



Effect of early thermal environment on the morphology and performance of a lizard species with bimodal reproduction

Iván Beltrán¹ · Victorien Durand^{1,2} · Rebecca Loiseleur^{1,2} · Martin J. Whiting¹

Received: 21 February 2020 / Revised: 11 August 2020 / Accepted: 9 September 2020 / Published online: 20 September 2020
© Springer-Verlag GmbH Germany, part of Springer Nature 2020

Abstract

Early developmental environments influence life-history traits and survival of reptiles. In fact, rising global temperatures have already caused widespread extinctions among lizards. Viviparous species might be more susceptible to increasing temperatures because of their inability to meet their energetic demands following rapid environmental changes. Nevertheless, we do not yet fully understand how lizards with different reproductive modes can respond to climate change. We investigated the effect of both maternal thermal environment during pregnancy and incubation temperature on hatchling morphology and physiological performance of two populations of the lizard *Saiphos equalis* differing in their mode of reproduction, to test whether reproductive mode affects the ability to buffer against rising temperatures. Gravid females from both populations were subjected to current or projected end-of-century (future) thermal environments, to evaluate differences in the body size, growth rate, thermal preference, and locomotor performance of their offspring. Our results show that independently of the mode of reproduction, high temperatures accelerated gestation periods. Thermal environments did not affect hatchling thermal preference, but viviparous hatchlings consistently preferred lower temperatures. Unlike viviparous lizards, oviparous hatchlings incubated under future temperatures were smaller and had a lower growth rate compared to current-incubated hatchlings. Finally, thermal environments did not affect hatchling endurance and speed when controlling for body size. Our results show that global warming is likely to have a negative impact on *S. equalis*, but suggest that some of its effects may be ameliorated by maternal responses during pregnancy, particularly in viviparous populations.

Keywords Squamate · Phenotypic plasticity · Climate change · Viviparity · Growth rate · Incubation temperature

Introduction

Environmental temperature is arguably the most influential variable impacting an animal's physiological performance (Brattstrom 1979; Huey 1982; Noble et al. 2018). For example, temperature affects protein functioning and limits the development, physiology, and behaviour of an animal, particularly in ectotherms such as reptiles, whose body temperature commonly fluctuates depending on external sources (Huey 1982; Somero 2004). For instance, incubation temperature strongly influences embryonic development in chelonians (Deeming and Ferguson 1991; Booth 2000), crocodilians (Webb and Cooper-Preston 1989; Birchard and Marcellini 1996) and squamates (Deeming and Ferguson 1991; Deeming 2004), with important consequences for the fitness and survival of hatchlings (Elphick and Shine 1998; Burger 1998; Booth 2000). The early thermal environment that the developing embryo experiences largely depends on female behaviour, whether it is the temperature of the female

Communicated by Kathrin Dausmann.

Victorien Durand and Rebecca Loiseleur have contributed equally to the work.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s00360-020-01312-2>) contains supplementary material, which is available to authorized users.

✉ Iván Beltrán
ic.beltran196@gmail.com

¹ Department of Biological Sciences, Macquarie University, Sydney, NSW, Australia

² Faculty of the Sciences of Life, University of Strasbourg, Strasbourg, Alsace, France

in utero (viviparity) or the female's choice for the location and depth of the nest she constructs (oviparity) (Packard et al. 1977). Although most squamates are oviparous, viviparity has evolved independently more than 100 times within this group (Blackburn 2006; Van Dyke et al. 2014).

This repeated convergent evolution has been explained by two main hypotheses. The 'maternal manipulation' hypothesis states that egg retention results in higher offspring viability because the environment provided by females contributes to developmental benefits such as an increased growth rate or sprint speed (Shine 1995; Pincheira-Donoso et al. 2013). The second hypothesis, which has received more support and attention, is the 'cold-climate' hypothesis (Sergeev 1940; Shine 1985; Blackburn 2000), which states that cold-adapted populations are more likely to be viviparous because the stable temperatures provided by females protect embryo development from unsuitable temperatures (i.e. maternal buffering). In fact, viviparity (as an adaptation to cold environments) is often associated with colder thermal preferences which may increase the sensitivity of the embryos of viviparous species to warmer temperatures, compared to those of oviparous species (Sinervo et al. 2010; Meiri et al. 2013). Nonetheless, some studies have shown that ectotherms living at high latitude and elevation (often cold environments) may have a larger 'thermal-safety margin' (Sunday et al. 2014) compared to tropical ectotherms. The 'thermal-safety margin' measures the difference between a species' maximum tolerance to heat and the warm air temperatures it regularly experiences (Deutsch et al. 2008; Sunday et al. 2014), suggesting that tropical species might be more vulnerable to climate change than temperate species. Likewise, in thermally-constrained environments, lizards might benefit from reasonable warming (Adolph and Porter 1993; Chamaillé-Jammes et al. 2006). However, Sinervo et al. (2010) showed that extinction probability in lizards strongly correlates with the magnitude of warming in spring, but not other seasons. This suggests that extinction in this group is driven by energetic deficiency during the reproductive season when the energy demands are highest (Huey et al. 2010). Because embryos are particularly vulnerable to environmental stress, such as global warming, viviparity has been identified as a key risk factor in exacerbating recent lizard extinctions (Sinervo et al. 2010; Pincheira-Donoso et al. 2013). However, most of the studies examining this premise are based on computational modelling (Sinervo et al. 2010; Meiri et al. 2013; Pincheira-Donoso et al. 2013; Kubisch et al. 2015) and there is little experimental evidence that viviparous species are indeed at a greater risk of extinction (but see Wang et al. 2017). Moreover, the findings of these studies are often difficult to interpret or extrapolate because of the phylogenetic and/or ecological differences between the compared species (Meiri et al. 2013; reviewed by Noble et al. 2018). In a global warming scenario, species with low dispersal ability, such as lizards, are more likely to respond through adaptation or behavioural

and physiological plasticity (MacLean and Beissinger 2017). However, the extent to which lizards with different modes of reproduction are able to respond to global warming by modifying key life-history traits that will later determine their fitness is still unclear.

The Australian skink *Saiphos equalis* (Gray 1825), is one of only three reptile species known to exhibit geographic variation in reproductive mode (Heulin et al. 1993; Smith and Shine 1997; Fairbairn et al. 1998). However, due to its nocturnal and fossorial habits, little is known about its behaviour and ecology. *S. equalis* inhabits cool environments where it is commonly observed under sun-warmed rocks and logs during the day (Gray 1825; Wu et al. 2009) and foraging on the surface at night (*personal observations*). This species has viviparous populations along the Northern Tablelands of New South Wales (NSW) where females give birth to fully-developed hatchlings inside a membrane from which they emerge within a couple of days. The populations from the eastern coast of NSW, including the Sydney region, have prolonged uterine egg-retention (hereafter referred to as oviparous) (Bustard 1964; Smith and Shine 1997). These oviparous populations lay shelled eggs in which embryos continue developing outside the female's body for approximately 5–7 days (Smith and Shine 1997). While these oviparous populations have a considerably shorter incubation duration compared to other sympatric oviparous lizards (> 30 days) (Qualls and Shine 1998), it is well known that small differences in the incubation period can have profound effects on hatchling phenotype and fitness (Vince and Chinn 1971; Shine and Olsson 2003; Radder et al. 2008; While et al. 2009). Therefore, *S. equalis* represents a unique opportunity to test specific predictions on the interplay between global warming and reproductive mode, while controlling for relatedness.

Here, we examined the effect of different thermal environments during development on body size, growth rate, thermal preference, and locomotor performance of hatchling lizards from oviparous and viviparous populations of *Saiphos equalis*. We hypothesized that predicted end-of-century temperatures will have a negative impact in most, if not all, of the traits examined in hatchlings from both populations. However, given that viviparous species are considered to be at greater risk of extinction due to energetic deficiency during the reproductive season (Sinervo et al. 2010; Meiri et al. 2013; Pincheira-Donoso et al. 2013), we predicted that the effect of future thermal environments would be greater in offspring from viviparous populations.

Materials and methods

Animal capture and husbandry

Between September and November 2017, we collected gravid females of *S. equalis* by hand from two populations: Riamukka State Forest ($n = 12$), in northern NSW ($31^{\circ}20' \text{ S}$, $151^{\circ}39' \text{ E}$), and greater Sydney ($n = 32$), in southern NSW [Winston Hills ($33^{\circ}46' \text{ S}$, $150^{\circ}59' \text{ E}$), Willoughby ($33^{\circ}48' \text{ S}$, $151^{\circ}11' \text{ E}$), Tempe ($33^{\circ}55' \text{ S}$, $151^{\circ}08' \text{ E}$) and Hurstville ($33^{\circ}58' \text{ S}$, $151^{\circ}05' \text{ E}$)]. Animals were found under rocks, logs, and leaf litter. We scored lizards as gravid based on appearance and light abdominal palpation of eggs/embryos and transported all lizards to Macquarie University within 48 h of capture. For each lizard, we measured the snout-vent length (with a transparent ruler to the closest $\pm 1 \text{ mm}$) and weight ($\pm 0.1 \text{ g}$). Each female was housed in a separate plastic enclosure ($200 \times 150 \times 90 \text{ mm}$) with moist potting soil to a depth of 5 cm. Each cage contained a $100 \times 100 \text{ mm}$ wooden shelter and a water dish. Animals were fed three times a week with 5–6 small crickets ($\sim 10 \text{ mm}$) each time. Crickets were dusted with vitamins (aristopet Repti-vite®) and calcium (URS Ultimate Calcium®) once per week. Females

were kept in these conditions for 3 days before they were allocated to one of the thermal environments.

A considerable proportion of the females with whom we started the study turned out not to be gravid and had to be removed from the incubation experiments. Consequently, our final sample size included 13 females from the oviparous population and 8 females from the viviparous population (Table 1).

