Universidad Autónoma del Estado de México

Facultad de Medicina Veterinaria y Zootecnia

Tesis

Respuesta morfofisiológica asociada al gradiente altitudinal en la lagartija *Sceloporus grammicus*

Que para obtener el grado de Maestría en Ciencias Agropecuarias y Recursos Naturales

Presenta:

Juan Carlos González Morales

Comité tutoral:

Director: Dr. Víctor Manuel Fajardo Guadarrama

Tutores: Dr. Aníbal Helios Díaz de la Vega Pérez

Dra. Erendira Quintana Sánchez

El Cerrillo Piedras Blancas, Toluca, Estado de México. Octubre 2017

AGRADECIMIENTOS

A mis padres Juan y Mary por su apoyo incondicional en todo momento y aliento a ser mejor cada día.

A mis hermanos Mario, Fernanda y Vanesa por su apoyo y corajes provocados jajaja, a mi sobrina Romina por ser la nueva integrante de la familia.

A Jimena Rivera por todos los momentos en campo y por todos los momentos especiales que hemos compartido.

A Gabriel y Macotela por todas esas reuniones y discusiones, además de su apoyo incondicional.

A Adelaida, Jesus, Alejandro y el resto de compañeros con lo que he pasado grandes momentos.

Al Dr. Víctor Fajardo por darme la oportunidad de ser miembro de su laboratorio y asesorarme durante la maestría.

Al Dr. Anibal por aceptarme en su laboratorio y su apoyo durante todo la parte de termorregulación. A la Dr. Erendira y Dr. Palestina por sus consejos.

A CONACyT por la beca de estudios de posgrado.

ÍNDICE

RESUMEN

Los ambientes de altas elevaciones proporcionan terreno fértil para el estudio de posibles variaciones morfofisiológicas en vertebrados, esto debido a cambios en parámetros ambientales como bajas temperaturas y una menor disponibilidad de oxígeno, entre otras. En el caso de reptiles resulta especialmente interesante, ya que estos organismos al ser ectotermos no pueden producir calor corporal de forma autónoma, por lo que dependen de la temperatura ambiental en conjunto con estrategias morfológicas y conductuales para mantener su temperatura corporal en un rango funcional. *Sceloporus grammicus* es una especie de lagartija perteneciente a la familia Phrynosomatidae, presenta una amplia distribución altitudinal que supera los 4000 m. por lo que representa un buen modelo estudio para estudiar potenciales variaciones morfofisiológicas. El objetivo de este trabajo es comparar las características hematológicas y algunos rasgos de termorregulación en la lagartija *Sceloporus grammicus* en un gradiente altitudinal que abarca poblaciones a 2500, 3400 y 4300 m. En la parte hematológica se evaluó el conteo de eritrocitos, porcentaje de hematocrito, concentración de hemoglobina, área celular, electrolitos, presión de gases, lactato y glucosa; para termorregulación se tomó en cuanta la temperatura de selección, índices de termorregulación de Hertz, temperaturas críticas, velocidad de calentamiento y reflectancia de la coloración dorsal. Los resultados indican que los rasgos sanguíneos se comportan de forma asintótica, mientras que los valores de gasometría no muestran diferencias significativas. Los rasgos de termorregulación muestran que a pesar de los ambientes de alta elevaciones son menos adecuados para las lagartijas no muestran cambios en la temperatura de selección y velocidad de calentamiento, sin embargo la población media muestra mayor resistencia a bajas y altas temperaturas, por otra parte la reflectancia de la coloración dorsal es menor a bajas temperaturas y altas elevaciones, por lo que la población más alta es más oscura. En conclusión *Sceloporus grammicus* presenta cambios morfofisiológicos para mantener la homeostasis en respuesta a cambios en las condiciones ambientales de altas elevaciones.

ABSTRACT

High altitude vertebrates exhibit morphological and physiological adaptation to survive at less avalibility oxygen and low temperature. Reptiles not producing autonomus heat so that generally obteined heat from environment throught morphological and behavioral strategyes and consecuently are called ectotherms. Sceloporus grammicus is a lizard of generus Phrynosomatidae and has a large altitudinal range, including populations from above 4000 m so that a good model study of potential morphophysiological variation. This work has objetive comparing hematological characteristics and thermal traits along altitudinal gradient in populations of Sceloporus grammicus at 2500, 3400 and 4300 m. Hematological measured was erythrocyte count, erythrocytr size, hematocrit, hemoglobin concentration, mean corpuscular hemoglobin concentration, cell volumen, pressures gases, lactate and glucose; for thermoregulation was temperatura selection, Hertz index, critical temperatura, heat rate and reflectance of dorsal coloration. Results showed that hematological traits differ significantly between population and had a siginificant nonlinear relationship with altitude. Values of gasometry not showed differences in any of these variables among population. Some termal traits as temperatura selection and heat rate not differ among population or altitudes although the thermal environment is worse with the elevation. On other hand middle population showed more resistense at temperature critique and minor dorsal refflection. In conclusion *Sceloporus grammicus* present morphophysiological changes that has been propouse to manteined homestatic conditions.

I. INTRODUCCIÓN

Los ambientes de gran altitud proporcionan terreno fértil para el estudio de potenciales adaptaciones fisiológicas. Para los vertebrados que residen en ambientes de gran altitud la hipoxia representa un factor de estrés que generalmente va acompañado de bajas temperaturas ambientales relativas con respecto a entornos de tierras bajas en latitudes similares, por lo que los organismos tienen el reto de mantener la producción de calor, a pesar de la disminución de la disponibilidad de O_2 y de la temperatura ambiental (Storz 2010).

Los vertebrados pueden ser clasificados de acuerdo a la forma en que producen y mantienen su temperatura corporal en dos grandes grupos: ectotermos y endotermos, los cuales presentan tres grandes diferencias entre sí: 1) Los ectotermos adquieren su calor corporal por medio de la exposición a fuentes calóricas del ambiente, por el contrario los endotermos producen su calor corporal mediante procesos metabólicos que implican transformar el alimento ingerido en calor, por lo que los costos tróficos son más altos para estos últimos. 2) Cuando la temperatura ambiental disminuye los ectotermos disminuyen su temperatura corporal y con ello su metabolismo, por ejemplo, durante la noche los ectotérmos utilizan solo una tercera parte de la energía que utilizan durante el día, sin embargo para la mayoría de los endotermos la disminución de la temperatura en la noche, juega un papel inverso, debido a que están obligados a mantener una temperatura corporal constante, por lo que se ven en la necesidad de aumentar su producción de calor. 3) La necesidad de mantener la temperatura corporal hace que un organismos endotermo invierta más tiempo forrajeando y de esta manera consumir suficiente alimento para mantener su temperatura corporal, en el caso de los ectotermos invierten una menor cantidad de tiempo y necesiten menor cantidad de alimento (ya que la temperatura la modulan en función del ambiente), por lo que ese tiempo extra lo invierten en reproducción y fertilidad (Pough 1980, Pough et al. 2001)

A pesar de que los organismos ectotermos presentan varias ventajas sobre los endotermos, existen importantes costos asociados con la termorregulación ectotermica, entre la más prominente esta la limitación impuesta por las temperaturas ambientales a la posibilidad de aumentar su distribución espacial (en latitud y/o altitud), lo contrario a lo que sucede con la regulación interna de los endotermos que les permite distribuirse casi ilimitadamente en el espacio, lo cual explica la existencia de endotermos en ecosistemas donde la temperatura ambiental es sumamente baja durante todo el año como en las zonas polares (Pough 1980, Pough et al. 2001), sin embargo a partir de que Hock sugirió que la hipoxia no era un factor que limitara la distribución de los reptiles en ambientes de alta altitud se han realizado una gran cantidad de estudios tratando de entender que cambios presentan estos organismos para sobrevivir en estos ambientes (ver Snyder y Weathers 1997).

Partiendo del hecho de que el oxígeno es esencial para mantener la vida tanto de animales ectotermos como endotermos, ambos deben presentar una serie de mecanismos que les permita aclimatarse o adaptarse a ambientes con hipoxia. En ectotermos estas adaptaciones incluyen una gran variedad de cambios morfofisiológicos en el sistema cardiovascular y respiratorio (Bouverot 1985), así como ajustes en la termorregulación y en la tasa metabólica (Storz y Moriyama 2008).

II. ANTECEDENTES

II.I. Respuesta hemática

Con la excepción de algunas especies de peces que habitan en aguas árticas, en la mayoría de los vertebrados el oxígeno es transportado desde las estructuras de intercambio gaseoso (pulmones, branquias y/o piel) en la hemoglobina presente en los glóbulos rojos (Hawkey, 1991).

Los datos hemáticos de una gran variedad de vertebrados residentes de altas y bajas elevaciones muestran cambios representados en una gran variedad de estrategias de aumento o disminución de varios rasgos como en el conteo de eritrocitos (*Erc* por sus siglas en inglés "Erythrocyte count"), porcentaje de hematocitro (*Hct*), concentración de hemoglobina (*[Hb]*) y recientemente en el área de los eritrocitos (*Ers* por sus siglas en inglés "Erythrocyte size"), sin embargo, estos ajustes hemáticos no son uniformes en todas las clases de vertebrados (Ruíz et al. 19993), por ejemplo, en mamíferos y aves (salvo pocas excepciones) la respuesta hemática consiste en aumentar los valores hemáticos en relación a la altitud; en anfibios la respuesta hemática es inversa, varios valores como *Erc* aumentan pero *Ers* disminuye (Ruíz et al. 1989); en el caso de los reptiles es difícil establecer un patrón claro, ya que los datos disponibles no son suficientes para establecer una idea concisa (González-Morales et al. 2015).

En 27 especies del genero *Liolaemus*, ampliamente distribuido en América del Sur la altitud no muestra relación alguna con las características hemáticas de los animales, incluso en varias especies en las que se compararon a individuos presentes en baja y alta elevación no fue posible identificar cambios hemáticos (Ruíz et al. 1993).

Por otra parte, en ciertas especies del género *Sceloporus* se ha establecido que la altitud si influye en ciertas características hemáticas, por ejemplo en *S. occidentalis* y *S. jarrovi* se registraron valores más altos en Erc y Hct respecto a poblaciones de las mismas especies a baja elevación (Vinegar y Hillyard 1972), recientemente González-Morales et al. (2015) mostraron que en la lagartija *S. torquatus* los rasgos hemáticos variaban en diversas estrategias dependiendo de la altitud a la cual se encontraba la población a lo largo de un gradiente altitudinal (1600, 1727, 2500, 2727 m).

He et al. (2013) realizó un estudio experimental con la lagartija *Phrynocephalus vanglalii*, el cual consistió en describir los rasgos hemáticos de organismos de alta y baja elevación, después sometió a un grupo de lagartijas de baja elevación a condiciones de hipoxia en cámaras hiperbáricas igualando las condiciones a la que estaba sometida de forma natural la población de alta elevación, los resultados mostraron que las lagartijas aumentaron los valores en sangre hasta igualar a las lagartijas nativas de la zona alta, lo que indica que efectivamente se trataba de un proceso de plasticidad fenotípica.

Todo esto ha llevado a suponer que probablemente la respuesta hemática sea exclusiva del genero Sceloporus y de *Phrynocephalus vlangalii* (González-Morales et al. 2015).

II.II. Termorregulación

La temperatura es clave en la biología de los reptiles debido a que esta influye en gran medida en diversos rasgos de historias de vida como el crecimiento y la reproducción (Pough et al. 2001). Al ser ectotermos y no poder generan de forma intrínseca calor corporal hacen uso de estrategias morfológicas y conductuales para alcanzar y mantener temperaturas corporales en un rango constante y de esta manera realizar sus actividades biológicas, tales como cortejar, copular y forrajear (Bartholomew 1977; Hertz et al. 1993).

La interacción entre lagartijas y su ambiente termal proporcionan un terreno fértil para el estudio de posibles variaciones fisiológicas y conductuales que les permita sobrevivir en zonas de altitudes elevadas y los cambios ambientales que están presentan, entre otras la prevención de la formación del congelamiento de fluidos corporales y cambios metabólicos (McConnachie et al. 2007).

