UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS
INSTITUTO DE BIOLOGÍA

ECOLOGÍA

MODO DE PARIDAD, DESEMPEÑO FISIOLÓGICO, COMPORTAMIENTO TERMORREGULADOR Y DISTRIBUCIÓN GEOGRÁFICA EN LACERTILIOS ANTE EL CAMBIO CLIMÁTICO GLOBAL

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTOR EN CIENCIAS

PRESENTA:

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MÉXICO, D.F. OCTUBRE 2013

UNAM POSGRADO
Ciencias Biológicas

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

ATENTAMENTE
"POR MI RAZA HABLARA EL ESPIRITU"
Cd. Universitaria, D.F. a 17 de septiembre de 2013.

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Agradecimientos

Agradezco al Posgrado en Ciencias Biológicas de la UNAM por todo el apoyo y facilidades recibidos durante mis estudios de posgrado. Al Consejo Nacional de Ciencia y Tecnología (CONACyT) por la beca otorgada para la realización mi tesis doctoral. Al Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (DGAP-PAPIIT IN221208-3), por el financiamiento para trabajo en campo y adquisición de material de laboratorio que permitió realizar esta investigación. Al PAEP del posgrado UNAM por el respaldo económico para la asistencia a congresos académicos nacionales e internacionales.

A mi tutor el Dr. Enrique Martínez Meyer y a los miembros de mi comité tutoral los Doctores Oswaldo Téllez Valdés y Eduardo Morales Guillaumín quienes siempre aportaron su experiencia y valiosas observaciones que contribuyeron al desarrollo de este trabajo.

Agradecimientos a título personal

Deseo agradecer de manera especial al Dr. Enrique Martínez Meyer por bríndame la oportunidad de ser parte del Laboratorio de Análisis Espaciales, por su apoyo y excelente guía durante el desarrollo de mis estudios de posgrado que han contribuido significativamente a mi formación profesional, además de su amistad. A los miembros de mi comité tutoral el Dr. Oswaldo Téllez Valdés y el Dr. Eduardo Morales Guillaumín que aportaron siempre ideas y consejos oportunos que contribuyeron a la culminación exitosa de este trabajo.

A los Doctores Adrián Nieto Motes De Oca, José Jaime Zúñiga Vega, Andrés García Aguayo, José Marín García Varela, Constantino Macías García y Carlos Rafael Cordero Macedo por su ayuda y excelentes cometarios durante el examen de candidatura y en la etapa de revisión final que enriquecieron notablemente este trabajo.

Al Dr. Miguel Nakamura del Centro de Investigación en matemáticas (CIMAT) por su tiempo, hospitalidad e invaluable ayuda durante la fase de análisis de los datos aquí presentados. Al Dr. Eric N. Smith y la Dra. Martha Lucia Calderón Espinosa por su ayuda y consejos durante el trabajo de campo y por facilitarme las hipótesis filogenéticas de los grupos de lagartijas *S. formosus* y *S. Spinosus* de su autoría para implementar análisis cruciales para el desarrollo de esta tesis. Al M. en C. Antonio Muñoz Alonso por su colaboración y consejos durante mi estancia en Chiapas. Al M. en C. Sergio Padilla y al Biol. Ernesto Perera Trejo por su ayuda durante el trabajo de campo en Campeche y por su amistad A Cristian Leyva por su ayuda durante en la colecta de ejemplares en el estado de Guerrero. Al Ing. Alejandro Gonzales Serratos (CONAGUA) por proporcionar amablemente los datos climáticos utilizados en varios capítulos de esta tesis.

Al personal académico y administrativo del Posgrado en Ciencias Biológicas de la UNAM por todas sus atenciones y facilidades a lo largo de mi estancia en el programa de doctorado. Especialmente a Rocío Gonzales Acosta y Karina García Bustamante por su ayuda, paciencia y gentileza en la realización de trámites escolares y administrativos

A mis amigos y compañeros del Laboratorio de Análisis Espaciales Yajaira, Mariana, Edith, Karina, Caro, Samara, Anny, Delia, Yaya, Constantino, Armando, Baruch, Miguel, Julián y Andrés por las buenas conversaciones y su ayuda durante el trabajo de campo.

A mis buenos amigos Rodrigo Macip, Martha Calderón, Elisa Cabrera, Cinthya Mendoza, Rubí Meza, Gabriel Barrios, Enrique Venegas, Aníbal Díaz por las conversaciones aventuras y viajes a lo largo de este tiempo: Y de manera especial a Heidy Gabriela Herrera (Florecita) quien siempre se ha preocupado de mi suerte.

Por último deseo agradecer y dedicar este trabajo a mi familia, mi mamá, papá, hermana, tías y primos que aunque a la distancia sé que están al pendiente.

INDICE

RESUMEN

Se ha propuesto que el fenómeno de incremento contemporáneo de la temperatura ambiental podría causar la extinción de un gran número de especies debido a la velocidad a la que se está produciendo, por lo que representa una importante e inmediata amenaza para la biodiversidad. Esta amenaza puede ser particularmente grave para organismos ectotermos como las lagartijas del género *Sceloporus* de regiones tropicales, cuyas funciones fisiológicas básicas (crecimiento, desarrollo embrionario, digestión, locomoción y reproducción) dependen de intervalos de temperatura específicos. En estas lagartijas, incluso un incremento mínimo de la temperatura ambiental podría exceder el umbral máximo de tolerancia térmica de dichos rasgos y en consecuencia impactar el tamaño de sus poblaciones y provocar su desaparición. En este escenario, algunas especies podrían migrar a sitios en donde existan condiciones ambientales adecuadas; sin embargo, otras no son capaces de movilizarse grandes distancias a corto plazo, de modo que deben de enfrentar *in situ* los efectos negativos de nuevas condiciones ambientales a través de diferentes estrategias. Por ejemplo, podrían ajustar el umbral máximo de temperatura tolerado para los procesos mencionados si poseen la suficiente plasticidad, o exhibir cambios conductuales simples qué contribuyan a amortiguar condiciones climáticas adversas. En este contexto, el objetivo de este estudio fue explorar y comparar ajustes en rasgos fisiológicos clave como el límite térmico máximo para el desarrollo embrionario (34°C), así como diversos rasgos de comportamiento termorregulador y de anidación en función de la temperatura disponible registrada en las diferentes localidades que habitan dos grupos hermanos de lagartijas del género *Sceloporus* con amplia distribución geográfica y diferente modo de paridad, *S. formosus* (vivíparo) *S. spinosus* (ovíparo), utilizando análisis estadísticos que eliminan los efectos filogenéticos para ambos grupos de especies. En particular se evaluaron: (1) la variación del comportamiento termorregulador y temperatura corporal (T_b) en las especies vivíparas del grupo *S. formosus* durante el desarrollo de su descendencia en función de las características térmicas de los sitios que habitan; (2) cambios en rasgos del comportamiento termorregulador específicos (extensión del tiempo de actividad y tiempo de asoleo) en diferentes tratamientos bajo condiciones controladas de laboratorio en la especie vivípara *S. adleri*; y (3) las respuestas fisiológicas y comportamiento de anidación en cinco especies ovíparas pertenecientes al grupo *S. spinosus* para mitigar los efectos de temperaturas ambientales excesivamente elevadas sobre sus embriones en desarrollo dentro de nidos. Asimismo, se compararon las estrategias que han permitido a las

especies que ocupan localidades con temperatura históricamente elevada persistir en estos sitios. Los resultados obtenidos en campo y en laboratorio sugieren que las especies vivíparas modulan su comportamiento termorregulador dependiendo de las condiciones térmicas disponibles a través de cambios simples en rasgos como selección de microhábitat, el tiempo que permanecen asoleándose y la amplitud del horario de actividad, lo cual les permite amortiguar y enfrentar potenciales efectos negativos de temperaturas elevadas sobre ellos mismos y su descendencia. Por otra parte, las especies ovíparas del grupo *S. spinosus* podrían mitigar los potenciales efectos negativos de temperaturas elevadas dentro de sus nidos en diferentes escenarios de incremento de temperatura ambiental mediante la selección de sitios de anidamiento bajo cobertura vegetal que ofrezcan las características térmicas adecuadas para el desarrollo de su descendencia. Por lo tanto, la evidencia sugiere que especies de ambos grupos de lagartijas han lidiado con temperaturas que superan el umbral máximo de tolerancia para el desarrollo de su descendencia a través de cambios tanto en el comportamiento termorregulador como de anidación que les permiten explotar la heterogeneidad térmica del hábitat.

ABSTRACT

It has been suggested that contemporary environmental temperature rise may drive several species to extinction, thus representing a major threat to biodiversity. This may be particularly true for ectothermic species like tropical *Sceloporus* lizards, whose basic physiological functions (growth, embryo development, digestion, locomotion and reproduction) depend on specific temperature ranges. Thus, even a slight increase of environmental temperature may exceed the maximum threshold of thermal tolerance for above traits and therefore affecting their populations. In this scenario, some species might migrate to sites where appropriate environmental conditions prevail, but *Sceloporus* lizards do not move great distances, so they must face t environmental changes *in situ* through different strategies, such as adjusting the maximum tolerated temperature threshold if they hold sufficient plasticity or by simple behavioral changes to damp unfavorable climatic conditions. In this context, our aim was to explore and compare potential adjustments a key physiological trait, namely the maximum threshold to embryo development (34°C), and several thermoregulatory and nesting behavior traits in localities inhabited by two closely related *Sceloporus* lizards groups with broad geographic and elevational distribution and different parity modes, *S. formosus* (viviparous) and *S. spinosus* (oviparous), using phylogenetically controlled analyses. Particularly, we evaluated: (1) the potential variation of thermoregulation behavior and body temperature (T_b) exhibited by *S. formosus* lizards during the reproductive period; (2) the activity and basking times at different controlled temperatures in the viviparous *S. adleri* species; (3) the possible physiological and nesting behavior response of oviparous species to damp deleterious effects of high environmental temperatures on developing embryos inside the nest; and (4) the possible strategies that have allowed two species to persist in sites where historic environmental temperatures have overpassed the physiological threshold. Our results of field and laboratory work suggest that viviparous species modulate their behavioral thermoregulation consistently with thermal environment available using simple changes in microhabitat selection, activity and basking time traits. On the other hand, oviparous species faced high nest temperatures by selecting nesting sites under shade with suitable temperatures. Therefore, our evidence suggests that both species groups deal with potentially dangerous high temperatures by exploiting the thermal habitat heterogeneity via changes in thermoregulation and nesting behavior.

INTRODUCCIÓN

La temperatura media de la Tierra se ha elevado a escala global durante los últimos cien años, principalmente de 1976 a la fecha (Oechel *et al*., 1994; Kerr, 1995; Thomson, 1995; Jones *et al*. 2001), debido muy probablemente al incremento en las concentraciones de gases de efecto invernadero en la atmosfera (CO_2) a consecuencia de la actividad humana (IPCC, 2007). Este aumento térmico ha sido el más rápido registrado para los últimos 10,000 años (Walther *et al*., 2002; IPCC, 2007), como lo muestran el ascenso al de la temperatura máxima diaria en el hemisferio sur (Easterling *et al* 2000) y en las regiones boscosas tropicales (Houghton *et al*. 2001; Santer *et al*., 2003; Stott, 2004). Además, se espera que la inercia del calentamiento continúe en las décadas por venir -incluso reduciendo significativamente las emisiones de gases a la atmósfera- como lo muestran las proyecciones que simulan el aumento de la temperatura media de la superficie terrestre, pudiendo incrementarse entre 1.4 y 5.8 °C para 2100 con respecto al promedio de 1960-1990 (IPCC, 2007).

Esto representa una amenaza para la biodiversidad de proporciones todavía no bien comprendidas (Parmesan y Yohe, 2003), pero seguramente altamente riesgosas que comprometen su estabilidad y la permanencia de un alto número de especies en el largo plazo, pues otros factores de impacto como la sobrexplotación, alteración y fragmentación de los hábitat, entre otros, actúan en sinergia con el cambio climático, sometiendo a los sistemas naturales a presiones sostenidas cada vez mayores (Peters y Lovejoy, 1994). Se ha documentado en éste y otros eventos de cambio climático que las especies pueden sobrevivir por medio de diferentes respuestas; por ejemplo, mediante la colonización de sitios en donde se encuentran las condiciones ambientales adecuadas para mantener poblaciones (Parmesan, 1996). Sin embargo, esta posibilidad se ve determinada por la capacidad y velocidad de dispersión de las especies, aunadas a las características y resistencias del paisaje en donde viven, por lo que no todas pueden desplazarse en busca de dichas condiciones rápidamente (Parmesan et al., 1999). Así, las modificaciones en el ambiente pueden causar la reducción e incluso la desaparición de poblaciones y especies si sus estrategias desarrolladas *in situ* para enfrentar con las nuevas condiciones climáticas son insuficientes y si no pueden migrar (Chevin *et al*. 2010).

Los animales que regulan su temperatura corporal a partir de la temperatura ambiental -como los ectotermos terrestres- podrían ser más vulnerables al incremento de temperatura debido a que sus funciones fisiológicas básicas dependen de temperaturas específicas (Grant y Porter, 1992; Dunham, 1993; Deutch *et al*., 2008). Por ejemplo, en los reptiles la temperatura ambiental disponible es una variable que influye sobre rasgos clave como el crecimiento, desarrollo embrionario, digestión, locomoción y reproducción, los cuales funcionan dentro de intervalos de temperatura específicos. Los cambios recientes en los regímenes térmicos en varias regiones de México han llevado a la extinción de decenas de poblaciones de varias especies de lagartijas, y se estima que no solo más poblaciones sino también algunas especies puedan extinguirse en las próximas décadas (Sinervo *et al*., 2010).

Sin embargo, los registros históricos documentan repetidos cambios climáticos de gran magnitud durante los millones de años de existencia de los reptiles; incluso, la mayor parte de las especies actuales sobrevivieron al último gran cambio climático, el de la transición del Pleistoceno al Holoceno hace unos 12 mil años, por lo que se ha propuesto que estos organismos tienen numerosas estrategias adaptativas para sortear condiciones climáticas adversas que les han permitido sobrevivir, las cuales incluyen desde cambios conductuales simples hasta cambios regulados genéticamente en los intervalos de tolerancia en que los procesos referidos ocurren (Doody *et al*., 2006).

Considerando lo anterior, la investigación sobre las posibles repuestas al rápido incremento de la temperatura en lagartijas, cuyos patrones de comportamiento termorregulador son muy variados y eficientes (Andrews, 1998), representa una oportunidad para conocer cómo éstas han podido amortiguar el efecto del cambio ambiental para que persistan sus poblaciones a través del tiempo y del espacio (Bogert, 1949; Hertz, 1981; Van Damme, 1990; Huey *et al*., 2003). Para ello, en este estudio nos enfocamos en el análisis del comportamiento termorregulador y de anidación en lagartijas durante los periodos críticos para el desarrollo embrionario. Utilizamos como modelo de estudio a especies de dos grupos hermanos de lagartijas del género *Sceloporus* con diferente modo de paridad durante sus respectivos periodos reproductores: las especies del grupo *S. formosus*, que son vivíparas, y las del grupo *S. spinosus*, que son ovíparas. En ambos grupos el régimen térmico que experimentan sus embriones durante el desarrollo repercute en el fenotipo de las crías (Elphick y Shine, 1998; Qualls y Andrews, 1999), pues temperaturas que exceden los 34°C producen malformaciones y deficiencias que disminuyen sus posibilidades de sobrevivencia (Beuchat, 1986; Beuchat y Endler, 1987). Esto sugiere que éste es un rasgo conservado en el género *Sceloporus* (Mathies y Andrews, 1997). Ambos grupos de lagartijas poseen la capacidad de alcanzar y mantener la temperatura corporal adecuada para el desempeño de sus funciones fisiológicas básicas a través de mecanismos de regulación de la temperatura corporal y de seleccionar los sitios con las mejores condiciones físicas para anidar, respectivamente.

Por lo tanto, es razonable suponer que ante el incremento de temperatura en sus localidades, las especies de estos dos grupos de lagartijas con alta eficiencia termorreguladora son capaces de mantener a su descendencia en desarrollo en el intervalo térmico adecuado a través de cambios simples en sus estrategias de comportamiento termorregulador en el caso de las lagartijas del grupo *S. formosus* y de anidación por parte de las del grupo *S. spinosus*, de acuerdo a las características térmicas de los sitios que habitan (Huey et al., 2003).

Ante el escenario descrito surgen las siguientes preguntas cuya resolución contribuiría a comprender mejor tanto los efectos como las posibles reacciones de estos organismos al rápido incremento térmico: 1) ¿Qué respuestas fisiológicas y/o de comportamiento podrían exhibir estas lagartijas para enfrentar cambios relativamente rápidos en el ambiente térmico? 2) ¿Cómo podría influir el incremento de temperatura sobre la persistencia de estos organismos, considerando las implicaciones ecológicas y fisiológicas inherentes a su modo de paridad? y 3) Dado que las lagartijas ovíparas no pueden manipular activamente la temperatura a la que se desarrolla su descendencia, ¿qué estrategias de anidación les podrían ser útiles para protegerla de altas temperaturas ambientales? Para responder las preguntas anteriores se caracterizó y comparó intra e inter especies: 1) la calidad térmica disponible para las hembras reproductoras en diferentes microhábitats en el caso de las especies vivíparas, así como la de los sitios de anidación para las especies ovíparas; 2) las temperaturas corporales para ambos grupos de especies; y 3) las estrategias de comportamiento termorregulador y de anidación que exhiben durante sus respectivos periodos reproductores. Los datos obtenidos se analizaron controlando los potenciales efectos filogenéticos debidos al parentesco entre especies pertenecientes a un mismo género. Al mismo tiempo, comparamos las estrategias que han permitido la sobrevivencia de una especie de cada grupo en localidades con registros históricos de temperatura ambiental que exceden el umbral máximo tolerado para el desarrollo embrionario. Además, al conocer los límites fisiológicos para el desarrollo embrionario y las características térmicas actuales de los sitios en donde anidan las especies del grupo *S. spinosus*, fue posible predecir si la temperatura en dichos sitios excederá el límite deletéreo para los embriones considerando diferentes escenarios de cambio climático e inferir qué estrategias de anidación podrían exhibir los organismos para proteger a su descendencia de temperaturas elevadas. Lo anterior representa un enfoque metodológico diferente en donde se identifica la disponibilidad espacial de los requerimientos térmicos de las especies (Thomas et al., 2004), combinándolos con información sobre su capacidad fisiológica que influye sobre la persistencia de sus poblaciones (Kearney y Porter, 2004).

De esta manera, el objetivo de este trabajo fue evaluar y comparar tanto los efectos como las respuestas ecológicas, fisiológicas y de comportamiento, así como sus repercusiones sobre la distribución geográfica de dos grupos de lacertilios con distinto modo de paridad del género *Sceloporus* ante el impacto del calentamiento global, utilizando análisis estadísticos que incorporan las hipótesis filogenéticas para cada grupo de especies.

Con base en lo anterior, esta tesis se encuentra integrada por cuatro capítulos. En el primero se probó si las estrategias termorreguladoras de diez especies de lagartijas vivíparas del grupo *Sceloporus formosus* cambian durante su periodo reproductivo dependiendo de las condiciones térmicas de los sitios que habitan a lo largo de un gradiente altitudinal, registrando y analizando desde una perspectiva filogenética su temperatura corporal (T_b) y el comportamiento termorregulador para detectar posibles correlaciones entre rasgos de su comportamiento termorregulador, temperatura corporal y temperatura ambiental. En el segundo capítulo analizamos el comportamiento termorregulador de 40 hembras gestantes de la especie *S. adleri* bajo diferentes tratamientos de temperatura en condiciones controladas de laboratorio. En el tercer capítulo exploramos las estrategias potenciales (fisiológicas, reproductivas y de comportamiento) exhibidas por cinco diferentes especies de lagartijas pertenecientes al grupo *Sceloporus spinosus* para mitigar los efectos negativos de temperaturas ambientales elevadas sobre su descendencia en desarrollo dentro de los sitios seleccionados para anidar. Por último, en el cuarto capítulo se exploraron y compararon las estrategias de una especie de lagartija perteneciente a cada grupo que les han permitido habitar en sitios con temperatura que supera su umbral de tolerancia durante los últimos 50 años.

CAPITULO 1. ¿El comportamiento termorregulador podría permitir a las lagartijas vivíparas habitar un mundo cálido?

MANUSCRITO BAJO REVISIÓN EN ZOOLOGY

Would behavioral thermoregulation allow viviparous lizards to cope with a warmer world?

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Number of text pages: 34

Number of figures: 2

Keywords: *Sceloporus formosus*, thermal stress, climate change, altitudinal gradient, Mexico, global warming

ABSTRACT

Viviparous *Sceloporus* lizards depend on external heat sources to achieve the ideal temperature for performing physiological processes; temperatures higher than 34°C may be lethal to developing embryos. Therefore, thermoregulation is crucial for their survival. Given the recent rapid increase of global temperature, it is expected that the first compensatory response by ectothermic organisms to deal with higher temperatures will be behavioral thermoregulation. Here, we tested if viviparous lizards of the *Sceloporus formosus* species group exhibited different thermoregulatory patterns to achieve the appropriate body temperature for optimal performance regardless of local thermal conditions. We measured field active body temperature (T_b), thermoregulatory behavior mechanisms (activity time, microhabitat selection and basking time), and available microhabitat thermal conditions (i.e., operative temperature, T_e) for ten lizard species during gestation period in their native localities distributed along an altitudinal gradient. We identified thermoregulatory patterns from measured mechanisms, tested differences between species and looked for significant relationships between these and T_e using both conventional and phylogenetically controlled analyses to explore if physiological T_b or behavioral thermoregulation adjusts in response to different thermal conditions. These species showed no significant differences in T_b during gestation regardless of local thermal conditions in which each one inhabits. Conversely, they exhibited significant differences in their behavioral thermoregulation patterns associated to local environmental conditions. Based on these observations the different thermoregulatory patterns identified are interpreted as compensatory behavioral adjustment to local thermal conditions. Therefore, we conclude that species facing temperature stress may deal with it by modulating their thermoregulatory mechanisms included in their phylogenetic repertoire.

1. Introduction

Reptiles depend on external heat sources for raising their body temperatures to an optimal range. This is maintained regardless environmental thermal fluctuations mainly by means of behavioral thermoregulation, through restricting activity time, shuttling from sunny to shady spots, using postural adjustments, and less frequently, by physiological mechanisms, such as absorptive changes of skin, evaporative cooling and controlling blood flow (Stevenson, 1985; Huey et al., 2003; Angilletta et al., 2006).

Behavioral thermoregulation in reptiles enables them to maintain optimum performance for their basic functions, including metabolism, locomotion for feeding and escaping predators, food assimilation (Angilletta, 2009), and embryonic development, which is thermally sensitive in species of the genus *Sceloporus* (Beuchat, 1986). This is because when embryos of this genus are exposed to temperatures higher than 34°C (maximum thermal tolerance threshold) they frequently show physical abnormalities at birth, affecting locomotion performance and thus increasing mortality (Andrews and Rose, 1994; Beuchat, 1986; Beuchat and Ellner, 1987). Considering the above, the current climate warming episode becomes relevant for the long-term survival of viviparous *Sceloporus* lizards, since recently it has been documented that environmental temperature rise could increase overheating risk if lizards are out of their shelters. This compromises offspring development by reducing their daily activity and thus their foraging and reproduction opportunities, leading their populations to the extinction (Sinervo et al., 2010).

Interestingly, viviparous *Sceloporus* species has a narrow and stable range of field active body temperature (T_b) in which their physiological processes occur: 34.5-37.0°C for non-reproductive females and males, and 28.0-33.5°C for pregnant females (Bogert, 1949; Beuchat, 1988; Grant and Dunham, 1990; Mathies and Andrews, 1997). This range holds despite their wide variation in altitudinal and latitudinal distributions (Andrews, 1998), in contrast to other lizards, for example *Liolaemus*, whose average T_b varies between 27 and 36°C among different species (Rodriguez- Serrano, 2009); suggesting that viviparous *Sceloporus* lizards exhibit a precise thermoregulatory behavior (Adolph, 1990; Smith and Ballinger, 1994; Andrews, et al., 1999; Lemos-Espinal and Ballinger, 1995; Lemos-Espinal, et al., 1997). Pregnant *Sceloporus* females are thus imposed to have greater control for optimizing the thermal regimes experienced by their developing offspring and by themselves regardless of environmental temperature (Webb et al., 2006).

Therefore, it is reasonable to expect that lizards are able to exhibit and combine diverse behavioral thermoregulatory mechanisms included in their genetic repertory depending on environmental thermal conditions. Thus, in the current climate warming context, behavioral thermoregulation could be the simplest and fastest way to cope and damp potential negative effects of higher temperatures on them and their offspring, if their physiological tolerated thermal thresholds are not adjusted or evolve. Under this scenario intriguing questions arise, such as: (1) Does the physiological thermal limit for survival and development of offspring in viviparous lizards adjust to warmer environmental temperatures? (2) Are pregnant *Sceloporus* lizards able to exhibit different behavioral thermoregulation patterns that allow them to maintain a suitable body temperature and compensate for high environmental temperatures? If the latter occurs, (3) does the thermoregulatory behavioral repertoire of these lizards allow them to survive in areas where environmental temperatures exceed their physiological threshold? To address these questions, we evaluated and compared the field active body temperature (T_b) and different thermoregulatory mechanisms, including activity time (*AT*), microhabitat selection (*MS*) and basking time (*BT*) of ten species of the *Sceloporus formosus* group from areas with different thermal regimes.

The *Sceloporus formosus* group is particularly interesting because it holds 15-20 recognized species that live in a broad array of ecological conditions, from 0-4600m above sea level and under diverse climates and vegetation types (Bell et al., 2003), thus species present several morphological and ecological adaptations (Sites et al., 1992). For these reasons, we aimed to characterize the behavioral thermoregulatory strategies of lizards in environments under different thermal conditions in a > 2000m elevation gradient.

2. Materials and methods

To achieve our goals we followed four steps: (1) We studied ten species of the *S. formosus* group distributed in different localities along an altitudinal gradient of near 2500m. (2) In each site we measured different thermoregulatory behavior mechanisms exhibited by the lizards during the reproductive period and their field active body temperatures (T_b). (3) We characterized operative temperatures (T_e; i.e., the expected temperature of non-thermoregulating lizards, which can be understood as the thermal offer of the environment to organisms [Huey, 1991; Bakken, 1992; Hertz, 1992]) with biophysical models in microhabitats used by lizards. (4) Finally, we compared pregnant females' T_b and behavioral mechanisms for the ten species and studied their relationships with T_e recorded at each locality. Details of these steps are presented below.

