PHENOTYPES AND SURVIVAL OF HATCHLING LIZARDS

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

MASTER OF SCIENCE
in
Biology

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January 16, 2001
Blacksburg, Virginia

Key Words: Clutch effects, Growth rate, Incubation moisture, Running speed, *Sceloporus undulatus*, Survival, Yolk investment

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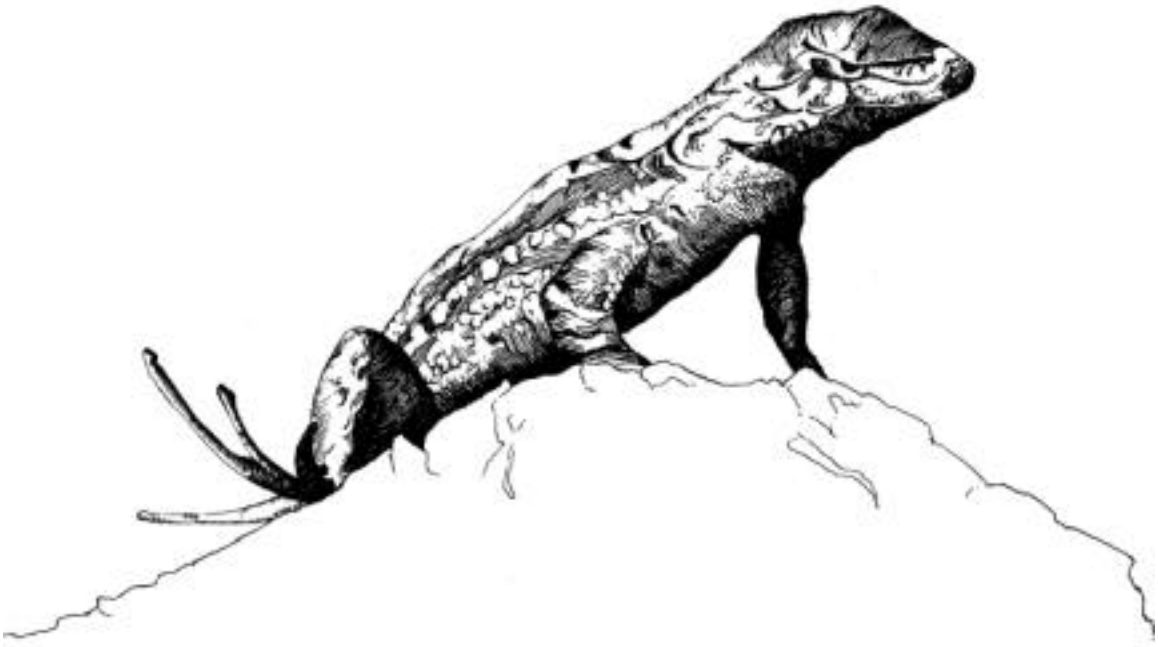

PHENOTYPES AND SURVIVAL OF HATCHLING LIZARDS

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(ABSTRACT)

The phenotypes of hatchling reptiles are influenced by the environmental conditions that embryos experience during incubation, by yolk invested into the egg, and by the genetic contributions of the parents. Phenotypic traits are influenced by these factors in ways that potentially affect the fitness of hatchlings. The physical conditions that embryos experience within the nest affects development, hatching success, and hatchling phenotypes. Thus, the nest site that a female selects can influence the survival of her offspring as well as her overall fitness. In Chapter 1, I addressed this issue through a nest site selection experiment designed to determine the substrate temperature and moisture conditions that female eastern fence lizards (*Sceloporus undulatus*) select when provided a range of conditions from which to choose. In general, I found that females selected nest sites with conditions that yield high hatching success.

In Chapter two, I investigated the relative contributions of incubation moisture conditions, maternal yolk investment, and clutch (genotype) to variation in hatchling phenotypes and survival under field conditions. Eggs from 28 clutches were distributed among two moisture treatments; wet (-150 kPa) and dry (-530 kPa). In another treatment, yolk was removed from eggs to determine the affect of yolk quantity on hatchling phenotypes. After hatching, several phenotypic traits (mass, snout-vent length, tail length, body shape, thermal preference, running speed, desiccation rate, and growth rate) were measured. Hatchlings were subsequently marked and released at a field site in southwest Virginia. Hatchlings were recaptured twice weekly prior to winter and the following spring to monitor growth and survival. I found that incubation moisture and yolk removal affected only hatchling body size; individuals from the dry and yolk removed treatments were smaller in body size than those from the wet treatment. However, clutch was the most important source of phenotypic variation; all phenotypes were affected by clutch. Significant clutch effects suggested the possibility that phenotypic variation had at least some genetic basis. In the field, survival was not affected by incubation moisture and yolk removal, and overall survival was not associated with hatchling body size. Survivors and nonsurvivors differed only in growth rate in the field and running speed measured in the laboratory. Survivors ran faster and grew more slowly than nonsurvivors. To examine the association of clutch with survival, I used clutch mean values to look at the relationship between phenotype and survival. Clutches that produced relatively slow growing individuals and fast runners had higher survival rates than clutches that produced relatively rapid

growing individuals and slow runners. In order to grow rapidly, an individual must eat more than slowly growing individuals. Thus, rapid growth rate may increase risk of predation through its association with foraging activity. Individuals that run fast should be capable of capturing prey and evading predators more effectively than individuals that run slowly. Overall, these results emphasize the importance of clutch to variation in phenotypes and survival in hatchling *Sceloporus undulatus.*

DEDICATION

To my parents, Charles and Mary Warner.

Without their love and support, I would not have made such accomplishments.
Little things, such as allowing me to keep unusual animals as a kid,
and taking me for hikes in the woods, have greatly influenced
my interest and appreciation for the natural world.

For that, I am forever grateful.

ACKNOWLEDGMENTS

I wish to express my thanks to my advisor, Dr. Robin Andrews. Her guidance, support, and advise during my graduate studies has helped me become a better researcher, communicator, and teacher. The hours upon hours of time that she has put into helping me with this project has greatly improved my interest and appreciation for science. I am grateful for the knowledge and direction of my other committee members, Drs. Tom Jenssen and Bob Jones. Their backgrounds and suggestions have greatly improved my thesis research and my overall graduate experience. I am grateful for the help of several undergraduate volunteers – Caran Astor, Danny Reinhardt, Julie Thomson, and Amber Waller. Their many hours of volunteer work is greatly appreciated and without their assistance in the laboratory and field this project would not have been possible. Thanks to Sara Crews for entering some of my data into the computer. Matt Lovern, Ryan McCleary, Kelly Passek, and Tonia Schwartz helped me on several occasions with collecting female lizards and/or recapturing hatchlings. Several insightful discussions about my research with Tom Bunt and Tonia Schwartz are greatly appreciated; these discussions helped me look at my research in new ways. Thanks to my brother, Vince Warner, for the illustration of the hatchling *Sceloporus undulatus* that is on the title page of this thesis. Thanks again to Tonia Schwartz for her support and friendship throughout the course of this research. I also express my thanks to my graduate student friends at Virginia Polytechnic Institute and State University for keeping me sane and making graduate school even more enjoyable and interesting.

This project was funded by a Sigma Xi Grant-in-Aid of research, by a Chicago Herpetological Society Grant, and by a Graduate Research Development Project Grant from the Graduate Student Assembly (GSA) of Virginia Polytechnic Institute and State University. I also received a Student Travel Award from the GSA to present my research at the joint meetings of the American Society of Ichthyologists and Herpetologists, Society for the Study of Amphibians and Reptiles, and the Herpetologists' League (June 2000). Thanks to the Department of Biology Chair, Dr. Joe Cowles, for providing me further financial support by matching each of these grants. Dr. Cowles also provided me with financial support to present my research at the meeting of the Society for Integrative and Comparative Biology (January 2001).

I want to acknowledge Luke and Rhonda Flowers for their permission to collect female lizards on their property and the Virginia Forest Service for granting me permission to work at the field site in Jefferson National Forest. All lizards in this study were collected under permit number 013057 of the Virginia Department of Game and Inland Fisheries. This project was approved by

the Virginia Polytechnic Institute and State University Animal Care Committee (Proposal number 99-054-BIOL).

During my undergraduate years, several people shared their knowledge with me and provided me with experience that has shaped my current research interests. Without this knowledge and experience, I would not have been so motivated to continue research in graduate school. I am grateful for the support that Dr. Fred Janzen has given me. The knowledge that he shared has enhanced my interest in ecology, evolutionary biology, and herpetology. Thanks to John Tucker who showed me how fun and exhausting field work can be, and how hard work will eventually pay off. Thanks to Robin Saunders for sharing her knowledge of herpetology. Her extensive knowledge made me realize how little I knew about this field, which, in turn, encouraged me to learn more about reptile and amphibian biology.

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BACKGROUND AND OBJECTIVES

The main objective of this research was to explore relationships among sources of phenotypic variation, phenotypes themselves, and hatchling survival in the eastern fence lizard (*Sceloporus undulatus*). Below, I provide a brief background and evidence for theories and ideas relevant to my thesis topic. Subsequently, I point out specific objectives of my research and explain how this thesis is organized. Finally, I provide background on the natural history of the study organism, *Sceloporus undulatus.*

In my thesis, I addressed three factors that influence phenotypic variation in hatchling reptiles. First, phenotypic traits are influenced by environmental temperature and moisture conditions experienced by the embryo during incubation. For instance, body size and proportions, sex, growth rate, temperature preference, and locomotor performance are just some hatchling traits that are influenced by incubation temperature and moisture (Burger et al., 1987; Werner, 1988; Janzen and Paukstis, 1991; Van Damme et al., 1992; Overall, 1994; Shine and Harlow, 1996). Second, maternal provisioning of yolk to developing embryos also influences phenotypic traits. Manipulations of yolk quantity indicate that the amount of yolk allocated to eggs affects the body size of hatchlings. This, in turn, can affect traits that are correlated with body size (Sinervo, 1990; Sinervo and Huey, 1990). Third genetic contributions of the male and female parents affect offspring phenotypes. Inter-clutch variation in phenotypes suggests the possibility of at least some genetic effects (Van Berkum and Tsuji, 1987; Brooks et al., 1991; Janzen et al., 1995; Rhen and Lang. 1995; Olsson et al., 1996; Shine et al., 1997b). However, clutch effects must be interpreted cautiously because genetic effects can be confounded by other maternal effects.

Because natural selection acts on phenotypes, variation in phenotypes reflects variation in fitness (Arnold, 1983; Garland and Losos, 1994). The three sources of phenotypic variation mentioned above influence hatchling reptiles in ways that seem likely to affect their survival, and thus the fitness of their parents. Therefore, selection should act on each of these sources of phenotypic variation. First, because environmental conditions during incubation (i.e. nest environment) affect hatchling phenotypes, female nesting behavior may play a role in the determination of hatchling fitness. If incubation-induced phenotypes are acted upon by selection, then, assuming nesting behavior is heritable, natural selection should influence maternal nest site selection. It would be expected that females are selected to construct nests in locations with conditions that produce the greatest hatching success and hatchlings fit for the local environment (Resetarits, 1996; Shine and Harlow, 1996). Second, because hatchling body size is an important predictor of fitness (reviewed by Packard and Packard, 1988), selection should also increase the

amount of yolk that females invest into their clutches. Third, for selection on phenotypes to have evolutionary consequences, variation in those phenotypes must have a genetic component (Arnold, 1986). Thus, phenotypes that are strongly influenced by genotype (seen as clutch effects) are more likely to have evolutionary consequences than phenotypes that are induced by environmental variation. Environmentally induced phenotypic variation is refered to as phenotypic plasticity. Moreover, the phenotypic response of an organism to environmental conditions (phenotypic plasticity) may be under genetic control as well. Thus, phenotypic plasticity itself has the potential to evolve like any other genetically based trait (reviewed in Stearns, 1989).

In reptiles, the influence of incubation conditions on phenotypes is well documented (Packard and Packard, 1988; Shine, 1995; Shine et al., 1997a; and references therein). The influence of yolk investment on hatchling phenotypes has received relatively less attention in the literature (Sinervo, 1990; Sinervo and Huey, 1990, Sinervo et al., 1992). The influence of clutch (or genotype) on hatchling phenotypes has received the least attention, despite studies that consistently indicate that clutch is a significant source of phenotypic variation (Van Berkum and Tsuji, 1987; Brooks et al., 1991; Janzen et al., 1995; Rhen and Lang. 1995; Shine et al., 1997b; Andrews et al., 2000). Understanding the relative contributions of each of these sources of phenotypic variation will provide insight into the mechanisms by which natural selection acts. For example, if variation in a particular trait is under genetic control and highly heritable, then the potential for that trait to evolve is greater than if variation is due largely to environmental factors (Arnold, 1986).

To determine the relationship between phenotype and fitness, the phenotypic traits that are measured should be chosen carefully. It is important to keep in mind that a phenotype is the integration of several attributes. For example, snout-vent length is an integration of body length, mass, shape, head length, etc. Moreover, the importance of particular traits should be interpreted carefully because many different traits can be correlated with each other. But, correlation between two traits does not always mean that one phenotype has a direct effect on the other. Therefore, identifying direct and indirect relationships can be difficult, and should be interpreted cautiously. For example, if selection acts on an unmeasured trait that is correlated with a measured trait, then the actual target of selection will be misidentified (Arnold, 1986). However, identifying direct and indirect relationships among phenotypes is a necessary component of understanding natural selection (Garland and Losos, 1994).

"Bigger is better" is a common hypothesis applied to hatchling reptiles (reviewed by Packard and Packard, 1988). Larger hatchlings may reach reproductive maturity faster than smaller hatchlings and therefore exhibit greater fecundity. In addition, larger hatchlings may be less

susceptible to predation than smaller hatchlings because they can run or swim faster than their smaller conspecifics (Miller et al., 1987; Sinervo, 1990; Sinervo and Huey, 1990). Large bodied individuals tend to hold quality territories and force smaller individuals into less favorable habitats were predation and starvation may be more likely (Fox, 1978). On the other hand, larger body size may enhance survival only if resources are low and competition is high (Ferguson et al., 1982; Ferguson and Fox, 1984), and the importance of body size may vary among years and populations (Sinervo et al., 1992; Forsman, 1993). In addition, a small individual may be as difficult a target for a predator as a large individual (Van Damme and Van Dooren, 1999).

Rapid growth is also considered important for fitness (Rhen and Lang, 1995). For males, large individuals have dominance in terms of establishing a territory (Fox, 1978). For females, large body size enhances clutch size, thereby increasing individual fecundity (Ferguson et al., 1983; Forsman, 1993). Thus, rapid growth to adult body size should be favored during juvenile stages. However, rapid growth rate does have risks. In order to grow rapidly, an individual must eat more than slowly growing individuals. Thus, rapid growth rate may increase the risks of predation through its association with foraging activity (Sorci et al., 1996).

Reptiles, in general, have been excellent models for addressing the above ideas. Many species produce large clutch sizes. Large clutch size allows investigators to subdivide clutches into several experimental treatments, allowing them to examine the relative contributions of treatment, clutch, and treatment by clutch interactions to phenotypic variation (Brooks et al., 1991; Janzen et al., 1995; Rhen and Lang, 1995; Shine et al., 1997b). The phenotypes of hatchling reptiles can be easily manipulated, which is critical for looking at the direct effect of one phenotype on another or on survival (Sinervo and Huey, 1990; Sinervo et al., 1992; Janzen, 1993). Lastly, the early life stages of many reptile species are subject to high mortality (Wilbur and Morin, 1988). Thus, juvenile stages provide an excellent time frame to study the mechanisms by which natural selection acts (Janzen, 1993).

Thesis Objectives and Organization

I used the eastern fence lizard (*Sceloporus undulatus*) to address three main objectives. In Chapter 1, I determine substrate temperature and moisture conditions selected by nesting females when provided a range of conditions from which to choose. Because the physical conditions that eggs experience within a nest affects egg survival and hatchling phenotypes, my findings will potentially provide explanations for observed nesting behaviors by females. In Chapter 2, I determine: (1) the relative contributions of incubation moisture conditions, maternal yolk investment, and clutch on variation in several phenotypic traits of hatchlings, and (2) determine the

relationship between phenotype and survival. These two objectives were addressed by an egg incubation experiment in the laboratory, followed by a release experiment in the field. By manipulating and measuring hatchling phenotypes in the laboratory and subsequently releasing hatchlings in the field, I was able to examine the relationship between phenotype and survival under natural conditions. The integration of both laboratory and field experiments is necessary for evaluation of natural selection (Arnold, 1986).

Sceloporus undulatus was an ideal organism to work with for several reasons. First, the demography and life history of this species is well studied (see below). Second, this species produces a relatively large clutch size that allowed me to subdivide single clutches into several experimental treatments. Third, hatchlings of *Sceloporus undulatus* usually do not travel long distances. This aspect of this lizard's natural history means that estimates of survival are not strongly confounded by dispersal (Ferguson et al., 1983; Jones et al., 1987; Niewiarowski and Roosenburg, 1993).

A Brief Natural History of *Sceloporus undulatus*

Sceloporus undulatus has been a model for numerous demographic, evolutionary, physiological, and behavioral studies (Ferguson and Bohlen, 1978; Rothblum and Jenssen, 1978; Ferguson et al., 1980; Tracy, 1980; Roggenbuck and Jenssen, 1986; Jones et al., 1987; Ferguson and Talent, 1993; Niewiarowski and Roosenburg, 1993; Klukowski et al., 1998). *Sceloporus undulatus* is found throughout the eastern two-thirds of the United States (Conant and Collins, 1991). This species is currently divided into seven subspecies (Wiens and Reeder, 1997). Because life history characteristics vary substantially in different parts its geographic range, it is difficult to make generalizations about the life history of *Sceloporus undulatus* (Gillis and Ballinger, 1992; Ferguson and Talent, 1993; Niewiarowski and Roosenburg, 1993; Niewiaroski, 1995).

My study focused on a Virginia population of *Sceloporus undulatus* (the subspecies *S. undulatus hyacinthinus*). This species is a rough-scaled lizard with a maximum snout-vent length (SVL) of 84 mm. Adult females grow to larger SVL's than males, showing strong sexual size dimorphism (Mitchell, 1994). Adult males possess patches of blue coloration along the ventral sides and throat, whereas females may only have a small patch of blue on the throat. Sex is distinguished among both juveniles and adults by the enlarged scales at the base of the tail near the cloaca in males; these scales are absent in females.

Sceloporus undulatus prefers edges along several habitat types, such as mixed deciduous forests, pine woods, and areas that have been disturbed by humans. Access to sunlight appears to

be an important requirement; thus, areas with exposed rocks, logs, and woody debri are suitable for this species (Mitchell, 1994). At night and overwinter, lizards retreat to hiding places under logs, rocks, or in rock crevices. Fence lizards are "sit-and-wait" predators, and feed on a wide variety of invertebrates (McGovern et al., 1986). They are also potential prey for a variety of predators, such as black rat snakes (*Elaphe obseleta*), copperheads (*Agkistrodon contortix*), domestic cats, and predatory birds (Mitchell and Beck, 1992; Mitchell, 1994).

Mating behavior begins as early as mid-April (Mitchell, 1994). Sexually mature males and females can be as small as 44 and 63 mm SVL, respectively. Nesting occurs from mid-May to mid-July, and clutch size ranges from 5 to 16 eggs (N=29, mean=10.6) (D. A. Warner and R. M. Andrews, unpublished data). Hatching typically occurs between late July and early September depending on environmental temperature and when oviposition took place (Mitchell, 1994, D. A. Warner and R. M. Andrews, unpublished data). Most females do not reach sexual maturity in their first year of life, but the few individuals that do, nest later in the season than females that were adult in spring (D. A. Warner and R. M. Andrews, unpublished data).

