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PARASITES, BUT NOT PALPATION, ARE ASSOCIATED WITH PREGNANCY FAILURE IN A CAPTIVE VIVIPAROUS LIZARD

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***Abstract.*—Successful production of offspring is vital for most captive management programs. Despite attempts to provide optimal conditions, pregnancies in captive viviparous lizards may still fail. This study tested the effect of abdominal palpation on embryonic survival within female McCann's Skinks (*Oligosoma maccanni*), documents a technique for successful removal of mites from pregnant females, and reports an association between ectoparasite presence and pregnancy failure. Contrary to anecdotal suggestions for other *Oligosoma* species, we found no evidence that palpation causes pregnancy failure in McCann's Skink. Abdominal palpation provided approximate estimates of mean litter size, but when used to estimate offspring production for population models, researchers should be aware that whether palpated or not, some pregnant females may not produce any viable offspring. Ectoparasitic scale mites were effectively removed from McCann's Skink using vegetable oil. Removal of mites was associated with increased pregnancy success: 80% of females had viable offspring in 2007, whereas when mites were not removed in 2004, females showed no morbidity yet only 6% had viable offspring. This study adds to a growing literature for captive reptiles, linking mites not just with morbidity, but also with reproductive failure. Experimental studies that control for infestation level and other possible variables (e.g., stress hormone levels) remain challenging given the difficulties of mite control, but are urgently needed.**

Key Words.—captive management; ectoparasite; mite; *Oligosoma maccanni*; reproduction.

INTRODUCTION

Successful production of offspring is vital for most captive management programs, as well as for laboratory research examining reproductive output, offspring growth, and survival. For viviparous lizards, thermal basking regimes, stress hormone concentrations, and nutrition influence whether offspring are viable or stillborn (e.g., Beuchat 1988; Doughty and Shine 1998; Cree et al. 2003). However, despite provision of otherwise seemingly optimal conditions, pregnancies can still fail. Some suggested causes for reproductive failure in captive lizards include abdominal palpation (Gartrell et al. 2002; Collen, R. 2007. Grand Skink (*Oligosoma grande*) and Otago Skink (*Oligosoma otagense*) husbandry manual. New Zealand Department of Conservation, Wellington, New Zealand.) and the presence of ectoparasitic mites (Atkins and Wapstra 2004).

Abdominal palpation has been widely used for decades to determine reproductive condition and to estimate clutch size in pregnant lizards, with viable offspring produced (Medica et al. 1971; Wapstra and Swain 2001; Holmes and Cree 2006). Abdominal palpation enables estimation of the size and shape of structures within the abdomen and pregnancy stage to be inferred (Holmes and Cree 2006). However, some conservation managers and herpetoculturists have recently raised concern that palpation of lizards might cause pregnancy failure (Collen *op. cit.*), presumably from the possibility of overly aggressive palpation rupturing yolk or follicles (Gartrell et al. 2002). Also, palpation has been associated with pregnancy failure in some mammals (e.g., Dairy Cows [*Bos Taurus*]; Thurmond and Picanso 1993). As approximately 19% of lizards are viviparous (Blackburn 1982), and many are threatened (IUCN, 2006), it is important to show that palpation does not have adverse effects on pregnancy as otherwise a potentially useful management technique could be discarded.

Another factor relevant to captive husbandry of reptiles and linked with morbidity and mortality is the presence of parasites. Parasites reduce fecundity in many animals (Tomkins and Begon 1999). The mite *Ophionyssus natrix* is the most common ectoparasite in captive reptile colonies, causing morbidity through anemia and transfer of haemoparasites in snakes and, to a lesser extent, lizards (Pasmans et al. 2008). Other mites in the genus *Ophionyssus* are similarly harmful, such as *O. scincorum*, which is a vector for haemoparasites in skinks (Allison and Desser 1981). Although parasitic infestations are linked with morbidity and mortality, their effects on reproductive output are less clear. Reproductive output of European Common Lizards (*Lacerta* [*Zootoca*] *vivipara*) captured in late-pregnancy and with high mite (family Laelapidae) infestations was not reduced, although many females suffered mortality (Sorci and Clobert 1995; Sorci et al. 1996). Conversely, a study on the Spotted Snow Skink (*Niveoscincus ocellatus*) noted an association between the presence of scale mites (*O. scincorum*) and pregnancy failure (Atkins and Wapstra 2004).

