EQUAL THERMAL OPPORTUNITY DOES NOT RESULT IN EQUAL GESTATION LENGTH IN A COOL-CLIMATE SKINK AND GECKO

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Abstract.—Successful development of embryos is important to captive breeding programs and studies of offspring phenotype. Here we compare the effects of three thermal regimes on gestation length and success in two viviparous lizards: McCann's Skink (*Oligosoma maccanni*), which is diurnal, and the Common Gecko (*Hoplodactylus maculatus*), primarily nocturnal. We hypothesized that large inter-specific differences in gestation length in the field would be reduced or eliminated under identical thermal regimes in the laboratory. Females were collected in early pregnancy and housed under regimes offering basking opportunity for 7, 5 or 3.5 d/wk (8 h/d). Gestation success was high in skinks under the two warmest regimes (78–83% of females produced viable offspring), but significantly lower under the coolest regime (53% success). Developmental success was high for geckos under all regimes (≥ 80% of females produced fully developed embryos), but parturition was not always spontaneous. Gestation length in geckos that delivered spontaneously was about 50% longer than in skinks, implying the involvement of non-thermal factors, including larger mass of the conceptus and offspring. Common Geckos are remarkable in recruiting the next season's clutch into vitellogenesis while still pregnant, and, in the field, in delaying parturition of fully developed embryos for months over winter; however, in the laboratory, prolonged gestation was sometimes associated with offspring death *in utero*. Thus, a phenomenon that appears adaptive in the wild can have costs under laboratory conditions. Although more research is needed to understand cues for parturition and consequences for offspring phenotypes, the results for these two model species should assist captive management for their respective genera.

Key Words.—clutch size; Common Gecko; *Hoplodactylus maculatus*; McCann's Skink; *Oligosoma maccanni*; parturition; pregnancy; vitellogenesis

INTRODUCTION

Laboratory thermal regimes can have profound effects on the outcome of gestation in viviparous lizards, including effects on offspring viability, birth date, physical and behavioral features, and in some cases even sex (e.g., Beuchat 1988; Shine and Harlow 1993; Wapstra et al. 2004). Such variation is of interest to evolutionary ecologists seeking to understand the effects of past selective pressures on different evolutionary lineages. Variation in pregnancy outcomes is also relevant in captive management, including the production of offspring for reintroduction to the wild. From the perspectives of both evolutionary ecology and conservation biology, an understanding of thermal effects on gestation is important to help predict the effects of climate change on life-history characteristics (e.g. Wapstra et al. 2009).

New Zealand has a large number of endemic, viviparous lizards living in some of the most southerly (cool) latitudes for reptiles anywhere in the world. These include 61 taxa (described and proposed species) ranked nationally as threatened with, or at risk of, extinction (Hitchmough, R., L. Bull, and P. Cromarty (compilers). 2007. New Zealand Threat Classification System Lists 2005. Department of Conservation, Wellington, New Zealand). Among the coolest mainland sites, including sub-alpine and montane regions of southern South Island, these include skinks in the genus *Oligosoma* (Scincidae), and geckos in the genus *Hoplodactylus* (Diplodactylidae).

To assist with future research and management of species in these genera, we are investigating thermal effects on offspring sex, morphology, and behavior in two model taxa (Fig. 1): a small, diurnal skink (McCann's Skink, *Oligosoma maccanni*), and a primarily nocturnal gecko (an undescribed member of the Common Gecko species complex '*Hoplodactylus maculatus*'). The gecko (known informally as *Hoplodactylus* aff. *maculatus* 'Otago/Southland large'; King et al. 2009) is hereafter referred to for convenience as the Common Gecko. Populations of both study taxa co-exist in tussock grassland at the subalpine site of Macraes Flat, a site of high diversity for lizards in mainland New Zealand (Towns et al. 2001). At high altitudes (500–710 m asl) at Macraes Flat, McCann's Skinks have summer pregnancies lasting 4–5 months (Holmes and Cree 2006), a pattern typical of diurnal New Zealand skinks (Cree 1994). In contrast, Common Geckos from the same altitudes have an unusual, biennial cycle in which pregnancies last up to 14 months; embryonic development is completed by six months, but females then carry offspring *in utero* over winter until birth the following spring, beginning the next vitellogenic cycle later that summer (Cree and Guillette 1995; Rock 2006). Shorter pregnancies of 3–5 months, with birth in late summer or early autumn, occur in Common Geckos at nearby lower altitudes and warmer sites elsewhere (Cree 1994; Girling et al. 1997).

FIGURE 1. Photos of the study species, McCann's Skink, *Oligosoma maccanni* (top) and a form of Common Gecko (*Hoplodactylus maculatus* species complex) from Otago, New Zealand (bottom). (Photographed by Alison Cree).

Here, we compare pregnancy success and gestation length, within and between the two species, among three thermal regimes that varied in access to selected body temperature. Selected temperature (T_{sel}, also known as preferred body temperature) is the body temperature that lizards select on a thermal gradient. Our null hypothesis was that inter-specific differences in gestation length in the field are thermally driven (e.g. through reduced access to selected temperature as a result of nocturnality in Common Geckos). Thus, we predicted that exposure to thermal regimes offering identical access to selected temperature should produce pregnancies of similar length for the two species in the laboratory. Previous studies on these species have documented pregnancy outcomes under various thermal regimes and collection dates (Cree et al. 2003; Rock and Cree 2003; Preest et al. 2005; Holmes and Cree 2006), but this is the first study to test three thermal regimes for either or both species simultaneously.

MATERIAL AND METHODS

Collection site and animals.—We collected both McCann's Skinks and Common Geckos during the austral spring (early-mid November 2005 and mid-late October 2007) from the Macraes Flat region in the southeastern South Island of New Zealand (45°S, 170°E; see Cree 1994 for map). These sympatric species live on schist rock outcrops in a habitat of rolling grasslands (a mixture of native tussocks and exotic pasture). We collected skinks from under rocks or by noosing (450–655 m asl), and geckos from under rocks (500–710 m asl).

