



UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS
FACULTAD DE ESTUDIOS SUPERIORES IZTACALA
ECOLOGÍA

ECOLOGÍA TÉRMICA DE *CROTALUS POLYSTICTUS* (SERPENTES:
VIPERIDAE) EN UNA POBLACIÓN DEL CENTRO DE MÉXICO

TESIS

(POR ARTÍCULO CIENTÍFICO)

The effect of thermal gradient design on the evaluation of
thermoregulation in snakes

QUE PARA OPTAR POR EL GRADO DE:

MAESTRO EN CIENCIAS BIOLÓGICAS

PRESENTA:

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Los Reyes Iztacala, Tlalnepantla, Estado de México Enero, 2019



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M. en C. Ivonne Ramírez Wence
Directora General de Administración Escolar, UNAM
Presente

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día **15 de octubre de 2018** se aprobó el siguiente jurado para el examen de grado de **MAESTRO EN CIENCIAS BIOLÓGICAS** en el campo de conocimiento de **Ecología y Manejo Integral de Ecosistemas** del alumno **FIGUEROA HUITRON RICARDO** con número de cuenta **308050376** por la modalidad de graduación de **tesis por artículo científico**, en el campo de conocimiento de **Ecología**, con la tesis titulada: **"The effect of thermal gradient design on the evaluation of thermoregulation in snakes"**, producto del proyecto realizado en la maestría que lleva por título **"Ecología térmica de *Crotalus polystictus* (Serpentes: Viperidae) en una población del centro de México"**, realizado bajo la dirección del **DR. HIBRAIM ADÁN PÉREZ MENDOZA** quedando integrado de la siguiente manera:

Presidente: **DR. JOSÉ JAIME ZUÑIGA VEGA**
Vocal: **DR. ANÍBAL HELIOS DÍAZ DE LA VEGA PÉREZ**
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Suplente: **DR. ÓSCAR ALBERTO FLORES VILLELA**
Suplente: **DRA. LETICIA MARGARITA OCHOA OCHOA**

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

ATENTAMENTE
"POR MI RAZA HABLARA EL ESPÍRITU"
Ciudad Universitaria, Cd. Mx., a 26 de noviembre de 2018


DR. ADOLFO GERARDO NAVARRO SIGÜENZA
COORDINADOR DEL PROGRAMA



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A MI papá Ricardo

ἰ ἔ ἔῃῃ ἔῃῃ ἰῃῃ

A MI mamá María Elena

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A MI HERMANA ELEONORA

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Resumen

La termorregulación es uno de los aspectos más importantes de la biología de los ectotermos, dado que la mayoría de los procesos fisiológicos se llevan a cabo dentro de un intervalo estrecho de temperaturas. Los reptiles han desarrollado diferentes mecanismos para regular su temperatura, aunque la estrategia particular utilizada por cada especie está relacionada con la calidad térmica del ambiente y la relación costo-beneficio de la termorregulación. El protocolo propuesto por Hertz et al. en 1993 ha permitido caracterizar la termorregulación de muchos ectotermos pequeños, sin embargo, su adaptabilidad es limitada. Una de las variables requeridas es el intervalo de temperaturas seleccionadas (T_{sel}), que se calcula monitoreando la temperatura corporal de los individuos dentro de un gradiente térmico. El protocolo menciona que este intervalo se debe calcular en condiciones controladas, sin embargo, esto no representa las condiciones ambientales que los individuos experimentan, lo que podría llevarlos a elegir temperaturas subóptimas. El objetivo de este trabajo fue analizar la estrategia y eficiencia de la termorregulación de *Crotalus polystictus* mediante el protocolo de Hertz et al., contrastando los resultados obtenidos con un gradiente térmico en laboratorio (GL) y un gradiente en semicautiverio (GSC). El trabajo se realizó en San Bartolo Morelos, una localidad del Estado de México. El intervalo intercuartil de T_{sel} obtenido con el GL fue mucho mayor (29 – 34.3 °C) que el calculado con el GSC (22.5 – 30.9 °C). La precisión en la termorregulación (d_b) y la calidad térmica del ambiente (d_e) fue menor en el GL, mientras que la eficiencia de la termorregulación (E) fue mayor en el GSC. Los intervalos de T_{sel} obtenidos son más amplios en comparación con los obtenidos estudiando otras serpientes, lo que sugiere que *C. polystictus* es euritérmica en esta localidad. El índice de Blouin-Demers y Weatherhead fue casi idéntico entre los dos gradientes. Al haber seleccionado temperaturas más altas en el GL, los resultados derivados de éste indican que *C. polystictus* es un termorregulador ineficaz y relativamente pasivo. En contraste, el GSC indica que es un termorregulador activo y eficaz. El GL puede representar un ambiente más estresante para las serpientes, y es probable que las serpientes hayan preferido mayores temperaturas en favor de tácticas antidepredatorias a costa de una termorregulación eficaz. Un gradiente térmico que se parezca más al hábitat original puede reducir el estrés asociado al cautiverio y ofrecer una mayor variabilidad térmica. Estas medidas permiten que las serpientes termorregulen de manera tan precisa como

lo hacen en su hábitat, lo que genera que los estimados de T_p obtenidos sean más robustos.

Abstract

Thermoregulation is one of the most important aspects of reptile biology, given that most of the physiological processes take place within a narrow range of body temperatures. Reptiles use a variety of mechanisms to regulate their temperature although the particular strategy used by each species is related to the thermal quality of the environment and the cost-benefit relation of thermoregulation. The protocol proposed by Hertz et al. in 1993 has allowed the characterization of many small ectotherms' thermoregulation, however, its adaptability is limited. One of the required variables is the selected temperature range (T_{sel}), which is calculated by monitoring the individuals' body temperature in a thermal gradient. The protocol states that the range should be calculated under controlled conditions, however, this does not represent the environmental conditions that the individuals experience, which could lead them to select suboptimal temperatures. The objective of this work was to analyse the strategy and thermoregulation efficiency of *Crotalus polystictus* using the Hertz et al. protocol contrasting the results obtained with a laboratory thermal gradient (LG) and a semi-captivity gradient (SCG). The research was conducted in San Bartolo Morelos, a locality in Estado de México. The interquartile range of T_{sel} from the LG was much higher (29 – 34.3 °C) than that from the SCG (22.5-30.9 °C). Accuracy of thermoregulation (db) and thermal quality of the environment (de) from the LG were consistently higher than those from the SCG. However, the efficiency of thermoregulation (E) was higher when calculated from the SCG. T_p estimates were wider than most that have been obtained from other snake species, suggesting that *C. polystictus* is eurythermic. The Blowin Demers and Weatherhead index was nearly identical in both gradients. Because the snakes chose higher temperatures in the LG, the results from that gradient indicated that *C. polystictus* is an inaccurate and inefficient thermoregulator. In contrast, the SCG suggested that it is a highly accurate and active thermoregulator. the LG could represent a stressful environment for snakes, and, as a consequence, they might select higher temperatures to increase anti-predatory performance at the expense of less efficient thermoregulation. Generally, a thermal gradient that more accurately replicates the

natural habitat of snake species should reduce stress and thereby result in more robust estimates of thermoregulatory variables.