Many treatments to eliminate reptile mites are available, varying in their ease of application, expense, efficiency, and danger to the animal and user (Fitzgerald and Vera 2006). Treatments include pyrethrins/pyrethroids, organophosphates, Ivermectin, Fipronil, Dichlorvos strips, water baths, and oil smears; all require parallel cage cleaning and sanitation of cage items (Fitzgerald and Vera 2006). Mites are extremely mobile, and if any contaminated object is left untreated, re-infestation can occur within 14–21 days (Fitzgerald and Vera 2006). Chemical treatments may be toxic (Atkins and Wapstra 2004), and are not 100% effective for all species and environments (Fitzgerald and Vera 2006). Vegetable oils are non-toxic and have been used effectively for removal of *O. natricis* from snakes and other mites (such as *Hirstiella pyriformis* from lizards, tortoises, and snakes) with only one treatment necessary (Espinoza et al. 1998).

McCann's Skinks (*Oligosoma maccanni*) are diurnal, viviparous lizards from southern New Zealand. They are common and non-threatened (Hitchmough et al. 2007) with an annual reproductive cycle in which pregnancy extends from September/October (austral spring) until January/February (mid-late summer; Holmes and Cree 2006). Females mature at 49 mm snout-vent length (SVL), all females > 56 mm SVL reproduce annually, and litter size (mean = 2.8 offspring; range 1–6) increases with SVL (Holmes and Cree 2006). McCann's Skinks are commonly infested with chigger mites (*Odontacarus lygosomae*) and scale mites (*O. scincorum*; Reardon and Norbury 2004). Scale mites generally congregate under the tail scales and reach higher numbers than chigger mites, which are generally found in the armpits (Reardon and Norbury 2004). Although smaller, McCann's Skink provides a valuable model for its larger endangered relatives (*O. grande* and *O. otagense*) as all three species are sympatric, viviparous, and have similar general biology.

To help establish procedures for effective captive husbandry of viviparous skinks, and to better understand potential reasons for pregnancy failures in *Oligosoma* spp., we asked the following questions using McCann's Skink: (1) Does abdominal palpation influence the outcome of pregnancy?; (2) Is palpation during early pregnancy an accurate method for determining the number of offspring obtained?; (3) Is vegetable oil an effective means of mite removal and, by comparing results of mite removal with an earlier study where mites were not removed, (4) are mites associated with pregnancy failure in captivity?

MATERIAL AND METHODS

Pilot study.—In 2004 research on the influence of maternal basking regime on offspring phenotype using McCann's Skink was begun; no skinks were treated for ectoparasites and all were palpated. As pregnancy success was low (see results), and mites were implicated in the failures, a pilot study was undertaken in 2005 to test differences in pregnancy success after removing mites using either topically applied vegetable oil or Ivermectin (data on the different treatments are not analyzed here due to small sample sizes).

Based on promising results (64% had viable offspring), in 2007 more skinks were caught, and all were treated for ectoparasites using the oil treatment. Half of these skinks were palpated to determine potential effects of palpation on pregnancy success. In this paper, we present results for both the 2007 and 2004 studies, beginning with the 2007 study in which the effect of palpation was experimentally tested.

