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THERMAL AND REPRODUCTIVE BIOLOGY OF THE LIZARDS SCELOPORUS
OCCIDENTALIS AND SCELOPORUS GRACIOSUS ALONG AN
ENVIRONMENTAL AND GEOGRAPHICAL GRADIENT

A Thesis
Presented to the
Faculty of
California State University,
San Bernardino

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
in
Biology

by
Scott Landsborough Parker
September 2001

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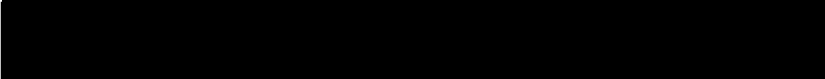
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ABSTRACT

The thermal environment exerts a strong influence on many aspects of squamate life history. Environmental temperatures constrain daily activity time, fecundity, growth rate, and also influences hatchling phenotype. Cold nest temperatures can slow the development of eggs deposited in the soil, cause developmental abnormalities or result in mortality of embryos. It is widely believed that viviparity in squamate reptiles evolved in response to cold environmental temperatures, and is referred to as the "cold climate model". In the present study, the lizards *Sceloporus occidentalis* and *Sceloporus graciosus* were used to evaluate the influence of reproductive condition on body temperature and the influence of elevation on reproductive life history traits. Mean selected body temperatures of gravid female *S. graciosus* were lower than non-gravid and male lizards, whereas there was no significant variation in mean selected body temperature between gravid female *S. occidentalis* and non-reproductive lizards. High elevation gravid female *S. graciosus* laid a significantly greater number of clutches at a more advanced developmental stage compared to gravid females from intermediate elevation, whereas there was no variation in embryonic stage of development

between populations of *S. occidentalis* at intermediate and low elevation. Mean clutch eggshell thickness did not differ between populations for either species. The mean clutch eggshell thickness was significantly thinner for *S. graciosus* (31.3 μ m) compared to *S. occidentalis* (49.28 μ m) however, it is presently unknown whether these differences are related to the capacity to support embryogenesis. The results of this study suggest that variation in thermal and reproductive biology of *S. occidentalis* and *S. graciosus* may partly explain the altitudinal distributions of these two species. Furthermore, these data also suggest that a variety of selective agents, including temperature, may be responsible for the evolution of squamate reproductive life history traits.

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CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

Life History Theory

Life history refers to the sum total of traits that constitutes an organism's life cycle (Pough et al. 1998). Combinations of life history characters often occur together, forming co-adapted clusters of traits (Schwarzkopf 1994). The existence of co-adapted complexes suggests that individual traits such as reproductive mode (oviparity/viviparity) and number of offspring are evolutionarily correlated (Pough et al. 1998). Natural selection operates to mold the suite of life history characters such that the reproductive fitness of individual phenotypes are maximized (Reznick 1985). Because life history traits are co-adapted, increasing the benefit of any one particular life history variable is likely to produce an associated cost in another related variable (Williams 1966; Schwarzkopf 1994). In this way, life history theory predicts that organisms evolve "optimal" life histories that are compromises between the benefits and associated costs of altering a specific character (Reznick 1985).

The compromise between costs and benefits in life history evolution are referred to as "tradeoffs". It is generally assumed that reproduction represents a cost, because increasing present fecundity or reproductive investment results in a decrease in the probability of future survival or fecundity of the reproducing individual (Williams 1966; Bell 1980; Reznick 1985). Most models therefore predict that life histories evolve so as to maximize reproductive rate, given the particular pattern of survivorship within a population (Reznick 1985).

Reproductive costs may be separated into two major categories; survival costs and energy allocation costs (Shine 1980). Survival costs consist of factors that potentially increase the mortality of reproducing individuals (Schwarzkopf 1994). For example, changes in behavior, or physiological performance associated with reproduction may reduce female survivorship. Energy allocation costs may reduce female survivorship and future fecundity due to the amount of resources devoted to reproduction rather than to growth and/or physiological maintenance (Bell 1980; Shine 1980). These costs may function separately or in combination to influence the evolution of life history characteristics (Brodie 1989; Schwarzkopf 1994).

