

Do female reproductive stage and phenotype influence thermal requirements in an oviparous lizard?



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ABSTRACT

Reproduction is an energetically costly activity that can alter thermal requirements in Squamata. This phenomenon has been largely studied in many viviparous species; however, little is known about the role of oviparous females in controlling temperature during vitellogenesis and embryo development before oviposition. Diversity of female phenotypes could be associated with diversity of thermoregulatory performance, since thermal requirements are frequently influenced by body shape. Furthermore, studying the thermoregulatory behaviour is fundamental to understand how females regulate their body temperature. Here, we aimed to determine whether reproduction alters thermal requirements in an oviparous model, *Tropidurus spinulosus*, and if the preferred body temperature and thermoregulatory behaviour vary among reproductive stages and in relation to the female phenotype. We characterized the female phenotypes according to multiple morphological traits (snout-vent length, inter-axillar length, abdominal perimeter, body condition and head width). Reproductive structures were diagnosed by portable ultrasound scanning. To determine body temperatures, the lizards were placed in an open-top terrarium, where a thermal gradient ranging from 18 to 50 °C was set up; we also recorded the permanence of individuals in cold, temperate and warm zones during each experiment. Reproductive females preferred higher temperatures and presented lower amplitude in thermal variability than non-reproductive females. Moreover, within reproductive females, females with wide abdominal perimeter and high body condition showed thermal stability. Females with oviductal eggs tended to select higher temperature and also show more thermal stability than females with vitellogenic follicles. The latter females used the temperate zone most frequently and to a lesser degree the warm one, whereas females with oviductal eggs used the temperate and warm zones with similarly high frequency. We conclude that reproductive stage and phenotypic traits influence the selection of preferred body temperatures and thermoregulatory behaviour in *T. spinulosus* females.

1. Introduction

Reproduction is an energetically costly activity that has the potential to alter the thermal preference of ectotherms (Graves and Duvall, 1993; Le Galliard et al., 2003; Dayananda et al., 2017). Thermoregulatory patterns can be influenced by physiological condition, with the possible occurrence of shifts in an animal's preferred body temperature during particular periods, such as breeding season (Gardner-Santana and Beaupre, 2009). Since body temperature is a key factor in physiological processes linked to reproduction (Huey, 1982; Huey and Pianka, 2007; Wapstra et al., 2004; Crane and Greene, 2008), the ability of ectotherms to maintain a particular range of body temperatures within the variation of the environmental temperatures is often critical for the reproductive performance of the individual (Huey and

Stevenson, 1979; Angilletta et al., 2002). In many viviparous species of Squamata, pregnancy may affect the selection of body temperature (Smith et al., 1993; Andrews et al., 1999; Rock et al., 2000; Labra and Bozinovic, 2002; Shine, 2004; Chiaraviglio, 2006); however, little is known about how reproductive processes modify female thermal requirements in oviparous species. Oviparous individuals are able to retain eggs in utero during a considerable period of embryogenesis (Shine, 1983; Braña et al., 1991; Blackburn, 1995). Consequently, it is interesting to know the role of oviparous females in controlling the temperature of developing embryos before oviposition (see review in Rodríguez-Díaz and Braña, 2011; Schwarzkopf and Andrews, 2012). In some species of oviparous lizards, gestating females exhibit different body temperatures from those of non-gestating females and/or males (Woolrich-Piña et al., 2015).

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Reproductive processes have an effect not only on mean incubation temperatures but also on the diel distribution of those temperatures (Shine, 2004). Previous works have reported that pregnant females exhibit higher and less variable body temperatures than non-reproductive females (Peterson et al., 1993; Dorcas Peterson, 1998; Chiaraviglio, 2006); in other words, reproductive females tend to select a stable body temperature. Gravid females often modify the mean or variance in body temperatures to benefit offspring development (Mathies and Andrews, 1997; Shine and Downes, 1999; Chiaraviglio, 2006; Rodríguez-Díaz and Braña, 2011; Toufarova and Gozdkik, 2016).

