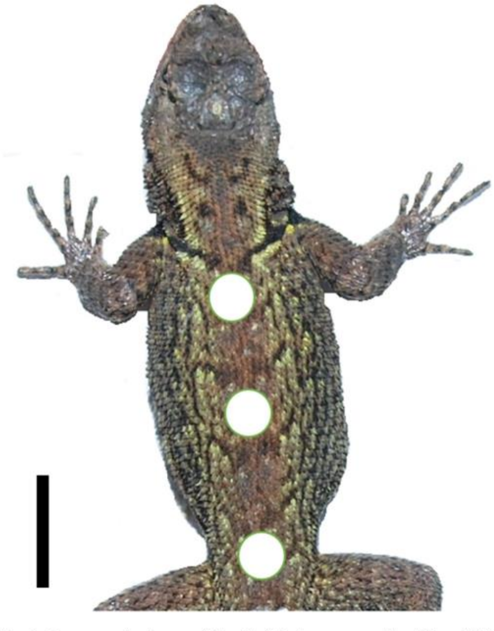

Fig. 1. Representative image of dorsal of *Sceloporus grammicus*. The solid line represents 1 cm in length. White circles show sample points for dorsal reflectance measurement.

2.5. Statistical analyses

Prior to all analyses, the assumptions of normality and homogeneity of variance were checked. Body mass fulfilled the assumptions with no transformation; SVL, leg hind-limb length and leg thickness met the assumptions after log transformation (Quinn and Keough, 2002). We conducted Ordinary Least Squares Linear Models. First, we analyzed the effect of elevation and sex on body mass, SVL, BCI, hind-limb length and leg thickness. Second, sprint speed and dorsal reflectance were analyzed separately using Linear Mixed Models (LMM), with altitude, sex, and body temperature as fixed factors and lizard ID as a random factor. When a test revealed a significant difference, the relevant post hoc tests (Bonferroni) were performed. Additionally, we used the Spearman rank correlation to evaluate the relationship between sprint speed and morphological measurements and dorsal reflectance at the three body temperature treatments used. Finally, we performed an ANCOVA for sprint speed at 20 and 30 °C *Tb* with dorsal coloration as a covariate to disentangle variation due to altitude and due to dorsal reflectance. Measurements at 10 °C were excluded because we did not find differences in sprint speed by altitude. The level of significance was established at $p < 0.05$. Statistical analyses were performed in JAMOVI (version 1.7.1)

2.6. Ethics statement

All experimental procedures were carried out following the guidelines of the Universidad Autónoma del Estado de México (UAEMéx), as well as the Mexican Federal Regulation for Animal Experimentation and Care (NOM-062-ZOO-2001; governmental approval SGPA/DGVS/02407/13).

3. Results

3.1. Morphological parameters

Lizards from HAP had a lower body mass than lizards from LAP and MAP (Table 1), and females had a lower body mass than males ($F1,54 = 15.33$, $P < 0.001$; Table S1). The interaction between altitude and sex was not significant ($F2,54 = 1.46$, $P = 0.24$). Similarly, lizards from LAP had greater SVL than MAP and HAP lizards (Table 1), but SVL was not affected by sex ($F1,58 = 1.12$, $P = 0.29$; Table S1) or the interaction of sex and altitude (altitude*sex: $F2,58 = 2.44$, $P = 0.11$). BCI differed among altitudes, with lizards from HAP having lower BCI than LAP and

Table 1
Morphological parameters of *Sceloporus grammicus* at different altitudes.

	LAP (2500 m)	MAP (3200 m)	HAP (4100 m)	F-values	*p*-values
Body mass (g)	6.0 ± 0.50^a	4.86 ± 0.27^{ab}	4.47 ± 0.23^b	6.08	0.004
SVL (mm)	59.0 ± 0.02^a	53.6 ± 0.01^b	50.6 ± 0.07^b	9.08	<0.001
BCI	0.00002 ± 0.49^a	-0.00001 ± 0.23^a	-0.002 ± 0.14^a	5.61	0.006
Hind limb length (mm)	34.9 ± 0.01^a	35.5 ± 0.01^a	30.2 ± 0.007^b	10.15	<0.001
Leg thickness (mm)	0.12 ± 0.005^a	0.13 ± 0.002^a	0.14 ± 0.029^a	24.39	<0.001

Note: letters show differences by altitude. Values are mean ± SE.

MAP lizards (Table 1), but males did not differ significantly from females ($F1,58 = 1.88$, $P = 0.17$; Table S1) nor was there a significant effect of the interaction of altitude and sex ($F2,54 = 2.61$, $P = 0.08$). Lizards of LAP and MAP had longer hind limbs than lizards from HAP (Table 1), but no differences associated with sex were found ($F1,53 = 1.26$, $P = 0.26$; Table S1), and the interaction between altitude and sex was not significant ($F2,53 = 0.94$, $P = 0.39$). Lizards of HAP had less thick legs than LAP and MAP lizards (Table 1), and females had thicker legs than males ($F1,53 = 15.32$, $P < 0.001$; Table S1). Males' leg thickness did not differ among altitudes, but females from MAP had thicker hindlimbs than females from LAP and HAP (altitude*sex interaction: $F2,53 = 17.10$, $P < 0.001$).

3.2. Dorsal reflectance

The interaction altitude*sex*Tb was not statistically significant ($F4,114 = 0.78$, $P = 0.53$). Dorsal reflectance varied with altitude, with lizards from HAP having lower reflectance (*i.e.*, being darker) than lizards from LAP and MAP (which were lighter) ($F2,57 = 40.39$, $P < 0.001$; Table 2). Females had lower dorsal skin reflectance than males ($F1,57 = 13.32$, $P < 0.001$). There was a significant effect of the interaction between Tb and altitude on dorsal reflectance; dorsal reflectance was not related to Tb in MAP and HAP, while lizards from LAP had their maximal reflectance at 20 °C (interaction altitude*Tb: $F4,114 = 3.21$, $P < 0.001$; Table 2).

3.3. Sprint speed

Since no sex differences in sprint speed were found, we pooled data from males and females for the analysis of sprint speed ($F1,57 = 0.57$, $P = 0.45$). Sprint speed was higher at 30 °C than at 10 and 20 °C ($F2,116 = 303.03$, $P < 0.001$, Fig. 2). The main effect of altitude on sprint speed was not significant ($F2, 57 = 0.83$, $P = 0.83$). However, at 20 °C, lizards of HAP had slower sprint speed than lizards of LAP and MAP, at 30 °C, lizards of HAP had faster sprint speed than lizards from the other two populations, and at 10 °C sprint speed did not differ by population (altitude*Tb interaction: $F4, 116 = 13.75$, $P < 0.001$). The analysis of covariance showed that when we used dorsal reflectance as a covariate, the effects of altitude and Tb remain significant. That is, HAP lizards were faster than LAP and MAP lizards when they ran at 30 °C ($F2,55 = 6.24$, $P = 0.003$), while at 20 °C, lizards from LAP and MAP were faster than HAP lizards ($F2,55 = 13.80$, $P < 0.001$).

The relationship between speed and morphological variables was only significant at 20 °C, with sprint speed increasing with body mass and SVL (Table 3). Similarly, speed sprint was positively correlated with hind-limb length and leg thickness (Table 3). We found a significant relationship between sprint speed and reflectance at 10 and 20 °C ($R = 0.34$, $P = 0.006$ and $R = 0.36$, $P = 0.003$), but not at 30 °C ($R = -0.18$, $P = 0.16$; Fig. 3). That is, darker lizards (with lower reflectance) had reduced sprint speed at 10 and 20 °C.

4. Discussion

Our results show that *S. grammicus* lizards from the HAP were faster and darker at 30 °C when compared with lizards from LAP and MAP, whereas, at 20 °C, lizards from HAP were slower than those from LAP and MAP. Moreover, the relationship between sprint speed and dorsal reflectance was statistically significant at 10 and 20 °C, with darker lizards being slower than lighter lizards The pressures on sprint speed in lizards depend on the ecological context. To obtain food, they can only use a percentage of the maximum speed available, while to escape from a predator or defend their territory, they could use their maximum speed (Irschick and Garland, 2001; Husak and Fox, 2006). Several studies have shown that, in lizards, the maximum sprint speed recorded under laboratory conditions is very similar to maximum speed in field conditions when escaping from a predator and is highly repeatable across years, which makes this a good indicator of survival probability (Huey and Dunham, 1987; Sinervo and Losos, 1991; Miles et al., 2000; Husak and Fox, 2006). Based on this, we consider the data obtained from *Sceloporus grammicus* to be a good indicator of the maximal sprint speed under natural conditions.

Table 2
Dorsal reflectance values from *Sceloporus grammicus* at different *Tb* conditions.

	10 °C	20 °C	30 °C
LAP	8.70 ± 1.03^{ABa}	9.90 ± 0.92^{Ba}	6.57 ± 0.57^{Aa}
MAP	7.04 ± 0.49^{Aa}	6.90 ± 0.42^{Ab}	4.64 ± 0.28^{Aa}
HAP	4.64 ± 0.26^{Ab}	5.13 ± 0.24^{Ac}	3.97 ± 0.28^{Ab}

Note: LAP: low altitude population, MAP: middle altitude population and HAP: high altitude population. Superscript capital letters show differences between *Tb* in the same population, superscript lowercase letters show differences for altitude in the same *Tb*. Values are mean ± SE.

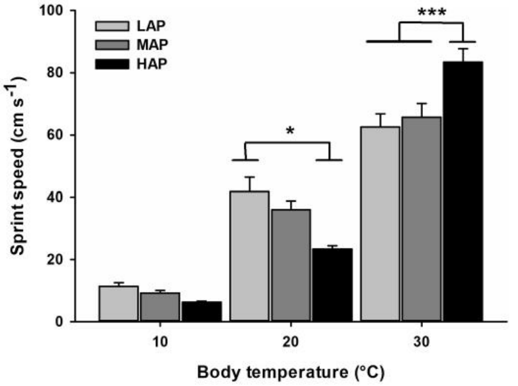

Fig. 2. Sprint speed of *Sceloporus grammicus* from different altitudes by *Tb*. Abbreviations are LAP: low altitude population; MAP: middle altitude population and HAP: high altitude population. Capital letters show differences by *Tb* between altitudes, lowercase letters show differences by altitude at the same *Tb*.

Table 3
Spearman correlation between sprint speed at different *Tb* and morphological parameters in *Sceloporus grammicus* lizards.

	Body mass (g)	SVL (mm)	BCI	Hind limb length (mm)	Leg thickness (mm)
Sprint at 10 °C	−0.07	−0.06	−0.06	−0.08	0.01
	0.60	0.62	0.62	0.56	0.93
Sprint at 20 °C	**0.51**	**0.36**	**0.36**	**0.33**	**0.37**
	<0.001	**0.004**	**0.004**	**0.010**	**0.003**
Sprint at 30 °C	0.04	−0.14	−0.14	−0.02	0.04
	0.73	0.27	0.27	0.88	0.73

Note: r and *p* values of morphological traits. Bold *p* values show significant correlations.

Thermoregulatory strategies are generally considered highly conserved in the genus *Sceloporus* (Hertz et al., 1993; Lemos-Espinal and Ballinger, 1995; Andrews, 1998). Our results from *Sceloporus grammicus* across an altitudinal gradient support that hypothesis. Although body temperature is highly variable in *Sceloporus grammicus* from different altitudes (see Table 1 in Woolrich-Piña et al., 2006), in our study sprint speed was highest at the same temperature (30 °C) in all three populations.

High-altitude environments are characterized by low environmental temperatures and lower atmospheric pressure (see Körner, 2007). In addition, temperatures available for lizard activity at high altitudes are

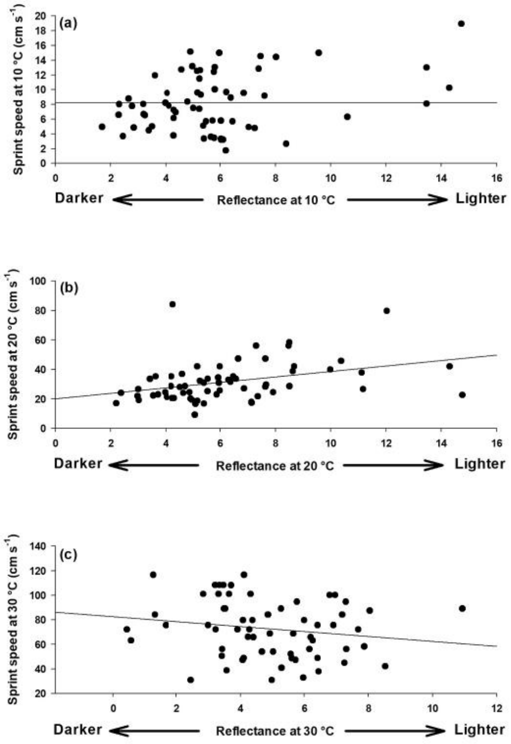

Fig. 3. Relationship between sprint speed and dorsal skin reflectance and 10 (a), 20 (b) and 30 °C (c) from *Sceloporus grammicus* from different altitudes.

usually lower and more variable than at lower sites (Gvoždík, 2002; Gutiérrez et al., 2010; Fierro-Estrada et al., 2019; Díaz de la Vega-Pérez et al., 2019). Additionally, in laboratory studies, *Sceloporus grammicus* from different altitudes showed similar thermal preferences (Díaz de la Vega-Pérez et al., 2019). Therefore, reaching the *Tb* at which sprinting is optimized could have greater associated costs in the higher-altitude populations, for example, requiring increased time basking and causing reduced growth and delayed sexual maturation (Lemos-Espinal and Ballinger, 1995: Sears and Angilleta, 2004). Indeed, although *S. grammicus* thermal preference does not vary with altitude, field body temperatures decreases with elevation (Díaz de la Vega-Pérez et al., 2019), suggesting heating is more costly at high altitude. Nonetheless, low dorsal reflectance significantly increases the heating rate of lizards in cold areas, so thermoregulatory costs could be compensated by this route (Clusella-Trullas et al., 2007; Moreno-Azócar et al., 2020; González-Morales et al., 2021).

Sprint speed is often related to morphological characteristics. For example, lizards with longer limbs typically have higher sprint speeds (Bauwens et al., 1995; Zamora- Camacho et al., 2014). However, we only found a relationship between sprint speed of *S. grammicus* and SVL, hind-limb length, or thickness of the leg at 20 °C. This may be because other morphological characteristics could have a greater influence, such as the frequency of leg movement, pelvic-girdle rotation or vertebral flexure (Sinervo et al., 1991; Sinervo and Losos, 1991; Martín and López, 2001; Husak, 2006). Physiological characteristics may also affect sprint speed, for example, the type of contraction and metabolic components of the muscle fibers involved in locomotion (Swoap et al., 1993; Bonine and Garland, 1999; Vanhooydonck et al., 2001). *Sceloporus grammicus* from the HAP had a greater sprint speed at 30 °C than lizards from the other two populations. This result could be due to a higher percentage of fast twitch fibers than that found in lower altitude populations (Bonine and Garland, 1999). Studies addressing physiological muscle characteristics are necessary to test this hypothesis.

Sprint speed at 20 °C was slower in lizards from the HAP than in lizards from the LAP or MAP. Lizards are usually more vulnerable to predation during the early morning hours when their *Tb* is low (Bulova, 1994), so keeping near shelters may be a strategy to compensate for low sprint speed (Van Damme, 1989; Carrascal et al., 1992). In another mountain system in which *S. grammicus* is present across a similar range of altitudes, lizards from LAP and MAP had lower survival probability than HAP, possibly because the HAP had lower thermal quality, causing lizards to be less active and reducing their exposure to predators (Domínguez-Godoy et al., 2020a). LAP and MAP lizards could be subjected to higher predation pressure, so not reducing their sprint speed as much could benefit their survival (Hertz et al., 1983; Domínguez-Godoy et al., 2020b). In lizards from the HAP, low dorsal reflectance could have multiple benefits, such as increasing the heating rate (González-Morales et al., 2021) and keeping hidden to avoid predators (Clusella-Trullas et al., 2007; see Bond, 2007; Smith et al., 2016a, 2016b; Orton and McBrayen, 2019).

Dorsal reflectance showed no relationship with *Tb* in lizards from MAPs and HAPs. In these sites, lizards typically must bask for longer than at low altitude sites (Lemos-Espinal and Ballinger, 1995; Martín et al., 1995; Gvoždík, 2002; Refsnider et al., 2018), hence the lack of variation in dorsal reflectance with *Tb* may help them increase their body temperatures quickly (Thermal Melanism Hypothesis [see Clusella-Trullas et al., 2007; Smith et al., 2016a]). In the case of lizards from the LAP, we found a significant increase of dorsal reflectance at 20 °C when compared against 10 and 30 °C. Reflectance transition is mediated by alpha melanin stimulant hormone (α-MHS) (Camargo et al., 1999; Moraes et al., 2014; Sheddon and Hews, 2020), which produces melanin dispersion (darkening the skin) or aggrupation (lightning the skin) depending on *Tb* (Sherbrooke, 1997). Changes in dorsal reflectance with *Tb* have been described in other lizards, such as *Phrynosoma cornutum*, *P. modestum* and *P. solare* (Sherbrooke, 1997) and *Pogona vitticeps* (Cadena et al., 2018), as well as in other animals such as the crab *Uca pugilator* (Silbiger and Munguia, 2008).

