



UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
POSGRADO EN CIENCIAS BIOLÓGICAS
INSTITUTO DE BIOLOGÍA
ECOLOGÍA

TERMORREGULACIÓN, TOLERANCIA AL FRÍO Y RIESGO DE EXTINCIÓN DE
Barisia imbricata

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTORA EN CIENCIAS

PRESENTA:

M. en C. NATALIA FIERRO ESTRADA

TUTOR PRINCIPAL DE TESIS: Dr. Fausto R. Méndez de la Cruz
INSTITUTO DE BIOLOGÍA, UNAM

COMITÉ TUTOR: Dra. Margarita Martínez Gómez
INSTITUTO DE INVESTIGACIONES BIOMÉDICAS, UNAM

COMITÉ TUTOR: Dr. Andrés García Aguayo
INSTITUTO DE BIOLOGÍA, UNAM

CD. MX.

OCTUBRE, 2019



Universidad Nacional
Autónoma de México



UNAM – Dirección General de Bibliotecas
Tesis Digitales
Restricciones de uso

DERECHOS RESERVADOS ©
PROHIBIDA SU REPRODUCCIÓN TOTAL O PARCIAL

Todo el material contenido en esta tesis esta protegido por la Ley Federal del Derecho de Autor (LFDA) de los Estados Unidos Mexicanos (México).

El uso de imágenes, fragmentos de videos, y demás material que sea objeto de protección de los derechos de autor, será exclusivamente para fines educativos e informativos y deberá citar la fuente donde la obtuvo mencionando el autor o autores. Cualquier uso distinto como el lucro, reproducción, edición o modificación, será perseguido y sancionado por el respectivo titular de los Derechos de Autor.

OFICIO CPCB/776/2019

Asunto: Oficio de Jurado para Examen de Grado.


M. en C. Ivonne Ramírez Wence
Directora General de Administración Escolar, UNAM
P r e s e n t e

Me permito informar a usted que en la reunión del Subcomité por Campo de Conocimiento de Ecología, Manejo Integral de Ecosistemas, Biología Evolutiva y Sistemática del Posgrado en Ciencias Biológicas, celebrada el día 8 de abril de 2019, se aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** de la alumna **FIERRO ESTRADA NATALIA** con número de cuenta **405059469** con la tesis titulada: "**Termorregulación, tolerancia al frío y riesgo de extinción de *Barisia imbricata***", realizada bajo la dirección del **DR. FAUSTO ROBERTO MÉNDEZ DE LA CRUZ**:

Presidente:	DR. MANUEL FERIA ORTIZ
Vocal:	DR. ISAIAS HAZARMABETH SALGADO UGARTE
Secretario:	DRA. MARGARITA MARTÍNEZ GÓMEZ
Suplente:	DR. DIEGO DE JESÚS CHAPARRO HERRERA
Suplente	DR. GUILLERMO ALFONSO WOOLRICH PIÑA

Sin otro particular, me es grato enviarle un cordial saludo.

A T E N T A M E N T E
"POR MI RAZA HABLARA EL ESPIRITU"
Cd. Universitaria, Cd. Mx., a 25 de junio de 2019.


DR. ADOLFO GERARDO NAVARRO SIGÜENZA
COORDINADOR DEL PROGRAMA



c.c.p. Expediente del (la) interesado (a).

COORDINACIÓN DEL POSGRADO EN CIENCIAS BIOLÓGICAS
UNIDAD DE POSGRADO
Edificio D, 1º Piso. Circuito de Posgrados, Ciudad Universitaria
Alcaldía Coyoacán. C. P. 04510 CDMX
Tel. (+5255)5623 7002 <http://pcbiol.posgrado.unam.mx/>

Agradecimientos Institucionales

Al Posgrado en Ciencias Biológicas de la UNAM, por brindarme la oportunidad de realizar mis estudios de posgrado.

Al CONACyT por la beca otorgada número 412836 para realizar mis estudios de doctorado. A PAPIIT por el proyecto: *Susceptibilidad de lagartijas ante el cambio climático y del uso de suelo*, con número IN210116, por el financiamiento concedido para la realización de los muestreos en campo y la adquisición de material para este estudio.

Agradezco profundamente al Dr. Fausto R. Méndez de la Cruz, por las oportunidades y las facilidades otorgadas para la realización de esta investigación. A los miembros de mi comité tutor, a la Dra. Margarita Martínez Gómez, por toda la ayuda brindada, por los comentarios y por las ideas para realizar este estudio. Al Dr. Andrés García Aguayo, por el apoyo y los conocimientos compartidos para mejorar esta investigación.

Agradecimientos Personales

Quiero agradecer primero a las 280 lagartijas que sin saberlo hicieron posible este estudio. Porque todos deberían de poder conocer los fabulosos mecanismos de una *Barisia imbricata*, pero sobre todo, porque todos deberían de dejar de tenerles miedo y conservarlas.

A mi tutor.

Dr. Fausto R. Méndez de la Cruz. Porque siempre ha creído en mí, porque siempre me ha apoyado en todos los proyectos que se me ocurre, por todas las veces que me acompañó a campo, por los viajes a Tlaxcala, porque siempre me ha motivado a ser una mejor investigadora, porque siempre me incluye en nuevas aventuras, por todos los viajes compartidos, pero en especial, porque siempre me incita a ser mejor persona.

A los miembros del jurado de tesis.

Dr. Isaías H. Salgado Ugarte, Dr. Manuel Feria Ortíz, Dr. Guillermo A. Woolrich Piña y Dr. Diego de J. Chaparro Herrera, por el tiempo invertido en la revisión de este escrito y por sus valiosas observaciones que sin duda nutrieron esta investigación.

Al Dr. Barry Sinervo and Dr. Donald Miles

I have no words to express my deep gratitude. I'm convinced that the world of science is much better with researchers like both of you. Thank you for solving all my doubts, thank you for guiding me, thank you for enriching this study and for the multiple revisions to the manuscripts.

A quienes estuvieron en campo.

Dr. Fausto, Aldo López, Adán Alvarado, Ana, Ángel Soto, Beto, Cyn, Diego, Edgar Peralta, Edgar Valera, Ezel, Franklin, Gabriel González, Hugo Siliceo, Jennyfer, Jonathan Olvera, Marcos, Rafael Barzalobre, Raúl, Saúl, Timo, Yasmin y a todos los que me auxiliaron en el muestreo, gracias chicos! Gracias por aguantar el frío y las muchas horas buscando lagartijas. Sin su ayuda esto no hubiera sido posible.

Gracias Almendra, David Pasten, Ezel, Enrique Lozano y Yasmin. Por su ayuda durante la realización de los experimentos en el cuarto frío, porque sé que fue muy cansado y sin duda no lo hubiera logrado sola.

Al Dr. Santiago Ramírez Barahona, por las ideas y orientación en la realización de los análisis estadísticos.

Al Dr. Amando Bautista, por todas las facilidades otorgadas para la realización de los experimentos en el cuarto frío.

Al Dr. Diego Arenas Aranda qepd, por aceptar amablemente ayudarme a buscar proteínas. A la Dra. Ruth Ruiz Esparza Garrido y el Dr. Miguel A. Velázquez Flores, por su colaboración en el procesamiento de extracción de proteínas. Esta idea no se pudo concretar, pero confío que algún día lo logremos.

A la Dra. Lidia Irene Cabrera, por orientarme y permitirme usar su equipo para homogenizar las muestras de tejido.

A Enrique Lozano, por ayudarme a obtener las muy difíciles y traumantes muestras de sangre.

A Francisco Muñoz, por tomarte el tiempo de revisar minuciosamente este escrito y mejorarlo con tus comentarios.

A Enrique Sosa y Ana Pérez, por las revisiones a mi escrito, su paciencia y sus ideas.

Al Dr. Eduardo Sanabria y la Dra. Lorena Quiroga, por compartirme sus conocimientos sobre mecanismos anticongelantes, hicieron de mi estancia en San Juan algo inolvidable, gracias por abrirme las puertas de su casa. A Yamil Rodríguez, por la paciencia, por las desveladas esperando a que los sapos se congelaran, por ayudarme y enseñarme. A Nati, Victoria, Mauricio, Juan, Paula y Cristina, por los buenos momentos compartidos, gracias chicos, fue una estancia increíble!

Al Taller de Escritura de Artículos Académicos en Inglés y en especial a la Dra. Katya Frank, por la orientación e innumerables revisiones del artículo de requisito.

A la Herpetological corner, que hicieron de ese un curso muy divertido y de mucho aprendizaje. En especial a Jacky, Eliza y Cristian gracias por su amistad chicos! Gracias Cristian por tu orientación en la realización de los GLMM.

A mi familia:

Agradezco infinitamente a mis papás: José Juan Fierro Vega y Rosa María Estrada Palacios.

Porque siempre me están apoyando, consintiendo y cuidando de mí y de mis crías.

¡Los admiro y amo infinitamente!

A mi abuelita Rosario Palacios, por tu inmenso cariño, por preocuparte siempre por mí,
porque siempre estas interesada en lo que hago.

A mi fabulosa hermana Lilián, por todo tu cariño, porque siempre me consientes y me cuidas a la distancia, pero sobre todo, gracias por encargarte de mis crías. A Maple que me enseñó a querer a los perros!

A mis crías: C, Z, S†, T†, A† y S†, porque sin duda, cuidarlos motivó y agrandó mi amor a los reptiles. A MA y Mafalda que me obligan a practicar todos los días la paciencia y me llena de motivos para sonreír.

A mis tíos y primos. Que siempre quieren saber de las aventuras en campo.

A mis amigos:

Yazmin Ledesma, gracias! Gracias por escucharme a cualquier hora, gracias por nunca dejar que decaiga mi ánimo, gracias por recordarme aún en los peores momentos que yo puedo. Gracias por compartir tanto conmigo, gracias por seguir siendo mi amiga aún a la distancia!

Ana, Aldo, Enrique (Tortu), Micho. Gracias chicos por tantos momentos divertidos en el laboratorio, por las comidas, por los miles de cafés, los momentos Pokémon, los paseos, por las levantadas de ánimo, por las celebraciones, Gracias! Porque sin duda, hicieron de mi estancia en el lab bastante comfortable. Gracias, porque ya saben cómo soy y aun así me aprecian.

A Micho por “mi” dibujo de *Barisia imbricata* para esta tesis. Porque todo es mejor con Barisias!!

Gracias Edith Flores por los ánimos, por las extensas charlas, por los consejos, por estar y por permitirme estar.

A Cristian Argueta, incontables gracias Bicho! Gracias por creer en mí, por los ánimos, por tu ayuda, por las salidas a campo, por todos los buenos momentos compartidos durante tantos años. Gracias por recordarme siempre que yo puedo.

A los Chuchos: Jesús y Catalina. Por ser un ejemplo, por estar siempre dispuestos a ayudarme, por las fiestas, los paseos, las comidas, las charlas, las palabras de aliento, las avenitas. Por cuidar a mis Barisias, porque están siempre al tanto de mis avances, por motivarme a seguir, por su amistad en los momentos más estresantes en la realización de un doctorado y por enseñarme a como no morir en el intento.

A Dennis, gracias morro por tu amistad. Por tus consejos, por escuchar tantas veces mi proyecto y por compartir conmigo los tuyos.

A Edgar Peralta y MA. Que me acompañaron a más de la mitad del muestreo, por las desmañadas, por los viajes, por sus ocurrencias, por sus ánimos, por los buenos ratos, por todo lo que me ayudaron, gracias! Al Sr. David Peralta, porque sin su ayuda jamás hubiera podido traer el chiller y el capítulo 2 no hubiera sido posible. Gracias por su interés en mi proyecto.

A Yasmin González, porque sin conocerme estuviste dispuesta a prestarme tu población de estudio, porque me ayudaste más de la mitad del muestreo, pero sobre todo porque me recibiste y nos alimentaste en los muestreos. A tu mamá y papá que en más de una ocasión nos auxiliaron. A tus tíos que muy amablemente nos abrieron las puertas de su casa para comer y descansar. A Gabriel González, por su invaluable ayuda en campo.

A Víctor, por tus ocurrencias, por los momentos para correr en las tardes que son simple pretexto para el “deestrés”, por los ánimos, por motivarme, por tu ayuda.

Al Coatí team: Aldo, Ana, Beto, Cynthia, Diego, Enrique Lozano, Enrique Sosa, Fabiola, Francisco, Isela, Micho, Raúl, Rufino, Sandra y Saúl. Por todos los viajes, las salidas a campo, las fiestas, los congresos, etc. Gracias chicos! Tengo millones de historias divertidas con ustedes.

A mis hermanos académicos mayores: Norberto, Rafa y Aníbal. Gracias por todo lo que me hay ayudado en estos 8 años. Gracias por sus enseñanzas, gracias por sus regaños, gracias por motivarme a hacer bien las cosas. Gracias por obligarme a ser mejor.

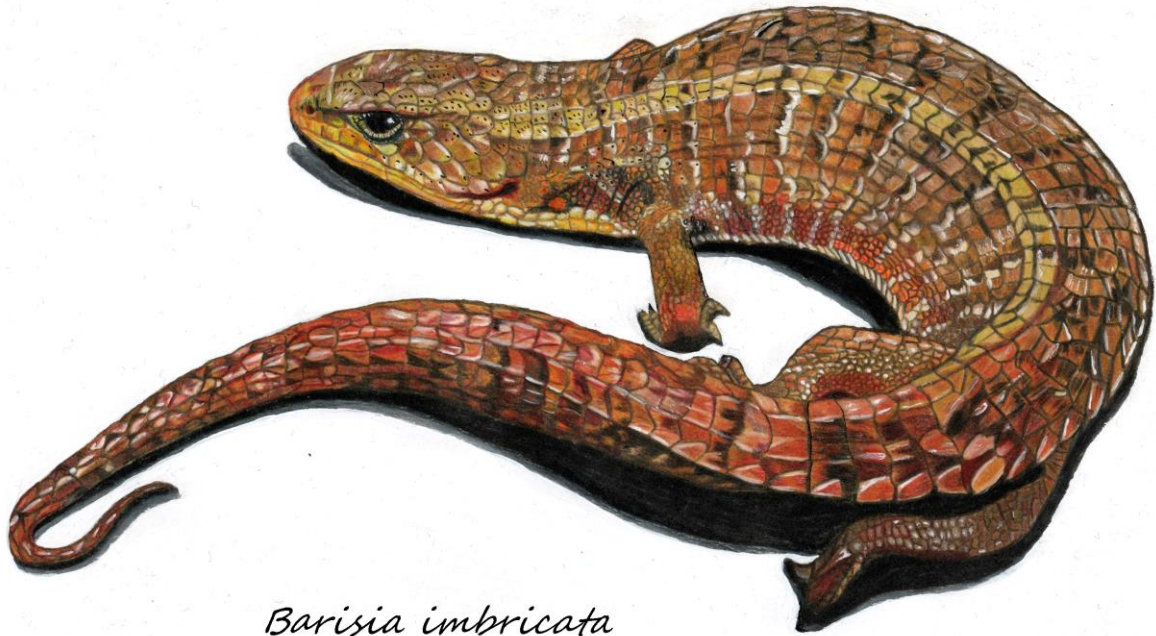
A Mixtli, por ser el mejor informante de la FES Zaragoza.

A mis amigos de inglés. A Karinita por animarme durante el sufrido proceso de aceptación del artículo. A Carlos, por todas las revisiones del manuscrito en inglés, gracias por levantarme el ánimo, gracias por la buena vibra.

Dedicatorias

A mi abuelito Carlos, porque sé que desde donde estas, estas orgulloso de mí, porque sé que siempre estás conmigo, este logro es para ti. Porque nos inculcaste a ser trabajadores, a cumplir metas, a alcanzar sueños, a que nada es imposible.

A mi sobrina María José, porque este estudio es parte de mi esfuerzo por construirte un mundo mejor. Porque espero que algún día esto te motive a hacer lo que sea que te guste y lo disfrutes tanto como yo disfruto estudiar reptiles.



Barisia imbricata

Micho

ÍNDICE

Resumen	1
Abstract	3
Introducción General.....	5
Termorregulación	6
Reptiles que toleran el frío.....	7
Riesgo de extinción.....	11
Objetivo General	16
Objetivo Particulares	16
Capítulo 1	17
Thermoregulation of the lizard <i>Barisia imbricata</i> at altitudinal extremes	17
Capítulo 2	30
Survival to freezing and glucose of the Mexican lizard <i>Barisia imbricata</i> (Squamata: Anguidae).....	30
Capítulo 3	46
An Ecophysiological Risk Assessment of Extinction Risk from Climate Change of the viviparous Mexican lizard <i>Barisia imbricata</i> (Squamata: Anguidae).....	46
Discusión General y Conclusiones.....	69
Literatura General.....	71

Resumen

La temperatura ambiental es un factor primario que afecta la fisiología y la actividad de los reptiles (Clase: Reptilia). El estudio de la ecología térmica de este grupo es relevante para comprender su distribución, historia de vida, ecología y requerimientos térmicos. La termorregulación implica una serie de mecanismos, los cuales mantienen la temperatura corporal de un organismo dentro de un intervalo de ajuste. Las lagartijas pueden termorregular fisiológicamente o conductualmente. De acuerdo a su eficiencia termorreguladora los organismos suelen denominarse “termorreguladores” o “termoconformistas”, y dependiendo del intervalo de temperaturas en las cuales los organismos mantienen actividad se dividen en “estenotermos” o “euritermos”. Además, la temperatura ambiental es un factor determinante en el rendimiento locomotor de las lagartijas. El rendimiento locomotor máximo se produce a una temperatura óptima. Si las temperaturas disminuyen, el rendimiento disminuye, y si las temperaturas ambientales alcanzan temperaturas de $-0\text{ }^{\circ}\text{C}$ los organismos pueden congelarse. Se sabe que las lagartijas pueden poseer dos mecanismos para sobrevivir a las bajas temperaturas, estos dos procesos dependen directamente de compuestos crioprotectores como la glucosa en sangre. Dicha molécula suele acumularse en otoño y elevar sus niveles en invierno, lo que les permite sobrevivir congeladas. Además, altos niveles de glucosa pueden estar relacionados con la actividad de algunas especies (*Zooteca vivipara*, *Podarcis muralis*, *Lacerta agilis*, etc) a bajas temperaturas corporales. Es necesario determinar cómo las especies pueden alcanzar temperaturas corporales activas en ambientes difíciles, para evaluar el riesgo de extinción debido al cambio climático, especialmente para las especies endémicas en peligro de extinción. Actualmente, el cambio climático es uno de los desafíos más importantes y apremiantes para las lagartijas, debido a su alta dependencia de la temperatura ambiental. De hecho, se han registrado extinciones de poblaciones de lagartijas, algunas de ellas de las montañas del centro de México. Uno de los organismos endémicos que habitan a lo largo del Eje Transvolcánico en el centro de México es la lagartija vivípara *Barisia imbricata*. En este estudio, se evaluó y comparó la estrategia termorreguladora de dos poblaciones de la lagartija *B. imbricata* de elevaciones

contrastantes (2,200 y 3,700 m). Los índices de eficiencia térmica se determinaron en función de las temperaturas corporales activas en el campo, las temperaturas preferidas (en un gradiente térmico) y las temperaturas ambientales operativas (según los modelos nulos). Las temperaturas del sustrato y del aire se registraron en el momento de la captura. La temperatura corporal media de ambas poblaciones mostró una correlación positiva y significativa con las temperaturas ambientales. Se encontraron diferencias estacionales significativas en la temperatura corporal en ambas poblaciones, y entre las temperaturas corporales de las dos poblaciones. También se tomaron los niveles de glucosa en sangre de las dos poblaciones durante todo el año, y se encontraron diferencias significativas en los niveles entre poblaciones, sin embargo, sólo la población de baja altitud presentó diferencias entre estaciones. Adicionalmente, se congelaron organismos de ambas poblaciones y se determinó el punto de cristalización de la especie. Además, se obtuvo el rendimiento locomotor de los organismos en siete temperaturas diferentes y se analizó la curva de rendimiento locomotor mediante la curva de Kumaraswamy, usando un modelo mixto aditivo generalizado con la longitud hocico-cloaca como covariable. Se utilizó la temperatura corporal, superior e inferior como umbral para calcular las horas de actividad, y las preferencias térmicas para calcular las horas de restricción de la especie. Con lo anterior, se modeló el riesgo de extinción de la especie ante el cambio climático en tres escenarios (RCP 2.6, 4.5 y 8.5) en la actualidad y para el año 2070. En conclusión, los resultados sugieren que *B. imbricata* es una especie euritérmica y puede termorregular activamente en cualquier momento. Sin embargo, cuando las temperaturas ambientales están dentro del intervalo de temperaturas preferidas, la especie no participa en un comportamiento termorregulador. *Barisia imbricata* tiene altos niveles de glucosa durante todo el año, lo que podría permitirle estar activa incluso a bajas temperaturas. Finalmente, *B. imbricata* enfrentará serios problemas ante el cambio climático en límites altitudinales bajos, pero las poblaciones a mayor altitud persistirán si el hábitat permanece.

Abstract

Environmental temperature is a primary factor that affects the physiology and activity of ectotherms (Class: Reptilia). The study of the thermal ecology of this group is relevant to understand their distribution, life history, ecology and thermal requirements. Thermoregulation involves a series of mechanisms to maintain the body temperature of an organism within a range of adjustment. The lizards can thermoregulate physiologically or behaviorally. According to their thermoregulatory efficiency the organisms are usually called "thermoregulators" or "thermoconformists", and depending on the temperature interval in which the organisms maintain activity, they are divided into "stenothermic" or "eurythermic". In addition, the environmental temperature is a determining factor in the locomotor performance in lizards. The maximum locomotor performance occurs at an optimum temperature. If the temperatures decrease, the performance decreases, and if the ambient temperatures reach temperatures below 0 °C, the organism can freeze. It is known that lizards can have two mechanisms to survive low temperatures, these two processes depend directly on cryoprotective molecules such as blood glucose. This molecule usually accumulates in autumn and increases its levels in winter, allowing them to survive frozen. Also, high levels of blood glucose could be related with the activity of some species (*Zooteca vivipara*, *Podarcis muralis*, *Lacerta agilis*, etc.) at low body temperatures. Determining how species can reach active body temperatures in difficult environments is necessary to assess the risk of extinction due to climate change, especially for endemic endangered species. Currently, climate change is one of the most important and pressing challenges for lizards, due to its high dependence on environmental temperature. Extinctions of some populations of lizards have already been reported, some of them from the mountains of central Mexico. One of the endemics organisms that occur along the Transvolcanic Axis in central Mexico is the viviparous lizard, *Barisia imbricata*. In this study the thermoregulatory strategy of two populations of the lizard *B. imbricata* of contrasting elevations (2,200 and 3,700 m) was evaluated and compared. The thermal efficiency indices were determined based on field active body temperatures, preferred temperatures (in a thermal gradient), and operative environmental temperatures (according to null models). The substrate and air

temperatures were recorded at the time of capture. Mean body temperature of both populations showed a positive correlation with environmental temperatures. Significant seasonal differences were found in body temperature in both populations, and between body temperatures of the two populations. The blood glucose of the two populations was also recorded throughout the year, and significant differences were found in levels between populations. Nevertheless, only the low altitude population showed differences among seasons. Additionally, organisms from both populations were frozen and the crystallization point of the species was determined. In addition, the locomotor performance of the organisms was obtained at seven different temperatures and the locomotor performance curve was analyzed using the Kumaraswamy curve, using an additive generalized mixed model with snout-vent length as a covariate. The upper and lower body temperatures were employed as an umbral to calculate the hours of activity, and thermal preferences to calculate the hours of restriction of the species. With all the above, the risk of extinction of the species in the face of climate change was modeled in three scenarios (RCP 2.6, 4.5 and 8.5) at present and for the year 2070. In conclusion, the results suggest that *B. imbricata* is an eurythermic species and can thermoregulate actively at any given time. However, when environmental temperatures are within the range of preferred temperatures the species does not engage in thermoregulatory behavior. *Barisia imbricata* has high glucose levels throughout the year, which could be allowing activity, even at low temperatures. Finally, the populations of *B. imbricata* will face serious problems at low altitudinal limits due to climate change (global warming), however populations at higher altitude will persist if the habitat remains.

Introducción General

Los términos “calor” y “temperatura” son a menudo confundidos. El calor es la energía que refleja el movimiento de los átomos y las moléculas, mismo que fluye desde un objeto con energía alta a otro con menor energía (Castellini, 2009). Por su parte, la temperatura es la cuantificación de la energía térmica de dicho objeto (Castellini, 2009), como una medida de la energía cinética promedio de las moléculas. Para los seres vivos existen dos fuentes principales de calor: la energía solar y el propio metabolismo celular (Willmer et al., 2005). Dependiendo de la principal fuente de obtención de calor, los organismos se clasifican en endotermos (aquellos que producen suficiente calor metabólico, como las aves y mamíferos) y ectotermos (aquellos que dependen de la temperatura de fuentes externas para obtener el calor suficiente, como anfibios y reptiles), mientras que la heterotermia constituye una condición intermedia entre las dos antes mencionadas (Castellini, 2009; Cowles and Bogert, 1944; Kearney and Porter, 2009; Willmer et al., 2005). Sin embargo, en la práctica todos los animales pueden generar algo de calor metabólico y ganar calor de fuentes externas (Willmer et al., 2005). Un ejemplo extremo en los reptiles es el Tegu Argentino (*Salvator merianae*), el cual mediante endotermia facultativa, logra mantener su temperatura corporal (T_c) hasta 10 °C por encima de la temperatura ambiental durante la temporada reproductiva (Tattersall et al., 2016).

También existe una categorización en función de la constancia de la T_c de los organismos. Los poiquilotermos son los animales con T_c variable, mientras que los homeotermos presentan T_c relativamente constante (Castellini, 2009; Whittow, 1966; Willmer et al., 2005). No deben confundirse estas divisiones, ya que puede haber organismos endotermos y poiquilotermos, como algunos mamíferos en hibernación o colibríes en torpor (Geiser, 2004), o ectotermos que son homeotermos a la vez, como el pez Opah (*Lampris guttatus*) que habita en aguas frías a 5 °C, pero que mantiene una T_c constante (Soyano and Mushirobira, 2018).

La T_c es un factor importante y determinante para los organismos, ya que de esta depende que se realicen correctamente las funciones fisiológicas, tales como la actividad, la alimentación, el cortejo, la evasión de los depredadores, la tasa de crecimiento, la

fecundidad, el rendimiento del aparato locomotor, los límites de la tasa metabólica en la que un organismo puede respirar, e incluso en la espermatogénesis y la maduración espermática (Darnell et al., 2015; Dubois et al., 2009; Elliott, 1994; Huey, 1982; Huey and Slatkin, 1976; Méndez de la Cruz et al., 2014).

Los reptiles obtienen el calor mediante tres fuentes. En función de la fuente principal, estos se dividen en dos grupos: heliotermos, aquellos organismos que obtienen calor por radiación solar directa, y tigmotermos, los cuales obtienen calor mediante la conducción por el contacto con rocas y sustrato o mediante convección del calor del aire (Castellini, 2009; Cowles and Bogert, 1944; Willmer et al., 2005). La constancia de la T_c depende del equilibrio entre la ganancia y la pérdida de calor.

Termorregulación

En animales ectotermos, el equilibrio térmico se puede lograr mediante la termorregulación, la cual tiene la finalidad de mantener la T_c lo más cercana a un intervalo de ajuste y lograr el intervalo óptimo de la T_c (Blouin-Demers and Nadeau, 2005; Castellini, 2009; Dubois et al., 2009; Hertz et al., 1993; Huey and Slatkin, 1976). Para esto, los animales deben buscar ambientes con temperaturas adecuadas o provocar ambientes internos óptimos. En los estudios de termorregulación en reptiles se evalúa el esfuerzo termorregulador de los organismos, el cual puede variar entre las especies en función de sus hábitats (Blouin-Demers and Nadeau, 2005). Los reptiles tienen una temperatura preferida o seleccionada (T_{sel}) en la cual se desempeñan adecuadamente. Esta T_{sel} suele encontrarse cercana a la temperatura óptima, es decir, a la temperatura a la cual se maximiza la aptitud de un organismo (Willmer et al., 2005). De igual forma la T_{sel} de una especie puede variar entre poblaciones, entre temporadas, entre sexos y en el mismo organismo pero realizando actividades diferentes (Hertz et al., 1993; Huey and Pianka, 1977).