Furthermore, variation in thermal requirements among the different stages of the reproductive process is predictable, since metabolic costs are different during vitellogenesis than during pregnancy (Boretto et al., 2015). Yolk energy content is the most significant component of reptilian reproductive effort and its production has a higher metabolic cost than pregnancy (Boretto et al., 2015). If the metabolic costs of vitellogenesis are a significant component of reproductive effort (Van Dyke and Beaupre, 2011), differences in female thermal requirements can be expected among the different stages of the reproductive processes.

Understanding the relationships among preferred body temperatures, reproductive processes and individual phenotypes is important, since selection of body temperatures could depend on phenotypic traits of the individuals (Medina et al., 2016; Heatwole et al., 1973; Zari, 1998). In addition, phenotypic diversity in female body shape has been related to reproductive potential in lizards (Cardozo et al., 2015). Diversity of phenotypes among females could be associated with diversity of thermoregulatory performances because thermal requirements are frequently influenced by body shape. For instance, large-bodied *Podarcis gaigeae* lizards achieve similar temperature to that of normal-bodied lizards, but the former prefer higher set-point temperatures and thermoregulate less effectively than the latter (Sagonas et al., 2013). The effectiveness of thermoregulation largely depends on body size and shape (Huey and Stevenson, 1979). Therefore, differences in body size would have a considerable impact on body temperature and would alter thermoregulatory behaviour (Stevenson, 1985; Woods, 2013; Nielsen and Papaj, 2015).

Lizards regulate their body temperature by alternating between hot and cold heat sources (Bowker et al., 2013); therefore, the spatial pattern behaviours have an important role for body temperature regulation by lizards (Castilla et al., 1999; Rocha et al., 2009). Individuals that exhibit variation in their reproductive condition might also vary their thermoregulatory behaviour (Cowgell and Underwood, 1979; Sievert and Hutchison, 1989; Braña, 1993), i.e., patterns of movements made by individuals along the thermal gradient to achieve the selected temperature (Bowker et al., 2013). For instance, in *Tupinambis* lizards, reproductive individuals behave more dynamically to thermoregulate than non-reproductive ones, i.e., the former perform more movements between distinct thermal zones of a temperature gradient than the latter (Cecchetto and Naretto, 2015).

The *Tropidurus* genus is one of the most widely distributed lizard genera in South America. The species are found from Venezuela to northern Argentina and Uruguay in the south (Carvalho, 2013). *Tropidurus spinulosus* is an interesting model because it occurs in the southernmost area of the genus distribution and is therefore subject to low average temperatures of temperate climate (Rodrigues, 1987; Rodrigues, 1988; Cei, 1993; Carvalho, 2013). In this oviparous species, females with vitellogenic follicles occur from late September to November and pregnant females from October to December (unpublished data). In a related species (*T. torquatus*) oviposition occurs at advanced stages (Py-Daniel et al., 2016); consequently, maternal body temperature is probably very important during pregnancy. Furthermore, mature females vary greatly in their body size, ranging from 84 mm to 113 mm snout-vent length (SVL). Accordingly, we hypothesize that reproduction alters thermal requirements in the model species; we predict that the preferred body temperature of reproductive females will differ from

that of males and non-reproductive females and finally, we presumed that the preferred body temperature will differ among females at different reproductive stages. To test our hypotheses, we estimated preferred body temperature and its variability in reproductive females, and compared it with that of males and non-reproductive females. We also compare thermal requirements between females with vitellogenic follicles and females with oviductal eggs, considering their phenotypic traits. Finally, we evaluated thermoregulatory behaviour in the use of thermal zones.