Se han establecido dos grandes vertientes en cuanto a la biología térmica de lacertilios en ambientes heterogéneos (como gradientes de altitud), la primera fue propuesta por Adolph y Porter (1993) y establece que los rasgos térmicos pueden ser adaptativos al ambiente, es decir que se pueden modificar por efecto de la estacionalidad, latitud y altitud, tal es el caso de la lagartija *Iberalocerta galani*, en la que la temperatura de selección es alta en primavera y baja en verano con la finalidad de minimizar los costos de termorregulación (Ortega et al. 2016); la segunda vertiente menciona que la biología termal es conservativa, es decir que no se modifica por efecto de la estacional, latitud y altitud, por lo que ajustes conductuales son suficientes para mitigar los cambios ambientales (Crowley 1985), por ejemplo *Sceloporus undulatus* en la que la velocidad de sprint y las temperaturas críticas no cambian en dos poblaciones de diferente altitud.

Para el género *Sceloporus* los datos actuales se ajustan de mejor manera a la visión estática, ya que la temperatura corporal y de actividad no se modifica por la altitud y latitud (Andrews 1998), esto ha llevado a la idea de que mantener este tipo de termorregulación en ambientes térmicamente desfavorables representa un costo bastante alto, por lo que las lagartijas que habitan en estos lugares deben de presentar una serie de compensaciones a diversos niveles de organización biológicos para sobrevivir y reproducirse en estos lugares (Lemos-Espinal 1995).

Recientemente la coloración dorsal se ha añadido como una variable más a considerar en la biología térmica de las lagartijas, se han establecido dos hipótesis no excluyentes sobre la función de la coloración dorsal, la primera establece que a altitudes y latitudes mayores los organismos se vuelven más negros para aumentar la velocidad de calentamiento y reducir los tiempos de termorregulación (hipótesis del melanismo térmico), sin embargo en ninguna especie de lagartija en la que se ha evaluado se ha encontrado esta relación, la cual si está presente en mamíferos (Clusella-Trullas et al. 2007). La segunda hipótesis establece que a altitudes mayores los organismos negros tendrían la ventaja de protegerse de la alta radiación UV por efecto del adelgazamiento de la atmosfera, y así evitar daños en el ADN y minimizar el estrés oxidativo a nivel celular (Matthews et al.

2016). Actualmente la hipótesis de protección UV tiene más sustento en lacertilios como se ha reportado recientemente en la lagartija *Psammodromus alguirus* (Reguera et al. 2015).

III. JUSTIFICACIÓN

Los gradientes altitudinales representan una oportunidad única para realizar estudios de variación morfofisiológicas con perspectivas ecológicas y evolutivas, más aun cuando se trata de la misma especie con presencia en todo el gradiente altitudinal debido a que condiciones como la temperatura y disponibilidad de oxígeno son menores a mayor altitudinal. Para especies que no pueden producir calor de forma autónoma como los reptiles, los ambientes de altas elevaciones representan un reto constante de supervivencia.

Sceloporus grammicus es una especie de lacertilio que se distribuye en gran parte de México, superando los 4000 m de altura, por lo que representa un buen modelo de estudio que nos ofrece la posibilidad de realizar estudios a diversos niveles estructurales.

El presente estudio pretende describir por primera vez algunos rasgos morfofisiológicas en la lagartija *Sceloporus grammicus*, así como la comparación de los mismos rasgos en poblaciones de diferentes elevaciones. Además pretende contribuir al conocimiento de las potenciales estrategias fisiológicas que permite a esta especie habitar zonas de elevaciones extremas.

IV. Objetivo

IV.I. General:

Comparar algunos rasgos morfofisiológicos de la lagartija *Sceloporus grammicus* a lo largo de un gradiente altitudinal

IV.II. Particulares

En *Sceloporus grammicus* provenientes de diferentes altitudes describir y comparar:

- Las características hemáticas y de gasometría, además conocer su relación con la altitud
- Algunos rasgos de la biología térmica y la coloración dorsal en función de la altitud y la temperatura corporal y ambiental.

V. Hipótesis

Si las características morfofisiológicas evaluadas se ven modificadas por efecto de la altitud, entonces *Sceloporus grammicus* presentará variaciones en las poblaciones presentes en un gradiente altitudinal

VI. Predicciones

- ➢ Hematología: Los valores hemáticos aumentaran con la altitud y se relacionaran positivamente con ella
- ➢ Termorregulación: Los rasgos evaluados serán termalmente conservativos, solo la coloración dorsal se verá modificada por la altitud y la temperatura

VII. Método

VII.I. Zona de estudio

Se estableció un gradiente con tres poblaciones suficientemente alejadas para evitar el flujo génico (Sears 2005), la primera fue en el Municipio de Texcalyacac, Estado de México la cual se encuentra a una altitud de 2500 m (Zona baja); la segunda se ubicó en la Las Lágrimas, Estado de México la cual se encuentra a 3400 m (Zona media); por último se usó una población de la ladera este del cráter del Nevado de Toluca, Estado de México que se encuentra a 4300 m (Zona alta) (Fig. 1).

Para cada uno de los estudios se emplearon tamaños de muestras diferentes, para la parte hematológica se emplearon 10 organismos de cada altitud, para Termorregulación se emplearon 30 organismos de cada zona, en todos los casos la proporción de machos y hembras fue la misma, además solo se usaron organismos adultos y no grávidos. La forma de captura consistió en el uso de cañas de pescar.

Fig. 1. Zonas de studio de *Sceloporus grammicus*. Zona baja (a), zona medía (b) y zona alta (c)

VII.II. Descripción de la especie, ¿por qué utilizar como modelo de estudio a *Sceloporus grammicus*?

Sceloporus grammicus es un complejo de especies que se distribuye desde Texas hasta la parte sur de México, ocupando una gran variedad de hábitats como zonas desérticas y montañas, además está presente en un rango altitudinal que va desde el nivel del mar hasta los 4600 m, aunque en México es poco frecuente en zonas menores a los 2500 m. Se han registrado 8 razas por lo que la variación cariotipica es alta, igual que la variación en rasgos de historia de vida, uso del hábitat y morfología (Ramírez-Bautista et al. 2004; 2012; Bastiaans et al. 2013).

Esta variación fenotípica y rango de distribución altitudinal hacen de *S. grammicus* un excelente modelo para realizar estudios de variaciones morfofisiológicas.

Fig. 2. Mapa de distribución de *Sceloporus grammicus* y fotografía de un organismo de la zona media (foto cortesia de Luis Macotela Colín)

VII.III. Hematología

Debido a que la cantidad de sangre que se puede obtener de cada lagartija sin sacrificarse no era suficiente para realizar todos los análisis hemáticos se optó por realizar dos muestreos en cada población. Para el primer grupo se tomaron 0.3 ml de sangre, de esta muestra se obtuvo el conteo de eritrocitos, porcentaje de hematocrito, concentración de hemoglobina y área de los eritrocitos. Con el segundo grupo se obtuvieron 0.1 ml, de este grupo se obtuvo mediante gasometría pH, presión de oxígeno y dióxido de carbono, carbonatos, electrolitos, glucosa y lactato. Todas las muestras fueron obtenidas mediante punción cardiaca y conservada en heparina hasta su traslado al laboratorio de Ecofisiología de Fauna Silvestre en la Facultad de Medicina Veterinaria y Zootecnia de la Universidad Autónoma del Estado de México. Las lagartijas también fueron transportadas al laboratorio durante un periodo de 3 días en los cuales se alimentaron e hidrataron *ad libitum* hasta su posterior liberación en la zona de muestreo.

El conteo de glóbulos rojos se hizo médiate el uso de cámaras de Neubauer. Para el área celular (Rbs) primero se realizaron frotis sanguíneos con tinción de Write, después se tomaron fotografías en el microscopio a 100x de zonas aleatorias y se analizaron con el software Sigma Scan 5 (González-Morales et al. 2015). El hematocrito (Hct) se determinó como el porcentaje de paquete celular, se obtuvo centrifugando la muestra a14890 g por 5 minutos, finalmente la hemoglobina se calculó mediante la técnica de cianometahemoglobina.

VII.IV. Termorregulación

VII.IV.I. Temperaturas operativas (*To*)

En cada una de las zonas de estudios se colocaron sensores de temperatura (Hobo were) conectados a modelos biofísicos que simulaban en tamaño y color a las lagartijas, estos modelos son representativos de las temperaturas corporales que experimentan las lagartijas en caso de que no termorregularan de forma activa, además muestran las temperaturas disponibles durante el periodo de actividad de cada una de las poblaciones (Hertz et al. 1993). Los modelos registraron las temperaturas durante un año (2016-2017)

VII.IV.II. Temperaturas de selección (*Tsel*)

Todos los organismos fueron capturados usando caña de pescar y solo fueron considerados si el tiempo de captura no era mayor a 15 minutos. Inmediatamente después de la captura se tomó la temperatura corporal (*Tc*), la temperatura del sustrato (*Ts*) y la temperatura del aire (*Ta*) del lugar en el que fue vista por primera vez la lagartija; todos los organismos capturados se transportaron al laboratorio de Fisiología de la Conducta en la Universidad Autónoma del Estado de México en sacos de manta. En el Laboratorio se colocaron en un gradiente térmico, el cual consiste en una caja de policarbonato con las siguientes dimensiones: 150 x 150 x 70 (Lara-Resendiz y Díaz de la Vega-Pérez 2013). Se colocaron focos de 120 W que daban como resultado un gradiente térmico de 20 a 50 °C, en este gradiente las lagartijas eran libres de elegir la temperatura que desearan. Se tomó la *Tc* cada 2 horas y solo consideran los datos del cuartil 25% al 75% para obtener *Ts.*

VII.IV.III. Indices de Termorregulación de Hertz y Blouin-Demers

Se calcularon los índices de termorregulación por medio del protocolo propuesto por Hertz et al. (1993) mediante el uso de 3 índices biofísicos: Calidad térmica del hábitat (*de*), precisión (*db*) y eficiencia (*E*), estos índices se calculan a partir de la *Tc*, *Tsel* y las *To*. Si la *Tc* o *To* < *Tsel*, entonces *db* = *Tsel* inferior-*Tc* y *de* = *Tsel* inferior-*To* y si la *Tc* o *To* > *Tsel* entonces *db*= *Tc* o *To* - *Tsel* superior y *de* = *To*-*Tsel* superior. Cuando la *Tc* o *To* está dentro del intervalo *Tsel* se considera *db* y *de* igual a 0. Valores altos de *db* y *de* indican precisión y calidad térmica baja, un valor igual o cercano 0 significa que los organismos son altamente precisos y representa ambientes térmicamente óptimos. Posteriormente se calcula el índice *E* con la ecuación E=1-(*db*/*de*), un valor cercano a 1 refiere termorregulación activa, si es igual o cercano a 0 es termorregulación pasiva. Finalmente el índice Blouin-Demers (Blouin-Demers y Nadeau 2005) se calcula mediante la ecuación *de* – *db*, el resultado son los grados de esfuerzo por parte de un organismos para llegar a *Tsel.*

VII.IV.IV. Velocidad de calentamiento

Para medir la velocidad de calentamiento cada lagartija era fijada a una tabla de madera con cinta adhesiva para evitar contribuciones posturales en la obtención de calor (Gvozdík 2002), después eran enfriadas a 19 °C y se les colocaba una termocupla tipo K conectada a un termómetro FLUKE 52 II, finalmente se colocaban debajo de un foco de 100 W suspendido a 20 cm. Cuando *Tc* alcanzaba 20 °C se registraba el tiempo que tardaba en llegar a 32 °C que es la *Tsel* reportada por Lara-Resendiz y Díaz de la Vega-Pérez (2013). Todas las mediciones se realizaron en un cuarto con la temperatura controlada a 20 °C (para más detalle de la metodología ver Gvozdik 1999).

VII.IV.V. Temperatura crítica mínima (*CTMin*) y máxima (*CTMax*)

La temperatura critica es considera como la temperatura a la cual el organismos pierde control sobre la locomoción y son incapaces de retomar la postura cuando se colocan en posición dorsal (Cowles y Bogert 1944). Para obtener *CTMin* se disminuyó gradualmente la temperatura con hielo depositado alrededor de una pecera sin que tuviera contacto directo con las lagartijas; para obtener *CTMax* se colocó un foco de 150 W suspendido a 30 cm de una pecera, cada cierto tiempo se colacaba a los organismos en posición dorsal y cuando fueron incapaces de retomar la postura se les tomó la *Tc*.