Animal collection and general husbandry (2007).—We captured 30 early-pregnant McCann's Skinks from 16–28 October 2007 at Macraes Flat, New Zealand (45°21'–45°28'S, 170°90'–170°50'E; elevation 448–655 m). We confirmed pregnancy status as < embryonic stage 25 via dissection of an individual euthanized via halothane overdose, and we based embryonic stage on that developed for the European Common Lizard in which fully developed embryos are at stage 40 (Dufaure and Hubert 1961). After capture, we returned skinks to the University of Otago, we weighed (± 1 mg) and measured (SVL, ± 1 mm) them, and treated them for mites (described below). We then separated females into two basking regimes, with basking opportunity available either 8 h/d, 7 d/wk (7 d/wk; N = 15) or 5 d/wk (N = 15). Ambient room temperatures simulated natural conditions at 15° C during the day and 12° C at night; when heat lamps were on, skinks could choose to bask at their preferred temperature range of 26.4–30.2° C (mean = 28.9° C; Hare et al. 2009) or remain at ambient room temperatures. These basking regimes formed part of other studies on the influence of maternal basking regime on gestation length (Cree and Hare 2010) and offspring phenotypes. As there was no difference in pregnancy success between 5 d/wk or 7 d/wk (χ^2 = 2.667, P = 0.264), data from the two basking regimes were pooled for subsequent analyses.

We housed females individually in 20-L plastic containers lined with paper towels. Enclosures contained an inverted terracotta saucer with a slot allowing access to a warm retreat site. The saucer doubled as a basking site via an incandescent 40W bulb. Rectangular tiles stacked at the other end of the cage provided a cool retreat site. Each cage also contained a dish of moist sphagnum moss and water. An Arcadia D3 reptile lamp (Croydon, UK) provided UV light 8 h/d (0900 to 1700 NZST). Ceiling-mounted fluorescent lights simulated a summer photoperiod (15:9 L:D).

We fed skinks three times a week with live crickets (*Teleogryllus commodus*), locusts (*Locusta migratoria*), wax-moth larvae (*Galleria mellonella*), mealworms (*Tenebrio molitor*), or with fruit (pear or apple based puree). We dusted insects lightly with a vitamin mixture (Repti-vite or Repti-Cal, Aristopet, Fortitude Valley, Australia). The progression of pregnancy was estimated by comparing mass gain of individuals every 28 days. At birth we measured offspring SVL and mass.

Behavioral observations (2007).—From 1 November 2007 until the first live birth on 9 January 2008, we recorded emergence behavior of females at random times throughout the day while heat lamps were available. We recorded whether a female was emerged and basking on the basking tile (or not) 3–4 times weekly.

Palpation (2007).—Palpation involved gently rolling sections of the lizard's abdomen between thumb and fingers to feel the size and shape of structures within the abdomen. Of the 30 skinks captured, we palpated 16 twice at capture (once by KMH and once by AC). In only one female was the estimation of number of embryos different (by $N = 1$ conceptus) between the two researchers. We did not palpate the remaining 14 skinks and no estimate of clutch size was made. We allocated eight palpated and seven non-palpated skinks to each basking regime.

Parasites (2007 and 2004).—We treated all skinks in 2007 for mites. Maintenance of an untreated (control) group was not possible because eradication of mites is a laborious process, the risk of re-infestation of treatment skinks from untreated skinks in the same room is too high, and offspring were wanted for other studies. We examined the freshly captured females under a stereomicroscope at 10x magnification by a single observer (KMH) for the number of raised scales (estimate for scale mite prevalence) and chigger mites in the pits of limbs. We minimized the time spent handling individuals (60–150 s per skink, including morphometric measures) to reduce any possible effects of stress. We used raised scales as a proxy for scale mite number. Where the skin is undamaged, uninfested, and not molting, the scales of McCann's Skink are smooth and overlapping.