A lizard's behavior when detecting a predator depends on its *Tb*. If its *Tb* is high, a lizard's usual behavior will be to run to escape the predator, whereas if its *Tb* is low, the lizard may be incapable of running and may instead exhibit aggressive behavior (Hertz et al., 1982; Van Damme et al., 1989; Herrel et al., 2009; de Barros et al., 2010). Variation in the thermal dependence of different muscles has been described in some lizards, with muscles related to locomotor performance (such as the caudofemoralis or iliofibularis) showing stronger thermal dependence than muscles related to aggressive behavior such as bite force (e.g., the adductor mandibulae externus superficialis; Herrel et al., 2007). These results suggest that the shift between flight or aggressive behavior is driven by the differential effect of body temperature on different muscles (Herrel et al., 2007). Although we did not observe any aggressive behavior in our study, we suggest future studies address this question more directly.

Biodiversity tends to decrease with altitude (Rahbek, 1995; Fosaa, 2004; Willing and Presley, 2016), which could imply that in high elevation environments the richness and abundance of potential predators of *S. grammicus* is lower (fewer species of birds or other reptiles as potential predators [Ballinger, 1979; Crowley, 1985; Willing and Presley, 2016, Domínguez-Godoy et al., 2020b]). It's possible that reduced predation pressure at high altitudes may partly explain why we found lower sprint speed in high-altitude lizards at the most ecologically relevant temperature we measured. However, other factors such as the vegetation cover of the substrate, the mode of predation or thermal quality of the environment may also influence sprint speed (Jones, 1981;

Van Damme and Vanhooydonck, 2001; Attum and Eason, 2006; Lara-Reséndiz et al., 2014). In high-altitude environments, the availability of prey may also be less than in low-altitude areas, so lizards may forage more actively (Huey et al., 1984).

In conclusion, sprint speed varied with body temperature in lizards. It was also related to dorsal reflectance and sprint speed, but only at 20 °C. Lizards were darker at higher elevations, and body size did not affect sprint speed. Lizards from our HAP are faster at temperatures near their preferred *Tb*, but they are slower at low *Tb* compared to individuals from our LAP or MAP.

Declaration of competing interest

The authors declare that they have no conflict of interest.

CRediT authorship contribution statement

Juan Carlos González-Morales: Conceptualization, Visualization, Investigation, Methodology, Writing - Original Draft, review & editing. **Jimena Rivera-Rea:** Investigation, Methodology, Data curation, Formal analysis, review & editing. **Gregorio Moreno-Rueda:** Visualization, Data curation, Software, Formal analysis, Validation, Writing - review & editing. **Elizabeth Bastiaans:** Methodology, Software, Data curation, Formal analysis, Visualization, Writing - original draft, Writing - review & editing. **Meily Castro-López:** Methodology, Data curation, Formal analysis, review & editing. **Víctor Fajardo:** Conceptualization, Investigation, Funding acquisition, Methodology, review & editing, Project administration.

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Appendix A. Supplementary data

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8. Capítulo III

Thermoregulatory varies with altitude and season in the Sceloporine Mesquite lizard.

González-Morales JC, Rivera-Rea J, Moreno-Rueda G, Bastiaans E, Macotela L, and Fajardo V. Por enviarse a Oecología.

El trabajo que se muestra a continuación contiene datos de las temperaturas operativas, temperatura corporal en campo y temperatura de selección en laboratorio e índices de termorregulación en tres poblaciones de diferente elevación y a lo largo de las estaciones del año en *Sceloporus grammicus*. El género *Sceloporus* ha sido considerado térmicamente conservativo, es decir, no se aprecian cambios en las preferencias térmicas entre especies cercanas en diferentes ambientes. Considerando que la termorregulación es principalmente conductual en lagartijas y que tiene un rol central en la biología de estos organismos, nosotros nos propusimos evaluar si la temperatura corporal en campo y la temperatura preferida en laboratorio diferían en las tres poblaciones a lo largo de las estaciones del año. Los resultados obtenidos muestran que las temperaturas operativas difieren por altitud y estación del año, siendo menores en las poblaciones de alta elevación y durante el otoño. En el caso de la temperatura corporal en campo y las preferencias térmicas en laboratorio, estas cambian tanto por altitud como por estación del año, las lagartijas de alta elevación tienen una menor temperatura corporal en campo, mientras que las tres poblaciones tienen preferencias térmicas más bajas durante el otoño respecto a la primavera y el verano. Finalmente, los índices de termorregulación fueron distintos entre elevaciones y estaciones del año. Como parte de nuestras conclusiones, consideramos que, al menos en nuestra de zona de estudio, no podemos considerar estrictamente térmicamente conservativo a *Sceloporus grammicus*, y que la variación en la biología térmica a lo largo de las estaciones del año podría tener ventajas en cuanto al uso del tiempo y costos asociados con la termorregulación.

Thermoregulatory varies with altitude and season in the Sceloporine Mesquite lizard.

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Abstract

In ectothermic animals, body temperature is the most important factor affecting physiology and behavioral processes. Reptiles depend on environment temperature to regulate their body temperature, so geographic variations in environmental temperature can affect the biology of these organisms in the short and long term, even more so when temperature is not stable year-round as in temperate zones. High-mountain temperate systems provide natural temperature gradients that allow studies of evolutionary and plastic variation in thermal ecology. The aim of the present study is to evaluate operative temperature with biophyscic models, active body temperature under field conditions, preferred temperature in the thermal gradient in the laboratory, and thermal indexes in *Sceloporus grammicus* lizards along an elevational gradient. We measured these traits in three populations at 2500, 3400, and 4100 m elevation at different seasons of the year (spring, summer, and autumn). Our results showed that operative temperature varied with season and elevation, being more pronounced at middle and high elevations. Body temperature and preferred temperature varied with altitude and season, while sex was not a significant factor. Thermal quality of the environment was lower in high-altitude populations and in the summer season. Thermoregulatory efficiency of the lizards was higher in the three populations in the autumn. Our results suggest that thermoregulations vary with elevation and season to face up thermal environment fluctuations around year, so *Sceloporus grammicus* lizards are not necessarily thermally conservative.

Keywords: thermoregulation, operative temperatures, altitudinal gradient, season, *Sceloporus grammicus*

Introduction

In ectotherms, as other vertebrates, body temperature (*Tb*) is the most important factor that affect physiological and behavioral processes (Clarck et al. 2005; Sun et al. 2012, Angilleta 2009). However, ectotherms such as reptiles depend on environmental temperature (*Te*) to regulate their *Tb* (Shabtay and Arad 2005; Shine 2005; Buckley et al. 2012). Reptiles have low metabolic rates compared to endotherms and they have limited physiological control of *Tb* (Huey and Kingsolver 1989). Thus, reptiles typically use behavioral strategies for maintaining *Tb* in an optimal temperature range (Hertz et al. 1993) in which physiological processes have been optimized (Pough 1980; Huey and Kingsolver 1989).

Environmental temperature shows geographic variation, which could impact the thermal biology in lizards. *Tb* can induce changes over short-terms as in locomotion (Bennett 1990, Zamora-Camacho et al. 2015, González-Morales et al. 2021), oxygen saturation (Tucker 1966) and metabolic rates (Sears 2005), as well as in long-term, affecting lizard growth, survival and reproduction (Porter 1996; Sears and Angilleta 2004; Adolph and Lu et al. 2018). For example, high-altitude lizards in temperate areas experience greater thermal variability compared to low and high elevation lizards on tropical places (Janzen 1967; Zamora-Camacho et al. 2016; Muñoz and Bodensteiner 2018). Hence, lizards are likely to show behavioral and physiological modifications at high altitudes to optimize their *Tb* (Angilletta 2009).

Furthermore, seasonal reduction of *Te* with altitude can restrict the lizard's activity periods too (Zamora-Camacho et al. 2013). For example, lizards from low altitude or tropical areas have more time available for behaviors like foraging, this being reflected in rapid growth and fast sexual maturation, while the lizards from high altitude or latitude would have less time available, they show slow growth and often delayed sexual maturation (Niewarowsky 2001; Sears and Angilleta 2004).

Accordingly, two major positions have been developed to explain the thermal biology of lizards: 1) *Labile view*: in line with this viewpoint, lizards can change thermal preferences (*Tpref*) according to the altitude, latitude or season they are experiencing (Rodríguez-Serrano 2009). For example, *Iberalocerta galani* selects lower temperatures in summer than spring (Ortega et al. 2016) and *Liolaemus* lizards decrease *Tb* with altitude (Rodríguez-Serrano et al. 2009). 2) *Static view*: this viewpoint suggests that thermal biology of lizard does not change with altitude, latitude or season, behavioral thermoregulation being sufficient to mitigate changes in *Te*. This strategy is called conservative thermoregulation (Crowley 1985; Andrews 1998). For example, *Sceloporus bicanthalis* at 4000 m asl does not show differences in *Tpref* with *Sceloporus aeneus* at 3000 m asl (Andrews et al. 1999).

Animals with conservative thermoregulation in cold areas, as high mountain systems, have large costs associated to its *Tb* regulation (Lemos-Espinal 1996; Gvozdik 2002; Díaz de la Vega-Pérez et al. 2019). For example, the time needed to obtain the *Tpref* could be longer, reducing the time for other activities such as foraging (Lemos-Espinal and Ballinger 1995; Gvozdik 2002) or lizards could be more exposed to predators (Gvozdik 2002; Huey and Slatkin 1976). These costs could limit the capacity of lizards to respond to changes in *Te*. Therefore, the study of how and when lizards may use behavioral plasticity to thermoregulate in environments with temperature fluctuation is important to understand how lizards face the climatic warming (Thomas et al., 2004; Sinervo et al. 2010; Bellard et al., 2012).

The goal of this study is to examine the connection of some thermal traits in field and laboratory conditions with thermal seasonality variation along altitudinal gradients in a natural population. We used as model of study the *Sceloporus grammicus* lizard, which inhabits from southern Texas (USA) to the Mexican state of Oaxaca and has a wide altitudinal distribution, including populations above 4000 m (González-Morales et al. 2017, González-Morales et al. 2022), occupying a wide variety of habitats including trees and rocky areas. Based on the available literature, we predicted that: 1) environment temperature will be lower and more fluctuating in high altitude sites than in lower altitude sites; and 2) *S. grammicus* lizard will not show fluctuations in *Tpref* or *Tb* by seasons, but thermoregulation index will be different in function of thermal environment conditions.

Methods

Study and sampling area

We studied three populations of *Sceloporus grammicus* in central Mexico at different altitudes: Low altitude population (LAP) is located in Texcalyacac at 2500 ± 1.64 m (19°07'37'' N, 99° 29' 41'' W); Middle altitude population (MAP) is located in Las Lágrimas at 3400 ± 2.69 m (19°06'82'' N, 99°49'48'' W) and high-altitude population (HAP) is located in Nevado de Toluca at 4100 ± 2.5 m (19°06'06'' N, 99°46'03'' W). More abiotic details of study areas are available in González-Morales et al. (2021a). We recorded altitude at all capture sites with a GPS (Etrax Vista, Garmin, Olathe, Kansas, USA). Sampling was carried out during April (spring, LAP_n= 12 males and 6 females, MAP_n= 11 males and 13 females, HAP_n= 6 males and 17 females), July (summer, $\mathrm{LAP}_{\mathrm{n}}$= 7 males and 6 females, MAP_n= 10 males and 13 females, HAP_n= 18 males and 11 females), October (autumn, LAP_n= 10 males and 6 females, MAP_n= 14 males and 9 females, HAP_n= 13 males and 13 females) and January (Winter) of 2018-2019 at each of the study sites for three consecutive weeks, in order not to leave too much time between each population. During the winter we did not observe active lizards, so we do not present data for this season. Lizards were captured by lasso during their daily activity period. Immediately after capture, we recorded body temperature (*Tb*) inside lizards´ cloacae, air temperature (shaded sensor 5 cm above the substrate, *Ta*), and substrate temperature (shaded sensor in contact with substrate, *Ts*) at the perch site with a digital quick reading thermometer (Fluke 52 II).

The lizards were transported in cloth bags to the Wildlife Fauna Ecophysiology laboratory belonging to Instituto para la Conservación de la Cordillera Neovolcánica ante el Cambio Climático. We used only adult males and females, identified by snout-vent length >40 mm and secondary sexual ventral coloration (Zuñiga-Vega et al. 2008). Animals were allotted in individual plastic terrariums of 20x13x9 cm, with water and food (*Tenebrio molitor* larvae) *ad libitum*. Lizard were exposed to the natural photoperiod (approximately 12:12). During the day, heat source was provided by a 70 W light bulb and rock sited in a corner in each individual terrarium to allow natural behavior thermoregulation. Lizards did not suffer physical damage and were returned to the field sites at the end of the studies (one week after capture). Prior to test, snout vent-length (SVL) and body mass were determined using a digital caliper (accuracy: 1 mm) and balance (accuracy: 0.01 g). The body condition index (BCI) was calculated from the residuals of a linear regression between log body mass and log SVL (Vervust et al. 2018). During all sampling seasons, the lizards were subjected to the same experimental procedure (see below) performed by the same persons (JCGM and JRR).

Field operative temperatures (*Top*)

We used biophysical models to determine the range of potential body temperatures available to lizards in the absence of thermoregulatory behavior (operative temperatures, *Top*; Bakken 1992; Hertz et al. 1993; Peterson et al. 1993). These models were cylindrical hallow polyvinylchloride (PVC) pipes with the ends sealed with cork and painted with gray primer. All models were of similar shape and size (7 cm long x 1.5 cm wide) to *S. grammicus*. The PVC models were previously calibrated under field conditions during the activity period of the lizards following methods of Adolph (1990), Sinervo et al. (2010) and Díaz de la Vega-Pérez et al. (2019). Biophysical models of lizards were connected to a data logger (HOBO pro v2-U23-003) with sensors channels. Recording took place during October of 2016 at February of 2020. Data loggers recorded the internal temperatures of the biophysical model at intervals of 30 minutes. Only the measurements taken during the lizards' activity period were used to estimate the *Top* at each study sited. Activity periods were determined according to the first and last field sighting from each site (*LAP*: 08:30-19:30; *MAP*: 09:30-18:30 and *HAP*: 10:30-17:30 h). To characterize the thermal environment, the PVC models were placed in typical perch sites used by lizards under different conditions (sun, shade and mosaic). Additionally, at all studies sited we changed the models to different perch sites during the years 2016-2020 to randomize the microclimatic characteristics.

Temperature preferred and thermal index

One day after capture, we estimated preferred temperature (*Tpref*) in a photothermal gradient (1.2 wide x 1.8 m long x 50 cm high). Six 100-W reflector bulbs were suspended 30 cm above the substrate as the source of heat and light, and the opposite side of the gradient was cooled from beneath with ice, substrate was clean peat moss and small rocks along track. Under these conditions, temperature available was 20-50 °C. Body temperature data in the thermal gradient were collected by manually capturing the animal and measuring *Tb* using a digital thermometer (Fluke 52 II), every 90 minutes between 9:00 to 18:00 h. The lizards were allowed to acclimate in the thermal gradient for one hour before the first temperature measurement (Hertz et al. 1993). Then we calculated each lizard´s *Tpref* as mean of those measurements. Also, we calculated set-point ranges as the central distribution of *Tpref*. We used values between 25 and 75% (50% central distribution). These values were compared as signal of amplitude of *Tpref* (*Tset*).

The information collected on *Tb*, *Tpref* and *Top* allowed us to calculate thermoregulation indexes widely used in thermal ecology of ectotherms (Hertz et al. 1993; Blouin-Demers and Weatherhead 2001). We calculated the following indexes: A) Thermal quality of the habitat (*de*): defined as average of the absolute values of deviations of *Top* relative to the nearest *Tpref* boundary, thermal quality of the habitat is lower as *de* index values moves away zero. B) Accuracy of the body temperature (*db*): defined as the average of the absolute vales of deviations of *Tb* relative to the nearest *Tpref* boundary; accuracy of body temperature of the habitat is lower as *db* index values moves away zero. C) Effectiveness of thermoregulation; we calculated two indexes, *E* (Hertz et al. 1993) calculated as 1-(*db*/*de*), where values close to 0 indicate thermoconformity, values close to 1 indicate thermoregulation, and values below 0 indicate avoidance of *Tpref*; and Blouin-Demers index (Blouin-Demers and Weatherhead 2001) calculated as *de-db*, defined as the effort in degrees Celsius required by the ectotherm to reach *Tpref*.