VII.IV.VI. Coloración dorsal por espectrofotometría

La reflectancia (*R*) de la zona dorsal fue medida de 300-700 nm usando un espectrofotómetro Jaz (Ocean Optics) debido a que esta zona es importante para la absorción de calor en la familia Squamata (Clusella-Trullas et al. 2007). Las lagartijas eran colocadas en peceras y las mediciones se realizaron a 10, 20 y 30 °C. Para cada lagartija se tomaron tres mediciones, en la parte inicial, media y final de la zona dorsal, siempre en la misma posición para todos los animales. *R* era calculada a partir de un blanco estándar, las datos crudos eran importadas al programa CLR-files para calcular *R* cada 20 nm y obtener un promedio general para cada lagartija (modificado de Matthews et al. 2016).

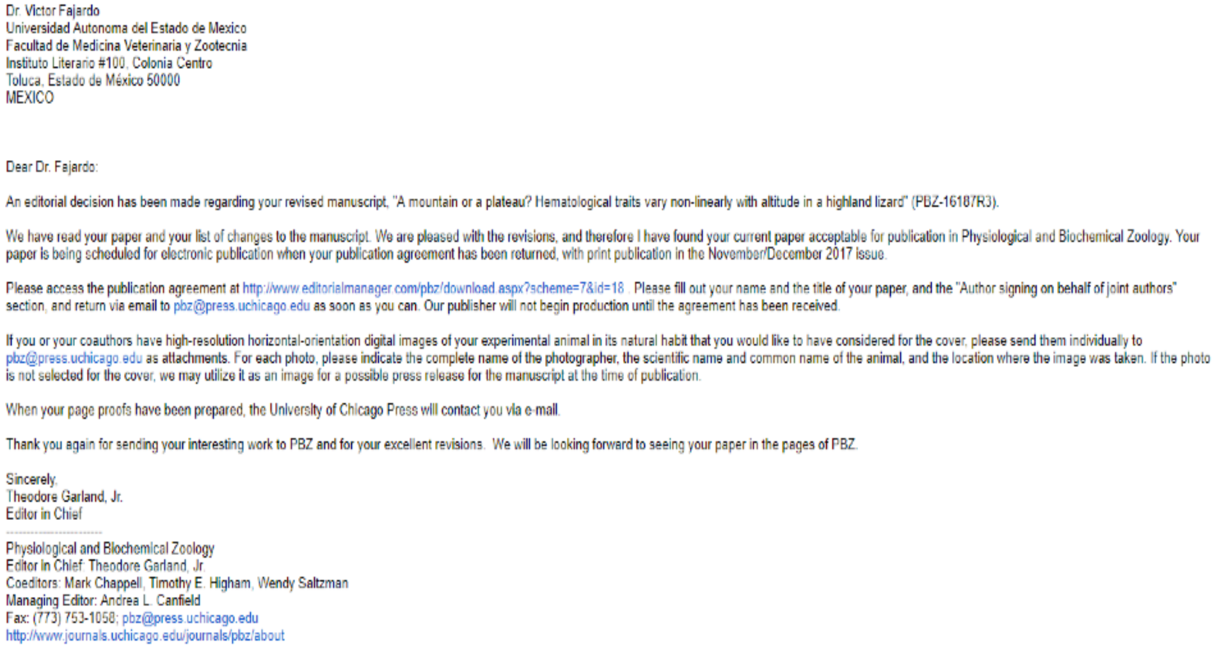
RESULTADOS

Capitulo I: Hematología

Aceptado para publicarse en Physiological and Biochemical Zoology

A mountain or a plateau? Hematological traits vary non-linearly with altitude in a highland lizard

González-Morales, J.C., Beamonte-Barrientos, R., Bastiaans E., Guevara.Fiore P. and V. Fajardo.



Dr. Victor Fajardo
Universidad Autonoma del Estado de Mexico
Facultad de Medicina Veterinaria y Zootecnia
Instituto Literario #100, Colonia Centro
Toluca, Estado de México 50000
MEXICO

Dear Dr. Fajardo:

An editorial decision has been made regarding your revised manuscript, "A mountain or a plateau? Hematological traits vary non-linearly with altitude in a highland lizard" (PBZ-16187R3).

We have read your paper and your list of changes to the manuscript. We are pleased with the revisions, and therefore I have found your current paper acceptable for publication in Physiological and Biochemical Zoology. Your paper is being scheduled for electronic publication when your publication agreement has been returned, with print publication in the November/December 2017 issue.

Please access the publication agreement at http://www.editorialmanager.com/pbz/download.aspx?scheme=7&id=18 . Please fill out your name and the title of your paper, and the "Author signing on behalf of joint authors" section, and return via email to pbz@press.uchicago.edu as soon as you can. Our publisher will not begin production until the agreement has been received.

If you or your coauthors have high-resolution horizontal-orientation digital images of your experimental animal in its natural habit that you would like to have considered for the cover, please send them individually to pbz@press.uchicago.edu as attachments. For each photo, please indicate the complete name of the photographer, the scientific name and common name of the animal, and the location where the image was taken. If the photo is not selected for the cover, we may utilize it as an image for a possible press release for the manuscript at the time of publication.

When your page proofs have been prepared, the University of Chicago Press will contact you via e-mail.

Thank you again for sending your interesting work to PBZ and for your excellent revisions. We will be looking forward to seeing your paper in the pages of PBZ.

Sincerely,
Theodore Garland, Jr.
Editor in Chief

Physiological and Biochemical Zoology
Editor in Chief: Theodore Garland, Jr.
Coeditors: Mark Chappell, Timothy E. Higham, Wendy Saltzman
Managing Editor: Andrea L. Canfield
Fax: (773) 753-1058; pbz@press.uchicago.edu
http://www.journals.uchicago.edu/journals/pbz/about

A mountain or a plateau? Hematological traits vary non-linearly with altitude in a highland lizard

Hematological traits in *Sceloporus grammicus*

Juan Carlos González-Morales[1], Rene Beamonte-Barrientos[2], Elizabeth Bastiaans[3], Palestina Guevara-Fiore[4], Erendira Quintana[5] and Victor Fajardo[6,*]

[1]Maestría en Ciencias Agropecuarias y Recursos Naturales. Universidad Autónoma del Estado de México. Instituto Literario No. 100, Col. Centro, Toluca, Estado de México, México.

[2]Centro Tlaxcala de Biología de la Conducta, Universidad Autónoma de Tlaxcala, Km. 1.5 Carretera Federal Tlaxacala-Puebla, Tlaxcala, Tlax., México.

[3]State University of New York College at Oneonta, 108 Ravine Parkway, Oneonta, NY 13820.

[4]Escuela de Biología, Benemerita Universidad Autónoma de Puebla, Bulevard Valsequillo y Avenida San Claudio, Edificio 112-A, Ciudad Universitaria, Puebla, México.

[5]Centro Universitario Amecameca, Universidad Autónoma del Estado de México. Instituto Literario No. 100, Col. Centro, Toluca, Estado de México, México

[6,*]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México. Instituto Literario No. 100, Col. Centro, Toluca, Estado de México, México.
Email: fajardo.vic@gmail.com

Highlights

Hematological changes resulting from high altitude environments are controversial in reptiles. Recently a correlation has been shown between hematologic parameters and altitude in lizards of the genera *Sceloporus* and *Phrynocephalus*.

This study shows that there is a quadratic correlation between several hematological parameters and altitude and a limit to the increase in erythrocyte count, hematocrit, hemoglobine concentration and erythrocyte size, but serum electrilites, glucose and lactate not showed response with altitude in *Sceloporus grammicus*.

A mountain or a plateau? Hematological traits vary non-linearly with altitude in a highland lizard

Abstract

High-altitude organisms exhibit hematological adaptations to augment blood transport of oxygen. One common mechanism is through increased values of blood traits such as erythrocyte count, hematocrit, and hemoglobin concentration. However, a positive relationship between altitude and blood traits is not observed in all high-altitude systems. To understand how organisms adapt to high altitudes, it is important to document physiological patterns related to hypoxia gradients from a greater variety of species. Here, we present an extensive hematological description for three populations of *Sceloporus grammicus* living at 2500, 3400 and 4300 m. We did not find a linear increase with altitude for any of the blood traits we measured. Instead, we found non-linear relationships between altitude and the blood traits erythrocyte number, erythrocyte size, hematocrit and hemoglobin concentration. Erythrocyte number and hematocrit leveled off as altitude increased, whereas hemoglobin concentration and erythrocyte size were highest at intermediate altitude. Additionally, lizards from our three study populations are similar in blood pH, serum electrolytes, glucose and lactate. Given that the highest altitude population did not show the highest levels of the variables we measured, we suggest these lizards may be using different adaptations to cope with hypoxia than lizards at low or intermediate altitudes. We discuss future directions that research could take to investigate such potential adaptations.

Keywords: high altitude, hypoxia, hematology, lizard, physiological constraints.

Introduction

All vertebrates require oxygen to survive, but high altitude vertebrates are challenged by hypoxia. Highland vertebrates thus usually have morphological and physiological adaptations to ensure sufficient oxygen is delivered to their cells. In some taxa, these adaptations involve changes in gas exchange surfaces, such as increased alveolar air space in mice (Monge and Leon-Velarde 1991) and thinner skin in amphibians (Hutchison et al. 1976, 1982). Lizards, however, typically adapt to hypoxia through changes to the cardiovascular system, such as increased hemoglobin concentration, hematocrit, or blood capillary density (He et al. 2013; González-Morales et al. 2015). Increased hematocrit at high altitude is also observed in mammals and birds (Monge and Leon-Velarde 1991; Weber 1995; Samaja et al. 2003).

Most previous work has been based on the assumption that increased concentrations of blood components should increase oxygen transport and thus be common in animals living in hypoxic environments (Vinegar and Hillyard 1972; Bennett and Ruben 1975; Ruiz et al. 1983; González-Morales et al. 2015). In support of this hypothesis, some ectotherms do show positive, linear relationships between altitude and blood traits such as erythrocyte count, hematocrit, and hemoglobin concentration. For example, western fence lizards (*Sceloporus occidentalis*) at higher altitudes have higher erythrocyte counts and greater hematocrit (Weathers and White 1972). Similarly, the congeneric Mexican lizard *S. torquatus* shows a positive relationship between altitude and hematocrit, hemoglobin concentration, erythrocyte count, and erythrocyte size in populations from approximately 1600 to 2700 m (González-Morales et al. 2015). This pattern is not unique to sceloporine lizards, as the Asian lizard *Phrynocephalus vlangalii* also showed a positive relationship between altitude and hematocrit, hemoglobin concentration, and mean corpuscular hemoglobin concentration (He et al. 2013).

In contrast to the linear increases observed in *S. occidentalis*, *S. torquatus*, and *P. vlangalii*, other lizards exhibit little or no correlation between blood traits and altitude (Weathers and White 1972; Snyder and Weathers 1977; Hillyard 1980). For example, a study of hematocrit and hemoglobin concentration in 27 South American lizards living at altitudes from sea level to 4600 m showed no correlation between these blood traits and altitude (Ruiz et al. 1993). Nonlinear correlations between blood traits and altitude have also been observed. For example, *Sceloporus jarrovi* from 1828 m had greater erythrocyte counts and hemoglobin concentration than those from 1554 m, but there was no difference between lizards from 1828 and 2621 m (Vinegar and Hillyard 1972). Thus, there appears to be no universal pattern in the relationship between altitude and blood traits. This lack of clarity results partly from variation in these relationships among even closely related species. However, a lack of studies of populations living in the most extreme high altitude environments (i.e., more than 3000 m) may also contribute to this uncertainty, because the values of blood traits may appear to increase linearly when comparing populations from low and intermediate altitudes but level off or decrease near the upper limit of a species' altitudinal range.

It appears likely that greater values of blood traits at high altitude are adaptive in at least some lizards, although it is not clear whether the observed differences result from genetic differences or phenotypic plasticity (Garland and Adolph 1991; He et al. 2013; González-Morales et al. 2015). In cases where blood traits level off or decrease at high altitudes, alternative mechanisms for oxygen delivery may be at play. One alternative mechanism to ensure delivery of oxygen to cells is by changes in blood traits that could enhance the amount of oxygen binding to hemoglobin, such as an increase in erythrocyte size and its consequent increase in hemoglobin concentration (González-Morales et al. 2015). Increased erythrocyte size in hypoxic conditions has been described for chicken embryos and lizards (Tazawa et al. 2012; He et al. 2013; González-Morales et al. 2015).