Daytime selected temperatures in pregnant females from the Macraes site are similar for the two species: 28.9 ± 0.7°C (mean ± SE) in McCann's Skinks (Hare et al. 2009) and about 28°C in Common Geckos (although geckos sometimes select lower temperatures at night; Rock et al. 2000). Environmental temperatures recorded in previous years at the Macraes site also reveal high stochasticity in whether thermal preference can be reached and for how long on any given day (for skinks, Hare et al. 2009; for geckos, Rock et al. 2000, 2002; Cree et al. 2003; Rock and Cree 2008). Therefore, in the laboratory, we varied the number of days per week that lizards were able to reach T_{sel} in three regimes providing heat for 7, 5 or a nominal 3.5 d/wk for 8 h/d (described in more detail below). Based on data from the field site for McCann's Skinks (Hare et al. 2009), we estimated that the 5 d/wk regime would provide similar mean basking temperatures to those at Macraes Flat during spring-summer. Overall, we tested the three basking regimes (7, 5 and 3.5 d/wk) for a total of 17, 18, and 23 skinks respectively and for 10, 10, and 10 geckos respectively. Most animals were collected in 2007 and below, we first describe procedures followed in that year. We then document minor differences for a few animals incorporated from a pilot study in 2005, whose responses were similar and whose inclusion yielded stronger statistical power.

Terminology.—Both McCann's Skinks and Common Geckos exhibit lecithotrophic viviparity, i.e., they ovulate large, yolky oocytes, with yolk being the main source for embryonic nutrition. In common with some other literature for viviparous squamates, we refer to the ovulated yolk plus embryo as a "conceptus", and to developing young as "embryos" from ovulation until birth; we also use "clutch" synonymously with "litter".

Skinks.—We collected females with distended abdomens (55–75 mm snout-vent length [SVL], 2.6–6.4 g) 16–28 October 2007. Most females (72%) we palpated were part of a partner study examining the effect of palpation on pregnancy success. There was no significant difference between palpated and unpalpated females in pregnancy success or litter size at birth (Hare et al. 2010), or in mean gestation length for females held under the same thermal regime ($F_{1,14} \leq 1.49$; $P \geq 0.242$; present study). Therefore, regardless of palpation status, data for females are combined here for analyses of thermal effects.

We transported skinks by vehicle to the University of Otago. One dissected female (euthanised with halothane) contained embryos at < stage 25 of development using the scheme developed for *Zootoca*
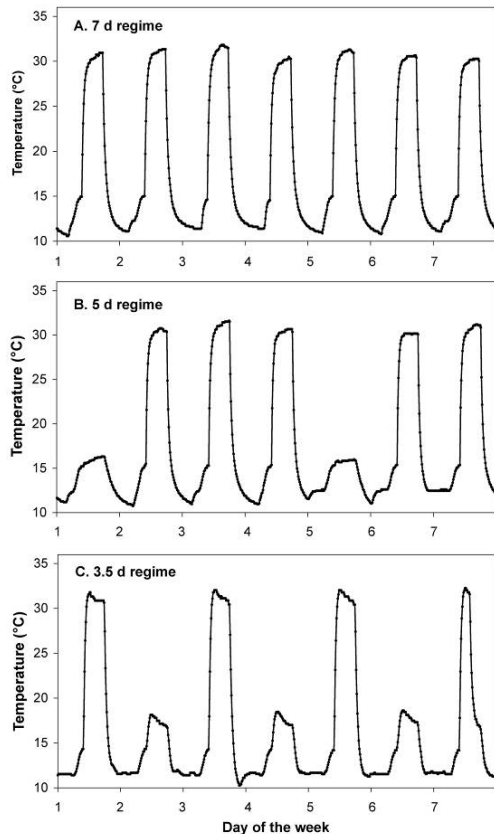
FIGURE 2. Lizards of both species were held under thermal regimes offering 7 days (A), 5 days (B) or 3.5 days (C) of basking opportunity per week (heat lamps on for 8 h/d). Room air temperature was programmed to provide an overnight temperature of 12°C and a daytime temperature of 15°C. Temperatures shown were measured on the under-surface of the top of the warm retreat (mean of two gecko cages per regime).

(*Lacerta*) *vivipara* by Dufaure and Hubert (Porter 1972). As previous experience had suggested a link in McCann's skinks between the presence of ectoparasitic scale mites (*Ophionyssus scincorum*) and pregnancy failure in captivity, we massaged all skinks with sunflower oil at capture and at intervals thereafter to eliminate mites (Hare et al. 2010). We maintained skinks individually in 20-L plastic bins, lined with paper towels and containing a warm retreat (an inverted terracotta saucer with a cut-out entrance), a cool retreat (terracotta tiles), a water dish, and a dish of damp sphagnum moss. Cages were held within a controlled-environment room programmed to provide a daily cycle in air temperature (15°C by day, 12°C by night) and photoperiod (15 L : 9 D, with a 2-h ramp period to simulate dawn and dusk within each photo-phase). Arcadia D3 reptile lights (Croydon, UK) positioned c. 0.27 m above the cages provided UV light 8 h/d, 7 d per week.