Las lagartijas termorregulan fisiológicamente o conductualmente. Fisiológicamente, lo logran mediante variaciones en el sistema cardiovascular, modificando la velocidad del flujo sanguíneo por cambios en la frecuencia cardíaca o en el diámetro de los vasos (Labra

et al., 2008) o mediante cambios rápidos de pigmentación de la piel, como consecuencia de la agregación o dispersión de melanosomas los cuales pueden absorber el calor de forma diferente de acuerdo a la hora del día y/o a la temperatura corporal (Sherbrooke et al., 1994). Conductualmente, se observa cuando los organismos están fríos y se exponen a fuentes de calor, o al retirarse de esta al detectar demasiado calor, mediante modificaciones en su postura, al alternar las superficies expuestas a la fuente de calor o mediante el ajuste de los tiempos de actividad (Blouin-Demers and Nadeau, 2005; Cowles and Bogert, 1944; Dubois et al., 2009; Hertz et al., 1993; Huey, 1982, 1974; Huey and Slatkin, 1976).

Usualmente se dividen a los organismos que son más precisos en su termorregulación en “termorreguladores” y a los que son pasivos a las condiciones ambientales en “termoconformistas”. Sin embargo, el termoconformismo es sólo uno de los extremos en el continuo de la termorregulación (Blouin-Demers and Nadeau, 2005; Hertz et al., 1993; Huey, 1974; Huey and Slatkin, 1976). Es así que las lagartijas se mantienen en el extremo termoconformista cuando el costo de termorregulación es mayor a los beneficios potenciales. Aunado a esto, existe una gama de tolerancias térmicas con las cuales se pueden observar dos estrategias: euritermia y estenotermia. Los organismos euritérmicos toleran y presentan actividad en una gama bastante amplia de T_c ; en cambio, los organismos estenotérmicos solo presentan actividad si su T_c está dentro de un intervalo preciso de temperaturas (Willmer et al., 2005). De esta forma, la euritermia puede aumentar los posibles tiempos de actividad, lo que supone más ventajas fisiológicas en contraste con la estenotermia (Huey, 1982, 1974).

Reptiles que toleran el frío

El umbral de temperaturas o las tolerancias térmicas se definen como el intervalo de T_c en las que un organismo puede estar activo, funcionar normalmente o sobrevivir indefinidamente a cierta temperatura (Huey and Stevens, 1979; Weeks and Espinoza, 2013; Willmer et al., 2005). Las tolerancias térmicas de los individuos están ampliamente correlacionadas con las temperaturas de los hábitats, por lo que establecen los límites donde pueden sobrevivir los organismos y por ende sus distribuciones geográficas (Huang et al., 2007). Por ejemplo, la tolerancia al frío o al calor podría influir en la distribución

latitudinal y altitudinal de los reptiles (Huang et al., 2007; Huey and Stevens, 1979; Tejedo et al., 2012). Dichas tolerancias térmicas pueden estar influenciadas por la aclimatación en laboratorio, o por la aclimatización en vida libre por la estacionalidad y por efectos geográficos (Weeks and Espinoza, 2013).

La mayoría de los organismos multicelulares funcionan en intervalos limitados de temperaturas. Estos intervalos se encuentran por lo general dentro de los -2 °C a los 60 °C. Cuando el intervalo de tolerancia de las células eucariotas se sobrepasa, comienza a deteriorarse el rendimiento de los organismos, la actividad enzimática y afinidad se ven afectadas, las membranas celulares se vuelven inestables y las células son obligadas a mantenerse mediante respiración anaeróbica (Weeks and Espinoza, 2013). En general, las lagartijas mexicanas presentan actividad entre los 19.2 °C (*Lepidophyma gaigeae*) y los 45.9 °C (*Dipsosaurus dorsalis*) (Arenas-Moreno et al., 2018; Lara-Reséndiz et al., 2019). Sin embargo, se ha observado que algunas especies de la familia Anguidae presentan actividad a T_c inferiores, como: *Elgaria multicarinata webbi* (*Gerrhonotus multicarinatus webbi*) a 11 °C (Cunningham, 1966), *Mesaspis monticola* (*Gerrhonotus monticolus*) a 8.6 °C (Vial, 1975), *Abronia taeniata* a 13 °C (Fierro-Estrada, 2013) y *Mesaspis gadovii* a 10.8 °C (Bautista del Moral, 2019).

Las temperaturas críticas (mínima o máxima) son la media aritmética de los puntos térmicos en el que la actividad locomotora se desorganiza, y el animal pierde su capacidad de escapar de las condiciones que rápidamente lo llevarán al punto anterior a su muerte (Beitinger et al., 2000; Lutterschmidt and Hutchison, 1997; Tejedo et al., 2012). Dichas temperaturas se pueden determinar mediante la pérdida del reflejo de enderezamiento, es decir, la temperatura más baja o alta a la cual los organismos son incapaces de reincorporarse luego de haberlos colocado sobre su dorso (Du et al., 2000; Huang et al., 2007; Weeks and Espinoza, 2013).

Cuando la T_c desciende a temperaturas cercanas al punto de congelación, se desencadena la formación de cristales de hielo en el citoplasma y esto ocasiona la muerte celular. La célula muere principalmente por la ruptura mecánica de las membranas, debido al crecimiento del cristal y al aumento de volumen asociado al cambio de fase del líquido intracelular (Costanzo and Lee, 2013; Storey and Storey, 2004). La excesiva deshidratación

es otra causa de muerte celular, ya que el hielo atrapa el agua necesaria para el funcionamiento celular. No obstante, si los cristales crecen en el espacio extracelular no siempre da lugar a la ruptura celular y es menos deletéreo para los organismos (Voituron et al., 2002).

Algunos organismos endotermos presentan estrategias que les ayudan a evadir temperaturas ambientales de congelamiento. Dichas estrategias pueden ser de tipo conductual, como la migración en ciertas especies de aves, o en mamíferos la construcción de madrigueras y la permanencia en ellas en hibernación durante los meses más gélidos. También pueden ser de tipo fisiológicas. Un ejemplo es la gruesa piel del oso polar o la capa de grasa en las focas (Castellini, 2009). En contraste, en muchos linajes de tetrápodos ectotermos el factor determinante de la T_c media al día es la temperatura ambiental, en especial en aquellos organismos que habitan en ambientes muy fríos (Carvajalino-Fernández et al., 2011; Voituron et al., 2002). Debido a esto, el estudio de los mecanismos de supervivencia a bajas temperaturas en organismos ectotermos, principalmente lagartijas, tortugas, ranas y salamandras, ha crecido en los últimos años.

En anfibios y reptiles se han descrito dos mecanismos para sobrevivir a las temperaturas bajo cero: 1) evitar congelación o sobreenfriamiento y 2) la tolerancia a la congelación (Claussen et al., 1990; Voituron et al., 2002). Aunque estas estrategias se creían mutuamente excluyentes, actualmente se ha descubierto que ambos fenómenos pueden ocurrir en conjunto. Inclusive se pueden dar en el mismo organismo, en la misma temporada, o en el mismo sitio, pero en diferentes organismos, y esto dependerá de la población, la época y la condición fisiológica (Costanzo et al., 2003; Costanzo and Lee, 2013; Grenot et al., 2000). Por ejemplo, ambas estrategias se han documentado en especies como la lagartija *Zootoca (Lacerta) vivipara* (Voituron et al., 2004). En el caso de la tortuga *Chrysemys picta*, se cree que pueden sobrevivir a la congelación en los nidos al inicio del invierno y, posteriormente, soportar el sobreenfriamiento cuando el hielo exterior se derrite al filtrarse al interior del nido, lo cual disminuye la temperatura (Costanzo et al., 2003; Costanzo and Lee, 2013; Packard and Packard, 2003).

El sobreenfriamiento es la condición metaestable en la que la temperatura del tejido se encuentra por debajo de su punto de congelación (punto de fusión) sin desencadenar la

formación de hielo (Costanzo et al., 1995b; Lowe et al., 1971; Pauly, 2012). Algunos reptiles como lagartijas y tortugas son capaces de escapar de los efectos letales de la congelación ambiental mediante sobreenfriamiento de todo el cuerpo. Dichos organismos pueden llegar hasta temperaturas de $-8\text{ }^{\circ}\text{C}$ a $-18\text{ }^{\circ}\text{C}$ (Costanzo and Lee, 2013; Lowe et al., 1971).

La tolerancia al congelamiento es la habilidad de un organismo que le permite sobrevivir ileso al congelamiento del agua en una parte de sus tejidos. Este mecanismo es posible debido a una combinación de adaptaciones moleculares, bioquímicas y fisiológicas. Dichas adaptaciones limitan la muerte celular por la contracción excesiva, daño a macromoléculas y membranas, perturbaciones metabólicas y estrés oxidativo. De esta forma, la tolerancia al congelamiento es una adaptación de supervivencia de algunos organismos durante el invierno, estos organismos pueden congelar sus tejidos gracias a la biosíntesis de carbohidratos crioprotectores y a una extensa deshidratación de órganos y tejidos (Costanzo et al., 1995b, 1995a, 1993; Costanzo and Lee, 2013; Pauly, 2012; Voituron et al., 2002). La respuesta crioprotectora de estas sustancias comienza con el inicio de la congelación, lo que ayuda a mitigar el estrés osmótico asociado a la concentración del citoplasma por congelación (Costanzo et al., 1995b). Si bien la tolerancia a la congelación se considera una estrategia para sobrevivir al invierno, se sabe que en algunas especies esta estrategia puede beneficiarlas al extender de manera segura su actividad en los meses más fríos, como se ha documentado en la tortuga *Terrapene carolina* y en la serpiente *Thamnophis sirtalis* (Costanzo and Lee, 2013).

El sobreenfriamiento y la tolerancia al congelamiento están fuertemente asociadas a moléculas crioprotectoras o anticongelantes, las cuales ayudan a la viabilidad de los tejidos cuando se someten a bajas temperaturas. Al respecto, se ha observado que la acumulación de osmolitos compatibles, incluyendo polioles, ciertos aminoácidos, altos niveles de glicerol, urea e hidratos de carbono de bajo peso molecular proporcionan crioprotección coligativa, disminuyen además el punto de equilibrio de congelación de los tejidos y fluidos corporales, con lo que limitan la formación de hielo (Costanzo et al., 2013; Costanzo and Lee, 2013; Voituron et al., 2002). También existe la presencia de proteínas específicas en la sangre que tienen acción anticongelante o de nucleación del hielo (“antifreeze proteins” AFP) (Costanzo and Lee, 1996; Grenot et al., 2000; Voituron et al., 2002), estas moléculas también

ayudan a aumentar la supervivencia después de la congelación, mediante la prevención de la formación de hielo intracelular, la estabilización de membranas y macromoléculas, además de servir como antioxidantes, sustratos y reguladores metabólicos (Larson et al., 2014; Storey and Storey, 1986).

Como ya se mencionó, la hiperglucemia es fundamental en la supervivencia de los reptiles y anfibios congelados. La glucosa se acumula durante otoño y principios de invierno, lo que permite a los individuos estar mejor preparados para enfrentar la temporada de frío (Costanzo et al., 2013). La tolerancia a la congelación y el sobreenfriamiento puede variar entre organismos de diferentes regiones o en diferentes estaciones debido a los niveles de producción de este crioprotector (Costanzo et al., 1993). La glucosa parece ser un buen crioprotector por su rápida producción y fácil transporte; también es un sustrato fundamental para la producción de energía anaeróbica, ya que es el único sustrato que puede mantener energéticamente congelado al tejido anóxico (Costanzo et al., 1991). Además, promueve la supervivencia a la congelación mediante la deshidratación celular, la reducción del punto de equilibrio de congelación y la disminución del contenido de hielo, e inclusive puede unirse al agua, lo que impide que esta se congele (Costanzo et al., 1995b). Cabe resaltar que diversas especies de lagartijas podrían presentar también altos niveles de glucosa, lo que les proporcionaría la capacidad de estar activas a bajas temperaturas, sin llegar a la congelación.

Por lo tanto, es importante conocer los límites de tolerancia térmica de una especie y la variación entre sus poblaciones. Esta información, en conjunto con el estudio de la termorregulación, puede ser útil para predecir la susceptibilidad, afectación o posibles respuestas de los organismos ante el cambio climático, sobre todo en aquellas poblaciones que se encuentran en los bordes de distribución (Currie et al., 1998; Sinervo et al., 2010; Weeks and Espinoza, 2013).

Riesgo de extinción

El cambio climático (CC) en los últimos 30 años ha producido numerosas modificaciones en la distribución, la abundancia, el comportamiento, las interacciones ecológicas, la fenología

y las extinciones de algunas especies (Thomas et al., 2004; Vicenzi et al., 2017). Se pronostica que el incremento de la temperatura ambiental podría provocar la extinción de hasta un 37% de especies en todo el mundo para el año 2050 (Thomas et al., 2004). La temperatura ambiental determina el número de horas al día en el cual los organismos pueden estar activos según sus requerimientos (Huey and Slatkin, 1976; Labra et al., 2008). Debido a esto y a la dependencia a la temperatura, los ectotermos, son posiblemente los organismos más propensos a los efectos del cambio climático (Deutsch et al., 2008; Vicenzi et al., 2017). En el caso específico de los reptiles, se estima que para el año 2080, hasta el 39% de todas las poblaciones de lagartijas podrían extinguirse, debido a un déficit en las horas aptas para la termorregulación (Sinervo et al., 2010).

Las lagartijas vivíparas podrían ser más vulnerables debido a los regímenes de actividad de las hembras preñadas (Shine, 2005; Vicenzi et al., 2017). El aumento de la temperatura ambiental en los periodos críticos para la reproducción (invierno y primavera) podría tener serias consecuencias, como afectar el periodo de gestación, el tiempo de reproducción, la supervivencia de los neonatos y de las madres, así como el desempeño de las crías (Shine, 2005; Sinervo et al., 2017; Vicenzi et al., 2017). Las especies vivíparas en altas latitudes son aún más susceptibles por la disminución del área de distribución y la fragmentación, y aún más aquellas poblaciones aisladas en regiones montañosas en altitudes elevadas (Chamaillé-Jammes et al., 2006; Sinervo et al., 2010; Vicenzi et al., 2017; Wang et al., 2017). Una teoría es que las lagartijas vivíparas han evolucionado de antepasados ovíparas como una adaptación a climas fríos (Andrews and Mathies, 2000; Wang et al., 2017), por lo que dichas lagartijas podrían presentar mayor riesgo de extinción, ya que como resultado de dicha invasión en climas fríos a gran altitud o latitud han evolucionado con una T_c y una tolerancia al calor reducida (Sinervo et al., 2018, 2010; Wang et al., 2017). Aunado a esto, podría aumentar la amenaza en las especies de montaña, debido a desplazamientos de especies generalistas térmicas de hábitats de menor altitud, los cuales podrían ascender como consecuencia del aumento de temperatura (Ortega et al., 2016; Sinervo et al., 2010). En el caso de algunas lagartijas del género *Sceloporus* se ha observado que especies vivíparas se están extinguiendo a una mayor tasa que sus contrapartes simpátricas ovíparas (Sinervo et al., 2018), y ya se ha observado que

Sceloporus aeneus (2,400-3,000 m) ha aumentado su distribución altitudinal 450 m, con lo cual está invadiendo el hábitat de *Sceloporus bicanthalis* (3,200-4,250 m) (Andrews et al., 1999; Sinervo et al., 2018, 2010).

Aunado a la viviparidad, posiblemente los organismos termoconformistas tropicales sean más susceptibles a la extinción, ya que también están más cerca de sus límites térmicos debido a su adaptación a vivir en hábitats más fríos (Sinervo et al., 2017). El riesgo de extinción también podría ser mayor en especies endémicas, como consecuencia a su distribución restringida, en especial aquellas que habitan en áreas montañosas (Chamaille-Jammes et al., 2006; Wang et al., 2017).

Los organismos tienen tres formas de responder ante los efectos del CC. 1) Por medio de la modificación de su distribución a ambientes térmicos más favorables. 2) Al ajustarse al nuevo clima local, ya sea por plasticidad de la aclimatación o por respuestas evolutivas adaptativas. 3) Mediante la extirpación de poblaciones o incluso la extinción de especies completas (Sinervo et al., 2018, 2017).

En la mayoría de las investigaciones sobre los efectos del CC, se utilizan las capas bioclimáticas actuales (temperatura, precipitación y estacionalidad) y la distribución actual de las especies. Con esto se estiman las condiciones bajo las cuales las poblaciones de una especie persisten en la actualidad, sobreviviendo a competidores y depredadores (Thomas et al., 2004). Dichas condiciones se extrapolan mediante diversos algoritmos sobre capas bioclimáticas de los distintos escenarios posibles del CC. Estos modelos son llamados correlativos y sólo relacionan los datos de presencia de la especie con datos ambientales (Kearney et al., 2010). Es decir, los enfoques correlativos vinculan estadísticamente los datos espaciales con los registros de distribución de una especie, con los cuales se puede inferir sobre los límites de distribución de la especie y la idoneidad de su hábitat (Kearney and Porter, 2009). Por otra parte, los modelos mecanicistas se basan en las restricciones fisiológicas, conductuales o demográficas de las especies (Kearney et al., 2010; Porter et al., 1973), ya que el impacto biológico del aumento de las temperaturas dependerá de la sensibilidad fisiológica de cada organismo (Deutsch et al., 2008). Es decir, un modelo mecanicista incluye comprensión sobre las restricciones de la distribución y la abundancia (Kearney and Porter, 2009). Por lo que, el integrar la respuesta fisiológica de los

organismos a los estudios aumenta la confianza para predecir los efectos del CC (Kearney et al., 2010; Vicenzi et al., 2017).

La distribución potencial de una especie puede predecirse de manera más acertada mediante una perspectiva térmica, para esto, se incluye un modelo biofísico basado en curvas de desempeño térmico para actividades vitales como la alimentación o la locomoción (Kearney and Porter, 2009). Las curvas de rendimiento térmico miden y expresan la respuesta fisiológica de un organismo a través de la variación de su desempeño en función a la temperatura (Angilletta Jr. et al., 2002; Kearney and Porter, 2009). Las temperaturas críticas pueden marcar límites de supervivencia, o reflejar temperaturas donde la supervivencia es posible pero algún proceso fisiológico clave no lo es (Kearney and Porter, 2009). Con esta información se pueden producir mapas certeros del hábitat para ese proceso fisiológico, lo cual restringe el rango potencial de distribución de las especies (Kearney and Porter, 2009).

La relación entre la T_c y el desempeño locomotor se describe en una función asimétrica conocida como "curva de desempeño locomotor" (Angilletta Jr. et al., 2002). Las curvas de desempeño locomotor inician con la temperatura crítica mínima (CT_{\min}), es decir la menor temperatura a la cual los organismos dejan de estar activos, desde ese punto, las curvas generalmente aumentan de forma gradual conforme la temperatura aumenta, hasta alcanzar su máximo en un punto conocido como temperatura óptima, que es el punto donde las funciones fisiológicas básicas de los organismos se desarrollan de forma adecuada y el rendimiento locomotor se maximiza. Después, las curvas de desempeño locomotor descienden tajantemente hasta alcanzar la temperatura crítica máxima (CT_{\max}) a la cual los organismos logran mantener actividad (Angilletta Jr. et al., 2002; Currie et al., 1998; Deutsch et al., 2008; Kearney and Porter, 2009). Las curvas de desempeño ofrecen el marco fisiológico de la especie con el cual se puede estimar el efecto directo de la temperatura en la aptitud de los organismos, dando una base fundamental para estudiar el efecto del CC en forma explícita y experimental (Deutsch et al., 2008).

Por otra parte, cuando la temperatura ambiental excede la CT_{\max} de los organismos puede provocar la muerte de estos. Para evitar morir de sobrecalentamiento los organismos estivan de forma estacional o se esconden en refugios térmicos diarios, los

cuales presentan temperaturas inferiores al resto del ambiente (Sinervo et al., 2018, 2017, 2010). Esto puede provocar que los organismos presenten horas de restricción (h_r), es decir, momentos dentro del intervalo de actividad diaria en los cuales las temperaturas sean tan altas que los organismos no puedan mantener su actividad. Las h_r pueden ser contraproducente, ya que al permanecer más tiempo en refugios térmicos provoca disminución del tiempo de forrajeo, limitación en procesos metabólicos y por ende la energía puede ser insuficiente para cubrir el costo de crecimiento o reproducción, pudiendo provocar una reducción en la población e incluso la extinción de las poblaciones y especies (Sinervo et al., 2018, 2010; Vicenzi et al., 2017). Por lo tanto, calcular las h_r que pueden llegar a presentar las especies evalúa el posible grado de afectación por el CC.

El Grupo Intergubernamental de expertos sobre el Cambio Climático (IPCC) propuso cuatro posibles futuros escenarios de emisiones del CC denominadas "Trayectorias de Concentración Representativas (Representative Concentration Pathways, RCPs)". Todos las RCPs presentan concentraciones atmosféricas de CO₂ al año 2100 más elevadas respecto a las de hoy en día. La RCP 2.6 es un escenario donde se espera que los esfuerzos de mitigación ayuden a disminuir la producción promedio de CO₂ y esta sea sólo de 421 ppm con un aumento de temperatura media global de 0.3° a 0.7°C. La RCP 4.5 es un escenario estable donde los niveles de CO₂ estarían por los 538 ppm con aumentos en la temperatura de 1.1° a 2.6°C. Y la RCP 8.5 es un escenario con un nivel creciente de emisiones de CO₂ de hasta 936 ppm el cual proyecta un aumento en la temperatura de 2.6° a 4.8°C en relación con las condiciones climáticas actuales (IPCC, 2013).

Para disminuir el riesgo de extinción es indispensable reducir las emisiones de gases de efecto invernadero y centrar la reforestación en áreas superiores a los 2,600 m, debido a que la altitud siempre que exista cubierta vegetal puede reducir uniformemente el riesgo de extinción tanto en organismos termoconformistas como en termorreguladores (Sinervo et al., 2017).

Objetivo General

Estudiar la ecología térmica, desempeño locomotor y mecanismos fisiológicos que permiten la actividad a bajas temperaturas de *Barisia imbricata*, y de acuerdo a su ecofisiología, estimar la susceptibilidad de la especie ante el cambio climático.

Objetivo Particulares

Evaluar la eficiencia térmica de *B. imbricata* comparando dos poblaciones de los extremos altitudinales de distribución.

Determinar y comparar la variación de los niveles de glucosa en sangre de *B. imbricata* en las diferentes épocas del año y entre poblaciones de los extremos altitudinales de distribución.

Determinar si los niveles de glucosa influyen en la actividad de los organismos a bajas temperaturas y en el punto de cristalización de la especie.

Desarrollar modelos de predicción de presencia de *B. imbricata*, con base en las horas de restricción, de actividad y el desempeño locomotor ante el cambio climático.

Capítulo 1

Thermoregulation of the lizard *Barisia imbricata* at altitudinal extremes



Thermoregulation of the lizard *Barisia imbricata* at altitudinal extremes

Natalia Fierro-Estrada^{1,2,*}, Yasmin Guadalupe González González³, Donald B. Miles⁴,
Margarita Martínez Gómez⁵, Andrés García⁶, Isaías Hazarmabeth Salgado-Ugarte⁷,
Fausto R. Méndez de la Cruz²

Abstract. Ambient temperature is a primary factor affecting the physiology and activity of reptiles. Thermoregulation involves a series of mechanisms to maintain an organism's body temperature within a narrow range. The study of thermal ecology of lizards is relevant for understanding their distribution, life history, ecology and thermal requirements. Moreover, determining how species are able to attain physiologically active body temperatures in challenging environments is necessary for assessing the risk of extinction due to climate change, especially for threatened endemic species. We evaluated and compared the thermal ecology of two populations of the viviparous lizard *Barisia imbricata*, at contrasting elevations (2200 and 3700 m). We obtained variation in thermal data from winter through autumn for multiple years. We determined thermal efficiency indices based on field active body temperatures, preferred temperatures (in a thermal gradient), and operative environmental temperatures (according to null models). We also recorded substrate and air temperatures at the time of capture. Mean body temperature of both populations showed a positive correlation with environmental temperatures. We found significant seasonal differences in body temperature in both populations, and between body temperatures of the two populations. Our results suggest that *B. imbricata* is an eurythermic species and can thermoregulate actively at any given time. However, when environmental temperatures are within the range of preferred temperatures, the species does not engage in thermoregulatory behavior. This information expands knowledge on the range of possible thermal responses to environmental variation within a species.

Keywords: body temperature, eurythermic, seasonal variation, thermal efficiency.

Introduction

Body temperature (T_b) in reptiles is an important factor for the optimum performance of diverse physiological functions, such as metabolic rate, locomotor performance, digestive efficiency, growth rate, egg production, spermatogenesis and sperm maturation (Huey and Slatkin, 1976; Angilletta, 2009; Dubois et al., 2009; Méndez de la Cruz et al., 2014). Variation in physiological performance in turn affects foraging success, evasion of predators, courtship behavior, and reproductive success. Maintenance of T_b depends on the balance between rates of heat gain and loss. Modulation of the equilibrium between heat gain and heat loss arises through behavioral thermoregulation, which facilitates an organism's ability to maintain a T_b within the range of temperatures bracketing the optimum temperature for physiological performance (Huey and Slatkin, 1976;

- 1 - Posgrado en Ciencias Biológicas, Instituto de Biología, Universidad Nacional Autónoma de México, A.P. 70-153, C.P. 04510 Ciudad de México, México
- 2 - Laboratorio de Herpetología, Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, A. P. 70515, C.P. 04510 Ciudad de México, México
- 3 - Laboratorio de Zoología, División de Biología, Instituto Tecnológico Superior de Zacapoaxtla, Carretera Acuaco Zacapoaxtla Km. 8, Col. Totoltepec, Zacapoaxtla, Puebla 73680, México
- 4 - Department of Biological Sciences, Ohio University, Athens, Ohio, USA
- 5 - Departamento de Biología Celular y Fisiología, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, México DF 04510, México
- 6 - Estación de Biología Chamela, Instituto de Biología, Universidad Nacional Autónoma de México, C.P. 48980 La Huerta, Jalisco, México
- 7 - Laboratorio de Biometría y Biología Pesquera, FES Zaragoza, Iztapalapa, México

*Corresponding author;

e-mail: natalia_fierro@hotmail.com

Hertz, Huey and Stevenson, 1993; Blouin-Demers and Nadeau, 2005; Dubois et al., 2009).

Thermoregulatory behaviours of lizards occur along a continuum and can range from perfect thermoconformers, where T_b fluctuates passively with ambient temperature, to perfect thermoregulators, where individuals show precise regulation with their body temperature maintained within a narrow breadth of temperatures (Huey, 1974; Huey and Slatkin, 1976; Hertz, Huey and Stevenson, 1993; Blouin-Demers and Nadeau, 2005). In addition to thermoregulatory behaviours, organisms can also be categorized according to the magnitude of temperature variation they can endure. Most lizards are stenothermic and regulate their T_b within a narrow range of temperatures (Huey and Webster, 1975; Kingsbury, 1993; Willmer et al., 2005). On the other hand, some other lizards are eurythermic and can remain active across a wider range of body and environmental temperatures (Huey and Webster, 1975; Kingsbury, 1993; Fierro-Estrada, 2013). The breadth of temperatures a species can tolerate may limit their distribution and the total number of hours suitable for activity (Huang et al., 2007).

Lizards often have a preferred body temperature (T_{pref}) that coincides with the temperature that maximizes physiological performance (Willmer, Stone and Johnston, 2005). Previous studies have demonstrated among population variation in T_b and T_{pref} within the same species, and among seasons in the same population (Huey and Pianka, 1977; Hertz, Huey and Stevenson, 1993; Labra, Soto-Gamboa and Bozinovic, 2001; Gutiérrez, Krenz and Ibarguengoytía, 2010; Caruccio et al., 2011). Other studies have found differences in thermal traits between sexes of the same species, between reproductive and nonreproductive females, between ill and healthy individuals, fasted and fed lizards, and ontogenetic variation (Smith and Ballinger, 1994; Mathies and Andrews, 1997; Rock, Andrews and Cree, 2000; Beal, Lattanzio and Miles, 2014; Gilbert and Miles, 2016; Zamora-Camacho, Reguera and

Moreno-Rueda, 2016). Thermal variation during the breeding season could possibly have a greater impact on fitness, i.e., survival and reproduction, when organisms are engaged in courtship and breeding behaviours (Smith and Ballinger, 1994; Mathies and Andrews, 1997).

