PHYSIOLOGICAL AND ECOLOGICAL CONSTRAINTS ON THE EVOLUTION OF VIVIPARITY IN SCELOPORINE LIZARDS

Scott L. Parker

Dissertation submitted to the faculty of Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY
In
Biological Sciences

Robin Andrews, Chairperson
D. Michael Denbow
Thomas Jenssen
Robert Jones
Anne McNabb

16 January 2006

Blacksburg, Virginia

Keywords: Squamate Reptiles, Sceloporus, Cold Climate Hypothesis, Physiological Constraints, Evolution, Distributional Limits

PHYSIOLOGICAL AND ECOLOGICAL CONSTRAINTS ON THE EVOLUTION OF VIVIPARITY IN SCELOPORINE LIZARDS

Scott L. Parker

ABSTRACT

Viviparity in reptiles evolves in response to cold environmental temperatures at high latitudes and high elevations through gradual increases in the duration of egg retention within the oviduct. The adaptive benefit of egg retention in cold climates is that embryos will develop faster at higher maternal body temperatures and therefore hatch earlier than embryos in eggs in a nest. In the majority of reptile species, however, egg retention past the normal time of oviposition results in retarded or arrested embryonic development. The paucity of reptile species that have the capacity to support embryonic development during extended egg retention suggest that physiological constraints prevent normal development from occurring during extended egg retention.

In Chapters one and two, I investigated the role of *in utero* oxygen availability as a constraint on the capacity to support embryonic development during extended egg retention. I incubated eggs of sceloporine lizards under conditions that simulated retention in the oviduct under a range of oxygen partial pressures. In Chapter one, I tested the hypothesis that embryos of the oviparous lizard *Sceloporus undulatus* from a high-latitude population are laid at more advanced developmental stages and have a higher developmental rate at low partial pressure oxygen (pO_2) under simulated *in utero* conditions than embryos from a low-latitude population. This hypothesis was rejected; embryos from the two populations did not differ in embryonic stage at oviposition or developmental rate when incubated under simulated *in utero* conditions at low pO_2. In Chapter two I tested the hypothesis that the degree of embryonic development attained by reptilian embryos *in utero* is directly related to *in utero* pO_2. The species chosen for the study differed in their capacity to support embryonic development during egg retention and were characterized by developmental arrest (*Urosaurus ornatus*), retarded development (*Sceloporus virgatus*), and normal development (*Sceloporus scalaris*) when eggs are retained

past the normal time of oviposition. The estimated *in utero* pO_2's for the three species increased in the order of *U. ornatus* (5-6 kPa) < *S. virgatus* (9-11 kPa) < *S. scalaris* (> 11 kPa). These results indicate that *in utero* oxygen availability is associated with interspecifc differences in the capacity to support embryonic development during extended egg retention.

Temperature is widely regarded as the primary factor responsible for the paucity of oviparous reptile species in cold climates. In Chapter three I tested the hypothesis that embryo thermal requirements set the northern distributional limit of *Sceloporus undulatus*, a widely distributed North American oviparous lizard species. To test this hypothesis, I incubated eggs of *Sceloporus undulatus* under conditions that simulated temperatures of nests within the geographic range of *S. undulatus* at 37 ° N and those of simulated nests beyond the northern distributional limit of *S. undulatus* at 42 and 44 °N. I evaluated the effects of incubation temperature on hatching success, incubation period, phenotypic traits (morphology, locomotor performance, growth), and hatchling survival. After hatching, snout-vent length (SVL), mass, tail length, body condition (SVL relative to mass), locomotor performance and growth rate were measured for each hatchling. Hatchlings were released at a field site to evaluate growth and survival under natural conditions. Incubation at temperatures simulating nests at 44 °N prolonged incubation and resulted in hatchlings with shorter SVL relative to mass, shorter tails, shorter hind limb span, slower growth and lower survival than hatchlings from eggs incubated at temperatures simulating nests at 37 and 42° N. I also predicted that the northernmost distributional limit of *S. undulatus* would be associated with locations that provide the minimum heat sum (degree-days) required to complete embryonic development. This prediction was upheld: 84% of location between 37-40 °N had ≥ 495 degree-days above a threshold of 17 °C accumulated during the summer incubation period (June-September) compared to 11% of locations between 41-50 °N. These results suggest that incubation temperatures at northern latitudes are not warm enough for a sufficient length of time to permit successful incubation of *S. undulatus* embryos. These results are consistent with the hypothesis that incubation temperature is an important factor limiting the geographic distributions of oviparous reptile species at high latitudes and high elevations.

DEDICATION

To my family, Bill, Joyce, and Robert and Karen.

Each one of you, in your own way, instilled in me the excitement of investigation and the passion for life-long learning. Without your unending love and encouragement, none of these accomplishments would have been possible.

ACKNOWLEDGEMENTS

I wish to express my heartfelt gratitude to Robin Andrews for giving me the opportunity to work in her lab. I could not have hoped for a better advisor and mentor to guide me through my doctoral research. Robin had the uncanny ability to know when to provide close supervision but also when to give autonomy. Robin's guidance has helped me to become a better scientist, writer, and educator. I will be forever grateful for the knowledge, skills, and diverse range of experiences she imparted to me. I would also like to extend very special thanks to Anne McNabb for the valuable mentorship she provided through out my graduate studies. Despite her numerous other responsibilities, Anne took the time to both challenge and encourage me. Anne has been an exemplary role model as a teacher, scientist, and human being. As a future faculty member, I can only hope to approximate Anne's exceptional energy level and enthusiasm. I am grateful for the support and encouragement of Bob Jones without which the diverse range of teaching and research experiences I have had at Virginia Tech would not have been possible. I also wish to thank my other committee members, Drs. Tom Jenssen, and Mike Denbow for their helpful suggestions, guidance, and patience over the past four years. Their questions and suggestions have challenged me to think broadly, and their input has been a profound influence on my development as a researcher. I would like to express my gratitude to Sue Rasmussen, who despite being asked the same questions from numerous other graduate students, patiently helped me to navigate through the paperwork and lists of requirement that are an integral part of graduate school. I am deeply grateful to Mary Schaeffer and Jack Cranford for providing me with the opportunity to teach a variety of interesting and challenging courses at Virginia Tech. I also thank Ellen Byus and Geoff Adams for assistance in the laboratory, and Harry Schwend for assistance in the field. Last but certainly not least, I would like to extend warm thanks to my wife Michelle Barthet, for her indispensable help with collecting lizards in the field, and for her unending patience and encouragement over the last three years.

I would like to thank the staff of the Rutgers Pinelands Field Station and the South Western Research Station of the American Museum of Natural History for their logistic support, and Luke and Rhonda Flowers for permission to collect lizards and conduct fieldwork on their property. This project was funded by a Sigma Xi Grant-in-Aid of research, a Graduate Research

and Development Project Grant from the Virginia Tech Graduate Student Assembly, and a Small Research Grant from the Virginia Academy of Sciences. Finally, I would like to extend thanks to Bob Jones for providing matching funds for each of these grants.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION AND BACKGROUND

Oviparity is the ancestral vertebrate reproductive mode (Packard and Seymour 1997). Among vertebrates, birds, crocodilians, turtles, and Sphenodontids (Tuataras) are entirely oviparous. While most amphibians are oviparous, viviparity has evolved in several lineages (Wake 1989). Mammals are characterized as viviparous, possessing one, or possibly two origins of viviparity (Guillette and Hutton 1986). In contrast to the modest number of origins of viviparity in amphibians and mammals, viviparity has evolved at least 100 times in squamate reptiles (Shine 1985). The high frequency of independent origins of viviparity within the Order Squamata has generated considerable interest in the selective factors underlying this shift in reproductive mode.

COLD CLIMATE MODEL FOR THE EVOLUTION OF REPTILIAN VIVIPARITY

Several hypotheses have been proposed (though few tested empirically) to explain the putative selective factors driving the evolutionary transition from oviparity to viviparity in squamate reptiles. The most broadly accepted hypothesis is that viviparity evolved in response to cold environmental conditions through gradual increases in the duration of egg retention within the oviduct (Packard et al. 1977; Shine and Bull 1979; Shine 1985). Because embryonic development in reptiles is temperature dependent, cold nest temperatures may slow the rate of embryonic development for eggs deposited in the soil, (Muth 1980), cause developmental abnormalities (Shine and Harlow 1993; Shine 1995; Qualls and Shine 1996; Qualls and Andrews 1999; Andrews et al. 2000), or result in mortality of embryos (Packard et al. 1977; Andrews 2000). According to the cold climate hypothesis, retention of eggs within the oviduct increases female fitness in cold climates because the embryos are warmer than they would be in a nest (Packard et al. 1977, Shine 1983). Under this model, even relatively short increases in the duration of egg retention would enhance female fitness because eggs would be more developmentally advanced, at oviposition, and thus require less time to hatch than non-retained eggs (Shine 1985). Selection for increasingly prolonged egg retention should therefore ultimately lead to complete intrauterine incubation of embryos resulting in fully developed offspring at oviposition/parturition.

The cold climate hypothesis is based in part by the correlation between environmental temperature and the proportion of viviparous species in squamate faunas (Shine and Berry 1978). The proportion of viviparous species generally increases with latitude and elevation. In North America, for example, about 29% of squamate species between 30-35° N are viviparous, compared to 63% between 50-55°N (Tinkle and Gibbons 1977).

The correlation between the distribution of viviparous species and climate, however, does not necessarily indicate the environmental conditions under which viviparity evolved (Shine and Berry 1979). It is possible that viviparity evolved in response to selective pressures other than temperature and that live bearing simply permitted viviparous species to colonize cold environments. That is, once viviparity has evolved, species possessing this reproductive mode may be able to survive and reproduce under a variety of different environmental conditions. The most compelling evidence supporting the hypothesis that viviparity evolved in cold climates is derived from studies of relatively recent origins of viviparity which compare closely related oviparous and viviparous species. The results of these studies demonstrated that the majority of recent origins of viviparity are associated with cold climates (Guillette et al. 1980; Shine 1985).

While cold temperatures may be a primary factor driving the evolution of reptilian viviparity, relatively few quantitative studies have been conducted to test the cold climate hypothesis directly. A fundamental prediction of the cold climate hypothesis is that gravid female squamates inhabiting cold climates should retain eggs to more advanced developmental stages compared to gravid females in warmer areas (Shine and Bull 1979; Shine 1983; Shine 1985). Recent studies in lizards, however, have shown that in many oviparous species, embryonic development is retarded or arrested when gravid females are experimentally induced to retain eggs past the normal time oviposition (Andrews and Rose 1994; Andrews 1997; Mathies 1998; Matheis and Andrews 1999; Mathies and Andrews 2000). In lizards, for example, the majority of oviparous species lay eggs within a relatively narrow range of developmental stages (26-31 of Dufaure and Hubert's staging system where 0 is fertilization and 40 is hatching) (Shine 1983; Blackburn 1995; Andrews and Mathies 2000). The observations that few lizard species appear capable of retaining eggs much past stage 31 coupled with the fact embryonic development is often retarded or arrested during egg retention suggests that physiological and/or

morphological constraints prevent normal development from occurring during egg retention. Selection for prolonged egg retention will thus not lead to viviparity unless the capacity to support embryonic development evolves prior to, or concurrently with egg retention (Mathies and Andrews 1999; Andrews 2002).

PHYSIOLOGICAL CONSTRAINTS ON THE EVOLUTION OF VIVIPARITY

Within the context of the evolution of reptilian viviparity, "egg retention" consists of two major components: (1) the ability to maintain the gravid state and, (2) the capacity to support embryonic development during egg retention (Mathies and Andrews 1999). The simplest model for the evolution of viviparity assumes that both traits evolve concurrently (Packard et al. 1977; Guillette et al. 1980; Shine and Guillette 1988). The wide variation among lizard species in both the capacity to retain eggs and to support *in utero* embryonic development during egg retention, however, suggests that these two processes are not necessarily linked. A major portion of this work is thus focused on understanding the putative constraints on the capacity to support embryonic development during extended egg retention.

In utero oxygen availability has been implicated as an important constraint on the evolution of reptilian viviparity (Packard et al. 1977; Guillette 1982; Shine 1985; Andrews 2002). Metabolic oxygen consumption increases throughout embryogenesis, and is particularly high during the later half of embryonic development (Dmi'el 1970). Oxygen supplied to the developing embryo must first diffuse from maternal circulation, through the fluid medium surrounding the egg, and across the eggshell before becoming available to the embryonic blood supply. Because diffusion of respiratory gases are much slower in water compared to air, the fluid saturating the channels of oviductal eggs forms a barrier to oxygen diffusion to the embryo (Packard et al. 1977). Prolonged egg retention should therefore be associated with physiological and/or morphological features that enhance oxygen delivery to developing embryos in an environment that becomes increasingly hypoxic as development proceeds.

INCUBATION TEMPERATURE AND GEOGRAPHIC DISTRIBUTIONS OF OVIPAROUS SPECIES

A fundamental assumption of the cold climate model is that cold environmental temperatures favor viviparous reproduction because embryos develop faster at higher maternal body temperatures compared to eggs in a nest. Conversely, cold temperatures are detrimental to oviparous reproduction by negatively affecting the length of incubation, survivorship, and fitness of hatchlings. If cold temperature is the primary factor responsible for the paucity of oviparous reptile species in these environments, the distributions of some oviparous species should be predictable based upon knowledge of embryonic thermal requirements. This information has important implications for the evolution of reptilian viviparity because it would provide direct evidence that the observed latitudinal and elevational trends in reproductive mode are determined by the thermal requirements of embryos.

OVERVIEW OF RESEARCH

Studies of historic events such as the evolution of viviparity are challenging because we cannot be certain of the environmental conditions under which viviparity originally evolved. Assuming, however, that similar selective pressures exist today as in the past, we can examine extant squamate species exhibiting intermediate stages of egg retention to elucidate potential environmental and physiological factors leading to the evolution of viviparity.

The present work focuses on three broad questions related to the evolution of viviparity in lizards: (1) do populations in cool climates at northern latitudes retain eggs longer and possess physiological adaptations to support embryonic development during extended egg retention, compared to populations at lower, and therefore warmer latitudes, (2) are interspecific differences in the capacity to support embryonic development during extended egg retention associated with differences in oviductal oxygen availability, and (3) do embryonic thermal requirements determine the distributional limits of oviparous species in cold climates?
In Chapters one and two, I use a comparative approach to evaluate whether intraspecific (Ch.1) and interspecific (Ch. 2) differences in the capacity to support embryonic development during

egg retention are related to *in utero* oxygen availability. The general experimental protocol employed in chapters 1 and 2 was to establish a standard curve relating the developmental rate of embryos incubated under simulated *in utero* conditions to partial pressure oxygen (pO_2). The developmental rate of embryos in the oviduct was determined by experimentally inducing gravid females to retain eggs past the normal time of oviposition. *In utero* pO_2 was then estimated from the standard curve.

Chapter three compliments material covered in previous chapters by examining the role of embryonic thermal tolerance in determining the distributional limits of oviparous lizard species. Specifically, I incubated lizard eggs under experimental temperature treatments that simulated the thermal environment eggs would experience if located at northern latitudes outside the species present geographic range. I then evaluated the effects of temperature treatment on phenotype, growth, and survival in the laboratory and in the field. Using data on embryonic developmental rates as a function of temperature, I also estimated the minimum number of degree-days (a measurement of physiological time) required for embryos to complete embryonic development. These data were used to predict the northern latitudinal limit of the species distribution in central and eastern USA.

EVOLUTION OF VIVIPARITY IN *SCELOPORUS* LIZARDS

The lizard genus *Sceloporus* (Phrynosomatidae) is the largest genus of North American reptiles (Wiens and Reeder 1997). The geographic range of *Sceloporus* extends from the southern edge of western Canada to as far south as Panama (Sites et al. 1992), with the highest species diversity occurring in southwestern USA and central Mexico (Wiens and Reeder 1997). The genus is comprised of approximately 80 species, about 30 of which are viviparous (Sites et al. 1992). Viviparity has apparently arisen four times in the genus, and is fixed in four of the 22 recognized species groups. Only one species group (the *scalaris* group) contains both oviparous and viviparous species (Méndez-de la Cruz et al. 1998). The small number of entirely viviparous species groups among a relatively large number of entirely oviparous species groups (17 out of 22) suggest that viviparity may evolve more easily in some lineages than in others (Andrews et al. 1999, Andrews and Mathies 2000).

Sceloporus lizards are an ideal model taxon for comparative studies on the evolution of egg retention and viviparity because the stage at which embryos are oviposited varies widely among species (Andrews 1997; Andrews and Mathies 2000; Mêndez-de la Cruz 1998). Moreover, they occur over a wide latitudinal and elevational range and thus occupy a variety of thermal environments (Sites et al. 1992). The majority of my research focused on members of the *undulatus* species group; an entirely oviparous lineage whose representatives are common over much of the United States. I also studied one member of the genus *Urosaurus*, because it is the sister genus to *Sceloporus* (Wiens and Reeder 1997) and is capable of prolonged egg retention (Mathies and Andrews 1999).

LITERATURE CITED

Andrews RM. 1997. Evolution of viviparity: variation between two sceloporine lizards in the ability to extend egg retention. *Journal of Zoology (London)* 243: 579-595.

Andrews RM 2002. Low oxygen: a constraint on the evolution of viviparity in reptiles. *Physiological and Biochemical Zoology* 75: 145-154.

Andrews RM, and Mathies T. 2000. Natural history of reptilian development: constraints on the evolution of viviparity. *Bioscience* 50: 227-238.

Andrews RM, Mathies T, Qualls CP, and Qualls FJ. 1999. Rates of embryonic development of Sceloporus lizards: do cold climates favor the evolution of rapid development? *Copeia* 1999: 692-700.

Andrews RM, and Rose. BR 1994. Evolution of viviparity: constraints on egg retention. *Physiological Zoology* 67: 1006-1024.

Blackburn DG. 1995. Saltationist and punctuated equilibrium models for the evolution of viviparity and placentation. *Journal of Theoretical Biology* 174: 199-216.

Dmi'el R. 1970. Growth and metabolism of snake embryos. *Journal of Embryology and Experimental Morphology* 23: 761-762.

Dufaure J, and Hubert J. 1961. Table de development du lezard vivpare: *Lacerta (Zootica) vivipara* Jaquin. *Archives Anatomie Microscopie Morphologie Expérimental* 50: 309-327.

Guillette LJ. 1982. The evolution of viviparity and placentation in the high elevation lizard *Sceloporus aeneus. Herpetologica* 38: 94-103.

Guillette LJ, Jones RE, Fitzgerald KT, and Smith HM. 1980 Evolution of viviparity in the lizard genus *Sceloporus*. *Herpetologica* 36: 201-215.

Guillette LJ, and Hutton N. 1986. The evolution of mammalian reproductive characteristics in therapsid reptiles. In: Hotten N, Maclean J, Roth J, and Roth EC, eds. *The ecology of mammal-like reptiles.* Washington, D.C.: Smithsonian University Press, 235-250.

Mathies T. 1998. Constraints on the evolution of viviparity in the lizard genus *Sceloporus.* PhD dissertation. Virginia Polytechnic Institute and State University, Blacksburg, VA.

Mathies T, and Andrews RM. 1999. Determinants of embryonic stage at oviposition in the lizard *Urosaurus ornatus. Physiological and Biochemical Zoology* 72: 645-655.

Méndez-De La Cruz FR, Villagrán Santa Cruz M, Andrews RM. 1998. Evolution of viviparity in the lizard genus *Sceloporus.* Herpetologica 54: 521-532.

Muth A. 1980. Physiological ecology of desert iguana (*Dipsosaurus dorsalis*) eggs: temperature and water relations. *Ecology* 61: 1335-1343.

Packard GC, Tracy CR, and Roth JJ. 1977. The physiological ecology of reptilian eggs and embryos and the evolution of viviparity within the Class Reptilia. *Biological Reviews* 52:71-105.

Packard MJ, and Seymour RS. 1997. Evolution of the amniote egg. In: Sumida S, Martin KM, eds. *Amniote Origins: Completing the Transition to Land.* San Diego: Academic Press, 265-290.

Qualls CP, and Shine R. 1996. Reconstructing ancestral reaction norms: an example using the evolution of viviparity. *Functional Ecology* 10: 699-697.

Qualls CP, Andrews RM (1999) Cold climates and the evolution of viviparity in reptiles: cold incubation temperatures produce poor-quality offspring in the lizard *Sceloporus virgatus*. *Biological Journal of the Linnean Society* 67: 477-491.

Qualls CP, and Andrews RM. 1999. Cold climates and the evolution of viviparity in reptiles: cold incubation temperatures produce poor-quality offspring in the lizard *Sceloporus virgatus. Biological Journal of the Linnean Society* 67: 477-491.

Shine R. 1983. 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. In: Gans C, Billet F, eds. *Biology of the Reptilia*. Volume 15. New York: Wiley and Sons, 605-694.

Shine R. 1995. A new hypothesis for the evolution of viviparity in reptiles. *The American Naturalist* 145: 809-823.

Shine R, and Berry JF. 1978. Climatic correlates of live-bearing in squamate reptiles. *Oecologia (Berlin)* 33: 261-268.

Shine R, and Bull JJ. 1979. The evolution of live-bearing in lizards and snakes. *The American Naturalist* 113: 905-923.

Shine R, and Guillette LJ. 1988. The evolution of viviparity in reptiles: a physiological model and its ecological consequences. *Journal of Theoretical Biology* 132: 43-50.

Shine R, and Harlow P. 1993. Maternal thermoregulation influences offspring viability in a viviparous lizard. *Oecologia* 96: 122-127.

Sites JW, Archie JW, Cole CJ, and Villela OF. 1992. A review of phylogenetic hypotheses for lizards of the genus *Sceloporus* (Phrynosomatidae): implications for ecological and evolutionary studies. *Bulletin of the American Museum of Natural History* 213: 1-110.

Tinkle DW, and Gibbons JW. 1977. The distribution and evolution of viviparity in reptiles. *Miscellaneous Publications Museum of Zoology, University of Michigan* 154: 1-55.

Wake MH. 1989. Phylogenesis of direct development and viviparity in vertebrates. In: Wake MH, Roth G, eds. *Complex Organismal Functions: Integration and Evolution in Vertebrates.* New York: Wiley and Sons, 235-250.

Wiens JJ, and Reeder W. 1997. Phylogeny of the spiny lizards (*Sceloporus*) based on molecular and morphological evidence. *Herpetological Monographs* 11: 1-101.

CHAPTER 1

EMBRYONIC RESPONSES TO VARIATION IN OVIDUCTAL OXYGEN IN THE LIZARD *SCELOPORUS UNDULATUS* FROM NEW JERSEY AND SOUTH CAROLINA

This work was originally published as:

Parker SL, Andrews RM, and Mathies, T. 2004. *Biological Journal of the Linnean Society* 83: 289-299. Blackwell Publishing

ABSTRACT

Viviparity in reptiles is hypothesized to evolve in cold climates at high latitudes and high elevations through selection for progressively longer periods of egg retention. Oxygen consumption of embryos increases during development, therefore, longer periods of egg retention should be associated with maternal or embryonic features that enhance embryonic oxygen availability. I tested the hypotheses that embryos of the oviparous lizard *Sceloporus undulatus* from a high latitude population in New Jersey are oviposited at more advanced developmental stages, and have a higher growth rate at low oxygen partial pressures (pO_2) than embryos from a low latitude population in South Carolina. These hypotheses were rejected; embryos from the two populations did not differ in embryonic stage at oviposition, survival, rate of differentiation, or growth in mass when incubated under simulated *in utero* conditions at low oxygen concentrations. I also estimated the effective pO_2 experienced by lizard embryos *in utero*. At an effective pO_2 of 8.6 kPa (9% O_2), development of *S. undulatus* embryos is arrested at Dufaure and Hubert stage 30 and at a dry mass of 0.8 mg. Physiological and morphological features of gravid females, embryos, or both, that facilitate oxygen uptake for developing embryos appear to be a critical early step during the evolution of reptilian viviparity.

INTRODUCTION

Reptilian viviparity is hypothesized to evolve in response to cold climates at high latitudes and high elevations through gradual increases in the retention of eggs within the oviduct (Packard et al., 1977; Shine & Bull, 1979; Shine, 1985). The putative benefit to extending egg retention in cold climates is that embryonic development is faster inside the thermoregulating female than in a nest (Packard et al., 1977; Shine, 1983). According to the cold climate hypothesis, even relatively short increases in the duration of egg retention would enhance female reproductive fitness because embryos would be more advanced developmentally at oviposition and, therefore, hatch in a shorter period of time than non-retained eggs (Shine, 1985).

The widespread acceptance of the cold climate model is, however, based largely on indirect observations. For example, viviparous species are relatively more common at high than low elevations and latitudes. In contrast, relatively few studies test the cold climate model directly. One example is Mathies and Andrews' (1995a) demonstration that gravid female *Sceloporus scalaris* from a high elevation population retained eggs longer and produced embryos that were more advanced developmentally compared to gravid females from a low elevation population. In another example, Shine (2002) simulated short-term egg retention in the laboratory by subjecting eggs of the skink *Bassiana duperreyi* to a two-week initial period of high incubation temperatures. Eggs subjected to simulated short-term egg retention at high temperatures developed more rapidly, had greater survivorship, and produced higher quality hatchlings, than eggs incubated at cool temperatures (simulating conditions in a nest) for their entire incubation period.

The majority of oviparous lizards lay eggs when 25 - 40% of the total embryonic development time has been completed (Shine, 1983; DeMarco, 1993) and embryos have reached approximately stage 30 (Andrews & Mathies, 2000) of Dufaure and Hubert's (1961) staging system, where stage 0 is fertilization and 40 is hatching. Relatively few oviparous lizard species retain eggs beyond stage 33, suggesting that the ability of females to retain eggs much past stage 30 is constrained (Mathies & Andrews, 1995a).

