Journal of Thermal Biology 113 (2023) 103526

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

Trade-off between thermal preference and sperm maturation in a montane lizard

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ARTICLE INFO

Keywords:
Acclimation
Ectotherms
Locomotor performance
Sperm parameters

ABSTRACT

Temperature is a key abiotic factor that influences performance of several physiological traits in ectotherms. Organisms regulate their body temperature within a range of temperatures to enhance physiological function. The capacity of ectotherms, such as lizards, to maintain their body temperature within their preferred range influences physiological traits such as speed, various reproductive patterns, and critical fitness components, such as growth rates or survival. Here, we evaluate the influence of temperature on locomotor performance, sperm morphology and viability in a high elevation lizard species (*Sceloporus aeneus*). Whereas maximal values for sprint speed coincides with field active and preferred body temperature, short-term exposure at the same range of temperatures produces abnormalities in sperm morphology, lower sperm concentration and diminishes sperm motility and viability. In conclusion, we confirmed that although locomotor performance is maximized at preferred temperatures, there is a trade-off with male reproductive attributes, which may cause infertility. As a consequence, prolonged exposure to preferred temperatures could threaten the persistence of the species through reduced fertility. Persistence of the species is favored in environments with access to cooler, thermal microhabitats that enhance reproductive parameters.

CRediT author statement

Rosa Isela Quintero-Pérez: Conceptualization, Investigation, Methodology, Software, Data curation, Formal analysis, Visualization, Writing - original draft, Writing - review & editing. Fausto Roberto Méndez-de la Cruz: Conceptualization, Investigation, Funding acquisition, Writing - review & editing. Donald B. Miles: Software, Data curation, Writing - original draft, Writing - review & editing. Mirna Crizel Vera Chávez: Investigation, Methodology, Data curation, Formal analysis, Writing - review & editing. Yolanda López-Ramírez: Investigation, Methodology, Writing - review & editing. Diego Miguel Arenas-Moreno: Conceptualization, Investigation, Methodology, Software, Data curation, Formal analysis, Visualization, Writing - original draft, Writing - review & editing. Edith Arenas-Ríos: Conceptualization, Investigation, Funding acquisition, Methodology, Formal analysis, Writing - original draft, Writing - review & editing.

1. Introduction

Temperature highly influences the environmental conditions necessary for thermoregulation at temporal and spatial scales in ectotherms such as reptiles (Angilletta, 2009). Behavioral thermoregulation allows reptiles to maintain their body temperatures within a narrow range during activity (Cowles and Bogert 1944; Cossins and Bowler, 1987;

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https://doi.org/10.1016/j.jtherbio.2023.103526
Received 15 September 2021; Received in revised form 3 February 2023; Accepted 22 February 2023

Domínguez-Guerrero et al., 2019). This temperature range, known as the normal activity range (Pough and Gans, 1982), can influence growth rates (Sinervo and Dunlap, 1995), physiological performance (Angilletta et al., 2002) and reproductive patterns (Licht, 1973).

Global climate change affects organisms in every biome and ecosystem. For lizards, local extinctions of ~20% are projected by the year 2080 accompanied by concomitant distributional changes are expected (Sinervo et al., 2010). As a consequence of increasing temperatures, the number of hours of activity could become limited (Huey et al., 2009). Given their dependence on temperature, reptiles are sensitive to environmental changes, which could be detrimental and even drive a population to extinction (Kearney et al., 2009). However, some organisms can manifest a favorable response to warmer temperatures by moving to habitats with higher thermal quality, when possible. As a consequence, either species distributions are altered by following spatial shifts in their thermal niche or they adapt by behavioral changes (Pereira et al., 2010).

In response to environmental changes, lizards may use short- and long-term acclimation responses to maintain physiological performance under local conditions (Pintor et al., 2016). Whereas current research primarily focuses on thermal performance curves that portray the dependence of physiological rates on temperature (Clusella-Trullas et al., 2011), few studies have examined how reproductive traits may respond to warmer environments. In mammals, it has been shown that high temperatures affect testicular, endocrine, and exocrine functions (Lue et al., 2006; Wang et al., 2007). For example, high temperatures interrupt spermatogenesis, which can generate giant, multinucleated cells (Paul et al., 2008); block the action of gonadotropins within the seminiferous tubules, which decreases testosterone synthesis (El-Hefnawy et al., 2000); alter the epithelial cycles; and cause direct damage to germ cells (Clegg, 1963; Dutta et al., 2013). However, the effect of temperature on sperm parameters in ectotherms, particularly in reptiles is still poorly understood (Gist et al., 2000; Tourmente et al., 2011). Another neglected aspect is the influence of high temperatures on the epididymis, an organ essential for influencing the fertilization capacity of sperm (Gist et al., 2000). In the epididymis, the sperm acquire the ability to move, recognize, and fertilize the oocyte, a process known as epididymal sperm maturation (Arenas-Ríos et al., 2017). This process, being androgen dependent (Robaire et al., 2006), could be affected by rising environmental temperatures. Past research has determined that physiological performance, in a myriad of different activities, is optimized at body temperatures close to or at the preferred body temperatures of a species. However, Méndez de la Méndez-de la Cruz et al. (2014) proposed that male lizards require lower temperatures to achieve efficient sperm maturation.

Here, we determined the effects of acclimation to different temperatures on epididymis morphology, and sperm parameters in adult male individuals of the lizard species *Sceloporus aeneus*. We compared the temperatures that maximize male reproductive traits with the preferred temperatures and optimal temperatures for locomotor performance.