Thermal environments

Gravid females from both populations were randomly allocated to one of two cycling incubators (PGR15/Growth chamber, Conviron®, Melbourne, Australia). The first treatment (hereafter referred to as ‘current’) was designed to match the preferred temperatures reported for gravid female *S. equalis* (sinusoidal daily curve of $22 \pm 1.6^{\circ} \text{ C}$ [mean $\pm$ SD], range = 20 – 25° C ; Table S1) (Wu et al. 2009). The second treatment (hereafter referred to as ‘future’) was set to have a mean increase in temperature of 3° C ($25 \pm 1.6^{\circ} \text{ C}$, range = 23 – 28° C ; Table S1) which corresponds to the expected increase in spring and summer temperatures by 2100 in south-eastern NSW (Dowdy et al. 2015). We checked for eggs twice a day. Eggs were weighed and then transferred to a 100-mL plastic jar with moist vermiculite

Table 1 General information about the pregnant/gravid female *Saiphos equalis* used in this study

Capture date	Population	Locality	Treatment	Female	Mass (g)	SVL (mm)	Total length (mm)	Birthing/oviposition date	Litter/clutch size	Days in treatment
11/11/17	Viviparous	Riamukka SF	Current	SeV17	4.4	80	131	15/1/18	3	62
11/11/17	Viviparous	Riamukka SF	Current	SeV04	5.6	77	165	16/1/18	5	63
11/11/17	Viviparous	Riamukka SF	Current	SeV13	6.1	84	173	18/1/18	4	65
11/11/17	Viviparous	Riamukka SF	Future	SeV06	4.5	76	147	6/1/18	3	53
11/11/17	Viviparous	Riamukka SF	Future	SeV18	3.4	72	97	7/1/18	3	54
11/11/17	Viviparous	Riamukka SF	Future	SeV09	3.7	83	111	8/1/18	2	55
11/11/17	Viviparous	Riamukka SF	Future	SeV20	5.9	82	168	11/1/18	4	58
11/11/17	Viviparous	Riamukka SF	Future	SeV19	5.1	79	160	12/1/18	1	59
28/11/17	Oviparous	Hurstville	Current	SeO36	4.3	72	149	31/1/18	3	61
28/11/17	Oviparous	Hurstville	Current	SeO31	3.3	77	105	3/2/18	3	64
28/11/17	Oviparous	Hurstville	Current	SeO40	4.9	79	169	21/2/18	1	82
29/11/17	Oviparous	Tempe	Current	SeO44	4.8	81	135	29/1/18	1	58
29/11/17	Oviparous	Tempe	Current	SeO43	4.6	77	163	5/2/18	2	65
1/11/17	Oviparous	Willoughby	Current	SeO47	4.6	78	167	27/1/18	3	84
28/11/17	Oviparous	Hurstville	Future	SeO33	4.4	72	154	20/1/18	4	50
28/11/17	Oviparous	Hurstville	Future	SeO35	4.2	80	144	20/1/18	3	50
28/11/17	Oviparous	Hurstville	Future	SeO41	6.6	81	175	22/1/18	4	52
29/11/17	Oviparous	Tempe	Future	SeO45	4.3	74	157	10/1/18	3	39
20/11/17	Oviparous	Willoughby	Future	SeO27	4.5	77	158	11/1/18	3	49
1/11/17	Oviparous	Willoughby	Future	SeO46	3.7	73	155	14/1/18	4	71
26/11/17	Oviparous	Winston Hills	Future	SeO30	4.2	81	126	12/1/18	4	44

(water potential of -200 kPa). Plastic jars were sealed with cling wrap to prevent desiccation and remained in the same incubators as their mothers until hatching. Temperature inside the incubators was recorded every 15 min using Thermocron iButton® loggers (DSG1921G, ± 0.5 °C, Maxim Integrated Products/Dallas Semiconductor, Texas, USA).

After females oviposited or gave birth, we released them at their original point of capture. Ovulation dates are unknown for the species; however, ovulation most likely occurs in early spring (October) for the oviparous population and possibly later in the viviparous population which inhabits a cooler montane environments (Michael Thompson, University of Sydney, *personal communication*). Because we were interested in the effect of the thermal environments on the gestation period rather than differences in gestation period between populations, we analysed this relationship for each population independently. Following Wapstra (2000) and While et al. (2009), we calculated the gestation days for each female as the elapsed number of days from a theoretical ovulation date (October 1st—spring in the southern hemisphere) until they gave birth or oviposited.

Hatchling morphology and growth

We weighed (± 0.001 g) and measured the snout-vent length (SVL) and tail length (both to ± 0.05 mm) of each newborn lizard using the software ImageJ Version 2.0 (RRID: SCR_003070; National Institute of Health, USA). For each lizard, we calculated its body condition index (BCI) using the residuals from a linear regression of $\log_{10}$ (mass) on $\log_{10}$ (SVL) (Cox et al. 2011). We determined sex at the end of the study following dissections of lizards at ~ 10 months of age, these results showed similar sex ratios between populations and thermal environments (Table S2).

Each hatchling was then transferred to a separate plastic enclosure ($175 \times 120 \times 80$ mm) containing moist potting soil to a depth of approximately 3 cm, a 45×45 mm ceramic tile shelter and a water dish. Animals were housed in a room (~ 21 °C) with a 12:12 light cycle. Enclosures were placed on wooden racks, with one end of the cage sitting on a timer-controlled heating cable to create a thermal gradient of 21 – 34 °C during the day, dropping to room temperature at night. We fed hatchlings three times a week with five pin-head crickets (~ 5 mm) and on 2 days the crickets were dusted with vitamins.

We repeated all body measurements every 4 weeks for 6 months to track differences in growth rate between populations and thermal environments. To reduce the number of predictive variables in the analysis, we estimated size-specific growth rate as the residuals of the linear regression of SVL as a function of age (weeks since hatching) (Sinervo 1990). The same procedure was applied for mass-specific and body condition-specific growth rates.

Thermal preference of hatchlings

Approximately 6 weeks after hatching, we measured the thermal preferences of all hatchlings in a thermal gradient consisting of six rectangular runways (each 900 mm $\times$ 45 mm). The arena was placed in a temperature-controlled room (~ 21 °C). The bottom of each runway was covered with 10 mm of potting mix for the lizards to move and bury themselves. A linear temperature gradient ranging from 8 – 40 °C was created by placing wide heating tape (BriskHeat®, Ohio, USA) on one end of the runway and a recirculating chiller bath (Haake F3-C, Germany) on the other end. The arena was insulated with Styrofoam to minimize temperature fluctuations. Temperatures inside the gradient were recorded every 10 min with iButton® loggers evenly distributed along each runway. Hatchlings were food deprived for 48 h before being placed in a runway. 1 day before the measurements were taken, a single lizard was placed in each of the six runways and allowed to habituate for at least 20 h (Daut and Andrews 1993; Wu et al. 2009). Animals were chosen randomly and the researcher measuring body temperature was blind regarding thermal environment. After habituation, the body temperature of each lizard was measured every hour over 10 h (12:00–22:00) using a thermocouple thermometer (± 0.1 °C; Digitech QM-1601, Hounslow, UK). Body temperature was measured by gently placing the thermocouple under the lizard at the midpoint of the body (Wu et al. 2009). The measurements appeared not to disturb lizards or to alter their behaviour. We calculated the mean preferred body temperature (T_{pref}) for each individual lizard across the 10 measurements. We repeated these measurements at 6 months of age to test for differences in T_{pref} due to acclimation to captive conditions.

Locomotor performance of hatchlings

Escape performance

At 7 weeks of age, we measured the escape performance of hatchlings in a racetrack (60×6 cm). The room was kept at ~ 21 °C which is the approximate preferred body temperature of these lizards in the laboratory (Wu et al. 2009; and the results section of this study). For each trial, we placed a hatchling on the racetrack, let it habituate for 3 min, and then tapped it on the base of the tail with a paintbrush to induce escape behaviour and measure their escape speed. Each animal was tested five times, with at least a 20-min rest between trials. Trials were recorded with a slow-motion camera (Sony® CMOS HDR-SR7). For each trial, we were blind to the thermal treatment. We determined the maximum speed (m/s), maximum acceleration (m/s^2), and maximum distance travelled (cm in the 60-cm racetrack) by the animal using the software Tracker (Open Source Physics, USA;

<https://physlets.org/tracker/>). To estimate these variables, the snout of the animal was used as reference point and was manually tracked along the racetrack in each video. Before each trial started, we recorded the lizards' body temperature (± 0.5 °C, with a handheld laser thermometer [Testo® 830-T1; Melbourne, Australia] pointed to the lizard's abdomen at a distance of ~1 cm). Maximum speed and maximum acceleration were significantly correlated (*Pearson correlation* = 0.82, $P < 0.01$) and when analysed independently, yielded similar results; thus, we only show the results for maximum acceleration (see Table S3 for results on maximum speed).

Endurance

At 9 weeks of age, animals were run on a small treadmill set to a fixed running speed of 0.25 km/h and placed in a room at ~21 °C. The treadmill had two parallel acrylic glass walls separated by 5 cm that prevented the lizard from turning around during a trial. All lizards were tested three times, with a 5-day rest between measurements. The order in which animals were tested was randomized for each measurement and researchers were blind regarding thermal environment. For each trial, lizards were placed in the first third (15 cm) of the treadmill, so that as they were getting tired we had enough time to tap them on the base of the tail (Garland Jr and Else 1987) with a small paintbrush before the lizard was carried along the track and pushed off the end. Endurance was assessed as the total time (s) that had elapsed until the hatchling fell off the belt a maximum of 6 times at which point it was exhausted (Huey et al. 1984). For each trial, we recorded the lizard's body size and body temperature (± 0.5 °C, with a handheld laser thermometer [Testo® 830-T1; Melbourne, Australia] pointed at the lizard's abdomen at a distance of ~1 cm).