One of the constraints on egg retention past stage 30 is oxygen availability for embryonic development *in utero* (Andrews & Mathies, 2000; Andrews, 2002). Because of the growth of the embryo (Dmi'el, 1970; Vleck & Hoyt, 1991), the oviduct should become increasingly hypoxic during late development when the size and the metabolism of the embryo increases dramatically. Oviposition should thus occur when the demand for oxygen by the embryo exceeds the ability of the female to supply it. For example, in most species of *Sceloporus* lizards, embryonic development is retarded when gravid females are experimentally induced to retain eggs past the normal time of oviposition (Andrews, 1997; Mathies & Andrews, 1999; Andrews and Mathies, 2000). Extended gestation in the oviduct must, therefore, be associated with mechanisms that enhance oxygen availability in an environment that becomes increasingly hypoxic as development proceeds.

Studies demonstrating variation in egg retention along geographic climatic gradients have typically been conducted on species in lineages comprised of both oviparous and viviparous populations (Guillette, 1982; Mathies & Andrews, 1995b; Smith & Shine, 1997; Qualls & Shine, 1998). In contrast, studies of species from entirely oviparous lineages also have the potential to provide support for the cold climate model. Demonstration that intermediate stages of egg retention are associated with low environmental temperatures independent of a phylogenetic history of viviparity would be particularly compelling. Moreover, intraspecific comparisons demonstrating variation in egg retention time along climatic gradients are unlikely to be confounded by species-specific adaptations unrelated to egg retention. *Sceloporus undulatus* (Latreille) is an appropriate species for studying the potential influence of climate on egg retention because it is a member of the entirely oviparous *undulatus* species group (Méndez-de la Cruz et al., 1998) and it has a wide latitudinal distribution over much of the central and eastern United States (Stebbins, 1985). Moreover, many life history characters differ between populations (Tinkle & Ballinger, 1972; Ferguson & Brockman, 1980). For example, females from New Jersey (NJ) have a shorter reproductive period (Figure 1.1) and produce two clutches of eggs per year at most (Angilletta et al., 2001), while females from South Carolina (SC) produce three clutches per year (Tinkle & Ballinger, 1972). These differences between populations are associated with lower environmental temperatures in NJ than in SC.

The first objective was to test two hypotheses related to the cold climate model. The first hypothesis was that gravid females from high latitude populations retain eggs to more advanced stages of embryonic development than females from low latitude populations. The second hypothesis was that embryos from high latitude populations have a higher developmental rate while *in utero* than embryos from low latitude populations. To test these two hypotheses, I contrasted egg retention and embryonic development *in utero* for a population of *S. undulatus* from near the northern latitudinal limit of the species distribution in New Jersey (NJ) with those of a population from South Carolina (SC).

The second objective was to estimate effective pO_2 experienced by lizard embryos *in utero*. To my knowledge, pO_2 experienced by lizard embryos *in utero* during egg retention has not been quantified at any time during development. To meet this second objective, therefore, I first established a standard curve relating the developmental rate of embryos to pO_2 under simulated *in utero* conditions. Data collected earlier (Mathies, 1998) provided data on the rate of development of embryos retained by females *in utero* past the time of normal oviposition. PO_2 *in utero* was then estimated from the standard curve (ie. the growth rates of embryos under known pO_2.

MATERIALS AND METHODS

COLLECTION AND MAINTENANCE OF GRAVID FEMALES

Gravid females of *Sceloporus undulatus* were captured at Sumter National Forest, Edgefield County, South Carolina during 31 May – 4 June 2002 (n = 16), and at Wharton State Forest, Burlington County, New Jersey during 27 June – 30 June 2002 (n=16). After capture, gravid females from both populations were placed in cloth bags and transported within five days of capture to Virginia Polytechnic Institute and State University (Virginia Tech), Blacksburg, Virginia. Females were measured for snout-vent length (SVL) and weighed to the nearest 0.1g before and after oviposition. Females were housed in plastic containers (73 x 48 x 22cm, 3 females per container) in an animal room at Virginia Tech. Ambient light was provided from windows and lizards were also provided with fluorescent Vita-lites (0800 - 1800). A 100-W

spotlight suspended at one end of each container (0900 - 1600) provided a temperature gradient that allowed females to thermoregulate. All containers were provided with boards and rocks for basking sites. Females were fed (crickets and mealworms dusted with vitamin-mineral supplement) and watered by misting daily.

COLLECTION OF EGGS AND INITIAL SAMPLING OF EGGS AND EMBRYOS

New Jersey and South Carolina females were initially assigned to one of two groups. Females in one group were provided with damp sand for oviposition. Females in the second group were provided only with dry sand in an attempt to induce them to retain eggs beyond the normal time of oviposition (Andrews & Rose, 1994). However, females did not retain eggs in response to the absence of a suitable nesting substrate; within populations, females that oviposited did so over the same period in the two substrate groups (NJ: 8d; SC: 7d) and same range of stages (NJ: 28 - 29.5; SC: 27 - 29.5). Therefore, I did not consider the initial oviposition substrates as part of the experimental design and eggs from both groups (i.e. damp and dry substrates) were pooled within each population for all subsequent analyses.

Containers were checked several times daily for eggs. When one-half of females had oviposited, the remainder were injected with oxytocin to induce oviposition: 14 June, five SC females; 5 July, six NJ females. The embryonic stage at oviposition for SC females injected with oxytocin did not differ from females that oviposited normally (t_8=1.68, P=0.130). The embryonic stage at oviposition for NJ females injected with oxytocin did, however, differ from females that oviposited normally (t_{10}=2.96, P=0.022). The difference in mean stage was relatively small (X=28.0 and X=28.6, injected versus normal oviposition, respectively), and both groups were therefore pooled in all subsequent analyses. Nine clutches (2 NJ and 7 SC) could not be used in experiments because eggs desiccated or because females did not have shelled eggs.

Eggs were weighed within a few hours of oviposition and numbered consecutively within each clutch. The surfaces of the eggshells were kept moist until placed under experimental conditions. A single egg from each clutch was dissected and the embryo staged according to

Dufaure & Hubert (1961). Half stages were assigned for embryos exhibiting intermediate suites of traits. After staging, embryos were dried to a constant mass at 40° C and weighed. A single egg from each NJ clutch was used to measure the area of the chorioallantois membrane (CAM). The extent of the CAM was visible through the eggshells at oviposition and therefore easily measured. The major axis and minor axis of each egg and diameter of chorioallantois of each egg sampled were measured using a dial calipers. The surface area of the eggshell was calculated using the formula for area of a prolate spheroid and the CAM surface area was calculated using the formula for area of a circle. Relative CAM area was the ratio the surface area of the CAM to the total eggshell surface area.

EXPERIMENTAL DESIGN AND MANIPULATION OF pO_2

Differentiation and growth of embryos were determined under simulated *in utero* conditions. To simulate conditions in the oviduct, eggs were incubated under conditions such that channels in the shell remained fluid filled during incubation as they would be normally in the oviduct. Using this procedure, oxygen must diffuse through the fluid filled eggshell before it is available to the embryo. This procedure may best simulate *in utero* conditions because in the oviduct, eggs are pressed against the walls of the uterus and are therefore in close proximity to maternal blood supply. One or two eggs were placed in 70ml specimen jars lined with Whatman® filter paper moistened with physiological saline (pH 7.4). The filter paper was re-moistened with saline at least every three days to ensure that the eggshell channels remained fluid filled during incubation (see also Seymour et al., 1991 for similar experimental approaches). I incubated eggs under a range of O_2 levels (target values: 4, 9, 15, and 21% O_2) (Table 1.1). Control eggs (simulating nest conditions) were placed in specimen jars with vermiculite moistened with distilled water (0.7:1.0g H_2O:vermiculite) corresponding to a water potential of –200 kPa (21% O_2 only). The only difference thus between the experimental and control eggs was that the shells of the former were fluid filled and those of the latter were filled with air.

Eggs were placed, according to treatment, into one of four airtight metal boxes. The boxes were flushed regularly with the appropriate gas mixture (O_2 and N_2) using a Cameron

Instruments, Model GF-3/MP gas mixing flow meter. Bubbling the gas mixture through distilled water saturated the air inside the boxes. Every time the boxes were flushed, the oxygen concentration inside the boxes was measured using an Applied Electrochemistry S-3A/II oxygen analyzer. Mean oxygen levels (in dry air) for the four oxygen treatments during the incubation period were 4.3%, 9.5%, 15.8%, and 20.4% respectively (Table 1.2). The actual values of pO_2 for the four oxygen treatments during the incubation period were 3.9, 8.6, 14.3, and 18.5 kPa, respectively, based upon a mean air pressure at Blacksburg (625 m) of 94.5 kPa (711 mmHg), a mean incubation temperature of 28 °C, and a P_{H2O} of 3.8 kPa of water vapor in air.

The boxes were placed in a single environmental chamber and incubated for 10 days at a mean of 28°C. Temperatures inside the environmental chamber ramped linearly for 4 hours between daily maximum and minimum temperatures (chamber temperature: mean daily maximum: 32.9°C, mean daily minimum: 22.9°C, overall mean: 27.7°C). The boxes were rotated within the chamber every 3 – 5 days to minimize position effects on embryonic development. After the experiment was completed, temperatures inside of the boxes were measured over a four-day period to determine the relationship between the temperatures within the boxes and temperatures within the environmental chamber. For simulated oviductal treatments, the temperature probe was placed at the bottom of the specimen jar in contact with the moistened filter paper. For simulated nest treatments, the temperature probe was placed in the center of the specimen jar and covered with vermiculite. During these observations, the mean temperatures of the simulated oviduct and simulated nest treatments were 0.5°C higher than the mean temperature inside the chamber ($X_{experimental}$ = 28.1 °C, $X_{control}$= 28.1 °C, $X_{chamber}$=27.6 °C).

ESTIMATION OF pO_2 *IN UTERO*

Data from Mathies (1998) were used to estimate actual pO_2 *in utero* for *S. undulatus*. Mathies (1998) provided one group of females collected near Blacksburg, VA with a substrate of damp sand in which to oviposit (referred to as “control” females). The normal time of oviposition (NTO) was defined as the time when approximately half of the control females laid eggs. A second group of gravid females was induced to retain eggs past the NTO by maintaining

them on a dry substrate (referred to as “retained females”). A dry substrate simulates drought conditions and females may respond by facultatively retaining eggs (Andrews & Rose, 1994). Embryonic stage and dry mass were obtained from retained clutches that were sampled at regular intervals for as long as 27 days during the period of retention. Daytime body temperatures of the retaining females (0800 - 1600h) averaged 32.6 °C and minimum body temperatures during inactivity (1700 – 0800h) were approximately 22 °C. Overall mean daily body temperature of retained females thus averaged approximately 27 °C.

Effective pO_2 *in utero* was estimated by first determining the developmental rate of *S. undulatus* embryos after 10d of retention *in utero* from previous studies (Mathies, 1998). A standard curve generated by the regression of embryonic developmental rate versus pO_2 determined by the experimental manipulations of oxygen levels (described above) was then used to predict the pO_2 associated with the *in utero* rate of embryonic development.

DATA MANIPULATION AND STATISTICAL ANALYSES

The effect of oxygen treatment on egg survival was similar between populations and data were therefore pooled for all subsequent survivorship analyses. The effect of oxygen treatment on egg survival among the 4, 9, 15, and 21% treatments was analyzed using a Chi-square Test of Independence (Freq Procedure, SAS Institute 1996). The effect of oxygen treatment on survival between the 21% treatment and control (simulated nest) treatment were analyzed using a Fisher’s Exact Test (FREQ Procedure, SAS Institute 1996).

The effect of oxygen treatment and population on embryonic stage (differentiation) and dry mass (growth) at 10 d was evaluated using two-factor analysis of covariance (ANCOVA) with embryonic stage and embryo dry mass at oviposition as covariates (GLM procedure; SAS Institute 1996). The 21% treatment and 21% control were contrasted using a one-way ANCOVA with initial stage or dry mass as the covariate. The effect of oxygen treatment on water uptake by eggs and growth of absolute or relative CAM area at 10 d was analyzed using single-factor ANCOVA with egg mass and absolute or relative area of CAM at oviposition as covariates.

Observations of relative area of CAM were arcsine-square root transformed prior to analysis. Water uptake by eggs in each treatment was assessed as the difference in egg mass at 10 days and at oviposition. *Post hoc* pair-wise comparisons were analyzed using a least significant difference test on least squared means. Probability values of less than 0.05 were considered significant. The assumption of homogeneity of slopes for all ANCOVA's was satisfied by testing for significance of the interaction of the covariate with treatment variables.

RESULTS

LIFE HISTORY DATA

Female SVL and mass (post-oviposition) did not differ between New Jersey and South Carolina (Table 1.3). While egg mass of New Jersey females was greater than that of South Carolina females, RCM and clutch size did not differ between populations. Moreover, embryonic stage at oviposition did not differ between populations. Although some females from each population were injected with oxytocin (NJ: 6/12; SC: 5/10, injected/non-injected), the difference in mean embryonic stage between oxytocin injected females and females that oviposited normally was small (< 0.6 stages) for both populations (see Materials and Methods). It is unlikely, therefore, that injection with oxytocin biased the comparison of embryonic stage at oviposition between the two populations.

SURVIVAL

Oxygen treatment affected embryo survival (Table 1.4). Overall, survival varied among the 4, 9, 15 and 21% treatments (χ^2=32.8, n=86, d.f.=3, P=<0.0001), and survival increased with pO_2. Survival of eggs in the 4% treatment was, however, substantially lower than in the 9, 15, and 21% treatments. Survival of control eggs did not differ from that of eggs in the 21% treatment (P=1.0, n=34, Fisher's Exact Test). Because of low survival of embryos in the 4% treatment, observations on embryonic differentiation and growth in this treatment could not be included in subsequent analyses.

EMBRYONIC DIFFERENTIATION AND GROWTH

Populations did not differ in embryonic responses to oxygen treatments (Tables 1.5 and 6). Oxygen treatment, however, affected both differentiation and growth. Embryonic differentiation was reduced at 9% O_2 and highest at 21% O_2 under simulated oviductal conditions (Figure 1.2). After 10 days of incubation at 9% O_2 embryos reached a mean stage of 29.6. This corresponds to an average increase of one stage over the 10-day incubation period. In contrast, embryos incubated at 21% O_2 reached a mean stage of 30.5, corresponding to an average increase of almost two stages. Differentiation, however, at 21% under simulated oviductal conditions was lower than in the 21% control under simulated nest conditions ($F_{1, 16}$= 21.0, $P < 0.001$, ANCOVA). Control embryos reached a mean stage of 32.1, corresponding to an increase of 3.5 stages over the incubation period.

Embryonic growth in mass was reduced at 9% O_2 and highest at 21% O_2 under simulated oviductal conditions (Figure 1.2). Embryos incubated at 9% O_2 reached a mean dry mass of 0.84 mg after 10 days of incubation corresponding to an average increase of 0.4 mg. Embryos incubated at 21% O_2 reached a mean dry mass of 1.9 mg corresponding to an average increase of 1.36 mg. Growth at 21% O_2 under simulated oviductal conditions was lower than in the 21% control under simulated nest conditions ($F_{1, 16} = 30.9$, $P < 0.001$, ANCOVA). Control embryos reached a mean dry mass of 3.7 mg corresponding to an average increase of 3.2 mg over the incubation period.

EGG SIZE AND CAM

For New Jersey females, the size of eggs at 10 days did not differ among treatments; water uptake was independent of oxygen treatment (Table 1.6). Water uptake at 21% O_2 under simulated oviductal conditions was lower than in the 21% control ($F_{1, 17} = 19.3$, $P < 0.001$, ANCOVA). Eggs incubated at 21% O_2 under simulated oviductal conditions reached a mean wet mass of 588 mg corresponding to an average increase of 149 mg over the incubation period. Control eggs reached a mean wet mass of 737 mg corresponding to an average increase of 267 mg.

Oxygen treatment affected both the absolute area and relative area of the CAM with larger areas associated with higher oxygen levels (Table 1.6). Pair-wise comparisons between treatments indicated that absolute and relative CAM area was larger in the 21% simulated oviduct than the 9% and 15% oxygen treatments. In the 9% O_2 treatment only 27% of the inner eggshell surface was covered by the CAM after 10 days of incubation, whereas the CAM covered more than 50% of the inner surface of the eggshell at 21% O_2 under simulated oviductal conditions. The CAM covered more than 90% of the inner surface of the eggshell in control eggs.

DISCUSSION

TESTS OF HYPOTHESES

Results of the experiments do not support the hypothesis that *S. undulatus* females from high latitudes have a greater capacity to retain eggs than those at low latitudes or the hypothesis that *S. undulatus* embryos from high latitudes have a higher developmental rate under hypoxic conditions than embryos from low latitudes. Embryonic stage at oviposition for NJ females was similar to that of SC females (NJ: mean stage=28.4 vs. SC: mean stage=28.8) (Table 1.3). The oxygen treatments affected embryo survival similarly in both populations (Table 1.4). Moreover, embryos from both populations had very similar differentiation and growth rates in the 9, 15, and 21% oxygen treatments (Table 1.5 and Figure 1.2).

One explanation for the lack of divergence in egg retention and embryonic differentiation and growth between the two populations could be that overall body temperatures (T_b's) of NJ and SC females do not differ. Despite the fact that average daytime air temperatures during May, June, and July are 3 – 5°C cooler in NJ than in SC (National Climate Data Center, Figure 1.1), females from the two populations maintain very similar T_b's when active (NJ: mean T_b=34.0, SC: mean T_b=33.1, Angilletta, 2001). Thus, NJ females are able to compensate for cooler air temperatures when active during the day by behavioral thermoregulation. In contrast, average minimum air temperatures during May, June, and July are 4 – 6 °C cooler in NJ than in SC. Because females cannot thermoregulate at night, nighttime T_b's of NJ females are likely to be

lower than those of SC females. The overall mean incubation temperature of *in utero* embryos could thus be several degrees lower in NJ than in SC.

Given that NJ females may have somewhat lower mean T_b's than SC females, why have the two populations not diverged in their reproductive biology as predicted? One reason may be that that costs of retaining eggs are greater than the potential thermal benefits of egg retention for NJ females. The physical weight of the clutch and distention of the abdomen caused by eggs may result in decreased sprint speed and reduced locomotor performance thus potentially increasing the female's risk of predation (Shine & Bull, 1979; Sinervo et. al., 1991; Miles et al., 2000). Furthermore, the thermal benefit of egg retention is likely to be strongest when the difference between the mean temperature of eggs retained *in utero* and the mean temperature of eggs in nests is relatively large. Observations on the montane lizard *Sceloporus virgatus* a close relative of *S. undulatus,* however, indicate that female T_b and nest temperatures are nearly identical (T_b =24.6 °C, nest=25.2 °C) (Andrews & Rose,1994). Thus, selection of thermally favorable nest sites and placement of nests at relatively shallow depths in the soil profile are alternative processes whereby females could compensate for relatively low ambient temperatures without extending egg retention (Andrews, 2000).

On the other hand, NJ embryos could compensate for relatively cool mean incubation temperatures if they had relatively high developmental rates at low temperatures. Because eggs were incubated at only one temperature in this experiment, I am unable to rule out such an adaptive response to temperature. Studies on other species of *Sceloporus*, however, indicate that embryos do not exhibit physiological adaptation to local temperature conditions. For example, within a given phylogenetic lineage, embryonic developmental rates are similar between populations in warm and cold climates (Andrews et al., 1997; Andrews et al., 1999).

The production of multiple clutches in a single reproductive season may also explain why *S. undulatus* females in NJ do not retain eggs longer than females in SC. Females in NJ typically produce 2 clutches per year. The first clutch is laid in May/June and the second clutch is laid in June/July (Angilletta et al., 2000). In lizard species that produce multiple clutches, prolonged egg retention would increase the interval between clutches. Prolonged egg retention could thus

prevent NJ females from producing a second clutch of eggs during a single reproductive season and reduce female fitness.

Finally, the lack of geographic variation in the length of egg retention and embryonic differentiation and growth may be due to phylogenetic constraint. Out of twenty-two species groups, viviparity has evolved four times and only one species group contains both oviparous and viviparous forms (Méndez de la Cruz et al., 1998). The fact that viviparity only occurs in a few lineages suggests that live-bearing may evolve more readily in some lineages than in others (Andrews et al., 1999). The similarity of NJ and SC populations may thus reflect a general inability of members of the *undulatus* species group to support embryogenesis *in utero* during extended egg retention.

IN UTERO pO_2

A fundamental assumption of the cold climate hypothesis for the evolution of viviparity is that there is a selective advantage for oviparous females to retain embryos *in utero* for progressively longer periods of time (Packard et al., 1977; Shine, 1985). In previous studies, experimentally induced egg retention in the lizard *Urosaurus ornatus* (sister genus of *Sceloporus*) for as long as 29 days past the normal time of oviposition resulted in embryonic development being arrested at stages 30-30.5 (Mathies & Andrews, 1999). In contrast, *U. ornatus* embryos laid at the normal time of oviposition were eight stages more advanced than retained embryos. In *S. undulatus* from Virginia, embryos retained for approximately 10 days past the normal time of oviposition had dry masses 4 to 8 times less than that of control embryos that were laid at the normal time of oviposition (Mathies, 1998). In these studies, egg retention inhibited embryonic development. Likewise, in the present study, hypoxia had a negative effect on embryonic development, water uptake, and growth of CAM. Reduced growth of the embryo has also been observed in turtle (Kam, 1992), alligator (Deeming & Ferguson, 1990), and chick (Black & Snyder, 1980) embryos incubated under hypoxic conditions. The reduced rate of embryonic development of retained eggs *in utero* and of eggs incubated under simulated oviductal conditions at low pO_2 supports the hypothesis that hypoxia inhibits embryonic development *in utero.*

What is the effective pO_2 for *in utero* embryos? The question can be answered by comparing the stage and dry mass of embryos that were incubated at known pO_2's under simulated *in utero* conditions (Figure 1.2) with the stage and dry mass of embryos that were actually retained *in utero* for a similar length of time (Figure 1.3, from Mathies 1998). In Mathies (1998) study, embryos retained *in utero* for 10 days at an average temperature of 27 °C reached a mean stage of 29.5 and a mean dry mass of 0.8 mg. These values correspond most closely to those of eggs incubated under simulated oviductal conditions at 9% O_2 and an average temperature of 28 °C which reached a mean stage of 29.6 and a mean dry mass of 0.84 mg.

Studies of a variety of vertebrate taxa indicate that embryonic development normally occurs at relatively low pO_2 (Ar & Mover, 1994). For example, pO_2 measured within the uterine lumen of rabbits averaged 7.9 kPa (Mastroianni & Jones, 1965) and between 3.4 – 6.3 kPa in rats (Kauffman & Mitchell, 1994). Similarly, the pO_2 of blood from 4-day-old chick embryos ranged between 6.0 – 10.8 kPa (Meuer & Baumann, 1987). The estimated pO_2 for *in utero S. undulatus* embryos is therefore consistent with values observed in other vertebrate taxa.

Eggs incubated at 21% O_2 under simulated oviductal conditions grew and differentiated more slowly than eggs incubated at 21% O_2 simulated nest conditions. Growth of embryos under simulated oviductal conditions was reduced by about half and differentiated by about two stages relative to controls. I assume that the major difference between treatments was whether eggshells were fluid or air filled. In this situation, the reduced rate of embryonic development in the 21% simulated oviductal treatment would be associated with substantially lower rate of diffusion of oxygen in water than air (Wangensteen, et al. 1970/71). While fluid in the eggshell impedes the flow of oxygen, factors other than simple diffusion (e. g. O_2 concentration gradient, shell membrane structure, etc.) affect the actual oxygen availability to embryos. For example, the hard-shelled eggs of the turtle *Emydura macquarii* that are fluid filled (at oviposition) have an O_2 conductance roughly one-thirtieth of eggs in which the eggshells are air filled (Thompson, 1985). In my study, which involved parchment-shelled eggs, development was affected when eggshells were fluid filled but did not appear to have constituted a dramatic impediment to O_2 availability.

Results of this study suggest that retained *S. undulatus* embryos develop until embryonic oxygen consumption exceeds oviductal oxygen availability. Differentiation and growth of retained *S. undulatus* embryos is arrested at an embryo stage of approximately 30 and at a dry mass of approximately 0.8 mg. At this point in development, an effective pO_2 of 9% is apparently sufficient to maintain embryonic metabolism, but not to support further differentiation or growth. In contrast, embryos of *Sceloporus scalaris* retained *in utero* for as long as one month developed at the same rate as control eggs incubated under simulated nest conditions (Mathies & Andrews, 1996). The capacity of *S. scalaris* to support prolonged embryonic development *in utero* with little or no detrimental effects suggests that this species possesses physiological or morphological features that enhance diffusion of respiratory gases between maternal and embryonic circulation. The physiological features most obviously implicated are eggshell structure, vascularity of the oviduct and extraembryonic membranes (Andrews & Mathies, 2000), and oxygen-binding affinity of embryonic blood (Ingermann, 1992). Of these features, eggshell structure does not appear to be associated with the capacity to support embryonic development in *Sceloporus* lizards (Mathies & Andrews, 2000). Comparative studies that isolate the contribution of maternal and embryonic physiological features associated with gas exchange would provide insight to the physiological and morphological changes that occur during the transition from oviparity to viviparity.

LITERATURE CITED

Andrews RM. 1997**.** Evolution of viviparity: variation between two sceloporine lizards in the ability to extend egg retention. *Journal of Zoology (London)* **243:** 579-595.