Heat lamps positioned above the warm retreat provided basking periods of 7, 5, or 3.5 d/wk for 15–20 skinks per regime in 2007. Heat lamps were on for 8 h/d for the full-day regimes; thus, the thermal regimes were equivalent to 56, 40, or 28 h of thermoregulatory opportunity per week. We adjusted heat lamps (40–60W incandescent bulbs) such that within 1 h of heat lamps coming on, the surface of the warm retreat reached a maximum, and thereafter, stable spot temperature of 31 ± 1°C (confirmed every few days using an infrared thermometer; Raynger® model ST80 ProPlus™, Raytek, Santa Cruz, California, USA). For further information on temperatures available on days with and without heat lamps, we taped the probe tip of a data logger (Stowaway™, Onset Computer, Pocasset, Mass.) to the under-surface of the hottest part of the warm retreat in cages without lizards (two per species per thermal regime); temperatures were recorded at 15 min intervals for 7 d (see Fig. 2 for examples from gecko cages). On the days that heat lamps were on, temperatures rose rapidly and were maintained at about 31°C throughout the basking period, and cooled rapidly once heat lamps were turned off. On the days without heat lamps, temperatures warmed slightly to about 16–18°C when heat lamps elsewhere in the room were on. Copper models, calibrated to match body temperatures of McCann's Skinks (Hare et al. 2009), were later positioned on top of the warm retreat with a probe inside the model. The models confirmed that temperatures available to lizards basking on the surface were about 2°C higher than on the under-surface (Fig. 2).

We fed skinks two to three times per week, with insects (mainly locusts, *Locusta migratoria*, and crickets, *Teleogryllus commodus*, plus occasional mealworm larvae, *Tenebrio molitor*, and wax-moth larvae, *Galleria mellonella*), as well as fruit (pear-based puree). We rotated cages within thermal regimes at weekly intervals, and we weighed skinks and transferred them to clean cages at two to four week intervals.

Under the coldest regime in 2007 (3.5 d/wk), the first seven females delivered late-stage abortions or stillbirths (stage 39–40, but often incompletely pigmented) at Days 100–143 of gestation. Video footage (Amanda Chamberlain, pers. comm.) plus dissections of two females revealed that non-viable offspring were sometimes eaten. At this point (Day 146), basking access for the 3.5 d/wk regime was raised to 5 d/wk in an attempt to obtain viable and/or offspring for which we could determine sex for other studies.

In combination with the data from 2007, we also include data from eight females in a pilot study in 2005. Although the collection date in 2005 (11 November, 2–3 skinks per regime) was about three weeks later than in 2007, field temperatures at Macraes Flat are frequently cool at this time (Hare et al. 2009). Embryos from a dissected female were slightly more advanced (stage 31) than in 2007, but still very small (mean mass 29 mg, about 9% of mean mass at birth). Thus, there was still ample opportunity

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for thermal effects on gestation length to be exhibited. We maintained females from 2005 in identical conditions to those in 2007, except that the coolest regime offered 3 d/wk rather than 3.5 d/wk of basking opportunity (the 3 d/wk regime appeared marginal for pregnancy success in skinks in 2005, so was increased to 3.5 d/wk in 2007). As gestation lengths for all regimes in 2005 were within the range for 2007, we pooled data from both years here; we refer to the nominal 3.5 d/wk regime as 3.5 d/wk hereafter. In both the 2007 data set and the combined data set, mean mass of females at collection did not differ among the three thermal regimes (2007: $F_{2,47} = 0.071$, $P = 0.932$; combined: $F_{2,55} = 0.137$, $P = 0.872$; n = 17–23 per regime).

We detected births in skinks by the presence of basking neonates and spent females during daily checks. In previous years, dissections of skinks from Macraes Flat revealed ovulation between September and October (Holmes and Cree 2006); the stages of development in the dissected females in the present study were consistent. Gestation length was therefore estimated from a nominal ovulation date of 1 October until the day of first birth. Comparative data are reported for skinks completing pregnancies in the field. We estimated gestation length for wild skinks based on visual appearance, palpations, and dissections of adult females, and the appearance of neonates, during January-February 1998–2003 (Holmes and Cree 2006), as well as from observations of females and neonates during January 2006 and 2008 (Karina Holmes, pers. comm.).

Geckos.—We collected female geckos (67–81 mm SVL; 7.3–13.0 g) between 19–28 October 2007. We assessed all females by palpation as carrying two conceptuses in early pregnancy (see Wilson and Cree 2003 for the high accuracy of palpation in this species). Clutch size is maximally two and not influenced by SVL in Common Geckos (Cree and Guillette 1995). We transferred geckos to the University of Otago, where we dabbed unidentified red chigger mites (present on about 25% of females) with sunflower oil. One female dissected within a day of collection carried embryos at < stage 25 of development.

We maintained geckos in controlled-environment rooms and were fed as for skinks. Cages (with a metal mesh lid to prevent escape) contained warm and cool retreats, dishes for water and moss, and a plastic climber over the cool retreat for nocturnal climbing. Occasional measurements of body temperatures (on days when cage cleaning coincided with basking days) confirmed that geckos often pressed themselves to the upper surface and were able to reach T_{sel} within warm retreats. Some geckos also basked directly on the surface of the warm retreat (especially under the 3.5 d/wk regime) and, as for skinks, could have achieved slightly higher body temperatures than shown in Figure 2. At 2 wks after collection, we checked geckos for mites (re-oiled when seen). Then and thereafter monthly, we weighed geckos and transferred them to clean cages, which were rotated at 2-weekly intervals. Mites had disappeared by 2.5 months after collection.

Space restrictions limited our study of geckos in 2007 to n = 7–8 per thermal regime (further studies are ongoing for examination of offspring phenotypes). Here, we also include data for eight geckos collected 11–14 November 2005 (n = 2–3 per regime), yielding n = 10 in total per regime. We maintained geckos identically in the two years, except for the changes noted above for skinks. Embryos in a dissected gecko at collection were slightly more advanced (stage 30) in 2005 than in 2007, but still very small (mean 30 mg, about 4% of mean neonate mass). Gestation lengths and success overlapped among geckos for the 3.5 d/wk and 3 d/wk regimes, and data for all regimes overlapped between years. In both the 2007 ($F_{2,19} = 0.012$, $P = 0.988$) and the combined data set, mean mass did not differ among regimes ($F_{2,27} \leq 0.167$; $P \geq 0.847$).