Statistical analysis

Model assumptions of normality of residuals and homogeneity of variance were verified using Shapiro–Wilk's tests and Levene's test, respectively (Zuur et al. 2010). We used linear (LMM) and generalized linear (GLMM) mixed models to evaluate the effect of thermal environment temperature (current vs. future), population (viviparous vs. oviparous), and their interaction (*population* $\times$ *thermal environment*) on our response variables (see below). Hatchling sex was not included in the models to avoid issues with statistical power (overparameterization) and because we did not expect differences in morphology and performance between male and female hatchlings of this species (Shine and Harlow 1996; Braña and Ji 2000). The models were structured as follows:

- (a) A GLMM using a Poisson error distribution ('log' link) was used to test differences in gestation period between thermal environments. Gestation periods were analysed independently for each population as stated above. Since the ovulation dates for the individual females were unknown, it is possible that the embryos were at different developmental stages when they entered the experimental treatments. Although this difference in exposure to the thermal environment between embryos can be a confounding factor, our low statistical power prevented us from testing it. Therefore, we recommend these results be interpreted with caution.
- (b) A GLMM using a Poisson error distribution ('log' link) was used to test differences in incubation duration between populations and thermal environments. This model included mother identity as random effect to account for non-independence in the data. Incubation duration for the viviparous lizards was calculated as the number of days in which neonates emerged from the membrane after birth.
- (c) A LMM was used to test differences in hatchling size at birth between thermal environments and populations. Mother identity was included as random effect. Egg mass (or the mass of the membrane containing the fully-developed lizards for the viviparous population) was included as a covariate in the model for SVL and body condition. Likewise, SVL was included as a covariate in the models for hatchling mass and tail length.
- (d) A LMM was used to test for differences in T_{pref} between thermal environments and populations. Mother identity was included as a random factor and SVL as a covariate.
- (e) A LMM was used to test for differences in mass- and size-specific growth rate between thermal environments and populations. Hatchling identity nested within mother identity was included as a random factor.
- (f) A LMM was used to test for differences in locomotor performance between thermal environments and populations. We used the data from all trials and accounted for within-individual variation (Careau and Wilson 2017), instead of estimating a maximum or average value per individual. Maximum distance travelled in the racetrack and endurance data were log-transformed to fit model assumptions. Trial number and hatchling identity nested within mother identity were included as random factors. Body temperature and SVL were used as covariates.

The selection of predictive variables was done by a step-wise backward elimination process, starting from the most complex global model and dropping the fixed effects that were not significant until a minimal adequate model was

reached (Harrison et al. 2018). Model selection was based on corrected Akaike Information Criteria (Burnham and Anderson 2002; Bolker et al. 2009) and when necessary, the simplest (i.e. most parsimonious) model was chosen. The R packages ‘lme4’ (Bates et al. 2015), ‘lmerTest’ (Kuznetsova et al. 2017) and ‘MuMIn’ (Bartoń 2018) were used to perform model analyses, calculate the corresponding *P*-values of the fixed effects in the mixed models, and in model selection, respectively. All analyses were carried out using the statistical software R (R Core Team 2018) at a significance level of $\alpha = 0.05$.

Results

Effect of thermal environments on gestation period and incubation duration

Hatching and birthing occurred between early-January and mid-February 2018 (Table 1). The variation in oviposition/birthing dates within experimental groups was relatively small (coefficient of variation range = 1.42–7.18%); however, high incubation temperatures reduced the gestation period of females. Oviparous females exposed to future temperatures oviposited earlier (mean $\pm$ s.d., future = 106.6 days $\pm$ 4.9 vs. current = 126.2 days $\pm$ 9.0; Fig. 1). Likewise, viviparous females exposed to future temperatures gave birth earlier, on average, compared to females exposed to current temperatures (future = 99.8 days $\pm$ 2.6 vs. current = 107.3 days $\pm$ 1.5; Fig. 1). This effect was statistically significant for the oviparous population ($\beta = -0.16 \pm 0.05$ (s.e.m.); $Z = -3.24$; $P < 0.01$) but not for the viviparous population ($\beta = -0.07 \pm 0.07$; $Z = -1.02$; $P = 0.31$), although these results must be interpreted with caution due to our low sample size. Regardless of the thermal environment, the incubation duration was shorter in the viviparous population compared to the oviparous population (0.56 days $\pm$ 0.58 vs. 7.56 days $\pm$ 2.94; *population*, $Z = -8.017$; $P < 0.001$); but elevated developmental temperatures did not affect the incubation duration in viviparous or oviparous populations (*thermal environment*, $P = 0.76$).

Effect of thermal environments on hatchling morphology and growth rate

Body size at birth

Birthing success was 100% in the viviparous population (25 lizards) while 30 out of 38 (78.9%) of the eggs from the oviparous populations hatched. From these 8 eggs that did not hatch, six belonged to the current treatment and two belonged to the future thermal treatment. Thus, our initial sample sizes were: current-oviparous ($n = 7$),

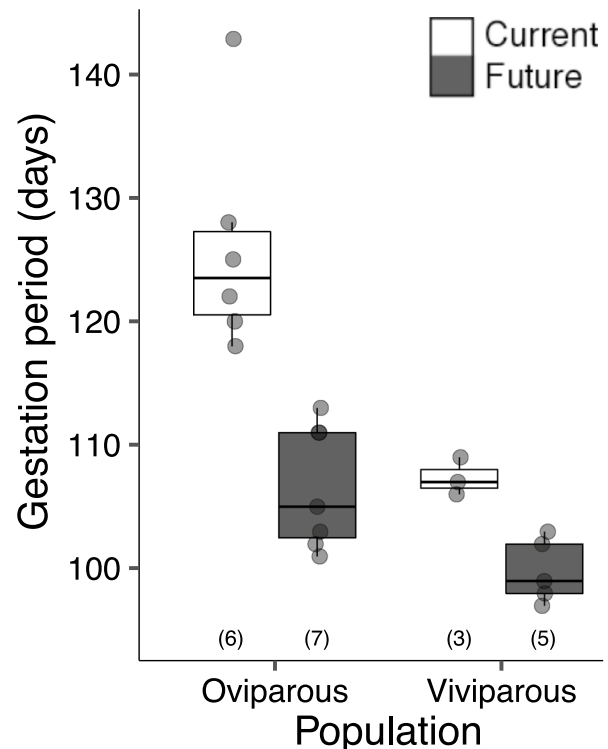


Fig. 1 Mean gestation period of females from two populations of *Saiphos equalis* subjected to different (current vs. future) thermal environments. Gestation period for each female was measured as the number of days between the 1st of October (theoretical ovulation date) until they gave birth or laid eggs. The number of females in each group is given on the bottom of each box

future-oviparous ($n = 23$), current-viviparous ($n = 12$), and future-viviparous ($n = 13$).

The thermal treatment had a significant effect on the body size of hatchlings (Table 2a). Due to our low sample size, the statistical model did not support an interaction between population and thermal environment; however, the effect of the treatment appears to be more pronounced in the oviparous population (Fig. 2a). Oviparous hatchlings incubated under future temperatures were smaller (mean $\pm$ s.d., 26.3 mm $\pm$ 1.4) compared to current-incubated oviparous hatchlings (28.1 mm $\pm$ 0.7; Fig. 2a), whereas viviparous hatchlings had similar body sizes regardless of their thermal treatment (future = 27.9 mm $\pm$ 0.9 vs. current = 28.1 mm $\pm$ 1.3; Fig. 2a).

When SVL was controlled for, tail length was similar between populations and incubation temperatures (*thermal environment*, $P > 0.89$; *population*, $P > 0.40$). Heavier eggs produced hatchlings with a larger SVL (*egg mass*, $P < 0.01$). After controlling for SVL, viviparous hatchlings were significantly heavier (mean $\pm$ s.d., 0.32 g $\pm$ 0.02) than oviparous hatchlings (0.23 g $\pm$ 0.04; Table 2b), regardless of the incubation temperature. Hatchlings from the future

Table 2 Outcome of the linear mixed models testing the effect of thermal environment (current vs. future) in several phenotypic traits of hatchlings from two populations (viviparous vs. oviparous) of *Saiphos equalis*

Response variable	Fixed term (reference level)	Estimate	Standard error	Degrees of freedom	<i>t</i> value	<i>P</i> value
(a) Hatchling body size (SVL)						
	<i>Intercept (Individual—oviparous current)</i>	23.810	1.405	46.647	16.945	< 0.001
	Population (viviparous)	0.190	0.595	18.211	0.320	0.753
	<i>Thermal environment (future)</i>	− 1.698	0.581	16.369	− 2.924	0.009
	<i>Egg mass</i>	9.196	3.059	46.449	3.006	0.004
(b) Hatchling body mass						
	<i>Intercept (Individual—oviparous current)</i>	− 0.100	0.076	48.188	− 1.312	0.196
	<i>Population (viviparous)</i>	0.072	0.009	14.606	7.768	< 0.001
	<i>Thermal environment (future)</i>	0.010	0.009	14.183	1.037	0.317
	<i>SVL</i>	0.012	0.002	49.156	4.412	< 0.001
(c) Mass-specific growth rate						
	<i>Intercept (Individual—oviparous current)</i>	0.007	0.014	26.513	0.489	0.628
	<i>Population (viviparous)</i>	0.030	0.018	15.083	1.654	0.118
	<i>Thermal environment (future)</i>	− 0.069	0.016	20.840	− 4.137	< 0.001
	<i>Population × thermal environment (viviparous × future)</i>	0.079	0.022	13.755	3.466	0.003
(d) Size-specific growth rate						
	<i>Intercept (Individual—oviparous current)</i>	0.015	0.010	31.104	1.524	0.137
	<i>Population (viviparous)</i>	0.002	0.012	16.545	0.199	0.844
	<i>Thermal environment (future)</i>	− 0.042	0.012	24.126	− 3.520	0.001
	<i>Population × thermal environment (viviparous × future)</i>	0.036	0.015	14.537	2.280	0.038
(e) Escape performance (max. acceleration)						
	<i>Intercept (Individual—oviparous current)</i>	0.592	0.657	46.492	0.900	0.373
	<i>Population (viviparous)</i>	− 0.245	0.075	12.430	− 3.260	0.007
	<i>Thermal environment (future)</i>	0.158	0.079	16.896	− 2.004	0.061
	<i>SVL</i>	0.025	0.020	45.913	1.257	0.215
(f) Endurance						
	<i>Intercept (Individual—oviparous current)</i>	5.216	0.697	89.506	7.489	< 0.001
	<i>Population (viviparous)</i>	− 0.230	0.043	9.506	− 5.364	< 0.001
	<i>Thermal environment (future)</i>	− 0.036	0.046	9.900	− 0.771	0.458
	<i>SVL</i>	0.034	0.012	39.791	2.760	0.009
	<i>Body temperature</i>	− 0.070	0.024	102.320	− 2.978	0.004

Significant values are in italics

thermal environment had similar body condition (*thermal environment*, $P = 0.07$). Viviparous hatchlings had a significantly higher body condition compared to oviparous hatchlings (*population [viviparous]*, $\beta = 0.26 \pm 0.04$; $t = 5.66$; $df = 17.97$; $P < 0.001$). Thus, viviparous hatchlings were born with a higher mass relative to their body size independent of incubation temperature.