We treated skinks for mites by gently smearing fresh sunflower oil (Sunfield, Te Atatu Peninsula, Auckland, Australia) over the entire body surface avoiding eyes and nares, particularly focusing on areas of raised scales (especially the tail) and generally in a posterior to anterior motion. The oil was smeared over the scales of the abdomen, but no attempt was made to feel for abdominal contents (c.f. palpation). We found no mites around eyes or nares of skinks examined under a dissecting microscope. We placed skinks individually in 2-L plastic containers with paper tissue to absorb excess oil and stored them overnight on a laboratory bench previously treated with Bugs Super (KiwiCare, Christchurch, New Zealand; product contains deltamethrin 0.3 g/l), a residual action insecticide that lasts up to 4 months. On one occasion, we examined the overnight boxes and tissues of 12 skinks and we counted all mobile and non-mobile mites. The day after oiling, we placed skinks in their individual enclosures, which were on trolleys also treated with Bugs Super.

We checked skinks for mites three more times: at approximately 14, 28, and 56 days post-capture (exact timing varied as all skinks on each nine-cage trolley were checked on the same day). During subsequent treatments, we removed all skinks from a trolley while the cage contents were searched for mites. We killed any free-roaming adult mites and we removed the paper towels and moss from the building. We scrubbed the cages, tiles, and dishes thoroughly in hot (65° C) water. The tiles were then soaked in hot water with Profoam disinfectant for at least 30 min, rinsed, and soaked again in hot water for at least 20 min. We also vacuumed the room holding the skinks and mopped it with hot water at each mite check, and mopped the room weekly to remove any mites that may have wandered to the floor. In the meantime, we brought the skinks to the laboratory, and checked them for chigger mites, raised scales, and evidence of live scale mites under the stereoscope (10x). We re-oiled all skinks at the second check, and only those with raised scales were oiled at the third check (~56 d post-capture), despite no evidence of live mites at the third check. No skinks were oiled at the fourth and final mite check. The entire mite check and oiling process took between 45 to 120 s per skink depending on the length of tail to scan.

From 8–14 October 2004, we captured 17 early-pregnant (< 25; Dufaure and Hubert 1961) McCann's Skinks from the same locations as 2007. All were palpated once by AC. We housed skinks using the same cage set-up as in 2007 and on a basking regime of 5 d/wk (light regime 16:8 L:D). All skinks from 2004 had scale mites, but we did not count mite load until pregnancy had failed; up until pregnancy failure began, ectoparasites were not thought to be a threat to pregnancy success based on previously successful pregnancies from females collected in late pregnancy without mite treatment (Holmes and Cree 2006). We also recorded observations of basking behavior in 2004.

Statistical analyses.—We analyzed data using version 2.5.1 of the statistical program R (Ihaka and Gentlemen 1996). All data were tested for normality and statistical significance was accepted at $P < 0.05$. Data are expressed as mean ± 1 SE unless otherwise stated.

We compared whether emergence and basking differed between the two basking regimes in 2007 (5 d/wk and 7d/wk) and between 2004 and 2007 (5d/wk only) using t-tests. We used a Fisher's Exact Probability Test for small sample sizes (< 5) or a Chi-squared Test to compare the proportion of successful

TABLE 1. Maternal and neonate characteristics for McCann's Skink (*Oligosoma maccanni*) captured in early pregnancy and subjected to mite or palpation treatments. Mites were present throughout pregnancy for individuals captured in 2004 and include chigger mites (*Odontacarus lygosomae*) and scale mites (*Ophionyssus scincorum*). Mites were removed from individuals captured in 2007. Abdominal palpation (AP) was undertaken at capture to estimate pregnancy status and clutch size. In the table, comb. includes all animals from 2007 (both palpated and non-palpated), Y = yes; N = no, and SVL = snout-vent length. Data are mean ± 1 SE. Mass is at capture. The asterisk denotes only one viable offspring and no SE could be calculated.

