

Constraints on the Evolution of Viviparity in the Lizard Genus *Sceloporus*

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(ABSTRACT)

I evaluated possible constraints on the evolution of viviparity in the lizard genus *Sceloporus* by experimentally extending the length of egg retention past the normal time of oviposition for a number of oviparous species. Observations also included a representative of the genus *Urosaurus*, the sister genus to *Sceloporus*. I determined the effects of retention on embryonic development, hatchlings, and gravid females. Results indicated that the proximate constraints on longer retention times and viviparity are 1) embryonic development becomes arrested or severely retarded *in utero*, and 2) the ability to maintain gravidity past the normal time of oviposition is limited in some species. Observations on *Urosaurus* further showed that extended egg retention results in hatchlings with traits that are associated with lower fitness.

I also tested the hypothesis that reproductive *Sceloporus* lower their body temperatures during activity because their normal body temperatures are detrimental to embryos. Observations on a viviparous species of *Sceloporus* indicated that the normal body temperature of the female was detrimental to embryonic development. This result indicates that viviparity would be constrained in some squamate lineages if maternal body temperatures are too high for successful embryonic development.

I also evaluated the hypothesis that the evolutionary transition from oviparity to viviparity involves a "reduction" of the eggshell concurrent with longer durations of egg retention. If this hypothesis is correct, then attributes of eggshells that should enhance

exchange of respiratory gases (i.e., thickness, density, permeability to water vapor) would be correlated with the maximal developmental stage that embryos are able to attain in the oviducts (i.e., stage of developmental arrest). The results of this study indicated that these features of shells do not determine the stage at which development becomes arrested. Thus, the results do not support the hypothesis that shell reduction occurs concurrently with longer periods of egg retention. The results are consistent with the alternative hypothesis that reduction of the eggshell occurs after viviparity has evolved.

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Table of Contents

Abstract	ii
Acknowledgments	iv
Introduction and Background	1
Chapter 1: Determinants of embryonic stage at oviposition in a squamate reptile (<i>Urosaurus ornatus</i>)	
Abstract	6
Introduction	7
Materials and Methods	10
Results	19
Discussion	31
Chapter 2: Constraints on the evolution of viviparity in a clade of sceloporine lizards	
Abstract	40
Introduction	41
Materials and Methods	43
Results	51
Discussion	61
Chapter 3: Variation among oviparous sceloporine lizards in the capacity to support embryogenesis in utero: influence of morphology and gas permeability of the eggshell	
Abstract	67
Introduction	68
Materials and Methods	71
Results	82
Discussion	98

Chapter 4: Influence of pregnancy on the thermal biology of the lizard, *Sceloporus jarrovi*:

why do pregnant females exhibit low body temperatures?

Abstract	105
Introduction	106
Materials and Methods	110
Results	120
Discussion	125
References	132
Curriculum Vitae	143

List of Figures

- Figure 1.1** Graph of incubation temperatures of control and retained eggs of *Urosaurus ornatus* 14
- Figure 1.2** Relationships between dry embryo mass, developmental stage, initial egg mass, days to hatch, and days past the normal time of oviposition for control and retained eggs of *Urosaurus ornatus* 21
- Figure 1.3** Relationships between live hatchling mass, snout-vent length, dry mass, hydration, and days retained past the normal time of oviposition for *Urosaurus ornatus* hatchlings 25
- Figure 1.4** Influence of incubation treatment and number of runs on locomotor performance of *Urosaurus ornatus* hatchlings in terms of mean sprint speed, fastest sprint speed, and number of stops 28
- Figure 2.1** Mean incubation temperatures of control eggs (eggs incubated in incubation media) and retained eggs (eggs retained *in utero* past the normal time of oviposition) of *Sceloporus undulatus hyacinthinus* and *Sceloporus undulatus consobrinus* 48
- Figure 2.2** Frequency distribution of the maximal embryo stage attainable *in utero* for four species of sceloporine lizards 53

Figure 2.3 Developmental stage and dry mass of <i>Sceloporus undulatus hyacinthinus</i> embryos in control and retained groups as a function of days past the normal time of oviposition	58
Figure 2.4 Developmental stage and dry mass of <i>Sceloporus undulatus consobrinus</i> embryos in control and retained groups as a function of days past the normal time of oviposition	60
Figure 3.1 Electron micrographs of eggshells of <i>S. u. hyacinthinus</i> and <i>S. clarkii</i> .	84
Figure 3.2 Electron micrographs of eggshells of <i>S. u. consobrinus</i> and <i>S. virgatus</i>	83
Figure 3.3 Electron micrographs of eggshells of <i>S. scalaris</i> , <i>U. ornatus</i> , and <i>S. clarkii</i> .	88
Figure 3.4 The relationship between mean eggshell thickness and the mean mass of the egg at the time of oviposition for six species of phrynosomatid lizards	95
Figure 4.1 Representative thermocouple probe temperatures on the thermal gradient for <i>Sceloporus jarrovii</i> measured at 20 cm intervals	112

Figure 4.2 Mean temperatures (air and body) experienced by *S. jarrovi*
in environmental chambers (35 °C and 32°C treatments) and
terraria that experienced ambient temperatures (TREG treatment) 115

Figure 4.3 Mean selected body temperatures of female *Sceloporus jarrovi*
when pregnant and postpartum measured at three times during the
main portion of the daily activity period 120

List of Tables

Table 2.1 Maximum embryonic stage attainable <i>in utero</i> for four species of sceloporine lizards	54
Table 3.1 Embryonic stage at oviposition and maximum stage attained <i>in utero</i> for six species of phrynosomatid lizards	74
Table 3.2 Water vapor conductance of eggs of four species of phrynosomatid lizards at low and high ambient water vapor pressure	83
Table 3.3 Thickness of shells of five species of phrynosomatid lizards	94
Table 3.4 Masses of components of shells of four species of phrynosomatid lizards	95
Table 4.1 Condition of offspring from female <i>Sceloporus jarrovi</i> that produced abnormal and/or dead embryos or neonates	123
Table 4.2 Characteristics of <i>Sceloporus jarrovi</i> neonates incubated under one of three temperature regimes	124

Introduction and Background

Evolutionary Constraints and the Evolution of Viviparity in Lizards

The identification of constraints and their underlying mechanisms has provided a useful means for gaining insight into the origins of key transitions in life history traits (Stearns 1989). Discontinuities among taxa in a particular trait may result from adaptive or developmental constraints. Differences in reproductive mode (e.g., oviparity and viviparity) among closely related taxa, for example, may arise from adaptive constraints; conditions in the environment favoring different modes of reproduction, but not the evolutionary intermediates.

Developmental constraints may arise from biomechanical principles that simply preclude the origin of certain traits, but more often they take the form of biases on the production of particular traits caused by the structure, character, composition, or dynamics of the developmental system (Stearns 1989). For example, viviparity may be constrained in turtles because 1) turtle embryos do not develop beyond the gastrula stage when eggs are retained in the oviducts past the normal time of oviposition (Ewert 1991) and, 2) unlike squamate eggs, eggs of most turtles die if they are turned during early development (Ewert 1984).

Most developmental constraints are the products of evolution and they are therefore, taxon-specific. Thus, developmental constraints (and adaptive constraints) often lead to phylogenetic constraints; key traits that become fixed, followed by organismal integration, and consequent irreversibility of the fixation. An organism's life history traits may

therefore be constrained by the phylogenetic position it occupies. The distribution of viviparity among squamates illustrates this phenomenon.

Viviparity has evolved many times in squamates. At least 15 families contain both oviparous and viviparous members and viviparity may have had at least 100 independent origins within these families. At least 63 of these origins are thought to have occurred in lizards (Shine 1985). However, the distribution of viviparity among the squamate lineages is not uniform; some families are entirely oviparous (*Teiidae*, *Varanidae*, *Pygododidae*, *Helodermatidae*, *Dibamidae*, *Leptotyphlopidae*). Thus, viviparity may be phylogenetically constrained in these oviparous lineages.

The large number of independent origins of viviparity indicates that viviparity has not been constrained in squamates as a whole, nevertheless, there is evidence that the transition from oviparity to viviparity is not "easy" even in those lineages where viviparity has evolved. Perhaps the most compelling evidence for this idea is that the stage of embryonic development at partition (oviposition or parturition) in lizards is bimodally distributed (Blackburn 1995). For the oviparous species, the developmental stage at oviposition ranges from about Stages 26-33 [staging according to Dufaure and Hubert (1961)] with a mode of Stage 30. In contrast, all viviparous species give birth to offspring that are at Stage 40 and are fully functional. Relatively few species exhibit prolonged egg retention, the retention of the eggs in the oviducts until embryos attain intermediate stages of development (~Stage 35). Thus, the stage at which partition occurs seems to be subject to adaptive constraints such that both reproductive strategies (oviposition at ~ Stage 30 or viviparity) are favored, but a transitional strategy (prolonged egg retention) is evolutionary unstable. The scarcity of species that retain eggs to intermediate stages of development and beyond further suggests that the oviductal environment may not meet the physiological needs of embryos after Stage 30.

The present work primarily concerns the identification of constraints on prolonged egg retention in lizards. This work was primarily directed towards identifying the presumed physiological problems that embryos of "typical" oviparous species might experience during the transition to prolonged egg retention and viviparity (Chapters 1 and 2). Thus, I focus on developmental constraints that could result from the change in incubation environment from that in a nest, to that within the maternal oviducts. My main objective was to investigate the effects of extended retention, *per se*, on development. A minor objective was to investigate possible ecological factors opposing prolonged egg retention (i.e., the "costs" of prolonged egg retention to females and effects of prolonged retention on hatchlings). The general experimental protocol used in Chapters 1-3 was to experimentally extend the duration of egg retention in oviparous species so that the effects of retention (or lack thereof) on embryos, offspring, and females are apparent. This protocol reasonably assumes that the effects of retention observed are the same as those that an oviparous species would experience given sufficient ecological pressure to retain eggs. Previously, investigations into the putative morphological and physiological modifications that occur during the transition to viviparity have been inferred from examinations of viviparous or oviparous species, or better, from a comparative approach using closely related species of both reproductive modes. While much has been gained from these approaches, both lack the power of the experimental approach as outlined above for identifying potential constraints on the evolution of viviparity.

One of the major developmental constraints on the evolution of prolonged egg retention and viviparity is the putative inhibition of embryogenesis *in utero* caused by the eggshell and its limiting effect on the exchange of respiratory gases (Blackburn 1995). That is, retention of eggs to intermediate stages of development may require reductions in the thickness of the eggshell (Shine 1985; Guillette 1993) as rates of gas exchange *in utero*

would otherwise be too low to support embryogenesis (Guillette 1982). Thus, there is a "conflict" between producing a shell that is thin enough to allow development while the egg is in the oviduct versus producing an eggshell that is thick enough to protect the egg until hatching after it is oviposited. In Chapter 3, I examine the idea that reductions in the eggshell are necessary for embryogenesis to progress to intermediate stages of development. The implications of this idea are of additional importance concerning the evolution of placentation because the general consensus is that simple placentation (i.e., a reduction of the shell) occurs *prior* to viviparity (Blackburn 1995), rather than *subsequent* to viviparity.

In Chapter 4, I examine a potential developmental constraint different from those considered in the previous chapters – that the preferred body temperature of the female is incompatible with that required by embryos for normal development (Shine 1985). Specifically, for one viviparous species, I experimentally evaluated a number of possible explanations as to why pregnant females exhibit lower body temperatures than non-pregnant females. The results of this work are consistent with the idea that some squamate lineages may be barred from evolving viviparity because maternal body temperatures are unfavorable to embryonic development.

Review of Oviparity and Viviparity in the Lizard Genus, Sceloporus

The genus *Sceloporus* is the largest genus of reptiles in North America with approximately 80 species currently recognized (Sites et al. 1992) and more recent phylogenetic analyses indicates that this number is conservative (Wiens and Reeder 1997). The genus is widespread, with representatives ranging from southern Canada to Panama, although they reach their highest diversity in central Mexico. They occur from sea level to

high elevations in most parts of their range, thus they occupy an extremely wide diversity of habitats.

A good phylogenetic framework exists for this genus (Wiens and Reeder 1997), and it is therefore possible to delineate those species groups in which viviparity has, and has not evolved. Despite the relatively large number of viviparous species in this genus (30 species), viviparity has apparently only evolved four times; once in a common ancestor of the *megalepiduras*, *torquatus*, and *grammicus* groups (viviparity fixed in all three groups), and twice in the *scalaris* group (Méndez-de la Cruz et al. 1998). Oviparity is the predominant mode of reproduction at the group level; oviparity is fixed in 17 of the 22 (77%) currently recognized species groups (Wiens and Reeder 1997). It is these groups that offer the most appropriate place to look for constraints on prolonged egg retention.

This work focused primarily on members of the *undulatus* species group. This oviparous lineage potentially contains more species than any other group in the genus (Wiens and Reeder 1997). Most of its members are endemic to the United States where they occupy a wide range of habitats. I also studied one member of the genus *Urosaurus* because this is the sister genus to *Sceloporus* (Reeder and Wiens 1996).

Chapter 1 Abstract

Most oviparous squamate reptiles oviposit when embryos reach Stage 30 of development. Determinants of embryonic stage at oviposition in the lizard, *Urosaurus ornatus* were investigated to gain insights into the causal base(s) of this phenomenon. Eggs that were experimentally retained *in utero* past the normal time of oviposition were compared with control eggs that were obtained at the normal time of oviposition and incubated in incubation medium. Retention resulted in developmental arrest of embryos at developmental Stages 30.0-30.5. Embryogenesis could be re-initiated at any time up to 29 days or longer past the normal time of oviposition by removing eggs from the oviducts and incubating them in incubation medium. Developmental arrest *per se*, did not affect the time it took eggs to hatch once eggs were removed from the oviducts, nor did it affect their hatching success. Snout-vent length, hydration of body tissue, and locomotor performance of hatchlings, were negatively related to the time spent in developmental arrest. Retention of eggs had no detectable effect on the body condition or locomotor performance of retaining females. Developmental arrest of embryos near Stage 30, if widespread among squamates, would account for the limited range of embryo stages at oviposition and may constitute a major constraint on the evolution of viviparity.

Chapter 1: Determinants of embryonic stage at oviposition in a squamate reptile (*Urosaurus ornatus*)

Introduction

One of the most interesting paradigms to emerge from studies of reptilian reproductive biology is that oviparous squamates typically oviposit when embryos are at developmental Stage 30 (Shine 1983a; DeMarco 1993; Blackburn 1995). Most species retain eggs within the oviducts until the embryos attain at least Stage 26, but very few retain eggs past Stage 33 [using the staging system of Dufaure and Hubert (1961), where Stage 40 is the stage at hatching]. Thus, a considerable amount of embryonic differentiation (65 to 80% of the total) occurs within the oviducts prior to oviposition. Because this period of pre-ovipositional development comprises only about one quarter of the total period of development (DeMarco 1993), the rate of differentiation in early stage embryos (pre-ovipositional) is relatively rapid compared to that of later stage (oviposited) embryos (Blackburn 1995, Fig. 1 and references therein). In contrast to the rate of differentiation, the absolute increase in embryo mass is greatest following oviposition (Xavier and Gavaud 1986; Shadrix et al. 1994).

The fact that most oviparous squamates deposit eggs with Stage 30 embryos indicates that there may be a constraint(s) on the stage at which oviposition occurs. Several processes associated with reproduction influence the stage at oviposition. First, the minimum stage at oviposition is presumably determined by the time required to shell the eggs. For example, in the lizard *Sceloporus woodi*, most of shelling occurs within 8 to 10 days of ovulation but deposition of the calcareous layer continues until the time of oviposition at Stage 27 (Palmer et al. 1993). Thus, the reason so few species oviposit eggs

with embryos at stages less than Stage 27 may be that the time needed to shell eggs is longer than the time needed to reach this stage (DeMarco 1993). Second, the stage at oviposition may be influenced by the shell if it presents a substantial barrier to respiratory exchange (Packard et al. 1977; Guillette 1982). Hypoxic conditions can retard (Ackerman 1981; Kam 1993) or arrest (Kennet et al. 1993) embryogenesis in reptilian eggs. Oviposition may therefore be timed to coincide with completion of the shell. Third, the stage at oviposition will depend, in part, on whether the timing of oviposition is obligate, or under facultative control. In cases where the timing is obligate and oviposition occurs soon after ovulation, embryos would be relatively undeveloped even though further embryonic development *in utero* might be physiologically possible. In contrast, the stage at oviposition might vary considerably in those taxa where the timing of oviposition is facultative. Lastly, the stage at oviposition may be influenced by space limitations within the female's body cavity or oviducts if such limitations adversely effect on embryonic development. Flexible shelled eggs of squamates typically undergo dramatic increases in water content during incubation (Packard et al. 1982b; Packard and Packard 1988; Shadrix et al. 1994) and at least some water uptake is necessary for successful hatching (Andrews and Sexton 1981; Packard and Packard 1988). In some lizards, such increases are either greatly limited (Mathies and Andrews 1996) or precluded (Andrews and Rose 1994; Andrews 1997) when eggs are retained in the oviducts past the normal time of oviposition.

Stage at oviposition may also be influenced in more subtle, indirect ways. First, incubation conditions are known to profoundly influence hatchling phenotype (Packard et al. 1981; Gutzke and Packard 1987; Whitehead and Seymour 1990; Shine and Harlow 1996; Mathies and Andrews 1997) and performance ability (Van Damme et al. 1992) in a wide variety of taxa. Thus, conditions experienced by embryos in the oviducts could influence the stage at oviposition if such conditions reduce hatching success or result in poor quality hatchling phenotypes. Second, gravidity per se, can impair sprint speeds of

females both during gravidity (Shine 1980; Bauwens and Thoen 1981) and for a period thereafter (Sinervo et al. 1991). If carrying the burden of the clutch imparts energetic costs and/or reduces female survival, then selection may favor oviposition of eggs at earlier developmental stages.

Recent studies in this laboratory have examined the effects of experimentally extended egg retention on embryonic development and hatchling phenotype as a means of identifying possible constraints on the evolution of viviparity (Andrews and Rose 1994; Mathies and Andrews 1996). These investigations focused on species known *a priori* to retain eggs past Stage 30 and have therefore sought to identify constraints acting "midway" between the transition from "typical" egg retention (i.e., oviposition of Stage 30 eggs) to viviparity. In the present study, I use the technique of experimentally extending the duration of egg retention to address the more basic question: what are the constraints that prevent a "typical" squamate from retaining eggs beyond embryo Stage 30?

The objective of this study was to identify possible determinants of embryonic stage at oviposition for a (presumably) "typical" oviparous lizard, *Urosaurus ornatus* (Baird and Girard). My specific aims were: (1) to determine the length of time females can maintain gravidity (i.e., is egg retention obligate or facultative?) and to evaluate the effects of extended egg retention on the physical condition and performance of females, (2) to determine the extent to which females can support development of embryos within the oviducts past the normal time of oviposition, and (3) to determine the effects of extended egg retention on incubation time, hatching success, hatchling phenotype, and hatchling performance.

Materials and Methods

Collection and initial maintenance of lizards

Urosaurus ornatus is a small (average adult SVL = 46 mm; Smith 1967) phrynosomatid lizard ranging from the border area common to Colorado, Utah, and Wyoming south throughout Arizona and western New Mexico and into Mexico to the Sea of Cortéz (Degenhardt et al. 1996). From New Mexico, it ranges east along both sides of the Rio Grande and into central Texas.

Gravid *U. ornatus* (n = 24) were collected by hand or noose near Animas, Hidalgo County, New Mexico on 1 July, 1996. Females that were collected had presumably ovulated recently because other similarly sized females encountered (but not included in this study) contained large follicles (as judged by external palpations and dissections). Females were weighed to 0.1 g and their snout-vent lengths (SVL) measured to the nearest 1 mm on the day they were collected. They were housed temporarily in terraria at the Southwestern Research Station, Portal, AZ. On 4 July, females were transported to our animal care facilities at Blacksburg, Virginia where they were housed until they were placed under experimental conditions. Females were fed crickets and provided with water daily (except on 4 July).

Experimental Design: oviposited eggs versus retained eggs

To assess the capacity of females to support oviductal embryonic development beyond the normal time of oviposition (NTO hereafter), I compared eggs that were retained *in utero* past the NTO (retaining treatment hereafter) with eggs that were obtained at the

NTO and incubated under normal conditions (control eggs hereafter). Gravid females were randomly assigned to treatments (n = 12, in each treatment). One female assigned to the retaining treatment was later found to contain infertile eggs, and data from this individual were therefore excluded from the analyses. Mean body mass did not differ between females in the control (3.59 g; SD = 0.64) and retaining (3.95 g; SD = 0.73) treatments (*t*-test: *t* = 1.45, *P* = 0.16). Likewise, mean SVL did not differ between females in the control (47.5 mm; SD = 2.36) and retaining (49.0 mm; SD = 2.91) treatments (*t*-test: *t* = 1.41, *P* = 0.17). Retention of eggs by females assigned to the retaining treatment was induced by keeping the substrates of their terrarias dry; such conditions have previously been shown to induce facultative egg retention in two other species of phrynosomatid lizard (Andrews and Rose 1994; Mathies and Andrews 1996).

The NTO for females in the population of *U. ornatus* I studied is not known. However, the NTO's of other species in this region coincide with the onset of the first heavy summer rains that usually begin in the first two weeks of July (Andrews and Rose 1994; Mathies and Andrews 1995). I therefore obtained clutches of eggs from the control females on 16 July by inducing oviposition with oxytocin. Control females (i.e., nongravid) were then placed under housing conditions identical to those described below for retaining females. One egg from each control clutch was dissected to determine the developmental stage of embryos in each clutch. I staged one egg per clutch since stage does not vary within clutches for a number of species of phrynosomatid lizards (DeMarco 1992) including *U. ornatus* (Mathies, unpub. data). Embryo stages were assigned following the criteria of Dufaure and Hubert (1961) with the modification that half stages were assigned if the embryos had characteristics intermediate between the Dufaure and Hubert stages. The mean embryonic stage of the control clutches was 29.5 (SD = 0.43) and ranged from Stage 29.0 to 30.0. Because females were randomly assigned to treatments, I assumed that embryos within retaining females were at similar stages at this time.

Maintenance of females

On the first day of the experiment (16 July), females assigned to the retaining treatment were transferred into individual terraria 12 X 27 X 15 cm so that they could be monitored closely. Each terrarium contained pieces of roofing tile that provided perches and refugia; cage substrates consisted of dry sand. Wire screen over the top of each terraria prevented escape and provided additional perch area. Photoperiod was determined by ambient light from the room windows. Terraria illumination was provided by two broad spectrum fluorescent light bulbs (Vita-lite™) suspended just above the top of each terraria. A heating cable situated beneath one end of each terraria was used to establish a temperature gradient in which females could behaviorally thermoregulate. Fluorescent lights were turned on and off at 0700 and 1800 hours EST, respectfully. The heating cable was turned on and off at 0800 and 1600 hours EST, respectively.

Females were fed a variety of live insects (crickets, mealworms, and wax moth larvae dusted with vitamin powder) once each day until satiated. To insure that the capacity to retain eggs was not influenced by an insufficient availability of water to females, water was provided temporarily once or twice each day by pooling small amounts on the flat surfaces of the roofing tile. Females readily drank water off these surfaces. I assumed that this allowed the female *U. ornatus* sufficient water given that rainfall is typically low and sporadic in the area where they occur and particularly during the time they are gravid.

Maintenance of control and retained eggs

On the day control eggs were obtained, they were placed individually into 72 ml glass jars containing a mixture of vermiculite and water (1.0 g dry vermiculite to 0.7 g distilled water, resulting in a water potential of approximately - 230 to - 200 kPa [Packard et al. 1987]), covered with plastic wrap secured with a rubber band, and placed on trays.

Control eggs experienced temperatures that fluctuated between 24 and 33°C ($X = 27.2^{\circ}\text{C}$) each day (Fig. 1.1). This temperature regime was chosen to approximate the temperatures experienced by eggs within retaining females. The temperature regime was produced by placing the trays of jars into a constant temperature chamber (Percival Model 1-30 BL) each morning at 0800 h. After placement into the chamber, control eggs equilibrated rapidly to a constant 33°C. Each day at 1600 h the trays of jars were removed from the constant temperature chamber and allowed to equilibrate with ambient room temperature. The temperature chamber was located in the same room as the terraria containing the retaining females. Temperatures within jars were periodically monitored using a temperature probe placed within a sealed jar (containing a vermiculite and water mixture only) situated near the center of a group of egg-containing jars on a tray. Probe temperatures were recorded every hour for 24 h and stored in a data logger. Jars and the temperature probe were rotated every three days among shelves to record temperatures at different positions within the chamber and to minimize position effects on embryonic development. Daily mean temperatures of control eggs did not vary over the course of the study except during the first ten days when daily means were approximately 2°C lower than at other times.

Temperatures experienced by eggs within retaining females were estimated for the periods each day when females were able, and unable, to thermoregulate. To estimate

temperatures during the period when thermoregulation was possible, body temperatures (cloacal) of each retaining female were measured between 1300-1600 h approximately every 7 days using a thermocouple thermometer. Each female was removed quickly from its terraria (taking care not to disturb the female in the adjacent terraria), its cloacal temperature recorded, and then returned to its terraria. Body temperature of retaining females averaged 35.2°C ($\text{SE} = 0.3^{\circ}\text{C}$) which is similar to body temperatures observed for this species in the field (Both sexes: mean = 35.6°C , $\text{SD} = 2.1^{\circ}\text{C}$; Pianka 1986).

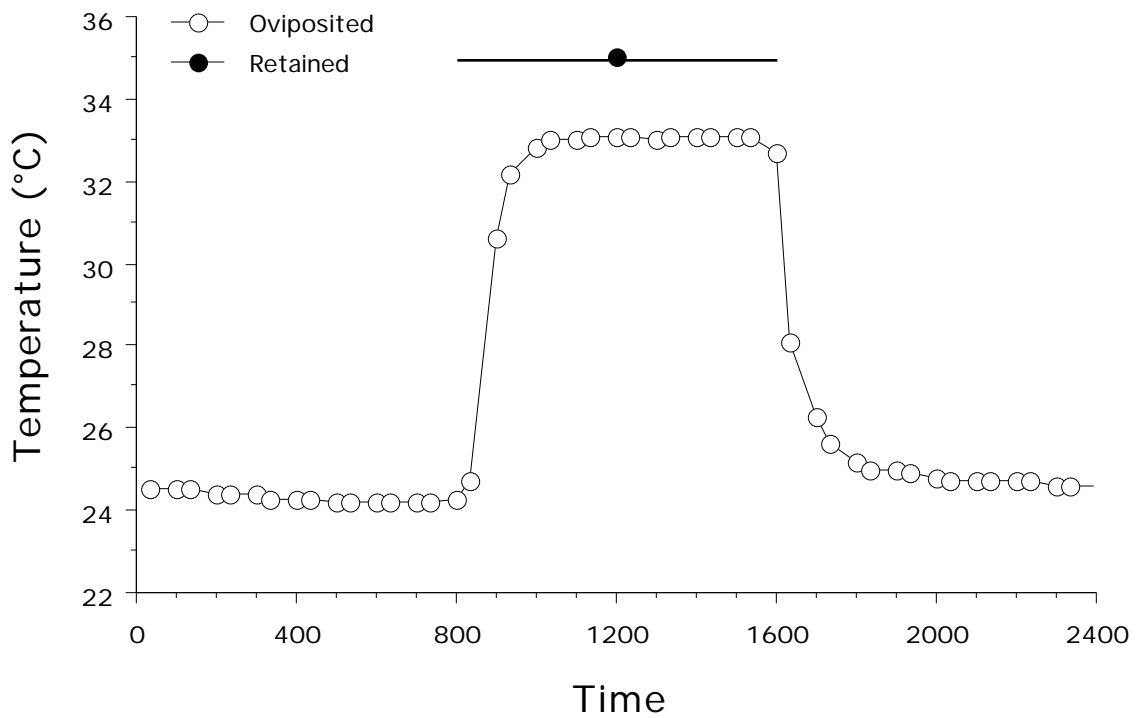


Figure 1.1 Incubation temperatures of eggs of *Urosaurus ornatus*. Open circles denote mean temperatures of control eggs incubated in incubation media. The horizontal bar and its length denotes the mean temperature of eggs incubated *in utero* and the length of the period at this temperature. Temperatures of retained eggs were similar to those of control eggs at all other times.

Thus, maximal temperatures experienced by retained eggs averaged about 3°C higher than those of control eggs during this period (Fig. 1.1). Because the temperature chamber was located in the same room as the terraria containing the retaining females, eggs in both treatments experienced similar temperatures during the period when females were not able to thermoregulate (approximately 1600 to 0800 h). During these hours retaining females usually rested on the terraria substrate and measurements of substrate surface temperatures were similar to those in the jars of control eggs. The *overall* mean incubation temperatures experienced by retained eggs was approximately 1°C higher than that of control eggs.

Sampling of control and retained eggs

Control eggs were sampled by randomly selecting one egg from one randomly selected clutch every 2 days starting on the seventh day past the NTO. Retained eggs were sampled on the same days as control eggs. Retained eggs were obtained after first weighing the female to the nearest 0.1 g, measuring SVL to the nearest 1 mm, and then determining the female's locomotor performance (below). Females were then killed by decapitation and the eggs immediately dissected out of the oviducts and weighed. At least one egg per clutch was dissected, the embryos staged as above, and the embryo and shell were dried at approximately 50°C for 24 h. Dry masses were determined to the nearest 0.01 mg. The mass of the shell fibril layer and the mass of the outer crystalline layer of the shell (in most squamates, the crystalline layer is composed of calcium carbonate in the form of calcite; Packard et al. 1982a; Packard and DeMarco 1991) were determined by decalcifying each dried shell in dilute (1N) HCL overnight, followed by a gentle rinse in distilled water (~ 2 h), after which the shell was re-dried and re-weighed. The mass of the crystalline layer was calculated as the difference between the initial dry shell mass and the dry shell mass after decalcification. It should be noted that the mass of "crystalline material" obtained using

these methods may also include imbedded or overlying organic constituents, when present. To facilitate comparisons among different sized eggs, I corrected for the amount of crystalline material on each shell by calculating the ratio of the mass of crystalline material to the mass of fibril material for each shell.

On the day each retained clutch was obtained, one to four eggs from that clutch were placed individually into jars and incubated (using the methods above) in order to evaluate the effect of retention on hatching success, hatchling phenotype, and hatchling performance. Eggs from some clutches of control eggs (1 egg each from 6 clutches) were also incubated to hatching.

Hatching success, hatchling phenotype, and hatchling performance

As the time of hatching approached, jars were checked for hatchlings twice each day, once in the morning and again in the late afternoon. Hatchlings were weighed to the nearest 0.1 mg and their SVL measured to the nearest 1 mm within a few hours of hatching, and again immediately following determination of locomotor performance (below). Hatchlings were held singly in terraria similar to those described above for retaining females until the day following hatching. Hatchlings were not given food or water.

The first hatchling from each clutch was used to determine locomotor performance. I quantified the locomotor performance of each hatchling on the afternoon following the day it hatched by chasing it down an electronically timed "racetrack". The racetrack was one meter long and five cm wide, and was made of wood with sand glued to its surface to increase traction. Five pairs of infra-red photocells positioned at 25 cm intervals along the

track were connected to an electronic stopwatch. Running speeds were measured in a walk-in controlled temperature chamber at a mean air temperature of 33.1°C (SD = 0.1°C). Hatchlings were given between 0.5 to 1.0 h to equilibrate to room temperature before the first run. After equilibrating, a hatchling was placed at the beginning of the track and then chased down the length of the track with a paintbrush. Each hatchling was run three times with only a brief pause between runs.

