



ELSEVIER

Contents lists available at ScienceDirect

Journal of Thermal Biology

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

Do sex, body size and reproductive condition influence the thermal preferences of a large lizard? A study in *Tupinambis merianae*

Nicolas Rodolfo Cecchetto^a, Sergio Naretto^{b,*}^a Universidad Nacional de Córdoba, Argentina^b Instituto de Diversidad y Ecología Animal (IDEA), CONICET and Laboratorio Biología del Comportamiento, Facultad de Ciencias Exactas Físicas y Naturales, Universidad Nacional de Córdoba, Vélez Sársfield 299, Córdoba CP: X5000JC, Argentina.

ARTICLE INFO

Article history:

Received 27 May 2015

Received in revised form

26 August 2015

Accepted 13 September 2015

Available online 5 November 2015

Keywords:

Temperature preference

Reproductive condition

Tupinambis merianae

Body size

Intraspecific variation

ABSTRACT

Body temperature is a key factor in physiological processes, influencing lizard performances; and life history traits are expected to generate variability of thermal preferences in different individuals. Gender, body size and reproductive condition may impose specific requirements on preferred body temperatures. If these three factors have different physiological functions and thermal requirements, then the preferred temperature may represent a compromise that optimizes these physiological functions. Therefore, the body temperatures that lizards select in a controlled environment may reflect a temperature that maximizes their physiological needs. The tegu lizard *Tupinambis merianae* is one of the largest lizards in South America and has wide ontogenetic variation in body size and sexual dimorphism. In the present study we evaluate intraspecific variability of thermal preferences of *T. merianae*. We determined the selected body temperature and the rate at which males and females attain their selected temperature, in relation to body size and reproductive condition. We also compared the behavior in the thermal gradient between males and females and between reproductive condition of individuals. Our study show that *T. merianae* selected body temperature within a narrow range of temperatures variation in the laboratory thermal gradient, with 36.24 ± 1.49 °C being the preferred temperature. We observed no significant differences between sex, body size and reproductive condition in thermal preferences. Accordingly, we suggest that the evaluated categories of *T. merianae* have similar thermal requirements. Males showed higher rates to obtain heat than females and reproductive females, higher rates than non-reproductive ones females. Moreover, males and reproductive females showed a more dynamic behavior in the thermal gradient. Therefore, even though they achieve the same selected temperature, they do it differentially.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

In reptiles, body temperature is a key factor in physiological processes, influencing ecology and performances such as development, growth and reproduction (Huey, 1982; Huey and Pianka, 2007; Wapstra et al., 2004; Crane and Greene, 2008). Many studies examined the importance of body temperature in the ecology of lizards and determined the optimal temperatures and their effects on physiology for different species (Angilletta et al., 2002); however, intraspecific variability remains less explored (Van Damme et al., 1986; Rock et al., 2000; Huey and Pianka, 2007) propose considering the potential differences between sexes when evaluating thermal parameters. Besides differences between males and

females, lizards with different body size may vary in their thermoregulatory biology and behavior (Castilla and Bauwens, 1991). Reproductive condition may also impose specific requirements on preferred body temperatures (Van Damme et al., 1987; Schwarzkopf and Andrews, 2012).

Body size influences many aspects of an organism's life history, such as its energy needs and interactions with abiotic and biotic components of the environment (Seebacher et al., 1999; Brandt and Navas, 2011). For instance, small lizards will show a higher surface-to-volume ratio and will heat and cool faster than larger ones (Vitt and Caldwell, 2009). As a consequence, thermal inertia is positively correlated with body size in lizards (Cruz et al., 2011; Maia-Carneiro and Duarte Rocha, 2013), and may influence the temperature that individuals select and how they achieve it (Seebacher and Shine, 2004).

In temperate lizards, reproductive events are affected by environmental factors such as seasonal changes in temperature and rainfall (Fitch, 1970; Duvall et al., 1982; Fitzgerald et al., 1993).

* Corresponding author.

E-mail addresses: nrcechetto@gmail.com (N.R. Cecchetto), naretto-sergio@gmail.com (S. Naretto).

Temperature could be considered a life history modeling factor in lizards (Adolph and Porter, 1993). For example, the offspring phenotype can be influenced by body temperature selected by gravid female in both oviparous and viviparous species (Shine, 2004a, 2004b; Lorigou et al., 2012; Lorigou et al., 2013; Paranjpe et al., 2013). Temperature regulates gonad maturation and reproductive processes, activating events such as spermatogenesis (Gribbins et al., 2006). Therefore, reproductive condition can modify the thermal requirements of individuals (Rock et al., 2000; Labra and Bozinovic, 2002; Shine, 2012).

If gender, body size and reproductive condition are underlying causes for different thermal requirements, then the preferred temperature may represent a compromise that optimizes the physiological functions that are more relevant at a given time (Castilla et al., 1999; Shine et al., 2000; Huey and Pianka, 2007). Selected temperature ranges are estimated as preferred body temperatures in an environment free of ecological costs (Huey and Slatkin, 1976). Therefore, it is expected that the body temperatures selected by lizards in a controlled environment reflect a temperature that maximizes their physiological needs.

Lizards regulate their body temperature by modifying aspects of their behavior, such as alternating between hot and cold heat sources (Bowker et al., 2013). Some lizards are very active and quickly move between heat sources, whereas others adjust their body temperature by making small movements (Pianka, 1966). In a laboratory thermal gradient, we can also evaluate the thermoregulatory behavior (Cowgell and Underwood, 1979; Sievert and Hutchison, 1989; Braña, 1993) and the way they achieve the selected temperature by monitoring the body temperatures and how the animals move along the gradient (Bowker et al., 2013). Body size and reproductive condition affect the locomotive performance in lizards (Winne and Hopkins, 2006; Tang et al., 2013; Des Roches et al., 2014) and will determine the rate at which they attain the preferred temperature.

Tupinambis merianae is an interesting oviparous species for studying thermal biology, since environmental temperature is an important factor that determines its distribution and behavior (Cardozo et al., 2012; Lanfri et al., 2013). The species has seasonal reproduction, hibernating in the cold months and mating in spring (Naretto et al., 2014). This lizard also has sexual dimorphism and wide ontogenetical variation in body size and, as reported by Cury de Barros et al. (2010), body size and temperature affect its behavior.