2. Materials and methods

2.1. Species and study area

Tropidurus spinulosus individuals (68 males and 39 females) were captured in Tanti, Córdoba province, Argentina (31°23'33"W, 64°35'48"S), corresponding to the phytogeographic region of Chaco mountain forest (Zak et al., 2004). We are authorized by the government environmental agencies for scientific capture. We collected mature individuals during the reproductive period (Martori and Aun, 1994; Cruz et al., 1997) between September and December (2014–2015). The phenotype of each individual was characterized according to the following morphological traits: snout-vent length (SVL), inter-axillar length (IL), and head width, which were measured to the nearest 1 mm with a digital caliper; abdominal perimeter was marked with thread and then measured with a digital caliper; and body mass (BM) was recorded to the nearest 0.01 g with a digital balance (Traveler OHAUS; precision: 0.01 g). We measured body condition by calculating residual scores from the general linear regression of ln-transformed body mass to SVL (Madsen and Shine, 1999; Bertona and Chiaraviglio, 2003). According to the criterion of the smallest reproductive male and female (Madsen et al., 2006), we determined that males reach sexual maturity at 82 mm SVL and females, at 84 mm SVL. Reproductive structures were diagnosed by portable ultrasound scanning (Sonosite 180 Plus, transducer 5–10 MHz). Females were considered 'reproductive' if they had vitellogenic follicles bigger than 5 mm diameter and/or oviductal eggs. Vitellogenic follicles were identified by their hyperechogenic aspect and the typical string distribution. Oviductal eggs were identified by their ellipsoid morphology, hyperechogenic mostly, thickened and laminar edges indicating calcareous deposition of the shell.

2.2. Estimation of preferred body temperatures

Body temperature preference experiments were conducted at the laboratory within the first week after capture. Room temperature was fixed at 18 ± 1 °C. Lizards were placed in an open-top terrarium (150 cm long, 15 cm wide and 35 cm high), where a thermal gradient ranging from 18 to 50 °C was set up. Lizard preferred body temperatures (Tpref) were measured every minute for 2 h, using ultra-thin catheter thermocouples (1 mm) located approximately 20 mm inside the cloaca and fastened to the base of the tail connected to a digital thermometer (Multiput Thermometer and Datalogger, ± 0.01 °C). Lizards were allowed to acclimate for 10 min at the beginning of each experiment; therefore, those values were not considered in statistical analysis. The mean of Tpref (Tsel) and the coefficient of variation (Tpref_CV) were calculated for each individual. The set-point range (Tset), considered as the temperatures within the interquartile range of the observations, was also noted because of neurophysiological evidence that ectotherms regulate between upper and lower set-point temperatures rather than around a single Tb (Barber and Crawford, 1977; Firth and Turner, 1982). In addition, to characterize the sequential pattern of Tb in relation to Tsel we calculated the number of runs: a run is defined as a sequence of sample values with a common characteristic preceded and followed by values that do not have that feature (Siegel, 1956). Thus, for each individual, we calculated the number of runs; a run

sequence was defined as the set of consecutive values of Tb above or below the mean sample (Tsel).

Thermal stability was considered by analyzing the relationship between Tpref_CV and the number of runs. We observed that Tpref_CV and the number of runs were negatively correlated ($p < 0.001$, $b = -0.57$) for the study population. Therefore, when Tpref_CV was low and the number of runs was high, we considered that the individuals show preferred body temperatures narrow their Tsel, showing thermal stability.

2.3. Evaluation of thermoregulatory behaviour

We evaluated the thermoregulatory behaviour of lizards by analyzing the use of thermal zones along the thermal gradient. We recorded time spent in cold, temperate and warm zones by individuals during each experiment. Six 25 cm long zones were established. The average temperatures of cold zones were: zone 1 = 19 °C and zone 2 = 20 °C; the average temperatures of temperate zones were: zone 3 = 22 °C and zone 4 = 27 °C; and the average temperature of the warm zones were: zone 5 = 35 °C and zone 6 = 45 °C. Lizard behaviour was recorded using a film recorder (Sony Digital HD, XR260V) located directly above the terrarium. The obtained videos were analyzed using the video-tracking software (ANY-Maze® Stoelting Co, USA), where we measured the time spent in each zone and then calculated the corresponding relative frequency in each zone (total time spent in each zone divided by the total time of the experiment).