Andrews RM. 2000. Evolution of viviparity in squamate reptiles (*Sceloporus* spp): a variant of the cold-climate model. *Journal of Zoology (London)*. **250:** 243-253.

Andrews RM. 2002**.** Low Oxygen: A constraint on the evolution of viviparity in reptiles. *Physiological and Biochemical Zoology* **75:** 145-154.

Andrews, RM, Rose BR. 1994. Evolution of viviparity: constraints on egg retention. *Physiological Zoology* 67: 1006-1024.

Andrews, RM, Mathies TM, Qualls, CP, Rose BR. 1997. Effects of low temperature on embryonic development of *Sceloporus* lizards. *Copeia* 1997: 827-833.

Andrews RM, Mathies TM, Qualls CP, Qualls FJ. 1999. Rates of embryonic development of *Sceloporus* lizards: do cold climates favor the evolution of rapid development? *Copeia* 1999**:** 692-700.

Andrews RM, Mathies TM. 2000. Natural history of reptilian development: constraints on the evolution of viviparity. *Bioscience* 50: 227-238.

Angilletta MJ. 2001. Thermal and physiological constraints on energy assimilation in a widespread lizard (*Sceloporus undulatus*). *Ecology* 82**:** 3044-3056.

Angilletta MJ, Winters RS, Dunham AE. 2000. Thermal effects on the energetics of lizard embryos: implications for hatchling phenotypes. *Ecology* 81: 2957-2968.

Ar A, Mover H. 1994. Oxygen tensions in developing embryos: system frequency or system requirement. *Israel Journal of Zoology* 40: 307-326.

Black CP, Snyder GK. 1980. Oxygen transport in the avian egg at high altitude. *American Zoologist.* 20: 461-468.

Deeming DC, Ferguson MJ. 1991. Reduction in eggshell conductance to respiratory gases has no effect on sex determination in *Alligator mississippiensis. Copeia* 1991: 240-243.

DeMarco V. 1993. Estimating egg retention time in sceloporine lizards. *Journal of Herpetology* 27: 453-458.

Dmi'el R. 1970. Growth and metabolism in snake embryos. *Journal of Embryology and Experimental Morphology* 23: 761-762.

Dufaure J, Hubert J. 1961. Table de developpment du lezard vivipare: *Lacerta* (*Zootica*) *vivipara* Jaquin. *Archives Anatomie Microscopie Morphologie Expérimental* 50**:** 309-327.

Ferguson GW, Brockman T. 1980. Geographic differences of growth rate of *Sceloporus* lizards (Sauria: Iguanidae). *Copeia.* 1980: 259-264.

Guillette LJ. 1982. The evolution of viviparity and placentation in the high elevation Mexican lizard *Sceloporus aeneus*. *Herpetologica* 38: 94-103.

Ingermann RL. 1992. Maternal-Fetal Oxygen Transfer in Lower Vertebrates. *American Zoologist* 32: 322-330.

Kam Y– C. 1992. Physiological effects of hypoxia on metabolism and growth of turtle embryos. *Respiration Physiology* 92: 127-138.

Kaufman DI, Mitchell JA. 1990. Alterations in intrauterine oxygen tensions during the estrous cycle of the rat and hamster and its regulation by ovarian steroid hormones: A comparative study. In: Piiper J, Goldstick TK, Meyer M, eds. *Oxygen transport to tissue. XII.* New York: Plenum Press, 745-750.

Mastroianni L, Jones R. 1965. Oxygen tension within the rabbit fallopian tube. *Journal of Reproduction and Fertility* 9: 99-102.

Mathies TM, Andrews RM. 1995a. Extended egg retention and its influence on embryonic development and egg water balance: implications for the evolution of viviparity. *Physiological Zoology* 61: 1021-1035.

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

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

Mathies TM. 1998. Constraints on the evolution of viviparity in the lizard genus *Sceloporus.* PhD dissertation. Virginia Polytechnic Institute and State University, Blacksburg, VA.

Mathies TM, Andrews RM. 1999. Determinants of embryonic stage at oviposition in the lizard *Urosaurus ornatus*. *Physiological and Biochemical Zoology* 72: 645-655.

Mathies TM, Andrews RM. 2000. Does reduction of the eggshell occur concurrently with or subsequent to the evolution of viviparity in phrynosomatid lizards? *Biological Journal of the Linnean Society* 71: 719-736.

Méndez-de la Cruz FR, Villagrán-Santa Cruz M, Andrews RM. 1998. Evolution of viviparity in the lizard genus *Sceloporus*. *Herpetologica* 54: 521-532.

Meur HJ, Baumann R. 1987. Oxygen supply of early chick embryo in normoxia and hypoxia. *Journal of Experimental Zoology Supplemental* 1: 203-207.

Miles DB, Sinervo, B, Frankino WA. 2000. Reproductive burden, locomotor performance, and the cost of reproduction in free ranging lizards. *Evolution*. 54: 1386-1395.

Packard GC, Tracy R, Roth JJ. 1977. The physiological ecology of reptilian eggs and embryos, and the evolution of viviparity within the Class Reptilia. *Biological Reviews* 52: 71-105.

Qualls CP, Shine R. 1998. *Lerista bougainvillii*, a case study for the evolution of viviparity in reptiles. *Journal of Evolutionary Biology* 11: 63-78.

SAS Institute. 1996. SAS version 8.2, SAS Institute, Cary, N.C.

Seymour RS, Geiser F, and Bradford DF. 1991. Gas conductance of the jelly capsule of terrestrial frog eggs correlates with embryonic stage, not metabolic demand or ambient O_2. *Physiological Zoology* 64: 673-687.

Shine R. 1983. 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. In: Gans C, Billet F, eds. *Biology of the Reptilia*. Volume 15. New York: Wiley and Sons, 605-694.

Shine R. 2002. Reconstructing an adaptationist scenario: What selective forces favor the evolution of viviparity in montane reptiles. *American Naturalist* 160: 582-593.

Shine R, Bull J. 1979. The evolution of live-bearing in lizards and snakes. *American Naturalist* 113: 905-923.

Sinervo, B, Hedges, R, and Adolph, SC. 1991. Decreased sprint speed as a cost of reproduction in the lizard *Sceloporus occidentalis*: variation among populations. *Journal of Experimental Zoology*. 155: 323-336.

Smith SA, Shine R. 1997. Intraspecific variation in reproductive mode within the scincid lizard *Saiphos equalis*. *Australian Journal of Zoology* 45: 435-445.

Stebbins RC. 1985. A field guide to western reptiles and amphibians. Second edition., Boston, Massachusetts: Houghton Mifflin.

Thompson MB. 1985. Functional significance of the opaque white patch in eggs of *Emydura macquarii*. In: Griggs G, Shine R, and Ehmann H, eds. *Biology of Australian frogs and reptiles.* New South Wales: Royal Zoological Society of New South Wales,387-395.

Tinkle DW, Ballinger RE. 1972. *Sceloporus undulatus*: a study of the intraspecific demography of a lizard. *Ecology* 53: 570-574.

Vleck CM, Hoyt DF. 1991. Metabolism and energetics of reptilian and avian embryos. In: Deeming DC, Ferguson MJ, eds. *Egg incubation: its effects on embryonic development in birds and reptiles.* Cambridge: Cambridge University Press, 285-306.

Wangensteen OD, Wilson D, and Rahn, H. 1970/71. Diffusion of gases across the shell of the hen's egg. *Respiration Physiology* 11: 16-30.

Table 1.1 Allocation of eggs from each clutch to control and experimental treatments. Eggs (2-5) of treatments were incubated under simulated oviductal conditions and control eggs (6) were incubated under simulated nest conditions.

Egg No.	$\%O_2$ (Treatment)
1	- (Sampled at oviposition)
2	4 (simulated oviduct)
3	9 (simulated oviduct)
4	15 (simulated oviduct)
5	21 (simulated oviduct)
6	21 (control)

Table 1.2. Mean percent oxygen concentration, standard error, and range for the four oxygen treatments measured during the 10 day incubation period. Total number of oxygen concentration measurements made during the incubation period for each treatment is given in parentheses.

Treatment (% O_2)	Mean oxygen concentration, %, (n)	Standard Error	Range
21%	20.4 (8)	0.06	20.1 – 20.6
15%	15.8 (8)	0.13	15.4 – 16.4
9%	9.5 (8)	0.29	8.2 – 10.4
4%	4.3 (6)	0.37	2.9 – 5.6

Table 1.3. Summary statistics: comparison of SVL, post-oviposition body mass, relative clutch mass (RCM), clutch size, egg mass, and embryonic stage at oviposition for female *S. undulatus*. Values represent means ± standard errors. Sample size is given in parentheses.

Variable	South Carolina	New Jersey	*t*	***P***
SVL (mm)	64.4 ± 1.05 (16)	65.5 ± 0.85 (16)	-0.139	0.890
Body mass (g)	7.83 ± 0.877 (10)	8.79 ± 0.409 (15)	-1.089	0.287
RCM	0.45 ± 0.058 (9)	0.34 ± 0.026 (16)	1.948	0.065
Clutch size	7.8 ± 0.56 (13)	6.6 ± 0.23 (14)	1.677	0.105
Egg mass (mg)	416 ± 17.5 (12)	470 ± 13.7 (12)	-2.405	0.025
Stage at oviposition	28.8 ± 0.25 (10)	28.4 ± 0.17 (12)	1.390	0.179

Table 1.4. Survival of *Sceloporus undulatus* embryos incubated at 4%, 9%, 15%, 21% O_2 simulated oviduct, and 21% O_2 control (simulated nest) treatments. S = survivors, NS = non-survivors. New Jersey (NJ), South Carolina (SC).

Treatment (%O_2)	4%	9%	15%	21%	21% Control
S (NJ/SC)	1/2	6/8	10/7	11/8	7/5
NS (NJ/SC)	12/6	5/1	3/0	2/0	1/0
% Survival (overall)	14.2	70	85	90.4	92.3

Table 1.5. Comparisons of *Sceloporus undulatus* eggs and embryos at 10 days of incubation. Means ± standard errors (n).

Variable	South Carolina	New Jersey
Stage		
9%	29.8 ± 0.21 (7)	29.5 ± 0.16 (8)
15%	30.1 ± 0.14 (7)	30.0 ± 0.19 (10)
21%	30.6 ± 0.18 (8)	30.4 ± 0.20 (11)
Control	32.4 ± 0.19 (5)	31.8 ± 0.21 (6)
Embryo dry mass (mg)		
9%	0.93 ± 0.180 (7)	0.86 ± 0.150 (8)
15%	1.10 ± 0.235 (7)	1.10 ± 0.280 (10)
21%	2.20 ± 0.230 (8)	1.60 ± 0.260 (11)
Control	3.60 ± 0.890 (5)	3.70 ± 0.190 (6)
Egg wet mass (mg)		
9%	---	561 ± 23.5 (8)
15%	---	578 ± 14.0 (10)
21%	---	588 ± 16.2 (11)
Control	---	737 ± 38.4 (6)
Absolute area of CAM (mm^2)		
9%	---	99.3± 17.11 (8)
15%	---	153.9 ± 44.35 (7)
21%	---	202.2 ± 42.19 (8)
Relative area of CAM	---	
9%	---	0.271 ± 0.045 (8)
15%	---	0.410 ± 0.104 (7)
21%	---	0.529 ± 0.103 (8)

Table 1.6. Statistical tests of responses of *Sceloporus undulatus* eggs and embryos after 10 days of incubation in 9%, 15%, and 21% oxygen treatments. Statistical analyses were two-factor ANCOVA's comparing treatment and population (New Jersey and South Carolina) except for analyses of egg wet mass and chorioallantoic membrane (CAM) which were one-factor ANCOVA's (New Jersey only).

	Statistical Test		
Response	Treatment	Population	Results:Treatment
Stage	$F_{2,46} = 11.4$, $P < 0.001$	$F_{1,46} = 1.2$, $P = 0.276$	9% < 15% < 21%
Embryo dry mass	$F_{2,41} = 8.7$, $P < 0.001$	$F_{1,41} = 2.5$, $P = 0.124$	9% = 15% < 21%
Egg wet mass	$F_{2,22} = 1.9$, $P = 0.175$	---	9% = 15% = 21%
Absolute area of CAM	$F_{2,19} = 3.7$, $P = 0.044$	---	9% = 15% < 21%
Relative area of CAM	$F_{2,19} = 3.5$, $P = 0.050$	---	9% = 15% < 21%

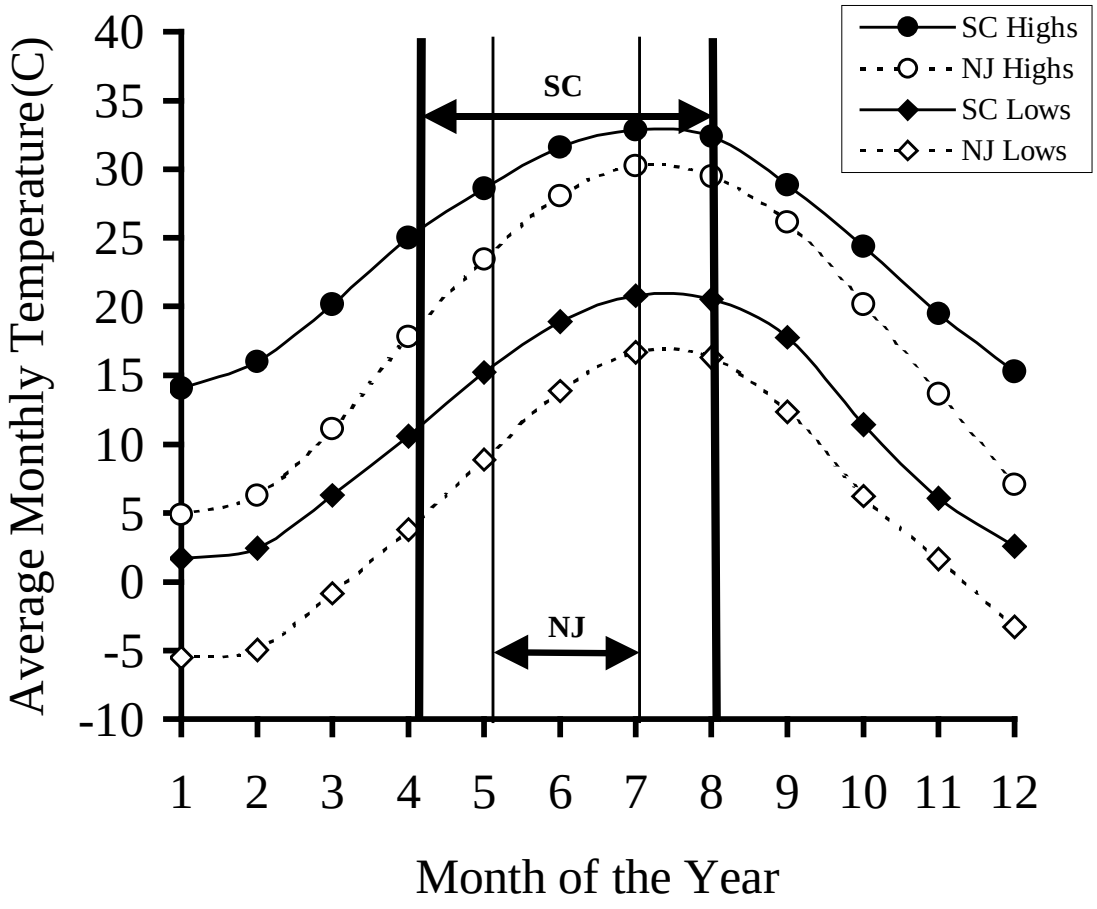


Figure 1.1 Average monthly maximum (circles) and minimum (diamonds) temperatures for Pemberton, NJ, (elevation 24 m, 39° 58' N, 074° 38' W), and Aiken SC, (elevation 158 m, 33° 34' N, 081° 44' W). Interval between fine vertical lines indicates months during which NJ females are gravid. Interval between bold vertical lines indicates months during which SC females are gravid. Monthly temperature data are 30-year averages obtained from the National Climate Data Center.

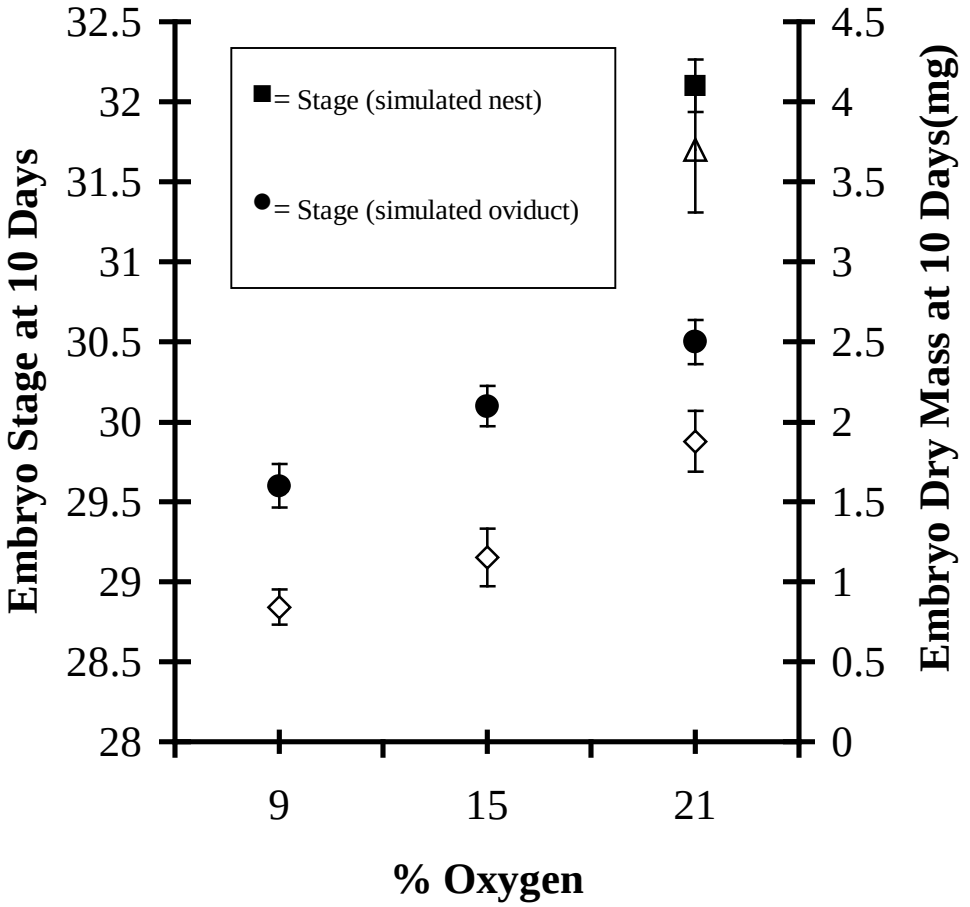


Figure 1.2 Embryonic responses (overall mean, SE) to incubation under 9%, 15%, 21% O_2 (simulated oviduct) and 21% O_2 control (simulated nest). NJ and SC populations were pooled for calculation of means. Filled circles represent embryonic stage, open diamonds represent embryonic dry mass. Results of statistical comparisons of treatment effects are presented in Table 1.6.

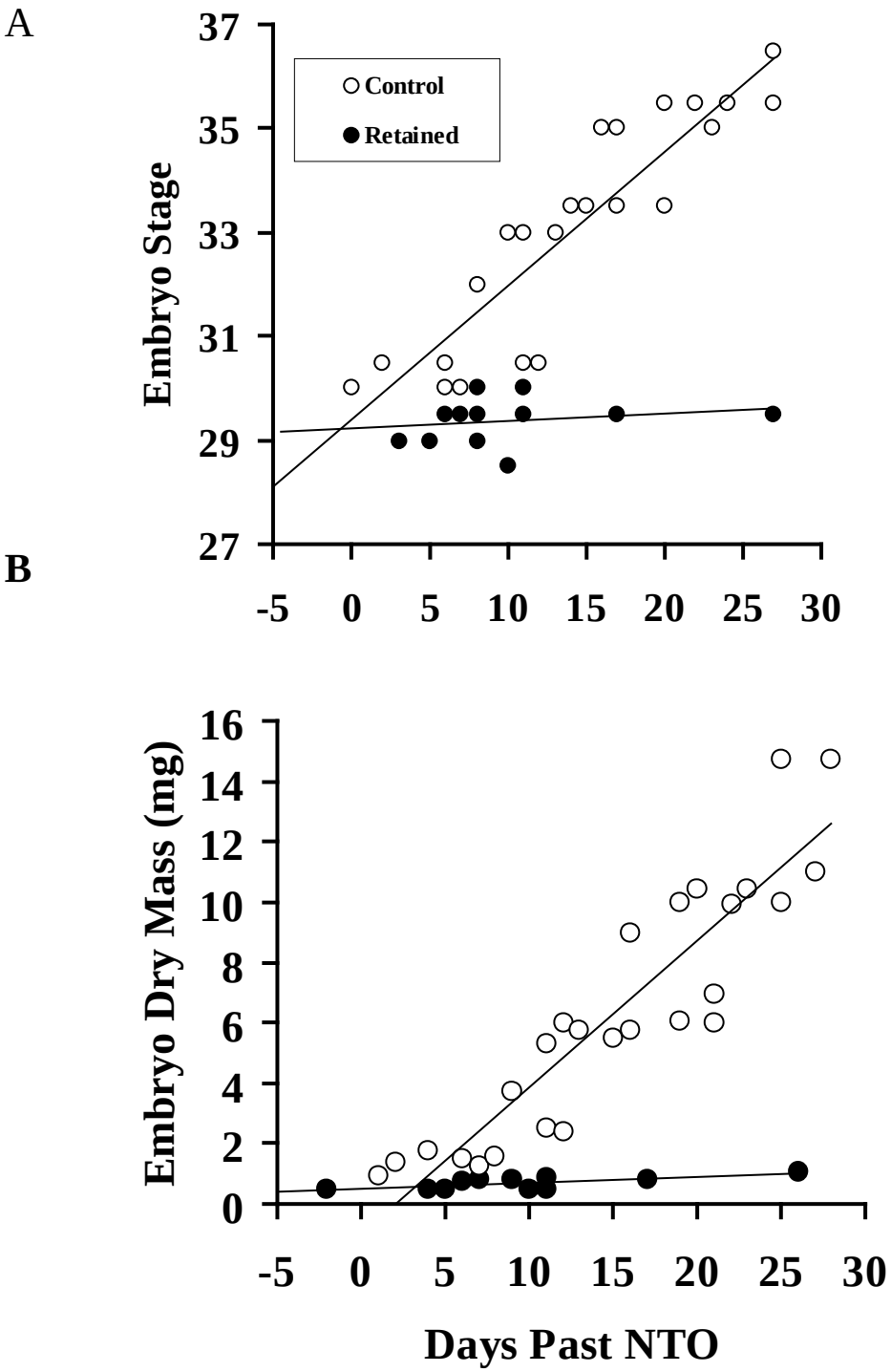


Figure 1.3. Embryo stage (A) and dry mass (B) of *Sceloporus undulatus* embryos in control (unfilled circles) and retained (filled circles) groups as a function of days past the normal time of oviposition (NTO) (Mathies, 1998).

CHAPTER 2

EVOLUTION OF VIVIPARITY IN SCELOPORINE LIZARDS: *IN UTERO* $p\mathbf{O_2}$ AS A DEVELOPMENTAL CONSTRAINT DURING EGG RETENTION

This work was published previously as:

Parker, SL, and Andrews, RM (2006) *Physiological and Biochemical Zoology*

University of Chicago Press

ABSTRACT

Reptilian viviparity evolves through selection for increasingly prolonged egg retention within the oviduct. In the majority of sceloporine lizard species, however, egg retention past the normal time of oviposition results in retarded or arrested embryonic development. In the present study, I tested the hypothesis that the amount of embryonic development normally attained *in utero* is directly related to *in utero* pO_2. The three species of sceloporine lizards I used are characterized by developmental arrest (*Urosaurus ornatus*), retarded development (*Sceloporus virgatus*), and normal development (*Sceloporus scalaris*) when eggs are retained. I incubated eggs of these species for 10 days under conditions that simulated retention in the oviduct at a range of experimental oxygen partial pressures (pO_2). I estimated *in utero* pO_2 from a standard curve generated from the stage and dry mass of experimental embryos incubated for 10 days at known pO_2. The standard curve was then used to predict the pO_2 associated with the observed rate of development of embryos retained *in utero*. The results of this study showed that the degree of embryonic development attained *in utero* during egg retention was positively associated with *in utero* pO_2. The results indicate that oxygen availability *in utero* is associated with interspecific differences in the capacity to support intrauterine development in sceloporine lizards.

INTRODUCTION

Reptilian viviparity putatively evolves through selection for increasingly prolonged retention of eggs within the oviduct (Packard et al. 1977; Shine 1983; Shine 1985). Recent studies in which gravid female lizards have been experimentally induced to retain eggs past the normal time of oviposition, however, have shown that embryonic development is often retarded or arrested during retention within the oviduct (Andrews and Rose 1994; Andrews 1997; Mathies 1998; Mathies and Andrews 1999; Mathies and Andrews 2000). Moreover, even if environmental conditions are not suitable for nesting, oviposition may still occur at the normal time. More generally, the majority of oviparous lizard species lay eggs at developmental stages 26-31, of Dufaure and Hubert's (1961) staging system where 0 is fertilization and 40 is hatching (Shine 1983; Blackburn 1995; Andrews and Mathies 2000). Eggs oviposited with embryos at stage 30 have completed approximately 25-40% of their total development (development time *in utero* plus development time in the nest) at the time of oviposition (DeMarco 1993; Shine 1983). The observations that few lizard species appear capable of retaining eggs much past stage 31, coupled with the fact that embryonic development is often retarded or arrested during extended egg retention, suggests that the capacity to support embryonic development much past stage 31 is constrained (Mathies and Andrews 1999; Mathies and Andrews 2000). Selection for prolonged egg retention will thus not lead to viviparity unless the physiological and morphological features necessary to support embryogenesis evolve concurrently with egg retention (Mathies and Andrews 1999; Andrews and Mathies 2000; Andrews 2002).