2.1 Species from the *S. formosus* group

The *Sceloporus formosus* species group is widespread throughout southern Mexico and Central America, inhabiting arboreal, saxicolous, or terrestrial habitats. Breeding and gestation occurs in September-March (Guillette and Sullivan, 1985; Sites et al., 1992; Ramirez-Pinilla et al., 2009). We selected 10 representative species of the group based on three criteria: (1) at least one species belongs to each clade of the group, according to the most robust phylogenetic reconstruction available (Smith 2001); (2) the elevational range of the group must be represented; and (3) females of target species were pregnant during the observational period. As such, species selected were: *S. adleri* (17.26 N, -99.73 W), *S. cryptus* (17.33, -96.48 W), *S. formosus* (16.16 N, -96.50 W), *S. smaragdinus* (15.03 N, -92.05 W), *S. subpictus* (17.06 N, -97.66 W), and *S. taeniocnemis* (16.75 N,-92.63 W) from coniferous forests; *S. internasalis* (18.36 N, -95.11 W), *S. internasalis 2* (15.35 N, -92.55 W) and *S. salvini* (17.63 N, -96.35 W) from tropical rain forest; and *S. stejnegeri* (16.46 N, -98.65 W) from tropical dry forest. The elevation range of selected the species was 299-2468m. The ten sampling localities for these species (one per species) were obtained from museum records and electronic databases (www.gbif.org and www.herpnet.org). Permits for field studies were issued by the Secretaria de Medio Ambiente y Recursos Naturales [SEMARNAT (permit number SGPA/DGUS/04949)].

2.2 Behavioral thermoregulation mechanisms per species

To identify behavioral thermoregulation patterns of each species we targeted each pregnant lizard when seen out of its shelter in the field, assigned with a unique identification number and observed for 10 minutes, recording the following thermoregulatory mechanisms: (1) Microhabitat selection (*MS*) or the most frequently microhabitat occupied by lizards among total shade (TSh), filtered sun (FS) or complete sun (CS); (2) Activity time (*AT*) recording sighting time for each lizard in a daylight period between 0800-1800 hr; and (3) Basking time (*BT*) in seconds that lizards spent in a specific microhabitat in order to compare average shuttling duration between species evaluated.

2.3 Field active body (T_b) and operative temperatures (T_e)

After recording behavioral thermoregulation mechanism for all females, we collected them with a thin cord attached to a fishing rod, and carefully measured their body temperature with a Miller and Weber® cloacal thermometer. None of the lizards was sacrificed or harmed during capture and handling. Simultaneously, we characterized the operative temperature (T_e) in each microhabitat where lizards were found (TSh, FS, and CS) by setting 30 hollow copper models —10 per microhabitat— of the size, shape and color (from brown to green) of each species analyzed (Hertz et al., 1993; Bauwens, et al., 1996) in the same location and orientation as the observed lizards (see Grant and Dunham, 1988; Hertz, 1992). We recorded T_e every 30 min inside established daylight period for six consecutive days (Bakken 1992) using outdoor Hobo U23 Pro v2 external temperature® data loggers (-40 to 70°C ± 0.2°C at 0° to 50°C calibration) plugged into the model central cavity with an external thermocouple (Hertz, 1992). To observe if temperature of the copper model and lizards varied simultaneously, we calibrated the models using live lizards exposed to full sun attached to a digital thermometer (Dzialowski, 2005). Then, we obtained the average T_e available for lizards between 08:00-1800 hr at each microhabitat per locality, denoting them by T_{TSh}, T_{FS} and T_{CS} (notice that *TSh* denotes the microhabitat, whereas T_{TSh} denotes its T_e). Once the above data were recorded, individuals were kept in captivity inside a cage during the working session to avoid recording the same lizard twice and released them at the same site at the end of fieldwork.

2.4 Statistical methods

The main issue at hand is to study the relationship between behavioral mechanisms (*AT*, *BT*, and *MS*) and physiological traits (T_b), with operative temperatures (T_{TSh}, T_{FS} and T_{CS}), while allowing for possible differences between lizard species. We applied two main types of analyses: (1) conventional methods that use raw data points, in order to establish possible differences, and (2) phylogenetically-controlled methods that are able to correct the historical non-independence of species data (Felsenstein, 1985; Harvey and Pagel, 1991; Martins, 2000), which are crucial for learning about causes of differences. For analysis purposes, in some instances we arbitrarily recoded TSh, FS, and CS values (which are ordinal with respect to amount of sunshine) to numerical 1, 2, 3 in order to enable the notion of an average for plotting purposes; however, the categorical nature of habitat selection will be explicitly taken into account in some of the analyses below.

Because of the differing nature of variables (categorical vs. numerical), we broke down conventional analysis into two main techniques: ordinary linear models, and multinomial regression, both implemented in R (R Core Team, 2012). The former was used to describe *AT*, *BT* and T_b as response variables, with species and T_e set as independent variables. For attaining necessary assumptions of normality in the linear models, variable *BT* was transformed to its square root, after having examined diagnostic plots (not shown).

Multinomial regression was called upon viewing *MS* is a categorical response variable with three possible classes. It was related to species and T_e. The setup allowed estimating the (three) probabilities of selecting any of the microhabitats as a function of T_e and species. The set of probabilities thus characterizes habitat selection for the species, and because the model involves T_e, it may also be used to make predictions under assumed changes in T_e. Multinomial regression (Yee and Hastie, 2003) is generally a lesser-known technique, so a few details are included in the Supporting Information. The end result is that the method provides estimates for the probabilities of each *MS* based on observed values of independent variables, via linear coefficients. Furthermore, it is interesting to note that because the three probabilities add up to one, a *triangle* or *ternary plot* was conveniently used for interesting graphical depictions of species or individuals. An example is shown in Figure 2.

We examined physiological (T_b) or behavioral (*AT*, *BT*, and *MS*) adjustments in response to T_{TSh}, T_{FS}, and T_{CS} by considering Pearson correlations through the origin after calculating Phylogenetically Independent Contrasts (PIC) (Grafen, 1989; Legendre and Desdevises, 2009) using PDAP: PTREE module in MESQUITE (Midford, et al., 2003; Maddison and Maddison, 2009). We have physiological and behavioral information for only 10 species of this group, thus the topology used for the PIC was a pruned *ad hoc* for these species version (Fig. 1in supplementary material), from the tree produced by Smith (2001). The phylogenetic hypothesis for the whole *S. formosus* group was generated based on mitochondrial DNA protein (ND4 741 bp) and tRNA (Histidine, Serine-AGY, and partial Leucine-UUR, 154bp) coding sequences and morphological characters. These datasets were combined and analyzed under parsimony criteria. Exclusion of mitochondrial data for the placement of most samples of *S. adleri* and all samples of *S. stejnegeri* in phylogenetic analyses is important because Smith (2001) provides evidence for strong mitochondrial DNA introgression in these species. We used this phylogenetic hypothesis for the PIC analysis because it matches the relationships observed in an alternative, more recent phylogenetic reconstruction based on nuclear DNA sequence data (Leaché, 2010), but the latter and a third phylogeny proposed by Wiens et al., (2010) were not useful for our purposes because they do not contain all the species included in our analyses. Smith (2001) summarized raw mitochondrial pairwise distances transformed into millions of years of separation between species. We used these distances for our tree branch lengths of the *S. formusus* group for the PIC analysis. We chose samples from among Smith's (2001) data that most closely approximated our study sites for each species.

3. Results

3.1 Descriptive analysis

In total, we captured 155 pregnant females with sample sizes ranging from 5 to 29 individuals per species (Table 1). Data is first summarized and displayed graphically (Table 1 and Figure 1) for descriptive purposes, using a multi-featured display. The following values are plotted by species: mean activity time (horizontal axis), standard deviation of activity time (vertical axis), mean basking time (color of species label), and mean habitat selection (size of circle plotted). In addition, four mean temperature values are superimposed using a starplot device [body temperature (black at 10 o'clock), and available temperatures for the three habitat types (blue at 1 o'clock; orange at 4 o'clock; and red at 7 o'clock)]. The plot is designed merely to describe and suggest observed differences between species; formal statistical testing of any differences follows below using conventional methods. Examples of interpretation of Figure 1 are: *S. salvini* tends to become active consistently (because standard deviation of *AT* is small) at 1415 hrs, has a rather uniform and intermediate temperature profile available across habitats, and tends to select FS ; *S. stegnejeri*, in contrast, tends to appear earlier in the day (on average at 1245 hrs) but with great variability, is subjected to higher available temperatures and gradients, and tends to choose TSh . The *BT* of these two species, however, is similar. By analyzing Figure 1, we observe that lizards use and combine three of the main behavioral thermoregulation mechanisms. They exhibit different thermoregulatory patterns consistent with thermal conditions available produced by local habitat structure at native localities, or T_e . Although some species behave similarly, each one used and regulated these mechanisms in a particular way.

3.2 Conventional analysis

Results of the linear model fit for sqrt(*BT*) are shown in Table 2. These indicate that there are very significant differences among species. The sign of estimated parameters indicates the direction of differences: positive means a greater mean *BT*. Thus, *BT* was extensive for *S. adleri*, *S. internasalis* 2 and *S. smaragdinus*, while for *S. salvini, S. cryptus, S. formosus, S. subpictus*, *S. internasalis*, *S. stegnejeri,* and *S. taecniocnemis, BT* was significantly shorter.

Results of linear model fit for *AT* are displayed in Table 3. Notice that temperature T_{FS} appears to contain information for predicting the mean value of *AT*. In addition, some, but not all species (*S. formosus*, and *S. stegnejeri*) are significantly different in regard to activity time. The interpretations of these results, as will be seen below, are likely confounded because species inhabit places characterized by specific values of T_{FS} .

Although differences in mean *AT* do exist, the spread or range of *AT* is also important biologically. The notion of spread, as summarized in the standard deviation of *AT* per species, is perceived in Fig. 1. Five species had relatively low *AT* values and were found out of their shelters only during some of the three first daylight periods, hiding through the last: *S. adleri*, *S. subpictus* and *S. smaragdinu*s were active in midday and afternoon mainly, *S. formosus* was active mostly during morning and midday, and *S. salvini* was observed between midday and afternoon only. On the other hand, *S. internasalis*, *S. taecniocnemis*, *S. cryptus*, *S. internasalis* 2, and *S. stegenejeri* had an extensive *AT*, although we only found active lizards in all day light periods for the last three species (from 0800 to 1800 hrs, Table3 and Fig. 1).

Estimation results of multinomial regression (see Supporting Information for details of multinomial logit regression for *MS*) are displayed in Table 4. Interpretation of the results indicate that temperatures T_e are indeed correlated with habitat selection, and that only *S. cryptus* is regarded to have a distinguishing difference for determining probability p_2 (which corresponds to T_{FS}). In addition, we observed differences between species because individuals of *S. adleri* and *S. subpictus* were found in the three microhabitats, although mainly using FS and TSh, respectively. *S. salvini* selected FS more frequently and *S. smaragdinus* preferred CS, but no lizard of these two species was observed in TSh. *S. formosus* was found exclusively in CS. In the case of *S. cryptus*, *S. internasalis* and *S. taecniocnemis*, they selected more frequently CS, TSh and FS, respectively. Females of *S. internasalis2* were more frequently observed in FS and *S. stejnegeri* preferred TSh microhabitats (Fig. 1, Table 1). Many of these features are discernible graphically from triangle plots, such as those included in Fig. 2.

Table 5 contains results of linear model fit for T_b. These results indicate that available temperatures in microhabitats have no effect on measured body temperature. There is some marginally evidence that *S. formosus* has a higher body temperature, and *S. internasalis2* a slightly lower temperature maybe because the first was observed only on CS microhabitat and the low availability of complete sun spaces for the last. However, these differences are irrelevant because lizard's females and developing offspring average T_b is within temperature range for optimal performance of both processes.

These results clearly indicate overall that differences in conduct observed visually in Fig. 1 are indeed significant, which is the main point we wished to establish at this stage. However, the origin of such differences is confounded due to experimental design, because specific species are associated to the specific environments they inhabit. We are thus unable to discern if such differences are caused by the environments or by species themselves. For example, *BT* appears related to species more than operative temperatures themselves, but this correlation may be spurious. In another case, *AT* appears to be related to T_{FS} , but this does not follow that this temperature is having a direct effect on *AT*. This issue of confounding is exactly the reason phylogenetic analysis (addressed in the following section) is required, that enables additional information regarding species to be included in order to isolate effects from environmental information. However, conventional analyses considered in this section is not to be disregarded, because it used raw data and is thus reliable, albeit inconclusive in regard to causal effects. These methods show that differences exist; in order to understand the nature of these differences, we next turn to phylogenetically controlled methods. For very specific differences obtained on a species-by-species basis, we included additional analysis in the Supporting Information section, obtained by restricting statistical comparisons to subsets of original data.

3.3 Phylogenetically controlled analysis

Phylogenetically controlled analyses (Table 6) showed non-significant correlations between field active body temperature (T_b) and activity time (*AT*) against T_e . However, these detected an inverse significant relationship between microhabitat selection (*MS*) and basking time (*BT*) with T_{TSh} , T_{FS} and T_{CS} available in the three microhabitats. This could be interpreted as compensatory behavioral adjust to local thermal conditions available at each locality.

4. Discussion

Because temperature is one of the main environmental variables influencing biological processes, its changes may exert strong selection pressures, especially on species with low dispersal rates (Brown et al., 2004; Davis et al., 2005). It has been proposed that some species may respond to increasing temperatures by shifting their seasonal activity to avoid unfavorable temperatures (Bradshaw and Holzapfel, 2008), whereas others may respond by extending their tolerance limits via developing biomolecular mechanisms of protein production at the cellular level to provide full corporal adaptation to hyperthermia (Grewal et al., 1996; Margulis and Guzhova 2000; Evgen'ev et al., 2007). However, evolutionary adaptation to new environmental conditions in physiological traits does not always take place (Holt 1990; Berteaux et al., 2004), especially when populations are exposed to abrupt and long-lasting changes in the environment, when favorable genetic modifications are not inherited (Lagerpetz, 2006), or when appropriate genetic variability is lacking (Bradshaw, 1991). Ultimately, when individuals are exposed to prolonged stress and their repertoire of adaptive strategies fails, fitness is reduced, populations decrease and the distribution of species may shrink to the extreme of collapse (Angilletta et al., 2004, Sinervo et al., 2010).

Our results suggest that different behavioral thermoregulation strategies are used by pregnant lizards to avoid damaging effects on adults and offspring produced by high temperatures, rather than adjusting their physiological limits of tolerance to high temperature (Bogert, 1949). We found no significant differences in field active body temperatures during the gestation period among the *Sceloporus formosus* group species, despite inhabiting a wide range of temperatures in a +2000 meter elevational gradient. Instead, behavioral differences exhibited by species to maintain ideal body temperatures for development of offspring were observed both when operative temperatures (T_e) did and did not exceed the deleterious temperature threshold for embryo development (34 °C). Using the same line of evidence, Huey et al. (2003) found differential behavior patterns to maintain body temperatures relatively constant (less variable than expected for thermoconforming lizards) among populations of *Anolis cristatellus* along an altitudinal gradient.

Reptiles control their body temperatures in different ways, e.g., hiding when environmental conditions are extreme (Kearney, 2002), shifting activity periods (Bauwens et al., 1996), selecting suitable microhabitat (Stevenson, 1985), and shuttling among microhabitats (Díaz, 1994; Webb and Shine, 1998). Among the ten species of the *Sceloporus formosus* group analyzed, we observed that each one exhibited different thermoregulation strategies during gestation, depending on the thermal offer at each locality. For instance, we expected that species with short *AT* and living in cool forested areas at high elevations (>2000 m) (i.e., *S. formosus*) remained in the sun for long periods of time, but this was not the case because this species remained for relatively short periods of time in their selected microhabitats. Certainly, the amount of heat available is enough for their thermoregulation, perhaps because habitat structure with plenty of patches at full sun allows them to achieve their ideal field active body temperature relatively fast. Other factors, such as predation risk and low food availability (Huey and Slatkin, 1976) may also influence this behavior, but further observations are needed to test alternative hypotheses. In contrast, other species of temperate sites with short *AT* (i.e., *S. adleri* and *S. smaragdinus*) spent more time at all microhabitats, probably because the thermal offer is more limited.

On the other hand, species like S*. cryptus*, *S. internasalis, S. taecniocnemis*, and *S. stegnejeri* that showed extensive *AT* live at different elevations and combine different thermoregulatory mechanisms to keep an appropriate body temperature, even at extreme temperatures. *S. stegnejeri*, lives in areas where T_e at complete sun (*CS*) exceeds the deleterious temperature for embryo development (34 °C) during most of the day. Females of this species move constantly across the mosaic of sun and shade, reducing the effective time they remained exposed to full sun, as described for *Psammodroum algirus* in the Mediterranean forests (Díaz and Cabezas-Díaz, 2004). This behavior allows lizards to take advantage of the microclimatic heterogeneity to buffer the impact of high temperatures, shuttling to thermal refuges at total shade (e.g., vegetation cover or large rocky outcrops) where T_e does not exceed the deleterious threshold. *S. stegnejeri* represents empirical evidence of how lizards can evade deleterious high temperatures using thermoregulatory behavior. However, if heat stress increases, shuttling behavior may not be enough and highest temperatures could reduce the time available for foraging and reproduction, thus impacting fitness. This is the argument that has been invoked to explain current populations' extinctions of other viviparous *Sceloporus* lizards in Mexico, presumably due to an energetic shortfall as a consequence of low food intake (Sinervo et al., 2010). Therefore, continuous temperature increases in tropical sites under current heat stress could threaten persistence of lizards in the long run if their thermoregulatory behavior is not sufficient to cope with high environmental temperatures (Kearney et al., 2009; Huey et al., 2009; Huey and Tewksbury, 2009).

Species of the *S. formosus* group hold a repertoire of different behavioral thermoregulation mechanisms that they use and combine depending on local thermal conditions. The phylogenetic controlled analyses showed significant inverse relationships between duration time in a specific microhabitat (*BT*) and microhabitat selection (*MS*) against available operative temperature (T_e) in all microhabitats. These results suggest that species of this group actively select the microhabitat and control the duration time in them, depending on local thermal conditions. Correlation values obtained after these analyses indicate that microhabitat selection and *BT* spent among microhabitats are two important behavioral components that have allowed the *S. formosus* lizards to maintain an adequate body temperature in response to different thermal conditions; these behavioral mechanisms contribute to regulate body temperature up to fourfold more than physiological mechanisms, and helps to avoid extreme temperatures

(Stevenson, 1985). Our results suggest that *BT* and *MS* are behavioral attributes of the *S. formosus* group that most contribute to buffer temperature changes, rather than a physiological adjustment in the maximum temperature tolerance limit to deal with warming environmental temperatures (Blomberg et al., 2003; Rezende and Garland, 2003).

The global trend in temperature rise over recent decades shows important regional and local differences (Le Treut et al., 2007), making it possible to search for behavioral and physiological responses of these lizards to current climatic change. Most future climate projections suggest that temperature will continue to increase during this century (IPCC, 2007). If so, it has been suggested that at sites where current maximum temperatures are close to deleterious limits for lizards, even small increases may compromise the long-term survival of populations by enhancing thermal stress (Williams et al., 2007; Deutsch et al., 2008; Huey et al., 2009), as demonstrated in a recent study for *Sceloporus* lizards in Mexico (Sinervo et al., 2010). Interestingly, our results indicate that such deleterious threshold may be evaded with behavior, as it happened in Tierra Colorada, Guerrero, where daily temperature reaches up to 40 °C in the *CS* microhabitat, exceeding physiological limits for developing healthy embryos during some periods of the day. Nonetheless, pregnant lizards of *Sceloporus stegnejeri* in this site are active most part of the day using thermoregulatory behavior, such as selecting mainly shaded microhabitats and shuttling between sun and shade constantly, remaining short duration times in full sun. Therefore, in localities where climate is changing, species may be already responding by adjusting some behavioral traits within their repertoire. On the other hand, species currently living in temperate areas and whose activity is limited by low temperatures may benefit with moderate temperature increases, allowing them to extend their activity period, as observed in the lizards of the Massif Central, France (Chamaillé-Jammes, et al., 2007).

Finally, literature on climate change biology has documented diverse physiological, demographic, and geographic responses of species to the current warming episode (Sinervo et al., 2010). Such responses represent further strategies when behavior is not enough to cope with environmental conditions becoming unsuitable. During stressful climatic changes, the first reaction is commonly behavioral, nonetheless it is frequently overlooked in studies aiming to analyze species' responses (Bradshaw and Holzapfel, 2006; Parmesan, 2006). This study represents one of the first assessments on the behavioral strategies of ectothermic animals facing climatic changes from an evolutionary perspective, which is probably the earliest means by which species respond to stressful temperatures (Bartholomew, 1987).

Acknowledgements

We thank Dr. Constantino Macías for his comments on behavioral observations and Dr. Norberto Martínez-Méndez for his valuable assistance in the development of the phylogenetic analyses. We also thank Jean Cristian Blancas-Hernández and Elizabeth Beltrán-Sánchez (UAG) for providing logistical and field support throughout our stay in the state of Guerrero. We thank Uri Omar García Vázquez (UNAM), Coleman M. Sheehy III, Robert C. Jadin, and Gloria Andrea Acevedo (UTA) for participating in preliminary fieldwork. We also thank Armando Escobedo, Edith Calixto, Enrique Venegas, Baruch Arroyo, and Antonio Muñoz for their assistance during intensive fieldwork. A. Townsend Peterson for his thorough review of an early draft of the manuscript, and Oscar Flores-Villela (UNAM) provided logistic support. We also thank the Posgrado en Ciencias Biológicas, UNAM for logistic support to S. L.-A. during his graduate studies. This work was supported by the Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPIIT) of the Universidad Nacional Autónoma de México (Project number: IN221208), the

Consejo Nacional para la Ciencia y la Tecnología (CONACYT) and also partially supported by a National Science Foundation grant (DEB-0416160 [ENS]) &Instituto Bioclon (ENS).

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Tables

Table 1. Mean overall average operative temperatures (T_e), average body temperature (T_b), average duration time in seconds at a specific microhabitat (*BT*), activity time (*AT*) calculated from standard deviation of sighting time records and microhabitat more frequently selected (*MS*) recorded per each species studied of the *Sceloporus formosus* group (Should appear in descriptive analysis subsection after line 216).

Species	**Locality**	**Sample size**	**T_e ($\bar{X}$) ±SD (min-max)**	**T_b ($\bar{X}$) ±SD (min-max)**	***BT* ($\bar{X}$) ±SD (min-max)**	***AT***	***MS***
S. adleri	Carrizal, Guerrero (2400 m)	29	21.2 ± 3.43 (14.8-29.6)	28.2 ± 2.9 (32.0-23.5)	248.8 ± 20.7 (225.4-265)	1.8	2.5
S. formosus	San Mateo, Oaxaca (2375m.)	22	19.8 ± 6.2 (9.5-34.7)	30.6 ± 2.1 (25.5-31.3)	187.0 ± 15.2 (171.-225)	1.7	3
S. smaragdinus	Chiquihuites, Chiapas (2468 m)	11	17.6 ± 2.0 (13.0-22.9)	28.7 ±1.73 (26.8-32.2)	363.3 ± 13.3 (350-363.)	1.5	2.8
S. salvini	La Esperanza, Oaxaca (1499m)	5	26.8 ± 1.9 (22.1-31.8)	28.7 ± 1.2 (26.8-30.2)	53.5± 12.4 (38.5-68.6)	1.4	2.4
S. subpictus	San Esteban, Oaxaca (1912m.)	9	23.8 ± 5.8 (10.0-33.9)	29.6 ± 1.2 (27.5-31.9)	62.4 ±10.6 (44.6-73.5)	1.6	1.8
S. cryptus	Ixtlán de Juárez, Oaxaca (2072 m.)	22	20.1 ± 2.9 (13.5-27.2)	29.4 ± .1.6 (25.5-33.0)	129.7 ±22 (92.3-152.2)	2.5	2.5
S. *internasalis*	Catemaco, Veracruz (442 m.)	7	30.1± 1.73 (26.1-34.2)	31.2 ± 1.13 (29.5-32.5)	184.2± 21.8 (162.5-211)	2.5	1.8
S.internasalis 2	El Quetzal, Chiapas, (1499 m.)	19	22.3 ± 2.8 (15.2-29.8)	26.8 ± 3.7 (21.7-33.5)	589.9 ±194.8 (421-900)	2.2	2.2
S. stegnejeri	Tierra Colorada, Guerrero (299 m)	9	33.8 ± 3.7 (25.9-41.8)	31.4 ± .97 (30.6-32.5)	10.8 ± 1.6 (8.5-13)	2.6	1.7
S. taeniocnemis	San Cristóbal, Chiapas (2189 m)	22	20.3 ± 4.2 (9.7-34.9)	28.8 ± 3.2 (19.3-32.5)	91.8 ± 31.4 (50.8-138)	2.1	2.1

Table 2. Results of lineal model for *sqrt(BT)*. *S. adleri* was used as a baseline value, having its mean *sqrt(BT)* estimated by $16.23403 + 0.02659T_{TSh} + 0.03427T_{FS} - 0.07887T_{CS}$. Other species used a specific shift. For example, mean *sqrt(BT)* for *S. cryptus* was estimated by $16.23403 - 4.25372 + 0.02659T_{TSh} + 0.03427T_{FS} - 0.07887T_{CS}$. Standard errors, *t*-values, and *p*-values for testing their significance are shown for each estimated coefficient. Significance codes are "***" for *p*<0.001, "**" for *p*<0.01, "*" for *p*<0.05, and "." for *p*<0.10 (Should appear in conventional analysis subsection after line 222).

Coefficients	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	16.23403	1.03842	15.633	< 2e-16 ***
T_{TSh}	0.02659	0.09242	0.288	0.7740
T_{FS}	0.03427	0.06575	0.521	0.6031
T_{CS}	-0.07887	0.05336	-1.478	0.1417
S. cryptus	-4.25372	0.58416	-7.282	2.40e-11 ***
S. formosus	-1.56175	0.67774	-2.304	0.0227 *
S. internasalis	-2.07275	0.80318	-2.581	0.0109 *
S. internasalis2	8.29373	0.49503	16.754	< 2e-16 ***
S. salvini	-8.36240	0.82896	-10.088	< 2e-16 ***
S. smaragdinus	2.94531	0.60570	4.863	3.15e-06 ***
S. stegnejeri	-12.06727	0.82276	-14.667	< 2e-16 ***
S.subpictus	-7.78048	0.68453	-11.366	< 2e-16 ***
S.taecniocnemis	-6.11908	0.57770	-10.592	< 2e-16 ***

Table 3. Results of lineal model for *AT*. *S. adleri* is used as a baseline value, having its mean *AT* estimated by $9.91714 - 0.08610 T_{TSh} + 0.17651 T_{FS} + 0.05093 T_{CS}$. Other species use a specific shift. For example, mean *AT* for species *cryptus* is estimated by $9.91714 + 0.15333 - 0.08610 T_{TSh} + 0.17651 T_{FS} + 0.05093 T_{CS}$. Standard errors, *t*-values, and *p*-values for testing their significance are shown for each estimated coefficient. Significance codes are "***" for *p*<0.001, "**" for *p*<0.01, "*" for *p*<0.05, and "." for *p*<0.10. (Should appear in conventional analysis subsection after line 236).

