

Small changes, big benefits: testing the significance of maternal thermoregulation in a lizard with extended egg retention

THOMAS FOUCART^{1,2}, BENOIT HEULIN¹ and OLIVIER LOURDAIS^{2,3*}

¹Station Biologique de Paimpont, UMR 6553 CNRS, F-35380, Paimpont, France

²Centre d'étude biologique de Chizé CNRS, F-79360, Villiers en Bois, France

³School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501, USA

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Early life stages are particularly vulnerable to environmental perturbations. Embryonic thermal sensitivity might be a driving force in the emergence of prenatal parental care, such as maternal thermoregulation. Viviparity has emerged on repeated occasions among squamate reptiles, and two main evolutionary hypotheses based on maternal thermoregulation have been proposed to explain these transitions, namely the 'cold climate hypothesis' and the 'maternal manipulation hypothesis'. Squamate embryos typically face important daily temperature fluctuations either in the nest or within the maternal body, but most experimental studies on development have relied on constant temperature. Therefore, we may have only limited insight on the effect of maternal thermoregulation on embryo development. We manipulated thermal conditions to compare the influence of a typical maternal temperature cycle (M) or nest thermal conditions (N) both during gravidity and during incubation in the oviparous form of a reproductively bimodal squamate (*Zootoca vivipara*). Although the two treatments had a similar mean temperature, we found that M treatment accelerated development, notably when applied during gravidity. Only limited effects were found when considering offspring phenotype and performance. Overall, our results suggest that small changes in thermal conditions can have a strong impact on reproductive phenology and might be a proximate target in the emergence of egg retention and, ultimately, of viviparity. Further studies are required to address long-lasting effects of maternal thermoregulation on offspring performance.

ADDITIONAL KEYWORDS: development – embryo – parental care – reproductive mode – viviparity.

INTRODUCTION

Early life-history stages are particularly sensitive to environmental conditions (Farmer, 2000; Shine, 2004a). The embryonic experience can have immediate consequences for survival or development but also long-lasting effects on offspring performance (Elphick & Shine, 1998; Lummaa & Clutton-Brock, 2002; Lummaa, 2003; Gorman & Nager, 2004). Recent developments in epigenetics have clearly demonstrated that environmental perturbations at early stages can also have multigenerational consequences (Wang *et al.*, 2017; Eaton *et al.*, 2018). Embryonic thermal sensitivity is therefore considered to be a driving force in the evolution of parental care, notably at early stages (i.e. prenatal parental

care). A wide diversity of taxa have developed specific behavioural or physiological strategies to buffer the effects of environmental factors (Farmer, 2000). Parents may also exert specific influences on the developmental trajectory depending on the environmental conditions they face (e.g. food level, predation risk; Mousseau *et al.*, 2009). Therefore, it appears essential to clarify embryonic sensitivity and the nature of parental influences to gain a better understanding of the evolution of parental care and reproductive modes (Clutton-Brock, 1991; Lorioux *et al.*, 2013).

Temperature is a crucial parameter (in either aquatic or terrestrial environments), and embryonic thermal sensitivity is a ubiquitous trait in vertebrates and invertebrates (Farmer, 2000). Thermal conditions during embryogenesis affect not only the duration of development and embryonic survival (Van Damme

*Corresponding author. Email: lourdais@cebc.cnrs.fr

et al., 1992; Lourdais *et al.*, 2004) but also a diverse range of phenotypic (e.g. body size, body condition) and behavioural (e.g. locomotor performances, feeding behaviour) traits in offspring (Burger, 1989; Webb *et al.*, 2001; Shine, 2004a; Rodríguez-Díaz & Braña, 2011a). Long-term effects on survival have also been reported (Shine & Harlow, 1993; Wapstra *et al.*, 2010; Le Henanff *et al.*, 2013). Embryonic thermal sensitivity may have been a driving force in the evolution of parental care, such as nesting behaviour, nest site selection (Brown & Shine, 2004; Löwenborg *et al.*, 2010), the transition to viviparity (Shine, 1995, 2002) and the emergence of endothermy in vertebrates (Farmer, 2000, 2003; Tattersall *et al.*, 2016).

According to Farmer's evolutionary scenario, even a small increment in maternal ability to thermoregulate (i.e. produce heat) during embryogenesis will be selected for if the reproductive benefits are high (Farmer, 2003). This assumption can be extended to different forms of thermal care, such as behavioural thermoregulation in ectotherms (brooding or egg retention) and the evolution of maternal thermoregulation to different thermal optima during gravidity (Rodríguez-Díaz & Braña, 2011b; Shine, 2012). Accessing optimal temperature probably constitutes a critical aspect for development (Huey *et al.*, 1999). Embryos have relatively narrow optimal temperature ranges (Angilletta, 2009), and maternal influences probably exist, such as minimizing deviation from the optimal temperature and optimizing the time spent within the preferred body temperature range (Lorioux *et al.*, 2012; Lourdais *et al.*, 2013). Embryonic development is composed of several stages that may differ in their sensitivity (Andrews, 2004). For instance, the early organizational phases that include neurogenesis and organogenesis are likely to be more sensitive to perturbations than the subsequent embryonic growth phase. High sensitivity at early developmental stages and a narrow optimal temperature range might have been a major driving force in the emergence of thermal parental care to the embryos (Braña & Ji, 2007; Lorioux *et al.*, 2012, 2013). The study of parental regulation of development should specifically address this stage-dependent embryonic sensitivity.