Hatchlings and juveniles enter hibernation before adults and emerge earlier (Mitchell, 1994). At my study site in Montgomery County, Virginia, individuals were captured as late as 14 November, and were captured as early as 5 March the following spring. However, dates when hatchlings and juveniles enter and emerge from hibernation vary depending on environmental temperature. For adults, precise dates of entrance and emergence from hibernation in Virginia have not been documented.

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CHAPTER 1

NEST SITE SELECTION OF FEMALE LIZARDS (*SCELOPORUS UNDULATUS*) IN RELATION TO TEMPERATURE AND MOISTURE

ABSTRACT

The development and survival of reptilian embryos is affected by the temperature and moisture conditions experienced within the nest. Moreover, temperature and moisture conditions that embryos experience during incubation also affect phenotypic traits of hatchlings. Thus, the location where a female constructs a nest will have an influence on her overall fitness. Females should select nest sites that enhance hatching success and produce hatchlings fit for the local environment. The objective of this study was to determine the substrate temperature and moisture conditions selected by female eastern fence lizards (*Sceloporus undulatus*) when provided a range of conditions from which to choose. In the laboratory, gravid females were housed in a large enclosure that provided suitable nesting substrate. A temperature gradient along the length of the nesting substrate was produced by overhead heat lamps. A moisture gradient was established perpendicular to the temperature gradient by varying the water content of the substrate. After females nested, temperature probes were placed within nests to monitor temperature for 48 hours after oviposition. Substrate samples were also taken from the nest to determine the moisture conditions within the nest. In general, females chose nest conditions within the range suitable for embryonic development. Mean nest temperatures during day-time hours ranged from 25.1 to 31.9 °C, when available temperatures ranged from 22.3 to 32.1 °C. Mean nest temperatures based on 48 hour means ranged from 23.8 to 28.2 °C. Nests were concentrated between –650 and –50 kPa, even though water potential ranged as low as –2000 kPa. Nest site choice appeared to be based on the female's familiarity with the thermal regime of the gradient prior to oviposition.

INTRODUCTION

Eggs of most oviparous reptiles are abandoned soon after oviposition. From this point on, the survival and development of the embryos depend primarily upon the physical conditions within the nest. Variation in environmental moisture and temperature in the nest is a major factor affecting development and survival of reptilian embryos. Flexible shelled reptile eggs must absorb substantial amounts of water during the course of incubation, but the absolute amount of water absorbed depends upon hydric conditions of the nest (Packard et al., 1980; Tracy, 1980; Packard and Packard, 1988). Hydric conditions, in turn, affect phenotypic traits of hatchlings. For example, eggs incubated under relatively wet conditions result in larger bodied hatchlings than eggs incubated under dry conditions (Packard and Packard, 1988; Packard et al., 1993), probably due to more efficient yolk intake at high water potentials than at low water potentials (Janzen et al., 1990). Hatchling growth rate is also affected by incubation moisture conditions (Overall, 1994). Hatchling traits that are correlated with body size, such as running speed (Sinervo, 1990), are indirectly affected by nest moisture. By affecting hatchling phenotypes, moisture conditions of the nest can also influence hatchling survival, and thus the overall fitness of the parents. For instance, large bodied hatchlings produced by moist incubation conditions have higher survival in the field than small individuals from relatively dry incubation conditions (Vleck, 1988). In addition, the hydric environment during incubation affects hatchling survival through its effect on the amount of yolk that is converted to fat before hatching (Christian et al., 1991).

Variation in incubation temperature also influences phenotypic traits of hatchling reptiles. This is of major importance for species with temperature dependent sex determination (Vogt and Bull, 1984; Schwarzkopf and Brooks, 1987; Janzen, 1994), but temperature also affects other phenotypic traits of hatchling reptiles, such as growth rate, body size and proportions, locomotor performance, and thermal preference (Shine, 1995; Rhen and Lang, 1995; Elphick and Shine, 1998; Qualls and Andrews, 1999). In addition, nest temperature influences the rate of embryonic development, thereby affecting the timing of hatching; embryos that experience warm temperatures develop faster, resulting in hatchlings that emerge sooner than those experiencing cool temperatures (Andrews et al., 2000). Depending on environmental conditions, date of hatching may affect survival (Ferguson and Bohlen, 1978; Andrews et al., 2000).

Previous studies demonstrate that physical conditions of the nest affect offspring phenotypes in ways that seem likely to influence post-hatching survival. The physical conditions of the nest can also influence hatching success. Egg desiccation under dry conditions and stress under high temperatures can be lethal (Muth, 1980; Angilletta et al., 2000). Extremely wet and cool

conditions can also negatively affect embryo survival and development. Cool temperatures can slow or arrest embryonic development (Sexton and Marion, 1974; Christian et al., 1986). Extremely moist conditions may increase chances of fungal infections or invasion of microorganisms (Tracy, 1980) or reduce oxygen exchange (Packard and Packard, 1984). These observations provide a selective basis for maternal nest site selection (Resetarits, 1996; Shine and Harlow, 1996; Shine et al., 1997a).

The objective of this study was to determine substrate temperature and moisture conditions selected by female *Sceloporus undulatus* when provided a range of conditions from which to choose. Based on results of other studies, I predicted that females would choose nest sites with physical conditions known to yield high hatching success and quality hatchlings. *Sceloporus undulatus* was ideal to use because the influence of incubation moisture and temperature on hatching success, hatchling phenotypes, and post-hatching survival are will known for this species (Sexton and Marion, 1974; Tracy, 1980; Andrews et al., 2000; Angilletta et al., 2000; Chapter 2).

MATERIALS AND METHODS

Collection and Husbandry of Gravid Females

Gravid female *Sceloporus undulatus* (n=28) were collected between 15 May and 26 June, 1999 in Montgomery county near Blacksburg, Virginia between 700 and 780 m elevation. Females were brought back to animal care facilities at Virginia Polytechnic Institute and State University. Females were permanently marked by unique toe clips and marked with paint on their dorsum so they could be identified from a distance. They were housed in a 1.5 x 1.5 m enclosure. The floor of the enclosure was partially covered with a 3 x 3 array of 9 plastic containers (46 long x 24 wide x 20 deep cm). The containers were filled with a mixture of vermiculite and peat moss to provide nesting substrate. Horizontal connecting branches on the array provided perches and allowed the lizards to move throughout the enclosure. The entire enclosure was illuminated by two 48” Vita-lites (daily photoperiod: 0700-1800 h) and heat lamps (daily photoperiod: 0800-1400 h) suspended over the nesting containers (see below). Heat lamps were also placed over branches and boards in one corner that provided additional perching and basking sites. Females were fed crickets and wax worm larvae dusted with a vitamin-mineral mix, and watered daily.

Experimental Design: Gradient in Substrate Temperature and Moisture

A temperature gradient was established by suspending three 100 W lamps approximately 30 cm above the surface along one side of the 3 x 3 array and three 100 W lamps approximately 60

cm above the surface over the middle of the gradient. No heat lamps were suspended above the cool end of the gradient. Because the thermal gradient was constructed by overhead heat lamps raised at different distances from the substrate, the hot end of the gradient was also the brightest. Diel fluctuations in temperature and the positive correlation between light intensity and temperature thus simulated natural conditions in the field.

A moisture gradient was established perpendicular to the temperature gradient by varying the water content of the substrate. Initially, the substrate water content ranged between 110% and 45% (-151.9 kPa to -472.9 kPa). A soil water retention curve (established phychrometrically using a Wescor Hygrometer/Psychrometer with a C-52 Sample Chamber) was used to convert water content to water potential. Small boards partially covered the substrate surface to reduce water evaporation and to provide nesting cover. I attempted to keep the moisture gradient as constant as possible by regularly adding measured amounts of water to the containers to replace the water that evaporated. Because of the higher rate of evaporation from the surface than the bottom, maintaining a constant gradient was difficult, particularly on the high temperature end of the thermal gradient.

Five temperature probes were buried 6 cm below the surface (approximate nest depth) at equal distances (34.5 cm) through the center of the array, perpendicular to the moisture gradient. Temperatures were monitored every hour over a total of 12 days during the experiment (4 days each at the beginning, middle, and end). Mean temperatures at 6 cm depth between 0800 and 1800 h ranged from 22.3 to 32.1 °C, and increased linearly from the cool end to the warm end of the gradient during the day (Figure 1.1). The mean temperature range along the gradient was narrow during early and late hours, and wide during mid-day (Figure 1.2). Thus, lizards had a broader range of nesting temperatures to choose from during mid-day than early or late hours. The overall range in temperature between 0800 and 1800 h was 21.1 to 37.0 °C. Night-time (1900 - 0700 h) mean temperature (21.1 °C) throughout the gradient was slightly lower than the minimum mean (0800 - 1800 h) temperature (22.3 °C). I regularly took substrate samples and measured the water content of each nesting container. Overall, water potential of the nesting substrate ranged between -50 kPa and -2000 kPa at 6 cm depth. A gradient from the surface to the bottom of the substrate provided additional variation in moisture.

Females were closely monitored for nesting activity from a blind in front of the enclosure to minimize disturbance to the females. If females exhibited nesting behavior (i.e. digging in the substrate), they were watched until I was sure that the females were in fact nesting. After females covered their nests, they were captured, weighed, measured, and eventually released at their location of capture. I carefully dug up each clutch immediately after oviposition, removed the eggs and

incubated them in vermiculite for another experiment (see Chapter 2). I measured the depth of each nest and took four substrate samples from the nest. The substrate samples were weighed, dried, and weighed again. The difference between these values provided the soil water content for each sample. Soil water content was converted to water potential using the soil water retention curve. Hobo temperature loggers placed in empty nest cavities monitored hourly temperatures for 48 hours after oviposition. The range of temperature available was calculated as the difference between maximum and minimum temperatures at the time of oviposition.

RESULTS

Moisture and temperature data were obtained for 17 nests because eight of 28 lizards oviposited on the surface of the nesting substrate, and 3 other lizards were injected with oxytocin to induce oviposition. Females that oviposited on the surface were in captivity (mean=27.5 days) longer than females that constructed nests (mean=16.8 days) ($F_{1,23}$=7.4, P=0.012; one-way ANOVA). Individuals that oviposited on the surface presumably retained their eggs longer prior to oviposition than females that made nests. For the 17 nests, oviposition dates ranged from 31 May to 1 July, 1999. Nests (88.2%) were concentrated between -650 and -50 kPa; only two nests (11.8%) were placed in drier sites (<-900 kPa) (Figure 1.3). Mean nest temperatures (based on day-time temperatures; 0800 - 1800 h) ranged from 25.1 to 31.9 °C (Figure 1.3). Mean nest temperatures for 48 hours after oviposition ranged from 23.8 to 28.2 °C. Most females nested at times (1000 - 1500) when a relatively wide range of temperatures was available (Figure 1.2). However, the range of temperatures available was not related to mean nest temperature (Figure 1.4).

In order to determine if nest or nesting characteristics were interrelated, I examined pair-wise correlation coefficients for all nest features (Table 1.1, means are reported in Table 1.2). Correlation analyses with time of day as one of the variables were corrected for declining temperatures after 1400 h; females that nested near 1400 and 1600 h were assigned times of 1300 and 1000 h, respectively. I found no significant correlation coefficients among nest and nesting characteristics after making Bonferroni adjustments. Mean nest temperature was not related to the variance in nest temperature or the time that females nested, nest depth was not related to water potential or mean temperature of the nest, and the number of days females were kept in lab prior to nesting was not related to nesting date.

DISCUSSION

Many studies provide evidence that female reptiles choose quite specific nest locations (Burger and Zappalorti, 1986; Bull et al., 1988; Plummer and Snell, 1988; Rauch, 1988; Burger, 1993; Wilson, 1998). In this study, most females chose nest sites with conditions within the range suitable for successful embryonic development. For *S. undulatus*, hatching success is high (>70%) at water potentials between -150 and -530 kPa, and hatching success is reduced (50%) at -590 kPa (Chapter 2; Tracy, 1980). Hatching success is high (>70%) at constant temperatures between 23 and 33 °C, and hatching success (0 - 28%) is reduced at 34 °C and above (Sexton and Marion 1974; Andrews et al., 2000; Angilletta et al., 2000). I found that most females placed nests within a moisture range of -95 to -604 kPa (Figure 1.3). However, two females deposited eggs in dry substrate (<-900 kPa), and those eggs would have desiccated if left in place. Females chose nest sites with day-time (0800 - 1800 h) mean temperatures (25.1 - 31.9 °C) and overall mean nest temperatures (23.8 to 28.2 °C) that are within the range suitable for embryonic development. In the field, one nest had a mean temperature of 27.0 °C, also within the range of those selected by females in this study (Andrews et al., 2000).

Nest site moisture and temperature may play an important role in determining hatchling phenotype and fitness. However, the nest water potential that most females selected ranged from -95 kPa to -604 kPa, and nests appeared to be randomly distributed within this range. For *Sceloporus undulatus*, while these moisture extremes produce variation in hatchling body size, the biological significance of this effect is questionable because variation in body size and developmental rate is small (Tracy, 1980; Chapter 2). Moreover, females chose sites with mean temperatures that ranged from 23.8 to 28.2 °C. Temperatures within this range have little effect on hatchling body size and growth, but have a large (about 1 month) effect on the rate of embryonic development (Andrews et al., 2000; Angilletta et al., 2000). To ensure hatching success, females should choose sites with mean temperatures that range from 23 to 33 °C. However, most females in this study selected sites with mean temperatures in the middle of this range, perhaps because high mean temperatures have high variance, and low mean temperatures would subject embryos to extremely long incubation periods. High temperature variance would expose embryos to lethal temperatures during part of the incubation period, and low mean incubation temperatures would cause hatchlings to emerge late in the season when competition is high (Ferguson and Bohlen, 1978).

Observations during this study suggested that nest site choice was based on the female's familiarity with the thermal regime of the gradient prior to oviposition. Females that nested early or

late did not have as wide a range of temperatures cues as females that nested during mid-day. Many females nested at times when a low range of temperatures was available, and these females still chose sites with mean temperatures that were similar to mean temperatures of sites chosen by females that nested when a wide range of temperatures were available (Figure 1.4).

Investigating nest site selection in the laboratory has advantages over field studies. First, under laboratory conditions I was able to closely monitor available nesting conditions. This would have been extremely difficult to do under field conditions. Second, measuring water potential of natural nests is particularly difficult because of the variety of substrate types found among nests and the difficulty of making accurate measurements (Packard and Packard, 1988; Packard et al., 1992). Third, locating *Sceloporus undulatus* nests in the field is next to impossible; I found only three natural nests over three years. On the other hand, data on natural nests in the field, when available, provides the best assessment of conditions that female actually select (Shine and Harlow, 1996; Shine et al., 1997a, 1997b; Qualls and Shine, 1998; Cagle et al. 1993 Packard et al., 1993).

A disadvantage of laboratory studies of nest site selection is that conditions may be perceived as abnormal or stressful to females and they may behave differently than under natural conditions. For example, stress may provide an explanation for the lack of nesting behavior by eleven of the 28 females in this study. *Sceloporus undulatus* can retain eggs for about 10 days beyond the normal time of oviposition, and oviposition occurs when embryos reach stage 28-30 (Andrews and Mathies, 2000). In the present study, females that did not make nests oviposited about 10 days later than those that nested normally. These females apparently postponed oviposition as long as it was physiologically possible. In the field, even short periods of egg retention may allow females to delay oviposition until suitable nesting sites are available (Andrews and Rose, 1994).

Females may choose nest sites based on other factors besides substrate temperature and moisture. Perhaps a female's priority is to choose a site that conceals the nest from predators, such as placing nests near or under vegetation (Wilson, 1998). Nest site selection could also be influenced by the timing of reproduction. For example, at the time of reproduction there could be a tradeoff between water availability for eggs and energy needs for hatchlings (Snell and Tracy, 1985). To fully understand maternal nesting behavior, many factors should be considered. Nevertheless, this study provided further evidence that substrate temperature and moisture conditions are important factors that females use when choosing a nest site.

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TABLE 1.1. Pearson correlation coefficients for nest and nesting characterstics (n=15). Females that oviposited on the surface were deleted from this analysis, as were the two outliers with water potentials <-900 kPa. No correlation coefficient was significant after making Bonferroni adjustments. Female in lab = number of days a female was kept in captivity before nesting, Date = julian day of nesting, Time = time (h) of nesting, Nest depth was measured in cm, Nest water potential was measured in kilopascals, Temp. (mean) = mean nest temperature over 48 h after oviposition, and Temp (std. dev.) = standard deviation of nest temperature measured over 48 h after oviposition.

	Date	Time	Nest depth	Nest water potential	Temp. (mean)	Temp. (std. dev.)
Female in lab	-0.352	0.078	-0.187	0.092	-0.347	-0.292
Date	-	0.481	-0.264	-0.123	0.415	0.268
Time		-	-0.031	0.073	-0.103	-0.412
Nest depth			-	-0.129	-0.035	-0.266
Water potential				-	0.295	-0.090
Temp. (mean)					-	0.625

TABLE 1.2. Descriptive statistics for mean nest temperature, temperature variance (std. dev.), water potential, and depth. The two outliers with water potentials <-900 kPa were deleted from the analyses. Nest temperature is based on hourly measurements over 48 h after ovipostion.

	N	Mean	Standard Deviation	Minimum	Maximum
Temperature (°C)	15	25.8	1.3	23.8	28.2
Temperature (std. dev.)	15	2.3	1.1	1.3	4.5
Water potiential (kPa)	15	-377.6	168.7	-604.0	-95.4
Nest Depth (cm)	15	7.3	1.9	4.5	10

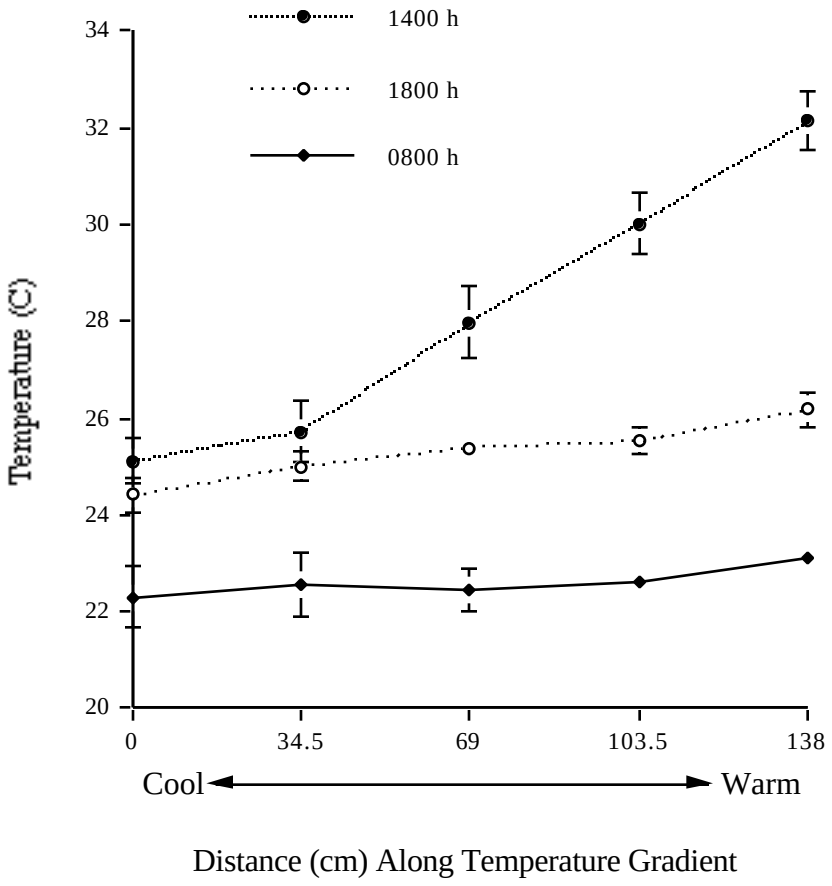

FIGURE 1.1. Temperature profile along the gradient at 0800, 1400, and 1800 h.