Year	Mites	AP	Maternal characteristics			Viable	Neonate characteristics	
			N	SVL (mm)	Mass (g)	offspring/♀	SVL (mm)	Mass (g)
2004/05	Y	Y	17	60.1 ± 1.2	3.48 ± 0.19	0.1*	24.0*	0.26*
2007/08	N	Y	16	62.8 ± 1.6	3.99 ± 0.28	2.7 ± 0.4	25.6 ± 0.2	0.31 ± 0.01
2007/08	N	N	14	65.8 ± 1.0	4.53 ± 0.18	2.5 ± 0.5	25.8 ± 0.2	0.32 ± 0.01
2007/08	N	comb.	30	64.2 ± 1.0	4.24 ± 0.17	2.6 ± 0.3	25.7 ± 0.1	0.30 ± 0.01

(viable offspring only), partially successful (at least one viable offspring and one stillbirth), and failed (stillbirths/no apparent births) pregnancies between groups of females that were palpated or not palpated. We cannot discount that some pregnancies classed as successful may include stillbirths that were eaten and should therefore be classed as partially successful. However, we noted that females that ate offspring always had messy stools a few days later and these distinctive stools were seen in no other situations. We used ANCOVA to test whether the number of offspring differed among palpated and non-palpated skinks (independent variables), with number of offspring born live as the dependent variable and maternal SVL as a covariate.

We compared the number of offspring predicted by palpation during early pregnancy with the number of offspring obtained at parturition among females that had at least one viable offspring using linear regressions. The number of conceptuses felt by palpation was compared with the number of offspring obtained at parturition (stillbirths or viable offspring). We compared the SVL and mass of neonates at parturition between individuals that were palpated or not palpated using linear mixed effects models. We included the size measure as a dependent variable and experience of palpation (palpation status as yes/no) as the independent variable. We included maternal SVL and basking regime as covariates and clutch as a random repeated variable.

For the 2004 data, we compared change in mass over time among skinks using linear mixed-effects models, with mass as the dependent variable, time as the independent variable, SVL as a covariate, and individual as a random repeated variable. For the 2007 data, change in mass over time also included pregnancy success (at least one viable offspring vs. no viable offspring) as an independent variable. We included mass of individuals up until the time when pregnancy ceased. The end of pregnancy was determined by the following outcomes coupled with palpation of mothers indicating that no embryos remained present in the oviducts: (1) live offspring; (2) still-births; (3) membranes and/or blood spots; (4) yolk discharges; or (5) large mass loss from last weighing coupled with presence of messy stools.

RESULTS

Behavior and changes in mass (2007).—Pregnant lizards from 2007 (mites removed) became habituated to experimental conditions and basked avidly when heat was available. There was no difference in basking frequency (on days that heat lamps were available) between females in the 5 d/wk (85% of females) or 7 d/wk (82% of females) basking regimes (t = 1.349, df = 39, P = 0.186). Skinks also fed readily, molted at regular intervals and appeared alert, and gained mass as pregnancy progressed. Mass of skinks that produced at least one viable offspring changed significantly over time ($F_{5,87}$ = 78.156, P < 0.001), peaking between 56 and 84 days after capture. Until the point of pregnancy failure, females that had unsuccessful pregnancies had a similar gain in mass over time as those that had successful pregnancies (interaction of time x pregnancy outcome: $F_{2,27}$ = 2.973, P = 0.096), with a slightly earlier drop in mass.

Palpation (2007).—Abdominal palpation did not influence success of pregnancies in females treated for mites (χ^2 = 2.606, P = 0.272). Eighty-one percent of females that were palpated (N = 16) and 78% of females that were not palpated (N = 14) had at least one viable offspring. Two palpated females had partially successful pregnancies, with one viable offspring and at least one stillbirth. Six females did not have viable offspring, yet gained mass and appeared to be pregnant until days 20 to 64 post-capture, when mass dropped and yolk discharges, abortions, and/or embryonic membranes and blood smears were observed. These females also had messy stools (more liquid than usual) within three days of deposits appearing in the cage as a consequence of eating birth products (including unviable offspring as seen from video footage; Amanda Caldwell, pers. obs.). Two of these females later went on to have partially successful pregnancies (one viable birth).