Viviparity has emerged repeatedly, notably among squamate reptiles, with > 100 independent transitions (Thompson & Blackburn, 2006; Van Dyke *et al.*, 2014). Therefore, it is not surprising that this group has generated considerable interest to clarify the benefit of maternal regulation of development (see Shine, 2004a, 2005 and references therein). Two main hypotheses have been formulated to explain these repeated transitions, both of which are related to thermal parental care. The first one, the 'cold climate hypothesis' (Tinkle & Gibbons, 1977), posits that maternal regulation is critical to minimize the duration of development in cold

conditions. In the second one, the 'maternal manipulation hypothesis', maternal regulation is critical to optimize offspring phenotypic quality, including morphology, locomotion and growth (Shine, 1995; Li *et al.*, 2009; Lorioux *et al.*, 2013). Importantly, these hypotheses are not mutually exclusive, and recent work clearly supports the idea that that females can optimize both reproductive phenology and offspring phenotypic quality (Rodríguez-Díaz *et al.*, 2010; Lorioux *et al.*, 2013).

Most oviparous squamates also retain their eggs for long periods, until one-third of the embryonic development is achieved. Therefore, critical steps of embryonic life (organogenesis and early embryonic growth) occur within the maternal body in these oviparous species (Andrews, 2004). During that period, embryos are exposed to temperature selected by the females, and numerous studies have demonstrated that they actively thermoregulate during gravidity (Shine, 2006; Lourdais *et al.*, 2008). The transition to viviparity may be based on the extension of maternal control that pre-existed in oviparous taxa (Shine, 2006). Therefore, prolonged egg retention may be important to optimize thermal developmental conditions during early embryonic life and provide the same advantages as demonstrated in viviparous taxa.

Among squamates, a few species show reproductive bimodality, with the coexistence of viviparous and oviparous populations (Heulin *et al.*, 1991; Qualls *et al.*, 1995; Smith & Shine, 1997). We studied the oviparous form of one of these species, the common lizard (*Zootoca vivipara*), which retains its eggs until embryos reach stages 30–35 (Heulin *et al.*, 1991; Rodríguez-Díaz & Braña, 2012). We manipulated developmental conditions in a cross-factorial experiment to test the effects of maternal vs. nest thermal regime applied during gravidity and the incubation periods. Specifically, we wanted to examine the following predictions:

1. A small change in thermal regime (increased time at thermal preference) should translate into significant reproductive benefits, including shorter development duration and enhanced offspring performance.
2. Thermal effects on embryonic development and offspring traits should be more marked when manipulating early embryonic stages (gravidity period) than the post-ovipositional incubation period.

MATERIAL AND METHODS

STUDY SPECIES

Zootoca vivipara (Lichtenstein, 1823) is a small [snout–vent length (SVL) 45–75 mm for adults], ground-dwelling Eurasian lacertid that generally lives

in humid habitats (heathland, peat bogs). This reproductively bimodal species presents distinct oviparous populations in southern France–northern Spain and in northern Italy–Slovenia. The oviparous form lays eggs at an advanced stage (30–35) which corresponds to 50% of the duration of development (Braña *et al.*, 1991; Heulin *et al.*, 1991; Rodríguez-Díaz & Braña, 2012).

Lizards (20 males and 30 females) were captured at Louvie, SW France, Pyrenees Mountains (43°06'N, 0°23'W, elevation 370 m a.s.l.), in the summer of 2013. The ecology and demography of this population have been previously described elsewhere (Heulin *et al.*, 1994, 1997).

HUSBANDRY AND REPRODUCTION

Lizards were reared in plastic terraria (length × width × height, 30 cm × 20 cm × 20 cm). Each terrarium was equipped with a PVC shelter and a 40 W bulb that provided a thermal gradient (from 18 to 40 °C) that allowed thermoregulation for 6 h day⁻¹. Water and food (mealworms) were provided *ad libitum*. We used a standard protocol (see Foucart *et al.*, 2014) to overwinter the lizards and to induce reproduction the following spring. Ultrasonography (Sonosite microMaxx, Inc., Bothell, WA, USA) was used to monitor reproduction, which enables a distinction between vitellogenesis and ovulation, in addition to identifying ovulation time (Gilman & Wolf, 2007). A total of 24 females engaged in reproduction and ovulated in early March 2014.

After ovulation, females were transferred into climatic chambers (Vötsch VP 600, Balingen, Germany) in plastic terraria (Fauna Box; length × width × height, 41 cm × 23 cm × 17 cm). A 12 h–12 h light–dark cycle was provided using a fluorescent tube controlled by a timer and placed in front of each climatic chamber. A damp sponge was placed under the shelter to increase humidity and to prevent the desiccation of clutches. We checked each female twice a day; therefore, the eggs were collected within a few hours after they were laid. Eggs were incubated on water-saturated sand using a previously described protocol (Heulin *et al.*, 1991).

