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RESEARCH ARTICLE

Maternal basking regime has complex implications for birthdate and offspring phenotype in a nocturnally foraging, viviparous gecko

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ABSTRACT

Maternal basking regime can affect gestation length in viviparous squamates, but effects on offspring phenotype in species with extended pregnancies and ability to delay the birth season are unclear. We investigated the effects of three maternal basking regimes on maternal thermoregulation, gestation length, pregnancy outcome and offspring phenotype in the gecko *Woodworthia* 'Otago/Southland'. This long-lived (30+ years), nocturnal forager has field pregnancies lasting up to 14 months, with fully developed offspring maintained *in utero* over winter. Within regimes, we also compared outcomes for spontaneous (early) deliveries with those from induced (late) deliveries to test the 'adaptive prolongation of pregnancy' hypothesis. Although a cool regime significantly extended gestation, the effect was reduced by increased maternal basking, and embryonic development under the cool regime was just as successful. Offspring from spontaneous births (but not induced births) were smaller and grew more slowly when from the cool regime. However, induced offspring did not differ in consistent ways from those delivered spontaneously and offspring from all groups had high viability, similar use of warm retreat sites and similar sprint speeds. Thus, consistent evidence for adaptive prolongation of pregnancy was lacking. Unusually for squamates, pregnant females can begin vitellogenesis before giving birth, and a profound drop in maternal body temperature near the end of pregnancy (~6.3°C, confirmed under the warm regime) may assist survival of embryos *in utero*. Female lizards that maintain fully developed embryos *in utero* have the potential to make complex trade-offs among birthdate, offspring phenotype and future reproduction.

INTRODUCTION

Time of birth or hatching ('birthdate') can profoundly influence the life history and fitness of offspring. Examples exist across varied taxa and parity modes, including an association of early birthdate with adoption of an alternative reproductive tactic in male fish (Fagundes et al., 2015), with greater survival and larger mass at first summer's end in penguin chicks (Stier et al., 2014), and with greater survival (at high population density) in squirrels (Williams et al.,

2014). In principle, effects on offspring from time of birth may arise from: (1) seasonal variation in the quality of the environment into which offspring are born; (2) variation in the quality of offspring themselves, arising from prenatal developmental effects; and (3) in those species that provide it, variation in parental care during the postnatal period (Wapstra et al., 2010; Stier et al., 2014). Birthdate can also influence a mother's readiness for future reproduction (e.g. Williams et al., 2014). Ultimately, offspring birthdate may influence a mother's lifetime reproductive fitness in multiple ways.

With at least 120 inferred origins of viviparity (Blackburn, 2015), squamate reptiles (lizards and snakes) offer an extraordinary opportunity to examine the influence of gestational factors on birthdate and offspring phenotype. Additionally, as postnatal parental care is typically not obligatory, small-bodied squamates enable thermal effects on developmental outcomes to be studied whilst controlling for maternal traits (Wapstra et al., 2010). One proposal regarding the significance of birthdate (and, potentially, developmental temperature) for squamates that merits broader evaluation is the 'adaptive prolongation of pregnancy' hypothesis (Shine and Olsson, 2003). As formulated for viviparous taxa, and supported with data from the skink *Niveoscincus microlepidotus*, this hypothesis proposes that mothers retain offspring *in utero* beyond the point of sufficient differentiation for birth, thus producing offspring with phenotypes (e.g. faster running speed) that have fitness advantages (e.g. greater survival) over those born earlier in the natural birth season. The hypothesis further proposes that the advantage of later birthdate (e.g. from improved neuromuscular development) need not involve an increase in offspring size. However, subsequent studies have not always revealed consistent patterns. For example, in a 3-year study of *N. microlepidotus*, later-born offspring were not faster runners, although they were sometimes larger or faster-growing (Atkins et al., 2006). Additionally, although reduced thermal opportunity during pregnancy often increases gestation length in lizards, consistent effects on offspring running speed and/or size are not apparent (e.g. Ji et al., 2006; Li et al., 2009; Cadby et al., 2014), and for a skink with asynchronous births, differences in running speed between first- and last-born offspring within a litter were not consistent across the birth season (While et al., 2009a). Extended gestation or delayed parturition can also have negative consequences (e.g. stillbirths or neonatal deaths; Guillette et al., 1991; Atkins et al., 2007).

Here, we investigated the effects of maternal basking regime on pregnancy outcomes in a live-bearing gecko with facultative control of birth date. *Woodworthia* 'Otago/Southland' is a cool-climate diplodactylid from southern New Zealand (a proposed species of *Woodworthia* Garman 1901 within the species complex previously known as *Hoplodactylus maculatus*; Nielsen et al., 2011). These long-lived geckos (30+ years; Sheehan et al., 2004) are nocturnal

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List of symbols and abbreviations	
AVT	arginine vasotocin
SVL	snout–vent length
T_b	body temperature
T_{sel}	selected temperature

foragers that thermoregulate by day within rock retreats (Rock et al., 2002; Rock and Cree, 2008); they also bask cryptically in the field (Gibson et al., 2015) and laboratory (Cree et al., 2003; Rock and Cree, 2003). At Macraes Flat, pregnancies begin in spring and embryos are fully developed by autumn, but birth does not occur until the following spring, meaning that most females reproduce biennially (Cree and Guillette, 1995; Rock, 2006; S. Penniket, Trends in body size and female reproductive frequency with elevation and temperature in a primarily nocturnal, viviparous gecko, MSc thesis, University of Otago, 2012). In contrast, females in most lower-elevation populations appear to reproduce annually, with births in autumn after a 3–5 month pregnancy (Cree, 1994; Girling et al., 1997; S. Penniket, MSc thesis, 2012). The gestation of up to 14 months at Macraes Flat is the longest known for any squamate (Stewart and Blackburn, 2015). While the delay in birth between seasons at Macraes Flat has probable survival advantages (similar to *N. microlepidotus*, which also defers parturition from autumn to spring; Olsson and Shine, 1998), laboratory studies reveal the potential for negative effects, including stillbirths from possible 'overgestation' (Rock and Cree, 2003; Cree and Hare, 2010).