2.4. Statistical analyses

The morphological variables (SVL and BM) and thermal parameters Tsel, Tset min, Tset max, Tpref_CV and number of runs were compared between reproductive females and males, between reproductive and non-reproductive females, and between females with vitellogenic follicles and those with oviductal eggs using Wilcoxon test. The covariates (SVL and BM) were not included in the comparisons of thermal parameters because they were non-significant, with the exception of number of runs, which was subjected to a non-parametric test with covariate (Conover and Iman, 1982). We related multiple thermal requirements and phenotypic traits by comparing reproductive and non-reproductive females as well as comparing reproductive females with vitellogenic follicles and with oviductal eggs via a principal component analysis (PCA). To interpret the principal components (PCs) obtained, we correlated their scores with thermal requirements and phenotypic traits using Spearman's correlation (r_s). We evaluated variations among different female conditions with Wilcoxon test using the PC scores. Within reproductive females, to test the relationship between female phenotype and thermal performance we performed Spearman's correlations (r_s) between abdominal perimeter and Tpref_CV and between abdominal perimeter and body condition.

We compared the relative frequency of time spent in the different zones of the gradient among non-reproductive females, females with vitellogenic follicles and females with eggs in oviduct using a Kruskal Wallis test. We also performed a chi-square test to evaluate whether there are differences between groups regarding the time spent in the different thermal zones. All statistical analyses were done using the software INFOSTAT, 2015 version (Universidad Nacional de Córdoba) (Di Rienzo et al., 2015) and SPSS16.0 (SPSS 16.0 Inc., Chicago, IL, USA) (Norusi, 2012). The significance level was set to 0.05.

3. Results

3.1. Thermal requirements and phenotypic traits between groups

No differences were detected in thermoregulatory requirements between reproductive females and males (Tsel, Tset min and Tset max) nor were there differences in thermal stability (Tpref_CV and number of runs) (Table 1).

Table 1

Thermal preferences of reproductive females and males of *Tropidurus spinulosus*, assessed using a Wilcoxon test (W); significant differences are indicated with *. The SVL and BM were not significant covariates in the comparisons of thermal parameters.

	Reproductive Females	Males	W	P
Tsel ± SD	33.79 ± 1.42	33.50 ± 2.27	1523.5	0.7666
Tset min ± SD	32.40 ± 1.85	32.02 ± 3.10	1482.50	0.9846
Tset max ± SD	35.34 ± 1.40	35.07 ± 2.04	1548.00	0.6271
Tpref_CV ± SD	6.35 ± 3.38	6.84 ± 4.10	1401.5	0.5173
Number of runs ± SD	13.07 ± 7.18	11.38 ± 5.54	1571	0.49
n	30	68		

Table 2

Loadings of morphological and thermal variables on principal components in females of *Tropidurus spinulosus*. PC1: First Principal Component; PC2: Second Principal Component.

	Females	
	PC1	PC2
Tsel	0.48	-0.01
Tset min	0.52	-0.01
Tset max	0.28	-0.02
Tpref_CV	-0.45	-0.03
Number of runs	0.29	-0.22
SVL	-0.08	0.56
IL	-0.02	0.54
Abdominal Perimeter	0.19	0.43
Head Width	-0.03	0.35
Body Condition	0.29	0.18

The PCA of reproductive and non-reproductive females yielded two significant axes that explained 60,7% of the data variability (Table 2). Some temperature variables (Tsel, Tset min, number of runs) and body condition were positively related to PC1, whereas thermal variability (Tpref_CV) was inversely related to PC1 (Table 3). The morphological variables SVL, IL, abdominal perimeter and head width were positively related to PC2 (Table 3). Reproductive females showed better body condition and wider abdominal perimeter, preferred higher temperatures (Tsel and Tset min) and presented lower amplitude in thermal variability (Tpref_CV) than non-reproductive females (Fig. 1 and Table 4), with these variations being significant according to PC1 scores (Wilcoxon test $W = 85.00$, $p = 0.0019$). Moreover, within reproductive females, females with wide abdominal perimeter and high body condition showed thermal stability, since in those females Tpref_CV was low (Spearman's correlation (r_s) between abdominal perimeter and Tpref_CV: $P = 0.02$, $r = -0.43$; Spearman's correlation (r_s) between abdominal perimeter and body condition: $P = 0.0009$, $r = 0.63$).