EXPERIMENTAL DESIGN

In order to address stage-dependent embryonic thermal sensitivity we considered two developmental periods:

1. 'Gravidity': this period corresponds to the fraction of development that occurs within the maternal body between ovulation and egg-laying. The embryonic stage at oviposition ranges between 31 and 35; therefore, the gravidity period encompasses major steps of organogenesis up to early

embryonic growth (Andrews, 2004). During that period, gravid females are known to thermoregulate actively (Rodríguez-Díaz & Braña, 2011a, b).

2. 'Incubation': this period is the fraction of development that occurs in the nest site and corresponds to embryonic growth and fetal life. During this period, embryos are exposed to thermal conditions experienced in the nest.

DAILY TEMPERATURE CYCLES

We wanted to compare, in the oviparous form, the effect of two daily thermal cycles (Fig. 1), reflecting either nest or maternal conditions, applied at two different periods (gravidity or incubation). The two daily thermal cycles were as follows.

Nest cycle ('N')

This regime was based on nest temperatures previously recorded in 1992 by one of the authors (B.H.) in a lowland population of the oviparous form (Heulin *et al.*, 1994). This population (Louvie) corresponds to where we captured the lizards used in the present study. The temperature data were derived from four nest sites (2 cm deep in south-facing tufts of *Sphagnum* sp.) monitored over 86 days (temperature recorded every hour) during the incubation period (June–September) in 1992. According to official meteorological recording (MeteoFrance) available for this region, the mean air temperature from June to September was 19.2 °C in 1992, which was very close to the mean value of 19.8 °C observed from 1980 to 2016. This indicates that the 1992 data set used to design our N regime is representative of a rather normal year for this population. We set our experimental N regime in such a way that it had the same distribution frequency of temperatures (hours per day) and the same mean daily temperature (18.9 °C) as those recorded in the nest sites of the natural population.

Maternal cycle ('M')

This thermal regime was designed by: (1) considering that during sunny days behavioural thermoregulation allows lizards to reach their preferred body temperature (i.e. T_{set}) for ≥ 6 h day⁻¹ (Rodríguez-Díaz & Braña, 2011b); and (2) using the published literature on thermal preferences (30–32 °C) observed both in gravid females of the oviparous form (Rodríguez-Díaz *et al.*, 2010; Rodríguez-Díaz & Braña, 2011a, b) and in pregnant females of the viviparous form (Le Galliard *et al.*, 2003) of *Z. vivipara*. Hence, our M regime was designed in such a way that it differed from our N regime only by a longer duration of access to the $T_{set} = 31$ °C (6 h in M vs. 1 h in N) during the middle part of the day.

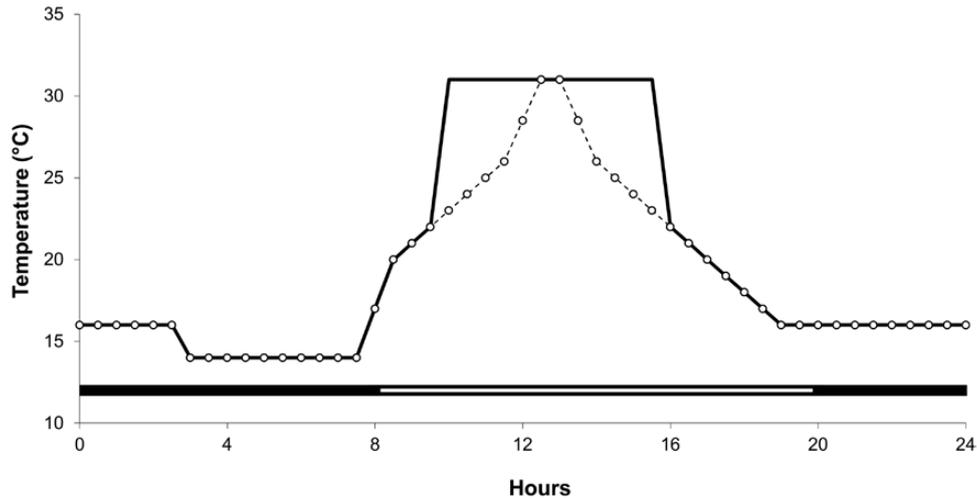


Figure 1. Thermal treatments were based on two different daily temperature cycles applied during gravidity and incubation. In the warm daily cycle, which mimicked the thermal cycle of reproductive females in the wild (continuous line), females reached their preferred body temperature ($T_{\text{set}} = 31.0\text{ }^{\circ}\text{C}$) for 6 h. The cool cycle (dashed line) mimicked the thermal regime recorded in a nest site in a lowland population; conditions in the nest were mild, because the temperature rose above $28\text{ }^{\circ}\text{C}$ for 3 h a day and reached maternal T_{set} for 1 h. Neon lighting was provided through the window of the climatic chamber from 08.00 to 20.00 h (bottom scale, open bar).

During other parts of the day (late afternoon, night and early morning), the two regimes were strictly identical. This implies that we assume that, once they retreat to their shelter (within litter) the females have body temperatures very likely to be close to the nest temperatures. This design results in only a small difference in mean daily temperature between the two regimes ($20 \pm 7\text{ }^{\circ}\text{C}$ for M vs. $19 \pm 5\text{ }^{\circ}\text{C}$ for N). The two thermal cycles were designed with SIMPATI software (version 2.06; Industrietechnik) and applied using four climatic chambers (VP 600; Vöetsch). Each thermal cycle was replicated in two chambers.