We investigated relationships among maternal basking regime, maternal basking behaviour, delivery mode and offspring phenotype in the Macraes Flat population of *Woodworthia* 'Otago/Southland'. At Macraes Flat, pregnant geckos routinely experience large daily fluctuations in thermoregulatory opportunity; thus, body temperature can vary by 20°C or more on sunny days (Rock and Cree, 2008). For ecological realism, we manipulated maternal basking regime rather than body temperatures directly, in common with recent studies of diurnal skinks from cool climates (e.g. Wapstra et al., 2010; Hare and Cree, 2010; Cadby et al., 2014). Specifically, we asked the following questions. (1) Does maternal basking regime influence maternal basking behaviour and the outcome of pregnancy (including gestation length)? (2) Does maternal basking regime, delivery mode or their interaction affect offspring morphology or performance? In particular, do offspring that are born early (spontaneously) differ consistently across regimes in phenotype from those that are born later, by induction (as predicted by the 'adaptive prolongation of pregnancy' hypothesis)? And (3) do morphological or behavioural characteristics of the mother explain whether births occur spontaneously?

MATERIALS AND METHODS
Animal collection and husbandry
We studied effects of three maternal basking regimes (hereafter 'regimes') on *Woodworthia* 'Otago/Southland'. Females were collected in early pregnancy from Macraes Flat, New Zealand (~45°23′S, 170°70′E; elevation 509–721 m a.s.l.) during 19–28 October of 2007 and 2008. Basking lamps were provided for 3.5, 5 or 7 days week^{-1}, for 8 h day^{-1} (equivalent to 28, 40 or 56 h week^{-1}; hereafter referred to as the cool, intermediate and warm regimes, respectively). The intermediate regime approximates basking opportunity at Macraes Flat in mid-summer (Hare et al., 2009). Our study builds on a pilot study with female geckos from

2005, which also reported on gestation for females in 2007 (Cree and Hare, 2010). All data presented here on maternal basking behaviour, maternal body temperature (T_b) and offspring phenotypes are new. For gestation length, pregnancy success and vitellogenic status at birth, we combine new data from 2008 with data from 2007 (Cree and Hare, 2010). Thus, we provide a comprehensive picture of pregnancy outcomes in relation to maternal characteristics and offspring phenotypes across the 2-year sample. Research was performed under University of Otago Animal Ethics Committee approval 71/07.

At collection, all females were carrying two conceptuses (maximum clutch size; for palpation accuracy, see Wilson and Cree, 2003). Geckos were treated for ectoparasitic mites using sunflower oil (Cree and Hare, 2010). One gecko dissected at collection in each year yielded embryos at ≤stage 20 (relative to the 40-point scheme for *Zootoca vivipara*; Dufaure and Hubert, 1961), confirming early pregnancy. Geckos were allocated randomly to each regime (seven to eight per regime in 2007; five per regime in 2008); mean body mass was similar among regimes ($P=0.988$). Females were maintained individually in 20-l plastic cages that contained warm and cool retreats fashioned from ceramic tiles, a plastic climber over the cool retreat, and dishes of water and sphagnum moss. A metal-mesh lid prevented escape. Cages were held within a controlled-environment room providing an air temperature of 15°C by day and 12°C by night; the photoperiod was 15 h:9 h light:dark (including 2-h ramps simulating dawn and dusk). Arcadia D3 reptile lights (Croydon, UK) provided UV light 8 h day^{-1}, 7 days week^{-1}.

Basking opportunity was delivered via a heat lamp above the warm retreat. Within 1 h, the lamp warmed the retreat's upper surface to a stable maximum temperature of 31±1°C (Cree and Hare, 2010), enabling geckos within the retreat to reach the highest daytime selected temperature (T_{sel}; mean 28°C) observed on a thermal gradient for pregnant females from this population (Rock et al., 2000). Geckos were fed two to three times per week with vitamin-dusted insects or fruit puree. Cages were rotated weekly within regimes.

Maternal basking behaviour and body temperatures
Many females basked openly within a few days of capture. Thereafter, for 3 months (and before births occurred), we recorded the posture of females basking on the surface of the warm retreat upon quiet entry into the room. Observations were made within 4 h of the heat lamps turning on ($N=40$, 59 and 82 days for the cool, intermediate and warm regimes, respectively). Each basking female was recorded as either 'flat' (ventral surface contacting the top surface of the warm retreat) or 'on toes' (body elevated on all four limbs, with abdomen held above the surface of the warm retreat).

Body temperatures achieved on days with basking opportunity were recorded twice: once during November (second month of pregnancy) and once during early March (sixth month of pregnancy; parturition under the warm regime began 8 days later). Skin temperatures were recorded between 4 and 7 h after heat lamps came on, using an infrared thermometer directed at the dorsal pelvis. In a separate colony, temperatures taken with an infrared thermometer, an infrared camera or a cloacal thermocouple did not differ ($P=0.645$; G. Moore and A.C., unpublished data); thus, we consider skin temperature to represent T_b (see also Hare et al., 2007). The position of the gecko within the cage (warm versus cool retreat; none were basking at this time of day) was also recorded.

Gestation length, pregnancy success and delivery mode

Females were checked for spontaneous deliveries by lifting the retreat-site tiles weekly from day 160 of gestation until the first birth, and thereafter twice weekly (more frequent disturbance was avoided from concern it might inhibit births). Gestation length was calculated in days from 1 October (the nominal date of ovulation; Cree and Hare, 2010). Females that did not deliver within 3–5 weeks of the first spontaneous birth under that regime were induced to deliver by administering arginine vasotocin (AVT) at 150 ng g^{-1} i.p. (Cree and Guillette, 1991; Cree and Hare, 2010 for method). This time-frame was chosen to achieve a mean gestation length for induced births longer than in spontaneously delivering females under the same regime, but sufficiently short to minimize deaths from 'overgestation' (Cree and Hare, 2010). Most induced females delivered complete litters following a single injection; however, one female failed to respond to three injections and was euthanised, allowing two viable neonates to be recovered by dissection. One induced offspring was also later recovered from a female that had spontaneously delivered a stillbirth. Following parturition, females were weighed (±1 mg) and measured [snout–vent length (SVL) and tail length ±1 mm], then palpated to assess whether vitellogenic follicles were present (an indication that the next ovarian cycle had begun; Cree and Hare, 2010).