2. Materials and methods

2.1. Study species and site characteristics

The southern bunchgrass lizard (*Sceloporus aeneus* Wiegmann, 1828) is a slender, small-sized lizard with a maximum snout-vent length (SVL) of 78 mm, endemic to central Mexico (Bryson et al., 2012). The species inhabits temperate forests and other open areas at elevations between of 2300 m and 3400 m (Kölher and Heimes, 2002). The species is a diurnal, terrestrial predator of small invertebrates. The reproductive cycle is characterized by mating in spring, when environmental temperature increases, and reaches a maximum in May (Manríquez Morán, 1995). Maximum testicular activity occurs from March to May (Hernández-Gallegos et al., 2014), which is concomitant with the increase in environmental temperatures. Females have a fixed spring reproductive pattern (Manríquez-Morán et al., 2013). Therefore, mating (copulations) occurs during the hottest months of the year (April, May). Oviposition takes place during the summer (June to September), during lower environmental temperatures and monsoon storms. Oviposition occurs during this time when nest conditions are ideal with soil temperatures and sufficient moistures for successful embryonic development.

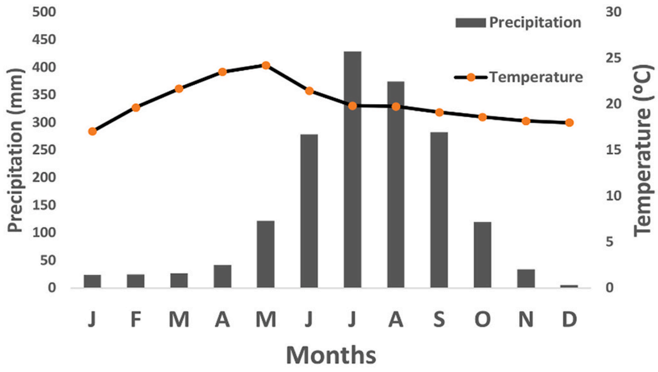

Fig. 1. Monthly variation in air temperature and precipitation at the study site. Data source: Weather and Climate – The Global Historical Weather and Climate Data.

The study site was the Volcán Coatzontle in San Miguel Ajusco, south of Mexico City (19°14′ 22″ N, 99° 12’ 38” W, 2900 m elev.). The average low temperature varies between 14 and 16 °C, and the average high temperature varies between 26 and 29 °C) during the hottest monthly interval (April–June, Fig. 1). The site has a summer rain regimen (May to October) with a mean annual precipitation of 1340 mm (Schmitter, 1953; Rzedowski, 1954).

2.2. Ecophysiological patterns

2.2.1. Fieldwork

We captured lizards during the reproductive season (March to May 2019) during their activity period (0900–1700 h, GMT-6). We searched for lizards among the microhabitats known to be used by the lizards (i.e., rocks, *Agave* spp. plants, and shrubs). We captured lizards by either a noose or hand (Harlow, 1996). We recorded the body (cloacal) temperature (T_b), within 10 s after the capture, using a digital thermometer Fluke® 54-II (±0.1 °C) (Lara-Reséndiz et al., 2013). Males were sexed by eversion of the hemipenes. We measured the SVL of the individuals using a digital caliper. The minimum SVL recorded was 40 mm. The mean size at sexual maturity is ~39 mm, thus we considered all of the lizards to be adults (Rodríguez-Romero, 2014).

2.2.2. Operative environmental temperature

We estimated operative environmental temperatures (T_e), i.e., the equilibrium temperature attained by an animal in the absence of temperature regulation (Bakken 1992). We first validated the appropriate operative temperature model following the protocol of Domínguez-Guerrero et al. (2019) on *Sceloporus torquatus*. We constructed three biomimetic models connected to data loggers (Thermochron iButton®; DS1921G). These models were validated before the measurements of T_e using the criteria proposed by Dzialowski (2005). Our calibration involved estimating the comparing the heating and cooling rates under laboratory conditions (10-min intervals of shade and artificial incandescent light, 100 W) with an adult male individual of *S. aeneus* and three operative temperature models. We used polyvinyl chloride (PVC) pipes similar in size to *S. aeneus* and painted different shades of gray as potential models (Dzialowski, 2005; Domínguez-Guerrero et al., 2019). We chose the PVC model with the highest value of R^2 (in this case was $R^2 = 0.88$), because this model best emulates the thermodynamic properties of the *S. aeneus* (Dzialowski, 2005).

The model consisted of a PVC pipe painted gray 33 with a size of 50 mm length × 20 mm diameter. We placed the models in microhabitats where the lizards were observed to be active (e.g., rocks, under and on top of Agave plants, and under shrubs), and recorded T_e once per hour for 90 days.

2.2.3. Laboratory work

Captured individuals were transported to a laboratory and maintained in separate terraria (34.5 × 21 × 12.5 cm) with peat moss as a substrate, controlled 8 h photoperiods matching the capture locality, and 30–40% humidity. We fed individuals every other day with 2–3 *Acheta domestica* per individual, supplemented with calcium. Lizards were provided with water *ad libitum.*

2.2.4. Selected temperature, critical thermal limits, and locomotor performance

We recorded the selected temperatures (T_{sel}) of 37 individuals during the species' activity period (0900–1700 h, GMT-6). The measurement of T_{sel} was done using a thermal gradient box (100 × 100 × 30 cm) with a 0.5 cm substrate (*peat moss)*. At one end of the gradient, we suspended 100 W light bulbs ~30 cm above the box and ice bags on the other end, resulting in a temperature gradient between 20 °C – 50 °C. Prior to recording T_{sel}, we habituated the lizards by placing them in the gradient for 1 h. We recorded the body temperature of each individual (cloacal temperature) every hour using a Fluke® 54-II digital thermometer (±0.1 °C). To avoid dehydration, we sprinkled water on the substrate (Arenas-Moreno et al., 2018). We used the mean body temperature in the thermal gradient as our estimate of T_{sel}. We also calculated the interquartile range (T_{sel} 25–75%), which was used as the T_{sel} set points, i.e., upper and lower T_{set}.

The critical minimum (CT_{min}) and maximum (CT_{max}) temperatures refer to the lower and upper temperatures that compromises locomotion (Beitinger et al., 2000). We determined the critical temperatures based on the loss of righting response (Brusch et al., 2016). For CT_{min} we placed a lizard in a perforated box, partially covered with ice, and monitored every 3 min. Whereas for CT_{max}, we put a lizard in a terrarium with a 100W incandescent light bulb suspended above. We measured the body temperature of the individual once we observed the loss of righting (Huey and Stevenson, 1979; Arenas-Moreno et al., 2018). We used different individuals for measuring CT_{min} ($N = 10$) and CT_{max} ($N = 9$).