Further data from lizard populations at different altitudes, particularly those at extremely high altitudes, are critical to our understanding of hematological adaptations to hypoxia and the trade-offs that shape them. Here, we present hematological data from the highland lizard *Sceloporus grammicus* at three different altitudes, including one population from above 4000 m. *Sceloporus grammicus* is a species complex of small lizards 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 large altitudinal range, including populations from above 4600 m in central Mexico (Lemos-Espinal 1992; Bastiaans et al. 2014). Our study compares individuals from populations at 2500, 3400, and 4300 m. We measured erythrocyte count, erythrocyte size, hematocrit, hemoglobin concentration, mean corpuscular hemoglobin concentration (MCHC), cell volume, pH, serum electrolytes, glucose, and lactate. We measured a large number of blood traits because the relationships between all these variables are not clear. In addition, we do not know how these variables and their interrelationships may vary with altitude. A positive relationship between hemoglobin concentration and erythrocyte size and a negative relationship between erythrocyte size and erythrocyte number have been observed in many vertebrates, but these relationships may change under hypoxia (Hawkey et al. 1991). Furthermore, most of what we know about blood traits and their relationships is based on measurements done on single populations, rather than those that compare individuals of the same species living at different altitudes (Dessauer 1970; Garland and Adolph 1991).

We predicted that *S. grammicus* would show a pattern similar to what was observed in the closely related species *S. jarrovi*, in which blood traits initially increased with altitude but leveled off near the upper limit of the species' range (Vinegar and Hillyard 1972). Additionally, we predicted that serum electrolytes, glucose and lactate would correlate positively with altitude because it has been suggested that such an increase is adaptive under hypoxia (see Jackson 2007) and a positive correlation was observed in *S. torquatus* from 1500 to 2714 m (Gonzalez-Morales et al. 2015).

Methods

Ethics statement

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

Field sites and animals

Our study populations were: 1) *Low altitude population*, located at Texcalyacac, Estado de México, 19°07′37″N, 99°29′41″W, 2500 ± 1.64 m altitude; 2) *Middle altitude population*, located at Las Lágrimas, Estado de México, 19°06′82″N, 99°49′48″W, 3400 ± 2.69 m; and 3) *High altitude population*, located at Nevado de Toluca, Estado de México, 19°06′06″N, 99°46′03″W, 4300 ± 2.5 m. We recorded altitude at all capture sites with an Etrex Vista GPS (Garmin, Olathe, Kansas, USA). *Sceloporus grammicus* occurs at lower elevations elsewhere in its range (Bastiaans et al. 2014), but populations below 2500 m in central Mexico are usually at low density (JGM, VF, and EB, pers. obs.; Bastiaans 2013) and were therefore excluded. Our three populations are sufficiently far enough from each other to suggest gene flow between them is unlikely (Texcalyacac is 39.31 km and 27.75 km from Las Lágrimas and Nevado de Toluca respectively, and Las Lágrimas is 27.76 km from Nevado de Toluca; Sears 2005).

Because only one blood sample can be used for each analysis, we carried out two sample collections, one to measure blood traits, and the second one for a gasometry analysis (see below). For the first analysis (August-September 2015), we collected 10 adult lizards, five females and five males, from each of our three study populations (first batch of individuals). For the second analysis (August-September 2016) we collected 6 adult lizards, 3 females and 3 males (second batch of individuals). Individuals were not marked, so we cannot be certain some animals from the second batch had not previously been part of the first batch. In both cases, we captured animals by hand or noose and placed them in cages with lamps at 25°C. After 20-30 min of acclimation, we anesthetized the lizards using intramuscularly injected ketamine (70 mg/kg) to measure body mass, snout-vent length (SVL) and collect ca. 200 µL of blood by cardiac puncture into heparinized sterile tubes. We stored samples in a cooler and transported them and the lizards to the Laboratory of Behavioral Physiology at the Facultad de Medicina Veterinaria y Zootecnia, UAEM. For the second batch of lizards, the pH, serum electrolytes, glucose and lactate were measured at the capture site. Lizards were housed for three days to allow for full recovery, during which time we fed them with crickets (*Acheta domesticus*) and water *ad libitum*. After recovering for three days, lizards were released at their capture sites.

Blood traits

Using the blood samples collected from the first batch of individuals, we measured erythrocyte count, erythrocyte size, and hemoglobin concentration. We calculated mean corpuscular hemoglobin concentration and cell volume following the methodology used by González-Morales et al. (2015). We used a light microscope (model BX41TF; Olympus Corporation, Tokyo, Japan) to evaluate erythrocyte size, and a digital camera system with detail enhancement (model E-330; Olympus Corporation, Tokyo, Japan) to micro-photograph seven fields of blood smears stained with Wright's stain to reveal erythrocytes. A minimum of 70 haphazardly selected erythrocyte cells were measured per animal at each altitude, using the Sigma Scan Pro software version 4 for Windows (Systat Software Inc., San Jose, California, USA). We determined hematocrit (Hct) as a percentage of packed cell volume by centrifuging blood samples in a microhematocrit tube for 7 minutes at 14,890g. We determined hemoglobin concentration [Hb] as cyanmethemoglobin using the Hycel cyanmethemoglobin reagent and a Bausch and Lomb Spectronic 20 colorimeter (He et al. 2013).

Using blood samples from the second batch of individuals, we determined pH, serum electrolytes (Na^+, K^+ and Ca^{2+} (mmol/L), PO_2, PCO_2, and $CHCO_3$), glucose and lactate using an EPOC blood analysis system (ALERE company, USA) following the methodology used by González-Morales et al. (2015) and Ding et al. (2014).

Statistical analysis

We conducted our analysis using two approaches. First, we treated altitude as a categorical variable and compared the blood traits we measured among lizards from low, middle, and high altitude populations. Next, we treated altitude as a continuous variable and performed linear and nonlinear regression analyses with model comparison to identify the regression model providing the best explanation for the observed relationships.

We used a Kruskal-Wallis ANOVA by ranks test with a Dunn's post-hoc test to compare erythrocyte counts among populations, because this variable was not normally distributed. After testing to confirm normality, we used one-way ANOVA with Tukey-Kramer post-hoc tests to compare erythrocyte size, hematocrit, hemoglobin concentration, MCHC, volume cell, pH, serum electrolytes (PO_2, PCO_2, and $CHCO_3$), glucose and lactate. The significance of regressions between altitude and erythrocyte count, erythrocyte size, hematocrit, and hemoglobin concentration were tested using linear and quadratic models. After model comparison with Akaike´s Information Criterion, the most parsimonious model for explaining the variance was a non-lineal model: the quadratic regression. All tests were two-tailed, and we considered differences regressions to be significant when $p < 0.05$. We observed no differences by sex in any blood traits, so we pooled data from males and females in all analyses. We used JMP (version 12 for Windows, SAS Institute Inc., Cary, North Carolina, USA) for all statistical analysis. Descriptive statistics are given as mean ± SE.

Results

Morphology

Average SVL and mass for lizards from our three study populations are summarized in Table 1. *Sceloporus grammicus* from our high altitude study site had significantly lower SVL than lizards from the middle or low altitude study sites ($F_{2,27} = 4.0$, $P<0.05$). Lizards from our middle altitude study site had significantly greater body mass than lizards from our low altitude study site ($F_{2,27} = 4.2$, $P<0.05$). Lizards from the high altitude study site did not differ significantly in body mass from lizards from the low or middle altitude sites.

Blood traits

Erythrocyte count and hematocrit had similar patterns of variation among our three populations, with lizards from the low altitude population showing significantly lower values than lizards from the middle or high altitude populations (erythrocyte count: $H_{2,27} = 13.123$, $P<0.001$, Table 2; hematocrit: $F_{2,27} = 22.005$, $P<0.001$, Table 2). We found significant non-linear relationships (quadratic relationships) between erythrocyte count and altitude ($r^2 = 0.76$, $P<0.001$; Fig. 1) and between hematocrit and altitude ($r^2 = 0.67$, $P<0.001$; Fig. 2).

All three populations differed significantly from each other in average hemoglobin concentration values. Lizards from our middle altitude population had the highest hemoglobin concentrations, followed by those from the high altitude population. Lizards from our low altitude population had the lowest hemoglobin concentrations ($F_{2,27} = 43.99$, $P<0.001$; Table 2). Hemoglobin concentration was significantly non-linearly correlated with altitude ($r^2 = 0.80$, $P<0.001$; Fig. 3).

Erythrocyte size had a similar pattern of variation to hematocrit. Our middle altitude population had significantly higher average erythrocyte size than our low or high altitude populations, which did not differ significantly from each other (erythrocyte size: $F_{2,27} = 38.83$, $P<0.001$, Table 2). Erythrocyte size had a significant non-linear relationship with altitude (erythrocyte size: $r^2 = 0.80$, $P<0.001$, Fig. 4).

MCHC showed similar patterns as erythrocyte size and hematocrit ($P<0.05$). Cell volume did not differ significantly between the low and middle population, but the high population had lower average cell volume than the low or middle altitude populations ($P<0.05$)

Values of pH, serum electrolytes (PO_2, PCO_2, and $CHCO_3$), glucose and lactate are summarized in Table 3. There were no significant differences in any of these variables among populations.

Discussion

Reptiles are a diverse group (Dzal et al. 2015), nonetheless, in contrast with other taxa, comparative studies about reptiles' physiology are scarce. Furthermore, most of the hematological descriptions are done on only one population per species and thus do not consider the possibility of intraspecific variation in blood traits. We compared hematological traits among three populations of wild *Sceloporus grammicus* from different altitudes. Erythrocyte size and hematocrit increased significantly with altitude, but leveled off at extremely high altitude. In contrast, hemoglobin concentration and erythrocyte size were highest at intermediate altitude (3400 m) and lower at both high and low altitudes

(4300 m and 2500 m, respectively). Our medium and high altitude populations showed similar erythrocyte counts and hematocrit, yet the high altitude population had lower hemoglobin concentration in comparison to the middle altitude population; while the low and the high altitude populations had similar erythrocyte size but different hemoglobin concentration. Surprisingly, hematocrit did not show a significant decrease in the high population despite the low cell volume and erythrocyte size. The lack of a significant difference in this case may reflect a lack of statistical power due to our small sample size. We recommend collecting larger samples in future studies.

Similar patterns of variation in erythrocyte count and hematocrit occur in *S. jarrovi* (Table 2; Vinegar and Hillyard 1972). That is, lizards from the lower part of this species' altitudinal range had the lowest values of the measured blood traits, whereas lizards from the middle and upper parts of each species' altitudinal range had similar values. In contrast, a study of four populations of *S. torquatus* found that erythrocyte size, erythrocyte count, hemoglobin concentration, and hematocrit increased linearly with altitude (González-Morales et al. 2015). Increases in erythrocyte count and hematocrit are a common response to altitude in other vertebrate taxa (mammals: Monge and Leon-Velarde 1991; amphibians: Ruiz et al. 1983), but in reptiles the relationship appears to be more complicated.

Increased hemoglobin concentration at greater altitude has been reported in several species of the genus *Sceloporus*: *S. jarrovi*, *S. poinsetti, S. virgatus,* and *S. scalaris* (Vinegar and Hillyard 1972; Newlin and Ballinger 1976). Increased hematocrit and hemoglobin concentration with altitude have also been reported in a toad (*Bufo spinulosus*; Ruiz et al. 1989) and the yak (*Bos grunniens*; Ding et al. 2014). In contrast, there was no change in hemoglobin concentration with altitude in *Urosaurus ornatus*, a phrynosomatid lizard species closely related to the genus *Sceloporus* (Newlin and Ballinger 1976).
Hemoglobin concentration and erythrocyte size were highest in our middle altitude (3400 m) population (Table 2), even though the population living at 4300 m faces greater hypoxia. In addition, erythrocyte count and hematocrit were greater in our middle altitude population than our low altitude population, but did not continue to increase as we moved to our high altitude population (Table 2). These results support the idea that animals from extremely

high altitudes may use a different strategy to cope with hypoxia than animals from middle altitudes, possibly involving an increase in anaerobic metabolism or a decreased metabolic rate, as in the lizard *Sceloporus graciosus* (Sears 2005).