I recorded the time taken to reach the end of the racetrack and the number of times a hatchling stopped per run. For each hatchling at each run, I then calculated the fastest mean speed over one meter, and the fastest speed over any 25 cm segment of track. Endurance of hatchlings was evaluated by looking for trends in each measure of performance when plotted against the three sequential runs.

Hatchlings were re-weighed, killed (by freezing) immediately after quantifying their locomotor performance, and then dried to a constant mass as above for egg components. The hydration of each hatchling was calculated as the ratio of body water mass to dry body mass.

Body condition and performance of retaining females

Each control and retaining female was weighed to 0.1 g, SVL measured to the nearest 1 mm, and its locomotor performance quantified on the afternoon its clutch was sampled. Thus, a control and a retaining female were sampled every two days starting on the seventh day past the NTO. Nongravid mass of retaining females was calculated as the gravid mass of the female minus the total wet mass of the clutch. The physical condition of

control and retaining females was assessed by their body condition (i.e., SVL-adjusted nongravid body mass).

Locomotor performance for control and retaining females was quantified similarly to that of hatchlings (above) except that females were given between one to two h to equilibrate to room temperature before the first run and the number of stops a female made was not recorded. The manner in which females were run (i.e., no rest between runs) was not expected to tax their performance capabilities since females were relatively large compared to the length of the racetrack. Since analysis of the data supported this expectation (see Results), Run number was not included in any further analyses. Analyses of female locomotor performance were thus based on the fastest mean speed over *any* one meter and the fastest speed over *any* 25 cm segment of track.

Statistical analyses

All analyses were conducted using the statistical packages StatView® 4.5, Power PC Version Abacus Concepts Inc. or SuperANOVA, v1.11, Abacus Concepts Inc. Standard statistical methods (linear regression, backward step-wise regression, Students *t*-test (two-tailed), ANOVA, ANCOVA) were used to identify relationships between variables. ANCOVA was performed only when slopes were judged homogeneous (P 's > 0.05). Analyses for hatchlings and components of eggs are based on clutch means. Calculations of hatching success were based on individual eggs. Means are given \pm 1 SD unless indicated otherwise.

I took two different approaches to the analysis of hatchling phenotypes. When data for only the retained treatment were used, regressions of a phenotypic trait on time past the

NTO were generally not statistically significant. In contrast, when data for the control treatment were included with those for the retained treatment, most regressions were significant. Combining the data for both treatments in this manner is not unreasonable in the sense that the treatments are not qualitatively different; like the retained eggs, control eggs were also "retained" up to the NTO, and sampling of the retained eggs commenced shortly thereafter. Therefore, the first type of analysis included the data for both control and retained treatments. However, calculations for the slope of a regression are relatively sensitive to observations (particularly multiple observations) taken near the end point of the observed range of the independent variable (in this case, the control treatment). Therefore, in the second type of analysis, I simply compared the means of the control and retained treatments using *t*-tests.

Results

Capacity of females to retain eggs and support development

None of the females in the retaining treatment laid eggs over the course of the study and the last female in the retaining treatment was sampled 29 days after the NTO. Thus, female *U. ornatus* exhibit a substantial capacity to facultatively retain eggs. Because retaining females were killed to obtain eggs, I do not know whether they could have oviposited naturally. However, all retaining females seemed healthy, a contention that is further supported by the relationship between size-adjusted nongravid mass (i.e., body condition) and days past NTO (below).

Dry embryo mass of control eggs increased linearly over the sampling period (Fig. 1.2A; $F_{1,10} = 169.4$, $P < 0.0001$). In contrast, dry embryo mass of retained eggs was not

related to the number of days eggs were retained ($F_{1,10} = 1.04$, $P = 0.33$). Thus, an increase in embryo mass over time was not detectable within retained eggs.

Stage of embryonic development within control eggs increased linearly over the sampling period (Fig. 1.2B; $F_{1,10} = 255.7$, $P < 0.0001$) as did stage of embryonic development within retained eggs ($F_{1,10} = 7.45$, $P = 0.02$). However, the rate of embryonic differentiation was considerably slower in retained than control eggs (heterogeneity of slopes test: $F_{1,20} = 112.43$, $P = 0.0001$). The mean embryonic stage within retained eggs (30.2 ± 0.44) was similar to, but significantly higher, than that of control eggs (29.5 ± 0.43) sampled at NTO (t -test: $t = 3.75$, $P = 0.001$). Thus, retained embryos underwent some differentiation, but no additional growth *in utero*. More importantly, the extent of embryonic differentiation occurring in retaining females reached a maximum of Stage 30.5 and this maximum was reached within 17 days of the NTO; all but one of the embryos obtained from the remaining 6 females over the following 12 days was at Stage 30.5 (Fig. 1.2B). All retained embryos examined were nonetheless alive as indicated by regular muscular contractions of the heart. Some embryos (1 embryo each from 4 clutches) exhibited one or more deformities (i.e., disproportionately elongated trunk, protrusion of the mesencephalon, incomplete closure of the cranial region of the neural crest, and disruption of bilateral symmetry of the head). However, only one hatchling that resulted from these clutches was deformed (below).

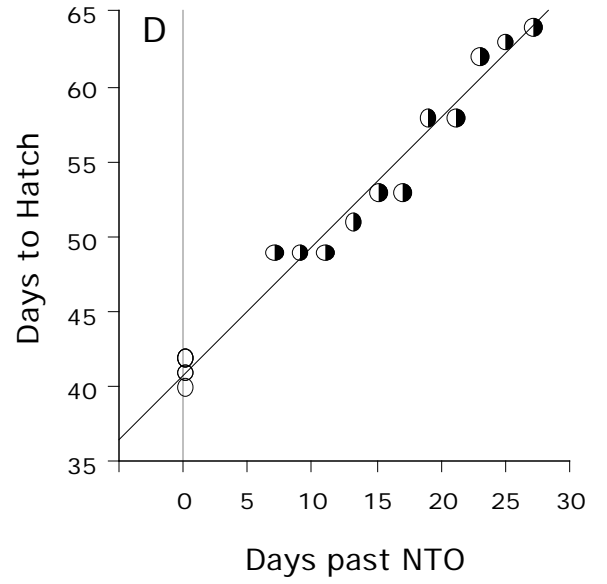
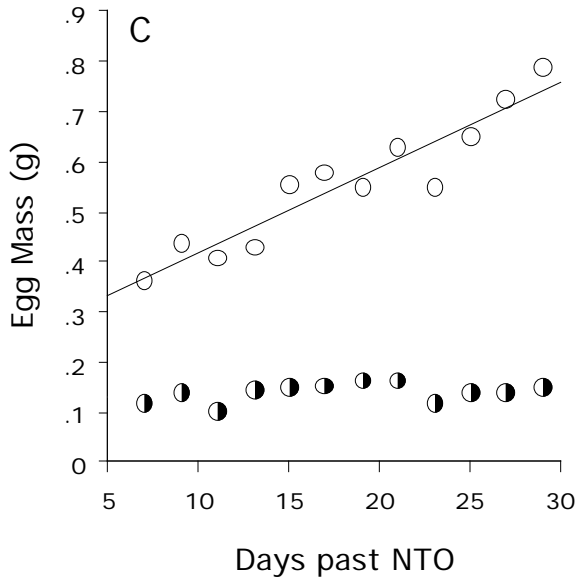
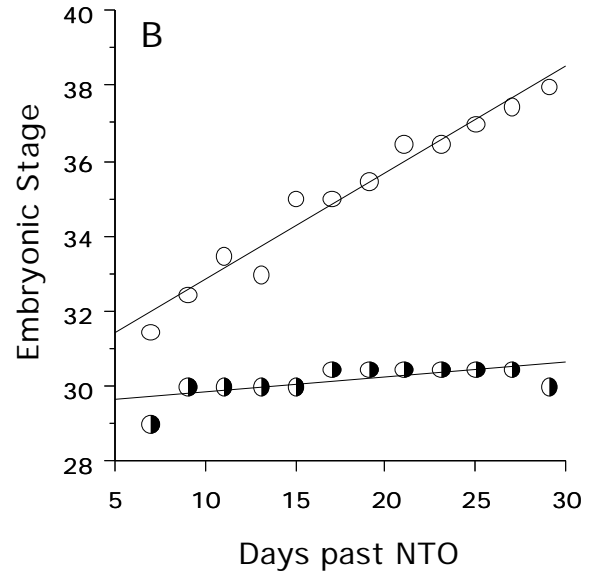
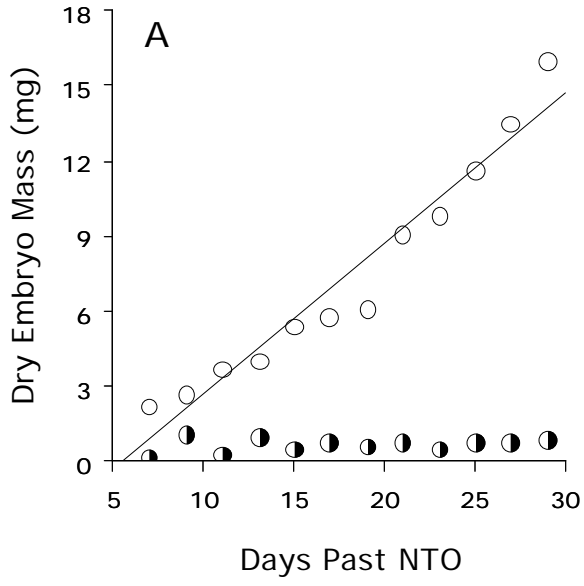


Figure 1.2 (A) Relationship between dry embryo mass and days past the normal time of oviposition (NTO) for control ($y = 0.6X - 3.29$, $R^2 = 0.94$) and retained ($y = 0.01X + 0.15$, $R^2 = 0.09$) eggs of *Urosaurus ornatus*. (B) Relationship between embryonic stage of development and days past the NTO for control ($y = 0.29X + 29.98$, $R^2 = 0.96$) and retained ($y = 0.04X + 29.44$, $R^2 = 0.43$) eggs. (C) Relationship between wet egg mass and days past the NTO for control ($y = 0.17X + 0.25$, $R^2 = 0.89$) and retained ($y = 0.001X + 0.12$, $R^2 = 0.11$) eggs. (D) Relationship between incubation time of retained eggs and days retained past the NTO ($y = 0.86X + 40.76$, $R^2 = 0.95$; data for control eggs not included in the regression calculation). In all panels, the *open circles* represent eggs that were obtained at the NTO (control eggs) and sampled later. The *half-filled circles* represent eggs that were retained past the NTO.

Mass of control eggs increased linearly over the course of the study (Fig. 1.2C; $F_{1,10} = 80.76$, $P < 0.0001$) whereas the mass of retained eggs did not vary over this period ($F_{1,10} = 1.23$, $P = 0.29$). Mass of the fibril layer of the shell did not vary over time for control ($F_{1,10} = 0.44$, $P = 0.52$) or retained ($F_{1,10} = 0.98$, $P = 0.34$) eggs. Moreover, no additional deposition of crystalline material occurred *in utero*; the ratio of crystalline layer mass to fibril layer mass of retained eggs did not vary over time ($F_{1,10} = 0.027$, $P = 0.87$). Shells of control eggs, however, lost crystalline material; the crystalline layer mass to fibril layer mass of control eggs was inversely related to time past NTO ($F_{1,10} = 10.60$, $P = 0.009$). Thus, the dampening effect of retention on embryonic development was not due to continued deposition of material to shells of retained eggs (i.e., additional material could reduce the gas exchange capabilities of the shell); shelling was presumably complete by the time this study was initiated.

Hatching success and incubation time

Hatching success was high for both control (n = 6: 100%) and retained eggs (n = 30: 86.7%). Control eggs hatched between 40-42 days (Mean = 41.33 ± 0.82) after the NTO. The six hatchlings representing the six control clutches seemed normal in terms of morphology and locomotor behavior, suggesting that the temperature regime control eggs experienced was appropriate for *U. ornatus*. Most hatchlings from retained eggs also seemed normal but a few (6 hatchlings from 5 different clutches) exhibited one or more of the following morphological abnormalities: bent tail, small body size relative to head, and/or one eye underdeveloped or absent. The only retained egg that failed to hatch was obtained from the last female to be sampled (i.e., 29 days after the NTO). Dissection of this egg revealed that the embryo had died at Stage 40 (i.e., the stage at hatching). Postovipositional incubation time for retained eggs was somewhat reduced relative to control eggs; incubation time scaled less than proportional ($b = 0.86$) to the number of days they were retained past the NTO (Fig. 1.2D; $F_{1,9} = 164.11$, $P < 0.0001$). However, the slope of this regression did not differ from 1 (t -test: $t = 2.10$, $P > 0.05$).

Hatchling phenotype and performance

Retention past the NTO affected the phenotype (morphology) and performance of hatchlings. Hatchling SVL was negatively related to the time eggs were retained past the NTO (Fig. 1.3B. $F_{1,15} = 15.94$, $P = 0.0012$). The mean SVL of hatchlings differed between the control (19.13 ± 0.58 mm) and retained (17.94 ± 0.94 mm) treatments (t -test: $t = 2.80$, $P = 0.014$). The live mass of hatchlings on the day of hatching (Day 1) was also negatively related to the time eggs were retained past the NTO (Fig. 1.3A. $F_{1,15} = 25.08$, $P = 0.0002$). The mean live mass of hatchlings differed between the control (242.12 ± 20.38

mg) and retained (181.27 ± 21.86 mg) treatments (*t*-test: $t = 5.61$, $P < 0.0001$). However, the dry mass of hatchlings was not related to the time eggs were retained (Fig. 1.3C. $F_{1,15} = 0.16$, $P = 0.70$) and mean dry mass of control hatchlings (33.50 ± 2.45 mg) and retained hatchlings (33.06 ± 4.10 mg) did not differ (*t*-test: $t = 0.24$, $P = 0.82$). Thus, the difference in live hatchling mass between treatments was presumably due to differences in body hydration. In accordance with this supposition, hydration of hatchlings on Day 1 was negatively related to the time eggs were retained (Fig. 1.3D; $F_{1,15} = 55.86$, $P < 0.0001$).

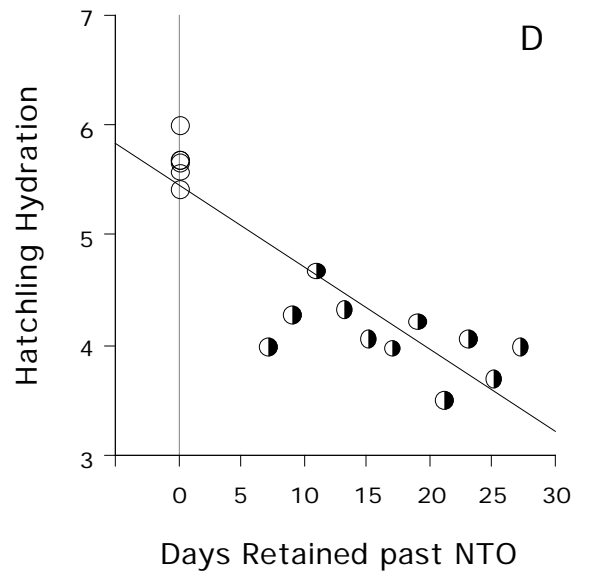
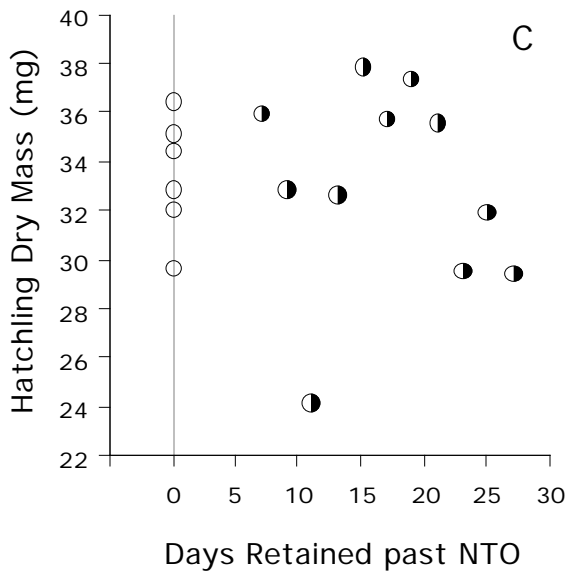
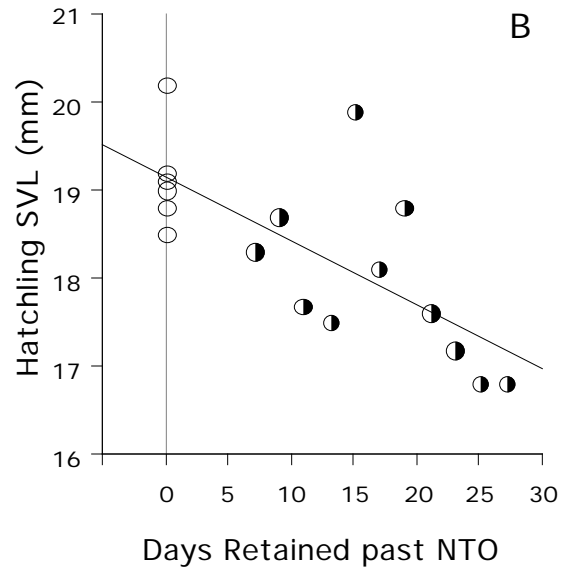
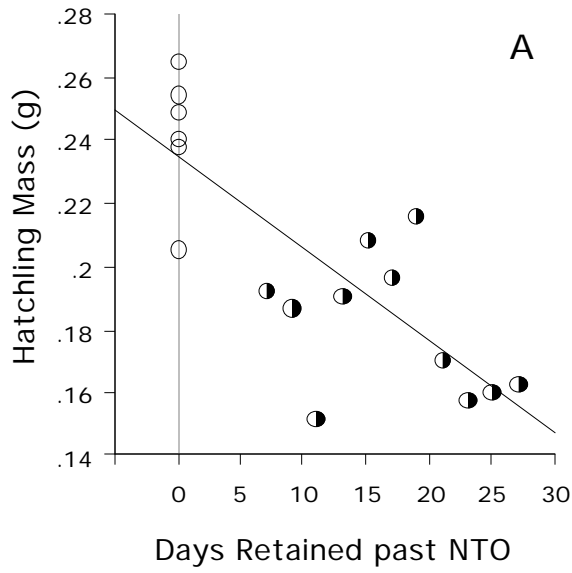


Figure 1.3 (A) Relationship between live hatchling mass (day 1) and days retained past the normal time of oviposition (NTO) for *Urosaurus ornatus* hatchlings ($y = -2.92X + 234.83$, $R^2 = 0.63$). (B) Relationship between SVL of hatchlings and days retained past the NTO ($y = -0.73X + 19.16$, $R^2 = 0.52$). (C) Relationship between dry mass of hatchlings and days retained past the NTO. (D) Relationship between body hydration of hatchlings on day of hatching and days retained past the NTO ($y = -0.075X + 5.47$, $R^2 = 0.79$). The hydration of each hatchling was calculated as the ratio of body water mass (mg) to dry body mass (mg). In all panels, the *open circles* represent hatchlings from eggs that were obtained at the NTO (control). The *half-filled circles* represent hatchlings from eggs that were retained past the NTO.

Did retention also influence *post*-hatching water loss (and thus hydration) of hatchlings? To investigate this possibility, I calculated daily water loss for each hatchling as the difference between its live mass on Day 2 and Day 1. I then size-adjusted daily water loss by calculating the residuals from the regression of daily water loss on hatchling dry mass. Size-adjusted rates of water loss were negatively related to the time eggs were retained past the NTO ($F_{1,15} = 6.36$, $R^2 = 0.30$, $P = 0.02$). Thus, longer retention times were associated with a *decreasing* propensity for post-hatching water loss. In summary, minimal retention times were associated with well hydrated hatchlings that tended to lose water relatively quickly whereas long retention times were associated with less hydrated hatchlings that tended to lose water relatively slowly.

Because live hatchling mass depended partly on body hydration, so might (though less conceivably) SVL. To investigate this possibility I performed a backwards step-wise regression using SVL as the dependent variable and hydration, hatchling dry mass, and days retained past NTO as the independent variables. SVL was negatively related to days past NTO and positively related to hatchling dry mass ($F_{2,14} = 15.59$, $P = 0.0003$). Days

retained past NTO explained most of the variance in SVL (52%), whereas hatchling dry mass explained only an additional 18%; more importantly, hydration did not enter the model.

Locomotor performance of hatchlings was first analyzed using *t*-tests to identify differences between treatments at each run. The alpha level (0.05) was Bonferroni corrected for the number of tests (3) in each analysis and differences were thus considered significant at $P < 0.017$. For means that differed at a particular run, I used the data for that run in a backwards step-wise regression to identify sources of variation in hatchling performance.

Treatment effects were detectable only at Run 3; neither measure of hatchling locomotor performance, or the number of times a hatchling stopped, was affected by retention at Runs 1 or 2 (*t*-tests, P 's > 0.20). At Run 3, hatchlings from the control treatment ran faster than those in the retained treatment in terms of their mean speed (Fig. 1.4A) and fastest segment speed (Fig. 1.4B). The mean speeds for control and retained hatchlings were $0.470 \pm 0.061 \text{ m s}^{-1}$ and $0.301 \pm 0.093 \text{ m s}^{-1}$, respectively (*t*-test: $t = 3.99$, $P = 0.0012$). The fastest segment speeds for control and retained hatchlings were $0.741 \pm 0.218 \text{ m s}^{-1}$ and $0.403 \pm 0.183 \text{ m s}^{-1}$, respectively (*t*-test: $t = 3.41$, $P = 0.0039$). Hatchlings from the control treatment also stopped less than those from the retained treatment (Fig. 1.4C). The mean number of stops per run for control and retained hatchlings was 3.00 ± 0.63 and 6.54 ± 2.54 , respectively (*t*-test: $t = 3.31$, $P = 0.0047$). Within treatments, neither mean sprint speed ($F_{2,45} = 1.25$, $P = 0.30$), maximum sprint speed ($F_{2,45} = 1.59$, $P = 0.22$), or the number of stops a hatchling made ($F_{2,45} = 0.05$, $P = 0.95$), differed among runs.

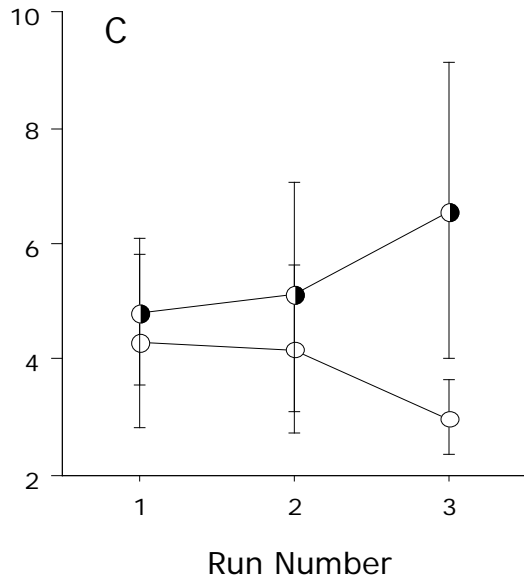
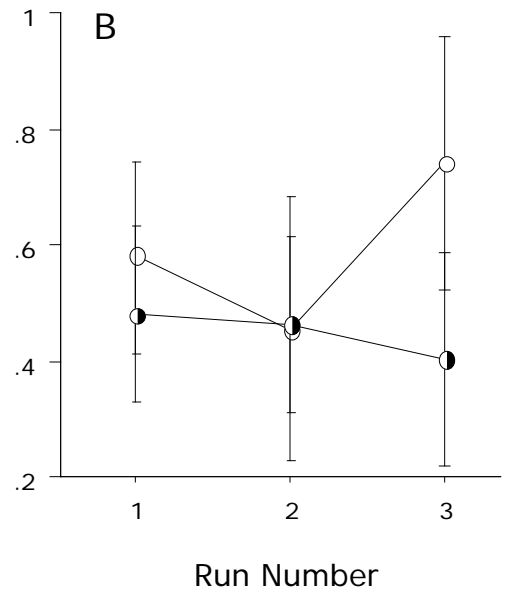
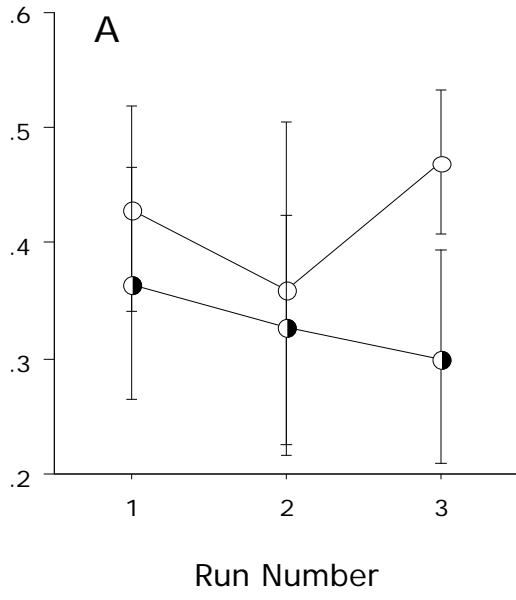


Figure 1.4 Influence of incubation treatment and number of runs on locomotor performance of *Urosaurus ornatus* hatchlings in terms of (A) the mean speed over a 1 meter interval, (B) the fastest speed over a 25 cm interval, and (C), the number of stops made over a 1 meter interval. Error bars indicate ± 1 SD. In all panels, the *open circles* represent hatchlings from eggs that were obtained at the normal time of oviposition (control). The *half-filled circles* represent hatchlings from eggs that were retained past the normal time of oviposition.

Backward step-wise regressions were performed using either mean speed or fastest segment speed as the dependent variable. Independent variables were SVL, hatchling hydration, days retained past NTO, and the number of stops per run. Mean speed was negatively related to the number of stops and positively related to SVL ($F_{2,14} = 46.09$, $P < 0.0001$). The number of stops explained 63% of the variance in mean speed and SVL explained an additional 24%; no other variables were retained in the model. Fastest segment speed was also negatively related to the number of stops and positively related to SVL ($F_{2,14} = 30.67$, $P < 0.0001$). In this case, however, the number of stops explained only 15% of the variance whereas SVL explained 66%. As in the previous analysis, no other variables were retained in the model.

Female body condition and performance

Time past the NTO did not affect the body condition of control or retaining females (ANCOVA: treatment as the factor, residuals from a regression of nongravid body mass on SVL as the dependent variable, and time past NTO as the covariate); the covariate was not significant (SVL: $F_{1,20} = 0.99$, $P = 0.33$). Furthermore, mean nongravid body mass of control and retaining females did not differ (ANCOVA: treatment as the factor, nongravid

body mass as the dependent variable, and SVL as the covariate; $F_{1,20} = 0.02$, $P = 0.998$). At the grand covariate mean SVL (47.4 mm), the adjusted mean nongravid body masses of control and retaining females were 3.31 ± 0.46 g, and 3.08 ± 0.44 g, respectively.

Mean sprint speed was higher for control females than retaining females (two-way ANOVA: treatment and run number as factors, and mean speed as the dependent variable; $F_{1,66} = 20.43$, $P < 0.0001$) but did not vary among runs ($F_{2,66} = 1.66$, $P = 0.20$). Likewise, the fastest segment speed was higher for control females than retaining females (two-way ANOVA: treatment and run number as factors, and fastest segment speed as the dependent variable; $F_{1,66} = 11.01$, $P = 0.0015$). In this case, fastest segment speed did vary (marginally) among runs ($F_{2,66} = 3.20$, $P = 0.047$). However, the fastest segment speeds for both control and retaining females were highest on Run 3. Therefore, the manner in which females were run did not negatively affect their locomotor performance on Run 3. To evaluate possible determinants of locomotor performance, the following analyses were based on the fastest mean speed for *any* run and the fastest speed for any 25 cm segment of track from *any* run.

Control females ran faster than retaining females in terms of fastest mean speed (*t*-test: $t = 3.47$, $P = 0.002$) and fastest segment speed (*t*-test: $t = 2.35$, $P = 0.028$). The fastest mean speeds for control and retaining females were 1.28 ± 0.30 m s⁻¹ and 0.86 ± 0.28 m s⁻¹, respectively. The fastest segment speeds for control and retaining females were 2.06 ± 0.99 m s⁻¹ and 1.30 ± 0.54 m s⁻¹, respectively. Most importantly, the time eggs were retained past the NTO did not affect the fastest mean speeds of control or retained females (ANCOVA: treatment as the factor, and time past NTO as the covariate); the covariate was not significant (time past NTO: $F_{1,20} = 0.55$, $P = 0.55$). Nor did it affect the fastest segment speeds of control or retained females (ANCOVA: treatment as the factor,

and time past NTO as the covariate); the covariate was not significant (time past NTO: $F_{1,20} = 3.39$, $P = 0.080$).

None of the other traits I measured that might be expected to influence locomotor performance (i.e., body condition, SVL, or the residuals from a regression of total wet clutch mass on nongravid body mass) explained significant amounts of the variance in either measure of performance in either retaining or control females (backwards step-wise regressions; P 's > 0.05).

Discussion

My data show that the developmental stage at oviposition for *U. ornatus* is approximately Stage 30, the same as that for most other squamate reptiles. The data also enable me to identify the probable determinants of stage at oviposition in this species. First, I consider the effects of egg retention on embryos, hatchlings, and females, and evaluate their relative contributions to determining the stage at oviposition. I then consider the importance of these effects in the context of the evolution of viviparity.

Determinants of stage at oviposition

Effects of retention on embryos. Egg retention had a number of unexpected effects on embryonic development. First and foremost, embryo stage at oviposition in *U. ornatus* is proximally determined by the arrested development of the embryo at Stage 30.0-30.5 (Fig. 1.2B). Developmental stage is not influenced by the extent to which females are able to control the timing of oviposition (although it would have been if embryos had continued to

develop *in utero*). At 29 days past the NTO (i.e., ~ 70% of the normal incubation time at 27°C), embryos within retaining females had advanced only one stage unit beyond that at the NTO and exhibited no detectable increase in mass. How developed *should* have embryos been by this time if retention had no inhibitory effect on development? Judging from the stage and embryo mass of control eggs at 29 days past the NTO, embryos should have attained at least Stage 38 and undergone a 436% increase in dry mass.

Developmental arrest occurs in many species of turtles but it occurs early in development when embryos are in the late gastrula stage (Ewert 1991). Evidence for diapause in squamates, however, is equivocal and apparently limited to some species of chamaeleonid lizards (Ewert 1991; and references therein) which also seem to have a diapause at the gastrula stage. Is developmental arrest of squamate embryos at more advanced stages uncommon among squamates? — maybe not. Developmental arrest at the stage at which oviposition occurs could be fairly widespread, just difficult to detect. In those species where egg retention has little or no facultative component, the time at which oviposition becomes physiologically inevitable might be timed to occur at, or shortly after embryos undergo developmental arrest. In such cases, there would be no outward indication of developmental arrest to an investigator because oviposited eggs would (necessarily) contain embryos at the "proper stage" for that species and incubated eggs might immediately resume development, and hatch in the expected number of days. Indeed, if not for the fact that *U. ornatus* exhibits substantial facultative egg retention, developmental arrest would have been much more difficult to detect. Thus, part of the reason developmental arrest at Stage 30 in squamates may have gone undetected is that, unlike turtles where developmental arrest can be quite lengthy (Ewert 1991), developmental arrest in most squamates might be relatively brief. If *U. ornatus* is a "typical" squamate with regards to the effect of retention on embryonic development, then the question, "why

do most squamates oviposit when their embryos reach Stage 30?" should perhaps be restated as: why does embryonic development slow or cease as embryos near Stage 30?