We hypothesize that life history traits influence the thermal biology of *Tupinambis merianae*. Therefore, we expect that sex, body size and reproductive condition determine differences in the preferred temperatures and/or in the way these lizards achieve them. In the present study we evaluate intraspecific variability of thermal biology of *T. merianae*. We determined the selected temperature and the rate at which the selected temperature is attained by males and females, in relation to body size and reproductive condition. Moreover we compared the behavior in the thermal gradient between males and females and considering reproductive conditions.

2. Material and methods

2.1. Species and study area

Tupinambis merianae is a large diurnal lizard that reaches sexual maturity at 29.5 cm snout-vent length (SVL) for males and 32 cm SVL for females, with reproduction occurring between October and December (Naretto et al., 2014). The specimens used in this study were captured with the help of local people in the Espinal region (Brown et al., 2006) of central Argentina (31°28'W,

63°38'S to 31°45'W, 63°15'S). All individuals used in this study were adults collected between October and December (2012–2013).

2.2. Morphology and reproductive condition

For each individual we measured the SVL to the nearest 0.5 cm with a ruler and body mass (BM) to the nearest 50 g with a balance (Pesola Macro Line Spring, Switzerland). Sex and reproductive condition were diagnosed by inspecting gonads using portable ultrasound scanning (Sonosite 180 Plus, United State of America). Females were considered reproductive if they had vitellogenic follicles bigger than 7 mm (Cardozo et al., 2015). We were able to determine the reproductive condition in 24 of 26 females evaluated (11 reproductive and 13 non-reproductive). Males were considered reproductive if they had presence of sperm in epididymis. Males were used in spermatology study (Blengini et al., 2014) and samples of seminal fluid were examined under a phase contrast Nikon eclipse Ti microscope (Nikon Instruments Inc, Tokyo, Japan). We were able to determine the reproductive condition in 22 of 24 males evaluated (15 reproductive and 7 non-reproductive). We were authorized by the government environmental agencies for scientific capture (Resolución Córdoba N° 865/2012).

2.3. Estimation of selected body temperatures

Body temperature preference experiments were conducted two days after capture. Individuals were kept at stable room temperature (19–21 °C) before trials. Lizards were placed in an open-top terrarium (200 cm long, 50 cm wide and 50 cm high) with a layer of sand cover. Thermal gradient was produced by a line of four infrared lamps overhead (two 250 W and two 150 W), adapted from Tsai and Tu (2005) and Medina et al. (2009), and the lamps were adjusted to different heights to make a linear substratum gradient from 21 to 55 °C. Room temperature ranged from 19 to 21 °C. Lizard body temperatures were measured every 1 min during 4 h, using ultra-thin (1 mm) catheter thermocouples located approximately 20 mm inside the cloaca and fastened to the base of the lizard's tail. We used a digital thermometer (TES 1302 thermometer, TES Electrical Electronic Corp., Taipei, Taiwan, ±0.01 °C). The experiments lasted 4 hours because we observed in previous trials, that during this period, temperature stabilizes for most individuals of *Tupinambis merianae*, and because the same amount of time was used by other big reptiles to reach their preferred temperature asymptote (Di Cola, 2012). Lizards were allowed to habituate for 10 min at the beginning of each experience; therefore, temperature data obtained during this period were not considered in statistical analysis. The mean and range of the selected body temperature (T_{set}) were estimated for each individual. The set-point range (T_{set}) was considered the temperatures within the interquartile range of the observations (Barber and Crawford, 1977; Firth and Turner, 1982). For analyses we used the lower and higher margin of that range ($T_{set\ min}$ and $T_{set\ max}$, respectively). To determine the rate at which they attain the preferred temperature we calculate the heating rate. This parameter represents the mixture of behavioral and physical aspects because the lizards are free to move along the gradient. The data were reviewed to check the amount of time it took lizards to attain the T_{set} . Then we determined that the period of time for analyzing that parameter would be from minute 10 to minute 40. Therefore, rates to obtain heat was calculated with the following formula = (Body temperature °C at the 40th minute – Body temperature °C at the 10th minute)/30. Additionally, we performed trials of two reproductive females that were subjected to the thermoregulation experiment during pregnancy (oviductal calcareous eggs) and

after laying the eggs. Therefore we described variability of these trials.

2.4. Evaluation of thermoregulatory behavior

Before the trials we established and marked 5 equal zones of 40 cm long each along the gradient. We obtained the average temperature of each zone by measuring temperature at different points of each zone with a thermometer. Those average temperatures ranged from zone 1 (the coldest) to zone 5 (the hottest) in the following order: 1 (21 °C); 2 (31 °C); 3 (35 °C); 4 (41 °C); and 5 (50 °C). We recorded a video to analyze the behavior of individuals during the trial. A film recorder (Sony Digital HD, XR260V) located directly above the terrarium recorded the movements of the lizards across the gradient during each individual trial. In the obtained videos, the location of the individuals in relation to the different zones was recorded once per minute during the whole experience. We recorded the time spent by lizards in each zone before they moved to another zone.

2.5. Statistical analyses

The mean and range of T_{sel} and $T_{set\ min}$ and $T_{set\ max}$ were estimated using the values obtained for each individual lizard. The parameters T_{sel} , $T_{set\ min}$ and $T_{set\ max}$ were compared between sexes and between reproductive conditions within each sex with an ANCOVA using SVL as a covariate, and ANOVA when the covariate was non-significant. Rate to obtain heat was compared between sexes and between reproductive conditions within sexes using ANCOVA, with SVL as a covariate. Thermal parameters were regressed on SVL. Amount of time spent in the different zones of the gradient was compared between sexes and between reproductive conditions within sexes by estimating frequency distribution, using a Chi square test. Finally, the average amount of time spent on the same zone of the gradient before moving to another zone was compared between males and females and between reproductive conditions in each sex. All statistical analyses were done using the software INFOSTAT, 2012 version (Universidad Nacional de Córdoba) and SPSS16.0 (SPSS 16.0 Inc., Chicago, IL, USA).