In previous studies, geckos at Macraes Flat ovulated between September and October (Cree and Guillette 1995), consistent with the stage of embryonic development in dissected females in the present study. Gestation length in geckos was therefore estimated relative to a nominal ovulation date of 1 October. We expected that, under at least the warmest two regimes, geckos would deliver by autumn, as they do in warm field locations. We therefore lifted retreat-site tiles at weekly intervals from 160 d of gestation onwards to determine whether births had occurred, and twice weekly once the first birth had been detected.

Although spontaneous births began under the 7 d/wk regime in 2005 by 174 d, two females were still carrying fully developed embryos (as assessed by palpation) at 231 d, well beyond the expected time for delivery. These females were induced to deliver by administering the hormone arginine vasotocin (AVT) as an IP injection (Cree and Guillette 1991; Rock 2006). The hormone (arg[8]-vasotocin acetate salt, V-0131, Sigma, St Louis, Mo.) was dissolved in 0.8% saline to deliver 150 ng/g body mass in an injected volume of 10 μl/g. The induced neonates (two live, one dead) were fully developed but with signs of "over-gestation" (including an upward distortion of the spine in the pelvis suggesting confinement *in utero* for too long). Given the potential for over-gestation leading to inviable offspring (an outcome that would have conflicted with our intention of comparing offspring phenotype and performance), we subsequently administered AVT to all geckos that did not spontaneously deliver within 3-5 weeks of the first spontaneous parturition under each thermal regime. We treated females on a basking day and thereafter we maintained them on a 7 d/wk basking regime. This intervention was successful, as most of the remaining 20 AVT-treated females delivered fully developed, viable offspring within 6 h of hormone injection.
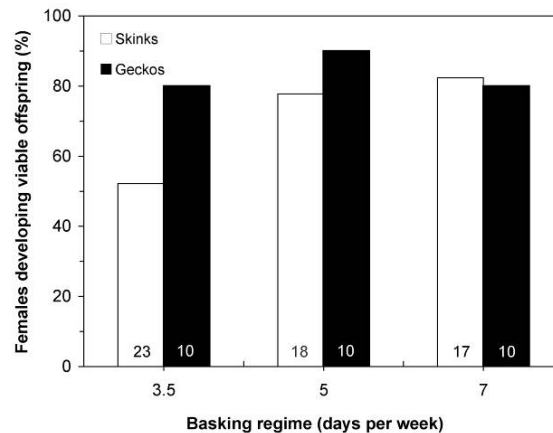
FIGURE 3. Gestation success in McCann's Skink (*Oligosoma maccanni*) and in an Otago-form of Common Gecko (*Hoplodactylus maculatus* species complex) under three thermal regimes. We collected lizards in early pregnancy and received 7, 5, or 3.5 days of basking opportunity per week (heat lamps on for 8 h/d). We assessed success in skinks as the percentage of females that spontaneously delivered at least one viable offspring. Success in geckos was the percentage of females developing at least one full-term, viable embryo (including those that were delivered spontaneously and those induced via injection of AVT). The nominal 3.5 d/wk regime included three skinks and two geckos under a 3 d regime. Total sample sizes are shown at the base of the bars.

However, one female under the 5 d/wk regime required dissection (following halothane euthanasia) to obtain her two viable neonates, and one female under the 3.5 d/wk regime required two AVT treatments nine days apart before delivering a viable neonate. We palpated post-partum females to assess whether vitellogenic follicles were present.

We therefore report gestation length in geckos in two ways: (1) as the mean for those geckos that spontaneously delivered; and (2) as the mean for all females, including those that were induced to deliver. We compared estimates of gestation length under the three thermal regimes with development time (the period by which fully developed, viable offspring have been observed *in utero*) in the field at Macraes Flat in previous years (Cree and Guillette 1995; Rock 2006; Rock and Cree 2008).

Statistical analyses.—For skinks, we compared gestation success under the coolest regime with the two warmer regimes using a chi-square test (pooling of the two warmest regimes was necessary to achieve expected frequencies of at least five in each category). We compared mean clutch size in successful pregnancies among regimes using analysis of covariance (ANCOVA), with SVL as a covariate given that clutch size is influenced by body size in McCann's Skinks (Holmes and Cree 2006). For the 3.5 d/wk regime (the only one with large samples of failed pregnancies), we examined whether gestation success was influenced by SVL or body condition using binary logistic regression. Body condition was calculated as standardized residuals from the linear regression between log post-partum mass and SVL. We compared gestation length among regimes using one-way analysis of variance (ANOVA), after confirming that variances were homogeneous.

For Common Geckos, sample sizes were too small for chi-square analysis of gestation success under the three thermal regimes. Instead, we compared the distribution of the number of viable offspring per female (0, 1 or 2) among thermal regimes using a Kruskal-Wallis test. We compared mean gestation length for female geckos delivering spontaneously between the 5 d/wk and 7 d/wk regimes using one-way ANOVA (there were insufficient spontaneous births to include the 3.5 d/wk regime). We used a two-way ANOVA to compare gestation length of spontaneous deliveries between skinks and geckos under the same two regimes. We also compared gestation lengths (pooling induced and spontaneous births) for geckos among all three regimes using a non-parametric Kruskal-Wallis test because of unequal variances. Analyses were made using SPSS 16.0 for Mac and significance was accepted when $P \leq 0.05$.