The second unexpected effect of retention on embryos was that upon removal from the oviducts, all retained eggs resumed development, and embryogenesis in most eggs proceeded normally thereafter (but see below: Effects on hatchlings). The length of time spent in developmental arrest did not affect total incubation time once embryogenesis was re-initiated (Fig. 1.2D), and hatching success of these eggs was high (86.7 %). This ability to restart development was still present even in eggs retained 29 days past the NTO.

The occurrence of developmental arrest *in utero*, followed by the resumption of development immediately following oviposition, suggests that some difference between the two incubation environments triggers the cessation and resumption of development. A number of possible mechanisms for maintaining or ending developmental arrest are unlikely or can be ruled out. First, it is unlikely that developmental arrest was maintained by the relatively high temperatures that embryos experienced during the period each day when retaining females were active; oviposited eggs of *U. ornatus* embryos develop rapidly and normally at even higher incubation temperatures (Andrews et al. submitted). Second, it is not oviposition *per se* that re-initiates development; eggs were surgically removed from retaining females. Third, the uterus of *U. ornatus* may secrete some inhibitory substance that maintains developmental arrest, as in some mammals (Mead 1993), but there is no evidence for such a complex control system in reptiles. Lastly, it has been suggested that continued normal development by embryos beyond Stage 30 requires substantial water uptake by the egg (Shadrix et al. 1994). The reasoning for this idea follows from the observation that flexible-shelled eggs begin taking up water immediately after oviposition (Packard et al. 1982b). In concordance with this reasoning, oviposited eggs of *U. ornatus* exhibited a pattern of water uptake similar to that of flexible-shelled eggs of other species

(see Fig. 1.2C: note the apparent large initial mass increase between the NTO and the seventh day past NTO). In contrast, eggs within retaining females did not take up additional water (Fig. 1.2C) suggesting that a lack of water could have led to developmental arrest. However, two other species of phrynosomatid lizards, continued embryogenesis *in utero* is *not* precluded (although possibly inhibited) by a failure of eggs to take up water (Andrews and Rose 1994; Andrews 1997).

One simple mechanism for initiating and maintaining developmental arrest in squamate embryos is that embryonic metabolism becomes limited by insufficient oxygen *in utero*. Such a mechanism has often been invoked (but never demonstrated) in discussions on the evolution of viviparity to explain why most squamates do not retain eggs past their NTO's (Packard et al. 1977; Guillette 1982). Data on eggshells of *U. ornatus* provide insight into two aspects of such a limitation. First, if shelling of retained eggs were to be continued past the NTO (i.e., if shells became thicker or denser than normal), then the rate of diffusion of oxygen across the such shells would presumably decrease with longer durations of retention. However, in *U. ornatus*, shelling is apparently complete by the NTO; the mass of the outermost shell layer (crystalline layer) of eggs retained past the NTO did not vary over time. Then perhaps a normal shell presents a substantial enough barrier to gas exchange to cause developmental arrest. Circumstantial evidence is consistent with this idea. Studies of *U. ornatus* eggshells using scanning electron microscopy have revealed that at embryo Stage 27, the large diameter fibrils are still being secreted around the shells but the smaller diameter fibrils and crystalline material have yet to be deposited (Mathies unpub. data). Even at this relatively advanced stage of embryonic development, such a shell would presumably be more conductive to gases than it would be after the denser, outermost layers are deposited. Nonetheless, it still seems unlikely that a low level of oxygen in utero in *U. ornatus* could completely halt development unless there is some minimum threshold level below which development stops.

Effects of retention on hatchlings. Retention had a number of potentially deleterious effects on hatchlings and the severity of these effects increased as a function of the time that eggs were retained beyond the NTO. Retention apparently interfered with morphogenesis of *U. ornatus* embryos such that longer retention times resulted in hatchlings that were smaller in terms of SVL and live mass (but not dry mass), less hydrated, and ran slower than control hatchlings. No factors other than those associated with the time spent in developmental arrest could have caused these effects since the incubation conditions that retained eggs experienced *after* they were obtained from females did not differ from those of control eggs (i.e., temperature, initial water content of eggs, water potential of incubation media, incubation time). In general, the time spent in developmental arrest was the best predictor of hatchling phenotype. For example, contrary to what might be expected, time retained past the NTO explained considerably more of the variance in SVL than did hatchling dry mass.

Because control and retained eggs experienced slightly different temperatures during incubation, I cannot determine whether the observed effects of retention on hatchlings are due to retention, temperature or both. However, it is important to recognize that the effects of extended retention on hatchlings are ecologically relevant because retaining females in the laboratory regulated body temperatures similar to those of free-ranging *U. ornatus*. How are the effects of retention on hatchlings relevant as possible determinants of stage at oviposition? Although developmental arrest is the proximate determinant of stage at oviposition in *U. ornatus*, the effects of retention past the NTO on hatchlings are also important because they might tend to counter selection for a more advanced stage at oviposition. That is, assuming the transition from developmental arrest to

some additional development *in utero* occurs gradually, hatchlings would still likely incur some of the deleterious phenotypic effects I observed.

Effects of retention on females. The physiological costs of gravidity to female *U. ornatus* were minimal as judged by the measures used here, and are thus unlikely to exert an influence on the stage at oviposition. This would also be true even if these costs were higher because the stage at oviposition becomes invariant near the NTO (i.e., ovipositing a little later would not alter the stage at oviposition). The apparent lack of negative effects of retention on females is, nonetheless, interesting regarding the presumed benefit of facultative egg retention to females (next section) because it suggests that there are no major additional physiological costs to females for retaining eggs past the NTO. Although retaining females ran slower than control females in terms of both measures of locomotor performance, retention past the NTO did not result in progressively slower speeds. Thus, there was no detectable cumulative effect of retention on female locomotor physiology and therefore no increasing impetus from gravidity to oviposit as soon as possible. That is not to say, however, that carrying the burden of the clutch for a longer period of time would not increase other costs to the female (i.e., increased chance of predation or difficulty in obtaining food). In addition, retention past the NTO had no detectable effect on the body condition of females. At any time during the study, retaining females were just as robust as they were at the outset of the study (i.e., body condition did not vary with time past the NTO) and also in comparison to the control females. Because females received plenty of food and water in captivity, these results may not apply to field-active females experiencing more stringent conditions. Nevertheless, they indicate that under "ideal" field conditions, the body condition of females would not be reduced during the period of egg retention.

Implications for the evolution of viviparity

The results of this study bear on several important issues regarding the evolution of viviparity in squamates. Most current models posit that viviparity evolves through selection for increasingly longer durations of egg retention *in utero* (Tinkle and Gibbons 1977; Packard et al. 1977; Shine 1985). When discussed in this context, the term "egg retention" actually comprises two different components: 1) the capacity to hold eggs within the oviducts, and 2) the capacity to support continued embryogenesis within the oviducts. It has generally been assumed that both components evolve concurrently (Weekes 1935; Packard et al. 1977; Guillette et al. 1980; Shine 1991). My observations for *U. ornatus*, however, indicate that the capacity to facultatively retain eggs for substantially longer periods of time can evolve independently of increases in the capacity to support intrauterine embryogenesis. Given that *U. ornatus* females can retain eggs for at least 29 days past the NTO, and incubation time for oviposited eggs is 42 days, females could theoretically support embryogenesis *in utero* for at least 70% of the postovipositional incubation time. Thus, it is clear from the dichotomous nature of "egg retention" exhibited by *U. ornatus*, that the selective forces behind evolution of facultative egg retention and those for supporting continued embryogenesis *in utero* can be quite different.

While it is not possible to know the historical conditions that favored the evolution of facultative egg retention in *U. ornatus*, such ability to retain eggs is adaptive in its seasonally dry environment. For example, in years when the rains that permit nesting are delayed, females could facultatively retain eggs, oviposit them after the first major rain, and then immediately initiate vitellogenesis and produce another clutch. Under the same conditions, a species without facultative egg retention (i.e., timing of oviposition is obligate) would experience at least one bout of reproductive failure because eggs would desiccate following oviposition. Thus, facultative egg retention allows female *U. ornatus* to

maximize their reproductive output (i.e., multiple clutches: up to six clutches annually [Tinkle and Dunham 1983]) in years when the onset of favorable nesting conditions are unpredictable or discontinuous during the nesting season.

The observation of substantial facultative egg retention in the absence of continued embryonic development permits new insights into how viviparity might evolve. Consider an oviparous ancestral species that facultatively retains eggs to the extent exhibited by *U. ornatus*. Given the appropriate circumstances, prolonged egg retention and viviparity might evolve relatively "easily" in such taxa because it is presumably easier to evolve one trait (e.g., the capacity to support embryogenesis *in utero*) rather than coordinate the concurrent evolution of two traits (e.g., facultative egg retention *and* the capacity to support embryogenesis). In taxa already possessing *some* capacity to support embryogenesis past the NTO, such an evolutionary transition would be easier still. In addition, unlike selection for an increased ability to support intrauterine embryogenesis which may tend to operate primarily in "cold climates" (Sergeev 1940; Tinkle and Gibbons 1977; Guillette et al. 1980; Shine 1983b), selection for facultative egg retention could operate in any environment where the onset of suitable nesting conditions is unpredictable. Data for *U. ornatus* suggest that it would not even be unreasonable if the duration of facultative retention evolved *exceeded* that of the "normal" incubation time. Thus, the "first" component of retention (the capacity to maintain gravidity), after evolving in one geographical area (for reasons unrelated to the evolution of viviparity), might then allow that species to move into to a previously unoccupied geographical area that favors the evolution of the "second" component of retention (the capacity to support intrauterine embryogenesis). The general applicability of such scenarios, however, awaits more information on the prevalence and extent of facultative retention among squamates.

Perhaps the most important ramification of the effects of retention on *U. ornatus* embryos pertains to the ease and frequency with which viviparity is likely to evolve. Depending upon how ubiquitous developmental arrest at Stage 30 is among squamates, developmental arrest *in utero* may constitute a major constraint on the evolution of viviparity, particularly in lineages without facultative retention. In such lineages, the presumed intermediate stages of egg retention would require not only the modifications needed to support continued normal embryogenesis, but also a *concurrent* increase in the capacity to retain those eggs. This process would be further complicated if the factor(s) that cause developmental arrest at Stage 30 differ qualitatively from those that retard intrauterine embryogenesis later on in development.

Chapter 2 Abstract

Viviparity has not evolved in many lineages of squamate reptiles although it is common in others. I suggest that the evolution of viviparity is often constrained because most species within these lineages are unable to facultatively prolong egg retention past the normal embryo stage at oviposition. To test this idea, I investigated the retention abilities of four species of *Sceloporus*, three in the *undulatus* species group, and one species in the *graciosus* species group. No individual of any species was able to retain eggs past embryo Stage 31, and the average stage each species was able to retain eggs to was essentially Stage 30, the modal stage at normal oviposition in squamates. Focal studies on two species within the *undulatus* group showed that females were only able to maintain gravidity for about ten days past the normal time of oviposition. Moreover, during this period, retention caused embryogenesis to slow drastically, or cease. Hence, a direct and substantial inhibitory effect of uterine retention on embryogenesis offers a plausible explanation why viviparity has not evolved in the *undulatus* species group, and possibly other squamate lineages as well.

Chapter 2: Constraints on the evolution of viviparity in a clade of sceloporine lizards

Introduction

Most oviparous squamate reptiles oviposit when the embryos reach developmental Stages 26-33 [Blackburn (1995), staging according to Dufaure and Hubert (1961)] and the remainder of development occurs in a nest. In contrast, viviparous species have evolved the capacity to retain eggs *in utero*, support embryogenesis throughout development, and give birth to fully formed young (i.e., Stage 40). Because of the high number of independent origins of viviparity in reptiles (Shine 1985), considerable research has been directed towards identifying the physiological (Packard et al. 1977; Guillette 1982; Guillette 1985; Shine and Guillette 1988) and morphological (Weekes 1935; Guillette and Jones 1985; Masson and Guillette 1987; Qualls 1996; Heulin 1990) modifications that accompany the transition from oviparity to viviparity, as well as the evolutionary mode and tempo by which this transition occurs (Packard et al. 1977; Shine 1985; Shine and Guillette 1988; Shine 1995; Blackburn 1995).

The most widely accepted view for the evolution of viviparity is that it evolves gradually (see references in Blackburn 1995; Qualls et al. 1997), and thus entails intermediate forms that retain the developing eggs in the oviducts for increasingly longer durations. Each increase in the duration of retention thus results in an increase in the proportion of embryogenesis that is completed *in utero*. Such "intermediate" oviparous forms are said to exhibit prolonged egg retention. Species of lizards known to exhibit prolonged retention include oviparous populations of *Lacerta vivipara* (Stages 31-34: Braña et al. 1991), montane populations of *Sceloporus scalaris* (Stages 36-37: Mathies and

Andrews 1995), oviparous populations of *Lerista bougainvillii* (Stages 36-39: Qualls et al. 1995), *Liolaemus scapularis* (Stage 36: Ramirez Pinilla 1994), and most notably, *Saiphos equalis* [hatching in 7-9 days (presumably Stage 40): Smith and Shine 1997]. However, the ability to prolong egg retention is probably variable among and within most species. For example, when conditions for nesting are not appropriate, female *S. scalaris* from a low elevation population are capable of *facultatively* prolonging egg retention considerably longer, and to later stages (Stage 39) than normally exhibited by montane females (Mathies and Andrews 1996). At least two oviparous squamates exhibit some degree of facultative egg retention in response to unsuitable nesting conditions (Stamps 1976; Cuellar 1984) as is presumably the case for most species.

The ability to facultatively retain eggs is thought to play an important role in facilitating the evolution of viviparity (Shine and Guillette 1988; Mathies and Andrews 1996). Under conditions favoring egg retention, a shift to a longer duration of retention could occur relatively *quickly* in populations where there is substantial, preexisting variance in this trait. Thus, the evolution of longer durations of retention and viviparity may be potentially efficacious (and thus more likely to occur) in populations where at least some (or particularly all) females exhibit a marked ability to facultatively retain eggs. Indeed, a low intra-population variance in the ability to facultatively retain eggs may provide a plausible explanation why viviparity has *not* evolved in some lineages.

This study concerns the evolution of viviparity in the lizard genus *Sceloporus*. This genus currently comprises twenty-two species groups, and oviparity is apparently fixed in seventeen of these groups (Wiens and Reeder 1997). Viviparity may have evolved only four times in this genus (Sites et al. 1992; Mink and Sites 1996; Méndez de la Cruz et al. 1998), once in the *formosus* group, once in the lineage including the *grammicus*, *torquatus*, and *megalepidurus* groups, and twice in the *scalaris* group. Only the *scalaris*

group contains both oviparous and viviparous members. Thus, the evolution of viviparity in most lineages this genus seems to have been constrained.

My general aim was to gain insight into why viviparity has (apparently) never evolved in these oviparous lineages. To do so, I focused on members of the *undulatus* and *graciosus* groups. Both groups are entirely oviparous and the *undulatus* group is particularly speciose. I hypothesized that viviparity may not have evolved in the *undulatus* group because few species within this lineage are able to facultatively prolong egg retention. To evaluate this prediction, I quantified the ability to facultatively prolong retention for three species within the *undulatus* species group, and one species within the *graciosus* group. A member of the *graciosus* group was included because the phylogenetic placement its group is basal to the *undulatus* group (Wiens and Reeder 1997).

Methods and Materials

Collection and maintenance of females

Gravid females were collected in spring just prior to the time of natural oviposition. Gravid *Sceloporus occidentalis* (n = 9) and *Sceloporus graciosus* (n = 6) were collected near Wrightwood in the San Gabriel Mountains of southern California 18-21 June 1996 (see Adolph 1987). Female *Sceloporus undulatus hyacinthinus* (n = 22) were collected near Blacksburg on the eastern slope of Brush Mountain in southwestern Virginia, between 28 and 31 May 1997. Female *Sceloporus undulatus consobrinus* (n = 19) were collected between Rodeo and Hachita in southwestern New Mexico, between 25 and 30 June 1997. A recent analysis suggests that these named subspecies of *S. undulatus* are different species

(Wiens and Reeder 1997) and they are considered such here. We conventionally refer to them as *Sceloporus undulatus hyacinthinus* and *Sceloporus undulatus consobrinus*. *Sceloporus graciosus* belongs to the *graciosus* species group; all other species herein belong to the *undulatus* species group.

All females were transported to our animal care facility at Virginia Polytechnic Institute within six days of collection. Female *S. occidentalis* and *S. graciosus* were housed in terraria (60 X 38 X 22 cm) in groups of six females. Female *S. u. hyacinthinus* and *S. u. consobrinus* were housed singly in terraria (12 X 27 X 15 cm). Each terraria contained pieces of roofing tile that provided perches and refugia. Cage substrates initially consisted of dry sand, but water was later added to the substrates of some terraria (see below). Photoperiod was determined by ambient light from the room windows. Illumination was provided by two broad spectrum fluorescent light bulbs (Vita-lite™) suspended just above the top of each terrarium. A 100-W incandescent flood lamp positioned over one end of each terrarium provided a temperature gradient in which females could behaviorally thermoregulate. Fluorescent lights were turned on and off at 0700 and 1700 hours EST, respectively. Incandescent lamps were turned on and off at 0800 and 1600 hours EST, respectively.

Females were fed a variety of live insects (crickets, mealworms, and wax moth larvae dusted with vitamin powder once each day until satiated). Water was provided once or twice each day by pooling small amounts on the flat surfaces of the roofing tile. Females readily drank from these surfaces.

Experimental Design

A comprehensive understanding of the ability to facultatively prolong egg retention requires knowledge of four features of a population's reproductive biology. One feature is the maximum developmental stage that embryos are able to attain within the oviducts. This parameter can be determined by experimentally inducing females to facultatively retain eggs past the normal time of oviposition (NTO, hereafter). Facultative retention can be induced by housing females on a dry terraria substrate. Under these conditions, females lose their ability to retain eggs after brief to long periods. Embryos are staged at this time. The second feature is the length of time that a female is able to facultatively retain eggs (i.e., maintain gravidity). To determine the length of this period requires knowing the NTO and the date each retaining female loses its ability to retain eggs. The third feature is the capacity of the female to support embryogenesis past the NTO. That is, the extent of development that occurs between the NTO and the day the female loses the ability to retain eggs. This can be assessed if the stage of embryonic development at the NTO is known and compared with the maximum developmental stage attainable *in utero*. The fourth feature is the rate at which eggs develop when they are retained past the NTO. The effect of retention on developmental rate (if any) can be determined by comparing developmental rates of eggs that are experimentally retained past the NTO with those of eggs that were oviposited at the NTO, and incubated under the same temperature regime as retained eggs. Thus, by determining the developmental rates of eggs that were oviposited and incubated under relatively normal conditions, one can determine how rapidly retained eggs *should* have been developing *if* retention had had no effect on embryogenesis.

I determined the maximum developmental stage that embryos are able to attain within the oviducts for all four the above species. The remaining features were obtained for two of the four species, *S. u. hyacinthinus* and *S. u. consobrinus*. The methods I used to

determine the maximum developmental stage attainable *in utero* were the same for all species herein, they are described below for *S. u. hyacinthinus* and *S. u. consobrinus*.

Gravid female *S. u. hyacinthinus* and *S. u. consobrinus* were assigned to one of two groups. Females in one group were housed on a dry terraria substrate (sand) as explained above. A dry housing substrate is an ecologically realistic means of inducing facultative egg retention and this method has worked well for at least two other species of sceloporine lizards (Andrews and Rose 1994; Mathies and Andrews 1996). Each female in this group eventually lost its ability to retain eggs (i.e., eggs were deposited in, or laid on top of terraria substrate). I refer to this day as the "day of retention failure". Females and eggs in this group are hereafter referred to as "retaining females" or "retained clutches". Females assigned to the other group were housed and maintained under conditions identical to those given above except that water was periodically added to the terrarias to keep the sand substrate moist. Such conditions presumably allowed females in this group to oviposit at the appropriate time. These females and their eggs will be referred to hereafter as "control females" and "control clutches". Eggs from control clutches were used to determine the NTO, the stage at oviposition, and the baseline developmental rates of eggs for each species (below).

Water was added to the substrates of the terraria that housed the control females at the appropriate time during the reproductive season. Suitable nest sites for *S. u. hyacinthinus* in southwestern Virginia are generally always available because precipitation is high, prior to, and throughout the nesting period. Therefore, I first added water to the terrarias containing *S. u. hyacinthinus* control females on the day each control female was placed in its terrarium. In contrast, suitable nest sites for *S. u. consobrinus* in southwestern New Mexico are probably not available until the onset of the summer monsoons which

generally arrive between late June and mid-July. Therefore, I first added water to the terraria containing the control females of *S. u. consobrinus* on 10 July.

Maintenance of control and retained clutches

Terraria of control and retaining females were checked at least three times daily for eggs. Eggs of control clutches were placed individually in 72 ml glass jars containing a mixture of vermiculite and water (1.0 g dry vermiculite to 0.7 g distilled water, resulting in a water potential of approximately - 230 to - 200 kPa [Packard et al. 1987]), and covered with plastic wrap secured with a rubber band.

Incubation temperatures of control eggs fluctuated between 22 and 33°C ($X = 26.2^\circ\text{C}$) each day (Fig. 2.1). This temperature regime was chosen to approximate the temperatures experienced by eggs within the retaining females of both species. The temperature regime was produced by placing the jars into a constant temperature chamber (Percival Model 1-30 BL) each morning at 0800 h. After placement into the chamber, control eggs equilibrated rapidly to approximately 33°C. Each day at 1600 h the jars were removed from the constant temperature chamber and allowed to equilibrate with ambient room temperature. The temperature chamber was located in the same room as the terraria that contained the retaining females. Temperatures within jars were recorded at two day intervals using a temperature probe placed within a sealed jar (containing a vermiculite and water mixture only) situated near the center of a group of egg-containing jars. Probe temperatures were recorded every hour for 24 h and stored in a data logger. Jars and the temperature probe were rotated every three days among shelves to record temperatures at different positions within the chamber and to minimize position effects on embryonic development.

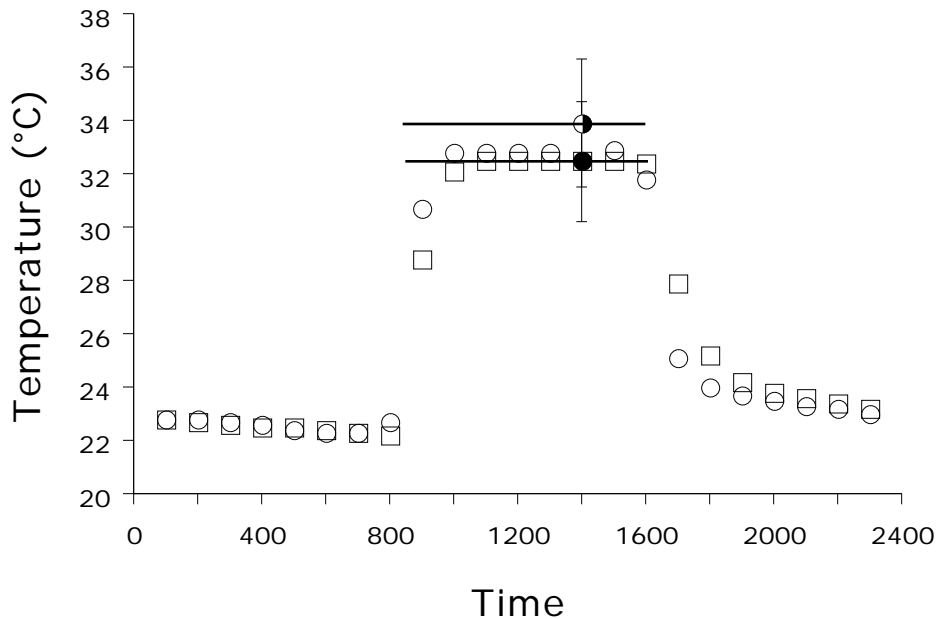


Figure 2.1 Mean incubation temperatures of control eggs (eggs incubated in incubation media) and retained eggs (eggs retained *in utero* past the normal time of oviposition). Mean incubation temperatures experienced by control eggs of *Sceloporus undulatus hyacinthinus* and *Sceloporus undulatus consobrinus* are denoted by squares and unfilled circles, respectively. Mean incubation temperatures (± 1 SD) experienced by retained eggs of *S. u. hyacinthinus* and *S. u. consobrinus* during the period each day when females were active (able to thermoregulate) are denoted by a filled square and a half-filled circle, respectively. The lengths of the horizontal bars denote the lengths of the daily activity periods. Temperatures of retained eggs were similar to those of control eggs at all other times.

Because development rates of reptile eggs are temperature dependent, a meaningful comparison of developmental rates of eggs in the control and retained groups requires that all eggs be incubated at similar temperatures. I therefore measured body temperatures (cloacal) of each retaining females with a thermocouple thermometer once, every 2 days, at 1400-1430 h. Body temperatures of female *S. u. hyacinthinus* and *S. u. consobrinus*

averaged 32.6°C (SD = 2.3°C) and 33.9°C (SD = 2.2°C), respectively, during the activity period. Thus, during the activity period, temperatures experienced by retained eggs of *S. u. hyacinthinus* were slightly lower than their controls, and temperatures experienced by retained eggs of *S. u. consobrinus* were slightly higher than their controls (Fig. 2.1).

Because the temperature chamber containing the control eggs was located in the same room as the terraria containing the retained clutches, eggs in both groups experienced similar temperatures at all other times (i.e., from approximately 1600 to 0800 h). During inactivity, females rested on the terraria substrates and substrate surface temperatures were similar to temperatures in the jars of control eggs.

Sampling of control and retained clutches

One egg from each control clutch was sampled on the day it was oviposited and additional eggs were sampled every two days thereafter. One egg from each retained clutch was sampled on the day the clutch was laid. Eggs were selected randomly and the embryo dissected free of its extraembryonic membranes. Embryos were assigned developmental stages following the criteria of Dufaure and Hubert (1961) with the modification that half stages were assigned if the embryos had characteristics intermediate between the Dufaure and Hubert stages. Embryos were dried at approximately 50°C for 24 h and then weighed to the nearest 0.01 mg.

Determining the length of retention and its affect on embryogenesis

I recorded the date that each of the retaining females lost its ability to retain eggs. The maximum length of time that each retaining female was able to facultatively retain its eggs was calculated by subtracting the mean NTO (for a female's population) from its day of retention failure. I then calculated the mean maximum length of facultative retention by averaging the individual values of maximum retention length for each species.

Female capacity to support embryogenesis beyond the NTO was determined by comparing embryos of retained clutches obtained on the day of retention failure with that of control clutches obtained at the NTO and incubated under standard incubation conditions.

Statistical comparisons of treatment effects using analyses such as the analysis of covariance, require that all groups within a factor have the same independent variable (i.e., days past the NTO in this study). For each control egg that was sampled, I knew exactly how many days had passed since it was oviposited. However, for each clutch of retained eggs, I had to estimate the number of days that had passed since the time it *should* have been oviposited (above). Therefore, to obtain the same independent variable for both groups, I also "estimated" number of days eggs were retained past the NTO for control eggs. This was accomplished by subtracting the mean NTO (for a female's species) from the day that each one of a control female's eggs were sampled. While this procedure introduces unrealistic variation into the analyses (e.g., the relationship between embryo mass and time for oviposited eggs is much tighter than is indicated in Figures 2.3 and 2.4), it permits comparison of the developmental rates of the control and retained eggs. This approach is conservative in that it reduces the likelihood of detecting differences between groups.

Statistical Analyses

All analyses were conducted using the statistical packages StatView® 4.5, Power PC Version Abacus Concepts Inc. or SuperANOVA, v1.11, Abacus Concepts Inc. Data were evaluated statistically using one-way ANOVA and Bonferroni/Dunn tests, unpaired *t*-tests (two-tailed), a two-tailed variance ratio test, linear regression, and one-factor ANCOVA. A significance criterion of $P < 0.05$ was used in all analyses other than the Bonferroni/Dunn tests ($P < 0.008$). Means are given ± 1 SD.

Results

Loss of the ability to facultatively retain eggs

All retaining females eventually laid eggs in, or on, the dry terraria substrates. Some females seemed to "sense" an impending loss of the ability to retain eggs. For example, female *S. occidentalis* spent considerable time each day "burrowing" through the terraria substrate in an apparent attempt to locate suitable areas to construct nests. Just prior to egg laying, female *S. u. consobrinus* were often observed scrambling over the inside of the terraria lids in an apparent attempt to get out. However, individuals of other species (particularly *S. u. hyancinthinus*) often times gave no such indications of impending egg laying. In any case, all females eventually either tried to construct a nest (not possible in the loose, dry sand), in which case eggs were sometimes partially buried in the sand, or they simply deposited their eggs on top of the sand. I considered both of these outcomes as evidence of a females' loss of the ability to retain eggs.

I observed the onset of laying in some of the retaining females. In each case, oviductal contractions would commence and continue until the entire clutch was laid. Sometimes a female would simply be sitting passively on a perch when contractions began. Each retaining female deposited its eggs on one day, generally over a period of about 30 minutes. These observations suggest that at some point after the NTO, some physiological change occurs that causes oviductal contractions and egg laying to begin regardless of whether or not a nest had been constructed.

Maximum embryonic stage attainable in utero

The maximum embryonic stage attainable *in utero* was approximately Stage 30 for all species (Fig. 2.2; Table 2.1). All eggs that were sampled contained live embryos. Differences among species in the mean maximum embryonic stage attainable *in utero*, though similar, were significant (one-way ANOVA: $F_{1,33} = 6.44$, $P = 0.001$). No female of any species I examined, however, was able to support embryogenesis beyond Stage 31. Thus, the capacity to support intrauterine embryogenesis among the members of the *undulatus* species group I examined is very limited, though inter-species differences in this trait were apparent.

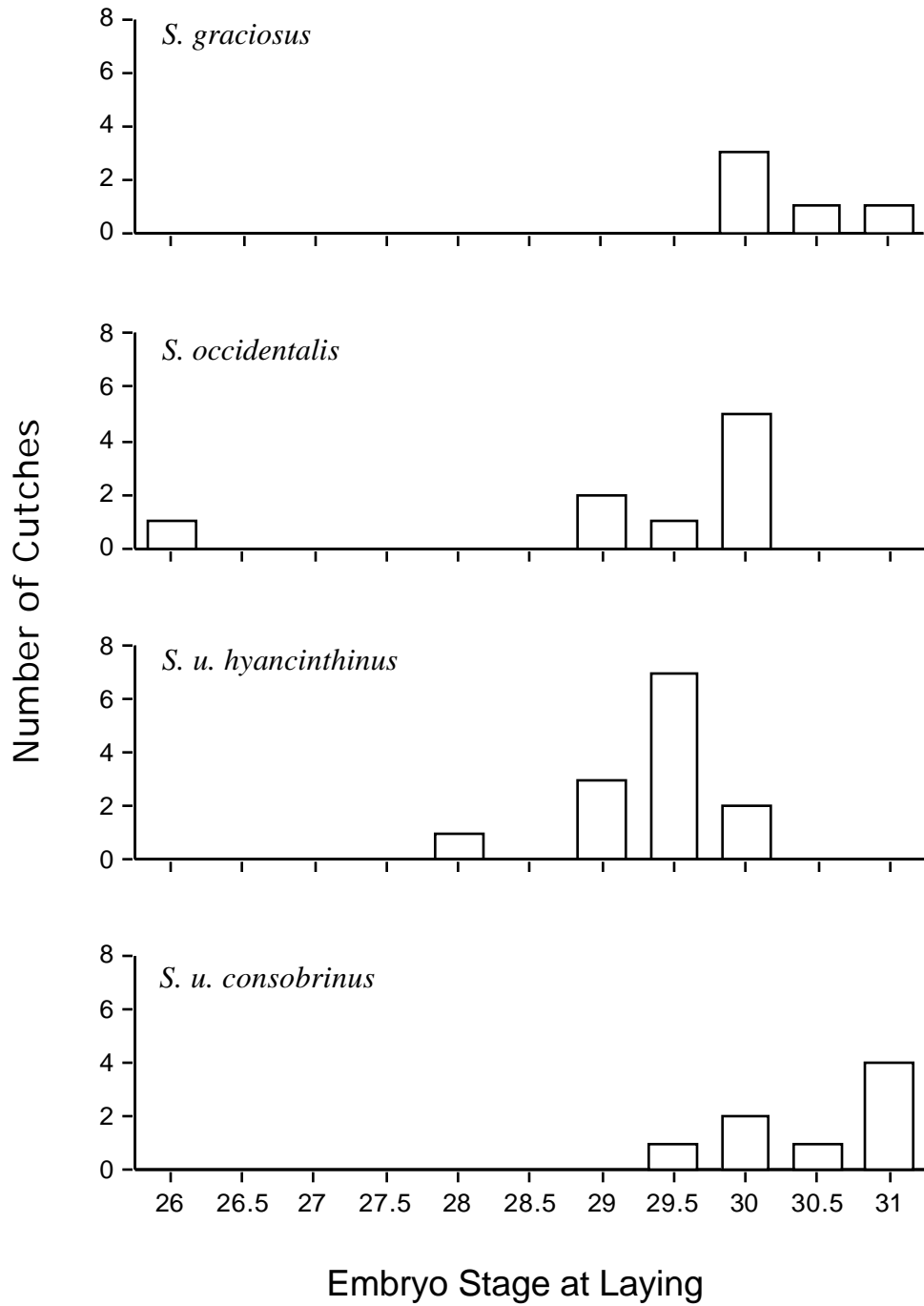


Figure 2.2 Frequency distribution of the maximal embryo stage attainable *in utero* for four species of sceloporine lizards.