Coefficients	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	9.91714	1.33277	7.441	1.02e-11 ***
T_{TSh}	-0.08610	0.11861	-0.726	0.4691
T_{FS}	0.17651	0.08439	2.092	0.0383 *
T_{CS}	0.05093	0.06848	0.744	0.4584
S. cryptus	0.15333	0.74975	0.205	0.8383
S. formosus	-1.79803	0.86986	-2.067	0.0406 *
S. internasalis	-2.02510	1.03085	-1.964	0.0515
S. internasalis2	-0.09147	0.63536	-0.144	0.8857
S. salvini	0.33418	1.06394	0.314	0.7539
S. smaragdinus	-0.03583	0.77740	-0.046	0.9633
S. stegnejeri	-2.26720	1.05598	-2.147	0.0336 *
S.subpictus	-0.59927	0.87856	-0.682	0.4963
S.taecniocnemis	-1.08290	0.74146	-1.461	0.1465

Table 4. Results of multinomial logit regression for *MS*. Let p_i denote the probability of selecting microhabitat i, for $i = 1, 2, 3$. Predictions for the two quantities $\log(p_1 / p_3)$ and $\log(p_2 / p_3)$, are given as $\log(p_j / p_3) = a_j + b_j \text{Species} + c_j T_{TSh} + d_j T_{FS} + e_j T_{CS}$ for $j = 1, 2$. See supplementary material for more details and examples. The table shows estimated coefficients, standard errors and *z*-values. Significance codes are "***" for *p*<0.001, "**" for *p*<0.01, "*" for *p*<0.05, and "." for *p*<0.10 (Should appear in conventional analysis subsection after line 248).

Coefficients	**Estimate**	**Std. Error**	**z value**
(Intercept):1	-7.041892	2.76095	-2.550536*
(Intercept):2	-1.680074	1.75846	-0.955425
S. cryptus:1	-1.270958	1.25198	-1.015158
S. cryptus:2	2.325471	-1.04069	-2.234556*
S. formosus:1	-16.818578	609.49240	-0.027594
S. formosus:2	-18.243640	658.84774	-0.027690
S.internasalis:1	0.086762	1.42497	0.060887
S.internasalis:2	0.538424	1.33990	0.401839
S.internasalis2:1	1.267729	0.88203	1.437280
S.internasalis2:2	-0.312097	0.81702	-0.381996
S. salvini:1	-15.250814	972.37635	-0.015684
S. salvini:2	0.868617	1.13853	0.762925
S. smaragdinus:1	-13.882041	743.64702	-0.018668
S. smaragdinus:2	-0.413346	0.99862	-0.413919
S. stegnejeri:1	-0.948350	1.72158	-0.550861
S. stegnejeri:2	0.307475	1.38102	0.222643
S. subpictus:1	0.344252	1.56807	0.219538
S. subpictus:2	1.112855	1.11199	1.000781
S.taecniocnemis:1	-0.125289	1.18310	-0.105899
S.taecniocnemis:2	0.384177	0.85540	0.449119
T_{TSh} :1	-0.508004	0.22676	-2.240261*
T_{TSh} :2	-0.437068	0.18280	-2.390967*
T_{FS} :1	0.485625	0.18975	2.559339*
T_{FS} :2	0.203924	0.16508	1.235294
T_{CS} :1	0.236036	0.14394	1.639778
T_{CS} :2	0.247091	0.12605	1.960328*

Table 5. Results of lineal model for T_b . *S. adleri* is used as a baseline value, having its mean T_b estimated by $24.28276 + 0.02676T_{TSh} + 0.09853T_{FS} + 0.05491T_{CS}$. Other species used a specific shift. For example, mean T_b for *S. cryptus* was estimated by $24.28276 + 1.36443 + 0.02676T_{TSh} + 0.09853T_{FS} + 0.05491T_{CS}$. Standard errors, *t*-values, and *p*-values for testing their significance are shown for each estimated coefficient. Significance codes are "***" for *p*<0.001, "**" for *p*<0.01, "*" for *p*<0.05, and "." for *p*<0.10 (Should appear in conventional analysis subsection after line 255).

Coefficients	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	24.28276	1.70697	14.226	<2e-16 ***
T_{TSh}	0.02676	0.15192	0.176	0.8604
T_{FS}	0.09853	0.10808	0.912	0.3636
T_{CS}	0.05491	0.08771	0.626	0.5323
S. cryptus	1.36443	0.96026	1.421	0.1576
S. formosus	2.67902	1.11409	2.405	0.0175 *
S. internasalis	1.42283	1.32028	1.078	0.2831
S. internasalis2	-1.47152	0.81374	-1.808	0.0728
S. salvini	-0.44254	1.36265	-0.325	0.7459
S. smaragdinus	1.31480	0.99566	1.321	0.1889
S. stegnejeri	0.51049	1.35246	0.377	0.7064
S.subpictus	0.91373	1.12524	0.812	0.4182
S.taecniocnemis	0.85589	0.94964	0.901	0.3690

Table 6. Correlation between behavioral thermoregulation mechanism and field active body temperature of lizards against operative temperature (T_e) available in different microhabitats, performed after Phylogenetic Independent Contrast (PIC). (Should appear in Phylogenetically controlled analysis subsection after line 277).

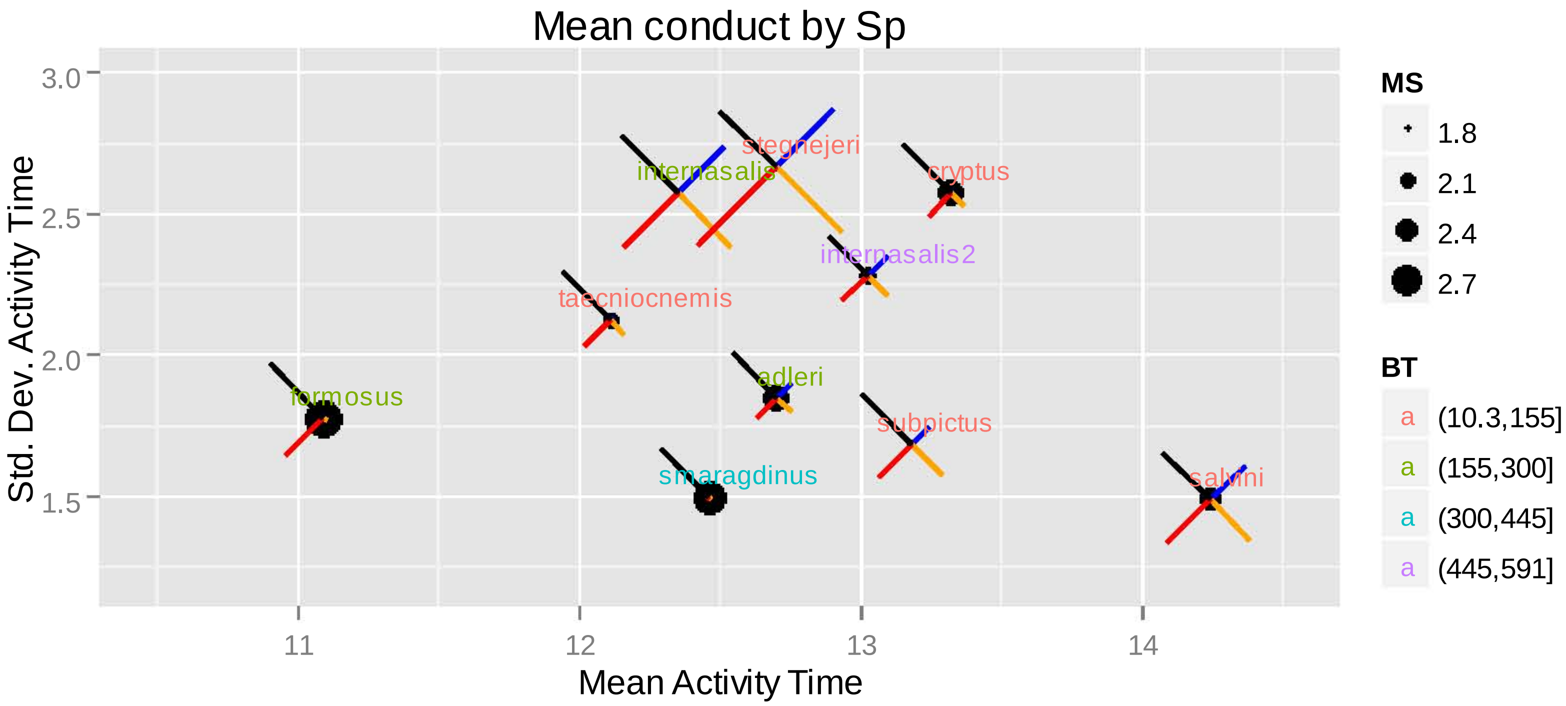
Pairwise correlations	**PIC**	***P***
	R	
AT vs T_{TSh}	0.417	0.230
AT vs T_{FS}	-0.223	0.553
AT vs T_{CS}	-0.465	0.174
MS vs T_{TSh}	-0.760	0.010*
MS vs T_{FS}	-0. 820	0.003*
MS vs T_{CS}	-0. 806	0.004*
BT vs T_{TSh}	-0.720	0.018*
BT vs T_{FS}	-0.757	0.011*
BT vs T_{CS}	-0. 634	0.048*
T_b vs T_{TSh}	0. 574	0.082
T_b vs T_{FS}	0. 601	0.066
T_b vs T_{CS}	0. 548	0.100

Figure Legends

Figure 1. Behavior map for the *Sceloporus formosus* group showing different behavioral strategies followed by species to keep a T_b without significant variations in diverse climatic conditions. Time of the day is on the x-axis and standard deviation of activity time (*AT*) is in y. Size of circles denote microhabitat selection (*MS*), e.g., species with small circles selected shaded microhabitats, color in species name indicates *BT* extent, e.g., species in blue spent short periods in any microhabitat. Blue, orange and red bars around circles indicate average T_e temperature in T_{TSh}, T_{TSh} and T_{CS} microhabitats, respectively, and the black bar represents average T_b for all species. (Should appear in conventional analysis subsection after line 236).

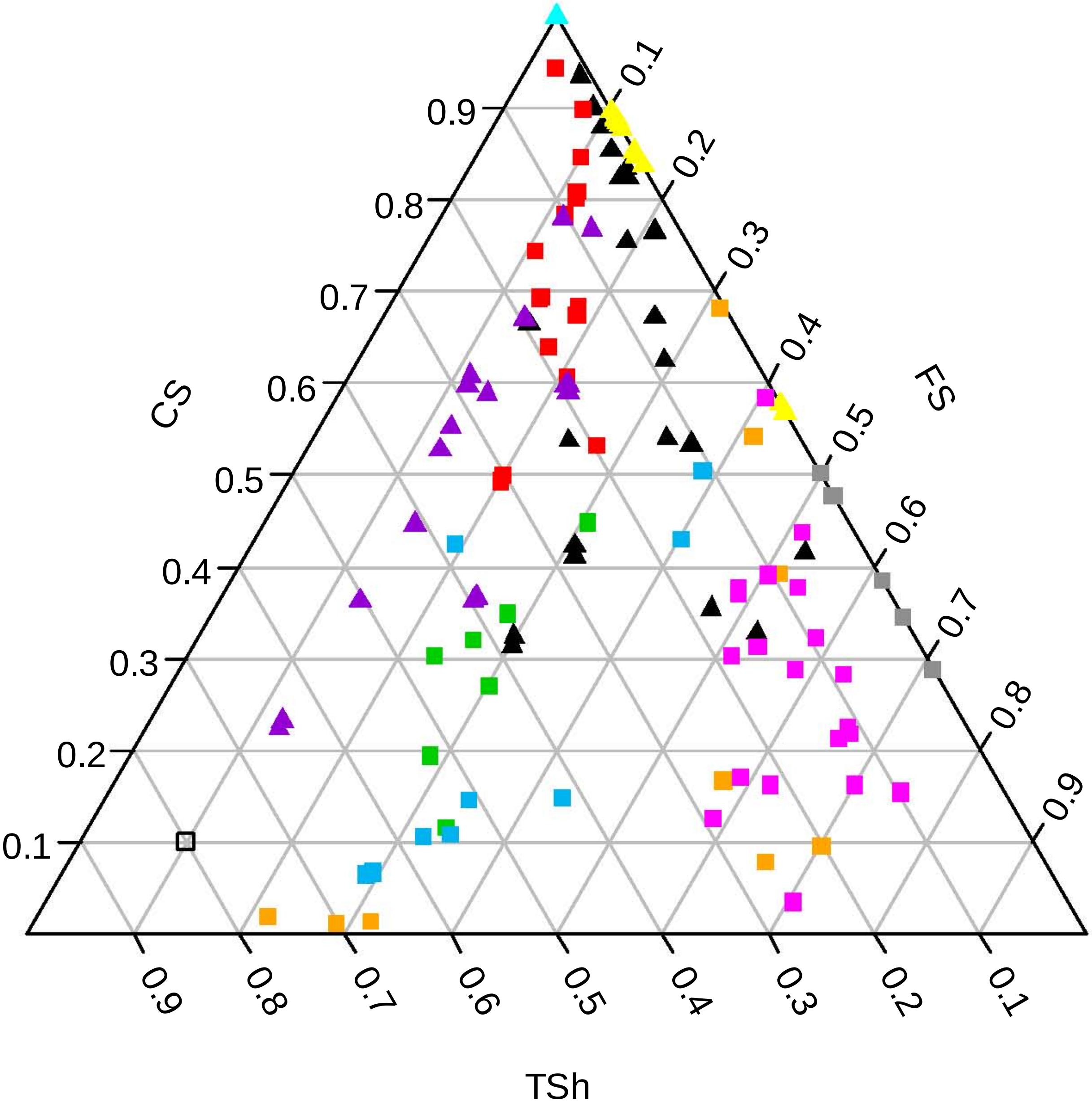
Figure 2. *Triangle (ternary) plots* for sampled individuals from all ten species. The device shows the three estimated probabilities (from multinomial regression) of a specimen being found in *TSh*, *FS*, and *CS* represented by a single point within the triangle. Gridlines correspond to the orientation of each set of tickmark scale labels. Any 3-way intersection of gridlines is associated with three corresponding probabilities that add up to 1. For example, the empty square plotted near the lower-left corner corresponds to 80% *TSh*, 10% *FS*, and 10% *CS.* Colors correspond to species as follows: *S. adleri* (black), *S. cryptus* (red), *S. formosus* (cyan), *S. internasalis* (green), *S. internasalis* 2 (purple), *S. salvini* (gray), *S. smaragdinus* (yellow), *S. stegnejeri* (blue), *S. subpictus* (orange), *S. taecniocnemis* (magenta). Species with short *BT* are marked with squares, and species having long *BT* with triangles. (Should appear in conventional analysis subsection after line 248).

Figure 1.


Mean conduct by Sp
Std. Dev. Activity Time
Mean Activity Time
3.0
2.5
2.0
1.5
11
12
13
14
formosus
smaragdinus
taecniocnemis
internasalis
stegnejeri
adleri
internasalis2
cryptus
subpictus
salvini
MS
1.8
2.1
2.4
2.7
BT
a (10.3,155]
a (155,300]
a (300,445]
a (445,591]

Microhabitat Selection probabilities

Figure 2.



SUPPLEMENTARY DATA

Details of multinomial logit regression for MS

Let p_i denote the probability of selecting microhabitat i, for $i = 1, 2, 3$; let T_{TSh}, T_{FS}, and T_{CS} be the measured environmental temperatures associated to each of the microhabitats. Function vglm in the R package VGAM (Yee, 2008) sets the third category, that is *MS*=*CS,* as a baseline. The model creates predictions for the two quantities $\log(p_1 / p_3)$ and $\log(p_2 / p_3)$ as $\log(p_j / p_3) = a_j + b_j \text{Species} + c_j T_{TSh} + d_j T_{FS} + e_j T_{CS}$ for $j = 1, 2$, where $a_j, b_j, c_j, d_j,$ and e_j are parameters to be estimated based on observed data. Table 4 shows estimated coefficients and corresponding standard errors. For example, an individual of the *S. salvini*, exposed to $T_{TSh} = 24.3$, $T_{FS} = 25$, and $T_{CS} = 26$ would give rise to the following estimates:

$$\log(p_1 / p_3) = -7.0419 - 15.2508 - (0.5080)(24.3) + (0.4856)(25) + (0.2360)(26) = -16.3611$$

and $\log(p_2 / p_3) = -1.6801 + 0.8686 - (0.4371)(24.3) + (0.2039)(25) + (.2471)(26) = 0.0891.$

By incorporating the additional equation $p_1 + p_2 + p_3 = 1$ one can solve to obtain the following estimates of probabilities: $p_1 = 3.7499e - 8$, $p_2 = 0.5225$, and $p_3 = 0.4775$, and these would agree regardless of which category was set as baseline. In Table S1, model coefficients having *z*-values that exceeded 1.96 in absolute value were regarded as significant at the 5% level (indicated with an asterisk in Table 4).

Statistical comparisons to subsets of original data

We performed more detailed analyses of *BT* and T_e through of daylight periods, thus the daylight period was divided into four intervals: morning (0800–1100 hrs), midday (1100–1400 hrs), afternoon (1400–1600 hrs), and evening (1600–1800 hrs; Marin and Bateson, 1989), and among microhabitats inside each species by one way ANOVA, with exception of BT daylight intervals to *Sceloporus salvini*, and microhabitats for *S. smaragdinus* because these only were active in two; hence we used a Paired *t*-test for independent samples (Zar, 1999).

BT was extensive and without significant differences throughout daylight periods and among microhabitats for *S. adleri*, (ANOVA: $F_{2,21} = 0.738$, $P = 0.490$ and $F_{2,21} = 0.168$, $P = 0.847$), S*. formosus* (ANOVA: $F_{2,18} = 2.681$, $P = 0.096$ only for daylight periods because it was observed only in T_{CS}) and *S. smaragdinus* (ANOVA: $F_{2,8} = 1.559$, $P = 0.268$ and Paired t-test $t_9 = 0.616$, $P = 0.553$). While *BT* was short and uniform and with significant differences between species both among daylight periods and between microhabitats for *S. salvini* (Paired t-test: $t_3 = 0.418$, $P = 0.704$ and $t_3 = 3.132$, $P = 0.052$) and *S. subpictus* (ANOVA: $F_{2,6} = 2.397$, $P = 0.172$ and $F_{2,6} = 4.010$, $P = 0.078$).

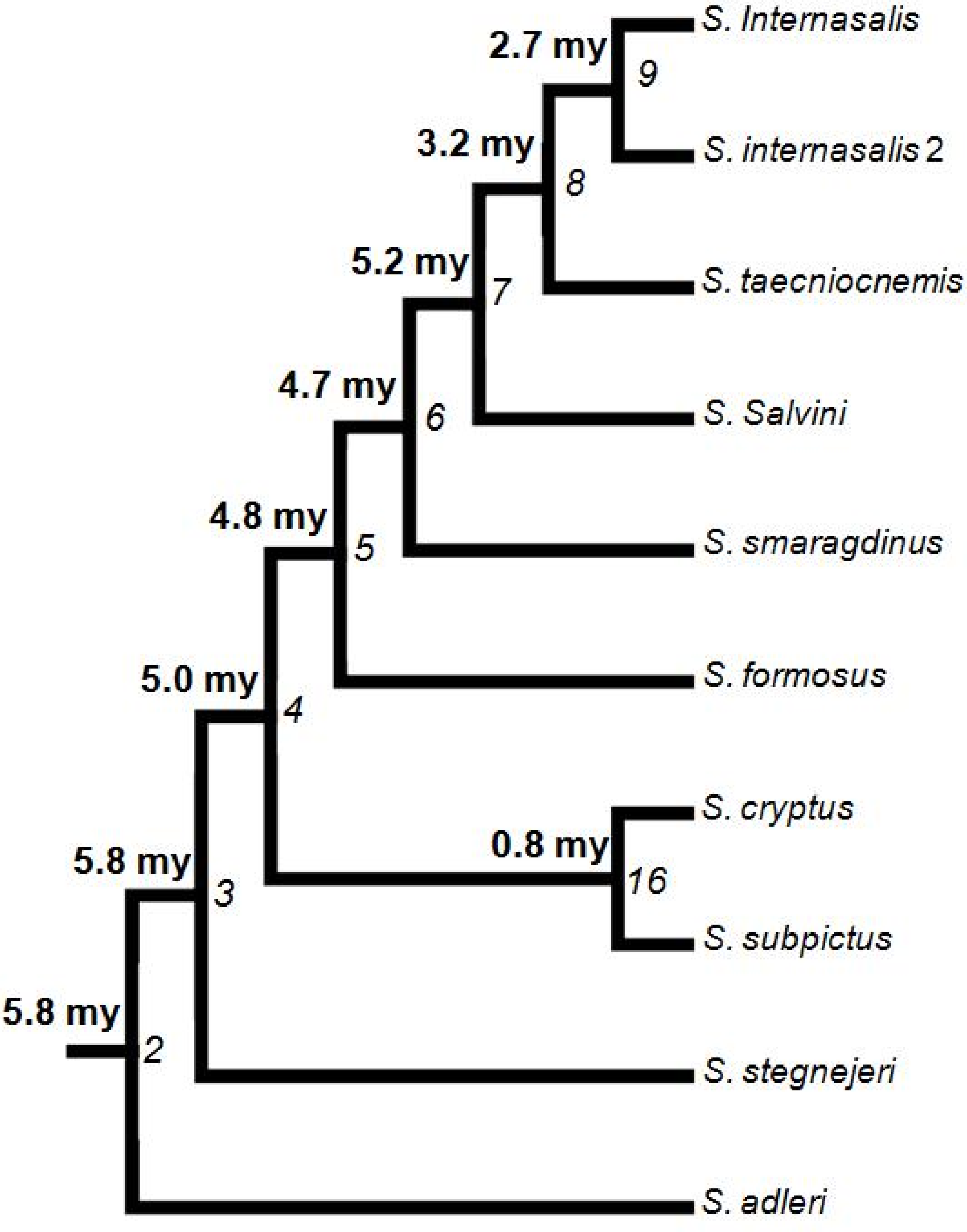
The *BT* for *S. cryptus* had no significantly differences at any specific microhabitat (ANOVA: $F_{2,19} = 0.838$, $P = 0.448$), but they remained significantly less time in 800-1100 hrs than in the rest daylight periods (ANOVA: $F_{3,18} = 4.961$, $P = 0.011$; Tukey test $P = 0.030$). In the case of *S. internasalis* and *S. taecniocnemis*, *BT* was not different for the former along the daylight periods (ANOVA: $F_{2,4} = 0.0567$, $P = 0.946$), whereas the latter remained significant longer periods of time in each microhabitat during the morning than the rest of the day (ANOVA: $F_{2,19} = 16.753$, $P = 0.001$; Tukey test $P = < 0.001$). Both species remained significantly shorter *BT* at T_{CS} (ANOVA: $F_{2,6} = 175.606$, $P = <0.001$; Tukey test $P = < 0.001$ and ANOVA: $F_{2,19} = 6.154$, $P = 0.009$; Tukey test $P = 0.025$), *S. internasalis* exhibited the longest average *BT* observed of all studied species with no significant differences among microhabitats and daylight periods (ANOVA: $F_{2, 16}$ = 0.402, P = 0.675 and ANOVA: $F_{3, 15}$ = 0.296, P= 0.828, respectively). In contrast, *S. stegnejeri* showed the shortest *BT* at any specific microhabitat with no differences throughout the day (ANOVA: $F_{3, 6}$ = 3.976, P = 0.086; Table 1, Table 2); moreover, *BT* was significantly longer in T_{TSh} than in the other two microhabitats (ANOVA: $F_{2, 6}$ = 9.625; P = 0.013; Tukey test P= 0.001), suggesting that individuals of this species thermoregulate by shuttling more frequently among microhabitats than the other species, thus exploiting the thermal heterogeneity offered by the landscape to avoid hiding underground or overheating when they are active.

Finally, T_e was different for each species and did not approach deleterious limits in any microhabitat at any time of day (i.e., 34 °C; Fig. 1Table 1and Table S1) and for *S. salvini* was significantly higher in *CS* than in the other two microhabitats ($F_{2, 30}$= 4.218, P = 0.024; Tukey test P= 0.020). For females of *S. cryptus* T_e was significantly higher (ANOVA: $F_{2, 30}$ = 14.924, P = 0.001; Tukey test P = 0.001), albeit without reaching deleterious levels. Where T_e was significantly higher for both species (*S. internasalis* ANOVA: $F_{2, 30}$ = 3.842, P = 0.033; Tukey test P= 0.026 and *S. taeniocnemis* $F_{2, 30}$ = 7.050; P = 0.003; Tukey test P= 0.036) than in the other two microhabitats, without reaching the maximum tolerance threshold in any microhabitat or time of day (Fig 1, Table 1, and Table S1). T_e for *S. internasalis2* did not differ among microhabitats (ANOVA: $F_{2, 30}$ = 1.021, P= 0.373). T_e at T_{CS} for *S. stegnejeri* was significantly higher (ANOVA: $F_{2, 30}$ = 6.493, P= 0.005; Tukey test P= 0.008), exceeding the 34 °C deleterious threshold for embryonic development.

Table S1. Mean field active temperature (T_b), duration time (*BT*) in a specific microhabitat and operative temperature (T_e) of *Sceloporus formosus* group species.