Statistical analyses

Prior to analyses, the assumptions of normality and homogeneity of variance were checked using Tlamatini R package (Sandoval-Molina 2021). *Tset* was sqrt transformed, the rest of the variables fulfilled the assumptions (Quinn and Keough, 2002). We conducted ordinary least square linear models. First, we compared in individual models the effects of altitude, season and sex on body mass, SVL, BCI, *Tpref*, *Tset*, *db*, *de*, *E* and *B-D* index. Second, we compared *Top* by altitude and season. When a test revealed a significant difference, the relevant post hoc test (Bonferroni) was performed. Finally, we used a Pearson correlation to evaluate the relationships between *Tb*, *Ta* and *Ts* in each season by altitude. The level of significance was established at $p < 0.05$. Statistical analyses were performed in R (version 4.1.1).

Results

Body size

Summary of body size data are available in Table 1. Lizards from middle altitude population (MAP) and high altitude population (HAP) had minor body mass than lizards from low altitude population (LAP; $F_{2, 177}= 58.47$, $p <0.001$). Body mass did not differ between summer and autumn, although it was lower in spring ($F_{2,177}= 11.18$, $p <0.001$). Body mass varied by season in function of altitude (interaction altitude × season= $F_{4,177}= 4.21$, $p < 0.001$). Body mass of lizards from LAP did not vary by season, while lizards from MAP and HAP had an increment in body mass during summer respect to spring and autumn.

Lizard from LAP had greater SVL than MAP and LAP lizards ($F_{2,177}= 29.89$, $p < 0.001$). Males were larger than females ($F_{1,177}= 8.62$, $p = 0.004$). Although the altitude × sex interaction was not significant ($F_{2,177}=2.13$, $p = 0.12$), we detected sexual dimorphism in HAP (pos hoc Bonferroni test = -3.08, $p = 0.03$.).

BCI did not differ between MAP and HAP lizards, however they had lower BCI than LAP lizards ($F_{2,177}= 24.49$, $p < 0.001$). In summer, lizards had greater values of BCI than in spring and autumn ($F_{2,177}= 35.78$, $p < 0.001$). Sex × season interaction was significative ($F_{4,177}= 8.96$, $p < 0.001$), in general terms, females had higher values of BCI in autumn, intermediated values in summer and lower values in spring. For males, they had lower values of BCI in autumn. The altitude × season × sex interaction was significant ($F_{4,177}= 4.68$, $p <0.001$). Seasonal variation of BCI was different by sex in each altitude population. Female and males' lizards from LAP not showed differences across seasons; MAP males had a similar pattern as LAP lizards, however, females had lower BCI in spring than in summer and autumn. Finally, HAP lizards had a same pattern in both sexes, during summer, lizards had greater values of BCI than in spring and autumn.

Operative temperatures

The PVC models accurately mimicked the thermal characteristics of the lizards ($r = 0.82$, $p < 0.001$, $n = 59$). *Top* statistically differed among altitudes ($F_{2,\,41057} = 667.09$, $p < 0.001$), HAP had lower *Top* than LAP and MAP. In summer, *Top* was lower than in other seasons, spring and winter did not significantly differ ($F=_{3,41057} = 295.08$, $p < 0.001$). In LAP, *Top* decreased along the year, winter season having the lowest *Top* of the year. MAP and HAP had similar patterns; in summer, *Top* decreased respect to spring and autumn, while winter had a higher *Top* of the complete year (interaction altitude × season: $F_{6,41057} = 424.62$, $p < 0.001$, fig. 1).

Field body temperature and relationship between *Tb*, *Ta*, *Ts* and SVL

Sex was not a significant factor affecting *Tb* (sex: $F_{1,178} = 1.08$, $p = 0.29$ and altitude × season × sex: $F_{4,178} = 0.24$, $p = 0.21$). Lizards from LAP and MAP had higher *Tb* than HAP lizards ($F_{2,178} = 7.55$, $p < 0.001$). Lizards had higher *Tb* in spring than in summer and autumn ($F_{2,178} = 14.33$, $p < 0.001$). Interaction altitude × season showed that in three populations *Tb* was lower in autumn than in spring and summer ($F_{4,178} = 7.40$, $p < 0.001$, Fig. 2).

The relationship between *Tb*, *Ta*, *Ts* and SVL varied with altitude and season. In spring, lizards from LAP and HAP had a stronger relationship between *Tb* and *Ts*, so those lizards use a thigmothermy strategy. In summer and autumn, the strongest relationship was *Tb* and *Ta*, suggesting that this season lizards use a heliothermic strategy. Lizards from MAP had not relationship between *Tb*, *Ta*, *Ts* and SVL an any season (Table 2).

Temperature preferred and temperature set points

No sex differences in *Tpref* were found, so we pooled data from males and females for the analyses of *Tpref* (sex: $F_{1,176} = 3.60$, $p = 0.62$, sex × altitude: $F_{2,176} = 1.15$, $p = 0.31$ and sex × altitude × season: $F_{4,176} = 0.25$, $p = 0.90$) and *Tset* (sex: $F_{1,176} = 0.55$, $p = 0.45$, sex × altitude: $F_{2,176} = 0.02$, $p = 0.97$ and sex × altitude × season: $F_{4,176} = 1.13$, $p = 0.34$). The lizards from HAP had lower *Tpref* than lizards from LAP, while lizards from MAP did not show differences with

LAP and MAP ($F_{2, 176}$= 5.96, $p = 0.003$). *Tpref* differed by season ($F_{2,176}$= 39.50, $p < 0.001$). All populations selected lower *Tpref* in autumn than in spring and summer. In LAP and HAP, during spring, *Tpref* had an intermediated values compared to summer and autumn (altitude × season: $F_{4,176}$= 4.08, $p = 0.017$, fig 2 and 3). *Tset* was lower in HAP than in MAP ($F_{2,175}$= 4.79, $p = 0.01$). We found different patterns by altitude and season, lizards from LAP had a narrower *Tset* in spring and summer than autumn. Lizards from MAP had narrower *Tset* in spring than summer and autumn, and lizards from HAP not showed differences by season ($F_{2,175}$= 10.48, $p < 0.001$; altitude × season: $F_{4,175}$= 3.44, $p < 0.001$).

Thermal Indexes

db index differed among altitudes ($F_{2,81}$= 6.06, $p < 0.001$) and seasons ($F_{2,81}$= 25.28, $p < 0.001$). Lizards from LAP and HAP had higher *db* values in summer than spring and autumn (lower accuracy of thermoregulation), while lizards from MAP had an equally low value (higher accuracy of thermoregulation) of *db* in all seasons (altitude × season: $F_{4,81}$= 22.68, $p < 0.001$, fig. 4a). *de* index was higher (lower thermal quality) in HAP than LAP and MAP ($F_{2,81}$= 29.87, $p < 0.001$). *de* index was lower (higher thermal quality) in autumn than spring and summer ($F_{2,81}$= 41.93, $p < 0.001$). All populations had lower *de* values in autumn and higher values in summer (interaction altitude × season: $F_{4,81}$= 14.90, $p = 0.008$, fig. 4b).

The *E* index did not differ by altitude ($F_{2,81}$= 2.86, $p = 0.06$) but differed by season ($F_{2,81}$= 10.68, $p < 0.001$), lizards thermoregulated less efficiently in summer than spring and autumn. Lizards from LAP thermoregulated more efficiently in spring than summer and autumn. MAP lizards did not vary in *E* values across seasons, they had high values of *E* all year, while HAP lizards thermoregulated more efficiently in autumn than spring and summer (interaction altitude × season: $F_{4,81}$= 21.10, $p < 0.001$, fig. 4c). Similar to *E* index, *B-D* index differed by altitude, tending to increase with altitude ($F_{2,81}$= 13.03, $p < 0.001$). *B-D* index was lower in autumn than spring and summer ($F_{2,81}$= 12.71, $p < 0.001$). In general terms, lizards from LAP had higher *B-D* values in spring than summer and autumn. MAP lizards had higher values in summer than spring and autumn, and HAP lizards not showed differences by season in *B-D* values (interaction altitude × season: $F_{4,81}$= 23.22, $p < 0.001$, fig. 4d).

Discussion

Our results showed that *S. grammicus* lizards had lower field body temperatures in autumn than spring and summer and *Tpref* varied with altitude and season; lizards preferred lower temperatures in autumn than other seasons. In addition, lizards at high and low altitude used different thermoregulatory strategies in each season; in spring they used tigmothermy and in summer and autumn they used heliothermy, while lizards from MAP did not show a relationship between field body temperature and *Ta* or *Ts*. Finally, thermoregulatory indexes differed in each altitude and season, lizards thermoregulating more efficiently in autumn.

In this study, *S. grammicus* presents a smaller body mass and SVL at high altitude (similar result was reported by González-Morales et al. 2021 and Díaz de la Vega-Pérez et al. 2019). Body mass varied by season. In LAP, we did not observe differences in body mass or BCI between seasons or sexes, while in MAP and HAP we recorded a lower body mass and BCI during spring in females, while only in males of HAP was this effect observed. In spring many females may have just given birth, so the cost associated with the maintenance of the offspring is reflected in this season, with a lower body mass and BCI (Smith et al. 1995). During the summer, lizards experienced an increase in body mass and BCI. In the study region, summer is characterized by being rainy and the abundance of prey increases (Ballinger 1979; Ferguson et al. 1990; Smith et al. 1995). In the autumn, a decrease of body mass was observed in HAP lizards. In high altitude areas the activity time available for the lizards is scarce (Gvozdik 2002, Anguilleta and Sears 2004) and in this season most of Sceloporines are in courtship phases (Guillette and Casas-Andreu 1980; Lozano and Ramírez-Bautista 2015; Ramírez-Bautista et al. 2019). Hence, HAP lizards, with limited time available, could be spending much time for the courtship and copulation at expense of time for foraging (Ruby 1997). A trade-off between reproduction and foraging may emerge in autumn, which would be more intense in high-elevation environments. More studies, recording the behavior in relation to the time given to each activity per season and altitude are necessary to validate this idea.

Furthermore, lizards are shorter at high elevations, so, in our study model, *S. grammicus* follows an inverse pattern to Bergmann's rule; similar results have been described for the same species in a different study area (Díaz de la Vega-Pérez et al. 2019). A previous study has shown in these populations that small lizards gain heat faster than larger lizards, in this way, small lizards having a higher overall heat gain than large lizards (González-Morales et al. 2021a). Hence, at cooler sites, such as high altitude, small body size is likely beneficial in this lizard. Therefore, the fact that *S. grammicus* body size follows the inverse of Bergmann's rule is consistent with small body size being selected by cold temperatures at high altitudes (Pincheira-Donoso et al 2008).

Our first prediction was fulfilled, at higher altitude quality of the thermal environment is poorer than at lower altitudes (higher values of *de*). High altitude mountains have lower temperatures, and overall, others abiotic factors as solar radiation and atmospheric pressures occurs in gradients from low to highlands (Körner 2007). Inclusively, time availability during activity lizard´s period can be restricted at high altitude (Zamora-Camacho et al. 2013) resulting in thermoregulation and phenological variation as reproduction, parturition or hibernation (Mathies and Andrews 1995; Zamora-Camacho et al. 2013). Despite the disadvantages that at first glance result from habitat in high elevation environments, *S. grammicus* is a common lizard in these zones. Higher survivals rates and lower depredation have been described in high altitude than low and middle altitudes in *S. grammicus* (Domínguez-Godoy et al. 2020a; Domínguez-Godoy et al. 2020b), in addition, biodiversity decreases with altitude (Rahbek 1995; Fossa 2004), so that, potential depredators and competence can be less, and inhabiting in high mountains can results advantageous in some cases (Comas et al. 2014).

Thermal indexes showed differences by altitude and seasons. The *Top* was lower in HAP, which is reflected in a low thermal quality during all seasons (higher values of *de*) with respect to the two low zones, in which during the summer the highest values of *de* were recorded, while that in spring and autumn the *de* was better (lower values of *de*); similar results have been described in works associated with altitude in *S. grammicus* in another study area (Díaz de la Vega-Perez et al. 2019), as well as for *Zootoca vivipara* (Gvozdik 2002) and *Iberalocertha aurelioli* (Ortega et al. 2017), among others. It has also been described seasonally in *I. galani* (Ortega et al. 2016), among others. Regarding the *E* index, it showed that during the autumn the lizards tend to be more active (values closer to 1) than in the spring and summer, that is, during this season the lizards seek to be more precise and efficient in thermoregulation.

Seasonal variation in thermal biology may be able to achieve body temperature closer to their thermal preferences for each season (Ortega et al. 2016). Temperate areas have greater temperature variation among seasons, which could reflect a broad temperature range over a day or a year. By contrast, tropical areas have lower variation in temperature and experience less seasonality, producing a thermic biology specialization in lizards (Muñoz and Bodensteiner 2018). Lizards have two adjust mechanism to face up thermal seasonality variation: 1) fast adjustments include behavioral changes as postures, microsites selection or changes in dorsal reflectance to absorb more efficiently solar radiation, and 2) slow adjustments as modification of temperature preference, metabolic rates and thermal critical limits. Interaction between behavioral and physiological adjustments are complex and can greatly illuminate our knowledge of how lizards can respond to climate warming (Kearny et al. 2019; Seebacher et al. 2015 and Domínguez-Guerrero et al. 2020).

Body temperature of lizards depends on both the magnitude of temperature variation in the environment and the lizards' ability to regulate heat exchange with the environment. Usually, when lizards depend on solar radiation they are called "heliotherms" and when they depend on warming through contact with substrate are called "thigmotherms". Although they seem to be two different strategies, the body temperature of lizards is based on a combination of these heat sources (see Belliure and Carrascal 2022). Surprisingly, the relationship between *Tb*, *Ta* and *Ts* was not equal across seasons. In spring, the relations between *Tb* and *Ts* was stronger than *Tb* and *Ta*, suggesting that lizard thermoregulation relied mainly on thigmotermy. By contrast, in summer and autumn, *Tb* was mainly correlated to *Ta*, suggesting that lizard thermoregulation relied in this case on heliothermy. In summer and autumn relative humidity in central Mexico is higher than in spring, doing substrate probably less suitable to thermoregulate. Hence, lizards seem to use substrate and air differentially along the year, choosing whichever most conveniently contribute to their thermoregulatory behavior.

Regarding our second prediction, we predicted based on the literature that *S. grammicus* will have a conservative thermoregulation (Andrews 1998, 1999), so would not present differences in *Tpref* among altitude and seasons. However, this prediction was not supported in our study area. *Tpref* was lower in MAP and HAP compared to LAP, and during the autumn the *Tpref* was lower than in spring and summer in all populations. Currently the genus *Sceloporus* is considered thermally conservative (Andrews 1998), however, much of this evidence comes from the comparison between data from different species and in periods of time that overlap several seasons, so the season could be a factor inadequately addressed in other studies (Andrews 1998; Díaz de la Vega-Pérez et al. 2019). In several species of other genus of lizards, it is common to observe differences in *Tpref* by season (Van Damme et al. 1986; Díaz and Cabezas-Díaz 2004; Díaz et al. 2006). For example, *Psammodromus algirus* and *Iberalocertha galani* show lower *Tpref* during spring, in order to minimize costs associated with thermoregulation (Díaz et al. 2006; Ortega et al. 2016). Our results, contradictory with other studies of *S. grammicus* (Díaz de la Vega-Pérez et al. 2019 and Lara-Resendíz et al. 2014), may be a consequence of local effects. Replicated studies between mountains are necessary to clarify these phenomena (Schall and Pianka 1978; Garland and Adolph 1991). In fact, others phenotypic traits as hematic values are not totally replicable between mountain systems in *S. grammicus* (González-Morales et al. submitted).

Thermal indexes show differences by altitude and seasons. The *Top* was lower in HAP, which is reflected in a low thermal quality during all seasons (higher values of *de*) with respect to the two low zones, in which during the summer the highest values of *de* were recorded, while that in spring and autumn the *de* was better (lower values of *de*); similar results have been described in works associated with altitude in *S. grammicus* in another study area (Díaz de la Vega-Perez et al. 2019), as well as for *Zootoca vivipara* (Gvozdik 2002) and *Iberalocertha aurelioli* (Ortega et al. 2017), among others. It has also been described seasonally in *I. galani* (Ortega et al. 2016). Regarding the *E* index, it shows that during the autumn the lizards tend to be more active (values closer to 1) than in the spring and summer, that is, during this season the lizards seek to be more precise and efficient in thermoregulation.