Table 2.1: Maximum embryonic stage attainable *in utero* in four species of sceloporine lizards

Species	Maximum embryonic stage attainable <i>in utero</i>			
	n	Mean	SD	Range
<i>Sceloporus occidentalis</i>	9	29.3 A	± 1.3	26.0-30.0
<i>Sceloporus undulatus</i> <i>hyacinthinus</i>	13	29.3 A	± 0.5	28.0-31.0
<i>Sceloporus undulatus</i> <i>consobrinus</i>	9	30.5 B	± 0.5	29.5-31.0
<i>Sceloporus graciosus</i>	6	30.3 A B	± 0.2	30.0-31.0

Note. Means followed by different letters are significantly different at the 0.008 level (ANOVA, followed by Bonferroni/Dunn tests). P-values for comparisons of *S. graciosus* vs. *S. occidentalis* and *S. graciosus* vs. *S. hyacinthinus* were less than 0.017.

Because eggs of retaining females desiccated rapidly after laying, I cannot be certain whether all embryos within these eggs were alive at the time they were laid. However, all embryos that I examined from retained eggs that were not desiccated, were alive, as

evidenced by regular contractions of the heart. One clutch of retained eggs was incubated to hatching (the last retaining female *S. u. consobrinus* to lay eggs); all eggs from this clutch hatched and the hatchlings seemed normal.

Ability of females to facultatively retain eggs past the NTO

The ability of *S. u. hyacinthinus* and *S. u. consobrinus* to facultatively retain eggs past their NTO's was limited and did not differ between species. The mean maximum length of retention was $10.4 \text{ d} \pm 12.53$ for *S. u. hyacinthinus* ($n = 9$), and $9.54 \text{ d} \pm 7.10$ for *S. u. consobrinus* ($n = 13$)(unpaired *t*-test; $t = 0.22$, $P = 0.83$). Females of both species were thus able to retain their eggs an average of 10 days past the NTO.

Variance in the ability to retain eggs also did not differ between species (two-tailed variance ratio test: $F_{8,12} = 3.11$, $P = 0.10$). While most females laid eggs within approximately 10 d of the NTO, there were, however, a few individuals that retained their eggs for much longer periods (Figs. 2.3 and 2.4). For example, one *S. u. hyacinthinus* female retained its eggs for 27 d past the NTO and one *S. u. consobrinus* female retained its eggs for 39 d past the NTO. These observations indicate intra-population variability in the ability to facultatively retain eggs in both species.

Capacity of females to support embryogenesis past the NTO

The degree of embryonic development at the NTO was similar for *S. u. hyacinthinus* and *S. u. consobrinus*. The mean embryo stage at the NTO did not differ between species (*t*-test; $t = 1.45$, $P = 0.16$), nor did mean dry embryo mass (*t*-test; $t =$

0.40, $P = 0.89$). Females of both species were able to support intrauterine development of embryos past the NTO. For *S. u. hyancinthinus*, the mean maximum stage of retained eggs was greater than the mean stage of control eggs sampled at the NTO. The mean embryo stages of control and retained eggs were 28.6 ± 0.68 and 29.3 ± 0.56 , respectively (t -test: $t = 2.68$, $P = 0.014$). The mean dry mass of retained embryos, however, did not differ from that of the control eggs sampled at the NTO. The mean dry embryo masses of control and retained eggs were $0.447 \text{ g} \pm 0.160$ and $0.363 \text{ g} \pm 0.183$, respectively (t -test: $t = 1.14$, $P = 0.27$). For *S. u. consobrinus*, the mean maximum stage of retained eggs was also greater than the mean stage of control eggs sampled at the NTO. The mean embryo stages of control and retained eggs were 29.2 ± 1.06 and 30.5 ± 0.56 (t -test: $t = 3.34$, $P = 0.004$), respectively. However, unlike the parallel analysis for *S. u. hyancinthinus*, the mean dry mass of retained embryos was greater than that of the control eggs sampled at the NTO. The mean dry embryo masses of control and retained eggs were $0.379 \text{ g} \pm 0.273$ and $0.965 \text{ g} \pm 0.341$, respectively (t -test: $t = 4.02$, $P = 0.001$). Thus, in both species, some differentiation took place between the NTO and the day each female lost its ability to retain eggs, but only embryos of *S. u. consobrinus* exhibited growth (i.e., increase in mass) during this period.

How rapidly *should* have embryogenesis been progressing *in utero* if retention had no adverse effects on embryogenesis? To investigate this question, I compared the rates of embryonic development (slopes) of retained eggs and the control eggs that were incubated in incubation medium. In both species, developmental rates of retained eggs were strikingly slower than control eggs (Figs. 2.3 and 2.4). In *S. u. hyancinthinus*, development within retained eggs progressed slower than control eggs in terms of differentiation (ANCOVA: $F_{1,35} = 30.65$, $P < 0.0001$) and mass increase (ANCOVA: $F_{1,35} = 51.51$, $P < 0.0001$). Similarly, in *S. u. consobrinus*, development within retained eggs also progressed slower than control eggs in terms of differentiation (ANCOVA: $F_{1,39} = 19.65$, $P < 0.0001$) and

mass increase (ANCOVA: $F_{1,38} = 23.74$, $P < 0.0001$). Thus, retention past the NTO by these species has a pronounced inhibitory effect on further embryogenesis *in utero*.

How pronounced was this inhibitory effect on embryogenesis? Embryos that were retained *in utero* (both species) exhibited little, or no further development, after reaching the NTO. Embryonic stage was not related to the number of days eggs were retained past the NTO for *S. u. hyancinthinus* (Fig. 2.3A; linear regression, $F_{1,11} = 3.71$, $P = 0.08$; $R^2 = 0.25$) or *S. u. consobrinus* (Fig. 2.4A; linear regression, $F_{1,7} = 2.56$, $P = 0.15$; $R^2 = 0.27$). Dry embryo mass of retained eggs was also not related to the number of days past the NTO for *S. u. hyancinthinus* (Fig. 2.3B; linear regression, $F_{1,11} = 4.22$, $P = 0.064$; $R^2 = 0.28$). This result is not unexpected given the fact that there was no difference in mean embryo mass at the NTO and approximately 10 days past the NTO (above). This relationship was, however, significant for retained eggs of *S. u. consobrinus* (Fig. 2.4B; linear regression, $F_{1,7} = 12.18$, $P = 0.01$, $R^2 = 0.64$). Thus, embryogenesis was arrested by the NTO in *S. u. hyancinthinus*, but continued very slowly in *S. u. consobrinus*.

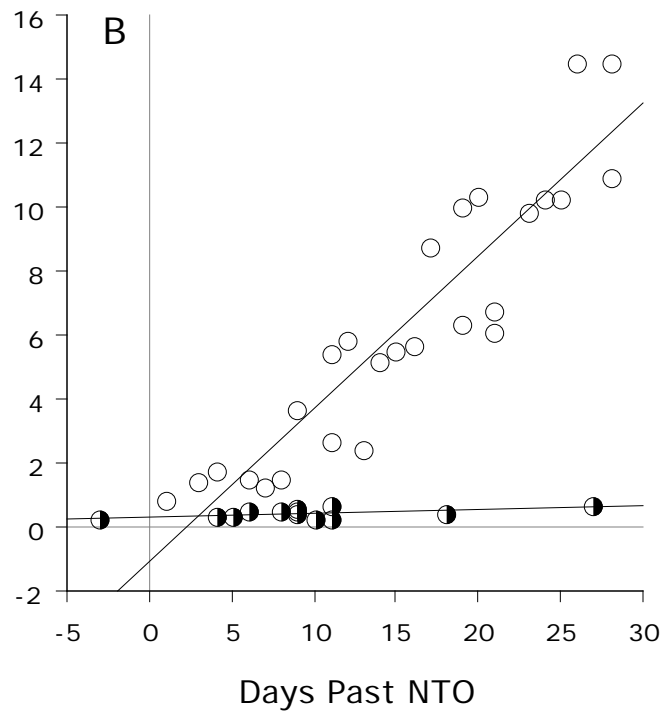
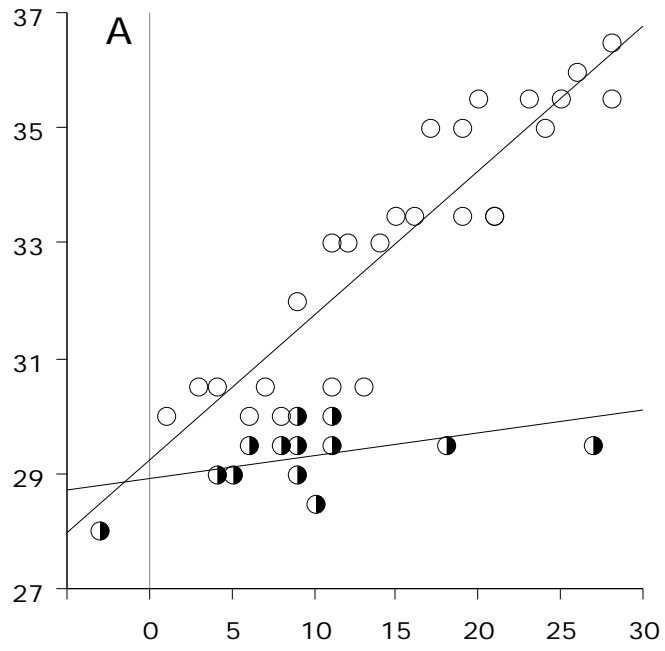


Figure 2.3 Embryo stage (**A**) and dry mass (**B**) of *Sceloporus undulatus hyacinthinus* embryos in control (*unfilled circles*) and retained (*half-filled circles*) groups as a function of days past the normal time of oviposition (NTO). The slopes of the regressions for embryo stage vs. days past the normal time of oviposition differed significantly between control (Stage = $29.2 + 0.25$ (days past NTO)) and retained eggs (Stage = $28.9 + 0.04$ (days past NTO)). The slopes of the regressions for dry embryo mass vs. days past the normal time of oviposition also differed significantly between control (mass = $-1.08 + 0.48$ (days past NTO)) and retained eggs (mass = $0.33 + 0.01$ (days past NTO)).

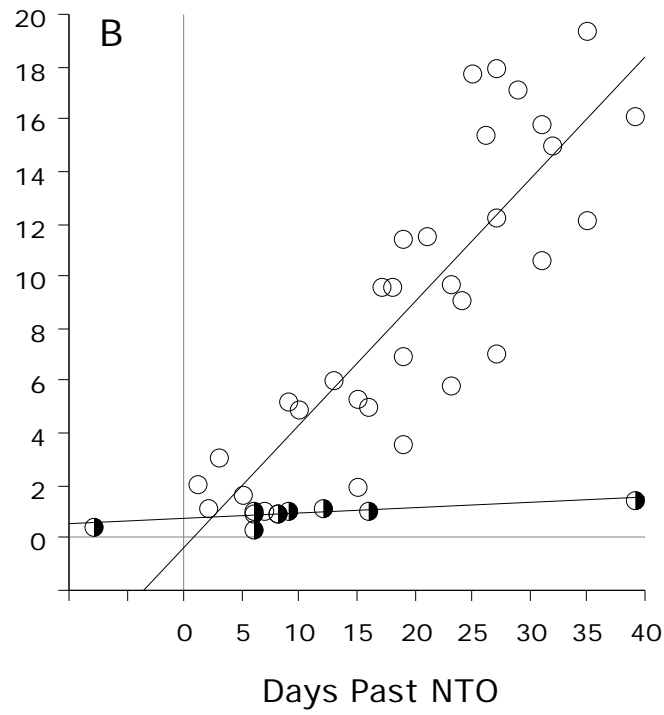
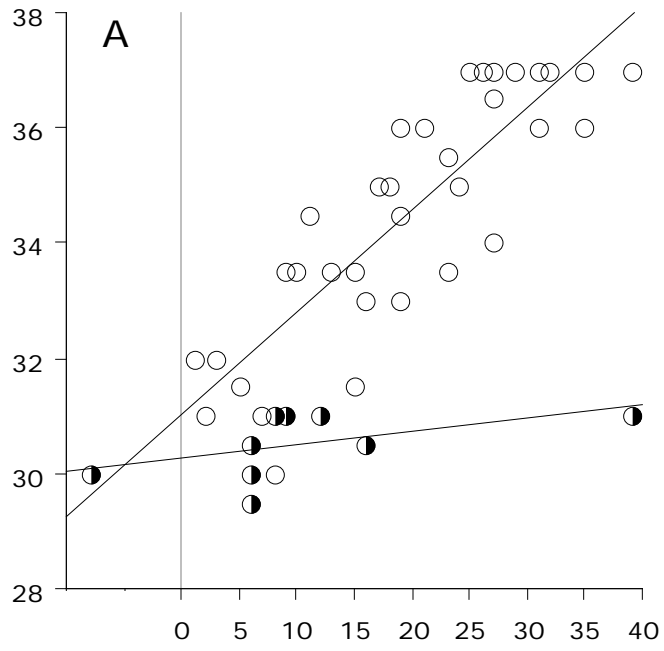


Figure 2.4 Embryo stage (**A**) and dry mass (**B**) of *Sceloporus undulatus consobrinus* embryos in control (*unfilled circles*) and retained (*half-filled circles*) groups as a function of days past the normal time of oviposition (NTO). The slopes of the regressions for embryo stage vs. days past the normal time of oviposition differed significantly between control (Stage = 31.0 + 0.18 (days past NTO)) and retained eggs (Stage = 30.3 + 0.02 (days past NTO)). The slopes of the regressions for dry embryo mass vs. days past the normal time of oviposition also differed significantly between control (mass = -0.36 + 0.47 (days past NTO)) and retained eggs (mass = 0.74 + 0.02 (days past NTO)) (see text).

Discussion

Frequency of facultatively prolonged retention within the undulatus group

Until this study, information on the retention capabilities of members of any squamate lineage was limited. At least one member of the undulatus group, *Sceloporus virgatus*, is capable of prolonging retention to at least Stage 36, although embryonic development is retarded (Andrews and Rose 1994; Andrews 1997). The only other member of this group for which there is information is *Sceloporus undulatus hyacinthinus* in Georgia and Missouri. This species is apparently unable to prolong retention; females that retained eggs past the normal time of oviposition produced eggs containing embryos at approximately Stage 30 (Crenshaw 1955; Sexton and Marion 1974). These findings are consistent with that reported here for *S. u. hyacinthinus* in Virginia and indicate that my findings on *S. u. hyacinthinus* are not just limited to the population I studied. The three species within the *undulatus* group that I examined exhibited no capacity to facultatively prolong egg retention; the mean maximum embryonic stage attainable in utero was approximately Stage 30 for all three species, and no individuals were able to support

embryogenesis past Stage 31. Thus, of the four species within the *undulatus* group whose retention capabilities have been investigated (*Sceloporus virgatus*, *S. u. hyacinthinus*, *S. u. hyacinthinus*, *S. occidentalis*) three exhibit no capacity to prolong egg retention. This comparison is somewhat biased because Andrews and Rose (1994) specifically chose to investigate *S. virgatus* because of its suspected ability to prolong egg retention. Regardless of this bias, the results presented herein support the hypothesis that viviparity has not evolved in the *undulatus* group because few members of this group are able to facultatively prolong egg retention. Lastly, *S. graciosus*, a member of the *graciosus* group, was also unable to support embryogenesis past Stage 31. This finding, along with those above, further suggests that the inability to facultatively prolong egg retention is the ancestral condition in the *undulatus* species group.

Are there members of the *undulatus* species group other than *S. virgatus* that are capable of prolonged retention? This question is difficult to assess partly because the taxonomy of this group is unclear (Wiens and Reeder 1997). There are six recognized species within this group, but Wiens and Reeder's analyses indicate that some named subspecies of *S. occidentalis* and *S. undulatus* may deserve species status.

My results for *S. u. hyacinthinus* and *S. u. consobrinus* provide insights into the reason why members of the *undulatus* group are unable to prolong egg retention. Specifically, these data permit me to discern whether embryogenesis did not progress past Stage 31 because females lost the ability to retain eggs before embryos had time to attain more advanced stages, or whether embryogenesis was inhibited when eggs are retained past the NTO, or both. Moreover, these data allow me to evaluate the relative contributions of these two influences on the ability to prolong retention (below).

Physiological limitations on the ability to prolong egg retention

My data for *S. u. hyacinthinus* and *S. u. consobrinus* shows that the ability to facultatively prolong retention has two components: the ability to maintain gravidity (i.e., facultatively retain eggs), and the capacity to support intrauterine embryogenesis. It is the combined influence of these two components that determines the maximal developmental stage attainable *in utero*. I first discuss both components of retention separately below, and then discuss their implications for the evolution of viviparity in the last section.

Ability to facultatively retain eggs. The ability to facultatively retain eggs was extremely limited in both species; on average, females were only able to retain eggs for ten days past the NTO. Thus, most females were physically unable to retain eggs long enough for embryos to reach developmental stages commensurate with those of species that exhibit prolonged retention. Such minimal retention times were not unexpected for *S. u. hyacinthinus* because it occurs in a region where rainfall is high throughout the nesting season, thus, appropriate nesting conditions are presumably generally available. Such environmental conditions would be unlikely to favor an ability to facultatively retain eggs for a long period of time. However, the observation that *S. u. consobrinus* females were not able to facultatively retain eggs longer than *S. u. hyacinthinus* females was unexpected. *Sceloporus u. consobrinus* occurs in a region where the timing of the rains that permit successful nesting are unpredictable. In addition, at least three other species that exhibit substantial abilities to facultatively retain eggs occur in this region (Andrews and Rose 1994; Mathies and Andrews 1996; Chapter 1,3). Furthermore, there is sufficient variation in this trait upon which selection could act. One of the nine retaining *S. u. consobrinus* females retained its eggs for 39 days past the NTO, or 29 days longer than most other *S. u. consobrinus* females. The observed general lack of ability to retain eggs

by *S. u. consobrinus* females may simply mean that the selective forces responsible for the substantial retention abilities observed in sympatric species, are not as relevant to *S. u. consobrinus*. A less likely alternative is that the hormonal mechanisms thought to control the length of retention (timing of oviposition) are not as responsive to selection as has been proposed (Guillette 1985; Shine and Guillette 1988).

Capacity to support embryonic development *in utero*. While most female *S. u. hyacinthinus* and *S. u. consobrinus* were only able to retain eggs for only ten days past the NTO, this short period does not mean that the amount of embryonic development that would have normally occurred over this period is insignificant. Judging from the regression equations for embryo stage *vs.* days past the NTO, embryos that had been retained ten days past the NTO should have advanced approximately three developmental stages more than was observed (i.e., from ~ Stage 29 to ~Stage 32). Similarly, the dry mass of embryos should have increased 4-8-fold over this same interval. Retained eggs of both species were still developing at the NTO (e.g., the mean maximum stage attainable *in utero* was greater than the mean stage at NTO for both species), although development had essentially become arrested by about ten days after the NTO. Thus, in *S. u. hyacinthinus* and *S. u. consobrinus* females, the maximum stage attainable *in utero* is determined primarily by a cessation of embryonic development shortly after the NTO. Further retention of eggs would result in little or no increase in embryonic development. I do not know whether development was similarly inhibited in retained eggs of *S. occidentalis* and *S. graciosus*, but the maximum stage attained *in utero* for these species was consistent with this possibility.

Implications for the evolution of viviparity

The modal embryonic stage at oviposition in squamates is Stage 30 (Shine 1983a; Blackburn 1995). This consistency in stage at oviposition in so many species occupying a wide diversity of habitats suggests that a single general factor is responsible. My results raise the possibility that many oviparous species are simply physiologically unable to support development *in utero* beyond Stage 30. The maximum embryo stage attainable *in utero* for all four species I examined was essentially Stage 30, and the data for *S. u. hyancinthinus* and *S. u. consobrinus* demonstrate that embryogenesis slows markedly, or ceases, soon thereafter. The proximate mechanism(s) for this phenomenon is unknown, although it is clear that the major constraint on further intrauterine development has a physiological basis. Thus, for *S. u. hyancinthinus* and *S. u. consobrinus*, at least, there is no need to invoke ultimate (evolutionary) explanations for an inability to prolong egg retention (e.g., "costs" of egg retention; Shine (1983a)).

The implication of my results for the evolution of reptilian viviparity is that the transition from oviparity to viviparity is probably not "easy", particularly in those lineages where both the ability to facultatively retain eggs and the capacity to support embryogenesis *in utero* are limited. For example, selection for prolonged retention in *S. u. consobrinus* would require modifications to help alleviate the inhibitory effects of retention on post-Stage 30 embryos, as well as modifications that would enable females to retain eggs for longer periods of time. Selection for one component of retention without the other would not lead (at least not directly) towards prolonged retention and viviparity.

The reproductive biology of *Sceloporus undulatus* has been well studied throughout the extensive range of this species (Gillis and Ballinger 1992, and references therein). Why then, have no workers observed inhibition of embryogenesis near the NTO? Is this

limitation peculiar to the two species I studied, or is it a general feature of many squamates? If it is widespread, why then has it not yet been observed in any other species? I propose that this inhibitory effect is widespread, and suggest that the reason it has not been observed is that it is not readily apparent and can be difficult to detect even when looked for. There are two reasons for this. First, it may not be apparent from the examination of freshly oviposited eggs whether development has been inhibited unless these embryos can be compared with those of eggs that were oviposited at the normal time, and incubated under the same temperature regime as the retained eggs. If retention does cause arrested development, then eggs that have been retained eggs past the NTO will necessarily contain embryos that are at the "proper" developmental stage regardless of when they are oviposited. Furthermore, they are likely to be alive, and they may hatch at the expected time. Second, if a species has a limited capacity to facultatively retain eggs (e.g., ~10 days for the two species in this study that were examined in detail), then the window of time in which one can detect this effect is relatively narrow.

The experimental protocols for evaluating the effects of retention on embryonic development are not complicated or overly laborious. More studies on the effects of retention on embryonic development for a wide diversity of taxa are clearly needed to assess whether the degree of inhibition of embryogenesis I observed is common in squamates and phylogenetically conserved in some lineages. If such an effect is ubiquitous, then taxonomically focused studies on species that *are* capable of facultatively prolonging retention and those that are not, should provide important advances in reconstructing the evolution of viviparity.

Chapter 3 Abstract

Viviparity and placentation have evolved many times within squamate reptiles, but the attendant morphological modifications and the sequence in which they occur remains unclear. In particular, it is unclear whether a "thinning" of the egg shell occurs concurrently with longer periods of egg retention, or whether such thinning occurs after viviparity has evolved. I therefore tested the hypothesis that shell morphology and permeability vary systematically with the capacity to support embryogenesis *in utero* (as judged by the maximum embryonic stage attainable *in utero*) in six species of oviparous phrynosomatid lizards. Despite major differences in the capacity to support uterine embryogenesis among these species, none of the features of eggshell morphology (general structure, thickness, density) or physiology (permeability to water vapor) varied as predicted. Hence, these results do not support the hypothesis that a thinning of the shell occurs concurrently with the evolution of viviparity. This suggests the interesting possibility that other features associated with simple placentation (e.g., increased oviductal and chorioallantoic vascular density) evolve concurrently with longer periods of egg retention and viviparity and that shell reduction occurs after the evolution of viviparity.

Chapter 3: Variation among oviparous sceloporine lizards in the capacity to support embryogenesis *in utero*: influence of morphology and gas permeability of the eggshell

Introduction

The evolution of reptilian viviparity and placentation has held the interest of biologists for over half a century (Weekes 1929; Fitch 1970; Packard et al. 1977; Blackburn 1982; Shine 1985; Stewart 1989; Guillette 1993; Qualls 1996), and the sequence of events culminating in these features has been an ongoing point of discussion (Weekes 1935; Panigel 1951; Tinkle and Gibbons 1977; Guillette 1982; Billett et al. 1985; Blackburn 1995; Qualls et al. 1997). The most widely accepted model for the transition from oviparity (egg laying) to viviparity (young fully formed at birth) in squamates posits that viviparity evolves through selection for increasingly longer periods of egg retention which results in an increase in the amount of embryonic development that takes place in the oviducts prior to oviposition. This model also posits that a "thinning" of the eggshell occurs concurrently with longer periods of retention (Guillette 1982; Shine 1985; Guillette 1991). Thinning of the egg shell would eventually culminate in what is termed a simple placenta (Weekes 1935); a close apposition of the chorioallantois of the embryo (and the shell membrane or remnant thereof) and the uterine tissues. Such an arrangement is thought to facilitate respiratory exchange between maternal and fetal tissues (review in Blackburn 1993).

Evidence to support the idea that thinning of the shell occurs concurrently with longer periods of retention, is limited. First, no known viviparous species posses a relatively complete eggshell, an occurrence that might be expected if thinning of the shell

occurs *subsequent* to evolving viviparity (Panigel 1951; Blackburn 1995). In every viviparous species examined to date, the shell is considerably reduced or absent (Stewart 1993; Blackburn 1993). Nonetheless, these observations do not negate the possibility that shell reduction occurs after viviparity is in place. Stronger supporting evidence for this idea is provided by the existence of oviparous species of lizards that retain their eggs to relatively advanced stages of embryonic development and produce shells that are thinner than those of conspecifics that retain eggs to less advanced stages of development (Mathies and Andrews 1996; Qualls et al. 1997; Smith and Shine 1997).

The idea that thinning of the shell occurs concurrently with longer periods of retention has gained widespread acceptance only relatively recently, although the initial formulations of this idea can be traced back at least as far as Weekes (1935). However, an alternate view, and one that was more prevalent in historical discussions on this topic, is that shell reduction and simple placentation occur subsequent to viviparity (Panigel 1951; Neill 1964; Tinkle and Gibbons 1977; Billett et al. 1985). The rationale behind this model is that a progressive thinning of the shell prior to viviparity would result in eggs that become more prone to dehydration (Weekes 1935; Packard 1966; Tinkle and Gibbons 1977). Thus, if longer periods of egg retention and thinning of the eggshell occur concurrently, then any selective benefit of shell reduction *in utero* would tend to be offset by selection against ovipositing such an egg in a nest, particularly in arid environments (Weekes 1935). Most oviparous squamates produce flexible-shelled eggs that lose water rapidly if oviposited in dry substrates (Andrews and Sexton 1981; Ackerman et al. 1984). Thus, unlike the rigid-shelled eggs of many crocodylians, some chelonians, and some lizards (Packard et al. 1982b), flexible shelled eggs, in general, are not suited for protection against desiccation. Nonetheless, the shells of flexible-shelled eggs may exhibit lower permeability to water vapor under conditions of low ambient humidity than high ambient humidity (Ackerman per. com.). This raises the possibility that under adversely dry

conditions, the structure of the shell may mechanically adjust in such a way that limits water loss from the egg (Feder et al. 1982). If so, then ovipositing thinly shelled eggs may not be as problematic as thought.

One way to evaluate whether thinning of the eggshell occurs concurrently with or subsequent to the evolution of viviparity, is to focus on the patterns of covariation in shell structure and permeability, and the capacity of females to support continued intrauterine embryogenesis among related oviparous species. For example, if shell reduction and longer periods of retention occur concurrently, a species that oviposits eggs with embryos at the modal stage of embryonic development in squamates (Shine 1983a; DeMarco 1993; Blackburn 1995), should produce a shell that is typical in gross morphology and permeability. In contrast, a species that oviposits eggs at relatively advanced stages of embryonic development should produce a shell that is relatively permeable to gases and/or exhibit morphological features associated with increased shell permeability (e.g., decreased shell thickness and/or density).

In this paper I describe shell structure and the permeability of the shell to water vapor for six species of phrynosomatid lizards (five species of *Sceloporus*, one species of *Urosaurus*). Differences among these species in their capacity to support embryogenesis *in utero* are substantial, ranging from embryo Stage 30, the modal stage at oviposition in lizards, to embryo Stage 40, the stage at hatching or parturition (Andrews and Rose 1994; Mathies and Andrews 1996; Andrews 1997; this paper). An important corollary to this information is that viviparity (and hence simple placentation) has had a minimum of four independent origins in sceloporine lizards (Sites et al. 1992; Mink and Sites 1996; Méndez de la Cruz et al. 1998). Thus, this group provides a taxonomically focused and relatively robust framework for evaluating whether the ability to support embryogenesis *in utero* is correlated with eggshell morphology and permeability.

The main objective of this study was to test the general hypothesis that shell morphology, shell permeability to water vapor, and shell density vary systematically with the capacity to support embryogenesis *in utero*. I also investigated the possibility that shells of flexible-shelled eggs are able to limit loss of water from the egg under conditions of low ambient water vapor pressure through a reduction in the shell's permeability to water vapor.

Material and Methods

Experimental Design

I examined eggs of five species of sceloporine lizards from three species groups. Members of the *undulatus* species group were *Sceloporus virgatus* Smith, *Sceloporus undulatus hyacinthinus* Green, and *S. undulatus consobrinus* Bosc and Daudin. A recent analysis suggests that these named subspecies of *S. undulatus* are actually different species and that *S. u. consobrinus* is more closely related to *S. virgatus* than it is to *S. u. hyacinthinus* (Wiens and Reeder 1997). I also examined one species in the *scalaris* species group (*Sceloporus scalaris* Wiegmann) and one species in the *clarkii* species group (*Sceloporus clarkii* Baird and Girard). Observations on *Urosaurus ornatus* Baird and Girard were also included because *Urosaurus* is the sister-genus of *Sceloporus* (Reeder and Wiens 1996).

Gravid females were collected in spring just prior to the time of natural oviposition. Female *S. u. hyacinthinus* were collected on the southern slope of Brush Mountain in Montgomery County, Virginia, between 28 and 31 May 1997. Female *S. virgatus* were

collected between 25 and 30 June 1997 in Cochise County, Arizona. Female *S. scalaris* were collected between 25 June and 2 July 1993 at the Appleton-Whittel Research Ranch Sanctuary, Santa Cruz County, Arizona. Female *S. u. consobrinus*, *S. clarkii*, and *U. ornatus* were collected between 25 and 30 June 1997 between Rodeo and Hachita, New Mexico. Females collected in Virginia were brought into our animal care facilities at Virginia Polytechnic Institute on the day they were collected. Females collected in Arizona and New Mexico in 1993 and 1997 were housed temporarily in terraria at the Southwestern Research Station in Portal, Arizona, and were transported to Virginia Polytechnic Institute on 4 July and 1 July of those years, respectively.