Species	**Locality**	T_b			*BT*			T_e		
		T_{TSh} ($\tilde{X}$) ±SD (min-max)	T_{FS} ($\tilde{X}$) ±SD (min-max)	T_{CS} ($\tilde{X}$) ±SD (min-max	T_{TSh} ($\tilde{X}$) ±SD (min-max)	T_{FS} ($\tilde{X}$) ±SD (min-max)	T_{CS} ($\tilde{X}$) ±SD (min-max	T_{TSh} ($\tilde{X}$) ±SD (min-max)	T_{FS} ($\tilde{X}$) ±SD (min-max)	T_{CS} ($\tilde{X}$) ±SD (min-max)
S. adleri	Carrizal, Guerrero	25.8 ± 1.5 (24.1-28.0) n= 4	29.1 ± 2.1 (25-30.4) n = 20	27.2 ± 2.0 (24.5-29.5) n= 5	246.0 ± 22.6 (230-262) n= 2	248.1 ± 20.1 (202-265) n= 18	252.4 ± 15.5 (225-264) n= 4	20.5 ± 3.5 (15.1-27.9) n= 1260	20.8± 3.0 (14.8-24.6) n= 1260	22.5 ± 3.5 (15.2-29.6) n= 1260
S. formosus	San Mateo, Oaxaca	–	–	30.6 ± 2.1 (25.5-31.3) 21	_		187.0 ± 15.2 (171.-225) n= 22	17.5 ± 4.0 (10.0-24.8) n= 1260	17.8 ± 4.2 (10.1-24.6) n= 1260	24.7 ± 8.1 (9.5-34.7) n= 1260
S. smaragdinus	Chiquihuites, Chiapas	–	27.3 ± 0.7 (26.8-27.8) n= 2	29.1±1.7 (27.8-32.2) n= 9	_	354.5 ± 0.7 (354-355) n= 2	356.3 ± 5.4 (350-363) n= 9	17.3 ± 1.7 (13.0-20.2) n= 1260	17.5 ± 2.1 (13.6-22.1) n= 1260	18.1 ± 2.3 (15.2-22.9) n= 1260
S. salvini	La Esperanza, Oaxaca	–	27.9 ± 1.4 (26.8-28.9) n= 2	29.3 ± 0.8 (28.5-30.2) n= 2	_	61.3 ± 8.1 (52.5-68.6) n= 3	41.7 ± 4.5 (38.5-45.0) n= 2	25.6 ± 1.3 (22.1-28.6) n= 1260	27.0 ± 2.0 (23.7-30.2) n= 1260	27.9 ± 1.73 (25.2-31.8 n= 1260
S. subpictus	San Esteban, Oaxaca	30.1± 1.0 (28.9-30.9) n= 3	29.2± 0.2 (29.0-29.5) n= 4	29.7± 3.1 (27.5-31.9) n= 2	69.4 ± 2.9 (67-72.1) n= 3	53.8 ± 10.3 (44.6-63.5) n= 4	69.2 ± 6.0 (65-73.5) n= 2	21.1 ± 4.3 (11.5-26.1) n= 1260	24.3± 6.53 (10.2-31.9) n= 1260	25.3 ± 6.42 (11.28-32.09) n= 1260
S. cryptus	Ixtlán de Juárez, Oaxaca	27.3± 2.5 (25.5-29.1) n= 2	28.6± 2.1 (26.2-30.1) n= 3_	29.7 ± 1.6 (27.0-33.0) n= 17	142.1 ± 12.9 (128-145) n= 2	120.3±10.5 (110-131) n= 3	129.7 ± 22.0 (92.3-152.2) n= 17	17.3 ± 2.1 (13.5-22.9) n= 1260	20.3 ± 1.6 (14.1-22.6) n= 1260	22.8 ± 2.1 (18.0-27.2) n= 1260
S. internasalis	Catemaco, Veracruz	30.7 ± 1.6 (29.5-32.5) n= 3	32.0 ± 10.14 (31.9-32.1) n= 2	31.1 ± 0.63 (30.6-31.5) n= 2	207.3 ± 3.2 (205-211) n= 3	169.0 ± 1.4 (168-170) n= 2	164. 7± 3.1 (162.5-167) n= 2	28.5 ± 0.96 (27.1-30.2) n= 1260	30.6± 1.63 (26.1-33.8) n= 1260	30.9 ± 1.53 (28.0-34.2) n= 1260
S.internasalis 2	El Quetzal, Chiapas	26.7±4.3 (21.7-31.5) n= 6	26.5 ± 4.0 (22.0-29.5) n= 3	26.9 ± 3.6 (21.9-33.5) n=10	607.8 ± 228.5 (425-900) n= 6	491.3 ± 59.4 (456-560) n= 3	608.8 ± 205.6 (421-900) n= 9	21.6 ± 2.7 (16.1.1-25.4) n= 1260	21.9 ± 3.0 (15.2-27.2) n= 1260	23.4 ± 2.5 (17.2-29.8) n= 1260
S. stegnejeri	Tierra Colorada, Guerrero	31.4 ± 0.4 (31.1-31.8) n= 4	31.4 ± 0.7 (30.6-32.5) n= 3	31.8 ± 1.06 (31.0-32..5) n= 2	12.3± 0.5 (12.0-13.0) n= 2	9.5 ± 0.7 (9.0-10.0) n= 4	8.8 ±0.3 (8.5-9.0) n= 2	31.2 ± 2.43 (25.9-34.6) n= 1260	33.5± 3.2 (26.4-36.9) n= 1260	36.4 ± 3.6 (28.94-41.8) n= 1260
S. taeniocnemis	San Cristóbal, Chiapas	25.7 ±3.3 (23.1-29.4) n=3	28.3 ±3.4 (19.3-32.5) n= 14	29.3 ± 2.4 (26.0-32.5) n= 6	64.0 ± 1.7 (62-65) n= 3	107.92± 30.4 (57-138) n= 14	70.9 ±16.5 (50.8-92.0) n= 6	17.7 ± 3.44 (9.72-22.35) n= 1260	20.4 ± 3.22 (10.24-25.0) n= 1260	23.2 ± 4.66 (12.5-34.9) n= 1260

Figure S1. Topology used for the PICs based on the pruning of the preferred tree of Smith (2001). This phylogeny was developed with mitochondrial DNA and morphological characters under the parsimony criterion. Numbers at the left of nodes refers to the length of each branch in millions of years. Numbers in italics at the right indicate the number of standardized independent contrasts.



CAPITULO 2. ¿El comportamiento termorregulador contribuye a que las lagartijas gestantes *S. adleri* enfrenten el incremento de la temperatura ambiental?

MANUSCRITO ACEPTADO PARA PUBLICACIÓN EN HERPETOLOGICAL JOURNAL

Does behavioral thermoregulation help pregnant *Sceloporus adleri* lizards in dealing with fast environmental temperature rise?

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Running title: Thermoregulation in *Sceloporus adleri*

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ABSTRACT

Physiological performance of ectotherms, such as reptiles, is subject to specific body temperature ranges, which are frequently similar between closely related species despite inhabiting sites with different thermal conditions. This is known as conservative thermal physiology and *Sceloporus* lizards exhibit this mechanism. Pregnant females should be particularly efficient for thermoregulation because healthy embryos develop at a relative narrow body temperature (T_b) range. In the context of current fast global warming, this could represent a risk because thermal conditions may become unsuitable in the short-term. Hence, we hypothesize that to dampen the potential negative effects of warmer temperatures, lizards should exhibit immediate and simple adjustments of behavioral thermoregulation traits, such as daily activity and basking time (*BT*). To test this idea we set up an experiment under controlled conditions with 40 pregnant *Sceloporus adleri* viviparous lizards and evaluated the above thermoregulatory traits at different thermal treatments (22, 24, 26 and 28°C) for six consecutive days. Lizards exhibited significant variation between treatments in their *BT*, being shorter in the warmest treatments. The probability that a lizard was active (out of its shelter) was significantly higher in the earliest two time periods of day (8:00–10:30 and 10:30–13:00hrs) when compared to later hours (15:30–18:00hrs) for all treatments, while between these the probability decreased at 24 and 26°C. Unexpectedly, the probability of activity for lizards increased at 28°C, becoming more similar to the control treatment (22°C). Finally, all lizards maintained a body temperature (T_b) without significant differences across treatments. These results suggest that *S. adleri* pregnant females are able to adjust behavioral thermoregulation mechanisms in different thermal environments and in a short time periods of time in order to reach an adequate T_b for key physiological processes, such as the development and growth of their offspring.

Key words: Reptiles, Ectotherms, Climate Change, Laboratory, thermal treatments**.**

INTRODUCTION

Frequently, performance of basic biological and ecological functions (feeding, maintaining water balance and breeding) in closely related species of reptiles occurs at similar ranges of field body temperature (T_b), regardless of habitation sites having different temperature conditions (Bogert, 1949; Bowker, 1993). This is known as conservative thermal physiology (Seebacher & Shine 2004; Glanville & Seebacher, 2006). Good examples of conserved thermobiology are *Sceloporus* lizards because they exhibit similar T_b range through diverse ecological and geographic conditions, in which all governed physiological functions, embryo development included, perform (Avery, 1982; Huey, 1982; Crowley, 1985; Crowley, 1987; Mathies & Andrews, 1997; Andrews, 1998). These lizards reach and maintain their preferred T_b range via thermoregulatory behavior.

Gravid females need to be particularly efficient for thermoregulation because healthy embryos develop at a relative narrow range (28–34°C; Beuchat & Ellner, 1987; Beuchat, 1988). Thus, pregnant females display a variety of effective and precise behavioral mechanisms, such as adjustment of daily activity time, microhabitat selection or shuttling between sun and shade to ensure an adequate thermal environment for their offspring (Andrews, et al., 1999; Angilletta, 2000; Angilletta, 2009; Webb et al., 2006).

The fast environmental warming episode we are experiencing (Berteaux et al., 2004) may pose a challenge for suitable thermoregulation in small-sized tropical *Sceloporus* lizards, potentially affecting their physiological performance and, ultimately, their survival (Deutsch et al., 2008; Sinervo et al., 2010). In this context, effective thermoregulation of pregnant *Sceloporus* females would be key for maintaining their body temperature within the preferred range for physiological performance and offspring development (Beuchat, 1986; Qualls & Andrews, 1999; Mathies & Andrews, 1997; Andrews et al., 2000). Consequently, these lizards should exhibit simple changes in behavioral traits that appear to be the most critical in determining T_b, such as adjustments in daily activity time and basking frequency (Stevenson, 1985; Adolph, 1990; Bauwens et al., 1996; Kearney, 2002), as one way to contend with warmest environmental temperatures (Huey et al., 2003).

Therefore, we hypothesize that *Sceloporus* pregnant females are able to manifest fast changes in behavioral thermoregulation traits for damping the negative effects of warmer temperatures on themselves and their developing offspring (Angilletta et al., 2002). To investigate this hypothesis, we evaluated the ability of a highland wild population of viviparous *Sceloporus adleri* pregnant lizards to reach and maintain their preferred T_b range, both for physiological performance and healthy development of embryos under different experimental thermal conditions projected for the future in tropical regions by climate models. We aimed to test if *S. adleri* pregnant lizards were able to reach and maintain similar body temperatures across different thermal environments and simultaneously testing if in doing so they manifested changes in two behavioral traits: (1) activity time and (2) basking time (*BT*) in four different controlled thermal treatments. We expected that as temperature rises, activity time was reduced and restricted to certain hours along the day, and basking time was shortened.

MATERIALS AND METHODS

Fieldwork

To compare the behavioral thermoregulation of female *S. adleri* lizards under different controlled thermal regimes, we collected 40 pregnant individuals in good physical condition (within 50–54mm of snout-vent length SVL, Smith & Savitzky, 1974) from the locality Carrizal de Bravos, in the

State of Guerrero, Mexico (17.6N, 99.8W at 2400m elevation). Lizards were noosed, and their field body temperature (T_b) was immediately measured with a Miller and Weber® quick-reading cloacal thermometer. We detected pregnant lizards by gently palpating the abdomen to feel for embryos and avoiding impairment (Gartrell et al., 2002). Lizards were then transported to the laboratory for experimental procedures. After the experiments, all lizards gave birth, confirming that the assessment of pregnancy was correct. Fieldwork was conducted in December, when neurulation, organogenesis and embryo growth occurs (Guillette & Sullivan, 1985), and when females of the *Sceloporus formosus* species group (to which *S. adleri* belongs) are highly sensitive to temperatures over 34°C (Beuchat, 1988).

Laboratory procedures and experimental design

Once in the laboratory, a room with a stable temperature regime ($\overline{X}$ = 19.7 ± 0.39) and large windows that supplied the natural daylight period as in the field (8:00–18:00hrs), lizards were randomly housed into four experimental plastic containers [150cm length x 100cm width x 25cm height] in equal numbers (10 per container). Lizards were marked on their backs with consecutive numbers from 1 to 10 for convenient identification within each treatment, using epoxy paint.

Containers were previously disinfected with a 5% dilution of sodium hypochlorite. Furthermore, to prevent other possible infection sources, lizards remained isolated from other organisms during all captivity time (Pasmans et al., 2008). Containers were filled with a 2cm-deep layer of substrate collected in the field (a mixture of soil, sand and leaf litter) and divided lengthwise into two equal sections: one for basking with a heat source at different available temperature per treatment, and the other for retreating and shelter with small rocks and wood fragments to provide shade and cover. To control temperature of basking area, two 75W incandescent reflectors (Phillips BR25 125V E26 ES) were suspended 30cm above substrate targeted to radiate completely only this area in each treatment container. The irradiated temperature produced by the reflectors in each basking section was regulated by a programmed digital pyrometer (TERMACRON JK-999-UR) plugged into a power relay (OMROM MGN2A-AC120), and a type J thermocouple with ±1°C accuracy (Honeywell SCTC-TCJ100). The thermocouple worked as a temperature sensor and was set at half of the basking area 1cm above the substrate. Prior to this, we measured the temperature along the basking section every 15cm to verify that temperature was uniform. When temperature decreased or increased beyond the treatment temperature, the system switched the reflectors on/off in order to maintain it constant.

Control treatment was maintained at 22°C (with an operative temperature range of $\bar{X} = 22.07 \pm 0.02$ °C and $\bar{X} = 19.3 \pm 0.60$ °C between basking and retreating areas). We chose this baseline temperature because it represented the recorded mean daytime environmental temperature in the field ($\bar{X}$ = 22.1 ± 2.8°C) during the first stages of reproductive period. Thus, in order to represent the gradual temperature increase of 6°C predicted at high altitudes in tropical regions under the A2 emissions scenario by the end of the century (IPCC, 2007), experimental treatments were fixed at 24°C [$\bar{X}$ = 24.05°C ± 0.13 (basking) and $\bar{X}$ = 19.2 ± 0.86°C (retreat)], 26°C [($\bar{X}$ =26.09 ± 0.04°C (basking) and $\bar{X}$ 19.3 ± 0.44°C (retreat)] and 28°C [($\bar{X}$ =28.07 ± 0.02°C (basking) and $\bar{X}$ =19.5 ±0.44°C (retreat)]. The thermal treatments operated from 8:00 to 18:00 hours and remained inactive at night. Lizards were acclimated at captivity conditions in a thermal regime of 22ºC in all treatments for ten days prior to start the trials (Kelley et al., 2006).

Lizards of the same treatment were kept together in their container because no aggressive or competitive behavior between females was detected neither in the field, even when they occupied reduced spaces (rocky outcrops) for basking, looking for preys and while retreating, as described for other *Sceloporus* species (Ruby, 1977; Ruby, 1978; Davies & Ford, 1983; Contreras-Lozano et al. 2011), nor during acclimation. Basking area was wide enough and thermally uniform so that it allowed several small lizards to thermoregulate simultaneously. Thus, we sustain that no socially induced conditions or other non-temperature variables, such as habitat characteristics and enclosure dimensions influenced thermoregulation behavior of individuals (Carrascal et al., 1992; Bulova, 1994; Mathies & Andrews, 1997).

Behavioral traits measured were: (1) the number and identification of active lizards and (2) basking time (in seconds) of each active lizard (*BT*) that remained directly exposed to the experimental temperature in its designated basking area. In each treatment we recorded behavioral traits between 8:00 to 18:00hrs during the whole period dividing this into four bins of two and half hours following Martin & Bateson (1993), i.e., based on natural light phases throughout the day, as follows: morning (8:00–10:30hrs), midday (10:30–13.00hrs), afternoon (13:00–15:30hrs), and evening (15:30–18:00hrs). This provided us with a framework to compare behavioral thermoregulation data between phases of day. These time periods are henceforth denoted by letters A, B, C and D, respectively.

We surveyed lizards' behavior simultaneously in the four treatments at a distance of 2.5m from the containers to minimize disturbances (this was the tolerated distance before hiding as observed in the field). We recorded body temperature (T_b) of active lizards with a Miller & Webber® cloacal thermometer once a day only during the B period after recording the corresponding behavioral observations to prevent excessive disturbances in this and subsequent periods. The rationale of measuring T_b in period B was to allow lizards enough time to raise and achieve their preferred body temperature after emerging from their shelters. Measurements were recorded for six consecutive days and only consisted of *BT* and T_b quantifications corresponding to active lizards in each time period; these measures were compared across time-of-day, days and treatments (Zar, 1999). Lizards were handled carefully and were fed daily with crickets (*Acheta domestica*) and meal worms (*Tenebrio molitor*), and supplied water *ad libitum* and organic debris (food and feces) were removed after the six-day trials. None of the lizards were euthanized or harmed during capture or experiments; the hatchlings born in captivity showed a healthy condition and survived until their release in the field. At the end of the experiments all lizards were returned to their locality and released (Sexton & Marion, 1974; Andrews et al., 2000; Anguilletta et al., 2000). This study was developed under the approval and permits of the Secretaría del Medio Ambiente y Recursos Naturales of the government of Mexico (SEMARNAT-GPA/DGVS/04949).

Data analysis

Three main issues were explored via analysis of experimental data. First, we investigated how the distribution of variable *BT* changed as a function of treatment, time-of-day, and day. Second, we analyzed variations in the state of being active/inactive as a function of treatment and time-of-day. Together, these two analyses enabled us to describe and test differences in behavioral traits. Third, we examined T_b data to study its mean behavior across treatments and days.

For models and tests, the experimental design described above introduces statistical complexity. This is due to two main facts. Firstly, the resulting design is highly unbalanced, because the number of active lizards observed is random, uncontrolled, and is not equal across treatments and times. More importantly, the same group of ten individuals is followed through time, albeit always located in the same treatment. To account for the effect of measuring the observations in the same lizards along time (and thus a possible dependence structure in the response variables), we used repeated measures models (Laird & Ware, 1982; Pinheiro & Bates, 2011). Two main specific models were considered: linear models for continuous responses, and logistic regression for analyzing the binary state of being active or not. Hypotheses are tested with usual statistics as in ordinary linear models and Anova (such as *F* tests, *t*-tests, or normal approximation *z*-tests), but making technical allowances in order to conserve validity in the results even under repeated measurements on the same subjects. See Supporting Information Section for further details and rationale regarding the statistical models and procedures.

All statistical computations were performed with R software version 2.12.1 (R Development Core Team, 2012). Discussion of results assumes the standard $\alpha = 0.05$ unless specified otherwise. Some of the graphical displays were produced with the ggplot2 R package (Wickham, 2009).

RESULTS

Basking time (*BT*)

We quantitatively observed progressively lower values of *BT* as one changes from treatment 22°C to 28°C (see Fig. S1 and Table 1). Activity changed with respect to time-of-day, and also the rate of activity between treatments. For lizards that were active sporadically during successive periods of time, there did not appear to be a strong correlation in the time series (Fig. S1). The 95% confidence interval for the autoregressive parameter resulted between -0.26 and 0.47, suggesting that zero correlation is plausible (as surmised above by visual means from Fig. S1); but we have maintained the possible correlative structure to be conservative.

Testing for these findings was as follows. With Anova tests in mixed-effects models, we observed that available temperature ($F_{3,213} = 356.3$, $P < 0.001$) and time-of-day ($F_{3,213} = 11.4$,

$P < 0.001$) significantly explained the *BT* exhibited by lizards. Day was not significant ($F_{5,213} = 1.7$, $P = 0.117$), which was an expected result due to intentional homogeneity of experimental conditions. We did not probe into the possibility or hint of a treatment-time interaction, since it is not strongly significant at the 5% level ($F_{9,157} = 1.9$, $P = 0.045$).

Table 2 displays numerical values of parameter estimates for significant main effects and corresponding 95% confidence intervals for the fitted mixed-effects linear model for sqrt(*BT*) (see Fig. S2). Our results indicate that time-of-day had a less dramatic effect on *BT* than treatment did. For all treatments we found that Times B-D were not significantly different, but were significantly smaller than Time A. Moreover, *BT* in Treatments 24–28°C were significantly shorter than in 22°C, but in 24°C and 26°C they were not significantly different. In sum, evidence indicates that basking time was shorter at 24°C, 26°C and 28°C Treatments and at the last three periods in each treatment, B, C, and D (Figure 2).

Activity of lizards and probability

Parameter estimates for population effects using the mixed-effects logistic regression model for the state of being active or not are shown in Table 3. As before, treatment and time-of-day elements had significant effects on lizard's probability of being active (except for Time B). As mentioned above, the fitted logistic regression model implicitly specifies estimated probability values (denoted by P in the supplementary information) for the condition of being active, and these are plotted in Figure 2. Thus, the highest estimated probabilities for lizards being active (i.e., out of shelter area) occurred in Treatment 22°C through most of the day, albeit it decreased at Time D. In upper thermal regimes (24 and 26°C) this probability was higher in Times A-B than C-D. Surprisingly, the probability of lizards being active increased again at Treatment 28°C during Times

A-D. These data suggest that *S. adleri* lizards can adjust separately or simultaneously the extent of daily activity and *BT* in response to the available temperature within treatments.

Body temperature (T_b) of lizards

Daily values of temperature (T_b) recorded once a day (at Time B) in the laboratory, both graphically represented (see Fig. S3) and subsequently analyzed by Anova in the mixed-effects lineal model, suggest that the mean T_b attained by lizards did not change significantly between treatments ($F_{3,36} = 0.516, P = 0.6742$) and days ($F_{5,108} = 0.395, P = 0.8511$; Table 1 and Fig. 3). The same statistical modeling also provided a 95% confidence interval for the mean T_b in the laboratory, given by (27.4°C, 29.8°C) (with point estimate 28.6°C). Data obtained in the field ($\bar{X}$ = 28.2 ± 2.64°C) yields the interval (27.4°C, 29.0°C). Because these two intervals did overlap, then we conclude that T_b in the laboratory and in the field were not significantly different.

DISCUSSION

The climate is changing and species are expected to respond in different ways, including behaviorally (Kearney et al., 2009), physiologically (Bradshaw & Holzapfel, 2010), by adjusting the timing of biological processes such as reproduction (Parmesan, 2006), or migrating (Pounds et al., 2005). When such response strategies fail, populations will decline and species may collapse in the long run (Sinervo et al., 2010). In ectothermic species, thermoregulation behavior is a key trait which is probably the first one used when environmental temperatures increase; here lies the importance of understanding the specific mechanisms that these species use to fine-tune behavioral traits under warmer temperatures.

Pregnant *Sceloporus adleri* lizards in the four temperature treatments did not show significant differences in T_b during the six days of experiment, suggesting that they achieve similar body temperatures by regulating their activity and basking times (two of the main behavioral thermoregulatory mechanisms in ectotherms) separately or combined, depending on the thermal conditions experienced locally (Stevenson, 1985; Adolph, 1990). Our results show that *S. adleri* lizards spent significantly more time out of their shelters throughout the day at the lowest (control) temperature regime (22°C), than lizards at 24 and 26°C (only during A and B periods). This partially validates our first prediction regarding the restriction of their activity out of their refuges to specific hours of the day. Furthermore, lizards in 24°C and 26°C Treatments significantly reduced their *BT*. This finding supports our second prediction in regard to the decrease of basking time, suggesting the possibly that lizards in all these treatments raise their preferred T_b equally fast, but at warmer temperatures they needed to bask less time to maintain it and thus spent some time to explore, search for preys and feeding before retreating in the last two periods. Both results suggest that lizards evade long exposure at warmer environmental temperatures to prevent overheating.

Notably, lizards in Treatment 28°C were out of their shelters at all times of day, more similar to lizards in the control temperature (22°C) than the ones at 24°C and 26°C Treatments. According to previous studies, lizards at higher environmental temperatures should continue to reduce their activity time significantly, until remaining hidden in their shelters most of day to escape of warmest environmental temperatures (Huey & Tewksbury, 2009). If this behavior were adopted, it would restrict foraging and reproduction opportunities (courtship and mating) to the point of having negative impacts for populations (Angilletta, 2009; Sinervo et al., 2010). The lizards *S. adleri* in Treatment 28°C adopted a different strategy: they did not restrict their activity time by hiding in the last periods of the day; in turn, they reduced their *BT* using a mechanism known as shuttling, i.e., moving constantly from full sun to shade, which allowed them to be active for feeding and exploration. When compared with the lizards of the other treatments, individuals at Treatment 28°C exploited available thermal environment by shuttling more frequently between retreating and basking areas throughout the day (Carrascal et al., 1992), reducing the effective time they were directly exposed to warmer temperatures.

In sum, pregnant females of *Sceloporus adleri*, showed similar preferred body temperature across different thermal treatments, which was very close to T_b recorded in the field and fall within the suitable temperature range (28–34°C) for performing physiological processes and developing healthy embryos reported for other viviparous *Sceloporus* species (Beuchat, 1988; Mathies & Andrews, 1997). Our results suggest that *S. adleri* pregnant females achieved this preferred body temperature through behavioral thermoregulation, even under a 6°C increase scenario, as other lizards do (Hertz 1981; Stevenson, 1985; Van Damme et al., 1987; Adolph 1990; Bauwens et al., 1996; Díaz, 1997). Similar results have been documented for other pregnant lizards of the same genus, like *Sceloporus jarrovi* (Beuchat & Ellner, 1987). Therefore, although experimental temperatures to which lizards were exposed did not exceed the maximum tolerated thermal threshold reported for *Sceloporus* embryo development (Beuchat, 1988), from these results we can expect that in a warmer world, *S. adleri* may be able to deal with highest temperatures in sites where local conditions offer a mosaic of sun and shade, without compromising their physiological condition or the viability of their offspring (Huey et al., 2003; Herczeg et al., 2008; Huey & Tewksbury, 2009). On the contrary, in cooler sites at the upper distributional limits, populations may even benefit from a moderate warming (Chamaillé-Jammes et al., 2007). This study represents a step forward towards understanding some potential behavioral responses of a tropical lizard to a local environmental temperature increase.

ACKNOWLEDGMENTS

We thank Enrique Venegas Flores, Armando Escobedo Galván, and Constantino González Salazar for their help in fieldwork. Baruch Arroyo Peña made important comments to early drafts of this manuscript. This work was funded by the Dirección General de Asuntos del Personal Académico of the Universidad Nacional Autónoma de México (DGAPA-PAPIIT project number IN221208). The Consejo Nacional para la Ciencia y la Tecnología (CONACYT) provided doctoral scholarships to SLA and RMR. We thank to the Programa de Posgrado en Ciencias Biológicas of UNAM for logistic support to S. L-A. This paper is a requirement for obtaining the Ph.D in Biological Sciences. Permit for fieldwork was issued by the Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT GPA/DGVS/04949).

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TABLES

Table 1. Descriptive statistics for basking time (*BT*) and active body temperature (T_b) observed for *S. adleri* in each temperature treatment. Total lizards for all treatments were 10 individuals, but since the number of lizards being active at a given time was varying and data considered here is compounded over six days and four times-of-day, the number of observations (*n*) in each treatment is not constant.