Introducción

La termorregulación es uno de los aspectos más importantes de la biología de los organismos ectotermos. La temperatura corporal de los individuos determina en gran medida cómo se llevan a cabo tanto procesos fisiológicos como patrones conductuales de actividad (Lelièvre et al., 2013; Shine et al., 2000). La mayoría de los procesos fisiológicos se llevan a cabo dentro de un intervalo estrecho de temperaturas, por lo que es importante para los reptiles mantener su temperatura corporal en o cerca del óptimo fisiológico (Blouin-Demers & Weatherhead, 2001; Brown & Weatherhead, 2000; Harvey & Weatherhead, 2011).

Los reptiles han desarrollado diferentes mecanismos para regular su temperatura, y las dos estrategias principales son el termoconformismo y la termorregulación activa (Molina & Leynaud, 2017; Navarro-García et al., 2008). La estrategia particular utilizada por cada especie está relacionada con la calidad térmica del ambiente y la relación costo-beneficio de la termorregulación, que son factores inherentes a cada localidad (Herczeg et al., 2007). En el termoconformismo la temperatura corporal de los individuos no está regulada por patrones conductuales, sino que está determinada principalmente por la temperatura ambiental (Molina & Leynaud, 2017; Woolrich-Piña et al., 2006). En ambientes tropicales con poca variación de temperaturas, el termoconformismo se ve favorecido (Shine & Madsen, 1996). La termorregulación activa, por otro lado, implica un ajuste conductual en el que los individuos modifican su posición dentro del hábitat para maximizar la ganancia o pérdida de calor (Navarro-García et al., 2008; Vitt & Caldwell, 2014; Woolrich-Piña et al., 2006).

La termorregulación activa es la estrategia más comúnmente empleada por los reptiles; sin embargo, ésta normalmente implica una elevada exposición a la luz solar en espacios abiertos, lo que también puede tener a su vez un efecto negativo en la supervivencia al aumentar el riesgo de depredación (Lelièvre et al., 2013; Webb & Whiting, 2005). Una manera común para evitar la depredación es la retirada hacia refugios; sin embargo, la elección de refugios también puede estar mediada por preferencias térmicas (Amo et al., 2004). Muchos reptiles escamados utilizan refugios que presentan variabilidad

térmica, lo que les ayuda a regular su temperatura (Cox et al., 2018). De esta manera, podemos notar que las estrategias termorregulatorias que presentan las especies pueden ser complejas, ya que en la mayoría de los casos existe una disyuntiva (“trade-off”) importante entre los costos y beneficios de la termorregulación (Huey et al., 1989; Huey & Slatkin, 1976).

El protocolo estandarizado más utilizado actualmente para analizar las estrategias térmicas de ectotermos pequeños y qué tan eficientes son para mantener su temperatura dentro del óptimo fisiológico es el propuesto por Hertz et al. (1993). Este protocolo ha permitido la caracterización de la termorregulación en diversas especies de lagartijas (Díaz De La Vega-Pérez et al., 2013; Lara-Reséndiz et al., 2015; Navarro-García et al., 2008; Sartorius et al., 2002), sin embargo, su aplicabilidad es limitada, excluyendo animales grandes (Seebacher & Shine, 2004). El protocolo utiliza tres variables principales: temperaturas corporales (T_c), temperaturas operativas ambientales (T_o) y temperaturas seleccionadas por los individuos en un gradiente térmico (T_{sel}). El intervalo de T_{sel} representa las temperaturas que los individuos intentan alcanzar en la naturaleza (Hertz et al., 1993), y es una variable central para calcular los índices de calidad térmica del ambiente (d_e), precisión en la termorregulación (d_b) y eficiencia térmica de la especie (E). El valor de E puede tomar cualquier valor entre 0 y 1, siendo estos dos extremos la representación de un individuo completamente termoconformista y un individuo que solamente utiliza la termorregulación activa. Sin embargo, Blouin-Demers y Weatherhead (2001) señalan las desventajas que puede tener este índice debido a ser una proporción, por lo que proponen simplemente usar la diferencia entre d_e y d_b como un índice de la magnitud en la que los individuos se alejan del termoconformismo.

El protocolo menciona que la estimación de T_{sel} se debe realizar en condiciones controladas, en las que ningún factor pueda limitar la termorregulación. Sin embargo, la mayoría de los hábitats presentan características que pueden evitar que los individuos termorregulen de manera efectiva, tales como la estructura de la vegetación, la calidad térmica del hábitat y las interacciones bióticas, particularmente, la depredación (Vitt & Caldwell, 2014; Weatherhead et al., 2012). Un gradiente térmico en condiciones de laboratorio no replica completamente estas condiciones ambientales (Hertz et al.,

1993), además de que las condiciones de cautiverio generan estrés en los animales (Dayger et al., 2013; Dickens et al., 2010). La respuesta conductual ante el estrés y la implementación de conductas antidepredatorias puede propiciar que los individuos no se comporten de manera natural y, por ende, seleccionen temperaturas subóptimas. Este problema se acentúa al trabajar con serpientes, que comúnmente sufren de prolongados picos de estrés en cautiverio (Sparkman et al., 2014). En su hábitat natural, las serpientes pueden pasar gran parte del día bajo refugios (Fitzgerald et al., 2003), especialmente si su estrategia de forrajeo es la emboscada, como es el caso de diversas víboras de cascabel (Clark, 2004; Reinert et al., 1984). Debido a estas razones, es necesario interpretar con precaución los estimados de T_p obtenidos con un gradiente en condiciones de laboratorio, siendo preferible utilizar un gradiente térmico que considere los aspectos ecológicos y conductuales de la especie para estimar el intervalo de T_p de una serpiente.

Crotalus polystictus es una serpiente de tamaño mediano, perteneciente a la familia Viperidae, endémica del centro de México (Mociño-Deloya et al., 2009; Setser et al., 2010). Se distribuye a lo largo del sur del altiplano mexicano y la sierra madre occidental; y habita generalmente en pastizales húmedos y subhúmedos, matorrales o praderas entre los 1450 y los 2739 m de altitud (Meik et al., 2012b; Mociño-Deloya et al., 2009). El sur del altiplano mexicano es una de las zonas de país con mayor cambio de uso de suelo para la agricultura y asentamientos humanos; por lo que la distribución de la especie ha sido fragmentada y algunas de sus poblaciones están en declive (Meik et al., 2012a). Esta especie está comúnmente asociada a áreas rocosas en las que puede utilizar madrigueras de mamíferos y rocas como refugios o como sitios de emboscada para cazar (Campbell & Lamar, 2004; Meik et al., 2012a). Las características de este tipo de microhábitats posiblemente afectan el comportamiento termorregulatorio de *C. polystictus*, y deben ser consideradas a la hora de evaluar la eficiencia en la termorregulación de la especie. El objetivo de este trabajo fue analizar la estrategia y eficiencia de la termorregulación de *Crotalus polystictus* mediante el protocolo de Hertz, contrastando los resultados obtenidos con un gradiente térmico en laboratorio (GL) y un gradiente en semicautiverio (GSC).