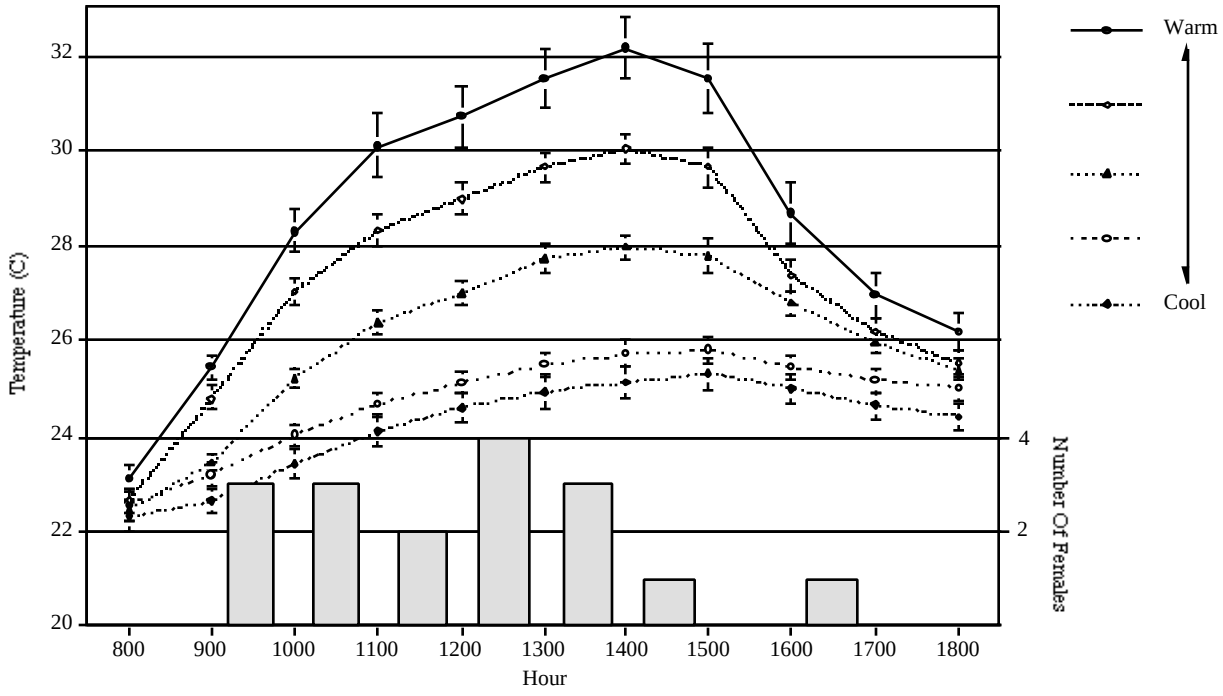

FIGURE 1.2. Mean hourly temperatures at 6 cm depth for the temperature gradient as a function of time of day. Vertical bars represent 1 standard error. The right vertical axis represents the number of females that nested during one hour intervals.

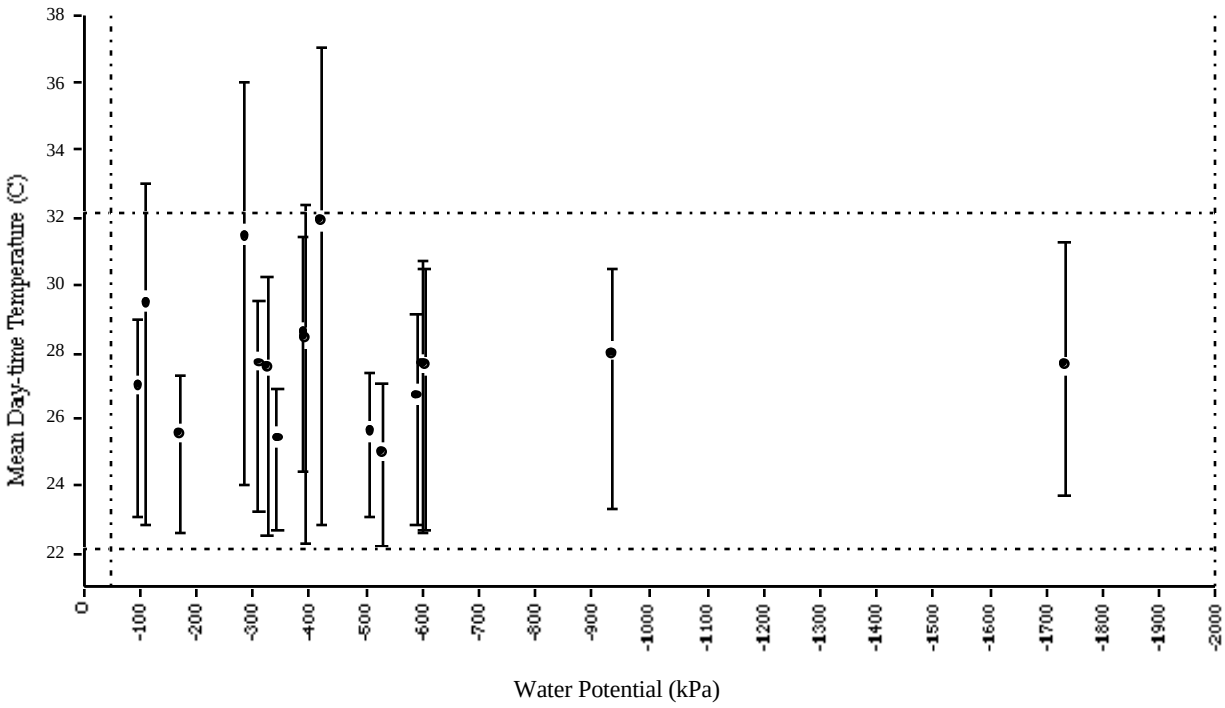

FIGURE 1.3. Mean day-time temperatures and water potentials of selected nest sites. Bars represent extremes in nest temperature during day-time hours (0800 - 1800 h). Dotted lines represent the range of mean temperatures and water potentials that was available.

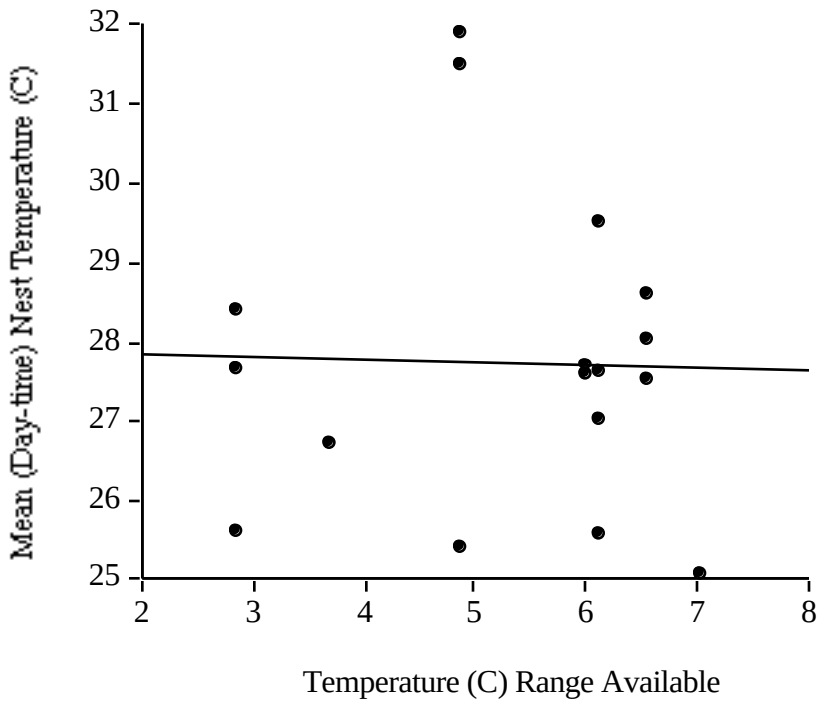

FIGURE 1.4. Relationship between mean nest temperature (based on day-time temperatures; 0800 - 1800 h) and temperature range available (r^2=-0.030, P=0.908).

CHAPTER 2

SOURCES OF VARIATION IN PHENOTYPES AND SURVIVAL OF HATCHLING LIZARDS (*SCELOPORUS UNDULATUS*)

ABSTRACT

To understand the process of natural selection, the relationship between phenotype and fitness and the sources of phenotypic variation must be known. I examined the relative importance of incubation moisture conditions, maternal yolk investment, and clutch to phenotypic variation in hatchlings of the lizard *Sceloporus undulatus.* Eggs were distributed between two moisture treatments (wet [-150 kPa] and dry [-530 kPa]) to determine the effect of incubation moisture on hatchling phenotypes. In another treatment, yolk was removed from eggs to evaluate the effect of yolk quantity on hatchling phenotypes. After hatching, mass, snout-vent length, tail length, body shape, thermal preference, running speed, desiccation rate, and growth rate were measured for each hatchling in the laboratory. Hatchlings were then marked and released at a field site in order to monitor growth and survival prior to winter and the following spring. Incubation moisture and yolk removal affected hatchling body size; individuals from the dry and yolk removed treatments were smaller than those from the wet treatment. However, clutch was the most important source of phenotypic variation; all phenotypes were affected by clutch. Significant clutch effects suggest that phenotypic variation has at least some genetic basis. In the field, survival was not affected by incubation moisture or yolk removal, and survival was not associated with hatchling body size. Survivors and nonsurvivors in the field differed only in growth rate and running speed. Survivors grew more slowly and ran faster than nonsurvivors. To examine the association of clutch with survival, I used clutch mean values to look at the relationship between phenotype and survival. Clutches that produced relatively slow growing individuals and fast runners had higher survival rates than clutches that produced relatively rapid growing individuals and slow runners. In order to grow rapidly, an individual must eat more than slowly growing individuals. Thus, rapid growth rate may increase risk of predation through its association with foraging activity. Individuals that run fast should be capable of capturing prey and evading predators more effectively than individuals that run slowly. Overall, these results emphasize the importance of clutch to variation in phenotypes and survival in hatchling *Sceloporus undulatus.*

INTRODUCTION

The relationship between phenotype and fitness is critical for evaluation of the mechanisms by which natural selection acts. Because natural selection acts on phenotypes, variation in phenotype reflects variation in fitness (Arnold, 1983; Garland and Losos, 1994). However, to evaluate selection, both direct and indirect influences of phenotype on fitness as well as the influence of one phenotype on another must be assessed. Moreover, to fully understand the processes of natural selection, the sources of phenotypic variation must also be known (Figure 2.1). If phenotypic variation has a genetic basis, then selection on phenotypes will affect allelic frequencies within a population and allow for genotypic adaptation to local environments. On the other hand, if phenotypic variation is induced entirely by environmental variation (phenotypic plasticity) the potential for long-term adaptations to evolve is less than if variation is genetically based. However, phenotypic plasticity itself has a genetic basis (environment × genotype interaction); i.e. the way an organism responds to its environment is under genetic control. Therefore, phenotypic plasticity can evolve like any other genetically based trait.

Phenotypes of hatchling reptiles are affected by environmental conditions experienced by the embryo during incubation. Variation in temperature during incubation affects such features as body mass and proportions, sex, growth rate, selected body temperature, locomotor performance, and the ability to detect or escape predators (Burger et al., 1987; Janzen and Paukstis, 1991; Van Damme et al., 1992; McKnight and Gutzke, 1993; Shine, 1995; Shine and Harlow, 1996; Elphick and Shine, 1998; Qualls and Andrews, 1999; Downes and Shine, 1999). Because temperature affects the rate of development and thus the length of incubation, variation in incubation temperature results in commensurate variation in the time of hatching. Hatchlings may, thus encounter different environments depending on when they hatch. For instance, resource competition may be higher late in the hatching season than earlier (Ferguson and Bohlen, 1978).

Moisture availability during incubation is the major factor responsible for changes in mass of flexible shelled reptile eggs (Packard et al., 1980a, 1980b; Tracy, 1980). Eggs absorb substantial amounts of water during the course of incubation depending upon hydric conditions of the nest. Relatively large hatchlings result from incubation in moist environments and relatively small hatchlings result from incubation in dry environments (Gutzke and Packard, 1987; Packard and Packard, 1988; Phillips et al., 1990; Cagle et al., 1993; Janzen et al., 1995). The association between small bodied hatchlings and low moisture is possibly due to inefficient yolk intake at low water potentials; more unabsorbed yolk remains in the shell under dry than wet conditions (Packard et al., 1980b; Packard et al., 1988; Janzen et al., 1990). In contrast to variation in temperature,

variation in moisture has only a negligible effect on length of incubation and thus time of hatching (Gutzke et al., 1987; Miller et al., 1987; Phillips et al., 1990; Overall, 1994). One study suggests that large hatchlings of *Sceloporus virgatus* from well hydrated eggs have greater survival in the field than the small hatchlings from poorly hydrated eggs (Vleck, 1988). Moreover, larger bodied hatchlings typically run faster and thus may be better at evading predators or capturing prey than smaller hatchlings (Sinervo and Adolph, 1989; Sinervo, 1990). Moisture conditions during incubation can also affect the subsequent growth rate of hatchling lizards (Overall, 1994).

Hatchling phenotypes are also influenced by the nutritional contribution of the maternal parent, that is, the quantity or quality of yolk. Yolk investment varies seasonally and annually in association with resource availability (Ballinger, 1977) and variation in egg size is associated with hatchling size (Gutzke et al., 1987; Sinervo, 1990; Brooks et al., 1991; Packard et al., 1987). Experimental manipulations of egg size through removal of yolk have shown that egg size directly affects offspring body size. Eggs with more yolk produce larger hatchlings than eggs with less yolk, and hatchling size affects other phenotypic traits, such as locomotor performance (Sinervo, 1990; Sinervo and Huey, 1990; Sinervo et al., 1992; Sinervo and Doughty, 1996). Yolk investment may directly or indirectly affect survivorship of hatchlings, at least in some years (Sinervo et al., 1992). Yolk quality (caloric and nutrient content) may also affect hatchling phenotypes, but this aspect of maternal provisioning has not been studied in reptiles.

Finally, hatchling phenotypes are affected by the genetic contributions of their parents (Van Berkum and Tsuji, 1987; Olsson et al., 1996). Typically, however, studies on the impact of environmental conditions on hatchling phenotypes attempt to remove the effect of genotype through experimental designs that randomly allocate eggs to treatments (Packard et al., 1985; Miller et al., 1987; Gutzke and Packard, 1987; Packard et al., 1987, 1988). As a consequence, the relative contribution of genotype to hatchling phenotypes is poorly known. Studies that have included clutch (as a surrogate for genotype) in their experimental design have found that this factor contributes substantially to phenotypic variation (Van Berkum and Tsuji, 1987; Brooks et al., 1991; Bobyn and Brooks, 1994; Janzen et al., 1995; Rhen and Lang, 1995; Shine et al., 1997; Sorci and Clobert, 1997; Madsen and Shine, 1998; Andrews et al., 2000). However, differences among clutches can reflect strictly maternal effects (e.g. quality or quantity of egg provisioning) as well as genetic effects.

The two main objectives of this study were to (1) explore the effects of incubation moisture, maternal yolk investment, and clutch on phenotypes of hatchling lizards and (2) to determine the relationship between phenotype and survival when hatchlings are released in the field. Based on

previous studies, I anticipated that hatchlings from eggs incubated under wet conditions would be larger in body size than those from dry conditions (Gutzke et al., 1987; Packard and Packard, 1988; Janzen, 1993). Hatchlings from eggs with relatively large quantities of yolk should be larger than those with smaller amounts of yolk (Sinervo, 1990). In either case, larger bodied individuals should run faster than smaller bodied individuals and have higher survival as well (Ferguson and Fox, 1984; Miller et al., 1987; Sinervo and Adolf, 1989; Sinervo et al., 1992; Janzen, 1993; Sorci and Clobert, 1999). I also predicted that clutch should be an important source of phenotypic variation (Van Berkum and Tsuji, 1987; Brooks et al., 1991; Janzen et al, 1995; Shine et al., 1997; Madsen and Shine; 1998; Andrews et al., 2000) and should be linked to survival in the field. Significant clutch effects would suggest at least some genetic contribution to phenotypic variation.

I also addressed three additional questions concerning variation in offspring phenotypes and survival. First, is body size related to growth rate, performance, or survival independent of incubation moisture, yolk investment, or clutch? Second, if body size is correlated with other phenotypic traits, does variance in moisture during incubation or yolk quantity affect these traits and survival independent of body size *per se*? Third, does variation in incubation moisture have a similar affect on phenotypes and survival as does variation in maternal yolk investment?

I used the eastern fence lizard, *Sceloporus undulatus*, to investigate the above hypotheses and questions. *Sceloporus undulatus* is an ideal organism to work with for several reasons. First, the life history and demography of *Sceloporus undulatus* are well known (Tinkle, 1972; Tinkle and Ballinger, 1972; Ferguson and Brockman, 1980; Ferguson, et al., 1980, 1982, 1983; Jones et al., 1987; Ferguson and Talent, 1993; Niewiarowski and Roosenburg, 1993; Niewiarowski, 1995). Second, clutch size (5-16 eggs) is relatively large which allows subdivision of single clutches into several experimental treatments. This experimental design allowed me to evaluate whether phenotypes were affected by treatment, whether they differed among clutches, and whether individuals from different clutches responded differently to the treatments. Third, hatchlings of *Sceloporus undulatus* usually do not travel long distances. This aspect of this lizard's natural history means that estimates of survivorship are not strongly confounded by dispersal (Ferguson and Bohlen, 1978; Ferguson et al., 1983; Jones et al., 1987; Niewiarowski and Roosenburg, 1993).

MATERIALS AND METHODS

Collection and Husbandry of Gravid Females

Gravid female *Sceloporus undulatus* were collected between 15 May and 26 June, 1999, on the south face of Brush Mountain near Blacksburg, Virginia (Montgomery County) between 700 and 780 m elevation. Females were housed in a 1.5 x 1.5 m enclosure until oviposition. The enclosure contained tubs of peat moss and vermiculite for nesting, and wood slabs and logs for basking. The enclosure was illuminated by Vita-lites (0700-1800 h) and 100 watt heat lamps (0800-1400 h). See Chapter 1 for a detailed description of the enclosure. Females were fed (crickets and wax worm larvae dusted with a vitamin-mineral mix) and watered daily. The enclosure was checked several times daily for nesting females. After oviposition, females were weighed and measured, and then marked by toe clipping and released at the location of their capture.

Oviposition occurred between 31 May and 5 July. One egg from each clutch was sampled in order to determine the stage of the embryo (staged according to Dufaure and Hubert, 1961). Embryonic stage at oviposition (mean = 29; range: 28-31) was relatively uniform among clutches. The remaining eggs from each clutch were distributed evenly among experimental treatments.

Experimental Design and Protocols

Egg incubation: Eggs were incubated at a constant 28 °C in a single environmental chamber. The four experimental treatments were incubation at: 1) high moisture (-150 kPa, wet treatment), 2) low moisture (-530 kPa, dry treatment), 3) high moisture for eggs that had yolk removed (yolk removed treatment), and 4) high moisture for eggs that were punctured as a control for the yolk removed treatment (punctured treatment).