RESULTS

Skinks.—Under the two warmest regimes, 78–83% of skinks delivered at least one viable offspring (Fig. 3). Gestation success under the nominal 3.5 d/wk regime (53%) was significantly lower than for the two warmer regimes combined ($\chi^2 = 5.021$, df = 1, $P = 0.025$). However, when successful pregnancies only are considered, mean clutch sizes for viable offspring did not differ significantly among the three regimes ($F_{2,36} = 0.149$, $P = 0.862$, with maternal SVL as a covariate). Marginal means (± SE) for clutch size were 3.2 ± 0.3 (7 d/wk), 3.1 ± 0.3 (5 d/wk) and 3.3 ± 0.4 (3.5 d/wk; all evaluated at a maternal SVL of 63 mm). Pooling all regimes, 17/18 (94%) of the completely failed pregnancies provided evidence that females had indeed been pregnant (i.e., yolk discharges, declines between months of 10–29% in body mass, late-stage abortions or stillbirths, or messy
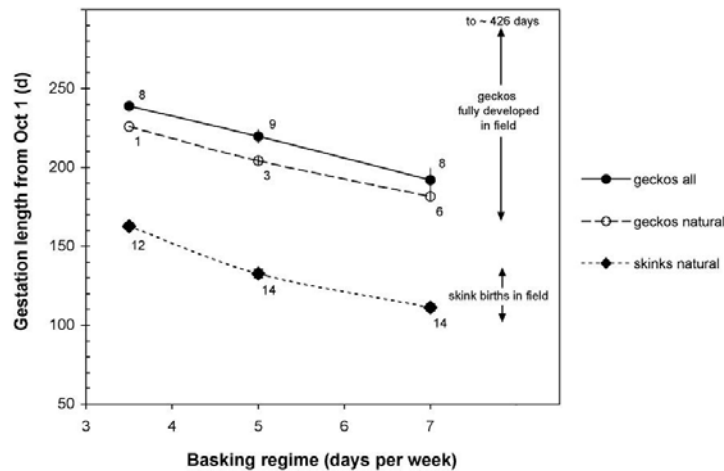
FIGURE 4. Gestation length in McCann's Skink (*Oligosoma maccanni*) and in an Otago-form of Common Gecko (*Hoplodactylus maculatus* species complex) under three thermal regimes (see Fig. 3 for details). The symbols show mean gestation length for females developing viable offspring, estimated from a nominal ovulation date of 1 October, with sample sizes indicated alongside. All skinks delivered spontaneously. Data for geckos are shown for those that delivered spontaneously (open circles) and for all females, including those that were induced hormonally (closed circles). Standard errors (vertical bars) are in most cases too small to show beyond the symbols. For comparison with laboratory gestations, the figure also illustrates the time period over which skinks deliver neonates and geckos carry fully developed embryos (viable if induced) at the Macraes Flat field site (data from Cree and Guillette 1995; Holmes and Cree 2006; Rock 2006; Rock and Cree 2008; Karina Holmes, pers. comm.).

stools indicative of ingestion of stillbirths). Within the nominal 3.5 d/wk regime, neither maternal SVL nor body condition were significant predictors of whether a pregnancy would fail completely or not ($P \geq 0.137$). Mean gestation length in skinks (in days from 1 October until the first live birth) showed a strong effect of basking regime ($F_{2,37} = 125.85$, $P < 0.001$; Fig. 4). At the Macraes field site over recent years, although neonates have been seen as early as mid-January, most births occur in late January or early February yielding an estimated gestation length of 103–137 days. Mean values for gestation length under the 7 d/wk and 5 d/wk regimes overlapped with this range (Fig. 4); however, the nominal 3.5 d/wk regime produced much longer pregnancies (148–173 days, including one female delivering successfully in 2005 under a 3 d/wk regime).

Geckos.—As noted above, gestation success in geckos is assessed based on the development, rather than spontaneous delivery, of viable offspring. At least 8/10 mothers under each regime developed at least one viable offspring (Fig. 3), and at least 7/10 developed the maximum clutch size of two. The number of viable offspring per female (0, 1 or 2) did not vary significantly among regimes (Kruskal-Wallis $\chi^2 = 0.175$, df = 2, $P = 0.916$). Failed pregnancies (conceptuses) were observed as effective stillbirths (three under the 7 d/wk regime; see below), a discharged yolk mass (one under the 5 d/wk regime), or disappeared without trace (two under the 7 d/wk regime, three under the 5 d/wk regime and four under the 3.5 d/wk regime). Mothers tended to increase in mass throughout pregnancy. Although the largest

mass loss occurred in a female whose pregnancy failed completely (12.5%, between the first and second month), similar mass losses (up to 10.9%, between the fourth and fifth month) also occurred in several females that subsequently delivered two viable offspring. Thus, mass loss in geckos was not a certain indicator of a failed pregnancy.

Only some geckos developing full-term embryos under each regime delivered spontaneously (6/10 under the 7 d/wk regime, 3/9 under the 5 d/wk regime and 1/8 under the 3.5 d/wk regime). Pregnancies in these females lasted 174–226 days. Among females delivering spontaneously from the 7 d/wk and 5 d/wk regimes, there was a clear effect of basking regime on gestation length ($F_{1,7} = 26.06$, $P = 0.001$; Fig. 4). These geckos also had substantially longer pregnancies than skinks (two-way ANOVA, species $F_{1,33} = 428.87$, $P < 0.001$), but the effect of basking regime ($F_{1,33} = 28.86$, $P < 0.001$) was similar for the two species (no significant interaction; $P = 0.518$). Mean gestation length for geckos under all regimes fell within the range of dates that females have been observed carrying fully developed, viable embryos in the field (between mid-March and December, i.e., 166–426 days from 1 October; Fig. 4).

