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Experimental litter size reduction reveals costs of gestation and delayed effects on offspring in a viviparous lizard

Josefa Bleu[1,*], **Manuel Massot**[1], **Claudy Haussy**[1] and **Sandrine Meylan**[1,2]

[1]*CNRS, UPMC, ENS—UMR 7625, Laboratoire Ecologie et Evolution, 7 Quai Saint Bernard, 75005 Paris, France*
[2]*IUFM de Paris—Université Sorbonne Paris IV, 10 rue Molitor, 75016 Paris, France*

Experimental studies have often been employed to study costs of reproduction, but rarely to study costs of gestation. Disentangling the relative importance of each stage of the reproductive cycle should help to assess the costs and benefits of different reproductive strategies. To that end, we experimentally reduced litter size during gestation in a viviparous lizard. We measured physiological and behavioural parameters during gestation and shortly after parturition, as well as survival and growth of females and their offspring. This study showed four major results. First, the experimental litter size reduction did not significantly affect the cellular immune response, the metabolism and the survival of adult females. Second, females with reduced litter size decreased their basking time. Third, these females also had an increased postpartum body condition. As postpartum body condition is positively related to future reproduction, this result indicates a gestation cost. Fourth, even though offspring from experimentally reduced litters had similar weight and size at birth as other offspring, their growth rate after birth was significantly increased. This shows the existence of a maternal effect during gestation with delayed consequences. This experimental study demonstrates that there are some costs to gestation, but it also suggests that some classical trade-offs associated with reproduction may not be explained by gestation costs.

Keywords: costs of reproduction; gestation; litter size reduction; maternal effect; surgery; viviparity

1. INTRODUCTION

Costs of reproduction are the trade-offs that exist between reproductive investment and survival and/or future reproduction [1,2]. In recent years, studies have focused on the ecological and physiological basis of these costs [3,4]. Such costs include increased oxidative stress (e.g. [5]) and metabolism (e.g. [6]), impaired immune function, and decreased growth, energy stores (e.g. [7]) and endurance (e.g. [8]). Reproductive effort can also have intergenerational effects: offspring quality may decrease when reproductive investment increases, owing to a trade-off between offspring number and quality [2]. Experiments have shown the existence of such a trade-off in mammals, reptiles and birds. For example, offspring from enlarged litters [9,10] or miniaturized eggs [11] have been shown to have a lower survival [10,11], be more stressed and have a lower immune performance [9]. Therefore, parental reproductive effort is linked to offspring quality. Moreover, small offspring can show a period of accelerated growth to compensate for their small initial size, but this catch-up growth may adversely affect several traits during their lifetime [12].

Different stages of the reproductive cycle may be associated with different costs. For example, in mammals, lactation is more costly than gestation (e.g. [13–16]), and in birds, most studies have investigated the costs associated with chick rearing, although egg production and incubation also incur fitness costs [17]. Disentangling the relative importance of each stage may be important to assess the costs and benefits of different reproductive strategies. Viviparity (live-bearing) has evolved from oviparity (egg-laying) many times independently in vertebrates, and can be found in fishes, amphibians, mammals and squamate reptiles (lizards and snakes) [18]. Among squamate reptiles, viviparity has evolved more than 100 times independently [18]. This group is particularly well-suited for the study of gestation costs because: (i) viviparity has evolved relatively recently, and oviparous and viviparous females can even be found in the same species [18]; (ii) in most species, there is no parental care [19]; and (iii) most nutrients for embryo development are provided in the egg yolk (lecithotrophic viviparity [18]). Thus, the cost of resource investment in terms of egg and litter size and the cost of gestation can be decoupled.

Gestation costs in viviparous squamates may be attributed to an increase in metabolism, a shift in thermal preference and locomotor impairment [20–23]. It is also possible that some trade-offs documented between reproductive investment and other functions (e.g. immune function) may be explained by the effort during gestation. The study of gestation costs is generally based on comparisons between reproductive and non-reproductive females, or between reproductive and post-reproductive females [20–23]. However, in the first comparison, females may differ according to other variables than their reproductive state. In reptiles, non-reproductive females often have

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*Author for correspondence (josefa.bleu@snv.jussieu.fr).

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lower body condition than reproductive females (e.g. [24]), for example owing to poor health or because they belong to a different age class. When comparing performances of the same female before and after parturition, other confounding effects arise, such as seasonal variation in traits. More importantly, in all of these studies, differences in reproductive investment among females with different litter size are not independent of other differences among these females. For example, we can measure a positive correlation between reproductive investment and immune function, even if a trade-off (i.e. a negative correlation) exists between these traits, because they are both correlated with another female trait (such as resource-acquisition efficiency) [25,26]. Thus, without experiments, we cannot make definitive conclusions regarding causality. This is why the production of experimental phenotypes has dramatically enhanced the understanding of life-history evolution [27,28]. For example, it has been used to study clutch size and reproductive investment [29–34], but, to our knowledge, it has never been used to specifically investigate gestation costs. The rare experiments dealing with gestation costs have consisted in applying an experimental burden to non-reproductive females to investigate the causes of the decrease in locomotor performances during gestation [21,35].