Populations within a habitat can experience fluctuations in environmental temperatures at multiple temporal scales. Short-term fluctuations include diurnal temperature cycles or short-term events, such as heat waves. To compensate for such fluctuations organisms use behavior to thermoregulate, by either basking during cool periods or seeking thermal refugia, such as shade and burrows during hot periods (Cowles and Bogert, 1944; Huey, 1974, 1982; Huey and Slatkin, 1976; Huey and Pianka, 1977). Furthermore, lizards can use different postures or substrates when exposed to seasonal variation in environmental conditions (Huey, 1974) or even change their activity time (Huey, 1974, 1982; Huey and Slatkin, 1976; Hertz, Huey and Stevenson, 1993; Lemos-Espinal and Ballinger, 1995; Blouin-Demers and Nadeau, 2005; Dubois et al., 2009). Long-term fluctuations are driven by seasonal or annual variability where an organism may acclimatize to deal with prevailing thermal conditions and modify their thermoregulatory basal value points (Huey and Slatkin, 1976; Huey and Bennet, 1987; Hertz, Huey and Stevenson, 1993; Gvozdík, 2002). However, thermoregulatory responses to seasonal variability in some organisms could be different along latitudinal and altitudinal gradients (Gvozdík, 2002; Catenazzi, Carrillo and Donnelly, 2005; Artacho et al., 2017a), while in other organisms the response is similar throughout the year (Lemos-Espinal and Ballinger, 1995; Sepúlveda et al., 2008).

Most studies have evaluated the thermoregulatory effort of the organisms during a single season or for only a single population (Kingsbury, 1993; Lemos-Espinal, Smith and Ballinger, 1998; Muñoz-Brito, 2013). However, this effort may vary through time and among

populations or species depending on the thermal quality of their habitat (Blouin-Demers and Nadeau, 2005). Considering these sources of variation, understanding the thermoregulatory behaviour of an organism should entail a long-term study that spans over several seasons and multiple years. It is also important to determine intrapopulation variation in thermal preferences and tolerances. Comparative studies enhances our ability to determine how variation in the abiotic environment understand could affect life history patterns of different populations (Lemos-Espinal and Ballinger, 1995; Mesquita et al., 2016).

In this study, we investigated the thermal ecology of the viviparous lizard *Barisia imbricata* (family Anguillidae). This species is endemic to México and lives in a variety of habitats along an elevational gradient from 2100 to 4000 m (Guillette and Smith, 1982; Guillette and Casas-Andreu, 1987; Lemos-Espinal, Smith and Ballinger, 1998). Evaluating the susceptibility and potential responses to climate change requires determination of variation in the thermal ecology of multiple populations of a species. In particular, populations at the margins of their distribution should be included in a study as they are expected to be more susceptible to fluctuations in abiotic and biotic factors (Currie, Bennett and Beitingger, 1998; Sinervo et al., 2010; Weeks and Espinoza, 2013; Lara-Reséndiz et al., 2014). Species that occupy habitats along a broad altitudinal gradient throughout the year, should experience differences in thermal availability that will determine the strategy for regulating body temperature, e.g., thermoconformer to thermoregulator (Muñoz and Losos, 2018). To examine the dependence between altitude, thermal quality and thermoregulatory strategy, we evaluated the thermal ecology of *Barisia imbricata*, by comparing two populations from extreme altitudes throughout the year.

Materials and methods

Study area and study species

The study focused on two populations of *Barisia imbricata* that occupy two distinct sites at elevations that bracket the margins of its altitudinal distribution. The first population was located in Ocotepéc, Puebla México at 2200 m (referred to as a low altitude population) (N19°33'17.8'' W97°39'07.8''). Individuals from this locality were mainly captured on or under *Agave* leaves and under rocks. The second study site was in Paso de Cortés, Estado de México at 3700 m (referred to as high altitude population) (N19°05'13.0'' W98°38'46.5''). The vegetation at this site was dominated by bunchgrass (*Festuca toluensis*). Our study lasted between 2014–2017. We captured individuals of *B. imbricata* from Paso de Cortés in Autumn 2014, Spring, Summer and Autumn 2015, and Spring and Winter of 2016. At Ocotepéc, we captured lizards during Spring, Summer and Autumn 2016 and Winter 2017. We only captured individuals that were active. We recorded the age (juvenile/adult), sex, and reproductive state of females (pregnant, not pregnant). Individuals were brought to the laboratory, individually marked by toe-clipping and maintained in terraria to perform the experiments. At the end of the experiment all lizards were returned to their capture site.

Measurement of thermal efficiency

The body temperature (T_b) of each individual (juveniles, males, non-reproductive females or pregnant females) was recorded within 20 seconds of capture using a quick-reading cloacal thermometer. Furthermore, we recorded substrate temperature (T_s) and air temperature (T_a , at 5 cm above where the lizards were caught) with a digital thermometer (Fluke® 51-II) with a sensor type T ($\pm 0.1^\circ\text{C}$). We also recorded the time of capture.

We measured the thermal preference (T_{pref}) of individuals each season using a thermal gradient. The thermal gradient was built with medium-density wooden fiberboard with dimensions $180 \times 100 \times 10$ cm (length \times width \times height), and was divided into ten tracks with an area of 1800 cm^2 . As in other studies, we established a temperature gradient from 18° to 35°C using incandescent lights at one end and ice packs at the other (Rock, Andrews and Cree, 2000; Lara-Reséndiz, Arenas-Moreno and Méndez-de la Cruz, 2013; Lara-Reséndiz et al., 2015; Gilbert and Miles, 2017; Diele-Viegas et al., 2018). We conducted measurements of thermal preference between one-two days after capture. Individual lizards were placed in the center of the gradient and allowed one hour to acclimate. We then recorded the T_b of each lizard every hour during the activity period (from 10 to 17 h). Our estimate of T_{pref} was the interquartile range (between the 25% and 75% quartile) (Hertz, Huey and Stevenson, 1993).

We estimated an index of thermal efficiency following the protocol of Hertz et al. (1993). This protocol is based on the accuracy of thermoregulation (d_b) and the thermal quality of the habitat (d_e). We calculated d_b as the mean of the absolute deviation between T_b and the quartile range of

T_{pref} . If $T_b < T_{\text{pref}}$, then $d_b = T_{\text{pref}25} - T_b$ and if $T_b > T_{\text{pref}}$ quartile range, then $d_b = T_b - T_{\text{pref}75}$. When T_b values were within the quartile range of T_{pref} , d_b was set to zero. High values of d_b indicate a low accuracy of thermoregulation and values equal to or near zero indicate high accuracy of thermoregulation. We determined the thermal quality of the environment (d_e) using operative environmental temperatures (T_e) obtained during the periods of lizard activity (see below). High values of d_e indicate low thermal quality of the habitat and values equal to or near zero indicate ideal thermal environments. We calculated the effectiveness of thermoregulation (E) using the equation $E = 1 - (d_b/d_e)$, using the averages of (d_b) and (d_e), we calculated E for each season for each population. An E value equal or close to zero indicates that the organisms is a thermoconformer, whereas values of E close to 1.0 indicates that the organisms is a perfect thermoregulator. However, negative values are possible when organisms avoid favorable thermal microhabitats, i.e., mean $d_b > \text{mean } d_e$ (Brown and Weatherhead, 2000; Medina et al., 2016). Avoidance of favorable thermal microhabitats may be a consequence of the presence of predators or reduced food availability (Hertz, Huey and Stevenson, 1993; Blouin-Demers and Nadeau, 2005). In addition, we calculated the index of thermoregulation proposed by Blouin-Demers and Weatherhead (2001), which estimates the effectiveness of thermoregulation by determining the magnitude of deviation from thermoconformity by subtracting to the accuracy of thermoregulation from the thermal quality of the environment ($d_e - d_b$). Finally, we compared E values between populations and among seasons using bootstrap estimates. To do so we obtained 1000 E values by bootstrapping using d_b average for each season and 1000 iterations of d_e (Hertz, Huey and Stevenson, 1993; Lara-Reséndiz et al., 2015; Ortega, Mencía and Pérez-Mellado, 2016).

Operative environmental temperatures and calibration

We obtained operative temperatures (T_e) using a Hobo®ProV2 data logger and lizard models. One data logger with two sensors was deployed in each population in the sites used by *B. imbricata*; one sensor was placed exposed to the sun and the other one in the shade, we assumed that there was a spectrum of temperature between these two extremes. Each sensor was connected to a model mimicking the size and reflectivity of *B. imbricata*. The models were placed in microsites where we observed active lizards (Hertz, Huey and Stevenson, 1993; Besson and Cree, 2010; Sinervo et al., 2010; Lara-Reséndiz, Arenas-Moreno and Méndez-de la Cruz, 2013; Lara-Reséndiz et al., 2014). T_e was recorded every 10 minutes. We sampled T_e at Ocotepéc from spring 2016 to winter 2017 and obtained 17 783 temperature records (865 in spring, 1756 in summer, 5388 in autumn and 9774 in winter). We deployed lizard models at Paso de Cortés from autumn 2015 to summer 2016 and recorded 19 974 temperature records (5772 in autumn, 5678 in winter, 7070 in spring, and 1454 in summer). Our estimates of T_e are based on sampling seasons consecutively. That is, at Ocotepéc we used T_e from spring, summer and autumn 2016 and T_e from winter (December of 2016 to March of

2017). At the Paso de Cortés population we recorded T_e from autumn 2015 to summer 2016.

We compared multiple types of models to as part of our calibration study. We constructed ten types of models using copper or polyvinylchloride (PVC) tubing. Some models were painted gray and filled with silicone whereas others were not. The models were calibrated by comparison with equilibrium T_b of a lizard during their times of activity. We recorded the temperature of two lizards and the different models every 30 minutes between microhabitats that had direct exposure to the sun and areas with shade. A comparison based on a correlation of Pearson revealed the best null model was a PVC cylinder filled with silicone and painted 33% gray. The correlation of the null model with T_b was significant and positive ($r = 0.961$, $P < 0.01$; $r^2 = 0.923$, $y = 0.3514 + 1.0244 * x$).

Statistical analyses

Prior to conducting the statistical analyses, we verified that the data met the assumptions of linear models. We tested assumptions of normality using a Kolmogorov-Smirnov test and for homogeneity of variances using a Levene's test. Data for T_b were normally distributed and homoscedastic, with the exception of T_b of the low altitude population, which were normal but not homoscedastic. We assessed the association between the following pairs of thermal variables $T_e - T_b$, $T_b - T_a$, and $T_b - T_c$ using Pearson product-moment correlations. We had thermal data for each population from multiple years. However, our objective was to examine thermal adaptation between populations. In particular we were interested in determining whether thermal traits varied among seasons and sex/reproductive state. Therefore, we compared the variation in T_b among populations using a Generalized Linear Mixed Model (GLMM). We used T_b as a response variable. We included population, sex and season as fixed effects. We treated year as a random effect in the model. This approach allowed us to sequester annual variation prior to comparing differences among the fixed effects. We used the `lmer` function in the R package `nlme` (Pinheiro et al., 2018) to complete the glmm analysis. We determined the significance of the random effect using the function `exactLRT` in the package `RLRsim` (Scheipl and Bolker, 2016).

Kruskal-Wallis tests were used to compare mean T_{pref} among populations. A Multivariate ANOVA with repeated measures was used when the T_{pref} were normal. The original data of d_b , d_e , and E were subsequently resampled by bootstrapping, and the distribution of E from each population were compared using a Kolmogorov-Smirnov test. We used `Statistica-7`, `Stata-13.0`, and `R version 3.5.1` (R Core Team, 2018) for statistical analyses.

Results

Our surveys resulted in the capture of 268 individuals of *B. imbricata*, 125 from the low altitude population and 143 in the high altitude

Table 1. Sample size for T_b (n_{T_b}), mean body temperature with standard deviation (T_b), minimal active body temperature (T_b min), sample size for T_{pref} (n_{pref}), quartile range for T_{pref} : (T_{pref25} - T_{pref75}) effectiveness of thermoregulation (E), accuracy of thermoregulation (d_b), habitat thermal quality (d_e) (Hertz, Huey and Stevenson, 1993) and index of Blouin-Demers and Weatherhead (2001) ($d_e - d_b$), for the low altitude population Ocotepc (2200 m) and high altitude population Paso de Cortés (3700 m).

	n_{T_b}	$T_b \pm SD$ °C	T_b min °C	n_{pref}	T_{pref25} - T_{pref75} °C	E	d_b	d_e	$d_e - d_b$
2200 m									
Spring	37	20.9 ± 4.51	15.2	37	28.1-30.6	0.31	7.2	10.4	3.2
Summer	26	21.5 ± 5.58	12	21	28.1-30.6	0.12	6.8	7.8	1
Autumn	25	20.1 ± 4.54	14.5	26	25-30.3	0.25	5.56	7.38	1.82
Winter	23	17.6 ± 6.60	9.5	24	28.5-31.3	-0.20	11.32	9.40	-1.92
3700 m									
Spring	38	19.0 ± 5.73	9.6	32	26-30.7	0.47	7.5	14.2	6.7
Summer	28	24.8 ± 3.96	17.2	27	25.8-30.9	0.86	2.2	15.9	13.7
Autumn	58	25.5 ± 4.04	9.1	41	25.2-30.7	0.92	1.3	16.8	15.5
Winter	12	24.8 ± 3.96	18.5	11	26.8-30.3	0.84	2.7	17.6	14.9

population. Only 247 individuals were included in the analyses (table 1). We did not find a significant difference in T_b between the sexes and reproductive categories (juvenile, males, non-reproductive females and pregnant females) in either population ($F_{2,94} = 0.44$, $P = 0.6436$ for the low altitude population, and $F_{3,112} = 0.66$, $P = 0.5816$ for the high altitude population).

The generalized linear mixed model resulted in a significant random effect for year. A likelihood ratio test comparing the mixed model with a fixed effects model only, based on 1000 simulations, resulted in rejecting the latter model (LRT = 11.39, $P < 0.0001$). The GLMM verified no difference in T_b between sex and reproductive category ($\chi^2 = 0.89$, $df = 1$, $P = 0.35$). However, there was a significant population \times season interaction ($\chi^2 = 15.97$, $P < 0.0001$). The mean T_b of the high altitude population varied between $19.0^\circ\text{C} \pm 5.73$ and $25.5^\circ\text{C} \pm 4.04$ and paradoxically was higher than the low altitude population whose mean T_b varied between $17.6^\circ\text{C} \pm 6.60$ and $21.5^\circ\text{C} \pm 5.58$ (table 1). In the low altitude population the mean T_b in winter ($17.6^\circ\text{C} \pm 6.60$) was different that in spring ($20.9^\circ\text{C} \pm 4.51$, $P < 0.05$) and in summer ($21.5^\circ\text{C} \pm 5.58$, $P < 0.05$), but similar in autumn ($20.1^\circ\text{C} \pm 4.54$, $P = 0.354$). In the high altitude population the T_b was different only during spring $19^\circ\text{C} \pm 5.73$, while during summer ($24.8^\circ\text{C} \pm 3.96$, $P < 0.05$), autumn

($25.5^\circ\text{C} \pm 4.04$, $P < 0.05$) and winter ($24.8^\circ\text{C} \pm 3.96$, $P < 0.05$) lizard had similar T_b (table 1). Notably, we detected no differences in mean T_b between populations during spring.

Active individuals in the low altitude population were observed at temperatures between 9.5°C - 32.3°C , and in the high altitude population between 9.1°C - 36.6°C . It is relevant to note that 25 individuals from the low altitude population and 15 from the high altitude population showed temperatures lower than 16°C (fig. 1) during the day and not only in the early morning.

Both populations showed positive and significant correlation between environmental temperatures throughout the four seasons, with the exception of T_b - T_s for the high altitude population during winter (table 2).

We compared T_{pref} among the demographic categories for each season and could not detect a significant difference between the populations. The results for the low altitude population were: spring ($H_2 = 0.424$, $P = 0.809$), summer ($H_2 = 0.907$, $P = 0.953$), autumn ($H_2 = 1.407$, $P = 0.495$) and winter ($H_2 = 1.518$, $P = 0.468$); and for the high altitude population, spring ($H_2 = 1.897$, $P = 0.387$), summer ($H_2 = 1.508$, $P = 0.471$), autumn ($H_2 = 5.722$, $P = 0.057$) and winter ($F_{3,4} = 1.683$, $P = 0.307$). Therefore, we pooled the

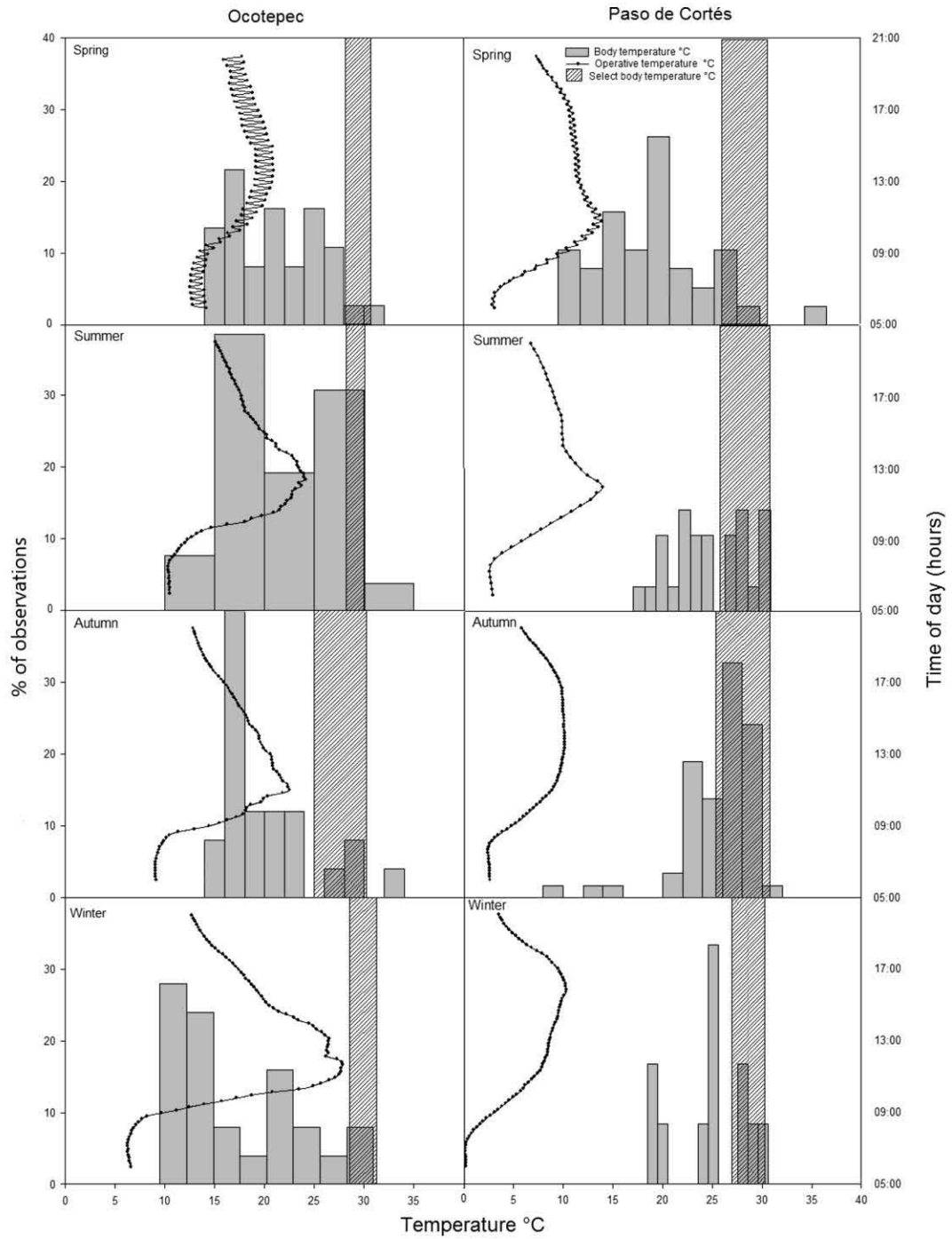


Figure 1. Percentage of observations for T_b (bars), mean of T_e for a typical day from each season (points), quartile range of T_{pref} (striped columns). Values for the Ocotepéc population are presented and the Paso de Cortés population on the right. Each graph represents a different season of the year.

Table 2. Correlations between environmental temperatures (substrate: T_s , and air: T_a), and body temperature (T_b) of *Barisia imbricata*.

	T_b-T_a	T_b-T_s
2200 m		
Spring	$r = 0.7009, P < 0.05$	$r = 0.7007, P < 0.05$
Summer	$r = 0.8373, P < 0.05$	$r = 0.8238, P < 0.05$
Autumn	$r = 0.5604, P < 0.05$	$r = 0.7865, P < 0.05$
Winter	$r = 0.7986, P < 0.05$	$r = 0.5764, P < 0.05$
3700 m		
Spring	$r = 0.6393, P < 0.05$	$r = 0.6953, P < 0.05$
Summer	$r = 0.4533, P < 0.05$	$r = 0.5691, P < 0.05$
Autumn	$r = 0.4356, P < 0.05$	$r = 0.5873, P < 0.05$
Winter	$r = 0.6762, P < 0.05$	$r = 0.5438, P = 0.067$

T_{pref} data among seasons to quantify the thermal indices. Note that with the exception of individuals captured in autumn at the high altitude site, the mean values of field T_b were lower than T_{pref} .

The periods of activity through the seasons differed and this was evaluated with respect to T_e , which we used to calculate the thermoregulation indices (fig. 1). The low altitude population showed low effectiveness of thermoregulation in spring, summer, autumn and winter with values of E all below 0.5. Note the value for thermoregulatory effectiveness in winter was negative. In contrast, individuals from the high altitude population had values of E consistent with a pattern of behavioral thermoregulation in summer, autumn and winter. Yet, these individuals showed low accuracy of thermoregulation (d_b) and low thermal quality of the environment (d_e). In contrast, during spring the individuals thermoregulated less and showed an even lower d_b and d_e (table 1; Hertz, Huey and Stevenson, 1993; Blouin-Demers and Weatherhead, 2001). The efficiency indices obtained with the bootstrap were statistically different among seasons in both populations (supplementary tables S1 and S2).

Discussion

Our analyses revealed distinct seasonal patterns of thermoregulation between populations

of *Barisia imbricata*. No differences were found in T_b between individuals in different demographic categories, which is consistent with results from Lemos-Espinal et al. (1998) and Muñoz-Brito (2013). These studies found no differences in T_b among males, non-reproductive females and pregnant females. Nevertheless, we only found pregnant females in high altitude during winter and spring. Pregnant females were more precise in their T_b (from 15.3° to 22.5°C), resulting in the highest values for T_b min (table 1). Also, it is important to note that the winter samples had T_b values lower than during the rest of the year, because of the low environmental temperatures, which reached 0°C during the activity period. We suggest additional studies that focus on pregnant females to determine whether their thermoregulatory behaviour is different from nonpregnant females.

In both populations, lizards exhibited positive and significant correlations between body and environmental temperatures. These results are also consistent with those reported by Lemos-Espinal et al. (1998), where a *B. imbricata* population at 3400 m had a higher correlation of T_b with T_s ($r^2 = 0.43$) than with T_a ($r^2 = 0.30$). In a separate study, Muñoz-Brito (2013) reported a slightly higher correlation of T_b with T_a ($r = 0.453$) than with T_s ($r = 0.427$).

There were significant differences in T_b among seasons for both populations. This pattern is congruent with that of Lemos-Espinal et al. (1998), who reported a mean T_b of 26.6°C (with a range from 13.4° to 34.1°C), although their estimate was slightly higher than ours. On the other hand, Muñoz-Brito (2013) reported an average T_b of 22.43°C ($n = 45$), which is within the range observed in this study. Yet, Muñoz-Brito (2013) did not find a significant difference between seasons.

Two general approaches have been proposed to explain the differences in the thermal biology of lizards. The first approach proposes that optimal temperatures are evolutionarily conserved within species. However,

thermoregulatory behaviour through microhabitat selection can allow individuals to compensate for thermal differences among sites along an elevation gradient. That is, behavioral plasticity buffers individuals against environment variation (Huey, 1982; Lemos-Espinal and Ballinger, 1995; Huey, Hertz and Sinnero, 2003; Angilletta, 2009). Thus, a species should not exhibit different T_b at different altitudes in different seasons, because they select different microhabitats that modulates variation in T_b . For example, two populations of *Sceloporus grammicus* exhibited similar values for T_b (31.5°C) (Lemos-Espinal and Ballinger, 1995). In contrast, a second hypothesis suggests that thermal biology of lizards should reflect local adaptation to different thermal environments (Lemos-Espinal and Ballinger, 1995; Angilletta, 2009; Rodríguez-Serrano, Navas and Bozinovic, 2009; Gabirot et al., 2013). Therefore, some populations could exhibit differences in T_b when occupying different habitats, as is the case with *B. imbricata* in our study.

Some individuals of *B. imbricata* were active even at low temperatures (table 1), which may have been an advantage for reducing the risk of predation (fig. 2). We observed a species of rattlesnake at each site during the study, which is a possible predator. At the high altitude site, individuals of *Crotalus triseriatus triseriatus* were observed only when T_a was benign and there was not much wind. In contrast, we observed individuals of *Crotalus ravus* at the low altitude population when the sun had already heated the substrate. There is little information about these species in literature. However, it is known that the mean active T_b of *C. triseriatus triseriatus* is 22.3°C (Lara-Reséndiz et al., 2014) and the minimum active body temperature ($T_{b \text{ min}}$) is 10°C (A.J.L. Jaramillo, pers. comm.); for *C. ravus* the mean active T_b is 25.6°C and $T_{b \text{ min}}$ is 23.2°C (G.A. Woolrich-Piña, pers. comm.). Another explanation is that predators adjust their activity patterns to coincide with the eurythermal behaviour of *B. imbricata*. We find this

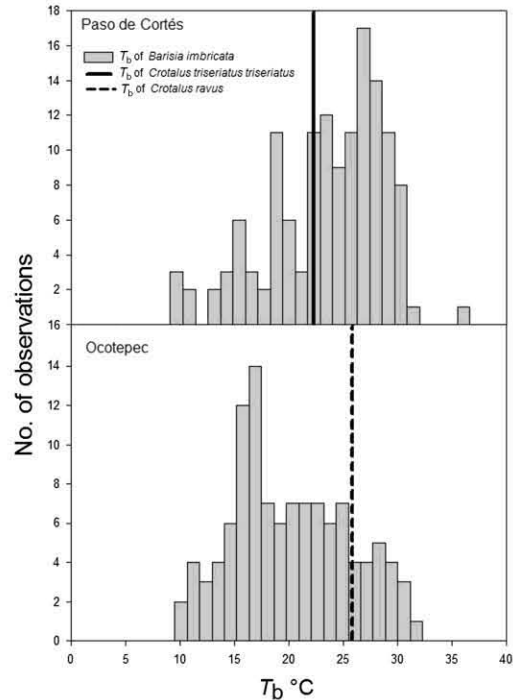


Figure 2. Observations of *Barisia imbricata* body temperatures (T_b), mean of T_b from *Crotalus triseriatus triseriatus* and *Crotalus ravus*. Paso de Cortés (top) and Ocoteppec (bottom).

topic extremely interesting and suggest this issue should be the focus of future research.

Our results suggest that *B. imbricata* is an eurythermic species. This corresponds with other species in the lizard family Anguillidae. For example, *Elgaria multicarinata* exhibits a broad range of T_b 's during activity, which may range from 18° to 33°C (Kingsbury, 1993). The T_b of *Abronia taeniata* spans from 13° to 30.6°C; moreover, this species exhibits significant differences in T_b across seasons (Fierro-Estrada, 2013). The eurythermic behaviour of *B. imbricata* could provide an advantage in relation to sympatric species of lizards at each site. We recorded the presence of four different species of *Sceloporus* and a skink species, *Plestiodon copei*, coexisting with *B. imbricata*. Our results showed that *B. imbricata* could initiate activity at T_b as low as 9.1°C and had T_{pref} values between 25° and 31.3°C (table 1). Meanwhile, the syntopic lizards did not have active T_b 's

Table 3. Comparison of species in sympatry with *B. imbricata*. Populations PC (Paso de Cortés) or Oc (Ocotepéc), mean of body temperature (T_b), minimal active body temperature ($T_{b \text{ min}}$), the thermal preferences (T_{pref}) and no data (ND).