The moisture treatments were selected to represent extreme moisture regimes of nests that result in high egg survival (Rose, 1993), and produce hatchlings that differ in body size (Vleck, 1991). These moisture conditions were also near the extremes that female *Sceloporus undulatus* select for nesting in the laboratory (see Chapter 1). Water potential was determined from a soil water retention curve established by a Wescor Soil Hygrometer/Psychrometer equipped with a C-52 Sample Chamber. For the yolk removed treatment, yolk was withdrawn from eggs with a 1.0 26G sterile syringe. As result of yolk removal, egg mass was reduced by an average of 22% (std. dev. = 7%).

Eggs were placed individually in 65 ml glass jars containing vermiculite and covered with clear plastic wrap sealed with a rubberband. Eggs were completely buried in vermiculite throughout

incubation. Racks with the jars were rotated to different positions within the chamber three times a week to eliminate effects due to possible temperature gradients within the environmental chamber. In order to monitor water uptake, eggs were weighed regularly throughout incubation. Eggs incubated at -150 kPa were weighed once per week. Eggs in the dry treatment (-530 kPa) were weighed three times per week to ensure that eggs did not experience negative water balance. Vermiculite was changed if these eggs decreased in mass. For all other eggs, vermiculite was changed at approximately half way through incubation.

Morphology and husbandry of hatchlings: Environmental chambers were checked twice daily for hatchlings. Hatching occurred between 15 July and 29 August. After hatching, I recorded the date, mass, snout-vent length (SVL), tail length (TL), and sex and gave each hatchling a unique toe clip for identification.

Hatchlings were housed in plastic containers (46 long x 24 wide x 20 deep cm) with sand substrate and small boards for basking and retreat sites. Each container was illuminated by Vita-lites (0700-1800 h), and heat was provided by 100 watt overhead heat lamps (0800-1400 h) placed at one end of each container. Each container housed 10 to 15 hatchlings. Hatchlings were fed crickets, wax moth larvae and flour beetle larvae (dusted with a vitamin-mineral mix) and provided with water twice a day. Hatchlings were not fed on the day performance traits were measured (see below). Hatchlings were maintained under these conditions for an average of 9 days (std. dev. = 2.2) before they were released.

Measurements of hatchling performance traits: Body temperature selected by hatchlings was measured at one to two days after hatching on a thigmo-thermal gradient. For a detailed description of the thermal gradient see Qualls and Andrews (1999). Nine thermocouples spaced at 10 cm intervals and located 5 mm above the floor of the gradient were used to record air temperatures along the length of the gradient. Air temperatures ranged from 26 to 40 °C, and varied linearly over the length of the gradient. Body temperature was assumed to be that of the air temperature at the position of the lizard within the gradient. This assumption was tested by placing freshly killed hatchlings on the gradient near a thermocouple and measuring body temperature (via a fine thermocouple inserted into the cloaca and taped in place) and air temperature. Temperatures were measured at 16 positions on the gradient. Measurements were made after 10 minutes of acclimation. Hatchling body temperature was closely related to air temperature (slope=0.99, r^2=0.97, P<0.001). This indirect method of measuring body temperature was used because temperatures of small lizards change rapidly. Direct measurement via a cloacal thermometer takes too long for accurate measurement (Andrews, 1994).

Hatchlings were placed individually in a lane of the gradient one-half hour for acclimation to the gradient before their positions were recorded. The position of a hatchling was then recorded every 10 minutes for one hour. Air temperatures along the gradient were recorded at the beginning, middle, and end of the hour. Position was converted to temperature using regression analysis (see Qualls and Andrews 1999).

Running speed at three to four days of age was measured by placing hatchlings in a 1 m long electronically timed racetrack (see Qualls and Andrews, 1999). Five infrared photocells (connected to an electronic stopwatch) were spaced at 25 cm intervals along the length of the track. The racetrack was in a walk-in environmental chamber set at a constant 30 °C. Hatchlings were given a half hour to acclimate in the environmental chamber before their speed was measured. Hatchlings were placed at the beginning of the track and prodded gently with a paintbrush if they did not run. Running performance was measured 3 times for each individual with at least a two minute resting period between trials. Running speed was recorded as the time (in seconds) it took hatchlings to cover each 25 cm interval and 1 m distance.

Desiccation tolerance was measured at six to seven days of age as short-term evaporative water loss. Body mass was recorded before hatchlings were placed in a desiccator at 0% relative humidity and a constant 30 °C and was recorded again two hours later. If hatchlings defecated while in the desiccator, their desiccation rate was measured the following day. After two hours in the desiccator, lizards were returned to their holding tubs and given food and water.

Mass, SVL, and tail length of each hatchling were remeasured prior to release to obtain data on growth and body proportions.

Release and Recapture of Hatchlings in the Field

The release site included a 1500 m^2 forest clearing that was originally used as a logging deck (surrounded on two sides by a gravel road), a smaller clearing (~500 m^2) immediately across the gravel road, and an abandoned dirt road (~80 m) extending from the northwest end of the site. These open areas were characterized by large piles of woody debris, tree stumps, fallen logs, and scattered small shrubs, which provided suitable habitat for hatchlings. These areas were surrounded by dense forest which acted as a natural boundary because hatchling *Sceloporus undulatus* typically do not disperse through the forest (personal observation; J. Matter, personal communication).

Release dates ranged from 24 July to 8 September. All 220 hatchlings were released on the same wood pile in the large clearing. I searched the study area and the periphery of the forest for hatchlings about twice weekly from 31 July to 5 December, 1999 and weekly from 5 March to 6 July, 2000. The perimeter of the release area was searched thoroughly, and few hatchlings were found in the forest surrounding the field site. In fact, hatchlings were not seen in the forest until 28 October, 1999 (after leaf fall). Altogether, only 19 of 296 (both field and laboratory) individuals were found in the forest. Only one of 49 individuals released in 1999 that were captured in 2000 had not been seen since release, and most of these 49 individuals were recaptured numerous times in 2000 suggesting that few lizards were overlooked. After recapture, hatchlings were identified by their toe clip, weighed, and measured (SVL and TL) and then released where they were captured. Location of capture was recorded to obtain data on movement.

I assumed the disappearances of hatchlings from the field site were due to death rather than dispersal. This assumption was supported by the recapture rates of *S. undulatus* hatchlings in an enclosed area where dispersal was not possible (Niewiarowski and Roosenburg, 1993). Their recapture rate of about 50% at 6 weeks after release was very similar to my recapture rate at six weeks (45%, see Results). Moreover, when Wilson (1991) factored emigration rates into survival analyses for *Uta stansburiana*, emigration was so rare that survival estimates were not biased by off-site captures. Weather conditions were not extreme during this study (see below), and insect abundance appeared to be high. Thus, dehydration and starvation were unlikely causes of death. Dead lizards were never found at the study site. Potential predators observed at the site included blue jays (*Cyanocitta cristata*), American crows (*Corvus brachyrhynchos*), rufous-sided towhees (*Pipilo erythrophthalmus*), copperheads (*Agkstrodon contortrix*), black rat snakes (*Elaphe obseleta*), and large mantids and spiders.

Individuals that hatched on the site were also captured, measured, and toe-clipped. A total of 76 field hatched individuals was captured; 42 of these that were similar in size to the laboratory reared hatchlings at the time of release were brought back to the laboratory for measurement of performance traits (thermal preference, running performance, and desiccation rate were measured as described above for the laboratory hatchlings). Individuals brought back to the laboratory were collected between 11 August and 7 October, 1999. They were maintained in the laboratory for an average of 7.9 days (std. dev. = 2.3) before they were released at their location of capture. These individuals were used as natural controls for the laboratory reared hatchlings.

Temperature and Rainfall at the Study Site

Two rain gauges and four Hobo temperature loggers were placed at the field site during the study period. Two temperature loggers were placed in open areas and two were in areas shaded by trees. These temperature loggers monitored shaded air temperatures 15 cm above the surface of the ground. The rain gauges were placed in the open at either end of the large clearing. Rain gauges and temperature loggers were removed from the field site on 5 December, 1999. Long-term weather data were provided from the Blacksburg Airport (located 11 km from the field site at an elevation of 700 m).

Temperature and rainfall during the study was similar to past years. Mean monthly temperatures at the field site were 20.3, 17.5, 11.9, 9.9 °C for August, September, October, and November, respectively. Rainfall during these months was 77.5, 175.5, 47.4. and 20.5 mm, respectively. Thirty year (1961-1990) mean temperatures for these respective months were 20.8, 17.2, 11.0, and 6.2 °C. Mean rainfall for these months was 95.7, 89.1, 92.2, and 73.4 mm.

Data Manipulation and Analysis

Laboratory data: Puncture and sex effects on hatchling phenotypes were evaluated with two-way analysis of variance (ANOVA) and covariance (ANCOVA) (with sex and treatment or clutch as class variables). These analyses indicated that puncturing eggs had no effect on any morphological or performance traits. Therefore, hatchlings from the punctured treatment were combined with those from the wet treatment. Preliminary analyses also indicated that sex did not affect hatchling morphology, performance, or post-hatching survival. Sex was, therefore, not considered in further analyses. See Appendices A, B, and C for a summary of these analyses.

Comparisons of relative egg mass (mass of the egg relative to its mass at oviposition after yolk removal for the yolk removed treatment) among the dry, wet, and yolk removed treatments at 10, 20, 30, and 40 days of incubation were made with four separate one-way ANOVA's. Repeated measures analysis was not appropriate because hatching time differed among clutches. Thus, measurements of eggs from all clutches did not overlap perfectly at each time period. A posteriori comparisons among treatments were made with Ryan-Eliot-Gabriel-Welsch multiple range tests. Sample sizes were too small for valid comparisons at day 50 because eggs had started to hatch by this time.

The effects of incubation moisture, yolk removal, and clutch (class variables) on incubation period, morphology and performance (dependent variables) were assessed using ANOVA and ANCOVA. Two separate contrasts were made; hatchlings from the dry treatment (-530 kPa) were

compared with those from the wet treatment (-150 kPa), and hatchlings from the yolk removed treatment (-150 kPa) were compared with those from the wet treatment (-150 kPa). Because of egg mortality or small clutch sizes, certain clutch/treatment combinations contained one or no hatchlings. Therefore, these particular clutches were removed from the analyses. Egg mass was used as a covariate in analyses of the effect of treatment and clutch on incubation period and hatchling body size (mass and SVL). The effects of treatment and clutch on tail length were evaluated using SVL at hatching as a covariate. Body shape (mass relative to SVL) at hatching was $mass^{0.3}/SVL$. Mass was raised to the 0.3 power to adjust for the non-linear relationship between mass and length (Andrews, 1982). Thermal preference was evaluated as the mean body temperature over a one hour period for individual lizards. Standard deviations of the six temperature observations for each hatchling were used as an index of thermoregulatory precision and frequency of shuttling. Running performance was assessed from the fastest speed (m/s) recorded for each individual over 25 cm and the entire 1 m interval. The effects of treatment and clutch on running speed were analyzed both with a covariate (SVL) and without a covariate. Treatment and clutch effects on desiccation rate over two hours were assessed with body mass as a covariate. Growth in the laboratory in both SVL and mass was calculated as a size specific growth rate; the difference between an individual's natural log transformed SVL or mass at the time of release and hatching divided by the number of days between measurements.

For all ANCOVA's, if the interaction of the main effects with the covariate was not significant ($P>0.05$), the analysis was rerun without this interaction term in the model. The interaction between the two main effects was always kept in the model. For all phenotypes that were adjusted by using a covariate (ANCOVA), mean values were reported as least squares means. All statistical analyses were conducted with SAS software (SAS institute, Inc., Vers. 6.12, 1997).

Field Data: The effects of treatment (either incubation moisture or yolk removal), clutch, and their interaction on growth rate in the field were analyzed as described for growth rate in the laboratory. Growth in the field was measured as the difference between an individual's natural log transformed SVL or mass at the last recapture prior to winter and the time of release divided by the number of days between measurements.

Performance traits of 42 field incubated hatchlings were compared to those of laboratory incubated hatchlings. Because incubation conditions that field hatchlings experienced were unknown, I combined laboratory individuals from the wet and the dry treatment to compare with field individuals. Because the clutch of origin for the field hatchlings was unknown, I used individual values rather than clutch means. Statistical analyses for thermal preference, running

performance, desiccation rate, and growth (in the laboratory and field) for the field hatchlings are the same as those described above for the laboratory hatchlings. I did not compare morphological traits because the morphology of field individuals at hatching was unknown.

Hatchling movement was analyzed in two different ways. For hatchlings, the distance traveled was measured from the location of release (laboratory hatchlings) or first capture (field hatchlings) to the location of last capture (displacement). Total distance traveled was measured as the sum of distances between successive captures. The number of days between release (laboratory hatchlings) or first capture (field hatchlings) and last capture was used as a covariate when analyzing treatment and clutch differences in displacement and total distance traveled. Movement was only analyzed prior to winter.

Survival of laboratory reared hatchlings was determined for three time periods beyond release date; survival up to 6 weeks, to 12 weeks, and to the following March, 2000; individuals captured at any time period were assumed alive at previous time periods. Chi-square tests were used to determine the association between survival at each of these periods with treatment (moisture and yolk removal) and clutch. Only clutches with eight or more hatchlings were used in these analyses. Survival of field hatchlings was compared with that of laboratory hatchlings. The first date of capture for field hatchlings was considered equivalent to the day of release for laboratory hatchlings.

Hatching in the laboratory, and the date of release, occurred over approximately a month and a half. As a consequence, survival could have been affected by date of release. Therefore, I divided the release dates into three ranges: early (24 July - 7 August), middle (8 August - 22 August), and late (23 August - 8 September). Chi-square tests used to determine the association between release date and survival were not significant (all P's > 0.09; see Appendix D).

In order to assess the overall association between phenotype and fitness, laboratory hatchlings were divided into two groups for each time period: the groups were (1) individuals that survived to 6, and 12 weeks, and up to March and (2) individuals that did not survive to these dates. Separate ANOVA and ANCOVA for each phenotypic trait at each time period were used to contrast survivors and nonsurvivors, irrespective of treatment and clutch. For growth in the field, the following convention was used to reduce potential bias associated with comparing individuals of different ages. Individual growth rates measured within the first 3 weeks after release over intervals of at least 15 days were used in the analyses. Therefore, analyses did not include data for individuals that were recaptured for their first time after 3 weeks following release.

In order to assess the genetic (clutch) contribution to fitness, the relationship between percent survival for each clutch and clutch means for phenotypic traits was assessed with correlation analysis (PROC CORR in SAS). To reduce bias introduced by small sample size, only clutches with eight or more hatchlings and with two or more hatchlings per clutch/treatment combination were used in this analysis (n = 16 clutches). For growth rate in the field, only clutches with growth data for five or more hatchlings were used (n = 8 clutches).

RESULTS

Treatment and Clutch Effects on Phenotypes and Survival

Water uptake, egg survival, and incubation length: All eggs increased in mass throughout incubation. However, the relative amount of water uptake differed among incubation treatments (all P's<0.001; Figure 2.2). Eggs from the yolk removed treatment increased in mass at the fastest rate and by day 40 were 4.2 times heavier than their mass at oviposition. Yolk removal presumably reduced pressure within the egg, thus allowing relatively fast water uptake. Eggs from the wet treatment increased in mass more slowly than those from the yolk removed treatment, and eggs were 3.5 times heavier by day 40 than their mass at oviposition. Eggs from the dry treatment increased in mass relatively slowly, and by day 40 were only 2.8 times heavier than their mass at oviposition.

Egg survival did not differ between the dry and wet treatments (χ^2=0.4, df=1, P=0.527) or between the yolk removed and wet treatments (χ^2=1.0, df=1, P=0.317). Overall, 222 of 269 eggs hatched (82.5% survival). Egg survival was associated with clutch (χ^2=39.8, df=23, P=0.016). Post hatching survival in the laboratory was not associated with treatment or clutch. Only two of the 222 hatchlings died in the laboratory (99.1% post hatching survival).

Mean incubation length was about 50 days for each treatment, and did not differ between the dry and wet treatments ($F_{1,82}$=2.0, P=0.155) (Tables 2.1 and 2.2) or between the yolk removed and wet treatments ($F_{1,85}$=0.2, P=0.658) (Tables 2.3 and 2.4). However, incubation length differed among clutches for both contrasts (P<0.001).

Developmental abnormalities: Bent tails were common among hatchlings; overall, 36.2% of the hatchlings had tails with slight to strong bends (kinks). However, bent tails were not associated with treatment: incubation moisture (χ^2=0.4, df=1, P=0.530) or yolk removal (χ^2=0.5, df=1,

P=0.469). The frequency of bent tails differed among clutches (χ^2=71.8, df=27, P<0.001). Performance was not affected by bent tails. For example, individuals with bent tails ran at similar speeds as those with normal tails ($F_{1,27}$=0.4, P=0.528), and bent tails were not associated with hatchling survival in the field at any time period (all P's>0.225, see Appendix E). Therefore, individuals with bent tails were left in all analyses except for tail length. No field hatchling had bent tails.

Hatchling morphology: Hatchlings from the dry treatment were smaller in mass and SVL than hatchlings from the wet treatment (Tables 2.1 and 2.2). Treatment differences were small; hatchlings from the dry treatment were only 1.3% shorter in SVL and 2.3% lighter than those from the wet treatment. Differences in hatchling body size did not persist to release 9 days later. The incubation moisture by clutch interaction for body mass at hatching was marginally significant ($F_{20,80}$=1.3, P=0.040), suggesting that the response of embryos to moisture conditions differed among clutches, but this interaction was not significant at release. Relative tail length and body shape at hatching and release did not differ between hatchlings from the dry and wet treatments.

The 22% reduction in egg mass by yolk removal resulted in hatchlings that were 23% lighter and 7% shorter than those without yolk removed (Tables 2.3 and 2.4). Differences in mass and SVL at hatching persisted to the time of release. The yolk removal by clutch interaction for body mass at hatching was significant ($F_{20,84}$=3.2, P<0.001), suggesting that the response of embryos to yolk removal differed among clutches, but this interaction was not significant at release. Hatchlings from the yolk removed treatment had shorter tails than those from the wet treatment and the difference in tail length persisted up to the time of release. Hatchling body shape did not differ between hatchlings from the yolk removed and the wet treatments.

Most of the variation in hatchling morphology was due to clutch, and significant clutch effects persisted to the time of release for all morphological traits (Tables 2.1 and 2.3).

Thermal preference: Thermal preference did not differ between hatchlings from the dry and wet treatments or between hatchlings from the yolk removed and wet treatments (Tables 2.2 and 2.4). The standard deviations associated with mean selected body temperature for hatchlings from the dry treatment (mean std. dev. =1.50) and wet treatment (mean std. dev. = 1.21) did not differ ($F_{1,38}$=3.0, P=0.091) indicating that moisture treatment did not affect shuttling behavior. Similarly, the standard deviations for hatchlings from the yolk removed treatment (mean std. dev. = 1.24) and hatchlings from the wet treatment (mean std. dev. = 1.31) did not differ ($F_{1,40}$=0.2, P=0.623). Mean standard deviations were low overall, suggesting that lizards were efficient at maintaining their

preferred body temperature. Most of the variation in hatchling thermal preference was due to clutch.

Running performance: Absolute and size adjusted running speed over 25 cm and 1 m did not differ between hatchlings from the dry and wet treatments or between hatchlings from the yolk removed and wet treatments. Despite similar speed overall, individuals from the yolk removed treatment stopped more frequently, suggesting that individuals from this treatment actually ran faster than those from the wet treatment. Most of the variation in running speed was due to clutch (Tables 2.1 and 2.3).

Desiccation rate: The rate of water loss did not differ between hatchlings from the dry and wet treatments or between hatchlings from the yolk removed and wet treatments. On average, hatchlings lost 0.9% of their initial body mass after two hours in the desiccator. Most of the variation in the rate of water loss was due to clutch (Tables 2.1 and 2.3).