Artículo sometido

The effect of thermal gradient design on the evaluation of thermoregulation in snakes

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Key words: behaviour, *Crotalus polystictus*, preferred temperatures, snakes, refuges, thermal efficiency

Abstract

Hertz et al. designed what is now the most widely used protocol to analyse the thermal strategies and efficiency of small squamates. Preferred temperature range (T_p) is one of the most important variables required for determining the thermal efficiency index, and is calculated by monitoring the body temperature of the individuals in an enclosure containing a thermal gradient. Although thermoregulation studies of lizards have traditionally employed thermal gradients under laboratory conditions, this approach is not suitable for snakes given that such thermal gradients don't accurately represent their natural thermal environment and thus may result in snakes selecting suboptimal temperatures. Here, we compare the results of this thermal efficiency protocol using a laboratory thermal gradient (LG) and a semi-captivity thermal gradient (SCG) in the rattlesnake *Crotalus polystictus*. T_p from the LG was much higher (29 – 34.3 °C) than that from the SCG (22.5-30.9 °C). Values for the accuracy of thermoregulation (d_b) and thermal quality of the environment (d_e) indices from the LG were consistently higher than those from the SCG. However, the efficiency of thermoregulation (E) was higher when calculated from the SCG. T_p estimates were wider than most that have been obtained from other snake species, suggesting that *C. polystictus* is eurythermic. The Blowin Demers and Weatherhead index was nearly identical in both gradients. Because the snakes chose higher temperatures in the LG, the results from that gradient indicated that *C. polystictus* is an inaccurate and inefficient thermoregulator. In contrast, the SCG suggested that it is a highly accurate and active thermoregulator. We suggest that the LG could represent a stressful environment for snakes, and, as a consequence, they might select higher temperatures to increase anti-predatory performance at the expense of less efficient thermoregulation. Generally, a thermal gradient that more accurately

replicates the natural habitat of snake species should reduce stress and thereby result in more robust estimates of thermoregulatory variables.

Introduction

In non-avian reptiles, behaviour and functioning of physiological processes depend mainly on temperature (Webb & Whiting, 2005). Thus, it is critically important for reptiles to maintain their body temperature (T_b) at or near their physiological optimum (Blouin-Demers & Weatherhead, 2001; Brown & Weatherhead, 2000; Harvey & Weatherhead, 2011). To achieve that, reptiles use a variety of thermal strategies, ranging from passive thermoconformity to stenothermy (Fitzgerald et al., 2003; Molina & Leynaud, 2017; Shine & Madsen, 1996). Behavioural thermoregulation, wherein individuals modify their position in the environment to maximize heat gain or loss, is the most frequent thermoregulatory strategy used by reptiles to regulate their T_b (Navarro-García et al., 2008; Vitt & Caldwell, 2014; Woolrich-Piña et al., 2006). Hertz et al. (1993) designed what is now the most widely used protocol to analyse the thermal strategies of small ectotherms and how efficient they are in maintaining their temperature within the optimal range (Lara-Reséndiz et al., 2015). This protocol requires three basic sets of data: operative environmental temperatures (T_e), the organisms' preferred temperature range (T_p) and *in situ* active T_b . By comparing the T_p range with both T_b and T_e , researchers can calculate indices of accuracy of thermoregulation (d_b), thermal quality of the environment (d_e) and efficiency of thermoregulation (E) (Hertz et al., 1993).

T_p is the range of body temperatures that ectotherms prefer for optimal behavioural and physiological performance. T_p is calculated by monitoring the body temperature of individuals in an enclosure containing a thermal gradient. The assumption is that,

without biological interactions and environmental constraints, individuals will select temperatures most suitable for them. It is important to note that the Hertz et al. (1993) protocol states that T_p is calculated under laboratory conditions, where no factor could limit thermoregulation. However, most habitats possess a variety of features that could limit the ability of individuals to thermoregulate effectively, including habitat structure (e.g. presence of rocks, logs, and vegetation), the thermal quality of the environment (Weatherhead et al., 2012), and biological interactions (Vitt & Caldwell, 2014). As Hertz et al. (1993) mention, a laboratory setting for the thermal gradient will never perfectly replicate the natural environment of a species.

Researchers should be cautious when interpreting the biological significance of T_p estimates obtained using a laboratory thermal gradient, as individuals might behave unnaturally in such artificial settings. This problem is even more salient in snakes, as they commonly experience prolonged bouts of stress in captivity (Sparkman et al., 2014) and, in their natural habitats, can often spend substantial lengths of time within refuges or retreat sites (Fitzgerald et al., 2003). When basking, some rattlesnakes use ground cover to avoid detection from predators or potential threats (Shoemaker & Gibbs, 2010). Additionally, reptiles can bask in open areas for other reasons besides thermoregulation, such as increase exposure to UV radiation to stimulate vitamin D₃ production (Seebacher & Franklin, 2005) or to induce immune responses (Iglesias-Carrasco et al., 2016). For these reasons, we propose that a thermal gradient used to obtain the T_p of a snake species should consider its ecological and behavioural tendencies.

Here, we describe and test a new approach to estimate the thermal efficiency of snakes using a semi-captivity thermal gradient (SCG) and compare its results with those obtained using a traditional laboratory thermal gradient (LG). We estimated thermal

efficiency in the rattlesnake *Crotalus polystictus*, a species that is commonly associated with rocky areas and uses mammal burrows beneath them as shelters (Campbell & Lamar, 2004; Meik, Setser, Mociño-Deloya, & Lawing, 2012). As these microhabitat associations likely affect the species thermoregulatory behaviour, we expect estimates of thermal efficiency to differ depending on the type of thermal gradient used.

Materials and methods

Species and study site

Crotalus polystictus is a medium sized viperid endemic to central Mexico (Mociño-Deloya et al., 2009; Setser et al., 2010). Individuals are commonly between 50 and 60 cm in total length, but some large individuals can reach 80 cm. Their dark spotted colour pattern over a pale brown or yellow base colour is unique among rattlesnakes (Campbell & Lamar, 2004). It inhabits dry and humid grasslands, pine-oak forests and scrublands of the Mexican Plateau between 1450 and 2739 m (Meik et al., 2012). They can be active at both day and night on some localities (Campbell & Lamar, 2004), but it is very unlikely to find them active at night on the highest locations of its altitudinal distribution, like our study site. However, the activity pattern of this species has not yet been formally described.