Offspring husbandry and phenotype

Neonate size was recorded at birth (SVL and tail length ±0.5 mm; mass ±1 mg; N=62). Neonates (individually toe-clipped) were housed in 17-l cages in groups of four, based on their birthdate and size (neonates with overlapping birthdates and sizes from a laboratory colony were occasionally used to maintain equal numbers per cage). Cages had contents similar to those for mothers, but with more complex retreats, allowing all offspring to simultaneously use crevices within each warm or cool retreat. All neonates were held on the intermediate (40 h week^{-1}) basking regime so that differences in postnatal performance could be attributed solely to prenatal factors. Vitamin-dusted insects (~45 mg neonate^{-1} week^{-1}) and fruit puree (*ad libitum*) were provided.

Offspring size was re-measured at 2 months. Choice of daytime retreat, with adjacent tile surfaces classed as either warm (24–32°C) or cool (16–19°C), was recorded on two occasions at least 2 days apart (small offspring size prevented us recording T_b directly). Maximal sprint speed was measured during the day (12:00–14:00 h) at age 2–3 months for a subset of 51 offspring encompassing all regimes. After being held for 3 days without food, and then 2–3 h in an incubator at 25°C, each individual was raced three times (with 15–20 min rest between trials) down a plastic racetrack (length 1 m, width 0.08 m). Four paired photocells placed 0.25 m apart and 4 mm high over 0.75 m allowed speed to be calculated. Pauses within each 0.25 m segment were recorded. The fastest speed over 0.25 m across the three trials was used in analyses. Three juveniles that refused to run were excluded from analyses. To assess whether speed under warm daytime conditions was correlated with speed under cooler nighttime conditions, we re-ran 25 offspring (representing all regimes) during darkness at 20:00–21:00 h on a separate day. Geckos were incubated for 3 h at 15°C, a red light was used for illumination, and the remaining procedures were as for daytime measures.

Offspring were later euthanised (via halothane vapour) and dissected to confirm sex. Sex was eliminated as a potential influence on morphology and speed of offspring, as described below (effects of basking regime on sex ratios will be reported separately).

Statistical analyses

For analyses of absolute proportions (e.g. maternal retreat site use, conceptus viability), Fisher's exact tests were performed (McDonald, 2009; http://udel.edu/~mcdonald/statfishers.html). For the analysis of basking behaviour, we compared the mean incidence of basking (mean proportion of observation days on which basking was seen, either regardless of posture or specifically in the on-toes position) among regimes using linear models. In the most complex models, we examined whether basking was also influenced by delivery mode (spontaneous or induced), vitellogenic condition at delivery or maternal size variables (as covariates).

Mean T_b (for females that later delivered viable offspring) was compared between the second and sixth months of pregnancy. Following confirmation of sphericity using Mauchly's test, a linear mixed analysis with repeated measures was performed with fixed effects of regime, month and regime×month. Differences within each regime between the two months were examined using paired t-tests. Differences in mean gestation length were first examined using a linear model with regime and delivery mode as fixed factors, after confirming normality of residuals and homogeneity of variances. Additional analyses examined the effects of regime for each delivery mode, with *post hoc* tests (least significant difference) performed to identify significant pairwise differences among basking regimes for spontaneous births.

Measures of juvenile size at each age, and of growth over the first 2 months, were analyzed using linear mixed models. Where appropriate, response variables were transformed (square or log) to improve normality of residuals. Regime, delivery mode and their interaction were included as fixed effects, and maternal ID as a random effect. Year (initially included as a fixed effect) was eliminated as a non-significant factor for most final models, as was sex for all models. For offspring SVL, we included maternal post-partum SVL as a covariate as maternal size influences neonate size (e.g. Rock and Cree, 2003). For vent–tail length, we included offspring SVL at the same age as well as maternal postpartum SVL as potential covariates. Body condition was calculated as the residuals from a linear regression of log mass on log SVL, with maternal postpartum SVL as a covariate. Growth in SVL (mm day^{-1}) and growth in mass (mg day^{-1}) were analysed with offspring SVL or mass at birth and maternal postpartum SVL as covariates. Where significant effects of, or interactions with, regime were identified, we examined the effects of regime in separate analyses for spontaneous births and for induced births to identify significant pairwise differences.

Maximum values for daytime speed at 2–3 months were log transformed to achieve normality of residuals and homogeneity of variances. Fixed factors were regime, delivery mode and regime×delivery mode, with number of pauses a covariate and maternal ID a random effect. Preliminary analyses showed that offspring SVL, age, year and sex had no significant effects, and these were excluded from the final model. Using GLMM, we also examined whether the number of pauses varied with the same factors or covariates. We then tested for correlation between speed during the day and night. After allowing for the number of pauses (see Results), a linear model was developed and residuals were examined using Spearman's non-parametric test.

The position of offspring within retreat sites (scored in binary code: 0=sometimes/always in the cool retreat, 1=always in the warm retreat) was analysed in relation to regime, delivery mode and size measures (SVL, tail length, mass or body condition) using GLMM.

For comparisons of maternal T_b, gestation length, and offspring morphology and speed, we used IBM SPSS Statistics version 22.

For the remaining analyses, version 2.5.1 of R (R Foundation for Statistical Computing, Vienna, Austria; Ihaka and Gentlemen, 1996) was used. Statistical significance was accepted at $P \leq 0.05$ and data are expressed as means±1 s.e.m. unless otherwise stated. Where significant effects of regime were detected, effect sizes (Cohen's d; http://www.uccs.edu/~lbecker/) were calculated to compare representative responses between cool and warm regimes, where 0.2 is considered a 'small' effect, 0.5 a 'medium' effect and 0.8 a 'large' effect.

RESULTS

Maternal basking behaviour and body temperatures

Most female geckos (27/37) were seen basking at least once. The mean incidence of basking (flat plus on toes) across all observation days varied significantly among regimes ($F_{2,31}$=3.908, P=0.031; Fig. 1), being most frequent for the two regimes offering the least opportunity for basking (t=−2.627, P=0.013; effect size for cool versus warm=1.584). On-toes basking, although infrequent (<6% of all observations), also varied significantly among regimes ($F_{2,31}$=3.399, P=0.046; Fig. 1), occurring most frequently in the two coolest regimes (P=0.033). Basking was not influenced by female SVL, tail length, mass or body condition (P>0.05 in all cases).