In conclusion, *S. grammicus* did not show a conservative thermic biology along altitudinal gradient and over seasons. In autumn, coinciding with the reproduction, lizards had lower *Tpref* and exhibited an increment in efficiency of thermoregulation. Additionally, field body temperatures decreased with altitude and their relationship with air and substrate differed along seasons, suggesting that lizards use different thermoregulatory behavior (thigmothermy or heliothermy) along the year. Finally, studies replicating gradients in different mountain systems are necessary for clarify if observed differences are product of methodological process, phenotypic plasticity or local adaptation.

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Figure 1. Operative temperatures registered by biophysical models during 2016-2020 at each population along the altitudinal gradient. Letters show differences by altitude and numbers show differences among seasons within same altitude. Values are represented by mean ± SE.

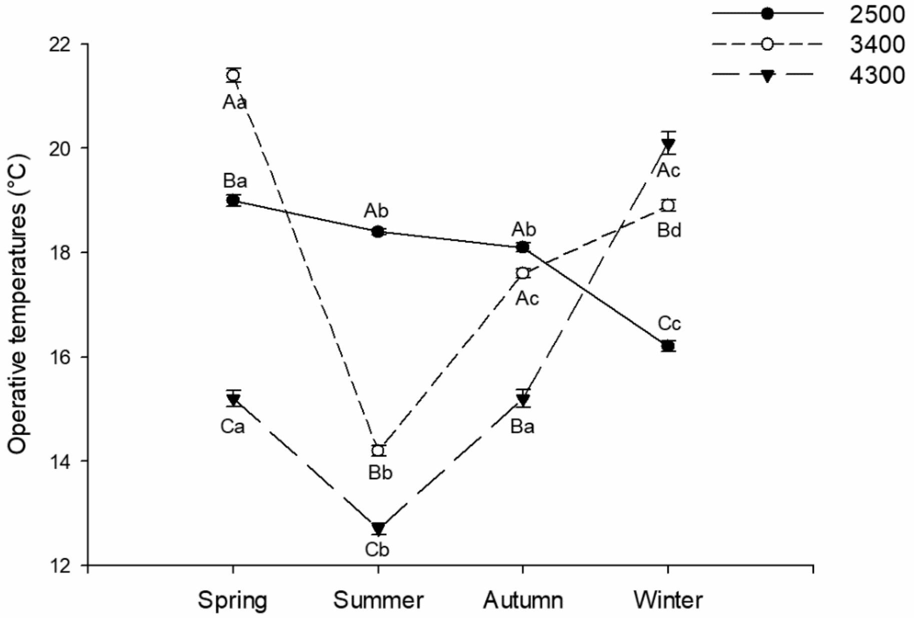


Figure 2. Distribution of operative temperatures by altitude and season during 2016-2020. The gray area indicates the interquartile *Tset* range for each population measured in a thermal gradient and the arrow indicates the average of body temperature in field conditions.

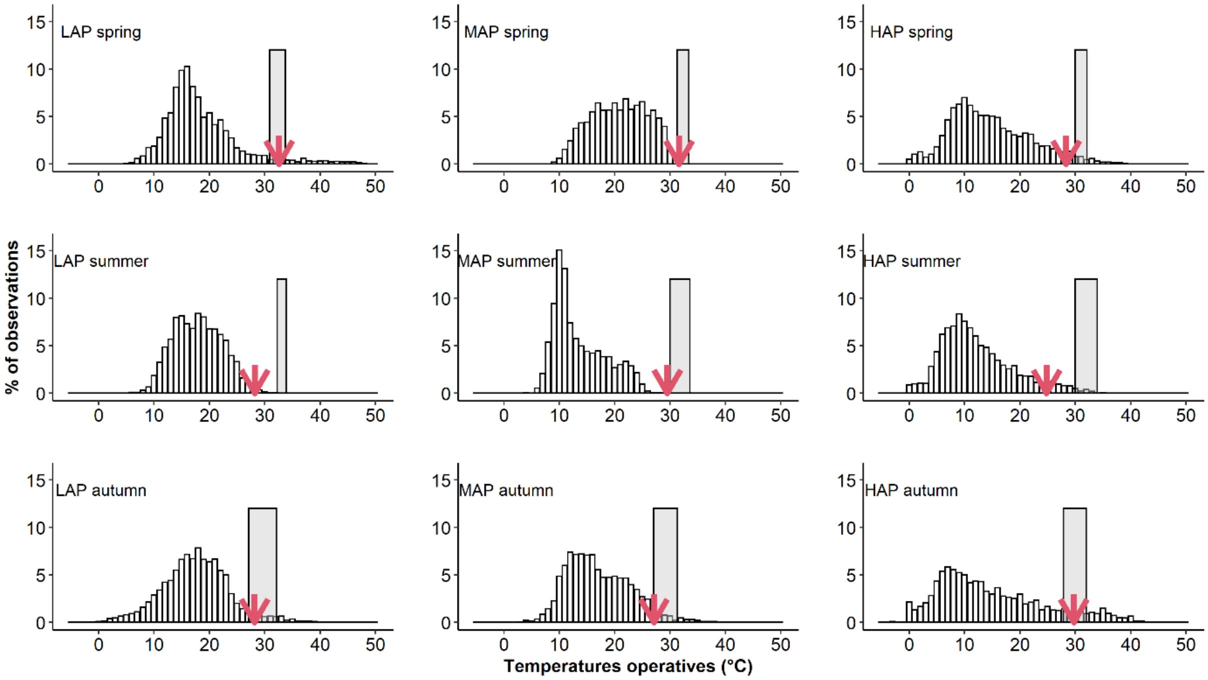


Figure 3. Average of preferred temperature in the laboratory (*Tpref*) of *Sceloporus grammicus* between altitudes and seasons. Letters show differences by season. Values are represented by mean ± SE.

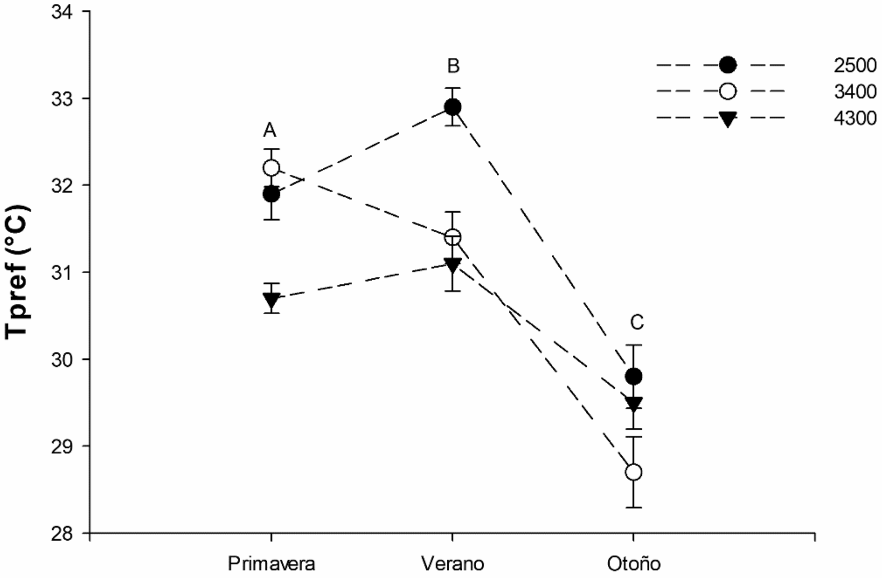
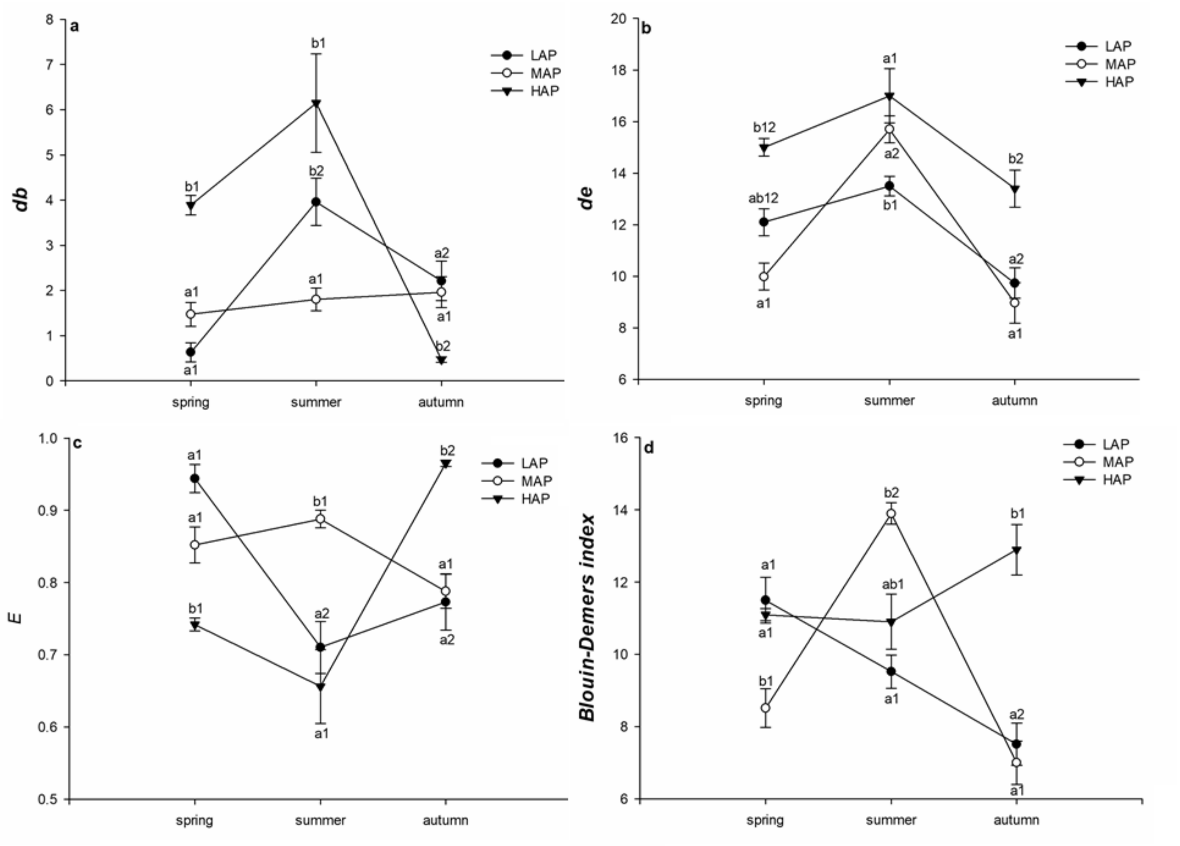


Figure 4. Thermal indexes of *Sceloporus grammicus* by altitude and season along the altitudinal gradient. Only for graphical representation we used raw data. a) Accuracy of the thermoregulation (*db*), b) thermal quality of the habitat (*de*), c) Effectiveness of thermoregulation (*E*) and d) Blouin-Demers index. Letters show differences by altitude and numbers show differences by season in the same altitude. Values are represented by mean ± SE.



9. Capítulo IV

González-Morales JC, Rivera-Rea J, Moreno-Rueda G, Bastiaans E, Plasman M and Fajardo V.

Dorsal skin reflectance and thermic rates are not repeatable between season along altitudinal gradient in mesquite lizards. Por enviarse a Journal of Experimental Biology

El capítulo que se muestra a continuación contiene datos de la reflectancia dorsal a tres tratamientos de temperatura corporal (10, 20 y 30 °C) y tasas térmicas (calentamiento, enfriamiento, ganancia neta de calor y retención de calor) en tres poblaciones de diferente elevación a lo largo de las estaciones del año. Con base en la revisión de la literatura disponible, notamos que la variación de la reflectancia dorsal y las tasas térmicas entre altitudes y a lo largo de las estaciones del año era un tema poco explorado, más aún considerando que la variación de dichos rasgos podría tener grandes implicaciones en la biología de pequeños ectotermos como *Sceloporus grammicus*. Nuestros resultados muestran que la variación intra especifica de la reflectancia dorsal difiere entre altitudes y estaciones del año. La reflectancia dorsal fue menor durante el verano, estación del año en la que previamente habíamos registrado las temperaturas operativas más bajas (ver capítulo 3). Las tasas térmicas fueron más altas durante el verano y el otoño respecto de la primavera. Finalmente, en todas las estaciones del año, los organismos más oscuros presentaron una mayor tasa de calentamiento y ganancia neta de calor. Nuestros resultados abren interrogantes acerca del posible costo asociado con la variación a corto (temperatura corporal) y largo (estaciones del año) plazo de la reflectancia dorsal.

Dorsal skin reflectance and thermic rates are not repeatable between season along altitudinal gradient in mesquite lizards

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Abstract

Temperature is the most important factor in the life histories of ectotherms, as body temperature has an undeniable effect on growth, activity, and reproduction. Lizards have a wide variety of strategies to acquire and maintain body temperature in an optimal range. The “Thermal Melanism Hypothesis” proposes that a dark coloration enables darker individuals to heat up faster than lighter conspecifics, because darker individuals absorb more solar radiation and thus being darker may be advantageous in cold habitats. Changes in dorsal skin reflectance at short (body temperature) and long term (season) may play a key role, especially in lizards that inhabit areas with a large temperature variation during the day and throughout the year. Here, we show how the variation in dorsal reflectance fluctuates with body temperature and varies among seasons, as well as its relationship to the rate of heat. We compared dorsal skin reflectance at three body temperature treatments, and measured thermal rates (i.e., heating and cooling rates, thermic lapse, and net heat gain) along an elevation gradient (2500-4100 m) and season (spring, summer, and autumn) in the Mezquite lizard. Our results show that dorsal skin reflectance was low during the months with the lowest environment temperatures. The rate of obtaining and retaining heat also differed during the year, being higher during the reproductive season. Our results indicate that the variation of dorsal skin reflectance and thermal rates follows a complex pattern in lizard populations along an elevational gradient as a function of season.

Keywords: reflectance, elevation gradient, season, heat rate, cool rate

Introduction

The environment gradually changes with elevation (Korner, 2007), and organisms distributed along an elevational gradient can show local adaptations or phenotypic plasticity processes generating clines for some traits (Welter-Schultes, 2000; Blackburn and Ruggiero, 2001; Ashton and Feldman, 2003). Organisms are subject to daily or seasonal variation in environmental conditions; which is more evident in temperate areas than in the tropics (Janzen, 1967; Muñoz and Bodensteiner, 2019). Particulary in ectotherms, body temperature has an undeniable effect on performance, growth, and survival (Angilleta, 2009; Sun et al., 2012; Brewster et al., 2013, González-Morales et al., 2021a). Consequently, they use a wide variety of strategies to regulate their body temperature within an optimal temperature range (Hertz et al., 1993; Angilleta, 2009). Generally, lizards behaviorally regulate their body temperature in a narrow temperature range by shuttling among sun exposed areas, shady patches, and refuges or by modifying body posture (Huey, 1974; Van Damme, 1989; Adolph, 1990; Bauwens et al., 1996; Díaz and Díaz, 2004).

High-elevation environments are sites with low oxygen pressure, high solar radiation, and low environmental temperature (Korner, 2007; Zamora-Camacho, et al., 2013; Reguera et al., 2014a; González-Morales et al., 2017). The environmental temperature decreases with elevation by approximately 5 °C every 1000 m (Korner et al., 2007; Zamora-Camacho et al., 2013). High elevation lizards can adjust their temperature acquisition in this poor thermal quality habitat through behavioral adjustments, for example by increasing the basking time.

However, this limits other activities such as foraging and increases the risk of predation (Lemos-Espinal and Ballinger, 1995; Gvozdík, 2002).

The body color affects many aspects of the lizard´s biology. It plays a central role in communication, homeostasis, sexual selection, and crypsis (Espmark et al., 2000; Andersson and Simmons, 2006; Geen and Johnston, 2014; Da Silva et al., 2013; Stuart-Fox et al., 2004). Dorsal skin reflectance (*DSR*) can have an important influence in the heating balance of lizards (Clusella-Trullas et al., 2007, González-Morales et al., 2021b). The Thermal Melanism Hypothesis (*TMH*) predicts that dark animals could benefit by the increased absorption of solar radiation compared to lighter conspecifics (Porter and Norris, 1969; Clusella-Trullas et al., 2007). *TMH* has received mixed support, while in some lizards lower reflectance results in higher heat rates (Tanaka, 2005; Smith et al., 2016; Clusella-Trullas et al., 2009, González-Morales et al., 2021b), in other species this is not evident (Crisp et al., 1979; Bittner et al., 2002; Zamora-Camacho et al., 2014).