Species	Area	T_b °C	$T_{b \text{ min}}$ °C	T_{pref} °C	References
<i>Sceloporus aeneus</i>	PC	32	14.6	33.8-35.3	Andrews et al., 1999 Lara-Reséndiz et al., 2014
<i>Sceloporus grammicus</i>	Oc, PC	31.5	28.6	30.8-35.0	Lemos-Espinal and Ballinger, 1995 Woolrich-Piña et al., 2006 Lara-Reséndiz et al., 2014
<i>Sceloporus bicanthalis</i>	PC	30	23.1	34.7-35.2	Andrews et al., 1999
<i>Sceloporus megalepidurus</i>	Oc	31.3	28.5	34.0-35.9	F.R. Méndez-de la Cruz (unpubl. results)
<i>Plestiodon copei</i>	PC	ND	ND	32.1-35.1	Lara-Reséndiz et al., 2014
<i>Crotalus triseriatus triseriatus</i>	PC	22.3	10.0	ND	Lara-Reséndiz et al., 2014 A.J.L. Jaramillo (pers. comm.)
<i>Crotalus ravus</i>	Oc	25.6	23.2	ND	G.A. Woolrich-Piña (pers. comm.)

as low: *Sceloporus spp.* (mean field active T_b 31.2°, T_{pref} 30.8°-35.9°C) and *Plestiodon spp.* (T_{pref} 32.1°-35.1°C). It is possible that *B. imbricata* has more hours of activity available, which grant an advantage over other species of lizards (table 3), or may be they occupy different microhabitats to avoid competition.

We used one data logger with two null models for each study site, but we followed the methodology proposed in previous studies by other researchers and we measured temperatures under extreme conditions such as sun and shade assuming that the measures of contrasting conditions showed the complete range of temperatures from each site (Besson and Cree, 2010; Sinervo et al., 2010; Lara-Reséndiz et al., 2015; Artacho et al., 2017b). In addition, we would like to point out that we considered that the whole range of available temperatures was recorded using these two models. The lack of gaps between the data showed that we captured the range of available operative temperatures. Nevertheless, the more models deployed, the more data will characterize the available operative temperatures.

In this study, the individuals at the low altitude population tend to be thermoconformers. This may be a consequence of the high thermal quality in this environment. The value for d_e was higher in comparison to the individuals of the high altitude population. It is striking to note that the high altitude lizards demonstrated

active thermoregulation despite having the lowest temperatures recorded (0°C) during the activity period. This agrees with Lara-Reséndiz et al. (2014), who reported higher d_e of populations of *B. imbricata* in forests ($d_e = 7.1$ at 2320 m and 5.4 at 2540 m) compared to montane populations ($d_e = 14.0$ at 3220 m and 11.9 at 3530 m). Lara-Reséndiz et al. (2014) used the interval of T_{pref} reported by Muñoz-Brito (2013) that varied between 26.1° to 30.5°C, which matches our results. This behaviour is similar to that of *S. grammicus*, whose individuals at a low altitude experience more benign thermal conditions, which results in a thermoconformer behaviour. On the other hand, high altitude lizards must face harsh environmental conditions and behave as a thermoregulator to maintain a T_b that permits optimal to near optimal physiological performance (Lemos-Espinal and Ballinger, 1995).

The negative value of effectiveness of thermoregulation observed during winter suggests that during this season, lizards avoided favorable thermal habitats. One explanation is that individuals selected lower quality thermal habitats that had a lower risk of encountering predators (Hertz, Huey and Stevenson, 1993; Blouin-Demers and Nadeau, 2005). This pattern has been reported in *Liolaemus magellanicus*, where the females have a negative value for E , while the males have a positive value (Ibargüen-goytía et al., 2010).

In conclusion, the lizard *Barisia imbricata* can actively thermoregulate at any given time. However, when environmental temperatures reach the preferred temperatures, individuals may thermoregulate less or not at all. Our data suggest *B. imbricata* is an eurythermic species with mean body temperatures differing among seasons and between populations. However, we found no evidence for differences in preferred temperatures even in populations located in the extremes of its distribution.

Acknowledgements. We thank Posgrado en Ciencias Biológicas, UNAM, and CONACyT for the scholarship awarded to N. Fierro-Estrada (412836) and UNAM PAPIIT/DGAPA (IN210116) for financial support. We thank E. Peralta, C. Flores, D. Arenas and J. Arellano that provided help during the fieldwork, F. Muñoz for the help in the statistical analyses, and C. Tec for providing helpful comments on the manuscript. O. Maldonado provided the facilities for fieldwork. We also thank L. Jaramillo and G. Woolrich for providing body temperature data of rattlesnakes. Permits for the research were approved by CONANP (DRCEM/PNIP/823/2014) for the Parque Nacional Iztaccihuatl-Popocatepetl. The project was submitted to SEMARNAT and permission was granted (01629) to FRMC. Miles was supported by a grant from the National Science Foundation (EF1241848). This article is a requirement for obtaining the degree of Doctora en Ciencias del Posgrado en Ciencias Biológicas, UNAM for Fierro-Estrada N. The manuscript has been improved by the comments from several reviewers.

References

- Andrews, R.M., Méndez-de la Cruz, F.R., Villagrán-Santa Cruz, M., Rodríguez-Romero, F. (1999): Field and selected body temperatures of the lizards *Sceloporus aeneus* and *Sceloporus bicanthalis*. *J. Herpetol.* **33**: 93-100.
- Angilletta, M.J. (2009): *Thermal Adaptation*. Oxford University Press.
- Artacho, P., Saravia, J., Perret, S., Bartheld, J.L., Le Galliard, J.F. (2017a): Geographic variation and acclimation effects on thermoregulation behavior in the widespread lizard *Liolaemus pictus*. *J. Therm. Biol.* **63**: 78-87.
- Artacho, P., Saravia, J., Perret, S., Bartheld, J.L., Le Galliard, J.F. (2017b): Geographic variation and acclimation effects on thermoregulation behavior in the widespread lizard *Liolaemus pictus*. *J. Therm. Biol.* **63**: 78-87.
- Beal, M.S., Lattanzio, M.S., Miles, D.B. (2014): Differences in the thermal physiology of adult Yarrow's spiny lizards (*Sceloporus jarrovi*) in relation to sex and body size. *Ecol. Evol.* **2**: 4220-4229.
- Besson, A.A., Cree, A. (2010): A cold-adapted reptile becomes a more effective thermoregulator in a thermally challenging environment. *Oecologia* **163**: 571-581.
- Blouin-Demers, G., Nadeau, P. (2005): The cost-benefit model of thermoregulation does not predict lizard thermoregulatory behavior. *Ecology* **86**: 560-566.
- Blouin-Demers, G., Weatherhead, P.J. (2001): Thermal ecology of black rat Snakes (*Elaphe obsoleta*) in a thermally challenging environment. *Ecology* **82**: 3025-3043.
- Brown, G.P., Weatherhead, P.J. (2000): Thermal ecology and sexual size dimorphism in northern water snakes, *Nerodia sipedon*. *Ecol. Monogr.* **70**: 311-330.
- Caruccio, R., Vieira, R.C., Verrastro, L., Machado, D.M. (2011): Thermal biology, activity, and population parameters of *Cnemidophorus vacariensis* (Squamata, Teiidae), a lizard endemic to southern Brazil. *Iheringia, Série Zool.* **101**: 283-295.
- Catenazzi, A., Carrillo, J., Donnelly, M. (2005): Seasonal and geographic eurythermy in a coastal Peruvian lizard. *Copeia* **2005**: 713-723.
- Cowles, R.B., Bogert, C.M. (1944): A preliminary study of the thermal requirements of desert reptiles. *Am. Museum Nat. Hist.* **83**: 261-296.
- Currie, R.J., Bennett, W.A., Beitinger, T.L. (1998): Critical thermal minima and maxima of three freshwater gamefish species acclimated to constant temperatures. *Environ. Biol. Fishes* **51**: 187-200.
- Diele-Viegas, L.M., Vitt, L.J., Sinervo, B., Colli, G.R., Werneck, F.P., Miles, D.B., Magnusson, W.E., Santos, J.C., Sette, C.M., Caetano, G.H.O., Pontes, E., Ávila-Pires, T.C.S. (2018): Thermal physiology of Amazonian lizards (Reptilia: Squamata). *PLoS One* **13**: 1-23.
- Dubois, Y., Blouin-Demers, G., Shipley, B., Thomas, D. (2009): Thermoregulation and habitat selection in wood turtles *Glyptemys insculpta*: chasing the sun slowly. *J. Anim. Ecol.* **78**: 1023-1032.
- Fierro-Estrada, N. (2013): *Ecología térmica de Abronia taeniata* (Reptilia: Anguillidae) y su susceptibilidad ante el calentamiento global. Universidad Nacional Autónoma de México.
- Gabirot, M., Ballerí, A., López, P., Martín, J. (2013): Differences in thermal biology between two morphologically distinct populations of Iberian wall lizards inhabiting different environments. *Ann. Zool. Fennici* **50**: 225-236.
- Gilbert, A.L., Miles, D.B. (2016): Food, temperature and endurance: effects of food deprivation on the thermal sensitivity of physiological performance. *Funct. Ecol.* **30**: 1790-1799.
- Gilbert, A.L., Miles, D.B. (2017): Natural selection on thermal preference, critical thermal maxima and locomotor performance. *Proc. R. Soc. B Biol. Sci.* **284**: 1-8.
- Guillette, L.J., Smith, H.M. (1982): A review of the Mexican lizard *Barisia imbricata*, and the description of a new subspecies. *Kansas Acad. Sci.* **85**: 13-33.
- Guillette, L.J., Casas-Andreu, G. (1987): The reproductive biology of the high elevation Mexican lizard *Barisia imbricata*. *Herpetologica* **43**: 29-38.

- Gutiérrez, J.A., Krenz, J.D., Ibargiengoytia, N.R. (2010): Effect of altitude on thermal responses of *Liolaemus pictus argentinus* in Argentina. *J. Therm. Biol.* **35**: 332-337.
- Gvozdík, L. (2002): To heat or to save time? Thermoregulation in the lizard *Zootoca vivipara* (Squamata: Lacertidae) in different thermal environments along an altitudinal gradient. *Can. J. Zool.* **80**: 479-492.
- Hertz, P.E., Huey, R.B., Stevenson, R.D. (1993): Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. *Am. Nat.* **142**: 796-818.
- Huang, S., Huang, S., Chen, Y., Tu, M. (2007): Thermal tolerance and altitudinal distribution of three *Trimeresurus* snakes (Viperidae: Crotalinae) in Taiwan. *Zool. Stud.* **46**: 592-599.
- Huey, R.B. (1974): Behavioral thermoregulation in lizards: importance of associated costs. *Science* **184**: 1001-1003.
- Huey, R.B. (1982): Temperature, physiology, and the ecology of reptiles. In: *Biology of the Reptilia*, p. 25-91. Gans, C., Pough, F.H., Eds, Academic Press, London.
- Huey, R.B., Bennet, A.F. (1987): Phylogenetic studies of coadaptation: preferred temperature versus optimal performance temperatures of lizards. *Evolution (N.Y.)* **41**: 1098-1115.
- Huey, R.B., Hertz, P.E., Sinervo, B. (2003): Behavioral drive versus behavioral inertia in evolution: a null model approach. *Am. Nat.* **161**: 357-366.
- Huey, R.B., Pianka, E.R. (1977): Seasonal variation in thermoregulatory behavior and body temperature of Diurnal Kalahari lizards. *Ecology* **58**: 1066-1075.
- Huey, R.B., Slatkin, M. (1976): Cost and benefits of lizard thermoregulation. *Q. Rev. Biol.* **51**: 363-384.
- Huey, R.B., Webster, P. (1975): Thermal biology of a solitary lizard: *Anolis marmoratus* of Guadeloupe, Lesser Antilles. *Ecology* **56**: 445-452.
- Ibargiengoytia, N.R., Marlin Medina, S., Fernández, J.B., Gutiérrez, J.A., Tappari, F., Scolaro, A. (2010): Thermal biology of the southernmost lizards in the world: *Liolaemus sarmientoi* and *Liolaemus magellanicus* from Patagonia, Argentina. *J. Therm. Biol.* **35**: 21-27.
- Kingsbury, B.A. (1993): Thermoregulatory set points of the eurythermic lizard *Elgaria multicarinata*. *J. Herpetol.* **27**: 241-247.
- Labra, A., Soto-Gamboa, M., Bozinovic, F. (2001): Behavioral and physiological thermoregulation of Atacama desert-dwelling *Liolaemus* lizards. *Ecoscience* **8**: 413-420.
- Lara-Reséndiz, R.A., Arenas-Moreno, D.M., Méndez-de la Cruz, F.R. (2013): Termoregulación diurna y nocturna de la lagartija *Phyllodactylus bordai* (Gekkotata: Phyllodactylidae) en una región semiárida del centro de México. *Rev. Chil. Hist. Nat.* **86**: 127-135.
- Lara-Reséndiz, R.A., Gadsden, H., Rosen, P.C., Sinervo, B., Méndez-De la Cruz, F.R. (2015): Thermoregulation of two sympatric species of horned lizards in the Chihuahuan Desert and their local extinction risk. *J. Therm. Biol.* **48**: 1-10.
- Lara-Reséndiz, R.A., Larraín-Barrios, B.C., Díaz De La Vega-Pérez, A.H., Méndez-De La Cruz, F.R. (2014): Calidad térmica a través de un gradiente altitudinal para una comunidad de lagartijas en la sierra del Ajusco y el Pedregal de San Ángel, México. *Rev. Mex. Biodivers.* **85**: 885-897.
- Lemos-Espinal, J., Smith, G.R., Ballinger, R.E. (1998): Temperature relationships of the lizard, *Barisia imbricata*, from México. *Amphibia-Reptilia* **19**: 95-99.
- Lemos-Espinal, J.A., Ballinger, R.E. (1995): Comparative thermal ecology of the high altitude lizard *Sceloporus grammicus* on the eastern slope of the Iztaccihuatl Volcano, Puebla, Mexico. *Can. J. Zool.* **73**: 2184-2191.
- Mathies, T., Andrews, R.M. (1997): Influence of pregnancy on the thermal biology of the lizard, *Sceloporus jarrovi*: why do pregnant females exhibit low body temperatures? *Funct. Ecol.* **11**: 498-507.
- Medina, M., Fernández, J.B., Charruau, P., Méndez de la Cruz, F., Ibargiengoytia, N. (2016): Vulnerability to climate change of *Anolis allisoni* in the mangrove habitats of Banco Chinchorro Islands, Mexico. *J. Therm. Biol.* **58**: 8-14.
- Méndez de la Cruz, F., Marínquez Moran, N.L., Arenas Ríos, E., Ibargiengoytia, N. (2014): Male reproductive cycles in lizards. In: *Reproductive Biology and Phylogeny of Lizards and Turtles*, p. 302-339. Rheubert, J., Siegel, L., Trauth, D.S., Eds, CRC Press.
- Mesquita, D.O., Costa, G.C., Colli, G.R., Costa, T.B., Shepard, D.B., Vitt, L.J., Pianka, E.R. (2016): Life-history patterns of lizards of the world. *Am. Nat.* **187**: 689-705.
- Muñoz-Brito, A. (2013): Termoregulación en *Barisia imbricata* (Sauria: Anguillidae). Universidad Nacional Autónoma de México.
- Muñoz, M.M., Losos, J.B. (2018): Thermoregulatory behavior simultaneously promotes and forestalls evolution in a tropical lizard. *Am. Nat.* **191**: E15-E26.
- Ortega, Z., Mencia, A., Pérez-Mellado, V. (2016): Behavioral buffering of global warming in a cold-adapted lizard. *Ecol. Evol.* **6**: 4582-4590.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Heisterkamp, S., Van Willigen, B. (2018): nlme. Linear and Nonlinear Mixed Effects Models Description. version 3.1-137.
- Rock, J., Andrews, R.M., Cree, A. (2000): Effects of reproductive condition, season, and site on selected temperatures of a viviparous gecko. *Physiol. Biochem. Zool. Ecol. Evol. Approaches* **73**: 344-355.
- Rodríguez-Serrano, E., Navas, C.A., Bozinovic, F. (2009): The comparative field body temperature among *Liolaemus* lizards: testing the static and the labile hypotheses. *J. Therm. Biol.* **34**: 306-309.
- Scheipl, F., Bolker, B. (2016): RLRsim. R package for fast and exact (restricted) likelihood ratio tests for mixed and additive models. Version 3.1-3.
- Sepúlveda, M., Vidal, M.A., Fariña, J.M., Sabat, P. (2008): Seasonal and geographic variation in thermal biology of the lizard *Microlophus atacamensis* (Squamata: Tropiduridae). *J. Therm. Biol.* **33**: 141-148.
- Sinervo, B., Méndez-de-la-Cruz, F., Miles, D.B., Heulin, B., Bastiaans, E., Villagran-Santa Cruz, M., Lara-Reséndiz, R., Martínez-Mendez, N., Calderon-Espinosa, M.L.,

- Meza-Lazaro, R.N., Gadsden, H., Avila, L.J., Morando, M., De la Riva, I.J., Sepulveda, P.V., Rocha, C.F.D., Ibarquengoytia, N., Puntriano, C.A., Massot, M., Lepetz, V., Oksanen, T.A., Chapple, D.G., Bauer, A.M., Branch, W.R., Clobert, J., Sites, J.W. (2010): Erosion of lizard diversity by climate change and altered thermal niches. *Science* **328**: 894-899.
- Smith, G.R., Ballinger, R.E. (1994): Temperature relationships in the high-altitude viviparous lizard, *Sceloporus jarrovi*. *Am. Midl. Nat.* **131**: 181-189.
- Weeks, D.M., Espinoza, R.E. (2013): Lizards on ice: comparative thermal tolerances of the world's southernmost gecko. *J. Therm. Biol.* **38**: 225-232.
- Willmer, P., Stone, G., Johnston, I. (2005): *Environmental Physiology of Animals*. Blackwell Publishing.
- Woolrich-Piña, G., Lemos-Espinal, J., Oliver-López, L., Calderón Méndez, M.E., González-Espinoza, J.E., Correa-Sánchez, F., Montoya Ayala, R. (2006): Ecología térmica de una población de la lagartija *Sceloporus grammicus* (Iguanidae: Phrynosomatinae) que ocurre en la zona centro-oriente de la ciudad de México. *Acta Zoológica Mex.* **22**: 137-150.
- Zamora-Camacho, F.J., Reguera, S., Moreno-Rueda, G. (2016): Elevational variation in body-temperature response to immune challenge in a lizard. *PeerJ* **4**: e1972.

Submitted: October 26, 2018. Final revision received: December 21, 2018. Accepted: February 5, 2019.
Associate Editor: Miguel A. Carretero.

Capítulo 2

Survival to freezing and glucose of the Mexican lizard *Barisia imbricata* (Squamata: Anguidae).

Survival to freezing and glucose of the Mexican lizard *Barisia imbricata* (Squamata: Anguidae).

Natalia Fierro-Estrada^{a,b}, Donald B. Miles^c, Barry Sinervo^d and Fausto R. Méndez de la Cruz^b

^aPosgrado en Ciencias Biológicas, Instituto de Biología, Universidad Nacional Autónoma de México, A.P. 70-153, C.P. 04510 Ciudad de México, México.

^bLaboratorio de Herpetología, Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, A. P. 70515, C.P. 04510 Ciudad de México, México.

^cDepartment of Biological Sciences, Ohio University, Athens, Ohio, USA.

^dDepartment of Ecology and Evolutionary Biology, Coast Biology Building, 130 McAllister Way, University of California, Santa Cruz, CA 95060.

Correspondence: natalia_fierro@hotmail.com

ABSTRACT

Environmental temperature determines locomotor performance in ectotherms. Maximum performance occurs at close to optimum temperature. When environmental temperatures decrease, locomotor performance is expected to decrease, up to the point when temperatures reach freezing temperatures for an individual. Lizards have two mechanisms to survive freezing and these two processes depend on cryoprotective molecules, such as glucose. High levels of glucose produce that organisms have lower crystallization points and this level can double after freezing. We measured glucose levels seasons in the viviparous lizard *Barisia imbricata* and compared the values among seasons and between two populations at different elevations (2,200 and 3,700 m). Also, we froze lizards in autumn and winter from the two populations. We did not find differences in glucose levels among seasons or between populations. The mean crystallization point of *B. imbricata* was -4.13°C . Glucose levels in the lizards increased after the first freeze in autumn. In addition, all lizards survived freezing. Our results suggest that *B.*

imbricata has antifreeze mechanisms, even in populations that are not exposed to subzero temperatures and that high glucose levels allow *B. imbricata* to be active at low temperatures.

Keywords

Locomotor performance, freezing, crystallization point

1. Introduction

Environmental temperature influences the range of body temperatures attained by ectotherms, therefore, it defines the hours of activity per day and may constrain, their ability to meet critical ecological requirements (Carvajalino-Fernández et al., 2011; Huey and Slatkin, 1976; Labra et al., 2008; Voituron et al., 2002). Temperature is probably the most important environmental factor in the physiological performance of reptiles (Angilletta et al., 2002). Organisms are assumed to exhibit maximal physiological performance at their optimum body temperature (T_{opt}). Performance exhibits a gradual decline as body temperatures drop below T_{opt} and a rapid decrease at temperatures above T_{opt} (Angilletta et al., 2002; Currie et al., 1998; Miles, 1994).

Most multicellular organisms are active within a narrow range of temperatures. When the upper tolerance range of eukaryotic cells is exceeded, the performance of an organism deteriorates through the disruption of enzymatic activity and affinity and instability of cell membranes. Ultimately, cells are forced to use anaerobic respiration (Weeks and Espinoza, 2013). When temperatures decrease, the cellular water can change phase and transform into ice, if the ice grows the cell can break and the animals die (Costanzo et al., 1995a). Lizard species inhabiting montane environments often encounter subzero temperatures, which has the potential to affect activity and lower survival, because of freezing. There are two mechanisms by which lizards can survive at freezing temperatures: supercooling and freeze tolerance (Claussen et al., 1990; Costanzo et al., 1995b; Costanzo and Lee, 2013; Lowe et al., 1971; Pauly, 2012; Voituron et al., 2002, 2000). It is known that both processes depend on cryoprotective molecules that are synthesized during autumn and used in winter (Costanzo et al., 2013, 1995b).

In general, in Mexican lizards the range of body temperatures during activity varies between 19.2° to 44.9°C. Nevertheless, there are some reports of activity in lizards species in the

family Anguidae at temperatures below 19.2°C, such as: *Elgaria multicarinata webbi* (= *Gerrhonotus multicarinatus webbi*) at 11°C (Cunningham, 1966), *Mesaspis monticola* (= *Gerrhonotus monticolus*) at 8.6°C (Vial, 1975), *Abronia taeniata* at 13°C (Fierro-Estrada, 2013), *Mesaspis gadovii* at 10.8°C (Bautista del Moral, 2019) and *Barisia imbricata* at 9°C, but it is unknown whether some species could survive when temperatures are at or below freezing. One species, *Barisia imbricata* lives along a wide altitudinal gradient (from 2100 to 4000 m) at sites with temperate and subtropical climates, Populations at very high altitudes experience environmental temperatures at or below 0°C (Dashevsky et al., 2013; Guillette and Smith, 1982; Guillette and Casas-Andreu, 1987; Lemos-Espinal et al., 1998; Zaldivar-Riverón et al., 2005).

Levels of blood glucose is one key adaptation for surviving temperatures that drop below 0°C in lizards (Costanzo et al., 1995b; Grenot et al., 2000), both during supercooling and during freezing (Costanzo et al., 1991, 1993, 1995b; Storey and Storey, 1986). Therefore, we predict that high levels of glucose have two outcomes. First, high glucose levels facilitate activity of lizards at low environmental temperatures. Second, elevated glucose levels enhance survivorship of individuals. To test these predictions, we estimated seasonal variation in blood glucose levels, measured the locomotor performance in different temperature treatments and determined the crystallization point of *Barisia imbricata*.

2. Material and Methods

2.1 Area and Study Species

We studied *Barisia imbricata* at two different altitudes: Paso de Cortés, Estado de México at 3,700 m (N19°05'13.0" W98°38'46.5") and Ocoatepec, Puebla at 2,200 m (N19°33'17.8" W97°39'07.8"). We collected individuals when active by hand at four times during the year: winter, spring, summer and autumn. After capture we weighed each individual (to the nearest gram). Our sample consisted of males, females (both pregnant and non-reproductive) and juveniles. We sampled individuals for glucose levels at Paso de Cortés during autumn 2014, spring, summer and autumn 2015 and spring and winter 2016. We determined the crystallization point of individuals during autumn 2018 and winter 2019. Our samples of glucose levels for *B. imbricata* from Ocoatepec occurred during spring, summer and autumn 2016 and winter 2017. We determined crystallization point in winter 2017 and spring 2018.

Lizards were brought to the laboratory for the experiments and kept in terraria in a common room with the ambient temperature maintained between 20° - 22°C at their natural photoperiod. We kept the individual during three weeks for locomotor performance experiment and during two days for freeze experiment, during this time we provided them water and food. The organisms were transferred for experiments of locomotor performance to Centro Tlaxcala de Biología de la Conducta. At the end of the experiments all lizards were returned at their capture site.

2.2 Crystallization point

We collected twelve lizards and we measured their blood glucose level (see Glucose reading). One or two days after the capture, the lizards were acclimated for 12 hours at 6.5°C. Subsequently, each lizard was attached to a thermocouple sensor with a type-T, and the thermocouple was plugged to the Thermocouple Data Acquisition Module (Omega TC08[®]). Each lizard was placed in a closed mesh sack, to immobilize it and transferred to a glass container, which was introduced to a Circulating Chiller (Thermo Neslab RTE 7). Temperature in the chiller started at 6.5 °C and we decreased it by 0.5 °C each 30 min to the crystallization point. This point was recorded when the readings of the temperature of the lizard showed an exothermic reaction. Subsequently, the lizards were removed and we again measured blood glucose levels. Finally, all the organisms were placed in their terrarium at 20° - 22 °C to warm. We recorded the mass of each individual prior to the experiment.

2.3 Blood Glucose Levels

We recorded the level of glucose of each organism on the day of capture. We extracted 0.1 ml of blood from the base of the tail with a syringe. We read the glucose level with a manual glucose monitoring system (One Touch Select Simple[®]). Which was previously calibrated by reading 0.1 ml of glucose at a known concentration of 100 mg/dL and the system threw a reading of 92 mg/dL. For each individual we recorded the mass (g) and snout-vent length (SVL) in mm.

2.4 Thermal preferences

We obtained the thermal preferences (T_{pref}) of *B. imbricata* one or two days after the captured. We exposed the lizards to a thermal gradient from 18° to 35° C in individual tracks, which was built in a wood medium-density fiberboard box with dimensions 1.80x1.00x.10m (length, width and height) (Lara-Reséndiz et al., 2015, 2013; Rock et al., 2000). We determined that the mean of T_{pref} was 28° C.

2.5 Locomotor performance

We registered the total distance traveled for each organism during one minute at different temperatures (Angilletta et al., 2002). We built a circular track with circumference external of 1.70 m, internal of 1.5 m, with 0.25 m of height and peat moss as substrate. We first measured performance of all individuals at a T_b of 28°C. We then measured performance a second time, but at a temperature higher than their T_{pref} (31° or 34°C). We measured performance a third time, but at a temperature below than T_{pref} (3°, 6°, 9°, 12° or 15°C). Locomotor performance at T_{pref} was measured two days after exposure to the thermal gradient. Subsequent measurements of performance at the higher temperatures were done two or three days after the T_{pref} measurement. Measurement of performance at low temperatures was carried out three weeks later. All the experiments were performed for each season and the organisms were chosen at random for each treatment. T_b was read prior to each experiment, and then each lizard was placed at the chosen experimental temperature until its T_b reach the temperature of the treatment.

2.6 Thermal performance curve

We estimated the thermal performance curve with a Kumaraswamy function (Sheth and Angert 2014, Mike Sears, pers. Comm.) using the nlsLM function in the R package minpack.lm (Elzhov et al. 2013, R Core Team 2019, R 3.6.0). We anchored the TPC using 2°C and 37°C as the CT_{min} and CT_{max} , respectively. We used the individual values for distance run to characterize the thermal performance curve. Based on the fitted curve we generated estimates for maximum performance, the optimal temperature for performance (T_{opt}) and the thermal performance breadth (B_{80}) as the temperature range where an individual attained 80% of maximum performance.