The lack of experimental approaches motivated our study, in which we experimentally reduced litter size during gestation. The aim of this study was to assess behavioural, physiological and survival gestation costs of large litters, and to test whether litter size during gestation has intergenerational effects in a short-lived viviparous lizard, *Zootoca vivipara*. To this end, we surgically removed one of the two oviducts (‘half-hysterectomy’) of pregnant common lizard females. Costs of reproduction have already been studied in this species. Normal gestation is associated with a decrease in locomotor performance and a selection of lower body temperatures [36]. An effect of the treatment on female traits would reveal gestation costs of large litters, whereas an effect of the initial litter size (litter size before surgery) would suggest vitellogenesis costs (i.e. a correlation with the initial investment of the female). We specifically addressed the following questions. (i) Can we detect some physiological functions affected by gestation of large compared with small litters? If gestation is costly, we expect a higher immune response, a higher postpartum body condition and a lower metabolic rate in females with reduced litter size compared with control females. We did not expect a change in the thermoregulatory behaviour after litter size manipulation because remaining embryos may still have the same thermal requirements. (ii) Is there a survival cost of gestation of large compared with small litters? If this cost exists, females with a reduced litter size would have a better survival than the other females. (iii) Are offspring larger at birth when litter size is reduced? Indeed, a female might transfer additional nutrients to the developing embryos (e.g. calcium [37]), or space might be a constraint on offspring development. (iv) Is there an effect of litter size reduction during gestation on the survival and growth of offspring?

2. MATERIAL AND METHODS

(a) *Model species*

Zootoca vivipara is a small (45–70 mm adult snout–vent length, SVL) ground-dwelling lizard, widely distributed across Eurasia. It includes both oviparous and viviparous reproductive forms in allopatric populations [38]. We studied viviparous populations located in the Massif Central mountain range (southeastern France). In this area, activity starts for adult males around mid-April and for adult females in early May. Mating may occur as early as 0–3 days after female emergence, and reproductive investment (vitellogenesis) occurs on average during the first three weeks of activity [39]. During gestation, a primitive chorioallantoic placenta allows respiratory, aqueous and mineral exchanges between mother and embryos [37,40]. Parturition occurs when embryos are fully developed, after an average gestation period of two months. The mean litter size is 5 (range from 1 to 12). Live offspring hatch immediately from their soft-shelled eggs and are thereafter autonomous. Lizards enter into hibernation gradually in late September.

(b) *Capture and rearing conditions*

We captured 56 and 46 pregnant females in mid-June 2009 and 2010, respectively, at Mont-Lozère (1420 m a.s.l., 44°23′01″ N, 3°52′18″ E). Females were marked by toe-clipping and brought to a field laboratory until parturition (mid-July to early August). Females were kept in individual terraria (18 × 12 × 12 cm) with a shelter, first with damp soil as substrate, and then with paper (see below). A 25 W spotlight provided opportunities for thermoregulation for 6 h daily (from 09.00 to 12.00 h and from 14.00 to 17.00 h), creating a thermal gradient from 24.7°C to 37.4°C in the terrarium. Water was provided *ad libitum* and one *Pyralis* sp. larva (average live weight ± s.d. = 0.189 ± 0.051 g, $n = 30$; average dry weight ± s.d. = 0.075 ± 0.025 g, $n = 30$) was offered per week. Immediately after parturition, mothers and their offspring were separated and measured. Within 4 days, the females were released at their capture point and offspring were released randomly at four different points on the site. Experimental procedures and rearing conditions were identical for both experimental years.

(c) *Experimental treatment: litter size reduction during gestation*

Females were randomly allocated to each treatment group: operated females from which approximately half of the eggs were removed, hereafter called litter-reduced (LR) females ($n = 30$ in 2009 and $n = 12$ in 2010); sham-operated females from which no eggs were removed, hereafter called sham (S) females ($n = 11$ in 2009 and $n = 10$ in 2010); and control females that were not manipulated, hereafter called control (C) females ($n = 15$ in 2009 and $n = 24$ in 2010). The S females allowed us to control for the effect of surgery. The experimental groups did not differ in SVL, body weight or average litter size at the beginning of the experiment (all $p > 0.14$). The manipulation was performed around mid-gestation (embryos removed were at stages 29–34 [41]).