DSR often present phenotypic plasticity, lizards use changes in *DSR* to mitigate changes in environment over short and long time periods (Stuart-Fox et al., 2006; Smith et al., 2016; Cadena et al., 2018; González-Morales et al., 2021a). Quick changes in *DSR* are usually caused by aggrupation (lighter *DSR*) or dispersion (darker *DSR*) of melanophores (Bagnara et al., 1968; Sherbrook, 1997). Over longer periods (for example, ontogenetic or seasonal variation), *DSR* may change over several months as a result of an increase (darker) or decrease (lighter) of the pigment melanin (Green, 1964; Cadena et al., 2018). Many lizard species show *DSR* variation within a short time, from a few minutes to several hours. Some studies have shown that a dark *DSR* can be an auxiliary mechanism for compensating low environmental temperatures in high elevation or latitude areas (Langkilde and Boronow, 2012; Stephenson et al., 2017; Cadena et al., 2018; González-Morales et al., 2021a, 2021b). However, changes in *DSR* induced by body temperature in different seasons and the relationship with thermal rates along altitudinal gradients are not fully clarified.

We predicted lizards to be darker (lower *DSR*) when environmental temperatures are lower (i.e., higher elevation, colder seasons), that lizards will present higher plasticity in their dorsal coloration when they inhabit a more variable thermal environment, and that darker lizards will have a higher thermal heat rate. Here, we studied the *DSR* of the lizard *Sceloporus grammicus* over seasons and an altitudinal gradient. *S. grammicus* is a medium sized lizard averaging 52 mm snout-vent length and 4.6 g body mass (Lemos-Espinal et al., 1992). It is common from southern Texas, USA to central Mexico and it is particularly interesting because it resides and is abundant above 4000 m asl at several volcanoes in Mexico (Sites et al., 1992; González-Morales et al., submitted).

Methods

Fieldwork and animal maintenance

Adult mesquite lizards, *Sceloporus grammicus*, were captured by hand or lasso in spring (April), summer (July), and autumn (October) in three populations along an altitudinal gradient from Nevado de Toluca in Central Mexico. We did not obtain lizards in the winter because we did not find active lizards in this season. The study sites were Texcalyacac (low elevation population, LEP; 19°07’37’’ N, 99° 29’ 41’’ W) at 2500 m asl, Las Lágrimas (middle elevation populations, MEP; 19°06’82’’ N, 99°49’48’’ W) at 3400 m asl, and Nevado de Toluca (high elevation populations, HEP; 19°06’06’’ N, 99°46’03’’ W) at 4100 m asl. We only included adult lizards (SVL > 35 mm; Zuñiga-Vega et al., 2008) in this study. Lizards were transported in cotton bags to Instituto para la Conservación de la Cordillera Neovolcanica ante el Cambio Climático (approximately a 1.5 h drive). Mesquite lizards were individually housed in terraria (23 × 41 × 21 cm; w × l × h) with rocks placed in one corner and peat moss as substrate. During the day (8:00 to 18:00 h) heat was provided by a 60 W light bulb (temperature range was 20–35 ºC in each individual terrarium). Before each test, we measured body mass with a balance (precision: 0.01 g) and SVL and hind limb length with a digital caliper (precision: 1 mm). We calculated the residual values of a linear regression of log-mass vs. log-SVL to calculate body condition index (BCI; Schulte-Hostedde et al., 2005). The same individuals were used for all tests described below.

Dorsal skin reflectance (*DSR*)

Before the measurement of *DSR*, each lizard was maintained in a thermal chamber set for acclimation at 10, 20, or 30 °C for 2 h, on three different days. These temperatures were selected according to the range of temperatures experienced by these lizards in their natural habitat: Texcalyacac: 11–24.5 °C, Las Lágrimas: 10–25.9 °C, Nevado de Toluca: 5–28.2 °C (González-Morales et al., submitted b). We randomized the order in which each lizard experienced these temperatures. We measured *DSR* at three points in the dorsal region. A small mark with non-toxic paint was made next to the site measured to ensure that the same site was used for all *DSR* measurements. These sites were located in the upper, medium, and lower dorsal region (for more details see González-Morales et al., 2021a and 2021b). Reflectance was measured within the 300–700 nm range using a spectrophotometer (Jaz Ocean Optics) with a Pulsed Xenon Light Source (Jaz-PX) connected to an optical fiber (Ocean Optics Inc., Dunedin, FL, USA). The probe was mounted within a holder that ensured readings were taken in a circular area 1 mm in diameter at a distance of 3 mm from the skin surface with a 45 ° angle (Megía-Palma et al., 2016). All measurements were relative to a white standard (99% WS-1). Raw spectra data were imported into the software CLR, and smoothed using a running average computed over 20 nm intervals, and the average reflectance was considered the dorsal skin reflectance. For each lizard, all reflectance measurements within a temperature test were made in less than 1 min.

Thermal rates

For the estimation of thermal rates and transitional lapses, we followed the protocol proposed by Zamora-Camacho et al., 2014. We immobilized lizards individually on a thermally stable synthetic plaque, using lace knotted around their chest and limbs. Next, in a laboratory at 20 °C room temperature, we placed the lizards 13 cm below a 60 W bulb. We started the trials when the lizards had attained a body temperature of 21 °C and switched off the light when their body temperature reached 33 °C. This body temperature range is within the preferred temperature range reported for this species (31.1 ± 3.7 °C) and does not approach the critical thermal maximum (40 °C) (Díaz de la Vega-Pérez et al., 2019). Once the light was switched off, the test continued until body temperature decreased to 27 °C, because this temperature is below the preferred temperature range (González-Morales et al., submitted b).

The body temperature of each lizard was monitored with a small T-type thermocouple inserted 4 mm into the cloaca and connected to a thermometer (Fluke 52-II). Body temperature was recorded every 30 s during the whole process. The heating rate was calculated as the linear regression slope of body temperature increasing over time during the heating period. The transitional lapse was calculated as the time (min) between switching off the light until the body temperature started to decrease. The cooling rate was calculated as the linear regression slope of body temperature decreasing over time during the cooling period. The net heat gain (*NHG*) was obtained by subtracting the cooling rate from the heating rate; this relationship expresses the total heat gain (Zamora-Camacho et al., 2014). All measurements were performed between 08:00 and 10:00 h. To make sure that the lizards were not harmed, they were kept in the laboratory for three days before we released them at their capture site.

Statistical analyses

Prior to all analyses, the assumptions of normality and homogeneity of variance were checked. *DSR*, heating and cooling rates, and lapse met the assumptions after log transformation (Quinn and Keough, 2002). We compared morphological parameters and thermal rates by elevation, sex, and season using an Ordinary Least Square Linear Model, BCI and body mass were considered as covariables in the thermal rates comparations. We examined differences in *DSR* using a Linear Mixed Model (LMM). *DSR* was considered as the dependent variable, and elevation (2400,3400, and 4100 m), body temperature (10, 20, and, 30 °C), sex (male and female), and season (spring, summer, and autumn) and their interactions as independent variables (fixed factors). Lizard identity was included as a random factor to account for repeated measurements on individuals. When we detected significant differences, we performed a post hoc pairwise comparison analysis using a Bonferroni post hoc test. *DSR* was highly correlated at 20 and 30 °C ($r = 0.53$, $P < 0.001$) so in the following analyses of correlation, we only show results at 30 °C. Results at 20 °C is availability in supplemental material. We used Pearson correlations to estimate the association between heating rate, cooling rate, and transitional lapse with dorsal reflectance at 30°C and body mass. Finally, to evaluate the effect of each predictive variable on thermal rates, we performed linear models with each of the thermal rates as dependent variables, elevation, sex, and season as factors, and body mass and dorsal reflectance as covariates. We performed all analyses with R software (v. 4.4.1), LMM was performed with *lme4* package (Bates et al., 2015). Post hoc analyses were performed with *emmeans* package (Lenth, 2018).

Results

Morphological parameters

The summary of body size data is available in Table 1. Lizards of MEP and HEP had lower body mass than lizards from LEP ($F_{2, 177}$= 58.47, p <0.001). Body mass varied by season in function of elevation (interaction elevation × season= $F_{4,177}$= 4.21, p < 0.001). Body mass of LEP did not vary with season, whereas MEP and HEP had an increment in body mass during the summer with respect to spring and autumn.

Lizards from LEP had greater SVL than MEP and HEP lizards ($F_{2,177}$= 29.89, p < 0.001). Males were larger than females in all populations ($F_{1,177}$= 8.62, p = 0.004). Although the elevation × sex interaction was not significant ($F_{2,177}$=2.13, p = 0.12), we detected sexual dimorphism in HEP (post hoc Bonferroni test z= -3.08, p = 0.03).

BCI differed by elevation, season and sex (elevation × season × sex interaction: $F_{4,177}$= 4.68, p <0.001). Females and males from LEP did not show differences in BCI across seasons. MEP males had a similar pattern as LEP lizards, however, females had lower BCI in spring than summer and autumn. Finally, HEP lizards had the same pattern in both sexes, during summer lizards had a higher BCI than in spring and autumn.

Dorsal skin reflectance

Lizards showed differences in *DSR* by elevation, season, body temperature, and sex (Table 2). Females had lower DSR than males in all populations (Table 2). Lizards from HEP had lower *DSR* (e.g., were darker) than lizards from LEP and MEP (post hoc test: estimate = 0.226, t = 3.74, p < 0.001, and estimate = 0.168, t = 3.34, p = 0.003, respectively). The interaction elevation × season × body temperature (Table 2) showed in spring, lizards from LEP had higher values of *DSR* at 30 °C than at 10 and 20 °C (fig. 1a), while lizards from MEP had lower *DSR* at 20 °C than at 10 and 30 °C (fig. 1b), and lizards from HEP had higher *DSR* at 20 and 30 °C than 10 °C (fig. 1c). In summer, lizards from LEP had lower *DSR* values at 30

°C than 10 and 20 °C (fig 1a), whereas lizards from LEP and MEP did not display variation in *DSR* among body temperatures (Fig. 1a and 1b). In autumn, lizards from LEP and HEP did not show variation in *DSR* among body temperatures (fig. 1a and 1c), whereas lizards from MEP had a light increment in *DSR* at 20 °C compared to *DSR* at 10 and 30 °C (fig. 1b).

DSR of lizards from LEP varied at 30 °C across seasons, but not at 10 or 20ºC (fig. 1a). Lizards from MEP did not show variation in *DSR* across seasons (fig. 1b), while lizards from HEP varied in *DSR* across seasons in all body temperatures, with significantly lower DSR in summer than spring and autumn (fig. 1c).

Thermal rates

Heating rate varied by elevation ($F_{2,71}$= 28.65, p < 0.001), season ($F_{2,71}$= 39.22, p < 0.001) and sex ($F_{1,71}$= 26.77, p < 0.001). Differences among seasons within a population showed that in summer lizards from LEP had a higher heating rate than in spring and autumn, lizards from HEP had lower heating rate in spring than summer and autumn, whereas lizards from MEP did not show differences in heating rate across seasons (interaction elevation × season [$F_{4,71}$= 2.96, p = 0.02; fig 2a]). Regarding the differences between elevation within a season, the MEP and HEP lizards had remarkably higher values during autumn compared to the LEP lizards. The BCI index was a significant covariable ($F_{1,71}$= 5.81, p = 0.018). Heating rate decreased with body mass (r = -0.39, P < 0.001; Fig. 3a) and with dorsal reflectance (r = -0.48, P < 0.001; Fig. 4a), meaning that smaller and darker lizards warmed up faster.

Cooling rate varied by elevation ($F_{2,71}$= 25.59, p < 0.001), season ($F_{2,71}$= 48.54, p < 0.001) and sex ($F_{1,71}$= 17.57, p < 0.001), and we found an interaction of elevation × season ($F_{4,71}$= 4.30, p = 0.003; fig. 2b). Differences within the same elevation over seasons show that cooling rate did not differ in lizards from LEP, while lizards from HEP and LEP had higher cooling rates in the autumn and summer than in the spring. Regarding the differences between elevation by season, cooling rate did not differ during summer and autumn, but LEP and MEP lizards had higher cooling rate in spring than lizards from HEP. BCI index was not a significant covariable ($F_{1,71}$= 2.43, p = 0.13). Cooling rate decreased with body mass (r = -0.28, P < 0.01; fig. 3b), but was not affected by dorsal reflectance (r = 0.009, P = 0.177; fig.

4b). Hence, smaller lizards lost temperature faster, but dorsal reflectance did not affect heat loss.

Net heat gain (*NHG*) varied by elevation ($F_{2,71}$= 14.84, p < 0.001), season ($F_{2,71}$= 49.80, p < 0.001) and sex ($F_{1,71}$= 16.29, p < 0.001), and we found an interaction of elevation × season ($F_{4,71}$= 4.11, p = 0.004; fig 2c). Differences within an elevation over seasons showed that *NHG* was lower in summer than spring and autumn in all populations. Regarding the differences between elevation by season, *NHG* did not differ during summer and autumn, while during spring, lizards from MEP had lower *NHG* than lizards from LEP and HEP. BCI was not a significant covariable ($F_{1,71}$= 2.23, p = 0.13). Pearson correlations showed that *NHG* decreased with body mass (r = -0.31, P < 0.01; fig. 3c) and with dorsal reflectance (r = -0.50, P < 0.01; fig. 4c). That is, smaller and darker lizards gained more heat than larger and lighter colored lizards.

Thermic lapse varied by elevation ($F_{2,71}$= 4.50, p = 0.014) and season ($F_{2,71}$= 45.02, p < 0.001). Sex did not have a significant effect ($F_{1,71}$= 2.35, p = 0.12). We found a significant interaction of elevation × season ($F_{4,71}$= 4.16, p = 0.004; fig. 2d). Differences over seasons in the same elevation show that thermic lapse did not differ in lizards from LEP, while lizards from MEP and HEP had higher thermic lapse values in autumn than in spring and summer. Regarding the differences between elevation per season, we did not find differences in spring and summer, while lizards from MEP and HEP had higher thermic lapse values than lizards from LEP during autumn. BCI did not have a significant effect ($F_{1,71}$= 0.08, p = 0.77). We did not find a significant correlation between body mass or dorsal reflectance and transitional lapse (r = 0.15, P = 0.14 and r = 0.05, P = 0.12, respectively).

Discussion

This is the first study that compared dorsal skin reflectance induced by changes in body temperature across seasons in an elevation gradient in a species of lizard. Our results showed phenotypic plasticity in *DSR* in *S. grammicus*. According to our prediction, the lizards from the high (cold) elevation were darker than the lizards from lower elevations (Fig. 1). However, changes in *DSR* were not homogeneous over the seasons and differed among elevations, even when body temperature was controlled in laboratory condition. Although we expected lizards to be darker in the colder season (summer), this was only observed in HEP for all body temperatures and in LEP only at 30ºC. Furthermore, *DSR* did not always reduce with increasing body temperature (Fig. 1). As expected, lower values of *DSR* were related to higher heating rate and net heat gain. Finally, thermic rates of lizard varied over the seasons; in autumn, lizards had higher thermic rates, including higher heat retention, than in other seasons.

Thermal Melanism Hypothesis posits that organisms with low *DSR* (dark) can have a higher heating rate than lighter conspecific because dark organisms absorb solar radiation more efficiently (Porter and Norris, 1966; Clusella-Trullas et al., 2007). Lizards inhabiting cold areas are then more likely to be dark. Accordingly, in our study, we found that *S. grammicus* was darker at the cold, high elevation, than at the middle and low elevation. *DSR* can be an auxiliar mechanism in heat acquisition, so it may vary among seasons. In summer, lizards from LEP and HEP had lower *DSR* than spring and autumn. In central Mexico, rainy and cold days are common in summer and temperatures are lower than in spring and autumn (González-Morales et al., submitted). Seasonal variation in *DSR* is attributed to the dispersion or the amount of melanin in the dermis (Cadena et al., 2018). Delhey (2018) showed that there is a negative relationship between the environmental temperature and humidity and the dorsal reflectance in Australian birds, which may favor the immune response to latent pathogens, which may be more common when humidity is high. We cannot rule out the possibility that different selective pressures act simultaneously on the dorsal reflectance in *S. grammicus*, both for increased heat absorption at low temperatures, protection against UV radiation when solar radiation is strong, and for the protection of bacteria and pathogens (see Mackintosh, 2001; Sheehan et al., 2020). Overall, our results indicate a greater seasonal effect of reptile color change consistent with variation in the thermal benefits (Smith et al., 2016; Ortega et al., 2016)

We expected that lizards from high elevation were darker than lizards from low elevation and that lizards that inhabiting more variable thermal environment should had higher plasticity in *DSR*, however we did not find results that support this idea. Although higher elevation lizards were darker, body temperature-induced *DSR* changes in LEP and HEP lizards. Although temperature data available for the study sites show that middle elevation has greater temperature variation in respect to the low and high elevation (González-Morales et al., submitted), lizards from the intermediate elevation showed no changes in *DSR* dependent on body temperature. In a short time, many ectothermic vertebrates have the ability to rapidly change skin color for thermoregulation, crypsis, or communication (Norris, 1967; Hadley, 1973; Smith et al., 2016; Stuart-Fox et al., 2017). The mechanism responsible for changes in dorsal reflectance is based on the grouping (skin lightening) or dispersion (darkening) of melanin stored in melanophores (Bagnara et al., 1968). When the body temperature is low due to effect of a low environmental temperature, the α-melanocyte-stimulating hormone (α-MSH) is secreted to promote melanin dispersion, and lizards present a lower *DSR*, when body temperature increases, the affinity of the hormone reduces, which causes dorsal skin clearance (Bagnara and Hadley, 1973; Sherbrook, 1997). However, HEP lizards showed a dark dorsal coloration when body temperature was high (30 °C). At high body temperatures lizards are generally more active (Lemos-Espinal and Ballinger, 1995; Woolrich-Piña et al., 2006), therefore, they are exposed to the high UV radiation characteristic of high elevation areas (Körner, 2007), which could lead to DNA damage (Ravanat et al., 2001; Constantini, 2008) and oxidative stress (Chang and Zheng, 2003). The presence of melanin in the skin may reduce the negative effects of UV radiation (Melanin-based UV Protection Hypothesis; Porter and Norris, 1967). *Psammodromus algirus* is darker at high elevation (Reguera et al., 2014a), which has been linked to lower levels of oxidative stress compared to populations of the same species at low elevations (Reguera et al., 2014b).