The major function of erythrocytes is to transport hemoglobin-bound O_2 from the lungs to all tissues of the body. Increasing blood transport of O_2 maintains O_2 availability in hypoxic environments (Storz et al. 2010). Hemoglobin's affinity for O_2 can be modified by adjusting intra-erythrocytic conditions and, accordingly, oxygen concentration. For example, ions can modify intraerythrocytic pH and improve hemoglobin's affinity for oxygen (for more details see Nikinmaa 1997; Bogdanova et al. 2009) and changes to cell size can increase the amount of hemoglobin (Hawkey et al. 1991).

On the other hand, the concentration of organic phosphates may decrease hemoglobin´s affinity for oxygen (Herman and Ingermann 1996; Petschow et al. 1997). In addition, amino acid residues located in heme proteins can modulate the oxygen-hemoglobin affinity. Altitudinal variation can produce hemoglobin polymorphism; these isoforms facilitate the uptake of oxygen in environments with low PO_2 (Storz 2007). More detailed studies are necessary to determine if our study species exhibits different hemoglobin isoforms.

Higher erythrocyte count and hematocrit can also increase O_2 transport (Vinegar and Hillyard 1972; Ruiz et al. 1983). However, high hematocrit achieved through an increased erythrocyte count or increased erythrocyte size is not necessarily associated with high attainable aerobic power output (Crowell et al. 1959; Crowell and Smith 1967; Villafuerte et al. 2004; Schuler et al. 2010). The associated increase in blood viscosity produces a higher peripheral vascular resistance that might compromise cardiac blood output, thereby reducing the rate of O_2 consumption by tissues (Guyton and Richardson 1961; Connes et al. 2006). Continued increase in erythrocyte count and hematocrit may not be beneficial at extreme altitudes, so selection should favor alternative strategies in reptiles living in these conditions, either through genetic differences or phenotypic plasticity.

Interestingly, *S. grammicus* from different altitudes had similar blood pH, serum electrolytes, glucose and lactate (Table 3). Similar physiological values between populations at different altitudes have also been observed in humans where this consistency has been proposed to arise as a result of homeostatic mechanisms adapted to hypoxic conditions (Simonson et al. 2010; Dempsey et al. 2015). Although humans from lower altitudes exhibit a transient increase in hemoglobin concentration when exposed to hypoxia at high altitudes, humans and other vertebrates that have evolved for many generations at high altitude do not show increased levels of hemoglobin concentration or many other blood traits (Simonson et al. 2010). This may be because the increased blood viscosity associated with high levels of hematocrit may increase the risk of health problems such as pulmonary edema. However, we expected to observe increased levels of red blood count, hematocrit and hemoglobin concentration with altitude, given than *S. torquatus* from medium altitudes (ca. 2700 m) showed a significant increase in these values in comparison to lower altitude populations (ca. 1600; González-Morales et al. 2015). The altitudinal gradient we tested for *S. grammicus* included higher elevations (ca. 2500-4300 m) and thus greater hypoxia. One of our future objectives is to analyze blood traits of individuals at lower altitudes (below < 2500 m) as they could be helpful in defining the physiological changes with altitude and contributing to hypotheses about the possible functional significance of these changes.

Finally, we also found that lizards had the greatest body mass per unit body length in our highest elevation population (Table 1). *Sceloporus occidentalis* populations also increase in body mass with altitude (Weathers and White 1972), but neither *S. torquatus* nor *Phrynocephalus vlangalii* populations vary in body mass with altitude (He et al. 2013; González-Morales et al. 2015). Higher body mass per unit body length may increase survival at extreme altitudes (Angilletta et al. 2004), and longer bodies may also be disadvantageous in matters of thermoregulation in cold environments (Pincherira-Donoso et al. 2008). Our results do not address the effects of body mass on fitness or whether the differences we observed result from genetic variation or phenotypic plasticity. However, at least one previous study (Lancaster et al. 2010) found that body shape (defined as the residuals of a regression of body mass vs. SVL) was heritable in another phrynosomatid lizard, *Uta stanburiana*, so it is possible that this trait has evolved in response to selection.

Further research is warranted into the question of how body mass varies with altitude across ectotherms, as well as what mechanisms underlie this phenotypic variation.

Although our experiment did not measure any evidence of selection, one possibility that could explain the data observed is that directional selection favoring increased blood traits under hypoxia could be constrained by physiological costs in *S. grammicus* experiencing extreme high altitudes. This pattern may explain why we observed asymptotes and nonlinear regression in the blood traits and altitude we measured (Figs. 1-4). Rather than increasing O_2 delivery via any of the mechanisms we tested, animals at extremely high altitudes may exhibit more anaerobic metabolism, relative to aerobic metabolism, than animals in low or mid-altitude populations. At extreme altitudes, it may not be possible for animals to further increase the capture and consumption of oxygen, but suppressing metabolic oxygen demand may be an effective strategy. However, oxygen pressure varies nonlinearly with altitude, such that the decrease in oxygen concentration with altitude at extremely high elevations is less drastic than at lower altitudes (Gamboa 1998). Future research into physiological adaptations to hypoxia should measure oxygen pressure directly, rather than relying on altitude as a proxy for reduced oxygen pressure.

These physiological adjustments may manifest at multiple biological levels of organization, from cardiopulmonary organ systems to the molecular level of oxidative metabolism (Hochachka 1986; Sears 2005; Storz and Moriyama 2008). Future studies should further investigate the metabolism of highland lizards, for example, using a respirometer. We also suggest common garden experiments to determine whether the differences we observed result from evolutionary adaptation or phenotypic plasticity.

Acknowledgments

We are grateful to the Consejo Nacional de Ciencia y Tecnología (grant 178723 and master degree scholarship JCGM 586443) for financial support and the Secretaría del Medio Ambiente y Recursos Naturales for the permits to collect animals (SGPA/DGVS/02407/15). We thank Jimena Rivera Rea and Jorge Mata for field and technical assistance and the Estación de Investigación Nevado de Toluca SEPANAF-CICBA for support. We also thank the two anonymous referees for their comments and suggestions, which significantly contributed to improving the quality of the publication.

Literature Cited

Angilletta Jr, M.J., P.H. Niewiarowski, A.E. Dunham, A.D. Leaché, and W.P. Porter. 2004. Bergmann´s Clines in Ectotherms: Illustrating a Life-History Perspective with Sceloporine Lizards. Am Nat 164: E168-E183.

Bastiaans E. 2013. Variation in Reproductive Behavior and Sexual Signals Within and Among Populations of an Incipiently Speciating Lizard. PhD Dissertation, University of California, Santa Cruz, CA, USA.

Bastiaans E., M.J. Bastiaans, G. Morinaga, J.G. Castañeda-Gaytan, J.C. Marshall, B. Bane, F. Méndez de la Cruz, and B. Sinervo. 2014. Female Preference for Sympatric vs Allopatric Male Throat Color Morphs in the Mesquite Lizard (*Sceloporus grammicus*) Species Complex. Plos ONE 9(4): e93197.

Bennett A.F. and J. Ruben. 1975. High altitude adaptation and anaerobiosis in sceloporine lizards. Comp Biochem Part A Physiol 50:105-108.

Bogdanova A., M. Berenbrink, and M. Nikinmaa. 2009. Oxygen-dependent ion transport in erythrocytes. Acta Physiol 195: 305-319.

Connes P., O. Yalgin, O. Baskert, J.E. Brun, and M. Hardeman. 2006. In health and in a normoxic environment, VO2 max is/is not limited primarily by cardiac output and and locomotor muscle blood flow. J Appl Physiol 100(6): 2099.

Crowell J.R., R.G. Ford, and V.M. Lewis. 1959. Oxygen transport in hemorrhagic shock as a function of the hematocrit radio. Am Physiol 196:1033-1038.

Crowell J.W. and E.E. Smith. 1967. Determinant of the optimal hematocrit. J Appl Physiol 22:501-504.

Dempsey J.A. and B.J. Morgan. 2015. Humans in hypoxia: a conspiracy of maladaptation?!. Physiology 30: 304-316.

Dessauer H.G. 1970. Blood chemistry of reptilians. *In* Biology of the Reptilia. *Edited by* C.Gans and T. Parson. Academic Press, New York. pp. 156-158.

Ding X.Z., C.N. Guo, X.Y. Wu, H.B. Wang, K.A. Johnson, and P. Yan. 2014. Physiological insight into the high-altitude adaptations in domesticated yaks (*Bos grunniens*) along the Qinghal-Tibetan Plateau altitudinal gradient. Livest Sci 162:233-239.

Dzal Y.A., S.E.M. Jenkin, S.L. Lague, M.N. Reichert, J.M. York, and M.E. Pamenter. 2015. Oxygen in demand: How oxygen has shaped vertebrate physiology. Comp Biochem Physiol A 186: 4-26.

Gamboa R. 1998. Cardiovascular physiology and physiopathology during the acute exposition at high altitude. Acta andin 7(1): 35-46.

Garland Jr T. and S.C. Adolph. 1991. Physiological differentiation of vertebrate populations. Ann Rev Ecol Syst 22: 193-228.

González-Morales J.C., E. Quintana, H. Díaz-Albiter, P. Guevara-Fiore, and V. Fajardo. 2015. Is the erythrocyte size a strategy to avoid hypoxia in Wiegmann´s Torquate Lizards (*Sceloporus torquatus*)? Field evidence. Can J Zool 93:377-382.

Guyton A.C. and T.Q. Richardson. 1961. Effect of hematocrit on venous return. Circ Res 9:157-164.

Hawkey C.M., P.M. Bennet, S.C. Gascoyne, M.G. Hart, and J.K. Kirkwood. 1991. Erythrocyte size, number and hemoglobin content in vertebrates. Br J Haematol 77: 392-397.

He J., M. Xiu, X. Tang, F. Yue, N. Wang, S. Yang, and Q. Chen. 2013. The different mechanisms of hypoxia acclimatization and adaptation in lizard *Phrynocephalus vlangalii* living on Quinghai-Tibet Plateau. J Exp Zool 319(3):117-123.

Herman J.K. and R.L. Ingermann. 1996. Effects of hypoxia and hyperoxia on oxygen-transfer properties of the blood of a viviparous snake. J Exp Biol (199): 2061-2071.

Hillyard S.D. 1980. Respiratory and cardiovascular adaptations of amphibians and reptiles to altitude. In Environmental Physiology: Aging, Heat and Altitude, ed. S. M. Horvath, M. K. Yousef, pp. 363-77. New York: Elsevied North-Holland. 468 pp.

Hochachka P.W. 1986. Defense strategies against hypoxia and hypothermia. Science 231:234-241.

Hutchinson V.H. 1982. Physiological ecology of the telmatobiid frogs of Lake Titicaca. Nat Geogr Soc Res Rep 14:357-361.

Hutchinson V.H., H.B. Haines, and G. Engbretson, 1976: Aquatic life at high altitude: respiratory adaptations in the Lake Titicaca frog, *Telmatobius coleus*. Respir Physiol 27:115-129.

Jackson D. 2007. Temperature and hypoxia in ectothermic tetrapods. J Therm Biol 32: 125-133.

Lancater L.T., A.G. McAdam, and B. Sinervo. 2010. Maternal Adjustment of Egg Size Organizes Alternative Escape Behaviors, Promoting Adaptive Phenotypic Integration. Evolution 64(6): 1607-1621.

Lemos-Espinal J.A. 1992. Ecology and comparative demography of *Sceloporus grammicus*: life history of an altitudinal generalist on the eastern slope of the Iztaccihuatl Volcano, Puebla, Mexico. Ph D. dissertation, University of Nebraska, Lincoln.

Monge C. and F. Leon-Velarde. 1991. Physiological adaptation to high altitude: oxygen transport in mammals and birds. Physiol Rev 71:1135-1172.

Newlin M. and R. Ballinger. 1976. Blood hemoglobin concentration in four species of lizards. Copeia 1976:392-394.

Nikinmaa M. 1997. Oxygen and carbon dioxide transport in vertebrate erythrocytes: an evolutionary change in the role of membrane transport. J Exp Biol 200(2):369-380.