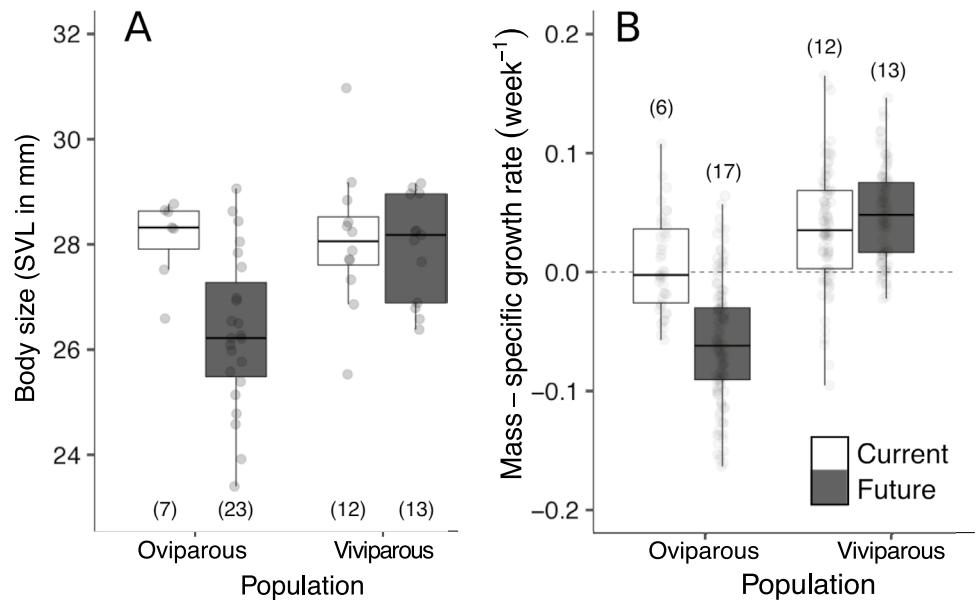
Hatchling growth rate

The mortality during in the first 6 months was considerably higher in the oviparous population subjected to the future thermal environment; only 17 (~ 74%) of 23 lizards were

alive at the end of the experiments. In fact, only one of the oviparous hatchlings from the current thermal environment died, while none of the viviparous hatchlings had died at the end of the experiments.

The thermal environment had a significant effect on the increase in body mass of hatchlings over time (*thermal environment*, $F = 6.627$; $df = 13.756$; $P = 0.022$; Fig. 2b). Contrary to the viviparous population, the increase in body mass of oviparous hatchlings was lower in the future-incubated lizards ($- 0.061 \text{ g/week} \pm 0.052$) compared to the current-incubated ones ($0.007 \text{ g/week} \pm 0.043$; Table 2c). We found the same pattern for size-specific growth rate. The thermal environment had a significant effect on the

Fig. 2 Effect of early thermal environment (current vs. future) on the **a** mean body size at birth and **b** mass-specific growth rate of hatchlings from two populations of *Saiphos equalis*. The number of individuals in each group is given on top or bottom of each box



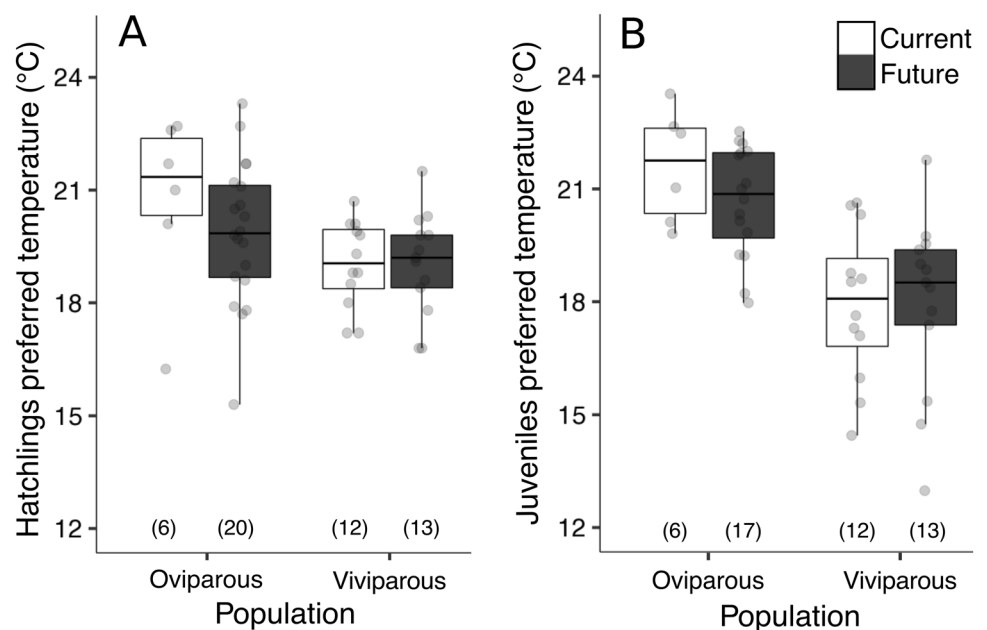
increase in body size of hatchlings over time (*thermal environment*, $F = 9.267$; $df = 14.537$; $P = 0.008$; Fig. 2b). Oviparous hatchlings incubated under future temperatures had lower size-specific growth rates (-0.026 mm/week ± 0.055) compared to current-incubated oviparous hatchlings (0.016 mm/week ± 0.048), whereas viviparous hatchlings had similar growth rates regardless of their thermal treatment (future = 0.012 mm/week ± 0.048 vs. current = 0.018 mm/week ± 0.053 ; Table 2d). Future thermal environments did not significantly affect the relative change in body condition of hatchlings over time (*thermal environment*, $P = 0.22$).

Effect of thermal environments on hatchling temperature preference

Viviparous hatchlings had a significantly lower mean T_{pref} ($19^\circ\text{C} \pm 1.2$; mean $\pm$ s.d.) compared to oviparous hatchlings ($20^\circ\text{C} \pm 2$; *population*, $t = -2.24$; $df = 48$; $P = 0.03$) when data from both current and future treatments were pooled. However, T_{pref} did not differ significantly between thermal environments in either group (*thermal environment*, $P = 0.49$; Fig. 3a).

The difference in T_{pref} between oviparous and viviparous hatchlings became stronger when lizards were measured

Fig. 3 Preferred body temperatures of **a** hatchling and **b** 6-month juvenile lizards from two populations of *Saiphos equalis* incubated under different thermal (current vs. future) environments. The number of individuals in each group is given on the bottom of each box



at the age of 6 months. Viviparous hatchlings preferred a considerably lower mean body temperature ($17.9\text{ }^{\circ}\text{C} \pm 2.2$; mean $\pm$ s.d.) compared to oviparous hatchlings ($20.9\text{ }^{\circ}\text{C} \pm 1.5$; *population*, $t = -5.40$; $\text{df} = 44$; $P < 0.001$). As observed in hatchlings, the body temperatures preferred by juvenile lizards did not differ between thermal environments (*thermal environment*, $P = 0.52$; Fig. 3).

Effect of thermal environments on hatchling locomotor performance

Escape performance

Future- and current-incubated lizards travelled similar maximum distances in the 60 cm-racetrack (*thermal environment*, $P = 0.38$). Likewise, the maximum distance travelled by viviparous or oviparous hatchlings was not significantly different (*population*, $P = 0.15$). Regardless of the population, thermal environment had a small, although not significant, negative effect on escape acceleration (Table 2e; Fig. 4a). When SVL is removed from the analysis, the effect of incubation temperature on escape acceleration becomes significant ($P = 0.039$), meaning that differences in acceleration could be partially explained by the larger SVL of current-incubated lizards. Interestingly, viviparous populations showed a significantly lower escape acceleration on the racetrack (Table 2e).