Thermal rates differed among seasons. Heating rate and net heat gain were higher in summer and autumn than spring season, in addition, thermic lapse was higher in MEP and HEP during autumn than spring season, it is evident that lizards use physiological mechanisms only when necessary and according to the activities they perform, during the coldest season (summer). During the summer, in the rainy season, environmental temperatures are often low, and the higher thermoregulation effort necessary may reduce the time for foraging compared to the warmer seasons (spring and autumn). A higher heating rate could reduce the time spent on thermoregulation and optimize the use of the available time (Clusella-Trullas et al., 2007), which may explain why in summer heating rate was high in all populations. Reproductive period of *S. grammicus* is during autumn, and the lizards spend time protecting their territories, courting, or copulating (Guillette and Casas-Andreu, 1980), and a reduced heating rate may free up time for these activities. Interestingly, the heat retention (thermal laps) was twice as high in the MEP and HEP lizards than in the LEP lizards. Similar results have been observed in the tegu lizard, in which, during the mating season, the lizards have a more efficient control of their body temperature and a lower cool rate (Tattersall et al., 2016). Increased heat retention during autumn could be associated with increased vasodilation (Sanders et al., 2015), increased metabolic rate (Sears, 2005), and even with muscular thermogenesis, as described in pythons (Harlow and Grigg, 1984).

We found a negative relationship between *DSR* and heating rate and net heat gain in *S. grammicus*. Several studies have found that lower *DSR* in cold environments, such as at high elevations, can allow individuals to gain heat faster (Clusella-Trullas et al., 2009; Geen & Johnston, 2014). For example, *Cordylus niger* at relative high elevation had lower *DSR* and a higher heating rate than *C. cordylus* at low elevation (Clusella-Trullas et al., 2009). Biophysical simulation of heat transfers in *Pogona vitticeps* showed that temperature-dependent color changes facilitate a more rapid heat acquisition (Smith et al., 2016). In addition, the thermal melanism hypothesis is supported in polymorphic lizards such as *Tiliqua scincoides,* in which a dark morph has a faster heating rate than lighter colored morphs (Geen & Johnston, 2014). However, other studies did not find a *DSR* effect in heat gain, for example, Zamora-Camacho et al. (2014) did not find a direct relationship between dorsal coloration and heating rate in *Psammodromus algirus*, despite that this lizard is darker at higher elevations. Similarly, Gvozdík (1999) failed to find a positive effect of melanism on thermal properties of *Zootoca vivipara*. We do not yet have an adequate understanding of the effects of melanism in lizards at extreme elevations, so more studies involving the energetic cost of short-term and long-term color changes are needed.

In conclusion, *DSR* varies with elevation, season, and body temperature in *Sceloporus grammicus* in a complicated pattern. Heating rate and net heat gain were higher in cool seasons and related negatively with *DSR,* strongly supporting the thermal melanism hypothesis.

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Figure legends

Figure 1. Dorsal skin reflectance by elevation and season in *Sceloporus grammicus* lizard, letters show differences among body temperatures within the season; a) low elevation population, b) middle elevation population, and c) high elevation population. Average is indicated by dot, triangle and square for low, middle, and high elevation respectively. Vertical bars represent mean ± SEM.

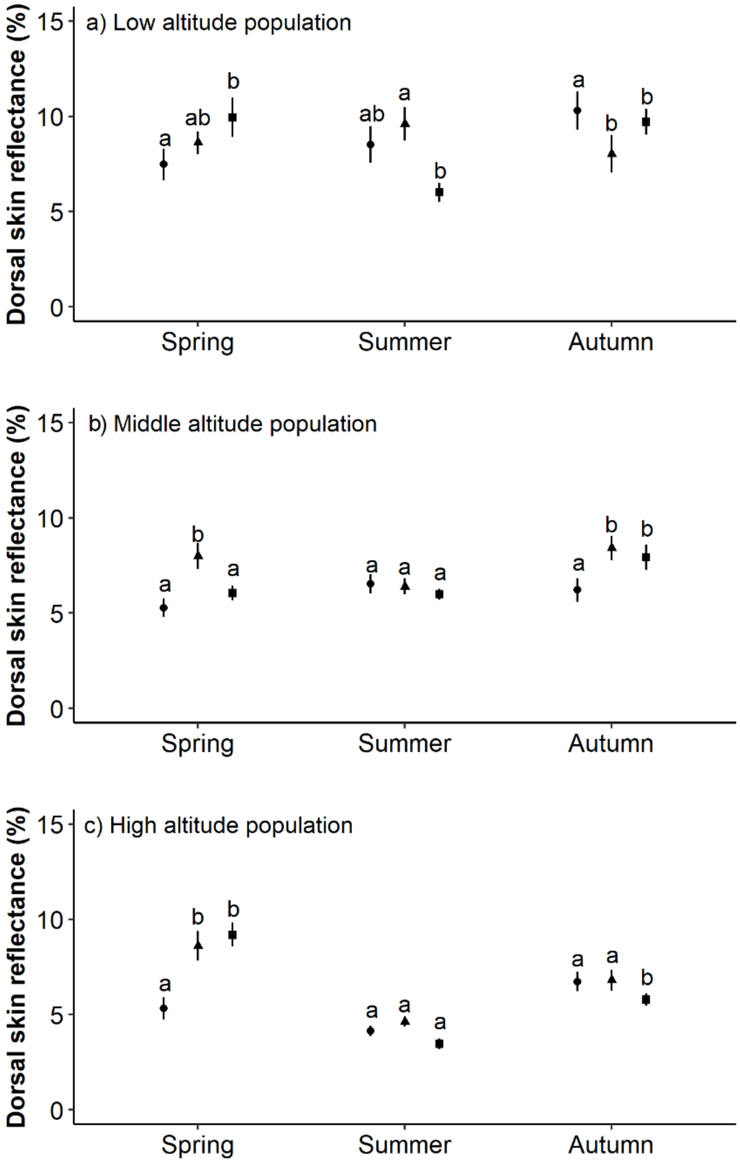

Figure 2. Thermal rates by elevation and season in *Sceloporus grammicus* lizards; a) heating rate, b) cooling rate, c) net heat gain, and d) thermic lapse. Dot, triangle, and square indicate the low, middle, and high elevation respectively. Capital letters show differences among seasons within a population and lower case letters show differences among populations within a season.

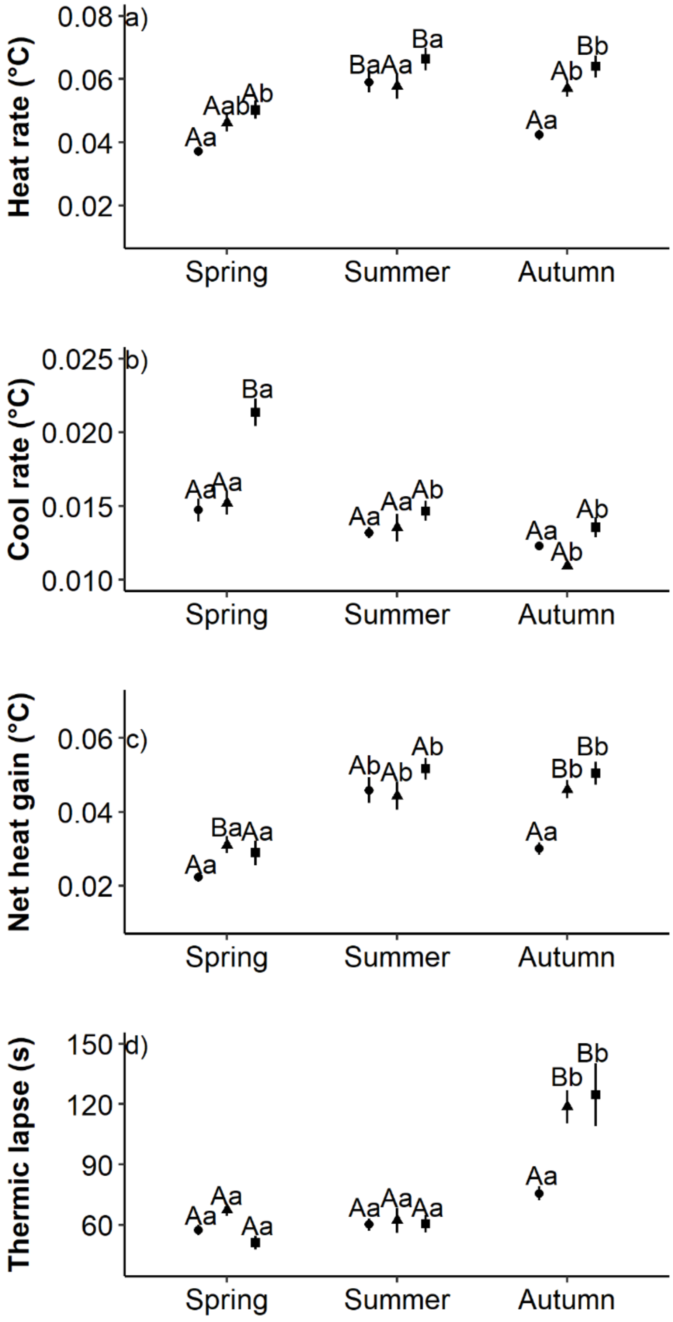

Figure 3. Relationship between body mass and a) heating rate, b) cooling rate, and c) net heat gain in *Sceloporus grammicus* lizard. Note that as body mass increased the value of thermal traits decreased.

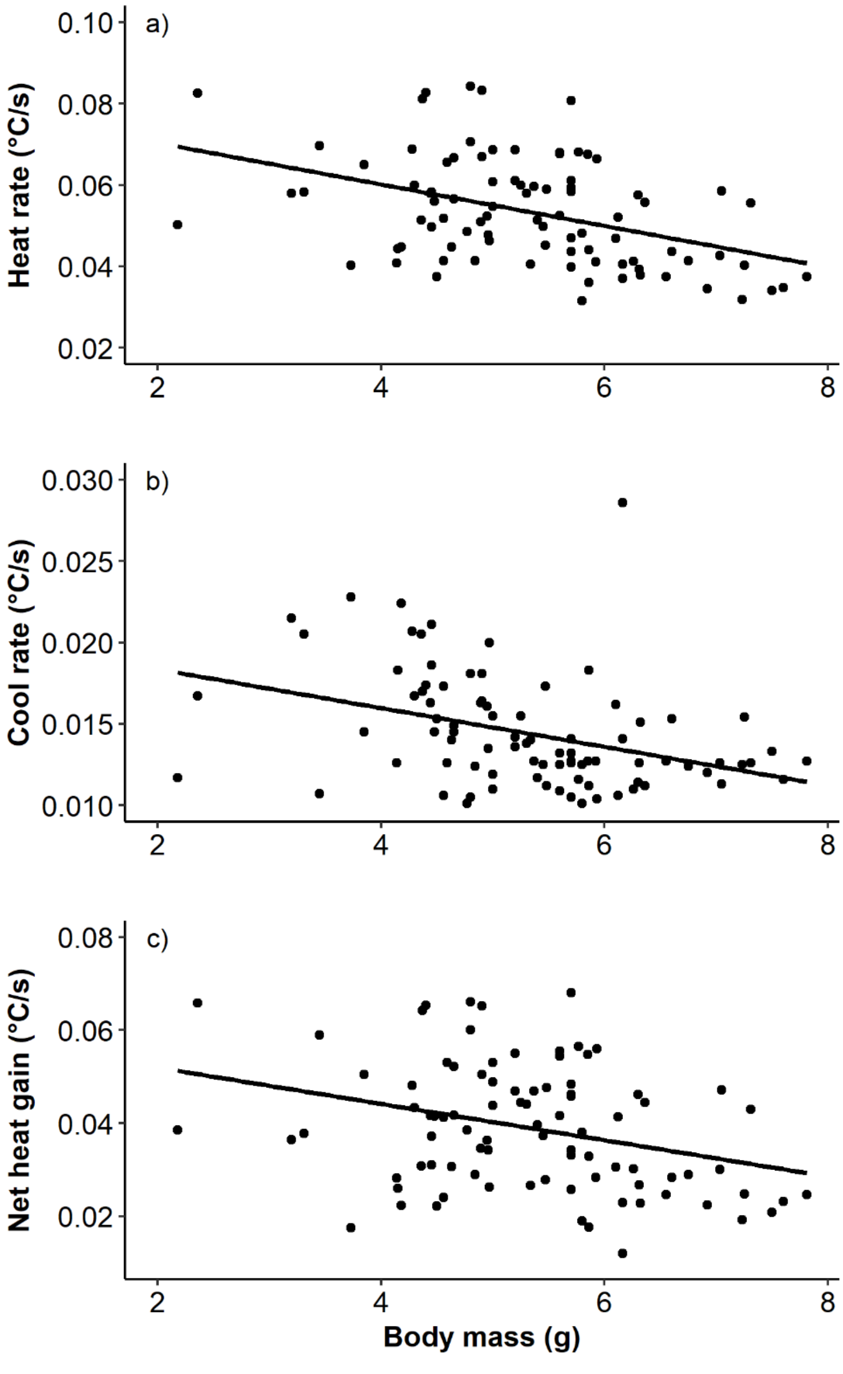

Figure 4. Relationship between dorsal skin reflectance and a) heating rate, b) cooling rate, and c) net heat gain in *Sceloporus grammicus*.