Of the 16 palpated females, seven gave birth to the number of offspring inferred by abdominal palpation during early pregnancy, three had more offspring and three had fewer offspring than inferred by palpation, and three had complete pregnancy failures. Two of the females that had fewer offspring than inferred by palpation discharged a yolky mass at 29 and 35 days post-capture (59 and 79 days prior to parturition). No

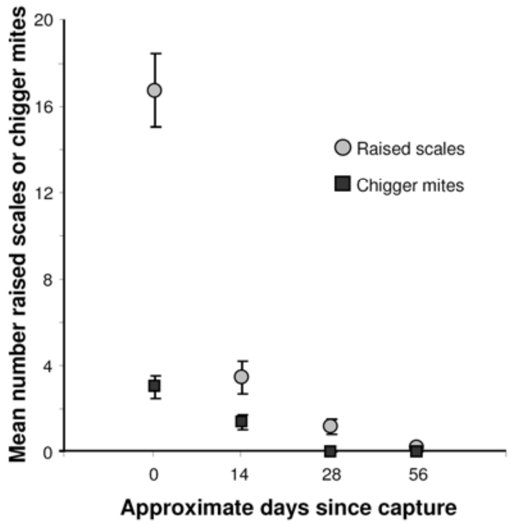

FIGURE 1. Mean number of raised scales (estimate of *Ophionyssus scincorum*) and chigger mites (*Odontacarus lygosomae*) on 30 McCann's Skinks (*Oligosoma maccanni*) at capture (day 0, 2007) and approximately 14, 28, and 56 days after first applying vegetable oil to eliminate ectoparasitic mites. No live mites were present 28 days after first treatment/capture. Error bars are ± 1SE.

discharges were noted from the other female. The females with more offspring than inferred by palpation had large litters (> 4) with one to two more offspring than estimated during early pregnancy (probably because palpation did not extend to a sufficiently anterior location). When data are averaged across the 13 palpated females with at least one viable offspring, the estimated litter size (by palpation; mean = 3.2 ± 0.2 embryos/female; range = 2–6) did not differ significantly from the total number of birth products at parturition (number of viable offspring + abortions; mean = 3.3 ± 0.3 offspring/female; range = 2–6) ($F_{1,11}$ = 0.103, P = 0.755). Litter size (viable offspring) of females that had at least one viable offspring did not differ between skinks that were palpated (N = 13, mean = 3.3 ± 1.4 offspring/ female; range = 2–6) or not palpated (N = 11, mean = 3.2 ± 2.0 offspring/female; range = 1–5; $F_{1,21}$ = 0.070, P = 0.794). Size of offspring at birth was not different among females that were palpated or not palpated (SVL, $F_{1,20}$ = 1.849, P = 0.189; mass, $F_{1,20}$ = 0.124, P = 0.728; Table 1).

Parasites (2004 and 2007).—In 2007, the use of vegetable oil to remove mites from skinks was successful after two applications. Twenty-nine of the 30 skinks had at least one raised scale (maximum = 55). Twenty-one skinks also had at least one chigger mite (maximum = 10) at capture. We checked overnight boxes for 12 skinks, and 11 contained free-roaming and/or non-moving, dead, or dying mites. One skink that had no raised scales at capture had four very small, dead scale mites in the overnight box. The average number of mites we found in the overnight boxes was 5.7 ± 1.3 mites/box (range = 0–17 mites/box), with higher values tending to be from females with more raised scales. Only one mite was observed under each raised scale in 2007 (in some skinks from 2004, two small mites were observed beneath a single scale).

One treatment with oil was insufficient to remove all mites from the skinks and cages. At the 14-day check, five skinks had live mites within their enclosures (1–6 mites/enclosure) and nine skinks still had live mites beneath raised scales (Fig. 1). After two treatments (including two entire cage and contents cleanings) the mites were completely eliminated from both skinks and cages. By 28 days after treatment no skinks had chigger mites, and no live mites were seen in enclosures; only dead mites and other debris were present under the few raised scales. Three skinks still had 3–5 raised scales at 56 days after treatment (Fig. 1), but this was associated with obvious scale damage and no live mites were seen at 56 days post-capture or at parturition (115 to 142 days post-capture).