Five males were selected to determine the thermal performance curve. We ran lizards at six temperatures 22°, 25°, 28°, 31°, 34° and 37°C to determine the thermal sensitivity of sprint speed. We randomized the order of temperatures. We exposed lizards to a 60 W incandescent light for 30–40 min until reaching the desired body temperature. We verified T_b was by inserting a thermocouple connected to a digital thermometer (Fluke 50 Series II®, sensor type K, precision ± 0.1 °C) approximately 10 mm into the cloaca. The speed was determined using a video analysis of the lizards running on a wood track (1.20 × 0.15 x 0.20, m; long, wide, and high, respectively) covered with a mesh (natural fiber) to provide traction. The floor of the track had a visible reference each 5 cm (Husak et al., 2006). We induced lizards to run by gently tapping the lizard at the base of the tail. Each individual lizard was run three times at each body temperature. Lizards were allowed to rest for at least 30 min between successive runs (Wang et al., 2007).

Lizards were filmed as they ran down the track with a GoPro→ video 4K camera (16 megapixels at 30 FPS) suspended above the track. Videos were processed using to the editor ®Avidemux to estimate sprint speed. We used the fastest time among the three trials as our estimate of maximum sprint speed.

We estimated the thermal performance curve using general additive mixed models (GAMM), which account for the nonlinear distribution of the data. We used the function "gamm" in the package "mgvc" v1.8-31 (Wood et al., 2016) as implemented in the package Mapinguari 0.4.1 (Caetano et al., 2017). We included SVL as a covariate and individual id as a random effect (Romero-Báez et al., 2020). We tested different correlation structures that account for the non-independent nature (i.e., repeated measures) of the performance data. We selected the best supported correlation structure based on the Akaike Information Criterion and Bayesian Information Criterion (BIC). We plotted the best supported model with the function "visgam" in "mgvc" (Wood et al., 2016). We calculated the predicted maximum speed, optimal temperature (T_o) for sprint speed, and the thermal performance breadth (i.e., 85% of the maximum performance, B_{85}; Huey and Stevenson, 1979). All analyses were conducted in the R computing environment v 4.0.2 (R Development Core Team, 2022).

2.3. Histology and sperm parameters

2.3.1. Histology

We collected an additional nine male individuals at the same site (mean SVL = 52. ± 2.6 mm; range 48 mm - 56 mm) to assess how exposure to different temperatures affected the micro-anatomy of the epididymis. We acclimated three lizards each at one of three temperatures (24 °C, 28 °C, and 32 °C, i.e., N = 3 for each temperature) for a period of seven days. Lizards were kept at these temperatures during the activity period (0900–1700 h). All lizards were maintained at a temperature of 15 °C during the inactive, nocturnal period. This temperature pattern is similar to that recorded at the study site. The sample size for this experiment was selected to minimize the number of sacrificed individuals. After the acclimation period, lizards were euthanized by an overdose of intraperitoneal injection of pentobarbital. We removed the right epididymis for histological analysis. The epididymides were kept for two days in 10% neutral stabilized formalin and dehydrated with an ascending concentration of ethanol (40%, 50%, 60%, 70%, 80%, 90%, 96% and 100%). Following dehydration, we added xylol to clear the tissue and embedded the epididymis in paraffin. We cut 5 μm slices, longitudinally, using a rotary microtome (Reichert HistoSTAT 820). We stained the tissue using hematoxylin and eosin (Sigma H-9627, E-4009) to facilitate differentiating the nucleus and other extra-cytoplasmic elements, such as collagen Photomicrographs were taken by region: *head*, *body*, and *tail*, using an Axioskop II optical microscope (Zeiss®), with an axioCamMRc5 camera at 40x magnification. Photomicrographs were processed using Axiovision (version 4.8). The morphometric analysis was conducted by region of the epididymal ducts; the epithelial area and spermatic area of the epididymal tubule were estimated as follows: nine epididymal tubules were reviewed in three sections of two lamellae. The inclusion criteria were to consider only round tubules.

The analysis of the epididymal ducts by region (*head*, *body*, and *tail*) included the area of the epithelium (AE), the space occupied by the epithelium in the epididymal tubule and the spermatic area, the space occupied by the sperm in the lumen of the epididymal tubule. The area of the epididymal epithelium at each region was obtained using the following formula: AE = DB – DA (where AE – area of the epididymis in $\int m^2$, DB = basal domain, and DA = apical domain).

2.3.2. Sperm traits

We extracted sperm from the left epididymis of lizards used in the histological analysis. We had used three lizards per temperature. The epididymis was divided into three regions: *head*, *body*, and *tail*. The sperm parameters analyzed were concentration, viability, morphology, and motility. Sperm parameters were analyzed the same day, following the techniques described in the World Health Organization manual for the evaluation of human semen (World Health Organization.WHO, 2010), with modifications considered for lizard sperm, which are described below. We added 20 μl of Ringer's solution [NaCl (MEYER), KCl (SIGMA), KH_2PO_4 (Productos Quimicos Monterrey) and $CaCl_2$ $2H_2O$ (Baker Analyzed)] to each region of the epididymis and then macerated the tissue. The collected pellet was resuspended in Ringer's solution and used to determine the core sperm parameters.

We calculated the sperm concentration of 5 μl of 1:20 diluted

Table 1
Sceloporus aeneus thermal parameters. Body temperature (T_b); preferred temperature (T_{pref}); interquartile range (T_{pref} 25–75%); critical thermal minimum (CT_{min}); critical thermal maximum (CT_{max}); thermal tolerance range (TTR; CT_{max}-CT_{min}). Temperatures (° C) are shown as mean ± SD.