Eggs used to determine the normal embryonic stage at oviposition were obtained in two ways. Female *S. u. hyacinthinus* and *S. u. consobrinus* were placed into individual terraria and allowed to oviposit naturally. Eggs of *S. virgatus*, *S. scalaris*, and *U. ornatus* were obtained at the time that oviposition occurs in the field by inducing oviposition with an intraperitoneal injection of oxytocin. Eggs were weighed to 0.1 mg within a few hours of oviposition. All eggs were stored briefly at room temperature in closed plastic containers containing moistened vermiculite. On the day each clutch was obtained, one egg from each clutch was selected for determination of embryonic stage at oviposition. Eggs were cut open and the embryos were dissected free of their extraembryonic membranes. Embryos were assigned stages following the criteria of Dufaure and Hubert (1961) with the modification that half stages were assigned if the embryos had characteristics intermediate between two developmental stages.

The capacity of females to support embryogenesis *in utero* was determined as the maximum stage of embryonic development that is attained *in utero* for each species (Table 3.1). This data was obtained from a second set of females. The maximum stage attainable *in utero* was determined by inducing these females to facultatively retain eggs past the

normal time of oviposition. Egg retention was induced in all species by keeping terraria substrates dry. The maximum stage attainable *in utero* was determined either by periodically sacrificing a retaining female and sampling an egg from her clutch, or by sampling eggs obtained at the time a female lost the capacity to maintain gravidity (i.e., when eggs were "dumped" on, or oviposited in the dry terraria substrate).

Data on the maximum stage attainable *in utero* for *S. clarkii* are based on one female that was gravid when it was field collected on 28 June. Although this female was housed under conditions that facilitated oviposition in a sympatric species (*S. u. consobrinus*), this female did not oviposit. Oviposition was therefore induced with oxytocin on 25 July and embryo stage was determined.

Basis for formulating predictions

Predictions for shell attributes for each of the above species were generated by ordering the six species according to their capacity to support extended embryogenesis *in utero*:

S. clarkii, *S. u. hyacinthinus* ~ *U. ornatus*, *S. u. consobrinus*, *S. virgatus*,
S. scalaris.

where

the maximum stage attainable *in utero* increases from left to right
(data in Table 3.1).

Table 3.1. Embryonic stage at normal oviposition and maximum embryo stage attainable *in vitro* for six species of polytocous lizards.

Species	Stage at Oviposition			Maximum Stage Attainable <i>in Vitro</i>	
	n	Mean \pm SD	Reference	Maximum	Reference
<i>Sceloporus undulatus hyacinthinus</i>	9	28.6 0.68	Chapter 2	30.0	Chapter 2
<i>Sceloporus undulatus concolorinus</i>	9	29.2 1.06	Chapter 2	31.0	Chapter 2
<i>Sceloporus virgatus</i>	12	31.4 1.44	Mathies, unpub. data	37.0	Mathies, unpub. data
<i>Sceloporus scalaris</i>	5	32.8 1.04	Mathies and Andrews 1995	40.0	Mathies, unpub. data
<i>Sceloporus clarkii</i>	1	29.5 ...	This Chapter	29.5	This Chapter
<i>Urosaurus ornatus</i>	12	29.5 0.43	Chapter 1	30.5	Chapter 1

Note. Stage at Oviposition: n refers to the number of clutches from which embryos were obtained. Maximum Stage Attainable *in Vitro* : sample sizes were variable.

Measuring the Water Vapor Permeability of Shells

Water vapor permeability (K_{H_2O}) was measured only for eggs of *S. u. hyacinthinus*, *S. u. consobrinus*, *S. virgatus*, and *U. ornatus*. All eggs used for this part of the study were obtained at the normal time of oviposition and K_{H_2O} was measured on the day eggs were laid, usually within 10 h of oviposition. K_{H_2O} was measured under two sets of experimental conditions: "low" or "high" ambient relative humidity. A bed of Drierite in one desiccator maintained the relative humidity between 11 and 23% while measurements were made. This treatment will be referred to hereafter as the low water vapor pressure treatment (P_{low}). Data obtained under these conditions was used to investigate the possibility that shells of flexible shelled eggs may undergo a reduction in permeability in response to conditions of low external water vapor pressure. A saturated salt solution in a second desiccator maintained the relative humidity between 74 and 91% while measurements were made. This treatment will be referred hereafter as the high water vapor pressure treatment (P_{high}). Data obtained under these conditions was used to investigate the possible relationship between the capacity to support embryogenesis *in utero* and the permeability of the shell.

For *S. u. hyacinthinus* and *S. u. consobrinus*, two eggs were randomly chosen from each clutch and randomly assigned to a treatment. For *S. virgatus*, and *U. ornatus*, one randomly chosen egg from each of 6 randomly chosen females was assigned to the P_{low} treatment; one randomly chosen egg from each of 5 or 6 randomly chosen females was assigned to the P_{high} treatment.

The temperature experienced by eggs was controlled by placing both desiccators in a common water bath. The temperature of the shell surface, the relative humidity within the desiccators, and the barometric pressure outside the desiccators were measured directly.

Both desiccators were vented, thus the water vapor pressure inside the desiccators varied with ambient barometric pressure. Just prior to placing an egg into a desiccator, the egg was heated to approximately the same temperature in the desiccator by placing it in a small sealed jar containing moistened vermiculite, and then placing the jar in the water bath to equilibrate. The vapor pressure at or near the inner shell membrane was calculated as the saturation vapor pressure at the temperature of the shell's outer surface. The assumption that the temperature at inner shell membrane did not differ from that at the outer shell membrane is probably justified because shells of *Sceloporus* eggs are thin (~ 25 μm , see Results).

Temperature measurements in each desiccator were made with two 30 -ga. copper-constantan thermocouples connected to an Omega data logger that recorded temperatures every 5 min. The shell temperature measurement was made by placing thermocouple against the surface of the eggshell. Surface temperatures of eggs averaged 30.8 (SE = 0.1) $^{\circ}\text{C}$. The relative humidity inside the desiccators was measured using HOBOTM-RH (Onset Instruments Corp. Pocasset, MA) relative humidity loggers that recorded the relative humidity every 8 seconds. Eggs assigned to the P_{low} treatment were weighed (± 0.1 mg) on a Mettler AE 240 electronic balance and quickly placed over a hole in the ceramic plate situated just above the Drierite in the desiccator. Eggs assigned to the P_{high} treatment were handled similarly except that they were supported on a thin wire stand approximately 1 cm above the ceramic plate. Within species, the mean initial mass of the egg did not differ between treatments (two-factor ANOVA: species and treatment as factors, and initial mass of the egg as the dependent variable ($F_{1,51} = 2.21$, $P = 0.14$)). After placement into a desiccator, each egg or egg and stand unit was removed from its desiccator every 15 minutes, weighed, quickly returned to its desiccator, and the barometric pressure recorded. Eggs lost water rapidly under both sets of conditions during preliminary observations, and

therefore, each egg was weighed no more than four times. Thus, each egg spent approximately 45 min. in a desiccator.

The water vapor conductance (G_{H_2O}) for each shell was calculated as $G_{H_2O} = MH_2O / (P_I - P_A)$ from changes in the mass of the egg or egg and stand unit (Ar et al. 1974). Here, MH_2O is the loss in mass in mg per day from each egg or egg and stand unit, P_I the water vapor pressure (kPa) in the desiccator, and P_A the water vapor pressure (kPa) at or near the inner shell membrane which was determined using the temperature of the shell's outer surface. MH_2O was corrected to a standard barometric pressure of 101.3 kPa.

The surface area of eggs (cm^2) was estimated from the relationship $A = 4.835M^{0.662}$ derived from data for avian eggs where M is the mass (g) of the egg (Paganelli et al. 1974). This relationship provides a good approximation for surface area when compared with calculated values (Ackerman et al. 1985). The permeability (K_{H_2O}) of each egg was determined by dividing its G_{H_2O} by its surface area.

Measuring and Describing Microscopic Features of Shells

The terminology used to describe eggshells follows Packard and DeMarco (1991). The term "eggshell" refers to all layers of the shell. The inner boundary refers to a thin layer immediately exterior to the albumen of the egg. The shell membrane comprises a relatively thick layer of proteinaceous fibers of variable diameter overlying the inner boundary. A crystalline layer (in most squamates, the crystalline layer is composed of calcium carbonate in the form of calcite: Packard et al. 1982a, Packard and DeMarco 1991), if present, overlies the shell membrane and is often variable in morphology.

Shells were examined using scanning electron microscopy. Shells of *S. u. hyancinthinus*, *S. u. consobrinus*, *S. virgatus*, and *U. ornatus* were obtained from the clutches used to determine embryonic stage at oviposition. One shell was randomly selected from its clutch and fixed on the day it was oviposited.

Eggshells of all the species (except *S. scalaris*, see below) were fixed by placing the egg in 3% gluteraldehyde (~ 1 h) and then in 70% ethyl alcohol (~ 1 h). Immersion in ethyl alcohol, in addition to further fixing the shell, caused the shells to become translucent enough to determine the embryonic pole of the egg. Each eggshell was cut into two halves by making a cut down the long axis of the egg. The shell half at the embryonic pole was rinsed clean with distilled water and any remaining extraembryonic membranes were carefully dissected away. One strip of shell was cut from the equator region of this shell half (i.e., directly adjacent to the embryo). The shell strips were then air dried in specially constructed shell holders that prevented curling and excess shrinkage of the strips as they dried. Each shell holder consisted of a microscope slide with recessed slots formed by gluing strips from glass cover slips at intervals along the length of the slide. Shell strips were placed in these slots and a regular microscope slide was affixed to the top of the slotted slide without placing excessive pressure on the shell strips. Shell strips were air dried inside the holders. To obtain a radial view of the shell suitable for assessing shell structure, shell strips were removed from their shell holder, dipped briefly into liquid nitrogen, and then snapped across their short axes into two pieces. This method avoided compression and distortion of the shell that results from cutting or tearing the shell.

To dissolve the overlying crystalline layer of the shell, one piece of shell was placed back in the shell holder and the shell holder was immersed in a decalcifying agent [dilute HCL (1N)] overnight. The shell pieces were rinsed by transferring the shell holders from HCL into distilled water overnight. Shell pieces were dried again in the shell holders.

Shell pieces (untreated and decalcified) were mounted with their fractured edge up on aluminum stubs using silver conducting paint. Shell pieces were coated with gold (~12 angstroms thick) in a Anatech Ltd. Hummer X Sputter Coater, and examined with a Philips 505 scanning electron microscope at an accelerating voltage of 12-20 kV. The fractured edge of each shell piece and a representative view of the outer shell surface for each species or subspecies was photographed at 406-1620 X.

Data on *S. scalaris* shells and preparation methods used (similar to those given above) comes from Mathies (1994); additional micrographs of these shells are presented here.

In two of the six species examined (*S. u. consobrinus* and *S. virgatus*), the shell thickness of untreated shells varied considerably and regularly over the shell because the crystalline material on the surface of the shell was not distributed evenly. This arrangement was made obvious, in part, because the shell strips naturally tended to fracture along thin areas that transversed the shell. Therefore, both the minimum and maximum thicknesses of shells (untreated only) were measured for all species (except *S. scalaris*, and *S. clarkii*, maximum only).

Shell thickness of untreated and decalcified shells was measured to 0.1 μm by taking five evenly spaced, but otherwise randomly placed, measurements from the Polaroid prints of each specimen using dial calipers. Means of the five measurements were used to represent the thickness of each shell strip halve. Thickness of the shell membrane was the mean thickness of a decalcified shell strip halve. Values for the thickness of the crystalline layer are not reported because it was not possible to measure the thickness of the crystalline layer on shells of those species where the crystalline material was unevenly distributed.

The eggshells of all five species of *Sceloporus* examined exhibited a thin, continuous, layer of organic material covering the outside of the shell which I refer to as the cuticle. Eggshells of some avian and testudian species exhibit a similar layer of material (Tyler 1965; Ewert 1985; Thompson and Goldie 1990). The thickness of the cuticle was measured for all the species of *Sceloporus* except *S. scalaris*. The cuticle on the shell of this species is difficult to measure because it is so thin. No cuticle was observed on shells of *U. ornatus*.

Measuring the Masses of Constituents of Shells

Eggshells were obtained from the same eggs used to determine stage at oviposition (above) and only one shell per clutch was used. Masses of shell components were not obtained for *S. scalaris* and *S. clarkii*, thus it was not possible to compute shell densities (below) for these species. After removing the contents of an egg, the shell was rinsed clean with distilled H₂O, dried at approximately 50°C for 24 h, and weighed to the nearest 0.01 mg using an analytical balance. The mass of the shell membrane and the mass of the outer crystalline layer of the shell (including imbedded or overlying organic constituents, if any) were determined by decalcifying each dried shell in dilute (1N) HCL overnight, followed by a gentle rinse in distilled water (~ 2 h), after which the shell was re-dried and weighed. The mass of the crystalline layer was calculated as the difference between the initial dry shell mass and the dry shell mass after decalcification.

Calculating Densities of Shells

Two measures of the density of shells were obtained: the density of the untreated shell and the density of the shell membrane. To calculate the density of an untreated shell, I first estimated its volume by multiplying the surface area (cm^2) of the egg times the mean of the maximum and minimum thicknesses (cm) of the untreated shell. Density of the untreated shell was expressed as the ratio of the dry untreated shell mass (mg) to shell volume (cm^3). Density of the shell membrane was calculated similarly by first multiplying the surface area (cm^2) of an egg times the mean thickness (cm) of its *decalcified* shell to obtain an estimate of the shell membrane volume. Density of the shell membrane was then expressed as the ratio of the dry decalcified shell mass (mg) to shell membrane volume (cm^3). The amount of crystalline material for each shell was expressed in two ways; as the ratio of the mass of crystalline material to the mass of fibril material X 100, and in terms of its coverage of the shell in mg of crystalline material cm^{-2} .

Statistical Analyses

All analyses were conducted using the statistical packages StatView® 4.5, Power PC Version and SuperANOVA, v1.11, Abacus Concepts Inc. Data were evaluated statistically using paired *t*-tests, linear regression, ANCOVA, ANOVA, and Student-Newman-Keuls (SNK) range tests). Percentages were converted to their arcsine equivalents for analyses. Analyses for embryos and shells are based on clutch means. Means are given ± 1 SD unless indicated otherwise.

Results

KH₂O of Shells

The KH₂O of shells varied among species and treatments (Table 3.2: two-factor ANOVA: species and treatment as factors, and KH₂O as the dependent variable: $F_{7,51} = 18.75$, $P < 0.0001$). Shells of *U. ornatus* eggs exhibited the highest mean KH₂O, and the mean KH₂O of shells of *S. u. consobrinus* eggs was just slightly (but not significantly) lower. The mean KH₂O of shells of *S. virgatus* eggs in the P_{high} treatment was significantly lower than those of *S. u. consobrinus*, but did not differ significantly from those of *S. u. hyancinthinus*. Thus, the observations of KH₂O are not in the order predicted by the capacity to support embryogenesis *in utero* among species.

The KH₂O's of shells of all species were consistently lower in the P_{low} treatment than in the P_{high} treatment although treatment differences within species were significant only for *S. u. consobrinus* and *U. ornatus* (Table 3.2). Only shells of *S. u. consobrinus* exhibited a large difference in KH₂O between treatments. However, these differences were partly due to the fact that the KH₂O's of the shells of these species were comparatively high under conditions of high relative humidity, not because they exhibited particularly low KH₂O 's in response to low relative humidity. The KH₂O of shells of *S. u. hyancinthinus*, *S. u. consobrinus*, and *S. virgatus* in the P_{low} treatment did not differ.

Table 3.1. The KH_2O of eggs of phrynosomatid lizards at low (P_{low}) and high (P_{high}) ambient water vapor pressure

Species and Treatment	Number of eggs	Initial Egg Mass (g)	Egg Surface Area (cm^2)	KH_2O ($\text{mg d}^{-1} \text{kg}^{-1} \text{cm}^{-2}$)
<i>Sceloporus undulatus</i> <i>hyacinthinus</i>				
P_{low}	9	0.442 ± 0.034	2.82 ± 0.14	167.66 ± 16.38 A, a
P_{high}	9	0.448 ± 0.027	2.84 ± 0.12	179.13 ± 18.56 A, 1
<i>Sceloporus undulatus</i> <i>concolorinus</i>				
P_{low}	9	0.364 ± 0.036	2.47 ± 0.16	161.18 ± 11.50 A, b
P_{high}	9	0.368 ± 0.037	2.49 ± 0.26	242.51 ± 30.73 B, 2
<i>Sceloporus</i> <i>virgatus</i>				
P_{low}	6	0.355 ± 0.061	2.43 ± 0.27	193.79 ± 20.97 A, a
P_{high}	6	0.398 ± 0.061	2.62 ± 0.26	201.96 ± 12.02 A, 1
<i>Urosaurus</i> <i>oreanus</i>				
P_{low}	5	0.166 ± 0.015	1.47 ± 0.09	236.18 ± 34.16 A, c
P_{high}	6	0.183 ± 0.027	1.56 ± 0.15	233.89 ± 29.74 B, 2

Note. P_{low} and P_{high} refer to the experimental conditions under which water vapor conductance was measured. P_{low} denotes a relative humidity of 11-23%, P_{high} denotes a relative humidity of 74-91%. Initial egg mass refers to the mass of the live egg just prior to time its water vapor conductance was measured. Within species, the initial mass of eggs did not differ between treatments (see Methods). Uppercase letters indicate comparisons between treatments for a species. Lowercase letters indicate comparisons among species in the P_{low} treatment. Numbers indicate comparisons among species in the P_{high} treatment. All values are means ± 1 SD. Within a column, values that are followed by different uppercase letters (within species comparisons only), lower case letters, or numbers, are significantly different at the 0.05 or higher level (ANOVA followed by SNK analyses).

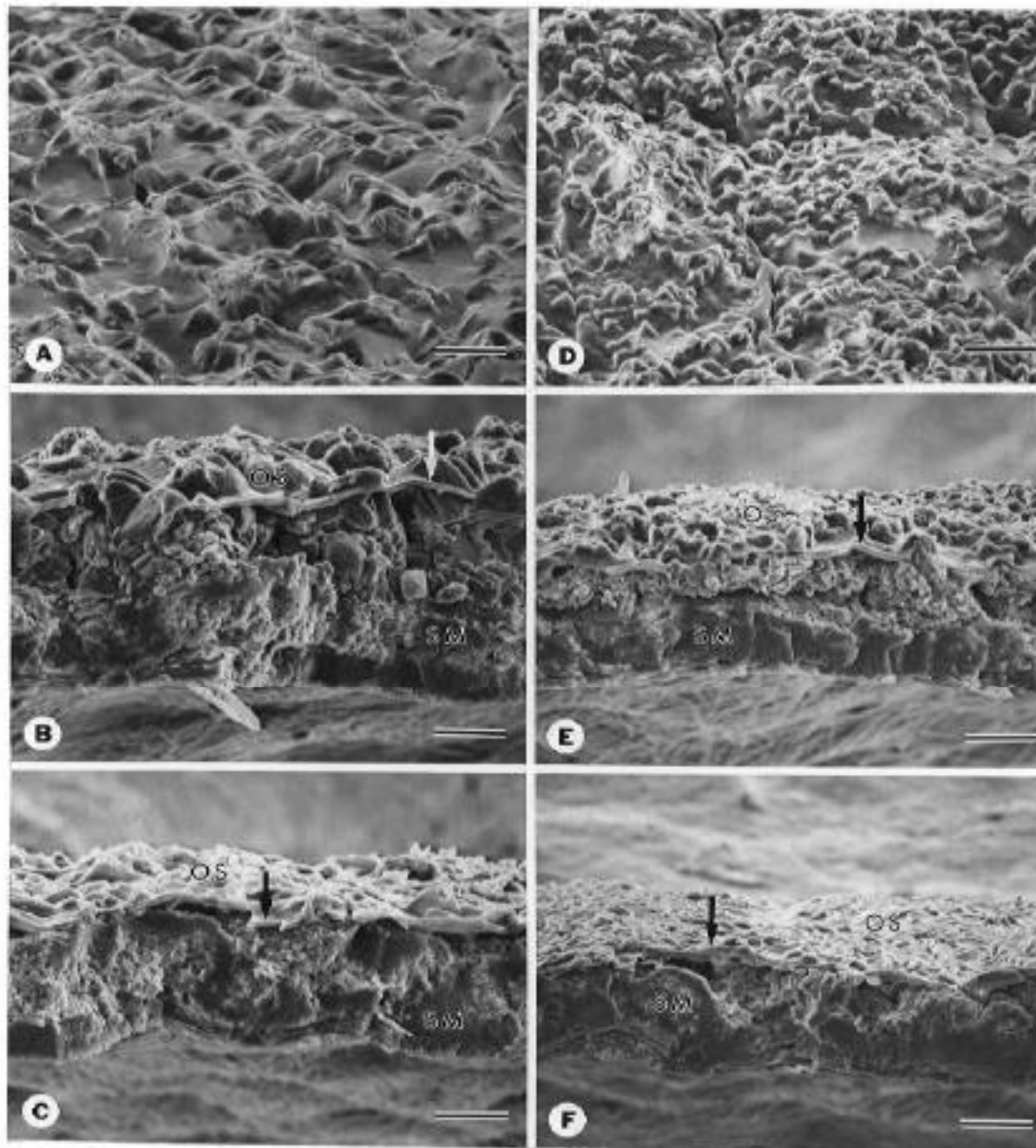


Figure 3.1 Scanning electron micrographs of eggshells of *Sceloporus undulatus hyacinthinus* (A-C) and *Sceloporus clarkii* (D-F). *S. u. hyacinthinus* and *S. clarkii* can support embryogenesis in utero up to embryo Stages 30 and 29.5, respectively. (A) Outer surface of eggshell showing the continuous coverage by the cuticle and underlying blocks of crystalline material. Cracks in the cuticle are presumably an artifact of shell preparation. (B) Radial section of the eggshell, with outer surface to the top of the picture, showing the cuticle (*arrow*) which overlies a layer of crystalline material, and the shell membrane. Note that the cuticle does not contact the shell membrane. (C) Radial section of the eggshell with the crystalline material removed. Outer surface is towards the top of picture. Note that the cuticle (*arrow*) is still present but now rests directly on the shell membrane. (D) Outer surface of eggshell showing the continuous coverage by the cuticle and underlying blocks of crystalline material. (E) Radial section of the eggshell, with outer surface to the top of picture, showing the cuticle (*arrow*) which overlies a layer of crystalline material, and the shell membrane. Note that the cuticle does not contact the shell membrane. (F) Radial section of the eggshell with the crystalline material removed. Outer surface is towards the top of picture. Note that the cuticle (*arrow*) is still present but now rests directly on the shell membrane. OS, outer surface; SM, shell membrane. Scale bars: 30 μm (A, B, C, D, E, F). thickness of the crystalline layer of *U. ornatus* shells appeared to be as thin as that on shells of *S. scalaris*. Examination of radial surfaces of shells of all species (except possibly *U. ornatus*), revealed that there were empty spaces between the units of crystalline material.

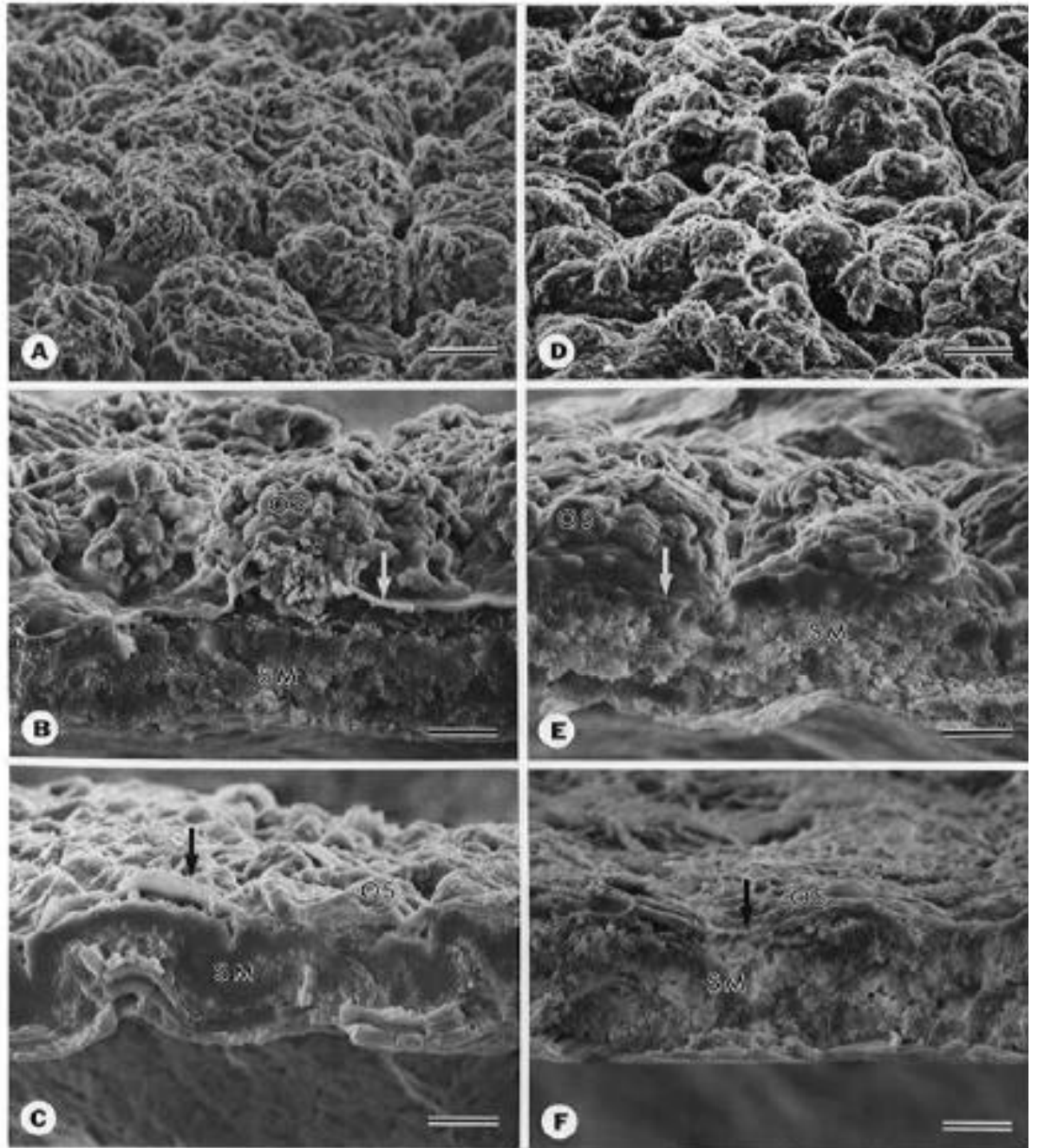


Figure 3.2 Scanning electron micrographs of eggshells of *Sceloporus undulatus* (**A-C**) *consobrinus* and *Sceloporus virgatus* (**D-F**). *S. u. consobrinus* and *S. virgatus* can support embryogenesis in utero up to embryo Stages 37 and 31, respectively. (**A**) Outer surface of eggshell showing crystalline material in the form of nodules that are further organized into discrete "clumps" Coverage of the crystalline material by the cuticle is continuous, though not apparent in surface views. (**B**) Radial section of the eggshell, with outer surface to the top of the picture, showing the cuticle (*arrow*), clumps of crystalline material, and the shell membrane. Note that the cuticle comes into contact with the shell membrane between the clumps crystalline material. (**C**) Radial section of the eggshell with the crystalline material removed. Outer surface is towards the top of picture. Note that the cuticle (*arrow*) is still present. (**D**) Outer surface of eggshell showing the coverage and organization of the crystalline material into "clumps". Coverage of the crystalline material by the cuticle is continuous, though not apparent in surface views. (**E**) Radial section of the eggshell, with outer surface to the top of the picture, showing the clumps of crystalline material and the shell membrane. Only the outer surface of the cuticle is apparent in this view (*arrow*); its upper and lower edges are not discernible. Note that the cuticle comes into contact with the shell membrane between the clumps crystalline material. (**F**) Radial section of the eggshell with the crystalline material removed. Outer surface is towards the top of picture. Note that the cuticle (*arrow*) is still present. *OS*, outer surface; *SM*, shell membrane. Scale bars: 30 μm (A, D), 15 μm (B, C, E, F).

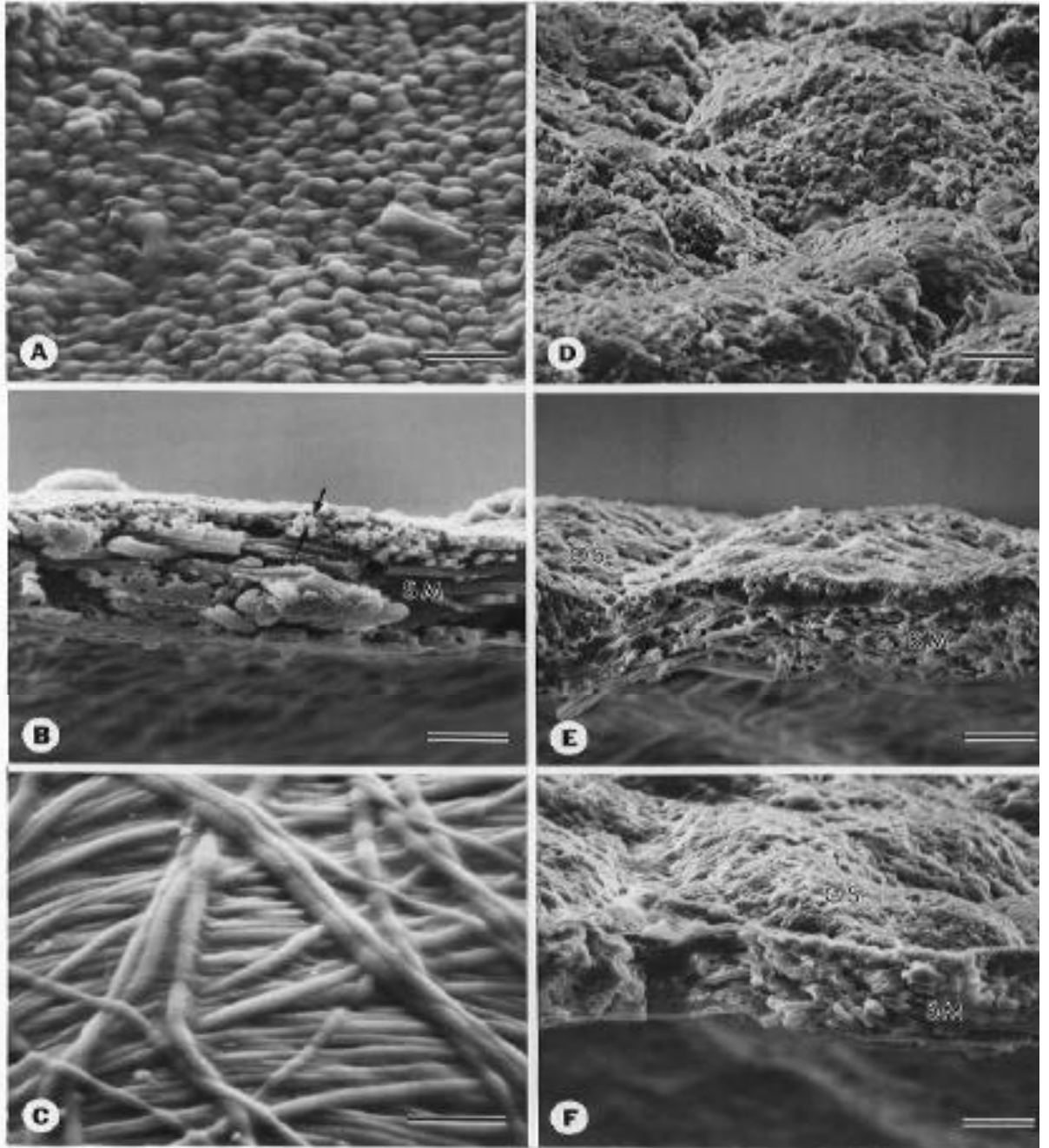


Figure 3.3 Scanning electron micrographs of eggshells of *Sceloporus scalaris* (**A-B**), *Sceloporus clarkii* (**C**), and *Urosaurus ornatus* (**D-F**). *S. scalaris* and *S. clarkii* can support embryogenesis in utero up to embryo Stages 40 and 29, respectively. (**A**) Outer surface of eggshell showing the continuous coverage by the cuticle and underlying crystalline material in the form of "spheroids". (**B**) Radial section of the eggshell, with outer surface to the top of the picture, showing crystalline material in the form of spheroids (*arrows*), and the shell membrane. (**C**) Representative view of the inner surface of the inner boundary (*S. clarkii*) showing the characteristic indentations of the overlying fibers. (**D**) Outer surface of eggshell showing a continuous coverage of crystalline material. (**E**) Radial section of the eggshell, with outer surface to the top of the picture, showing the shell membrane and overlying crystalline material. (**F**) Radial section of the eggshell with the crystalline material removed. Outer surface is towards the top of picture. The thin, but densely interwoven mat of small diameter fibers, is most apparent from the view of the outer surface of the shell. *OS*, outer surface; *SM*, shell membrane. Scale bars: 8 μm (A), 10 μm (B, C), 15 μm (D, E, F).