The study was conducted on San Bartolo Morelos, located in the municipality of Morelos, north of Estado de Mexico (19.77 N, 99.65 W). This area is composed of croplands surrounded by natural and induced grasslands and oak forest patches at 2660 meters above sea level. The weather is temperate sub-humid with summer rains (INEGI, 2009). Rocky areas between croplands provide suitable and numerous shelters for rattlesnakes.

Fieldwork

We sampled the locality 13 times between March 2016 and July 2018. Each visit was performed by 3-4 people during three days. Active searches for rattlesnakes ran from 0900 to 1900. Within the first 30 seconds after the capture, we measured the T_b with a digital thermometer (Fluke model 51-II) whose sensor was inserted 1cm into the cloaca. We also measured the substrate temperature (T_s) and the air temperature (T_a) 10 cm above the substrate of the capture site. We recorded time of capture, mass (g), snout-vent length (SVL, mm), sex and geographic coordinates (Garmin Etrex GPS).

Operative environmental temperatures (T_e)

We used biophysical models to determine the range of potential body temperatures available to snakes in the absence of thermoregulatory behaviour (Bakken, 1992; Hertz et al., 1993; Peterson, Gibson, & Dorcas, 1993). These models were green coloured cylindrical hollow polyvinylchloride (PVC), filled with tile adhesive and sealed with tape, with similar shape and size (50 cm long x 2 cm wide) to *C. polystictus*. The PVC models were previously calibrated under field conditions during the activity period of the snake following the method of Adolph (1990), Díaz De La Vega-Pérez, Jiménez-Arcos, Manríquez-Morán and Méndez-De La Cruz (2013), Lara-Reséndiz, Jezkova, Rosen and Méndez-De La Cruz (2014) and Sinervo et al. (2010). Our models accurately mimic the gain and lose of heat by *C. polystictus* in our study site ($r^2 > 0.95$, $P < 0.01$). We deployed two biophysical models at the study site under shaded and sunlit conditions. The models were connected to a data-logger sensor (HOBO® Pro V2) which was set to record T_e every 15 minutes from June 2017 to April 2018. For the thermoregulation analyses we selected only the T_e data corresponding to the activity period of the snakes based on our *in-situ* observations (09:00 – 18:30).

Preferred temperature range (T_p)

The LG was built in three adjacent 120 x 40 x 40 cm crystal enclosures, each containing a layer of approximately 2 cm of potting soil (Figure 1). Using cardboard barriers, we divided each tank into four racks, each 10 cm wide, to prevent behavioural interactions or competition for a particular basking site. At one end we hanged six heat lamps at 55 cm above the soil and placed three heat mats under the enclosures. At the other end of the tanks, on the outside, we placed 15 frozen refrigerant gel packs, which we replaced every four hours during the experiments. These measures generated a thermal gradient from ~ 15 °C to 45 °C. The gradient was set up at 0730 and all organisms were acclimated for 1.5 hours prior to the first temperature measurement. Preferred body temperatures were recorded every two hours from 0900 to 1900. We used an infrared thermometer (Fluke 561) to take temperature recordings to minimize time, handling and, therefore, rattlesnakes' stress during the experiment. Unpublished data from a similar study with *Crotalus triseriatus* showed no significant difference between the temperature lectures made with the Fluke 51-II and Fluke 561 thermometers. Every measurement was made in the middle region of the body, in the shade, and at a distance of 15 cm away from the snake. Each day of the LG test, we moved the snakes to a different rack and cleaned the tanks superficially to minimize the possible effect of olfactory signals.

To make the SCG, we built a 3 m diameter circular enclosure bounded by polycarbonate plates (Figure 1). The enclosure was placed approximately 4 km away from the study site, on a flat, uncovered area. The enclosure was prepared with multiple rocks of similar size and shape than those found on the snakes' natural habitat. The rocks functioned as refuges in which the rattlesnakes could thermoregulate as normally as possible, without most of the stress associated with captivity in a LG. We placed six heat lamps on one extreme of the gradient, at approximately 100 cm from the ground,

which also was uncovered from sunlight. We covered the other extreme of the gradient to provide shade during the whole experiment. The conditions described above generated a thermal gradient from ~ 23 °C to ~ 45 °C. Just like with the LG, rattlesnakes were subjected to an acclimation period prior to the test. We conducted five different temperature preference trials on May, June, August, and September 2017, and April 2018; each one was performed with an average of 13 rattlesnakes. T_p were recorded using the same device and time intervals as described for the LG.

Thermoregulation indices and statistical analyses.

We calculated indices for thermal quality of the environment (d_e) and accuracy of thermoregulation (d_b) (Hertz et al., 1993). Both d_e and d_b are the mean of all individual deviations between T_e and T_b and the interquartile range of T_p , respectively. Deviations were calculated as follows: if T_e or $T_b > T_p$ interquartile range, then $d_b = T_b - T_p$ and $d_e = T_e - T_p$. If T_e or $T_b < T_p$, then $d_b = T_p - T_b$ and $d_e = T_p - T_e$. If T_e or T_b lie within the interquartile range of T_p , then both d_b and d_e are equal to zero. d_b and d_e values equal to or similar to zero represent high thermoregulatory accuracy and ideal thermal environments for the organisms. High values of the indices indicate low thermoregulatory accuracy and environments with low thermal quality.

We then calculated the Hertz et al. (1993) index for efficiency of thermoregulation: $E = 1 - (\bar{d}_b/\bar{d}_e)$. An E value close to one indicates that environmental temperatures available for the individuals don't match their physiological requirements, and therefore active thermoregulation is necessary to achieve optimal temperatures. In contrast, an E value close to zero indicates that the organisms find themselves in an ideal thermal environment and therefore must resort to thermoconformity. We also calculated the index developed by Blouin-Demers and Weatherhead (2001), which represents the

effectiveness of thermoregulation and the deviation from thermoconformity. This index is expressed directly in °C and is calculated simply by subtracting d_b from d_e .

We used Spearman correlations to test whether T_s and T_a are correlated with T_b . We tested for a difference between male and female T_b using a student's t -test. We used a Mann-Whitney's test to analyse differences between male and female T_p within each gradient and differences in overall T_p between gradients. We tested normality and homoscedasticity with Shapiro-Wilk and Bartlett tests, respectively. All analyses were made in the software R, version 3.3.2 (R Core Team, 2018).

Results

During the sampling period, we captured and recorded T_b for 93 females and 36 males. The activity period of *C. polystictus* was between 0900 and 1830; the highest peak of activity was between 1000 and 1200. We detected a second lower activity peak from 1600 to 1800. Mean T_b , T_a and T_s are presented in Table 1. We found no significant differences between male and female T_b 's ($t = -1.446$, $P = 0.152$). T_b was significantly related with T_a ($\rho = 0.218$, $P < 0.05$) and T_s ($\rho = 0.584$, $P < 0.05$). T_p did not differ significantly between sexes neither in the LG ($W = 11266$, $P = 0.192$) nor in the SCG ($W = 9848$, $P = 0.265$). Nevertheless, overall T_p differed significantly between gradients ($W = 77830$, $P < 0.001$). Below we present the results from each thermal gradient type separately.