Field thermal parameters	Laboratory thermal parameters				
T_b	T_{pref}	$T_{pref\ 25\%–75\%}$	CT_{min}	CT_{max}	TTR
33.84 ± 3.68 22.2–40.4 n = 179	32.71 ± 5.45 21.2–38.3 n = 37	28.3–36.95	6.70 ± 1.22 5.2–9.2 n = 10	42.98 ± 1.54 40.4–45.6 n = 9	36.28

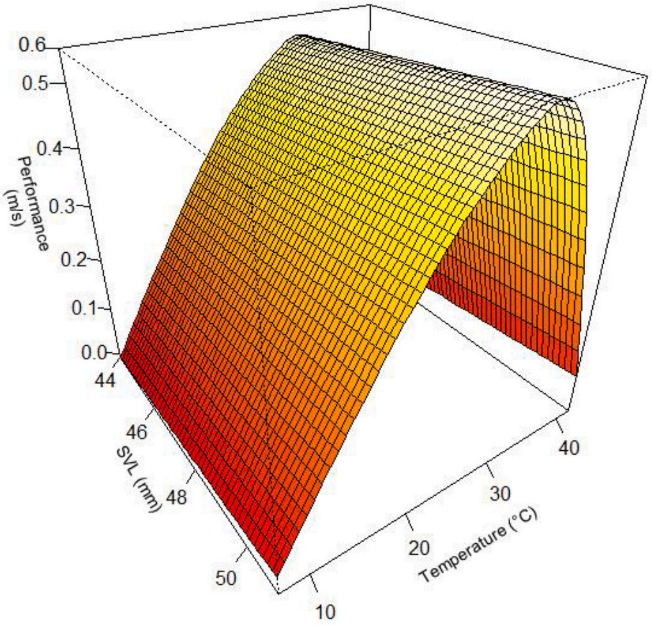

Fig. 2. Locomotor performance curve of *S. aeneus* generated by a general additive mixed model (GAMM).

samples in a Neubauer chamber at 400x magnification. We calculated the sperm concentration using the formula N/n x (1:20) where x = dilution factor, N = total number of the sperm count in the chambers, and n = counted lines. We used a smear test to estimate sperm viability. A 5 μl aliquot of sperm was mixed with 2 μl EspermaVit (Laboratorios FertiMexico S.A de C.V). We determined the sperm count of 100 cells at 1000x magnification. We estimated sperm morphology after mixing sperm and EspermaVit. We recorded the number of normal and abnormal sperm. Abnormal sperm were identified by either the presence of a coiled and stump tail, curved head, or in some cases, the presence of cytoplasmic droplets. To analyze the sperm motility, we mixed 5 μl sperm and 10 μl Tyrode and identified the mobile and motionless sperm at a 400x magnification in each region of the epididymis. Motility is given as the percent of mobile sperm in the sample.

2.3.3. Data analysis

The histology results and the sperm parameters were analyzed using Kruskal-Wallis and Tukey and Dunn's test, α = 0.05. Statistical analyses were performed in SigmaPlot v 11.0 (Systat Software, 2011).

All experiments were conducted following the standards of ARRIVE (Animal Research: Reporting of In Vivo Experiments) (Kilkenny et al., 2010), and permits of Secretaría del Medio Ambiente y Recursos Naturales (Approval: 01629 and 005406/18).

Table 2
Summary of models from a GAMM analysis to describe the thermal performance curve in *Sceloporus aeneus*. Values are presented for Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC). Scores ΔAIC = Delta Akaike Information criterion, are taken as the difference between a specific model and the null model of performance and temperature. Values in boldface indicate the best supported model.

Model	GAMM correlation structure class	AIC	ΔAIC	BIC	ΔBIC
1	corAR1 (form = ~1|id)	−19.60185	0.7486787	−11.413921	0.0081933
2	corAR1 (0.1,form = ~1|id)	−19.60185	0.7486787	−11.413921	0.0081933
3	corCAR1 (0.1,form = ~1|id)	−19.52274	0.8277891	−11.334811	0.08730369
4	corGaus (form = ~1|id)	−19.52274	0.8277891	−11.334811	0.08730369
5	corGaus (form = ~1|id, nugget = TRUE)	−17.52274	2.8277891	−7.697225	3.72488985
6	corExp (form = ~1|id)	−19.52274	0.8277891	−11.334811	0.08730369
7	corExp (form = ~1|id, nugget = TRUE)	−17.52274	2.8277891	−7.697225	3.72488985
8	corRatio (form = ~1|id)	−19.52274	0.8277891	−11.334811	0.08730369
9	corRatio (form = ~1|id, nugget = TRUE)	−17.52274	2.8277891	−7.697225	3.72488986
10	corSpher (form = ~1|id)	−19.52274	0.8277891	−11.334811	0.08730369
11	corSpher (form = ~1|id, nugget = TRUE)	−17.52274	2.8277891	−7.697225	3.72488985
12	corARMA (form = ~1|id,p = 0,q = 1)	−19.61005	0.7404854	−11.422114	0
13	corARMA (form = ~1|id,p = 1,q = 0)	−19.60185	0.7486787	−11.413921	0.0081933
14	corARMA (form = ~1|id,p = 1,q = 1)	−17.61483	2.7357018	−7.789312	3.63280256
15	corARMA (form = ~1|id,p = 1,q = 2)	−15.8217	4.5288287	−4.358599	7.06351562
16	corARMA (form = ~1|id,p = 2,q = 1)	−20.35053	0	−8.887427	2.53468692