Growth rate in the laboratory and field: Growth rate in the laboratory and in the field did not differ between hatchlings from the dry and wet treatments or between hatchlings from the yolk removed and wet treatments (Tables 2.1 and 2.3). Clutch was associated with growth rate in the laboratory, but not with growth rate in the field for the moisture treatment contrast. This was likely due to a reduced sample of clutches in this contrast (23 clutches had ≤1 hatchling per clutch/treatment combination and were removed from the analysis). However, clutch was associated with growth rate in the field for the contrast between the yolk removed and wet treatments.

Movement in the field: Displacement and total distance traveled by hatchlings in the field did not differ between individuals from the dry and wet treatments or between individuals from the yolk removed and wet treatments (Tables 2.1 and 2.3). Clutch was not associated with the length of displacement and total distance traveled by hatchlings.

Survival in the field: Survival did not differ between the dry and wet treatments or between the yolk removed and wet treatments at any time period (Table 2.5, Appendix F). In addition, overwinter survival did not differ between the dry and wet treatments (χ^2=0.2, df=1, P=0.620) or between the yolk removed and wet treatments (χ^2=1.0, df=1, P=0.756). The association between clutch and survival was not significant at any time period (Table 2.5). However, at 12 weeks after release, the association between clutch and survival was marginal (P=0.067). Furthermore, survival varied greatly among clutches despite these insignificant results; survival to 6 weeks varied from 11% to 75%, survival to 12 weeks varied from 0% to 67%, and survival to March varied from 0% to 44%.

Contrasts Between Field and Laboratory Hatchlings

Performance, growth, and movement: Most traits did not differ between field and laboratory hatchlings (Table 2.6). Thermal preference did not differ between field hatchlings and laboratory hatchlings. Running speed did not differ between field hatchlings and laboratory hatchlings, except for absolute speed over 1 m. However, laboratory hatchlings stopped more frequently when running over a 1 m distance than did the field hatchlings suggesting that laboratory hatchlings actually ran faster than field hatchlings. Desiccation rate did not differ between field hatchlings and laboratory hatchlings.

Growth rate differed between field and laboratory hatchlings. Under both laboratory conditions and in the field, laboratory hatchlings grew significantly faster than field hatchlings (Table 2.6; Figure 2.3). Displacement did not differ between field and laboratory hatchlings, however, total distance traveled for field hatchlings was greater than that for laboratory hatchlings (Table 2.6).

Survival in the Field: Field hatchlings consistently had higher survival than laboratory hatchlings (Figure 2.4), but differences were only significant at the March time period (χ^2=7.1, df=1, P=0.008); field hatchlings had 31% survival and laboratory hatchlings had 17% survival at this period. In addition, overwinter survival of field hatchlings was greater than that of laboratory hatchlings (χ^2=10.7, df=1, P=0.001). Of the field and laboratory hatchlings that survived to 12 weeks, 100% and 67% survived over winter, respectively.

Survival: Independent of Treatment

Overall contrasts between survivors and nonsurvivors: Growth rate in the field and running speed (over 1 m) were the only traits that differed between survivors and nonsurvivors consistently across all time periods (Table 2.7). Individuals that survived ran faster and grew more slowly than those that did not survive (Figure 2.5). The few other traits that were marginally significant or were not consistently significant across time periods (Table 2.7) were not considered further.

Association between clutch means and survival: The only significant correlations (P<0.05) among clutch means for phenotypic traits (including percent survival) were between survival and growth rate in the field (in SVL and mass) and between survival and running speed (over 25 and 1 m) (Table 2.8, Appendix G). Growth was negatively correlated with survival and running speed was positively correlated with survival (Figure 2.6). Moreover, growth rate and running speed were negatively correlated (r^2=-0.689, P=0.011).

DISCUSSION

Two objectives were addressed in this study. First, this study was designed to determine the relative importance of incubation moisture conditions, maternal yolk investment, and clutch to phenotypic variation in hatchling *Sceloporus undulatus.* I made two main predictions: 1) eggs incubated under dry conditions and those with yolk removed should produce smaller hatchlings than those incubated under relatively wet conditions and without yolk removed. Thus, the effects of these two manipulations to body size (and other phenotypes) should be similar to each another. 2) Clutch should be an important source of phenotypic variation. Significant clutch effects would demonstrate the possibility of a genetic basis for phenotypic variation.

My second objective was to determine the relationship between hatchling phenotypes (and their source of variation) and survival by releasing individuals and monitoring their subsequent growth and survival under field conditions. I predicted that 1) large hatchlings from wet incubation conditions should have higher survival than small individuals from dry incubation conditions and those with reduced yolk. Large bodied individuals should also have high survival independent of incubation treatment or clutch. 2) Traits that are associated with survival should have a significant genetic component (clutch), which allows genotypic adaptations to local environments.

Moisture and Yolk Effects on Phenotypes in the Laboratory

In this study, body size (mass and SVL) was the only trait affected by incubation moisture conditions. As expected, dry incubation conditions resulted in relatively small hatchlings compared to those from wet incubation conditions. However, differences in body size between moisture treatments were small and they did not persist to 9 days after hatching. Furthermore, incubation moisture did not affect hatchling thermal preference, running speed, desiccation rate, or growth rate. Perhaps more extreme (drier) moisture conditions would have increased phenotypic variation. It seems likely, however, that drier conditions would have resulted in high egg mortality. In general, little evidence exists that moisture levels in the incubation substrate translate to significant long-term phenotypic effects on hatchlings (Tracy, 1980; Miller et al., 1987; Brooks et al., 1991; Bobyn and Brooks, 1994).

In contrast to incubation moisture, reduction in the quantity of yolk from eggs had strong effects on hatchling body size, and the effect of yolk removal persisted up to the time of release, probably because yolk removal had a large (23% reduction in body mass) impact on hatchling size. Mass, SVL, and tail length were reduced by yolk removal. No traits were indirectly affected by yolk removal. For example, sprint speed was not related to body size even when speed was not size

corrected. This was not expected because other studies show a significant positive relationship between body size and running speed for size manipulated hatchlings (Sinervo, 1990). In this study, however, it is possible that variation in body size induced by manipulations of incubation moisture and yolk removal was not large enough to have an effect on running speed.

Genetic (Clutch) Effects on Phenotypes in the Laboratory

I found that clutch was the most important source of variation in phenotypes of hatchling *Sceloporus undulatus.* I assumed that the phenotypic differences among clutches has at least some genetic basis for the following reasons. I reduced environmental differences among gravid females, eggs, and hatchlings by maintaining relatively uniform housing conditions. However, females were collected at different stages of gravidity, thus, some were kept in captivity for longer periods before oviposition than others. However, the length of time females were held in captivity was not related to clutch means for any hatchling phenotype (Correlation Coefficients all P's > 0.117; see Appendix H). Egg size at oviposition differed among clutches ($P<0.05$) suggesting inter-female variation in yolk allocation. This maternal effect (yolk allocation) was statistically removed from the analyses by adjusting hatchling body size for initial egg mass at oviposition. Clutch still had strong effects on hatchling body size after this adjustment. In addition, I found significant clutch effects on size adjusted running speed, which suggests a genetic basis for inter-clutch variation in running speed. Furthermore, by removing yolk from some eggs within a clutch, I was able to separate the effect of yolk quantity from the effect of genotype. Variation in hatchling phenotypes was still largely due to clutch, even after removing yolk from eggs. I also found no significant relationship between female morphology (mass, SVL, and shape) and offspring phenotypes (all P's>0.199; see Appendix H). I found little genetic variation in the way embryos responded to their incubation environment as indicated by insignificant clutch × treatment (incubation moisture and yolk removal) interactions. On the other hand, I did not address the possibility of variation in yolk quality (nutritive and caloric content of yolk), and therefore cannot rule out the possibility that yolk quality contributed to some of the variation in hatchling phenotypes.

For selection on particular phenotypes to have evolutionary consequences, variation in those traits must have a genetic component (Arnold, 1986). Several studies indicate that variation in growth, performance, morphology, and even survival has a significant genetic (clutch) component (Van Berkum and Tsuji, 1987; Brooks et al., 1991; Bobyn and Brooks, 1994; Janzen et al, 1995; Rhen and Lang, 1995; Madsen and Shine, 1998; Andrews et al., 2000). Interpretations of such clutch effects should be done cautiously, however, because experimental designs in which hatchlings are maternally related can confound genetic causes with other maternal effects (Arnold, 1986; Van Berkum and Tsuji, 1987). Moreover, the influence of paternal genotype on hatchling

phenotypes (Olsson et al, 1996), and the frequency of multiple paternity within populations are other concerns. For *Sceloporus undulatus*, however, genetic studies indicate that multiple paternity is uncommon, if it occurs at all (G. J. Haenel, L. C. Smith, H. B. John-Alder, submitted).

Moisture and Yolk Effects on Phenotypes in the Field

In this study, both the moisture and the yolk removal treatments reduced body size. The effect of incubation moisture on body size at hatching was small and did not persist up to the time of release. For variation in incubation moisture to have biological meaning, selection would have to act on body size soon after hatching. Because hatchlings were not released into the field until after the effects of incubation moisture had disappeared (Table 2.1), I was unable to assess the survival consequences of incubation moisture immediately after hatching. In contrast, the effect of yolk removal on body size (mass, SVL, and TL) was relatively large, and differences between the yolk removed and wet (control) treatments persisted to the time of release. Nonetheless, reduction in size by moisture or yolk removal was not related to survival in the field (Table 2.5), and neither of the treatments affected hatchling performance in the laboratory (see above) or performance (growth, displacement, and total distance traveled) in the field.

Body size is a commonly studied trait and is often thought of as an important predictor of survival. The idea that "bigger is better" is a frequently used hypothesis (reviewed by Packard and Packard, 1988), and several experimental studies support this (Ferguson and Fox, 1984; Vleck, 1988; Sinervo et al., 1992; Janzen, 1993; Sorci and Clobert, 1999). Large bodied individuals may be less susceptible to predation than smaller individuals for several reasons. For instance, predators with a limited range of acceptable prey size may not be able to feed on large individuals (Dunham, 1978). Large bodied individuals may be more efficient at foraging or evading predators because they can run or swim faster than their smaller conspecifics (Miller et al., 1987; Sinervo and Adolf, 1989; Sinervo, 1990; Rhen and Lang, 1995). Body size affects the probability of obtaining and holding quality territories and home ranges. Because large individuals tend to hold quality territories, they presumably force small individuals into less favorable habitats where predation or starvation may be more likely (Fox, 1978). Larger individuals may reach reproductive maturity faster than smaller individuals, thereby potentially increasing their fecundity (Madsen and Shine, 1992; Forsman, 1993).

Large body size, however, may not always enhance survival. For instance, large bodied hatchlings may have significant survival advantages only during periods of low resource abundance when competition is high (Ferguson et al., 1982; Ferguson and Fox, 1984), and the survival advantage of large individuals may vary among years and populations (Sinervo et al., 1992;

Forsman, 1993). Moreover, small individuals do not necessarily have higher mortality than large individuals (Van Damme and Van Dooren, 1999). Rhen and Lang (1995) suggest that growth rates may be more important than initial hatchling size. For example, individuals with rapid growth may reach reproductive maturity earlier than those with slow growth.

Phenotypes and Survival

In this study, manipulations of body size (by incubation moisture or yolk removal) did not affect performance or survival (Table 2.5). Therefore, to examine the relationship between phenotype and fitness, I made overall comparisons of the phenotypes of survivors and nonsurvivors irrespective of treatment (Table 2.7). In this analysis, running speed over 1 m and growth rate in the field during the first month after release were consistently associated with survival. Survivors grew more slowly and ran faster than nonsurvivors at all time periods (Figure 2.5). In parallel with these results, field hatchlings grew slower than laboratory hatchlings, both in the field and in the laboratory (Figure 2.3) and field hatchlings consistently had higher survival than laboratory hatchlings (Figure 2.4). These patterns are indicative of a strong influence of growth and running speed on fitness.

While the results of overall contrasts between survivors and nonsurvivors indicated that phenotypes were related to survival of hatchlings, these contrasts are potentially biased. Clutch effects were highly significant, and, in the overall analyses, clutches were represented by only a few, or as many as 16 individuals. Significant differences might thus reflect the influence of certain clutches. Therefore, at the cost of greatly reduced sample size, I eliminated this bias by examining the relationship between phenotypes and fitness using means for clutches that were represented by a relatively large number of hatchlings.

Clutch Effects on Survival

The associations between growth, running speed, and survival documented by the overall comparisons, were even more pronounced when the analysis was restricted to the eight clutches with at least 5 hatchlings. High survival was associated low growth rate in the field and with fast running speed, and running speed and growth rate were inversely related (Figure 2.6). Both running speed and growth rate are considered important measures of fitness. Running speed incorporates both morphological and physiological traits (Garland and Losos, 1994). Fast running speed is thought to be associated with high survival because fast runners are presumably more effective at capturing prey and evading predators than slow runners (Sinervo and Adolf, 1989; Sinervo, 1990).

Rapid growth is considered important for fitness of adults. For males, large individuals have a dominance advantage in terms of establishing a breeding territory. For females, large body size enhances clutch size, thereby increasing individual fecundity (Madsen and Shine, 1992; Forsman, 1993). The importance of large adult body size suggests that selection should maximize the growth of juveniles. However, rapid growth has risks (Forsman, 1993; Sorci et al., 1996). In order to grow rapidly, an individual must eat more than slowly growing individuals. Thus, if growth rate is positively associated with foraging activity, rapidly growing individuals may suffer high predation. For *Sceloporus undulatus* and *Lacerta vivipara*, activity levels and growth are positively correlated (Gerwien and John-Alder, 1992; Lorenzon et al., 1999), and snapping turtles (*Chelydra serpentina*) that move infrequently are more cryptic and have higher survival than individuals that move frequently (Janzen, 1995). In addition, for *Uta stansburiana*, relatively active individuals have lower survival than less active individuals (Fox, 1978).

To be ecologically meaningful, an assessment of the relationship between phenotype and fitness should take into account environmental and demographic variation from year to year (Dunham, 1980; Ferguson and Fox, 1984; Sinervo et al., 1992; Sinervo and DeNardo, 1996). While growth rate and running speed were significant predictors of survival in this study, these phenotypes may not reflect survival all years. Nonetheless, my results documented the overriding influence of clutch, and presumably genetic, effects on phenotypes and fitness.

By integrating both laboratory and field experiments, I demonstrated links among sources of phenotypic variation, phenotypes, and fitness (Figure 2.7). Incubation moisture conditions had a small, but significant effect on hatchling body size. Yolk investment, however, had a large effect on hatchling body size, but body size was still not associated with survival. Clutch explained most of the variation in all phenotypes that were measured, and clutch was the only source of phenotypic variation that was associated with fitness. By examining clutch means for each phenotype, I found that growth rate in the field was inversely related to running speed, and clutches that produced fast running and slow growing individuals had high survival in the field. Future studies should closely examine genetic (clutch) effects on variation in phenotypes and fitness. Heritability estimates would provide insight on the degree to which phenotypes are genetically based. Furthermore, measurement of lifetime reproductive success in the field would provide the best assessment of the relationship between phenotype and fitness.

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TABLE 2.1. Effects of moisture treatment (dry [-530 kPa], and wet [-150 kPa]), clutch, and their interaction on hatchling phenotypes. All analyses were two-way ANOVA or ANCOVA. Associated mean values and standard errors are reported in Table 2.2.

Trait	Covariate	Moisture	Clutch	Interaction
Incubation Period (days)	egg mass	$F_{1,81}$=1.6, P=0.209	$F_{20,81}$=6.0, **P<0.001**	$F_{20,81}$=1.6, P=0.083
Snout-vent length (mm)				
At hatching	egg mass	$F_{1,81}$=6.0, **P=0.017**	$F_{20,81}$=1.3, P=0.193	$F_{20,81}$=0.9, P=0.564
At release	egg mass	$F_{1,81}$=0.4, P=0.512	$F_{20,81}$=6.5, **P<0.001**	$F_{20,81}$=1.5, P=0.094
Body mass (g)				
At hatching	egg mass	$F_{1,80}$=11.2, **P=0.001**	$F_{20,80}$=5.8, **P<0.001**	$F_{20,80}$=1.8, **P=0.040**
At release	egg mass	$F_{1,81}$=0.3, P=0.619	$F_{20,81}$=8.4, **P<0.001**	$F_{20,81}$=1.3, P=0.189
Tail length (mm)				
At hatching	SVL	$F_{1,54}$=3.6, P=0.063	$F_{14,54}$=5.4, **P<0.001**	$F_{14,54}$=1.3, P=0.249
At release	SVL	$F_{1,52}$=2.5, P=0.124	$F_{15,52}$=4.9, **P<0.001**	$F_{14,52}$=1.5, P=0.142
Body shape ($mass^{0.3}$/SVL)				
At hatching	-	$F_{1,81}$=1.9, P=0.172	$F_{20,81}$=2.6, **P=0.002**	$F_{20,81}$=1.3, P=0.214
At release	-	$F_{1,82}$=0.3, P=0.602	$F_{20,82}$=2.4, **P=0.004**	$F_{20,82}$=0.7, P=0.838
Thermal preference (°C)	-	$F_{1,79}$=0.1, P=0.756	$F_{19,79}$=3.1, **P<0.001**	$F_{19,79}$=0.7, P=0.842
Running speed (m/s)				
Over 25 cm	-	$F_{1,82}$=0.1, P=0.708	$F_{20,82}$=2.7, **P<0.001**	$F_{20,82}$=1.5, P=0.122
Over 1 m	-	$F_{1,82}$=0.5, P=0.466	$F_{20,82}$=3.5, **P<0.001**	$F_{20,82}$=1.1, P=0.398
Over 25 cm	SVL	$F_{1,81}$=0.2, P=0.702	$F_{20,81}$=2.4, **P=0.004**	$F_{20,81}$=1.4, P=0.132
Over 1 m	SVL	$F_{1,81}$=0.5, P=0.484	$F_{20,81}$=3.2, **P<0.001**	$F_{20,81}$=1.1, P=0.412
Stops over 1m	-	$F_{1,77}$=0.5, P=0.492	$F_{20,77}$=2.8, **P<0.001**	$F_{20,77}$=1.7, P=0.050
Desiccation rate (Δmg/h)	Mass	$F_{1,74}$=0.0, P=0.886	$F_{18,74}$=2.9, **P<0.001**	$F_{18,74}$=1.1, P=0.377
Growth in laboratory				
SVL (logΔmm/day)	-	$F_{1,82}$=2.6, P=0.109	$F_{20,82}$=2.8, **P<0.001**	$F_{20,82}$=1.7, P=0.061
Mass (logΔg/day)	-	$F_{1,81}$=1.1, P=0.296	$F_{20,81}$=4.6, **P<0.001**	$F_{20,81}$=1.1, P=0.407
Growth in field				
SVL (logΔmm/day)	-	$F_{1,14}$=0.1, P=0.800	$F_{4,14}$=1.9, P=0.162	$F_{4,14}$=0.8, P=0.536
Mass (logΔg/day)	-	$F_{1,14}$=0.0, P=0.859	$F_{4,14}$=2.1, P=0.139	$F_{4,14}$=1.3, P=0.319
Displacement (m)	Days	$F_{1,13}$=0.5, P=0.482	$F_{4,13}$=2.0, P=0.148	$F_{4,13}$=0.4, P=0.842
Total distance traveled (m)	Days	$F_{1,13}$=0.5, P=0.512	$F_{4,13}$=2.7, P=0.081	$F_{4,13}$=0.8, P=0.543

TABLE 2.2. Mean values and standard errors for hatchling phenotypes from the dry and wet incubation treatments. Least square means are reported for traits that were adjusted (see Table 2.1 for adjusted traits and statistical tests).