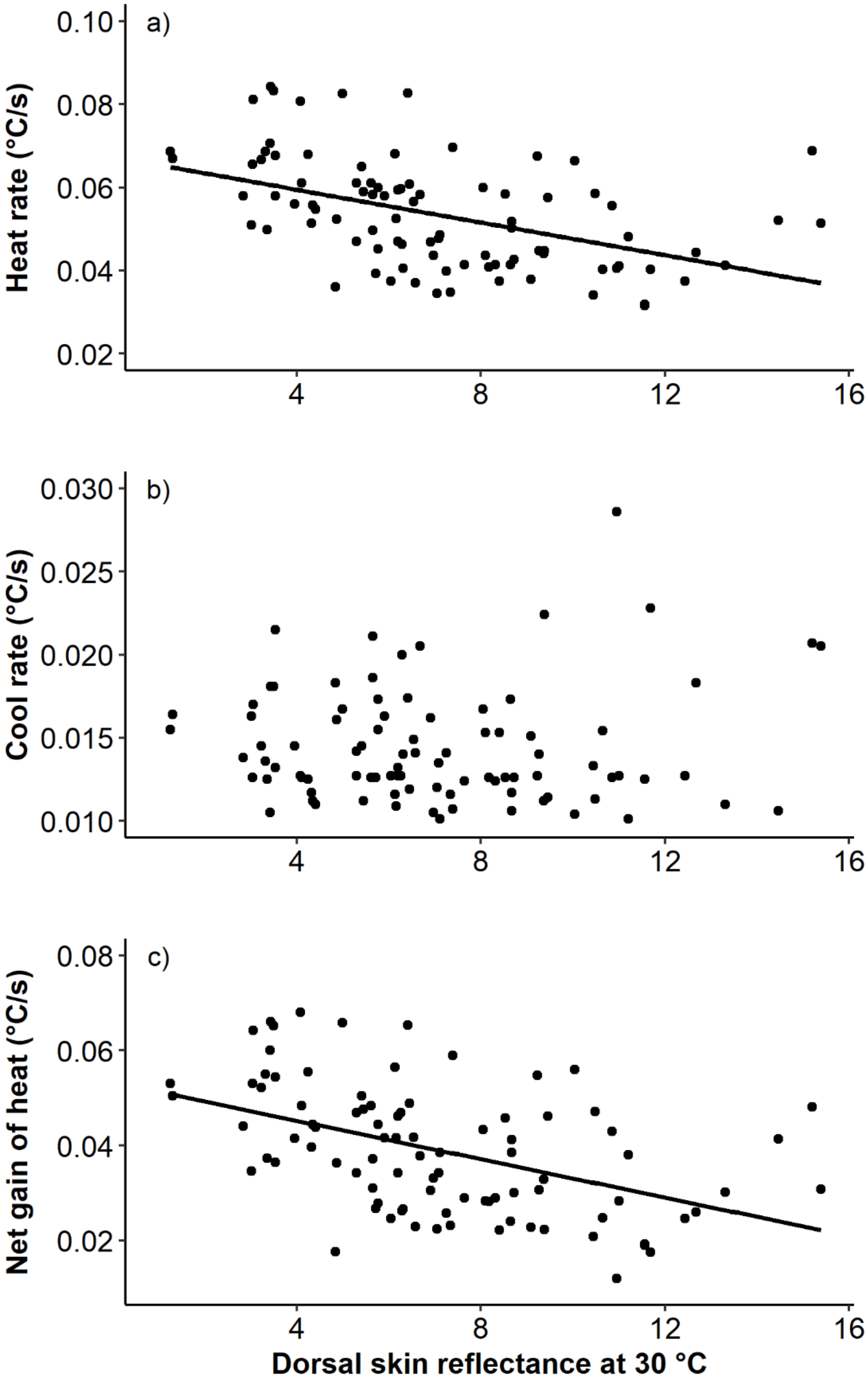

Table 1. Morphological parameters of *Sceloporus grammicus* by altitude and season

		Body mass (g)		BCI		SVL (mm)	
		♂	♀	♂	♀	♂	♀
LAP (2500 m)	Spring	6.61 ± 0.31^{Aa}	5.31 ± 0.23^{Aa}	1.28 ± 0.27^{Aa}	-0.005 ± 0.25^{Aa}	5.66 ± 0.27^{Aa}	5.65 ± 0.03^{Aa}
	Summer	5.89 ± 0.19^{Aa}	5.92 ± 0.42^{Aa}	0.11 ± 0.49^{Aa}	0.45 ± 0.28^{Aa}	6.29 ± 0.49^{Aa}	5.85 ± 0.37^{Aa}
	Autumn	6.09 ± 0.30^{Aa}	7.18 ± 0.63^{Aa}	0.73 ± 0.22^{Aa}	1.72 ± 0.45^{Aa}	5.70 ± 0.22^{Aa}	5.85 ± 0.25^{Aa}
MAP (3400 m)	Spring	5.19 ± 0.36^{Ab}	5.31 ± 0.24^{Ab}	-0.010 ± 0.28^{Ab}	-1.32 ± 0.13^{Aa}	5.47 ± 0.11^{Ab}	4.92 ± 0.12^{Ab}
	Summer	5.29 ± 0.23^{Aa}	5.92 ± 0.17^{Ba}	0.40 ± 0.32^{Aa}	0.76 ± 0.11^{Ba}	5.04 ± 0.43^{Ab}	4.72 ± 0.34^{Ab}
	Autumn	4.82 ± 0.43^{Aab}	4.94 ± 0.41^{ABa}	-0.022 ± $0.29A^{ab}$	-0.09 ± 0.35^{Aab}	5.25 ± 0.19^{Ab}	5.25 ± 0.10^{Ab}
HAP (4300 m)	Spring	3.60 ± 0.14^{Ab}	4.11 ± 0.22^{Aab}	-1.14 ± 0.16^{Ab}	-0.62 ± 0.16^{Aa}	4.83 ± 0.19^{Ab}	4.84 ± 0.12^{Ac}
	Summer	5.23 ± 0.07^{Ba}	4.78 ± 0.13^{Aa}	0.26 ± 0.12^{Ba}	1.05 ± 0.12^{Ba}	5.15 ± 0.24^{Ab}	3.42 ± 0.18^{Ac}
	Autumn	4.30 ± 0.28^{ABb}	3.86 ± 0.23^{Aab}	-0.60 ± $0.16A^{Bb}$	-0.90 ± 0.16^{Ab}	5.07 ± 0.10^{Ab}	4.87 ± 0.12^{Ac}

Note: Values are represented as mean ± SE. Capital letters show differences by season in the same altitude; lowercase letters show differences by season between altitudes. ♂= males, ♀= females.

Table 2. Linear Mixed Model results of dorsal skin reflectance of *Sceloporus grammicus*

Factor	D.F.	F-value	p-value
Altitude	2	18.47	**<0.001**
Season	2	9.59	**<0.001**
Sex	1	11.13	**<0.001**
Body temperature	2	9.31	**<0.001**
Altitude*Season	4	5.56	**<0.001**
Altitude*Sex	2	2.01	0.13
Altitude*Body temperature	4	1.88	0.11
Season*Sex	2	0.50	0.60
Season*Body temperature	4	11.34	**<0.001**
Sex*Body temperature	2	0.87	0.42
Altitude*Season*Sex	4	0.45	0.77
Altitude*Season*Body temperature	8	5.28	**<0.001**
Altitude*Sex*Body temperature	4	0.18	0.94
Season*Sex*Body temperature	4	1.62	0.16
Altitude*Season*Sex*Body temperature	8	0.510	0.85

Note: Significant results are in bold

10. DISCUSIÓN GENERAL

La temperatura de selección como rasgo complejo y los índices de termorregulación, reflectancia dorsal y tasas de calentamiento como rasgos subordinados presentaron diferencias significativas por altitud y estación del año. Dichos resultados indican que *S. grammicus* no sigue una estrategia de termorregulación conservativa. En ambientes en los que las condiciones ambientales no son constantes a lo largo del año, mantener las mismas preferencias térmicas, puede tener costos asociados altos, por lo que modificar tanto las preferencias térmicas como los mecanismos de obtención de calor, puede resultar en una estrategia con mejor balance costo/beneficio.

Las zonas de estudio en las que se realizaron los muestreos a lo largo del año muestran variación estacional. Las temperaturas operativas (*Top)* en las elevaciones alta y media registraron temperaturas altas durante el invierno, similar a lo reportado por Díaz de la Vega-Pérez y cols. (2019). El invierno en altas elevaciones en la zona centro de México se caracteriza por la ausencia de nubes y la alta radiación solar, dando como resultado días soleados y secos, mientras que en verano las *Top* son muy bajas respecto al invierno, ya que en esta estación las lluvias y la alta nubosidad dan como resultados días con poca radiación solar y fríos (Sinervo y cols. 2010). Por otra parte, la cobertura vegetal puede afectar negativamente la radiación solar al nivel del sustrato (Lara-Reséndiz y cols. 2014), caso parecido al que sucede en la elevación baja, en donde los árboles y vegetación en general son más abundantes respecto a la zona alta.

La temperatura de selección (*Tsel)* mostró diferencias por altitud y estación del año (capítulo 3). De manera general, el género *Sceloporus* ha sido considerado térmicamente conservativo (Andrews 1998), no obstante, los trabajos recientes en lo que se aborda desde una perspectiva estacional o a lo largo del año, han mostrado variación en ciertos rasgos como la *Tsel* o las temperaturas críticas mínimas o máximas, por lo que, tanto la flexibilidad como la plasticidad fenotípica podrían tener un impacto mayor a lo que se ha considerado en la termorregulación del género *Sceloporus* (Domínguez-Guerrero y cols. 2019).

Mantener las mismas preferencias térmicas independientemente de la altitud y la estacionalidad, podría implicar grandes costos asociados a la termorregulación, ya que al considerar que la conducta es suficiente para alcanzar la temperatura corporal óptima, se reduciría el tiempo disponible para otras actividades, sobre todo en ambientes en donde la heterogeneidad térmica es alta, como las altas elevaciones, en donde el cambio en la *Top* puede ser de 8 °C. Modificar la *Tsel* de acuerdo con las condiciones ambientales (estaciones del año) podría favorecer ciertos aspectos de los organismos, como reducir los costos asociados con la termorregulación en inversión de tiempo durante la obtención de calor o ampliar el rango de temperatura en el cual pueden desempeñarse durante la búsqueda de alimento (Díaz y cols. 2006; Ortega y cols. 2016). Por ejemplo, altos valores de *Tsel* durante la primavera y el verano podrían estar relacionados con la obtención y digestión del alimento, mientras que valores bajos durante el otoño podrían relacionarse con la disminución del uso de energía y la pérdida de agua, para dedicar más tiempo al cortejo y las cópulas (Crowley 1985).

La temperatura corporal influye en gran medida en la biología de los ectotermos, modificando el punto óptimo y rendimiento de diversos rasgos como la digestión, captación de oxígeno y la velocidad de sprint (Hertz y cols. 1983; Angilletta 2009). Esta última suele estar implicada en la supervivencia de algunas especies de lagartijas, de ella puede depender el escapar con éxito de un depredador o capturar presas durante el forrajeo (Garland y Losos 1994). Nuestros resultados mostraron que existen diferencias significativas en la velocidad de sprint en función de la temperatura corporal y la altitud, además de que la relación que presentó con la reflectancia dorsal fue diferente para cada altitud. A 20 °C de *Tc*, las lagartijas con mayor reflectancia (más claras) tenían una mayor velocidad de sprint, mientras que a 10 y 30 °C no encontramos relación entre estas variables (Artículo 2). Las lagartijas de la zona baja y media presentaron una mayor reflectancia dorsal (i.e. eran más claras) respecto a las lagartijas de la zona alta. Varios estudios han reportado que los datos obtenidos en condiciones de laboratorio de la velocidad de sprint son semejantes a los reportados en condiciones naturales, por lo que es viable inferir las consecuencias a partir de los datos de laboratorio cuando las lagartijas escapan de algún depredador o como un buen indicador de la probabilidad de sobrevivir (Huey y Dunham 1987; Sinervo y Losos 1991; Miles y cols. 2000).

La velocidad de sprint fue mayor a 30 °C de *Tc* en las tres poblaciones estudiadas, partiendo del hecho de que tomaría más tiempo alcanzar esa temperatura corporal en zonas de alta elevación, es probable que las lagartijas residentes de estos sitios tengan que invertir más tiempo en la termorregulación que en otras actividades como la obtención de alimento, cortejar parejas o exponerse más a depredadores (Lemos-Espinal y Ballinger 1995; Gvozdik 2002). Sin embargo, a mayor elevación, el número de potenciales depredadores y la competencia entre especies podría ser menor respecto a zonas de elevaciones menores en latitudes similares, puesto que la biodiversidad tiende a disminuir con la altitud (Willing y Presley 2016; Domínguez-Godoy y cols. 2020b). Recientemente, Domínguez-Godoy y cols. (2020a) han reportado que la probabilidad de supervivencia es mayor a grandes altitudes en *Sceloporus grammicus*, por lo que de acuerdo con Hock (1967), es probable que los ambientes de alta elevación no sean tan agrestes para los lacertilios como siempre se ha considerado.

Otros factores como la morfología y la fisiología pueden afectar la velocidad de *sprint* de los lacertilios (Bonine y Garland 1999). Generalmente, entre más largo es el cuerpo y las extremidades posteriores de las lagartijas, mayor es la velocidad de *sprint* que pueden alcanzar (Bauwens y cols. 1995; Zamora-Camacho y cols. 2014b). En nuestro modelo de estudio no se cumple dicha premisa. A 30 °C de *Tc* la velocidad de *sprint* fue mayor en las lagartijas de alta elevación respecto a la zona media y baja, esto a pesar de tener las extremidades posteriores más cortas. Otras características morfológicas como la posición de la columna vertebral o el movimiento de las patas durante la carrera, incluso ciertas características fisiológicas como la proporción de fibras blancas o rojas, así como la contracción de los músculos asociados con la locomoción, podrían tener un efecto mayor a la longitud de las patas traseras en la velocidad de *sprint* (Bonine y Garland 1999, Quintana y cols. 2014). Es necesario realizar estudios comparativos de las características a nivel de tejido, contracción muscular y uniones neuromusculares para corroborar esta idea en relación a la velocidad de *sprint*.

Si bien, la longitud de las extremidades es relevante durante la velocidad de *sprint* en varias especies de lagartijas, otros rasgos fenotípicos como el tamaño corporal pequeño y la baja reflectancia parecen ser atributos que benefician la ganancia de calor en altitudes extremas, a pesar de que la tasa de enfriamiento es mayor, la ganancia neta de calor indica que es ventajoso seguir esa estrategia. Puesto que el tamaño corporal pequeño da una relación mayor entre el ambiente y la superficie de contacto, sería más factible obtener calor rápido (Pincheria-Donoso y cols. 2008). Todos estos factores podrían reducir el tiempo necesario para alcanzar la temperatura de selección sin tener que reducir el tiempo de otras actividades como el forrajeo o el cortejo (Clusella-Trullas y cols. 2007). Por otra parte, un tamaño corporal pequeño también podría tener consecuencias negativas en la aptitud, por ejemplo, el número de crías por camada o su tamaño podrían reducirse (Sinervo 1990).

El aumento del lapso térmico durante el otoño en las lagartijas de la elevación alta indica que la retención de calor por parte de las lagartijas es más alta, es posible que *S. grammicus* posea algún mecanismo fisiológico que le permita retener calor por más de dos minutos, aun cuando las lagartijas fueron sujetadas a la tabla de plástico para eliminar contribuciones conductuales al mantenimiento del calor. Un mecanismo causal de este fenómeno podría ser el aumento de la tasa metabólica. Plasman y cols. (2020) mostraron que *S. grammicus* en altas elevaciones presentan una tasa metabólica mayor. Por otra parte, la lagartija *Salvator meriani* muestra un incremento en la tasa metabólica y la temperatura corporal durante cierta parte del año que coincide con la época de apareamiento (Tattersall y cols. 2016). Es necesario realizar más estudios de la tasa metabólica en *S. grammicus* en diferentes estaciones para sustentar esta idea.

La baja reflectancia dorsal favorece la ganancia de calor, caso parecido a *Cordylus niger*, que al ser más oscuro presenta una mayor velocidad de calentamiento que *Cordylus cordylus* que es más claro (Clusella-Trullas y cols. 2009). La reflectancia dorsal depende de la *Tc* de la lagartija, mostrando variación entre las temperaturas usadas durante las pruebas, una variación que puede ser producto de la flexibilidad fenotípica (Smith y cols. 2016) Es necesario evaluar si la melanización tiene efecto benéfico sobre la aptitud con un aumento en la tasa de crecimiento o fecundidad en zonas de altas elevaciones (Stuart-Fox y cols. 2017).

Nuestros resultados indican que existe variación en la reflectancia dorsal en función de la temperatura corporal y altitud, además de que dicha variación no es homogénea entre estaciones del año. Como se ha mencionado anteriormente, las lagartijas de la elevación alta tienen una menor reflectancia respecto a las lagartijas de la elevación baja y media, por otra parte, durante el verano se registraron los valores más bajos de reflectancia, dicha estación coincide con el registro de las temperaturas operativas más bajas.

La reflectancia dorsal puede modificarse a corto (ruta fisiológica) o largo plazo (ruta morfológica). En primera instancia, cuando el organismo detecta que la *Tc* es baja, la glándula pituitaria secreta la hormona estimulante de la melanina (HSM), la cual provoca una desagrupación de la melanina en el tegumento de las lagartijas, pero cuando la *Tc* incrementa, la sensibilidad de la HSM disminuye, provocando la agrupación de la melanina y un incremento de la reflectancia (Sherbrook 1997). A largo plazo, la reflectancia dorsal se modifica por la deposición de pigmentos de melanina en el tegumento, por lo que solo se observan cambios en la reflectancia por estación u ontogenia (Sherbrook 1997). Los cambios en la reflectancia dorsal se asocian con ajustar o mejorar el proceso de la termorregulación y se ha observado en diversas especies de lagartijas (Smith y cols. 2016), no obstante, se desconoce si los cambios a corto o largo plazo en la reflectancia dorsal tengan algún costo energético asociado.

El cambio de coloración por estación en *S. grammicus* podría tener otro tipo de contribución ante otras presiones selectivas, la protección ante la radiación UV podría ser uno de ellos, tal como lo han descrito Reguera y cols. (2014a y 2014b) en la lagartija *Psammodromus algirus*, o para pasar desapercibido ante los depredadores como señalan Marshall y cols. (2015).