T_p and thermoregulatory indices derived from the laboratory gradient

We conducted five temperature preference trials with 12 rattlesnakes (nine females and three males). The snakes were captured between April 20-22, 2018 and five trials were made daily from May 1-5, 2018. Mean T_p and the interquartile range of T_p are presented in Table 1. Only 13% of field T_b fell within this range, while 87% were below it and

none above it. The overlap of T_e with the T_p interquartile range was minimal (3%) while 86% of T_e fell below it and 11% above it (Figure 2). Both d_b and d_e were high values, indicating a low accuracy of thermoregulation and a habitat of thermal quality (Table 1). The efficiency index from Blouin-Demers and Weatherhead suggests that individuals are active thermoregulators. The index E , on the other hand, suggests that individuals have a tendency towards thermoconformism.

T_p and thermoregulatory indices derived from the semi-captivity gradient

Mean T_p and the interquartile range of T_p are presented in Table 1. A total of 52% of field body temperatures fell within this range, 43% below it and only 5% above it. Only 12% of T_e fell within the T_p interquartile range, while 13% fell above it and 75% below it. (Figure 2). The d_b indicates relatively accurate thermoregulation, while the d_e suggests that the habitat is of low thermal quality habitat, but considerably more benevolent than that estimated from the LG (Table 1). E was considerably higher than that calculated from the LG, indicating a preference for active thermoregulation, which is consistent with the value of the Blouin-Demers and Weatherhead index.

Discussion

Due to the manifold biological implications of T_b for reptiles, robust estimates of this physiological trait are critically important for ecological and evolutionary studies.

Although T_a and T_s have been widely proposed to provide possible predictors of T_b , some researchers have found them of little use (Seigel & Collins, 1993). We found a positive relationship between T_b and both T_a and T_s , but the relationship was stronger with T_s , suggesting that temperature regulation is tigmothermal.

To accurately describe the thermal ecology of a species, we need to go beyond estimating T_b and its predictors or determinant factors. Analysis of T_p is crucial for

understanding the implications of variation in T_b and how efficiently a species can thermoregulate. Therefore, a robust estimation of T_p is necessary for analysing the thermal ecology of a species. Our findings illustrate that the outcome of the thermal indices derived from the Hertz et al. (1993) protocol critically depends on the accurate estimation of T_p .

C. polystictus chose a lower T_p interquartile range in the SCG than in the LG. Indeed, the upper limit of the SCG's range (30.9 °C) barely exceeds the lower limit of LG's range (29 °C). The range obtained from the SCG was 8.4 °C, the widest interquartile range yet to be reported among thermal studies of snakes (Blouin-Demers & Weatherhead, 2001; Brown & Weatherhead, 2000; Fitzgerald et al., 2003; Harvey & Weatherhead, 2011). The T_p range calculated from the LG was considerably lower (5.6 °C) but was still wider than most T_p 's estimated from other snake species. These findings indicate that *Crotalus polystictus* is eurythermic at our study site. Eurythermy is commonly favoured when the energetic costs of thermoregulation are high and the individuals live in thermally variable climates (Powell & Russell, 1985).

The operative environmental temperatures obtained with the biophysical models are indeed highly variable compared to T_b in our study site. Our estimates of the thermal quality of the environment are low, specially the one obtained from the results of the LG (Table 1). Thus, the evidence indicates that San Bartolo Morelos does not provide a thermally ideal habitat for *C. polystictus*.

The differences between both gradients are even more evident based on the estimates of the accuracy and efficiency of thermoregulation. Based on the results of the LG, *C. polystictus* is considered an inaccurate and inactive thermoregulator ($d_b = 5.84$ °C, $E = 0.42$). In contrast, according to the data obtained from the SCG, *C. polystictus* is considered a highly accurate and active thermoregulator ($d_b = 1.63$ °C, $E = 0.72$).

Interestingly, estimates of the Blouin-Demers and Weatherhead index are nearly identical based on the data obtained from both gradients, where the snakes have to compensate 4.2 °C to reach T_p . The scale of the d_b and the d_e differs between gradients, despite the actual difference between both indexes within each gradient being minimal. This finding indicates that there is a preference for active thermoregulation over thermoconformity by individuals in this population.

The important differences found in E are directly caused by the higher temperatures the snakes chose on the LG. However, stress might explain why individuals selected those high temperatures. Generally, reptiles often exhibit stress responses to handling and captivity (Franklin et al., 2003; Moore et al., 1991; Schuett et al., 2004). Many studies have confirmed that blood levels of corticosterone increase after capture and confinement in several snake species (Bailey et al., 2009; Dayger et al., 2013; Herr et al., 2017; Mathies et al., 2001; Schuett et al., 2004; Sykes & Klukowski, 2009).

Behavioural and anti-predator responses in reptiles depend on temperature (Mori & Burghardt, 2001). Snakes generally prefer escaping and showing more aggressive behaviours like striking when the temperatures are higher and motor abilities are optimal (Citadini & Navas, 2013; Llewelyn et al., 2010; Mori & Burghardt, 2001; Passek & Gillingham, 1997; Prior & Weatherhead, 1994; Schieffelin & De Queiroz, 1991). When cold, snakes rely more on crypsis given that motor capability in ectotherms decreases with lower temperatures (Brodie III & Russell, 1999; Keogh & DeSerto, 1994; Prior & Weatherhead, 1994). It is likely that, due to increased stress, snakes chose higher temperatures in the LG to enhance their ability to perform anti-predatory displays (Currin & Alexander, 1999). On the other hand, the addition of refuges to the SCG might have helped to reduce stress in the snakes.

Besides the clear effect that refuges have in avoiding detection by predators, the use of refuges has also thermoregulatory considerations (Todd et al., 2016). Some have suggested that many squamates choose specific retreat sites that provide thermal variation and could be used for thermoregulatory purposes (Amo et al., 2004; Cox et al., 2018). For example, Cox et al. (2018) found that solitary *Diadophis punctatus* individuals select rocks that allow them to maintain a stable range of T_b within their preferred range. Webb and Shine (1998) found that *Hoplocephalus bungaroides* selects thin rocks in spring, but changes to thicker rocks in summer as the temperatures under the refuges increase. Similarly, Huey et al., (1989) showed that *Thamnophis elegans* chooses intermediate-thickness rocks that improve energy gain and temperature regulation.