Table 3

Spearman's correlations (r_s) between the principal components and thermal and morphological variables in females of *Tropidurus spinulosus*; significant differences are indicated with * ($P < 0.05$). PC1: First Principal Component; PC2: Second Principal Component.

Variables	r_s		r_s	
	PC1	P	PC2	P
Tsel	0.83	1.4E - 10*	-0.05	0.76
Tset min	0.91	0.00*	-2.6E - 03	0.99
Tset max	0.44	0.01*	-0.04	0.83
Tpref_CV	-0.78	8.3E - 09*	-0.09	0.58
Number of runs	0.59	9.9E - 05*	-0.34	0.04*
SVL	-0.22	0.19	0.90	5.1E - 08*
IL	-0.09	0.56	0.86	1.5E - 07*
Abdominal Perimeter	0.38	0.02*	0.73	9.3E - 06*
Head Width	-0.04	0.82	0.61	5.0E - 05*
Body Condition	0.50	2.2E - 03*	0.31	0.06

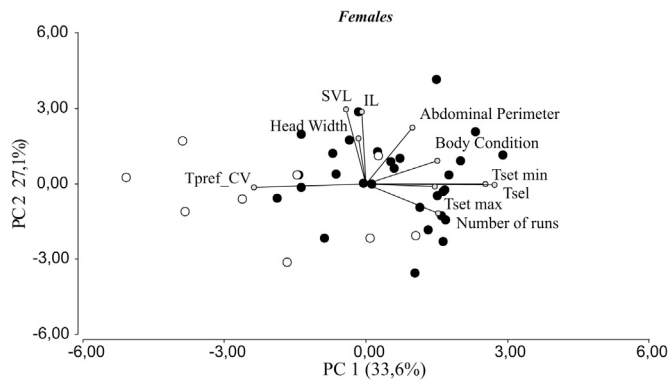


Fig. 1. Principal Component Analysis relating morphological traits and thermal parameters in females of *Tropidurus spinulosus* (black dots indicate reproductive females and white dots, non-reproductive females).

Table 4

Thermal preferences of reproductive females and non-reproductive females of *Tropidurus spinulosus* assessed using a Wilcoxon test (W); significant differences are indicated with *. The SVL and BM were not significant covariates in the comparisons of thermal parameters, except for Number of runs which was tested with a non-parametric test with covariate (Conover and Iman, 1982).

	Reproductive Females	Non-Reproductive Females	W	P
Tset ± SD	33.79 ± 1.42	31.43 ± 2.35	99.00	0.0069*
Tset min ± SD	32.40 ± 1.85	29.09 ± 3.41	99.00	0.0069*
Tset max ± SD	35.34 ± 1.40	33.91 ± 2.10	125.00	0.0665
Tpref_CV ± SD	6.35 ± 3.38	10.20 ± 4.28	258.5	0.0093*
Number of runs ± SD	13.07 ± 7.18	9.00 ± 5.41	133	0.039*
N	30	9		

Considering reproductive stages, females with vitellogenic follicles and females with oviductal eggs also differed in their thermal requirements. The PCA yielded two significant axes that explained 56.6% of the data variability (Table 5). The morphological variables SVL, IL, abdominal perimeter and head width were related to PC1. The thermal variables Tset, Tset min, and number of runs were positively related to PC2 and amplitude in thermal variability (Tpref_CV) was inversely related to PC2 (Table 6). Females at different stages of the reproductive cycle were separated by PC2, indicating that females with oviductal eggs preferred a higher temperature and presented a higher number of runs and a lower Tpref_CV than females with vitellogenic follicles (Fig. 2), with these variations being significant, according to PC2 scores (Wilcoxon test W = 150.00, P = 0.0485). The tendency of females with oviductal eggs to present a higher number of runs than females with vitellogenic follicles was also detected in univariate analyses (Table 7).

Table 5

Loadings of morphological and thermal variables on principal components in reproductive females of *Tropidurus spinulosus*. PC1: First Principal Component; PC2: Second Principal Component.