Treatments	***BT* (mean) ± SD (min-max) (*n*)**	**T_b (mean) ± SD (min-max) (*n*)**
22°C	256.7 ± 82.3 (105.0-576.0) (130)	28.6 ± 1.9 (24.8-32.1) (44)
24°C	142.4 ± 43.7 (57.0-280.0) (96)	28.6 ± 2.0 (24.6-32.1) (46)
26°C	115.3 ± 35.7 (45.7-197.0) (60)	28.4 ± 2.3 (24.1-32.5) (20)
28°C	56.9 ± 22.9 (10.0-152.0) (105)	29.4 ± 1.5 (25.9-32.5) (43)

Table 2: Parameter estimates and end points of 95% confidence intervals for fitted mixed-effects linear model for studying sqrt(*BT*). Interactions are not displayed here because they were not significant. These intervals are plotted in Figure S2.

Main effects	**Lower**	**Est.**	**Upper**
22°C (Intercept)	16.19929348	16.96744434	17.73559521
24°C	-5.35987204	- 4.48252661	-3.60518118
26°C	-6.36933091	-5.55307805	-4.73682518
28°C	-10.08326628	-9.29988275	-8.51649922
TimeB	-2.17275389	-1.27362011	-0.37448633
TimeC	-3.20258905	-2.15143108	-1.10027310
TimeD	-4.01552583	-2.02690255	-0.03827927

Table 3. Summary of estimated parameter results for logistic regression with random effects applied to lizards' state of being active or not.

Main effects	**Estimate**	**Std Error**	**z value**	**p**
22°C (Intercept)	1.6406	0.2154	7.616	< 0.001
24°C	-0.8645	0.2294	-3.768	< 0.001
26°C	-1.7889	0.2405	-7.438	< 0.001
28°C	-0.6398	0.2285	-2.800	< 0.001
TimeB	-0.2032	0.2018	-1.007	0.314
TimeC	-2.0169	0.2156	-9.356	< 0.001
TimeD	-3.6913	0.3115	-11.851	< 0.001

FIGURE LEGENDS

Figure 1: Box plots for *BT* [not sqrt(*BT*), for ease of interpretation] exhibited by active lizards, grouped by time and treatment.

Figure 2: Estimated probabilities of lizards being active, as a function of time-of-day (8:00–10:30, 10:30–13:00, 13:00–15:30 and 15:30–18:00hrs represented by letters A, B, C, and D on the horizontal axis) and treatment, as computed by logistic regression with random effects for lizards.

Figure 3: Box plots for T_b raw data recorded to active lizards, grouped by day and treatment (see also Table1).

Figure 1.

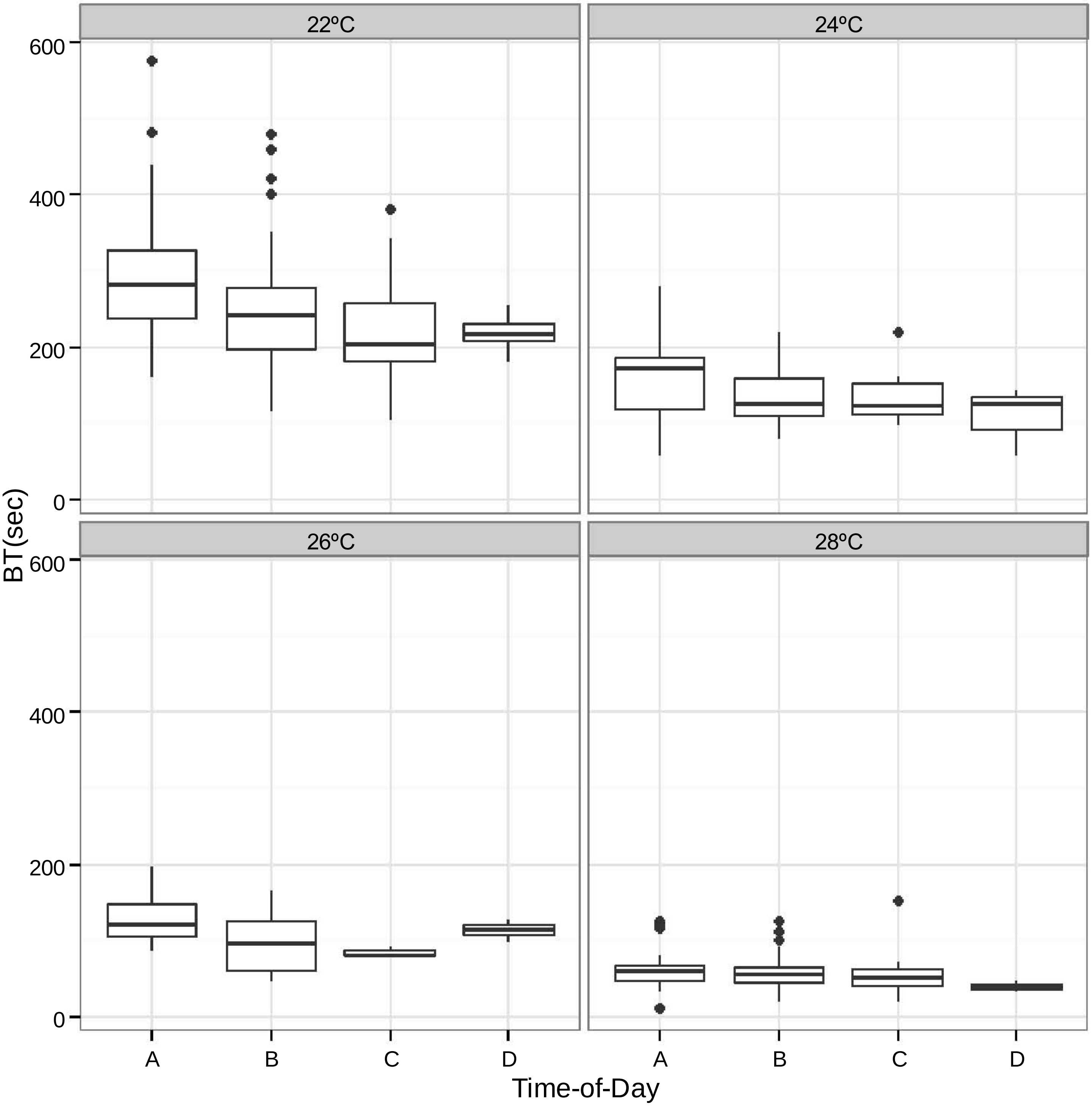
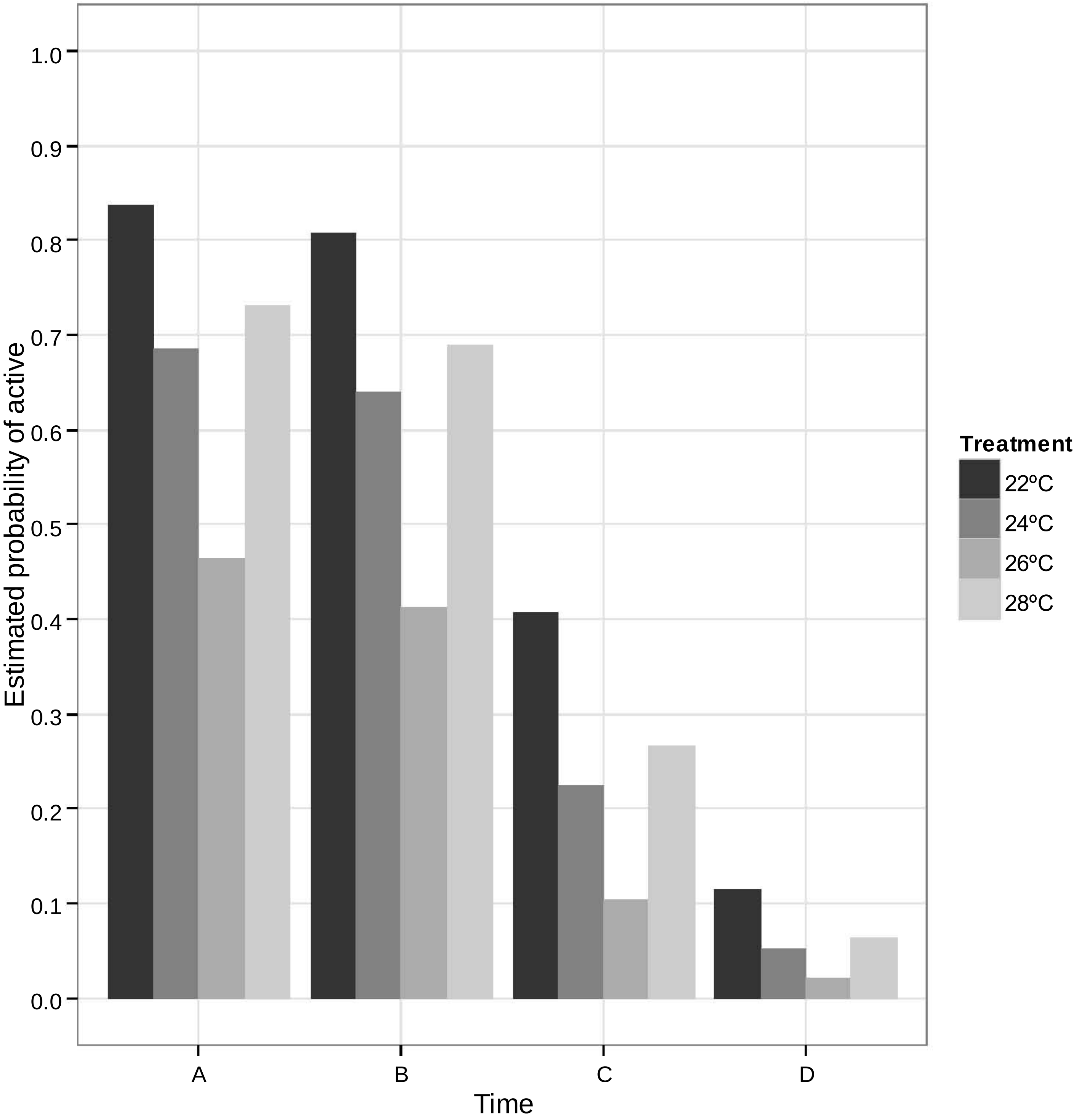
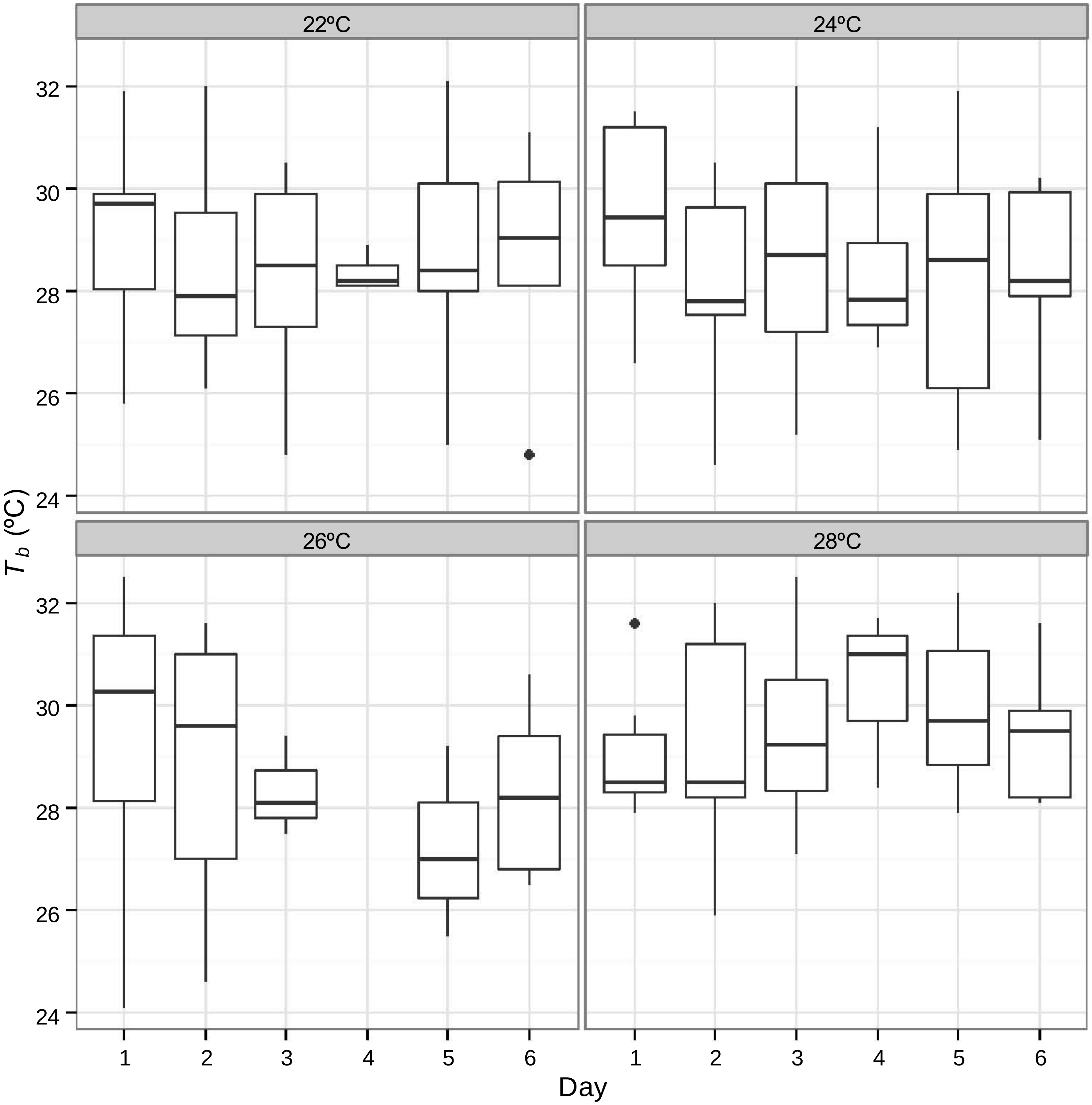

22ºC
24ºC
26ºC
28ºC
600
400
200
0
600
400
200
0
BT(sec)
A
B
C
D
A
B
C
D
Time-of-Day

Figure 2



Estimated probability of active
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
A
B
C
D
Time
Treatment
22ºC
24ºC
26ºC
28ºC

Figure 3



SUPPORTING INFORMATION

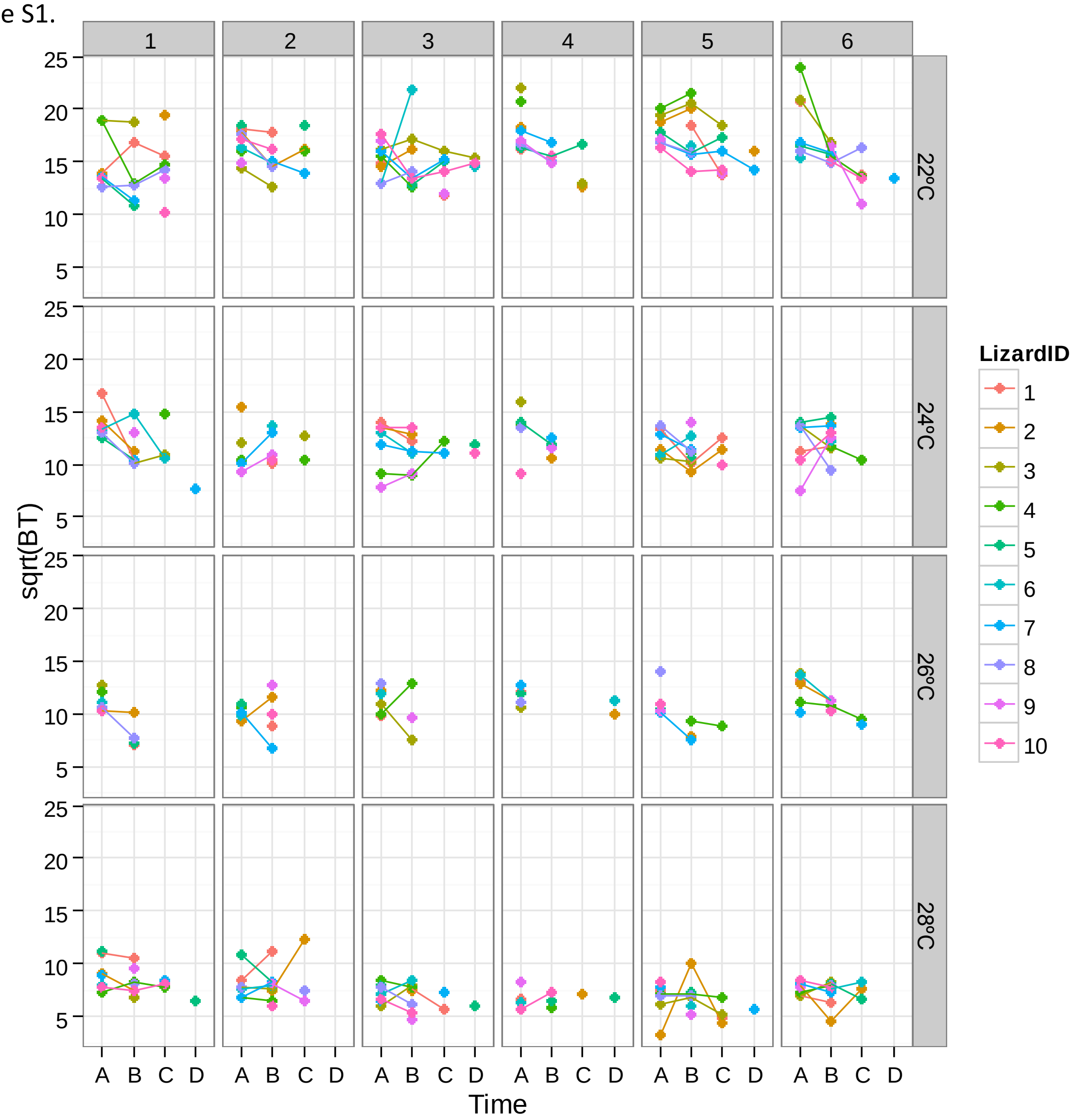
Rationale of statistical analyses

Raw data for basking time (*BT*), which were transformed to their square root for attaining statistical assumptions in the models described below, were graphically represented for a first visual inspection (see Fig. S1). For analyzing *BT* more thoroughly, we fitted a linear random-effects model as implemented in the lme function of the R package nlme (Pinheiro et al., 2012). Specifics of the fitted model follow, employing the terminology of Laird &Ware (1982) and R package nlme. The response variables are the sequence of multiple sqrt(*BT*) measurements for each active lizard through time. Fixed population effects involve day and treatment*time (the asterisk indicates that individual effects for treatment and time are considered, as well as interactions). The random effects are assumed to be normally distributed constants with mean zero for each lizard on each day, which amounts to conceiving that each individual lizard either adds or subtracts a random daily constant from the overall population mean sqrt(*BT*). The estimated value of the standard deviation of these random effects was 0.34 (and an interval for it was very wide). For describing the repeated measurements on each lizard through times on a given day, a covariance structure must be specified, and we have chosen a simple autoregressive structure of order 1, which amounts to saying that for each lizard, sqrt(*BT*) at a time interval is correlated with sqrt(*BT*) at the previous time interval only via a multiplicative constant. Finally, motivated by varying grades of data spread apparent in Fig. S2, in the model we have allowed for the variance of sqrt(*BT*) to be different in each treatment, a provision which proved to be worthwhile because variances turned out to be significantly different (specifically, relative variances for Treatments 2, 3, 4 are 76%, 62%, and 60% with respect to the variance for Treatment 1).

For analyzing the binary state of being active or inactive, we used logistic regression, also with the consideration of random effects for lizards as implemented in the glmer function of the R package lme4 (Bates et al., 2011). This provided a model for predicting the probability, P of being active as a function of explanatory variables treatment and time-of-day. The model we implemented incorporates treatment and time as main effects (with interactions removed because they did not result significant), with random effects specified for lizards. Day was likewise removed from the final model, because as expected, it did not result in having significant effects. The final model adopted is $\ln[P/(1-P)] = \text{treatment} + \text{time} + \text{lizard}$ (where treatment and time are fixed population effects, and lizard is a random effect).

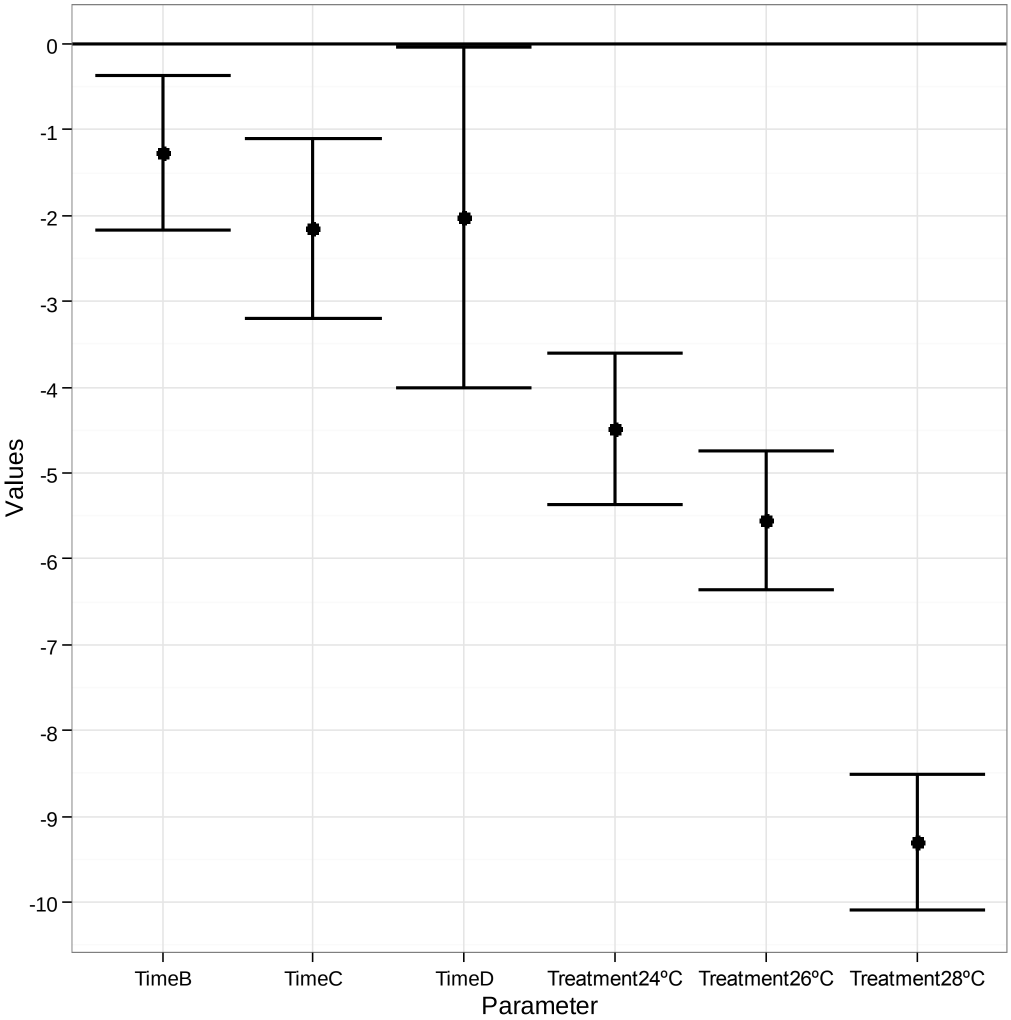
Daily values of body temperature (T_b) recorded only once a day (at Time B) for lizards in all treatments were graphically represented for a first visual inspection (see Fig. S3), and subsequently also analyzed through a linear mixed-effects model. Again, because individual lizards may have been observed multiple times in different days, random effects for lizards were also considered, with possible temporal correlations specified by day, instead of time-of-day as described above for the *BT* analysis (because time-of-day was non-varying when T_b was measured). The relevance of including a random effect is made clear by examining Fig. S3 closely; a few lizards were incidentally active all days, providing us with a valuable chance to notice a clear idiosyncratic behavior in their measured T_b (some individuals gravitate rather persistently to different characteristic levels of T_b). R package nlme was used, specifying T_b as a response variable, treatment and day as population effects, and lizard as a random effect. No transformation of T_b was here prescribed because analysis of residuals implied the normality assumption was valid. Variances of T_b were also allowed to differ between treatments, as before.

Figure S1.



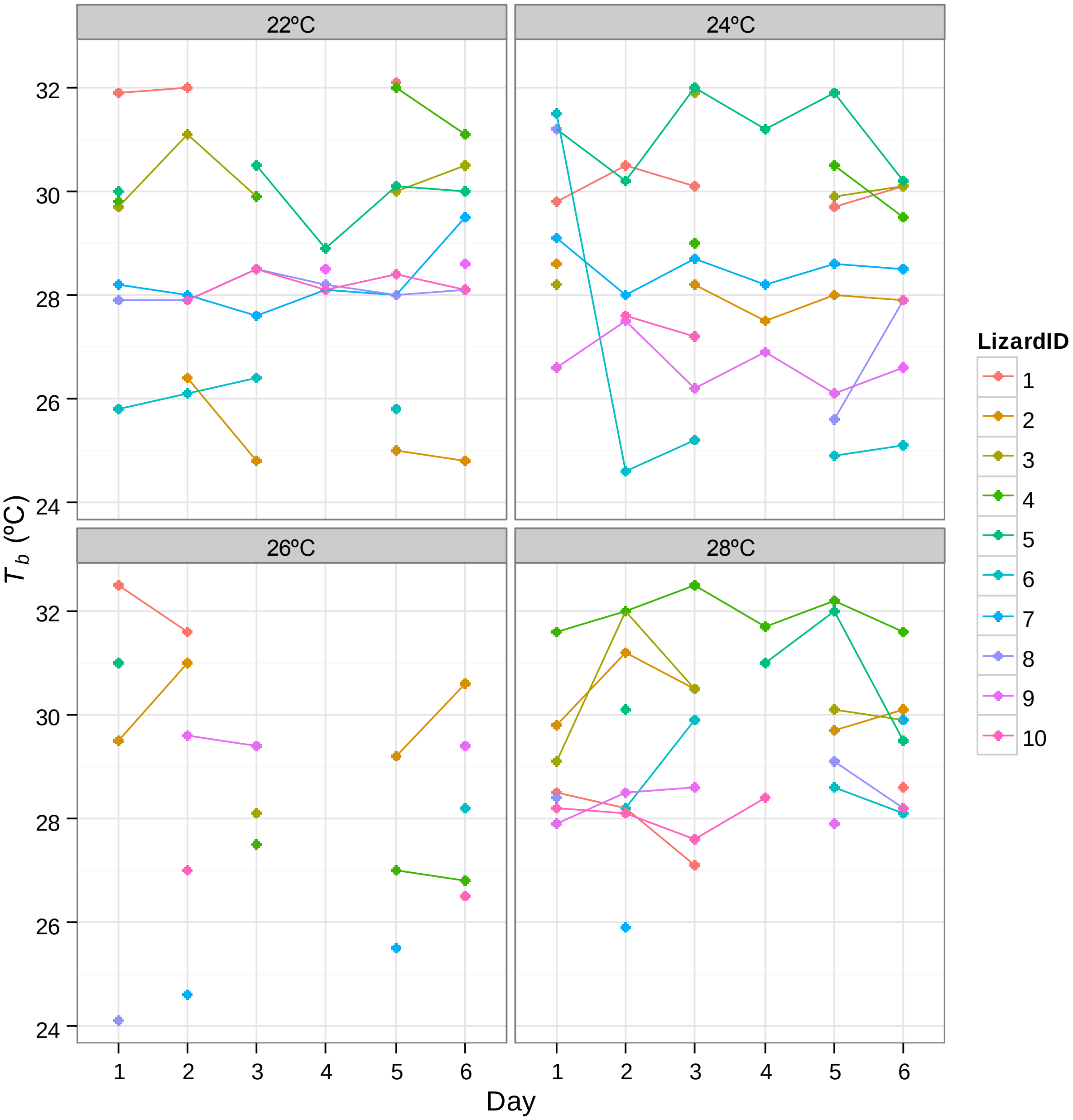
Square root of *BT* as a function of time-of-day. There are separate panels for each combination of treatment and day. Letter codes A, B, C, and D represent different times-of-day (see Materials and Methods section). Since each treatment was represented by the same individuals, we use lines of different color to connect successive observations pertaining to the same lizard. If points are missing, it is because particular individuals were inactive at that time of day so that no *BT* was measured.