2.7 Statistical analysis

Prior to conducting the statistical analyses, we verified that the data met the assumptions of linear models. We tested assumptions of normality using a Kolmogorov-Smirnov test and for homogeneity of variances using a Levene's test. As the glucose levels were not normal, we used a log 10 transformation to achieve normality. A generalized linear mixed model (GLMM) was used to compare the glucose levels between two populations, among seasons and among sex/age. Our mixed model analyses employed the lmer function in the R (v 3.5.1, R Development Core Team) lmer package. We used glucose concentration as a response variable and population, season, and sex/age (i.e., juvenile, adult male, pregnant female, non-reproductive female) as fixed effects. We include body mass in the model as a covariate. Year was treated as a random effect.

3. Results

We froze twelve organisms, two in spring, two in autumn and eight during winter, from the two populations. The average of crystallization point was -4.13°C . The mean crystallization point for individuals from Paso de Cortés ($n=6$) was -3.30°C and from Ocotepc ($n=6$) was -4.74°C . Glucose levels increased by 26% ($n=9$) after exposure to freezing temperatures in autumn and winter, decreased by 13% ($n=1$) in winter, and 12% ($n=2$) in spring (Table 1). All lizards survived the freeze experiment.

We obtained 124 measurements of blood glucose levels from Ocotepc and 130 from Paso de Cortés (Table 2). We found significant differences between the populations ($X^2_{1,241}=28.85$, $P < 0.0001$). There was no influence of body mass ($X^2_{1,241}=0.74$, $P = 0.39$) or sex/age ($X^2_{1,241}=1.52$, $P = 0.68$) on blood glucose levels. We did not find significant differences among glucose levels among the different seasons in Paso de Cortés ($X^2_{3,130}=6.54$, $P = 0.88$), however, the Ocotepc population exhibited seasonal differences in glucose ($X^2_{3,111}=13.02$, < 0.001) (Fig.1). The blood glucose levels of lizards from Ocotepc were higher in different in spring than summer and autumn. We did not find significant differences among glucose levels of different sex/age in two populations, Paso de Cortés ($X^2_{3,130}=2.13$, $P = 0.55$) and Ocotepc ($X^2_{2,111}=4.70$, $P = 0.10$) (Fig.2).

The thermal performance curve yielded a T_{opt} value of 22.6°C . The performance breadth (B_{80}) had a value of 19.4°C (range 12.1°C - 31.5°C).

4. Discussion

The lizard *Barisia imbricata* can survive freezing. Our experiment showed the species had an average crystallization point of -4.13°C . The only other species of lizard in Mexico with data on freeze tolerance was the species *Sceloporus jarrovi*, which had an estimated freezing point between -0.586° to -0.680°C (Halpern, 1979; Lowe et al., 1971). Lowe et al. (1971) reported freezing points for several other species of Anguils: *Mesaspis monticola* (= *Barisia monticola*) -0.61°C , *Mesaspis moreletii* (= *Barisia moreleti*) -0.55°C , *Elgaria coerulea* (= *Gerrhonotus coeruleus*) -0.60°C and *Elgaria multicaudata* (= *Gerrhonotus multicaudatus*) -0.53°C . In contrast, this mechanism has been extensively studied in the European common lizard *Zootoca* (= *Lacerta*) *vivipara*, which freezes to -6°C (Costanzo et al., 1995a; Costanzo and Lee, 2013; Grenot et al., 2000; Gvozdík, 2002; Voituron et al., 2004, 2002). It is also known that the lizard *Podarcis* (= *Lacerta*) *muralis* supports freezing at temperatures below -1°C (Claussen et al., 1990), and *Lacerta agilis* can reach -3°C (Costanzo et al., 1995b). We found that *B. imbricata* can be experimentally frozen, until -6.15°C , which is a lower temperature than previously reported for the species (Table 1).

The crystallization point differs among seasons and between populations. The degree of tolerance to freezing may depend on the climatic diversity regimes of the area inhabited by the individual (Costanzo et al., 2013). For example, *S. jarrovi* exhibited differences in freezing point between seasons (Lowe et al., 1971). In addition, the frog *Lithobates* (= *Rana*) *sylvatica* shows a tolerance to freezing ranging from -18° to -3°C depending on the population (Costanzo et al., 2013). Our data, even though we had too few individuals to compare among seasons, showed the crystallization point of the population of higher altitude was lower than in the population of lower altitude (Table 1).

The cryoprotective response of glucose begins with the onset of freezing and helps to mitigate osmotic stress associated with the concentration of the cytoplasm generated by freezing (Costanzo et al., 1995b). Moreover, glucose levels can increase during freezing, because it also enhances survival after freezing by preventing intracellular ice formation, serves as an antioxidant, substrate, metabolic regulator, and stabilizes membranes and macromolecules (Larson et al., 2014; Storey and Storey, 1986). For example *Z. vivipara* maintains high glucose levels even after thawing (Costanzo et al., 1995b; Grenot et al., 2000). In the frog *L. sylvatica*, it

is known that glucose levels increases several times in a few minutes at the beginning of freezing, from 270 mg/dL to over 540 mg/dL (Costanzo et al., 1995b, 1993, 1991; Storey and Storey, 1986). We found *B. imbricata* has the capacity to raise glucose levels 92% after freezing in autumn and winter (Table 1).

Although our sample size of individuals for the freezing experiment was low among seasons, the global data show consistent patterns, since we obtained data from two populations at the altitudinal extremes in the distribution of the species. Our study revealed differences in the crystallization point between populations. Note that research permits for conducting these manipulative experiments are usually very restrictive, if not denied. However, we recommend more studies of the freeze tolerance should be done with Mexican lizards, with focus on montane lizards at high elevations in general and Anguids, in particular.

Tolerance to freezing may also vary between organisms from different regions due to glucose production levels (Costanzo et al., 1993). In *L. sylvatica* for example it has been observed that the amount of hepatic glycogen deposit (reservoir and glucose producer) is significantly higher in Alaskan populations near the northern limit of its range, compared to populations of a more temperate environment, located in southern Ohio, USA (Costanzo et al., 2013). We also found significant differences in glucose levels between the two populations of *B. imbricata* with the high elevation population having higher levels. Also, glucose levels can vary among seasons. As recorded for the turtle *Chrysemys picta*, in which the levels of glycemia in winter exceed those of autumn up to 15 times (to 60 mmol l⁻¹), while in *Z. vivipara* can increases 245% in winter (at 50 mmol l⁻¹) (Costanzo and Lee, 2013; Voituron et al., 2000). The frog *L. sylvatica* accumulates glucose during the fall and the early winter (Costanzo et al., 2013). In *B. imbricata* we found significative seasonal differences levels of glucose in only the population at 2,200m. However, in the population at 3,700m were found glucose levels in winter exceeded those from the other seasons (Fig. 1).

An important factor for the survival of organisms at low temperatures is the microsite they use as a refuge during winter. Some organisms hibernate as a behavioral strategy to evade low temperatures. For example *Podarcis sicula* does not have tolerance to freezing, since it can be buried more than 24cm deep to avoid freezing (Burke et al., 2002). It is known that they occupy hibernacula for periods of weeks to months. Hibernaculum protect them from low environmental

temperatures, providing constant cold temperatures, but without freezing (Burke et al., 2002; Halpern, 1979). At the same time, the magnitude of cold tolerance depends on geographic origin of the population (Voituron et al., 2000). *Z. vivipara* lives in areas with a minimum average temperature of 2.5 °C and a maximum of 12.5 °C, so it needs to shelter in hibernacula during the colder season (November to February), and survive frozen without feeding (Grenot et al., 2000). We found that individuals of *B. imbricata* remain active during winter; however, we also observed lower density in the Paso de Cortés population at 3,700 m. Therefore, we do not rule out the possibility that during this season, which reaches freezing temperatures with some snowfall, the lizards can take refuge in hibernacula and return to the surface when the substrate is dry. We found some freezing soil temperatures during sunny days, after 8-10 days of the snowfall. On the other hand, some other species, e.g., *S. jarrovi*, that may be active during the winter due to the increase in blood hemoglobin. It has been suggested that it is an adaptation that allows a higher metabolic rate to compensate for the decrease in heat during that season (Halpern, 1979). An increase in hemoglobin could be a mechanism used by *B. imbricata*. We do not have data for this, however we observed that its blood was very viscous and coagulates quickly in the field, so we consider it interesting to delve into this topic.

Tolerance to freezing is often considered as a strategy to survive the winter. However, it is known that freeze tolerance in some species allows individuals to remain active during the coldest months of winter, e.g., the eastern box turtle (*Terrapene carolina*) and the garter snake (*Thamnophis sirtalis*) (Costanzo and Lee, 2013). Another species of lizard, *Pseudocordylus melanotus melanotus*, can survive short periods of subzero temperatures, such as frost during the night, especially if they are in suitable, dry shelters and the organisms are in a state of fasting (Mcconnachie et al., 2007). We consider that this phenomenon may occur in *B. imbricata* during the cold months of winter. We suggest this may occur during periods without snowfall and ambient temperatures reach 6°C, which is a temperature well below its selected temperature (28°C). Yet we have recorded this temperature when lizards have been observed to be active (Fierro-Estrada et al., 2019). Indeed, the broad range of temperatures at which performance is 80% of maximum suggest lizards can remain active at low ambient temperatures without a substantial decrease in locomotor performance. High glucose levels throughout the year and a high level of performance even at this temperature suggest that organisms can maintain activity even at unfavorable temperatures.

Acknowledgments

We thank Hugo Siliceo, Rafael Barzalobre, Franklin Chavarria, Ezel Galindo, Alberto Rodriguez and Cristian Argueta for help during the field and laboratory work. Amando Bautista provided the facilities for experimental work. We thank Posgrado en Ciencias Biológicas, UNAM, and CONACyT for the scholarship awarded to N. Fierro-Estrada (412836) and UNAM PAPIIT/DGAPA (IN210116) for financial support. The permits for the research were granted by SEMARNAT (01629) and for the Parque Nacional Iztaccihuatl-Popocatepetl CONANP (DRCEN / PNIP / 823/2014) and SEMARNAT (005406/18) to FRMC. This article is part of PhD Thesis of the Fierro-Estrada N.

5. References

- Angilletta, M.J., Niewiarowski, P.H., Navas, C.A., 2002. The evolution of thermal physiology in ectotherms. *J. Therm. Biol.* 27, 249–268.
- Bautista del Moral, A., 2019. Termorregulación en *Mesaspis gadovii* (Squamata : Anguillidae) en Carrizal de Bravo , Guerrero”. Universidad Autónoma de Guerrero.
- Burke, R.L., Hussain, A.A., Storey, J.M., Storey, K.B., 2002. Freeze Tolerance and Supercooling Ability in the Italian Wall Lizard, *Podarcis sicula*, Introduced to Long Island, New York. *Copeia* 3, 836–842.
- Carvajalino-Fernández, J.M., Bonilla Gomez, M.A., Navas, C.A., 2011. Freezing Risk in Tropical High-Elevation Anurans: An Assessment Based on the Andean Frog *Pristimantis nervicus* (Strobomantidae). *South Am. J. Herpetol.* 6, 73–78. <https://doi.org/http://dx.doi.org/10.2994/057.006.020>
- Claussen, D.L., Townsley, M.D., Bausch, R.G., 1990. Supercooling and freeze-tolerance in the European wall lizard, *Podarcis muralis*, with a revisional history of the discovery of freeze-tolerance in vertebrates. *J. Comp. Physiol. B* 160, 137–143. <https://doi.org/10.1007/BF00300945>
- Costanzo, J.P., do Amaral, M.C.F., Rosendale, A.J., Lee, R.E., 2013. Hibernation physiology, freezing adaptation and extreme freeze tolerance in a northern population of the wood frog. *J. Exp. Biol.* 216, 3461–3473. <https://doi.org/10.1242/jeb.089342>
- Costanzo, J.P., Grenot, C., Lee, R.E., 1995a. Supercooling, ice inoculation and freeze tolerance in the European common lizard, *Lacerta vivipara*. *J. Comp. Physiol. B* 165, 238–244. <https://doi.org/10.1007/BF00260815>
- Costanzo, J.P., Lee Jr, R.E., Wright, M.R., 1991. Effect of cooling rate on the survival of frozen wood frogs, *Rana sylvatica*. *J. Comp. Physiol.* 161, 225–229.
- Costanzo, J.P., Lee, R.E., 2013. Avoidance and tolerance of freezing in ectothermic vertebrates. *J. Exp. Biol.* 216, 1961–1967. <https://doi.org/10.1242/jeb.070268>
- Costanzo, J.P., Lee, R.E., DeVries, A.L., Wang, T., Layne, J.R., 1995b. Survival mechanisms of vertebrate ectotherms at subfreezing temperatures: applications in cryomedicine. *FASEB J.* 9, 351–358. <https://doi.org/10.1096/FASEBJ.9.5.7896003>
- Costanzo, J.P., Lee, R.J., Lortz, P.H., 1993. Glucose Concentration Regulates Freeze Tolerance in the Wood Frog *Rana sylvatica*. *J. Exp. Biol.* 181, 245–255.
- Cunningham, J.D., 1966. Thermal Relations of the Alligator Lizard *Gerrhonotus multicarinatus webbi*. *Herpetologica* 22, 1–7.
- Currie, R.J., Bennett, W.A., Beitinger, T.L., 1998. Critical thermal minima and maxima of three freshwater game-fish species acclimated to constant temperatures. *Environ. Biol. Fishes* 51, 187–200. <https://doi.org/10.1023/A:1007447417546>
- Dashevsky, D., Meik, J.M., Mociño-Deloya, E., Setser, K., Schaack, S., 2013. Patterns of sexual dimorphism in Mexican alligator lizards, *Barisia imbricata*. *Ecol. Evol.* 3, 255–261. <https://doi.org/10.1002/ece3.455>
- Fierro-Estrada, N., 2013. Ecología térmica de *Abronia taeniata* (Reptilia: Anguillidae) y su susceptibilidad ante el

- calentamiento global. Universidad Nacional Autónoma de México.
- Fierro-Estrada, N., González-González, Y.G., Miles, D.B., Martínez-Gómez, M., García, A., Salgado-Ugarte, I.H., Méndez-de la Cruz, F.R., 2019. Thermoregulation of the lizard *Barisia imbricata* at altitudinal extremes. *Amphibia-Reptilia*. <https://doi.org/10.1163/15685381-20191155>
- Grenot, C.J., Garcin, L., Dao, J., Hérold, J.P., Fahys, B., Tséré-Pagès, H., 2000. How does the European common lizard, *Lacerta vivipara*, survive the cold of winter? *Comp. Biochem. Physiol. - A Mol. Integr. Physiol.* 127, 71–80. [https://doi.org/10.1016/S1095-6433\(00\)00236-1](https://doi.org/10.1016/S1095-6433(00)00236-1)
- Guillete, L.J., Smith, H.M., 1982. A Review of the Mexican Lizard *Barisia imbricata*, and the Description of a New Subspecies. *Kansas Acad. Sci.* 85, 13–33.
- Guillette, L.J., Casas-Andreu, G., 1987. The Reproductive Biology of the High Elevation Mexican Lizard *Barisia imbricata*. *Herpetologica* 43, 29–38.
- Gvozdík, L., 2002. To heat or to save time? Thermoregulation in the lizard *Zootoca vivipara* (Squamata : Lacertidae) in different thermal environments along an altitudinal gradient. *Can. J. Zool.* 80, 479–492. <https://doi.org/10.1139/z02-015>
- Halpern, E.A., 1979. Supercooling in the Iguanid Lizard *Sceloporus jarrovi* Author. *Physiol. Zool.* 52, 190–204.
- Huey, R.B., Slatkin, M., 1976. Cost and benefits of lizard thermoregulation. *Q. Rev. Biol.* 51, 363–384. <https://doi.org/10.1086/409470>
- Labra, A., Vidal, M.A., Solís, R., Penna, M., 2008. Ecofisiología de anfibios y reptiles, in: Vidal, M.A., Labra, A. (Eds.), *Herpetología de Chile*. Springer Verlag, pp. 483–516.
- Lara-Reséndiz, R.A., Arenas-Moreno, D.M., Méndez-de la Cruz, F.R., 2013. Termorregulación diurna y nocturna de la lagartija *Phyllodactylus bordai* (Gekkota : Phyllodactylidae) en una región semiárida del centro de México. *Rev. Chil. Hist. Nat.* 86, 127–135.
- Lara-Reséndiz, R.A., Gadsden, H., Rosen, P.C., Sinervo, B., Méndez-De la Cruz, F.R., 2015. Thermoregulation of two sympatric species of horned lizards in the Chihuahuan Desert and their local extinction risk. *J. Therm. Biol.* 48, 1–10. <https://doi.org/10.1016/j.jtherbio.2014.11.010>
- Larson, D.J., Middle, L., Vu, H., Zhang, W., Serianni, A.S., Duman, J., Barnes, B.M., 2014. Wood frog adaptations to overwintering in Alaska: new limits to freezing tolerance. *J. Exp. Biol.* 217, 2193–2200. <https://doi.org/10.1242/jeb.101931>
- Lemos-Espinal, J., Smith, G.R., Ballinger, R.E., 1998. Temperature relationships of the lizard, *Barisia imbricata*, from México. *Amphibia-Reptilia* 19, 95–99.
- Lowe, C.H., Lardner, P.J., Halpern, E.A., 1971. Supercooling in reptiles and other vertebrates. *Comp. Biochem. Physiol. -- Part A Physiol.* 39, 125–135. [https://doi.org/10.1016/0300-9629\(71\)90352-5](https://doi.org/10.1016/0300-9629(71)90352-5)
- Mcconnachie, S.Á., Alexander, G.J., Whiting, M.J., 2007. Lower temperature tolerance in the temperate, ambush foraging lizard *Pseudocordylus melanotus melanotus*. *J. Therm. Biol.* 32, 66–71. <https://doi.org/10.1016/j.jtherbio.2006.10.002>
- Miles, D.B., 1994. Population differentiation in locomotor performance and the potential response of a terrestrial organism to global environmental change. *Am. Zool.* 34, 422–436. <https://doi.org/10.1093/icb/34.3.422>
- Pauly, G.B., 2012. Evidence for Freeze Tolerance in the Eastern Fence Lizard (*Sceloporus undulatus*). *Southwest. Nat.* 57, 472–473. <https://doi.org/10.1894/0038-4909-57.4.472>
- Rock, J., Andrews, R.M., Cree, A., 2000. Effects of Reproductive Condition, Season, and Site on Selected Temperatures of a Viviparous Gecko. *Physiol. Biochem. Zool. Ecol. Evol. Approaches* 73, 344–355.
- Storey, K.B., Storey, J.M., 1986. Freeze tolerance and intolerance as strategies of winter survival in terrestrially-hibernating amphibians. *Comp. Biochem. Physiol. -- Part A Physiol.* 83, 613–617. [https://doi.org/10.1016/0300-9629\(86\)90699-7](https://doi.org/10.1016/0300-9629(86)90699-7)
- Voituron, Y., Hérold, J.P., Grenot, C., 2000. Metabolic Adaptations of Overwintering European Common Lizards (*Lacerta vivipara*). *Physiol. Biochem. Zool. Ecol. Evol. Approaches* 73, 264–270.
- Voituron, Y., Heulin, B., Surget-Groba, Y., 2004. Comparison of the Cold Hardiness Capacities of the Oviparous and Viviparous Forms of *Lacerta vivipara*. *J. Exp. Zool.* 301A, 367–373. <https://doi.org/10.1002/jez.a>
- Voituron, Y., Storey, J.M., Grenot, C., Storey, K.B., 2002. Freezing survival, body ice content and blood composition of the freeze-tolerant European common lizard, *Lacerta vivipara*. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* 172, 71–76. <https://doi.org/10.1007/s003600100228>
- Weeks, D.M., Espinoza, R.E., 2013. Lizards on ice: Comparative thermal tolerances of the world's southernmost gecko. *J. Therm. Biol.* 38, 225–232. <https://doi.org/10.1016/j.jtherbio.2013.02.005>
- Zaldivar-Riverón, A., Nieto-Montes De Oca, A., Laclette, J.P., 2005. Phylogeny and evolution of dorsal pattern in the Mexican endemic lizard genus *Barisia* (Anguillidae: Gerrhonotinae). *J. Zool. Syst. Evol. Res.* 43, 243–257. <https://doi.org/10.1111/j.1439-0469.2005.00308.x>

Table 1. Glucose levels of *Barisia imbricata* before and after freezing and crystallization point.

Population	Season	Sex	Glucose before	Crystallization point	Glucose after	Difference in Glucose
			mg/dL	°C	mg/dL	mg/dL
2,200 m	Winter	M	168	-6.15	207	39
		M	198	-3.63	252	54
		F	271	-5.64	283	12
		F	192	-5.05	252	60
	Spring	F	234	-4.06	217	-17
		M	291	-3.93	245	-46
3,700 m	Autumn	F	166	-2.56	187	21
		M	121	-2.5	233	112
	Winter	F	219	-2.16	191	-28
		M	266	-4.81	269	3
		F	294	-4.46	345	51
		M	204	-4.56	247	43

Table 2. Number of individuals of *Barisia imbricata* captured during each season. We provide blood glucose levels by Age, Sex and Reproductive Status. Values presented are means \pm standard deviation (SD).

Season	<i>n</i> total	Juveniles	Males	Females	Reproductive females
	mg/dL \pm SD	<i>n</i> , mg/dL \pm SD	<i>n</i> , mg/dL \pm SD	<i>n</i> , mg/dL \pm SD	<i>n</i> , mg/dL \pm SD
Spring	76, 216.5 \pm 65.5	27, 200.8 \pm 54.8	23, 224.1 \pm 72.54	16, 234.5 \pm 68.73	10, 211.7 \pm 69.64
Summer	51, 180.6 \pm 43.0	10, 169.2 \pm 34.52	16, 181.5 \pm 41.95	25, 184.4 \pm 47.17	
Autumn	91, 208.6 \pm 79.6	14, 186.6 \pm 40.75	32, 208.6 \pm 61.97	45, 215.6 \pm 97.58	
Winter	36, 206.1 \pm 53.4	13, 201.4 \pm 28.68	12, 192.3 \pm 56.60	7, 205.6 \pm 76.0	4, 272.3 \pm 18.04

Fig.1. Glucose levels of *Barisia imbricata* of the two populations in different season.

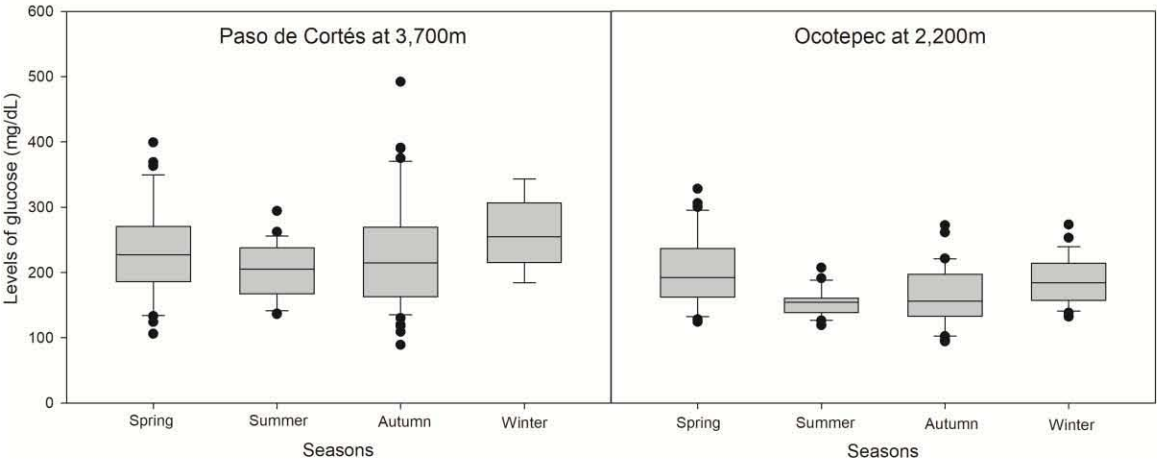
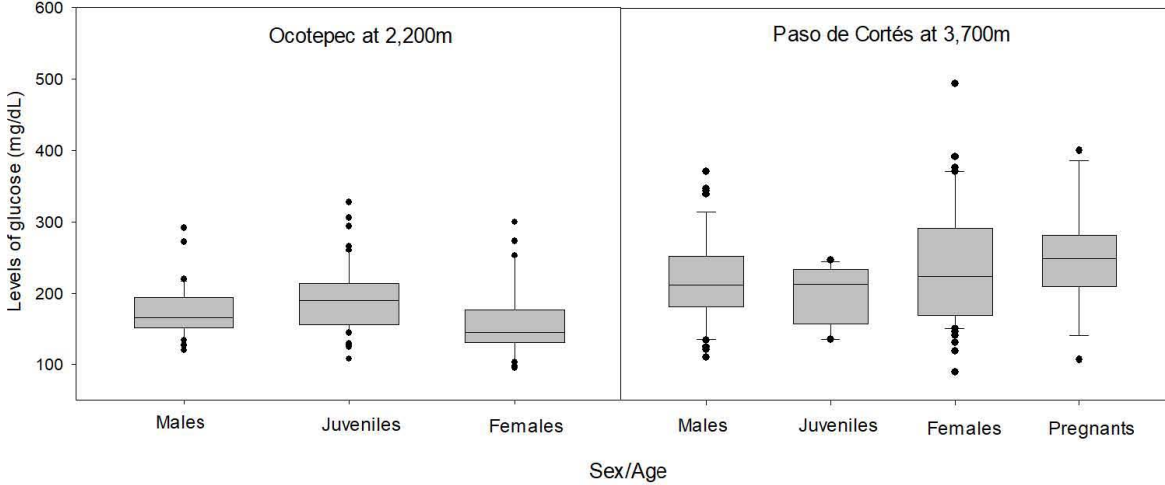


Fig.2. Glucose levels of *Barisia imbricata* of the two populations in different sex or age.



Capítulo 3

An Ecophysiological Risk Assessment of Extinction Risk from Climate Change of the viviparous Mexican lizard *Barisia imbricata* (Squamata: Anguidae).

(Manuscrito sometido a revisión)

An Ecophysiological Risk Assessment of Extinction Risk from Climate Change of the viviparous Mexican lizard *Barisia imbricata* (Squamata: Anguidae).

Natalia Fierro-Estrada^{a,b}, Fausto R. Méndez de la Cruz^b, Norberto Martínez-Méndez^c, Donald B. Miles^d and Barry Sinervo^e

^aPosgrado en Ciencias Biológicas, Instituto de Biología, Universidad Nacional Autónoma de México, A.P. 70-153, C.P. 04510 Ciudad de México, México

^bLaboratorio de Herpetología, Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, A. P. 70515, C.P. 04510 Ciudad de México, México

^cLaboratorio de Bioconservación y Manejo, Departamento de Zoología de la Escuela Nacional de Ciencias Biológicas del Instituto Politécnico Nacional, Ciudad de México, 11340, México

^dDepartment of Biological Sciences, Ohio University, Athens, Ohio, USA

^eDepartment of Ecology and Evolutionary Biology, Coast Biology Building, 130 McAllister Way, University of California, Santa Cruz, CA 95060

Correspondence: natalia_fierro@hotmail.com

ABSTRACT

1. Climate change is one of the most important and pressing challenges for animals, and in particular for ectotherms, due to their high dependence on environmental temperature. Extinctions of some populations of lizards have already been reported, some of them from the mountains of Central México. One of the lizards that occurs along the Transvolcanic Axis in Central México is the viviparous, *Barisia imbricata*.
2. We collected *B. imbricata* from two populations that characterize its distributional elevation limits. We recorded body temperature (T_b) at the time of capture and recorded operative temperatures with data-loggers at the collection sites, but we also obtained environmental temperatures (T_e) from microclim. In the laboratory, we obtained locomotor performance of lizards at seven test temperatures, along with Critical Thermal Maximum (CT_{Max}) and Minimum (CT_{Min}), the upper and lower limits of the right response, and analyzed thermal performance curve (TPC) by a Generalized Additive Mixed Model with snout-vent length as a covariate as well as with TPC modeled with the Kumaraswamy curve. We also used the lowest (T_b) as a threshold to calculate hours of activity and thermal preference (T_{pref}) as a threshold for hours of restriction of the species through a mixed model (Mechanistic-Correlative).