	Reproductive Females	
	PC1	PC2
Tset	-0.15	0.42
Tset min	-0.01	0.55
Tset max	-0.28	0.05
Tpref_CV	-0.12	-0.52
Number of runs	-0.09	0.32
SVL	0.51	-0.09
IL	0.49	-0.01
Abdominal Perimeter	0.42	0.25
Head Width	0.39	-0.14
Body Condition	0.21	0.22

Table 6

Spearman's correlations (r_s) between the principal components and thermal and morphological variables in reproductive females of *Tropidurus spinulosus*; significant differences are indicated with * ($P < 0.05$). PC1: First Principal Component; PC2: Second Principal Component.

Variables	r_s		r_s	
	PC1	P	PC2	P
Tset	-0.29	0.12	0.68	4.6E - 05*
Tset min	-0.08	0.69	0.85	5.1E - 09*
Tset max	-0.49	0.01	0.17	0.39
Tpref_CV	-0.27	0.16	-0.72	1.3E - 04*
Number of runs	-0.07	0.74	0.61	4.1E - 04*
SVL	0.80	2.3E - 05*	-0.31	0.10
IL	0.82	1.5E - 05*	-0.14	0.45
Abdominal Perimeter	0.75	6.7E - 05*	0.39	0.04
Head Width	0.61	1.3E - 03*	-0.14	0.45
Body Condition	0.34	0.08	0.37	0.05*

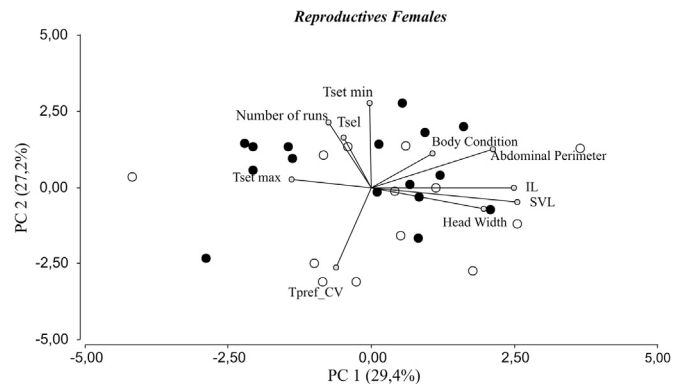


Fig. 2. Principal Component Analysis relating morphological traits and thermal parameters in reproductive females of *Tropidurus spinulosus* (black dots indicate females with oviductal eggs and white dots, females with vitellogenic follicles).

Table 7

Thermal preferences of females with vitellogenic follicles and females with oviductal eggs of *Tropidurus spinulosus*, assessed using a Wilcoxon test (W); significant differences are indicated with *. The SVL and BM were not significant covariates in the comparisons of thermal parameters.

	Females with Follicle	Females with Eggs	W	P
Tset ± SD	33.48 ± 1.31	33.89 ± 1.44	179.00	0.4828
Tset min ± SD	31.91 ± 1.80	32.61 ± 1.75	173.00	0.3343
Tset max ± SD	35.32 ± 1.25	35.28 ± 1.57	197.50	0.9126
Tpref_CV ± SD	7.50 ± 3.72	5.54 ± 2.98	224.00	0.2035
Number of runs ± SD	10.62 ± 7.10	14.44 ± 6.75	152.5	0.0612
N	13	16		

3.2. Thermal behaviour between groups

Regarding the use of thermal zones along the thermal gradient, non-reproductive females used mainly the temperate zone 4, followed by the warm zone 5 and even used the cold zone 1 (Kruskal Wallis H = 22.39, P = 0.0004); females with vitellogenic follicles frequently used the temperate zone 4, followed by the warm zone 5 (Kruskal Wallis H = 43.54, P < 0.0001), whereas females with oviductal eggs used the temperate zone 4 and the warm zone 5 with similarly high frequency, and even used the warm zone 6 (Kruskal Wallis H = 50.60, P < 0.0001) (Fig. 3). However, the Chi-square test showed non-significant differences between groups regarding the time spent in the different thermal zones ($\chi^2 = 10.84, P = 0.3703$).