Oxygen availability for the developing embryo has been implicated as an important constraint on the evolution of reptilian viviparity (Packard et al. 1977; Guillette 1982; Shine 1985). The latter half of embryonic development is characterized by substantial growth in mass (Xavier and Gavaud 1986), and a concomitant large increase in metabolic oxygen demand by the embryo (Dmi'el 1970; Vleck and Hoyt 1991; Birchard et al. 1984). During the later stages of development, the uterine environment becomes increasingly hypoxic as a result of the metabolic demands of the growing embryo (Webb and Brent 1972). Embryonic development should thus become arrested when embryonic oxygen demand exceeds intra-uterine oxygen availability. The maximum embryonic stage at which squamate eggs are laid thus may be determined by the

oxygen supply of the oviduct. Accordingly, species capable of supporting embryonic development during prolonged egg retention should possess physiological and morphological features to enhance oxygen availability to developing embryos during gestation.

Studies on the oviparous lizard *Sceloporus undulatus* provide direct evidence that oxygen is the proximate factor that constrains the degree of embryonic development *in utero* (Andrews 2002; Parker et al. 2004). For example, Andrews (2002) demonstrated that embryonic growth in mass and morphological differentiation were retarded by incubation at low (7 kPa pO_2) oxygen partial pressure under simulated nest conditions. In a subsequent study, Parker et al. (2004) incubated eggs of *S. undulatus* under a range of oxygen partial pressures under conditions that simulated retention in the oviduct. In this latter study, the rate of growth and differentiation of *S. undulatus* embryos incubated at 8.6 kPa pO_2 under simulated *in utero* conditions was similar to that of the reduced rate of growth and differentiation of *S. undulatus* embryos retained in the oviduct for 10 days past the normal time of oviposition. The results of these studies suggest that the rate of embryonic development *in utero* is directly related to oxygen availability.

Comparative studies of species that differ in capacity to support embryonic development during egg retention have the potential to provide further insights regarding the influence of oxygen availability as a developmental constraint during egg retention. Sceloporine lizards are an ideal model taxon for comparative studies on the physiology of reptilian egg retention because the stage at which embryos are oviposited varies widely among species (Andrews 1997; Mathies and Andrews 2000; Méndez-de la Cruz 1998). Most species lay eggs with embryos at stages 28-30 (Mathies and Andrews 2000). A few species of *Sceloporus*, however, have the capacity to retain eggs for extended periods, with some species (e.g. *Sceloporus scalaris*) capable of retaining eggs until development is nearly completed (Andrews 1997; Mathies and Andrews 2000).

The objective of this study was to test the hypothesis that that the degree of embryonic development attained by reptilian embryos *in utero* is directly related to *in utero* oxygen availability. To meet this objective, I evaluated responses of embryos (survival, growth in mass, and morphological differentiation) of three species of sceloporine lizards in response to

incubation at a range of pO_2s under conditions that simulated retention in the oviduct. These data plus an assessment of actual embryonic development *in utero* were used to estimate the *in utero* pO_2 experienced by embryos during egg retention. The lizard species chosen for the study were selected based on their differing capacities to support embryonic development during extended egg retention. *Urosaurus ornatus* (Baird) (sister genus of *Sceloporus*) is capable of retaining eggs facultatively for nearly one month past the normal time of oviposition (Mathies and Andrews 1999). During egg retention, however, development of embryos is arrested at stages 30-30.5. *Urosaurus ornatus* embryos resume normal development once oviposition occurs. *Sceloporus virgatus,* (Smith) a member of the *undulatus* species group, has the capacity to retain eggs to stages 36-37, however, development of retained embryos is retarded compared to embryos laid at the normal time of oviposition (Andrews and Rose 1994; Andrews 1997). Finally, *Sceloporus scalaris,* (Smith) a member of the *scalaris* species group, has the capacity to retain eggs until development is nearly completed (i.e. stage 39). Moreover, during egg retention *S. scalaris* embryos develop at nearly the same rate as those laid at the normal time of oviposition (Mathies and Andrews 1995; 1996). Based upon knowledge of the developmental stage attained by embryos of the three species when retained past the normal time of oviposition, I predicted that the *in utero* pO_2 increases in the order of *U. ornatus*<*S. virgatus*<*S. scalaris.* I also predicted that survival and development of embryos would increase in the same order as a function of *in utero* pO_2.

MATERIALS AND METHODS

COLLECTION AND MAINTENANCE OF GRAVID FEMALES

Gravid females of *Urosaurus ornatus* (n=20), *Sceloporus virgatus* (n=20), and *Sceloporus scalaris* (n=2), were captured at the Chiricahua Mountains, Cochise County, Arizona during 30 June-5 July 2003. Gravid females of *U. ornatus* and *S. virgatus* were captured along canyons and dry washes approximately 2 km northwest of the American Museum of Natural History's Southwestern Research Station (SWRS). Gravid females of *S. scalaris* (n=2), were captured in the vicinity of Barfoot and Rustler Park. On the day of capture, females were measured for snout-vent length (SVL) and weighed to the nearest 0.1 g. Females were

maintained in glass terraria supplied with bark and branches in an animal facility at SWRS. The open-air animal facility was shaded and screened, and thus provided a natural photoperiod and diel temperature range. Females were watered by misting and fed with a variety of insects captured in the vicinity of SWRS daily. On 6 July, females were placed singly in cloth bags for transport by car to Virginia Polytechnic Institute and State University (Virginia Tech), Blacksburg, Virginia. From 8 July, 2003 on, females were maintained in an animal facility at Virginia Tech. *Sceloporus virgatus* and *S. scalaris* were housed in plastic containers (73 x 48 x 22cm, 2-3 females per container), and females of *U. ornatus* were housed in 94 x 51 x 50 cm plastic containers (10 females per container). Daily photoperiod was provided by ambient light from animal room windows and also from full spectrum Vita-lites (0800-1900). A 100-W spotlight suspended at one end of each container (0900-1900) provided a temperature gradient that allowed females to thermoregulate. Containers were provided with rocks and boards for basking. Females were fed crickets and mealworms daily. Water was provided daily by misting rocks, boards, and sides of terraria. Shallow plastic dishes were also placed in each container to collect water. Lizards were thus able to drink water accumulated on surfaces within the terraria while the shallow sand substrate remained dry. Experimental and animal care protocols were approved by the Virginia Tech Animal Care Committee (proposal # 02-101-Biol).

EXPERIMENTAL DESIGN: *UROSAURUS ORNATUS AND SCELOPORUS VIRGATUS*

Urosaurus ornatus and *S. virgatus* females were assigned to one of two groups. Individuals were selected such that size classes of females were uniformly distributed between the two groups. Gravid females of one group (*U. ornatus*: n=10, *S. virgatus*: n=10) were obtained at the normal time of oviposition of *U. ornatus* and *S. virgatus* populations in Arizona. Oviposition of both species occurs with the onset of summer monsoon rains (Andrews and Rose 1994). In 2003, summer rains began during the second week of July. Accordingly, female *U. ornatus* and *S. virgatus* were killed by decapitation and eggs and oviducts surgically removed on 12 and 14 July, respectively. Eggs were used to determine the rate of embryonic development over 10 days at a range of oxygen partial pressures (pO_2) (see following section on "manipulation of pO_2") under simulated *in utero* conditions (hereafter referred to as "experimental group").

A second group was used to determine developmental rate of embryos retained *in utero*. Females from this group (*U. ornatus*: n=10, *S. virgatus*: n=10) were induced to retain eggs for 10 days past the normal time of oviposition by maintaining them on a thin, dry sand substrate to inhibit oviposition (hereafter referred to as "retained group"). A dry substrate simulates drought conditions and females facultatively respond by retaining eggs (Andrews and Rose 1994). Females from the retained group were killed by decapitation on 22 and 24 July, respectively, and eggs and oviducts removed.

Developmental rates of embryos in the experimental and retained groups are comparable only if they are incubated at the same temperature. Therefore, body temperatures of gravid females of both species were measured during their activity period (0900-1900 hrs) prior to oviposition. To minimize disturbance, body temperatures were measured using a Raytek Ranger® ST 60™ infrared temperature sensor. For a sub-set of measurements, body temperatures were also measured cloacally with a thermocouple thermometer (Physi-Temp® Bat-12); on average, the two sets of measurements differed by less than 0.3 °C. The body temperature of each female was measured 1-4 times, and no female was measured twice within the same day. Overall, average activity body temperatures were calculated for each species based upon the mean body temperatures of each individual. Activity body temperatures averaged 33.0 ± 0.42 °C and 32.7 ± 0.39 °C for *U. ornatus* and *S. virgatus*, respectively. I assumed that body temperature declined to that of ambient temperature when females were inactive and unable to thermoregulate. Mean body temperatures during inactivity (1900-0900) were estimated by placing temperature probes in the substrate at the bottom of the enclosures and recording substrate temperatures at hourly intervals. The mean temperatures inside the enclosures during inactivity averaged 24.4 ± 0.01 °C (*U. ornatus*) and 24.5 ± 0.05 °C (*S. virgatus*). Overall body temperatures of female *U. ornatus* and *S. virgatus* during egg retention were calculated based on daily mean body temperatures, 27.8 ± 0.10 °C and 27.6 ± 0.09 °C, respectively.

EXPERIMENTAL DESIGN: *SCELOPORUS SCALARIS*

Female *S. scalaris* were maintained on a dry substrate to inhibit oviposition as described above. Because only two females were captured, I did not assign females to experimental and retained groups. Both females were therefore induced to retain eggs on a dry substrate until 26 July. At this time water was added to the substrate of their enclosures. Both females constructed nests, one oviposited on 26 July and the other on 29 July. Eggs were thus laid approximately two weeks after the time when oviposition would have occurred in Arizona. One egg from each clutch was sampled for embryo stage and the remainder allocated among experimental treatments (see below).

SAMPLING OF EGGS AND EMBRYOS

Eggs were weighed to the nearest 0.01 g within two hours of removal from the female and numbered consecutively within each clutch. Eggs were maintained in contact with moist Kimwipes® to ensure that the eggshells remained wet until placed under experimental conditions. A single egg from each clutch was used to measure the area of the chorioallantoic membrane (CAM) at oviposition. The extent of the CAM is visible through the moist eggshell, and was therefore easily measured. The major and minor axes of each CAM were measured to the nearest 0.1 mm using dial calipers and the surface area of the CAM estimated using the formula for an ellipse. If the CAM covered more than 50% of the eggshell surface, the major and minor axes of the region of eggshell not covered by the CAM were measured, and the area not covered by the CAM subtracted from the total surface area of the egg. The surface area of the egg was estimated from egg mass using the formula, $\text{Area}=4.835\ \text{Mass}^{0.662}$ (Panganelli et al. 1974) where area is in cm^2 and mass is in grams. The size of the CAM was expressed as absolute area and as a percentage of the surface area of the egg.

After completion of CAM measurements, one to two eggs were dissected to determine the embryonic stage at oviposition according to Dufaure and Hubert (1961), where stage 0 is fertilization and 40 is hatching. Half stages were assigned for embryos exhibiting intermediate suites of traits. After staging, embryos were dried to a constant mass at 40 °C and weighed.

MANIPULATION OF PO_2

Growth and differentiation of experimental embryos were determined under conditions that simulated retention on the oviduct. To simulate oviductal conditions, eggs were incubated in contact with a moist substrate such that the channels of the eggshell remained fluid filled as would normally occur within the oviduct. One or two eggs were placed in 70ml glass specimen jars lined with Whatman® filter paper moistened with distilled water (pH adjusted to 7.4). The filter paper was remoistened at least every three days to ensure that the channels of the eggshell remained fluid filled throughout incubation (see also Seymour et al. 1991 for similar experimental approaches). This procedure may best simulate *in utero* conditions because during egg retention, eggs are pressed against the walls of the oviduct and are thus in close apposition to the maternal blood supply (Seymour, *pers. communication*). Eggs from each clutch were allocated among four oxygen treatments (target values: 5%, 9%, 15%, and 21%) (Table 2.1). As a control (simulating nest conditions), one or two eggs from each clutch were placed in specimen jars partially filled with vermiculite moistened with distilled water (0.7:1.0g H_2O:vermiculite) corresponding to a water potential of –200 kPa (determined by thermocouple psychrometry, 21% O_2 only). Experimental and control eggs in the 21% O_2 treatment were thus treated identically except that the shells of experimental eggs remained fluid filled during incubation whereas the shells of control eggs remained dry and filled with air.

Experimental and control eggs were placed, according to treatment into one of four airtight metal boxes, and the boxes flushed with the appropriate gas mixture (O_2 and N_2) using a Cameron Instruments, Model GF-3/MP gas mixing flow meter. Air inside the boxes was humidified by bubbling the gas mixture through distilled water. Boxes were flushed at least every three days, and every time the boxes were flushed the oxygen concentration inside the boxes was measured using an Applied Electrochemistry S-3A/II oxygen analyzer. Mean oxygen concentrations (in dry air) over the course of the study period were 5.8%, 8.7%, 15.3%, and 20.4% respectively. Based upon a mean air pressure at Blacksburg (625 m) of 94.5 kPa (711 mmHg), a mean incubation temperature of 28 °C, and a P_{H2O} of 3.8 kPa of water vapor in air, the actual values of pO_2 for the four oxygen treatments during the study period were 5.3, 7.9, 13.8, and 18.5 kPa pO_2, respectively.

The boxes were placed in a single programmable temperature chamber and incubated for 10 days under a fluctuating temperature regime. The temperature regime was selected to match the body temperatures of females in the retained group. Temperatures inside the boxes varied linearly for 4 hours between daily maximum and minimum temperatures (mean daily maximum: 32.8 °C, mean daily minimum: 23.1 °C, overall mean: 27.9 °C). Incubation temperatures were measured inside the boxes where the eggs were maintained: for simulated oviductal treatments, the temperature probe was placed at the bottom of the specimen jar in contact with the moistened filter paper. For simulated nest treatments, the temperature probe was placed in the center of the specimen jar and covered with vermiculite. The boxes were rotated within the chamber every 3 – 5 days to minimize position effects on embryonic development.

ESTIMATION OF *IN UTERO* PO_2

In utero pO_2 was estimated by comparing the developmental rate of embryos retained *in utero* (where pO_2 is unknown) with the developmental rate of embryos incubated under simulated *in utero* conditions at known pO_2s. After 10 days of incubation/retention, eggs from experimental and retained groups were dissected to determine CAM area, embryo stage, and embryo dry mass (described above). To estimate pO_2 *in utero*, a standard curve was generated from the stage and dry mass of experimental embryos incubated for 10 days under known pO_2. The standard curve was then used to predict the pO_2 associated with the observed *in utero* rate of embryonic differentiation (stage) and growth (dry mass).

DATA MANIPULATION AND STATISTICAL ANALYSES

Statistical analyses were conducted using the SAS Statistical Package (SAS Institute 1996). Because no embryos of *U. ornatus* survived in the 5.3 and 7.9 kPa pO_2 treatments, the effect of oxygen treatment on survival of embryos between the 13.8 and 18.5 kPa pO_2 treatments were analyzed using a Fishers Exact Test. For *S. virgatus,* the effect of oxygen treatment on egg survival among the 5.3, 7.9, 13.8, and 18.5 kPa treatments was analyzed using a Chi-square Test of Independence (FREQ Procedure). The effect of oxygen treatment on embryo survival between the 18.5 kPa pO_2 treatment and control (simulated nest) was analyzed using a

Fisher's Exact Test. Contrasts of embryonic features and water uptake by eggs for oviposited (day 0) and retained groups (day 10) were analyzed using a Student's T-test (T-test Procedure). The effect of oxygen treatment on embryonic features and water uptake by eggs at 10 days was evaluated using analysis of covariance (ANCOVA) (GLM Procedure) with values at oviposition of embryo dry mass (when dry mass was the dependent variable), stage (when stage was the dependent variable), or wet egg mass (when CAM area was the dependent variable) as a covariates. Analyses of treatment effects were based on clutch means. No *U. ornatus* embryos survived in the 7.9 kPa treatment, although embryos increased substantially in dry mass and stage suggesting that embryos survived until late in the 10-day observation period. Dead *U. ornatus* embryos from this treatment were thus included in my analyses on the effect of oxygen treatment on embryonic growth and differentiation which otherwise only included live embryos. To assess the consequences of using dead embryos in analyses, I also conducted separate analyses of treatment effects using combined live and dead *U. ornatus* and *S. virgatus* embryos (stage only, mass could not be determined reliably for embryos that died early in the 10-day period). Observations of relative area of CAM were arcsine transformed prior to analysis. When the covariate was not significant, single-factor analysis of variance (ANOVA) was used to evaluate treatment effects. Prior to ANCOVA analyses, the assumption of homogeneity of slopes was tested. For all ANCOVAs, *post hoc* pair-wise comparisons were made using a least significant difference test on least squared means. For all ANOVAs, *post hoc* pair-wise comparisons were made using a Tukey's honestly significant difference test. Because only two *S. scalaris* clutches were available, sample sizes were too low for statistical analysis. Data from *S. scalaris* eggs and embryos, however, were reported for comparative purposes. Data are reported as the mean ± SEM unless otherwise reported, and probability values less than 0.05 were considered significant.

RESULTS

SURVIVAL OF EMBRYOS RETAINED *IN UTERO*

Survival of embryos differed among species (Table 2.2). After 10 days of retention, survival by *U. ornatus* embryos was lower than that of *S. virgatus* embryos when assessed as the

number of clutches containing dead embryos (Fisher's exact test, n=20 P=0.033, data not shown in Table 2.2) and as total embryo survival (Fisher's exact test, n=38 P=0.007). Fifty percent of retained *U. ornatus* clutches contained dead embryos, and overall survival of retained embryos was 67%. In contrast, all retained embryos of *S. virgatus* survived. All embryos of *S. scalaris* survived 14 and 17 days of retention *in utero* before they were transferred to the experimental treatments.

SURVIVAL OF EXPERIMENTAL EMBRYOS

Survival of embryos varied both as a function of treatment and of species (Table 2.2). Survival increased with pO_2 and was lowest for *U. ornatus* and highest for *S. scalaris*. Survival of *U. ornatus* and *S. virgatus* embryos varied among oxygen treatments (*U. ornatus:* χ^2=33.1, n=62, d.f.=3, P<0.001; *S. virgatus:* χ^2=39.6, n=65, d.f.=3, P<0.001). Survival of *U. ornatus* embryos was lower than *S. virgatus* embryos in the 7.9 kPa pO_2 treatment (Fisher's exact test n=33, P=0.018). Survival of *U. ornatus* and *S. virgatus* embryos did not differ between either the 13.8 (Fisher's exact test, n=31, P=0.066), or the 18.5 kPa pO_2 treatments (Fisher's exact test, n=31, P=0.10). For *S. scalaris*, survival of embryos was 50% in the 5.3 kPa treatment, and 100% in the 7.9, 13.8, and 18.5 kPa treatments. Because of low survival of *U. ornatus* and *S. virgatus* embryos in the 5.3 kPa treatment, observations on embryonic differentiation and growth in this treatment were not included in subsequent analyses.

EFFECTS OF RETENTION I*N UTERO* ON EGGS AND EMBRYOS

The effect of retention *in utero* on embryonic development varied among species (Table 2.3). After 10 days of retention *in utero*, embryos of *U. ornatus* did not differ in stage or dry mass from that of the stage and dry mass at the normal time of oviposition (day 0). Embryos at day 0 and at day 10 had a mean stage of about 30 and a mean dry mass of about 0.6 mg. In contrast, after 10 days of retention *in utero*, *S. virgatus* embryos had advanced from a mean stage of 32 to a mean stage of 33.8 and had nearly doubled in dry mass (2.3 to 4.2 mg, respectively). I could not compare the amount of embryonic growth and differentiation between oviposition and retention for embryos of *S. scalaris* because they were not sampled at the normal time of

oviposition. Both females laid eggs with embryos at stage 39, that is, embryos were almost fully developed. Observations by Mathies and Andrews (1995) on embryos of *S. scalaris* at the time of normal oviposition suggest that embryos would have advanced from stage 34-36 to stage 39 during the roughly two weeks of retention that I observed.

In contrast to embryonic development during egg retention, egg wet mass, absolute, and relative area of CAM did not differ between embryos at day 0 at day 10 for either *U. ornatus* or *S. virgatus*. Eggs therefore did not take up water and the CAM did not increase in size during the 10 days of retention.

GROWTH AND DIFFERENTIATION OF EXPERIMENTAL EMBRYOS

Development of embryos varied as a function of treatment (Tables 2.4 and 2.5, Figures 2.1 and 2.2). Growth in mass of embryos of all species increased with oxygen availability (Table 2.5, Figures 2.1 and 2.2). For both *U. ornatus* and *S. virgatus*, growth of experimental embryos incubated at 18.5 kPa pO_2 was reduced compared to the growth of embryos incubated under simulated nest conditions at 18.5 kPa. In contrast, growth of experimental *S. scalaris* embryos incubated at 18.5 kPa pO_2 was similar to that of embryos incubated at 18.5 kPa pO_2 under simulated nest conditions (Figure 2.2). Treatment affects on differentiation (stage) paralleled those of growth in mass (Table 2.5, Figure 2.1). ANOVAs using combined live and dead *U. ornatus* and *S. virgatus* embryos gave the same significance levels for treatment contrasts as those presented in Table 2.5.

Of the three species, *S. scalaris* embryos were the least affected by incubation at low pO_2. For example, three of four embryos in the 5.3 kPa pO_2 treatment attained stage 40, as did all experimental embryos incubated at 7.9, 13.8, 18.5 kPa, and control embryos incubated at 18.5 kPa pO_2 during the 10 day experimental period. Seven (of the 8) *S. scalaris* eggs that remained under experimental conditions after the 10-day sample hatched after a total of 12-16 days of incubation. There was a trend of increasing incubation period with decreasing pO_2. On average, the incubation period was shortest in the 18.5 kPa pO_2 simulated oviductal treatment (mean=12.5 days $\pm$ 0.5) and longest in the 5.3 kPa pO_2 treatment (mean=15.5 $\pm$ 0.5). Hatchling masses

increased as a function pO_2. Mean hatchling masses in the simulated oviductal treatments after 12-16 days of incubation were 36.2 mg ± 0, 49.9 mg ± 8.5, 59.7 mg ± 4.8, and 66.5 mg ± 4.3 (5.3, 7.9, 13.8, and 18.5 kPa pO_2 treatments, respectively), and 63.1 mg ± 0, in the 18.5 kPa pO_2 simulated nest treatment.

MASS AND CHORIOALLANTOIS OF EXPERIMENTAL EGGS

Eggs of all three species exhibited similar patterns of water uptake as judged by the wet mass of eggs after 10 days of incubation (Tables 2.4, 2.5). Mass of eggs increased in parallel with oxygen availability, and eggs in the simulated nest treatment took up less water than eggs in any of the experimental treatments.

The size of the chorioallantois paralleled that of wet egg mass. In general, the absolute and relative areas of the CAM increased with increasing oxygen. The absolute area of the CAM of eggs incubated in vermiculite was lower than that of eggs incubated in water at 18.5 kPa pO_2. Because CAM coverage was essentially complete, eggs that took up less water would have had lower surface areas and thus absolutely smaller CAM's. This is why, for example, the relative areas of the CAM in the 18.5 kPa treatment was almost identical (about 100%) for all species at the end of the 10 day period.

IN UTERO PO_2

Estimates of *in utero* pO_2 projected from growth in dry mass and stage differed slightly (1-2 kPa pO_2) for both *U. ornatus* and *S. virgatus*. Similarly, estimates of *in utero* pO_2 based upon embryo stage differed when live and dead embryos were combined and used to generate the standard curve. Nonetheless, lower estimates for *U. ornatus* than *S. virgatus* supported my initial prediction (Figure 2.1). Embryos of *U. ornatus* retained *in utero* for 10 days had a mean dry mass of 0.6 mg and a mean stage of 30 (Table 2.3). These values correspond most closely to those of *U. ornatus* embryos incubated under simulated oviductal conditions at 5-6 kPa pO_2 (6-7% pO_2). In contrast, embryos of *S. virgatus* retained *in utero* for 10 days had a mean dry mass

of 4 mg and a mean stage of 34 (Table 2.3). These values correspond most closely to those of *S. virgatus* embryos incubated at 9-11 kPa (10-12% pO_2) under simulated oviductal conditions.

The results of my experiments suggest that development of *S. scalaris* embryos is not limited by *in utero* oxygen availability during extended egg retention. My conclusion is based on four main observations. First, gravid female *S. scalaris* retained eggs until embryonic development was nearly completed (i.e. stage 39). Second, I did not observe differences in growth between experimental embryos and embryos incubated under simulated nest conditions at 18.5 kPa pO_2. Third, eggs hatched in all treatments while under experimental conditions. Finally, in previous studies, *S. scalaris* embryos retained *in utero* developed at the same or nearly the same rate as embryos incubated under standard conditions (Mathies and Andrews 1996; Andrews 1997).