For our study population of *Crotalus polystictus*, refuges appear to provide an important microhabitat for thermoregulation. Most of the individuals that we captured were found under rocks instead of basking out in the open, regardless of the time of day. Traditionally, snakes located under shelters would be considered inactive (Whitaker & Shine, 2002). However, this characterization fails to consider that some snakes could prioritize the use of refuges for thermoregulatory reasons. *C. polystictus*, in particular, is known to prefer burrowing rodents as prey (Meik et al., 2012), which might explain its high preference for dwelling under rocks as sit-and-wait predators. However, a trade-off between selecting refuges for foraging and thermoregulatory purposes can exist in some species (Huey et al., 1989). Thus, additional study of the effects that refuges have on the thermoregulation of *C. polystictus* is needed.

Overall, we suggest that a LG like the one used in most thermoregulatory studies in reptiles is not suitable for snakes. A thermal gradient in semi-captivity that generally resembles the original habitat can reduce the stress associated with captivity while also

providing more thermal variability than a laboratory gradient. The addition of refuges also allows snakes to use the same substrates that they would naturally thermoregulate on. Most thermal gradients in snakes studies have been made with wood and covered with various substrates (Aubret & Shine, 2010; Blouin-Demers & Weatherhead, 2001; Fitzgerald et al., 2003; Harvey & Weatherhead, 2011; Whitaker & Shine, 2002). However, different materials conduct heat at different rates; thus, using substrates from the natural habitat of a species is ideal for estimating their T_p . Providing a more naturalistic thermal gradient allows snakes, and potentially vertebrate ectotherms in general, to thermoregulate as accurately as they would in their natural habitat, which, in turn, enhances the accuracy of preferred temperature range estimates.

We can conclude, therefore, that *Crotalus polystictus* is an accurate thermoregulator despite living in a low thermal quality habitat. Although it is an active thermoregulator, it has a wider set of preferred temperatures than most other snakes that have been studied to date (Blouin-Demers & Weatherhead, 2001; Brown & Weatherhead, 2000; Fitzgerald et al., 2003; Harvey & Weatherhead, 2011). Our results also emphasize the importance of tailoring the design of thermal enclosures to each study species. In sum, our study shows that the Hertz et al. (1993) protocol can be robustly adapted to more species, thereby increasing the quality of data on thermoregulation obtained from a variety of non-avian reptiles.

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Ecología térmica de una población de la lagartija *Sceloporus grammicus* (Iguanidae: Phrynosomatinae) que ocurre en la zona centro-oriente de la ciudad de México. *Acta Zoológica Mexicana*, 22(2), 137–150.

Tables

Table 1. Descriptive thermoregulatory data and thermoregulation indices for *Crotalus polystictus* in San Bartolo Morelos, Estado de México. Means and SD are shown.

Variables	Temperature (°C)	
	Field body temperature (T_b)	23.2 ± 4.5
Air temperature (T_a)	20.4 ± 4.1	
Substrate temperature (T_s)	20.2 ± 3.6	
Operative environmental temperatures (T_e)	22.5 ± 10.3	
	Thermal gradients	
	Laboratory gradient (LG)	Semi-captivity gradient (SCG)
Mean preferred temperature range (T_p)	30.9 ± 4.9	26.5 ± 6.1
T_p interquartile range (T_{p25} – T_{p75})	29 – 34.3	22.5 – 30.9
Accuracy of thermoregulation (d_b)	5.84 ± 4.3	1.63 ± 2.3
Thermal quality of the environment (d_e)	10.08 ± 5.3	5.88 ± 4.9
Thermoregulatory efficiency (d_e – d_b)	4.24	4.25
Thermoregulatory efficiency (E)	0.42	0.72

Figures

Figure 1. Diagram of the laboratory thermal gradient (A) and the semi-captivity thermal gradient (B) used to test the T_p range of *Crotalus polystictus*. Heat lamps, heat mats and refrigerant gel packs in the laboratory gradient were used on all three enclosures.