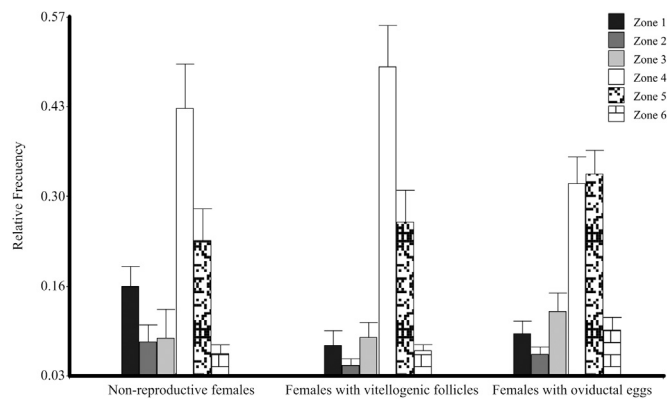


Fig. 3. Frequency distribution of time spent in different zones of the thermal gradient of non-reproductive females and reproductive females (oviductal eggs and vitellogenic follicles) of *Tropidurus spinulosus*.

3.3. Differences in the morphology between groups (SVL and Body Mass)

As regards morphological variables we found significant results in SVL and body mass between reproductive females and males, and between reproductive and non-reproductive females (Table 8). Significant differences were not found between females with vitellogenic follicles and females with oviductal eggs (Table 9).

4. Discussion

Our results showed that reproductive stage and phenotypic traits of *Tropidurus spinulosus* females influence the selection of body temperature and thermoregulatory behaviour. These results are consistent with our hypothesis that reproduction alters thermal requirements of females of this oviparous model and that the preferred body temperature will differ among the different stages of the female reproductive cycle, being also associated with female phenotype.

We did not find differences in thermal requirements between males and reproductive females, meaning that this species shows no sex-related effects on T_{pref} . In Squamate, reproductive males might also have specific thermal requirements, since there are evidences about the effects of temperature in sperm motility and velocity (Tourmente et al., 2011). Woolrich-Piña et al. (2015) did not find differences in body temperature between gravid females and males of *Anolis nebulosus*, but they found differences in thermoregulatory efficiency. Lara-Resendiz et al. (2013) did also not find differences in body temperature between males and females of *Phyllodactylus bordai*, but they indicate the importance of considering gravid females separately. Therefore, reproductive condition may involve specific thermal requirements.

The role of female thermoregulation in oviparous species has been scarcely recognized; however we demonstrated that in the oviparous model *Tropidurus spinulosus* reproductive females have specific thermal requirements and higher thermoregulatory stability than non-reproductive females; i.e., reproductive females preferred higher body temperatures and maintained the minimum ($T_{set\ min}$) body temperature three degree Celsius higher than the non-reproductive females. Furthermore, reproductive females presented more thermal stability than non-reproductive ones because they narrow the preferred body

Table 8

Differences in the morphology between groups of *Tropidurus spinulosus*, assessed using a Wilcoxon test (W).

Traits	Males	Reproductive Females	Non-reproductive Females	W	P
SVL (mean \pm SD)	112.62 \pm 9.51	97.69 \pm 6.38	95.17 \pm 7.93	699.00	< 0.0001 (Male-ReproFemale)
				155.00	< 0.0001 (ReproFemale-NonReproFemale)
Body Mass (mean \pm SD)	47.53 \pm 11.86	32.03 \pm 5.73	26.14 \pm 6.90	751.00	< 0.0001 (Male-ReproFemale)
				112.00	< 0.0001 (ReproFemale-NonReproFemale)

Table 9

Differences in the morphology between different reproductive stages of females of *Tropidurus spinulosus*, assessed using a Wilcoxon test (W).

Traits	Females with Vitellogenic Follicles	Females with Oviductal Eggs	W	P
SVL (mean \pm SD)	99.35 \pm 7.21	96.39 \pm 5.73	222.00	0.2364
Body Mass (mean \pm SD)	32.38 \pm 6.50	31.58 \pm 5.37	199.00	0.8608

temperatures (lower amplitude of variation of T_{pref}). Moreover, the reproductive stage within the female reproductive cycle influenced the selection of body temperatures. The comparison of different stages along the reproductive cycle of females showed that females with oviductal eggs tended to narrow preferred body temperatures.