Figure S2.



Parameter estimates for significant main effects for sqrt(*BT*) model (dots), and 95% confidence intervals (whiskers). Significance is evident when such intervals do not contain zero (the horizontal line at value zero). The reference overall estimated mean value for 22°C Treatment and Time A is 16.97, and the values depicted here are interpreted as differences from this overall mean. For example, the effect of changing to 24°C Treatment is a reduction in 4.48, and the change from 26°C Treatment to 28°C Treatment is significant because the corresponding intervals do not overlap. Treatments at 24 and 26°C, however, are not significantly different because of overlap.

Figure S3.



Daily measurements of body temperature for active lizards grouped by temperature treatment and ordered by day. Observations pertaining to the same individuals on successive days are joined by lines of different colors. Average T_b and SD are shown in Table 1 and Fig. 3.

CAPITULO 3. ¿Qué pueden hacer las lagartijas cuando la temperatura en los sitios de anidación aumenta? Una aproximación experimental en el grupo *Sceloporus spinosus* (Squamata: Phrynosomatidae)

MANUSCRITO BAJO REVISIÓN EN JOURNAL OF THERMAL BIOLOGY

What would lizards do when nesting temperature goes up? An experimental approximation in the *Sceloporus spinosus* (Squamata: Phrynosomatidae) group

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Running headline: Lizard´s responses to nests warming

ABSTRACT

Current environmental temperature increases may restrict available nesting sites in the tropics for females of *Sceloporus* lizards, threatening the viability of their populations; lizards may be expected to respond strategically to prevent clutch exposure to lethal temperatures in the nest. To test this hypothesis we (1) analyzed maximum environmental temperature (T_{max}) trends over the recent decades at five localities inhabited by different species of the *Sceloporus spinosus* group; (2) recorded and compared environmental (T_{env}) and artificial nest temperatures at different depths exposed to full sun and under shade within and among localities, and related them to the body temperature (T_b) of active gravid females, which represents the temperature that embryos would experience under normal gestation conditions or the temperature for normal embryonic development (T_{ned}); and (3) analyzed potential future thermal scenarios for developing embryos at each site, via climate change forecast for years 2030 and 2050. T_{env} has increased significantly at three of five localities in the recent decades. For two of these located above 1000 m, environmental, full sun and shade exposed nest temperature, were lower than the thermal threshold for embryos. Whereas temperature at sun exposed nest, of a third locality (580 m, Xalitla), exceeded the thermal limit for embryos (≥34°C), and was significantly higher than temperature of nests built under shaded. Nest temperatures in two remain localities below <1000m under sun and shaded conditions, were close to thermal thresholds for developing embryos. Climatic forecast to 2030 and 2050 showed that the anticipated increase of T_{env} during the nesting period would not exceed thermal thresholds for normal embryo development at most sites, except for the locality where it is already happening as at Xalitla. However, lizards of that site could prevent embryo exposure to deleterious temperatures nesting at shaded sites. Finally, phylogenetically controlled analyses between T_{ned} of gravid females with T_{env} and temperature of sun exposed nests at each locality, suggested that thermal ranges for healthy embryo development could be adjusted in function to these. Then, we conclude future temperature increases do not represent immediate threats to successful development of embryos of *S. spinosus* at nesting sites.

Key words: Nesting behavior, Oviparous Lizards, Temperature rise

1. Introduction

Global environmental temperatures of earth have shown rapid increases over the last half-century (IPCC 2007). It has been suggested that this phenomenon threat on wild populations of sensitive ectothermic vertebrates such as tropical lizards, (Deutsch et al., 2008; Huey and Tewksbury, 2009; Huey et al. 2009; Cadena et al., 2011). In this regard it has studied the potential effect of temperature increase on viviparous *Sceloporus* lizard´s populations by modeling their influence on diurnal activity time (Sinervo et al., 2010). *Sceloporu*s genus lizard's also includes oviparous species and has been considered thermally conservative, because exhibit similar field body temperatures thought different geographic and ecological conditions (Avery, 1982; Huey, 1982; Sites, et al., 1992; Andrews, 1998; Andrews, et al., 1999), and the developmentally safe temperatures for embryos both viviparous and oviparous *Sceloporus* species do not exceed 34°C, because highest temperatures can have strong and negative effects on offspring phenotype and survival (Beuchat, 1988; Overall, 1994; Smith and Ballinger, 1994; Andrews, et al., 2000; Angilletta et al., 2000). Then in a warmest world nesting strategies that guarantee favorable thermal conditions could play also a key role to survival of tropical *Sceloporus* oviparous lizard populations (Warner and Andrews, 2002).

Has been reported that several *Sceloporus* oviparous species form temperate latitudes are able to locate sites where their embryos grow and develop under beneficial thermal environment, through carefully microhabitat selection and adjusting the nest depth. This reduces the risk of damage from overheating and would optimize their developing time and size at emergence (Kerster, 1964; Bernardo, 1996; Anguilletta et al., 2000; Andrews, 2000; Angilletta et al., 2009). Thus, oviparous *Sceloporus* lizards from tropical regions in a warming environmental temperatures scenario could compensate for unfavorable thermal incubation conditions simply using efficient behavioral nesting strategies, as already outlined, before adjusting other traits as reproductive phenology or physiology as thermal adjustment of normal development at temperatures beyond the thermal threshold (Huey et al., 2003).

Here, we test above ideas analyzing thermal conditions experienced by five species of oviparous *Sceloporus spinosus* group distributed along an elevational gradient (0-2400 m) to analyze thought this the potential alternatives to lizards achieve maintain to their offspring at appropriate temperature ranges. Our aim was to determine (1) historical trends of local environmental temperatures to, (2)whether nests building under different solar radiation conditions (full sun versus shaded microhabitats) and at different depths would keep developing embryos under appropriate thermal conditions (below maximum thermal deleterious threshold of 34°C), (3) we also analyze indirectly potential adjusts in thermal ranges for a healthy embryonic development for this species group, it is, if developmental temperature would change with increasing environmental and nesting temperature. We did this by evaluating the relationship between gravid body temperatures (T_b) or temperature for normal development of embryos (T_{ned}), with T_{env} and nest temperature at different localities where *Sceloporus spinosus* group occurs and finally (4) whether forecasted climatic scenarios for coming decades would lead deleterious nest temperatures for embryos in each one of these species.

2. Materials and Methods

2.1. Study species and environmental temperature trends

We obtained data to five localities inhabit for *Sceloporus spinosus* group from HerpNet (www.herpnet.org; see Acknowledgments for specific data sources) and from review o the scientific literature (Calderón-Espinosa et al., 2006). We selected one locality per species for fieldwork at elevations ranging from sea level to 2400 m to cover the altitudinal and thermal range of the group. Sampled localities and species than inhabit were: San Mateo in Oaxaca for *S. edwartaylori* (LN 16.2000; LW -95.0000; 0 m), Chamela in Jalisco for *S. horridus albiventris*, (LN 19.5333; LW -105.0833; 11 m), Xalitla in Guerreo for S*. h. horridus* (LN 18.0167; LW -99.5500; 580 m), Mitla in Oaxaca for *S. s. apicalis* (LN 16.9167; LW -96.4000; 1682 m) and Las Minas, in the estate of Puebla (LN 18.5667; LW -97.3833; 2400 m) for *Sceloporus spinosus spinosus*.

For each locality, we obtained historical records of maximum environmental temperature ($T_{\max}$) from the nearest climatological stations administered by Mexico's Comisión Nacional del Agua (CNA), then we take the daily available maximum temperature recorded during July-August and obtained the average for each year in these period. Latter, we explored the temporal trends of at each locality in the above period which corresponds to the lizard´s reproductive season (Valdéz-González and Ramírez-Baustista, 2002) via an abnormalities graph (IPCC, 1994) see appendix 1. If annual $T_{\max}$ exceeds at least one standard deviation its global mean in recent years, it was considered a signal of a warming trend; these trends were tested via regression analyses, also we compared $T_{\max}$ records between localities with One-way ANOVA test.

2.2. Relationship among environmental, nests and gravid lizard body temperatures

We conducted fieldwork at each locality during the oviposition period at July-August months (Valdéz-González & Ramírez-Baustista, 2002). In the field are seldom found natural nest, because *Sceloporus* lizards construct their nest full buried in a soil column (Shine, 1988; Warner and Andrews, 2002). Thus eggs temperature is regulated by the temperature of surrounding soil. Therefore soil temperature is good approximation of temperature experienced by embryos in the nests (Campbell and Norman, 1998; Ackerman and Lott 1999; Shine, 2002). Then to measure soil temperature at which developing embryos are exposed we built artificial nests, randomly distributed with five meters of distance among them both under canopy cover areas where gravid adult females were detected and in contiguous open sandy areas potentially used by lizards to nesting, at each survey localities (Angilletta et al., 2009). At which we dig perpendicularly to ground surface to build a cavity to set the temperature sensors where the eggs should be and subsequently filled. Then, we set 19 temperature sensor Hobo pendant® UA-002-08 data loggers at each locality, 18 for soil temperature at three sun exposed nest and three shaded (T_{esn} and T_{shn}) conditions respectively, nine sensors were set inside the same number of sun exposed nest at different depths in the ground: (a) three at 6.4 cm ($T_{\mathrm{esn6.4}}$) [the known nest depth at which is known at least two *Sceloporus* lizards species (*S. undulatus* and *S. virgatus*) lay their eggs in sun exposed conditions (Andrews, 2000; Warner and Andrews, 2002; Anguilletta et al., 2009)], (b) three at 8.4 cm ($T_{\mathrm{esn8.4}}$), and (c) three at 10.4 cm ($T_{\mathrm{esn10.4}}$). Another nine sensors were set at shaded nest at depths of (a) three at 4.7cm ($T_{\mathrm{shn4.7}}$), (b) three at 6.7 cm ($T_{\mathrm{shn6.7}}$), and (c) three at 8.7 ($T_{\mathrm{shn8.7}}$); we chose these nest depths because nests in shaded microhabitats observed in other *Sceloporus* lizards are known to be shallower than those under full sun (Anguilletta, 2009) and furthermore one to record environmental temperature (T_{env}) or air temperature at 1cm above the ground at full sun. Sensors recorded temperatures every 30 min. between 8:00-19:00 hrs and for seven days. We used these data to estimate average hourly temperature and then compared available temperatures between nest in shaded and full sun microhabitats and among nests depths via nested ANOVAS test.

We captured 76 gravid females of each species at its site (Table 1) and recorded field active body temperatures (T_{b}), with a fast reading cloacal thermometer (Miller &Weber®). We used this temperature as measure of temperatures tolerated by developing embryos of each species, we will call this T_{b} as temperature for normal embryonic development (T_{ned}) although we were well aware that such sample will not indentify tolerance extremes. We then inferred potential adjustment in measured T_{ned} range exhibited by lizards in response to T_{env} and $T_{\text{esn6.4}}$, $T_{\text{esn8.4}}$ and $T_{\text{esn10.4}}$ (recorded environment and nest temperature s) available at each locality by Pearson correlations (forced through the origin) after obtain Phylogenetically Independent Contrasts (PIC) through Felsestein´s method to correct correlation test for historical non-independence of species (Felsenstein, 1985; Harvey and Pagel, 1991; Rezende and Garland, 2003; Ashton, 2004). Positive relationships indicate that healthy embryo development could occur at wide environmental and nest temperature range, which could be interpreted as a physiological thermal adjustment of embryo development (Andrews and Schwarzkopf, 2012).

The phylogenetic hypothesis used to perform PICs was a pruned version of the phylogeny of the *Sceloporus spinosus* group, present by Calderón-Espinosa et al. (2006; Fig 3). This was generated with 1857 base pairs of two mitochondrial characters of ND4 and Citb genes and with *S. melanorhinus* as external group. Uncorrected genetic distances in the *ad hoc* tree (minimum evolution) were, used for the Phylogenetic Independent Contrasts (PIC). The topology generated is congruent with a more complex analysis based on more samples and one additional mitochondrial fragment (Calderón-Espinosa, personal communication). The tree was reconstructed with uncorrected *p* distances and pairwise deletion options only for the subset of species for which we had data of T_{ned}, T_{env} and nest temperatures. We compared T_{ned} (T_b of pregnant females) during pre-oviposition stage among localities by one way ANOVA test (Zar, 1999).

2.3. Forecasting nest temperatures under different green house gas emissions

We used linear regression analysis to evaluate if T_{env} at each locality determined nest temperature under full sun and shaded conditions; we used $T_{\text{esn6.4}}$ and $T_{\text{shn4.7}}$ because are the two know depths of nesting in *Sceloporus* lizards at sun and shade conditions respectively and represent a first step towards understanding effects of environmental temperature increase that would be experienced within nests from studied localities, under four different green house gas emissions (A1, A2, B1, and B2) for years 2030 and 2050, produced by the ECHAM5/MPI, UKHADGEM1, and GFDL CM 2.0 general circulation models (GCM), which were obtained from the Centro de Ciencias de la Atmósfera of the Universidad Nacional Autónoma de México webpage (www.atmosfera.unam.mx; Conde et al., 2008). For each scenario we averaged expected temperature increases for the nesting period (July-August) across GCMs and added then in a regression model used to explain the relationships between current environmental temperatures and nest at each locality, thus forecast temperatures available within these nests for years 2030 and 2050.

All statistical analyses were performed with Statistica for Windows Version 8.0® (Stat Soft, 2007). We checked normality and homocesdasticity assumptions for all regression analyses. Phylogenetic analyses were conducted with the PDTREE module (Garland and Ives, 2000) of Mesquite version 2.71 (Madison and Maddison, 2009; Midford et al., 2003).

3. Results

3.1. Environmental temperature trends at each locality

Maximum temperature records through time at highland localities showed no significant trend to increase (regression analyses $F_{1, 37} = 0.391$; $R^2 = 0.0105$; $P = 0.536$ and $F_{1, 8} = 3.758$; $R^2 = 0.320$; $P = 0.089$ for Las Minas and Mitla respectively). In contrast, two of three lowland localities (<1000m), exhibited a significant increasing trend of $T_{\max}$ (regression analyses $F_{1, 27} = 48.181$; $R^2 = 0.641$; $P = <0.001$ for Chamela and $F_{1, 61} = 4.487$; $R^2 = 0.0685$; $P = 0.038$ for San Mateo). Current $T_{\max}$ at these localities approach maximum deleterious thermal thresholds for developing offspring (34°C). Temperatures at Xalitla, a lowland locality, showed no significant trends (regression analyses $F_{1, 27} = 0.233$; $R^2 = 0.0.008$; $P = 0.633$), but maximum environmental temperature at this locality was significantly higher than the maximum temperature observed in other four localities (one way ANOVA test $F_{4, 165} = 524.8$; $P = <0.001$; Tukey test $P = < 0.05$). Historical records showed that maximum temperature at this site exceeds 35°C (see Fig. 1A).

3.2. Nest temperatures and relationship with environmental and developmental temperatures

Highland localities showed environmental and nest temperatures below the maximum thermal threshold tolerated by developing embryos (34°C). Temperatures of sunny exposed nests were significantly higher than those recorded for nests under shaded conditions (nested ANOVA test $F_{1, 132} = 112.21$; $P = <0.001$ for Las Minas and $F_{1, 132} = 79.93$; $P = <0.001$ for Mitla). Additionally, nest, temperatures did not differ among depths under either sun or shaded conditions (nested ANOVA test $F_{4, 132} = 0.2538$; $P = 0.9069$ and $F_{4, 132} = 0.6714$; $P = 0.6129$, Las Minas and Mitla respectively). On the other hand, environmental and nest temperatures from lowland localities, Chamela and San Mateo, approach the 34°C threshold and did not differ between sun exposed and shaded nests (nested ANOVA test $F_{1, 132} = 2.6846$; $P = 0.1037$ and $F_{1, 132} = 0.1846$; $P = 0.6680$, respectively), or among different depths (nested ANOVA test $F_{4, 132} = 0.2764$; $P = 0.\ 8927$; for Chamela, $F_{4, 132} = 0.0563$; $P = 0.\ 9933$ for San Mateo). In contrast, nest temperature under sun exposed condition, exceeded the maximum thermal embryo’s threshold at Xalitla in all depths with no statistical differences among them (nested ANOVA test, $F_{4, 132} = 0.3336$; $P = 0.8549$); nevertheless, temperature of these nest were significantly higher than recorded inside nests built under shaded nest (nested ANOVA test, $F_{1, 132} = 66.135$; $P < 0.001$; Table 1 and Fig. 1).

Phylogenetically controlled analyses detect a significant positive relationship between T_{ned} (temperature tolerated by embryos) and T_{env}, $T_{\mathrm{esn6.4}}$, $T_{\mathrm{esn8.4}}$, and $T_{\mathrm{esn10.4}}$ available at each locality (Table.3). Gravid females of *S. s. spinosus* exhibited T_{ned} significantly lower than other species (one way ANOVA, $F_{4, 66}$ =16.94; P < 0.001; Tukey test <0.05). Regression models indicated significant relationships between T_{env} and nest temperatures at two depths for sunny and shaded conditions ($T_{esn6.4}$ and $T_{shn4.7}$) for Las Minas, Mitla, Chamela, and San Mateo localities; similar relationship was observed in Xalitla, but only between T_{env} and $T_{esn6.4}$ (for statistics see Table 2).

3.3. Available nest temperatures under different scenarios of temperature rise

We only used the regressions between $T_{ens6.4}$ and T_{env}, substituting maximum temperature increases predicted for each locality for 2030 and 2050 for T_{env} (2.5°C) in linear regression models, to evaluate effects on nest temperature at each studied locality, because the resulting estimated temperatures of the most shallow nests exposed to full sun at most localities would not exceed the thermal threshold for embryo development during the oviposition period, except for Xalitla, which is currently already over the thermal threshold; then, nests from this locality would experience deleterious temperatures under any scenario (Fig. 2).

4. Discussion

Worldwide climate forecasts anticipate that magnitude of temperature increases expected in tropical regions will be lower than those in temperate zones (IPCC, 2007; Williams et al., 2007). However, impact on biotas may prove be stronger at tropics, because organisms there are already close to maximum thermal threshold (Deutch et al., 2008; Huey et al., 2013). Therefore, even moderate increases in environmental temperatures may drive many species to extinction of their wild populations (Huey and Tewksbury, 2009). For instance, Sinervo et al. (2010) evaluated effects of temperature increases on population viability of *Sceloropus* species in Mexico. Results based on models that included time activity restrictions of lizards suggested that viviparous and high elevation species are under greater extinction risk. We have explored the potential effect of temperature change on a group of *Sceloporus* lizard species, but from a different perspective. We evaluated environmental temperature trends and their likely effects on available temperatures inside potential lizard nests, at different nest depths, microhabitats and localities, for oviparous species belonging to the same lineage. We interpreted these results and proposed hypothesis of how would lizards cope temperature rise of these potential nests sites.

Our results suggest that potential environmental temperature increase at different sites where *Sceloporus spinosus* species occur will not threat population persistence at most localities evaluated, at least from nesting thermal conditions perspective. At high elevation localities, those above 1000 m (Las Minas and Mitla), recorded temperature s even shallowest sun exposed nests (6.4 cm depth) were 21.7 and 25.2, respectively; hence, a forecasted increase between 1° and 2.5°C will not take those nests at temperatures even close to the thermal threshold of 34°C. Besides, higher incubation temperature (≥ 25°C) in other *Sceloporus* species, (*Sceloporus undulatus* and *S. virgatus*), accelerates embryo development, so young hatch during high productivity season and then accumulate fats and grow fast, which might has a positive effect on its survival through dry season contributing to increase population density (Qualls and Andrews, 1999; Chamaillé-Jammes et al., 2006; Parker and Andrews, 2007; Buckley et al., 2008). Physiological effects of nesting temperature increase, even below thermal threshold, should be evaluated for *S. spinosus* species group, to suggest stronger hypothesis about possible effects of temperature change on wild population persistence.

In contrast to highland environments, developing embryos from lowland *Sceloporus* populations (<1000 m) already experience temperatures close to the thermal threshold thought incubation period. Indeed, those potential nests located under sun exposed conditions at Xalitla could be presently facing temperatures above the maximum deleterious thermal threshold. Lizards at this locality would have four different alternative strategies to mitigate negative effects of potentially high incubation temperatures: 1) a physiological adjustment of embryonic development at high temperatures, 2) egg retention to protect embryos from lethal temperatures through female thermoregulation, 3) modification of timing of oviposition, and 4) behavioral strategies to select nesting sites exposed to lower temperatures (shaded sites).

The significant positive relationship of $T_{n\mathrm{ed}}$ (temperatures of gravid females before oviposition) with environmental and nest temperatures and significant lower $T_{n\mathrm{ed}}$ recorded for *S. s spinosus* suggest that embryos could develop normally within a wide range of temperatures currently available in sun exposed nest (21.7-35.8°C), which change as a function of environmental temperatures, as in other *Sceloporus* species (Andrews and Schwarzkopf, 2012). However, it is unknown whether normal embryonic development could be occurring at temperatures above 34°C (Beuchat and Ellner, 1987; Mathies and Andrews, 1997), as those experienced inside potential sunny nesting sites in Xalitla. It is, we do not know if this physiological adjustment to high incubation temperatures has evolved in these species.

Egg retention, another possible strategy to compensate high deleterious developing temperature has been recorded in females of this species group (Calderón-Espinosa et al., 2006). However, females that retain eggs belonged to populations where environmental and nest temperatures were below and above the thermal limit, according to this study. Then, egg retention is not exclusive of females from localities with potential deleterious nesting temperatures and then, could have evolved in response to a different factor.

Oviposition timing in lizards is known to be adjusted to environmental conditions (Telemeco et al., 2009). All females used to record body temperatures were caught gravid in pre ovipositon stage through July – August months, when this phase cycle is known to occurs (Valdéz-

González and Ramírez-Baustista, 2002), so females from this species group have not modify their oviposition timing as a response of potentially high nesting temperatures.

Therefore, we suggest that females inhabiting places with potentially warm (>34°C) nesting sites as Xalitla, could have developed behavioral strategies to cope with deleterious effects of high incubation temperatures. Lizards are known to exhibit fast variation in their behavioral strategies (Leal and Powell, 2011). Besides, *Sceloporus* species are known to have great skill to find sites with appropriate thermal conditions that not exceed thermal threshold for developing offspring (Warner and Andrews, 2002; Warner and Shine, 2008; Anguilleta et al., 2009). Variation in female behavioral strategies during gravidity and oviposition could not only prevent embryos exposition to deleterious temperatures but guarantee thermal shelter for adults (Kearney et al., 2009). Previous studies on the nesting behavior in oviparous lizards showed that lizards at lower elevations, where temperature is higher, build deeper nests (Doody, 2009). Our results suggest that this would not be an optimal strategy for *Spinosus* lizards, since temperature did not change among nest depths evaluated, even under increasing temperature scenarios. Besides, Campbell and Norman (1998) observed that a significant thermal buffering occurs at 30-50 cm from nest surface. Thus, it would be impossible for small lizard females such as those of the *Spinosus* group to dig such a deep nest.

Then, we suggest that an optimal behavioral strategy of female lizards to mitigate potential negative effects of high nesting temperature, as those historically and currently experienced in Xalitla locality, would be to select shaded nesting sites, as those covered by vegetation. Nesting behavior at this locality is then an interesting aspect to evaluate to test our hypothesis about strategies implemented by female lizards that face high temperature nesting conditions.

Our study evaluated a key aspect in the persistence of wild lizard´s populations under temperature rise scenarios, and then, provide hypothesis about how female of oviparous lizards could handle potentially high thermal embryo developmental conditions. Then, it would be interesting to integrate this information in the evaluation of extinction risk of lizard´s populations, which would provide more realistic models for oviparous lizard´s species.

Acknowledgments

We thank to C. Benítez, C. Enríquez, E. Venegas, A. H. Escobedo and B. Arroyo for their support during field work season. Dr. Andrew Townsend Peterson for his thorough review of an early draft of the manuscript. Data set for species localities were provide by Museum of Comparative Zoology, Harvard University, University of Colorado Museum of Natural History, Museo de Zoología "Alfonso L. Herrera" Facultad de Ciencias (MZFC) UNAM, Colección Nacional de Anfibios y reptiles (CNAR) del Instituto de Biología UNAM and Comisión Nacional Para el Conocimiento y Uso de la Biodiversidad (CONABIO). We also thank to the Posgrado en Ciencias Biológicas, UNAM, for logistic support to S. L. A. during his graduate studies. Funding was provided by the Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPIIT) of the Universidad Nacional Autónoma de México (Project number: IN221208) and the Consejo Nacional para la Ciencia y la Tecnología (CONACYT), who grant a doctoral scholarship to S. L. A. Permits for field studies were issued by the Secretaria de Medio Ambiente y Recursos Naturales (SEMARNAT).

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Table 1. Mean body temperature, environmental temperature, inside nest temperature at different depths inside full sun and shade exposed conditions recorded for five localities in Mexico inhabit by *Sceloporus spinosus* group lizards.