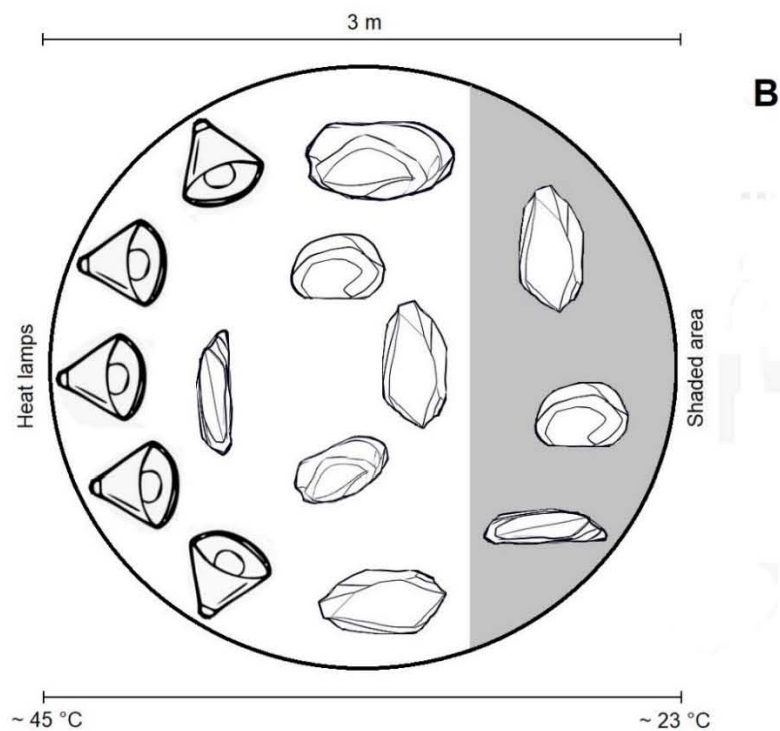
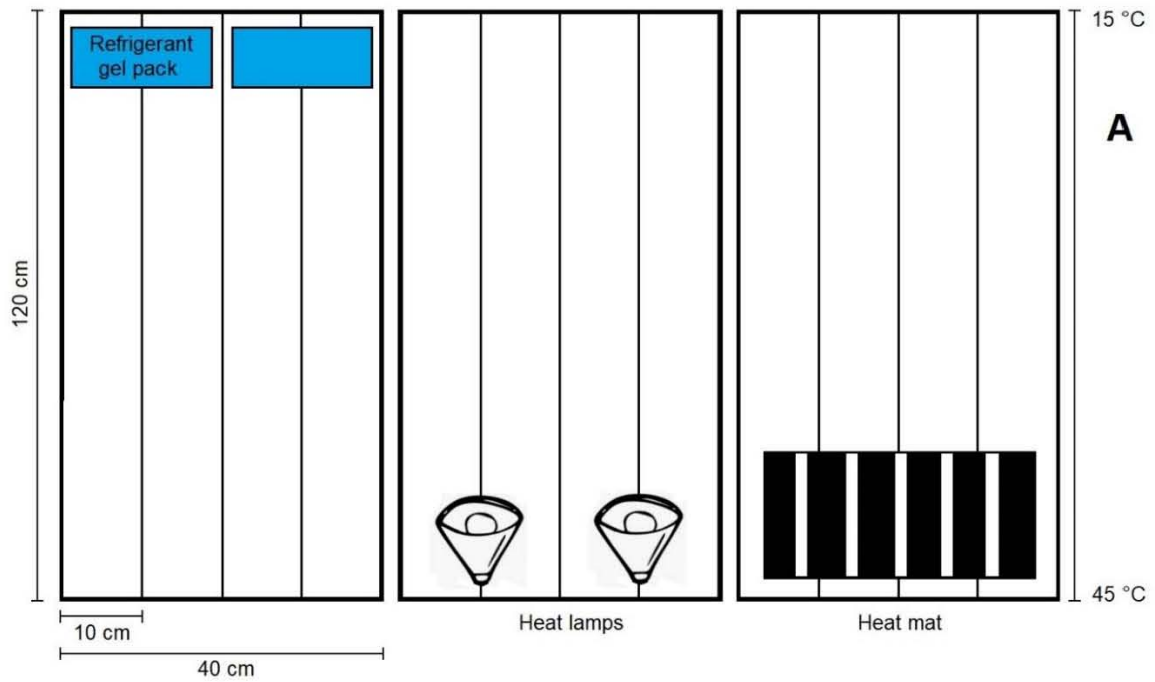
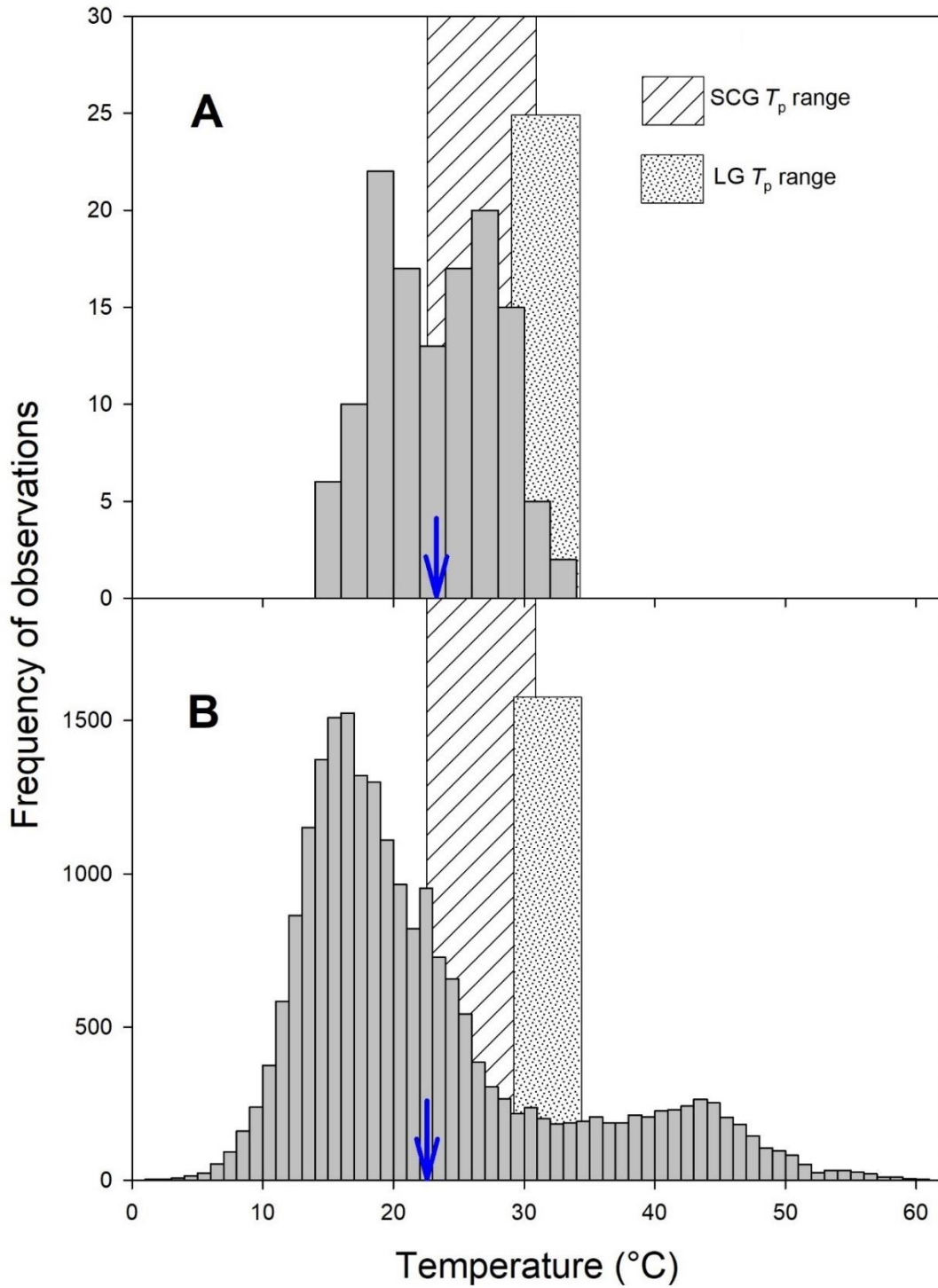


Figure 2. Distribution of field body temperatures (A) and operative temperatures (B) of *Crotalus polystictus* at San Bartolo Morelos, Estado de México. The arrows point to each temperature distribution's mean. The interquartile of the preferred temperature range obtained with the semi-captivity gradient (22.5 – 30.9 °C) is represented by diagonal lines, whereas the one obtained with the laboratory gradient (29 – 34.3 °C) is represented by a dotted area.



Comprobante de requisitos para titulación por artículo

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Hibraim Perez-Mendoza:

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Kind Regards,

Dr Rachael Antwis

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Discusión

Para describir con precisión la ecología térmica de las especies, es necesario ir más allá de simplemente estimar la temperatura corporal de los individuos y posibles predictores de ésta. Una estimación robusta del intervalo de T_{sel} es de suma importancia para comprender las implicaciones de la variación de T_c y qué tan efectiva es la termorregulación de una especie. Este trabajo ilustra que el resultado de los índices térmicos derivados del protocolo de Hertz et al. (1993) depende ampliamente de una estimación precisa del intervalo de T_{sel} . El intervalo intercuartil de T_{sel} seleccionado por *C. polystictus* en el GSC fue significativamente menor que el derivado del GL. La amplitud del intervalo del GSC fue de 8.4 °C; es el intervalo más amplio reportado hasta el momento entre los estudios de termorregulación de serpientes (Blouin-Demers & Weatherhead, 2001; Brown & Weatherhead, 2000; Fitzgerald et al., 2003; Harvey & Weatherhead, 2011). La amplitud del intervalo de T_{sel} obtenido a partir del GL, en cambio, fue de 5.6 °C, aunque sigue siendo mayor que la mayoría de los intervalos obtenidos en los estudios previamente mencionados. Estos resultados indican que la población estudiada de *C. polystictus* es euritérmica. Powell y Russell (1985) mencionan que la euritermia es favorecida cuando los costos energéticos de la termorregulación son altos y los individuos viven en ambientes térmicamente variables. Las temperaturas operativas en San Bartolo Morelos son muy variables en comparación con la distribución de las T_c de *C. polystictus*. Esto se ve reflejado en los bajos estimados de la calidad térmica del ambiente obtenidos con ambos gradientes térmicos, que indican que San Bartolo Morelos no representa un ambiente térmico ideal para *C. polystictus*.