	Dry treatment			Wet treatment		
Trait	Clutches (N)	Means	1 SE	Clutches (N)	Means	1 SE
Incubation period (days)	21	50.3	0.1	21	50.5	0.1
Snout-vent length (mm)						
At hatching	21	24.0	0.1	21	24.3	0.1
At release	21	26.3	0.1	21	26.5	0.1
Body mass (g)						
At hatching	21	0.5376	0.0027	21	0.5501	0.0026
At release	21	0.6789	0.0151	21	0.6892	0.0146
Tail length (mm)						
At hatching	15	27.5	0.3	15	28.4	0.3
At release	15	30.1	0.4	15	30.9	0.4
Body shape ($mass^{0.3}$/SVL)						
At hatching	21	0.0346	0.0002	21	0.0343	0.0002
At release	21	0.0336	0.0002	21	0.0335	0.0002
Thermal preference (°C)	20	34.0	0.4	20	33.8	0.4
Running speed (m/s)						
Over 25 cm	21	0.722	0.078	21	1.371	0.751
Over 1 m	21	0.279	0.029	21	0.243	0.896
Over 25 cm (adjusted)	21	0.723	0.815	21	1.370	0.784
Over 1 m (adjusted)	21	0.279	0.017	21	0.244	0.016
Stops over 1m	21	8.9	1.7	21	8.1	0.9
Desiccation rate (Δmg/h)	19	2.6	0.1	19	2.6	0.1
Growth in laboratory						
SVL (logΔmm/day)	21	0.0101	0.0009	21	0.0088	0.0008
Mass (logΔg/day)	21	0.0213	0.0035	21	0.0181	0.0036
Growth in field						
SVL (logΔmm/day)	5	0.0092	0.0013	5	0.0086	0.0014
Mass (logΔg/day)	5	0.0273	0.0036	5	0.0257	0.0035
Displacement (m)	5	27.7	7.2	5	34.8	6.7
Total distance traveled (m)	5	30.9	7.4	5	37.8	6.9

TABLE 2.3. Effects of yolk removal, clutch, and their interaction on hatchling phenotypes. All analyses were two-way ANOVA or ANCOVA. The yolk removal effect is a comparison of hatchling phenotypes from the wet treatment (-150 kPa) and the yolk removed treatment (-150 kPa). Associated mean values and standard errors are reported in Table 2.4.

Trait	Covariate	Yolk removal	Clutch	Interaction
Incubation Period (days)	egg mass	$F_{1,84}$=0.1, P=0.750	$F_{20.84}$=8.1, **P<0.001**	$F_{20,84}$=1.3, P=0.217
Snout-vent length (mm)				
At hatching	egg mass	$F_{1,84}$=150.2, **P<0.001**	$F_{20.84}$=2.8, **P<0.001**	$F_{20,84}$=11.5, P=0.098
At release	egg mass	$F_{1,84}$=63.3, **P<0.001**	$F_{20.84}$=4.4, **P<0.001**	$F_{20,84}$=0.7, P=0.842
Body mass (g)				
At hatching	egg mass	$F_{1,84}$=550.1, **P<0.001**	$F_{20.84}$=5.8, **P<0.001**	$F_{20,84}$=3.2, **P<0.001**
At release	egg mass	$F_{1,84}$=39.9, **P<0.001**	$F_{20.84}$=4.6, **P<0.001**	$F_{20,84}$=0.8, P=0.710
Tail length (mm)				
At hatching	SVL	$F_{1,62}$=4.4, **P=0.003**	$F_{14.62}$=5.7, **P<0.001**	$F_{14,62}$=0.5, P=0.908
At release	SVL	$F_{1,62}$=4.2, **P=0.045**	$F_{14.62}$=6.4, **P<0.001**	$F_{14,62}$=0.7, P=0.750
Body shape ($mass^{0.3}$/SVL)				
At hatching	-	$F_{1,85}$=1.8, P=0.181	$F_{20.85}$=2.6, **P=0.001**	$F_{20,85}$=1.1, P=0.336
At release	-	$F_{1,85}$=0.1, P=0.758	$F_{20.85}$=2.1, **P=0.009**	$F_{20,85}$=0.9, P=0.619
Thermal preference (°C)	-	$F_{1,85}$=0.1, P=0.830	$F_{20.85}$=2.2, **P=0.006**	$F_{20,85}$=0.5, P=0.942
Running speed (m/s)				
Over 25 cm	-	$F_{1,86}$=3.7, P=0.058	$F_{20.86}$=2.5, **P=0.002**	$F_{20,86}$=0.8, P=0.755
Over 1 m	-	$F_{1,86}$=3.0, P=0.859	$F_{20.86}$=3.4, **P<0.001**	$F_{20,86}$=0.9, P=0.588
Over 25 cm	SVL	$F_{1,85}$=1.2, P=0.286	$F_{20.85}$=1.9, **P=0.027**	$F_{20,85}$=0.8, P=0.767
Over 1 m	SVL	$F_{1,85}$=2.3, P=0.135	$F_{20.85}$=2.9, **P<0.001**	$F_{20,85}$=0.9, P=0.596
Stops over 1m	-	$F_{1,82}$=4.5, P=0.037	$F_{20.82}$=2.7, **P=0.001**	$F_{19,82}$=1.1, P=0.410
Desiccation rate (Δmg/h)	Mass	$F_{1,77}$=0.2, P=0.677	$F_{19.77}$=2.6, **P=0.002**	$F_{19,77}$=0.8, P=0.679
Growth in laboratory				
SVL (logΔmm/day)	-	$F_{1,85}$=1.0, P=0.317	$F_{20.85}$=2.4, **P=0.003**	$F_{20,85}$=0.7, P=0.771
Mass (logΔg/day)	-	$F_{1,85}$=2.4, P=0.129	$F_{20.85}$=2.7, **P=0.001**	$F_{20,85}$=1.0, P=0.486
Growth in field				
SVL (logΔmm/day)	-	$F_{1,30}$=2.2, P=0.145	$F_{10.30}$=3.4, **P=0.005**	$F_{10,30}$=0.8, P=0.612
Mass (logΔg/day)	-	$F_{1,31}$=0.9, P=0.361	$F_{10.31}$=3.4, **P=0.004**	$F_{10,31}$=0.8, P=0.606
Displacement (m)	Days	$F_{1,31}$=0.2, P=0.632	$F_{10,31}$=0.8, P=0.660	$F_{10,31}$=1.3, P=0.289
Total distance traveled (m)	Days	$F_{1,31}$=0.3, P=0.608	$F_{10.31}$=0.7, P=0.707	$F_{10,31}$=1.1, P=0.426

TABLE 2.4. Mean values and standard errors for hatchling phenotypes from the yolk removed and wet treatments. Least square means are reported for traits that were adjusted (see Table 2.3 for adjusted traits and statistical tests).

	Yolk removed treatment			Wet treatment		
Trait	Clutches (N)	Mean	1 SE	Clutches (N)	Mean	1 SE
Incubation period (days)	21	50.3	0.1	21	50.4	0.1
Snout-vent length (mm)						
At hatching	21	22.6	0.1	21	24.3	0.1
At release	21	24.7	0.2	21	26.4	0.1
Body mass (g)						
At hatching	21	0.4243	0.0041	21	0.5528	0.0036
At release	21	0.5030	0.0159	21	0.6736	0.0138
Tail length (mm)						
At hatching	15	26.5	0.3	15	28.5	0.4
At release	15	29.3	0.3	15	30.3	0.3
Body shape ($mass^{0.3}$/SVL)						
At hatching	21	0.0342	0.0001	21	0.0344	0.0002
At release	21	0.0334	0.0002	21	0.0335	0.0002
Thermal preference (°C)	21	33.9	0.3	21	33.8	0.4
Running speed (m/s)						
Over 25 cm	21	0.502	0.052	21	1.380	0.750
Over 1 m	21	0.199	0.021	21	0.241	0.020
Over 25 cm (adjusted)	21	0.530	1.009	21	1.352	0.971
Over 1 m (adjusted)	21	0.197	0.018	21	0.243	0.017
Stops over 1m	21	10.7	1.3	21	8.2	0.9
Desiccation rate (Δmg/h)	19	2.5	0.2	19	2.4	0.2
Growth in laboratory						
SVL (logΔmm/day)	21	0.0099	0.0008	21	0.0089	0.0007
Mass (logΔg/day)	21	0.0226	0.0037	21	0.0169	0.0032
Growth in field						
SVL (logΔmm/day)	11	0.0089	0.0008	11	0.0077	0.0001
Mass (logΔg/day)	11	0.0266	0.0027	11	0.0242	0.0029
Displacement (m)	11	19.1	4.5	11	22.3	4.4
Total distance traveled (m)	11	20.3	5.0	11	24.1	4.9

TABLE 2.5. Effect of incubation moisture, yolk removal, and clutch on survival of laboratory hatchlings at 3 time periods beyond release.

	Moisture	Yolk removal	Clutch
Survival at 6 weeks	χ^2=1.1, df=1, P=0.285	χ^2=1.5, df=1, P=0.215	χ^2=20.9, df=15, P=0.134
Survival at 12 weeks	χ^2=2.5, df=1, P=0.114	χ^2=2.1, df=1, P=0.152	χ^2=23.9, df=15, P=0.067
Survival to March	χ^2=2.3, df=1, P=0.126	χ^2=1.0, df=1, P=0.311	χ^2=19.4, df=15, P=0.197

TABLE 2.6. Comparison between laboratory hatchlings and field hatchlings. Least squares means are reported for traits that were adjusted. For laboratory hatchlings, only the combined dry and wet treatments were used in the analyses. One-way ANOVA and ANCOVA were used for all analyses; clutch was not used as a factor because clutch of origin was unknown for field hatchlings.

		Field hatchlings		Laboratory hatchlings		
Trait	Covariate	N	Mean ± 1 SE	N	Mean ± 1 SE	Statistical test
Thermal preference (°C)	-	42	34.3 ± 0.4	147	33.9 ± 0.2	$F_{1,187}$=1.0, P=0.309
Running speed (m/s)						
Over 25 cm	-	40	0.823 ± 0.062	148	1.060 ± 0.419	$F_{1,186}$=3.8, P=0.052
Over 1 m	-	40	0.377 ± 0.031	148	0.247 ± 0.012	$F_{1,186}$=12.3, **P<0.001**
Over 25 cm	SVL	40	0.286 ± 0.990	148	1.205 ± 0.416	$F_{1,185}$=0.5, P=0.481
Over 1 m	SVL	40	0.297 ± 0.032	148	0.269 ± 0.013	$F_{1,185}$=0.2, P=0.696
Stops over 1m	-	39	5.6 ± 0.9	143	8.9 ± 0.7	$F_{1,180}$=5.5, **P=0.020**
Desiccation rate (Δmg/h)	Mass	42	2.8 ± 0.2	140	2.7 ± 0.1	$F_{1,179}$=0.1, P=0.739
Growth in laboratory						
SVL (logΔmm/day)	-	42	0.0042 ± 0.0006	148	0.0095 ± 0.0004	$F_{1,188}$=38.7, **P<0.001**
Mass (logΔg/day)	-	42	0.0068 ± 0.0037	147	0.0187 ± 0.0017	$F_{1.187}$=10.01, **P=0.002**
Growth in field						
SVL (logΔmm/day)	-	49	0.0021 ± 0.0020	81	0.0072 ± 0.0004	$F_{1,128}$=7.6, **P=0.007**
Mass (logΔg/day)	-	49	0.0075 ± 0.0069	81	0.0219 ± 0.0014	$F_{1,129}$=6.6, **P=0.012**
Displacement (m)	Days	49	29.1 ± 4.9	81	25.5 ± 3.2	$F_{1,116}$=0.4, P=0.543
Total distance traveled (m)	Days	49	43.9 ± 5.6	81	29.3 ± 3.6	$F_{1,117}$=4.6, **P=0.033**

TABLE 2.7. Statistical tests of contrasts between survivors and nonsurvivors at three time periods. ANOVA and ANCOVA were used independent of treatment and clutch.

Trait	Covariate	At 6 weeks	At 12 weeks	To March
Incubation Period (days)	egg mass	$F_{1,212}$=0.1, P=0.741	$F_{1,212}$=1.0, P=0.318	$F_{1,212}$=0.3, P=0.616
Snout-vent length (mm)				
At hatching	egg mass	$F_{1,211}$=0.2, P=0.690	$F_{1,211}$=0.1, P=0.722	$F_{1,211}$=0.1, P=0.718
At release	egg mass	$F_{1,211}$=1.2, P=0.274	$F_{1,211}$=2.5, P=0.114	$F_{1,211}$=2.1, P=0.150
At hatching	-	$F_{1,212}$=0.4, P=0.511	$F_{1,212}$=0.1, P=0.715	$F_{1,212}$=0.5, P=0.468
At release	-	$F_{1,212}$=0.0, P=0.955	$F_{1,212}$=0.5, P=0.472	$F_{1,212}$=0.6, P=0.461
Body mass (g)				
At hatching	egg mass	$F_{1,210}$=0.0, P=0.902	$F_{1,210}$=2.3, P=0.129	$F_{1,210}$=1.9, P=0.171
At release	egg mass	$F_{1,211}$=0.1, P=0.750	$F_{1,211}$=0.9, P=0.358	$F_{1,211}$=0.4, P=0.538
At hatching	-	$F_{1,211}$=1.4, P=0.245	$F_{1,211}$=0.0, P=0.907	$F_{1,211}$=0.0, P=0.975
At release	-	$F_{1,212}$=0.2, P=0.636	$F_{1,212}$=0.1, P=0.781	$F_{1,212}$=0.0, P=0.886
Tail length (mm)				
At hatching	SVL	$F_{1,132}$=0.1, P=0.817	$F_{1,132}$=0.2, P=0.663	$F_{1,132}$=0.2, P=0.639
At release	SVL	$F_{1,132}$=0.0, P=0.880	$F_{1,132}$=0.4, P=0.526	$F_{1,132}$=1.4, P=0.236
At hatching	-	$F_{1,133}$=0.1, P=0.810	$F_{1,133}$=0.8, P=0.372	$F_{1,133}$=0.7, P=0.417
At release	-	$F_{1,133}$=0.0, P=0.905	$F_{1,133}$=0.1, P=0.814	$F_{1,133}$=0.1, P=0.741
Body shape ($mass^{0.3}$/SVL)				
At hatching	-	$F_{1,211}$=0.6, P=0.432	$F_{1,211}$=0.1, P=0.710	$F_{1,211}$=1.3, P=0.258
At release	-	$F_{1,212}$=4.6, **P=0.033**	$F_{1,212}$=2.7, P=0.101	$F_{1,212}$=2.5, P=0.112
Thermal preference (°C)	-	$F_{1,211}$=0.3, P=0.573	$F_{1,211}$=0.1, P=0.805	$F_{1,211}$=0.2, P=0.669
Running speed (m/s)				
Over 25 cm	-	$F_{1,213}$=0.4, P=0.540	$F_{1,213}$=2.3, P=0.130	$F_{1,213}$=1.1, P=0.306
Over 1 m	-	$F_{1,212}$=3.4, P=0.068	$F_{1,212}$=4.4, **P=0.038**	$F_{1,212}$=4.0, **P=0.047**
Over 25 cm	SVL	$F_{1,212}$=0.2, P=0.654	$F_{1,212}$=2.2, P=0.140	$F_{1,212}$=0.8, P=0.381
Over 1 m	SVL	$F_{1,211}$=2.9, P=0.088	$F_{1,211}$=4.2, **P=0.041**	$F_{1,211}$=3.6, P=0.061
Stops over 1m	-	$F_{1.204}$=6.1, **P=0.015**	$F_{1,204}$=3.7, P=0.054	$F_{1,204}$=0.7, P=0.415
Desiccation rate (Δmg/h)	Mass	$F_{1,199}$=0.0, P=0.952	$F_{1,199}$=5.3, **P=0.022**	$F_{1,199}$=3.4, P=0.066
Growth in laboratory				
SVL (logΔmm/day)	-	$F_{1,212}$=0.0, P=0.974	$F_{1,212}$=0.6, P=0.448	$F_{1,212}$=1.8, P=0.186
Mass (logΔg/day)	-	$F_{1,211}$=0.8, P=0.362	$F_{1,211}$=0.6, P=0.461	$F_{1,211}$=0.4, P=0.546
Growth in field				
SVL (logΔmm/day)	-	$F_{1,46}$=6.3, **P=0.016**	$F_{1,64}$=16.1, **P<0.001**	$F_{1,64}$=5.8, **P=0.019**
Mass (logΔg/day)	-	$F_{1,46}$=4.8, **P=0.034**	$F_{1,64}$=4.8, **P=0.033**	$F_{1,64}$=2.1, P=0.148
Displacement (m)	Days	$F_{1,124}$=0.1, P=0.818	$F_{1,124}$=0.2, P=0.637	$F_{1,124}$=0.0, P=0.936
Total distance traveled (m)	Days	$F_{1,123}$=0.0, P=0.836	$F_{1,123}$=0.1, P=0.743	$F_{1,123}$=0.2, P=0.658

TABLE 2.8. Pearson correlation coefficients for the traits (growth rate and running speed) that were significantly correlated with survival. Correlations were based on clutch mean values. The relationships between growth in SVL and survival, and running speed (over 25 cm) and survival are in Figure 2.6.

	Survival to 6 weeks	Survival to 12 weeks	Survival to March
Running speed (m/s)			
Over 25 cm	0.534 (P=0.173)	0.845 **(P=0.008)**	0.858 **(P=0.006)**
Over 1 m	0.862 **(P=0.006)**	0.580 (P=0.132)	0.562 (P=0.147)
Growth in field			
SVL (logΔmm/day)	-0.819 **(P=0.013)**	-0.817 **(P=0.013)**	-0.822 **(P=0.012)**
Mass (logΔg/day)	-0.597 (P=0.119)	-0.856 **(P=0.007)**	-0.812 **(P=0.014)**

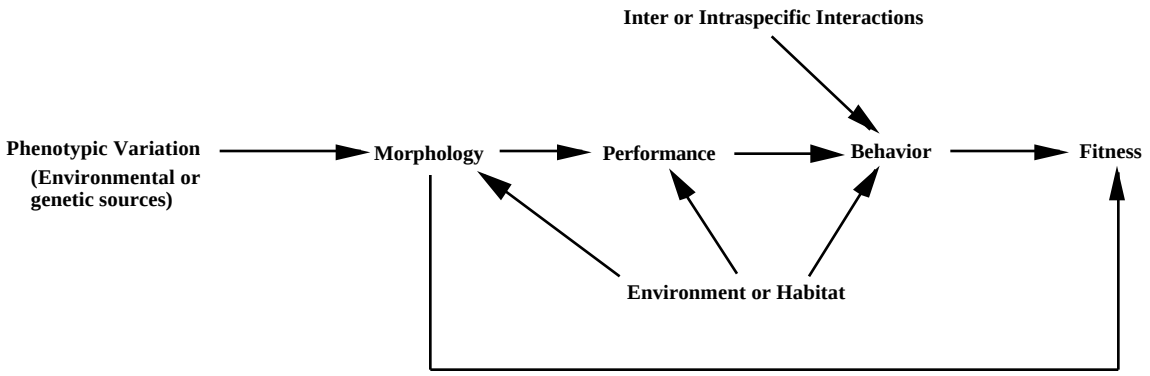

FIGURE 2.1. A modified version of Garland and Losos' (1994) fitness paradigm (originally from Arnold [1983]). This simplified diagram illustrates theoretical paths of direct and indirect effects of phenotypes on fitness. It is obvious that other factors and links can be used to demonstrate causal effects on fitness, but this diagram simply demonstrates possible factors that natural selection acts on, either directly or indirectly. I included a link between genetic or environmental influences on phenotypic variation with morphology. To gain a more complete understanding of the processes of natural selection, it is necessary to investigate the relative contributions of genotype and environment to phenotypic variation, which is equally as important as the relationship between phenotype and fitness as well as the influence of one phenotype on another.