Petschow D., I. Wurdinger, R, Baumann, J. Duhm, G. Braunitzer, and C. Bauer. 1997. Causes of high blood O_2 affinity of animals living at high altitude. J Appl Physiol 42(2): 139-143.

Pincheira-Donoso D., D.J. Hodgson, and T. Tregenza. 2008. The evolution of body size under environmental gradients in ectotherms: why should Bergmann´s rule apply to lizards?. BMC Evol Biol 8(68): 1-13.

Ruiz G., M. Rosenmann, and A. Veloso. 1983. Respiratory and haemotological adaptation to high altitude in *Telmatobius* from Chilean Andes. Comp Biochem Part A Physiol 78:109-114.

Ruiz, G., M. Rosenmann, and A. Veloso. 1989. Altitudinal distribution and blood values in the toad, *Bufo spinulosus* Wiegmann. Comp Biochem Part A Physiol 94(4):643–646.

Ruiz G., M. Rosenmman, and H. Nuñez, 1993. Blood values in South American lizards from high and low altitudes. Comp Biochem Part A Physiol 106(4):713-718.

Samaja M., T. Crespi, M. Guazzi, and K.D. Vandergriff. 2003. Oxygen transport in blood at high altitude: role of the hemoglobin-oxygen affinity and impact of the phenomena related to hemoglobin allosterism and red cell function. Eur J Appl Physiol 90(3-4):351-39.

Schuler B., M. Arras, S. Keller, A. Rettich, C. Lunby, J. Vogel, and M. Gassmann. 2010. Optimal hematocrit for maximal exercise performance in acute and chronic erythropoietin treated mice. Proc Natl Acad Sci U.S.A. 107:419-423.

Sears M.W. 2005. Resting metabolic expenditures as a potential source of variation in growth rates of the sagebrush lizard. Comp Biochem Physiol Part A 140:171-177.

Simonson T.S., Y. Yang, C.D. Huff, H. Yung, G. Qin, D.J. Z. Bai, F.R. Lorenzo, and J. Xing. 2010. Genetic Evidence for High-Altitude Adaptations in Tibet. Science 329 (72): 72-74.

Snyder G. K. and W.W. Weathers. 1977. Activity and oxygen consumption during hypoxic exposure in high altitude and lowland sceloporine lizards. J Comp Physiol 117:291-301.

Storz J.F. 2007. Hemoglobin function and physiological adaptation to hypoxia in high-altitude mammals. J Mammal 88(1): 24-31.

Storz J.F. and H. Moriyama. 2008. Mechanims of hemoglobin adaptation to high altitude hypoxia. High Alt Med Biol 9(2):148-157.

Storz J.F., G.R. Scott, and Z.A. Cheviron. 2010. Phenotypic plasticity and genetic adaptation to high-altitude hypoxia in vertebrates. J Exp Biol 213(24): 4125-4136.

Tazawa H., S.J. Andrewartha, and W.W. Burggren. 2012. Acute regulation of hematocrit and blood acid-base balance during hypoxia challenges in late chicken embryos (*Gallus gallus*). Resp Physiol Neurobiol 184:86-96.

Villafuerte F.C., R. Cárdenas, and C.C. Monge. 2004. Optimal hemoglobin concentration and high altitude: a theoretical approach for Andean men at rest. J Appl Physiol 96:1581-1588.

Vinegar A. and S.D. Hillyard. 1972. The effect of altitude on oxygen-binding parameters of the blood of the iguanid lizards *Sceloporus jarrovi* and *Sceloporus occidentalis*. Comp Biochem Physiol Part A Physiol 43:317-320.

Weathers W.W. and F.N. White. 1972. Hematological observations on populations of the lizard *Sceloporus occidentalis* from sea level and altitude. Herpetologica 28:172-175.

Weber R.E. 1995. Hemoglobin adaptations to hypoxia and altitude: the phylogenetic perspective. *In* Hypoxia and the brain. *Edited by* J.R. Sutton, C.S. Houston, and G. Coates. Queen City Printers, Burlington, VT. Pp. 31-44.

Table 1. Morphological parameters of *Sceloporus grammicus*

	Low altitude (ca. 2500 m asl)	Middle altitude (ca. 3400 m asl)	High altitude (ca. 4300 m asl)
Snout-vent lenght (SVL;mm)	5.3±0.2^{a}	6.0±0.4^{a}	4.1±0.5^{b}
Body mass (g)	4.8±1.2^{a}	5.5±0.9^{b}	5.2±0.2^{ab}

Note: Values are means ± SE. asl, above sea level

Table 2. Hematological traits in populations of *Sceloporus grammicus* lizard.

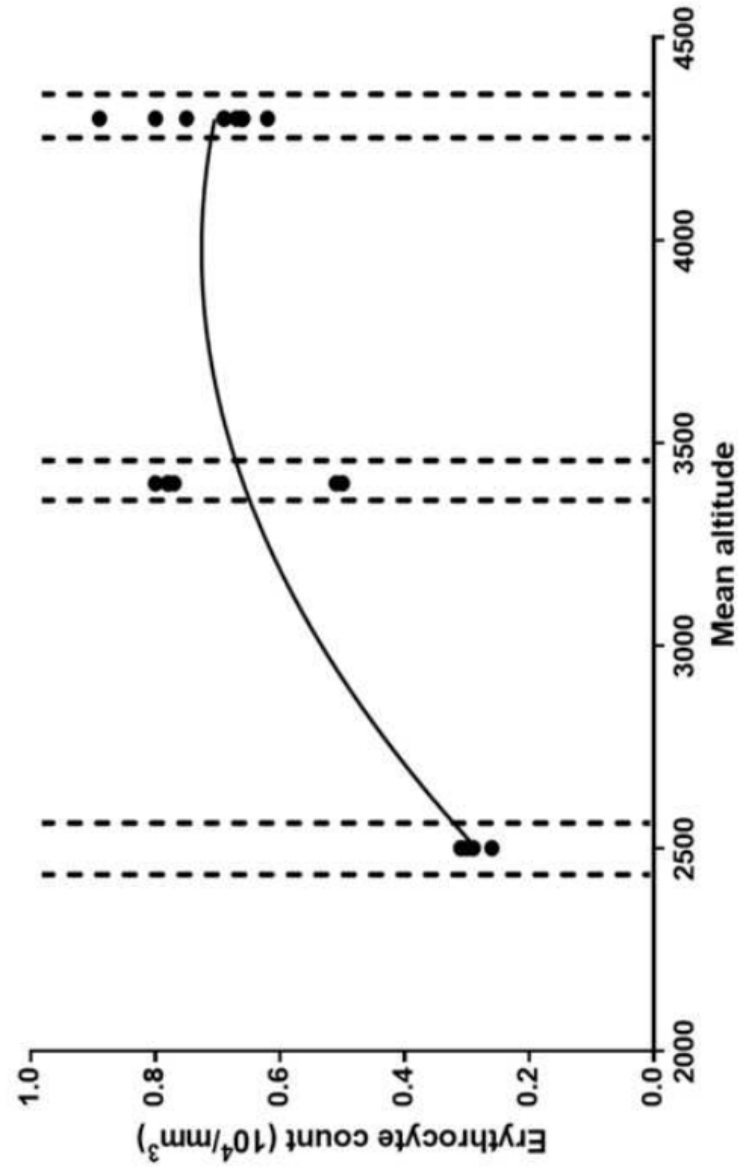
	Low altitude (ca 2500 m asl)	Middle altitude (ca 3400 m asl)	High altitude (ca 4300 m asl)	H-F values exept for eryrhrocyte count (H)	*p* values
Erythrocyte count (10^4 cells/mm^3)	0.26-0.3^a	0.50-0-7^b	0.66-0.6^b	13.12	<0.001
Hematocrit (%)	26.7±1.0^a	54.8±3.4^b	49.1±1.8^b	39.52	<0.001
Hemoglobin concentration (g/L)	7.6±0.6^a	25.5±0.9^b	16.4±1.4^c	69.78	<0.001
Erythrocyte size (μm^2)	77.1±1.5^a	95.5±2.4^b	69.3±1.8^a	46.32	<0.001
MCHC (g/L)	29.4±3.1^a	47.5±2.2^b	33.3±2.5^a	12.47	<0.001
Volume cell (fl)	94.4±4.0^a	91.7±7.1^a	71.1±3.0^b	6.44	<0.05

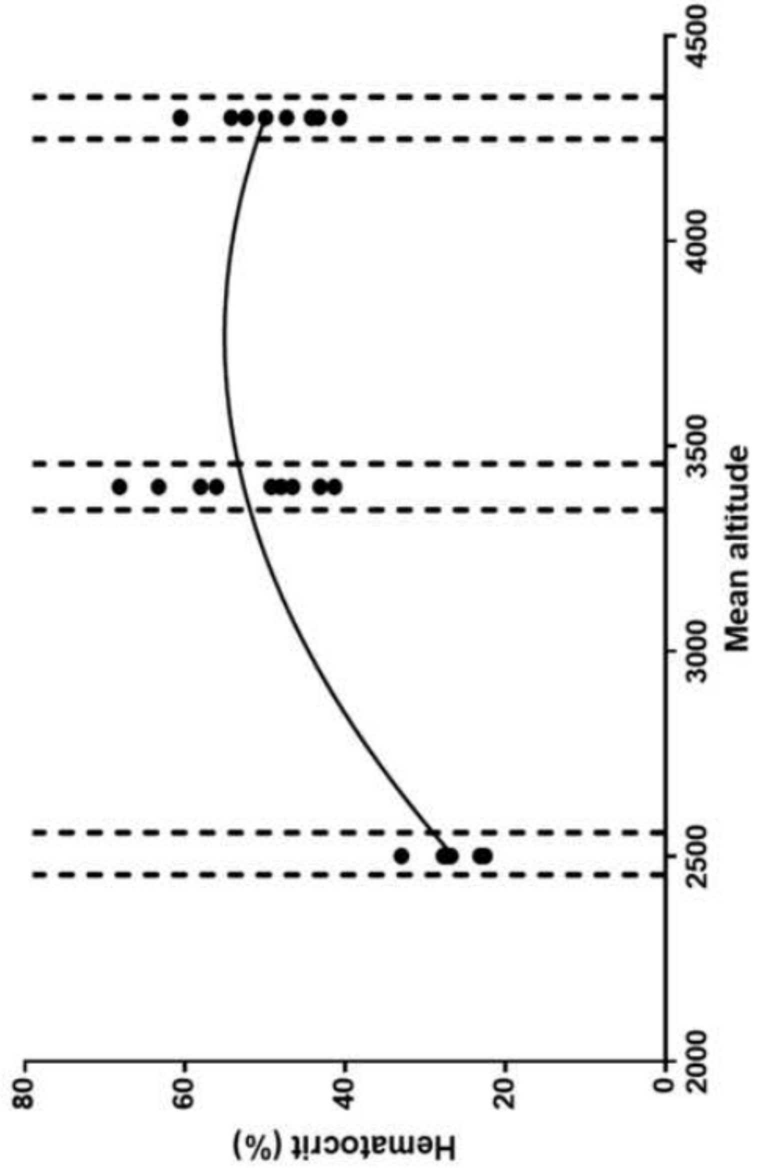
Note: Values are means ± SE exept for erythrocyte count that representes the ranges used for the Kruskall-Wallis analysis. H values are presented only for erythrocyte count whereas F vaules are presented for all other blood characteristic.