DISCUSSION

INTERSPECIFIC COMPARISONS OF PO_2 *IN UTERO*

The embryonic stages at which eggs of the three species were oviposited in the laboratory represent the normal range of stages for eggs oviposited in the field. Because development of *U. ornatus* embryos is arrested at about stage 30, gravid females oviposit eggs with embryos at stages no greater than 30 (Mathies and Andrews 1999). Females of *Sceloporus virgatus* normally oviposit at stages 31-33 (Andrews and Mathies 2000) and *S. scalaris* from high elevation populations (where I also collected females) oviposit at stages 35-37 (Mathies and Andrews 1995). As a consequence, I cannot directly compare developmental rates among the three species. Nonetheless, my results provide comparative estimates of *in utero* pO_2.

As predicted, *U. ornatus* embryos had the lowest *in utero* pO_2 as well as the lowest survival at low pO_2 of the three species (Table 2.2). *Urosaurus ornatus* embryos did not develop at 5.3 kPa pO_2. Some development occurred at 7.9 kPa pO_2 although no embryos survived the 10-day experimental period. Development of *U. ornatus* embryos thus became arrested at an *in utero* pO_2 of about 6 kPa (Figure 2.1) when embryos reached stage 30 (Table 2.3).

Developmental arrest presumably occurred when oxygen demands of the embryos exceeded oviductal oxygen availability. Embryo survival was reduced at 13.8 kPa pO_2, compared to the18.5 kPa pO_2 simulated oviductal treatment although there was no difference in growth and differentiation between the two treatments.

In contrast, *S. virgatus* embryos developed at oxygen partial pressures as low as 7.9 kPa, although survival, growth, and differentiation were much reduced compared to the 13.8 and 18.5 kPa pO_2 treatments (Table 2.2 and Figure 2.1). Moreover, two *S. virgatus* eggs sampled after 15 days of incubation at 7.9 kPa pO_2 contained living embryos at stage 35 (unpublished data). In previous studies, embryos of *S. virgatus* retained *in utero* for as long as one month beyond the normal time of oviposition developed as far as stage 37. Development of retained embryos, however, was retarded and survival to hatching was reduced compared to control eggs laid at the normal time of oviposition (Andrews and Rose 1994; Andrews 1997). For example, after 30 days of retention *in utero*, the dry masses of *S. virgatus* embryos were less than half that of eggs laid at the normal time of oviposition. Similarly, in the present study, *S. virgatus* embryos incubated at 7.9 kPa pO_2 were less advanced developmentally, by about 3 stages and had dry masses less than half those of embryos incubated under simulated nest conditions (Figure 2.1). Embryos of *S. virgatus* are still developing, albeit relatively slowly, at a predicted *in utero* pO_2 of 10-11 kPa. Whether this pO_2 would be sufficient to support the metabolic demands of the embryo throughout development until hatching is not known, however.

Sceloporus scalaris embryos exhibited the highest survival and greatest growth at low pO_2 of the three species (Tables 2.2, 2.4 and Figure 2.2). Because my observations were restricted to two clutches of eggs, I cannot rule out the possibility that my results may not represent a typical response of *S. scalaris* embryos to incubation at low pO_2. My results (albeit based upon small sample size) are supported, however, by observations from previous studies demonstrating that development of *S. scalaris* embryos is not retarded during prolonged egg retention (Mathies and Andrews 1996; Andrews 1997). Unlike embryos of *U. ornatus* and *S. virgatus*, *S. scalaris* embryos incubated in the 5.3 kPa pO_2 treatment survived and increased in mass over the 10-day experimental period. Furthermore, hatching occurred in all treatments including the 2 eggs in the 5.3 kPa pO_2 treatment, and the 1 egg in the 7.9 kPa pO_2 treatment.

Finally, in contrast to *U. ornatus* and *S. virgatus* embryos, the dry masses of experimental embryos in the 18.5 kPa pO_2 treatment were very similar to those of control embryos incubated at 18.5 kPa pO_2 (18.5 kPa experimental: 60.8 mg ± 1.15 vs. 18.5 kPa control: 58.3 mg ± 2.91, Figure 2.2). Experimental embryos thus grew nearly the same amount as embryos incubated under simulated nest conditions. Retained embryos from both *S. scalaris* clutches were laid at stage of 39, thus embryos had completed the majority of their development *in utero* by the time oviposition occurred.

While I was not able to directly estimate *in utero* pO_2 for *S. scalaris*, the ability of embryos to survive and develop at 5.3 and 7.9 kPa pO_2 suggests that *S. scalaris* possess physiological features that enhance oxygen uptake during egg retention. Because oxygen consumption by embryos increases throughout development, the negative effects of low oxygen on embryo survival and growth should be greatest late in development when oxygen demand of embryos is highest (Dmi'el 1970; Vleck and Hoyt 1991; Birchard et al. 1984). For example, when eggs of *S. undulatus* were incubated under simulated nest conditions at low (7 kPa) pO_2 during the later half of development (≥ stage 38), survival and growth of embryos were reduced relative to that of embryos incubated at low pO_2 during the first half of development (Andrews 2000). In contrast, *S. scalaris* embryos were nearly fully developed (stage 39) when placed under experimental conditions, yet embryos survived and increased in mass at low pO_2.
The results of my experiments confirmed the prediction that the degree of embryonic development attained during egg retention is directly related to *in utero* pO_2. Because my method of estimating *in utero* pO_2 is indirect, however, my results provide a relative estimate of the pO_2 experienced by lizard embryos retained *in utero*. Estimated *in utero* pO_2 was lowest for *U. ornatus* (5-6.5 kPa), followed by *S. virgatus* (10-11 kPa), and highest for *S. scalaris* (>11 kPa). Moreover, my results are consistent with observations that embryonic development of *Sceloporus undulatus* becomes arrested at stage 30 and at a partial pressure of 8.6 kPa (9% O_2) when retained past the normal time of oviposition (Mathies 1998; Parker et al. 2004). Thus, the relatively low estimate of *in utero* pO_2 for retained *U. ornatus* embryos is consistent with values observed in *S. undulatus*. Unlike *U. ornatus, S. virgatus,* and *S. undulatus,* extended egg retention has relatively little effect on growth and development of *S. scalaris* embryos. For example, when, *S. scalaris* embryos were experimentally retained *in utero* past the normal time

of oviposition, embryos grew at the nearly the same rate as control embryos incubated at ambient pO_2 (Mathies and Andrews 1996; Andrews 1997). These observations suggest that *in utero* pO_2 is substantially higher in *S. scalaris* than the other species.

OXYGEN AVAILABILITY AND EMBRYONIC DEVELOPMENT

Previous studies on embryos from a wide variety of taxa including insects (Frazier et al. 2001; Woods and Hill 2004), frog (Seymour et al. 2000), turtle (Kam 1992), alligator (Deeming and Ferguson 1991), and chick (Black and Snyder 1980), have demonstrated that embryonic development is reduced or arrested at low pO_2. For example, development of *Drosophila* embryos is completely arrested when embryos are incubated under severe hypoxic conditions, but reinitiate development when oxygen content is increased (Teodoro and Farrell 2003). The results of my study implicates *in utero* oxygen availability as the primary factor responsible for interspecific differences in the capacity to support intrauterine development in sceloporine lizards. The stage attained by embryos retained *in utero* is apparently determined by the ability of gravid females to provide sufficient oxygen to meet the metabolic demands of developing embryos. Thus, embryonic development is presumably arrested when the oxygen demand of the growing embryos equals or exceeds the oxygen availability in the oviduct.

What physiological and morphological features affect *in utero* pO_2 for embryos retained past the normal time of oviposition? Oxygen must first diffuse from oviductal capillaries, through the fluid film surrounding the egg, and across the eggshell before being taken up by the embryonic blood supply in the chorioallantois. Thus, a combination of both maternal and embryonic features could potentially affect oxygen availability *in utero*. Of these features, a reduction in eggshell thickness, associated with extended egg retention has been observed in several species (Guillette and Jones 1985; Mathies and Andrews 1995; Qualls and Shine 1998; Heulin et al. 2001). Reduced eggshell thickness could enhance oxygen availability to the embryo by decreasing the diffusion distance between maternal and embryonic circulation. In phrynosomatid lizards, however, eggshell structure and thickness is not associated with the capacity to support embryonic development during extended egg retention (Mathies and Andrews 2000). The most likely features mediating gas exchange in the oviduct are thus the

vascularized respiratory surfaces of the oviduct and CAM, and oxygen binding properties of embryonic blood.

The oviduct and CAM are both believed to play an integral role in gas exchange (Guillette and Jones 1985; Masson and Guillette 1987; Yaron 1985; Blackburn 1993; Stewart and Thompson 1993). In two closely related species of *Sceloporus* lizards, for example, the vascular density of the oviduct is higher in the viviparous species (*S. bicanthalis*) than the oviparous species (*S. aeneus*) (Guillette and Jones 1985). The CAM covers the inner surface of the eggshell and functions as the primary respiratory membrane for the late stage embryo. In this study, because the embryo stage at oviposition varied dramatically between species (mean stage at oviposition: *U. ornatus* 29.6, *S. virgatus* 32.1, *S. scalaris* 39), I could not directly compare the absolute and relative size of the CAM among the three species. A previous study, however, demonstrated that at comparable embryonic stages, both absolute and relative CAM area was larger for *S. scalaris* than *S. virgatus* (Andrews 1997). The larger CAM of *S. scalaris* eggs would provide a greater surface area for gas exchange, and thus enhance oxygen availability to the embryo. In addition to surface area, vascular density of the CAM could also play an important role in embryonic gas exchange. To my knowledge, no comparative studies of CAM vascular density have been conducted on any species of squamate reptile.

Oxygen binding affinity of embryonic blood could also facilitate increased oxygen uptake of embryos retained in the oviduct. Several studies on viviparous squamates have demonstrated that the oxygen binding affinity of fetal blood is greater than that of maternal blood (Grigg and Harlow 1981; Birchard et al. 1984; Holland et al. 1990; Ragsdale and Ingermann 1991). Increased blood oxygen affinity has been documented in chick embryos when incubated under hypoxic conditions (Baumann et al. 1983; Ingermann 1992). The oxygen binding properties of embryonic blood in oviparous reptiles is less well documented (Ingermann 1992) and whether oxygen binding affinity of embryonic blood in oviparous reptile species differs from that of adults is unknown.

The results of this study support the hypothesis that selection for progressively longer periods of egg retention will not lead to viviparity unless features that enhance oxygen

availability to embryos evolve concurrently with egg retention. The contributions of maternal and embryonic physiological features and of shell morphology associated with gas exchange, however, have yet to be studied in a broadly comparative sense. Future studies should examine the mechanisms of embryonic gas exchange both among squamate families with diverse reproductive natural histories and among closely related taxa within these families.

LITERATURE CITED

Andrews R.M. 1997. Evolution of viviparity: variation between two sceloporine lizards in the ability to extend egg retention. *Journal of Zoology (London)* 243:579-595.

Andrews R.M. 2002. Low oxygen: a constraint on the evolution of viviparity in reptiles. *Physiological and Biochemical Zoology* 75:145-154.

Andrews R.M. and B.R. Rose. 1994. Evolution of viviparity: constraints on egg retention. *Physiological Zoology*. 67:1006-1024.

Andrews R.M. and T.M. Mathies. 2000. Natural history of reptilian development: constraints on the evolution of viviparity. *BioScience* 50:227-238.

Baumann R., S. Padeken, E.–A. Haller, and T. Brilmayer. 1983. Effects of hypoxia on oxygen affinity, hemoglobin pattern, and blood volume of early chicken embryo. *American Journal of Physiology* 244:R733-R741.

Birchard G.F., C.P. Black, G.W. Schuett, and V. Black. 1984. Influence of pregnancy on oxygen consumption, heart rate and hematology in the garter snake: implications for the "cost of reproduction" in live bearing reptiles. *Comparative Biochemistry and Physiology* 77A:519-523.

Blackburn D.G. 1993. Chorioallantoic placentation in squamate reptiles: structure, function, development, and evolution. *Journal of Experimental Zoology* 266:414-430.

Blackburn D.G. 1995. Saltationist and punctuated equilibrium models for the evolution of viviparity and placentation. *Journal of Theoretical Biology* 174:199-216.

Black C.P. and G.K. Snyder. 1980. Oxygen transport in the avian egg at high altitude. *American Zoologist* 20:461-468.

Deeming D.C. and M.J. Ferguson. 1991. Reduction in eggshell conductance to respiratory gases has no effect on sex determination in *Alligator mississippiensis. Copeia*:1991:240-243.

DeMarco V. 1993. Estimating egg retention time in sceloporine lizards. *Journal of Herpetology* 27:453-458.

Dmi'el R. 1970. Growth and metabolism of snake embryos. *Journal of Embryology and Experimental Morphology* 23:761-762.

Dufaure J. and J. Hubert. 1961. Table de development du lezard vivipare: *Lacerta (Zootica) vivipara* Jaquin. *Archives Anatomie Microsopie Morphologie Expérimental* 50:309-327.

Frazier M.R., H.A. Woods, and J.F. Harrison. 2001. Interactive effects of rearing temperature and oxygen on the development of *Drosophila melanogaster*. *Physiological and Biochemical Zoology* 74:641-650.

Grigg G.C. and P. Harlow. 1981. A fetal-maternal shift of blood oxygen affinity in an Australian viviparous lizard, *Spenomorphus quoyii* (Reptilia, Scincidae). *Journal of Comparative Physiology* 142:495-499.

Guillette L.J. Jr. 1982. The evolution of viviparity and placentation in the high elevation 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. Journal of Morphology* 184:85-98.

Heulin B., G. Samuele, N. Vogrin, Y. Surget-Groba, and C.P. Guillaume. 2002.Variation in eggshell characteristics and intrauterine egg retention in the lizard *Lacerta vivipara*: insight into the oviparity-vivparity continuum in squamates. *Journal of Morphology* 252:255-262.

Holland R.A.B., J.F. Hallam, M.B. Thompson, R. Shine, and P. Harlow. 1990. Oxygen carriage by blood of gravid and nongravid adults, and in embryos and newborn, of a viviparous Australian elapid snake, *Pseudiechis porphyriacus. Physiologist* (Aug) 1990:A68.

Ingermann R.L. 1992. Maternal-fetal oxygen transfer in lower vertebrates. *American Zoologist* 32:322-330.

Kam Y-C. 1992. Physiological effects of hypoxia on metabolism and growth of turtle embryos. *Respiration Physiology* 92:127-138.

Masson G.R. and L.J. Guillette. 1987. Changes in the oviductal vascularity during the reproductive cycle of three oviparous lizards *(Eumeces obsoletus, Sceloporus undulatus*, and *Crotaphytus collaris*). *Journals of Reproduction and Fertility* Ltd. 80:361-371.

Mathies T. 1998. Constraints on the evolution of viviparity in the lizard genus *Sceloporus*. PhD dissertation. Virginia Polytechnic Institute and State University.

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. *Physiological Zoology* 69:1021-1035.

Mathies T. and R.M. Andrews. 1999. Determinants of embryonic stage at oviposition in the lizard *Urosaurus ornatus. Physiological and Biochemical Zoology* 72:645-655.

Mathies T. and R.M. Andrews. 2000. Does reduction of the eggshell occur concurrently with or subsequent to the evolution of viviparity in Phrynosomatid lizards? *Biological Journal of the Linnean Society* 71:719-736.

Méndez-de la Cruz F.R., M. Villagrán-Santa Cruz, and R.M. Andrews. 1998. Evolution of viviparity in the lizard genus *Sceloporus. Herpetologica* 54:521-532.

Packard G.C., R. Tracy, and J.J. Roth. 1977. The physiological ecology of reptilian eggs and embryos and the evolution of viviparity within the Class Reptilia. *Biological Reviews* 52:71-105.

Panganelli C.V., A. Olszwaka, and A. Ar. 1974. The avian egg: surface area, volume, and density. *Condor* 76:319-325.

Parker S.L., R.M. Andrews, and T. Mathies. 2004. Embryonic responses to variation in oviductal oxygen in the lizard *Sceloporus undulatus* from New Jersey and South Carolina, USA. *Biological Journal of the Linnean Society* 83:289-299.

Qualls C. P. and R. Shine. 1998. *Lerista bougainvillii*,a case study for the evolution of viviparity in reptiles. *Journal of Evolutionary Biology* 11:63-78.

Ragsdale, F.R. and R.L. Ingermann. 1991. Influence of pregnancy on the oxygen affinity of red cells from the northern Pacific rattlesnake *Crotalus viridis oreganus. Journal of Experimental Biology* 159:501-505.

SAS Institute. 1996. SAS Version 8.2. SAS Institute, Cary, NC.

Shine R. 1983. Reptilian viviparity in cold climates: testing the assumptions of an evolutionary hypothesis. *Oecologia* 57:397-405.

Seymour, R.S., J.D. Roberts, N.J. Mitchell, and A.J. Blaylock. 2000. Influence of environmental oxygen on development and hatching of aquatic eggs of the Australian frog, *Crinia georgiana. Physiological and Biochemical Zoology* 73: 501-507.

Shine R. 1985. The evolution of viviparity in reptiles: an ecological analysis. Pp 605-694 in: C. Gans and F. Billet, eds. *Biology of the Reptilia.* Vol. 15. Development B. Wiley, New York.

Stewart J.R. and M.B. Thompson. 1993. A novel pattern of embryonic nutrition in a viviparous reptile. *Journal of Experimental Biology* 174:97-108.

Teodoro R.O. and P.H. O'Farrell. 2003. Nitric oxide-induced suspended animation promotes survival during hypoxia. *The EMBO Journal* 22:580-587.

Vleck C.M. and D.F. Hoyt. 1991. Metabolism and energetics of reptilian and avian embryos. Pp 285-306 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.

Webb P.W. and J.R. Brent. 1972. Oxygen consumption of embryos and parents, and oxygen transfer characteristics within the ovary of two species of viviparous sea perch, *Rhachochilus vacca*, and *Embiotoca lateralis. Journal of Fish Research Board of Canada* 29:1543-1553.

Woods H.A. and R.I. Hill. 2004. Temperature-dependent oxygen limitation in insect eggs. *The Journal of Experimental Biology* 207:2267-2276.

Xavier F. and J. Gavaud. 1986. Oviparity-viviparity ontinuum in reptiles: physiological characteristics and relation with the environment. Pp. 79-93 in I. Assemacher and J Bossin, eds. *Endocrine Regulations as Adaptive Mechanisms to the Environment*. Centre National de la Recherche Scientifique, Paris.

Yaron Z. 1985. Reptile placentation and gestation: structure, function, and endocrine control. Pp. 527-603 in C. Gans and F. Billett, eds. *Biology of the Reptilia*. Vol. 15. Development B. Wiley, New York.

Table 2.1. Allocation of eggs from each clutch into treatments.

Egg no.	kPa pO_2 (treatment)
1	--- (sampled at oviposition)
2	5.3 (simulated oviduct)
3	7.9 (simulated oviduct)
4	13.8 (simulated oviduct)
5	18.5 (simulated oviduct)
6	18.5 (simulated nest)

Note. Eggs (nos. 2-5) of each clutch were incubated under simulated *in utero* conditions, and egg no. 6 was incubated in vermiculite, simulating nest conditions. When clutches had more than six eggs, eggs were systematically allocated among simulated oviductal treatments.

Table 2.2 Percent survival of *Urosaurus ornatus*, *Sceloprous virgatus*, and *Sceloporus scalaris* embryos (experimental and retained groups).

	Percent Survival of embryos (no. embryos)		
Experimental Group	*U. ornatus*	*S. virgatus*	*S. scalaris*
Number of clutches	10	10	2
5.3 (simulated oviduct)	0 (16)	0 (16)	50 (4)
7.9 (simulated oviduct)	0 (16)	35 (17)	100 (4)
13.8 (simulated oviduct)	47 (15)	81 (16)	100 (4)
18.5 (simulated oviduct)	80 (15)	100 (16)	100 (4)
18.5 (simulated nest)	100 (10)	100 (11)	100 (3)
Retained Group	67 (18)	100 (20)	100 (19)

Note. Embryos from experimental groups were incubated for 10 days past the normal time of oviposition at 5.3, 7.9, 13.8, and 18.5 kPa pO_2. Embryos from the retained group were retained *in utero* for 10 days (*U. ornatus* and *S. virgatus*) and about two weeks (*S. scalaris*) past the normal time of oviposition.

Table 2.3. Comparisons of stage, embryo dry mass, wet egg mass, absolute and relative area of chorioallantois (CAM) at day 0 and after 10 days of retention for *Urosaurus ornatus* and *Sceloporus virgatus*.

	Day 0	Day 10	*P*
U. ornatus			
Stage	29.6 ± 0.41 (10)	30.2 ± 0.164 (10)	0.156
Embryo dry mass (mg)	0.55 ± 0.064 (10)	0.61 ± 0.061 (10)	0.497
Egg mass (g)	0.18 ± 0.008 (10)	0.19 ± 0.007 (9)	0.479
Absolute area of CAM (mm^2)	33.2 ± 2.42 (9)	37.9 ± 2.28 (9)	0.169
Relative area of CAM (mm^2)	21 ± 0.013 (9)	24 ± 0.015 (9)	0.229
S. virgatus			
Stage	32.1± 0.208 (10)	33.8 ± 0.316 (10)	<0.001
Embryo dry mass (mg)	2.33 ± 0.187 (10)	4.19 ± 0.313 (10)	<0.001
Egg mass (g)	0.35 ± 0.010 (10)	0.36 ± 0.009 (10)	0.611
Absolute area of CAM (mm^2)	115.7 ± 2.80 (10)	125.6 ± 4.74 (10)	0.079
Relative area of CAM (%)	48 ± 0.007 (10)	50 ± 0.004 (10)	0.067

Note. Values are clutch means ± SEM (number of clutches). Statistical tests of egg and embryonic features were made using *t*-tests.

Table 2.4. Comparisons of wet egg mass, absolute and relative area of chorioallanotoic membrane (CAM) at 10-days of incubation for *Urosaurus ornatus*, *Sceloporus virgatus*, and *Sceloporus scalaris*.

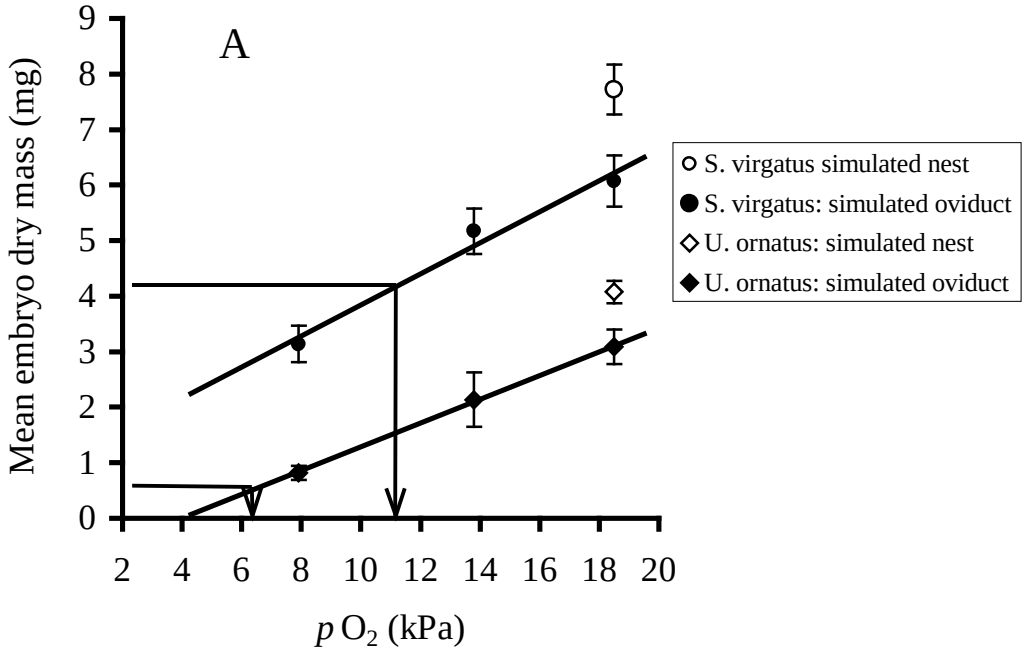
Variable	*U. ornatus*	*S. virgaus*	*S.scalaris*
Egg wet mass (g)			
5.3	---	---	1.12 ± 0.014 (2)
7.9	---	0.86 ± 0.022 (4)	1.67 ± 0.229 (2)
13.8	0.46 ± 0.050 (5)	0.98 ± 0.049 (9)	1.62 ± 0.146 (2)
18.5	0.50 ± 0.038 (8)	1.02 ± 0.051 (10)	1.65 ± 0.295 (2)
18.5 (simulated nest))	0.32 ± 0.031 (10)	0.65 ± 0.024 (6)	1.06 ± 0.007 (2)
Absolute area of CAM (mm^2)			
5.3	---	---	521.2 ± 5.78 (2)
7.9	48.6 ± 4.70 (5)	353.8 ± 9.08 (4)	676.1 ± 61.87 (2)
13.8	213.1 ± 35.53 (5)	431.7 ± 20.54 (9)	665.9 ± 39.64 (2)
18.5	285.9 ± 19.53 (8)	465.8 ± 20.85 (10)	670.7 ± 80.11 (2)
18.5(simulated nest)	223.9 ± 11.05 (10)	363.4 ± 9.14 (6)	503.4 ± 22.02 (2)
Relative Area of CAM (%)			
5.3	---	---	100 ± 0 (2)
7.9	33 ± 0.124 (5)	81 ± 0.009 (4)	100 ± 0 (2)
13.8	86 ± 0.047 (5)	90 ± 0.018 (9)	100 ± 0 (2)
18.5	92 ± 0.031 (7)	95 ± 0.020 (10)	100 ± 0 (2)
18.5 (simulated nest)	99 ± 0.0002 (10)	100 ± 0 (6)	100 ± 0 (2)

Note. Values are clutch means ± SEM (number of clutches). Simulated nest indicates clutches incubated in vermiculite. Values for embryo stage and dry mass are presented in Figures 2.1 and 2.2.