EXPERIMENTAL TREATMENTS

Gravid females were exposed to thermal treatments at ovulation. The assignment of females to each treatment (M, 13 individuals; and N, 12 individuals) and to the four climatic chambers was random. For each female, as soon as egg-laying was observed, eggs were allocated to one or the other thermal regime (Table 1). Using a cross-factorial design, the following thermal treatments were obtained (see Table 1):

1. MM: gravidity period and incubation at the maternal cycle. This treatment reflects permanent access to favourable thermal conditions. It corresponds to the body temperature maintained in the viviparous form having access to sunny days during the entire duration of development.
2. MN: gravidity period at the maternal cycle and incubation at the nest cycle. This treatment corresponds

to conditions in the oviparous form where females actively thermoregulate during gravidity. Eggs were exposed to the average nest temperature cycle observed in the lowland population.

3. NN: gravidity and incubation at the nest temperature cycle. This treatment corresponds to incubation conditions applied during the entirety of development and provides a control by removal of maternal thermal influences.
4. NM: gravidity at the nest cycle and incubation at the maternal cycle. This treatment corresponds to altered thermoregulation at early stages (gravidity), with subsequent favourable conditions during incubation.

Four females (three from the M gravidity treatment and one from the N gravidity treatment) produced undeveloped ova. One female died during the experiment for unknown reasons, and another showed substantial mass loss and was removed from the experiment. Therefore, a total of 19 females (ten in the M and nine in the N gravidity treatment) produced fertile eggs that were later incubated (see Table 1).

VARIABLES MEASURED

We measured different parameters related to reproductive phenology, reproductive success and offspring traits.

Duration of development

The ovulation ordinal date was determined for each female by ultrasonography. We also collected the

Table 1. Experimental design and sample size used for the study

Experimental Design		Variables measured		
Egg laying	Hatching	<u>Reproduction traits</u>		
<u>Gravidity</u>	<u>Incubation</u>	Development duration Incubation duration Hatching success Sex-ratio		
Treatment M (n=10)	Treatment M (n=23) Treatment N (n=21)	<u>Offspring traits</u>		
		<u>J-0 Phenotype</u>	<u>J-1 Locomotor performances</u>	<u>J-2 Exploratory behavior</u>
		Scale abnormalities	Stamina	Number of visited compartments
		Body mass	Run duration until first stop	Number of visited shelters
		Head length	Number of stops	Time under shelters
		Body condition		
Treatment N (n=9)	Treatment M (n=19) Treatment N (n=20)			

Thermal treatments (M and N) are as depicted in [Figure 1](#). Offspring traits were recorded on the day of hatching (J-0). Locomotor performances and exploratory behaviour, respectively, were collected 1 day (J-1) and 2 days (J-2) after hatching.

oviposition date and, for each egg, the hatching date. We considered duration of gravidity (number of days from ovulation to laying), the duration of incubation (from laying to hatching) and the total duration of development (from ovulation to hatching).

Reproductive effort

For each female, we determined the clutch size (total number of eggs laid) and clutch mass (total mass of eggs laid). We also determined the number of infertile eggs produced per clutch.

Hatching success and offspring phenotype

We considered the influence of thermal treatment on the number of eggs that produced healthy neonates. At birth, we collected SVL (± 0.1 mm), head length, total length (TL; ± 0.1 mm) and body mass (BM; ± 1 mg). Body condition was derived from the residuals of $\log(\text{BM})$ against $\log(\text{SVL})$. For each offspring, a high-resolution scan (600 dpi) of the ventral side was obtained (CanoScan 8800F; Canon). Body size measurements were collected on the scanned images using Inkscape software (open source software, licensed under the general public license, v. 0.48.5). Ventral scales were counted on the two medioventral lines between the anal plate and the gular collar to determine offspring sex and to record abnormalities in ventral scalation (i.e. asymmetries). This sex assignment technique has previously been used successfully in this species ([Lecomte et al., 1992](#); [Heulin et al., 1997](#)). The count of ventral scale abnormalities was defined as the sum of scales duplicated on the right and the left medioventral lines.

Locomotion

We measured offspring stamina 1 day after hatching in a temperature-controlled room (mean temperature 27.04 ± 0.61 °C). This parameter offers a reliable index of individual quality, because it is correlated with subsequent growth, activity and survival in the study species ([Clobert et al., 2000](#); [Le Galliard et al., 2004](#)). Before the test, each individual was acclimated for 1 h in an opaque container to minimize stress. We used a circular runway (internal diameter 50 cm and external diameter 60 cm) as described by [Clobert et al. \(2000\)](#). The runway was delimited by a 15-cm-high perspex wall, and the surface was covered with sandpaper to facilitate locomotion. Individuals were placed on the runway and stimulated using a gentle tap on the tail with a soft paintbrush. Stamina was derived from the running time until individuals were no longer responding to repeated stimulations (more than ten in a row). We also measured the duration of the run until the first stop and the number of stops during the run.