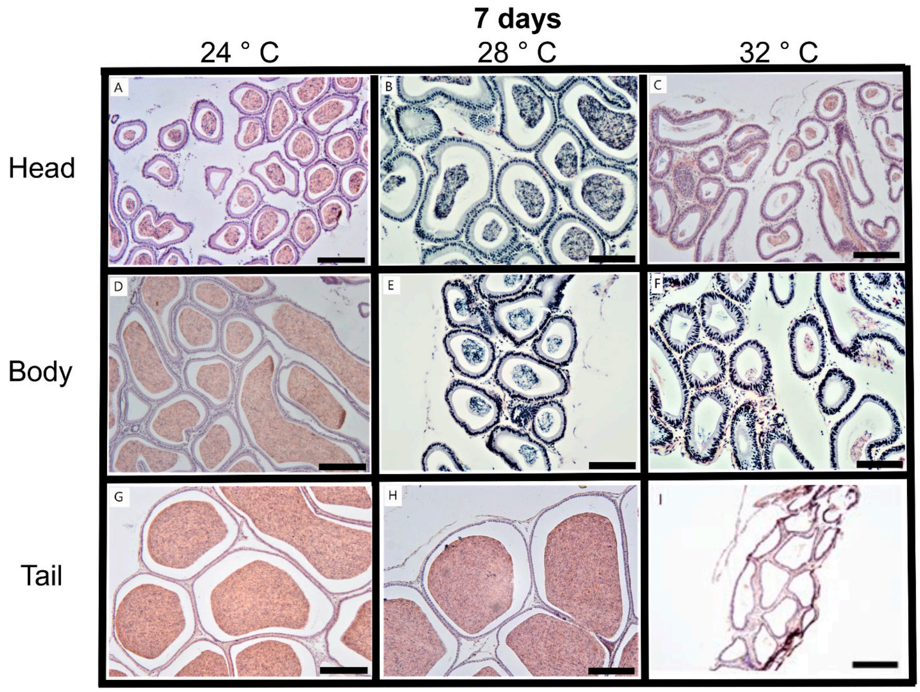

Fig. 3. Micrographs of transverse sections of the epididymis regions (*head*, *body*, and *tail*) of *S. aeneus*, after acclimation to 24 °C, 28 °C, and 32 °C. Hematoxylin-eosin staining 10 μm scale bars and 100x magnification.

3. Results

3.1. Ecophysiological patterns

The results of the thermal parameters are summarized in Table 1. The field active T_b of *S aeneus* is similar to their T_{sel} (32.84 °C and 33.84 °C, respectively, Table 1). The mean operative environmental temperature (T_e) during the hours of activity was 19.67 °C and 15 °C at night. These values were obtained during the reproductive season.

Individuals of *S. aeneus* are capable of activity between the temperatures of 6.70°C and 42.98°C, which yields a thermal tolerance range of 36.3 °C. Sprint speed increased as T_b approached ~34 °C, which is similar to the species T_{sel} (Fig. 2). The thermal performance breadth (B_{85}) was at 28–34 °C. The best model presented an optimal temperature (T_o) for performance at 34°C °C with a speed of 0.54 m/s (AIC = -20.35, Δ AIC = 0.00 and BIC = -11.42, Fig. 2). Table 2 presents summary of the models estimated from the GAMM analysis.

3.2. Sperm parameters

An example of the stained sections of the epididymis (*head*, *body*, and *tail*) is shown in Fig. 3. Quantitative morphometric analysis of the three epididymis regions shows significant differences in the treatment. The *head* region (H = 8.926, df = 2, p = 0.012), shows an increased thickening of the epithelium at 28 °C (23 809.24 μm^2), the *body* region (H = 29.713, df = 2, p = 0.001), shows a larger epithelial area (31 569.44 μm^2) at 24 °C and the *tail* region (H = 15.206, df = 2, p = 0.001), a larger area (33 516.66 μm^2) at 32 °C (Fig. 4).

We found significant statistical differences in sperm area, regardless of the treatment. The head region (H = 52.785, df = 2, p = 0.001) showed a larger area at 24 °C (15 320.09 μm^2), the *body* region (H = 47.458, df = 2, p = 0.001) at 28 °C, (35 071.96 μm^2) and the *tail* (H = 30.618, df = 2, p = 0.001) at 24 °C (16 2470.53 μm^2) (Annex Fig. 3). Whereas temperatures below T_{sel} favored sperm area, temperatures over 32 °C resulted in a striking decrease in area. At 32 °C the sperm area decreases while the luminal area increases, which is opposite to what was observed at 24 and 28 °C (Fig. 4).

We obtained the sperm count per epididymal region. The concentration found in the treatment dropped in the *body* region (γ = 0.050: 1.000; p = 0.001). Both the *head* (γ = 0.050: 0.339; p = 0.091) and *tail* regions (γ = 0.050: 0.462; p = 0.057), showed no statistical differences (Fig. 5).

The percentage of viable sperm significantly dropped at 24 °C, in every analyzed region, *head* (80.33%, γ = 0.050: 1.000; p = 0.001), *body* (72%, γ = 0.050: 1.000; p = 0.001) and *tail* (85.33%, γ = 0.050: 1.000; p = 0.001) (Fig. 5).

We found significant statistical differences in the three epididymal regions, regarding sperm normality. At 32 °C showed a lower percentage of normal sperm morphology in the *head* (γ = 0.050: 0.973; p = 0.004), *body* (γ = 0.050: 1.000; p = 0.001), and *tail* (γ = 0.050: 0.930 p = 0.006), 7.66%; 2.33% and 4.66%, respectively (Fig. 6). Our results show that a 7-day exposure to preferred temperatures (32 °C) increases sperm abnormality.

Sperm motility also showed significant difference in the *head* (γ = 0.050: 0.521; p = 0.046), *body* (H = 6.006; df = 2; p = 0.025) and *tail* (H = 7.200; df = 2; p = 0.004) regions of the epididymis. Exposure to 24 °C, showed percentages of 48% motile sperm and at 28 ° C 61% motile sperm (Fig. 6). The treatment provides evidence that temperatures below preferred temperatures (24 °C and 28 °C) favor sperm motility.