Endurance

Current- and future-incubated hatchlings had similar endurance (*thermal environment*, $P = 0.46$; Fig. 4b); however, the

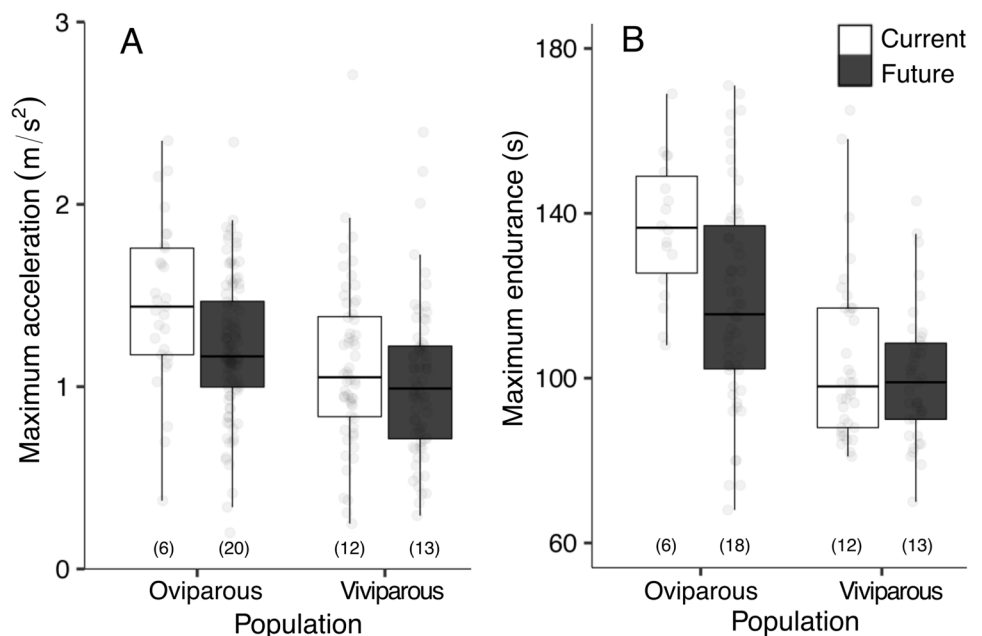
endurance of viviparous lizards was considerably lower than oviparous lizards, even when SVL was included as a covariate (Table 2f). Body size (SVL) had a positive effect on hatchlings endurance (Table 2f), suggesting that bigger hatchlings ran for longer. Conversely, hatchlings with higher body temperature ($23\text{ }^{\circ}\text{C} \pm 0.6$; range = $21.5\text{--}24.5\text{ }^{\circ}\text{C}$) had lower endurance (Table 2f).

Discussion

Experimental evidence on how early thermal environments affect hatchling phenotype is necessary to predict the vulnerability of viviparous and oviparous lizards to environmental changes, such as global warming. Here, we show that both viviparous and oviparous populations of *Saiphos equalis* are affected by elevated developmental temperatures. Regardless of the reproductive mode, future-incubated lizards hatched earlier and were smaller at birth compared to current-incubated lizards. Elevated temperatures reduced hatchling growth rate in future-incubated oviparous, but not viviparous, hatchlings. However, the thermal environments did not affect thermal preference although viviparous hatchlings consistently preferred cooler temperatures in a thermal gradient compared to oviparous hatchlings. Finally, the thermal environments had no effect on hatchling maximum acceleration, speed or endurance, when body size was controlled for.

Key physiological functions, including embryonic development, have a high dependence on environmental conditions in ectotherms such as lizards (Deeming and Ferguson 1991; Gillooly and Dodson 2000). For example, elevated

Fig. 4 Effect of early thermal environment (current vs. future) on the **a** maximum acceleration over a 0.6 m racetrack and **b** maximal endurance capacity of hatchling lizards from two populations of *Saiphos equalis*. The number of individuals in each group is given on the bottom of each box



temperatures accelerate the gestation process in ectotherms, including both oviparous (Van Damme et al. 1992; Burchard and Marcellini 1996; Burger 1998) and viviparous (Beuchat 1988; Shine and Harlow 1993) squamates. Here, we found that the mean gestation period of both oviparous and viviparous populations of *Saiphos equalis* was reduced by higher temperatures, although this effect was statistically significant only for oviparous lizards. Thermal environments did not affect post-laying incubation duration in *S. equalis*, contrary to what has been reported in many other reptile species (Werner 1990; Van Damme et al. 1992; Downes and Shine 1999). Because the incubation duration in *S. equalis* is considerably short, it is possible that the effect of temperature on these late stages of development is minimal (Smith and Shine 1997; Angilletta et al. 2000; Qu et al. 2014). Nevertheless, because the gestation period was accelerated in future-incubated *S. equalis*, there could be fitness implications for wild populations because offspring would hatch sooner (mean for viviparous: 7.5 days; mean for oviparous: 16.7 days). Early hatching could be beneficial for juvenile lizard survival because they benefit from a longer activity season and attain a larger body size prior to their first hibernation (Adolph and Porter 1993; Wapstra 2000; Booth 2006; Warner and Shine 2007). However, in the wild, hatchlings from both populations of *S. equalis* appear during the peak of summer (late-January and mid-February), thus, they only experience high environmental temperatures for a relatively short period of time (*personal observations*). The low thermal preference (Wu et al. 2009; Fig. 3) and low critical thermal limits (Greer 1980) of *S. equalis* suggest that longer exposure to high temperatures during mid-summer, as a consequence of an earlier hatching, could impact hatchlings physiology and behaviour (Huey and Slatkin 1976; Kearney 2002; Vickers et al. 2011). However, considering our low statistical power, further studies are needed to understand the effect of high developmental temperatures on the hatching times of *S. equalis*.

Oviparous hatchlings incubated under future temperatures were smaller (SVL) but had similar mass and body condition than current-incubated hatchlings, which is consistent with what has been found in most lizards (Van Damme et al. 1992; Downes and Shine 1999; Braña and Ji 2000; Deeming 2004) and other ectotherms (Gillooly and Dodson 2000; Sheridan and Bickford 2011). In lizards, this process is considered to be a result of an accelerated metabolic rate that reduces the total amount of yolk material converted to tissue during a shorter incubation period (Booth 2000). Although it is difficult to predict the adaptive value of a reduced body size because the fitness of this change will largely depend on its match with future environments, in many lizard species, smaller individuals have higher predation rates (Husak 2006) and lower physiological performance capacity (Irschick and Garland 2001; Dayananda et al. 2017). This suggests that

elevated developmental temperatures may have a negative impact on the survival of *S. equalis* hatchlings in the wild.

Unlike viviparous lizards, oviparous hatchlings reared under future environmental conditions had a lower growth rate compared to current-incubated hatchlings. Mass-specific measurements of growth rate control for the effect of initial body size, suggesting that the long-lasting negative effect of developmental temperatures was not related to the smaller body size at birth of future-incubated oviparous lizards (Sinervo 1990). Instead, this reflects a negative effect of thermal environments on the physiology and/or behaviour of oviparous hatchlings (Wapstra 2000). In our study, *S. equalis* hatchlings from both thermal environments were not food-restricted, had access to the same temperatures, and preferred similar body temperatures, thus, it is likely that the differences in growth rate are related to a higher metabolic rate (i.e. energy expenditure) from the future-incubated hatchlings (Coomber et al. 1997; Angilletta et al. 2000).

Hatchlings from different thermal environments did not differ in their T_{pref} at 6 weeks or at 6 months of age. These results show that the thermal preferences of *S. equalis* have a low capacity to respond to early thermal environments, which may be disadvantageous in a climate warming scenario (Visser 2008; Paranjpe et al. 2013). This is further supported by a post hoc analysis showing that the T_{pref} of *S. equalis* changed significantly within the populations, between hatchling and juvenile stages (*population* $\times$ *stage*, $F = 10.96$, d.f. = 47.68, $P = 0.001$). In other words, the T_{pref} of oviparous *S. equalis* was similar between hatchlings and juveniles, while the T_{pref} of viviparous *S. equalis* decreased even more after 6 months of age. This pattern could reflect a physiological adaptation to the environmental temperatures where each population occurs; since the viviparous population inhabits cooler montane environments, whereas the oviparous population lives along the warmer coast of NSW (Smith and Shine 1997; Garcia-Porta et al. 2019).

In terms of locomotor performance, our results show that future- and current-incubated lizards had similar maximum acceleration and stamina. Although the negative effect of early thermal environments on locomotor performance has been reported in many other reptiles (Van Damme et al. 1992; Shine 1999; Sim et al. 2015; Dayananda et al. 2017), this sometimes, as in this study, is a consequence of the smaller body size of hot incubated animals (Amiel et al. 2014). This suggests that the lower acceleration of *S. equalis* incubated under future conditions is partially explained by their smaller body size. Due to its fossorial habits, *S. equalis* will most likely escape from a predator by burying itself quickly (Wu et al. 2015). Moreover, in the laboratory, these lizards behave as ambush predators (*personal observations*); thus, high acceleration and speed are likely crucial for both anti-predator and foraging behaviours in *S. equalis*, and therefore, elevated developmental temperatures might

have a negative effect on hatchling survival. Unexpectedly, we found that viviparous hatchlings of *S. equalis* had a significantly lower maximal endurance compared to oviparous hatchlings. This difference, however, was not related to the larger body size and greater mass of viviparous hatchlings. Given that viviparous *S. equalis* have a low T_{pref} (19 °C), it is possible that their reduced performance was caused by the relatively high temperature of the experimental room (~21 °C). Further studies are needed to understand the effect of environmental temperature on the locomotor performance of *S. equalis*.

Viviparity has been identified as a factor exacerbating the recent extinction of many lizard populations because they have evolved lower preferred body temperatures and heat tolerance (Sinervo et al. 2010; Meiri et al. 2013; Pincheira-Donoso et al. 2013). Live-bearing species are thought to be more vulnerable to global warming however, there is not enough experimental and empirical evidence supporting this premise (but see Wang et al. 2017). Our study suggests that both viviparous and oviparous populations of *S. equalis* will be significantly affected by elevated developmental temperatures. We found that high temperatures had a stronger negative effect on oviparous *S. equalis*, particularly on their growth rate and survival. Of the 23 oviparous lizards that hatched successfully under high temperatures, 6 (26%) of them died within the 6 months that the experiment lasted. Although the growth rate and survival rate of viviparous hatchlings were not significantly affected by high incubation temperatures, our results show that viviparous *S. equalis* have a strong preference for low temperatures, which might increase their vulnerability to global warming as suggested before (Sinervo et al. 2010; Pincheira-Donoso et al. 2013). Regarding the incubation-induced effects on offspring phenotype, our results suggest that viviparous females are somehow buffering the negative effects of high temperatures on their offspring. Behavioural thermoregulation is the most probable mechanism that viviparous females will use to mitigate the effect of high temperatures on their offspring (Beuchat 1986; Shine and Harlow 1993). It is unlikely that soil temperatures change considerably in the relatively shallow enclosures in which females were kept; however, we did not measure soil temperatures and/or female depth under the soil during the gestation period, thus we cannot exclude this possibility. Another possibility is that the differences in phenotypic plasticity observed in hatchlings are due to prenatal maternal effects (Fox and Mousseau 1998; Sheriff and Love 2013), which have been proposed as an important source of adaptive (or maladaptive) variation (Marshall and Uller 2007; Love and Williams 2008). The gene expression profiles in the placenta of viviparous and oviparous populations of *S. equalis* is significantly different, particularly in genes important for

uterine remodelling, respiratory gas and water exchange, and immune regulation (Foster et al. 2020). A similar case has been reported in *Phrynocephalus* lizards, where closely related species with different modes of reproduction have important changes in genes related to eggshell degeneration and inhibited maternal immune rejection of the embryo, among others (Gao et al. 2019). Therefore, although the origin of viviparity in *S. equalis* is relatively recent (Smith et al. 2001), it is possible that their simple placenta could act, to some degree, as a means to protect offspring from hormonal diffusion from the mother (Welberg and Seckl 2001; Painter et al. 2002).