Females from 2004 showed similar basking and behaviors to the 2007 females, basking on 81% of occasions when heat lamps were available (P > 0.05). Pregnancy success of 2004 and 2007 females was not related to the size (mass adjusted for length) of females at capture ($F_{2,43}$ = 0.570, P = 0.570). Gain in mass over time for females captured in 2004 was similar to gain in mass over time for those captured in 2007 ($F_{36,167}$ = 1.503, P = 0.142). In 2004, mass gain of skinks changed significantly over time ($F_{5,75}$ = 24.45, P < 0.001) and peaked at around 71 days post capture. Pregnancy failures of the 2004 females (N = 17) became evident from early January 2005 and only one skink had a viable neonate (6% partially successful pregnancies and 94% completely failed; Table 1). This result contrasts with that of the 2007/2008 breeding season: when ectoparasites were removed (all palpation treatments pooled) from the females (N = 30), 73% had successful pregnancies, 7% had partially successful pregnancies, and 20% had unsuccessful pregnancies.

DISCUSSION

Palpation of McCann's Skink in early pregnancy did not influence reproductive output but the presence of mites on captive skinks was a possible factor associated with pregnancy failure. Female McCann's Skinks that had pregnancy failures did not appear to behave differently from those that had successful pregnancies, with mass increasing and peaking at about 70 days post-capture regardless of the outcome of pregnancy. Gestation length of the basking regimes used in this study overlaps the 103–137 days seen in wild populations (Cree and Hare, unpubl. data). Pregnancies failed at different times, with some females discharging yolk soon after capture and others discharging premature fetuses.

Palpation.—Abdominal palpation is a useful tool for detecting follicular development, ovulation, and pregnancy status in reptiles. It has been used in many species of viviparous lizards, with viable offspring produced (e.g., Swain and Jones 2000; Wapstra and Swain 2001; Holmes and Cree 2006). The result that abdominal palpation did not influence pregnancy success in McCann's Skink, including offspring morphology, provides valuable reassurance for captive management programs of threatened species. However, safe and effective palpation requires training and practice to lower the risk of false-negatives, or accidentally inferring pregnancy from food items or large fat bodies.

Abdominal palpation during early pregnancy provides some estimate of final litter size, although some unfertilized and/or deformed ova will be lost during pregnancy (Holmes and Cree 2006). In this study, palpation during early pregnancy correctly estimated final litter size of only 54% of females, mainly due to subsequent embryonic losses. Further, the accuracy of palpation in estimating litter size (as confirmed by dissections) depends on the time of the reproductive cycle, with accuracy being greatest in early pregnancy (86%) and falling in late pregnancy (29%) when the enlarged conceptuses distend the abdomen (Holmes 2004). In this study, and in that by Holmes (2004), palpation inaccuracies were generally an under- or over-estimation of the larger litters by one or two conceptuses. However, some females will have complete pregnancy failures, with no offspring, and in these cases any estimate of final litter size will be an over-estimate. When palpation is used to estimate offspring production for population models researchers should be aware that some females may not produce any viable offspring. Although not directly quantified, some failure of embryos in wild females is likely. Research comparing litter size estimates using palpation and dissection of females throughout the reproductive cycle showed a decrease in mean litter size in wild females from early-pregnancy to late-pregnancy (Holmes 2004; Holmes and Cree 2006).