Among vertebrates, lizards are particularly well suited for studies relating to the costs of reproduction and other aspects of life history evolution (Schwarzkopf 1994). Squamate (lizards, snakes, and amphisbaenians) life histories are highly variable, and therefore allow a variety of questions concerning the costs of reproduction to be addressed. Most reptiles and all birds lay eggs (Shine and Bull 1979). However, the reproductive mode of squamates spans a continuum from oviparity (egg-laying) to complete viviparity (live bearing). Many squamates retain eggs for varying lengths of time and in some cases until embryonic development is complete. Many viviparous squamates possess placentation with maternal-fetal nutrient transfer, and total absence of calcified egg membranes (Shine 1985; Blackburn 1992). Additionally, many squamate genera contain both oviparous and viviparous forms (Shine 1985). This allows direct comparative analysis of the "benefits" and "costs" associated with each of these life history "strategies" relative to environmental and species characteristics (Shine 1985; Dunbrack and Ramsay 1989).

It is widely accepted that oviparity is the ancestral mode of reproduction in reptiles (Packard et al. 1977). Within the class reptilia, three of the orders

(Testudines, Crocodylia, and Rhynchocephalia) are exclusively oviparous. In contrast, within the order squamata, viviparity has arisen a minimum of 30 times in snakes, twice in amphisbaenians, and 63 times in lizards (Shine 1985). The existence of viviparity in several different squamate families has prompted considerable investigation of the selective factors responsible for its evolution (Packard et al. 1977). Most hypotheses concerning the evolutionary transition from oviparity to viviparity address the fitness "benefit" (in terms of lifetime reproductive output) versus the associated "costs" of the two reproductive modes (Dunbrack and Ramsay 1989). The relative advantages and disadvantages of these two life history traits are dependent upon the selective constraints imposed by the environment as well as the morphological and physiological characteristics of the individual species (Tinkle 1969; Shine 1985; Qualls and Shine 1998).

Model for the Evolution of Viviparity

Several hypotheses have been proposed to explain the underlying selective pressures responsible for the evolution of viviparity in squamates. The most generally accepted hypothesis is that viviparity evolved in response

to cold climatic conditions, and is referred to as the "cold climate model" (Packard et al. 1977, Shine and Bull 1979). Temperature exerts a strong influence on many aspects of squamate life history (Porter et al. 1973; Adolph and Porter 1993, 1996). The rate of embryonic development in reptiles depends upon temperature (Shine 1983; Packard and Packard 1988), therefore cold environmental temperatures may prolong the incubation period of eggs deposited in the soil (Muth 1980), or potentially result in increased mortality to the embryos (Packard et al. 1977). Additionally research has demonstrated that eggs incubated at cold nest temperatures produce hatchling phenotypes with low viability (Shine 1995; Shine and Harlow 1993; Qualls and Shine 1996; Qualls and Andrews 1999; Andrews et al. 2000). Heliothermic reptiles are capable of maintaining elevated and relatively constant body temperatures by behavioral thermoregulation, regardless of dramatic fluctuations in ambient temperature (Huey 1974, 1982; Huey and Stevenson 1979; Shine 1985; Adolph 1990; Hertz et al. 1993). Extending the period of egg retention in the oviduct allows eggs to develop more rapidly because they are maintained at higher maternal body temperatures than would be possible at soil nest temperatures (Packard et al.

1977; Shine 1983). Even relatively small increases in the duration of egg retention would permit embryos to develop more rapidly, and therefore hatch earlier than embryos from non-retained eggs (Packard et al. 1977). Research conducted by Shine (1983) on heliothermic montane skinks demonstrated that eggs retained for approximately half of the embryonic development time shortens the total incubation period from 160 to 110 days. Additionally, prolonged uterine egg retention may be an advantage in cold seasonal climates because hatchlings have increased viability (Shine 1995), emerge earlier, and therefore possess a greater opportunity to feed and grow before the onset of hibernation (Shine 1985; Qualls and Shine 1996; Burger 1998).

The cold climate hypothesis is also supported by the strong correlation between climate and the distribution of viviparous species. The proportion of viviparous squamate taxa increases both with increasing latitude and elevation (Packard et al. 1977; Shine 1985). Moreover, in both Australia and North America, the proportion of viviparous species increases substantially where summer mean minimum temperatures are below 15°C (Shine 1985).