3. Our Species Distribution Model (numerically) integrates all salient ecophysiological traits (T_b minimum, T_{pref} , CT_{Min} , CT_{Max} , TPCs) with climate variables (Minimum temperature, Maximum temperature, Precipitation).
4. Finally, we built a mixed model to project probability of persistence (1-extirpation probability) of the species under three scenarios of climate change at present time and by the year 2070.
5. We obtained 71% in the scenario RCP 2.6, 60% in the RCP 4.5 and 37% in the RCP 8.5 of probability presence. *B. imbricata* will face serious problems at low altitudinal limits, but populations at higher altitude will persist if the habitat remains intact.

Keywords: Body temperature; environmental temperature; performance curve; mechanistic models

1. Introduction

In the last 30 years climate change (CC) has produced numerous modifications in the distribution, abundance, behavior, ecological interactions, phenology and extinction of some species (Thomas et al., 2004; Vicenzi, Corbalán, Miles, Sinervo, & Ibarquengoytía, 2017). It is estimated that as a consequence of the increase in the environmental temperature (T_e) up to 37% of species in the world could become extinct by the year 2050 (Thomas et al., 2004). The T_e is a key abiotic variable, due to this, dramatic impacts can influence ectotherms, because their biological activities must be performed within a certain range of temperature (Dubois, Blouin-Demers, Shipley, & Thomas, 2009; Elliott, 1994; Hertz, Huey, & Stevenson, 1993; Huey, 1982; Huey & Slatkin, 1976; Sinervo et al., 2010). This intimate dependence that the ectotherms have on T_e makes them more prone to suffer impacts of CC (Deutsch et al., 2008; Gunderson & Leal, 2012; Vicenzi et al., 2017). In the case of reptiles, it is estimated that by the year 2080, up to 39% of all lizard populations can become extinct in tropical regions, due to a decrease in the hours that are suitable for thermoregulation (Sinervo et al., 2010). Viviparous lizards may be even more vulnerable because of the thermal requirements of pregnant females, the increase of T_e may influence in the gestation period and viability and locomotion in hatchlings (Shine, 2005; Vicenzi et al., 2017). Populations of viviparous lizards in mountainous regions with high altitudes are more susceptible to CC because the decrease of distribution areas, and fragmentation (Chamaillé-Jammes, Massot, Aragón, & Clobert, 2006; Sinervo et al., 2010; Vicenzi et al., 2017), since they occur in upland zones with a greater amplitude of thermal oscillation than in lowlands (Lara-Reséndiz, Larraín-Barrios, Díaz De La Vega-Pérez, & Méndez-De La Cruz, 2014).

The T_e determines the number of hours per day in which organisms can be active to achieve its requirements (Huey & Slatkin, 1976; Labra, Vidal, Solís, & Penna, 2008). In fact, the lizards are active within a precise range, determined by preferred temperatures (Sinervo et al., 2010). In the periods of the day when T_e exceeds the maximum critical temperature (CT_{max}), lizards must hide in shelters to avoid such ecophysiological limits (Sinervo et al., 2018). This response has been termed hours of restriction (h_r). However, the time spends in thermal refuges limit basic biological activities of lizards; e.g. shorter foraging time may decrease the energy achievement to cover the reproductive cost. The lizard *Sceloporus mucronatus* in the Transvolcanic region of Mexico ceased reproduction during a particular severe warm spell, and this species went locally extinction or was extirpated in the subsequent decade (Sinervo, Miles, Martinez-Mendez, Lara-Resendiz, & R, 2011). Thus under the demographic collapse hypothesis the hours of restriction model is projected to have cumulative effects, resulting in extirpations of populations at low elevation and low latitude range, limits, culminating in species level extinction (Sinervo et al., 2010, 2011, 2018; Vicenzi et al., 2017). Accordingly, by calculating the h_r along the distribution of a species it is possible to assess the potential degree of impacts due to CC.

An approach to study effects of CC involves the use of correlative models merged to mechanisms of ecophysiology, based on environmental data where the species occurs (Kearney & Porter, 2009; Kearney, Wintle, & Porter, 2010; Sinervo et al., 2018), climate surfaces data in the contemporary period (temperature, precipitation and seasonality) and data on the current distributions of the species, as a proxy to estimate conditions under which the populations persist in the present day in the face of competitors and predators (Thomas et al., 2004). The mechanistic models are based on the ecophysiological restrictions of the species (Gunderson & Leal, 2012; Kearney & Porter, 2009; Kearney et al., 2010). Because, the biological impact of the increase in temperatures will depend on the physiological sensitivity of each organism (Deutsch et al., 2008). Therefore, a better proxy to more precisely estimate the reliability of the future projections of the CC effects is to determine the physiological response of the organisms to the increase in global temperature and its effects on fitness (Kearney et al., 2010; Pontes-da-Silva et al., 2018; Sinervo et al., 2018; Vicenzi et al., 2017). One of best proxies of fitness in the context of thermal biology is to develop a mechanistic model based on the thermal performance curve (TPC) (Angilletta, Niewiarowski, & Navas, 2002).

The TPC describes the relationship between body temperature and locomotor performance, and it is used to measure the physiological response of an organism through the variation of its performance (Angilletta et al., 2002; Sinclair et al., 2016). The curves start with the minimum critical temperature (CT_{Min}), that is, the lowest temperature at which organisms are active; from that point a TPC increases gradually as the temperature increases, until reaching its maximum at a point known as optimal

temperature (T_o), i.e., the point where whole-organism physiological functions promoting locomotor performance is maximized. Thereafter, the curve descends sharply until it reaches the CT_{Max} at which the organisms are unable to maintain activity, as indexed by the loss of the right response (Angilletta et al., 2002; Currie, Bennett, & Beitinger, 1998; Deutsch et al., 2008; Sinclair et al., 2016). The TPC offers physiological framework of the species, which can be integrated over time, using data on T_e . Therefore, this ecophysiological framework is fundamental to studies on the effect of climate in ectotherms (Deutsch et al., 2008; Gunderson & Leal, 2012; Miles, 1994; Sinclair et al., 2016).

Here we merge the correlative and mechanistic approaches and for the first time and use the full set of ecophysiological parameters that might govern species distribution including, the minimum T_b for activity observed in the field, the thermal preference (T_{pref}) measured in the laboratory, CT_{Min} and CT_{Max} along with TPC. The Intergovernmental Panel on Climate Change (IPCC) proposed four possible future emission scenarios for the CC called "Representative Concentration Pathways (RCPs)". All RCPs consider CO₂ atmospheric concentration by the year 2100 higher than those of today. RCP 2.6 is a scenario of mitigation, RCP 4.5 is a stable scenario, and RCP 8.5 is a scenario increasing of CO₂ in comparison to the current (IPCC, 2013). To evaluate the contemporary probability of presence, we obtained: the aforementioned future scenarios for monthly T_{max} , T_{min} and precipitation and 1975 (contemporary) worldclim data, T_e data at (contemporary) occurrence sites of the species. Here we assess the utility of h_r and activity as well as the integrated TPC, to predict the contemporary distribution of this species in a Species Distribution Modeling framework, and used this model to project the probability of presence of *B. imbricata* under the three scenarios noted above by the year 2070, and we also modeled T_e using microclim (Kearney et al., 2014; Sinervo et al., 2010, 2018).

2. Materials and Methods

2.1 Area and organisms of study

Barisia imbricata is a viviparous lizard endemic to México, it lives in temperate and subtropical regions from 2100 to 4000 m of elevation in the Transvolcanic Mexican Belt (Dashevsky, Meik, Mociño-Deloya, Setser, & Schaack, 2013; Guillete & Smith, 1982; Guillete & Casas-Andreu, 1987; Lemos-Espinal, Smith, & Ballinger, 1998; Zaldivar-Riverón, Nieto-Montes De Oca, & Laclette, 2005). The study sites comprised two populations. First population was in Ocotepc, Puebla México (N19°33'17.8" W97°39'07.8" at 2,200 m), at the low elevation range of the species. The second population was in Paso de Cortés, Estado de México (N19°05'13.0" W98°38'46.5" at 3,700 m), near the high elevation range of the species. We captured organisms at Ocotepc during spring, in summer and in autumn of 2016, plus winter 2017. Whereas at Paso de Cortés during autumn of 2014, in spring, in summer and in autumn of

2015, as well as in winter and spring of 2016. We took the individuals to the laboratory and performed measurements of ecophysiological traits, and afterward we returned them to their habitat at the point of capture.

2.2 Environmental and body temperatures

We manually captured active *B. imbricata*. In the first 20s after capture, we took its body temperature (T_b) by inserting in the cloaca a type-T thermocouple sensor ($\pm 0.1^\circ\text{C}$) connected to a digital quick reading thermometer (Fluke ® 51-II). The environmental temperatures (T_e) were registered each 10 min with one data-loggers HOBO®_{PROV2} in each site. Each data-logger had two sensors and each sensor was connected to a physical model. We deployed one physical model exposed to the sun and the other in the shade, to collect the range of temperatures that the lizards could be exposed (Besson & Cree, 2010; Sinervo et al., 2010, 2011). The physical models were constructed from polyvinylchloride (PVC), filled with silicone, painted gray and calibrated previously for the species ($r = 0.961$, $P < 0.001$; $r^2 = 0.923$) (Hertz et al., 1993; Kubisch, Corbalán, Ibarzüengoytía, & Sinervo, 2016; Pontes-da-Silva et al., 2018; Sinervo et al., 2010). We also recorded air temperatures (T_{air}) with a data-logger HOBO®_{PROV2} for temperature and humidity which had an exposed sensor in each site. Both data-loggers for each site were deployed in the microsites where organisms were observed (Pontes-da-Silva et al., 2018). Finally, the temperatures were recorded at Ocotepc from spring 2016 to spring 2017, and at Paso de Cortés from autumn 2015 to winter 2017.

2.3 Laboratory experiments

In the laboratory, animals were maintained in terraria with water and food, with a natural photoperiod of light, which also provided opportunity for thermoregulation. To determine the thermal preferences (T_{pref}) of *B. imbricata*, we tested subjects in a thermal gradient two days after capture. For this, we built a thermal gradient in a wood medium-density fiberboard box with dimensions 1.80 x 1.00 x .10m (length, width and height), and we exposed the lizards to a temperature range between 18° to 35° C. The mean of preferred temperatures of *B. imbricata* was 28.4°C (Hertz et al., 1993; Sinervo et al., 2010), and based on this data we determined the temperatures treatments for the locomotor performance experiments.

2.3.1 Locomotor performance experiments

We placed lizards on a circular track and we induced them to run during one minute to register the total distance traveled by each organism (Angilletta et al., 2002). The circular track had an external

circumference of 1.70m, internal circumference of 1.5m and walls with a height of 0.25m, the base was made of cardboard and covered with peat moss as substrate. Each subject was tested three times at each temperature. The animals rested 48 h between each experiment, with water and food (*Acheta domesticus*) provided *ad libitum*. Prior to and between experiments, all the organisms were kept in terraria at the same room temperature (20° - 22° C). Lizards were randomly selected for each experiment and acclimated to treatment temperature 1 h prior to the experiment. The experiments were carried out in the following order: first all organisms ran at preferred temperature 28° C. The second time, each organism ran at higher temperature than the preferred: 31° or 34 °C. Finally each organism ran at a lower temperature than the preferred temperature: 15°, 12°, 9°, 6° or 3° C. Prior to the experiments we recorded the snout-vent length (SVL) and the weight for each individual. We also estimated CT_{Min} (n=3) and CT_{Max} (n=3) for a smaller group of animals to establish the basal levels of the TPC.

2.4 Extinction risk

We modeled maps of probability of presence of the species with the library "biomod2" of the Rstudio program, following the script *Mapinguari* v0.4.1, and code as listed in Sinervo et al. 2018, but we modified the code to run on a high performance computer to integrate correlative and mechanistic models (Caetano, Santos, Miles, & Sinervo, 2017). The methods were as follows.

2.4.1 Collection sites.

We searched and verified georeferencing points of *B. imbricata* of the National Collection of Amphibians and Reptiles (CNAR) of the Institute of Biology and of the Global Biodiversity Information Facility (GBIF.org, 21 July 2019). We eliminated repeated points (within a 1x1 km cell with the clean points function of the R package *Mapinguari*, Caetano et al. 2017), erroneous points outside the distribution or altitude of the species and in total we used 221 georeferenced points for the species.

2.4.2 Choice and cutting of bioclimatic surfaces.

We obtained surfaces of monthly climatic variables of precipitation, minimum temperature, maximum temperature and altitude for the present scenario with a resolution of 30s from the Global Climate Data WorldClim (<http://www.worldclim.org/>). Also, we downloaded surfaces of precipitation, minimum temperature and maximum temperature for three possible future scenarios (RCPs: 2.6, 4.5 and 8.5) of the Max Planck Institute for Meteorology (MPI-ESM_LR) (Caetano et al., 2017; Vicenzi et al., 2017). This

climate model is the one that best captures observed patterns of temperature and precipitation during the control period (Anav et al., 2013) and, thus, may predict future climate change (Sinervo et al., 2018).

2.4.3 Environmental and body temperatures.

We used body temperatures (T_b) to determine the range of temperatures at which *B. imbricata* can be active (thermal threshold). We used the lowest T_b ($T_{b, MinActivity}$) recorded in the field in our models of ecophysiology and behavioral thermoregulation. We used this range because, as in other anguids, *B. imbricata* is an eurythermic species, a mixture of thigmothermic, thermoconforming and heliothermic behaviors (Sinervo et al., 2010). Also, we used the environmental temperatures (T_e) and temperature of air (T_{air}) registered in the study sites according to the data-loggers deployed.

2.4.4 Construction of raster surfaces of hours of activity and restriction.

We calculated potential hours of activity (h_a) and potential hours of restriction (h_r) of *B. imbricata* with sigmoidal equations (Richards Growth Equation) of hours above the maximum air temperature taken by the data loggers (Sinervo et al., 2018) and we compared these empirical data with h_a and h_r computed from microclim rasters (Kearney, Munns, Moore, Malishev, & Bull, 2018). We made a generalized logistic regression using the hours of activity or restriction as binary outcomes (1/0), and air temperature as independent variable, we applied to the model a WorldClim maximum temperature surface to predict the hours of restriction and activity in the entire projected area (Pontes-da-Silva et al., 2018). Following the methodology of Sinervo et al. (2010, 2018), we used the T_e registered with the physical models and the thermal threshold ($T_{b, MinActivity}$ and T_{pref}). We considered that the h_a were the number of hours per day during activity time in which the temperature were above $T_{b, MinActivity}$ and h_r as the number of hours per day in which temperatures were above T_{pref} (Pontes-da-Silva et al., 2018; Sinervo et al., 2010, 2018; Vicenzi et al., 2017).

2.4.5 Locomotor performance curve

We fit the locomotor performance curve by means of a Kumaraswamy curve using code developed by Michael Sears (personal communication) for the R package (Note to reviewers: all R code will be placed in a supplementary online materials upon final acceptance of the manuscript).

2.4.6 Construction of new raster surfaces using ecophysiological data.

We constructed three “daily” integrals over time, t , for 12 months of the year in analyses of raster surfaces for h_r with $T_{pref} = 28.4^\circ\text{C}$, h_a with $T_{b,MinActivity} = 9.1^\circ\text{C}$ using Richard’s functions, f , and for raster surfaces of TPC with $CT_{Min} = 1.89^\circ\text{C}$ and $CT_{Max} = 37.0^\circ\text{C}$, t (24-h day) using the Kumaraswamy function, k :

$$h_{r,m} = \int_{t=0}^{24} f(T_{Max,m} - T_{pref}) dt \Rightarrow \int_{t=sunrise}^{sunset} f(T_{Max,m} - T_{pref}) dt \quad \text{Eqn. 1,}$$

$$h_{a,m} = \int_{t=0}^{24} f(T_{Min,m} - T_{b,MinActivity}) dt \Rightarrow \int_{t=sunrise}^{sunset} f(T_{Min,m} - T_{b,MinActivity}) dt \quad \text{Eqn. 2,}$$

$$TPC_m = \int_{t=0}^{24} k(CT_{Min}, CT_{Max}) dt \Rightarrow \int_{t=sunrise}^{sunset} k(CT_{Min}, CT_{Max}) dt \quad \text{Eqn. 3.}$$

Given that *B. imbricata* is a diurnally active heliotherm/thigmotherm it is only active and foraging during the day, we computed integrals between sunrise and sunset for Eqns. 1-3 with up crossings and down crossings over the thermal thresholds (e.g., $T_{pref} = T_e$) fitted to 24-h scenarios of microclim (Sinervo et al. 2018). We computed numerical integrals using a total of N_e hourly rasters of microclim T_e scenarios for each month, m , of the year in a “Lebesgue” integral I , which uses T_{pref} as the threshold for integration (i.e., values above T_{pref} are non-zero) across each of the N_e scenarios for $T_{e,i}(m, h)$ ($i = 0\%, 25\%, 50\%, 75\%, 100\%$ shade for soil and sand substrate at both 0 cm and 1cm, Kearney et al. 2014) of the 24-h of the day (e.g., 24 rasters, one for each hour). For each scenario, we computed h_r as the number of hours above the T_{pref} threshold (with up-crossings and down crossings), yielding monthly averages $\bar{h}_{r,m}$:

$$\bar{h}_{r,m} = \frac{N_e \sum_{i=1}^{h=sunset} \sum_{h=sunrise} I(h_a(T_{e,i}(m,h) > T_{pref}))}{N_e} \quad \text{Eqn. 4.}$$

Our model assumes that lizards in nature move among T_e habitats (of microclim scenarios) and its body temperature would be the hourly average of all T_e scenarios as it works its way across the local landscape.

A similar function was used for $\bar{h}_{a,m}$, the average number of hours above $T_{b,MinActivity}$ threshold:

$$\bar{h}_{a,m} = \frac{N_e \sum_{i=1}^{h=sunset} \sum_{h=sunrise} I(h_a(T_{e,i}(m,h) > T_{b,MinActivity}))}{N_e} \quad \text{Eqn. 5.}$$

These values were computed for each of the known $N=221$ occurrence records at microclim raster cells. This data, along with temperature maximum or minimum (T_{Max} or T_{Min}) at each raster cell for monthly rasters of worldclim, was then used to fit a non-linear Richards function (in JMP 14) for hours of restriction and activity:

$$h_{r,m}(T_{Max} - T_{pref}) = \tau_{r,1} (1 + \tau_{r,2} \exp[-\tau_{r,3} (T_{Max,m} - T_{pref})])^{(1/\tau_{r,4})} \quad \text{Eqn. 6,}$$

$$h_a(T_{Min,m} - T_{b,MinActivity}) = \tau_{a,1} (1 + \tau_{a,2} \exp[-\tau_{a,3} (T_{Min,m} - T_{b,MinActivity})])^{(1/\tau_{a,4})} \quad \text{Eqn. 7.}$$

A partitioning of T_{Min} with h_a is intuitive: when average $T_{e,i}(m, h)$ exceeds $T_{b,MinActivity}$ the animals would wakeup at the start of the day or retreat at the end of the day. A partitioning of T_{Max} with h_r is intuitive: when average $T_{e,i}(m, h)$ exceeds T_{pref} animals would be forced into mid-day retreat sites.

These two functions were then used to downscale h_r and h_a for each month of the year for worldclim rasters at the 5x5 km resolution for use in preliminary species distribution models (SDMs) for forest cover (Prevedello, Winck, Weber, Nichols, & Sinervo, 2019) and altitude (worldclim.org). We used AIC to eliminate variables that do not provide sufficient information. We then re-fitted the best model at 1x1 km resolution for present-day and future scenarios (e.g., 2070 RCP 8.5, 4.5, or 2.6).

In our SDM fitting procedure, we also used raster output from a Kumaraswamy TPC as a function of the T_e experiment from the laboratory data was used to fit parameters a, b, c, CT_{Min} and CT_{Max} :

$$TPC(T_e) = ab \left(\frac{(T_{e,i} - CT_{Min})}{(CT_{Max} - CT_{Min})} \right)^{a-1} \left(1 - \frac{(T_{e,i} - CT_{Min})}{(CT_{Max} - CT_{Min})} \right)^{(b-1)c} \quad \text{Eqn. 8.}$$

Given that microclim is at a resolution of 20 x 20 km, we had to express this function in terms of T_{Min} and T_{Max} to allow us to downscale the TPC to 1 x 1 km climate data, as was done previously for h_r (Sinervo et al., 2018). In highly montane environments such as the habitats of *B. imbricata*, the 20 x 20 km resolution of microclim (Kearney et al., 2018) does not provide sufficient resolution for modeling topographical changes in climate, even though the curves fitted from 20 x 20 km should still be unbiased and also span the temperature ranges, suitable for modeling climate impacts in the future. The equation for numerical integration of \overline{TPC}_m for each month is given by the mean across all $T_{e,i}$ scenarios by month:

$$\overline{TPC}_m = \sum_{i=1}^{N_e} \sum_{h=sunrise}^{sunset} \frac{TPC_{h,m}(T_{e,i})}{N_e} \quad \text{Eqn. 9.}$$

This data for \overline{TPC}_m , computed at all N=221 occurrence records for each month of the year, was then used to obtain a fitted function of the climate variables T_{Min} and T_{Max} , analogous to Richard's functions fitted to $\bar{h}_{r,m}$ and $\bar{h}_{a,m}$ data. Intuitively, T_{Min} and T_{Max} should be related to CT_{Min} and CT_{Max} , respectively, daily air temperature excursions and the roots of the TPC function. We fit the \overline{TPC}_m data as a function of linear terms (T_{Min}, T_{Max}) and quadratic terms (T_{Min}^2, T_{Max}^2) as well as an interaction term ($T_{Min} \times T_{Max}$):

$$\overline{TPC}(T_{Min}, T_{Max}; \text{parameters} = u, v, w, x, y, z) \\ = u + vT_{Min} + wT_{Max} + xT_{Min}^2 + yT_{Max}^2 + zT_{Min}T_{Max}$$

Eqn. 10.

2.4.7 Construction of present and future distribution maps.

To generate a mixed maps of possible presence of the *B. imbricata* for scenarios RCP 2.6, 4.5 and 8.5 for the present time and the year 2070 (Caetano et al., 2017; Sinervo et al., 2018; Vicenzi et al., 2017), we used generalized linear models (GLMs) and generalized additive models (GAMs). We used these surfaces generated for h_a , h_r , TPC (average per day across the year) and the current bioclimatic surfaces (1950 - 2000) as predictor variables and using the Akaike criterion to choose the best model. Because *B. imbricata* is pregnant from August to May we used the summation of all months of the year, given that after parturition females would need a period of recovery in mid-summer. Previous models of h_r have generally been restricted to reproductive periods (Sinervo et al., 2010) but in this case the reproductive period spans nearly the entire year. We also added precipitation from worldclim to generate additional models to assess potential impacts of moisture on the species range limits, and we compared SDMs with precipitation across the year, or binned in the dry (May-September) versus wet seasons (October to April) observed across *B. imbricata*'s species range. In our modeling we tested whether the choice of pseudoabsence selection limits had impacts on the modeling (biomod2 vignette: 20000 inner ring, 2000000 outer ring, vs. 50000 inner ring, 20000000 outer ring,) as suggested by Elith et al. (2011) and Barbet-Massin et al. (2012). We found no effects between the two pseudo-absence scales. In all models we ran 10 replicates (NbRunEval=10, in the BIOMOD_Modeling function of the biomod2 package) and with quadratic and interaction terms.

2.4.8 Extraction of values and percentages.

With the generated rasters, we created new maps in ArcMap, added an altitude layer and extracted the probability values of persistence for each future climate scenario.

3. Results

3.1 Temperatures

We collected 125 organisms in Ocotepc and 143 in Paso de Cortés, and we determined that the thermal threshold for activity for *Barisia imbricata* was $T_{b,MinActivity} = 9.1^{\circ}\text{C}$ and mean field body temperature (\bar{T}_b) was $= 22.0^{\circ}\text{C}$ (Figure 1A). The distribution data for thermal preference (T_{pref}), was $= 28.4^{\circ}\text{C}$ (Figure 1B). We registered 181,109 environmental temperatures with the null models and 77,314 air temperatures for two populations.

3.2 Hours of restriction and of activity

The fitted hours of restriction (h_r) and the fitted hours of activity (h_a) for *B. imbricata* using Richard's Growth equation is given in Figure 2AB.

3.3 Performance curve.

Barisia imbricata had non-zero performance from 6° to 34°C, and the peak of activity was located between 23° to 30°C for the GAMM (Figure 1c). The fitted Kumaraswamy TPC, with $CT_{Min} = 1.89^\circ\text{C}$ and $CT_{Max} = 37.00^\circ\text{C}$, is provided in Figure 1d, which we used in modeling TPC integrated over the course of the activity day and averaged for each of the 12 months of the year. The fitted curves for h_r and h_a using the function *RichHobo* of the R package *Mapinguari* (Caetano et al., 2017) are overlaid in Figure 2AB, on top of the fitted curves based on $T_{e,i}$ rasters of microclim (Kearney et al., 2014).

3.4 AIC comparisons of models with different parameters.

We first compared SDMs at a 5x5 km resolution to take advantage of a new climate product from satellite data premised on Land Surface Temperature (LST), that shows in forested environments, deforestation increases air temperatures at a local scale (of 5x5 km cells in a 25x25km area) and thus may also impact extinction risk through elevated T_{max} (Prevedello et al., 2019).

We also fitted SDMs with altitude (m) a raster from (worldclim.org) to assess whether the lizard might be limited by elevation in some way that is not built into our rasters premised on thermal physiology. In models with the larger pseudo-absence bins, we first compared a model with h_a and h_r and annual precipitation (AIC = 733.3), with a model that additionally included TPC, which indicated a far better fit with the inclusion of TPC (AIC = 450.6). We next compared the last model with precipitation summed across the year (e.g. AIC = 450.6) with a model where the precipitation was split into a Dry-West periods, which indicated a far better fit with the splitting of Dry-Wet seasons (AIC = 335.6). However, we found that inclusion of forest levels did not improve the fit (AIC=584), nor did inclusion of altitude (AIC=664). Therefore, the species range of *B. imbricata* is best fit by ecophysiological variables (h_r , h_a , TPC, prec_{Wet} , prec_{Dry}) but no other biotic (forest) or abiotic factors (altitude).

We then fitted the SDM at a higher resolution of 1x1 km to obtain better resolution for all climate variables in the montane environments where *B. imbricata* was found (forest layers require local impacts to be estimated at 5 x 5 km, see, Prevedello et al., 2019). The parameters for the final fitted model are in

Table 1. We used this model for assess contemporary climate (worldclim.org) and estimate the future climate scenarios (Fig. 3).

3.5 Maps of presence of the *B. imbricata*

The projection for the contemporary time period (1975) shows that a single occurrence record has less than 10% of probability of presence, 179 points have more than 80% of probability of presence and on average the species has 90% of presence at the know occurrence. The projection with the RCP 2.6 shows that 3 occurrence records will have zero probability of presence, 118 points have more that 80% of presence and the species on average has 71% of presence. The projection with the RCP 4.5 shows 4 points will have zero probability of presence, 96 points have more that 80% of presence and on average the species has 60% of probability of presence. In contrast, the projection with the RCP 8.5 shows that 31 points will have zero of presence, 51 points have more that 80% probability of presence and in general the species has an overall 37% probability of presence. The current projection showed that 98 out of 221 collection points are at sites higher than 2,600 m altitude, of those 98 points; 88 points have more that 80% probability of presence for RCP 2.6, 77 for RCP 4.5 and 51 for the RCP 8.5.

4. Discussion

For species modeling, inclusion of the integrals for the Thermal Performance as a function of T_e for *B. imbricata* complemented the integrals for hours of restriction and hours of activity based on T_e . This is intuitively appealing: the integration for hours of activity and restriction operate at cool and high temperature ecophysiological limits: periods when activity is possible above the minimum field body temperature and periods when activity becomes restricted above the thermal preference. The integral for TPC is more nuanced supplying information on the interior of these extrema for ecophysiology of the Critical Thermal Limits, as daily T_e oscillates.