We found that high developmental temperatures had a stronger effect on oviparous *S. equalis*. However, this does not necessarily mean that the oviparous population might be more vulnerable to global warming. First, we must consider that the oviparous population of *S. equalis* is not truly oviparous (Smith and Shine 1997). It has a considerably shorter incubation duration (5–10 days, *this study*) compared to other sympatric oviparous species whose eggs are incubated for more than 30 days (Qualls and Shine 1998). Thus, it is not possible to directly measure the relative advantage of a longer incubation or a developed eggshell (Hallmann and Griebeler 2015). Second, in this study, females were only able to oviposit in a 5-cm deep layer of substrate, which could explain the high egg mortality in the oviparous population. The actual depth at which oviparous females of *S. equalis* lay their eggs is unknown but considering the nesting behaviour of sympatric and related skinks, it is probably more than 5 cm (Shine 1999). It is therefore possible that nest site selection by oviparous females in the wild may buffer offspring from elevated temperatures (Telemeco et al. 2009; reviewed by Urban et al. 2013). Finally, we are aware of the limitations we can draw from our sample size and the fact that population and mode of reproduction are confounding factors in our study. Although it is difficult to make generalizations on the effect of global warming in other species, most of the effect sizes related to the influence of the thermal environment that we report here are large (Cohen's $d > 0.8$) and highly significant ($P \leq 0.01$), showing that there is a strong effect of elevated developmental temperatures on *S. equalis* life-history traits. Likewise, the between-individual variation explained by mother identity (i.e. family-related) was low in most analyses, showing that the effect of developmental temperatures was consistent and strong across litters. For these reasons, we consider our results are noteworthy given the relevance of the study.

To the best of our knowledge, this is the first experimental study testing the effect of global warming on a species with bimodal reproduction. We provide new insights into the extent to which lizards with different reproductive mode can modify key life-history traits in response to global warming. Our study shows that, like other reptiles, *S. equalis* is highly

vulnerable to global warming, but also suggests that viviparous females may be able to mitigate some of these effects via behavioural and/or physiological responses.

Acknowledgements We thank Sergio Naretto and Cooper Van Der Waal for their assistance in the field and the laboratory, as well as Catarina Vila Pouca and Birgit Szabo for their helpful suggestions during the writing of the manuscript. We thank Camilla Whittington, Michael Thompson and Scott Parker for their advice regarding animal biology, capture and identification. Finally, we are grateful to two anonymous reviewers for the helpful comments which improved the manuscript.

Author contributions MJW and IB conceived and designed the experiments. IB conducted fieldwork. IB, VD and RL performed the experiments. IB, VD and RL analysed the data. IB and MJW acquired the funding for the research. IB, VD and RL wrote the first draft of the manuscript. IB and MJW reviewed and edited the final manuscript.

Funding Financial support for this research was provided by the Australasian Society for the Study of Animal Behaviour (2018 Student Grants), the Australian Museum (2018/19 Peter Rankin Trust Fund for Herpetology), and Macquarie University. This work was performed in the Plant Growth Facility (PGR) at Macquarie University. IB was supported by an international Research Training Program (iRTP) scholarship from the Australian Government and Macquarie University. VD and RL were supported by Le CROUS (Centre Régional des Oeuvres Universitaires et Scolaires) de Strasbourg and University of Strasbourg (France).

Data availability The datasets generated during the current study (raw data files and R scripts) are available in the Open Science Framework repository (<https://osf.io/6jzv8>).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures and protocols were approved by the Macquarie University Animal Ethics Committee (ARA No. 2017/029) and collection of animals was approved by the New South Wales National Parks and Wildlife Service, Office of Environment and Heritage (OEH; licence No. SL101962).

References

Adolph SC, Porter WP (1993) Temperature, activity, and lizard life histories. *Am Nat* 142:273–295. <https://doi.org/10.1086/285538>

Amiel JJ, Lindström T, Shine R (2014) Egg incubation effects generate positive correlations between size, speed and learning ability in young lizards. *Anim Cogn* 17:337–347. <https://doi.org/10.1007/s10071-013-0665-4>

Angilletta MJ, Winters RS, Dunham AE (2000) Thermal effects on the energetics of lizard embryos: implications for hatchling phenotypes. *Ecology* 81:2957–2968. [https://doi.org/10.1890/0012-9658\(2000\)081\[2957:TEOTEO\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2000)081[2957:TEOTEO]2.0.CO;2)

Bartoń K (2018) MuMIn: multi-model inference. Version R package version 1.42.1. R package version 1.42.1. <https://CRAN.R-project.org/package=MuMIn>. Accessed 3 Sept 2019

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using *lme4*. *J Stat Softw* 67:1–48. <https://doi.org/10.18637/jss.v067.i01>

Beuchat CA (1986) Reproductive influences on the thermoregulatory behavior of a live-bearing lizard. *Copeia* 1986:971–979. <https://doi.org/10.2307/1445294>

Beuchat CA (1988) Temperature effects during gestation in a viviparous lizard. *J Therm Biol* 13:135–142. [https://doi.org/10.1016/0306-4565\(88\)90024-1](https://doi.org/10.1016/0306-4565(88)90024-1)

Birchard GF, Marcellini D (1996) Incubation time in reptilian eggs. *J Zool* 240:621–635. <https://doi.org/10.1111/j.1469-7998.1996.tb05312.x>

Blackburn DG (2000) Reptilian viviparity: past research, future directions, and appropriate models. *Comp Biochem Physiol A Mol Integr Physiol* 127:391–409. [https://doi.org/10.1016/S1095-6433\(00\)00272-5](https://doi.org/10.1016/S1095-6433(00)00272-5)

Blackburn DG (2006) Squamate reptiles as model organisms for the evolution of viviparity. *Herpetol Monogr* 20:131–146

Bolker BM, Brooks ME, Clark CJ et al (2009) Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol Evol* 24:127–135. <https://doi.org/10.1016/j.tree.2008.10.008>

Booth DT (2000) Incubation of eggs of the Australian broad-shelled turtle, *Chelodina expansa* (Testudinata: Chelidae), at different temperatures: effects on pattern of oxygen consumption and hatchling morphology. *Aust J Zool* 48:369. <https://doi.org/10.1071/ZO000055>

Booth DT (2006) Influence of incubation temperature on hatchling phenotype in reptiles. *Physiol Biochem Zool* 79:274–281. <https://doi.org/10.1086/499988>

Braña F, Ji X (2000) Influence of incubation temperature on morphology, locomotor performance, and early growth of hatchling wall lizards (*Podarcis muralis*). *J Exp Zool* 286:422–433. [https://doi.org/10.1002/\(SICI\)1097-010X\(20000301\)286:4<422:AID-JEZ10>3.0.CO;2-D](https://doi.org/10.1002/(SICI)1097-010X(20000301)286:4<422:AID-JEZ10>3.0.CO;2-D)

Brattstrom BH (1979) Amphibian temperature regulation studies in the field and laboratory. *Integr Comp Biol* 19:345–356. <https://doi.org/10.1093/icb/19.1.345>

Burger J (1998) Antipredator behaviour of hatchling snakes: effects of incubation temperature and simulated predators. *Anim Behav* 56:547–553. <https://doi.org/10.1006/anbe.1998.0809>

Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretic approach, 2nd edn. Springer, New York

Bustard HR (1964) Reproduction in the Australian rain forest skinks, *Saiphos equalis* and *Sphenomorphus tryoni*. *Copeia* 1964:715. <https://doi.org/10.2307/1441458>

Careau V, Wilson RS (2017) Of uberfleas and krakens: detecting trade-offs using mixed models. *Integr Comp Biol* 57:362–371. <https://doi.org/10.1093/icb/ixc015>

Chamaillé-Jammes S, Massot M, Aragón P, Clobert J (2006) Global warming and positive fitness response in mountain populations of common lizards *Lacerta vivipara*. *Glob Change Biol* 12:392–402. <https://doi.org/10.1111/j.1365-2486.2005.01088.x> Citations:121

Coomber P, Crews D, Gonzalez-Lima F (1997) Independent effects of incubation temperature and gonadal sex on the volume and metabolic capacity of brain nuclei in the leopard gecko (*Eublepharis macularius*), a lizard with temperature-dependent sex determination. *J Comp Neurol* 380:409–421. [https://doi.org/10.1002/\(SICI\)1096-9861\(19970414\)380:3<409:AID-CNE9>3.0.CO;2-6](https://doi.org/10.1002/(SICI)1096-9861(19970414)380:3<409:AID-CNE9>3.0.CO;2-6)

Cox RM, Duryea MC, Najarro M, Calsbeek R (2011) Paternal condition drives progeny sex-ratio bias in a lizard that lacks parental care. *Evolution* 65:220–230. <https://doi.org/10.1111/j.1558-5646.2010.01111.x>

Daut EF, Andrews RM (1993) The effect of pregnancy on thermoregulatory behavior of the viviparous lizard *Chalcides ocellatus*. *J Herpetol* 27:6–13. <https://doi.org/10.2307/1564898>