Likewise, the PCA showed that females with oviductal eggs preferred warmer temperatures and presented lower amplitude in $T_{pref\ CV}$ than females developing follicles. Pregnant females of *T. spinulosus* are capable of retaining eggs in utero for approximately one month (as mentioned above, oviposition occurs at stage 28 in *T. torquatus*, Py-Daniel et al., 2016); consequently, maternal body temperature is probably important in the process of pregnancy. These results also support our hypothesis that thermal requirements change along the reproductive cycle of females and that maternal body temperature in oviparous species would be very important since the start of the embryogenesis, as previously found in viviparous species. Embryogenesis proceeds normally only within a limited and species-specific range of temperatures (Thompson, 1990; Vinegar, 1974; Shine, 1999; Shine and Thompson, 2006); therefore, lower variance in body temperatures of gravid females is usually attributed to more precise thermoregulation (Stewart, 1984; Beuchat, 1986; Charland and Gregory, 1990; Schwarzkopf and Shine, 1991; Dayananda et al., 2017). Moreover, higher temperatures accelerate embryogenesis and may enhance offspring viability (Shine and Thompson, 2006; Woolrich-Piña et al., 2015). Maternal temperature has been widely reported to affect traits influencing offspring (Beuchat, 1988; Shine and Harlow, 1993a, 1993b; Mathies and Andrews, 1997; Wapstra, 2000); therefore, females are expected to narrow their T_{pref} during pregnancy, which is supported by our data.

Female phenotype also influences thermoregulatory performance. Reproductive females with wide abdominal perimeter and high body condition were able to narrow preferred body temperatures, demonstrating thermal stability. In fact, Cardozo et al. (2015) showed that phenotypic diversity in female lizards was directly related to body condition; in particular, abdomen width was associated with the weight of fat bodies. Body condition is an indicator of the amount of energy stored in the form of fats (Peig and Green, 2009, 2010). A wide abdomen provides females with large body cavities for storing fat reserves, which are important for many physiological processes, such as reproductive and thermal performances (Lourdais et al., 2006). Deen and Hutchinson (2001) showed that the lizards that chose high body temperatures had a greater energy base (i.e., higher body mass) than those that chose low body temperature. Hence, considering the results obtained in this study, the abdominal perimeter and the body condition would be good indicators of the ability of females to performed thermal stability during the reproductive process.

Our results show that females at different reproductive stages vary in the use of the zones of the thermal gradient, indicating that there is a relationship between the thermoregulatory behaviour and female reproductive stage. The analyses of the behaviour helped us to understand how reproductive females are able to attain the specific range of temperatures required. As we explained previously, females with vitellogenic follicles as well as females with oviductal eggs used the temperate and warm zones more intensely than non-reproductive females. This agrees with the fact that reproductive females preferred higher body temperature (Tsel and Tset min) than non-reproductive females. Moreover, pregnant females used frequently even the warmest zone, which probably allows them to reach higher body temperatures required during embryogenesis. Cecchetto and Naretto (2015) also showed that reproductive individuals behave differently in the use of thermal zones than non-reproductive ones, i.e., reproductive males and females presented a dynamic behaviour, since they achieved higher frequencies in the cooler and hotter zones. To sum up, our results indicate that lizard females might control the range of temperatures required during reproductive processes by increasing the time spent in suitable thermal zones.

5. Conclusion

The reproductive stage and the phenotypic traits of females influence the selection and stability of body temperatures. Reproductive females preferred higher temperatures and were thermally more stable than non-reproductive females. Body condition is an important trait for maintaining thermal stability. Females with oviductal eggs tend to narrow the preferred temperatures. Finally, thermoregulatory behaviour might control the range of temperatures required during reproduction.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.jtherbio.2017.11.013>.

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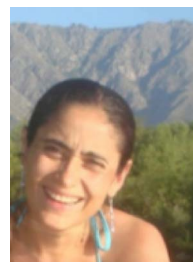
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