Gecko offspring from all regimes were fully developed and viable (whether induced or not) with the exception of three fully developed but non-viable offspring induced from different mothers under the 7 d/wk regime. The latter offspring were effective stillbirths with signs of over-gestation (enclosed in apparent molt skin, with pelvic flexion pronounced in one case; a weak heartbeat at dissection was the only sign of life). One viable offspring from one of the
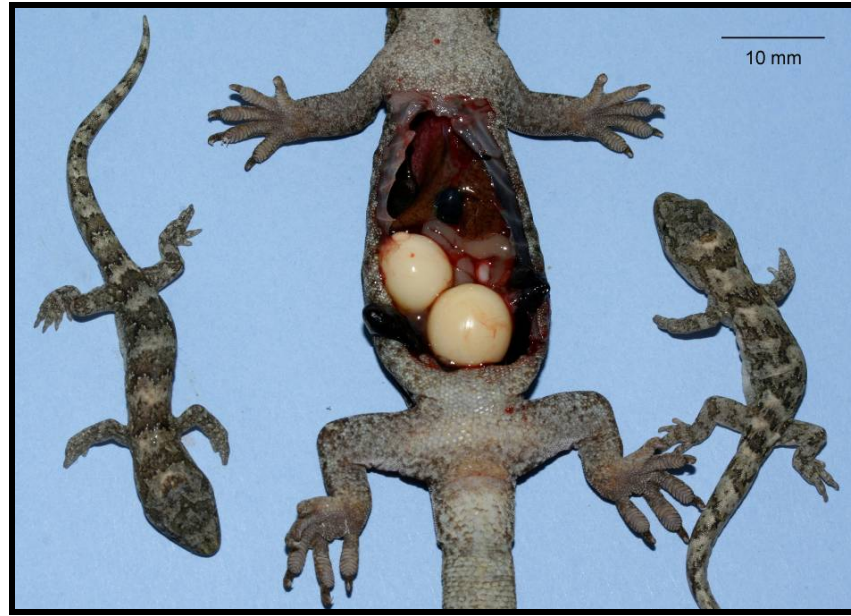
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FIGURE 5. Some females of the Common Gecko (*Hoplodactylus maculatus*) recruited follicles into vitellogenesis prior to parturition. The female shown (78 mm SVL) failed to respond to AVT, and was euthanized to obtain two viable offspring. Vitellogenic follicles (8 mm in diameter) were already close to the size at ovulation. (Photographed by Alison Cree).

same females also exhibited minor flexion of the pelvis, as did three offspring from two other mothers (one from the 7 d/wk and one from the 5 d/wk regime), but this diminished within a few days. When data for all geckos producing viable offspring are examined, gestation length clearly increased as basking opportunity decreased across the three regimes (Kruskal-Wallis $\chi^2 = 16.80$, df = 2, $P < 0.001$; Fig. 4).

However, as the time of intervention was discretionary, these data provide only an indication of the maximum gestation length required to produce viable young. We detected vitellogenic folliclesby palpation in 41% of post-partum geckos (7/10 under the 7 d/wk regime, 2/9 under the 5 d/wk regime and 2/8 under the 3.5 d/wk regime), including both females that delivered spontaneously and those that were induced to give birth. Follicle diameter reached 7–8 mm in two dissected females (Fig. 5).

DISCUSSION

Husbandry regimes that support successful pregnancies are an essential step towards understanding relationships among temperature, gestation length, and offspring quality in lizards. This is true whether such information is sought for captive management of threatened species or to answer fundamental questions in evolutionary ecology. Here we confirm high developmental success under at least two thermal regimes for McCann's Skinks and Common Geckos collected in early pregnancy. We also document differences between these two species

in the incidence of spontaneous parturition and in gestation length for spontaneous births.

McCann's Skinks are typical of diurnal viviparous skinks in New Zealand (Cree 1994) and cool-temperate Australia (Swain and Jones 2000; Wapstra 2000) in having annual pregnancies spanning about 4–5 months of the spring-summer period in wild habitats. In the laboratory, gestation success of McCann's Skinks was temperature-dependent, with success rates under the two warmest regimes (78-83% of females delivering at least one viable offspring) comparable with that in a previous study when females were not collected until late pregnancy (83%; Holmes and Cree 2006). Clutch sizes for successful pregnancies under the two warmest regimes (marginal means 3.1–3.2) also compare favorably with a clutch size of 2.9 at the same SVL for females collected in late pregnancy (Holmes and Cree 2006). Even in the wild, some conceptuses of McCann's skinks fail to develop, apparently because of developmental anomalies or lack of fertilization (Holmes and Cree 2006).

Gestation success was reduced in McCann's skinks under the coolest regime (nominal 3.5 d/wk of basking opportunity). However, those females that did produce offspring under this regime had clutch sizes comparable with those of females experiencing pregnancy under warmer laboratory regimes or in the field. This suggests that female skinks make essentially an all-or-nothing response to cool temperature. Either the pregnancy is abandoned (often very late in development), or the pregnancy is successful with clutch size unaffected. The factors that lead to this difference remain unclear; for example, those females in which pregnancies failed

TABLE 1. Gestation length under variable basking regimes in the laboratory for small diurnal skinks.