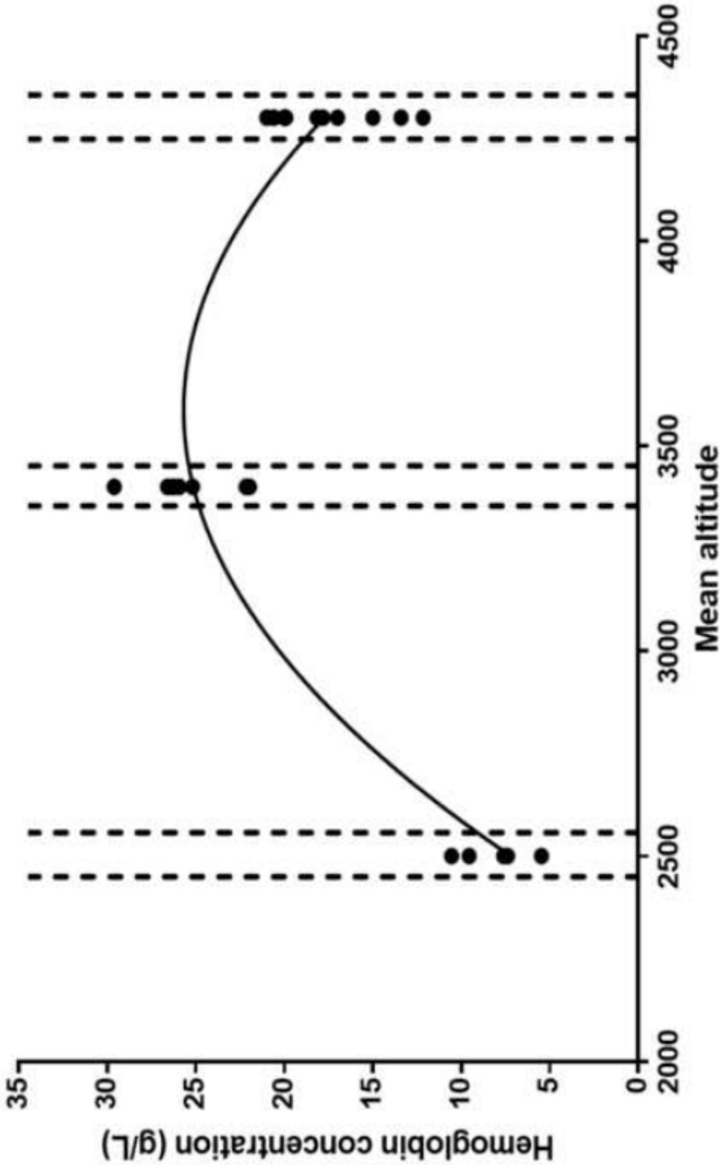
Table 3. PO_2 and CO_2 pressure, carbonates, oxygen saturation, electrolyte concentrations and pH, in populations of *Sceloporus grammicus* lizards.

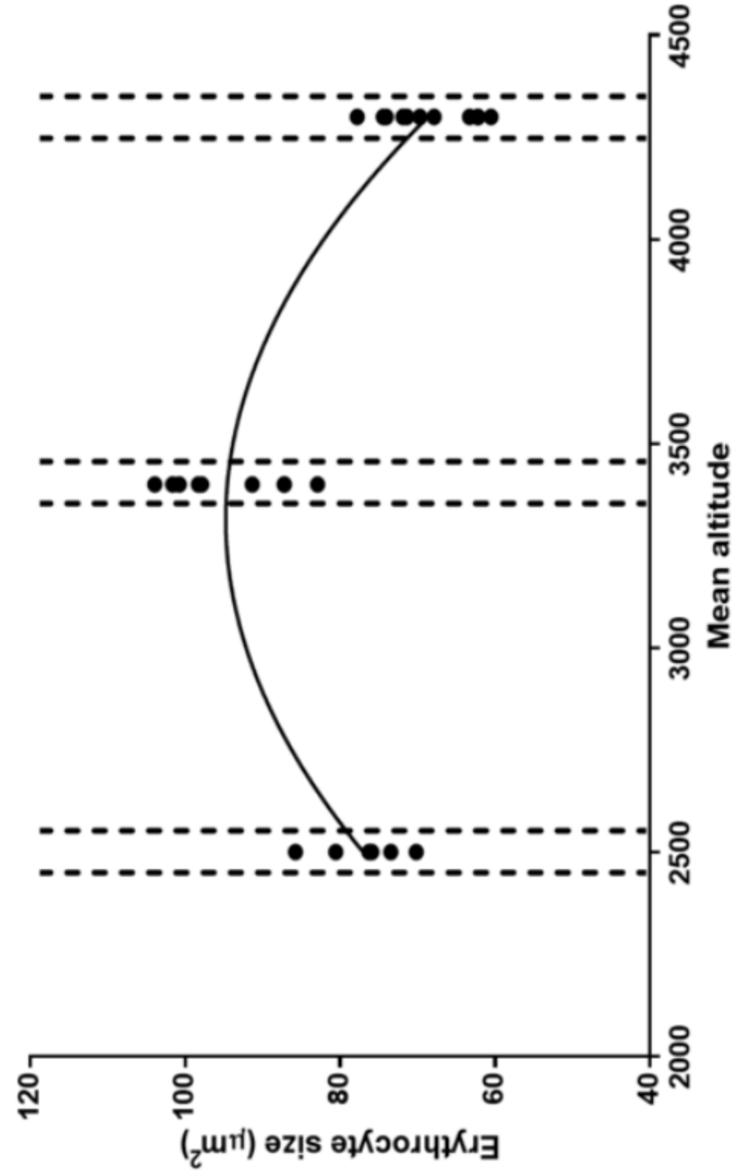
	Low altitude (ca 2500 m asl)	Middle altitude (ca 3400 m asl)	High altitude (ca 4300 m asl)	F values	*P* values
pH	7.1 ± 0.0	7.23 ± 0.1	7.26 ± 0.1	0.78	0.44
PO_2	52.9 ± 1.7	75.3 ± 16.6	43.2 ± 5.4	2.33	0.15
PCO_2	12.7 ± 2.1	8.6 ± 1.7	12.0 ± 5.3	0.34	0.71
$CHCO_3$	5.0 ± 2.3	4.1 ± 1.0	5.7 ± 2.7	0.78	0.44
SO_2	74.0 ± 8.2	70.3 ± 11.3	71.4 ± 8.1	0.19	0.83
Na^+	99.3 ± 9.0	92.0 ± 4.0	97.0 ± 9.5	0.15	0.86
K^+	3.1 ± 0.6	5.6 ± 0.6	3.8 ± 0.5	4.22	0.05
Ca^{++}	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.58	0.58
Glucose	161.3 + 26.5	130.2 ± 14.3	14.50 ± 26.2	0.44	0.65
Lactate	9.4 ± 2.2	9.9 ± 0.9	7.3 ± 0.6	1.22	0.34

Note: Values are means ± SEM, differences are significantly when $p<0.05$; PO_2 and PCO_2 (mmHg), $CHCO_3$, Na^+, K^+, Ca^{++} and Lactate (mmol/L), SO_2 (%), Glucose (mg/dL)


Erythrocyte count (10^4/mm^3)
1.0
0.8
0.6
0.4
0.2
0.0
2000
2500
3000
3500
4000
4500
Mean altitude


Hematocrit (%)
0
20
40
60
80
Mean altitude
2000
2500
3000
3500
4000
4500


Hemoglobin concentration (g/L)
35
30
25
20
15
10
5
0
2000
2500
3000
3500
4000
4500
Mean altitude


Erythrocyte size (µm²)
120
100
80
60
40
2000
2500
3000
3500
4000
4500
Mean altitude

Manuscrito en preparación para enviarse a la revista Journal of Evolutionary Biology

Static or labile view? Evaluation of some thermal traits in high altitude mezquite lizard *Sceloporus grammicus*

González-Morales J.C., A.H. Díaz de la Vega-Pérez, E. Bastiaans, J. Rivera-Rea, J. Manjarrez and V. Fajardo.

INTRODUCTION

In vertebrates physiological processes are only possible at certain range of body temperature, fishes, amphibians and reptiles are ectothermy species thus its Tb is highly dependent of environmental temperature (Shabtay and Arad 2005; Shine 2005; Buckley et al. 2012). High altitude environments exhibit lower temperatures respectively to low altitude ones, it is calculated that each 100 m the temperature decrease 0.3 °C (Hammond et al. 2004; Zamora-Camacho et al. 2014). Thus, the presence of lizards at high altitude environments has aroused a great interest in understand how could survive at extreme thermal conditions (Pough 1980, González-Morales et al., 2015 and 2017).

In that sense, two major positions have been developed to explain the thermal biology of lizards: 1) labile view: lizard could changes termal preferences with altitude, for example, in the generus *Liolaemus*, traditional analysis and phylogenetical approaches indicated that Tb varies consistently with temperature environment, suggesting that thermal biology of Liolaemus lizard had a evolutionary flexibility (Rodríguez-Serrano et al. 2009). 2) Static view: Termal biology of lizard not present changes for effect to altitude, only changes of termoregulatory behavior are sufficient to mitigate changes in the environment (Adolph 1989; Crowley 1985), for example, *Sceloporus jarrovi* not showed changes in sprint and critical temperatures in two population of different altitude (Crowley 1985). Lemos-Espinal and Ballinger (1995) showed that *Sceloporus grammicus* lizard presents the same Tb in field active lizard at 3700 and 4100 m asl, even with the major costs of thermoregulation that would suppose in the population of higher altitude since the period of activity is smaller. This has given basis to what the genus *Sceloporus* is consider thermally conservative (Crowley 1985; Andrews 1998). However other studies sowed that *Sceloporus* species from high elevation habitats, especially at tropical latitudes, exhibit low Tb compared to other ones at low elevations (see Andrews et al., 1997). This observation prompts the question of whether *Sceloporus* at high elevations exhibit physiological adaptations to low environment temperatures.

On the other hand, recently, lizard dorsal coloration has been added as one more element to consider within the thermal biology of lizards (Matthews et al. 2016), as an option to increase the heating rate by increasing melanism at high altitudes (thermal melanism hypothesis (Crustella-Trullas et al. 2008)) or as protection against the increase of the UV radiation product of the decrease of the atmosphere to great elevations (Hypothesis of the UV protection (Porter and Norris 1969)). Gvozdik (2002) showed that the *Zootoca vivipara* lizard does not show a higher heating rate in high altitude lizards; Zamora-Camacho et al. (2014) does not find an increase in the heating rate in higher altitude populations of *Psammodromus algirus*. Reguera et al. (2014) showed that there is a negative relationship between dorsal reflectance and UV radiation in the *Psammodromus algirus* lizard, even lizards were darker at high altitudes.

Sceloporus grammicus is a complex of lizards that live at altitudes higher than 4300 m, so it represents a good study model to observe how different traits potentially changes in its thermal biology. Here we present data obtained from the selection temperature, Hertz thermoregulation indices, maximum and minimum critical temperature, as well as the reflectance of the dorsal area in three populations that form a gradient at 2500, 3400 and 4300 m. We predict that all thermal traits, with the exception of dorsal coloration, do not present differences between populations since it has been established that the genus *Sceloporus* is thermally conservative, in addition to that the populations at higher altitude will be darker.

METHODS

Ethics statement

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

Field sites and animals

Our study populations were: 1) Low altitude population, located at Texcalyacac, Estado de México, 19°07′37″N, 99°29′41″W, 2500 ± 1.64 m altitude; 2) Middle altitude population, located at Las Lágrimas, Estado de México, 19°06′82″N, 99°49′48″W, 3400 ± 2.69 m; and 3) High altitude population, located at Nevado de Toluca, Estado de México, 19°06′06″N, 99°46′03″W, 4300 ± 2.5 m. We recorded altitude at all capture sites with an Etrex Vista GPS (Garmin, Olathe, Kansas, USA). Our three populations are sufficiently far enough from each other to avoid gene flow (Sears 2005; Texcalyacac is 39.31 km and 27.75 km from Las Lágrimas and Nevado de Toluca respectively, and Las Lágrimas is 27.76 km from Nevado de Toluca).

Field and environmental measurements

The lizards were captured by nose or hand, we measured body temperatures (Tb) of all lizards caught within 30 s after capture, we using a 1 mm diameter termocuple assembled to a thermometer (Fluke), after we recorded the environmental (Te) and substrate (Ts) temperature. Next we measurement body mass and SVL. For each population we used 30 lizards.

Also, following the protocol described by Lara-Resendiz et al. (2014), the Te's were recorded (each 30 m) in the there studies zone from may 2016 to abril 2017, we used models of PVS (with similar size and color of lizards) connected to a temperature sensors (HOBO ProV2). Previously we verified the relationship between PVS models and living lizards under different thermal conditions.