Mean T_b when basking lamps were on was high under all regimes in the second month of pregnancy (~28°C; Fig. 2), but fell significantly by the sixth month of pregnancy ($F_{1,30}$=59.604, P<0.001). The decline was most dramatic (reaching a mean T_b of 21.2±1.0°C, a drop of 6.3°C) for the warm regime (month×regime, $F_{2,30}$=16.923, P<0.001; effect size for cool versus warm at 6 months=2.040). In the second month of pregnancy, all females were in the warm retreat 4–7 h after heat lamps came on. In the sixth month of pregnancy, all females from the two coolest regimes, but only 36.4% from the warm regime, were in the warm retreat (P<0.001).
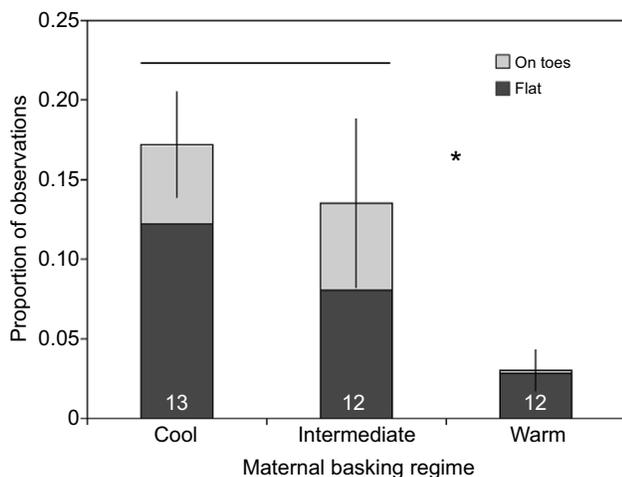
Fig. 1. Mean (±s.e.m.) proportion of observation days on which pregnant geckos (*Woodworthia* 'Otago/Southland') were seen basking. Pregnant geckos were held under one of three maternal basking regimes: cool (28 h week⁻¹ of basking opportunity), intermediate (40 h week⁻¹) or warm (56 h week⁻¹). The posture of geckos seen basking was recorded as either flat (dark grey) or on toes (light grey; s.e.m. bars shown only for the combined total; number of geckos per group at base of bar). The mean incidence of basking varied among regimes (P=0.031 and 0.046 for total and on toes, respectively) and was greater under the cool and intermediate regimes than for the warm regime (asterisk, P=0.013 and 0.033); the cool and intermediate regimes did not differ from each other (horizontal bar, P=0.652 and 0.981).
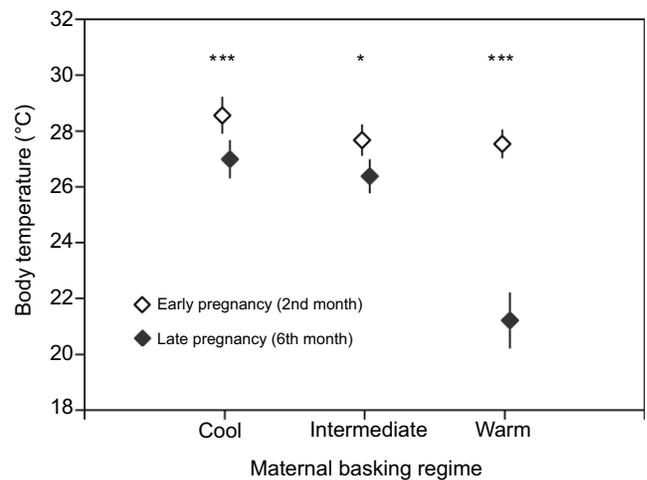
Fig. 2. Mean (±s.e.m.) body temperatures of pregnant geckos (*Woodworthia* 'Otago/Southland') on days with basking opportunity during the second and sixth months of pregnancy. Geckos from three maternal basking regimes selected similar and high body temperatures during the second month, and lower body temperatures in the sixth month. However, by late pregnancy, those under the warm regime had become more strongly heat-avoiding compared with the other two groups (linear mixed model with repeated measures; P<0.001). Data are for females that produced at least one viable neonate (N=11 per regime). For paired t-tests between months, within regimes: *P<0.05, ***P<0.001.

Pregnancy success, gestation length and delivery mode

Within each regime, most females (84.6–91.7%) developed one to two viable offspring. We analysed success at the level of conceptuses because the outcome for the two conceptuses could differ within a female. The proportion of all conceptuses that were delivered by any means (≥84.6%), and the proportion of delivered conceptuses that were viable (live, fully developed and mobile; ≥91.3%), did not differ among regimes (P≥0.517; Fig. 3A). The proportion of viable conceptuses delivered spontaneously had a non-significant tendency to be higher under the warmest regime (P≥0.088; Fig. 3A).

Mean gestation length for females producing viable offspring was strongly influenced by regime ($F_{2,27}$=70.313, P<0.001; Fig. 3B) and delivery mode ($F_{1,27}$=21.838, P<0.001), with no interaction (P=0.916). For spontaneous births, mean gestation length differed significantly among regimes (P≤0.013), ranging from 178 days (~6 months) under the warm regime to 223 days (~7.4 months) under the cool regime (effect size for cool versus warm=4.903). Within regimes, females that basked more often were not more likely to deliver spontaneously than to require induction (P=0.935). Similarly, maternal size variables (SVL, tail length, postpartum mass and body condition) did not explain delivery mode (P>0.05 in all cases).

Eight failed conceptuses disappeared without trace during mid-pregnancy. Four others were delivered as stillbirths (two spontaneous and two induced). Relative to maternal SVL, stillbirths did not differ in mean SVL or mass (35.0±0.7 mm, 0.87±0.04 g) from live neonates (33.5±0.2 mm, 0.84±0.01 g; P≥0.2). Stillbirths appeared fully and normally developed, with no signs of residual yolk or 'overgestation' (e.g. no pelvic flexion); despite having no muscle tone or reflexes, two stillbirths exhibited slow heart beats (once every 2–4 s) at dissection.