Manipulated (LR and S) females were anaesthetized for about 15 min, inhaling 3 per cent isoflurane (Forene, Abbott, France), wiped with 70 per cent ethanol on the abdomen and placed on a sterile surgical board. A 10–15 mm-long incision was made 5 mm laterally of the mid-ventral line. For LR females, one of the two oviducts was lifted out of the incision by gently pressing the abdomen and cut (half-hysterectomy) to remove eggs. The oviducts of S females were not manipulated. Females were sutured using a surgical thread (coated Vicryl Rapide, Covéto, France; reference 665593) and wiped with Betadine (povidone-iodine). They were allowed to wake up in a separate room in a terrarium with paper as substrate and were brought back to the rearing room when completely awake, about 30 min after surgery. From then on, all females were kept with paper as the substrate (to prevent infection and healing problems after surgery).

(d) *Short-term effect of litter size reduction*

(i) *Behaviour*

One week after the treatment, the activity of each lizard was measured seven times by a naive observer over 5 days, one or two times per day between 10.00 and 11.00 h or 15.00 and 16.00 h. The observer noted whether the lizard was sheltering (under the shelter or the substrate, i.e. the paper), full-basking or half-basking (the head under the light and the rest of the body hidden; as described in the study of Cote *et al.* [42]). We distinguished half-basking from full-basking as the behaviours may differ in thermoregulatory efficiency (female body temperature) or 'predation risk'.

(ii) *Metabolism*

Standard metabolic rate (SMR) was measured on average 5 days before parturition for a subset of females from each treatment (2009: $n = 15$ (LR), 7 (S), 8 (C); 2010: $n = 5$ (LR), 7 (S), 15 (C)). SMR was measured as the minimum rate of oxygen consumption under post-absorptive conditions in the inactive phase of the daily cycle [43]. We measured the volume of dioxygen consumed per mass unit and per time unit (ml O_2 g^{-1} h^{-1}). This methodology assumes a constant ratio between energy production and oxygen consumption, a condition currently accepted [44]. Oxygen consumption was measured using an open airflow respirometer from Sable Systems (Las Vegas, NV, USA), comprising a two-channel pump PP-2, two mass flow controller electronic units MFC-2, an eight-channel multiplexer TR-RM8, an FC-10a Oxygen analyser and the subsampler/pump/mass flow meter unit TR-SS3. We placed animals individually in 200 ml darkened chambers with airflow of 20 ml min^{-1}. Oxygen consumption was measured at room temperature every minute over 30 min. The temperature could not be controlled and was on average 29.0°C (s.d. = 1.7°C). The volume of oxygen consumed by each animal was calculated from the difference between the oxygen rate in the ambient air and that at the exit of the chamber. The value used for each individual was the minimal measurement. All lizards had been deprived of food for at least 3 days before the measurements and were placed inside the chamber at least 1 h before the beginning of the observations to acclimate.

(iii) *Proinflammatory potential*

Immunocompetence was estimated after parturition with the phytohaemagglutinin-induced skin-swelling test. Phytohaemagglutinin (PHA) swelling response is complex, including both innate and adaptive components of the immune system [45,46]. Thus, PHA swelling response is not solely based on T-cell proliferation; it evaluates the general ability of an individual to mount an inflammatory response [45]. One day after parturition, we injected subcutaneously 0.04 ml of a solution of phosphate-buffered saline (Sigma-Aldrich, St Louis, MO; reference D5773) containing 2.5 mg ml^{-1} of PHA (Sigma-Aldrich; reference L8754) in the right posterior leg (2009: $n = 27$ (LR), 10 (S), 14 (C); 2010: $n = 12$ (LR), 9 (S), 23 (C)). Differences in sample sizes reflect a missing value. Before and 12 h after the injection, we measured thickness of the leg to the nearest 0.01 mm with a spessimeter (ID-C Absolute Digimatic, Mitutoyo, France; reference 547-301). PHA swelling response was calculated as the change in thickness of the leg between the two measurements.

(iv) *Corticosterone measurement*

Surgery can be stressful for females. It has been shown previously that a prolonged increase of maternal stress during gestation modified the overall offspring phenotypes ([47] and references therein). Thus, two weeks after the manipulation, we checked whether LR and S females were more stressed than the C females by measuring circulating blood levels of corticosterone (2009: $n = 15$ (LR), 7 (S), 9 (C); 2010: $n = 11$ (LR), 10 (S), 23 (C)). Differences in sample sizes reflect a missing value owing to insufficient blood volume. Blood samples (40–60 µl whole blood) were collected from the post-orbital sinus using two to three 20 µl microhaematocrit tubes. They were taken within 3 min of the removal of an animal from its cage to avoid handling-induced increases in plasma corticosterone levels [48]. Blood samples were centrifuged. The plasma was transferred to 0.5 ml Eppendorf tubes and stored in a freezer at −20°C until analysis. Corticosterone was assayed using a competitive enzyme immunoassay with corticosterone EIA kits (IDS, France; reference AC-14F1).