- Dayananda B, Penfold S, Webb JK (2017) The effects of incubation temperature on locomotor performance, growth and survival in hatchling velvet geckos. *J Zool* 303:46–53. <https://doi.org/10.1111/jzo.12460>
- Deeming DC (2004) Post-hatching phenotypic effects of incubation in reptiles. In: Deeming DC (ed) *Reptilian incubation environment, evolution and behaviour*. Nottingham University Press, Nottingham, pp 229–252
- Deeming DC, Ferguson MJW (1991) Physiological effects of incubation temperature on embryonic development in reptiles and birds. *Egg incubation: its effects on embryonic development in birds and reptiles*. Cambridge University Press, Cambridge, pp 147–171
- Deutsch CA, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC, Martin PR (2008) Impacts of climate warming on terrestrial ectotherms across latitude. *Proc Natl Acad Sci USA* 105(18):6668–6672. <https://doi.org/10.1073/pnas.0709472105>
- Dowdy A, Abbs D, Bhend J, East Coast Cluster Report, Bhend J, Chiew F, Church J, Ekström M, Kirono D, Lenton A, Lucas C, McInnes K, Moise A, Monselesan D, Mpelasoka F, Webb L, Whetton P et al (2015) Climate change in Australia projections for Australia's natural resource management regions: cluster reports. CSIRO and Bureau of Meteorology, Melbourne, pp 1–53
- Downes SJ, Shine R (1999) Do incubation-induced changes in a lizard's phenotype influence its vulnerability to predators? *Oecologia* 120:9–18. <https://doi.org/10.2307/422353>
- Du W-G, Elphick M, Shine R (2010) Thermal regimes during incubation do not affect mean selected temperatures of hatchling lizards (*Bassiana duperreyi*, Scincidae). *J Therm Biol* 35:47–51. <https://doi.org/10.1016/j.jtherbio.2009.10.007>
- Elphick MJ, Shine R (1998) Long-term effects of incubation temperatures on the morphology and locomotor performance of hatchling lizards *Bassiana duperreyi*, Scincidae). *Biol J Linn Soc* 63:429–447. <https://doi.org/10.1111/j.1095-8312.1998.tb01527.x>
- Fairbairn J, Shine R, Moritz C, Frommer M (1998) Phylogenetic relationships between oviparous and viviparous populations of an Australian lizard (*Lerista bougainvillii*, Scincidae). *Mol Phylogenet Evol* 10:95–103. <https://doi.org/10.1006/mpev.1997.0468>
- Foster CSP, Thompson MB, Dyke JUV, Brandley MC, Whittington CM (2020) Emergence of an evolutionary innovation: gene expression differences associated with the transition between oviparity and viviparity. *Mol Ecol* 29:1315–1327. <https://doi.org/10.1111/mec.15409>
- Fox CW, Mousseau TA (1998) Maternal effects as adaptations for transgenerational phenotypic plasticity in insects. *Maternal effects as adaptations*. Oxford University Press, New York, pp 159–177
- Gao W, Sun YB, Zhou WW et al (2019) Genomic and transcriptomic investigations of the evolutionary transition from oviparity to viviparity. *Proc Natl Acad Sci USA* 116:3646–3655. <https://doi.org/10.1073/pnas.1816086116>
- Garcia-Porta J, Irisarri I, Kirchner M et al (2019) Environmental temperatures shape thermal physiology as well as diversification and genome-wide substitution rates in lizards. *Nat Commun* 10:4077. <https://doi.org/10.1038/s41467-019-11943-x>
- Garland T Jr, Else PL (1987) Seasonal, sexual, and individual variation in endurance and activity metabolism in lizards. *Am J Physiol Regul Integr Comp Physiol* 252:R439–R449. <https://doi.org/10.1152/ajpregu.1987.252.3.R439>
- Gillooly JF, Dodson SI (2000) The relationship of neonate mass and incubation temperature to embryonic development time in a range of animal taxa. *J Zool* 251:369–375. <https://doi.org/10.1111/j.1469-7998.2000.tb01087.x>
- Gray JE (1825) A synopsis of the genera of reptiles and Amphibia, with a description of some new species. *Ann Philos Br Mus* 10:193–217
- Greer A (1980) Critical thermal maximum temperatures in Australian scincid lizards: their ecological and evolutionary significance. *Aust J Zool* 28:91. <https://doi.org/10.1071/ZO9800091>
- Hallmann K, Griebeler EM (2015) Eggshell types and their evolutionary correlation with life-history strategies in squamates. *PLoS ONE* 10:e0138785. <https://doi.org/10.1371/journal.pone.0138785>
- Harrison XA, Donaldson L, Correa-Cano ME et al (2018) A brief introduction to mixed effects modelling and multi-model inference in ecology. *PeerJ* 6:e4794. <https://doi.org/10.7717/peerj.4794>
- Heulin B, Guillaume C-P, Bea A, Arrayago M (1993) Interprétation biogéographique de la bimodalité de reproduction du lézard *Lacerta vivipara*: un modèle pour l'étude de l'évolution de la viviparité. *Biogeographica* 69:1–11
- Huey RB (1982) Temperature, physiology, and the ecology of reptiles. In: Gans C, Pough FH (eds) *Biology of the reptilia*. Academic Press, London, pp 25–91
- Huey RB, Slatkin M (1976) Cost and benefits of lizard thermoregulation. *Q Rev Biol* 51:363–384. <https://doi.org/10.1086/409470>
- Huey RB, Bennett AF, John-Alder H, Nagy KA (1984) Locomotor capacity and foraging behaviour of Kalahari lacertid lizards. *Anim Behav* 32:41–50. [https://doi.org/10.1016/S0003-3472\(84\)80322-X](https://doi.org/10.1016/S0003-3472(84)80322-X)
- Huey RB, Losos JB, Moritz C (2010) Are lizards toast? *Science* 328:832–833. <https://doi.org/10.1126/science.1190374>
- Husak JF (2006) Does speed help you survive? A test with Collared lizards of different ages. *Funct Ecol* 20:174–179. <https://doi.org/10.1111/j.1365-2435.2006.01069.x>
- Irschick DJ, Garland T (2001) Integrating function and ecology in studies of adaptation: investigations of locomotor capacity as a model system. *Annu Rev Ecol Syst* 32:367–396. <https://doi.org/10.1146/annurev.ecolsys.32.081501.114048>
- Kearney M (2002) Hot rocks and much-too-hot rocks: seasonal patterns of retreat-site selection by a nocturnal ectotherm. *J Therm Biol* 27:205–218. [https://doi.org/10.1016/S0306-4565\(01\)00085-7](https://doi.org/10.1016/S0306-4565(01)00085-7)
- Kubisch EL, Corbalán V, Ibarguengoytia NR, Sinervo B (2015) Local extinction risk of three species of lizard from Patagonia as a result of global warming. *Can J Zool* 94:49–59. <https://doi.org/10.1139/cjz-2015-0024>
- Kuznetsova A, Brockhoff PB, Christensen RHB (2017) *lmerTest* package: tests in linear mixed effects models. *J Stat Softw* 82:1–26. <https://doi.org/10.18637/jss.v082.i13>
- Love OP, Williams TD (2008) The adaptive value of stress-induced phenotypes: effects of maternally derived corticosterone on sex-biased investment, cost of reproduction, and maternal fitness. *Am Nat* 172:E135–E149. <https://doi.org/10.1086/590959>
- MacLean SA, Beissinger SR (2017) Species' traits as predictors of range shifts under contemporary climate change: a review and meta-analysis. *Glob Change Biol* 23:4094–4105. <https://doi.org/10.1111/gcb.13736>
- Marshall DJ, Uller T (2007) When is a maternal effect adaptive? *Oikos* 116:1957–1963. <https://doi.org/10.1111/j.2007.0030-1299.16203.x>
- Meiri S, Bauer AM, Chirio L et al (2013) Are lizards feeling the heat? A tale of ecology and evolution under two temperatures. *Glob Ecol Biogeogr* 22:834–845. <https://doi.org/10.1111/geb.12053>
- Noble DWA, Stenhouse V, Schwanz LE (2018) Developmental temperatures and phenotypic plasticity in reptiles: a systematic review and meta-analysis. *Biol Rev* 93:72–97. <https://doi.org/10.1111/brv.12333>
- Packard GC, Tracy CR, Roth JJ (1977) The physiological ecology of reptilian eggs and embryos, and the evolution of viviparity within the class Reptilia. *Biol Rev* 52:71–105. <https://doi.org/10.1111/j.1469-185X.1977.tb01346.x>
- Painter D, Jennings DH, Moore MC (2002) Placental buffering of maternal steroid hormone effects on fetal and yolk hormone levels: a comparative study of a viviparous lizard, *Sceloporus*