Our data for empirical T_e from two sites from populations at low and high elevation parts of the species range did correspond to the T_e curves computed from microclim (Kearney et al., 2018; Sinervo et al., 2018), but the high-temperature range for the empirical data was not as great as that seen for microclim rasters. The microclim based curves sample across all known occurrence records for a species range. Thus, a Richard's equation for modeling h_a and h_r under future scenarios would be greatly underestimated with empirical T_e estimates, while the biophysical T_e model is far more robust, spanning very low and very high temperatures found across the range of a given species.

Similar empirical and theoretical T_e curves, premised on climate variables (T_{Max}) and ecophysiology (T_{pref}) were used to compute extinction risk models of *Phrynocephalus* species spanning the Arabian Peninsula to the Qinghai-Tibetan plateau. These new biophysical-ecophysiological models provide a unified view of the evolution of the thermal physiology across 21 species. The empirical T_e data from PVC models also calibrated the biophysical estimates of T_e (Sinervo et al., 2018). We also found that T_e curves plotted against the climate parameter T_{Max} (i.e., ignoring T_{pref}) suggest a complex set of curves governs species differences (Figure 4, Sinervo et al., 2018) but plotting h_r against $f(T_{Max} - T_{pref})$ shows that all species lie on a single line. The use of $h_r = f(T_{Max} - T_{pref})$ also has a simple biophysical interpretation: the difference between maximum air temperature and preferred body temperature drives all of the key biophysical processes of conduction, convection, and radiation involved in heat transfer between the organism and its thermal environment (Kearney et al., 2014; Stevenson, 1985).

The new ecophysiological model we present here, with a Richard's equation for $h_r = f(T_{Max} - T_{pref})$ and $h_a = f(T_{Min} - T_{b, MinActivity})$, along with precipitation summed into a Dry and a Wet season segments spans the 3 key climate variables that are standard in rasters for climate change (T_{Max} , T_{Min} , $prec$) as well as key ecophysiological variables for the lower limits of activity in nature and thermal preference in the laboratory. The addition of the TPC as a function of biophysical models of T_e provides a simple set of integrated variables for predicting the future impacts of climate and physiology into a set of ecophysiological limits (T_{pref} , $T_{b, MinActivity}$, CT_{Min} , CT_{Max}) related to salient climate variables (T_{Max} , T_{Min}).

As mentioned by Gunderson and Leal (2012), studies of susceptibility of CC that mix correlative and mechanistic approaches present a valuable tool, being able to predict thermal conditions and their physiological consequences based on the complex interactions between thermal environment, thermal physiology and the behavior of a species. In this study, we included physiological and microenvironmental data from two populations with altitudinal extremes to have a complete approach of the physiological capacity of this species in the projections, and provide one of the most comprehensive set of physiological traits tested (h_r , h_a , TPCs, precipitation). We studied locomotor performance as a physiological index for two different populations of *B. imbricata* and we found that the interval for activity from this species is from 6° to 34°C, these data are important, because, it has been reported that the locomotor performance can be different between populations (Miles, 1994). The environmental conditions could be different in the different locations, therefore the climate sensitivity could be distinct, and the organisms could respond differently to the CC (Miles, 1994; Pontes-da-Silva et al., 2018), perhaps due to evolved genetic differences in TPCs.

Barisia imbricata is exemplary in having one of the widest plateaus for high activity among those TPCs reported in the literature, but it also has a very low CT_{Max} (37°C). Corresponding to this wide

plateau for high performance, the observed field T_b for active lizards is also quite broad (Figure 1). We used the thermal preference of 28.4° C as the thermal threshold for *B. imbricata* and calculated 3.2 h_r as the critical limit for this viviparous species. In contrast, in previous studies of the Anguidae family the researchers used a T_b range of 21.4° to 32.3 ° C and calculated 5.6 h_r (Sinervo et al., 2010), pooling results across viviparous and oviparous species. As we showed the measure of the interval threshold temperatures for each species is a better proxy in the projections to assess the distributional effects of the CC and better understanding of the species along its distribution.

Despite the breadth of its thermal performance and thermoregulatory behaviors, this species is under an extremely high risk of extinction, with a persistence probability of 37% to 2070 (RCP 8.5). Sinervo et al. (2017) reported an 80 to 95% of persistence probability for the Anguidae family by 2080 (RCP 4.5 and 8.5 respectively), however, in our projection we include the locomotor performance and physiological limits for the species, so the prediction for the family in general could be underestimated.

Nevertheless, our projections of *B. imbricata* shows that the less affected populations could be those that are at higher altitudes. High altitude uniformly reduces the risk of extinction and species can persist if the areas are covered with forests (Sinervo et al., 2017). This result, agrees with Sinervo et al. (2017), who implemented a simpler model based on h_r of Sinervo et al. 2010, since they found that species occurring in high altitude distributions have lower extinction risk than lowland populations as they have better thermal quality and more refuges to face of CC. Sinervo et al. (2017) propose protecting areas adjacent to species at risk above 2,600 m, which could potentially increase persistence of species at risk. This included in climate-risk version of GAP Analysis (Scott et al., 1993) to analyze specific species at the highest risk, but did not have protected areas that might act as future climate refugia (see also Sinervo et al., 2018). If CO₂ production were reduced as projected in RCP 2.6, 88 (40%) current occurrence records that are above 2,600 m would a persistence probability greater than 80%. However, if all sites at altitudes higher than 2,600 m were protected, then 98 points (44% of total points) could be protected with probability of presence greater than 67%. Although the study area at 3,700 m is part of a National Park, we consider it important to emphasize the protection of high altitudinal sites in general, because, some adjacent areas are unprotected and suffer from active deforestation, which could isolate the gene exchange of the protected population and severely affect long-term species persistence.

There is a higher extinction risk in viviparous species than in oviparous because evolved much lower heat tolerances, during the invasion of cold climates at high altitudes or latitudes (Sinervo et al., 2010, 2018, 2017; Wang, Ma, Shao, & Ji, 2017). Sinervo et al. (2017) reported that two thirds of the current extinctions in Mexican *Sceloporus* lizards were due to thermal causes such as low T_b related to viviparity. Wang et al. (2017) support the hypothesis that viviparous lizards are more vulnerable to CC,

however, they report that this is because warming dramatically limits their activity, not due to the decrease in T_b and thermal tolerance. We found that *Barisia imbricata* can be active between 9.1° to 36.6° C, nevertheless, their activity decreases dramatically above 30° C. Considering their activity at wide range of temperatures and its viviparity, *B. imbricata* could be more affected than projected in this study, so we suggest future studies on the local thermal adaptations of the species. If organisms fail to adapt physiologically to the new environmental conditions, they must modify their daily activity pattern or their range of distribution (Kearney, Shine, & Porter, 2009; Sinervo et al., 2018; Vicenzi et al., 2017), and this can cause that organisms to reduce their activity, and even during extreme warm spells, we will expect a total cessation of reproduction causing locals extinctions due to the energetic cost (Sinervo et al., 2010; Sinervo et al., 2011).

In conclusion, inclusion of TPC as function of T_e increases the power of our ecophysiological model and was essential to assess the possible effects of climate change on a eurythermic lizard as *B. imbricata*. We demonstrated that in the worst climate change scenario *B. imbricata* will have serious problem in low areas, and the habitats suitable for the species will be present only at the highest altitudes. According to this scenario it is necessary to focus more effort in the protection and conservation of the species at high-elevation sites of its contemporary range.

5. Acknowledgements

We thank Gabriel González, Aldo López, Ana Pérez, Ángel Soto, Raúl Gómez-Trejo and Timo Budesheim for help during the field work. This article is part of PhD Thesis of the Fierro-Estrada N., at the Programa de Posgrado en Ciencias Biológicas, UNAM, funded by a scholarship awarded to N. Fierro-Estrada CONACyT (412836). We thank UNAM PAPIIT/DGAPA (IN210116) for financial support to FRMC. B. Sinervo and D.B. Miles were supported by an NSF grant in the Emerging Frontiers program (EF-1241848). The permits for the research were granted by SEMARNAT (01629) and for the Parque Nacional Iztaccihuatl-Popocatepetl CONANP (DRCEN / PNIP / 823/2014) and SEMARNAT (005406/18) to FRMC.

6. Statement to archive the data

We will archive the data and R code in Open Science Framework when the article is published.

7. References

- Anav, A., Friedlingstein, P., Kidston, M., Bopp, L., Ciais, P., Cox, P., ... Zhu, Z. (2013). Evaluating the Land and Ocean Components of the Global Carbon Cycle in the CMIP5 Earth System Models. *Journal of Climate*, 26(18), 6801–6843. doi: 10.1175/JCLI-D-12-00417.1
- Angilletta, M. J., Niewiarowski, P. H., & Navas, C. A. (2002). The evolution of thermal physiology in ectotherms. *Journal of Thermal Biology*, 27, 249–268.
- Besson, A. A., & Cree, A. (2010). A cold-adapted reptile becomes a more effective thermoregulator in a thermally challenging environment. *Oecologia*, 163(3), 571–581. doi: 10.1007/s00442-010-1571-y
- Caetano, G., Santos, J. C., Miles, D., & Sinervo, B. (2017). Mappinguari v0.0.1 (Version v0.0.1). *Zenodo*, September(8). doi: 10.5281/zenodo.887962
- Chamaillé-Jammes, S., Massot, M., Aragón, P., & Clobert, J. (2006). Global warming and positive fitness response in mountain populations of common lizards *Lacerta vivipara*. *Global Change Biology*, 12, 392–402. doi: 10.1111/j.1365-2486.2005.01088.x
- Currie, R. J., Bennett, W. A., & Beitinger, T. L. (1998). Critical thermal minima and maxima of three freshwater game-fish species acclimated to constant temperatures. *Environmental Biology of Fishes*, 51, 187–200. doi: 10.1023/A:1007447417546
- Dashevsky, D., Meik, J. M., Mociño-Deloya, E., Setser, K., & Schaack, S. (2013). Patterns of sexual dimorphism in Mexican alligator lizards, *Barisia imbricata*. *Ecology and Evolution*, 3(2), 255–261. doi: 10.1002/ece3.455
- Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., & Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences*, 105(18), 6668–6672.
- Dubois, Y., Blouin-Demers, G., Shipley, B., & Thomas, D. (2009). Thermoregulation and habitat selection in wood turtles *Glyptemys insculpta*: Chasing the sun slowly. *Journal of Animal Ecology*, 78, 1023–1032. doi: 10.1111/j.1365-2656.2009.01555.x
- Elliott, A. (1994). A comparison of thermal polygons for british freshwater teleosts. *Journal of the Freshwater Biological Association*, 5(3), 178–184.
- Guillete, L. J., & Smith, H. M. (1982). A Review of the Mexican Lizard *Barisia imbricata*, and the Description of a New Subspecies. *Kansas Academy of Science*, 85(1), 13–33.
- Guillette, L. J., & Casas-Andreu, G. (1987). The Reproductive Biology of the High Elevation Mexican Lizard *Barisia imbricata*. *Herpetologica*, 43(1), 29–38.
- Gunderson, A. R., & Leal, M. (2012). Geographic variation in vulnerability to climate warming in a tropical Caribbean lizard. *Functional Ecology*, 26(4), 783–793. doi: 10.1111/j.1365-2435.2012.01987.x
- Hertz, P. E., Huey, R. B., & Stevenson, R. D. (1993). Evaluating Temperature Regulation by Field-Active Ectotherms: The Fallacy of the Inappropriate Question. *The American Naturalist*, 142(5), 796–818.
- Huey, R. B. (1982). Temperature, Physiology, and the Ecology of Reptiles. In F. H. Gans, C. and Pough (Ed.), *Biology of the Reptilia* (Vol. 12, pp. 25–91). doi: 10.1016/j.dsr.2014.07.003
- Huey, R. B., & Slatkin, M. (1976). Cost and benefits of lizard thermoregulation. *The Quarterly Review of Biology*, 51(3), 363–384. doi: 10.1086/409470
- IPCC. (2013). *Cambio Climático 2013. Bases físicas* (T. F. Stocker, D. Qin, G.-K. Plattner, M. M. B. Tignor, S. K. Allen, J. Boschung, ... P. Midgley, eds.). doi: 10.1017/CBO9781107415324.
- Kearney, M. R., Munns, S. L., Moore, D., Malishev, M., & Bull, C. M. (2018). Field tests of a general ectotherm niche model show how water can limit lizard activity and distribution. *Ecological Monographs*, 88(4), 672–693. doi: 10.1002/ecm.1326
- Kearney, M. R., & Porter, W. (2009). Mechanistic niche modelling: Combining physiological and spatial data to predict species' ranges. *Ecology Letters*, 12(4), 334–350. doi: 10.1111/j.1461-0248.2008.01277.x
- Kearney, M. R., Shamakhy, A., Tingley, R., Karoly, D. J., Hoffmann, A. A., Briggs, P. R., & Porter, W. P. (2014). Microclimate modelling at macro scales: a test of a general microclimate model integrated with gridded continental-scale soil and weather data. *Methods in Ecology and Evolution*, 5(3), 273–286. doi: 10.1111/2041-210X.12148
- Kearney, M. R., Shine, R., & Porter, W. P. (2009). The potential for behavioral thermoregulation to buffer “cold-blooded” animals against climate warming. *Proceedings of the National Academy of Sciences*, 106(10), 3835–3840. doi: 10.1073/pnas.0808913106
- Kearney, M. R., Wintle, B. A., & Porter, W. P. (2010). Correlative and mechanistic models of species distribution provide congruent forecasts under climate change. *Conservation Letters*, 3(3), 203–213. doi: 10.1111/j.1755-263X.2010.00097.x

- Kubisch, E. L., Corbalán, V., Ibarquengoytía, N. R., & Sinervo, B. (2016). Local extinction risk of three species of lizard from Patagonia as a result of global warming. *Canadian Journal of Zoology*, *94*(1), 49–59. doi: 10.1139/cjz-2015-0024
- Labra, A., Vidal, M. A., Solís, R., & Penna, M. (2008). Ecofisiología de anfibios y reptiles. In M. A. Vidal & A. Labra (Eds.), *Herpetología de Chile* (pp. 483–516). Springer Verlag.
- Lara-Reséndiz, R. A., Larraín-Barrios, B. C., Díaz De La Vega-Pérez, A. H., & Méndez-De La Cruz, F. R. (2014). Calidad térmica a través de un gradiente altitudinal para una comunidad de lagartijas en la sierra del Ajusco y el Pedregal de San Ángel, México. *Revista Mexicana de Biodiversidad*, *85*, 885–897. doi: 10.7550/rmb.42249
- Lemos-Espinal, J., Smith, G. R., & Ballinger, R. E. (1998). Temperature relationships of the lizard, *Barisia imbricata*, from México. *Amphibia-Reptilia*, *19*, 95–99.
- Miles, D. B. (1994). Population differentiation in locomotor performance and the potential response of a terrestrial organism to global environmental change. *American Zoologist*, *34*(3), 422–436. doi: 10.1093/icb/34.3.422
- Pontes-da-Silva, E., Magnusson, W. E., Sinervo, B., Caetano, G. H., Miles, D. B., Colli, G. R., ... Werneck, F. P. (2018). Extinction risks forced by climatic change and intraspecific variation in the thermal physiology of a tropical lizard. *Journal of Thermal Biology*, *73*(February), 50–60. doi: 10.1016/j.jtherbio.2018.01.013
- Prevedello, J. A., Winck, G. R., Weber, M. M., Nichols, E., & Sinervo, B. (2019). Impacts of forestation and deforestation on local temperature across the globe. *PLoS ONE*, *14*(3), 1–18. doi: <https://doi.org/10.1371/journal.pone.0213368>
- Scott, J. M., Davis, F., Csuti, B., Noss, R., Butterfield, B., Groves, C., ... Wright, R. G. (1993). Gap Analysis: A Geographic Approach to Protection of Biological Diversity. *Wildlife Monographs*, (123), 3–41.
- Shine, R. (2005). Life-History Evolution in Reptiles. *Annual Review of Ecology, Evolution, and Systematics*, *36*(1), 23–46. doi: 10.1146/annurev.ecolsys.36.102003.152631
- Sinclair, B. J., Marshall, K. E., Sewell, M. A., Levesque, D. L., Willett, C. S., Slotsbo, S., ... Huey, R. B. (2016). Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? *Ecology Letters*, *19*(11), 1372–1385. doi: 10.1111/ele.12686
- Sinervo, B., Mendez-de-la-Cruz, F., Miles, D. B., Heulin, B., Bastiaans, E., Villagran-Santa Cruz, M., ... Sites Jr, J. W. (2010). Erosion of Lizard Diversity by Climate Change and Altered Thermal Niches. *Science*, *328*, 894–899. doi: 10.1126/science.1184695
- Sinervo, B., Miles, D. B., Martinez-Mendez, N., Lara-Resendiz, R., & R. M.-D. la C. F. R. (2011). Response to Comment on “Erosion of Lizard Diversity by Climate Change and Altered Thermal Niches.” *Science*, *332*(6029), 537. doi: 10.1126/science.1195348
- Sinervo, B., Miles, D. B., Wu, Y., Méndez de la Cruz, F. R., Kirchoff, S., & Qi, Y. (2018). Climate change, thermal niches, extinction risk and maternal-effect rescue of Toad-headed lizards, *Phrynocephalus*, in thermal extremes of the Arabian Peninsula to the Tibetan Plateau. *Integrative Zoology*, *13*, 450–470. doi: 10.1111/1749-4877.12315
- Sinervo, B., Resendiz, R. A. L., Miles, D. B., Lovich, J. E., Ennen, J. R., Muller, J., ... Méndez-de la Cruz, F. R. (2017). Climate Change and Collapsing Thermal Niches of Mexican Endemic Reptiles Sinervo. *EScholarship*, 21. Retrieved from <https://escholarship.org/uc/item/4xk077hp>
- Stevenson, R. D. (1985). Body Size and Limits to the Daily Range of Body Temperature in Terrestrial Ectotherms. *The American Naturalist*, *125*(1), 102–117. Retrieved from <https://www.jstor.org/stable/2461610> Accessed:
- Thomas, C. D., Cameron, A., Green, R. E., Bakkenes, M., Beaumont, L. J., Collingham, Y. C., ... Williams, S. E. (2004). Extinction risk from climate change. *Nature*, *427*(8), 145–148.
- Vicenzi, N., Corbalán, V., Miles, D., Sinervo, B., & Ibarquengoytía, N. (2017). Range increment or range detriment? Predicting potential changes in distribution caused by climate change for the endemic high-Andean lizard *Phymaturus palluma*. *Biological Conservation*, *206*, 151–160. doi: 10.1016/j.biocon.2016.12.030
- Wang, Z., Ma, L., Shao, M., & Ji, X. (2017). Are viviparous lizards more vulnerable to climate warming because they have evolved reduced body temperature and heat tolerance? *Oecologia*, *185*(4), 573–582. doi: 10.1007/s00442-017-3979-0
- Zaldivar-Riverón, A., Nieto-Montes De Oca, A., & Lacleste, J. P. (2005). Phylogeny and evolution of dorsal pattern in the Mexican endemic lizard genus *Barisia* (Anguillidae: Gerrhonotinae). *Journal of Zoological Systematics and Evolutionary Research*, *43*(3), 243–257. doi: 10.1111/j.1439-0469.2005.00308.x

Tables

Table 1. Coefficients of GLM models.

Degrees of Freedom: 1220 Total (i.e. Null); 1207 Residual

Null Deviance: 2913

Residual Deviance: 548.4 AIC: 576.4

	Coefficients
Intercept	-2.999e+0
I(ha ²)	-7.476e+00
hr	3.164e-01
TPC	6.727e+01
I(TPC ²)	-3.934e+01
prec _{Dry}	-5.721e-02
ha	3.657e+01
I(hr ²)	-1.121e-03
TPC: prec _{Dry}	6.355e-02
hr: prec _{Dry}	9.986e-05
hr:TPC	-4.721e-01
TPC:ha	-3.271e+01
prec _{Dry} :ha	1.116e-02
hr:ha	-6.772e-02

Figures

Fig.1 A) The distribution of field body temperature data for *Barisia imbricata* with $T_{b,MinActivity} = 9.1^{\circ}\text{C}$ and $\bar{T}_b = 20.0^{\circ}\text{C}$. B) The distribution of data on thermal preference ($\bar{T}_{pref} = 28.4^{\circ}\text{C}$) as measured in the laboratory.

Fig.2. A) The fitted Richard's growth equations for hours of restriction for *Barisia imbricata*, h_r , ($\tau_{r,1} = 12$; $\tau_{r,2} = 3.9596$; $\tau_{r,3} = 0.17868$; $\tau_{r,4} = 1.3344$, $T_{pref} = 28.4$) and B) hours of activity, h_a ($\tau_{a,1} = 12$; $\tau_{a,2} = 38.8630$; $\tau_{a,3} = 0.43694$; $\tau_{a,4} = 4.4204$). During the fitting (JMP 14) $\tau_{r,1}$ was constrained to the same value as $\tau_{a,1} = 12$ h. C) The fitted Kumaraswamy thermal performance curve of *B. imbricata* ($a = 1.25259$, $b = 1.19027$, $c = 3.37983$, $CT_{Min} = 1.8647$, $CT_{Max} = 37.00$).

Fig.3. Maps of probability of presence of *Barisia imbricata*, the contemporary time (A), RCP 2.6 (B), RCP 4.5(C) and 8.5(D).

Fig.1

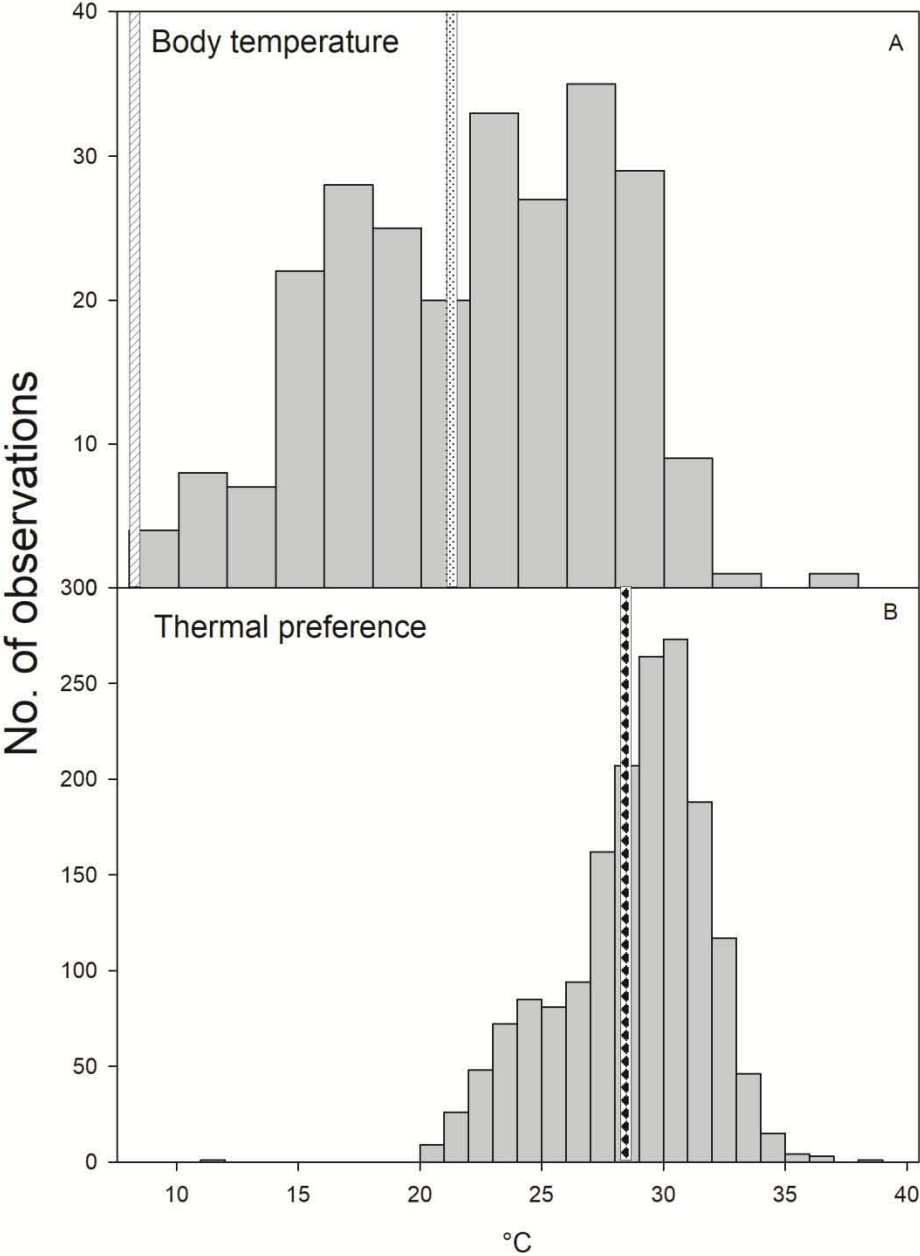


Fig. 2

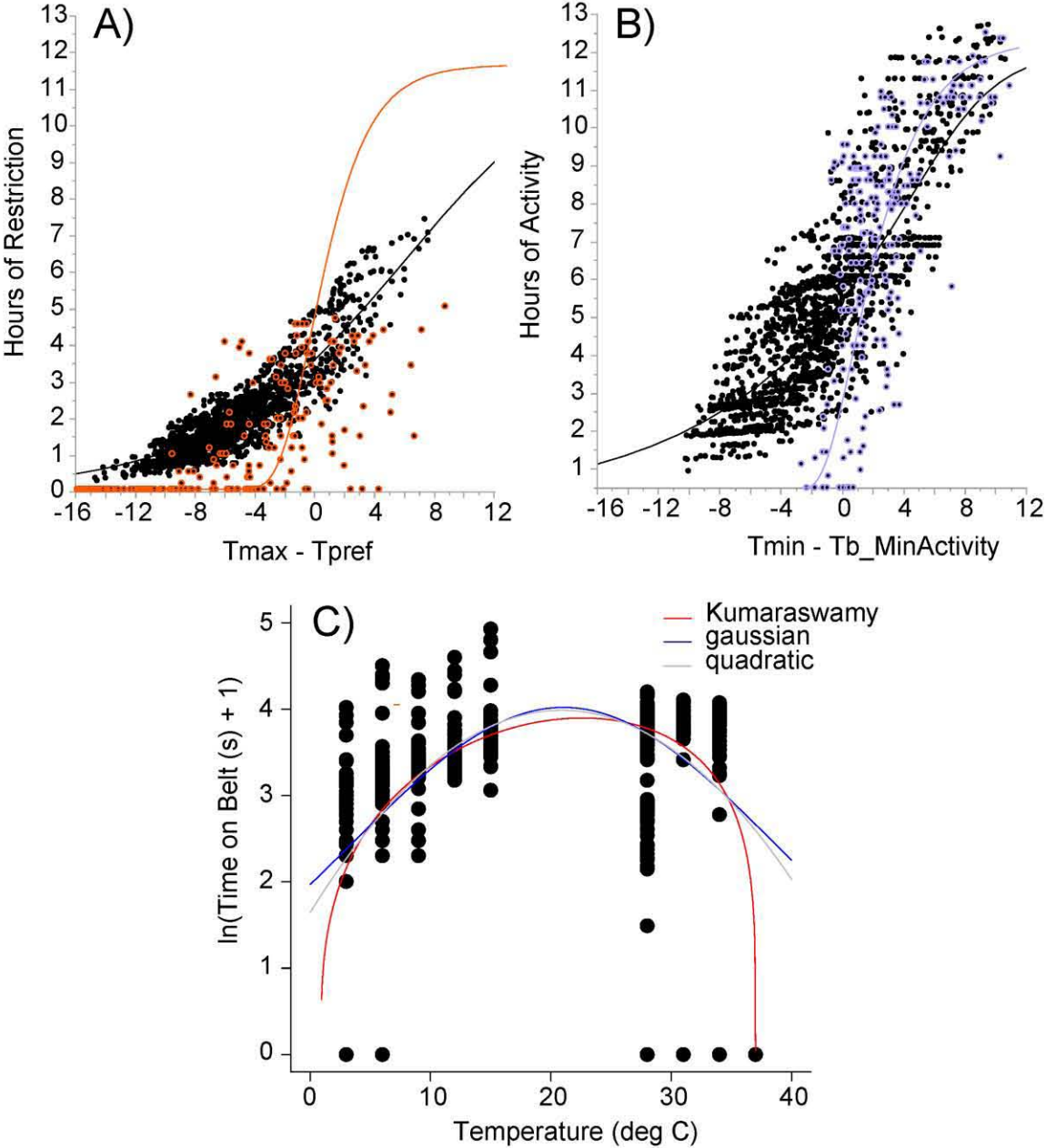
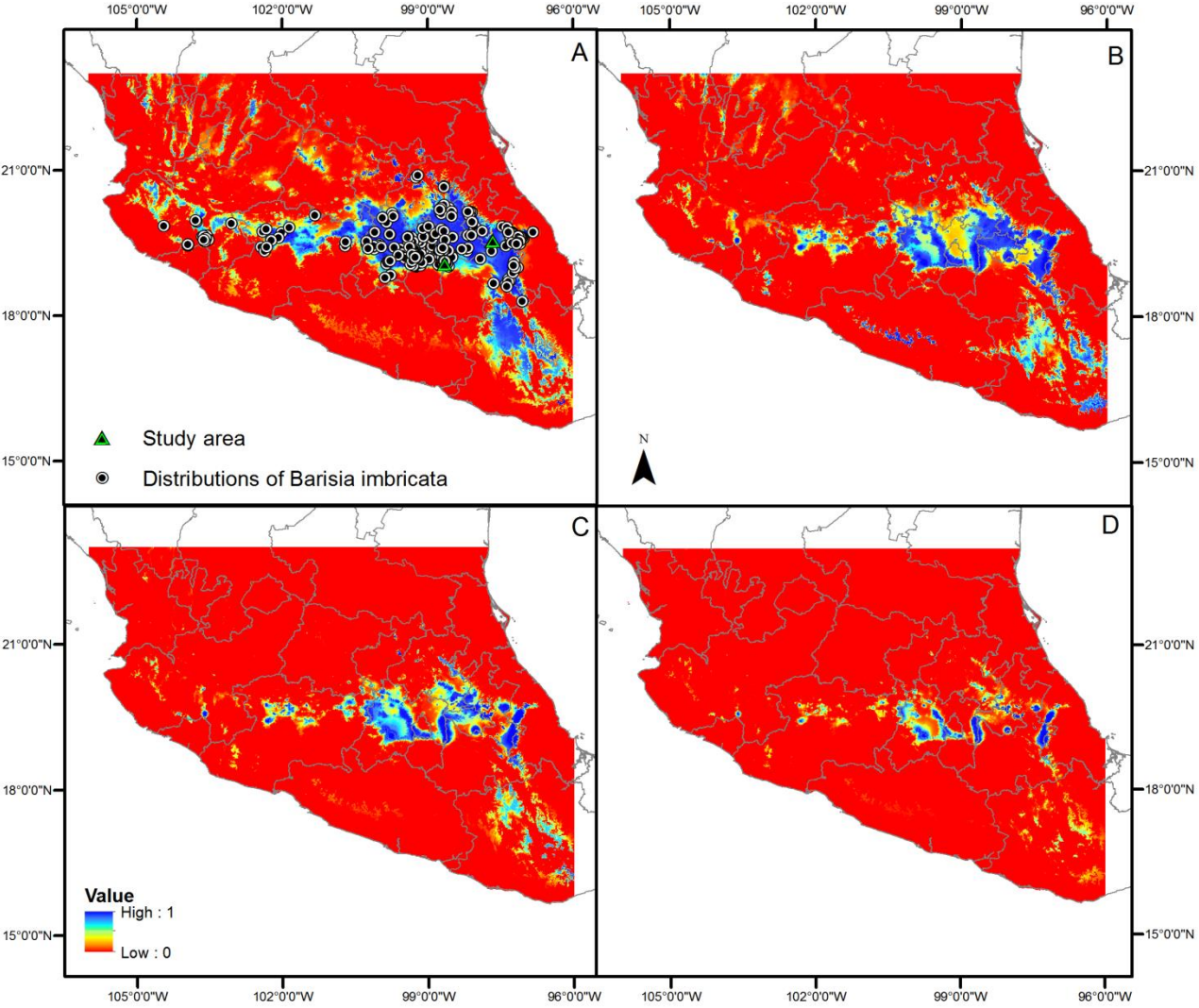