Table 2.5. Statistical tests of responses of *Urosaurus ornatus* and *Sceloporus virgatus* eggs and embryos to incubation at 7.9, 13.8, and 18.5 kPa pO_2 for10 days.

U. ornatus	*In utero* contrasts	Results:	*In utero* vs. nest contrasts (18.5 kPa pO_2)	Results:
Stage	$F_{2, 16}$=36.6, P=<0.001	7.9<13.8=18.5	$F_{1, 15}$=4.8, P=0.045	18.5<control
Embryo dry mass	$F_{2, 17}$=14.3[a], P<0.001	7.9<13.8=18.5	$F_{1, 15}$=10.4, P=0.006	18.5<control
Egg wet mass	$F_{1, 10}$=5.9, P= 0.036	13.8<18.5	$F_{1, 15}$=156.2, P=<0.001	18.5>control
Absolute area of CAM	$F_{2, 12}$=56.1, P<0.001	7.9<13.8=18.5	$F_{1, 13}$=7.6, P=0.016	18.5>control
Relative area of CAM	$F_{2, 14}$=16.4[a], P<0.001	7.9<13.8=18.5	$F_{1, 15}$=8.7[a], P=0.010	18.5<control
S. virgatus				
Stage	$F_{2, 19}$=10.1, P=<0.001	7.9<13.8=18.5	$F_{1, 13}$=4.1, P=0.065	18.5=control
Embryo dry mass	$F_{2, 19}$=16.8, P=<0.001	7.9<13.8<18.5	$F_{1, 13}$=7.1, P=0.019	18.5<control
Egg wet mass	$F_{2, 19}$=7.9, P=<0.003	7.9<13.8=18.5	$F_{1, 14}$=28.8, P=0.001	18.5>control
Absolute area of CAM	$F_{2, 20}$=5.1[a], P=<0.016	7.9=13.8, 7.9<18.5, 13.8=18.5	$F_{1, 13}$=18.7, P<0.001	18.5<control
Relative area of CAM	$F_{2, 20}$=7.7[a], P=<0.003	7.9=13.8, 7.9<18.5, 13.8=18.5	$F_{1, 14}$=6.9[a], P=0.019	18.5<control

Note. Tests were ANOVAs and ANCOVAs comparing treatment effects for eggs incubated under simulated *in utero* conditions (first two columns), and simulated *in utero* conditions at 18.5 kPa pO_2 vs. simulated nest conditions at 18.5 kPa pO_2 (third and fourth columns). *Post hoc* pair-wise comparisons were made using a Tukey's honestly significant difference test (ANOVAs) or a least significant difference test on least squared means (ANCOVAs).


A
Mean embryo dry mass (mg)
0
1
2
3
4
5
6
7
8
9
2
4
6
8
10
12
14
16
18
20
$p\,O_2$ (kPa)
S. virgatus simulated nest
S. virgatus: simulated oviduct
U. ornatus: simulated nest
U. ornatus: simulated oviduct

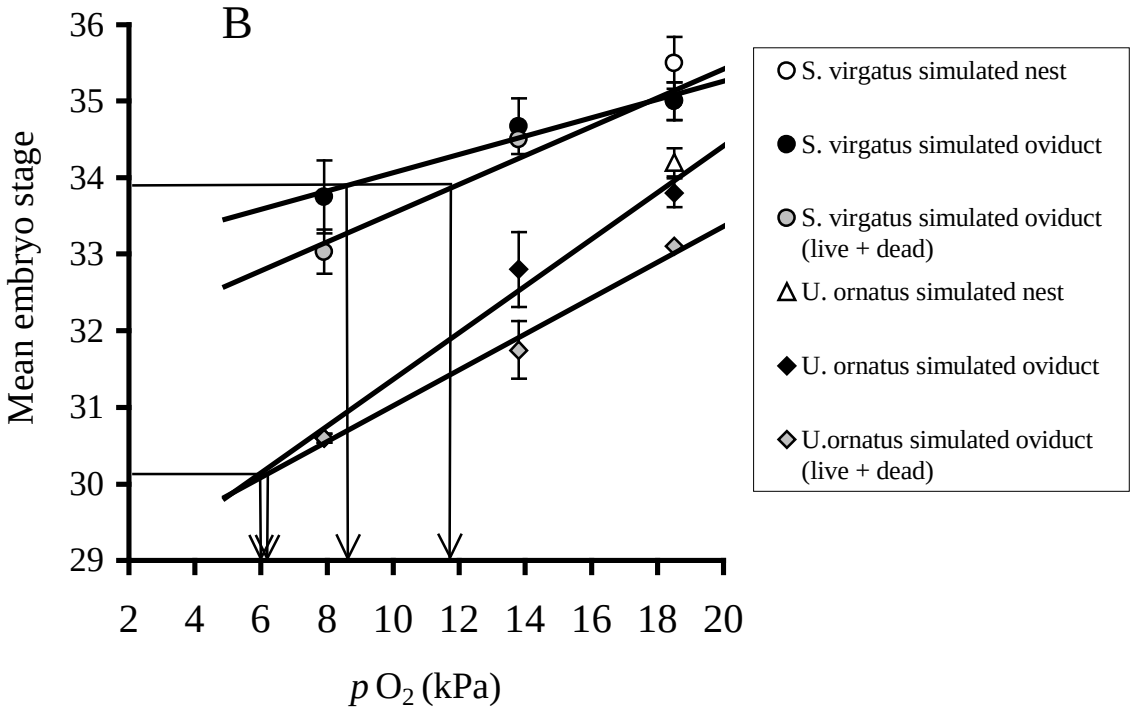

B
Mean embryo stage
29
30
31
32
33
34
35
36
2
4
6
8
10
12
14
16
18
20
$p\,O_2$ (kPa)
S. virgatus simulated nest
S. virgatus simulated oviduct
S. virgatus simulated oviduct (live + dead)
U. ornatus simulated nest
U. ornatus simulated oviduct
U.ornatus simulated oviduct (live + dead)

Figure 2.1 Embryonic growth at 10 days (A) and stage (B), (mean ± SEM) in response to incubation at 7.9, 13.8, and 18.5 kPa pO_2 (simulated oviduct, filled symbols), and 18.5 kPa pO_2 control (simulated nest, open symbols) treatments. Light gray filled symbols represent values based on combined live and dead embryos. Circles represent *Sceloporus virgatus*, and diamonds *Urosaurus ornatus.* Intersection of horizontal line with the Y-axis indicates the mean embryo dry mass or stage attained after 10 days of retention *in utero*. Arrows indicate estimated pO_2 associated with the degree of *in utero* development of lizard embryos during egg retention. Statistical comparisons of treatment effects are presented in Table 2.5.

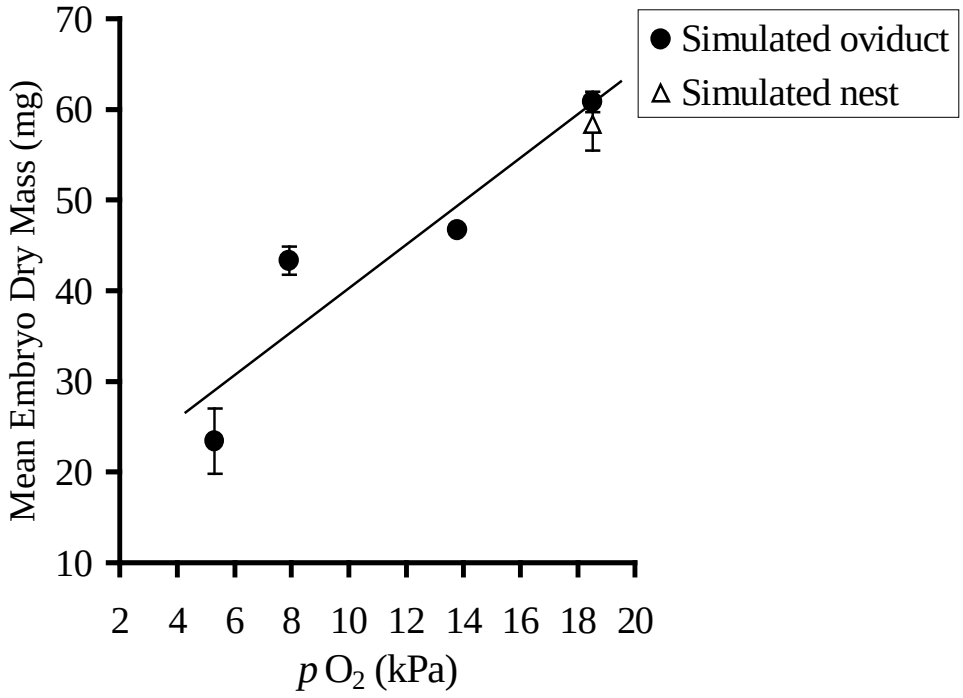


Figure 2.2 Dry mass of *Sceloporus scalaris* embryos at 10 days in response to incubation at 5.3, 7.9,13.8, 18.5 kPa pO_2 (simulated oviduct, filled symbols), and 18.5 kPa control (simulated nest, open symbols) treatments. Stage is not illustrated because all embryos were at stage 39.

CHAPTER 3

INCUBATION TEMPERATURE AND PHENOTYPIC TRAITS OF HATCHLING *SCELOPORUS UNDULATUS*: IMPLICATIONS FOR THE NORTHERN LIMITS OF DISTRIBUTION

ABSTRACT

Cold environmental temperatures at high latitudes and elevations are detrimental to oviparous reproduction by prolonging incubation period, negatively affecting embryonic developmental processes or by killing embryos in eggs directly. Because cool soil temperatures may prevent successful development of embryos in eggs in nests, the geographic distributions of oviparous species may be influenced by the thermal requirements of embryos. In the present study, I tested the hypothesis that low incubation temperature is a major factor determining the northern distributional limit of the oviparous lizard *Sceloporus undulatus*. To compare the effects of incubation temperature on incubation length, survival and hatchling phenotypic traits Iincubated eggs of *S. undulatus* under temperature treatments that simulated the thermal environment that eggs would experience if located in nests within their geographic range at 37 °N and at latitudes north of the species present geographic range at 42, and 44 °N, respectively. After hatching, snout-vent length (SVL), mass, tail length, body condition (SVL relative to mass), locomotor performance and growth rate were measured for each hatchling. Hatchlings were released at a field site to evaluate growth and survival under natural conditions. Incubation at temperatures simulating nests at 44 °N, prolonged incubation and resulted in hatchlings with shorter SVL relative to mass, shorter tails, shorter hind limb span, slower growth and lower survival than hatchlings from eggs incubated at temperatures simulating nests at 37 and 42° latitude. I also evaluated the association between environmental temperature and the northern distribution of *S. undulatus*. I predicted that the northernmost distributional limit of *S. undulatus* would be associated with locations that provide the minimum heat sum (degree-days) required to complete embryonic development. My prediction that the northern distributional limit of *S. undulatus* is associated with the minimum heat sum required for successful development of *S. undulatus* embryos was upheld: 84% of location between 37-40 °N had ≥ 495 degree-days above a threshold of 17 °C accumulated during the summer incubation period (June-September) compared to 11% of locations between 41-50 °N. Results suggest that soil temperatures at northern latitudes are not warm enough for a sufficient length of time to permit successful incubation of *S. undulatus* embryos. These results are consistent with the hypothesis that incubation temperature is an important factor limiting the geographic distributions of oviparous reptile species at high latitudes and elevations.

INTRODUCTION

Cold climate is the putative selective factor for the evolution of viviparity in reptiles (Packard et al. 1977; Tinkle and Gibbons 1977; Shine and Bull 1979; Shine 1985). The adaptive benefit of viviparity in cold climates is that embryonic development is faster inside the thermoregulating female than in a nest (Packard et al. 1977; Shine and Bull 1979; Shine 1983). Support for this hypothesis (referred to as the cold climate model) is based in part on observations that the proportion of viviparous species in squamate faunas increases in cold climates at high latitudes and high elevations (Weeks 1933; Sergeev 1940; Greer 1968; Tinkle and Gibbons 1977; Shine and Berry 1978). In the coldest environments, 100% of squamate species are viviparous (Tinkle and Gibbons 1977, Shine 1985), and the proportion of viviparous species declines with decreasing latitude and elevation. In North America, for example, about 29% of squamate species between 30-35° N are viviparous, compared to 63% between 50-55°N (Tinkle and Gibbons 1977). The small proportion of oviparous compared to viviparous species in cold climates and occurrence of recent origins of viviparity in cold climates suggests that the geographic distributions of some oviparous species may be constrained by the thermal requirements of embryos (Shine 1985; Shine 1987; Porter et al. 2002).

Cold environmental temperatures are thought to be detrimental to oviparous reproduction by affecting the length of incubation and survivorship of hatchlings (Qualls and Andrews 1999; Shine 2002). Because embryonic development in reptiles is temperature dependent, cold environmental temperatures may prolong, or prevent successful incubation of eggs in nests (Packard et al. 1977; Muth 1980). Temperate zone squamate reptiles typically oviposit eggs in the late spring and early summer, and hatchlings emerge during late summer and early fall (Shine 1985). Prolonged incubation as a result of cool incubation temperatures would thus delay fall emergence, leaving hatchlings with little time to feed and grow before cold temperatures preclude activity (Packard et al. 1977). Moreover, cold temperatures during incubation may also adversely affect embryonic developmental processes, resulting in hatchlings with phenotypic traits associated with low fitness (Qualls and Andrews 1999, Shine 2002). For example, Qualls and Andrews (1999) demonstrated that lizard eggs incubated at cool temperatures resulted in

smaller hatchlings with reduced growth rates and slower sprint speeds compared to hatchlings from eggs incubated at warmer temperatures.

Incubation temperature appears to be an important determinant of geographic distributions of some reptiles (Muth 1980; Shine 1999; Porter et al. 2002; Kearney and Porter 2004). For example, the geographic distribution of oviparous species of *Pseudechis* (Elapidae), closely correspond to regions where mean summer ambient temperatures are greater than 22 °C (the minimum temperature required for successful development of *Pseudechis* embryos). Shine (2002) demonstrated that eggs of the montane oviparous skink *Bassiana duperreyi* had reduced hatching success and hatchling viability when incubated at cool temperatures simulating nests at altitudes exceeding the elevational limit of the species' natural distribution. Similarly, hatching success and hatchling viability of *B. duperreyi* eggs were also reduced when incubated in the field in artificial nests at altitudes exceeding the elevational limit of the species natural distribution. The distributional limits of some oviparous species at high latitudes and elevations should therefore be predictable based upon knowledge of egg incubation temperatures, embryonic thermal tolerances, and embryonic developmental rates as a function of temperature.

Oviparous squamate species with wide latitudinal distributions provide an opportunity to study the association between the thermal tolerances of embryos and geographic distribution. The oviparous lizard *Sceloporus undulatus*, for example, has a wide latitudinal distribution across the United States and is one of the northern-most members of the genus *Sceloporus* (Figure 3.1). In the central and eastern United States the geographic range of *S. undulatus* extends maximally to about 40 °N (Sites et al. 1992; Stebbins 1985). *Sceloporus undulatus* occurs in a wide range of habitats, from arid deserts in the southwestern U.S. to temperate deciduous forests in the eastern U.S. No obvious geographical or ecological barriers prevent a northern expansion of *S. undulatus* and viviparous squamate species extend considerably further north than S. undulatus. It is unlikely that soil moisture plays an important role in determining the distributional limit of *S. undulatus* because precipitation in the U.S. changes on a longitudinal, not latitudinal gradient. Moreover, individuals thus do not appear to have specialized dietary requirements. While the possibility that factors other than incubation temperature may influence the geographic distribution of *S. undulatus* cannot be ruled out, the

above considerations implicate temperature as an important determinant of its geographic distribution.

The objective of this study was to test the hypothesis that the thermal requirements for embryonic development determines the northernmost distribution of *S. undulatus*. Incubation temperature could affect survival directly (e.g. by affecting developmental processes) or indirectly (by affecting time of hatching). I therefore, addressed this hypothesis in several ways. The experimental protocol was to incubate eggs of *S. undulatus* under treatments that simulated temperatures of nests within the geographic range of *S. undulatus* and those of nests outside the northern distributional limits of *S. undulatus*. I then evaluated the affect of incubation temperature on hatching success, incubation period, hatchling phenotypic traits (morphology and performance) and post hatching survival. I predicted that hatchlings from eggs incubated at cool temperatures simulating nests at latitudes outside the northernmost distributional limit would have reduced hatching success, longer incubation period, reduced performance and lower survival than hatchlings incubated at temperatures simulating nests within the geographic distribution of *S. undulatus*.

I also determined the association between environmental temperature and the northern distribution of *S. undulatus*. Because successful incubation of reptilian embryos is temperature dependent, I predicted that the northernmost distributional limit of *S. undulatus* would be associated with locations that provide the minimum heat sum (degree-days) required to complete embryonic development. I tested this prediction by assessing the geographic variation in environmental temperature at sites within and outside the northern distributional limits of *S. undulatus*.

MATERIALS AND METHODS

COLLECTION AND MAINTENANCE OF GRAVID FEMALES

Gravid females of *S. undulatus* (n=19) were collected from 17 June to 6 July 2005 from sites within 35 km of Blacksburg, Montgomery County, Virginia. Females were placed singly in

cloth bags and transported to Virginia Polytechnic Institute and State University (Virginia Tech), Blacksburg, VA. From the field, eggs from a single clutch were also collected on the day of oviposition. Females were weighed to the nearest 0.1 g, and measured for snout-vent length (SVL) before and after oviposition. They were housed in plastic containers (73 L x 48 W x 22 H, 2-3 females per container) in the laboratory until oviposition occurred. The containers housing females were supplied with a layer of sand to provide a suitable substrate for nesting. The sand substrate at one end of the container was kept moist to facilitate nesting and to prevent desiccation of eggs. Daily photoperiod was provided by laboratory windows and also by fluorescent room lighting (0800-1800h). A 100-W flood lamp was suspended at one end of each container (0900-1700h) as a heat source, and all containers were provided with boards and rocks for basking and hiding places. Females where fed until satiation (crickets and mealworms dusted with mineral-vitamin supplement) every other day. Water was provided daily by misting the rocks, boards, and sides of container. Shallow ceramic dishes filled with water were also placed in each container. Containers housing gravid females were checked several times daily for nesting females and eggs. After oviposition, females were released at the location of their capture.

EXPERIMENTAL DESIGN

Eggs were weighed to the nearest 0.01 g within a few hours of oviposition, and numbered consecutively within each clutch using a non-toxic waterproof marker. A single egg from each clutch was dissected and the embryo staged according to the Dufaure and Hubert (1961) staging system The remaining eggs of each clutch were incubated individually in 70 mL specimen jars containing vermiculite moistened with a sufficient quantity of distilled water (0.7:1.0 g H_2O:vermiculite) to produce a water potential of –200 kPa (determined by thermocouple psychrometry). The jars were covered with plastic wrap and sealed with a rubber band.

Eggs from each clutch were allocated among three experimental temperature treatments. One of the treatments was based on temperatures observed in actual *S. undulatus* nests and the other two treatments were based on soil temperatures for localities north of the present distributional limit near 42 and 44 º latitude. Soil temperature data were obtained from the

National Climate Data Center for four localities (Chicago Botanical Garden, IL, Geneva Research Farm, NY, Freemont, OH, and Wanatah, IN) located at approximately 42 °N and three localities (Waseca, MN, NW Michigan Research Farm, MI, and Canton, NY) at approximately 44 °N. Soil temperatures at 44 and 42 °N were based on average maximum and minimum soil temperatures during June-August, measured over a period of 5 to 30 years, at a soil depth of 10 cm. The 22-32 °C (mean=27 °C) treatment represented actual temperatures observed in *S. undulatus* nests at 37 °N in Virginia (Andrews et al. 2000) and at 39 °N in New Jersey (depth of 6 cm) (Angilletta et al. 2000). The 19-25 (mean=22 °C) and 21-27 °C (mean=24 °C) treatments represented temperatures of simulated nests outside the northernmost distributional limit of *S. undulatus* at 44 and 42 °N, respectively, if eggs were buried at depths similar to those of typical *S. undulatus* nests at low latitude.

The coldest temperature treatment (simulating nests at 44 °N) was selected because 22 °C is similar to the lowest temperature regime where hatching could be successful. Eggs of *S. undulatus* fail to hatch when incubated at a constant temperature of 20 °C (Sexton and Marion 1974). Eggs of *S. virgatus* (a member of the *undulatus* species group), however, hatched when temperatures fluctuated around a mean of 20 °C (Andrews et al. 1997). Hatching can thus occur at a low average temperature as long as the diel cycle includes temperatures above the threshold temperature for development.

Eggs were placed, according to treatment, into programmable temperature chambers and incubated under a fluctuating temperature regime. Temperatures inside the chambers cycled linearly for 4 hours between daily maximum and minimum temperatures. Incubation temperatures were measured inside a 70 mL jar containing moistened vermiculite. The temperature probe was placed in the center of the jar and buried approximately 1 cm below the surface of the vermiculite (approximately the same depth as that of experimental eggs). The mean daily minimum, maximum, and overall mean temperatures for the 22, 24, and 27 °C treatments during the incubation period were 19.3, 25.1 and 22.3 °C, respectively; 21.3, 27.1, and 24.2 °C, respectively; and 21.7, 31.8, and 26.8 °C respectively. Racks of jars with eggs were rotated within the chambers every three days to minimize position effects on embryonic

development. To ensure that eggs did not experience negative water balance, the vermiculite was changed approximately 30 days after the start of incubation.

MORPHOLOGY AND HUSBANDRY OF LABORATORY HATCHLINGS

Hatchling sex, mass, snout-vent length (SVL), tail length (TL), and hind-limb span (HLS) (linear distance between the tip of the fourth digit on each hind limb), were recorded within 12 hours of hatching. Each hatchling was numbered using a non-toxic marker and given a unique toe-clip for identification. Hatchlings were housed in plastic containers (73 L x 48 W x 22 H cm), with 10-15 hatchlings per container. Hatchlings were fed (pinhead crickets and mealworms dusted with vitamin mineral supplement) and watered by misting the interior and sides of containers twice daily. Otherwise, husbandry was the same as for gravid females. Hatchlings were not fed on the day that locomotor performance trials were performed (see section below). Hatchlings were maintained in the laboratory for 10-13 days before release in the field. Mass, SVL, TL, and HLS were recorded at 10 days after hatching.

MEASUREMENT OF LOCOMOTOR PERFORMANCE AND GROWTH OF LABORATORY HATCHLINGS

Locomotor performance was measured at 3-4 days after hatching using a 1 m long electronically timed racetrack (Qualls and Andrews 1999, Warner and Andrews 2002). Five infrared photocells were connected to an electronic stopwatch; the five photocells were spaced at 0.25 m intervals along the length of the racetrack. The racetrack was placed in an environmental chamber (150 L x 80 W x 80 W cm) set at a constant 31 °C. Two electric fans were situated at opposite ends of the chamber to promote air circulation and to ensure a uniform temperature distribution within the chamber. Observations were made between 1100-1530 h. Hatchlings were acclimated for 30 min in the environmental chamber prior to locomotor performance trials. Locomotor performance was measured three times for each hatchling with at least a 2 min rest between trials. Hatchlings were placed at the beginning of the racetrack and prodded gently with a small paintbrush if they failed to run. Locomotor performance was assessed using three criteria: 1) the fastest running speed of the twelve 0.25 m intervals, 2) the fastest running speed

of the three 1 m trials, and 3) the number of times that a hatchling stopped during the trial. The time required for hatchlings to cover the prescribed distance was expressed as meters per second. The number of times that a hatchling stopped along the 1-m distance was recorded using a manual counter.

Growth in the laboratory was measured over 10 days and was assessed as the difference between an individual's natural log transformed SVL or mass at 10 days and at hatching divided by 10, the number of days between measurements.

MEASUREMENT OF LOCOMOTOR PERFORMANCE AND GROWTH OF FIELD HATCHLINGS

In order to determine if laboratory produced phenotypes were comparable to phenotypes produced in the field under comparable thermal conditions, I measured the phenotypes of hatchlings collected in the field ("field hatchlings"). These individuals (n=41) were captured from 24 August–19 September at one of the field sites. They were measured for mass, SVL, toe-clipped, and numbered with a non-toxic marker at the time of capture and at the time of release. They were returned to the laboratory for measurement of locomotor performance traits and growth rate. Field hatchlings were housed in separate containers from laboratory hatchlings but otherwise maintained under identical conditions (see section on "Morphology and husbandry of hatchlings" above). Locomotor performance traits were measured 2-3 days after capture (as described for laboratory hatchlings). Field hatchlings were also maintained in the laboratory for 10-13 days before they were released at their location of capture.

GROWTH AND SURVIVAL OF HATCHLINGS IN THE FIELD

Hatchlings (n= 47, 42, and 41 for the 22, 24, and 27 °C treatments, respectively) were released between 27 August and 6 November at the site where field hatchlings were collected on warm, sunny days between 1000-1600 h. The release site was an approximately 600 m^2 forest clearing located on private land in Montgomery County, VA. Piles of woody debris, and small shrubs scattered throughout the clearing provided suitable habitat for hatchlings. The clearing

was surrounded on all sides by forest except for a gravel access road that passed through the site. Hatchlings typically do not disperse through dense forest, and therefore tend to remain in the open areas where they hatched for at least the first month or two of life (Warner and Andrews 2002).

The clearing and periphery of the surrounding forest were searched thoroughly for hatchlings twice weekly from 27 August to 14 November. After capture, hatchlings were identified by their number and toe clip, weighed, and measured for SVL, TL and HLS. After measurements were recorded, hatchlings were released at their site of capture.