- jarrovi, and an oviparous lizard, *Sceloporus graciosus*. Gen Comp Endocrinol 127:105–116. [https://doi.org/10.1016/S0016-6480\(02\)00075-8](https://doi.org/10.1016/S0016-6480(02)00075-8)
- Paranje DA, Bastiaans E, Patten A et al (2013) Evidence of maternal effects on temperature preference in side-blotched lizards: implications for evolutionary response to climate change. Ecol Evol 3:1977–1991. <https://doi.org/10.1002/ece3.614>
- Pincheira-Donoso D, Tregenza T, Witt MJ, Hodgson DJ (2013) The evolution of viviparity opens opportunities for lizard radiation but drives it into a climatic cul-de-sac. Glob Ecol Biogeogr 22:857–867. <https://doi.org/10.1111/geb.12052>
- Qu Y-F, Lu H-L, Li H, Ji X (2014) Incubation temperature fluctuation does not affect incubation length and hatchling phenotype in the Chinese skink *Plestiodon chinensis*. J Therm Biol 46:10–15. <https://doi.org/10.1016/j.jtherbio.2014.09.008>
- Qualls CP, Shine R (1998) *Lerista bougainvillii*, a case study for the evolution of viviparity in reptiles. J Evol Biol 11:63–78. <https://doi.org/10.1046/j.1420-9101.1998.11010063.x>
- R Core Team (2018) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Radder RS, Elphick MJ, Warner DA et al (2008) Reproductive modes in lizards: measuring fitness consequences of the duration of uterine retention of eggs. Funct Ecol 22:332–339. <https://doi.org/10.1111/j.1365-2435.2007.01380.x>
- Sergeev M (1940) Researches in the viviparity of reptiles. Mosc Soc Nat (Jubilee Issue):1–34
- Sheridan JA, Bickford D (2011) Shrinking body size as an ecological response to climate change. Nat Clim Change 1(8):401–406. <https://doi.org/10.1038/nclimate1259>
- Sheriff MJ, Love OP (2013) Determining the adaptive potential of maternal stress. Ecol Lett 16:271–280. <https://doi.org/10.1111/ele.12042>
- Shine R (1985) The evolution of viviparity in reptiles: an ecological analysis. In: Gans C, Billett F (eds) Biology of the Reptilia. Wiley, New York, pp 605–694
- Shine R (1995) A new hypothesis for the evolution of viviparity in reptiles. Am Nat 145:809–823. <https://doi.org/10.1086/285769>
- Shine R (1999) Egg-laying reptiles in cold climates: determinants and consequences of nest temperatures in montane lizards. J Evol Biol 12:918–926. <https://doi.org/10.1046/j.1420-9101.1999.00093.x>
- Shine R, Harlow P (1993) Maternal thermoregulation influences offspring viability in a viviparous lizard. Oecologia 96:122–127. <https://doi.org/10.1007/BF00318039>
- Shine R, Harlow PS (1996) Maternal manipulation of offspring phenotypes via nest-site selection in an oviparous lizard. Ecology 77:1808–1817. <https://doi.org/10.2307/2265785>
- Shine R, Olsson M (2003) When to be born? Prolonged pregnancy or incubation enhances locomotor performance in neonatal lizards (Scincidae). J Evol Biol 16:823–832. <https://doi.org/10.1046/j.1420-9101.2003.00600.x>
- Sim EL, Booth DT, Limpus CJ (2015) Incubation temperature, morphology and performance in loggerhead (*Caretta caretta*) turtle hatchlings from Mon Repos, Queensland, Australia. Biol Open 4:685–692. <https://doi.org/10.1242/bio.20148995>
- Sinervo B (1990) Evolution of thermal physiology and growth rate between populations of the western fence lizard (*Sceloporus occidentalis*). Oecologia 83:228–237. <https://doi.org/10.1007/BF00317757>
- Sinervo B, Méndez-de-la-Cruz F, Miles DB et al (2010) Erosion of lizard diversity by climate change and altered thermal niches. Science 328:894–899
- Smith SA, Shine R (1997) Intraspecific variation in reproductive mode within the scincid lizard *Saiphos equalis*. Aust J Zool 45:435. <https://doi.org/10.1071/ZO97023>
- Smith SA, Austin CC, Shine R (2001) A phylogenetic analysis of variation in reproductive mode within an Australian lizard *Saiphos equalis*, Scincidae. Biol J Linn Soc 74:131–139. <https://doi.org/10.1111/j.1095-8312.2001.tb01382.x>
- Somero GN (2004) Adaptation of enzymes to temperature: searching for basic “strategies”. Comp Biochem Physiol B Biochem Mol Biol 139:321–333. <https://doi.org/10.1016/j.cbpc.2004.05.003>
- Sunday JM, Bates AE, Kearney MR, Colwell RK, Dulvy NK, Longino JT, Huey RB (2014) Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. P Natl Acad Sci USA 111:5610–5615. <https://doi.org/10.1073/pnas.1316145111>
- Telemeco RS, Elphick MJ, Shine R (2009) Nesting lizards (*Bassiana duperreyi*) compensate partly, but not completely, for climate change. Ecology 90:17–22. <https://doi.org/10.1890/08-1452.1>
- Urban MC, Richardson JL, Freidenfelds NA (2013) Plasticity and genetic adaptation mediate amphibian and reptile responses to climate change. Evol Appl 7:88–103. <https://doi.org/10.1111/eva.12114>
- Van Damme R, Bauwens D, Braña F, Verheyen RF (1992) Incubation temperature differentially affects hatching time, egg survival, and hatchling performance in the lizard *Podarcis muralis*. Herpetologica 48:220–228
- Van Dyke JU, Brandley MC, Thompson MB (2014) The evolution of viviparity: molecular and genomic data from squamate reptiles advance understanding of live birth in amniotes. Reproduction 147:15–26. <https://doi.org/10.1530/REP-13-0309>
- Vickers M, Manicom C, Schwarzkopf L (2011) Extending the cost-benefit model of thermoregulation: high-temperature environments. Am Nat 177:452–461. <https://doi.org/10.1086/658150>
- Vince MA, Chinn S (1971) Effect of accelerated hatching on the initiation of standing and walking in the Japanese quail. Anim Behav 19:62–66. [https://doi.org/10.1016/S0003-3472\(71\)80135-5](https://doi.org/10.1016/S0003-3472(71)80135-5)
- Visser ME (2008) Keeping up with a warming world; assessing the rate of adaptation to climate change. Proc R Soc B Biol Sci 275:649–659. <https://doi.org/10.1098/rspb.2007.0997>
- Wang Z, Ma L, Shao M, Ji X (2017) Are viviparous lizards more vulnerable to climate warming because they have evolved reduced body temperature and heat tolerance? Oecologia 185:573–582. <https://doi.org/10.1007/s00442-017-3979-0>
- Wapstra E (2000) Maternal basking opportunity affects juvenile phenotype in a viviparous lizard. Funct Ecol 14:345–352. <https://doi.org/10.1046/j.1365-2435.2000.00428.x>
- Warner DA, Shine R (2007) Fitness of juvenile lizards depends on seasonal timing of hatching, not offspring body size. Oecologia 154:65–73. <https://doi.org/10.1007/s00442-007-0809-9>
- Webb GJW, Cooper-Preston H (1989) Effects of incubation temperature on crocodiles and the evolution of reptilian oviparity. Am Zool 29:953–971. <https://doi.org/10.1093/icb/29.3.953>
- Welberg LA, Seckl JR (2001) Prenatal stress, glucocorticoids and the programming of the brain. J Neuroendocrinol 13:113–128. <https://doi.org/10.1111/j.1365-2826.2001.00601.x>
- Werner YL (1990) Do gravid females of oviparous gekkonid lizards maintain elevated body temperatures? *Hemidactylus frenatus* and *Lepidodactylus lugubris* on Oahu. Amphib-Reptil 11:200–204. <https://doi.org/10.1163/156853890X00627>
- While GM, Uller T, Wapstra E (2009) Offspring performance and the adaptive benefits of prolonged pregnancy: experimental tests in a viviparous lizard. Funct Ecol 23:818–825. <https://doi.org/10.1111/j.1365-2435.2009.01544.x>
- Wu Q, Parker SL, Thompson MB (2009) Selected body temperature, metabolic rate and activity pattern of the Australian fossorial skink, *Saiphos equalis*. Herpetol J 19:127–133

- Wu NC, Alton LA, Clemente CJ et al (2015) Morphology and burrowing energetics of semi-fossorial skinks (*Liopholis spp.*). J Exp Biol 218:2416–2426. <https://doi.org/10.1242/jeb.113803>
- Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common statistical problems. Methods Ecol Evol 1:3–14. <https://doi.org/10.1111/j.2041-210X.2009.00001.x>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Effect of early thermal environment on the morphology and performance of a lizard species
with bimodal reproduction

Submitted to Journal of Comparative Physiology B

Iván Beltrán^{1*}, Victorien Durand^{1,2,†}, Rebecca Loiseleur^{1,2,†} and Martin J. Whiting¹

¹ *Department of Biological Sciences, Macquarie University, NSW Australia*

² *Faculty of the Sciences of Life, University of Strasbourg, France*

*Corresponding author: ic.beltran196@gmail.com

Electronic Supplementary Material

Table S1. Setting up parameters used in the incubators during the thermal environment experiments. Relative humidity inside the incubators was kept constant at 70%. Photoperiod in the incubators was 13L:11D, the lights were on between 6:00 h and 19:00 h.

Month	Time	Current incubator	Future incubator
November	0 - 6 h	20	23
	6 - 10 h	21	24
	10 - 12 h	24	27
	12 - 14 h	23	26
	14 - 20 h	22	25
	20 - 24 h	20	23
December	0 - 6 h	20	23
	6 - 10 h	21	24
	10 - 12 h	24	27
	12 - 14 h	23	26
	14 - 20 h	22	25
	20 - 24 h	20	23
January	0 - 6 h	21	24
	6 - 10 h	22	25
	10 - 12 h	25	28

	12 - 14 h	24	27
	14 - 20 h	23	26
	20 - 24 h	21	24
	Mean	22	25
	SD	1.6	1.6

Table S2. Sex ratio of hatchlings from two populations of *Saiphos equalis* gestated under current and future temperatures.

Population	Incubation	Number of females	Number of males	χ^2 test	χ^2 test by Population	χ^2 test by treatment
Oviparous	Future	5	10	$\chi^2 = 0.6$, d.f. = 3, P = 0.89	$\chi^2 \approx 0$, d.f. = 1, P $\approx$ 1	$\chi^2 \approx 0$, d.f. = 1, P $\approx$ 1
	Current	3	3			
Viviparous	Future	5	8			
	Current	4	8			

Table S3. Effect of thermal environment (current vs. future) on the maximum speed over a 0.6 m racetrack of hatchlings from two populations of *Saiphos equalis*. When SVL is controlled for, elevated temperatures did not have a significant effect on maximum speed (thermal environment in model 1). However, thermal environment becomes significant when SVL is excluded from the analysis (model 2).

Model	Estimate of fixed effect ($\pm$ s.e.m)			P-value of fixed effect		
	Population	Thermal environment	SVL	Population	Thermal environment	SVL
Model1	-0.24 (0.07)	-0.15 (0.08)	0.02 (0.02)	0.006**	0.06	0.21
Model2	-0.25 (0.08)	-0.18 (0.08)	-	0.009**	0.04*	-