Species	**Locality**	**T_{ned} $\tilde{X}$ ±SD (min-max)**	**T_{env} $\tilde{X}$ ±SD (min-max)**	**$T_{esn6.4}$ $\tilde{X}$ ±SD (min-max)**	**$T_{esn8.4}$ $\tilde{X}$ ±SD (min-max)**	**$T_{esn10.4}$ $\tilde{X}$ ±SD (min-max)**	**$T_{shn4.7}$ $\tilde{X}$ ±SD (min-max)**	**$T_{shn6.7}$ $\tilde{X}$ ±SD (min-max)**	**$T_{shn8.7}$ $\tilde{X}$ ±SD (min-max)**
S. h. albiventris	Chamela	33.4 ± 1.91	31.8 ± 0.68	28.35 ± 0.48	28.3 ± 0.78	28.1 ± 1.12	28.1 ± 0.61	28.0 0.99 ±	27.9 0.89 ±
N= 15	11 m	(27.5-35.5)	(30.5-32.8)	(24.5-29.5)	(27.2-29.6)	(26.6-30.2)	(27.3-28.9)	(26.7-29.8)	(26.7-29.1)
S. s. spinosus	Las Minas	27.9 ± 3.52	26.9 ± 0.40	21.7 ± 3.22	21.6 ± 3.54	21.2 ± 3.16	17.1 ± 1.73	16.9 ± 1.48	16.67 ± 1.13
N= 7	2399 m	(22.0-31.5)	(26.5-27.5)	(15.5-25.0)	(15.4-25.1)	(16.0-24.6)	(14.4-22.1)	(14.22-18.23)	(14.86-17.80)
S. s.apicalis	Mitla	32.3 ± 0.96	27.5 ± 3.19	25.2 ± 3.68	25.2 ± 2.85	24.9 ± 3.22	21.9 ± 1.39	21.3 ± 1.01	20.8 ± 1.16
N= 18	1682 m	(31.0-33.8)	(23.0-32.8)	(18.5-29.3)	(20.6-28.3)	(19.9-28.7)	(19.4-23.4)	(19.4-22.5)	(19.0-22.9)
S. edwartaylori	San Mateo	33.2 ± 1.06	32.1 ± 0.85	29.9 ± 3.05	29.8 ± 2.84	29.6 ± 3.16	29.7 ± 3.03	29.5 ± 3.27	29.5 ± 3.26
N= 11	0 m	(31.0-34.5)	(31.0-34.5)	(26.0-34.3)	(26.2-33.6)	(25.0-33.7)	(26.3-34.2)	(26.3-34.6)	(26.1-34.8)
S. horridus	Xalitla	33.7 ± 1.28	36.8 ± 1.97	35.8 ± 6.19	35.3 ± 6.18	34.4 ± 5.28	29.2 ± 1.27	29.0 ± 2.4	29.0 ± 2.52
N= 25	588 m	(31.2-36.0)	(33.6-40.1)	(26.3-43.2)	(26.5-42.2)	(27.1-40.3)	(27.2-30.8)	(25.7-32.0)	(25.9-32.5)

Table 2. Relationships for environmental temperature and available temperature inside nest at different depths ($T_{esn6.4}$ and $T_{shn4.7}$); degrees of freedom are the same for all analyses.

Locality	T_{env} vs $T_{esn6.4}$			T_{env} vs $T_{shn4.7}$		
	$F_{1,21}$	R^2	P	$F_{1, 21}$	R^2	P
Las Minas	38.261	0.646	<0.001	13.436	0.390	0.001
Mitla	41.996	0.667	<0.001	15.964	0.432	<0.001
Chamela	24.08	0.534	<0.001	16.518	0.440	<0.001
San Mateo	25.864	0.552	<0.001	16.169	0.435	<0.001
Xalitla	47.745	0.695	<0.001	0.002	0.000	0.962

Table 3. Pearson correlation analysis after independent contrast (PIC'S), for normal embryo development temperature (T_{ned}) of lizards and different temperatures (T_{env}, $T_{\mathrm{ens6.4}}$, $T_{\mathrm{ens8.4}}$ and $T_{\mathrm{ens10.4}}$) available in the environment.

Pairwise correlations	**PIC**	
	R	***P***
T_{ned} vs T_{env}	0.8864	0.0186
T_{ned} vs $T_{\mathrm{esn6.4}}$	0.9425	0.0048
T_{ned} vs $T_{\mathrm{esn8.4}}$	0.9441	0.0045
T_{ned} vs $T_{\mathrm{esn10.4}}$	0.9479	0.0030

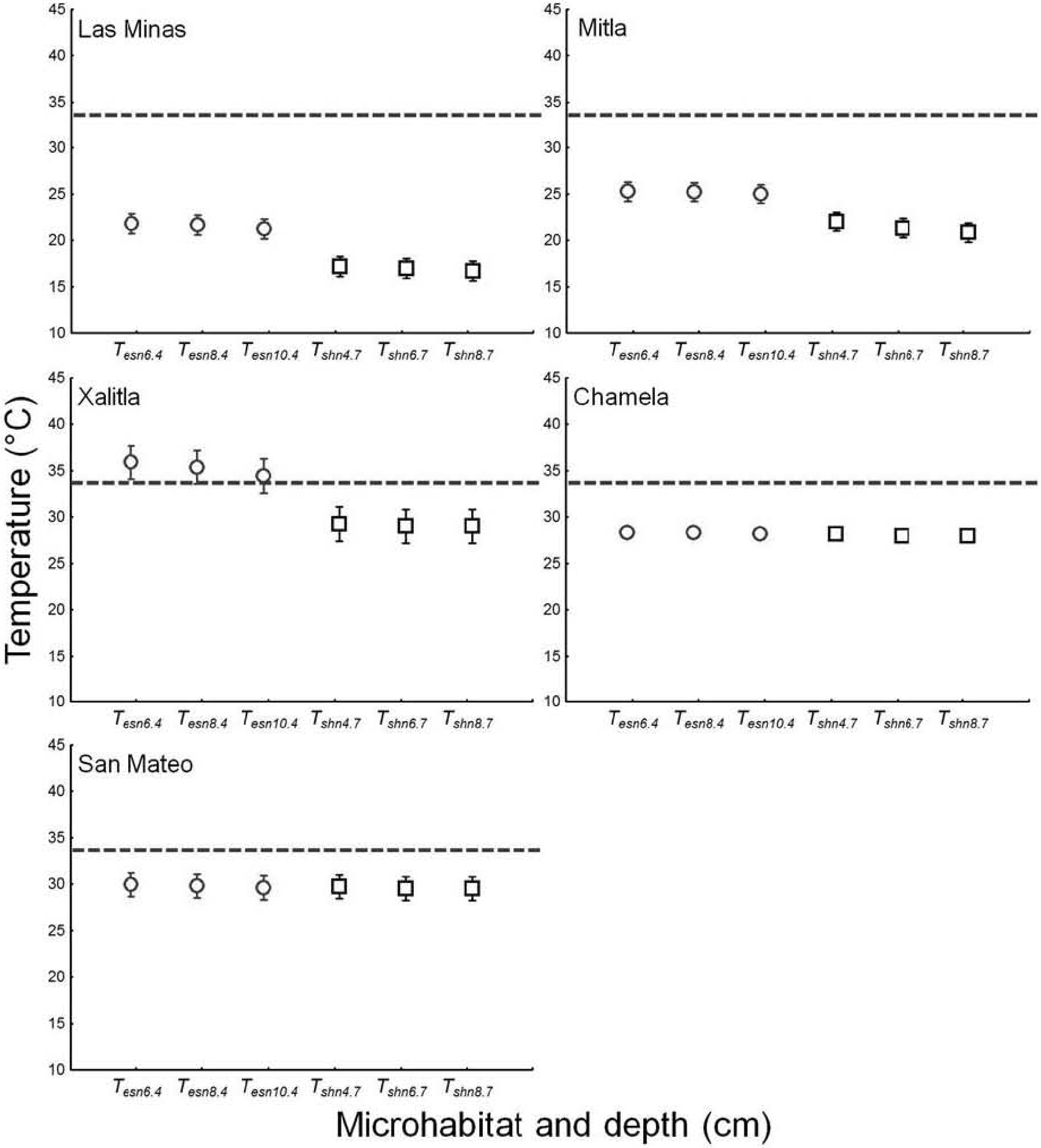
FIGURE CAPTIONS

Figure 1. Localities in México characterized in terms of available temperature inside artificial nest between microhabitats and between depths. Circles represent nests exposed to full sun; squares are nests under shade; the horizontal dash line represents the maximum threshold for embryonic development in *Sceloporus spinosus* lizards (34°C).

Figure 2. Inferences about available potential environmental temperatures in nest sites exposed to full sun at each study locality considering extreme and moderate climate change scenarios for 2030 and 2050 forecasted to México.

Figure 3. Uncorrected distances ad hoc tree (minimum evolution) used for the PICs constructed with 1858 mitochondrial characters of ND4 and Citb genes. Topology is consistent with hypothesis with more samples. Numbers at the left of nodes refers to the length of each branch. Numbers in italics at the right indicate the number of standardized independent contrasts.

Fig.1



Las Minas
Mitla
Xalitla
Chamela
San Mateo
45
40
35
30
25
20
15
10
$T_{esn6.4}$
$T_{esn8.4}$
$T_{esn10.4}$
$T_{shn4.7}$
$T_{shn6.7}$
$T_{shn8.7}$
Temperature (°C)
Microhabitat and depth (cm)

Fig. 2

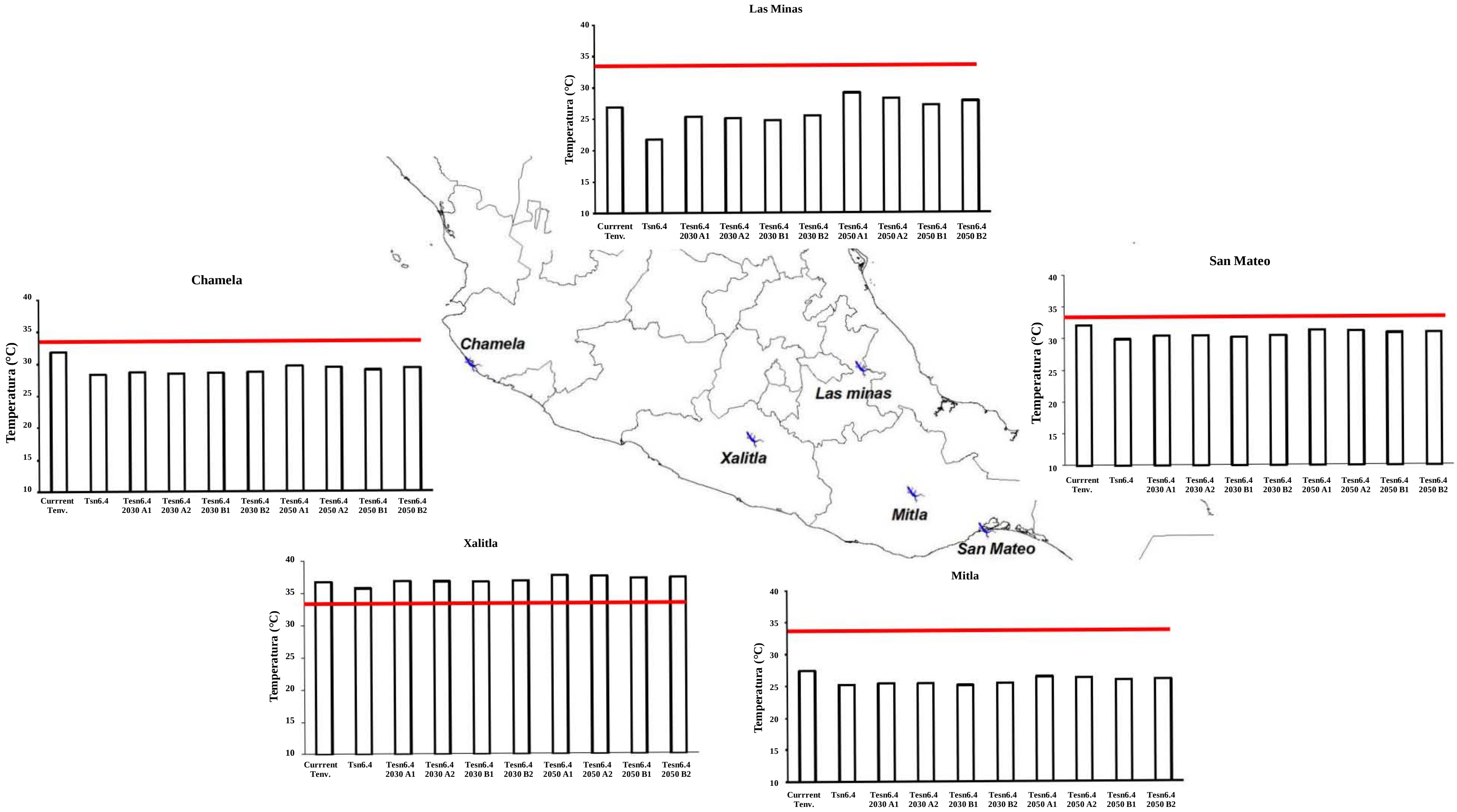

Las Minas
Chamela
San Mateo
Xalitla
Mitla
Las minas
Temperatura (°C)
10
15
20
25
30
35
40
Currrent Tenv.
Tsn6.4
Tesn6.4 2030 A1
Tesn6.4 2030 A2
Tesn6.4 2030 B1
Tesn6.4 2030 B2
Tesn6.4 2050 A1
Tesn6.4 2050 A2
Tesn6.4 2050 B1
Tesn6.4 2050 B2

Fig. 3.

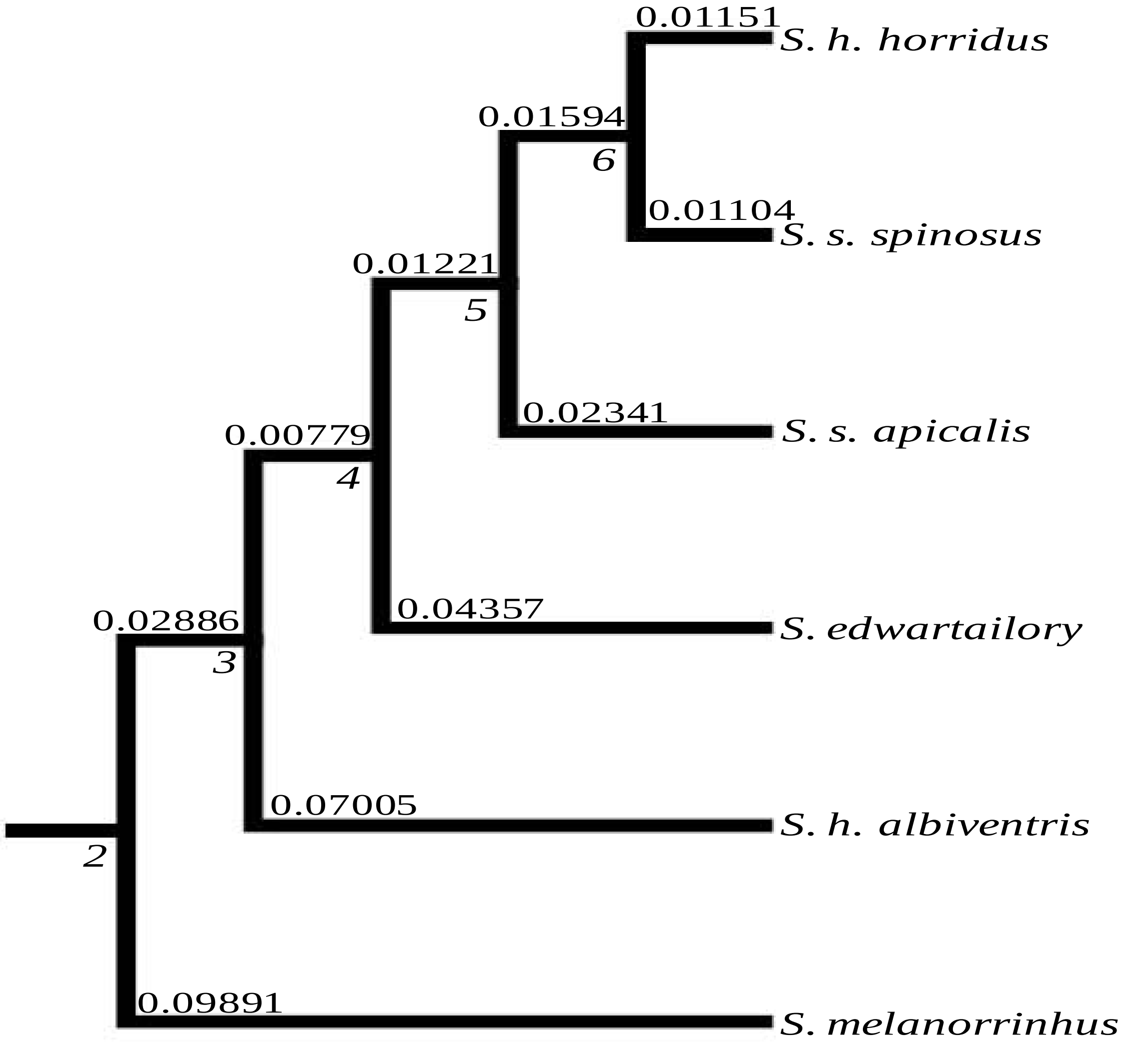
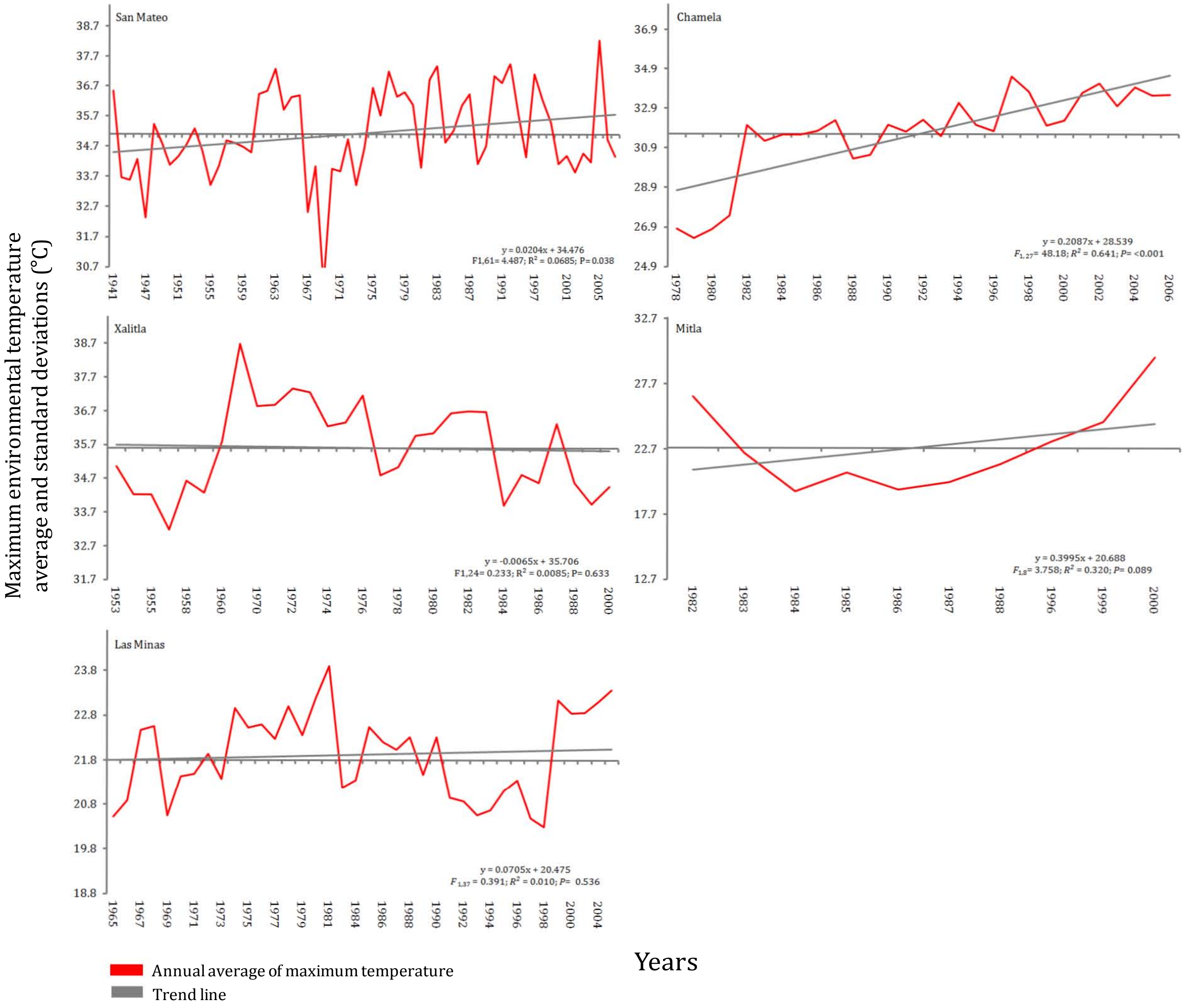

0.01151
S. h. horridus
0.01594
6
0.01104
S. s. spinosus
0.01221
5
0.02341
S. s. apicalis
0.00779
4
0.04357
S. edwartailory
0.02886
3
0.07005
S. h. albiventris
2
0.09891
S. melanorrinhus

Figure 1A. Historical trends of $T_{\max}$ to July-August period in localities inhabited by *S. spinosus* in México. San Mateo and Chamela localities showed significant changes in temperature trough time. Conversely, $T_{\max}$ in Xalitla, Mitla and Las Minas did not exhibit a significant temperature increase.



CAPITULO 4.

CAPITULO 4. ¿Lidiando con temoperaturas letales: ¿Que pueden decirnos las lagartijas del género *Sceloporus*?

Dealing with lethal temperatures: What *Sceloporus* lizards say?

Saúl López-Alcaide[1], Constantino Gonzáles-Salazar[1] & Enrique Martínez-Meyer[1]*

Summary

It has been suggested that contemporary temperature rise phenomenon might lead to the loss of several lizard species, especially in the tropics because even a minimum temperature increase would exceed their thermal threshold and consequently impacts their fitness and reduce populations' size. However, there are lowland tropical sites inhabited by species of *Sceloporus* lizards in which historic environmental maximum monthly temperatures have overpassed 34°C, which is the threshold for a healthy development of embryos. Here we investigated how these lizards have survived in environments with temperatures potentially lethal by means of: (1) analyzing the historic trend of maximum environmental temperatures (T_{env}), (2) recording the available temperature of sites of potential nesting for a oviparous species and microhabitats used by a viviparous species during their respective reproductive seasons, (3) monitoring temperatures in these sites throughout the day to detect the time periods when temperature overpassed the lethal threshold, (4) measuring field active body temperature of reproductive females for two species, and finally (5) we projected future environmental temperatures available in the localities inhabited by these species under two climate change scenarios. Our results indicated that in two localities T_{env} exceeded the threshold for developing embryos. Currently, available temperatures at the microhabitat level for viviparous lizards are lower in shaded areas (*Dsh*) than filtered sun (*FS*) and complete sun (*CS*). Also, these lizards can be exposed without risk of overheating for several hours a day in *Dsh* microhabitat. For oviparous species we found that temperatures in shaded nests were significantly lower and appropriate for developing embryos than sun-exposed nests at different depths (6.4 and 8.4 cm), where temperatures reached lethal. These data suggest that both species have available microhabitats and time periods to buffer the negative impact of high temperatures on themselves and their offspring. We conclude that behavioral strategies coupled with microhabitat heterogeneity allow adult lizards of the two species to display regular activity periods without risk of overheating and damage their embryos.

Introduction

In recent years, several studies have addressed the potential effects of the current episode of temperature increase (IPCC 2007; Williams et al. 2007) in the behavior, distribution, ecology, physiological performance, reproduction, and survival of ectothermal animals, such as insects and reptiles (Kearney et al, 2009; Huey & Tewksbury 2009). Some of these studies suggest that tropical species might be more vulnerable to higher temperatures because they have been historically exposed to thermal regimes closer to the maximum tolerated temperatures for adequate performance of key physiological traits (Deutch et al, 2008); therefore, even a small temperature increase would significantly reduce suitable daily time periods and sites to maintain regular activity for feeding and reproduction for several lizard species (Huey et al. 2009; Sinervo et al. 2010).

However, these studies represent generalizations of a global phenomenon that may manifest differently at local spatial and temporal scales (Le Treut et al. 2007), since local climate is largely determined by the local physiographic context (Wilbanks & Kates 1999). Therefore, it is important to gather data at the local scale to know the different strategies that species follow to deal with unfavorable environmental conditions. For example, developing embryos of genus *Sceloporus* lizards have a maximum thermal tolerance of 34 °C (Andrews & Rose 1994), when this threshold is exceeded embryos frequently exhibit physical abnormalities at birth that reduce their fitness and increase mortality (Beuchat 1986; Beuchat & Ellner 1987). Thus, under these circumstances, how do lizards have survived in environments with available temperatures potentially lethal?

To answer this question we explored historic records of maximum environmental temperature (T_{max}) for two localities with warm climate, each inhabited by an oviparous and a viviparous *Sceloporus* lizard species, respectively. Lizards of this genus are good models to study questions on thermoregulation strategies as they occupy a broad latitudinal and altitudinal range exposed to a great variety of environmental conditions exhibiting a diversity of ecological, ethological, morphological, and physiological adaptations (Sites et al. 1992). We also recorded temperatures at potential nest sites for oviparous species and in specific microhabitat occupied by viviparous species, available temperatures throughout the day, and field active body temperatures (T_b) of organisms with the aim to understanding the specific mechanisms that lizards use to cope with exceedingly high temperatures.

Methods

Lizard species and localities

We selected two *Sceloporus* lizard species with different reproductive mode that inhabit warm climate localities in the Balsas basin, in Guerrero state, in southern Mexico: the viviparous *S. stejnegeri* belonging to the *Sceloporus formosus* species group lives in Tierra Colorada, at 350 masl (type locality), and the oviparous *S. horridus* from the *Sceloporus spinosus* group in Xalitla, at 520m (Sites, et al 1992; Bell, et al 2003).

Environmental temperature analyses and forecast

We obtained maximum temperature (T_{max}) records from three nearby weather stations at each locality from the Comisión Nacional de Agua (CONAGUA) network, and built a database for the months corresponding to the reproductive period of each lizard species, namely October-March (Guillette & Sullivan, 1995), from 1961 to 2010 for *S. stejnegeri* at Tierra Colorada, and March-September from 1953 to 2000 (Valdéz-González & Ramírez-Baustista, 2002) for *S. horridus* at Xalitla, with the aim of knowing if lizards and have been exposed and for how long to environmental temperatures that exceed the thermal tolerance threshold of 34 ºC for embryonic development.