Table 1

Thermal preferences of males and females of *Tupinambis merianae* (T_{sel} and T_{set} , mean and S.D.). SVL as a covariate was non-significant when compared with ANCOVA.

	Males	Females	F	ANOVA P
T_{sel} (°C) ± S.D.	36.42 ± 1.18	36.07 ± 1.73	$F_{1,48}=0.67$	0.416
$T_{set\ min}$ (°C) ± S.D.	35.92 ± 1.37	35.02 ± 2.91	$F_{1,48}=1.89$	0.175
$T_{set\ max}$ (°C) ± S.D.	37.15 ± 1.05	37.27 ± 1.44	$F_{1,48}=0.10$	0.758
C.V.	3.16	5.09		
N	24	26		

Table 2

Thermal preferences for different reproductive conditions in males and females of *Tupinambis merianae* (T_{sel} and T_{set} , mean and S.D.).

	Males		F	ANOVA P	Females		F	ANOVA P
	Reproductive	Non-Reproductive			Reproductive	Non-Reproductive		
T_{sel} (°C) ± S.D.	36.28 ± 1.40	36.81 ± 0.62	$F_{1,20}=0.90$	0.353	36.51 ± 1.69	35.55 ± 1.77	$F_{1,22}=1.81$	0.192
$T_{set\ min}$ (°C) ± S.D.	35.75 ± 1.57	36.49 ± 0.67	$F_{1,20}=1.38$	0.254	35.92 ± 1.76	33.97 ± 3.51	$F_{1,22}=2.79$	0.109
$T_{set\ max}$ (°C) ± S.D.	37.02 ± 1.26	37.43 ± 0.58	$F_{1,20}=0.65$	0.428	37.41 ± 1.40	37.05 ± 1.53	$F_{1,22}=0.36$	0.553
C.V.	3.25	2.69			4.23	6.01		
N	15	7			11	13		

3. Results

3.1. Selected body temperatures

Tupinambis merianae selected their body temperature between 36.24 ± 1.49 °C ($N=50$). The values of T_{sel} did not vary between males and females (Table 1). Values for $T_{set\ min}$ and $T_{set\ max}$ were not different between sexes (Table 1). Temperature parameters did not differ between reproductive conditions (Table 2) but we observed higher values in the coefficient of variation in reproductive females compared to non-reproductive females. Table 3.

We did not find a relationship between T_{sel} and SVL in *T. merianae* (Linear regression: $F_{1,48}=2.53$; $R^2=0.05$; $P=0.118$). The relationship between T_{sel} and SVL was also not significant within each sex (Linear regression: T_{sel} males vs SVL $F_{1,24}=2.15$; $P=0.155$; Linear regression: T_{sel} females vs SVL $F_{1,22}=1.78$; $P=0.196$). Regressions between $T_{set\ min}$ and SVL, and between $T_{set\ max}$ and SVL were not significant (Linear regression: $T_{set\ min}$ vs. SVL $F_{1,48}=2.70$; $P=0.107$; $T_{set\ max}$ vs. SVL $F_{1,48}=0.80$; $P=0.375$).

Rate to obtain heat in the gradient was different between sexes, being higher for males (Males= 0.28 ± 0.08 °C/min; Females= 0.23 ± 0.09 °C/min; ANCOVA: sex effect term $F_{1,37}=8.06$, $P=0.007$; covariate SVL term $P < 0.001$). We observed a negative relationship between SVL and heating rate in males and females, (Males: $F_{1,20}=19.01$; $P=0.001$; $R^2=0.49$; Females: $F_{1,16}=5.56$; $P=0.031$; $R^2=0.26$). The slope was similar between sexes (ANCOVA: interaction effect $F_{2,37}=13.38$; $P < 0.001$). Larger lizards had lower rates to obtain heat than smaller ones (Fig. 1).

For males, rate to obtain heat was similar between reproductive conditions (reproductive males= 0.28 ± 0.07 °C/min ± S.D.; non-reproductive males= 0.28 ± 0.11 °C/min ± D.E; ANCOVA $F_{2,17}=8.99$; $P=0.686$; Covariate $P < 0.001$; $N=20$). Conversely, reproductive females had higher rates to obtain heat than non-reproductive females, with values of reproductive females being similar to those found in males (reproductive females= 0.28 ± 0.04 °C/min ± S.D.; non-reproductive females= 0.18 ± 0.03 °C/min ± S.D.; ANCOVA $F_{2,13}=10.03$; $P=0.012$; Covariate $P=0.039$; $N=16$).

The thermoregulation experiment was conducted with two gravid females (presence of calcareous eggs in the oviduct) before and after laying their eggs. Even though only one female changed her T_{sel} before laying eggs comparing with after lying eggs, we highlight the difference (for both females) in the stability of the selection of temperature while gravid, in comparison with the post-laying selection (Fig. 2).

In both sexes, frequency distribution of the zones used by lizards reach the T_{sel} was significantly different between reproductive conditions (Males: Chi Square Test: $\chi^2=183.4$; d.f.=4; $P < 0.001$; Females: Chi Square Test: $\chi^2=133.15$; d.f.=4; $P < 0.001$). Reproductive individuals achieved higher frequencies in the cooler and hotter zones (zones 1 and 5, respectively, 60% of the time), whereas non-reproductive individuals spent more time in zones 1 and 2 (60% of the time), showing a similar pattern for both sexes (Fig. 3).

Table 3
Heating rates in males and females of *Tupinambis merianae*.