Parasites.—Data presented here, along with data from the Spotted Snow Skink infested with the same species of scale mite (Atkins and Wapstra 2004), provide circumstantial evidence that mites reduce the success of pregnancy in captive skinks (but see Sorci and Clobert 1995; Sorci et al. 1996). Hormone levels can be associated with parasite prevalence. For example, testosterone increases the susceptibility of male lizards to ectoparasitic infestation (Salvador et al. 1996). Perhaps the stress of capture and confinement, resulting in an increase in corticosterone (Cree et al. 2003; Jones and Bell 2005; Verckena et al. 2007) with probable suppression of immunity (Cartledge et al. 2005), makes some skinks susceptible to the effects of mites. For example, handling of Heatwole's Skink (*Eulamprus heatwolei*) during general husbandry can elicit an increase in corticosterone levels (Langkilde and Shine 2006), and if an interaction between stress hormone and mite impacts occurs, this may be enough to tip the balance and promote stillbirths and pregnancy failures. Despite similar behaviors between skinks in 2004 and 2007, it may be that a moderate mite-induced anemia in the 2004 skinks was sufficient to cause embryonic mortality. Alternatively, it may be that an unidentified factor, unrelated to mite presence and not studied here (or by Atkins and Wapstra 2004), elicited the pregnancy failures. These may include slight difference in day-length between years (1 h more light available to the 2004 individuals), a catastrophic virus that had no obvious effect on the behavior of the mothers, or other unappreciated differences.

Deliberate, controlled infestation of skinks was not an option in this study, but will be required to empirically test the effect of parasites on pregnancy success. To do this, researchers would need at least two simultaneous treatment groups with identical but separated husbandry conditions to prevent movement of mites between treatments. Also of value would be to test whether an increase in corticosterone associated with capture and confinement influences the prevalence of mites and their effect on the host. The ethical and experimental difficulties associated with ensuring mites do not reach plague proportions in captivity could be overcome by cage hygiene. It would also be valuable to undertake simultaneous studies on mite prevalence, pregnancy outcome, and number of offspring in wild individuals, as well as links with endoparasites. From the annual recruitment of neonates in wild populations, mites presumably do not have such an influence on pregnancy in the wild; however, the effect of mites on wild populations has not been investigated.

One application of vegetable oil has been used effectively to remove mites on lizards, tortoises, and snakes due to the oil entrapping and suffocating the mites by occluding their respiratory passages (Espinoza et al. 1998). In this study, oil smears combined with thorough cage cleaning proved successful in reducing the incidence of infested skinks after the first treatment, but two treatments were required to completely eradicate mites. Also, one skink estimated to have no scale mites had four very small scale mites in the box the following day. These observations suggest that estimates of scale mites using raised scales may be underestimates, and emphasize the importance of thorough examination of lizards and their enclosures at intervals to enable re-treatment before mite numbers escalate. Cases of mites infesting a colony of reptiles from a person who earlier handled a reptile with mites (Fitzgerald and Vera 2006) further demonstrate the risk of having mites on any individuals within a colony. On the basis of the results of the present study, the vegetable-oil treatment has been successfully applied to critically endangered *O. grande* and *O. otagense* captured for a captive breeding program. These skinks also required two treatments of oil plus rigorous cage sanitization to eradicate all live mites (Lesley Judd, pers. comm.).

Conservation implications and conclusions.— Techniques that enable accurate evaluation of reproductive condition and offspring number are valuable tools for captive management, population estimates, and research on reproductive biology. This study provides evidence that abdominal palpation in early pregnancy does not influence pregnancy outcome of viviparous lizards. Secondly, palpation yields reasonable estimates of final litter size for a group of animals, although this estimate is less accurate at the individual level. Palpation estimates can likely be improved now that we know that large females may have conceptuses extending further anteriorly than was originally appreciated. However, some females will not have any viable offspring, and these females should be taken into account for population models.

Ectoparasitic mites increase morbidity and mortality in many captive reptiles, and are now associated with pregnancy failure without evidence of morbidity in at least two skinks (Atkins and Wapstra 2004; this study). Vegetable oil provides a safe and effective method for removing mites from animals needed for captive colonies. While the inferences about the effect of mites presented here remain circumstantial, they add to growing suspicions that ectoparasitic mites contribute to pregnancy failure in adult viviparous lizards in superficially healthy condition.

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