Survival estimates in the field were based upon the assumption that disappearance of hatchlings was largely due to mortality rather than dispersal. This assumption is supported by previous studies on *Uta stansburiana* and *S. undulatus* that indicate that emigration is a relatively rare event and does not bias survival estimates (Wilson 1991; Andrews et al. 2000; Warner and Andrews 2002).

CALCULATION OF DEGREE-DAYS

The heat sum accumulated during each treatment was expressed as:

$$S = \sum_{day=1}^{day=j} (t_m - t_o),$$

where S=sum of degree days, t_o= the threshold temperature for development, t_m=the mean daily temperature. Because development of *S. undulatus* embryos is arrested at 17 °C (Andrews et al. 1997), I used 17 °C as the minimum threshold temperature for degree-day calculations. Because embryos presumably would not complete development at a mean temperature less than 22 °C (see above), the 22 ºC treatment represents the minimum number of degree-days required for successful incubation of *S. undulatus* embryos.

The number of degree-days above 17° C was also estimated as a function of latitude using mean monthly air temperature data obtained from 50 recording stations ranging from 37-50° N. Mean monthly air temperatures (based on 30 year averages) during June, July, August, and September were obtained from the National Climate Data Center. The use of mean air

temperatures instead of soil temperatures in degree-day calculations is justified because at shallow depths (5-20 cm), soil temperature is similar to standard air temperatures (Shine 1983; Parton 1984). Degree-days accumulated from June to September were estimated by summing the number of degree-days above 17 °C over the 122 d period for each locality.

DATA MANIPULATION AND STATISTICAL ANALYSES

Analyses of incubation length, morphology, growth and locomotor performance

Statistical analyses were conducted using SAS Statistical Package version 9.1.2 (SAS Institute, 2003). The effects of temperature treatment on incubation length, morphology, growth, and locomotor performance were analyzed using analysis of covariance (ANCOVA) and analysis of variance (ANOVA) (GLM Procedure). Preliminary analyses indicated that sex did not affect hatchling morphology, performance, or survival. Sex was therefore not considered in further analyses. Stage at oviposition was used as a covariate in analyses of the effect of temperature treatment on incubation length. The effect of temperature treatment on SVL, HLS, TL, and body mass at hatching was evaluated using egg mass as a covariate. The effect of treatment on hatchling mass at 10 days was evaluated using initial hatchling mass as a covariate. Snout-vent length at hatching was used as a covariate in analyses of the effect of temperature treatment on morphology at 10 days (SVL, TL, HLS) and running speed. Body condition (mass relative to SVL) was assessed as $mass^{0.3}$/SVL (Andrews 1982). When the covariate was not significant ($P > 0.05$), single-factor analysis of variance (ANOVA) was used to evaluate treatment effects. Analyses of treatment effects were based upon clutch means. Prior to ANCOVA analyses, the assumption of homogeneity of slopes was satisfied by testing for significance of the interaction of the covariate with treatment variables. For all ANCOVAs *post-hoc* pair-wise comparisons were made using a least significant difference test on least squared means. For all ANOVAs, *post-hoc* pair wise comparisons were made using a Tukey's honestly significant difference test. The effect of temperature treatment on the frequency of kinked or bent tails was analyzed using a Chi-square test (Freq Procedure). Data are reported as mean ± SEM unless otherwise reported, and probability values less than 0.05 were considered significant.

Analyses of growth and survival in the field

The effect of temperature treatment on growth rate of hatchlings in the field was analyzed as described for growth rate in the laboratory. Because some hatchlings were not recaptured after release, no data on growth was obtained for some clutch/treatment combinations. Consequently, the number of clutches in the analysis was reduced to eight in the 22 °C treatment, 15 in the 24 °C treatment, and 13 in the 27 °C treatment, respectively. Growth at 10-25 days after release in the field was measured as the difference between an individual's natural log transformed SVL or mass at last capture and at the time of release in the field divided by the number of days between measurements. The effect of temperature treatment on hatchling survival at 10, 20, and 30 days after release in the field was analyzed using Chi-squared tests. The overall association between phenotype and survival independent of treatment was assessed for each phenotypic trait using ANOVAs and ANCOVAs. Separate analyses were used to contrast survivors versus non-survivors for each of the three time periods.

Contrasts of field and laboratory hatchlings

Growth and locomotor performance of field hatchlings were compared to laboratory hatchlings. Because incubation conditions and clutch of origin of field hatchlings were unknown, individual values rather than clutch means were used in statistical analyses. Nine of the laboratory hatchlings (n=5 in the 24 °C treatment and n=4 in the 27 °C treatment) were inadvertently released before first obtaining data on locomotor performance. The number of hatchlings used in performance analyses was therefore reduced to 37 in both the 24 and 27 °C treatments. The effect of temperature treatment on hatchling running speed was analyzed using ANCOVA with initial SVL hatchling mass as a covariate. I did not compare morphological traits (SVL, body mass, TL, HLS) between field and laboratory hatchlings because the morphology of field individuals at the time of hatching was unknown. Otherwise, statistical contrasts comparing growth and survival of field and laboratory hatchlings are the same as those described above for laboratory hatchlings (above).

RESULTS

EFFECT OF TEMPERATURE TREATMENT ON EGG SURVIVAL AND INCUBATION LENGTH

Survival of eggs was not affected by temperature treatment. Overall, 131 of 140 eggs hatched (94% survival), and survival exceeded 90% in all treatments. Survival of hatchlings in the laboratory was not related to temperature treatment. Only one (from the 22 °C treatment) of 131 hatchlings died prior to release (99.2% survival).

Incubation length differed among temperature treatments and was negatively associated with increasing temperature (Tables 3.1 and 3.2). Hatching occurred from 17 August to 6 September in the 27 °C treatment, 1 September to 28 September in the 24 °C treatment, and 29 September to 27 October in the 22 °C treatment, with mean incubations periods of 56, 74, and 99 days, respectively.

EFFECT OF TEMPERATURE TREATMENT ON HATCHLING PHENOTYPIC TRAITS

Hatchling SVL and body mass at hatching and at 10 days did not differ among temperature treatments (Tables 3.1 and 3.2). Body condition at hatching was not affected by temperature treatment. At 10 days, however, hatchlings from the 22 °C treatment heavier for their length than hatchlings from the 27 °C treatment. Tail length and hind limb span at hatching and at 10 days differed among temperature treatments (Tables 3.1 and 3.2). At hatching, the tail lengths of hatchlings from the 22 °C treatment were on average about 4 mm shorter than those of hatchlings in the 27 °C treatment and differences in tail length persisted over the 10 days that hatchlings were in the laboratory. Hind limb span at hatching and at 10 days was shorter by about 3 mm in the 22 °C treatment compared to the 24 and 27 °C treatments. Hind limb span did not differ between the 24 and 27 °C treatments. Hatchlings from the 22 °C treatment also exhibited a higher frequency of kinked tails than hatchlings from the 24 and 27 °C treatments (χ^2= 7.1, d.f.=2, n=130, *P* =0.03). Approximately 21% of hatchlings from the 22 °C had kinked

tails compared to 2% of hatchlings in the 24 °C treatment and 12% of hatchlings in the 27 °C treatment, respectively.

Hatchling growth in the laboratory over 10 days and locomotor performance did not differ among treatments (Tables 3.1 and 3.2). Hatchlings grew in SVL and mass at similar rates and ran at similar speeds irrespective of treatment.

GROWTH RATE AND SURVIVAL OF LABORATORY HATCHLINGS IN THE FIELD

Growth in mass during 10-25 days after release in the field was related to temperature treatment but growth in SVL was not (Tables 3.1 and 3.2). Hatchlings from the 22 °C treatment tended to grow more slowly in mass and SVL than those from the 24 and 27 °C treatments. Growth in mass of hatchlings incubated at 22 °C was about 50% slower than that of hatchlings incubated at 24 and 27 °C.

Survival of hatchlings in the field at 10, 20, and 30 days after release differed among temperature treatments (Table 3.3 and Figure 3.2) with lower hatchling survival in the 22 °C treatment than in the 24 and 27 °C treatments.

CORRELATES OF SURVIVAL OF LABORATORY HATCHLINGS IN THE FIELD

Overall, individuals that survived to 10 days of age had longer tails and higher average running speeds over 0.25 and 1 m than individuals that did not survive (Figure 3.3). While this pattern continued to 20 and 30 days after release, differences were not significant.

CONTRASTS BETWEEN FIELD AND LABORATORY HATCHLINGS

Field-incubated hatchlings had a mean SVL of 27 mm at the mean date of first capture (4-September). Given the overall mean SVL of 25 mm at hatching and the overall mean growth rate of about 0.3 mm/d of laboratory hatched individuals, the mean date of hatching in the field would have been 28 August. Assuming that oviposition of field eggs took place on 3 July (the

median date of oviposition in the laboratory), the mean incubation period in the field would have been 56 days.

LOCOMOTOR PERFORMANCE AND GROWTH

Running speed of field hatchlings did not differ from that of laboratory hatchlings (Tables 3.4 and 3.5). Field hatchlings, however, stopped more frequently over 1 m compared to laboratory hatchlings from the 22 °C treatment.

Under laboratory conditions, field hatchlings grew in SVL about 40% more slowly than did hatchlings from the 27 °C treatment, while growth in mass did not differ between field and laboratory hatchlings (Tables 3.4 and 3.5). In the field, growth of field hatchlings did not differ from that of laboratory hatchlings 10-25 days after release in the field (Tables 3.4 and 3.5). The average growth of field hatchlings was almost identical in both SVL and mass to that of laboratory hatchlings incubated at 24 and 27 °C.

Survival in the field of field hatchlings was similar to that of hatchlings from the 24 and 27 °C treatments at 10, 20, and 30 days after release (Table 3.3 and Figure 3.2). Survival of field hatchlings was, however, consistently higher than that of laboratory hatchlings from the 22 °C treatment, but differences were only significant at 20 and 30 days after release in the field. At 20 and 30 days after release, survival of field hatchlings was 48% and 36%, respectively, compared to 19% and 12% survival for hatchlings from the 22 °C treatment, respectively.

EFFECT OF LATITUDE ON INCUBATION LENGTH

The number of degree-days accumulated during each treatment ranged from 495 at 22 °C, 521 at 24 °C, to 564 at 27 °C. The number of degree-days above 17 °C decreased with latitude (r^2=0.56, F=63.0, P<0.001) and ranged from >900 at 37° N to 103 at 50° N (Figure 3.4). Eighty-four percent of the locations within the geographic distribution of *S. undulatus* between 37-40° N had ≥ 495 degree-days above 17° C accumulated during June-September. In contrast, only 11%

of locations at latitudes between 41-50° N outside the geographic distribution of *S. undulatus* had ≥ 495 degree-days accumulated over the same period of time. Based upon a minimum requirement of approximately 495 degree-days above 17° C to complete embryonic development, the predicted northern latitudinal limit of *S. undulatus* would lie at approximately 40.5° N.

DISCUSSION

A central question in ecological and evolutionary physiology is whether the variables measured in laboratory studies are ecologically relevant indicators of fitness under natural conditions (Irschick 2003). One difficulty is that the association between phenotypic traits and fitness is complex and poorly understood (Travis et al. 1999; Andrews et al. 2000). For example, phenotypic traits of hatchling lizards may exist transiently during ontogeny and may therefore have relatively little effect on hatchling fitness (Irschick 2000; Qualls and Shine 2000). Another difficulty is that studies examining the association between incubation temperature and phenotypic traits such as locomotor performance and growth rate often yield conflicting results. For example, cool incubation temperatures may be associated with increases (Qualls and Andrews 1999) or decreases (Elphick and Shine 1998) in running speed. A further complication is that lizards in nature may rarely achieve running speeds recorded under laboratory conditions (Braña 2003; Irschick 2003). Running speed may depend on multiple interacting factors such as distance from refugia, energy expenditure, benefits of prey capture, or the perceived threat of a predator. With this in mind, I determined whether the measures of fitness I selected were ecologically relevant by assessing the overall association between survival of laboratory hatchlings in the field and phenotype. Individuals that survived through the 30-day observation period had longer tails and faster average running speeds than individuals that did not survive this long (Figure 3.3). Relatively long tails and relatively fast speed were thus related to the survival of hatchlings.

My results also support a direct connection between the phenotypic traits produced in the laboratory and in the field, given comparable temperature regimes. For example, nest temperatures at our field sites near Blacksburg average about 27 °C (Andrews et al. 2000). I

therefore predicted that the incubation period and phenotypes of field hatchlings would be most similar to hatchlings from the 27 °C treatment. In general, my observations supported my prediction: field hatchlings and the hatchlings from the 27 °C treatment had similar incubation periods and survival in the field, while both groups differed from hatchlings from the 22 °C treatment. For most performance traits, field hatchlings did not differ from laboratory hatchlings from the 27 and 24°C treatments.

EGG INCUBATION TEMPERATURE AS A DETERMINANT OF GEOGRAPHIC DISTRIBUTIONS

Incubation of *S. undulatus* eggs at temperatures simulating nests at 44 °N (22 °C treatment) substantially increased incubation length, affected hatchling phenotypic traits, (Tables 3.1 and 3.2) and reduced survival in the field (Table 3.3 and Figure 3.2). In contrast, incubation at temperatures simulating nests at 42 °N (24 °C treatment) resulted in a relatively modest increase in incubation length, and with the exception of tail length, hatchlings did not differ in any aspect of phenotype, locomotor performance, or survival compared to hatchlings incubated under normal temperature conditions at a mean temperature of 27 °C.

My prediction that the northern latitudinal limit of *S. undulatus* would be associated with the minimum number of degree-days required for successful development of *S. undulatus* embryos was upheld (Figure 3.4). Based upon a minimum requirement of approximately 495 degree-days above a threshold of 17 °C, the predicted northern distributional limit of *S. undulatus* in the central and eastern U.S. would lie at approximately 40.5 °N. The predicted value of 40.5 closely corresponds to the observed northern distributional limit of approximately 40 °N.

How does incubation temperature limit populations of *S. undulatus* to approximately 40 °N, at least in the eastern U.S.? Cool soil temperatures could influence geographic distribution by affecting embryo survival in nests. In contrast to my prediction, however, hatching success was high (>90%) in all treatments, suggesting that eggs could at least survive to hatching in nests

at a mean temperature of 22 °C at latitudes as far as 44 °N. Eggs incubated at a mean temperature of 22 °C had an incubation period of approximately 100 d. At 44 °N, hatching would thus occur during the first part of September assuming eggs were laid at the beginning of June. Soil temperatures at that latitude, however, decrease toward the end of the summer (National Climate Data Center). For example, the average soil temperature at 44 °N decreased from a mean of about 22 °C in August to 19 °C in September. At high latitudes, therefore, the incubation period would presumably be even longer than in my experiments due to decreasing temperatures during September and October. Because embryos appear to be most susceptible to the effects of cold temperatures late in development prior to hatching, eggs in nests at high latitudes could be exposed to lethally low temperatures during late summer and early autumn (Yntema 1978; St. Clair and Gregory 1990; Bobyn and Brooks 1994).

Temperature-induced effects on physiological processes during development could also influence the geographic distribution of *S. undulatus.* Hatchlings from the 22 °C treatment had shorter SVL relative to mass, shorter tails, shorter hind limb span, and higher frequency of tail deformities compared to hatchlings in the 24 and 27 °C treatments (Tables 3.1 and 3.2). Contrary to my predictions, however, locomotor performance did not differ among temperature treatments. Nevertheless, relatively long tails and relatively high speeds enhanced juvenile survival in populations of *S. undulatus* in the field (Figure 3.3). In general, cool incubation temperature has a negative effect on hatchling fitness (e.g. Shine et al. 1997; Qualls and Shine 1998; Qualls and Andrews 1999; Andrews et al. 2000; Blouin-Demers et al. 2004). For example, hatchling *S. virgatus* incubated at a mean temperature of 20 °C were smaller and ran more slowly than hatchlings from eggs incubated at a mean temperature of 25 °C (Qualls and Andrews 1999). In other examples, hatchling black rat snakes incubated at 25 °C had reduced locomotor performance compared to hatchlings from eggs incubated at 30 °C and for pine snakes (*Pituophis melanolucus*), incubation at 23 °C resulted in abnormal hatchling behaviors such as increased emergence time from nests compared to hatchlings from eggs incubated at 28 °C (Blouin-Demers et al. 2004; Burger 1991).

Cool soil temperature could also affect hatchling survival indirectly, by prolonging the incubation period and delaying the time of hatching. The putative benefit of early hatching is that hatchlings experience more favorable environmental conditions and thus are able to grow more and accumulate greater fat stores than hatchlings that emerge late in the season (Ferguson and Fox 1984; Shine 1997; but see Andrews et al. 2000). In the present study, hatchlings from the 22 °C treatment that were released late in the season had substantially slower growth in mass and lower survival than hatchings from the warmer treatments (Tables 3.1, 3.2, and Figure 3.2). Because growth rate in the laboratory did not differ among treatments, the reduced growth and survival of hatchlings from the 22 °C treatment was presumably attributable to environmental factors such as reduced activity period, increased competition, and/or reduced food supply associated with hatching in October rather than August and September.

An unanswered question, however, is why does *S. undulatus* occur in localities such as Blacksburg which has a relatively cool climate for its latitude (37 °N). According to my model, approximately 364 degree-days are accumulated during June-September while about 495 degree-days are required for successful development. The explanation seems to be that gravid females compensate for low ambient temperature associated with high elevation (ca. 625 m) through selection of warm sites on both landscape and micro-habitat scales (Andrews 2000; Shine 2004). In mountainous southwestern Virginia, *S. undulatus* is typically found on south-facing slopes. Moreover, females oviposit in open areas where nest sites are exposed to the sun for most of the day (A. Roberts and R. Andrews, pers. comm.). Selection of warm microhabitats for oviposition by gravid females has been observed for other species of reptiles inhabiting high elevations and high latitudes, including *Sceloporu*s lizards (Andrews 2000), snakes (Blouin-Demers et al. 2004), skinks (Hecnar 1994; Shine 2004), and turtles (Litzgus 1998). Availability of thermally appropriate nesting sites would thus appear to be critically important for populations of oviparous species living in cool climates near their distributional limits.

IMPLICATIONS FOR THE EVOLUTION OF VIVIPARITY

The putative benefit of viviparity in cold climates is that development of *in utero* embryos is enhanced by higher and more stable body temperatures of the thermoregulating

female compared to eggs in a nest (Packard et al. 1977; Shine 1983; Shine 1985). The term "cold", however, is often used in a qualitative and subjective manner with regard to explanations of the distributions of oviparous and viviparous species. For example, in *Sceloporus* lizards, the majority of viviparous species are confined to high elevations at low latitudes, whereas the northernmost members of the genus are entirely oviparous (Sites et al. 1992; Méndez-De-La Cruz et al. 1998). In this example, viviparous species of *Sceloporus* lizards at tropical latitudes have activity seasons that may last the entire year even at high elevations. In contrast, oviparous species (such as *S. undulatus*) inhabiting northern latitudes have relatively short activity seasons due to cold temperatures during winter (Andrews 1998). Considering the substantial body of research on the evolution of reptilian viviparity, it is surprising that relatively few studies have attempted to quantify the environmental limits of oviparous reproduction.

Our research suggests that the distributional limits of oviparous species at high latitudes and elevations are determined both by absolute temperature and the length of time eggs are exposed to a favorable range of incubation temperatures. For example, even in relatively cool environments at high elevations, construction of shallow nests in warm micro-sites by gravid females can largely ameliorate the negative effects of low ambient air temperatures on embryonic development (Andrews 2000; Shine 2004). Thus, at intermediate latitudes and elevations there may be little thermal advantage to extended egg retention. Shallow nests, however, also expose eggs to potential risks such as extreme temperature fluctuations, desiccation, or predation (Andrews 2000). At high latitudes and elevations, increasing mortality of embryos would thus eventually offset the thermal benefits associated with a shift from deeper to shallower nests. With progressive increases in latitude or elevation, gravid females would eventually be unable to behaviorally compensate for declining air temperatures through nest site selection.

LITERATURE CITED

Andrews RM. 1982. Patterns of growth in reptiles. In: Gans C, Pough FH eds. *Biology of the Reptilia.* Volume 13. New York: John Wiley and Sons, 273-320.

Andrews RM. 1998. Geographic variation in field body temperatures of *Sceloporus* lizards. *Journal of Thermal Biology* 23: 329-334.

Andrews RM. 2000. Evolution of viviparity in squamate reptiles (*Sceloporus* spp): a variant of the cold-climate model. *Journal of Zoology (London)* 250: 243-253.

Andrews RM, Qualls CP, Rose BR. 1997. Effects of low temperature on embryonic development of *Sceloporus* lizards. *Copeia* 1997: 827-833.

Andrews RM, Mathies T, Warner DA. 2000. Effects of incubation temperature on morphology, growth, and survival of juvenile *Sceloporus undulatus*. *Herpetological Monographs* 14: 420-431.

Angilletta MJ, Winters RS, Dunham AE. 2000. Thermal effects on the energetics of lizard embryos: implications for hatchling phenotypes. *Ecology* 81: 2957-2968.

Bobyn ML, Brooks RJ. 1994. Incubation conditions as potential factors limiting the northern distribution of snapping turtles, *Chelydra serpentina. Canadian Journal of Zoology* 72: 28-37.

Blouin-Demers G, Weatherhead PJ, Row JR. 2004. Phenotypic consequences of nest-site selection in black rat snakes (*Elaphe obsoleta*). *Canadian Journal of Zoology* 82: 449-456.

Braña F. 2003. Morphological correlates of burst speed and field movement patterns: the behavioral adjustment of locomotion in wall lizards (*Podarcis muralis*). *Biological Journal of the Linnean Society* 80: 135-146.

Burger J. 1991. Effects of incubation temperature on behavior of hatchling pine snakes: implications for reptilian distribution. *Behavioral Ecology and Sociobiology* 28: 297-303.

Dufaure J, Hubert J. 1961. Table de development du lezard vivipare: *Lacerta* (*Zootica*) *vivipara* Jaquin. *Archives Anatomie Microscopie Morphologie Expérimental* 50: 309-327.

Elphick MJ, Shine R. 1998. Longterm effects of incubation temperatures on the morphology and locomotor performance of hatchling lizards *Bassiana duperreyi*, (Scincidae). *Biological Journal of the Linnean Society* 63: 429-447.

Ferguson GW, Fox SF. 1984. Annual variation of survival advantage of large juvenile side-blotched lizards, *Uta stansburiana*: its causes and evolutionary significance. *Evolution* 38: 342-349.

Greer AE, 1968. Modes of reproduction in the squamate faunas of three altitudinally correlated life zones in east Africa. *Herpetologica* 24: 229-232.

Hecnar SJ. 1994. Nest site distribution, site selection, and brooding in the five-lined skink (*Eumeces fasciatus*). *Canadian Journal of Zoology* 72: 1510-151.

Irschick DJ. 2000. Effects of behavior and ontogeny on the locomotor performance of a West Indian lizard: *Anolis lineatopus. Functional Ecology* 14: 438-444.

Irschick DJ. 2003. Measuring performance in nature: implications for studies of fitness within populations. *Integrative and Comparative Biology* 43: 396-407.

Kearney M, Porter WP. 2004. Mapping the fundamental niche: physiology, climate, and the distribution of a nocturnal lizard. *Ecology* 11: 3119-3131.

Litzgus JD, Brooks RJ. 1998. Reproduction in a northern population of *Clemmys guttata. Journal of Herpetology* 32: 252-259.

Méndez-De La Cruz FR, Villagrán Santa Cruz M, Andrews RM. 1998. Evolution of viviparity in the lizard genus *Sceloporus. Herpetologica* 54: 521-532.

Muth A. 1980. Physiological ecology of desert iguana (*Dipsosaurus dorsalis*) eggs: temperature and water relations. *Ecology* 61: 1335-1343.

Packard GC, Tracy CR, Roth JJ. 1977. The physiological ecology of reptilian eggs and embryos and the evolution of viviparity within the Class Reptilia. *Biological Reviews* 52:71-105.

Parton WJ. 1984. Predicting soil temperatures in a shortgrass steppe. *Soil Science* 138: 93-101.

Porter WP, Sabo JL, Tracy CR, Reichman OJ, Ramankutty N. 2002. Physiology on a landscape scale: plant animal interactions. *Integrative and Comparative Biology* 42: 431-453.

Qualls CP, Andrews RM. 1999. Cold climates and the evolution of viviparity in reptiles: cold incubation temperatures produce poor-quality offspring in the lizard *Sceloporus virgatus*. *Biological Journal of the Linnean Society* 67: 477-491.

Qualls FJ, Shine R. 1998. Geographic variation in lizard phenotypes: importance of the incubation environment. *Biological Journal of the Linnean Society* 64: 477-491.

Qualls FJ, Shine R. 2000. Post hatching environment contributes greatly to phenotypic variation between two populations of the Australian garden skink *Lampropholis guichenoti*. *Biological Journal of the Linnean Society* 71: 315-341.

SAS Institute. 2003. SAS Version 9.1.2 Cary, NC: SAS Institute

Sergeev AM. 1940. Researches on the viviparity in reptiles. *Moscow Society of Naturalists* (Jubilee Issue): 1-34.

Sexton OJ, Marion KR. 1974. Duration of incubation of Sceloporus undulatus eggs at constant temperature. *Physiological Zoology* 47: 91-95.

Shine R. 1983. Reptilian viviparity in cold climates: testing the assumptions of an evolutionary hypothesis. *Oecologia* 57: 397-405.

Shine R. 1985. The evolution of viviparity in reptile: an ecological analysis. In: Gans C, Billett F eds. *Biology of the Reptilia.* Volume 15. New York: John Wiley and Sons, 605-694.