	Males	Females	F	ANCOVA P and covariate (SVL) P
Heating rate mean ($\Delta^{\circ}\text{C}/\text{min}$) \pm D.E.	0.28 \pm 0.08	0.23 \pm 0.09	$F_{1,37}=8.06$	0.007 < 0.001
Heating rate Q3 ($\Delta^{\circ}\text{C}/\text{min}$)	0.33	0.27	$F_{1,47}=0.01$	0.932 0.408
Heating rate Q1 ($\Delta^{\circ}\text{C}/\text{min}$)	0.23	0.22	$F_{1,47}=3.68$	0.061 0.039
N	22	18		

In both sexes, mean lizard body temperature was similar in all the occupied zones (ANCOVA Males: $F_{5,87}=2.63$; $P=0.989$; Covariate SVL $P<0.001$; ANCOVA Females: $F_{5,65}=1.75$; $P=0.908$; Covariate SVL $P<0.001$). The time spent in the same zone before moving to another was similar for both reproductive condition in males (reproductive males = 5.83 ± 6.65 minutes \pm S.D.; non-reproductive males = 4.01 ± 1.21 minutes \pm S.D.; ANOVA $F_{1,17}=0.36$; $P=0.557$; $N=19$) and females (reproductive females = 3.16 ± 0.93 minutes \pm S.D.; non-reproductive females = 27.29 ± 57.34 minutes \pm S.D.; ANOVA $F_{1,12}=1.24$; $P=0.287$; $N=14$).

4. Discussion

Our results show similarity of thermal preferences with respect to sex, body size and reproductive condition, suggesting that these lizards have similar thermal requirements for a broad range of physiological functions. *Tupinambis merianae* selected body temperature within a narrow range of variation in a laboratory thermal gradient, the preferred temperature being $36.2 \pm 1.49^{\circ}\text{C}$. Moreover, there were no differences in thermal preferences (T_{set} and T_{set}) between sexes, body sizes and reproductive conditions. The selected body temperature may be a trade-off among several conflicting thermal priorities (Huey and Slatkin, 1976). Most aspects of the behavior and physiology of lizards are sensitive to body temperature (Huey and Stevenson, 1979; Huey, 1982), influencing the performance of individuals. For example, in males of *T. merianae*, aggression and bite performance are crucial because more aggressive individuals are often better competitors for

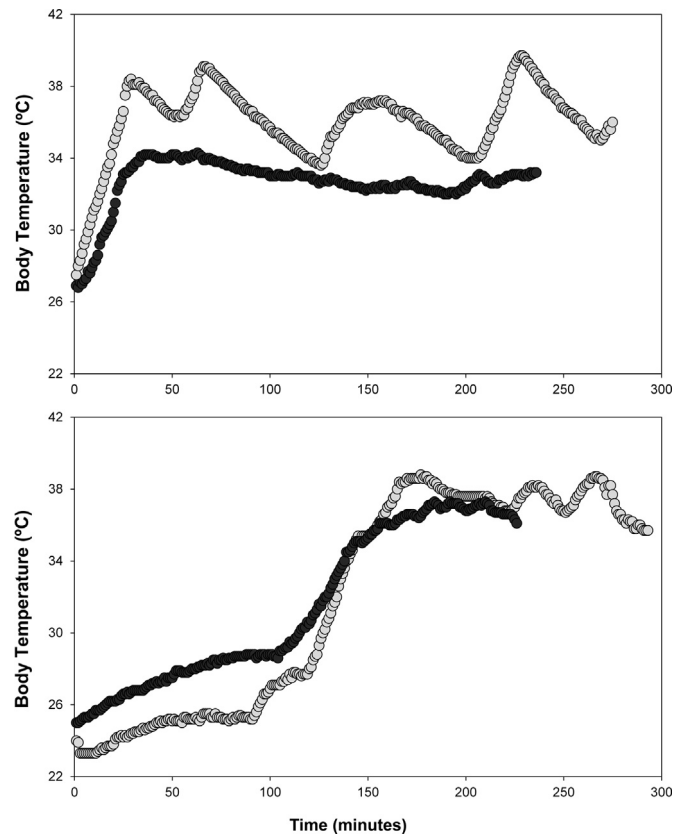


Fig. 2. Thermoregulation experience of two gravid females of *Tupinambis merianae*, before (black) and after laying their eggs (grey). Female of 36 cm SVL (above) and 42 cm SVL (below).

limited resources such as mates (Herrel et al., 2009). Large individuals of *T. merianae* are more aggressive and have bigger jaw muscle and a stronger bite than small individuals at the same temperature (Herrel et al., 2009; Naretto et al., 2014). However, Cury de Barros et al. (2010) found more behavioral differences between small and large males at body temperatures lower than 36°C (temperature near the T_{set} is obtained in this study). Thermal preferences are usually related to temperatures at which

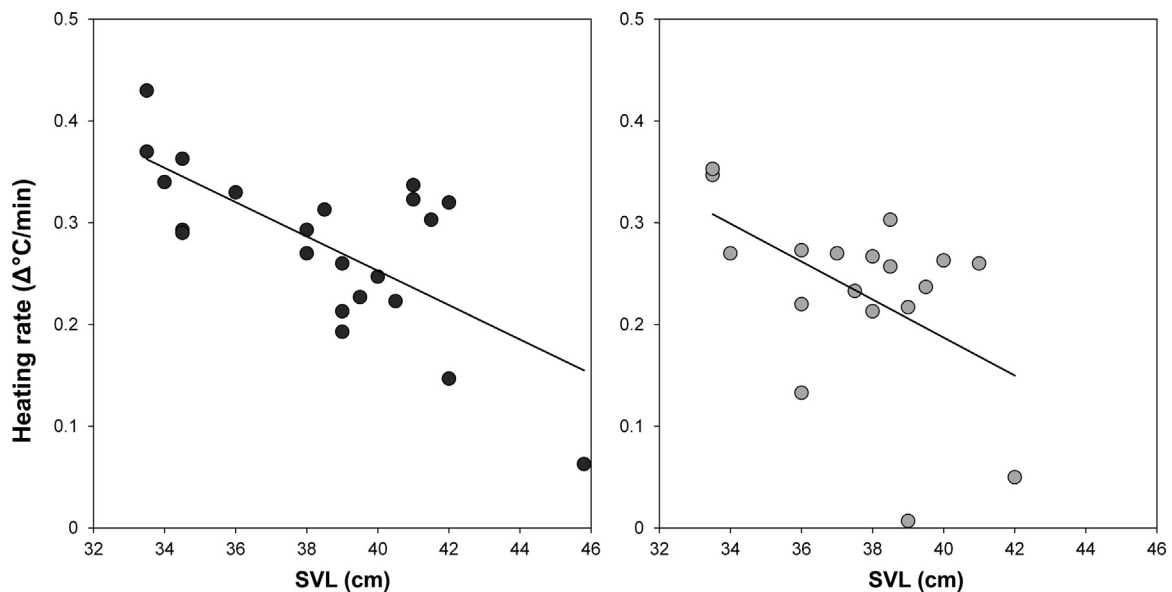


Fig. 1. Relationship between SVL and rate to obtain heat ($\Delta^{\circ}\text{C}/\text{min}$) in males (black) and females (grey) of *Tupinambis merianae*.