De acuerdo con los resultados del GL, *C. polystictus* presenta una baja eficiencia térmica y tiende más hacia el termoconformismo que hacia la termorregulación activa ($d_b = 5.84$ °C, $E = 0.42$). De manera contrastante, los resultados del GSC indican que *C. polystictus* es un termorregulador activo y eficiente ($d_b = 1.63$ °C, $E = 0.72$). Los estimados del índice de Blouin-Demers y Weatherhead obtenidos con ambos gradientes son casi idénticos, a pesar de que la escala de los valores de d_b y d_e difiere entre gradientes, siendo mayores en el GL. Ambos resultados muestran que la población debe compensar 4.2 °C para alcanzar las temperaturas preferidas. Este resultado indica que existe una preferencia

hacia la termorregulación activa sobre el termoconformismo por parte de los individuos en esta población.

Las diferencias importantes en los estimados de los índices de termorregulación se deben directamente a la elección de temperaturas más altas dentro del GL. Sin embargo, una posible explicación para esa selección es el estrés. Los reptiles expresan comúnmente una variedad de respuestas antidepredatorias ante el manejo y el cautiverio (Franklin et al., 2003; Moore et al., 1991; Schuett et al., 2004). Diversos estudios han documentado el aumento en los niveles de corticosterona en sangre de serpientes después de la captura y confinamiento (Bailey et al., 2009; Dayger et al., 2013; Herr et al., 2017; Mathies et al., 2001; Schuett et al., 2004; Sykes & Klukowski, 2009). Debido a que la capacidad motora disminuye conforme la temperatura baja, en estas condiciones, las serpientes dependen de la cripsis como táctica antidepredatoria (Brodie III & Russell, 1999; Keogh & DeSerto, 1994; Prior & Weatherhead, 1994). Sin embargo, las serpientes prefieren escapar y mostrar comportamientos más agresivos cuando las temperaturas son más altas y las habilidades motoras son óptimas (Citadini & Navas, 2013; Llewelyn et al., 2010; Mori & Burghardt, 2001; Passek & Gillingham, 1997; Prior & Weatherhead, 1994; Schieffelin & De Queiroz, 1991). Dado que el GL representó un ambiente muy diferente al hábitat natural de las serpientes, es posible que el nivel de estrés de los individuos haya aumentado con respecto al GSC, lo que pudo llevarlos a escoger temperaturas más altas para favorecer su capacidad de mostrar diversas conductas antidepredatorias (Currin & Alexander, 1999).

La adición de refugios en el GSC pudo haber disminuido el nivel de estrés de los individuos al disminuir el potencial riesgo de depredación que experimentan los individuos. Aunado a esto, los refugios les permitieron a las serpientes usar los mismos sustratos sobre los que termorregularía normalmente. La mayoría de los gradientes térmicos utilizados con serpientes se han hecho con madera y cubiertos con diversos sustratos (Aubret & Shine, 2010; Blouin-Demers & Weatherhead, 2001; Fitzgerald et al., 2003; Harvey & Weatherhead, 2011; Whitaker & Shine, 2002). Sin embargo, diferentes materiales conducen el calor a diferentes tasas, por lo que es ideal usar sustratos del hábitat natural de las serpientes para calcular su T_p .

El uso de refugios también puede tener un efecto importante sobre la elección directa de las temperaturas (Todd et al., 2016). Diversos reptiles escamosos seleccionan refugios con base en su variación térmica, lo que les ayuda a mantener la temperatura dentro del intervalo de T_{sel} (Amo et al., 2004; Cox et al., 2018). Diversos factores como la estructura y el grosor de las rocas y la época del año juegan un papel importante en la selección de refugios con fines termorregulatorios (Huey et al., 1989; Webb & Shine, 1998). En nuestra población de estudio, parece ser que los refugios proveen microhábitats importantes para la termorregulación de *C. polystictus*. La mayoría de los individuos capturados en este trabajo se encontraron bajo rocas, independientemente de la hora del día y la temperatura ambiental. Los resultados, a su vez indicaron que la T_c de *Crotalus polystictus* tiene una relación positiva significativa más fuerte con la temperatura del sustrato que con la temperatura del aire, lo que sugiere que la obtención de calor se da mayormente a través de la tigmotermia. Esta estrategia consiste en que la ganancia de calor se da al posicionarse sobre un sustrato caliente en lugar de la radiación directa del sol (Lara-Reséndiz et al., 2014). Es probable que individuos que pasan la mayor parte del tiempo bajo refugios recurran a esta estrategia. No obstante, los reptiles pueden presentar una combinación de ambas estrategias, dependiendo de las condiciones térmicas del hábitat (Pelegrin et al., 2013; Woolrich-Piña et al., 2006).

Otra explicación para la preferencia de *C. polystictus* por permanecer bajo refugios puede ser la obtención de alimento. *C. polystictus* tiende a preferir roedores enterradores como presa (Meik et al., 2012a), lo que puede explicar este comportamiento como parte de una estrategia de forrajeo por emboscada. Sin embargo, en algunas especies puede existir un *trade-off* entre la elección de refugios para termorregular o para forrajear (Huey et al., 1989). Por consiguiente, es necesario realizar estudios detallados sobre el efecto que tienen los refugios en la termorregulación de *C. polystictus*.

De manera general, un GL como el utilizado en la mayoría de los estudios de termorregulación con reptiles, no es adecuado para las serpientes. Un gradiente térmico en semicautiverio que se parezca más al hábitat original puede reducir el estrés asociado al cautiverio y proveer de una mayor variabilidad térmica al gradiente. Estas medidas

permiten que las serpientes termorregulen de manera tan precisa como lo hacen en su hábitat, lo que genera que los estimados de T_p obtenidos sean más robustos.

Conclusiones

El tipo de gradiente térmico utilizado determina diferencialmente los estimados de los índices de la termorregulación del protocolo de Hertz et al. (1993).

Crotalus polystictus es un termorregulador activo y preciso, según el gradiente en semicautiverio, a pesar de vivir en un ambiente con baja calidad térmica.

La estrategia termorregulatoria de *C. polystictus* en el sitio de estudio es la euritermia, con una tendencia hacia la tigmotermia.

Los gradientes en semicautiverio son ideales para conocer las preferencias térmicas de las serpientes, debido al menor nivel de estrés que sufren los organismos, a la mayor oferta térmica disponible y a la disponibilidad de sustratos habituales para la termorregulación de los individuos.

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