A greater percentage of females held under the warm regime contained vitellogenic follicles at parturition (83.3%) than under the two cooler regimes (18.2%; P<0.001). Within
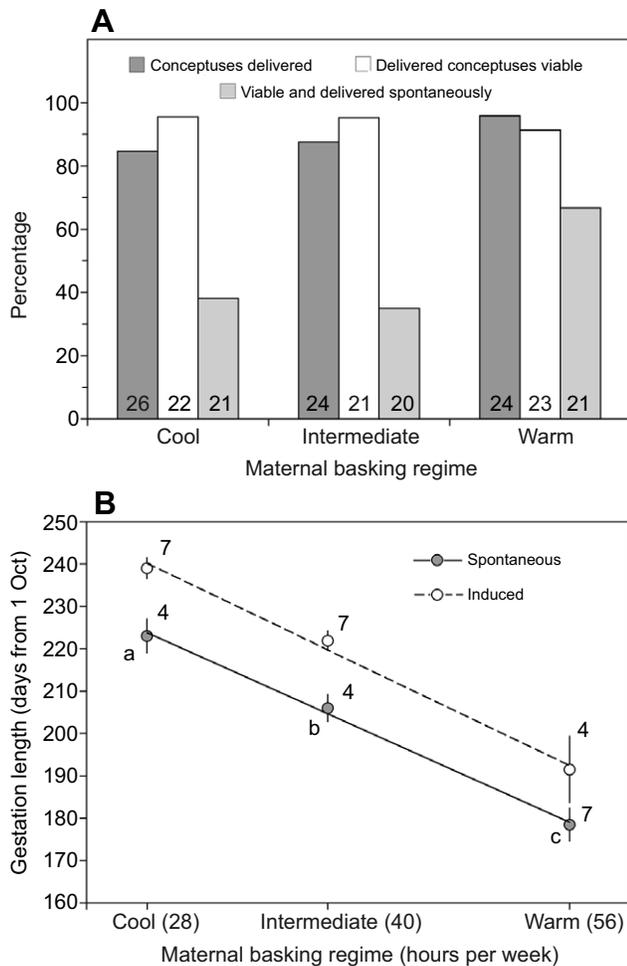
Fig. 3. Gestation outcome and gestation length in geckos (*Woodworthia* 'Otago/Southland') maintained under three basking regimes during pregnancy. (A) Proportion of all conceptuses delivered, proportion of delivered conceptuses that were viable, and proportion of viable conceptuses that were delivered spontaneously did not differ among regimes (Fisher's exact tests; $P \geq 0.088$). (B) Mean (±s.e.m.) gestation length for geckos that delivered viable offspring spontaneously was strongly dependent on basking opportunity (linear model; $P < 0.001$) and differed between each regime as shown by the different lowercase letters ($P \leq 0.013$). Delivery mode also had an effect on gestation length, as intended ($P < 0.001$). Sample sizes are shown at the base of bars in A and alongside each symbol in B. The figure presents new data for 2008 combined with expanded data for 2007 from Cree and Hare (2010).

regimes, females that basked more often were not more likely to be vitellogenic at parturition ($P \geq 0.063$). Similarly, whether females were vitellogenic at parturition was not influenced by size (SVL, tail length, mass or body condition; $P > 0.05$ in all cases).

Offspring phenotype

All offspring survived with intact tails to 2 months of age, apart from two that died in accidents. At birth, SVL was influenced by maternal regime ($P = 0.006$), by the interaction between regime and delivery mode ($P \leq 0.018$) and by maternal SVL as a covariate ($P = 0.005$; Fig. 4A, Table 1). Neonates were smallest from the cool regime, particularly when delivered spontaneously. At 2 months, the interaction term and the effect of maternal SVL remained significant ($P \leq 0.018$), and the effect of regime almost so ($P = 0.056$; Fig. 4B). Among spontaneously born offspring, SVL varied between the cool and intermediate regimes at birth and at

2 months ($P \leq 0.019$), and between the cool and warm regime at birth ($P < 0.001$; effect size 1.878), but never between the intermediate and warm regimes. Among offspring from induced births, no effects of regime on SVL were detected.

Vent–tail length was not affected by maternal regime, delivery mode or their interaction, with or without SVL as a significant covariate ($P < 0.001$; Table 1). At birth, unadjusted mass (Fig. S1) and body condition were also unaffected by regime, delivery mode or their interaction (maternal postpartum SVL was a significant covariate of body condition at birth; $P = 0.003$; and a year effect was detected: 2008>2007, $P = 0.027$; Table 1). At 2 months, unadjusted mass was almost influenced by regime ($P = 0.054$) and was influenced by the interaction term ($P = 0.050$; Fig. S1). However, when expressed as body condition (effectively controlling for SVL), these effects disappeared ($P \geq 0.309$; Table 1). Growth in SVL over the first 2 months was not influenced by any variables (Table 1). However, growth in mass was influenced by regime and by the interaction term (Fig. 4C); mass at birth or maternal postpartum SVL were not significant covariates, but a year effect was detected (2008>2007, $P \leq 0.014$; Table 1). Offspring from the cool regime, particularly those from spontaneous births, grew more slowly in mass than other groups. Differences for spontaneous births were significant only between the cool regime and other regimes ($P \leq 0.040$; effect size for cool versus warm=1.156); differences in growth among induced births were not detected.

By day, most juveniles (61%) were found in the warm retreat; retreat-site choice was not explained by regime, delivery mode or offspring size measures ($P > 0.05$). Daytime sprint speed at 25°C was faster for juveniles that paused less often ($P < 0.001$), but regardless of whether adjusted for the number of pauses, was not affected by regime, delivery mode or their interaction ($P \geq 0.323$; Fig. 4D, Table 1). As expected, mean sprint speed (adjusted for number of pauses and maternal identity) was faster by day at 25°C (0.43 ± 0.03 m s^{-1}) than by night at 15°C (0.22 ± 0.08 m s^{-1}; $P = 0.011$). Juveniles that ran quickly by day at 25°C also ran quickly by night at 15°C ($R^2 = 0.5$, $P = 0.01$).

DISCUSSION

Our study provides the first comprehensive assessment of links among maternal basking regime, thermoregulatory behaviour, delivery mode and offspring phenotype in a nocturnally foraging squamate with unusually flexible birthdates. In *Woodworthia* 'Otago/Southland' from Macraes Flat, New Zealand, regime had large effects on basking behaviour, maternal T_b at 6 months of pregnancy and gestation length (hence, birthdate), and also influenced whether females were vitellogenic at parturition. However, regime had little or no effect on the success of embryonic development, or on the proportion of females that delivered spontaneously.