(e) *Reproductive traits*

Parturition dates, realized fecundity (litter size at parturition) and litter success were recorded. Initial fecundity was calculated as the sum of the realized fecundity and the number of eggs removed by surgery. Live offspring ($n = 335$) were marked by toe-clipping, measured for SVL (to the nearest millimetre), weighed (to the nearest milligram) and sexed by ventral scale count [49]. Females were weighed after parturition.

(f) *Delayed effect of litter size reduction on growth and survival*

After being released in the field, some offspring and adult females belonging to the 2009 cohort were recaptured in September of the year of release and in June of the following year (table 1). The number of days of recapture was equal during these two recapture sessions (11 days). At each recapture, we weighed adults and measured all individuals. Juvenile growth rates before hibernation were calculated as the change in SVL (SVL at recapture − SVL at birth) divided by the time interval (date of recapture − date of birth). Adult weight gain before hibernation was calculated as the change in weight (weight at recapture − postpartum weight) divided by the time interval (date of recapture − parturition date).

(g) *Statistical analyses*

All models were implemented in R 2.13.0 statistical software (http://cran.r-project.org/). They included the following additive fixed effects: (i) treatment; (ii) female SVL; (iii) initial fecundity corrected for female SVL (standardized initial fecundity), which represents the effect of the initial investment of females; (iv) parturition date, because females may differ depending on their reproductive stage; and (v) their first-order interactions with treatment. The random part included a year effect. Models were simplified using backward elimination of the non-significant terms. For the behaviours, we first analysed the proportion of time spent basking (half- or full-basking), and then we analysed the proportion of time spent full-basking when the lizard was basking. These analyses were conducted with mixed-effects

Table 1. Sample sizes during the recapture sessions. Individuals from the experiment conducted in 2009 were recaptured at two different times: in September of the same year (before hibernation) and in June of the following year. The numbers of identified recaptured individuals are indicated.

	adult females			juveniles		
	July–August 2009 (release)	September 2009 (recapture)	June 2010 (recapture)	July–August 2009 (release)	September 2009 (recapture)	June 2010 (recapture)
C						
female	11	4	5	22	1	7
male				36	5	5
S						
female	11	2	3	19	4	5
male				28	5	5
LR						
female	30	13	13	23	6	6
male				43	9	8
total	52	19	21	171	30	36

logistic regressions including a binomial error term (*lmer* procedure). Parameters were estimated with a Laplace approximation of the maximum likelihood, and fixed effects were tested with χ^2-tests [50]. Litter success was analysed as a binomial variable (litters with all viable offspring versus litters with at least one failure) with a logistic regression including a binomial error term. Corticosterone levels, metabolism, postpartum female body condition, PHA response, parturition dates, SVL and body condition at birth of offspring were analysed with mixed effects linear models (*lme* procedure). Parameters were estimated with the restricted maximum-likelihood approach and fixed effects were tested with marginal *F*-tests [51]. The analysis of parturition dates included only an effect of the treatment and of female SVL. The analysis of metabolism also included an effect of the measurement chamber and an effect of the room temperature during measurement, because metabolism is affected by temperature. SVL of offspring at birth also included an effect of offspring sex. Offspring body condition at birth also included an effect of offspring sex and of offspring SVL. The random part of those two models included a maternal identity effect nested in the year effect.

Juvenile growth rates and adult weight gain before hibernation were analysed with linear models (*lm* procedure). We tested the following fixed effects: (i) experimental treatment, (ii) standardized initial fecundity and (iii) parturition date or birth date. For the analysis of juvenile growth rate, we also included juvenile sex and initial SVL to control for decelerating growth curves [52]. For the analysis of adult weight gain, we also included female SVL. However, the weight gain may also depend on the postpartum body condition (postpartum weight corrected for female SVL) of the female. As postpartum body condition and the experimental treatment are correlated (see §3), their effects cannot be tested in the same model. Thus, adult weight gain was analysed with two models, one including the treatment and one including the postpartum body condition. For those models, we did not test interactions because of the small sample sizes.

The assumptions of normality and homogeneity of variances were checked for all models. In one analysis (offspring body condition at birth) we detected heterogeneity of variances across the experimental groups. This heteroscedasticity was corrected using the varIdent function in the *lme* procedure [51].