4. Discussion

Ectothermic animals tend to maintain their active body temperature within a narrow range. A common assumption is that this range of temperatures maximizes performance and fitness (Huey and Bennett 1987). Nevertheless, there is evidence that ectotherms tend to select body temperatures below the optimal temperature for locomotor performance to avoid a collapse in fitness due to the risk of overheating (Martin and Huey, 2008). Furthermore, the comparison among selected body temperatures, optimal temperatures in locomotor performance and the temperatures that maximize the reproduction patterns in males, has received limited attention. In this study, we found that the values for T_{sel} and T_o were significantly higher than the optimal temperature for several sperm traits in *S. aeneus*.

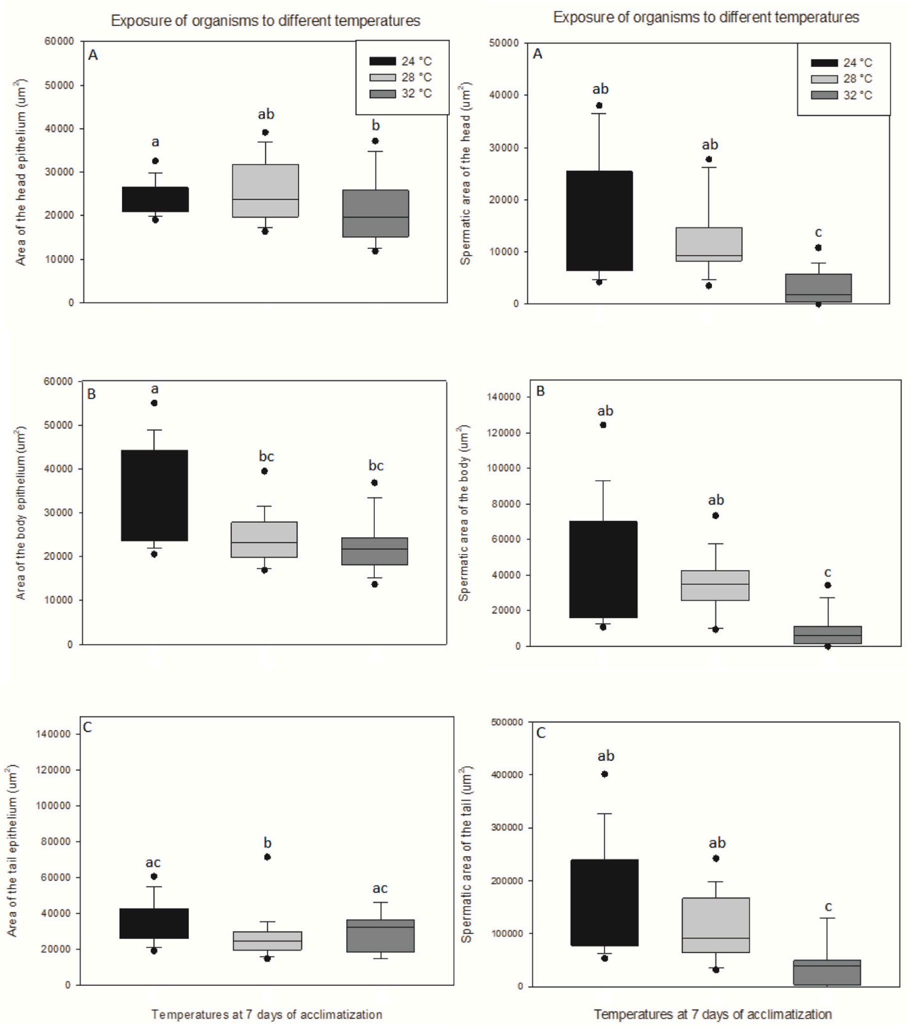

Fig. 4. Epididymal epithelium area on the left y-axis and spermatic area on the right y-axis. The three regions are represented: A. *Head*, B. *Body*, and C. *Tail*. Different letters represent significant statistical differences. Epithelium area (A. p = 0.001, B. p = 0.282, and C. p = 0.001). Sperm area (p = 0.001). Kruskal-Wallis and Dunn, n = 9.

The average T_{sel} for individuals at our study site is close to the field active body temperature, which suggests active thermoregulation of the organisms at the Coatzontle study site. The lizards regulate their behavior to keep a T_b within the T_{sel} range. Several studies on other species in the lizard family *Phrynosomatidae* have reported T_{sel} values similar to the present study (Güizado-Rodríguez et al., 2011, 2011; Ávila-Bocanegra et al., 2012; Lara-Reséndiz et al., 2014), which may suggest that the T_{sel} range is phylogenetically conserved, as proposed previously (Adolph, 1990; Andrews, 1998).

Warm temperatures (close to preferred temperatures), as our results indicate, promote efficient locomotor performance. However, these temperatures can also result in heat stress leading to unanticipated physiological repercussions (Pacak et al., 1998). Glucocorticoids are secreted in response to stress (Sapolsky et al., 2000) and this can lead to glycemia, which may alter the energy availability. It is important to point out that rising glucocorticoid levels always precedes a decrease in testosterone in males (Dhanabalan et al., 2011; García-Díaz et al., 2015).

Hyperthermia can induce short-term damage to Leydig cells, which are responsible for androgen synthesis and secretion and essential for reproductive functions. Cessation of testosterone production, by environmental factors, can lead to infertility or sterility (Papadopoulos, 2007). Previous studies show that high temperatures also alter spermatogenesis, promote multinucleated germ cell generation, block the action of gonadotropin on the seminiferous tubules, alter epithelial cycles, and cause damage to germ cells (Clegg, 1963; Dutta et al., 2013). Experiments using rodents and apes that hyperthermia can reduce sperm concentration due to suppression of spermatogenesis by apoptosis (Lue et al., 2006; Zhang et al., 2012).

The effect of tolerated and required temperatures have been long debated but it is well known that in scrotal mammals, higher temperatures can cause infertility in males (Papadopoulos 2007; Paul et al., 2008). Cowles and Burleson (1945) determined testicular damage in the males of the *Xantusia vigilis* lizards when they were exposed during a week at a maximum temperature range of 95% of T_o for locomotor performance (~36 °C) (Kaufmann and Bennett, 1989). In addition, most sperm and primary spermatocytes agglomerated, nuclear material disintegrated accompanied by increased macrophage activity, seminiferous tubules degenerated and almost complete atrophy was observed (Cowles and Burleson, 1945). In this study, we noticed that both the T_o and T_{sel} affect significatively the sperm parameters.