Maternal regime also influenced offspring SVL at birth and growth in mass over the first 2 months; however, effects were limited to spontaneously born offspring. Compared with their induced counterparts, spontaneously born offspring were relatively small and slow-growing when from the cool regime, but not under other regimes. Effects of delivery mode (and hence, delayed parturition) on size and growth were not consistent across regimes, and no effects on retreat-site use or sprint speed at 2–3 months were detected. Thus, support for the 'adaptive prolongation of pregnancy' hypothesis is limited to (possibly beneficial) increases in size and growth for induced births under the cool regime.
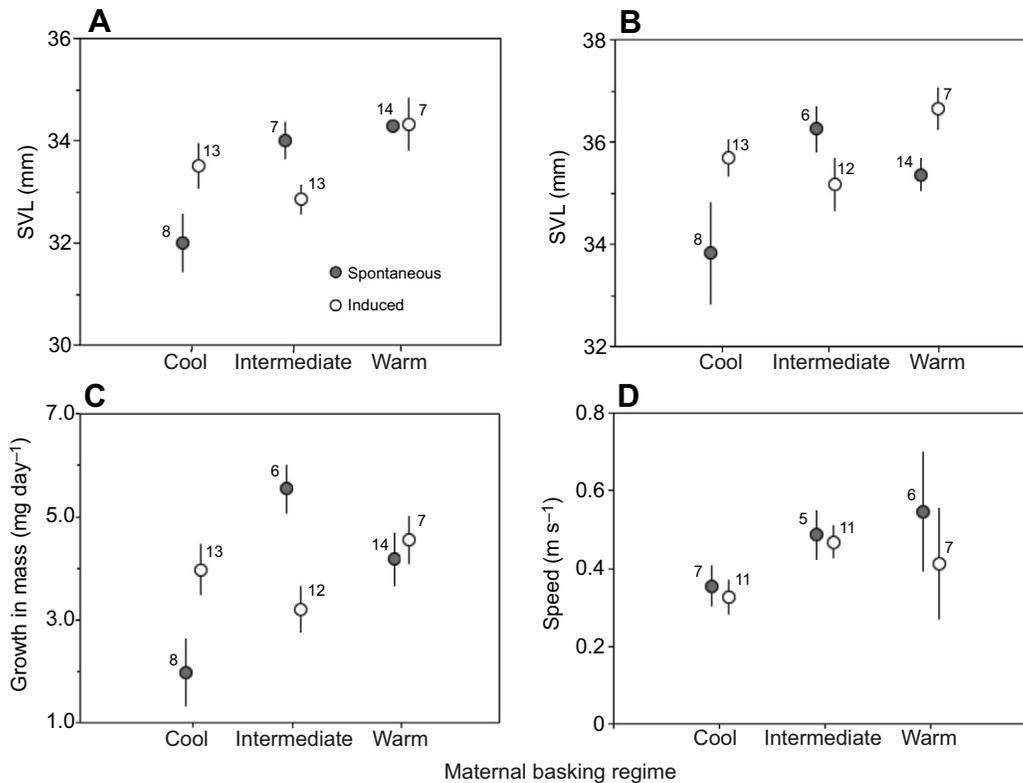
Fig. 4. Mean (±s.e.m.) snout–vent length (SVL), growth in mass and sprint speed of gecko offspring (*Woodworthia* 'Otago/Southland'), either born spontaneously or induced using the hormone arginine vasotocin after a longer gestation. For details of maternal basking regimes, see Fig. 1. From birth, all juveniles were maintained under an intermediate basking regime. (A) SVL at birth. (B) SVL at 2 months. (C) Growth in mass over the first 2 months. (D) Sprint speed at 2–3 months (recorded during the day at 25°C). Sample sizes are given alongside mean values. Maternal basking regime influenced SVL at birth and growth in mass, with a significant interaction between maternal regime and delivery mode for SVL at birth, SVL at 2 months and growth in mass. In particular, spontaneously born geckos from the cool regime were relatively small and slow-growing. However, sprint speed was not affected by maternal regime, delivery mode or their interaction (see Table 1 for details).

Maternal thermoregulatory behaviour and gestational outcomes

Basking opportunity had a large effect on basking behaviour. Despite being nocturnal foragers, Otago/Southland geckos openly basked during the first 3 months of pregnancy, especially under the two coolest regimes. Pregnant geckos that bask directly and raise themselves 'on toes' probably reach T_{sel} more quickly than those in retreats, which potentially hastens embryonic development. Diurnal skinks also increase aspects of thermoregulatory behaviour during pregnancy when basking opportunity is limited (e.g. Uller et al., 2011; Cadby et al., 2014); however, to our knowledge, use of an 'on-toes' posture for basking during pregnancy is seen only in *Woodworthia* 'Otago/Southland'. Our manual observations probably underestimated the frequency of basking in this wary species; in a field study using cameras, females basked up to several hours a day (Gibson et al., 2015).

As in earlier studies, increased maternal basking opportunity in *Woodworthia* 'Otago/Southland' resulted in shorter pregnancies (Rock and Cree, 2003; Cree and Hare, 2010). However, even under the warmest regime, pregnancies with spontaneous deliveries averaged 178 days (~6 months), an exceptional length compared with that in diurnal lizards with similar-sized offspring and similar basking opportunity (~51–71 days; Beuchat, 1988; Schwarzkopf and Shine, 1991; Li et al., 2009). Under the cool regime, mean gestation length in geckos increased by 25% (to 223 days), a smaller difference than might be expected given that basking opportunity

was reduced by 50%. The greater incidence of direct basking under the cool regime may have helped attenuate the difference. Additionally, females from the warm regime may have carried fully developed offspring for several weeks prior to birth. We infer this from the large reduction in T_b at 6 months in these females (see below for discussion), as well as the greater proportion of these females in vitellogenic condition at parturition. A further possibility is that some embryonic development may occur under all regimes below ~17°C when heat lamps are off (as, for example, in an oviparous gecko when eggs are incubated at 14–16°C; Ota, 1994). Studies to test effects of controlled temperatures for short periods would be valuable in *Woodworthia* if developmental rates can be quantified *in vivo*; however, we recommend against maintenance of these geckos under constant temperatures throughout pregnancy because of the risk in squamates of maternal ill health or mortality (additional to embryo mortality) if temperatures are unsuitable and/ or thermoregulation is not possible (e.g. Beuchat, 1988; Gao et al., 2010; Michel et al., 2013).