(i) *Survival analyses*

We performed separate survival analyses for adult females, juvenile males and juvenile females. Survival estimates were obtained independently of capture probabilities, using a capture–mark–recapture method based on the open population model of Cormack–Jolly–Seber. This model produces apparent survival estimates resulting from mortality and emigration. We tested the effect of the treatment and of the standardized initial fecundity in separate analyses. The standardized initial fecundity was transformed in a categorical variable: high (positive residuals) or low (negative residuals) standardized initial fecundity. We used the program MARK v. 6.0 to fit models [53], and models were compared with the Akaike information criterion corrected for small sample size (AICc [53]). The best model is the one most consistent with the data while using the fewest number of parameters (i.e. giving the lowest AICc). It is considered that two models differ when their difference of AICc is higher than 2 [54]. The goodness-of-fit of the time-dependent Cormack–Jolly–Seber models were tested with the bootstrap procedure (1000 simulations) provided by the program MARK [53], and we did not find significant over-dispersion in the data (all $p > 0.11$).

3. RESULTS

Our treatment reduced litter size by 49.6 per cent (s.e. = 11.4%, range = 25–80%). On average, LR females gave birth to 2.37 (s.e. = 0.21) fewer offspring compared with C and S females. Our treatment had no effect on litter success ($\chi^2 = 4.61$, $p = 0.10$), which was only negatively related to female SVL ($\chi^2 = 4.16$, $p = 0.042$). We had 35 per cent of the litters with at least one failure. Our treatment had no effect on parturition dates ($F_{2,96} = 0.38$, $p = 0.686$).

(a) ***Short-term effects of litter reduction on female performances***

(i) *Behaviour and physiological measurements*

The proportion of time spent basking was not significantly affected by the treatment ($\chi^2 = 3.10$, $p = 0.21$), and it depended only positively on female SVL ($\chi^2 = 6.71$, $p = 0.0096$) and parturition date ($\chi^2 = 23.90$, $p < 0.001$). Three females (two C and one S) were always

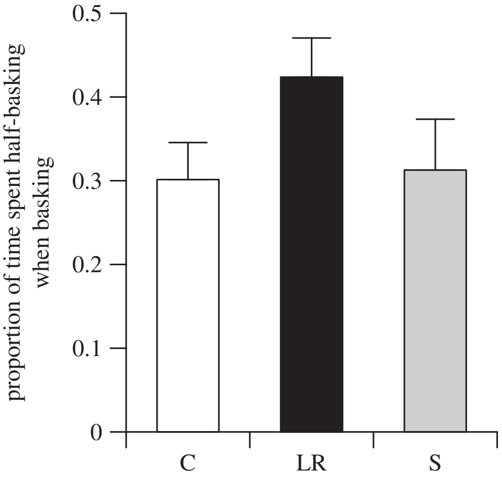

Figure 1. Mean (±s.e.) proportion of time spent half-basking when the female was basking per treatment group. Pairwise comparisons showed that LR females spent more time half-basking than S females ($p = 0.0027$) and C females ($p = 0.0022$). S and C females were similar ($p = 0.65$).

sheltering and were thus not included in the following analysis of the basking behaviour. The treatment significantly affected the proportion of time spent full-basking when the female was basking ($\chi^2 = 12.71$, $p < 0.001$). When basking, LR females spent more time half-basking than the other females (figure 1). The proportion of time spent full-basking when the female was basking did not significantly depend on parturition date ($\chi^2 = 0.002$, $p = 0.96$) or female size ($\chi^2 = 0.17$, $p = 0.68$). In none of these models were the interactions significant (all $p > 0.22$) nor was the standardized initial fecundity (all $p > 0.07$). Metabolism, corticosterone levels and PHA responses were not affected by the treatment (table 2).

(ii) *Reproductive traits*

Postpartum body condition (body mass statistically controlled for SVL) was influenced by the treatment ($F_{2,96} = 6.03$, $p = 0.0034$). LR females were more corpulent than S and C females (figure 2). Moreover, female postpartum body condition was negatively correlated with standardized initial fecundity ($F_{1,96} = 6.44$, $p = 0.013$). Interactions and parturition date were not significant (all $p > 0.27$). Concerning juvenile characteristics, body condition at birth was not affected by the treatment ($F_{2,87} = 1.41$, $p = 0.25$). Offspring body condition at birth was negatively correlated with female standardized initial fecundity ($F_{1,90} = 24.91$, $p < 0.0001$), and male offspring were more corpulent than females ($F_{1,240} = 15.21$, $p < 0.0001$). The other variables tested or the interactions were not significant (all $p > 0.11$). We observed the same pattern for offspring size: there was no effect of the treatment ($F_{2,88} = 1.25$, $p = 0.29$), but a significant effect of female standardized initial fecundity ($F_{1,90} = 8.75$, $p = 0.0039$) and sex ($F_{1,241} = 93.23$, $p < 0.0001$, males are shorter).