Exploratory behaviour

Neonates are autonomous at birth, and exploratory behaviour is a good indicator of the ability of the individual to find suitable microhabitats in a new environment ([Lorioux et al., 2013](#)). Two days after hatching, we assessed neonate exploratory behaviour using a 360 mm \times 270 mm polypropylene box. Two shelters (height \times length \times width, 30 mm \times 100 mm \times 60 mm) were provided, one on each side of the arena. Trials were conducted in a temperature-controlled room (25.87 ± 0.9 °C), and individuals were acclimated in

the middle of the arena under an opaque cover for 1 h. The cover was removed, and the behaviour of the lizard was recorded on video for 1 h (camera SONY Handycam Xr100/101). The space of the arena was subdivided into six virtual, equally sized compartments. We documented the number of compartments visited, the number of shelters visited, and time spent under shelter. Between trials, the entire system was cleaned with 70% alcohol to remove residual odours.

STATISTICAL ANALYSES

All analyses were performed with R software (R Development Core Team, v. 3.1.1) using the packages lme4, car and multcomp. The data were tested for normality (Shapiro–Wilk test) and homogeneity of variances (F -test) and \log_{10} transformed before analysis when required to meet the assumptions of parametric testing. Influences of thermal treatment on reproductive output were tested using general linear models, treating clutch traits as the dependent variable and maternal size (SVL) as a covariate. We analysed possible impacts on hatching success using a binomial model, treating individual egg success (zero or one) as the dependent variable and maternal identity as a random factor. A similar procedure was used to test the influences of treatment on offspring sex. We tested the effects of thermal treatment on offspring traits using a linear mixed model, with mother identity as a random factor and maternal traits (body size) as a cofactor. A Gaussian distribution was used for morphological traits, whereas a Poisson distribution was used for scale duplication counts. All pairwise comparisons were made by Tukey's *post hoc* HSD tests. All data are presented as means \pm SD. All results were tested for statistical significance at the $P < 0.05$ level. Denominator degrees of freedom for the F -statistic in mixed models were calculated using a Kenward–Roger correction (Kenward & Roger, 1997).

RESULTS

REPRODUCTIVE OUTPUT AND HATCHING SUCCESS

We found that clutch size was weakly related to female body size ($F_{1,16} = 3.41$, $P = 0.08$) and was not influenced by treatment (ANOVA, $F_{1,16} = 0.44$, $P = 0.51$). Clutch mass was positively related to female body size ($F_{1,16} = 13.16$, $P = 0.02$). When taking into account this allometric influence, we found a significant influence of gravidity treatment on relative clutch mass (ANCOVA, $F_{1,15} = 4.83$, $P = 0.04$), with higher relative clutch mass in gravid females exposed to the M thermal cycle compared with the N cycle (1038.5 ± 65.74 vs. 839.25 ± 73.5 g). We found that hatching success was higher in females exposed to the M thermal cycle when compared to the N cycle during gravidity. We found no influence of incubation treatment and no interaction was detected (Table 2).

REPRODUCTIVE PHENOLOGY

Oviposition occurred earlier when females were exposed to M vs. N thermal treatment during gravidity (respective oviposition ordinal date 88.30 ± 1.34 vs. 103.14 ± 2.87 ; $F_{1,15} = 26.73$, $P < 0.001$). The duration of gravidity was 37% shorter in females exposed to the M vs. N thermal cycle (25.4 ± 1.87 and 40.42 ± 2.58 days, respectively; $F_{1,15} = 23.40$, $P < 0.001$). We found an effect of treatment on the duration of incubation, which was 24% shorter when eggs were exposed to the M vs. N thermal cycle (30.87 ± 0.19 and 40.8 ± 0.34 days, respectively; ANOVA, $F_{1,52,14} = 1369.96$, $P < 0.001$). We also found a delayed influence of gravidity treatment (ANOVA, $F_{1,14,7} = 20.41$, $P < 0.001$), with a shorter duration of incubation when eggs were previously exposed to M cycle in the maternal body (30.47 ± 0.94 vs. 32.00 ± 0.63 days for MM vs. NM and 39.70 ± 1.52 vs. 42.26 ± 1.66 days for MN vs. NN; Fig. 2A). The difference tended to be more marked when eggs were incubated under the N thermal cycle (interaction

Table 2. Influence of thermal treatment on hatching success

Variable	Gravidity treatment		Incubation treatment		Interaction			
	N	M	N	M	NN	NM	MN	MM
Reproductive females (N)	9	10	–	–	–	–	–	–
Eggs incubated (N)	39	44	41	42	20	19	21	23
Eggs hatching (N)	30	43	37	36	17	13	20	23
Hatching success (%)	77	98	90	86	85	68	95	100
Z-test		0.04	0.29		0.12			

Reproductive females were kept in either N ($N = 9$) or M ($N = 10$) thermal treatments during gravidity. After oviposition, the eggs of each clutch were distributed equally into M and N treatments for incubation. Significant differences are reported in bold values.