On the other hand, we used climatic forecasts based on four emission scenarios downscaled for México by the Centro de Ciencias de la Atmósfera (UNAM) (www.atmosfera.unam.mx), two drastic (A1and A2) and two moderates (B1 and B2), for years 2030 and 2050 based on three general circulation models: ECHAM5/MPI, UKHADGEM1 and GFDL CM 2.0 (Conde et al. 2008). We averaged the expected temperature rise of the three general circulation models for each scenario and estimated the expected environmental temperature during the reproductive season of species at each locality and compared to the historical environmental temperature data.

Fieldwork

We estimated the actual environmental temperature at which active lizards could be exposed, called operative temperature (T_e), in November-December (Guillette and Sullivan, 1985), in the habitats where pregnant *S. stejnegeri* lizards were found (Deep shade [*Dsh*], Filtered sun [*FS*] and complete sun [*CS*]), by randomly setting 10 hollow cooper models in each habitat. Cooper models were constructed according to the size and shape of lizards and calibrated with alive lizards by comparing model T_e against lizard T_b, plugged into an outdoor Hobo pendant® data logger throughout an external thermocouple to measure the range of available T_e that lizards potentially could used for thermoregulation (Hertz, el al. 1993; Dzialowski, 2005).

To measure temperature in potential nesting sites used by oviparous *Sceloporus horridus* near rivers with sandy soil, , we placed nine Hobo pendant® data loggers in same number of artificial nests at following depths under full sun: Three at 4.7 cm, Three at 6.4 cm (known nesting depths at shade an full sun, respectively) and three more at 8.4 cm under full sun microhabitats

(Warner & Andrews, 2002; Anguilletta et al 2009a) during July-August, when is the time that embryos remain in the nest (Valdéz-González & Ramírez-Baustista, 2002). We recorded available temperatures both at the microhabitat level and nesting sites every 30 min from 08:00-18:00 hrs for seven consecutive days and then obtained the average hourly temperature. This allowed us to know how much time during the day embryos in the nest of oviparous lizards could be exposed to high temperatures in sunny and shady sites, and also the time that viviparous pregnant lizards could be active without exposing themselves and their offspring to lethal temperature in each microhabitat. Finally we recorded field active body temperatures (T_b) of captured active females of both lizard species with a Miller & Webber cloacal thermometer and duration time (*DT*) at the three microhabitats for viviparous species. All these data were compared with a parametric or non-parametric ANOVA, depending on the fulfillment of assumptions.

Results

Environmental temperature analyses and projections

Historic records from nearby weather stations showed that maximum T_{env} has frequently overpassed the 34°C threshold in the last 50 years during the reproduction activity months for the two species. In Tierra Colorada, average T_{max} for October-March period was 34.4 °C ±0.23 (29.7-40.0), whereas in Xalitla was 37.2 °C ± 1.5 (32.2-44.9) in March-September. The historic T_{max} average of Tierra Colorada did not differ significantly from the predicted T_{max} for 2030 (X=34.1 ± 1.3, min = 32.1, max= 37.0) and 2050 (X=34.9 ± 1.2, min = 32.7, max= 37.6) (ANOVA $F_{2,\,400}$= 2.38; P=0.093). We found a similar pattern for Xalitla, where historic T_{max} average during the reproductive season of *S. horridum* was significant higher than predicted for 2030 (X̃ =34.1 ± 2.53, min = 29.6, max= 38.5 and 2050 (X̃ =34.8 ± 2.53, min = 30.3, max= 39.3) (ANOVA $F_{2,\,501}$= 51.0; P=<0.001; All Pairwise Multiple Tukey Comparison P>0.001) (Fig. 1).

Microhabitats and nests available temperatures

Data from cooper models and artificial nests between 08:00-18:00hrs in each locality revealed that at the present time T_e available at the microhabitat level for *S. stegnejeri* was significantly lower in *Dsh* than in *FS* and *CS* (Kuskal-Wallis test H_2= 9.961; *P*= 0.007, *n* = 33; Dunn's test all pair wise comparisons *P*<0.005). In the case of *S. horridum*, we found that temperatures in nests under shade at 4.7 cm were significantly lower than nests at full sun at 6.4 and 8.4 cm deep, with no significant differences between these latest two (Kuskal-Wallis test H_2= 7.374; P= 0.025, *n*= 33 Dunn's test all pair wise comparisons Q= 2.646; P<0.005 and Q= 0.794; P>0.005 respectively) (Fig. 2). Also, both active gravid *S. horridus* and pregnant *S. stegnejeri* lizards maintained T_b below deleterious threshold temperatures for embryos (table1). *S. stegnejeri* lizards showed a significant lower duration time (*DT*) in *CS* ($\tilde{X}$ = 8.5 ± 0.5; 7.2-9.0) and *FS* ($\tilde{X}$ =10.6 ± 0.9; 10.0-12.0) than in *Dsh* ($\tilde{X}$ =12.5 ± 0.4; 12.0-13.0) microhabitats (ANOVA: $F_{2,18}$= 58.78; *P*= <0.001; all pairwise Tukey test comparisons *P*=<0.05).

Finally, we found that during *S. stegnejeri* reproductive period, pregnant lizards remain active for several hours during the day in *Dsh* microhabitat, because T_e in these sites only exceeds the thermal threshold between 14:00-1600 hrs. On the other hand, developing embryos of *S. horridus* in the nest could be exposed to lethal temperatures (>34°C) in nests under full sun (6.4 and 8.4 cm), whereas in nests built in the shade temperatures did not exceed deleterious threshold (Fig. 2).

Discussion

Climate warming could cause different biological responses by ecthothermic tropical species to cope with high temperatures, driven by heritable genetic changes (Bradshaw & Holzapfel, 2006) or by phenotypic plasticity (Pigliucci et al, 2006). However, among the first and simplest response is to modify their thermoregulatory behavior to keep appropriate range body field active temperature (T_b) without significant variation (Huey et al. 2003). However, it has been suggested that thermoregulatory behavior can be used effectively only if tropical ecthoterms have extensive shade available to buffer the impact of high temperatures (Huey & Tewksbury, 2009; Kearney et al. 2009).

Our field data suggest that the two *Sceloporus* lizards species studied here have sufficient suitable microhabitats for thermoregulation or selection of nesting sites to avoid exposing themselves and their offspring to lethal temperatures. For *Sceloporus stegnejeri*, temperatures registered from biophysical models in *DSh* were significantly lower than in *FS* and *CS*, which allow that pregnant lizards to thermoregulate via shuttling between microhabitats, but spending longer *DT* in *Dsh* than in the other two microhabitats (Bauwens, et al. 1996; Kearney, 2009). Moreover, temperature in *Dsh* only exceeds the thermal threshold for two hours at midday during November and December, when first stages of gestation occurs (Guillette & Sullivan, 1985),

On the other hand, arboreal oviparous *S. horridus* gravid lizards have ample availability of soil under shade to build nests (Parker, et al 2005) and complex topography of these localities produces that available canopy provides high potential solar radiation interception during driest months (Galicia et al. 1999). Our results showed that the available temperatures in artificial nests under full sun at different depths (6.4 and 8.4 cm.) were significantly higher and exceeded the maximum threshold tolerated by offspring than nest temperatures under vegetal cover. Therefore, it is probable that gravid *S. horridus* lizards buffer the negative impact of high environmental temperatures on their offspring by building their nests under shade rather than digging deeper nests under full sun (Warner and Shine, 2008; Anguilleta et al 2009b). Also, projections for 2030 and 2050 indicated that temperature would not see a significant rise neither in Tierra Colorada,nor in Xalitla, on the contrary, a reduction was observed (Fig.1).

In conclusion, the mosaic of shade and sun in the two localities seem to be the possible reason that have allowed *S. stegnejeri* and *S. horridus* lizards' populations to remain active most of the day and survive in sites where historical environmental temperature has overpassed the thermal threshold for appropriate embryo development, avoiding an energetic shortfall as a consequence of low food intake, as suggested for some extinct *Sceloporus* populations (Sinervo et al. 2010).

Tables

Table 1. Field body, microhabitat and nesting sites temperatures at different depths for viviparous *S. stegnejeri* and oviparous *S. horridus* lizards during their respective reproductive seasons.

	T_b ($\tilde{X}$) ±SD (min-max)	***TSh/$T_{shn4.7}$ ($\tilde{X}$)±SD (min-max)***	***FS/$T_{sn8.4}$ ($\tilde{X}$) ±SD (min-max)***	***CS/$T_{sn6.4}$ ($\tilde{X}$) ±SD (min-max)***
S. stegnejeri	31.4 ± .97 (30.6-32.5)	31.9 ±2.8 (26.7-34.6)	33.6±3.41 (26.9-37.5)	36.3 ±3.23 (29.4-40.4)
S. horridus	33.2±1.22 (31.2-36)	30.22±0.62 (29.0-30.7)	35.46±4.85 (28.5-40.9)	36.70±5.46 (29.3-42.9)

Figure legends

Figure 1. Historic mean monthly maximum environmental temperatures and forecast for 2030 and 2050 for Tierra Colorada for *S. stegenejri* and Xalitla for *S. horridus*, in Guerrero, Mexico during their reproductive seasons.

Figure 2. Available temperature in different microhabitats and times of the day for *Sceloporus stegenejeri* lizards in Tierra Colorada, Guerrero, and temperature at different depths and sun conditions for *Sceloporus horridus* lizards in Xalitla, Guerrero, during their respective reproductive seasons.

Figure 1.

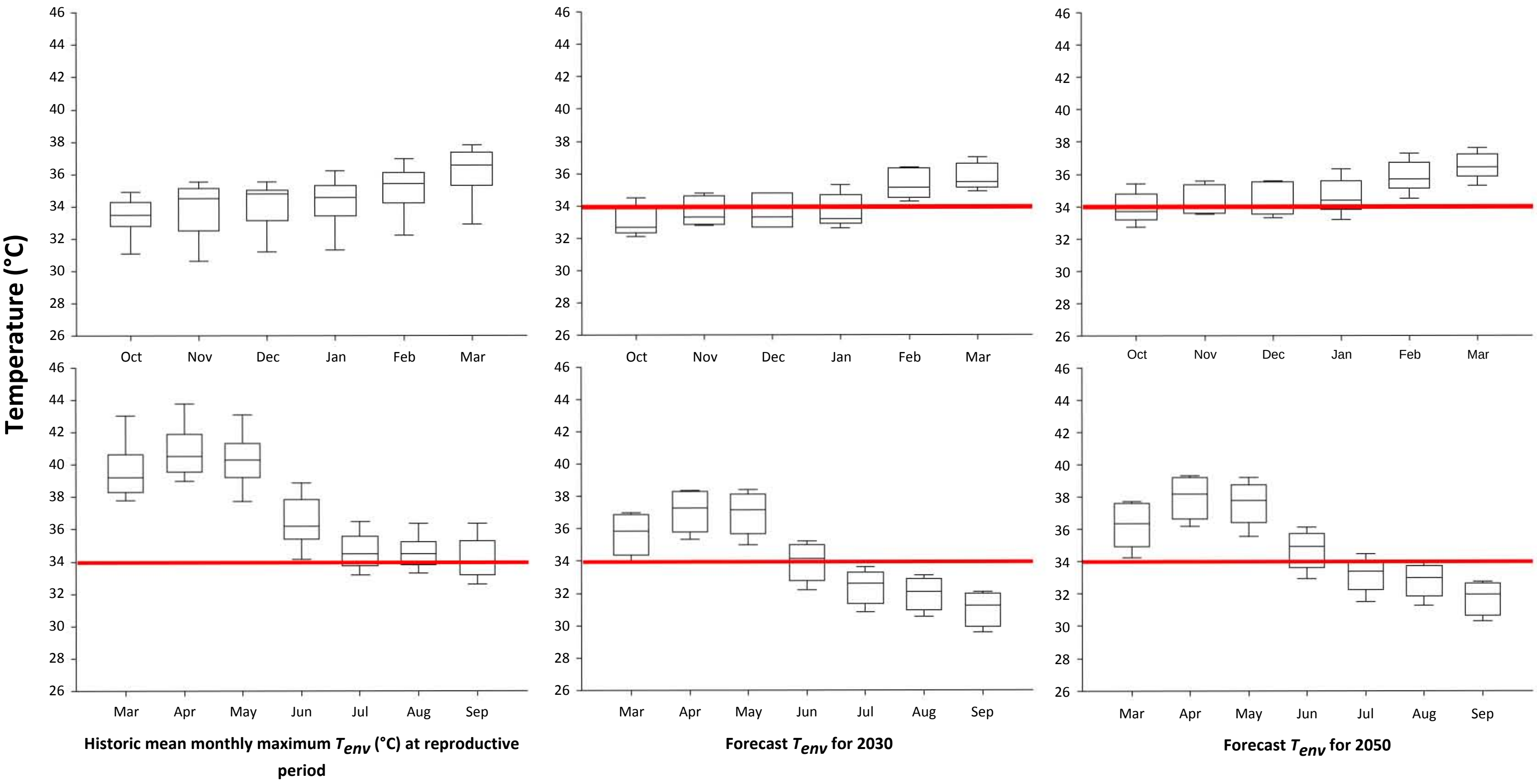
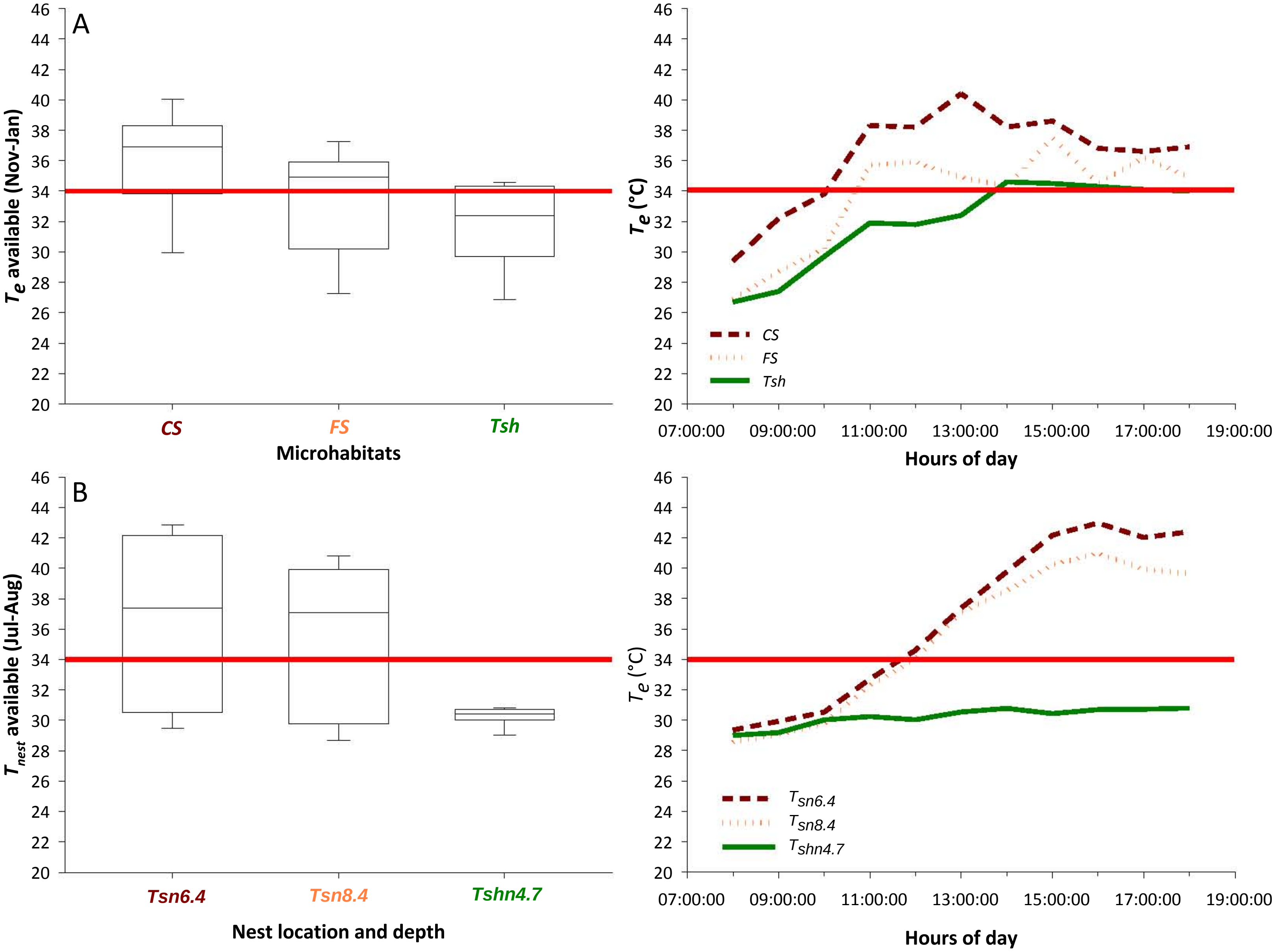

Temperature (°C)
46
44
42
40
38
36
34
32
30
28
26
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Historic mean monthly maximum T_{env} (°C) at reproductive period
Forecast T_{env} for 2030
Forecast T_{env} for 2050

Figure 2.


A
T_e available (Nov-Jan)
46
44
42
40
38
36
34
32
30
28
26
24
22
20
CS
FS
Tsh
Microhabitats
T_e (°C)
CS
FS
Tsh
07:00:00
09:00:00
11:00:00
13:00:00
15:00:00
17:00:00
19:00:00
Hours of day
B
T_{nest} available (Jul-Aug)
Tsn6.4
Tsn8.4
Tshn4.7
Nest location and depth
T_e (°C)
$T_{sn6.4}$
$T_{sn8.4}$
$T_{shn4.7}$
Hours of day

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DISCUSIÓN Y CONCLUSIONES GENERALES

Actualmente el clima del planeta está cambiando aceleradamente -en una escala de décadas-; este fenómeno se manifiesta de diferentes maneras y no sólo con promedios anuales más altos, sino también con la ocurrencia más frecuente de eventos extremos de calor y cambios en los regímenes estacionales térmicos y en los patrones locales de precipitación, que influyen en diversos aspectos de la biología y ecología de las especies, como la fenología (Menzel et al., 2006) e interacciones (Parmesan, 2006) que impactan su demografía (Chevin et al., 2010) y distribución geográfica (Peterson et al., 2002; Parra-Olea et al., 2005; Araújo et al., 2006). Estos impactos pueden ser particularmente severos para aquellas especies que no pueden desplazarse a sitios más favorables en el corto plazo debido a restricciones geográficas y/o a que su capacidad para movilizarse es reducida, así que deben enfrentar la variación ambiental en los sitios que habitan por medio de diversas estrategias conductuales y fisiológicas (Angilletta y Sears, 2011).

En el caso de animales ectotermos, estudios recientes han resaltado la necesidad de explorar si sus umbrales de tolerancia térmica podrían evolucionar, lo que les permitiría adaptarse a temperaturas ambientales elevadas o si, por otra parte, los cambios en su comportamiento termorregulador contribuyen a amortiguar los efectos negativos de éstas y por lo tanto les facilitarían mantener temperaturas corporales adecuadas (Huey y Tewksbury, 2009). En este estudio se aporta evidencia empírica sobre la capacidad de las especies de dos grupos de lagartijas del género *Sceloporus* con diferente modo de paridad para mantenerse a sí mismas y a su descendencia dentro del intervalo de temperaturas adecuadas para el sano desarrollo embrionario, a través de exhibir diferentes estrategias de comportamiento termorregulador y de anidación.

En el capítulo 1, el análisis desde un enfoque filogenético del comportamiento termorregulador, la temperatura corporal y la caracterización del ambiente térmico disponible en diferentes microhábitats para diez especies del grupo de lagartijas vivíparas del grupo *S. formosus* durante su periodo reproductor, sugiere que estas lagartijas modulan su comportamiento termorregulador dependiendo de las condiciones térmicas disponibles a través de cambios simples en rasgos como la selección del microhábitat, el tiempo que permanecen asoleándose y horario de actividad, lo que les permite amortiguar los efectos negativos del estrés térmico y ajustarse a condiciones climáticas adversas. Los resultados sugieren que las lagartijas del grupo *S. formosus* pueden utilizar las estrategias de comportamiento termorregulador contenidas dentro de su repertorio genético dependiendo de las características térmicas disponibles en sus localidades. Por ejemplo, las hembras gestantes de *Sceloporus stegnejeri*, que habitan zonas bajas y calientes, explotan la heterogeneidad térmica ambiental moviéndose constantemente entre sitios expuestos a pleno sol y con sombra, permaneciendo poco tiempo expuestas a temperaturas deletéreas. Esta estrategia les permite mantener periodos de actividad diaria extensos y al mismo tiempo evita el sobrecalentamiento y la exposición de su descendencia en desarrollo a temperaturas deletéreas, aún en sitios y horarios en donde la carga térmica ambiental rebasa sus umbrales máximos de tolerancia fisiológica. De esta forma no tienen que refugiarse bajo tierra la mayor parte del día, lo que reduciría su tiempo de forrajeo ocasionándoles un déficit energético a causa de la imposibilidad para adquirir alimentos (Sinervo et al., 2010).

En otro caso, *S. adleri*, que habita zonas de gran altitud con clima predominantemente frío, exhibe una estrategia termorreguladora diferente al seleccionar preferentemente microhábitats expuestos a sol pleno, permanecer periodos más extensos en ellos y refugiarse cuando la temperatura ambiental disminuye. Bajo tratamientos de temperatura controlada en laboratorio, el tiempo de asoleo entre tratamientos varió significativamente, siendo más extenso en los tratamientos con temperatura moderada y menor en temperaturas elevadas, sin detectarse diferencias significativas en su temperatura corporal (T_b). Esto representa evidencia sobre la flexibilidad del comportamiento termorregulador por parte de las hembras gestantes para conseguir y mantener la temperatura corporal adecuada para su desempeño fisiológico y el desarrollo de su descendencia en diferentes condiciones ambientales.

Sin embargo, dicha flexibilidad del comportamiento termorregulador puede favorecer el conservadurismo en rasgos fisiológicos, como lo indica el hecho de que la temperatura corporal registrada no varíe significativamente entre las diez especies estudiadas en respuesta a la temperatura disponible en las diferentes localidades. Lo anterior sugiere que no existe algún cambio regulado genéticamente en el umbral máximo de temperatura tolerado en estas lagartijas, por lo que si la temperatura ambiental continúa incrementándose en las próximas décadas hasta un nivel en el que los cambios en el comportamiento termorregulador sean insuficientes para enfrentarlo -como se cree que puede suceder especialmente en sitios ubicados en latitudes tropicales con baja altitud (Deutch et al., 2008)- y si las especies del grupo *S. formosus* que habitan dichos sitios no poseen la suficiente variabilidad genética para producir fenotipos con tolerancia a temperaturas más altas sobre los cuales actué la selección natural, éstas podrían extinguirse (Chevin, 2010).

Para el grupo de lagartijas ovíparas *S. spinosus*, el análisis y pronóstico de las temperaturas disponibles en los sitios de anidación en distintos microhábitats y profundidades en localidades distribuidas a lo largo de un gradiente latitudinal, considerando escenarios de calentamiento drásticos y moderados, reveló que actualmente y aún con un incremento drástico de la temperatura ambiental, la temperatura disponible dentro de los nidos expuestos a sol pleno no alcanzaría niveles deletéreos para los embriones. No solo eso; actualmente los nidos construidos en zonas templadas de gran elevación presentan temperaturas subóptimas para su crecimiento y desarrollo (< 28°C), por lo que incluso un incremento de la temperatura ambiental en estos sitios podría producir mejores condiciones para éstos. En caso de que la temperatura en los nidos expuestos a sol pleno superara el límite térmico para los embriones en desarrollo de las hembras ovíparas de zonas altas, éstas podrían responder modificando su comportamiento de anidación y construir sus nidos en microhábitats bajo la sombra producida por la cubierta vegetal.

Por otra parte, para las especies ovíparas que habitan zonas bajas, si la temperatura disponible en los nidos superara el límite térmico de los embriones, la estrategia para evitar el impacto de temperaturas letales sobre su descendencia no sería la de cavar nidos más profundos en sitios expuestos a sol pleno, sino construir sus nidos en sitios bajo la sombra de la cubierta vegetal para amortiguar el impacto de la temperatura máxima ambiental. Además, según los registros de las estaciones climatológicas cercanas a las localidades de baja altitud donde habitan lagartijas ovíparas (*S. horridus*) y vivíparas (*S. stegnejeri*), éstas han estado expuestas durante sus respectivos periodos reproductores a temperaturas ambientales que superan el umbral de tolerancia para el desarrollo de su descendencia al menos durante los últimos cincuenta años. Posiblemente la estrategia más simple que ha permitido a estas especies persistir ha sido la modificación descrita en el comportamiento termorregulador o de anidación, según el caso, que les permiten explotar la heterogeneidad térmica del hábitat. De esta forma los adultos pueden mantenerse activos por varias horas al día sin riesgo de sobrecalentarse y dañar a su descendencia, o encontrar lugares adecuados para anidar en el caso de las especies ovíparas. Sin embargo, los análisis filogenéticos sugieren que en las lagartijas ovíparas el intervalo de temperatura soportada por los embriones en desarrollo puede ser un rasgo flexible y constituir una vía de adaptación a las temperaturas elevadas en estos organismos.

Los resultados obtenidos en este estudio sugieren que las especies ovíparas y vivíparas de lagartijas del género *Sceloporus* tienen la capacidad para modular tanto su comportamiento termorregulador como de anidación, lo cual les permite amortiguar diferentes condiciones térmicas en los sitios que habitan que podrían perjudicar a su descendencia en desarrollo y a ellas mismas, aprovechando la heterogeneidad térmica del ambiente. Por lo tanto, es posible suponer que podrían persistir en un mundo con temperaturas ambientales más elevadas siempre y cuando los sitios que habitan les ofrezcan un mosaico de espacios soleados y con sombra (Huey y Tewksbury, 2009).

Sin embargo, las estrategias de comportamiento podrían tener una efectividad limitada si el incremento de la temperatura ambiental es excesivo. Por ello, es necesario explorar el potencial genético de rasgos fisiológicos (variabilidad, heredabilidad y plasticidad) que permitan a las especies adaptarse al cambio (Huey et al., 2013). Entonces, resultaría interesante integrar toda esta información para evaluar el riesgo de extinción de las lagartijas y proporcionar modelos más reales para estas especies. Este estudio representa un avance en la comprensión de las estrategias de comportamiento que exhiben los organismos ectotermos para enfrentar el estrés térmico, las cuales probablemente son las primeras en expresarse para responder al cambio climático.

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