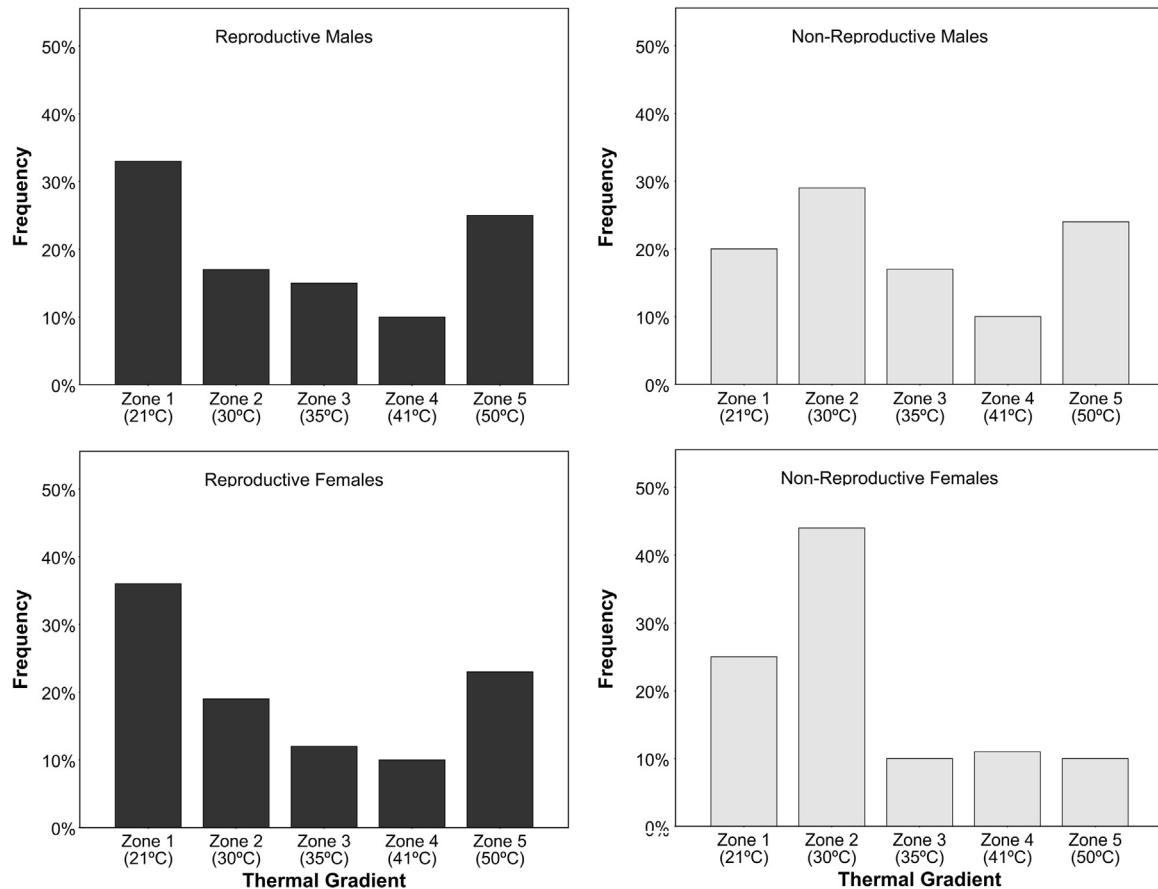


Fig. 3. Frequency distribution of time spent in different zones of the gradient of reproductive (black) and non-reproductive (grey) males (above) and females (down) of *Tupinambis merianae*.

physiological performance is also near optimal (Huey and Bennett 1987; Angilletta 2001). Therefore, differences in performance among individuals could also be minimized at the preferred temperature.

Although we did not observe differences in the mean T_{sel} , we observed a tendency to lower variability in reproductive females than in non-reproductive. We also observed differences in the thermal experiment of the same female before and after egg laying, where the parameter (T_{sel}) was more stable in gravid females than post-posture condition. Although both results are tentative due to low sample size, these observations suggest the importance of stability over thermal preference. New questions arise about the importance in gravid females of *T. merianae* to maintain less variable temperature than non-gravid as postulated in viviparous reproductive mode (Shine, 2004a, 2004b; Lourdaís et al., 2004; Crane and Greene, 2008).

The species distribution models showed that temperature shapes the ecological niche of *Tupinambis merianae* (Lanfri et al., 2013). Similar sized *Tupinambis nigropunctatus* showed similar thermal preferences (Bennett and John-Alder, 1984), even though this species has a more tropical distribution than our study species. A comparative study of performance among populations of species with large distributions such as *T. merianae*, which inhabits different latitudes and biogeographic regions, would contribute to the understanding of the relationship between thermal preference and environment. Similar thermal preferences were also found in similar-sized lizards, such as *Varanus* species in subtropical areas of Australia (Christian and Weavers, 1996). Curiously, smaller lizards inhabiting a completely different habitat, such as the Puna region or the Patagonia region, highland areas, showed similar

values of T_{sel} (Moreno Azócar et al., 2013; Valdecantos et al., 2013). Whereas the cost for maintaining body temperature would be very variable in different species, the preferred temperature of *T. merianae* is a usual temperature found in many in lizards. This information denotes the importance of comparative interspecific studies to evaluate the influence of the evolutionary history, body size and environments on thermal preferences and thermoregulation behaviors (Moreno Azócar et al., 2013).