(b) *Delayed effects on adult females and juveniles*

(i) *Adult weight gain and survival*

Female weight gain between parturition and hibernation was neither dependent on the treatment ($F_{2,14} = 0.17$, $p = 0.69$) nor on postpartum body condition ($F_{1,16} = 0.64$, $p = 0.44$). Moreover, it was not affected by the date of parturition or SVL (all $p > 0.40$). For the survival analysis regarding the experimental treatment, our best model included a time and a treatment effect (best AICc for model $\Phi_{\text{time+treatment}}$ and p_{time}; electronic supplementary material, S1A). S females had a lower probability of survival (0.36 ± s.e. = 0.18) than LR (0.89 ± s.e. = 0.16) and C (0.85 ± s.e. = 0.22) females. However, other models, with no effect of the treatment, had the same support in the data (electronic supplementary material, S1A). A specific likelihood-ratio test showed a marginally significant difference between the best model and a model dependent on time only ($\chi^2 = 5.35$, $p = 0.069$). Regarding the standardized initial fecundity, female survival before hibernation was not affected by this trait (see electronic supplementary material, S1B).

(ii) *Juvenile growth rates and survival*

Juveniles' growth before hibernation was dependent on the treatment of their mother ($F_{2,27} = 4.40$, $p = 0.022$), with juveniles from LR females exhibiting faster growth (figure 3). All the other variables tested were non-significant ($p > 0.20$). Survival of juvenile males before hibernation was not dependent on the standardized initial fecundity (electronic supplementary material, S2A) nor on the maternal treatment (electronic supplementary material, S3A). Survival of juvenile females was also independent of maternal standardized initial fecundity (best AICc for model Φ_{time} and p_{time}); however, other models had comparable AICc (electronic supplementary material, S2B). Specific likelihood-ratio tests showed no difference between the best model (Φ_{time} and p_{time}) and other models ($\chi^2 = 1.42$, $p = 0.23$ for $\Phi_{\text{time+fecundity}}$ and p_{time}, $\chi^2 = 1.39$, $p = 0.24$ for Φ_{time} and $p_{\text{time+fecundity}}$). When we tested the effect of the treatment, we found an effect (best AICc for model Φ_{time} and $p_{\text{treatment+time}}$; electronic supplementary material, S3B). This model was significantly different from the model with no effect of the treatment ($\chi^2 = 6.18$, $p = 0.046$). Juvenile females seemed to have different capture probabilities depending on their treatment (C = 0.069 ± s.e. = 0.069, S = 0.31 ± s.e. = 0.16, LR = 0.48 ± s.e. = 0.19).

4. DISCUSSION

We experimentally studied gestation costs in a viviparous lizard, using half-hysterectomy surgery, which consists of reducing the litter size by half. The act of surgery did not affect any of the traits analysed (sham-operated and control females were similar), and neither surgery nor egg removal affected litter success. Moreover, despite a definitive ablation, LR females were still able to breed the following year (11 LR females from the 2009 experiment were recaptured in 2010 and kept in the laboratory until parturition: their mean litter size was 3.0). Thus, we are confident that our treatment had no deleterious effects on gestation and reproduction abilities.

This study showed four major results concerning litter size reduction during gestation. First, litter size reduction did not significantly influence survival, cellular immune response and metabolism of the adult females. Second, we observed that the females with a reduced litter

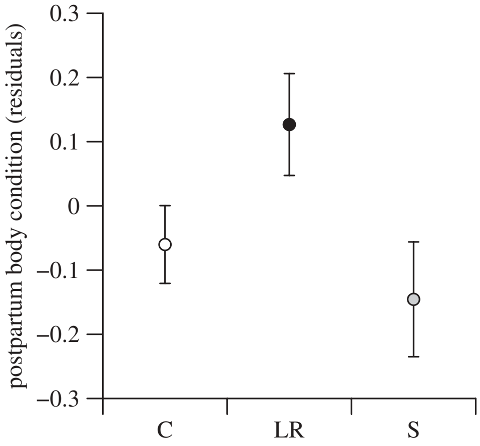

Figure 2. Mean (±s.e.) female body condition after parturition (residuals from a regression between female body weight and female SVL) per treatment group. LR females were more corpulent than S females ($p = 0.0011$) and C females ($p = 0.0338$). S and C females were similar ($p = 0.1335$).

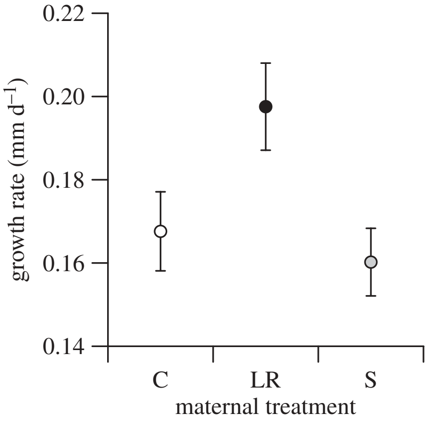

Figure 3. Mean (±s.e.) juvenile growth rates (mm d^{-1}) before hibernation per maternal treatment group. Pairwise comparisons showed that juveniles from LR females had a higher growth rate than juveniles from S females ($p = 0.011$) and than juveniles from C females ($p = 0.064$, marginally significant). Juvenile growth rates of juveniles from S and C females were similar ($p = 0.64$).