Fig. 3



Discusión General y Conclusiones

En los últimos años ha aumentado el conocimiento sobre la ecología térmica de lagartijas (Arenas-Moreno et al., 2018; Diele-Viegas et al., 2018; Lara-Reséndiz et al., 2015, 2014). Sin embargo, pocas investigaciones han abordado las diferencias termorreguladoras entre estaciones y entre poblaciones de la misma especie (Artacho et al., 2017; Lemos-Espinal and Ballinger, 1995). En este estudio, se encontró que los organismos de *Barisia imbricata* emplean mecanismos termorreguladores diferentes entre poblaciones. Los organismos de la población a gran altitud pueden termorregular activamente; en contraparte, cuando el ambiente presenta temperaturas similares a la temperatura preferida, como el caso de la población a baja altitud, los organismos tienden al extremo termoconformista.

Existen dos enfoques para explicar los mecanismos termorreguladores entre poblaciones. El primer enfoque propone que las temperaturas óptimas se conservan evolutivamente dentro de las especies. En contraparte, el segundo enfoque sugiere que la biología térmica de las lagartijas debería reflejar la adaptación local a diferentes ambientes térmicos (Angilletta Jr, 2009; Gabirot et al., 2013; Huey, 1982; Huey et al., 2003; Lemos-Espinal and Ballinger, 1995; Rodríguez-Serrano et al., 2009). Por lo cual, algunas poblaciones podrían mostrar diferencias en la temperatura corporal cuando ocupan hábitats diferentes, como es el caso de *B. imbricata* la cual presentó diferencias en el promedio de la temperatura corporal entre estaciones y entre poblaciones. No obstante, en el caso de su temperatura seleccionada no difirió entre poblaciones.

El amplio intervalo de las temperaturas corporales a las cuales se encontraron activos los organismos de *B. imbricata* refleja la euritermia de esta especie. Esta tendencia ha sido observada en otras especies de lagartijas de la familia Anguidae (Fierro-Estrada, 2013; Kingsbury, 1993).

Los organismos de *B. imbricata* mostraron actividad a bajas temperaturas corporales, dicha capacidad ha sido reportada en otras especies de la familia Anguidae (Bautista del Moral, 2019; Cunningham, 1966; Fierro-Estrada, 2013; Vial, 1975). Sin embargo, *B. imbricata* también presentó altos niveles de glucosa en sangre durante todo el año. Se sabe que almacenar grandes cantidades de glucosa puede proveer la capacidad de

sobrevivir al congelamiento; dicho mecanismo se ha estudiado ampliamente en algunos organismos, como la lagartija europea *Zootoca vivipara* y la rana *Lithobates sylvatica* (Costanzo et al., 1995b, 1995a; Grenot et al., 2000; Storey and Storey, 1986).

La población de mayor altitud de *B. imbricata* presentó niveles más altos de glucosa en comparación con la población de menor altitud, sin embargo, los niveles en esa población fueron similares entre las cuatro estaciones. En contraparte, la población de menor altitud presentó diferencias significativas en los niveles de glucosa entre estaciones. En algunos organismos los niveles de glucosa en sangre pueden variar entre poblaciones de diferentes regiones como en *L. sylvatica*, además, en esta especie se ha observado que la glucosa se acumula durante otoño y el comienzo de invierno, por lo que suele ser diferente entre estaciones (Costanzo et al., 2013, 1993).

Los organismos de *B. imbricata* provenientes de dos poblaciones con altitudes extremas sobrevivieron al congelamiento experimental, y en las estaciones más frías elevaron su nivel de glucosa después del congelamiento. Se sabe que dicho mecanismo el cual se presenta en *Z. vivipara* y *L. sylvatica*, ayuda a mitigar los daños producidos por la formación de cristales de hielo (Costanzo et al., 1995a, 1993, 1991; Storey and Storey, 1986).

Por último, las predicciones de presencia de *B. imbricata* al año 2070 ante las consecuencias del cambio climático (calentamiento global), muestran que la especie se verá seriamente afectada, y que las poblaciones que presentan mayor probabilidad de presencia son las que actualmente se localizan a mayor altitud, esto concuerda con Sinervo et al. (2017), quienes encontraron que las especies con distribuciones de gran altitud podrían tener mayores refugios ante el cambio climático.

Literatura General

- Andrews, R. M., Méndez-de la Cruz, F. R., Villagrán-Santa Cruz, M., & Rodríguez-Romero, F. (1999). Field and Selected Body Temperatures of the Lizards *Sceloporus aeneus* and *Sceloporus bicanthalis*. *Journal of Herpetology*, 33(1), 93–100.
- Angilletta, M. J. (2009). *Thermal Adaptation*. Oxford University Press.
- Angilletta, M. J., Niewiarowski, P. H., & Navas, C. A. (2002). The evolution of thermal physiology in ectotherms. *Journal of Thermal Biology*, 27, 249–268.
- Arenas-Moreno, D. M., Santos-Bibiano, R., Muñoz-Nolasco, F. J., Charruau, P., & Méndez-de la Cruz, F. R. (2018). Thermal ecology and activity patterns of six species of tropical night lizards (Squamata : Xantusiidae : Lepidophyma) from Mexico. *Journal of Thermal Biology*, 75, 97–105. doi: 10.1016/j.jtherbio.2018.06.001
- Artacho, P., Saravia, J., Perret, S., Bartheld, J. L., & Le Galliard, J. F. (2017). Geographic variation and acclimation effects on thermoregulation behavior in the widespread lizard *Liolaemus pictus*. *Journal of Thermal Biology*, 63(November 2016), 78–87. doi: 10.1016/j.jtherbio.2016.11.001
- Bautista del Moral, A. (2019). *Termorregulación en Mesaspis gadovii (Squamata : Anguidae) en Carrizal de Bravo, Guerrero* . Universidad Autónoma de Guerrero.
- Beitinger, T., Bennett, W. A., & McCauley, R. W. (2000). Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. *Environmental Biology of Fishes*, 58, 237–275. doi: 10.1023/A:1007676325825
- Blouin-Demers, G., & Nadeau, P. (2005). The Cost–Benefit Model of Thermoregulation Does Not Predict Lizard Thermoregulatory Behavior. *Ecology*, 86(3), 560–566. doi: 10.1890/04-1403
- Carvajalino-Fernández, J. M., Bonilla Gomez, M. A., & Navas, C. A. (2011). Freezing Risk in Tropical High-Elevation Anurans: An Assessment Based on the Andean Frog *Pristimantis nervicus* (Strobomantidae). *South American Journal of Herpetology*, 6(2), 73–78. doi: http://dx.doi.org/10.2994/057.006.020
- Castellini, M. A. (2009). Thermoregulation. In *Encyclopedia of Marine Mammals* (Second Edi, pp. 1166–1171). Academic Press.
- Chamaillé-Jammes, S., Massot, M., Aragón, P., & Clobert, J. (2006). Global warming and positive fitness response in mountain populations of common lizards *Lacerta vivipara*. *Global Change Biology*, 12, 392–402. doi: 10.1111/j.1365-2486.2005.01088.x
- Claussen, D. L., Townsley, M. D., & Bausch, R. G. (1990). Supercooling and freeze-tolerance in the European wall lizard, *Podarcis muralis*, with a revisional history of the discovery of freeze-tolerance in vertebrates. *Journal of Comparative Physiology B*, 160(2), 137–143. doi: 10.1007/BF00300945
- Costanzo, J. P., Baker, P. J., Dinkelacker, S. A., & Lee, R. E. (2003). *Endogenous and exogenous ice-nucleating agents constrain supercooling in the hatchling painted turtle*. (2001), 477–485. doi: 10.1242/jeb.00112
- Costanzo, J. P., do Amaral, M. C. F., Rosendale, A. J., & Lee, R. E. (2013). Hibernation physiology, freezing adaptation and extreme freeze tolerance in a northern population of the wood frog. *Journal of Experimental Biology*, 216(18), 3461–3473. doi: 10.1242/jeb.089342
- Costanzo, J. P., Grenot, C., & Lee, R. E. (1995). Supercooling, ice inoculation and freeze tolerance in the European common lizard, *Lacerta vivipara*. *Journal of Comparative Physiology B*, 165(3), 238–244. doi: 10.1007/BF00260815
- Costanzo, J. P., Lee Jr, R. E., & Wright, M. R. (1991). Effect of cooling rate on the survival of frozen wood frogs, *Rana sylvatica*. *Journal of Comparative Physiology*, 161, 225–229.
- Costanzo, J. P., & Lee, R. E. (2013). Avoidance and tolerance of freezing in ectothermic vertebrates. *The Journal of Experimental Biology*, 216, 1961–1967. doi: 10.1242/jeb.070268
- Costanzo, J. P., Lee, R. E., DeVries, A. L., Wang, T., & Layne, J. R. (1995). Survival mechanisms of vertebrate ectotherms at subfreezing temperatures: applications in cryomedicine. *The FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology*, 9(5), 351–358. doi: 10.1096/FASEBJ.9.5.7896003
- Costanzo, J. P., & Lee, R. E. J. (1996). Mini-Review: Ice Nucleation in Freeze-Tolerant Vertebrates. *Cryo-Letters*, 17, 111–118.
- Costanzo, J. P., Lee, R. J., & Lortz, P. H. (1993). Glucose Concentration Regulates Freeze Tolerance in the Wood Frog *Rana sylvatica*. *The Journal of Experimental Biology*, 181, 245–255.
- Cowles, R. B., & Bogert, C. M. (1944). A preliminary study of the thermal requirements of desert reptiles.

- American Museum of Natural History*, 83(5), 261–296.
- Cunningham, J. D. (1966). Thermal Relations of the Alligator Lizard *Gerrhonotus multicarinatus webbi*. *Herpetologica*, 22(1), 1–7.
- Currie, R. J., Bennett, W. A., & Beitinger, T. L. (1998). Critical thermal minima and maxima of three freshwater game-fish species acclimated to constant temperatures. *Environmental Biology of Fishes*, 51, 187–200. doi: 10.1023/A:1007447417546
- Darnell, M. Z., Nicholson, H. S., & Munguia, P. (2015). Thermal ecology of the fiddler crab *Uca panacea*: Thermal constraints and organismal responses. *Journal of Thermal Biology*, 52, 157–165. doi: 10.1016/j.jtherbio.2015.06.004
- Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., & Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences*, 105(18), 6668–6672.
- Diele-Viegas, L. M., Vitt, L. J., Sinervo, B., Colli, G. R., Werneck, F. P., Miles, D. B., ... Ávila-Pires, T. C. S. (2018). Thermal physiology of Amazonian lizards (Reptilia: Squamata). *PLoS ONE*, 13(3), 1–23. doi: 10.1371/journal.pone.0192834
- Du, W. G., Yan, S. J., & Ji, X. (2000). Selected body temperature, thermal tolerance and thermal dependence of food assimilation and locomotor performance in adult blue-tailed skinks, *Eumeces elegans*. *Journal of Thermal Biology*, 25(3), 197–202. doi: 10.1016/S0306-4565(99)00022-4
- Dubois, Y., Blouin-Demers, G., Shipley, B., & Thomas, D. (2009). Thermoregulation and habitat selection in wood turtles *Glyptemys insculpta*: Chasing the sun slowly. *Journal of Animal Ecology*, 78, 1023–1032. doi: 10.1111/j.1365-2656.2009.01555.x
- Elliott, A. (1994). A comparison of thermal polygons for british freshwater teleosts. *Journal of the Freshwater Biological Association*, 5(3), 178–184.
- Fierro-Estrada, N. (2013). *Ecología térmica de Abronia taeniata (Reptilia: Anguillidae) y su susceptibilidad ante el calentamiento global*. Universidad Nacional Autónoma de México.
- Gabirot, M., Balleri, A., López, P., & Martín, J. (2013). Differences in Thermal Biology Between Two Morphologically Distinct Populations of Iberian Wall Lizards Inhabiting Different Environments. *Annales Zoologici Fennici*, 50(4), 225–236. doi: <https://doi.org/10.5735/085.050.0406>
- Geiser, F. (2004). METABOLIC RATE AND BODY TEMPERATURE REDUCTION DURING HIBERNATION AND DAILY TORPOR. *Annual Review of Physiology*, 66, 239–274. doi: 10.1146/annurev.physiol.66.032102.115105
- Grenot, C. J., Garcin, L., Dao, J., Hérold, J. P., Fahys, B., & Tséré-Pagès, H. (2000). How does the European common lizard, *Lacerta vivipara*, survive the cold of winter? *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, 127(1), 71–80. doi: 10.1016/S1095-6433(00)00236-1
- Hertz, P. E., Huey, R. B., & Stevenson, R. D. (1993). Evaluating Temperature Regulation by Field-Active Ectotherms: The Fallacy of the Inappropriate Question. *The American Naturalist*, 142(5), 796–818.
- Huang, S., Huang, S., Chen, Y., & Tu, M. (2007). Thermal Tolerance and Altitudinal Distribution of Three *Trimeresurus* Snakes (Viperidae: Crotalinae) in Taiwan. *Zoological Studies*, 46(5), 592–599.
- Huey, R. B. (1974). Behavioral thermoregulation in lizards: importance of associated costs. *Science*, 184(4140), 1001–1003. doi: 10.1126/science.184.4140.1001
- Huey, R. B. (1982). Temperature, Physiology, and the Ecology of Reptiles. In F. H. Gans, C. and Pough (Ed.), *Biology of the Reptilia* (Vol. 12, pp. 25–91). doi: 10.1016/j.dsr.2014.07.003
- Huey, R. B., Hertz, P. E., & Sinervo, B. (2003). Behavioral Drive versus Behavioral Inertia in Evolution: A Null Model Approach. *The American Naturalist*, 161(3), 357–366. doi: 10.1086/346135
- Huey, R. B., & Pianka, E. R. (1977). Seasonal Variation in Thermoregulatory Behavior and Body Temperature of Diurnal Kalahari Lizards. *Ecology*, 58(5), 1066–1075. doi: 10.2307/1936926
- Huey, R. B., & Slatkin, M. (1976). Cost and benefits of lizard thermoregulation. *The Quarterly Review of Biology*, 51(3), 363–384. doi: 10.1086/409470
- Huey, R. B., & Stevens, R. D. (1979). Integrating Thermal Physiology and Ecology of Ectotherms: A Discussion of Approaches. *American Zoologist*, 19, 357–366.
- IPCC. (2013). *Cambio Climático 2013. Bases físicas* (T. F. Stocker, D. Qin, G.-K. Plattner, M. M. B. Tignor, S. K. Allen, J. Boschung, ... P. Midgley, Eds.). doi: 10.1176/CBO9781107415324
- Kearney, M. R., & Porter, W. (2009). Mechanistic niche modelling: Combining physiological and spatial data to predict species' ranges. *Ecology Letters*, 12(4), 334–350. doi: 10.1111/j.1461-0248.2008.01277.x
- Kearney, M. R., Wintle, B. A., & Porter, W. P. (2010). Correlative and mechanistic models of species distribution

- provide congruent forecasts under climate change. *Conservation Letters*, 3(3), 203–213. doi: 10.1111/j.1755-263X.2010.00097.x
- Kingsbury, B. A. (1993). Thermoregulatory Set Points of the Eurythermic Lizard *Elgaria multicarinata*. *Journal of Herpetology*, 27(3), 241–247.
- Labra, A., Vidal, M. A., Solís, R., & Penna, M. (2008). Ecofisiología de anfibios y reptiles. In M. A. Vidal & A. Labra (Eds.), *Herpetología de Chile* (pp. 483–516). Springer Verlag.
- Lara-Reséndiz, R. A., Gadsden, H., Rosen, P. C., Sinervo, B., & Méndez-De la Cruz, F. R. (2015). Thermoregulation of two sympatric species of horned lizards in the Chihuahuan Desert and their local extinction risk. *Journal of Thermal Biology*, 48(February), 1–10. doi: 10.1016/j.jtherbio.2014.11.010
- Lara-Reséndiz, R.A., Galina-Tessaro, P., Pérez-Delgadillo, A.G., Valdez-Villavicencio, J.H., Méndez-de la Cruz, F.R., 2019. Efectos del cambio climático en una especie de lagartija termófila de amplia distribución (*Dipsosaurus dorsalis*): un enfoque ecofisiológico. *Rev. Mex. Biodivers.* 1–11.
- Lara-Reséndiz, R. A., Larrain-Barrios, B. C., Díaz De La Vega-Pérez, A. H., & Méndez-De La Cruz, F. R. (2014). Calidad térmica a través de un gradiente altitudinal para una comunidad de lagartijas en la sierra del Ajusco y el Pedregal de San Ángel, México. *Revista Mexicana de Biodiversidad*, 85, 885–897. doi: 10.7550/rmb.42249
- Larson, D. J., Middle, L., Vu, H., Zhang, W., Serianni, A. S., Duman, J., & Barnes, B. M. (2014). Wood frog adaptations to overwintering in Alaska: new limits to freezing tolerance. *The Journal of Experimental Biology*, 217, 2193–2200. doi: 10.1242/jeb.101931
- Lemos-Espinal, J. A., & Ballinger, R. E. (1995). Comparative thermal ecology of the high altitude lizard *Sceloporus grammicus* on the eastern slope of the Iztaccihuatl Volcano, Puebla, Mexico. *Can. J. Zool.*, 73, 2184–2191.
- Lowe, C. H., Lardner, P. J., & Halpern, E. A. (1971). Supercooling in reptiles and other vertebrates. *Comparative Biochemistry and Physiology -- Part A: Physiology*, 39(A), 125–135. doi: 10.1016/0300-9629(71)90352-5
- Lutterschmidt, W. I., & Hutchison, V. H. (1997). The critical thermal maximum: history and critique. *Canadian Journal of Zoology*, 75, 1561–1574. doi: 10.1139/z97-783
- Méndez de la Cruz, F., Marínquez Moran, N. L., Arenas Ríos, E., & Ibagüengoytia, N. (2014). Male Reproductive Cycles in Lizards. In S. Rheubert, Justin L. Siegel, Dustin S. Trauth (Ed.), *Reproductive Biology and Phylogeny of Lizards and Turtles* (pp. 302–339). CRC Press.
- Ortega, Z., Mencía, A., & Pérez-Mellado, V. (2016). Behavioral buffering of global warming in a cold-adapted lizard. *Ecology and Evolution*, 6(13), 4582–4590. doi: 10.1002/ece3.2216
- Packard, G. C., & Packard, M. J. (2003). Natural freeze-tolerance in hatchling painted turtles ? *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, 134, 233–246.
- Pauly, G. B. (2012). Evidence for Freeze Tolerance in the Eastern Fence Lizard (*Sceloporus undulatus*). *The Southwestern Naturalist*, 57(4), 472–473. doi: 10.1894/0038-4909-57.4.472
- Porter, A. W. P., Mitchell, J. W., Beckman, W. A., & Dewitt, C. B. (1973). Behavioral Implications of Mechanistic Ecology . Thermal and Behavioral Modeling of Desert Ectotherms and Their Microenvironment. *Oecologia*, 13(1), 1–54. Retrieved from <https://www.jstor.org/stable/4214873>
- Rodríguez-Serrano, E., Navas, C. A., & Bozinovic, F. (2009). The comparative field body temperature among *Liolaemus* lizards : Testing the static and the labile hypotheses. *Journal of Thermal Biology*, 34, 306–309. doi: 10.1016/j.jtherbio.2009.04.002
- Sherbrooke, W. C., Castrucci, A. M. D. L., & Hadley, M. E. (1994). Temperature Effects on in vitro Skin Darkening in the Mountain Spiny Lizard, *Sceloporus jarrovi* : A Thermoregulatory Adaptation ? *Physiological Zoology*, 67(3), 659–672. Retrieved from <http://www.jstor.org/stable/30163763>
- Shine, R. (2005). Life-History Evolution in Reptiles. *Annual Review of Ecology, Evolution, and Systematics*, 36(1), 23–46. doi: 10.1146/annurev.ecolsys.36.102003.152631
- Sinervo, B., Mendez-de-la-Cruz, F., Miles, D. B., Heulin, B., Bastiaans, E., Villagran-Santa Cruz, M., ... Sites Jr, J. W. (2010). Erosion of Lizard Diversity by Climate Change and Altered Thermal Niches. *Science*, 328, 894–899. doi: 10.1126/science.1184695
- Sinervo, B., Miles, D. B., Wu, Y., Méndez de la Cruz, F. R., Kirchoff, S., & Qi, Y. (2018). Climate change, thermal niches, extinction risk and maternal-effect rescue of Toad-headed lizards, *Phrynocephalus*, in thermal extremes of the Arabian Peninsula to the Tibetan Plateau. *Integrative Zoology*, 13, 450–470. doi: 10.1111/1749-4877.12315
- Sinervo, B., Resendiz, R. A. L., Miles, D. B., Lovich, J. E., Ennen, J. R., Muller, J., ... Méndez-de la Cruz, F. R. (2017). Climate Change and Collapsing Thermal Niches of Mexican Endemic Reptiles Sinervo, *EScholarship*, 21.

- Retrieved from <https://escholarship.org/uc/item/4xk077hp>
- Soyano, K., & Mushirobira, Y. (2018). The Mechanism of Low- Temperature Tolerance in Fish. In M. Iwana-Inoue (Ed.), *Survival Strategies in Extreme Cold and Desiccation*. (p. 1081). doi: 10.1007/978-981-13-1244-1
- Storey, K. B., & Storey, J. M. (1986). Freeze tolerance and intolerance as strategies of winter survival in terrestrially-hibernating amphibians. *Comparative Biochemistry and Physiology -- Part A: Physiology*, 83(4), 613–617. doi: 10.1016/0300-9629(86)90699-7
- Storey, K. B., & Storey, J. M. (2004). Physiology , biochemistry and molecular biology of vertebrate freeze tolerance : the wood frog . In *Life in the Frozen State* (pp. 243–274).
- Tattersall, G. J., Leite, C. A. C., Sanders, C. E., Cadena, V., Andrade, D. V, Abe, A. S., & Milsom, W. K. (2016). Seasonal reproductive endothermy in tegu lizards. *Science Advances*, 2(1), 1–8. doi: 10.1126/sciadv.1500951
- Tejedo, M., Duarte, H., Gutiérrez-Pesquera, L. M., Beltrán, J. F., Katzenberger, M., Marangoni, F., ... Solé, M. (2012). El estudio de las tolerancias térmicas para el examen de hipótesis biogeográficas y de la vulnerabilidad de los organismos ante el calentamiento global. Ejemplos en anfibios. *Boletín de La Asociación Herpetológica Española*, 23(2), 2–27.
- Thomas, C. D., Cameron, A., Green, R. E., Bakkenes, M., Beaumont, L. J., Collingham, Y. C., ... Williams, S. E. (2004). Extinction risk from climate change. *Nature*, 427(8), 145–148.
- Vicenzi, N., Corbalán, V., Miles, D., Sinervo, B., & Ibargüengoytía, N. (2017). Range increment or range detriment ? Predicting potential changes in distribution caused by climate change for the endemic high-Andean lizard *Phymaturus palluma*. *Biological Conservation*, 206, 151–160. doi: 10.1016/j.biocon.2016.12.030
- Voituron, Y., Heulin, B., & Surget-Groba, Y. (2004). Comparison of the Cold Hardiness Capacities of the Oviparous and Viviparous Forms of *Lacerta vivipara*. *Journal of Experimental Zoology*, 301A, 367–373. doi: 10.1002/jez.a
- Voituron, Y., Storey, J. M., Grenot, C., & Storey, K. B. (2002). Freezing survival, body ice content and blood composition of the freeze-tolerant European common lizard, *Lacerta vivipara*. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, 172(1), 71–76. doi: 10.1007/s003600100228
- Wang, Z., Ma, L., Shao, M., & Ji, X. (2017). Are viviparous lizards more vulnerable to climate warming because they have evolved reduced body temperature and heat tolerance? *Oecologia*, 185(4), 573–582. doi: 10.1007/s00442-017-3979-0
- Weeks, D. M., & Espinoza, R. E. (2013). Lizards on ice: Comparative thermal tolerances of the world's southernmost gecko. *Journal of Thermal Biology*, 38, 225–232. doi: 10.1016/j.jtherbio.2013.02.005
- Whittow, G. C. (1966). Terminology of Thermoregulation. *Physiologist*, 9(1), 358–360.
- Willmer, P., Stone, G., & Johnston, I. (2005). Environmental Physiology of Animals. In *Environmental Physiology of Animals* (Second Edi). Blackwell Publishing.