Rate to obtain heat of *T. merianae* lizards is negatively related to body size in both sexes and in both reproductive conditions. Larger lizards took longer to obtain the selected body temperature, which is in agreement with the physics of thermal biology, because larger individuals have less relative available surface than smaller ones and more body mass to heat (Vitt and Caldwell, 2009; Cruz et al., 2011; Maia-Carneiro and Duarte Rocha, 2013). On the other hand, reproductive females presented higher a rate to attain preferred temperature than non-reproductive females. Physiological thermal requirements of reproductive condition affect offspring fitness (Martin and Huey, 2008); hence, reproductive females could require reaching T_{sel} as soon as possible to optimize gonadal development. These differences in rates to attain the preferred temperature affect basking behavior in the sense that individuals with lower rates will have to start basking earlier, for longer periods, or both (Seebacher and Shine, 2004). Differences in the mode of heating may have ecological implications, such as greater exposure or less time for activities. Moreover, the differences in heating rate is correlated with differences in thermal inertia in the sense that, by the physical properties of heat dissipation, larger objects will retain heat for longer than smaller objects (Garrick, 2008). Hence, larger individuals might maintain values close to T_{sel} for longer periods. This would allow

them to spend more time after basking without heat exposure, which could in turn improve performance in activities such as competition for mating, making these lizards excellent thermoregulators. If smaller males may offset the competition ability by reaching selected temperature faster, interaction between body size and strategies of thermoregulation could impose differences in daily activity and competition.

Lizards are subject to ecologic restrictions that affect thermoregulation constantly, such as climatic factors that affect reproductive cycles in seasonal species (Fitzgerald et al., 1993) and the structural characteristics of the environment (Crane and Greene, 2008), which is heterogeneous for *T. merianae* in our study area (Cardozo et al., 2012). Moreover, different sizes and reproductive conditions impose different restrictions on lizards to obtain the same preferred temperature in the field. Therefore, if lizards with different constraints need to reach the same temperature, they may appeal to different thermoregulation strategies in the field (different basking times, differential microsite selection and different basking behavior). Whereas knowledge of T_{sel} is useful for understanding thermoregulation, extrapolating the data from laboratory measurements to an environmental context is difficult. Further investigation is required, involving body and operative temperatures in the field, in order to determine accuracy, precision and effectiveness in thermoregulation of males and females of different reproduction conditions. In this work we have made an approach to thermoregulation behavior. Regarding time spent in each zone of the thermal gradient, histograms showed a similar tendency among reproductive individuals of both sexes to spend most of the time alternating between the ends of the gradient (the coldest and the hottest). We highlight that this shifting from one spot of thermal offer to another during the experiment instead of lizards staying in a heat source close to T_{sel} , suggests that there might be a more active thermoregulatory behavior in reproductive *T. merianae*. Finally, the comparison of time spent in a zone of the gradient before moving to another showed a tendency of a more dynamic behavior in males and reproductive females than in non-reproductive females. The amount of movement is related to body size and physiology, site choice, and the thermal characteristics of the zone or environment (Bowker, 1984). This reinforces the previous observation that, even though they share T_{sel} , the way they achieve it is different.

Thermoregulation is a complex process that involves many variables and is in turn affected by many factors, both internal and external. This fact proposes new challenges in the study of mechanisms used by lizards to obtain and retain body heat. This study found that intrinsic factors such as sex, body size and reproductive condition do not influence thermal preference in this species, but may play a role in lizard behavior to acquire the preferred temperature.

Acknowledgements

We are grateful to rural people from the study area for their invaluable assistance in the field, especially Carlos Beck of Villa del Rosario, Córdoba. We thank very much Dra. Vale Di Cola for her motivation to perform thermal experiences. We also thank biologist Mitchell Scott for his assistance with the English language and advice. This study was funded by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) N° 314, Fondo para la Investigación Científica y Tecnológica (FONCYT) BID-PICT N° 2011–1599, Secretaría de Ciencia y Tecnología (SeCyT) N° 05/I624 2011. NRC is biologist of the Universidad Nacional de Córdoba. SN is postdoctoral researcher and fellowship holders of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