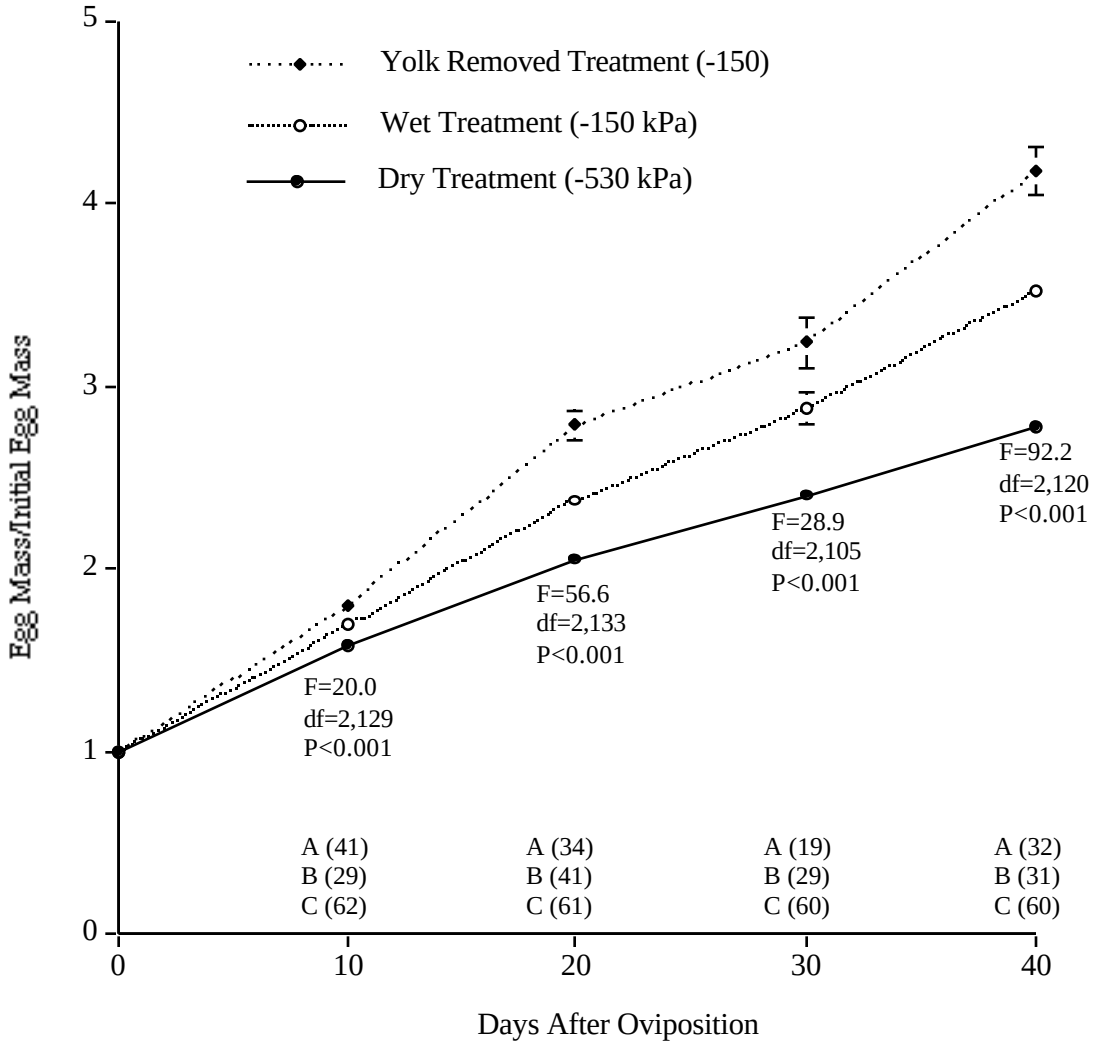

FIGURE 2.2. Water uptake by eggs during incubation expressed as the mass of the egg relative to its mass at oviposition (after yolk removal for eggs from the yolk removed treatment). Treatments with the same letter do not differ (P>0.05). Numbers in parentheses are sample sizes for each treatment for each time period.

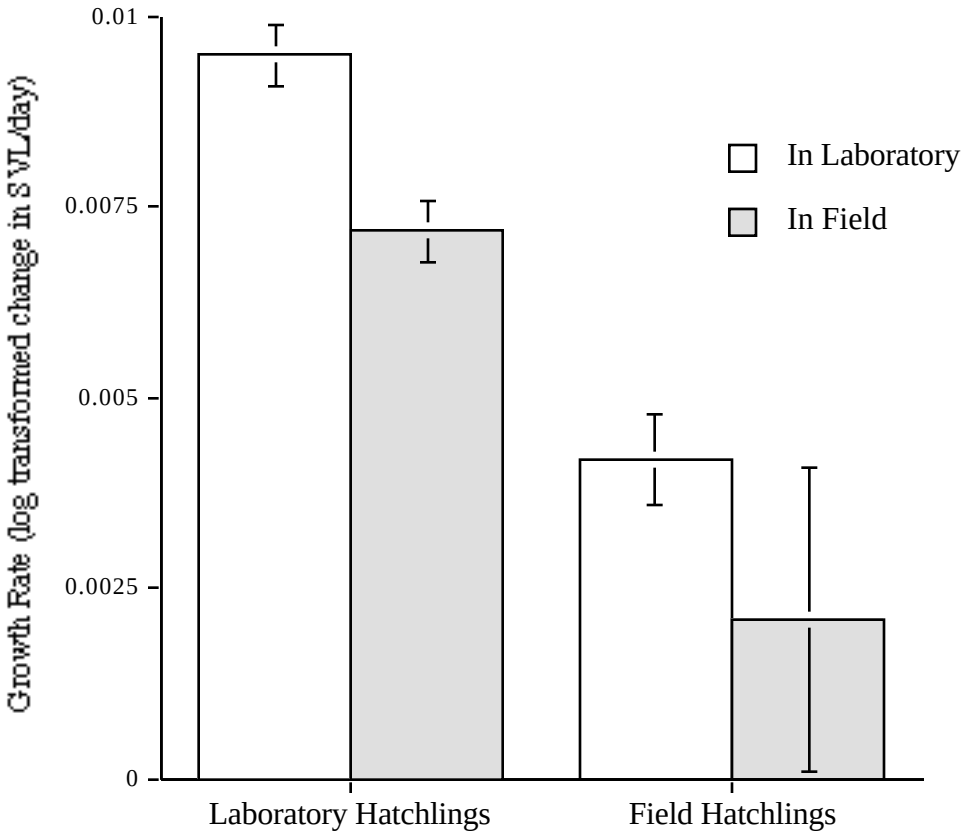

FIGURE 2.3. Contrasts of growth rate between laboratory hatchlings and field hatchlings. Under laboratory and field conditions, laboratory hatchlings grew significantly faster than those from the field. Laboratory hatchlings grew significantly faster while in captivity than in the field ($P<0.05$). Statistical tests are reported in Table 2.6.

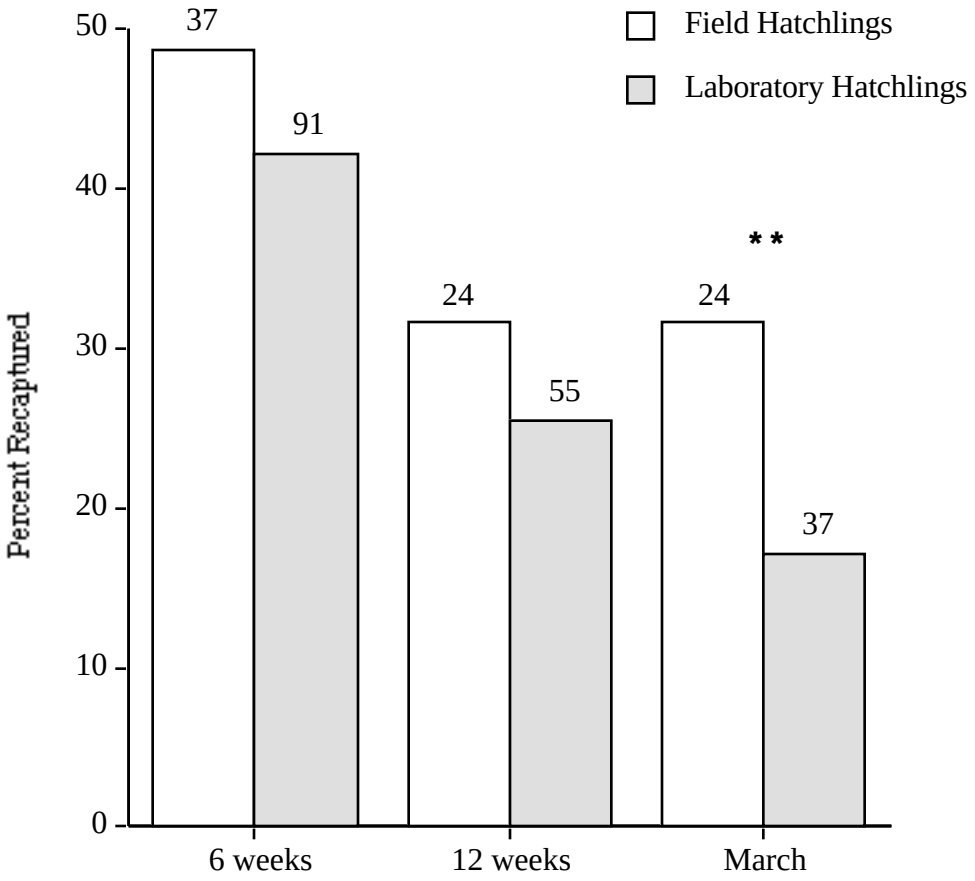

FIGURE 2.4. Recaptures of laboratory and field hatchlings at 6 weeks, 12 weeks, and March. A total of 220 laboratory hatchlings were released and 76 field hatchlings were captured. Numbers above the bars indicate the number of individuals recaptured at each time period. Field hatchlings had significantly higher survival than laboratory hatchlings at the March time period (**P=0.008).

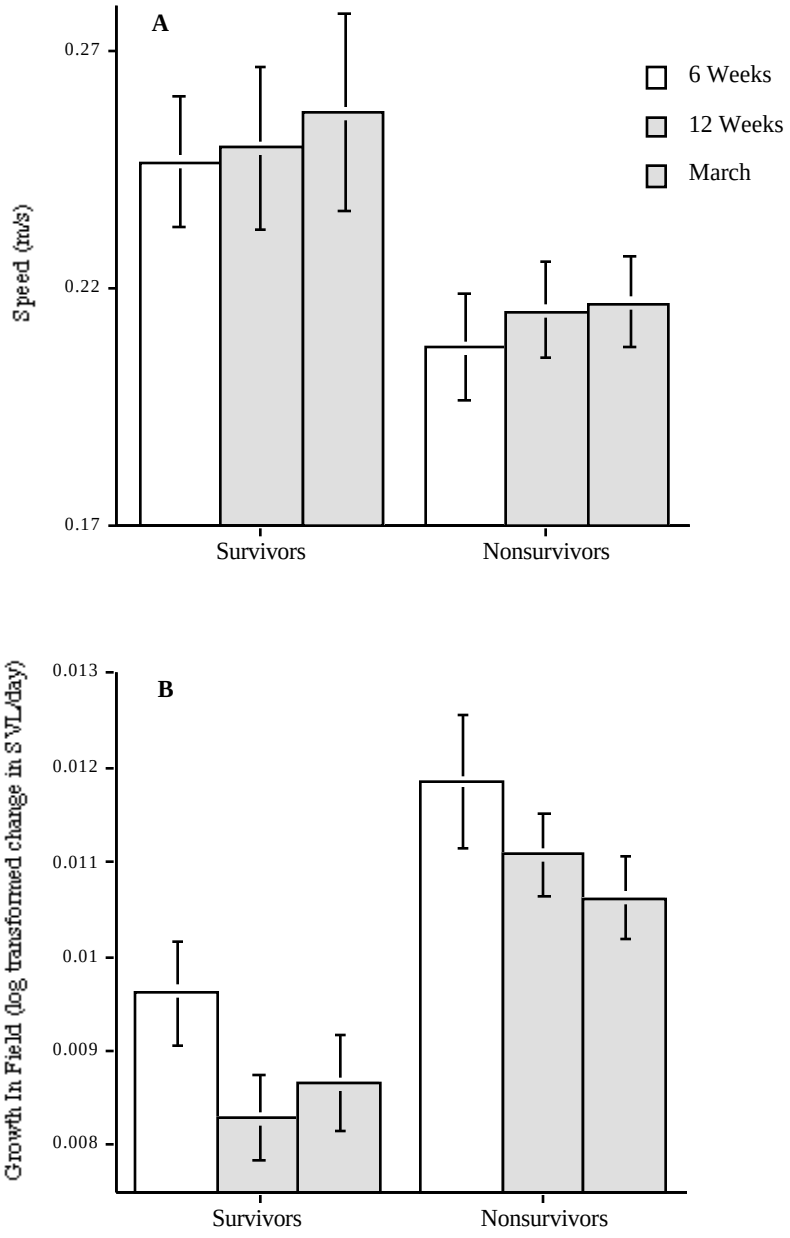

FIGURE 2.5. A) A comparison of running speed (over 1 m) between survivors and nonsurvivors of laboratory hatchlings after release in the field. Survivors ran faster than nonsurvivors across all time periods. B) A comparison of growth rate between survivors and nonsurvivors of laboratory hatchlings after release in the field. Survivors grew significantly slower than nonsurvivors across all time periods. Statistical tests are reported in Table 2.7.

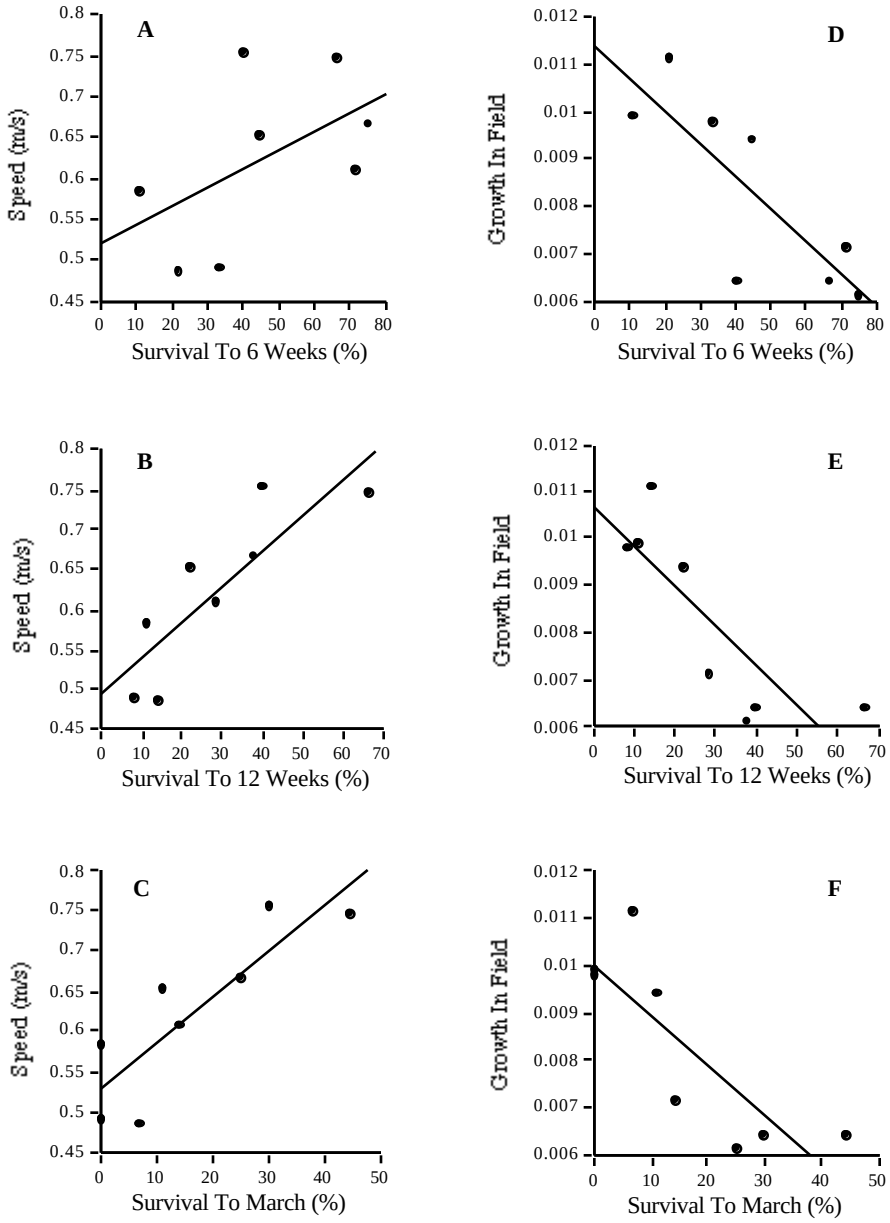

FIGURE 2.6. The relationship between running speed (over 25 cm) and survival of laboratory hatchlings based on clutch means. A) Survival to 6 weeks, B) Survival to 12 weeks, and C) Survival to March. Relationship between growth rate in the field (log transformed change in SVL/day) and survival for laboratory hatchlings based on clutch means. D) Survival to 6 weeks, E) Survival to 12 weeks, and F) Survival to March. Statistics are reported in Table 2.8.

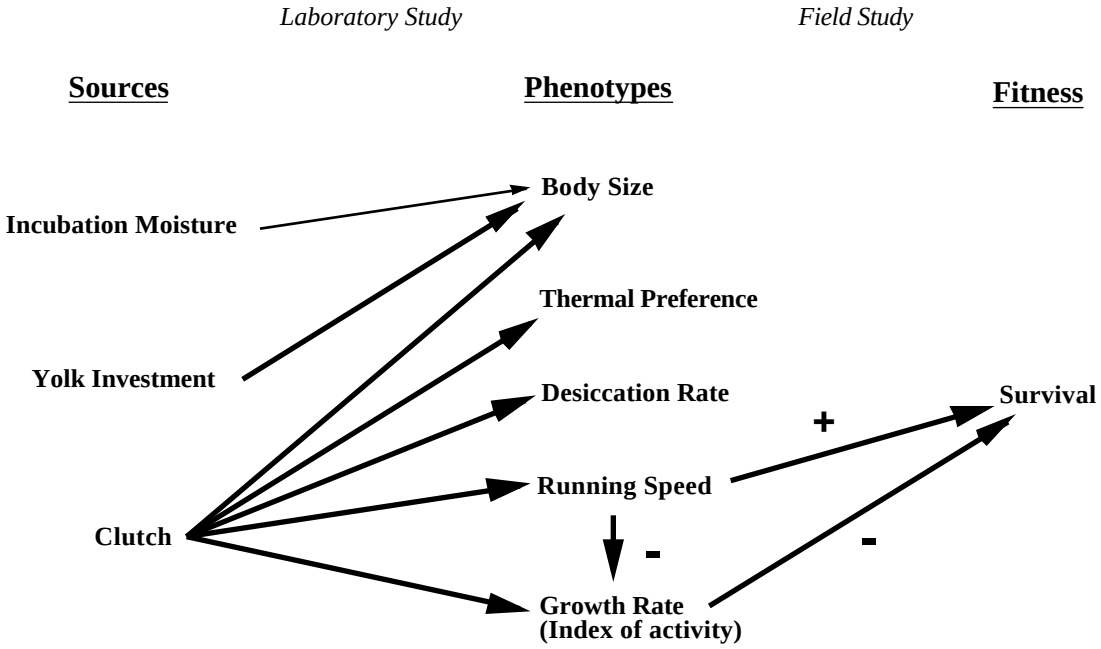

FIGURE 2.7. The relationships among three sources of phenotypic variation, phenotypes, and fitness. Incubation moisture conditions had a small, but significant effect on hatchling body size (indicated by a thin arrow). Maternal yolk investment had a strong effect on hatchling body size, but did not influence any other trait (indicated by a thick arrow). Clutch was the most important source of phenotypic variation (indicated by thick arrows). Clutch was associated with all phenotypes, and was the only source of phenotypic variation linked with survival. Growth rate under field conditions was negatively correlated (-) with running speed and survival. Running speed was positively correlated (+) with survival.