Table 2. Physiological parameters. Effect of treatment, female size (SVL), standardized initial fecundity, parturition date and room temperature (when relevant; see §2*g*) on the metabolism, the corticosterone levels and the PHA response. The interactions are not shown but have been tested and were not significant (all $p > 0.12$). Non-significant effects were eliminated sequentially; thus, in this table, we show the tests of the effects during model selection. Significant effects are in bold ($p < 0.05$), marginally significant effects are underlined ($p < 0.10$).

	treatment	SVL	standardized initial fecundity	parturition date	room temperature
metabolism	$F_{2,50} = 0.88$, $p = 0.420$	$F_{1,52} = 1.56$, $p = 0.218$	$F_{1,45} = 0.72$, $p = 0.402$	$\mathbf{F_{1,53} = 7.04}$, $\mathbf{p = 0.011}$	$\mathbf{F_{1,53} = 14.80}$, $\mathbf{p < 0.001}$
corticosterone level	$F_{2,69} = 1.68$, $p = 0.194$	$F_{1,68} = 0.87$, $p = 0.353$	$F_{1,71} = 2.29$, $p = 0.135$	$\underline{F_{1,72} = 3.18}$, $\underline{p = 0.079}$	—
PHA response	$F_{2,88} = 1.60$, $p = 0.207$	$\underline{F_{1,91} = 3.47}$, $\underline{p = 0.066}$	$F_{1,90} = 2.10$, $p = 0.151$	$\mathbf{F_{1,91} = 5.87}$, $\mathbf{p = 0.017}$	—

changed their thermoregulatory behaviour. Third, they also had an increased postpartum mass. Fourth, even though offspring from experimentally reduced litters had similar weight and size at birth to other offspring, their growth rate before hibernation was significantly increased. We discuss those results in the context of reproductive cost.

(a) ***Survival cost and physiological adjustments***

Survival has been shown to be influenced by reproductive effort, as suggested by experimental yolkectomy in reptiles [7,8,30,55], and specifically in the common lizard by a study showing that a higher investment in reproduction early in life is correlated with a shorter lifespan [56]. We could expect reduction of litter size during gestation to induce an increase in survival. Yet we observed no effect of our manipulation on survival. First, this result may be an artefact owing to our small sample sizes at recapture. Second, this could indicate that most of the reproduction cost on female survival is associated with vitellogenesis. This hypothesis is also supported by a study of oviparous and viviparous female, *Lerista bougainvillii* [57]. This study showed that survival rates after reproduction were similar for both reproductive modes in outdoor enclosures [57]. An experiment combining a manipulation of the reproductive effort during vitellogenesis and gestation in a viviparous lizard will be necessary to test this hypothesis.

We also found that the proinflammatory potential was not modified. PHA swelling response may be correlated with individual quality and is traded off with other functions [58], such as reproduction (recent experimental evidence [7]). In our study, the proinflammatory potential was not affected by our manipulation, suggesting that females with a reduced litter did not invest more energy in the part of the immune system triggered by the PHA swelling test. Gestation is also usually associated with an increase in the metabolic rate, for two reasons: embryos are metabolically active, and maternal physiology is changed to support the litter (this is called the maintenance cost of gestation [20]). However, mass-independent metabolism may be independent of litter size, as shown in a viper [22]. In our study, mass-independent metabolism was not different among females. This result therefore suggests that the maintenance cost of gestation is independent of litter size.

Contrary to survival rates, immune response and metabolism, thermoregulatory behaviour was significantly affected by litter size reduction. Females adjust (i.e. increase or decrease) their body temperatures during gestation to optimize embryonic development [19]. In *Z. vivipara*, pregnant females select lower body temperatures than non-pregnant females or males, both in a thermal gradient [36] and in the field [59]. This selection for lower temperatures may allow females to avoid too high temperatures (which may have detrimental effects on their embryos [60,61]) and/or to reduce the risk of predation. Considering constraints on embryos, we predicted that our experimental litter size reduction would not affect the thermoregulatory behaviour of females. Indeed, the embryos have the same thermal requirements whatever their number, as previously observed in a viper and in *Z. vivipara* (females selected temperatures independently of their litter mass [22,36]). Surprisingly, we showed that females with a reduced litter size spent more time in half-basking than control and sham females. The half-basking strategy is thought to be less efficient than the full-basking one. However, this does not imply that females using this strategy have a different temperature. Indeed, females with a reduced litter size are lighter, so their surface to volume ratio is higher than control females. They may thus heat faster [62] and need less basking time to reach the optimal temperatures for the embryos. In reptiles, and more particularly in the common lizard, gestation length is influenced by maternal thermoregulation [63]. As parturition dates were not different between females, it reinforces the idea that they did not select different temperatures. Finally, half-basking also reduces the exposure to predators. If this result also applies in a natural environment, females with reduced litter size may show an increased survival.