Despite effects of basking regime on gestation length, pregnancy success in *Woodworthia* 'Otago/Southland' was unaffected to the extent that most conceptuses developed successfully and were viable once delivered. Thus, under a cool regime with infrequent exposure to T_{sel}, embryonic development in *Woodworthia* 'Otago/ Southland' appears more successful than in cool-climate skinks, in which late-stage abortions, stillbirths or disappearance of conceptuses increased in frequency compared with warmer

Table 1. Results from statistical analyses of morphology and daytime sprint speed for offspring of *Woodworthia* 'Otago/Southland'

Response	Maternal basking regime	Delivery mode	Maternal basking regime×delivery mode	Covariate
SVL at birth (squared)	$F_{2,24.632}=6.298$ **$P=0.006$**	$F_{1,24.668}=0.076$ $P=0.784$	$F_{2,24.521}=4.716$ **$P=0.018$**	Maternal pp SVL: $F_{1,24.503}=9.568$, **$P=0.005$**
VTL at birth (log)	$F_{2,24.995}=0.853$ $P=0.438$	$F_{1,23.364}=0.099$ $P=0.756$	$F_{2,24.603}=0.646$ $P=0.533$	SVL at birth: $F_{1,53.662}=41.007$, **$P<0.001$**
Unadjusted mass at birth	$F_{2,26.088}=0.753$ $P=0.481$	$F_{1,26.089}=0.131$ $P=0.720$	$F_{2,26.088}=0.398$ $P=0.676$	–
Body condition at birth[a]	$F_{2,25.674}=1.114$ $P=0.344$	$F_{1,25.644}=0.050$ $P=0.824$	$F_{2,25.970}=1.825$ $P=0.181$	Maternal pp SVL: $F_{1,25.552}=10.386$, **$P=0.003$**
SVL at 2 months (squared)	$F_{2,22.815}=3.273$ $P=0.056$	$F_{1,22.781}=0.220$ $P=0.643$	$F_{2,22.818}=4.855$ **$P=0.018$**	Maternal pp SVL: $F_{1,22.346}=9.888$, **$P=0.005$**
VTL at 2 months	$F_{2,25.763}=0.824$ $P=0.450$	$F_{1,25.502}=0.003$ $P=0.959$	$F_{2,25.988}=0.046$ $P=0.955$	SVL at 2 months: $F_{1,42.584}=64.995$, **$P<0.001$**
Unadjusted mass at 2 months	$F_{2,24.147}=3.291$ $P=0.054$	$F_{1,24.148}=0.237$ $P=0.631$	$F_{2,24.147}=3.398$ **$P=0.050$**	–
Body condition at 2 months	$F_{2,25.167}=1.231$ $P=0.309$	$F_{1,25.168}=0.352$ $P=0.558$	$F_{2,25.167}=0.855$ $P=0.437$	–
Growth in SVL	$F_{2,25.075}=1.021$ $P=0.375$	$F_{1,25.077}=1.683$ $P=0.206$	$F_{2,25.075}=1.256$ $P=0.302$	–
Growth in mass[b]	$F_{2,78.706}=4.512$ **$P=0.014$**	$F_{1,78.588}=0.035$ $P=0.851$	$F_{2,77.782}=10.452$ **$P<0.001$**	–
Daytime sprint speed at 25°C	$F_{2,20.027}=0.583$ $P=0.567$	$F_{1,20.234}=1.027$ $P=0.323$	$F_{2,20.090}=0.027$ $P=0.974$	No. pauses: $F_{1,29.350}=26.269$, **$P<0.001$**

Responses were assessed at birth and at 2 months (morphology) or 2–3 months (sprint speed). Probabilities from linear mixed models are in bold where effects were significant. pp, post-partum; SVL, snout–vent length; VTL, vent–tail length.
[a]Significant effect also of year on body condition at birth: $F_{1,26.232}=5.484$, $P=0.027$.
[b]Significant effect also of year on growth in mass: $F_{1,76.657}=8.933$, $P=0.004$.

regimes (Cree and Hare, 2010; Cadby et al., 2014; disappearance of squamate conceptuses, probably via oviducal extrusion, has been confirmed in a snake using imaging: Bonnet et al., 2008). Differences in pregnancy success among lizard species may reflect differences in embryonic tolerance, as well as the geckos' ability to minimise the effects of reduced basking opportunity by basking 'on toes'.

Our comments on pregnancy success for *Woodworthia* 'Otago/Southland' need to be qualified by the fact that not all offspring were delivered spontaneously. The fact that any geckos required induction under the warm regime was surprising, as basking opportunity was greater than appears possible at Macraes Flat (Hare et al., 2009; Cree and Hare, 2010), and wild females at warmer locations nearby are capable of shorter pregnancies (~4–5 months; Girling et al., 1997; S. Penniket, MSc thesis, 2012). Clearly, factors other than maternal basking behaviour during early pregnancy, maternal size or access to T_{sel} influence whether geckos with fully developed embryos deliver their offspring.

A notable finding here was that female geckos under the warm regime showed a substantial decline in T_b (by 6.3°C) by the end of pregnancy. This reduction, which supports inferences from measurements of T_{sel} in field-fresh geckos (Rock et al., 2000), was much larger than under other regimes and is thus not simply a response to time in captivity (it might have been even greater if cooler retreats had been available). Such a large reduction in apparent T_{sel} appears novel for pregnant squamates, and may be limited to the time period (and species) when fully developed embryos are maintained *in utero*. Indications of altered T_{sel} or field T_b around the end of pregnancy in other squamates appear small (~2–3°C or less) and sometimes appear to span the period when births are occurring (Gier et al., 1989; Charland, 1995; Le Galliard et al., 2003). In a more rigorous comparison within pregnancy for a snake, T_{sel} remained unchanged between the first, middle and final phases (Lorioux et al., 2013). We predict that the reduced T_{sel} in *Woodworthia* 'Otago/Southland' benefits fully developed embryos

by reducing their metabolic demands, at a time when maternal lung volume appears constrained.

Effects of basking regime and delivery mode on offspring phenotypes

Live offspring of *Woodworthia* 'Otago/Southland' had high viability. However, among spontaneously delivered offspring, those from the cool regime were small (in SVL) and slow-growing (in mass). Small size and/or slow subsequent growth have sometimes been seen in offspring of diurnal lizards from cool maternal regimes (Swain and Jones, 2000; Wapstra, 2000; Li et al., 2009), though not always (e.g. patterns can vary between populations: Caley and Schwarzkopf, 2004; Cadby et al., 2014). Furthermore, some differences on lizard phenotypes at birth or hatching may be transitory (Buckley et al., 2007; Hare and Cree, 2010). Although we were only able to assess phenotype during a relatively short period of juvenile life and under one (intermediate) postnatal regime, the effects of a cool maternal regime on size of *Woodworthia* 'Otago/Southland' became stronger over 2 months, suggesting that they may be ongoing.