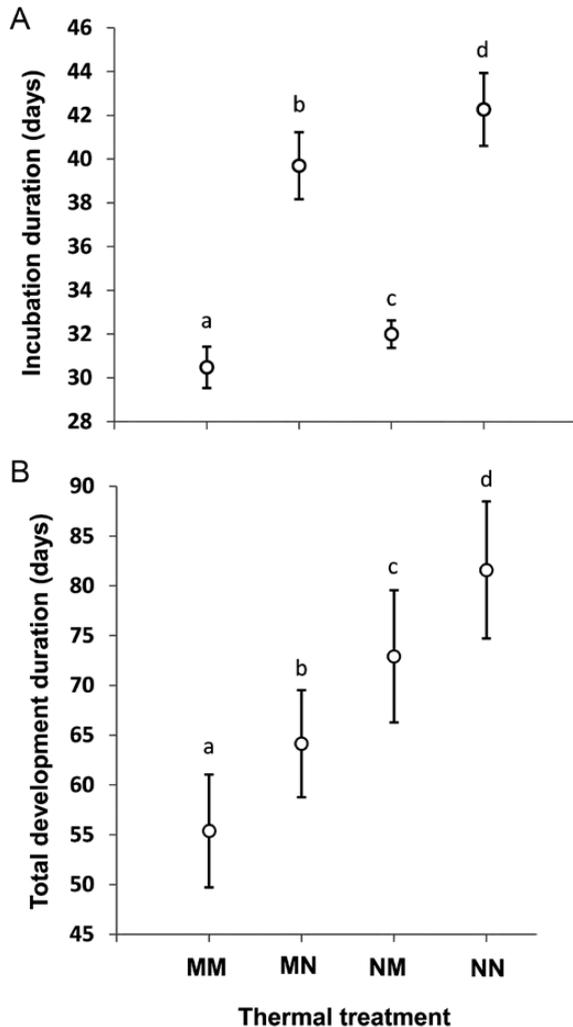


Figure 2. Influence of thermal treatment on duration of incubation (A) and total duration of development (B) in *Zootoca vivipara*. Thermal treatments MM, NN, MN and NM: the first and second letter, respectively, corresponds to the experimental conditions applied during gravidity and during incubation of the eggs, with M indicating a cycle mimicking maternal thermoregulation, and N mimicking a thermal regime in a nest. Error bars represent SE. Symbols not connected by the same letter are significantly different.

between gravidity and incubation treatments, ANOVA, $F_{1,52.14} = 3.44$, $P = 0.07$). The four experimental groups differed significantly in the duration of incubation (ANOVA, $F_{3,40.71} = 492.61$, $P < 0.001$; all Tukey's HSD *post hoc* test, $P < 0.05$; Fig. 2A).

When considering the total duration of development, we found no interaction between gravidity and incubation treatment (ANOVA, $F_{1,53.07} = 1.34$, $P = 0.25$). We found a significant influence of gravidity treatment, with a shorter development time under M vs. under N thermal cycles (59.46 ± 1.07 and 77.83 ± 1.45 days,

respectively; ANOVA, $F_{1,16.01} = 31.69$, $P < 0.001$). We also found an effect of incubation treatment, with a shorter duration of development under M than under N thermal cycle (61.72 ± 1.73 and 72.16 ± 1.75 days, respectively; ANOVA, $F_{1,53.07} = 1420.79$, $P < 0.001$). The four experimental groups differed significantly in the duration of development (ANOVA, $F_{3,43.18} = 493.83$, $P < 0.001$; all Tukey's HSD *post hoc* test, $P < 0.05$; Fig. 2B). They also differed in hatching dates (ordinal dates: MM, 118.47 ± 0.88 ; MN, 127.15 ± 0.9 ; NM, 134.69 ± 1.81 ; NN, 144.11 ± 1.99 ; $F_{3,43.14} = 494.66$, $P < 0.001$; all Tukey's HSD *post hoc* test, $P < 0.05$).

OFFSPRING MORPHOLOGY

We found no influence of thermal treatments on offspring sex (Table 3). Given that sex effects were not the main focus of the present study, males and females were pooled in subsequent analyses. We found a positive influence of maternal size on offspring mass and SVL. Controlling for maternal SVL, we found no influence of gravidity or incubation treatments on offspring body size (head length, SVL or TL) or body condition at birth (Table 3). A significant influence of incubation treatment on body mass was detected, with offspring being slightly heavier when incubated under N thermal cycle (ANCOVA, $F_{1,56.67} = 6.58$, $P = 0.013$). The occurrence of scale abnormalities varied significantly among our thermal treatments ($\chi^2 = 12.29$, d.f. = 3, $P = 0.006$), but pairwise comparisons revealed a lower occurrence of such abnormalities only in the MN group compared with the NN group (Tukey's HSD *post hoc* test, $P = 0.003$).

OFFSPRING BEHAVIOUR AND LOCOMOTION

We found an influence of incubation treatment on exploratory behaviour, with a higher number of compartments visited when incubated under the M thermal cycle (see Table 3). No differences were found when considering the number of shelters visited or the time spent under a shelter. We found a significant interaction between incubation and gravidity treatment when considering locomotion. Individuals in the MM group had higher stamina when compared with the others (Fig. 3; ANOVA, $F_{3,31.65} = 6.03$, $P = 0.002$; Tukey's HSD *post hoc* test, MM vs. any other group, $P < 0.05$).