Our histologic analysis confirmed our observations. In the acclimation treatments, at T_{sel} and higher temperatures, the lumen of seminiferous tubules lumen decreased in the area. As a consequence, sperm concentration, viability, motility, and morphology were affected. The potential effect is the reduction in fertilization capacity. In contrast, temperatures below T_{sel} and T_o decreased the lumen of seminiferous tubules, but increased the spermatic area which represents favorable conditions for sperm.

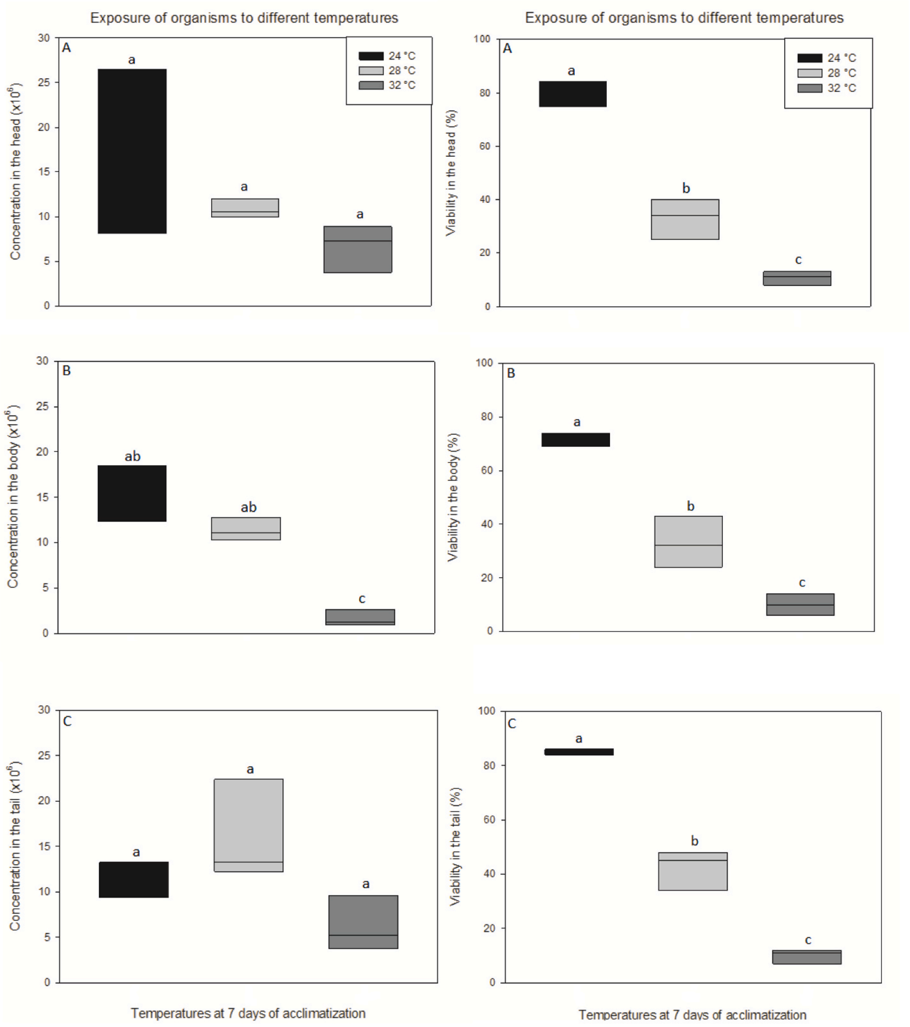

Fig. 5. Sperm concentrations (millions/epididymis) on the left y-axis and percentage of sperm viability on the right y-axis, by region (A. *Head*, B. *Body*, and C. *Tail*). Different letters represent significant statistical differences. Sperm concentration (A. $p = 0.004$, B. $p = 0.009$, and C. $p = 0.008$) and sperm viability ($p = 0.001$), according to Kruskal-Wallis and Tukey tests.

Androgens receptors are found along the epididymis in different species primarily in the main cells, although they can also be found at basal and apical cells (Zhu et al., 2001). Because main cells synthesize proteins required for sperm maturation in the epididymis, heat stress can induce lower testosterone levels, affecting epididymal epithelium cells (Arenas-Ríos et al., 2017). Hence, this damage may explain the results we obtained in the sperm parameters in the *head* and *body* regions. Therefore, sperm maturation is affected as a consequence of rising temperatures.

Temperature acts as an environmental cue of vital importance to the reproductive cycle in males (Kumar et al., 2011). In mammals, short-term exposure to high temperatures damages the seminiferous epithelium due to apoptosis and autophagia, in germ cells, and abolish spermatogenesis (Yin et al., 1997; Lue et al., 2006). However, this phenomenon is not well understood (Rockett et al., 2001). It is likely that the observed drastic effects, in the present work, are caused by temperature-induced cell death. Apart from the mentioned effects, heat stress can damage DNA (Banks et al., 2005; Paul et al., 2008) and alter oxygen levels, ion, and water transport, protein synthesis, and cell structure (Seiler et al., 2000). Our results are congruent with previous reports, that exposure to temperatures above preferred temperatures, results in a dramatic decrease in sperm concentration and damages the cells responsible for sperm production.

According to Gist et al. (2000), lower temperatures boost sperm motility and speed. This has been observed in some turtles with winter copulas. Gametes may be more active at lower temperatures which can improve fertilization success. This matches with our results, which show that temperatures under the T_{sel}, enhance sperm parameters. In contrast, lower temperatures decrease locomotor performance.