Species	Region of origin	Lat-itude	Mean snout-vent length (mm)	Mean clutch size	Mean gestation length (d)	Total basking opport-unity (h/wk)	Basking regime	Back-ground temp-erature (°C)	Comments (GL = gestation length)	Reference
Mabuya multifasciata	southern China	18° N	~103	~4	51	98	14 h/d, 7 d/wk	≤ 20	GL calculated from date of collection; group-housed	Ji et al. (2006a)
Spheno-morphus indicus	eastern China	30° N	~84	~7	85	98	14 h/d, 7 d/wk	≤ 20	GL calculated from date of collection; group-housed	Ji et al. (2006b)
Eulamprus tympanum	south-eastern Australia	34°S	~91	3-4	~51 ~58 ~81	56 28 14	8 h/d, 7 d/wk 4 h/d, 7 d/wk 2 h/d, 7 d/wk	20-22	GL calculated from date of collection (~stage 20); individually housed	Schwarz-kopf and Shine (1991)
Eulamprus heatwolei	south-eastern Australia	~35° S	~90	~3	59 73	56 14	8 h/d, 7 d/wk 2 h/d, 7 d/wk	23	GL calculated from date of collection (< ~2 wk post-ovulation); group-housed	Shine and Harlow (1993)
Niveoscincus ocellatus	Tasmania, Australia	42°S	~64	2-3	104 142	70 28	10 h/d, 7 d/wk 4 h/d, 7 d/wk	10-14	GL calculated from estimated ovulation date of 1 October; housed in pairs	Wapstra (2000)
Niveoscincus metallicus	Tasmania, Australia	~42° S	~52	2-3	92 145	70 20	10 h/d, 7 d/wk 10 h/d, 2 d/wk	12	GL calculated from estimated ovulation date of 1 November; "high-food" treatment; group-housed	Swain and Jones (2000)
Oligosoma maccanni	southern New Zealand	45°S	~63	2-3	111 133 163	56 40 28	8 h/d, 7 d/wk 8 h/d, 5 d/wk 8 h/d, 3.5d/wk	10-18	GL calculated from estimated ovulation date of 1 October; individually housed	Present study

were not significantly smaller than "successful" females.

Gestation length was clearly temperature-dependent in McCann's skinks. Under the two warmest regimes, gestation length spanned the range observed for wild skinks at Macraes Flat (Holmes and Cree 2006; Karina Holmes, pers. comm.). Although a (generally inverse) relationship between temperature and gestation length has repeatedly been observed in other viviparous lizards, comparisons of our results with those for other species are made challenging by the variety of thermal regimes employed. Regimes have included constant temperatures (Beuchat 1988; Ji et al. 2006a, b), variable temperatures but with no opportunity for behavioral thermoregulation (Ji et al. 2006a, b), variable temperatures providing opportunity to bask (and thus access to T_{sel}) on a daily basis but for differing amounts of time each day (Schwarzkopf and

Shine 1991; Shine and Harlow 1993; Wapstra 2000), and variable temperatures in which basking opportunity is provided but for differing numbers of days per week (Swain and Jones 2000; present study). For small-bodied, diurnal skinks in which thermoregulatory opportunity was provided, either daily or every few days, gestation lengths that we observed for McCann's skinks were at the highest end of those reported, but are broadly comparable with those in annually reproducing *Niveoscincus* spp. from similar latitudes in Tasmania, Australia (Table 1). Differences in background temperatures (those provided overnight and during other non-thermoregulatory periods) may have contributed to the variation in gestation length.

Our approach was to vary basking opportunity (and thus access to T_{sel}) among days per week. We believe that this approach offers the greatest ecological relevance for McCann's Skinks, given that T_{sel} is achievable in the field on only about 49% of days during pregnancy (Hare et al. 2009). However, future studies on cool-climate lizards should examine whether length and success of gestation are affected by differences in frequency versus duration of basking opportunity. For example, does 28 h of basking opportunity per week have the same effect if offered in bursts of 4 h/d, 7 d/wk as it does when offered in bursts of 8 h/d, 3.5 d/wk (present study)? To further assist inter-specific comparisons, we urge researchers to include accurate within-cage monitoring of temperatures (preferably body temperatures) throughout the 24-h period, because realized body temperatures will be affected by the efficiency of cooling and ventilation systems in the room (and potentially by social interactions, if group housing is employed).

Unlike McCann's Skinks, Common Geckos at the Macraes field site reproduce biennially, and have a remarkable capacity to carry fully-developed offspring *in utero* for about seven months over winter (Cree and Guillette 1995). During winter, pregnant females occupy deep crevices in rock outcrops where temperatures are typically only a few degrees above freezing (Rock 1999). Offspring remain *in utero* over winter without any apparent detriment in terms of body mass, abdominal fat-body mass or sprint speed (Rock 2006), and are eventually born during a season more favorable for growth, although survival differences in the field have not been compared. The Tasmanian skink, *Niveoscincus microlepidotus*, has a similar biennial cycle with fully developed offspring remaining *in utero* over winter. In *N. microlepidotus*, survival of offspring released in spring at the normal time of birth was greater than that for offspring induced by warm temperatures and released in autumn, which is consistent with a hypothesis of adaptive deferment of parturition (Olsson and Shine 1998). However, some reduction in offspring growth and sprint speed has been noted in this species when parturition is deferred until spring (Atkins et al. 2007).

In the laboratory, Common Geckos did not always deliver spontaneously when their embryos were fully developed. Common Geckos deliver in autumn at lower-altitude field sites near to Macraes Flat (Girling et al. 1997). Given this lability in the field, and that our lab-housed females were able to experience T_{sel} (for 28-56 h/wk) on a summer photoperiod, we did not anticipate that so many (especially under the two warmest regimes) would require induction to deliver. Pregnant geckos were almost always responsive to injected AVT, suggesting that some factor may have been inhibiting the release of the endogenous hormone. Access to warm daytime temperatures was not lacking (if anything, geckos on the 7 d/wk regime became heat-avoiding, often using the cold retreat by day during several weeks prior to birth). Perhaps some other thermal cue (e.g. warm overnight temperatures) was marginal for parturition. Alternatively, non-thermal factors (e.g., declining photoperiod, or an endogenous rhythm entrained by prior photo-thermal exposure) may be important. The involvement of non-thermal factors is suggested by the observation that six pregnant geckos, from a captive colony held in the same room on a 7 d/wk thermal regime (group-housed until shortly before parturition) all delivered viable offspring without the need for hormonal induction (cf. 60% of field-ovulated females on the same thermal regime).