DISCUSSION

The influence of temperature on embryonic development is a universal feature (Farmer, 2000; Angilletta, 2009). Although ectotherms typically face important daily fluctuations in their body temperature, to date the vast majority of experimental studies have used

Table 3. Influence of thermal treatment on offspring phenotype and behaviour

Offspring traits	Gravidity treatment			Incubation treatment			Interaction		
	M	N	Effect	M	N	Effect	Gravidity × incubation	Covariate (maternal SVL)	
Sex ratio (male/female)	21/21	17/14	$\chi^2 = 0.18$, d.f. = 1, $P = 0.67$	22/15	16/20	$\chi^2 = 1.49$, d.f. = 1, $P = 0.22$	$\chi^2 = 1.762$, d.f. = 1, $P = 0.18$	–	
Mass (mg)	222.53 ± 6.3	238.35 ± 5.90	$F_{1,36.27} = 3.6$, $P = 0.07$	226.34 ± 4.7	234.54 ± 4.73	$F_{1,53.71} = 7.16$, $P = 0.009$	$F_{1,54.01} = 1.78$, $P = 0.18$	$F_{1,144.81} = 13.17$, $P = 0.002$	
Head length (mm)	8.03 ± 0.1	7.90 ± 0.1	$F_{1,17.28} = 1.44$, $P = 0.24$	7.99 ± 0.07	7.94 ± 0.07	$F_{1,56.85} = 0.30$, $P = 0.58$	$F_{1,53.21} = 0.06$, $P = 0.81$	$F_{1,14.19} = 0.13$, $P = 0.72$	
SVL (mm)	20.33 ± 0.22	19.86 ± 0.24	$F_{1,12.08} = 1.42$, $P = 0.26$	20.06 ± 0.19	20.12 ± 0.19	$F_{1,53.66} = 0.01$, $P = 0.91$	$F_{1,52.97} = 2.04$, $P = 0.16$	$F_{1,14.04} = 7.22$, $P = 0.017$	
TL (mm)	46.79 ± 0.6	46.51 ± 0.64	$F_{1,25.71} = 0.11$, $P = 0.74$	46.90 ± 0.48	46.40 ± 0.40	$F_{1,51.63} = 2.41$, $P = 0.12$	$F_{1,51.87} = 2.41$, $P = 0.12$	$F_{1,14.67} = 2.43$, $P = 0.08$	
Exploratory behaviour (no. of compartments)	38.08 ± 4.73	33.7 ± 4.15	$F_{1,17.51} = 0.48$, $P = 0.49$	42.59 ± 4.23	29.19 ± 4.17	$F_{1,60.71} = 5.76$, $P = 0.01$	$F_{1,58.45} = 0.98$, $P = 0.32$	–	
Stamina (s)	71.87 ± 6.24	58.11 ± 6.99	$F_{1,22.5} = 2.16$, $P = 0.15$	76.47 ± 5.92	53.51 ± 5.83	$F_{1,61.28} = 10.55$, $P = 0.002$	$F_{1,59.74} = 14.73$, $P = 0.0003$	–	

Influences of treatment (M, maternal daily cycle; N, nest daily cycle) applied during gravidity and incubation using a 2 × 2 factorial design. We provide adjusted means derived from the main effects of the model. Significant differences are indicated in bold. A significant interaction between gravidity and incubation was found for stamina. Detailed values of stamina for each group are presented in Figure 3. Abbreviations: SVL, snout–vent length; TL, total length.

constant temperature (Bowden *et al.*, 2014), often imposing an important deviation from the temperature naturally experienced by the embryos (Braña & Ji, 2007). The ecological relevance of these approaches can be questioned (Bowden *et al.*, 2014), notably when addressing the functional significance of maternal thermoregulation (Shine, 2004a). For instance, imposition of a constant (either high or low) temperature will inevitably lead to deleterious effects on embryos if the regimes are outside the ecological range (Huey *et al.*, 1999). Herein, we imposed daily temperature cycles to test the impact of contrasted access time to the maternally preferred temperature in a lizard with extended egg retention. We discuss our findings below.

We detected a marked effect of maternal thermal regime (MM) on the duration of development, which was 25 days shorter when compared with nest incubation regimes (NN). Importantly, the magnitude of the effect was dependent on the reproductive stage. The duration of gravidity was 37% shorter under the maternal thermal regime, but the effect was less marked during incubation (24% reduction in duration). We also found that the duration of incubation was shorter when maternal thermal conditions were imposed during gravidity compared with nest thermal conditions (i.e. MM < NM and MN < NN). Therefore, the period of gravidity (which encompasses the first half of embryonic life) seems particularly sensitive to thermal conditions and access to thermal preferences. Reproductive phenology is an important determinant of reproductive performance in a variety of taxa (Visser & Both, 2005; Goutte *et al.*, 2011), notably in cold/temperate climatic conditions (Le Henanff *et al.*, 2013; Tomotani *et al.*, 2016). Hastened development and an early date of birth will optimize the time available for essential aspects, such as energy intake, and may have long-term positive effects on individual performance (Wapstra *et al.*, 2010; Le Henanff *et al.*, 2013).