Shine R. 1987. Reproductive mode may determine geographic distributions in Australian venomous snakes (*Pseudechis*, Eliapidae). *Oecologia (Berlin)* 71: 608-612.

Shine R. 1997. Seasonal timing of oviposition in sand lizards (*Lacerta agilis*): why earlier clutches are better. *Journal of Evolutionary Biology* 10: 369-381.

Shine R. 1999. Egg-laying reptiles in cold climates: determinants and consequences of nest temperatures in montane lizards. *Journal of Evolutionary Biology* 12: 918-926.

Shine R. 2002. Reconstructing an adaptationist scenario: what selective forces favor the evolution of viviparity in montane reptiles? *The American Naturalist* 160: 582-593.

Shine R. 2004. Incubation regimes of cold-climate reptiles: the thermal consequences of nest-site choice, viviparity and maternal basking. *Biological Journal of the Linnean Society* 83: 145-155.

Shine R, Berry JF. 1978. Climatic correlates of live-bearing in squamate reptiles. *Oecologia (Berlin)* 33: 261-268.

Shine R, Bull JJ. 1979. The evolution of live-bearing in lizards and snakes. *The American Naturalist* 113: 905-923.

Shine R, Madsen TRL, Elphick MJ, Harlow PS. 1997. The influence of nest temperatures and maternal brooding on hatchling phenotypes in water pythons. *Ecology* 78: 1713-1721.

Sites JW Jr, Archie JW, Cole CJ, Villela OF. 1992. A review of the phylogenetic hypotheses for lizards of the genus *Sceloporus* (Phrynosomatidae): implications for ecological and evolutionary studies. *Bulletin of the American Museum of Natural History* 213: 1-110.

St. Clair RC, Gregory PT. 1991. Factors affecting the northern range limit of painted turtles (*Chrysemys picta*): winter acidosis or freezing? *Copeia* 1990: 1083-1089.

Stebbins RC. (1985) *A field guide to western reptiles and amphibians*, 2nd edn. Boston: Houghton Mifflin.

Tinkle DW, Gibbons JW. 1977. The distribution and evolution of viviparity in reptiles. *Miscellaneous Publications Museum of Zoology, University of Michigan* 154: 1-55.

Travis JM, McManus MG, Baer CF. 1999. Sources of variation in physiological phenotypes and their evolutionary significance. *American Zoologist* 39: 422-433.

Warner DA, Andrews RM. 2002. Laboratory and field experiments identify the sources of variation in phenotypes and survival of hatchling lizards. *Biological Journal of the Linnean Society* 76: 105-124

Weekes HC. 1933. On the distribution, habitat and reproductive habits of certain European and Australian snakes and lizards, with particular regard to their adoption of viviparity. *Proceedings of the Linnean Society of New South Wales* 58: 270-274.

Wilson BS. 1991. Latitudinal variation in activity season mortality rates of the lizard *Uta stansburiana*. *Ecological monographs* 61: 393-414

Yntema CL. 1978. Incubation times for eggs of the turtle *Chelydra serpentina* (Testudines: Chelydridae) at various temperatures. *Herpetologica* 34: 274-277.

Table 3.1. Mean values and standard error (number of clutches) for incubation period, phenotype, locomotor performance, and growth of laboratory *Sceloporus undulatus* hatchlings incubated at 22, 24, and 27 °C. (Statistical tests are reported in Table 3.2)

Trait	22	24	27
Incubation period (days)	98.9 ± 0.7 (20)	74.4 ± 0.9 (19)	56.4 ± 0.3 (19)
SVL (mm)			
At hatching	25.3 ± 0.2 (20)	25.4 ± 0.2 (19)	25.4 ± 0.2 (19)
At release	27.8 ± 0.3 (20)	27.8 ± 0.3(19)	28.4 ± 0.4 (19)
Body mass (g)			
At hatching	0.587 ± 0.013 (20)	0.589 ± 0.014 (19)	0.580 ± 0.015 (19)
At release	0.761 ± 0.034 (20)	0.725 ± 0.024 (19)	0.714 ± 0.027(19)
Body shape ($mass^{0.3}$/SVL)			
At hatching	0.0336 ± 0.0001 (20)	0.0335 ± 0.0001 (19)	0.0333 ± 0.00021 (19)
At release	0.0329 ± 0.0002 (20)	0.0326 ± 0.0002 (19)	0.0318 ± 0.0004 (19)
Tail length (mm)			
At hatching	25.6 ± 0.6 (20)	28.2 ± 0.5 (19)	29.9 ± 0.47 (19)
At release	28.4 ± 0.7 (20)	30.6 ± 0.6 (19)	32.9 ± 0.56 (19)
Hind leg span (mm)			
At hatching	38.6 ± 0.9 (20)	41.5 ± 0.3 (19)	41.6 ± 0.4 (19)
At release	41.6 ± 0.4 (20)	42.8 ± 0.4 (19)	43.8 ± 0.4 (19)
Locomotion (m/s)			
Speed over 25 cm	0.692 ± 0.032 (19)	0.785 ± 0.063 (18)	0.828 ± 0.11 (18)
Speed over 1 m	0.417 ± 0.020 (19)	0.384 ± 0.020 (18)	0.421 ± 0.034 (18)
No. stops over 1m	3.0 ± 0.2 (19)	3.8 ± 0.2 (18)	3.7 ± 0.3 (18)
Growth in the field			
SVL (logΔmm/day)	0.0036 ± 0.0003 (8)	0.0056 ± 0.0008 (15)	0.006 ± 0.0005 (13)
Mass (logΔg/day)	0.0105 ± 0.0023 (8)	0.0162 ± 0.0020 (15)	0.021 ± 0.0012 (13)
Growth in laboratory			
SVL (logΔmm/day)	0.0091 ± 0.0008 (20)	0.0087 ± 0.0091 (19)	0.0108 ± 0.0012 (19)
Mass (logΔg/day)	0.0235 ± 0.0039 (20)	0.0198 ± 0.0024 (19)	0.019 ± 0.0035 (19)

Table 3.2. Statistical tests (ANOVAs and ANCOVAs) of effects of incubation at 22, 24, and 27 °C on incubation period, phenotype, performance, and growth of *Sceloporus undulatus* hatchlings. *Post hoc* pair-wise comparisons were made using a Tukey's honestly significant difference test (ANOVAs), or a least significant difference test on least-squared means (ANCOVAs). Significant differences are in bold type.

Trait	Covariate	Treatment	Results
Incubation period (days)	Embryo stage	$F_{2, 54}$=996.9, ***P* < 0.001**	22 > 24 > 27
SVL (mm)			
At hatching	Egg mass	$F_{2, 54}$=0.1, P =0.904	---
At release	SVL	$F_{2, 54}$=1.3, P =0.292	---
Body mass (g)			
At hatching	Egg mass	$F_{2, 54}$=0.2, P =0.855	---
At release	Mass	$F_{2, 54}$=0.8, P =0.433	---
Body shape ($mass^{0.3}$/SVL)			
At hatching	---	$F_{2, 55}$=0.8, P =0.465	---
At release	---	$F_{2, 55}$=3.8, ***P* =0.027**	22>27
Tail length (mm)			
At hatching	Egg mass	$F_{2, 54}$=18.9, ***P* < 0.001**	22 < 24 < 27
At release	SVL	$F_{2, 54}$=18.7, ***P* < 0.001**	22 < 24 < 27
Hind-limb span (mm)			
At hatching	Egg mass	$F_{2, 54}$=8.4, ***P* <0.001**	22 < 24 = 27
At release	SVL	$F_{2, 54}$=9.6, ***P* <0.001**	22 < 24 = 27
Locomotion (m/s)			
Speed over 25 cm	---	$F_{2, 51}$=0.9, P =0.434	---
Speed over 1 m	SVL	$F_{2, 51}$=0.6, P =0.569	---
No. stops over 1 m	---	$F_{2, 52}$=2.8, P =0.068	---
Growth in the field			
SVL (logΔmm/day)	---	$F_{2, 33}$=3.1, P =0.06	---
Mass (logΔg/day)	---	$F_{2, 33}$=4.9, ***P* =0.014**	22 < 27
Growth in the laboratory			
SVL (logΔmm/day)	---	$F_{2, 55}$=1.3, P =0.269	---
Mass (logΔg/day)	---	$F_{2, 55}$=0.4, P =0.682	---

Table 3.3. Statistical tests comparing survival of laboratory (contrasts of treatments) and field incubated (contrasts of field vs. laboratory) hatchlings of *Sceloporus undulatus* at 10, 20, and 30 days after release in the field. Overall contrasts of survival were performed using Chi-square tests. Significant differences are in bold type.

	Statistical test (overall)
Laboratory hatchlings	
Survival at 10 days	χ^2=7.1, d.f.=2, ***P*=0.028**
Survival at 20 days	χ^2=14.8, d.f.=2, ***P*<0.001**
Survival at 30 days	χ^2=13.4, d.f.=2, ***P*=0.001**
Field vs. lab hatchlings	
Survival at 10 days	χ^2=7.1, d.f.=3, *P*=0.067
Survival at 20 days	χ^2=15.5, d.f.=3, ***P*=0.001**
Survival at 30 days	χ^2=13.5, d.f.=5, ***P*=0.004**

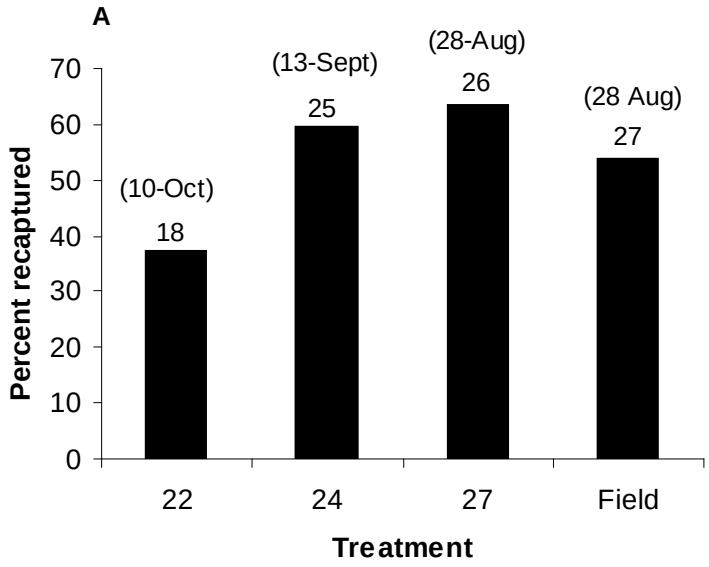
Table 3.4. Comparisons of performance and growth between laboratory hatchlings incubated at 22, 24, and 27 °C, and field incubated hatchlings of *Sceloporus undulatus*. Values are means ± SEM (number of hatchlings). (Statistical tests are reported in Table 3.5)

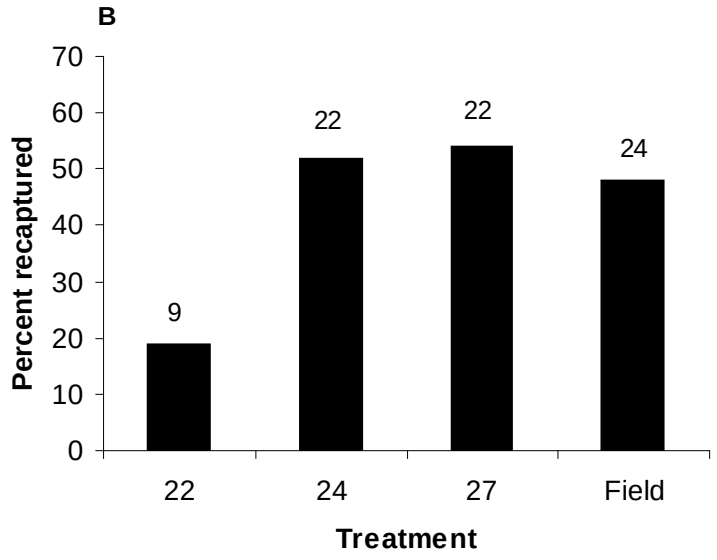
	Field Hatchlings	Laboratory Hatchlings		
Trait		22	24	27
Locomotion (m/s)				
Speed over 25 cm	0.842 ± 0.032 (41)	0.689 ± 0.026 (47)	0.815± 0.065 (37)	0.838 ± 0.081 (37)
Speed over 1 m	0.470 ± 0.018 (41)	0.410 ± 0.018 (47)	0.390 ± 0.020 (37)	0.418 ± 0.021 (37)
No. stops over 1 m	3.7 ± 0.16 (41)	3.2 ± 0.19 (47)	3.6 ± 0.19 (37)	3.7 ± 0.24 (37)
Growth in field				
SVL (logΔmm/day)	0.007 ± 0.0007 (22)	0.004 ± 0.0004 (10)	0.007 ± 0.001 (20)	0.007 ± 0.0006 (22)
Mass (logΔg/day)	0.019 ± 0.002 (22)	0.011 ± 0.002 (10)	0.018 ± 0.003 (20)	0.021 ± 0.001 (22)
Growth in lab				
SVL (logΔmm/day)	0.007 ± 0.0007 (41)	0.009 ± 0.0007(47)	0.008 ± 0.0008 (42)	0.011 ± 0.0009 (41)
Mass (logΔg/day)	0.022 ± 0.0022 (41)	0.022 ± 0.003 (47)	0.018 ± 0.002 (42)	0.021 ± 0.003 (41)

Table 3.5. Statistical tests (ANOVAs and ANCOVAs) between laboratory hatchlings incubated at 22, 24, and 27 °C and field incubated hatchlings. *Post hoc* pair-wise comparisons were made using a Tukey's honestly significant difference test (ANOVAs), or a least significant difference test on least-squared means (ANCOVAs). Because clutch of origin could not be determined for field hatchlings, statistical contrasts of laboratory and field hatchlings were conducted using individual values rather than clutch means. Significant differences are in bold type

	Covariate	Statistical test (laboratory vs. field)	Result
Trait			
Locomotion (m/s)			
Speed over 25 cm	---	$F_{3, 158}$ =2.1, P = 0.106	---
Speed over 1 m	SVL	$F_{3, 157}$ =0.7, P =0.538	---
No. stops over 1 m	SVL	$F_{3, 157}$ =3.1, ***P* = 0.028**	Field >22
Growth in field			
SVL (logΔmm/day)	---	$F_{3, 70}$ =2.0, P = 0.109	---
Mass (logΔg/day)	---	$F_{3, 70}$ =3.3, ***P* = 0.024**	22<27
Growth in lab	---		
SVL (logΔmm/day)	---	$F_{3, 167}$ =5.1, ***P* = 0.002**	Field < 27
Mass (logΔg/day)	---	$F_{3, 167}$ =0.3, P = 0.799	---

Figure 3.1. Geographic distribution of *Sceloporus undulatus* in the United States. Information on distributional boundaries was obtained from several sources (see text).


A
Percent recaptured
70
60
50
40
30
20
10
0
(10-Oct)
18
(13-Sept)
25
(28-Aug)
26
(28 Aug)
27
22
24
27
Field
Treatment


B
Percent recaptured
70
60
50
40
30
20
10
0
9
22
22
24
22
24
27
Field
Treatment

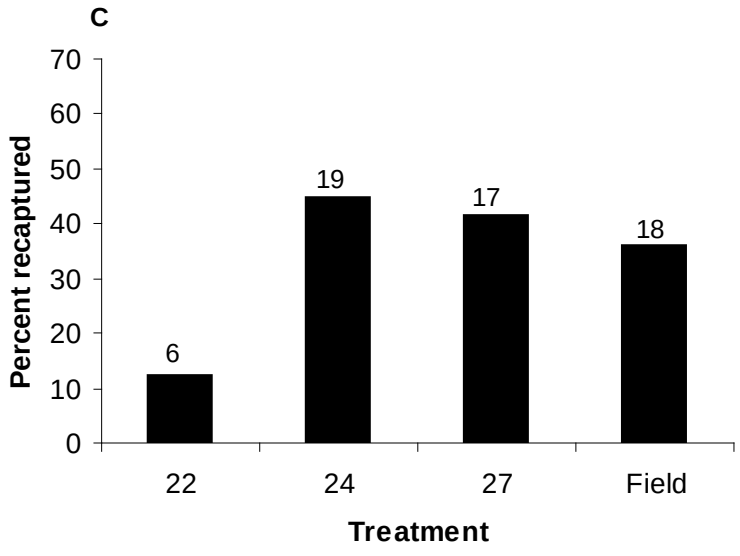
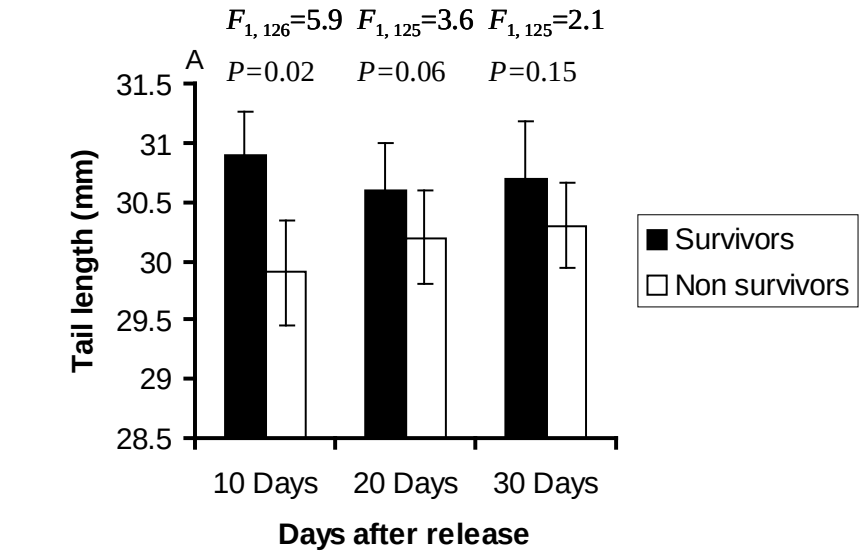
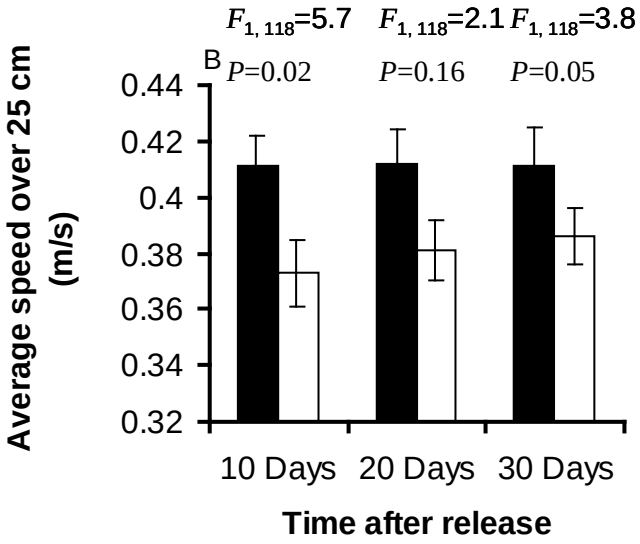

C
Percent recaptured
70
60
50
40
30
20
10
0
6
19
17
18
22
24
27
Field
Treatment

Figure 3.2. Percent survival of laboratory and field incubated hatchlings at (A) 10, (B) 20, and (C) 30-days after release in the field. Date above the bars in (A) indicate mean date of hatching for lab individuals and the mean date of capture for field individuals. Numbers above the bars (A-C) indicate the number of hatchlings recaptured at each time period from an initial release of 47, 42, and 41 hatchlings from the 22, 24, and 27 °C treatments, respectively and an initial capture of 50 field hatchlings.


$F_{1, 126}$=5.9 $F_{1, 125}$=3.6 $F_{1, 125}$=2.1
A
P=0.02 P=0.06 P=0.15
31.5
31
30.5
30
29.5
29
28.5
Tail length (mm)
Survivors
Non survivors
10 Days
20 Days
30 Days
Days after release


$F_{1, 118}$=5.7 $F_{1, 118}$=2.1 $F_{1, 118}$=3.8
B
P=0.02 P=0.16 P=0.05
0.44
0.42
0.4
0.38
0.36
0.34
0.32
Average speed over 25 cm (m/s)
10 Days
20 Days
30 Days
Time after release

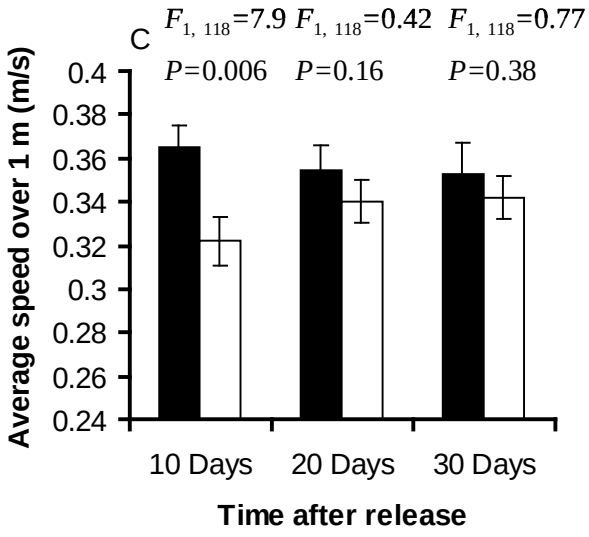

$F_{1, 118}$=7.9 $F_{1, 118}$=0.42 $F_{1, 118}$=0.77
C
P=0.006 P=0.16 P=0.38
0.4
0.38
0.36
0.34
0.32
0.3
0.28
0.26
0.24
Average speed over 1 m (m/s)
10 Days
20 Days
30 Days
Time after release

Figure 3.3. Comparisons of tail length and locomotor performance (overall mean, SE) independent of temperature treatment for hatchling *S. undulatus* that survived and did not survive to 10, 20, and 30 days after release in the field. (A) Tail length, (B) average running speed over 25 cm, (C) average running speed over 1 m.

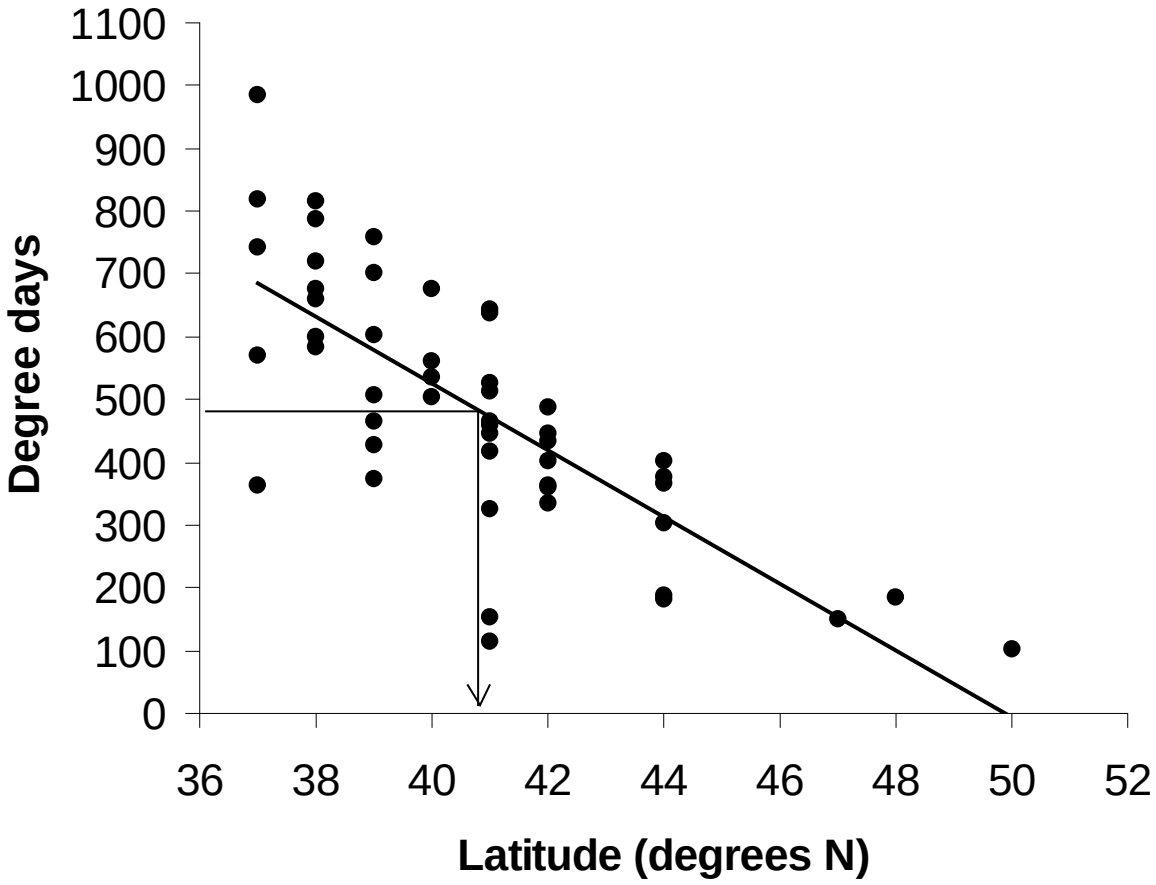


Figure 3.4. Relationship between number of degree-days above 17 °C accumulated over 122 days (June-September) as a function of latitude (37-50 °N). Intersection of horizontal line with Y-axis indicates the minimum estimated number of degree-days required for successful incubation of *Sceloporus undulatus* embryos. Arrow indicates the predicted northern latitudinal limit (approximately 40.5 °N) for *S. undulatus* based upon a minimum of 495 degree-days required for successful egg incubation.