References

- Adolph, S.C., Porter, W.P., 1993. Temperature, activity, and lizard life-histories. *Am. Nat.* 142, 273–295.
- Angilletta, M.J., 2001. Thermal and physiological constraints on energy assimilation in a widespread lizard (*Sceloporus undulatus*). *Ecology* 82, 3044–3056.
- Angilletta, M.J., Niewiarowski, P.H., Navas, C.A., 2002. The evolution of thermal physiology in ectotherms. *J. Therm. Biol.* 27, 249–268.
- Barber, B.J., Crawford Jr, E.C., 1977. A stochastic dual-limit hypothesis for behavioral thermoregulation in lizards. *Physiol. Zool.* 50, 53–60.
- Bennett, A.F., John-Alder, H.B., 1984. The effect of body temperature on the locomotory energetics of lizards. *J. Comp. Physiol.* 155, 21–27.
- Blengini, C.S., Naretto, S., Cardozo, G., Gjojalas, L.C., Chiaraviglio, M., 2014. Variability in sperm form and function in the context of sperm competition risk in two *Tupinambis* lizards. *Ecol. Evol.* 4, 4080–4092.
- Brandt, R., Navas, C.A., 2011. Life-History Evolution on Tropidurinae Lizards: Influence of Lineage, Body Size and Climate. *Plos One* 6, e20040.
- Braña, F., 1993. Shifts in body temperature and escape behaviour of female *Podarcis muralis* during pregnancy. *Oikos* 66, 216–222.
- Brown, A., Martínez Ortiz, U., Acerbi, M., Corcuera, J., 2006. La Situación Ambiental Argentina 2005. Ed Fundación Vida Silvestre Argentina, Buenos Aires.
- Bowker, R.G., 1984. Precision of thermoregulation of some African lizards. *Physiol. Zool.* 57, 401–412.
- Bowker, R.G., Bowker, G.E., Wright, C.L., 2013. Thermoregulatory movement patterns of the lizard *Podarcis carbonelli* (Lacertilia: Lacertidae). *J. Therm. Biol.* 38, 454–457.
- Cardozo, G., Naretto, S., Zak, M., Chiaraviglio, M., 2012. The Role of landscape in contact zones of sister species of lizards, in: Tiefenbach (Ed.), Perspectives on Nature Conservation, Patterns, Pressures and Prospects. (<http://www.in-techweb.org>), Croatia, pp.161–176.
- Cardozo, G., Naretto, S., Blengini, C.S., Chiaraviglio, M., 2015. Phenotypic diversity in female body shape is related to reproductive potential in *Tupinambis merianae* lizards. *Ann. Zool. Fenn.*, 52.
- Castilla, A.M., Bauwens, D., 1991. Thermal biology, microhabitat selection, and conservation of the insular lizard *Podarcis hispanica atrata*. *Oecologia* 85, 366–374.
- Castilla, A.M., Van Damme, R., Bauwens, D., 1999. Field body temperatures, mechanisms of thermoregulation and evolution of thermal characteristics in lacertid lizards. *Nat. Croat.* 8, 253–274.
- Christian, K.A., Weavers, B.W., 1996. Thermoregulation of monitor lizards in Australia: an evaluation of methods in thermal biology. *Ecol. Monogr.* 66, 139–157.
- Cowgell, J., Underwood, H., 1979. Behavioral thermoregulation in lizards: A circadian rhythm. *J. Exp. Zool.* 210, 189–194.
- Cury de Barros, F., de Carvalho, J.E., Abe, A.S., Kohlsdor, T., 2010. Fight versus flight: the interaction of temperature and body size determines antipredator behaviour in tegu lizards. *Anim. Behav.* 79, 83–88.
- Crane, A.L., Greene, B.D., 2008. The effect of reproductive condition on thermoregulation in female *Agkistrodon piscivorus* near the northwestern range limit. *Herpetologica* 64, 156–167.
- Cruz, F.B., Antenucci, D., Luna, F., Abdala, C.S., Vega, L.E., 2011. Energetics in Lio-laemini lizards: implications of a small body size and ecological conservatism. *J. Comp. Physiol.* 181, 373–382.
- Des Roches, S., Torresdal, J., Morgan, T.W., Harmon, L.J., Rosenblum, E.B., 2014. Beyond black and white: divergent behaviour and performance in three rapidly evolving lizard species at White Sands. *Biol. J. Lin. Soc.* 111, 169–182.
- Di Cola, V., 2012. Significado ecológico y evolutivo de los requerimientos de hábitat de ofidios (Serpentes) neotropicales. Universidad de Córdoba, Argentina, Tesis de Doctorado.
- Duvall, D., Guillette Jr, L.G., Jones, R.E., 1982. Environmental control of reptilian reproductive cycles. In: Gans, C., Pough, F.H. (Eds.), *Biology of the Reptilia* Vol 13. Academic Press, New York, pp. 210–231.
- Firth, B.T., Turner, J.S., 1982. Sensory, neural, and hormonal aspects of thermoregulation. In: Gans, C., Pough, F.H. (Eds.), *Biology of the Reptilia*. Academic Press, London, pp. 213–274.
- Fitch, H.S., 1970. Reproductive cycles in lizards and snakes. 52. *Univ. Kansas Mus. Nat. Hist. Publ.*, pp. 1–247.
- Fitzgerald, L.A., Cruz, F.B., Perotti, G., 1993. The reproductive cycle and the size at maturity of *Tupinambis rufescens* (Sauria: Teiidae) in the dry Chaco of Argentina. *J. Herpetol.* 27, pp. 70–78.
- Garrick, D., 2008. Body surface temperature and length in relation to the thermal biology of lizards. *Biosci. Horizons* 1, 136–142.
- Gribbins, K.M., Elsey, R.M., Gist, D.H., 2006. Cytological evaluation of the germ cell development strategy within the testis of the American alligator. *Alligator mississippiensis*. *Acta Zool.* 87, 59–69.
- Herrel, A., Andrade, D.V., de Carvalho, J.E., Brito, A., Abe, A., Navas, C., 2009. Aggressive behavior and performance in the Tegu Lizard *Tupinambis merianae*. *Physiol. Biochem. Zool.* 82, 680–685.
- Huey, R.B., Slatkin, M., 1976. Cost and benefits of lizard thermoregulation. *Q. Rev. Biol.* 51, 363–384.
- Huey, R.B., Stevenson, R.D., 1979. Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. *Am. Zool.* 19, 357–366.
- Huey, R.B., 1982. Temperature, physiology, and the ecology of reptiles. In: Gans, C., Pough, F.H. (Eds.), *Biology of the Reptilia*. Academic Press, London, pp. 25–91.
- Huey, R.B., Bennett, A.F., 1987. Phylogenetic studies of coadaptation: preferred temperatures versus optimal performance temperatures of lizards. *Evolution* 4,