Contrary to the effects on the duration of development, our thermal treatments had a limited effect on offspring morphology, with only a marginal effect on offspring mass. The lack of marked phenotypical effects contrasts with previous findings and, notably, experimental studies using more contrasted thermal conditions. For instance, previous work has reported the deleterious effect of exposing embryos to temperatures above maternal preference (Rodríguez-Díaz & Braña, 2011b). The limited impact on offspring morphology could be related to our study design based on only moderate constraints in the nest thermal regime (i.e. still with an access to T_{set}) when compared with the maternal thermal regime. A study in an oviparous snake, the Children's python (*Antaresia childreni*), demonstrated that accessing thermal preference during gravidity, even for a short duration, hastens

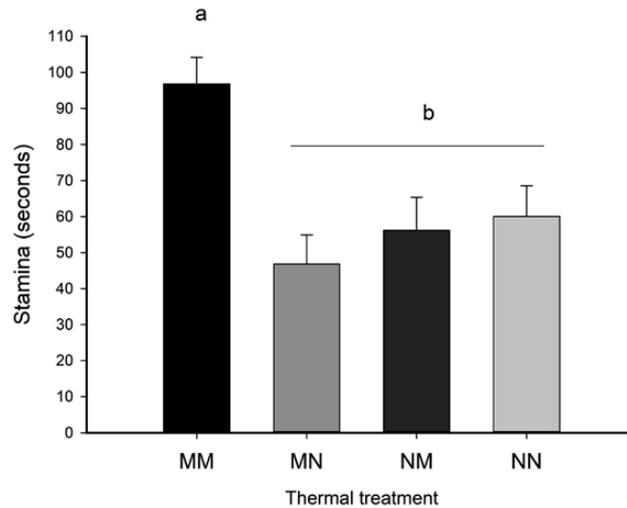


Figure 3. Influence of thermal treatment on offspring stamina (measured from the time delay before the first stop in the experimental arena). Thermal treatments MM, NN, MN and NM are as described in Figure 2. Error bars represent SE. Symbols not connected by the same letter are significantly different (Tukey's *post hoc* tests).

development and enhances offspring morphology and behaviour (Lorioux *et al.*, 2012). This experimental evidence provides support to the optimal developmental temperature hypothesis (Huey *et al.*, 1999).

We found that maternal thermal treatment during incubation stimulates neonatal activity when compared with nest conditions. Higher exploratory behaviour could be interpreted as beneficial for the neonate to optimize habitat selection and dispersion (Meylan *et al.*, 2002). Offspring exposed to maternal thermal regimes during gravidity and incubation had higher stamina than the other groups. Therefore, although limited effects on morphology were detected, significant influences on offspring performance may exist when the maternal thermal treatment (MM) is applied throughout development. Importantly, high environmental temperatures can also downregulate dispersion, as demonstrated in the viviparous form (Chamaillé-Jammes *et al.*, 2006). However, these effects probably reflect the impact of rising temperatures in relationship to global warming. Likewise, Rodríguez-Díaz & Braña (2011b) found that suboptimal (high) developmental temperatures alter locomotor performance in oviparous common lizards. Overall, the relationship between developmental temperature and locomotion is likely not to be linear, and the influence of access to preferred temperatures deserves more attention.

The significance of maternal thermoregulation in ectotherms has attracted considerable debate, with several competing explanations (Schwarzkopf & Andrews, 2012; Shine, 2012). According to the maternal manipulation hypothesis, a change in maternal thermoregulation is beneficial by optimizing

developmental conditions for the embryos. The selfish mother hypothesis posits that a maternal thermal shift primarily benefits the mother rather than the embryos. The accelerated development that we report typically supports the benefits of maternal manipulation (maternal phenological effects), because early hatching contributes to neonate fitness in a cold environment (Wapstra *et al.*, 2010; Le Henanff *et al.*, 2013). Previous studies have demonstrated that maternal thermoregulation can enhance both reproductive phenology and offspring phenotype (Rodríguez-Díaz *et al.*, 2010; Lorioux *et al.*, 2013). For instance, Lorioux *et al.* (2013) found, in a viviparous snake, that phenotypical effects were more pronounced at early stages, whereas maternal phenological influences were more continuous during pregnancy. However, Lorioux *et al.* (2013) imposed a deviation from thermal preferences rather than manipulating the duration of exposure to T_{set} .

We posit that the benefits of maternal regulation have to be considered within the framework of environmental constraints and microclimatic conditions in basking and nesting sites (Shine, 2004b; Hare & Cree, 2010; Wang *et al.*, 2014). Thermoregulatory strategies will depend on the thermal quality of the habitat, which varies in both space and time (Basson *et al.*, 2017). In cold climates, maternal thermoregulation provides an efficient way to minimize deviations from thermal preference (Gvozdík, 2002; Besson & Cree, 2010; Lourdais *et al.*, 2013) and to maintain temperatures that would hardly be accessible in a nest site (Shine, 2004c). The degree of embryonic retention is altitude dependent in female common lizards and is more important in high-elevation populations

facing colder thermal conditions (Rodríguez-Díaz & Braña, 2012). Results from our study suggest that this increase in retention will extend maternal control of developmental temperature and thereby provide substantial phenological benefits in cold climates.

CONCLUSIONS

We found that access to preferred maternal temperature significantly influences reproduction mainly through accelerated development when compared with nest conditions. Also, the maternal cycle during gravidity subsequently shortened the duration of incubation (i.e. delayed maternal effect). Only minor phenotypical effects were found, probably because embryos were also exposed to maternal thermal preferences in the nest treatment. Our results demonstrate that: (1) phenological effects are particularly sensitive to variations in the access to thermal preferences; and (2) a small maternal change of thermal conditions experienced by the embryos is relevant to optimize reproductive phenology. Further work is required to clarify the relationship between maternal thermoregulation, phenological and phenotypical effects.

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