Sceloporus grammicus es una lagartija ampliamente utilizada en estudios ecológicos y fisiológicos, sin embargo, nuestros resultados no concuerdan completamente con los reportados en otras zonas de estudio. Mientras que en otras montañas del centro de México los datos muestran que la termorregulación es conservativa a lo largo de gradientes altitudinales, en nuestra zona de estudio, nuestros datos sugieren firmemente que la termorregulación es lábil. Estas diferencias pueden deberse a aspectos simples como el método empleado durante la obtención de datos (el tipo de materiales usados en la elaboración del gradiente térmico y número de focos), la temporalidad de la colecta de los organismos, a aspectos complejos como variación en la historia de vida de las diferentes poblaciones o efectos locales en los rasgos evaluados. Es necesario realizar estudios a mayor escala para validar estas suposiciones.

12. CONCLUSIÓN

Los rasgos complejos evaluados (temperatura de selección y velocidad de sprint) mostraron cambios por efecto de la altitud y la estación, por lo que no podemos considerar los como conservativos en nuestro sistema de estudio. Los rasgos subordinados como la tasa de calentamiento y la reflectancia dorsal también mostraron cambios asociados con la altitud y estación. Es probable que las lagartijas modifiquen ambos rasgos de acuerdo con la prioridad que tengan sus actividades durante el año y su éxito en zonas de altas elevaciones se deba a la interacción entre ambos tipos de rasgos con las condiciones ambientales.

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ANEXO 1.

Artículo de divulgación publicado en la revista "¿Cómo ves?"

Sangre de colores: desde humanos hasta lagartijas.

Juan Carlos González-Morales y Jimena Rivera-Rea.

Sangre de colores:

desde humanos hasta lagartijas

Por **Juan Carlos González Morales** y **Jimena Rivera Rea**

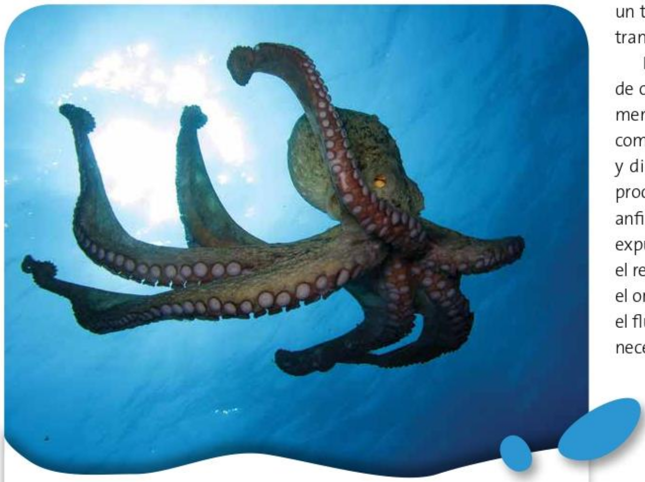
En los cuentos, los príncipes y las princesas tienen "sangre azul". Por supuesto, es solo una manera de hablar, pero hay otros animales cuya sangre sí es de ese color. También hay animales de sangre verde y hasta transparente. El color de la sangre de una especie está relacionado con su historia evolutiva.

Según el historiador John H. Kautsky en su libro *The politics of aristocratic empires* (*La política en los imperios aristocráticos*), los miembros de las familias más poderosas en el pasado buscaban maneras de demostrar una superioridad jerárquica y biológica sobre el resto de la población. Les gustaba encontrar características que los distinguieran del común de los mortales. Una muy socorrida era mostrar las venas de los brazos, que se veían de color azul pálido por el contraste entre lo rojo de la sangre y la palidez de la piel de personas que no tenían que laborar al rayo del Sol. Sus súbditos o vasallos generalmente tenían la piel tostada por las largas jornadas de trabajo y era menos probable que se les vieran azules las venas. Así, lo de la "sangre azul" de los aristócratas se refiere a la diferencia entre las clases que trabajaban en el campo y las clases ociosas, o que tenían otras ocupaciones.

¿Sangre azul? Tradicionalmente se representan las venas del sistema circulatorio en azul; si miramos las que están a flor de piel parecen azules, pero esto se debe a un efecto de la luz en la piel y no a la sangre misma.

Fotos: Shutterstock

No hay humanos con sangre azul, pero otros organismos sí pueden tener sangre de ese color, e incluso de otros colores. Los primeros organismos unicelulares en la historia de la vida en la Tierra obtenían nutrientes del ambiente por difusión simple (o sea, esperando a toparse con las sustancias necesarias) o fagocitosis (rodeando y capturando partículas de nutrientes con la membrana celular). Al aumentar la complejidad biológica, los organismos desarrollaron una serie de conductos en el interior de sus cuerpos para transportar oxígeno y nutrientes. En las esponjas de mar, organismos muy simples, la función de trans-



La sangre azul de los pulpos se debe a la oxidación del cobre presente en la metaloproteína conocida como hemocianina.

Foto: ©Julia Sunnarborg/iNaturalist

Si bien el escinco *Prasinohaema* virens tiene hemoglobina en la sangre, el color verde se debe a una alta concentración de biliverdina.

portar nutrientes la lleva a cabo el agua que hacen circular por el interior de su cuerpo. Los organismos más complejos, como los cefalópodos (pulpos, sepias y calamares) y los vertebrados, cuentan con un tejido especial para cumplir esas funciones de transporte.

La sangre es un tejido encargado del transporte de oxígeno y dióxido de carbono (CO_2) principalmente, aunque tiene otras funciones importantes como transportar nutrientes y hormonas o captar y disipar calor. En organismos que no pueden producir su propio calor corporal, como reptiles, anfibios y peces, el calor es absorbido por la piel expuesta a la luz solar, tras lo cual se difunde por el resto del organismo gracias a la sangre. Cuando el organismo necesita conservar el calor corporal, el flujo de sangre se reduce y, al contrario, cuando necesita disipar calor, el flujo aumenta.

La sangre se forma en tejidos especializados como la médula ósea roja (que se encuentra en el interior de los huesos) en mamíferos y los riñones en los peces, en un proceso conocido como hematopoyesis ("creación de sangre"). La sangre está compuesta por un tejido transparente llamado plasma y por células como los glóbulos rojos, células del sistema inmune y plaquetas, que son las encargadas de la reparación o cicatrización de zonas donde el tejido ha sido dañado. En la mayoría de los casos, en el interior de cada glóbulo rojo se encuentra una proteína que tiene la función de capturar moléculas de oxígeno. Esta proteína es un pigmento. El tipo de pigmento en cada grupo de organismos determina el color de la sangre.

Adaptación al frío

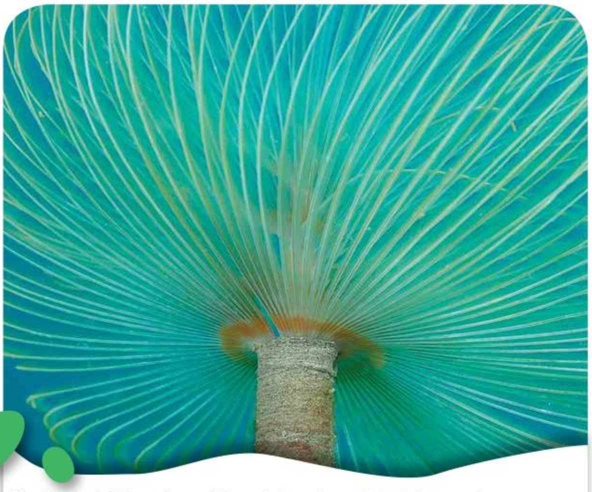
La mayoría de los vertebrados tienen sangre roja. Esta debe su color a la hemoglobina, sustancia compuesta por aminoácidos y un grupo "hemo" de gran afinidad con el oxígeno y que contiene un átomo de hierro. Al captar las moléculas de oxígeno la hemoglobina toma un color rojo parduzco.

La sangre es el primer tejido en sufrir cambios cuando se modifican las características del ambiente (temperatura y presión atmosférica, entre otras) o internas del organismo (por ejemplo, por edad o trastornos a la salud). Por eso se utiliza este tejido como indicador de salud y bienestar tanto en humanos como en animales. Con los avances de la medicina, se prevé que pronto será posible detectar un gran número de enfermedades con una sola gota de sangre.

Los primeros registros de animales que sí tienen la sangre azul datan de 1878, cuando el fisiólogo belga Léon Fredericq notó que la sangre de algunos cefalópodos (pulpos, por ejemplo) se volvía azul al pasar por las branquias. Posteriormente se descubrió que este fenómeno se debía a la oxidación del cobre presente en una proteína que se denominó hemocianina (actualmente está en debate el nombre, ya que esta proteína no contiene el grupo "hemo"). Existen varias diferencias entre los organismos de sangre roja y los de sangre azul en lo que se refiere a la función de la sangre.

Los organismos de sangre azul presentan el inconveniente de que su capacidad de transportar oxígeno es tres veces menor que la de los organismos de sangre roja. El oxígeno es esencial para generar energía, y esa energía se utiliza para realizar movimientos corporales u otras actividades como crecer y buscar alimento o pareja. La capacidad reducida de transportar oxígeno en los organismos de sangre azul ha llevado a que los cefalópodos del océano Antártico tengan una forma de vida sedentaria, marcada por poco movimiento y ausencia de cambios corporales durante su ciclo de vida. Actualmente a la comunidad científica le preocupa que estos organismos no puedan responder de manera adecuada al calentamiento global y desaparezcan. La principal razón es que la afinidad de la hemocianina con el oxígeno se reduce al aumentar la temperatura. Al parecer lo que era una adaptación al frío extremo es un inconveniente en la situación global actual, en que los mares se están calentando.

La sangre azul ha despertado gran interés en la comunidad científica por dos razones: la primera es que se desconoce si su aparición fue independiente en los grupos que tienen hemocianina (cefalópodos y artrópodos), ya que los hábitos de cada grupo y la cantidad de esta proteína que poseen no favorecen ningún patrón en particular. El protagonista de la segunda razón es el cangrejo herradura y el potencial uso médico de su sangre (ver *¿Cómo ves?* Núm. 227). Muchos avances científicos y tecnológicos provienen de la observación de los organismos en su ambiente. Los científicos notaron que, a pesar de vivir en el océano, los cangrejos herradura que tenían heridas no presentaban infección. Con el tiempo descubrieron que inyectar bacterias o partes de bacterias en los cangrejos herradura provocaba una respuesta de gelatinización en la sangre, lo que inactivaba los agentes tóxicos. Actualmente los científicos se han propuesto descifrar cómo funciona este sistema de protección y la posibilidad de usarlo en el tratamiento de enfermedades inmunes en los seres humanos.



El poliqueto *Sabella spallanzanii* tiene el pigmento respiratorio clorocruorina; con una estructura parecida a la hemoglobina este le da un tono verdoso a su sangre.

Órganos en los que se lleva a cabo la formación de glóbulos rojos en distintas clases de vertebrados

Clase de organismo	Ejemplo	Órgano hematopoyético
Ciclóstomos (No tienen mandíbula inferior)	Lampreas Mixinos	Submucosa intestinal
Elasmobranquios	Mantarrayas Tiburones	Órgano de Leydig Riñón cefálico Bazo
Teleósteos (Peces con esqueleto osificado)	Truchas	Riñón Bazo
Anfibios	Ranas Salamandras	Hígado Bazo Medula ósea
Aves, reptiles y mamíferos	Lagartijas Cuervos Humanos	Bazo Medula ósea roja

De la sangre de los cangrejos herradura se extrae lisado de amebocitos (son parte del sistema inmunológico de estos organismos), que son esenciales para detectar endotoxinas en la industria farmacéutica. Las empresas farmacéuticas usan el lisado de amebocitos para fabricar y probar vacunas contra la COVID-19. El número de cangrejos herradura que se requieren para probar y producir

Algunos invertebrados marinos como *Sipunculus nudus* tienen hemeritrina como pigmento respiratorio que, al oxigenarse, le da un tono rosa-violeta a la sangre.

Diferencias entre organismos de sangre roja y azul

Sangre roja	Sangre azul
Peces, anfibios, reptiles, aves y mamíferos	**Cefalópodos y artrópodos**
Sistema circulatorio cerrado (la sangre permanece dentro de venas y arterias, nunca entra en contacto directo con otros tejidos)	Sistema circulatorio abierto (la sangre entra en contacto directo con los tejidos)
La hemoglobina se encuentra dentro de los glóbulos rojos	La hemocianina se encuentra en el plasma
El hierro es la molécula que capta el oxígeno	El cobre es la molécula que capta el oxígeno
Mayor capacidad de captación de oxigeno	Poca capacidad de captación de oxigeno

las vacunas es inmenso; esto ha puesto en alerta a la comunidad científica, que teme por la población de estos fósiles vivientes.

Sangre verde

Ciertas especies de lagartijas de Nueva Guinea tienen la sangre verde. No se debe a que no tengan hemoglobina sino a una acumulación de biliverdina, el pigmento que da el color verde a la bilis. La biliverdina proviene de la desintegración de la hemoglobina y se encuentra en tales concentraciones en la sangre de estos reptiles, que el color verde predomina sobre el color de los glóbulos rojos y tiñe la sangre, los huesos, los músculos, la lengua y las mucosas.

En todos los demás vertebrados, este exceso de pigmento biliar en la sangre causa un problema patológico conocido como ictericia, que puede ocasionar graves daños a nivel motor y cerebral. Las lagartijas del género *Prasinohaema* de Nueva Guinea toleran concentraciones de este pigmento que serían altamente tóxicas para las personas (más de 100 veces lo reportado en humanos). Aunque la función del exceso de pigmentos de la bilis en lagartijas es desconocida, se especula que puede protegerlas contra los rayos ultravioleta, servir como tóxico en caso de que sean depredadas por otros animales y para la termorregulación; las lagartijas no pueden producir calor de forma autónoma como los mamíferos, por lo que tienen que exponerse a fuentes caloríficas externas, de ahí la frase "tomar el Sol como lagartija". En la última década, la biliverdina en organismos animales ha sido de interés por dos motivos: 1) la medicina tradicional china la emplea como antioxidante y 2) la ruta metabólica de la degradación de la hemoglobina podría ser útil para el tratamiento de la ictericia en humanos.

Además de estas lagartijas, también hay peces, ranas y una especie de insecto con sangre verde, pero la concentración de biliverdina en estas especies no es tan grande.

Peces de hielo

Ahora nos falta hablar de cierto grupo de peces cuya sangre es trasparente. En el océano Antártico, los llamados "peces del hielo" carecen de pigmento respiratorio en la sangre, por lo que esta se considera sin color o transparente. Aunque parece ilógico no tener pigmento respiratorio en la sangre, es probable que se deba a que el pigmento aumentaría la viscosidad del fluido sanguíneo, lo que en lugares fríos dificultaría la actividad de bombeo del corazón. Así, la selección natural habría favorecido la eliminación del pigmento en estos organismos. Por otra parte, en este tipo de hábitat la disponibilidad de oxígeno es muy alta, por lo que no es necesario tener pigmento respiratorio. Incluso algunos investigadores han mostrado que estos peces han perdido la base genética para producir proteínas del tipo globulinas funcionales. Una desventaja es que estos peces poseen una tolerancia muy baja a la hipoxia (concentración de oxígeno por debajo de lo normal) en comparación con peces de sangre roja.

El color no importa

El estudio de la sangre en organismos como los mamíferos (incluidos los humanos) está muy avanzado, pero en otros como los anfibios y reptiles no. De los organismos de sangre azul o transparente sabemos muy poco. Una probable causa de esta desigualdad se debe a la dificultad de trabajar con ciertos organismos: obtener muestras para los análisis tiende a complicarse en lagartijas de cinco gramos de peso o ranas de tres centímetros.

Con el calentamiento global, una cantidad considerable de especies podrían desaparecer. Se ha propuesto que una opción es que migren a zonas más frías que las que habitan actualmente; por ejemplo, a zonas más elevadas o latitudes más altas, lo cual es bastante difícil para los cefalópodos y peces del hielo que ya habitan zonas cercanas a los polos. Sin embargo, para mamíferos, anfibios y reptiles sí es probable, siempre y cuando tengan los mecanismos fisiológicos y conductuales necesarios. Por ejemplo, el aumento de los glóbulos rojos y la hemoglobina en la sangre, así como proteínas que impidan la congelación de los tejidos.

Foto: Uwe Kils

Los peces del hielo (*Chaenocephalus aceratus*) carecen de pigmento respiratorio en su sangre; esta es incolora o transparente.

Ahora que hemos platicado de la función de la sangre, así como de sus variaciones de color, debo decir que no se puede afirmar que un tipo de sangre sea mejor que otro. Simplemente, cada especie ha pasado por una serie de eventos de adaptación por selección natural que han moldeado a la especie de acuerdo con sus hábitos y biología en general. Los cefalópodos de sangre azul no son mejores organismos que los de sangre de otro color... y lo mismo se puede decir de los aristócratas en la especie humana, que otrora querían distinguirse por el supuesto color de su sangre. 👁

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