Structure of Shells

The crystalline layer. Examination of outer and radial surfaces of shells revealed that the shell membrane of all species is covered by a relatively thin layer of crystalline material (Figs. 3.1, 3.2, 3.3). This crystalline material effervesced strongly in, and was removed by, hydrochloric acid (Figs. 3.1C,F, 3.2C,F, 3.3F). The fine structure and arrangement of the crystalline material on the shell surface varied considerably among species, but the underlying shell membrane was never visible when viewing the outer surface of shells. Thus, the surfaces of shells were completely covered by crystalline material and, in some species, a cuticle as well (below). The arrangement of crystalline material on shells of *S. u. hyacinthinus* was much different from that of *S. u. consobrinus*. The crystalline material on shells of *S. u. hyacinthinus* eggs was in the form of "blocks" that are irregular in shape and orientation (Fig. 3.1B). These "blocks" were evenly distributed over the shell surface resulting in a relatively thick crystalline layer compared to that of *S. u. consobrinus*. In *S. u. consobrinus*, the crystalline material appeared to be in the form of nodules that were further organized into discrete clumps that were distributed fairly evenly over the surface of the shell (Fig. 3.2D,E). Radial sections revealed that there was little crystalline material on the shell membrane between these clumps. Thus, in contrast to eggs of *S. u. hyacinthinus*, the outer shell surface of eggs of *S. u. consobrinus* comes into close apposition to the underlying shell membrane at regular intervals over the shell. The fine structure and arrangement of crystalline material on shells of *S. virgatus* appeared to be identical to that of *S. u. consobrinus* (Fig 3.2D,E). The fine structure of the crystalline material on shells of *S. scalaris* differed from that of all other species. Here the crystalline material was in the form of nodules in the shape of prolate spheroids that were distributed thinly, but evenly over the shell membrane (Fig. 3.3A,B). The fine structure and arrangement of crystalline material on shells of *S. clarkii* was very similar to that of *S. u. hyacinthinus* although the thickness of this layer is much thinner than that of *S. u.*

hyacinthinus (Fig. 3.1E). The crystalline material on shells of *U. ornatus* was organized into irregular plaques which are pitted with holes and spaces (Fig. 3.3 D,E). These holes and spaces appeared to be "blind ended" and thus, are unlikely to function as pores. The thickness of the crystalline layer of *U. ornatus* shells appeared to be as thin as that on shells of *S. scalaris*. Examination of radial surfaces of shells of all species (except possibly *U. ornatus*), revealed that there were empty spaces between the units of crystalline material.

A thin amorphous coating of an unknown organic material (~ 1.8 µm thick) overlaid the crystalline layer of all species of *Sceloporus* that I examined. This "cuticle" was not altered when exposed to hydrochloric acid but simply collapsed down onto the shell membrane of treated shells (Fig. 3.1C,F, 3.2C,F, 3.3F). The cuticle was most apparent on shells of *S. u. hyacinthinus*, *S. clarkii*, and *S. scalaris* where the outlines of the underlying "blocks" and "spheroids" of crystalline material, respectively, were plainly visible beneath the cuticle (Figs. 3.1AD, 3.3A). The cuticle was not as readily visible from surface views of shells of species where the crystalline material is arranged into blocks (*S. u. consobrinus* and *S. virgatus*) but was much more obvious on radial views of these shells (Fig. 3.2B,E). Unlike the shells of the sceloporine species, shells of *U. ornatus* did not have a cuticle.

The shell membrane. Examination of radial surfaces of shells revealed that the shell membrane of all species was composed of fibers and that the diameters of the innermost fibers were relatively large compared to that of the outermost fibers (Figs. 3.1, 3.2, 3.3). It was not possible to clearly delineate particular layers of fibers in the shells of any of the species I examined, but the arrangement of these fibers was similar in all species. The innermost, large diameter fibers were relatively loosely packed with empty spaces often visible among fibers. In contrast, the smaller diameter fibers overlying these large diameter fibers appeared to be tightly woven into a dense mat. In most cases, these fibers were

packed so tightly that individual fibers were not visible using the SEM; in radial views of the shell, these areas were generally dark colored, and amorphous in appearance (Fig. 3.1C,E,F, 3.2B,C,E, 3.3E). The number and packing of the small diameter fibers in *S. scalaris* and *U. ornatus* differed somewhat from that of the shells of the other species. In shells of *S. scalaris*, there were few small diameter fibers and these appeared to be packed relatively loosely. In shells of *U. ornatus*, the small diameter fibers were relatively numerous and tightly packed as described above, but the overall thickness of this region of the shell membrane was relatively thin. Thus, in both *S. scalaris* and *U. ornatus*, the shell membrane was composed primarily of rather loosely packed, large diameter fibers.

The shell membrane of all species was organized into a series of alternating hills and troughs (Packard and DeMarco 1991) which usually, but not always, reflected the concerted undulations of underlying groups of large diameter fibers. This type of organization was most evident in the shells of *S. u. hyacinthinus*, *S. u. consobrinus*, *S. virgatus*, and *S. clarkii*, and to a much lesser extent, in shells of *S. scalaris* and *U. ornatus*.

The shells of all species exhibited an inner boundary covering the inner surface of the shell. This boundary was relatively thin insofar as its presence was discernible only because the outlines of large diameter fibers overlying it were clearly visible (Fig. 3.3C).

Thickness and Density of Shells

Thickness of Shells. Both the mean minimum (one-way ANOVA: $F_{3,24} = 117.46$, $P < 0.0001$) and mean maximum ($F_{3,24} = 90.85$, $P < 0.0001$) thicknesses of shells varied significantly among species (Table 3.3). The mean thickness of the shell membrane (one-way ANOVA: $F_{3,24} = 120.69$, $P < 0.0001$) and the mean thickness of the cuticle (one-way

ANOVA: $F_{2,14} = 5.71$, $P = 0.015$) also varied significantly among species and these values paralleled those for overall shell thickness (Table 3.3). That is, thick shells had a relatively thick shell membrane and cuticle.

These data and the fact that the thickness of avian egg shells scales with egg mass (Ar et al. 1974) suggest that shell thickness of squamate eggs might also be related to egg size.

Table 3.3 Thickness of shells and shell components (μm) of five species of phrynosomatid lizards

Species	Minimum Shell Thickness		Maximum Shell Thickness		Fibrel Layer Thickness		Cuticle Thickness	
	n	Mean \pm SD	n	Mean \pm SD	n	Mean \pm SD	n	Mean \pm SD
<i>Sceloporus undulatus</i>	8	84.76 A \pm 12.62	8	87.81 A \pm 10.84	8	50.84 A \pm 4.88	8	2.18 A \pm 1.42
<i>Ameiva ameiva</i>	7	25.89 B \pm 5.98	7	45.33 B \pm 7.38	7	24.92 B \pm 2.21	5	1.68 B \pm 0.45
<i>Sceloporus undulatus</i> <i>consobrinus</i>	6	22.93 B \pm 3.02	6	40.92 B \pm 8.09	6	24.55 B \pm 4.03	4	1.25 B \pm 0.43
<i>Sceloporus sclaris</i>	8	26.6
<i>Sceloporus clarki</i>	1	57.96	1	2.06
<i>Urosaurus ornatus</i>	7	18.37 B \pm 5.50	7	19.51 C \pm 5.18	7	17.43 C \pm 2.89	not present	

Note. All values are means \pm 1 SD. Within a column, values followed by different letters are significantly different at the 0.05 or higher level (ANOVA followed by SNK analyses).

I therefore performed a linear regression using mean shell thickness as the dependent variable and the mass of the egg at oviposition as the independent variable. Mean shell thickness was calculated by averaging the minimum and maximum thickness (where appropriate) for the data in Table 3.3. In this analysis, mean shell thickness was not significantly related to eggmass ($F_{1,4} = 2.34$, $P = 0.20$). Upon visual inspection of this data, it was apparent that shells of *S. u. hyancinthinus* are considerably thicker than those of the other species relative to the size of its egg (Fig. 3.4).

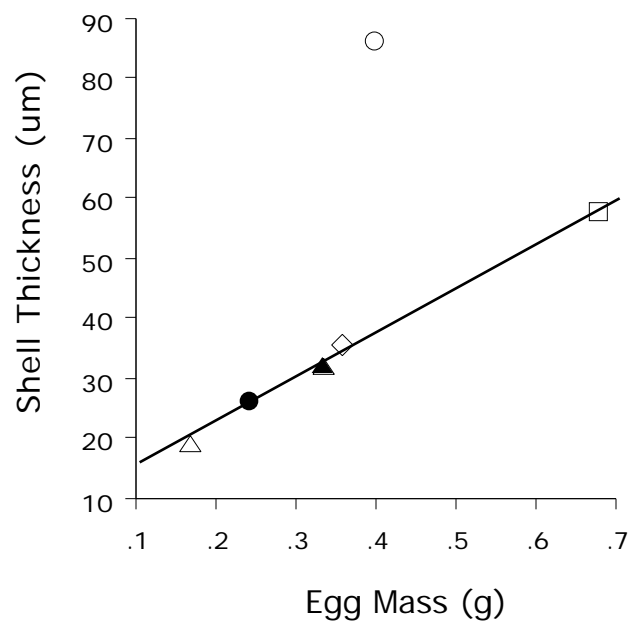


Figure 3.4 The relationship between mean eggshell thickness and the mean mass of the egg at the time of oviposition for six species of phrynosomatid lizards. Unfilled circle: *Sceloporus undulatus hyancinthinus*; unfilled diamond: *Sceloporus undulatus consobrinus*; filled triangle: *Sceloporus virgatus*; filled circle: *Sceloporus scalaris*; unfilled triangle: *Urosaurus ornatus*.

When this outlier was deleted from the data set and second regression analysis was performed, mean shell thickness was significantly related to eggmass (Fig. 3.4: $F_{1,3} = 605.02$, $P = 0.0001$). The regression equation is: mean shell thickness = $7.56 + 75.1(\text{eggmass})$, where $R^2 = 0.995$. Thus, when the differences in egg mass among species are taken into account, the differences in shell thickness among species are trivial.

Density of Shells and the Amount of Crystalline Material on Shells. The mean density of untreated shells did not vary among species (Table 3.4: one-way ANOVA: $F_{3,23} = 0.92$, $P = 0.44$). Similarly, the mean density of the shell membrane did not vary among species (Table 3.4: one-way ANOVA: $F_{3,23} = 1.65$, $P = 0.20$).

For each of the species I examined, the mean density of untreated shells was significantly greater than that of the mean density of the shell membrane (paired t -tests, P 's < 0.04). This result indicates that for each species, the crystalline layer of the shell is denser than the shell membrane. The mean % crystalline material of shells varied significantly among species (Table 3.4: one-way ANOVA: $F_{3,23} = 25.31$, $P < 0.0001$) and was highest in *S. u. hyacinthinus* and lowest in *S. virgatus* and *U. ornatus* (no difference between means for the latter two species). The mean % crystalline material of shells was significantly higher for *S. u. consobrinus* than *S. virgatus* although the magnitude of the difference was not large. Differences among species in the mean coverage of the shell by crystalline material were likewise significant (Table 3.4: one-way ANOVA: $F_{3,23} = 309.48$, $P < 0.0001$) and for the most part, paralleled those for % crystalline material of shells. Unlike the previous analysis, however, the mean coverage of the shell by crystalline material did not differ between shells of *S. u. consobrinus* and *S. virgatus*. Thus, these observations are not in the order predicted.

Table 3. 4 Dry shell mass, shell density, and amount of crystalline material on shells of four species of phrynosomatid lizards

Species	Number of Eggs	Dry Shell Mass (mg)	Unreated Shell Density (mg cm ⁻³)	Shell Membrane Density (mg cm ⁻³)	Crystalline Density (%)	Crystalline Material (mg cm ⁻²)	Crystalline Material (mg cm ⁻²)
<i>Sceloporus undulatus frontochlorus</i>	8	26.63 ± 2.59 A	1183.63 ± 99.54 A	898.73 ± 54.74 A	54.99 ± 2.22 A	5.57 ± 0.44 A	5.57 ± 0.44 A
<i>Sceloporus undulatus consociatus</i>	6	9.48 ± 0.48 B	1103.60 ± 172.29 A	909.14 ± 60.57 A	42.24 ± 4.33 B	1.65 ± 0.26 B	1.65 ± 0.26 B
<i>Sceloporus virgatus</i>	6	8.35 ± 1.97 B	1093.37 ± 130.03 A	934.71 ± 151.10 A	35.74 ± 7.46 C	1.28 ± 0.37 B	1.28 ± 0.37 B
<i>Urosaurus ornatus</i>	7	3.18 ± 0.47 C	1181.63 ± 124.35 A	826.63 ± 87.18 A	34.26 ± 5.05 C	0.76 ± 0.24 C	0.76 ± 0.24 C

Note. All values are means ± 1 SD. Within a column, values followed by different letters are significantly different at the 0.05 or higher level (ANOVA followed by SNK analyses). For each species or subspecies, the untreated shell density is significantly higher than the shell membrane density (P's < 0.002, paired t-tests).

Discussion

My data for six species of phrynosomatid lizards does not support the hypothesis that shell morphology and permeability vary systematically with the capacity to support embryogenesis *in utero*. By extension, my results do not support the hypothesis that a "thinning" of the eggshell would have to occur concurrently with evolution of viviparity (Shine 1985; Guillette 1991; Guillette 1993; Blackburn 1993). I also conclude that the eggshells of the species I examined are incapable of preventing rapid water loss through reductions in KH_2O . First, I consider characteristics of eggshells with regard to evaluating the hypothesis that a thinning eggshell and longer periods of egg retention evolve concurrently. I then consider the implications of my results regarding the evolution of placentation and viviparity.

Shell characteristics and the capacity to support intrauterine embryogenesis

The KH_2O 's of shells did not vary as predicted with the capacity to support uterine embryogenesis. Shells of *S. virgatus* did not exhibit the highest values of KH_2O (maximal stage in utero = 37.0). The mean KH_2O of shells of *S. virgatus* eggs was lower than that of *S. u. consobrinus*₂ (maximal stage in utero = 31.0) and did not differ from that of *S. u. hyancinthinus* (maximal stage in utero = 30.0). Moreover, the highest mean KH_2O I observed was for shells of *U. ornatus*, a species whose embryos undergo developmental arrest *in utero* at embryo Stages 30.0-30.5 (Chapter 1). These results suggest that the permeability of oxygen (KO_2) of shells does not vary in the manner predicted. KH_2O varies monotonically with KO_2 for eggs of avian species (Paganelli et al. 1978; Paganelli 1991). However, the relationship between KO_2 and KH_2O for avian eggs has not been useful for estimating KO_2 of flexible-shelled reptilian eggs because it yields values of KO_2 that are

much higher than empirically determined values for birds (see Deeming and Thompson 1991). This may mean that, unlike avian eggs, the channels for diffusion of water vapor and oxygen are not the same. However, a simpler explanation is that the diffusion paths for water vapor are "shorter" than those for oxygen vapor simply because reptilian eggshells typically have a relatively high water content (Thompson 1985; Kern and Ferguson 1997).

Second, the thicknesses of shells also did not decrease as predicted with an increase in the capacity to support uterine embryogenesis. Two species, *S. scalaris* and *S. virgatus*, are able to support embryogenesis to relatively advanced stages and thus deserve further comment. I suggested previously that the reason *S. scalaris* is able to support embryonic development *in utero* to such advanced stages (33-40; Table 3.1) is that its shell is relatively thin (Mathies and Andrews 1995). However, the allometry between mean shell thickness and egg mass indicates that neither *S. scalaris* or *S. virgatus* produce shells that are substantially thinner than species in which embryogenesis is greatly retarded or arrested *in utero*. Indeed, the relationship between shell thickness and egg mass predicts that relative to their mass, small eggs (e.g., *S. scalaris*) have *thick* shells. Clearly, the potential for allometric relationships between features of eggshells (and possibly the shell membrane of viviparous species as well) and the mass of the egg should be considered when making comparisons among species or populations.

As has been suggested for avian species, the thickness of flexible-shelled reptile eggs may have been selected primarily to provide sufficient structural support for the egg, rather than to prevent excessive water loss (Ar and Rahn 1985). For example, shells of *S. u. hyancinthinus* are much thicker than expected based on the initial mass of its egg (Figs. 3.1,3.4), yet their mean KH_2O does not differ from that of *S. virgatus*, a species with a much thinner shell (Table 3.2).

Lastly, the gross structure and density of shells did not vary as predicted with the capacity to support uterine embryogenesis; shells of all species were similar in gross structure and density. Other than the cuticle of shells of the sceloporine species, shell morphology was similar to that reported for other lizard species that produce flexible-shelled eggs (Packard et al. 1982a; Schleich and Kästle 1988; Packard and DeMarco 1991). There were, however, obvious differences in the fine structure of shells among species, primarily in the morphology of the crystalline layer.

The shells of *S. scalaris* exhibited two features that might result in increased gas permeability. First, the dense layer of small diameter fibers that underlie the crystalline layer in all the other species I examined was reduced or absent in shells of *S. scalaris*. This dense layer of small diameter fibers has been observed in most flexible-shelled eggs examined to date (Packard et al. 1982a; Packard et al. 1982b; Trauth and Fagerberg 1984; Schleich and Kästle 1988; Guillette et al. 1989; Packard and DeMarco 1991; Palmer et al. 1993). Second, the crystalline layer of shell of *S. scalaris* appears to be relatively permeable in that there are numerous empty spaces present between the nodules of crystalline material (Fig 3.3B).

Several observations tend to negate the idea that these features enhance the permeability of *S. scalaris* shells. The layer of small diameter fibers of shells of *U. ornatus* (Fig. 3.3E) is relatively thin, yet its embryos undergo developmental arrest *in utero* (Chapter 1). Moreover, even though this layer is relatively thick in shells of *S. virgatus* (Fig. 3.2E), this species exhibits substantial capacity to support embryogenesis *in utero* (albeit to a lesser extent than *S. scalaris*). Shells of *S. u. hyancinthinus* exhibit a considerable amount of crystalline material (Fig. 3.1B), yet the KH_2O for this species does not differ from that of *S. virgatus*, a species whose shells exhibit considerably less crystalline material. Thus, it is unlikely that these two features of the shell alone, are

responsible for the capacity of *S. scalaris* females to support embryogenesis *in utero*. Measures of the permeability of shells of *S. scalaris* would help resolve whether these attributes of its shell are partly responsible for the substantial degree of intrauterine embryogenesis observed in this species.

Environmental constraints on a "thinning" of the eggshell

Weekes (1935) was the first to point out that the "major difficulty" with the idea that evolutionary reductions of the shell occur prior to viviparity (Packard 1966; Tinkle and Gibbons 1977) is that eggs with thin shells would be much more likely to desiccate than eggs with thick shells. While such a conclusion seems intuitive, observations on water relations of flexible-shelled eggs show that rates of water loss from such eggs are exceedingly high regardless of the thickness of the shell (Andrews and Sexton 1981; Ackerman et al. 1984; present paper). While eggs of all the species I examined exhibited a reduced KH_2O under conditions of low ambient humidity, the resulting reductions in MH_2O were trivial. For example, an egg of *S. u. hyacinthinus* (which has a particularly thick shell), when exposed to an ambient relative humidity of 90% at 31°C, would lose 50% of its initial mass (mean initial mass = 0.45 g) in less than 24 h as judged from the regression equation for MH_2O and ambient relative humidity ($MH_2O = 1.17 - 0.011(\% \text{ relative humidity})$; $F_{1,8} = 10.84$, $P = 0.01$; $R^2 = 0.61$). Water loss of this magnitude is fatal; freshly oviposited eggs of *S. scalaris*, *S. virgatus*, and *U. ornatus* that have lost approximately 50% of their mass (do not hatch (Mathies, unpubl. data). It therefore seems likely that to ensure hatching, any small flexible-shelled egg, regardless of the thickness of its shell, would have to be oviposited in a place where it rarely, if ever, experiences water vapor pressures less than saturation. If this is true, then the perceived "conflict" between a

"thinning" of the eggshell vs. protection against desiccation (Weekes 1935; Tinkle and Gibbons 1977; Blackburn 1995) is a moot issue.

The constraints on thinly shelled eggs more likely involve structural considerations (as my data suggest), protection against small invertebrate predators (Andrews 1988), or invasion by microbial pathogens (Ferguson 1981). Indeed, if thicker shells were effective for reducing rates of water loss, then contrary to my observations, I would expect shells of species that occur in arid environments (e.g., *S. u. consobrinus*, *S. virgatus*, *S. scalaris*, *S. clarkii*, and *U. ornatus*) to be thicker than those in more mesic environment (e.g., *S. u. hyacinthinus*). I would not expect a relationship (much less a strong one) between shell thickness and egg size like that reported here; the vulnerability of small eggs to desiccation (based on considerations of surface area and volume), predicts that shells of small eggs should be thicker than those I observed.

Shell morphology, gas exchange, and the evolution of viviparity

I predicted that features of eggshells would be correlated with the maximal stage at oviposition. Several species exhibit the predicted negative correlation between eggshell thickness and embryo stage at oviposition. Females in oviparous populations of the skink, *Saiphos equalis*, oviposit "poorly calcified eggs" that remain in the nest for about seven to nine days before hatching (Bustard 1964; Greer 1989; Smith and Shine 1997). The skink, *Spenomorphus fragilis*, lays eggs with shells that are thin (~ 10 μm , Guillette 1992) compared to those of other similarly sized oviparous species, and somewhat opaque, that "hatch" in within a few hours of being laid (Greer and Parker 1979). Stronger evidence still is provided by within-species comparisons. The developmental stage of embryos and the thickness of the shell membrane of eggs oviposited by females in one population of the

skink, *Lerista bougainvillii*, are intermediate between those of "normal" oviparous and viviparous females (Qualls 1996). Lastly, female *S. scalaris* in a montane population oviposit eggs with more advanced embryos and thinner eggshells than females in a low elevation population (Mathies and Andrews 1995).

One possible reason for the nonconcordance of my findings with those above is that the species I examined that exhibit a substantial capacity to support embryogenesis *in utero* (*S. scalaris* and *S. virgatus*) may not be representative of those species that have made the transition from oviparity to viviparity. This is conceivable for *S. virgatus* since it belongs to a species group where oviparity is apparently fixed, but less likely for *S. scalaris* since it has close relatives that are viviparous (Sites et al. 1992; Wiens and Reeder 1997; Méndez-de la Cruz et al., 1998). Assuming that the species I examined are representative of those oviparous species that have made the transition to viviparity, how can I account for my observation that shell morphology and permeability varies independently of the capacity to support intrauterine embryogenesis over the entire range of embryonic development from the modal embryo stage at oviposition (Stage 30: Shine 1983a; DeMarco 1993; Blackburn 1995) to hatching or parturition (Stage 40)? If the variation in the capacity to support intrauterine embryogenesis is due to species-specific differences in the capacity to meet the gas exchange needs of embryos (O_2 and CO_2), then there must be other mechanisms besides shell reduction for facilitating adequate gas exchange. If so, what other mechanisms could account for the variation in the degree of intrauterine embryogenesis I observed?

One explanation is that such variation results from functional differences in one or both of the two main components of the tissues that form the simple placenta in viviparous species, the maternal oviduct and the fetal chorioallantois. Both structures are thought to play a major role in gas exchange (Guillette and Jones 1985; Yaron 1985; Blackburn 1993;

Stewart 1993) and there is circumstantial evidence for such functional differences among squamates. For example, in two closely related members of the *scalaris* group, the vascular density of the oviduct is higher in the viviparous species, *Sceloporus bicanthalis*, than in the oviparous species, *Sceloporus aeneus* (Guillette and Jones 1985). Furthermore, the chorioallantois of *S. scalaris* embryos covers a greater percentage of the inner surface area of the shell than that of *S. virgatus* embryos at similar stages of embryonic development, and intrauterine embryonic development is less retarded in *S. scalaris* than *S. virgatus* during extended egg retention (Andrews 1997). Intraspecific differences in the capacity to support embryogenesis *in utero* such as I observed might simply result from species-specific differences in an angiogenic response by the uterus and the chorioallantois to local hypoxia produced by the embryo (Guillette 1989). Thus, it is conceivable that "placentation" (albeit a crude form) evolves concurrently with increases in the duration of egg retention through increases in the vascular density of the oviduct and chorioallantois, but without a thinning of the eggshell (at least initially) as generally envisioned. Such modifications cannot produce a morphological arrangement that can be termed a simple placenta *sensu stricto*, but they would certainly meet the functional definition for this structure (Mossman 1937).

More comparative studies on the relationship between the capacity to support intrauterine embryonic development and oviductal and chorioallantoic vascularity are needed before we can better evaluate the idea that "functional placentation" (complete eggshell, but increased oviductal and chorioallantoic vascularity) evolves concurrently with egg retention and viviparity. Such studies on closely related oviparous species that exhibit similar eggshells, but differences in the capacity to support embryonic development *in utero* (e.g., *S. virgatus* and *S. u. consobrinus*) could be particularly insightful.

My observations on shell thickness, density, and permeability, for phrynosomatid lizards do not support the idea that shell reduction occurs concurrently with longer periods of egg retention. The maximum developmental stage embryos attain *in utero* was not correlated with these features of eggshells. These findings indicate that it would be premature to reject the view that a reduction of the eggshell occurs subsequent to the evolution of viviparity.

Chapter 4 Abstract

I measured selected body temperatures of female lizards, *Sceloporus jarrovi*, on a photothermal gradient during late pregnancy and again when postpartum, and subjected pregnant females to one of three fluctuating temperature regimes that simulated body temperatures of 1) pregnant females, 2) postpartum females, or 3) allowed normal thermoregulation. Overall, females selected lower body temperatures when pregnant (mean = 32.0°C) than when postpartum (mean = 33.5°C). Females regulated body temperature more precisely when pregnant than when postpartum as judged by their smaller variances in body temperature throughout the day. When pregnant, females selected a lower mean maximum body temperature (mean: pregnant = 32.8°C; postpartum = 34.5°C) than when postpartum, but selected mean minimum body temperatures did not differ. None of the experimental temperature treatments were detrimental to pregnant females. Female body length increased during pregnancy but the rate of increase did not differ among treatments. Moreover, length-adjusted body mass of postpartum females did not differ among treatments. The experimental temperature treatments were detrimental to offspring. Pregnant females that experienced postpartum body temperatures produced neonates that were smaller in body mass and length than pregnant females that experienced pregnant body temperatures and females that were allowed to thermoregulate. For neonates resulting from the postpartum body temperature treatment, the disparity in the body length, but not mass, was still observed at 9 days of age, although survival and growth of neonates was high and did not differ among treatments. My results demonstrate that pregnant females could maintain higher postpartum body temperatures without compromising their physical condition, but select relatively low body temperatures, presumably to avoid decrements in offspring fitness.

**Chapter 4: Influence of pregnancy on the thermal biology of the lizard,
Sceloporus jarrovi: why do pregnant females exhibit low body
temperatures?**

Introduction

Many reptiles regulate their body temperature within a relatively narrow range by using behavioral adjustments such as shuttling between different thermal microclimates (Cowles and Bogert 1944; Huey 1982). Regulation may include fine-grained adjustments that match body temperature to functions with physiological optima, such as digestion or sprint speed (Patterson and Davies 1978; Huey 1982; Stevenson et al. 1985). However, there may be other explanations for the association between body temperatures and physiological processes besides active temperature selection (Huey 1982). For example, shifts in body temperature could be the result of active selection of new thermal optima or the result of passive acceptance of higher or lower temperatures as the result of some ecological constraint on thermoregulation.

Temperature shifts (both upwards and downwards) associated with reproductive status are well documented among some species of lizards and snakes (Daut and Andrews 1993, and included references). A postulated benefit of an upward shift in body temperature by reproductive (gravid or pregnant) females is that developmental rates, which are temperature-dependent (Muth 1980), would increase. The resultant shortened incubation period could reduce costs of reproduction (e.g., decreased survival or future fecundity of females) by reducing the time over which these costs are incurred (Shine 1980; 1983b; Seigel and Fitch 1984). In addition, shortened incubation periods in temperate zone environments might enhance offspring fitness by increasing the time available for growth

and accumulation of energy reserves before cessation of activity in autumn. On the other hand, a postulated benefit of a downward shift in body temperature is enhanced survival of embryos; high incubation temperatures are detrimental to embryonic development (Vinegar 1974; Gutzke and Packard 1987). Thus, if normal body temperatures reduce offspring fitness, females would benefit from a shift to lower body temperatures when they become reproductive.

Are the body temperatures exhibited by reproductive females actively selected or passively accepted? Because observed shifts in body temperatures have presumptive physiological benefits, one explanation for the relatively high or low body temperatures of reproductive females is that they are actively selected. Alternatively, body temperatures may reflect ecological constraints (Hertz 1992; Hertz, Huey and Stevenson 1993). For example, if the burden of the clutch causes females to become more susceptible to predators (Shine 1980), females may compensate by adopting a more cryptic behavior(s) (e.g., reduce the frequency and extent of movement). Such an increase in cryptic behaviors could limit a female's thermoregulatory opportunities and thereby preclude regulation of body temperatures at preferred levels. Observations on lacertid lizards support this conjecture (Braña 1993).

I used data from laboratory studies on the viviparous lizard, *Sceloporus jarrovi* Cope (Phrynosomatidae), to gain insight into why pregnant females exhibit lower body temperatures than postpartum females. *Sceloporus jarrovi* is a particularly appropriate study species because its reproductive (Goldberg 1971) and thermal biology (Beuchat 1986) are well documented, and field-active females exhibit lower mean body temperatures when pregnant than when postpartum (32.0°C and 34.5°C, respectively, Beuchat 1986).