APPENDIX A. Effects of puncturing eggs. Two-way ANOVA and ANCOVA were used with puncture and clutch as class variables. The puncture effect was a comparison of the wet and punctured treatments. For growth in the field, one-way ANOVA was used because of a reduced sample for puncture/clutch combinations.

Trait	Covariate	Puncture	Clutch	Interaction
Incubation Period (days)	egg mass	$F_{1,15}$=2.0, P=0.178	$F_{6,15}$=11.8, **P<0.001**	$F_{6,15}$=0.9, P=0.520
Snout-vent length (mm)				
At hatching	egg mass	$F_{1,14}$=3.0, P=0.106	$F_{6,14}$=2.3, P=0.096	$F_{6,14}$=2.2, P=0.108
At release	egg mass	$F_{1,14}$=1.6, P=0.225	$F_{6,14}$=2.1, P=0.116	$F_{6,14}$=1.2, P=0.347
Body mass (g)				
At hatching	egg mass	$F_{1,14}$=0.0, P=0.881	$F_{6,14}$=2.6, P=0.066	$F_{6,14}$=1.5, P=0.244
At release	egg mass	$F_{1,14}$=1.4, P=0.259	$F_{6,14}$=2.8, P=0.053	$F_{6,14}$=0.7, P=0.645
Tail length (mm)				
At hatching	SVL	$F_{1,12}$=0.1, P=0.799	$F_{5,12}$=8.3, **P=0.001**	$F_{5,12}$=0.3, P=0.882
At release	SVL	$F_{1,12}$=0.4, P=0.548	$F_{5,12}$=7.8, **P=0.002**	$F_{5,12}$=0.2, P=0.938
Body shape ($mass^{0.3}$/SVL)				
At hatching	-	$F_{1,15}$=2.8, P=0.113	$F_{6,15}$=2.7, P=0.057	$F_{6,15}$=1.7, P=0.181
At release	-	$F_{1,15}$=0.6, P=0.451	$F_{6,15}$=4.2, **P=0.011**	$F_{6,15}$=0.7, P=0.642
Thermal preference (°C)	-	$F_{1,15}$=0.7, P=0.402	$F_{6,15}$=3.5, **P=0.024**	$F_{6,15}$=2.3, P=0.093
Running speed (m/s)				
Over 25 cm	-	$F_{1,15}$=0.3, P=0.598	$F_{6,15}$=1.2, P=0.369	$F_{6,15}$=0.7, P=0.679
Over 1 m	-	$F_{1,15}$=0.1, P=0.724	$F_{6,15}$=1.1, P=0.407	$F_{6,15}$=0.6, P=0.757
Over 25 cm	SVL	$F_{1,14}$=0.6, P=0.459	$F_{6,14}$=1.0, P=0.488	$F_{6,14}$=0.7, P=0.657
Over 1 m	SVL	$F_{1,14}$=0.4, P=0.526	$F_{6,14}$=0.9, P=0.499	$F_{6,14}$=0.6, P=0.708
Stops over 1 m	-	$F_{1,14}$=0.2, P=0.699	$F_{6,14}$=0.6, P=0.714	$F_{6,14}$=1.0, P=0.453
Desiccation rate (Δmg/h)	Mass	$F_{1,14}$=1.7, P=0.220	$F_{6,14}$=0.6, P=0.729	$F_{6,14}$=0.6, P=0.738
Growth in laboratory				
SVL (logΔmm/day)	-	$F_{1,15}$=0.5, P=0.510	$F_{6,15}$=2.1, P=0.113	$F_{6,15}$=1.3, P=0.325
Mass (logΔg/day)	-	$F_{1,15}$=3.1, P=0.099	$F_{6,15}$=2.6, P=0.063	$F_{6,15}$=0.4, P=0.870
Growth in field				
SVL ((logΔmm/day)	-	$F_{1,35}$=0.1, P=0.714	-	-
Mass (logΔg/day)	-	$F_{1,36}$=03, P=0.608	-	-

APPENDIX B. Sex differences for all phenotypic traits. Two way ANOVA and ANCOVA were used with sex and clutch as class variables.

Trait	Covariate	Sex effect	Clutch effect	Interaction
Incubation Period (days)	egg mass	$F_{1,150}$=5.7, **P=0.019**	$F_{23,150}$=8.8, **P<0.001**	$F_{23,150}$=1.0, P=0.492
Snout-vent length (mm)				
At hatching	egg mass	$F_{1,149}$=2.6, P=0.105	$F_{23,149}$=2.4, **P=0.009**	$F_{23,149}$=1.0, P=0.488
At release	egg mass	$F_{1,149}$=1.2, P=0.292	$F_{23,149}$=6.0, **P<0.001**	$F_{23,149}$=0.8, P=0.732
Body mass (g)				
At hatching	egg mass	$F_{1,147}$=0.1, P=0.905	$F_{23,147}$=5.1, **P<0.001**	$F_{23,147}$=1.6, P=0.052
At release	egg mass	$F_{1,149}$=2.9, P=0.088	$F_{23,149}$=7.2, **P<0.001**	$F_{23,149}$=0.7, P=0.822
Relative tail length (mm)				
At hatching	SVL	$F_{1,116}$=0.0, P=0.946	$F_{19,116}$=5.6, **P<0.001**	$F_{19,116}$=0.8, P=0.695
At release	SVL	$F_{1,119}$=1.7, P=0.195	$F_{19,119}$=5.5, **P<0.001**	$F_{19,119}$=1.5, P=0.111
Body shape ($mass^{0.3}$/SVL)				
At hatching	-	$F_{1,148}$=1.7, P=0.189	$F_{23,148}$=2.8, **P<0.001**	$F_{23,148}$=0.5, P=0.965
At release	-	$F_{1,150}$=0.3, P=0.575	$F_{23,150}$=3.1, **P<0.001**	$F_{23,150}$=0.9, P=0.569
Thermal preference (°C)	-	$F_{1,149}$=1.9, P=0.166	$F_{23,149}$=2.3, **P<0.001**	$F_{23,149}$=1.3, **P<0.001**
Running speed (m/s)				
Over 25 cm	-	$F_{1,148}$=0.3, P=0.596	$F_{22,148}$=1.7, **P=0.033**	$F_{22,148}$=0.5, P=0.984
Over 1 m	-	$F_{1,148}$=0.1, P=0.789	$F_{22,148}$=2.0, **P=0.009**	$F_{22,148}$=0.6, P=0.948
Over 25 cm	SVL	$F_{1,147}$=0.3, P=0.591	$F_{22,147}$=1.6, **P=0.046**	$F_{22,147}$=0.4, P=0.986
Over 1 m	SVL	$F_{1,147}$=0.1, P=0.808	$F_{22,147}$=1.9, **P=0.011**	$F_{22,147}$=0.6, P=0.949
Stops over 1 m	-	$F_{1,141}$=0.3, P=0.567	$F_{22,141}$=3.3, **P<0.001**	$F_{22,147}$=1.2, P=0.300
Desiccation rate (Δmg/h)	Mass	$F_{1,134}$=0.7, P=0.415	$F_{21,134}$=3.0, **P<0.001**	$F_{21,134}$=1.2, P=0.234
Growth in laboratory				
SVL (logΔmm/day)	-	$F_{1,150}$=1.7, P=0.200	$F_{23,150}$=2.8, **P<0.001**	$F_{23,150}$=0.8, P=0.745
Mass (logΔg/day)	-	$F_{1,148}$=0.8, P=0.367	$F_{23,184}$=4.2, **P<0.001**	$F_{23,148}$=0.9, P=0.655
Growth in field				
SVL (logΔmm/day)	-	$F_{1,47}$=0.1, P=0.748	$F_{11,47}$=3.5, **P<0.001**	$F_{11,47}$=2.5, P=0.013
Mass (logΔg/day)	-	$F_{1,47}$=0.0, P=0.990	$F_{11,47}$=3.4, **P=0.002**	$F_{11,47}$=1.7, P=0.115

APPENDIX C. Sex differences for all phenotypic traits and survival. One-way ANOVA and ANCOVA were used to assess differences between males and females for all phentypes. Chi-square tests were used to determine sex differences in survival.

Trait	Covariate	Sex effect
Incubation Period (days)	egg mass	$F_{1,46}$=1.3, P=0.268
Snout-vent length (mm)		
At hatching	egg mass	$F_{1,46}$=1.7, P=0.201
At release	egg mass	$F_{1,45}$=0.4, P=0.518
Body mass (g)		
At hatching	egg mass	$F_{1,45}$=0.0, P=0.959
At release	egg mass	$F_{1,45}$=0.9, P=0.355
Relative tail length (mm)		
At hatching	SVL	$F_{1,37}$=0.2, P=0.640
At release	SVL	$F_{1,37}$=0.9, P=0.347
Body shape ($mass^{0.3}$/SVL)		
At hatching	-	$F_{1,46}$=1.0, P=0.319
At release	-	$F_{1,46}$=0.2, P=0.688
Thermal preference (°C)	-	$F_{1,46}$=0.9, P=0.348
Running speed (m/s)		
Over 25 cm	-	$F_{1,44}$=0.3, P=0.585
Over 1 m	-	$F_{1,44}$=0.1, P=0.800
Over 25 cm	SVL	$F_{1,43}$=0.6, P=0.430
Over 1 m	SVL	$F_{1,43}$=0.2, P=0.661
Stops over 1 m	-	$F_{1,44}$=0.2, P=0.685
Desiccation rate (Δmg/h)	Mass	$F_{1,41}$=0.4, P=0.553
Growth in laboratory		
SVL (logΔmm/day)	-	$F_{1,46}$=0.9, P=0.351
Mass (logΔg/day)	-	$F_{1,46}$=0.3, P=0.562
Growth in field		
SVL (logΔmm/day)	-	$F_{1,23}$=0.0, P=0.882
Mass (logΔg/day)	-	$F_{1,23}$=0.0, P=0.994

Suvival	Sex effect
Survival at 6 weeks	χ^2=0.26, df=1, P=0.610
Survival at 12 weeks	χ^2=0.22, df=1, P=0.638
Survival to March	χ^2=0.00, df=1, P=0.956

APPENDIX D. Effect of release date on survival. Release dates were divided into three categories. Chi-square tests were used to assess differences in survival at each release period.

	Early (24 July - 7 Aug)	Middle (8 Aug - 22 Aug)	Late (23 Aug - 8 Sept)	Effect of release date
Survival to 6 weeks	38.5%	44.1%	43.3%	χ^2=0.50, df=2, P=0.778
Survival to 12 weeks	15.4%	28.8%	30.0%	χ^2=4.83, df=2, P=0.090
Survival to March	9.23%	18.64%	22.2%	χ^2=4.56, df=2, P=0.102

APPENDIX E. Effect of bent tails on survival. Chi-square tests were used to assess survival differences between hatchlings with and without bent tails.

	Statistical test
Survival to 6 weeks	χ^2=0.691, df=1, P=0.225
Survival to 12 weeks	χ^2=0.916, df=1, P=0.916
Survival to March	χ^2=0.992, df=1, P=0.319

APPENDIX F. Number and percent of survivors at each time interval for each treatment. The association between survival and treatment was not significant at any time period (see Table 2.6 for statistics for each treatment contrast).

	Dry Treatment	Yolk removed treatment	Wet treatment
Survival at 6 weeks	24 38.10%	27 39.71%	39 46.99%
Survival at 12 weeks	11 17.46%	16 23.53%	27 32.53%
Survival to March	7 11.11%	12 17.65%	18 21.69%

APPENDIX G. Pearson correlation coefficients between phenotypic traits and survival based on clutch means. Number in parentheses is the number of clutches used. Significant relationships ($P<0.05$) are marked by asterisks, and P-values for the significant relationships are reported in Table 2.8. ****** $P<0.01$

Trait	Survival to 6 weeks	Survival to 12 weeks	Survival to March
Incubation Period (days)	0.070 (16)	0.065 (16)	0.133 (16)
Snout-vent length (mm)			
At hatching	0.332 (16)	0.396 (16)	0.483 (16)
At release	0.017 (16)	0.158 (16)	0.201 (16)
Body mass (g)			
At hatching	0.348 (16)	0.300 (16)	0.272 (16)
At release	0.029 (16)	0.158 (16)	0.191 (16)
Tail length (mm)			
At hatching	0.019 (16)	-0.541 (16)	-0.006 (16)
At release	-0.008 (16)	0.005 (16)	0.084 (16)
Body shape ($mass^{0.3}$/SVL)			
At hatching	0.027 (16)	-0.162 (16)	-0.358 (16)
At release	-0.104 (16)	0.199 (16)	0.132 (16)
Thermal preference (°C)	-0.171 (16)	-0.222 (16)	-0.191 (16)
Running speed (m/s)			
Over 25 cm	0.534 (8)	0.845 (8)**	0.858 (8)**
Over 1 m	0.862 (8) **	0.580 (8)	0.562 (8)
Stops over 1 m	-0.355 (16)	-0.345 (16)	-0.467 (16)
Desiccation rate (Δmg/h)	0.209 (16)	-0.252 (16)	-0.109 (16)
Growth in laboratory			
SVL (logΔmm/day)	-0.050 (16)	-0.060 (16)	-0.213 (16)
Mass (logΔg/day)	0.010 (16)	0.166 (16)	0.145 (16)
Growth in field			
SVL (logΔmm/day)	-0.819 (8)**	-0.817 (8)**	-0.822 (8)**
Mass (logΔg/day)	-0.597 (8)	-0.856 (8)**	-0.812 (8)**
Displacement (m)	0.073 (16)	-0.174 (16)	-0.169 (16)
Total distance traveled (m)	0.060 (16)	-0.147 (16)	-0.129 (16)

APPENDIX H. Pearson correlation coefficients for hatchling phenotypes and maternal characteristics. Hatchling phenotypes were based on clutch mean values. Number in parentheses indicate the number of clutches used. Significant relationships ($P<0.05$) are marked by asterisks. ***** $P<0.05$, ****** $P<0.01$, ******* $P<0.001$

Trait	Female SVL (mm)	Female mass (g)	Female shape ($mass^{0.3}$/SVL)	# of days in captivity	Clutch size	Egg mass (g)
Clutch size	0.589 (16)**	0.348 (15)	0.003 (15)	-0.063 (16)	-	-
Egg mass (g)	0.193 (16)	0.072 (15)	-0.113 (15)	0.271 (16)	-0.330 (16)	-
Incubation Period (days)	0.267 (16)	-0.041 (15)	-0.262 (15)	-0.330 (16)	-0.145 (16)	0.156 (16)
Snout-vent length (mm)						
At hatching	0.206 (16)	0.134 (15)	-0.212 (15)	-0.116 (16)	-0.396 (16)	0.768 (16)***
At release	0.181 (16)	0.857 (15)	-0.165 (15)	-0.322 (16)	-0.112 (16)	0.783 (16)***
Body mass (g)						
At hatching	0.058 (16)	0.017 (15)	-0.120 (15)	-0.192 (16)	-0.449 (16)	0.856 (16)***
At release	0.097 (16)	-0.006 (15)	-0.171 (15)	-0.315 (16)	-0.118 (16)	0.728 (16)**
Tail length (mm)						
At hatching	-0.088 (16)	0.271 (15)	0.435 (15)	0.338 (16)	-0.299 (16)	0.106 (16)
At release	-0.233 (16)	0.320 (15)	0.548 (15)	0.496 (16)	-0.364 (16)	0.014 (16)
Body shape ($mass^{0.3}$/SVL)						
At hatching	-0.296 (16)	-0.196 (15)	0.188 (15)	-0.081 (16)	-0.110 (16)	0.097 (16)
At release	-0.447 (16)	-0.449 (15)	-0.106 (15)	-0.227 (16)	-0.264 (16)	-0.133 (16)
Thermal preference (°C)	-0.258 (16)	0.354 (15)	0.097 (15)	0.124 (16)	0.059 (16)	0.095 (16)
Running speed (m/s)						
Over 25 cm	-0.281 (16)	0.076 (15)	0.476 (15)	0.190 (16)	-0.344 (16)	0.122 (16)
Over 1 m	-0.015 (16)	0.085 (15)	0.064 (15)	-0.024 (16)	-0.516 (16)*	0.518 (16)
Stops over 1 m	0.100 (16)	-0.095 (15)	-0.176 (15)	-0.227 (16)	0.567 (16)*	-0.488 (16)
Desiccation rate (Δmg/h)	0.407 (16)	0.351 (15)	0.115 (15)	0.339 (16)	0.081 (16)	-0.079 (16)
Growth in laboratory						
SVL (logΔmm/day)	0.070 (16)	-0.275 (15)	-0.220 (15)	-0.557 (16)	0.209 (16)	0.437 (16)
Mass (logΔg/day)	0.001 (16)	-0.240 (15)	-0.045 (15)	-0.388 (16)	0.056 (16)	0.344 (16)
Growth in field						
SVL (logΔmm/day)	0.113 (8)	-0.279 (7)	-0.231 (7)	-0.316 (8)	0.683 (8)	-0.825 (8)**
Mass (logΔg/day)	0.261 (8)	-0.081 (7)	-0.347 (7)	-0.249 (8)	0.064 (8)	-0.892 (8)**
Displacement (m)	0.885 (8)**	0.851 (7)*	0.239 (7)	-0.117 (8)	0.614 (8)	0.076 (8)
Total distance traveled (m)	0.866 (8)**	0.890 (7)**	0.327 (7)	-0.091 (8)	0.600 (8)	0.095 (8)

CURRICULUM VITAE

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Professional Experience and Activities:

Graduate Teaching Assistant, Department of Biology, Virginia Polytechnic Institute and State University; General Biology Laboratory, Ornithology Laboratory, Mammalogy Laboratory (1999 - 2000).

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Laboratory and Field Assistant, Department of Zoology and Genetics, Iowa State University. Advised by Dr. Fredric Janzen (1996 - 1998).

Field Assistant, Great Rivers Field Station, Illinois Natural History Survey. Advised by John Tucker (1997).

Zoo Keeper Intern, Herpetology Department, Cincinnati Zoo and Botanical Garden (1996).

Field Assistant, Iguana Research Program, John G. Shedd Aquarium (1995).

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Grants and Awards Received:

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Travel Grant, Virginia Polytechnic Institute and State University, Graduate Student Assembly (2000).

Honorable mention for best student paper, annual meeting of The Virginia Academy of Science (2000).

Graduate Student Research Development Project Grant, Virginia Polytechnic Institute and State University, Graduate Student Assembly (1999).

Grant-in-Aid of Research, Sigma Xi (1999).

Graduate Research in Herpetology Grant, Chicago Herpetological Society (1999).

Undergraduate

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Study Abroad Scholarship (Costa Rica), Iowa State University (1998).

Gary C. White Award, Department of Animal Ecology, Iowa State University (1998).

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Department of Zoology and Genetics Summer Internship, Iowa State University (1997).

Undergraduate Research in Herpetology Grant, Chicago Herpetological Society (1997).

Study Abroad Scholarship (Ecuador and Galápagos Islands), Iowa State University (1997).

Publications:

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