(b) *Future reproductive cost and female body condition*

We observed that females with a reduced litter size were more corpulent after parturition. As females with a reduced litter size have fewer embryos to carry and maintain, they may allocate more energy to their own maintenance and growth. Another explanation could be that females have different feeding rates. Indeed, pregnant females have a digestive tract more constrained than non-reproductive females, and eat less (e.g. [57]). However, this hypothesis is not supported by our data, as the number of larvae eaten was not different among females ($F_{2,98} = 0.94$, $p = 0.39$). Corpulence is an essential aspect of female future reproductive effort because common lizard females breed annually and postpartum body condition is positively correlated with litter size of the following year (J. Bleu, J.-F. Le Galliard, P. S. Fitze, S. Meylan & M. Massot 2011, unpublished data). Females with a reduced litter size, which are able to reach a higher corpulence after parturition, may thus be able to invest more in their future reproduction.

Another observation of our study is that the investment at vitellogenesis (i.e. initial fecundity) was negatively correlated with female postpartum mass, independently of female treatment. Vitellogenesis requires a large amount of resources, mainly fat [64]. The fact that females were not able to compensate entirely for their investment during vitellogenesis suggests that egg investment is an important cost of reproduction, in particular as not even females with reduced litter size compensated for their initial investment. In our lecithotrophic viviparous species, the cost of egg investment and gestation can be decoupled. This is not the case in other viviparous species that ovulate small eggs and that transfer nutrients to their embryos during gestation, as in mammals and some reptiles. Moreover, in mammals, nutrients are also invested after birth in the offspring during lactation. This may explain why, in mammals, studies have focused on the cost of lactation [13–16,65–67] or on the total cost of reproduction [31,32]. Nevertheless, it seems that the investment in the offspring is more costly than the production of the offspring (i.e. vitellogenesis is more costly than gestation in lecithotrophic viviparous species, and lactation is more costly than gestation in mammals).

(c) *Intergenerational costs of gestation*

Juvenile size and weight at birth were not dependent on litter size reduction and were only correlated with initial fecundity. First, this suggests that space is not a constraint on embryo development, because embryos from operated females had more space available but did not become larger juveniles. A previous study on the same species has also suggested that space is not a constraint [68]. Furthermore, juvenile characteristics could depend on the amount of nutrients received. Even though most nutrients necessary for embryo development are provided in the egg yolk (during vitellogenesis), some other nutrients, such as calcium, are transferred by the mother during gestation [37]. Embryos of reduced litters may receive more of those transferred nutrients. As juveniles from reduced litters were not significantly larger, we have no concrete evidence that they received more nutrients.

Interestingly, juveniles from females with a reduced litter size grew faster between birth and hibernation. First, juveniles from reduced litters may have stored more nutrients and exploited those extra reserves after birth. This hypothesis is only applicable to nutrient stores that are not detectable through a measure of size or weight, such as a calcium store. Second, maternal effects may have also modified behavioural offspring traits, such as foraging strategy (active versus sit-and-wait foraging) or the ability to catch prey. Indeed, maternal effects during gestation can affect the whole offspring phenotype, including offspring behaviour (e.g. [69]). Third, in wild populations, the environmental conditions experienced by the mother during gestation can affect the growth rate of the juvenile after birth. In particular, juvenile growth rate is positively correlated with the amount of rainfall [63]. However, in our laboratory experiment, this parameter was fixed and cannot explain our observation.

Size has several implications for reptile life-history traits. There is a weak positive relationship between juvenile size and survival in the common lizard [63]; yet, in our study, juveniles from reduced litters did not show higher survival rates. Nevertheless, being larger gives undeniable advantages later in life as size is often correlated with age at maturity and with litter size for adult females [56,70]. Thus, if the difference in size persists long enough, juveniles from reduced litters may be more competitive adults. In any case, it is very hard to draw conclusions on the quality of juveniles from reduced litters with respect to their entire lifespan, because accelerated growth may also be associated with underlying physiological cost that will be expressed later in life, such as elevated metabolic rate in adulthood [71].

5. CONCLUSION

This experimental study reveals the existence of some gestation costs in a viviparous lizard. Litter size during gestation is associated with a decrease of female postpartum body condition, which is known to decrease the size of their subsequent litter. Moreover, females with reduced litters decrease their time spent exposed during thermoregulation, which might affect their survival during gestation in natural conditions. Furthermore, we have shown that offspring from reduced litters grow faster after birth. Thus, maternal effects during gestation can have delayed consequences and can shape their offspring phenotype after birth.

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