This study addresses two complementary questions: First, do pregnant females actively select, or passively accept, low body temperatures? This question can be answered by measuring the body temperatures that females select on a thermal gradient when pregnant and postpartum. Use of a thermal gradient in a laboratory setting insures that lizards can select body temperatures with minimal ecological costs and constraints. I predicted that if field-active pregnant females exhibit low body temperatures because postpartum body temperatures are physiologically stressful to the female, her offspring, or both, then I would expect pregnant females on a thermal gradient to select lower body temperatures than postpartum females. Alternatively, if field-active pregnant females exhibit low body temperatures because of exogenous constraints on thermoregulation, then we would expect pregnant females on a thermal gradient to select the same high body temperatures as postpartum females.

Second, if postpartum body temperatures are physiologically stressful, do they affect the pregnant female, the embryos, or both? I predicted that if postpartum body temperatures are stressful to pregnant females, then pregnant females maintained at postpartum body temperatures should exhibit signs of poor health or decreased survivorship. On the other hand, if postpartum body temperatures are stressful to embryos, then offspring incubated at postpartum body temperatures should exhibit features associated with low fitness.

Materials and Methods

Collection and assignment of females to experiments

Pregnant females ($n = 70$) were collected in the Chiricahua Mountains of southeastern Arizona in the vicinity of the Southwestern Research Station (SWRS) near Portal, Arizona between 30 April and 2 May 1995. Each female was given a unique toe clip and a corresponding number was painted on her back for identification. Females were randomly assigned to one of two experiments: 1) those used to measure selected body temperatures ($n = 18$) (next section), and 2) those used to determine the effect of postpartum versus pregnant body temperatures on pregnant females and their offspring ($n = 52$) (see Incubation experiment).

Measurement of selected body temperatures

Maintenance conditions. Pregnant females were housed in a 2.5 X 4.0 m dirt-floored outdoor enclosure. Three 16 X 32 X 34 cm cinderblock cairns within the enclosure provided numerous perches and refugia. Drinking water was provided daily by running water through a shallow trench that ran alongside the bases of the cairns. Females were fed daily with crickets dusted with vitamin powder.

On 5 July, all 18 females (all postpartum) were transported to indoor facilities at Blacksburg, Virginia, where they were maintained in two 33 X 77 X 47 cm slate-bottomed terraria. Room temperature was maintained at approximately 24°C and light from windows determined photoperiod. Illumination was provided by two broad spectrum fluorescent light bulbs (Vita-lite™, Duro-Light Corp., Fairfield, New Jersey, USA) resting on top of

the terraria. One heat lamp (150-W) suspended over the end of each terraria allowed behavioral thermoregulation. Lights were turned on and off each day at 0700 and 1700 h MST, respectively. Water was provided daily by squirting water into each cage to form a small temporary pool. Females were fed as above.

Testing apparatus. Selected body temperatures (see Pough and Gans 1982) of pregnant females were measured in Arizona in a room in which temperature was maintained at approximately 24.0°C. Light from a door at each end of the room determined photoperiod. Selected body temperatures of postpartum females were measured in Blacksburg in the same room where they were housed.

The testing apparatus consisted of a corrugated cardboard box with a 2.5 cm thick Styrofoam bottom to reduce temperature fluctuations. Cardboard strips were used to divide the box into six 37 X 200 cm runways. *Sceloporus jarrovi* normally uses rock substrates and pregnant females tend to remain close to crevices where they can take refuge (Smith and Ballinger 1994). I therefore simulated a rock surface by painting the runway floors with glue and then dusting them lightly with sand. Each runway was provided with a "continuous refugia". This consisted of a 16 cm wide cardboard strip that ran the entire length of the runway such that the top edge of the strip rested on the runway wall and the bottom edge projected 8.5 cm away from the wall at a height of 3.5 cm above the runway floor. This arrangement allowed females to hide beneath the strip any point along a runway. A thermal gradient was established by suspending a series of incandescent floodlamps of various wattages at various heights and spacings down the midline of each runway. Hollow aluminum probes with internal thermocouples were placed at evenly spaced intervals along the length of each runway floor and the floodlamps were adjusted so that probe temperatures ranged linearly from approximately 29 to 41°C (Fig. 4.1).

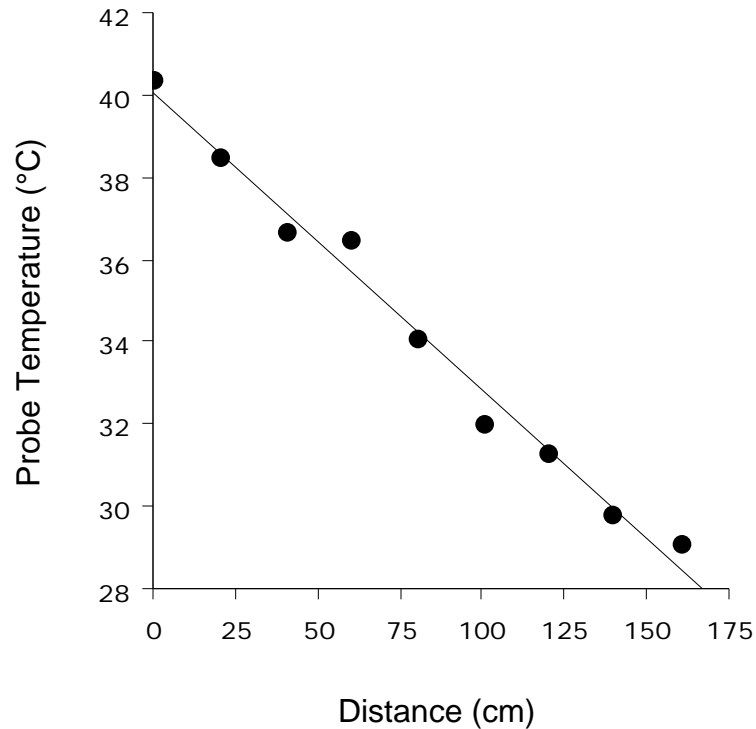


Figure 4.1 Representative thermocouple probe temperatures (T_p) on the thermal gradient measured at 20 cm intervals (d). The line indicates the least squares linear regression of probe temperature on probe position in the gradient. $T_p = 40.06 - 0.071(d)$, $R^2 = 0.98$, $P < 0.001$.

The average rate of temperature change in the gradient was $0.072^\circ\text{C}/\text{cm}$ or $0.53^\circ\text{C}/\text{mean female SVL}$. With few exceptions, females perched towards the center of the gradient. I therefore judged that the range in available body temperatures was considerably wider than necessary for normal thermoregulation.

Observations. I measured body temperatures during 15-19 May (pregnant) and 11-15 July (postpartum). Pregnant females were in their last trimester as judged by the first appearance of neonates in the field on 22 May.

The following protocol was used to measure body temperatures. On day 1 at 1530 h, six females were placed on the gradient, each in an individual runway, where they remained for the following 35.5 h (29.5 h of acclimation and 6 h of observation). Body (cloacal) temperatures were measured at 0900 h, 1200 h, and 1500 h on day 3 with a thermocouple thermometer (Physitemp digital laboratory thermometer, Model: BAT-12, Physitemp Instruments Inc., Clifton, New Jersey, USA). Each female was removed quickly from its runway (taking care not to disturb the female in the adjacent runway), its cloacal temperature recorded, and then returned to the gradient. This protocol was repeated for successive sets of six females. Females received no food or water while on the gradient. Gradient lights were turned on and off at 0700 and 1700 h MST, respectively.

Incubation experiment

Temperature treatments and maintenance conditions. During the day, field-active *Sceloporus* lizards typically exhibit a high and relatively constant body temperature, but at night, body temperatures converge on ambient temperatures. I used a cycling thermal regime that approximated the natural thermal environment of *S. jarrovi* because constant temperature regimes are stressful to squamate reptiles (Shine 1983b).

Pregnant females were randomly allocated to one of three experimental temperature treatments (n = 15, each treatment). Two of the treatment groups were placed into controlled light and temperature chambers (Percival model I-35L, Percival Manufacturing Co., Boone, Iowa, USA). Temperatures in both chambers were similar during the inactive period, falling to approximately 15°C by 2300 and remaining there until 0700 the following morning. In one chamber, the temperature during the activity period (0900 - 1600 h) was

35.7°C. This temperature is higher than the observed mean body temperature of field-active postpartum females (34.5°C, Beuchat, 1986), but falls within the 95% confidence interval for the mean. I used 35.7°C, as opposed to 34.5°C, because deleterious effects of temperature on physiological processes, if any, would more likely be induced by body temperatures near the extreme, rather than the mean. In the other chamber, the temperature during the activity period (0900 - 1600 h) was 32°C, the mean field-active body temperature of pregnant females (Beuchat, 1986). These two temperature regimes are hereafter referred to as the 35°C and 32°C treatments, respectively. Body temperatures (cloacal) of females in the chambers were measured to verify that females experienced the desired experimental temperature. Actual mean body temperatures of females in the 35°C and 32°C treatments were 35.4°C (n = 14, SE = 0.1°C) and 32.3°C (n = 12, SE = 0.1°C), respectively. Periodically during the experiment, ambient air temperatures in the chambers were recorded at half hour intervals for 24 h and stored in a data logger (OMEGA ® OM-550 DATALOGGER, Omega Engineering, Inc., Stamford, Connecticut, USA). Photoperiod in each chamber was the same as that in Arizona in May (13L:11D).

A third temperature treatment allowed females to behaviorally thermoregulate (TREG treatment, hereafter). Females in this treatment were maintained in individual terraria each fitted with a 60-W incandescent light bulb over one end. Lights were turned on and off each day at 0730 and 1600 h MST, respectively. These terraria were located in an open-air building where temperatures fluctuated with ambient temperatures. Thus, maintenance conditions experienced by females in the TREG treatment most closely reflected those experienced by pregnant females in the field. Ambient air temperatures at the cool and hot ends of the terraria were recorded (every half hour over 24 h) periodically during the experiment and stored in temperature loggers (HOBO-TEMP-XT™, Onset Instruments, Pocasset, Massachusetts, USA)(Fig. 4.2; temperatures shown are from cool ends of terraria). Temperatures at the hot end of the terraria generally exceeded 37°C during

the daily activity period. Direct measurements of body temperatures (cloacal) of females in the TREG treatment during the activity period verified that females were able to regulate body temperatures (mean body temperature = 32.8°C; SE = 0.2°C, n = 14) similar to those of pregnant females in the field. Photoperiod was determined by ambient light from windows.

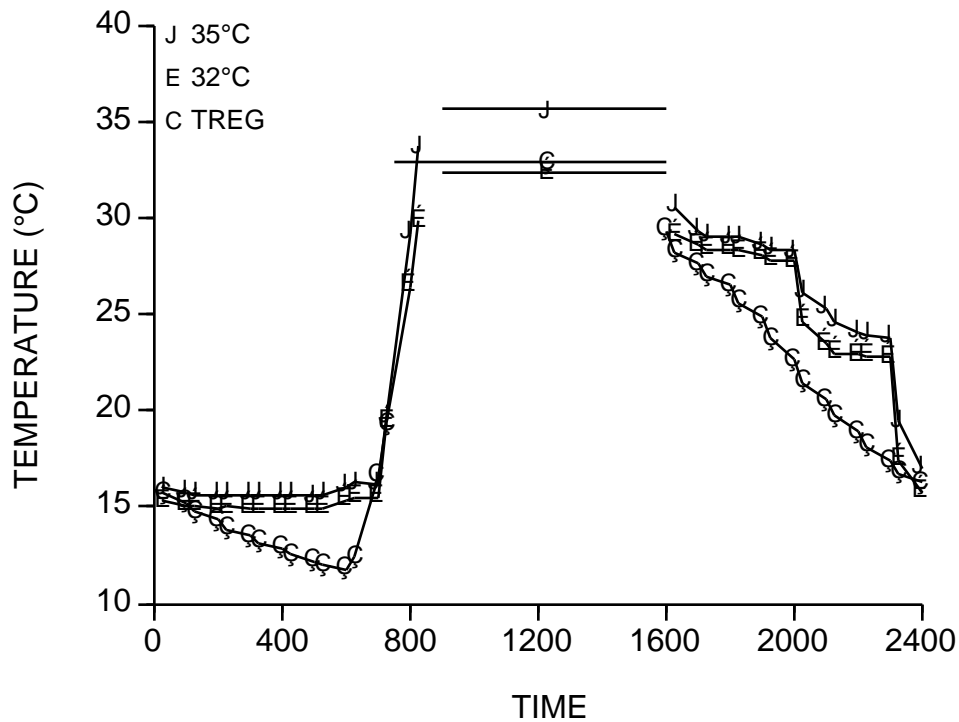


Figure 4.2 Mean temperatures (air and body) in environmental chambers (35 °C and 32°C treatments) and terraria that experienced ambient temperatures (TREG treatment). Letters connected by a line denote air temperatures for the period females were inactive. Solid horizontal bars represent average body temperatures for the period females were active. Standard errors of the mean body temperatures of females during activity in the 35°C, 32°C, and TREG treatments were ± 0.1 , ± 0.1 , and $\pm 0.2^\circ\text{C}$, respectively.

Average body temperatures for females in each of the three treatments were calculated as the means of chamber temperatures for each half hour interval of the inactive period and the actual mean body temperatures of females for each half hour interval of the activity period. Temperatures for the 35°C, 32°C, and TREG treatments averaged (mean of the 48 means for each treatment) 26.1°C, 24.4°C, and 23.7°C, respectively. Temperature profiles for the treatments are summarized in Figure 4.2.

On the day that females were put under experimental conditions (3 May), I weighed all females to 0.01 g on an electronic balance (Mettler model PM200) and measured snout-vent length (SVL) to the nearest 1 mm. At this time, the mean SVL of females in the 35°C (74.4 ± 1.5), 32°C (72.2 ± 1.4), and TREG (73.1 ± 1.0) treatments did not vary among treatments ($F_{2,41} = 0.68$, $P = 0.51$, one-way ANOVA). Females were placed into individual glass terraria (28 X 22 X 31 cm) provided with a large piece of bark, which females could use as a perch or refuge. Terraria were rotated among positions within treatments every three days to minimize position effects. Crickets dusted with vitamin powder were provided once or twice each day. Water was provided twice daily by squirting water into each cage to form a small temporary pool. Females were weighed and SVL was measured every 10-12 days and immediately after parturition.

Determination of initial developmental stage. I estimated the developmental stage of embryos near (30 April-5 May) the time that females were put under experimental conditions. To do so, I made a small incision in the abdomen of each of seven females (none included in the experiments) and everted a section of the oviduct containing one embryo. Embryos could be viewed through the oviduct and shell membrane and their developmental stage determined. Following determination of embryonic stage, embryos were put back in the body cavity, the incision closed with sutures, and the females later released. The mean developmental stage of these embryos was stage 34 and ranged from

stage 33-36 (following Dufaure and Hubert 1961; range of staging sequence: 1-40; parturition at stage 40). Thus, pregnancy was presumably relatively advanced in most females at the outset of the experiment.

Data collection. Terraria were checked daily for the appearance of neonates. On the day of parturition, I recorded the mass and SVL of the postpartum female and each neonate. Neonates were examined for morphological and behavioral (i.e., movement) abnormalities. One randomly selected neonate from each litter was killed and dried at 50°C for 24h and weighed to 0.01 g. Another randomly selected neonate was killed and preserved in 70% ethanol for later determination of residual yolk mass. After approximately 240 d, the residual yolk was removed from neonates, dried, and weighed to 0.01 mg. Up to five of the remaining neonates in each litter were used to measure growth rate (additional neonates were released). These neonates were first housed by litter in 40 X 25 X 26 cm terraria under similar conditions as females in the TREG group (above). At 3 days of age, each neonate was reweighed, remeasured, and transferred to a common outdoor enclosure identical to that described above, except that it contained numerous rocks scattered about the bases of the cairns. At 9 days of age, each neonate was reweighed and remeasured for a third time and then released. Neonates were fed ad libitum with live insects collected by sweep net and with 2-4 mm crickets dusted with vitamin powder. Water was provided once or twice each day by misting the sides of the terraria and by running water through a trench that ran alongside the bases of the cairns.

Statistical analyses

All analyses were performed using SAS statistical packages (SAS Institute Inc. 1985). Means or least-squared means and their standard errors are presented as mean \pm 1 SE. Differences were considered significant at $P < 0.05$ unless stated otherwise.

A repeated-measures ANOVA (repeated across the 3 times of day and repeated between reproductive conditions) was used to compare selected body temperatures of females when pregnant and when postpartum. Tukey's least significant differences was used to identify pairwise differences in the means for each time of day.

Variance in body temperature was used as an index of thermoregulatory precision (Sievert and Hutchison 1988; Hertz et al. 1993). Variances were calculated for pregnant and postpartum females at 0900, 1200, and 1500 h. F_{\max} tests were used to test for differences in thermoregulatory precision between pregnant and postpartum females at each of these three times of day. For this analysis and the above analysis of selected body temperatures, the alpha level (0.05) was Bonferroni corrected for the number of tests (3) in each analysis and differences were thus considered significant at $P < 0.017$.

The physical condition of females was assessed in two ways: by determining growth rate (change in SVL) and body condition (length-adjusted body mass; Bradshaw 1986) at parturition. Size-specific growth rate of pregnant females was calculated as

$$\text{Size-specific growth rate} = [\ln(\text{SVL}_2) - \ln(\text{SVL}_1)] / (t_2 - t_1),$$

where SVL_1 and SVL_2 denote the SVL on the day females were placed under experimental conditions and the last measurement of SVL before parturition, respectively and $t_2 - t_1$ denotes the time in days elapsed between the two measurements of SVL.

Treatment effects on body condition of postpartum females were determined with ANCOVA, using postpartum body mass (measured on the day of parturition) as the dependent variable and SVL at parturition as the covariate.

All analyses involving neonates were based on litter means except those for neonate dry mass, neonate water content, and dry residual yolk mass. Neonate water content was determined as the difference between neonate live and dry masses. One-way analyses of variance (ANOVA) were used to determine whether treatment affected date of parturition, neonate mass, neonate SVL, and neonate dry mass. Tukey's least significant differences tests were used to identify pairwise differences among the treatment means.

Analysis of covariance (ANCOVA) was used to determine whether treatment affected neonate "robustness"; that is, how heavy a neonate was relative to its SVL. Treatment effects on neonate water content and residual yolk mass were determined similarly using neonate dry mass as the covariate.

ANCOVA's were used to determine whether body mass and SVL of neonates differed among treatments at the end of each growth period (i.e., days 1-3 and days 3-9). For each analysis, the size measurement (averaged for each litter) immediately preceding the following size measurement was used as the covariate. For example, for the period day 3 to day 9, litter means for SVL on days 9 and 3 were used as the dependent variable and the covariate, respectively.

Results

Selected Body temperatures of females when pregnant versus postpartum

Females had significantly lower body temperatures when pregnant than when postpartum [Fig. 4.3; $F_{1,17} = 12.4$, $P = 0.001$, repeated-measures ANOVA]. Body temperature did not differ among the measurement times during the day for females in either reproductive condition ($P = 0.57$). Mean body temperatures of pregnant females were lower than those of postpartum females at 0900 h and 1200 h (P -values < 0.017), but not at 1500 h ($P > 0.017$). The overall mean body temperatures of females when pregnant and postpartum were $32.1 \pm 0.1^\circ\text{C}$ and $33.5 \pm 0.5^\circ\text{C}$, respectively.

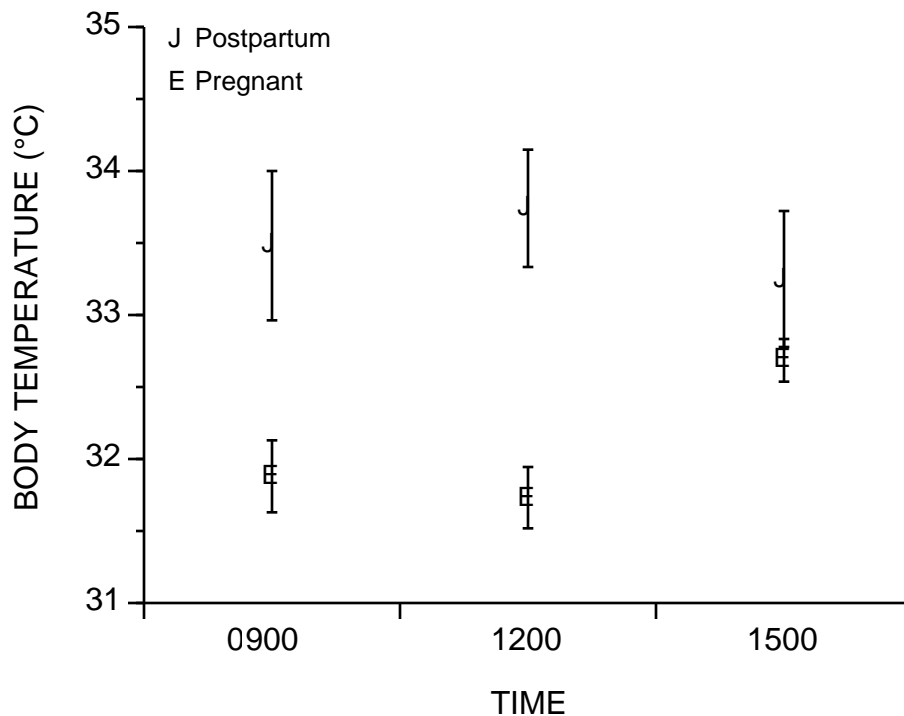


Figure 4.3 Mean selected body temperatures (± 1 SE) of female *Sceloporus jarrovii* ($n = 18$) when pregnant and postpartum measured at three times during the main portion of the daily activity period.

Females also regulated their body temperatures more precisely when pregnant than when postpartum (Fig. 4.3). Variance in body temperature when pregnant (0900 h: 1.1; 1200 h: 0.8; 1500 h: 0.4) was lower than when postpartum (0900 h: 4.9; 1200 h: 3.0; 1500 h: 4.0) at all three times of day (P-values < 0.017). Mean minimum body temperatures did not differ between pregnant ($31.3 \pm 0.2^\circ\text{C}$) and postpartum ($32.4 \pm 0.5^\circ\text{C}$) females (paired *t*-test; *t* = 1.8, *P* = 0.10). However, the mean maximum body temperature of pregnant females ($32.8 \pm 0.1^\circ\text{C}$) was lower than that of postpartum ($34.5 \pm 0.3^\circ\text{C}$) females (paired *t*-test; *t* = 5.1, *P* < 0.001).

Incubation experiment

Females gave birth between 20 and 50 days after initiation of experiments. The time to parturition varied among treatments ($F_{2,41} = 3.7$, *P* = 0.03). The mean time to parturition was 32.7 ± 1.9 d, 37.3 ± 1.9 d, and 40.0 ± 2.0 d for the 35°C, 32°C, and TREG treatments, respectively. Time to parturition for females in the 35°C treatment was shorter than that of females in the TREG treatment (*P* < 0.05). However, time to parturition did not differ between the 35°C and 32°C treatments or between the 32°C and TREG treatments (*P*-values > 0.05).

All females survived to the end of the experiment. On average, females in all treatments increased in SVL over the duration of the experiment. However, growth rates of females did not differ among treatments (ANOVA $F_{2,41} = 2.69$, *P* = 0.08). The mean size-specific growth rates of females were 0.0035 ± 0.0007 , 0.0044 ± 0.0007 , and 0.0060 ± 0.0008 mm/d for the 35°C, 32°C, and TREG treatments, respectively.

Treatment effects on female body condition, if any, should be most apparent at parturition. None were detected; the length-adjusted postpartum body mass of females did not differ significantly among treatments (ANCOVA $F_{2,39} = 1.4$, $P = 0.25$). At the grand covariate mean SVL (74.4 mm), the adjusted mean postpartum body masses of females were 11.5 ± 0.2 , 11.3 ± 0.2 , and 11.8 ± 0.2 g for the 35°C, 32°C, and TREG treatments, respectively.

Five females produced dead or abnormal offspring (Table 4.2). Abnormal neonates were weak and did not move normally. For the four females in the 35°C treatment that produced dead offspring, mortality varied within litters. Nearly all the offspring produced by two females were born dead whereas nearly all of those produced by the other two females were born alive and seemed normal. Of the three live, but abnormal, neonates born to females in the 35°C treatment, only one survived to day 9. All other neonates in the experiment survived, and seemed healthy on day 9.

Neonate mass (live) at parturition and maternal SVL were unrelated (linear regressions; P -values > 0.05), so female size was not used as a covariate in the following analyses. At parturition, neonates from the 35°C treatment were smaller in mass and SVL than neonates from the 32°C and TREG treatments (Table 4.1). Neonate size did not differ between the 32°C and TREG treatments. Neonates from the 35°C treatment were also smaller in dry mass although this difference was not significant. Neonates from the 35°C treatment had lower water content than neonates from the 32°C and TREG treatments. The small size of neonates from the 35°C treatment was not due to them being born "prematurely" (i.e., with a relatively large amount of internalized residual yolk); the least-squares means for dry mass of residual yolk did not differ significantly among treatments.

Table 4.1. Means and standard errors of dry mass, water content, dry residual yolk mass, morphology, and growth of offspring in the viviparous lizard *Sceloporus jarrovi* maintained under three incubation regimes

Trait	33° C Treatment	32° C Treatment	TREG Treatment	Statistical Test
Neonate dry mass at parturition (mg)	85.63 ± 2.36 a	91.63 ± 2.28 a	92.31 ± 2.36 a	ANOVA F = 2.45, df = 2,40 P = 0.10
Neonate water content (adjusted for neonate dry mass) at parturition (g)	0.52 ± .01 a	0.58 ± .01 b	0.57 ± .01 b	ANCOVA F = 5.35, df = 2,38 P = 0.009
Neonate dry residual yolk mass (adjusted for neonate dry mass) at parturition (µg)	6.18 ± 1.92 a	6.93 ± 1.78 a	6.35 ± 1.90 a	ANCOVA F = 0.05, df = 2, 30 P = 0.95
Neonate mass (g)				
At parturition	0.59 ± .02 a	0.66 ± .02 b	0.66 ± .02 b	ANOVA F = 7.37, df = 2,41 P = 0.002
At 9 days of age	0.89 ± .03 a	0.98 ± .03 a	0.96 ± .03 a	ANOVA F = 2.63, df = 2,39 P = 0.08
Neonate SVL (mm)				
At parturition	25.83 ± .19 a	26.85 ± .19 b	26.72 ± .20 b	ANOVA F = 8.56, df = 2,41 P < 0.001
At 9 days of age	29.45 ± .28 a	30.66 ± .28 b	30.33 ± .28 b	ANOVA F = 4.96, df = 2,39 P = 0.012
SVL-adjusted neonate mass (g)				
At parturition	0.63 ± .01 a	0.64 ± .01 a	0.64 ± .01 a	ANCOVA F = 0.11, df = 2,40 P = 0.90
At 9 days of age	0.96 ± .01 a	0.93 ± .01 a	0.94 ± .01 a	ANCOVA F = 1.19, df = 2,38 P = 0.31
Mass-adjusted (previous) neonate mass (g)				
At 3 days of age	0.66 ± .01 a	0.67 ± .01 a	0.66 ± .01 a	ANCOVA F = 0.35, df = 2,40 P = 0.71
At 9 days of age	0.94 ± .01 a	0.93 ± .02 a	0.95 ± .02 a	ANCOVA F = 0.19, df = 2,38 P = 0.83
SVL-adjusted (previous) neonate SVL (mm)				
At 3 days of age	26.94 ± .10 a	26.92 ± .10 a	26.81 ± .10 a	ANCOVA F = 0.51, df = 2,40 P = 0.61
At 9 days of age	30.02 ± .19 a	30.21 ± .19 a	30.21 ± .18 a	ANCOVA F = 0.31, df = 2,38 P = 0.74

Note. Table shows least-squares means ± SE; mean value ± SE are given for neonate dry mass at parturition, neonate mass, and neonate SVL. Interaction terms were not significant in any of the ANCOVA's. Means followed by the same letters were not significantly different (P > .05). TREG Treatment denotes pregnant females that were allowed to thermoregulate.

Table 4. 2 Condition of offspring from *Scotoplanus jarrovi* females that produced at least one abnormal decedembryo or neonate.

Treatment	Female #	Neonate/Embryo Condition at Parturition		
		Normal (n)	Abnormal (n)	Dead (n)
35°C	19	0	1	6 (Stage 40)
	33	10	0	1 (Stage 33)
	20	9	1	0
32°C	15	0	1	5 (Stage 40.2; Stage 33-36.3*)
	29	4	0	1 (Stage 33)

Number following the colon indicates the number of embryos dead at that developmental stage. * Embryos too decomposed to stage precisely

While neonates from the 35°C treatment were relatively small, SVL-adjusted neonate mass at parturition did not differ among treatments. Thus, neonates from the 35°C treatment were just as "robust" for their SVL as neonates from the other treatments.

Differences in neonate size among treatments persisted over time. At nine days of age, neonates from the 35°C treatment were still significantly smaller in SVL than those from the 32°C and TREG treatments. Mean body mass of neonates from the 35°C treatment was also still less than that of neonates from the other treatments, but the difference was not significant. As was true at parturition, length-adjusted neonate mass at nine days of age did not differ among treatments.

Neonates from all treatments increased in mass and SVL over the observation period at similar rates (Table 4.1). Neonate mass (adjusted for masses at the previous weighing) did not differ among treatments on days 3 and 9 (Table 4.1). Likewise, neonate SVL (adjusted for SVL's at the previous measuring) did not differ among treatments on these days.

Discussion

Selected body temperatures

Female *S. jarrovi* in the laboratory selected lower body temperatures when pregnant than when postpartum even though higher body temperatures would have been easily attainable. Furthermore, the mean selected body temperature of pregnant females in this study (32.1°C) was virtually identical to the mean body temperature of field-active pregnant females (Beuchat 1986: 32.0°C). These data suggest that field-active pregnant

females select low body temperatures and that ecological constraints (e.g., the encumbrance of the clutch) do not affect thermoregulatory behavior. However, without information on the operative field temperatures (see Hertz 1992; Hertz et al. 1993) available to pregnant and postpartum females, I cannot be certain as to how difficult it would be for pregnant females to maintain body temperatures as high as those of postpartum females. But since the difference in mean field-active body temperatures of postpartum and pregnant females is small, it seems likely that pregnant females could maintain higher field-active body temperatures if they wanted to. I also do not know the extent to which my estimates of selected body temperatures might have been affected by seasonal variation in selected body temperature as body temperatures of postpartum and pregnant females were measured at different times. However, selected body temperatures of *Sceloporus occidentalis* do not vary seasonally (McGinnis 1966).

Variances and ranges of selected body temperatures provided additional insights into the nature of the shift to low body temperatures by pregnant females. Females selected body temperatures that were less variable during pregnancy than when postpartum at each of the three measurement times during the day. Increased precision of thermoregulation by reproductive females (both in laboratory and field studies) is well documented (Stewart 1984; Beuchat 1986; Gier, Wallace and Ingerman 1989; Charland and Gregory 1990) although not always observed (Schwarzkopf and Shine 1991; Daut and Andrews 1993).

One explanation why females increase their precision of thermoregulation when reproductive is that offspring fitness is optimized at a species-specific temperature (Beuchat 1986). Therefore, reproductive females should regulate body temperatures closely around this temperature. However, a statistical (unintentional on the female's part) "increase in precision" could result if offspring fitness is adversely affected by a particular temperature extreme and females that are otherwise thermoregulating normally, simply avoid

temperatures approaching this extreme. Lizards do seem to thermoregulate between upper and lower set-point temperatures, rather than around a single body temperature (Berk and Heath 1975; Barber and Crawford 1977). Furthermore, set-points may vary with reproductive state (Patterson and Davies 1978; Sievert and Hutchison 1988). I used the maximum and minimum body temperatures selected by pregnant and postpartum *S. jarrovii* females on the thermal gradient to estimate these set-points. The upper set point was lowered during pregnancy; the mean maximum body temperature females when pregnant was lower than when postpartum. However, pregnancy did not influence the position of the lower set-point; there was no difference in the mean minimum body temperature of females when pregnant and when postpartum. Indeed, a lowering of the lower set-point temperature would not be expected if the length of gestation is related to costs of reproduction. Thus, pregnant females exhibit low mean body temperatures because they avoid upper temperature extremes that are tolerated when postpartum. Consequently, the relatively low variance in body temperatures exhibited by pregnant females may be the inevitable result of lowering the upper set-point temperature against a stationary lower set-point temperature rather than a reduction in variance about a single optimal temperature for development.