Temperature selected and set points

Lizard captured were transported at laboratory of Physiological Behavior at Facultad de Medicina Veterinaria of Universidad Autónoma del Estado de México and following the protocol described for Díaz de la Vega-Pérez (2013) we kept the lizards one day for their acclimatization in individuals terrariums with substrate. Next day we estimated termorregulatory set-points from measurement of preferred body temperatures (Tsel) in photothermal gradient (150 x 150 x 70) made of polycarbonate (Díaz de la Vega-Pérez et al. 2013). A 100-W bulbs was suspended 50 cm above sustrate as the source of heat gradient. After 2 hours for acclimation to the gradient, they were then removed quickly and their cloacal temperatura were measured with termocuple (Model) connected to termometer, measurements were taken only during the period of activity observed in field.

Thermoregulation Index

Following Hertz et al. (1993) we describe thermoregulation used: (1) Accuracy of thermoregulation (db): db = Tb – UBTset for Tb > UBTset, db = LBTset – Tb for Tb < LBTset, and db = 0 for LBTset = Tb = UBTset. This index indicates how closely lizards maintain their body temperature to Tset. The higher the mean db, the lower the accuracy of thermoregulation of lizards. (2) Thermal quality of a habitat (de), i.e., the mean deviation of Te's from the Tset range, was calculated analogously to db, with Te instead of Tb. (3) The effectiveness of thermoregulation (E) was calculated as E = 1 – db/de. Values of E approaching 1 indicate active thermoregulation, while values approaching 0 indicate thermoconformity. Blouin-Dammer index was: de-db.

Heating rate

We used 15 lizards of each population. Lizards were placed on plastic board and fixed it with transparent adhesive tape, a thermocouple connected to termometer was inserted into its cloaca. The lizards were then cooled to 19°C and placed under a 100 W bulb suspended at 20 cm above centre of its body. When the body temperature reached 20 °C, it was recorded the time it took to reach 33 °C. Heating rate was calculated as Tb1-Tb2/t, where Tb1 is body temperature at 20°C, Tb2 is body temperature at 33°C and t is the time it took to reach the temperature final.

Dorsal skin reflectance

Skin reflectance from upper, médium and lower dorsal regions of lizards were measured in the 200-700nm spectral range using a spectrophotometer (Jaz DPU® Module) and a Pulsed Xenon Light Source (Jaz-PX) connected to an optical fibre probe. The probe was fitted with a RPH Reflection Probe Holder to standar disemeasuring distance and shield outambient light. The probe was fixed with a RPH Reflection Probe Holder to standarize measuring distance and shield outambient light. During measurements, the probe was held at an angle of 45° (Endler 1990; Martín and López 2009; Bajer et al. 2010; Pérez de Lanuza and Font 2010). All the measurements were relative to a 99 % WS-1 white reflectance standard (all the components from Ocean Optics Inc., Dunedin, FL, USA). The spectral records were made with similar condition for avoid influence of environment. Reflectance was measured at 10, 20 and 30°C. The average reflectance of all measurements was calculated.

Minimun and maximun critical temperatures

This test was calculated at the end of the study, only 10 lizards were tested per population. The critical temperature is considered as the temperature at which the organisms loses control over the locomotion. For the minimum critical temperature (CTMin), the lizards were placed in a recipient in which the temperature gradually decreased aproximately 1 °C per minute. Bulbs were used for the critical critical temperature (CTMax) to gradually heat the conteiner aproximately 1 °C per minute.

RESULTS

Morphological parameters

Body mass decrease with altitude, population of middle and high altitude had minor body mass respectly to low altitude ($p < 0.05$), although this populations not had differences. SVL showed a similar pattern that body mass (table 1)

Temperature selected and set points

Operative temperature decrease with altitude, nevertheless between low and middle altitude this affect it is not marked, for high altitude temperature decrease 38% respectly low altitude (Table 2).

Temperature selection not changes with altitude, all population presentes range of temperature similar of 27-32 °C ($p > 0.05$)

Thermoregulation Index

db, de and Blouin-Damers index showed a similar pattern, high population showed higher values regarding the low and middle population, while between this two population not das significant differences (db: $F= 12.50$, $p < 0.05$; de: $F= 10.40$, $p < 0.05$; Blouin-Demers index: $F= 7.58$, $p < 0.05$). E not showed differences by altitude so that it can infered that all population are active termorregulation ($F= 0.193$, $p > 0.05$).

Heating rate

Populations of altitudinal gradient not showed diffences in heating rates ($F = 1.08$, $p > 0.05$), however it is positively correlated with SVL ($r = 0.38$, $p < 0.05$) and negatively correlated with body mass ($r = -0.59$, $p < 0.05$).

Skin reflectance

The two-way ANOVA test shows three things: 1) the middle and high populations show lower values of reflectance compared with low population, so they are darker ($F = 24.72$, $p < 0.05$); 2) temperature is not a significant factor ($F = 1.22$, $p > 0.05$) and 3) there is no significant interaction between altitude and temperature ($F= 1.93$, $p > 0.05$). However, when analyzing the data of the populations separately we find several patterns of dorsal coloration since the low population always maintains constant values of coloration despite the change of temperature ($F = 0.327$, $p > 0.05$); the middle population tends to be darker with increasing temperature ($F = 3.93$, $p < 0.05$), while the high population is always very darker regardless of the temperature ($F = 3.17$, $p > 0.05$)

Minimun and maximun critical temperatures

Population showed differences in TCMax ($F = 5.99$, $p < 0.05$), low and high population resist 1 °C less compared with middle altitude, on other hand CTMin also show differences for altitude ($F = 17.85$, $p < 0.05$), middle population had minor values of CTMin respectly low and middle altitude

DISCUSSION

Our results demonstrate that the Sceloporus grammicus lizards in the altitudinal gradient indicate that they are conservative in some thermal traits such as selection temperature and heating rate, however in other thermal traits such as critical temperature minimum and maximum and dorsal coloration showed differences by altitude effect.

All populations show the same selection temperature, thermoregulatory activity and heating rate, with the difference that the accuracy temperature and the thermal quality of the habitat is less with the altitude. High and low populations show the same levels of resistance at critical temperatures, although the high population is darker regardless of temperature, while the low population is gray, on the other hand the middle population is the most resistant to high and low temperatures, in addition to being as dark as the high population.

On the other hand, body size exhibits substantial variation in relation to the thermal differences of the environment (Anguilleta et al. 2004; Moreno-Azocar et al. 2014; Zamora-Camacho et al. 2014), although other factors can also affect, for example late maturation and predation among others (Atkinson 1994: Partridge and Coyne 1997). In vertebrates it has been related to the ability to acquire and maintain body heat with the efficiency of thermoregulation (), however, in reptiles the available data are controversial and there is no clear pattern of increase or decrease in size by effect Of altitude, for example in the gender The relationship between body size and altitude is negative, proposing as alternative hypothesis that greater body size is not desirable a large elevation, since it entails a higher metabolic expenditure (Pincheira-Donoso et al. 2010) The *Sceloporus graciosus* presents a larger body size of the mayor in the areas of high elevations, besides presenting a lower metabolic rate with respect to the same species in low elevations.

Although lizards have limited physiological means of regulating body temperature, many diurnal lizard species are known to regulate body temperature effectively by using thermoregulatory behaviors (Cowles and Bogert 1944; Heath 1965; Muth 1977; Avery 1982).

Regulatory behaviors and acclimatization both compensate for thermal variation within a habitat. Whether lizard populations or congeneric species occupying thermally distinct environments also compensate by genetic adaptation of thermal physiology is currently unresolved and the subject of considerably discussion (Hertz et al. 1983; Hertz 1983).

REFERENCES

Adolph S.C y W.P. Porter. 1993. Temperature, activity, and lizard life histories. Am Nat **142**: 273-295.

Andrews R.M. 1998. Geographic variation in field body temperature of *Sceloporus* lizards. J Therm Biol **23**: 329-334.

Andrews R.M., F.R. Méndez-de la Cruz, M. Villagrán-Santa Cruz and F. Rodriguez-Romero. 1999. Field and selected body temperatures of the lizards *Sceloporus aeneus* and *Sceloporus bicanthalis*. J Herpetology **33**:93-100.

Anguilleta M.J., P.H. Niewiarowski, A.E. Dunham, A.E. Leaché and A.D. Porter. 2004. Bergmanns's clines in ectotherms: ilustrating a life-history perspective with Sceloporine lizards. Am Nat **164**: 168-183

Blouin-Demers G. y P. Nadeau P. 2005. The cost-benefit model of thermoregulation does not predict lizard thermoregulatory behavior. Ecology **86**: 560-566.

Buckley L.B., A.H. Hulbert and W. Jetz. 2012. Broad-scale ecological implications of ectothermy and endothermy in changing environmetns. Global Ecol Biogeogr **21**: 873-885

Clusella-Trullas S., van Wyk J.H. y J.R. Spotila. 2007. Thermal melanism in ectotherms. J Therm Biol **32**: 235-245.

Cowles R. y C. Bogert. 1944. A preliminary study of the termal requeriments of desert reptiles. Amer Must Nat Hist Bull **83**(5): 265-296.

Crowley S.R. 1985. Thermal sensitivity of sprint-running in the lizard *Sceloporus undulatus*: support for a conservative view of termal physiology. Oecologia **66**: 219-225.

González-Morales J.C., E. Quintana, H. Díaz-Albiter, P. Guevara-Fiore, and V. Fajardo. 2015. Is the erythrocyte size a strategy to avoid hypoxia in Wiegmann´s Torquate Lizards (*Sceloporus torquatus*)? Field evidence. Can J Zool **93**:377-382.

González-Morales J.C., R. Beamonte-Barrientos, E. Bastiaans, P. Guevara-Fiore, E. Quintanda and V. Fajardo. 2017. A mountain or a plateau? Hematological traits vary non linear with altitude in Highland lizard. Physiol Biochem Zool. S/N.

Gvozdík L. 2002. To heat or to sabe time? Thermoregulation in the lizard *Zootoca vivípara* (Squamata: Lacertidae) in different termal environments along an altitudinal gradient. Canadian Journal of Zoology **80**: 479-492.

Hertz P.E., Huey R.B. y R. D. Stevenson. 1993. Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropiate question. Am Nat **142**: 796-818.

Lara-Resendiz R.A. y A.H. Díaz de la Vega-Pérez (2013) *Sceloporus grammicus* (Mesquite Lizard). Selected body temperature. Herpetol Rev **44**(2): 328.

Lemos-Espinal J.A. y R.E. Ballinger. 1995. Comparative termal ecology of the high-altitude lizard *Sceloporus grammicus* on the eastern slope of the Iztaccihuatl Volcano, Puebla, Mexico. Can J Zool **73**: 2184-2191.

Matthews G., Goulet C.T., Delhay K. y D.G. Chapple. 2016. The effect of skin reflectance on termal traits in a small heliothermic ectotherm. J Therm Biol **60**(2016): 109:124.

McConnachie S., Alexander G.J., y M.J. Whiting. 2007. Lower temperature tolerance in the temperate, ambush foraging lizard *Pseudocordylus melanotus melanotus*. J Therm Biol **32**: 66-71.

Moreno-Azocar S.L., M.F. Bonino, M.G. Perotti, J.A. Schulte, C.S. Abdala and F.B. Cruz. 2004. Effect of body mass and melanismo on heat balance in *Liolaemus* lizard of the *goetschi* clade. Exp Biol **219**: 1162-1171.

Ortega Z., Mencia A. y V. Pérez-Mellado. 2016. Adaptative seasonal shifts in the termal preferences of the lizard *Iberolacerta galani* (Squamata: Lacertidae). J Therm Biol **62:** 1.6.

Patridge L. and J.A. Coyne. 1997. Bergmann's rule in ectotherms: Is it adaptative?. Evolution **51**:632-635.

Pincheira-Donoso D., D.J. Hodgson and T. Tregenza. 2008. The evolution of body size under environmental gradients in ectotherms: why should Bergmann's rule apply to lizard?. **8**: 68

Pough F. 1980. The adventages of ectothermy for tetrapods. Am Nat **115**: 92-112.

Regera S., Zamora-Camacho F.J. y G. Moreno-Rueda. 2014. The lizard Psammodromus algirus (Squamata: Lacertidae) is darker at high altitudes. Biolo J Linnean Soc **112**: 132-141.

Rodriguéz-Serrano E., C.A. Navas and F. Bozinovic. 2009. The comparative field body temperatura among *Liolaemus* lizard: Testing the static and the labile hypotheses. J Therm Biol **34**: 306-309.

Shine R. 2005. Life History evolution in reptiles. Annu Rev Evol Syst **36**: 23-46