In other respects, offspring from the cool maternal regime seemed unaffected. For example, offspring of *Woodworthia* 'Otago/Southland' were proportionately shaped, in contrast with some lizard species in which cool-gestated offspring may have relatively short tails (Shine and Downes, 1999; Li et al., 2009) or relatively small or large mass (Shine and Harlow, 1993; While and Wapstra, 2009). In addition, at 2–3 months of age, geckos from different regimes were able to access a warm retreat as readily and to run by day as quickly. Among other viviparous lizards, effects of a low maternal basking regime on offspring speed appear variable: neonates may be relatively slow (While et al., 2009a; Li et al., 2009), relatively fast (Shine and Harlow, 1993; Swain and Jones, 2000) or show no difference (Shine and Downes, 1999), and differences may disappear within 2–3 months (Swain and Jones, 2000; Hare and Cree, 2010). In *Woodworthia* 'Otago/Southland', the faster mean

speed by day at 25°C than at night at 15°C is attributable to the effect of temperature rather than photoperiod: in adults of this species, sprint speed does not vary between day and night at constant temperature, whereas sprint speed varies by day between 25 and 15°C (Gaby et al., 2011). We also observed consistency in relative speeds among offspring tested by day (at 25°C) and by night (at 15°C). In diurnal lizards, faster sprint speed is sometimes associated with higher survival (e.g. Husak, 2006; but see Hare et al., 2012). Although the survival value for nocturnally foraging geckos is unknown, rapid locomotion might reduce predation risk when basking (Gibson et al., 2015) and when moving between daytime retreats (Rock, 2006).

With regard to induced births, our intervention produced the intended difference in mean gestation length compared with spontaneous births, with no interaction between delivery mode and regime. However, offspring morphology was affected not by delivery mode, but by the interaction between delivery mode and regime, in contrast to expectations from the 'adaptive prolongation of pregnancy' hypothesis. Induced offspring were only larger and faster-growing than spontaneously delivered offspring when they were from the cool regime, and there was no effect of delivery mode, or interaction with regime, on retreat-site choice or sprint speed at 2–3 months. The difference in size under the cool regime could indicate that delayed parturition under these circumstances allows more growth *in utero* (e.g. through improved yolk metabolism or placental transfer of nutrients), although we cannot rule out an alternative possibility: that differences in embryo size arose earlier and affected whether births under the cool regime were spontaneous.

Potential fitness implications of birthdate and offspring phenotype

Field studies on diurnal viviparous skinks from temperate climates suggest that birthdate and offspring phenotype can affect fitness. For example, offspring sometimes experience faster growth or higher survival from an early birthdate (While et al., 2009b; Wapstra et al., 2010) or from larger mass at birth (Uller et al., 2011). In *N. microlepidotus*, which (like our study species) has fully developed embryos by autumn but normally gives birth the following spring, variation in offspring survival with birth season, birthdate within a season, sprint speed and growth rate has been reported (Olsson and Shine, 1998, 1992; Shine and Olsson, 2003). Collectively, however, the direction and significance of the patterns observed in diurnal viviparous skinks across species, populations and/or years seems variable (Olsson and Shine, 1998, 2002; Shine and Olsson, 2003; While et al., 2009b; Wapstra et al., 2010; Uller et al., 2011).

Given this potential for variable effects, plus great longevity, the effects of thermal opportunity during pregnancy on fitness in *Woodworthia* 'Otago/Southland' would be extremely challenging to unravel. We nonetheless suggest that effects on gestation length (and thus birthdate) are likely to be more important than those on offspring phenotype for several reasons. First, the effect size for gestation length is much greater (perhaps reflecting that effects of thermal regime on gestation length in squamates appear additive throughout pregnancy whereas effects on offspring phenotype may arise mainly during early pregnancy; Lorioux et al., 2013). Second, in *Woodworthia* 'Otago/Southland', detected effects on offspring phenotype are limited to spontaneously born offspring under the cool regime, and there appears potential for these to be eliminated through delayed parturition (though with the risk of stillbirths if gestation is extended excessively, reasons for which remain unclear). Advantages for mothers of an early birthdate also need to be considered (Schwarzkopf and Andrews, 2012). These

potentially include a reduced physical burden, an earlier resumption of feeding at normal rates, and an earlier return to warmer T_{sel}, all of which potentially enhance maternal growth. In this species with invariant clutch size, larger maternal size is associated with larger offspring size, both within our population (Rock and Cree, 2003; Preest et al., 2005; Rock, 2006; present study) and among populations (Penniket and Cree, 2015). Thus, faster maternal growth would mean more rapid attainment of a size favouring larger offspring (and thus, potentially, lifetime reproductive success). An additional variable worth considering for future studies in this long-lived species is maternal age.

In conclusion, in the gecko *Woodworthia* 'Otago/Southland' from Macraes Flat, variation in maternal basking regime leads to both parallels and unusual outcomes for pregnancy and offspring compared with squamates from lineages with independently evolved viviparity. In the laboratory, pregnant geckos basked openly, especially under the two coolest regimes, including in an 'on-toes' posture that brings embryos close to the heat source. Maternal T_b was initially high, but a dramatic fall near the end of pregnancy (documented under the warm regime) suggests that a change in T_{sel} occurs that may assist survival of embryos *in utero*, whilst allowing vitellogenesis to resume. Increased basking opportunity shortened gestation length in females that spontaneously delivered, but even under the warmest regime pregnancies lasted more than twice as long as in other squamates of similar size. Among spontaneously delivered offspring, offspring from the cool regime were smaller and slower-growing than from other regimes, with no evidence of altered performance in other measures. Although induced offspring (with longer mean gestation length) were larger and faster-growing within the cool regime, consistent differences in phenotype between induced and spontaneously delivered offspring were not seen across regimes; thus, consistent support for the 'adaptive prolongation of pregnancy' hypothesis was lacking. Females of this population may have flexibility to make trade-offs among time of birth, offspring phenotype and future reproduction that may be adaptive under some situations.

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