The increasing environmental temperatures, due to global warming, can affect the reproductive physiology of ectotherms, including lizards. High temperatures limit the activity periods and distribution of ectotherms (Sinervo et al., 2010). Since global warming has direct effects on precipitation, temperature variations, reproductive seasons, and alters the reproduction physiology, particularly on pregnant females (Clusella-Trullas et al., 2011), it may be a direct cause of species extinction (Méndez de la Cruz et al., 2014). Therefore, we highlight the importance of studies focusing on thermal physiology, in particular sperm physiology.

In conclusion, we confirmed the tradeoff between the fact that T_{sel} favors the locomotor performance, however, they diminish lizards' reproductive aspects which may cause infertility, endangering these species populations. *Sceloporus aeneus* must be exposed to lower T_{sel} temperatures to efficiently complete its spermatic capacity and therefore, be able to reproduce.

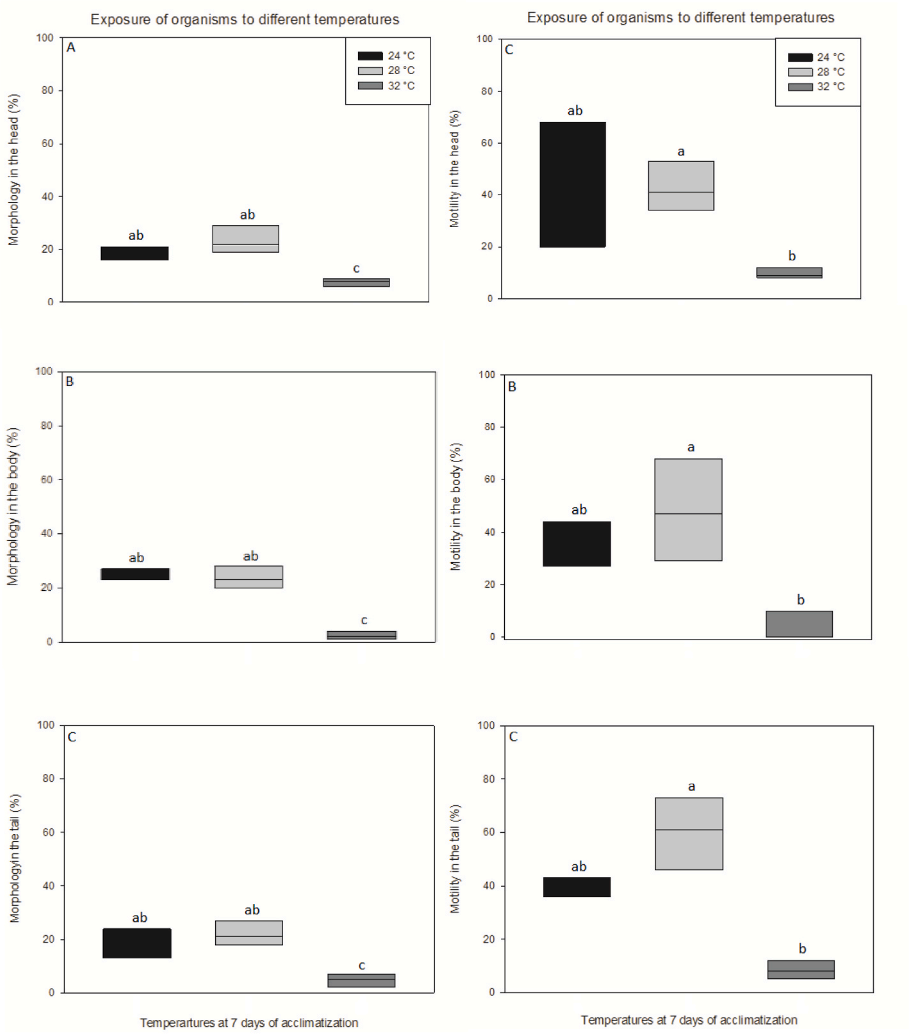

Fig. 6. Morphology of the sperm on the left y-axis and percentage of sperm motility on the right y-axis, by region (A. *Head*, B. *Body*, and C. *Tail*). Different letters represent significant statistical differences. Sperm morphology (A. $p = 0.002$, B. $p = 0.001$, and C. $p = 0.003$). Sperm motility (A. $p = 0.015$, B. $p = 0.004$, and C. $p = 0.001$) based a Kruskal-Wallis and Tukey test.

Funding

Funding for this research was provided by Universidad Nacional Autónoma de México (PAPIIT; IN 212119).

Declaration of competing interest

The authors report no conflict of interest.

Acknowledgments

We gratefully acknowledge the Posgrado in Ciencias Biológicas of the Universidad Nacional Autónoma de México. Funding for this research was provided by the Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPIIT; IN 212119). RIQP, YLR and DMAM were funded by CONACYT postgraduate scholarships (CVU 923587, 620409 and 412744 respectively). DBM was supported by NSF (DEB- 1950636). FRM was supported by DGAPA, PASPA, UNAM N° 015/2022. This work is part of the requisites for obtaining the master's degree of the Posgrado en Ciencias Biológicas (UNAM), as a first author. We are grateful to Lisette Chávez, Misael Garrido, Isaac Aguilar, and Cynthia Flores for their valuable assistance in the field work. We also thank Hugo Salinas for his help in the data analysis and to Aranza Quintana-Armas for proofreading the manuscript. We thank to Francisco Muñoz for their comments and suggestion to improve the manuscript. All lizards were collected by permission SGPA/DGVS/02523/19 issued to Fausto R. Méndez de la Cruz by the Secretaría de Gestión para la Protección Ambiente y Recursos Naturales (SEMARNAT).

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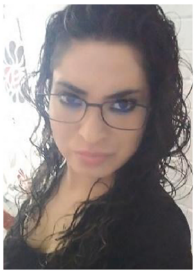

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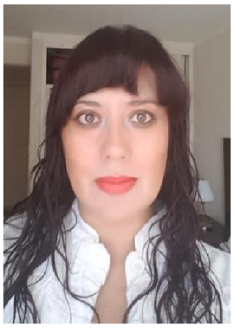

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