None of the geckos requiring induction exhibited physical signs linked in other reptiles with dystocia (failure of a female to complete the process of parturition or oviposition; DeNardo 2006): there was no evidence of an aborted attempt at parturition (e.g., cloacal prolapse) or of poor muscle tone or obstruction within the pelvis. One might reasonably ask whether activation of the hypothalamo-pituitary-adrenal (HPA or "stress") axis resulting in release of corticosterone could be a possible factor. However, in Common Geckos natural deferral of parturition from autumn to spring in the wild is not associated with elevated corticosterone (Girling and Cree 1995), and experiments to date have not provided evidence that increased concentrations of HPA hormones during pregnancy extend gestation (Cree et al. 2003; Preest et al. 2005). Similarly, application of exogenous corticosterone at various stages or durations of pregnancy did not affect parturition date in *Zootoca* (*Lacerta*) *vivipara*, a lizard with short annual pregnancies (Vercken et al. 2007).

Whatever the cause, the "over-gestation" seen in several geckos in the laboratory, in which embryos apparently remain too long *in utero* and eventually die, has not been observed in the field. Over-gestation (viewed here as a failure of the mother to deliver, rather than a failure of embryos to develop normally) may have been an unrecognized factor contributing to occasional stillbirths, typically delivered several weeks after viable offspring from other females, in previous laboratory studies on this species (Rock and Cree 2003; Cree et al. 2003; Preest et al. 2005). Perhaps

this phenomenon is a trade-off of having a labile parturition response; in other words, the ability to defer parturition may be adaptive in some circumstances but not all. Our observations highlight a need for more research on the thermal and other regimes that stimulate parturition, as opposed to embryonic development, in species with labile parturition dates.

Common Geckos are also unusual lizards in resuming vitellogenesis while still pregnant. Pregnancy and vitellogenesis typically involve different hormonal profiles (high progesterone versus high estradiol respectively) and have therefore been viewed as incompatible physiological states in reptiles (Ibargüengoytía and Cussac 1996). In wild Common Geckos reproducing biennially at Macraes Flat, the two processes seem independent: vitellogenesis is not observed in the population until late summer or autumn, about 1–3 months after parturition (Cree and Guillette 1995). However, in annually reproducing populations from lower altitudes an overlap is sometimes observed, within individuals, between the resumption of vitellogenesis in late summer or autumn and the completion of pregnancy, both in the wild (Girling et al. 1997) and in the laboratory (Rock and Cree 2003). Furthermore, a museum specimen of another viviparous New Zealand gecko, *Naultinus gemmeus*, contained large vitellogenic follicles 9 mm in diameter as well as two stage 40 embryos in the uteri (Wilson and Cree 2003). In that study we hypothesized that resumption of vitellogenesis within pregnant individuals is an adaptation of New Zealand geckos that allows annual reproduction under thermally marginal conditions (i.e., it effectively allows overlap between reproductive cycles in successive years; Wilson and Cree 2003). With information from the present study, we further suggest that the overlap is restricted to the period when embryos are fully developed. It is clearly possible for vitellogenic follicles to reach an enlarged size during this period (close to the ovulatory diameter of 8–11 mm in Common Geckos), and for parturition of viable offspring still to occur naturally. More research is needed on the extent to which pregnancy and vitellogenesis overlap in wild populations of viviparous geckos.

Despite uncertainty about the cues required for parturition, it is clear that Common Geckos were able to develop a high proportion of viable young under all thermal regimes. There is a suggestion that geckos may be more successful at maintaining pregnancies under the 3.5 d/wk regime than skinks, with less effect on gestation length, although the small sample precludes statistical testing. However, Common Geckos that deliver spontaneously clearly have much longer pregnancies (by about 50%) than McCann's skinks (or other diurnal skinks; Table 1) under similar thermal regimes. Given that access to selected body temperature was essentially identical in laboratory enclosures, non-thermal factors must be involved.

Non-thermal factors influencing rates of embryonic development among reptiles have received greatest attention in egg-laying species, for which developmental temperatures are most easily controlled. Among 67 species of oviparous lizards incubated at similar constant temperatures, initial egg mass has a clear influence on incubation period (larger eggs take longer to hatch; Birchard and Marcellini 1996). Although significant variation remains amongst families once initial egg mass is controlled for, this may at least partly result from differences in stage of development at oviposition (Birchard and Marcellini 1996). Among viviparous mammals (in which developmental temperatures would also be fairly constant), gestation length and neonate mass generally increase among species as maternal body mass increases (Blueweiss et al. 1978). The differences we observed in gestation length between McCann's Skinks and Common Geckos are not attributable to differences in stage of development at collection (both were at similar stages), but are consistent with differences in sizes of conceptuses and neonates. Conceptuses of McCann's Skink soon after ovulation (c. 120 mg; Holmes and Cree 2006) are smaller than those of Common Geckos (c. 450 mg), and neonates of McCann's Skinks (310 ± 66 mg, n = 37; unpubl. obs.) are also substantially smaller than those of Common Geckos (833 ± 17 mg, n = 25; unpubl. obs.). Thus, Common Geckos have to metabolize more yolk to produce neonates of larger absolute size. We also cannot rule out the possibility of additional, lineage-specific differences in developmental rates, or that Common Geckos that delivered spontaneously may have delayed parturition beyond the end of development.

Overall, our results provide valuable information about procedures yielding successful development of embryos for two viviparous New Zealand lizards when housed indoors. McCann's Skinks and Common Geckos thus represent model taxa against which other species in their genera may be compared, and may be considered, with caution, as surrogate species for endangered taxa that are now the subject of captive management (e.g., *Oligosoma grande* and *O. otagense*; Connolly and Cree 2008).

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