- 1098–1115.
- Huey, R.B., Pianka, E.R., 2007. Lizard thermal biology: do genders differ? *Am. Nat.* 170, 473–478.
- Labra, A., Bozinovic, F., 2002. Interplay between pregnancy and physiological thermoregulation in *Liolaemus* lizards. *Ecoscience* 9, 421–426.
- Lanfry, S., Di Cola, V., Naretto, S., Chiaraviglio, M., Cardozo, G., 2013. Understanding the ecological niche to elucidate spatial strategies of the southernmost *Tupinambis* lizards. *Amphibia-Reptil.* 34, 551–565.
- Lorioux, S., DeNardo, D.F., Gorelick, R., Lourdaís, O., 2012. Maternal influences on early development: preferred temperature prior to oviposition hastens embryogenesis and enhances offspring traits in the Children's python, *Antaresia childreni*. *J. Exp. Biol.* 215, 1346–1353.
- Lorioux, S., Vaugoyeau, M., Denardo, D.F., Clobert, J., Guillon, M., Lourdaís, O.B., 2013. Stage dependence of phenotypical and phenological maternal effects: insight into Squamate reptile reproductive strategies. *Am. Nat.* 182, 398–409.
- Lourdaís, O., Shine, R., Bonnet, X., Guillon, M., Naulleau, G., 2004. Climate affects embryonic development in a viviparous snake. *Vipera aspis*. *Oikos* 104, 551–560.
- Maia-Carneiro, T., Duarte Rocha, C.F., 2013. Influences of sex, ontogeny and body size on the thermal ecology of *Liolaemus lutzae* (Squamata, Liolaemidae) in a restinga remnant in southeastern Brazil. *J. Therm. Biol.* 38, 41–46.
- Martin, T.L., Huey, R.B., 2008. Why "Suboptimal" is Optimal: Jensen's inequality and ectotherm thermal preferences. *Am. Nat.* 171, 102–118.
- Medina, M., Gutierrez, J., Sclaro, A., Ibarguengoytia, N., 2009. Thermal responses to environmental constraints in two populations of the oviparous lizard *Liolaemus bibronii* in Patagonia, Argentina. *J. Therm. Biol.* 34, 32–40.
- Moreno Azócar, D.L., Vanhooydonck, B., Bonino, M., Perotti, M.G., Abdala, C., Schulte, J.A., Cruz F.B., 2013. Chasing the Patagonian sun: comparative thermal biology of lizards of the *Liolaemus goetschi* group (Liolaemini: Iguania) *Oecologia* 171, 773–788.
- Naretto, S., Cardozo, G., Blengini, C., Chiaraviglio, M., 2014. Sexual selection and dynamic of jaw muscle in *Tupinambis* lizards. *Evol. Biol.* 41, 192–200.
- Paranjpe, D.A., Bastiaans, E., Patten, A., Cooper, R.D., Sinervo, B., 2013. Evidence of maternal effects on temperature preference in side-blotched lizards: implications for evolutionary response to climate change. *Ecol. Evol.* 3, 1977–1991.
- Pianka, E.R., 1966. Convexity, desert lizards, and spatial heterogeneity. *Ecology* 47, 1055–1059.
- Rock, J., Andrews, R.M., Cree, A., 2000. Effects of reproductive condition, season, and site on selected temperatures of a viviparous gecko. *Physiol. Biochem. Zool.* 73, 344–355.
- Schwarzkopf, L., Andrews, R.M., 2012. Are moms manipulative or just selfish? Evaluating the "maternal manipulation hypothesis" and implications for life history studies of reptiles. *Herpetologica* 68, 147–159.
- Seebacher, F., Grigg, G.C., Beard, L.A., 1999. Crocodiles as dinosaurs: behavioural thermoregulation in very large ectotherms leads to high and stable body temperatures. *J. Exp. Biol.* 202, 77–86.
- Seebacher, F., Shine, R., 2004. Evaluating thermoregulation in reptiles: the fallacy of the inappropriately applied method. *Physiol. Biochem. Zool.* 77, 688–695.
- Shine, R., Harlow, P.S., Elphick, M.J., Olsson, M.M., Mason, R.T., 2000. Conflicts between courtship and thermoregulation: the thermal ecology of amorous male garter snakes (*Thamnophis sirtalis parietalis*). *Physiol. Biochem. Zool.* 73, 508–516.
- Shine, R., 2004a. Seasonal shifts in nest temperature can modify the phenotypes of hatchling lizards, regardless of overall mean incubation temperature. *Func. Ecol.* 18, 43–49.
- Shine, R., 2004b. Does viviparity evolve in cold climate reptiles because pregnant females maintain stable (not high) body temperatures? *Evolution* 58, 1809–1818.
- Shine, R., 2012. Manipulative mothers and selective forces: the effects of reproduction on thermoregulation in reptiles. *Herpetologica* 68, 289–298.
- Sievert, L.M., Hutchison, V.H., 1989. Influences of season, time of day, light and sex on the thermoregulatory behaviour of *Crotaphytus collaris*. *J. Therm. Biol.* 14, 159–165.
- Tang, X.L., Yue, F., He, J.Z., Wang, N.B., Ma, M., Mo, J.R., Chen, Q., 2013. Ontogenetic and sexual differences of thermal biology and locomotor performance in a lacertid lizard, *Eremias multiocellata*. *Zoology* 116, 331–335.
- Tsai, T., Tu, M., 2005. Postprandial thermophily of Chinese green tree vipers, *Trimeresurus s. stejnegeri*: Interfering factors on snake temperature selection in a thigmothermal gradient. *J. Therm. Biol.* 30, 423–430.
- Valdecantos, S., Martínez, V., Lobo, F., Cruz, F.B., 2013. Thermal biology of *Liolaemus* lizards from the high Andes: Being efficient despite adversity. *J. Therm. Biol.* 38, 126–134.
- Van Damme, R., Bauwens, D., Verheyen, R.F., 1986. Selected body temperatures in the lizard *Lacerta vivipara*: variation within and between populations. *J. Therm. Biol.* 11, 219–222.
- Van Damme, R., Bauwens, D., Verheyen, R.F., 1987. Thermoregulatory responses to environmental seasonality by the lizard *Lacerta vivipara*. *Herpetologica* 43, 405–415.
- Vitt, L.J., Caldwell, J.P., 2009. *Herpetology: An Introductory Biology of Amphibians and Reptiles*, third ed. Academic Press, San Diego.
- Wapstra, E., Olsson, M., Shine, R., Edwards, A., Swain, R., Joss, J.M., 2004. Maternal basking behaviour determines offspring sex in a viviparous reptile. *Proc. R. Soc. Lond.* 271, 230–232.
- Winne, C.T., Hopkins, W.A., 2006. Influence of sex and reproductive condition on terrestrial and aquatic locomotor performance in the semi-aquatic snake *Seminatrix pygaea*. *Funct. Ecol.* 20, 1054–1061.



Nicolas Cecchetto. Biologist graduated in 2014, Universidad Nacional de Córdoba, Argentina. Currently working at Universidad Nacional del Comahue. My field of study includes thermal biology and ecology in lizards. I am especially interested in thermoregulation, supercooling and freezing strategies in reptiles facing extremely cold weather.



Sergio Naretto. Ph.D. degree in 2014, Universidad Nacional de Córdoba, Argentina. I am a Postdoctoral fellow (CONICET). My fields of study include evolutionary biology, sexual selection and ecology in lizard models. My PhD Thesis was about reproductive strategies of *Tupinambis merianae* and *Tupinambis rufescens*. I am particularly interested in questions pertaining to sexual selection, secondary sexual traits and animal personality.