Effects of incubation temperature on females and offspring. Do female *S. jarrovii* select lower body temperatures when pregnant than when postpartum because postpartum body temperatures are detrimental to the female, to the embryos, or both? In general, I found that the physical condition of pregnant females was not affected by any of the experimental temperature treatments. However, the fitness of neonates that had been exposed to the 35°C treatment during embryogenesis was reduced.

The growth and survival of females did not differ among treatments. Growth (in SVL) of females in the 35°C treatment was the same as that of females in the other

treatments. In concordance with this result, treatment temperature had no effect on body condition (size-adjusted body mass). Postpartum females in the 35°C treatment had virtually the same body mass for their SVL as postpartum females in the other two treatments. Moreover, all females in this study survived to the end of the experiment. These results differ markedly from those of a parallel study on the effects of temperature during the gestation period of *S. jarrovi* (Beuchat 1988). Beuchat (1988) observed approximately 40% mortality of females in her 36°C and 32°C temperature treatments, and surviving lizards in her 36°C temperature treatment lost weight during the experiment. Unlike this study, however, lizards in Beuchat's (1988) study were maintained under constant temperature regimes. Constant high temperatures are particularly stressful to squamates, and cause high mortality (Licht 1965; Shine 1983b) suggesting why Beuchat's (1988) results differ so much from those reported in this paper.

In contrast to the absence of treatment effects on the physical condition of females, temperature treatment had a strong effect on embryonic development. Neonates produced by pregnant females from the 35°C treatment were smaller in live body mass and in SVL than those produced by pregnant females in the 32°C and TREG treatments. At parturition, neonates from the 35°C treatment were approximately 10.6 % lighter in mass and 3.6% shorter in SVL than neonates from the other treatments. Beuchat (1988) also observed a decrease in the body size of neonates that were exposed high incubation temperatures.

Mean incubation temperature was related to the mean time to parturition. Females in the 35°C treatment gave birth an average of 4 and 7 d, respectively, before females in the 32°C and TREG treatments. Thus, pregnant females could conceivably reduce costs of reproduction and increase offspring fitness by selecting high body temperatures. However, the detrimental effects of high body temperatures on neonate size probably outweigh these advantages. Indeed, the 4 d difference (not significant) in mean time to parturition for

females in the 35°C and 32 ° treatments seems trivial compared to the lasting effects of high incubation temperature on offspring body size.

I observed very few dead or abnormal offspring overall. However, four of the five litters with dead and or abnormal neonates were in the 35°C treatment, suggesting that embryos were subjected to some level of thermal stress during gestation. In contrast, Beuchat (1988) observed a large number of dead or abnormal offspring (> 60% in some treatments). Such high morbidities are even more striking considering females in Beuchat's (1988) study were field-collected and placed under experimental conditions much later in the season (~ 1 month later) than the females in this study; hence, these embryos were only under experimental conditions for a few weeks before parturition. These results presumably also reflect high levels of experimentally induced stress on pregnant females and their embryos caused by constant high temperatures.

The relatively low body mass of neonates from the 35°C treatment was not entirely due to their shorter SVL; these neonates also had relatively low water contents. In accord, neonates exhibited greater similarity in dry mass than wet mass among treatments (dry mass was, however, lowest for neonates in the 35°C treatment). The most likely site for the observed water deficiency is the urinary bladder. At parturition, the bladder of neonatal *S. jarrovii* contains dilute urine that comprises approximately 13.6 % of their total body mass (Beuchat, Vleck and Braun 1986). Using this value and the mean body mass of neonates in the 32°C and TREG treatments (0.66 g, Table 4.1), I predicted that the bladder of a fully hydrated neonate would contain a fluid content of 0.09 g. Actual body water content of neonates from the 35°C treatment was approximately 0.06 g less than that of neonates from the other two treatments. Thus, the deficiency in body water content exhibited by neonates from the 35°C treatment could have been due to an incompletely filled (~ 33.3% full) urinary bladder. Such a reduction in fluid content of the bladder could adversely affect

neonate survival because the bladder may serve as a reserve of reabsorbable water that is used to buffer body tissues against osmotic perturbation during the first few days following parturition (Beuchat et al. 1986).

Differences in SVL between neonates from the 35°C treatment and neonates from the 32°C and TREG treatments persisted until at least 9 days of age. The effect of treatment on body mass was lessened through time; body mass of neonates did not differ significantly among treatments at 9 d although neonates from the 35°C treatment were still lighter on average than neonates from the other treatments. However, despite their smaller body size, neonates from the 35°C treatment were no less robust than neonates from the other treatments. Thus, for the traits I measured, the overall effect of high incubation temperature (35°C treatment) on offspring phenotype was a reduction in body size (particularly SVL) of otherwise normally proportioned offspring.

Variation in body size of the magnitude reported here can affect neonate survival and fitness. Large juvenile lizards have been shown to survive better than small juveniles (Fox 1978; Ferguson and Fox 1984), occupy more optimal habitat (Fox 1978), and be socially dominant over conspecifics (Fox and Rostker 1982). Differential survival between smaller and larger juveniles can be amplified when competition for resources is increased (Ferguson and Fox 1984). Thus, it is possible that I did not observe differences in growth among neonates from the different temperature treatments because food was always available in excess.

My results demonstrate that while temperatures near the upper set-point for postpartum females are not detrimental to pregnant females, pregnant females actively select relatively low body temperatures. Observations on the effect of relatively high body temperature on embryonic development, indicate that pregnant females avoid high

temperatures during pregnancy because such temperatures are deleterious to their offspring. Specifically, high temperature during gestation results in relatively small, and presumably less fit, offspring.

References

- Adolph S. C. 1987. Physiological and behavioral ecology of the lizards *Sceloporus occidentalis* and *Sceloporus graciosus*. Dissertation. University of Washington. Seattle, Washington.
- Ackerman R. A. 1981. Oxygen consumption by sea turtle (*Chelonia*, *Caretta*) eggs during development. *Physiol. Zool.* 54:316-324.
- Ackerman R. A. 1981. Growth and gas exchange of embryonic sea turtles (*Chelonia*, *Caretta*). *Copeia* 1981:757-765.
- Ackerman R. A., R. Dmi'el, and A. Ar. 1984. Energy and water vapor exchange by parchment-shelled reptile eggs. *Physiol. Zool.* 58:129-137.
- Andrews R. M. and O. J. Sexton. 1981. Water relations of the eggs of *Anolis auratus* and *Anolis limifrons*. *Ecology* 62:556-562.
- Andrews R. M. and B. R. Rose. 1994. Evolution of viviparity: constraints on egg retention. *Physiol. Zool.* 67:1006-1024.
- Andrews R. M. 1997. Evolution of viviparity: variation between two sceloporine lizards in the ability to extend egg retention. *J. Zool., Lond.* 243:579-595.
- Ar A., C. V. Paganelli, R. B. Reeves, D. G. Greene, and H. Rahn. 1974. The avian egg: water vapor conductance, shell thickness, and functional pore area. *Condor* 76:153-158.
- Ar A. and H. Rahn. 1985. Pores in avian eggshells: gas conductance, gas exchange, and embryonic growth rate. *Respir. Physiol.* 61:1-20.
- Barber B. J. and E. C. Crawford. 1977. A stochastic dual-limit hypothesis for behavioral thermoregulation in lizards. *Physiol. Zool.* 50:53-60.
- Bauwens D. and C. Thoen. 1981. Escape tactics and vulnerability to predation associated with reproduction in the lizard *Lacerta vivipara*. *J. Anim. Ecol.* 50:733-743.

- Berk M. L. and J. E. Heath. 1975. An analysis of behavioral thermoregulation in the lizard *Dipsosaurus dorsalis*. J. Therm. Biol. 1:15-22.
- Beuchat C. A. 1986. Reproduction influences on the thermoregulation behavior of a live-bearing lizard. Copeia 1986:971-979.
- Beuchat C. A. 1988. Temperature effects during gestation in a viviparous lizard. J. Therm. Biol. 13:135-142.
- Beuchat C. A., D. Vleck, and E. J. Braun. 1986. Role of the urinary bladder in osmotic regulation of neonatal lizards. Physiol. Zool. 59:539-551.
- Billet F., C. Gans, and P. F. A. Maderson. 1985. Why study reptilian development? Pp.1-39 in F. Billet and P. F. A. Maderson, eds. Biology of the Reptilia. Vol. 15, John Wiley and Sons, New York.
- Blackburn D. G. 1982. Evolutionary origins of viviparity in the Reptilia. I. Sauria. Amphib.-Reptilia. 3:185-205.
- Blackburn D. G. 1993. Chorioallantoic placentation in squamate reptiles: structure, function, development, and evolution. J. exp. Zool. 266:414-430.
- Blackburn D. G. 1995. Saltationist and punctuated equilibrium models for the evolution of viviparity and placentation. J. theor. Biol. 174:199-216.
- Bradshaw S. D. 1986. Ecophysiology of Desert Reptiles. Academic Press, Sydney.
- Braña F. 1993. Shifts in body temperature and escape behavior of female *Podarcis muralis* during pregnancy. Oikos 66:216-222.
- Charland M. B. and P. T. Gregory. 1990. The influence of female reproductive status on thermoregulation in a viviparous snake, *Crotalus viridis*. Copeia 1990:1089-1098.
- Cowles R. B. and C. M. Bogert. 1944. A preliminary study of the thermal requirements of desert reptiles. Bull. Am. Mus. Natural His. 83:261-296.
- Crenshaw J. W., Jr. 1955. The life history of the southern spiny lizard *Sceloporus undulatus undulatus* Latreille. Am. Midl. Nat. 54:257-298.

- Cuellar O. 1984. Reproduction in a parthenogenetic lizard: with a discussion of optimal clutch size and a critique of the clutch weight/body weight ratio. *Am. Midl. Nat.* 11:242-258.
- Daut E. F. and R. M. Andrews. 1993. The effect of pregnancy on the thermoregulatory behavior of the viviparous lizard *Chalcides ocellatus*. *J. Herpetol.* 27:6-13.
- Deeming D. C. and M. W. J. Ferguson. 1991. Reduction in eggshell conductance to respiratory gases has no effect on sex determination in *Alligator mississippiensis*. *Copeia* 1991:240-243.
- Deeming D. C. and M. B. Thompson. 1991. Gas exchange across reptilian eggshells. Pp. 277-284 in D. C. Deeming and M. W. J. Ferguson, eds. *Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles*. Cambridge University Press, Cambridge.
- Degenhardt W. G., C. W. Painter, and A. H. Price. The Amphibians and reptiles of New Mexico. University of New Mexico Press, Albuquerque.
- DeMarco V. 1992. Embryonic developmental times and egg retention in four species of sceloporine lizards. *Funct. Ecol.* 6:436-444.
- DeMarco V. 1993. Estimating egg retention times in sceloporine lizards. *J. Herpetol.* 27:453-458.
- Dufaure J. P. and J. Hubert. 1961. Table de développement du lézard vivipare: *Lacerta (Zootica) vivipara* Jaquin. *Arch. Anat. Microsc. Morphol. Exp.* 50:309-328.
- Ewert M. A. 1985. Embryology of turtles. Pp. 75-267 in C. Gans ed. *Biology of the Reptilia*. Vol. 14, John Wiley and Sons, New York.
- Ewert M. A. 1991. Egg incubation: its effects on embryonic development in birds and reptiles. Pp. 173-191 in D. C. Deeming and M. W. J. Ferguson, eds. *Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles*. Cambridge University Press, Cambridge.

- Feder M. E., S. L. Satel, and A. G. Gibbs. 1982. Resistance of the shell membrane and mineral layer to diffusion of oxygen and water in flexible-shelled eggs of the snapping turtle (*Chelydra serpentina*). *Respir. Physiol.* 49:279-291.
- Ferguson G. W. and S. F. Fox. 1984. Annual variation of survival advantage of large juvenile side-blotched lizards, *Uta stansburiana*: its causes and evolutionary significance. *Evolution* 38:342-349.
- Fitch H. S. 1970. Reproductive cycles in lizards and snakes. *Univ. Kansas Mus. Nat. Hist. Publ.* 52:1-247.
- Fox S. F. 1978. Natural selection on behavioral phenotypes of the lizard *Uta stansburiana*. *Ecology* 59:834-847.
- Fox S. F. and M. A. Rosker. 1982. Social cost of tail loss in *Uta stansburiana*. *Science* 218:692-693.
- Gillis R. and R. E. Ballinger. 1992. Reproductive ecology of red-chinned lizards (*Sceloporus undulatus erythrocheilus*) in Southcentral Colorado: comparisons with other populations of a wide-ranging species. *Oecologia.* 89:236-243.
- Gier P. J., R. L. Wallace, and R. L. Ingerman. 1989. Influence of pregnancy on behavioral thermoregulation in the northern Pacific rattlesnake *Crotalus viridis oreganus*. *J. exp. Biol.* 145:465-469.
- Goldberg S. R. 1971. Reproductive cycle of the ovoviviparous iguanid lizard *Sceloporus jarrovi* Cope. *Herpetologica* 27:123-131.
- Greer A. E. 1989. *The Biology and Evolution of Australian lizards*. Surrey Beatty and Sons, Sydney, Australia.
- Guillette L. J. Jr., R. E. Jones, K. T. Fitzgerald, and H. M. Smith. 1980. Evolution of viviparity in the lizard genus *Sceloporus*. *Herpetologica* 36:201-215.
- Guillette L. J. Jr. 1982. The evolution of viviparity and placentation in the high-altitude, Mexican lizard *Sceloporus aeneus*. *Herpetologica* 38:94-103.

- Guillette L. J., Jr. and R. E. Jones. 1985. Ovarian, oviductal, and placental morphology of the reproductively bimodal lizard, *Sceloporus aeneus*. J. Morph. 184:85-98.
- Guillette L. J. Jr. 1989. The evolution of vertebrate viviparity: morphological modifications and endocrine control. Pp. 219-233 in D. B. Wake and G. Roth, eds. Complex Organismal Functions: Integration and Evolution in Vertebrates. John Wiley and Sons, Ltd.
- Guillette L. J. Jr. 1993. The evolution of viviparity in lizards. Bioscience 43:742-751.
- Guillette L. J. Jr., S. L. Fox, and B. D. Palmer. 1989. Oviductal morphology and Egg Shelling in the Oviparous Lizards *Crotophytus collaris* and *Eumeces obsoletus*. J. Morphol. 201:145-159.
- Gutzke W. H. N. and G. C. Packard. 1987. Influence of the hydric and thermal environments on eggs and hatchlings of bull snakes *Pituophis melanoleucus*. Physiol. Zool. 60:9-17.
- Hertz P. E. 1992. Evaluating thermal resource partitioning by sympatric *Anolis cooki* and *A. cristatellus*: a field test using null hypotheses. Oecologia 90:127-136.
- Hertz P. E., R. B. Huey, and R. D. Stevenson. 1993. Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. Am. Natur. 142:798-818.
- Heulin B. 1990. Étude comparative de la membrane coquillère chez les souches ovipare et vivipare du lézard *Lacerta vivipara*. Can. J. Zool. 68:1015-1019.
- Huey R. B. 1982. Temperature, physiology, and the ecology of reptiles. Pp. 25-92 in C. Gans and F. H. Pough, eds. Biology of the Reptilia. Vol 12, Academic Press, New York.
- Kam Y-C. 1993. Physiological effects of hypoxia on metabolism and growth of turtle embryos. Respir. Physiol. 92:127-138.

- Kennet R. M., K. Georges, and M. Palmer-Allen. 1993. Developmental arrest during immersion of eggs of a tropical freshwater turtle, *Chelodina rugosa* (Testudinata: Chelidae), from northern Australia. *Aust. J. Zool.* 41:47-52.
- Kern M. D. and W. J. Ferguson. 1997. Gas permeability of American alligator eggs and its anatomical basis. *Physiol. Zool.* 70:530-546.
- Licht P. 1965. The relation between preferred body temperature and testicular heat sensitivity in lizards. *Copeia* 1965:428-436.
- Masson G. R. and L. J. Guillette, Jr. 1987. Changes in the oviductal vascularity during the reproductive cycle of three oviparous lizards (*Eumeces obsoletus*, *Sceloporus undulatus*, and *Crotaphytus collaris*). *J. Reprod. Fert.* 80:361-371.
- Mathies T. and R. M. Andrews. 1995. Thermal and reproductive biology of high and low elevation populations of *Sceloporus scalaris*. *Oecologia* 104:101-111.
- Mathies T. and R. M. Andrews. 1996. Extended egg retention and its influence on embryonic development and egg water balance: implications for the evolution of viviparity. *Physiol. Zool.* 69:1021-1035.
- McGinnis S. M. 1966. *Sceloporus occidentalis*: preferred body temperature of the western fence lizard. *Science* 152:1090-1091.
- Mead R. A. 1993. Embryonic diapause in vertebrates. *J. exp. Biol.* 266:629-641.
- Méndez-de la Cruz F. R., M. Villagrán-Santa Cruz, and R. M. Andrews. 1998. Evolution of viviparity in the lizard genus *Sceloporus*. *Herpetologica*, In Press.
- Mink D. G. and J. W. Sites, Jr. 1996. Species limits, phylogenetic relationships, and the origins of viviparity in the scalaris complex of the lizard genus *Sceloporus* (Phrynosomatidae: Sauria). *Herpetologica* 52:551-571.
- Mossman H. W. 1937. Comparative morphogenesis of the fetal membranes and accessory uterine structures. *Carnegie Inst. Contrib. Embryol.* 26:129-246.
- Muth A. 1980. Physiological ecology of desert iguana (*Dipsosaurus dorsalis*) eggs: temperature and water relations. *Ecology* 61, 1335-1343.

- Neill W. T. 1964. Viviparity in snakes: some ecological and zoogeographical considerations. *Am. Natur.* 48:35-55.
- Packard G. C. 1966. The influence of ambient temperature and aridity on modes of reproduction and excretion of amniote vertebrates. *Am. Natur.* 100:667-682.
- Packard G. C., C. R. Tracy, and J. J. Roth. 1977. The physiological ecology of reptile eggs and embryos, and the evolution of viviparity within the class Reptilia. *Biol. Rev.* 52:71-105.
- Packard G. C., M. J. Packard, K. Miller, and T. J. Boardman. 1987. Influence of moisture, temperature, and substrate on snapping turtle eggs and embryos. *Ecology* 78:983-993.
- Packard M. J., L. K. Burns, K. F. Hirsch, and G. C. Packard. 1982a. Structure of shells of eggs of *Callisaurus draconoides* (Reptilia, Squamata, Iguanidae). *Zool. J. Linnean Society* 75:297-316.
- Packard M. J., G. C. Packard, and T. J. Boardman. 1982b. Structure of eggshells and water relations of reptilian eggs. *Herpetologica* 38:136-155.
- Packard M. J. and G. C. Packard. 1988. The physiological ecology of reptilian eggs and embryos. Pp. 523-605 in C. Gans, R. B. Huey, eds. *Biology of the Reptilia*. Vol. 16, Ecology B. Alan R. Liss, Inc., New York.
- Packard M. J. and V. G. DeMarco. 1991. Eggshell structure and formation in eggs of oviparous reptiles. Pp. 53-69 in D. C. Deeming and M. W. J. Ferguson, eds. *Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles*. Cambridge University Press, Cambridge.
- Paganelli C. V., A. Olszowka, and A. Ar. 1974. The avian egg: Surface area, volume and density. *Condor* 76:319-325.
- Patterson J. W. and P. M. Davies. 1978. Preferred body temperature: seasonal and sexual differences in the lizard *Lacerta vivipara*. *J. Therm. Biol.* 3:39-41.

- Pianka E. R. 1986. Ecology and natural history of desert lizards. Princeton University Press, Princeton, New Jersey.
- Palmer B. D., V. G. DeMarco, and L. J. Guillette, Jr. 1993. Oviductal morphology and eggshell formation in the lizard, *Sceloporus woodi*. J. Morphol. 217:205-217.
- Panigel M. 1951. Rapports anatomo-histologiques établis au cours de la gestation entre l'oeuf et l'oviducte maternel chez le lézard ovovivipare *Zootoca vivipara* W. (*Lacerta vivipara* J.) Bull. Soc. Zool. Fr. 76:163-170.
- Pough H. F. and C. Gans. 1982. The vocabulary of reptilian thermoregulation. Pp.17-23 in C. Gans and F. H. Pough, eds. Biology of the Reptilia. Vol. 12, Academic Press, New York.
- Qualls C. P. 1996. Influence of the evolution of viviparity on eggshell morphology in the lizard, *Lerista bougainvillii*. J. Morphol. 228:119-125.
- Qualls C. P., R. M. Andrews, and T. Mathies. 1997. The evolution of viviparity and placentation revisited. J. theor. Biol. 185:129-135.
- SAS Institute Inc. 1985. SAS Users Guide: Statistics, Version 5 Edition. Cary, NC, USA.
- Schleich, H. H. and W. Kästle. Reptile Egg-Shells SEM Atlas. Stuttgart: Gustav Fischer.
- Schwarzkopf L. and R. Shine. 1991. Thermal biology of reproduction in viviparous skinks, *Eulamprus tympanum*: why do gravid females bask more? Oecologia 88:562-569.
- Sergeev A. M. 1940. Researches on the viviparity of reptiles. Mosc. Soc. Nat. 1-34.
- Seigel R. A. and H. S. Fitch. 1984. Ecological patterns of relative clutch mass in snakes. Oecologia 61:293-301.
- Sexton O. J. and K. R. Marion. 1974. Duration of incubation of *Sceloporus undulatus* eggs at constant temperature. Physiol. Zool. 47:91-98.

- Shadrix C. A., D. R. Crotzer, S. L. McKinney, and J. R. Stewart. 1994. Embryonic growth and calcium mobilization in oviposited eggs of the scincid lizard, *Eumeces fasciatus*. *Copeia* 1994:493-498.
- Shine R. 1980. "Costs" of reproduction in reptiles. *Oecologia (Berl.)* 46:92-100.
- Shine R. 1983a. Reptilian reproductive modes: the oviparity—viviparity continuum. *Herpetologica* 39:1-8.
- Shine R. 1983b. Reptilian viviparity in cold climates: testing the assumptions of an evolutionary hypothesis. *Oecologia* 57:397-405.
- Shine R. 1985. The evolution of viviparity in reptiles: an ecological analysis. Pp. 605-694 in C. Gans, F. Billett, eds. *Biology of the Reptilia*. Vol. 15, John Wiley and Sons, New York.
- Shine R. 1991. Influences of incubation requirements on the evolution of viviparity. Pp. 361-369 in D. C. Deeming and M. W. J. Ferguson, eds. *Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles*. Cambridge University Press, Cambridge.
- Shine R. 1995. A new hypothesis for the evolution of viviparity. *Am. Natur.* 145:809-823.
- Shine R. and P.S. Harlow. Maternal manipulation of offspring phenotypes via nest-site selection in an oviparous lizard. *Ecology* 77:1808-1817.
- Sievert L. M. and V. H. Hutchison. 1988. Light vs. heat: thermoregulatory behavior in a nocturnal lizard (*Gecko gecko*). *Herpetologica* 44:266-273.
- Sinervo B., R. Hedges, and S. C. Adolph. 1991. Decreased sprint speed as a cost of reproduction in the lizard *Sceloporus occidentalis*: variation among populations. *J. exp. Biol.* 155:323-336.
- Sites J. W. Jr., J. W. Archie, C. J. Cole, and O. Flores Villela. 1992. A review of phylogenetic hypotheses for lizards of the genus *Sceloporus* (Phrynosomatidae). *Bull. Am. Mus. Nat. Hist.* 213:1-110

- Smith G. R. and R. E. Ballinger. 1994. Temperature relationships in the high-altitude viviparous lizard, *Sceloporus jarrovi*. *Am. Midl. Natur.* 131:181-189.
- Smith H. M. 1967. *Handbook of lizards*. Cornell University Press, Ithaca, New York.
- Smith S. A. and R. Shine. 1997. Intraspecific variation in reproductive mode within the scincid lizard *Saiphos equalis*. *Aust. J. Zool.* 45:435-445.
- Stamps J. A. 1976. Egg retention, rainfall and egg laying in a tropical lizard, *Anolis aeneus*. *Copeia* 1976:759-764.
- Stearns S. C. 1989. *The evolution of life histories*. Oxford University Press, New York.
- Stevenson R. D., C. R. Peterson, and J. S. Tsuji. 1985. The thermal dependence of locomotion, tongue flicking, digestion, and oxygen consumption in the wandering garter snake. *Physiol. Zool.* 58:46-57.
- Stewart J. R. 1984. Thermal biology of the live bearing lizard *Gerrhonotus coeruleus*. *Herpetologica* 40:349-355.
- Stewart J. R. 1989. Facultative placentotrophy and the evolution of squamate placentation: Quality of eggs and neonates in *Virginia striatula*. *Am. Natur.* 133:111-137.
- Stewart J. R. and M. B. Thompson. 1993. A novel pattern of embryonic nutrition in a viviparous reptile. *J. expl. Biol.* 174:97-108.
- Thompson M. B. and K. N. Goldie. 1990. Conductance and structure of eggs of Adelie Penguins, *Pygoscelis adeliae*, and its implications for incubation. *Condor* 92:304-312.
- Thompson M. B. 1985. The functional significance of the opaque white patch in eggs of *Emydura macquarii*. Pp. 387-385 in G. Grigg, R. Shine, and H. Ehmann, eds. *Biology of Australian Frogs and Reptiles*. Surrey Beatty Pty Ltd, Sydney.
- Tinkle D. W. and J. W. Gibbons. 1977. The distribution and evolution of viviparity in reptiles. *Misc. Publ. Univ. of Mich.* 154:1-55.

- Tinkle D. W. and A. E. Dunham. 1983. Demography of the tree lizard, *Urosaurus ornatus* in central Arizona. *Copeia* 1983:585-598.
- Trauth S. F. and W. R. Fagerberg. 1984. Ultrastructure and stereology of the eggshell in *Cnemidophorus sexlineatus* (Lacertilia: Teiidae). *Copeia* 1984:826-832.
- Tyler C. 1965. A study of the eggshells of the Sphenisciformes. *J. Zool. (Lond.)* 147:1-19.
- Vinegar A. 1974. Evolutionary implications of temperature induced anomalies of development in snake embryos. *Herpetologica* 30:72-74.
- Vitt L. J. 1977. Observations on clutch and egg size and evidence for multiple clutches in some lizards of the southwestern United States. *Herpetologica* 33:333-338.
- Weekes H. C. 1927. Placentation and other phenomena in the scincid lizard *Lygosoma (Hinulia) quoyi*. *Proc. Zool. Soc. N. S. W.* 52:499-554.
- Weekes H. C. 1935. A review of placentation among reptiles, with particular regard to the function and evolution of the placenta. *Proc. Zool. Soc. London.* 2:625-645.
- Wiens J. J. and T. W. Reeder. 1997. Phylogeny of the spiny lizards (*Sceloporus*) based on molecular and morphological evidence. *Herpetol. Monographs* 11:1-101.
- Xavier F. and J. Gavaud. 1986. Oviparity—viviparity continuum in reptiles, physiological characteristics and relation with the environment. Dans *Endocrine regulation as adaptive mechanisms to the environment*. Editeurs: I. Assenmacher et J. Boissin. Centre national de la recherche scientifique, Paris. pp. 79-93.
- Yaron Z. 1985. Reptile placentation and gestation: structure, function, and endocrine control. Pp. 527-603 in C. Gans, F. Billett, eds. *Biology of the Reptilia*. Vol. 15, John Wiley and Sons, New York.

Curriculum Vitae

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| 1991-1994 | Virginia Polytechnic Institute and State University, Master of Science, Biology, 1994 |
| 1988-1991 | University of Washington, Bachelor of Science, Zoology, 1991 |
| 1986-1988 | Seattle Central Community College |
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Professional Experience

1994-1997 Graduate Teaching Assistant, Virginia Polytechnic Institute
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Publications

Dunlap, K. D. and T. C. Mathies. 1994. The effects of nymphal ticks and their interaction with malaria on the physiology of male fence lizards. *Copeia*, 1994:1045-1048.

Mathies, T. and R. M. Andrews. 1995. Thermal and reproductive biology of high and low elevation populations of *Sceloporus scalaris*: implications for the evolution of viviparity. *Oecologia*, 104:101-111.

Mathies, T. and R. M. Andrews. 1996. Extended egg retention and its influence on embryonic development and egg water balance: implications for the evolution of viviparity. *Physiological Zoology*, 69:1021-1035.

Qualls C. P., Andrews R. M. and T. Mathies. 1997. Evolution of Viviparity Revisited. *Journal of Theoretical Biology*, 185:129-135.

Mathies, T. and R. M. Andrews. 1997. Influence of pregnancy on the thermal biology of the lizard, *Sceloporus jarrovi*: why do pregnant females exhibit low body temperatures? *Functional Ecology*, 11:498-507.

Manuscripts (submitted or in prep.)

Andrews R. M., Mathies T., Qualls C. P., and F. Qualls. Rate of embryonic development of *Sceloporus* lizards: do cold climates favor rapid development? Submitted.

Abstracts and Presentations

Mathies, T. C., and Andrews, R. M. 1993. Comparative reproductive biology of lowland and montane populations of *Sceloporus scalaris*. Programs and abstracts, Combined meetings of the American Society of Ichthyologists and Herpetologists' League, University of Texas, Austin, p. 212.

Mathies, T. and R. M. Andrews. 1994. Effects of extended egg retention on embryonic development in an oviparous lizard, *Sceloporus scalaris*. Abstracts, Combined meetings of the Herpetologists' League and the Society for the study of reptiles and amphibians, University of Georgia, Athens, p. 53.

Mathies, T. and R. M. Andrews. 1996. Influence of pregnancy on the thermal biology of the lizard, *Sceloporus jarrovi*: why do pregnant females exhibit low body temperatures? Regional meeting of the Divisions of Comparative Endocrinology and Comparative Physiology and Biochemistry, Mountain Lake, Virginia, p 5.

Mathies, T. and R. M. Andrews. 1998. Developmental arrest determines embryonic stage at oviposition in the lizard, *Urosaurus ornatus*. Combined meetings of the American Society of Ichthyologists and Herpetologists' League, University of Guelph, Guelph, Ontario, Canada.

Andrews, R. M., T. Mathies, C. P. Qualls, and F. J. Qualls. Embryonic development of *Sceloporus* lizards: do cold climates favor rapid development? Combined meetings of the American Society of Ichthyologists and Herpetologists' League, University of Guelph, Guelph, Ontario, Canada. Presented by R. M. Andrews.

Grant Proposals Submitted

Physiological and ecological bases for the thermal behavior of a lizard during pregnancy.

Graduate Research Development Project. Rejected April 1995.

Grant Proposals Funded

Evolution of squamate viviparity: the effects of egg retention on embryonic O₂ availability in utero. Sigma Xi: \$390.00, matched by department. Funded June 1993.

Thermal biology of a lizard during pregnancy: is thermal behavior physiologically or ecologically based? Sigma Xi: \$650.00, matched by department. Funded April 1995.

Altered thermal behavior of a lizard during pregnancy: a physiological or ecological basis? Theodore Roosevelt Memorial Fund: \$500.00, matched by department. Funded April 1995.

Memberships in Professional Associations

Society for the Study of Reptiles and Amphibians

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