The evolutionary transition from oviparity to viviparity is thought to have occurred through a gradual

increase in the duration of uterine egg retention (Packard et al. 1977). The anatomical and physiological modifications associated with this transition most likely preclude any abrupt saltational shift from egg laying to live-bearing. Rather, for viviparity to evolve from oviparity, there must be a selective advantage conferred to females that retain eggs for progressively longer periods of time (Shine and Bull 1979). As the duration of egg retention increases, a greater proportion of development occurs in the oviduct, and eggs are correspondingly laid at more advanced developmental stages. The transition to viviparity is achieved by offspring completing their incubation in the oviduct, and are fully developed at the time of oviposition/partuition (Packard et al. 1977; Shine and Bull 1979).

Costs of Reproduction and Constraints on Egg Retention

Consistent with life history theory, it is assumed that "tradeoffs" must exist between the fitness benefit conferred by extended egg retention, and the costs in terms of female survival or future reproduction (Shine and Bull 1979; Shine 1980; Shine 1985). Behavioral changes associated with reproduction may result in increased

mortality in gravid female lizards (Shine and Bull 1979; Shine 1980; Shine 1985; Brodie 1989). The results of a number of studies demonstrate that gravid female lizards have decreased mobility and sprint speed due the burden of eggs (Shine and Bull 1979; Shine 1980; Shine 1985; Brodie 1989; Sinervo et al. 1991). Few studies have shown that gravid females experience increased mortality as a direct result of reduced mobility. However, Shine (1980) demonstrated in laboratory trials that a significantly greater number of gravid skinks were preyed upon by white-lipped snakes (*Drysdalia coronoides*) compared to male lizards.

It is well documented that in some species of lizards and snakes, gravid females exhibit changes in thermoregulatory behavior (Packard et al. 1977; Shine 1985; Braña 1993). For example, gravid female lizards may spend more time basking compared to non-gravid females (Shine 1980). There is also evidence to suggest that some gravid squamates maintain higher body temperatures compared to non-gravid and male individuals (Packard et al. 1977; Shine 1980; Werner 1990; Daut and Andrews 1993). Conversely, other studies have shown that gravid female lizards may maintain lower body temperatures than non-gravid lizards (Smith and Ballinger 1994; Mathies and

Andrews 1997). Additionally, gravid female water snakes (*Natrix faciata* and *Natrix taxispilota*) have been shown to maintain their body temperatures within a narrower range than non-gravid members of the two species (Packard et al. 1977). Similarly, in a laboratory study using the viviparous spiny lizard *Sceloporus cyanogenys*, pregnant females terminated basking at lower body temperatures, and maintained a more stationary position in a thermal gradient, compared to non-gravid lizards (Garick 1974). This suggests that pregnant lizards are maintaining a more constant body temperature during their daily period of activity (Garrick 1974).

There are several reasons why these changes in thermoregulatory behavior are thought to occur. First, the increased body mass due to eggs results in thermal inertia, therefore it may take gravid female lizards longer to reach preferred body temperatures (Shine 1985). Gravid females also may select higher body temperatures both to increase the rate of embryonic development, and to compensate for reduced mobility (Shine 1980). In some cases, however, the preferred body temperature normally maintained by adult lizards may exceed the optimum temperature for embryonic development (Braña 1993). Higher than optimum temperatures are physiologically stressful to

embryos and can cause developmental abnormalities (Andrews et al. 1997; Andrews et al. 2000). Therefore, for some species, a tradeoff exists between the optimum physiological temperature for the gravid female and the optimum physiological temperature for the developing embryos. For this reason, gravid females may reduce their preferred body temperature to achieve a "compromise" temperature that is between the optimum for embryonic development and the physiological optimum for the adult female (Braña 1993). There is also evidence suggesting that lizard embryos can tolerate limited periods of relatively high temperatures such as those experienced during daily temperature cycles in nature (Shine and Harlow 1996). Laboratory studies conducted on skinks demonstrated that offspring from eggs incubated at a constant temperature of 30°C resulted in less active offspring with slower running speeds compared to eggs incubated at a mean temperature of 30°C but with a fluctuating temperature regime (Shine and Harlow 1996).

Gravid female lizards may also alter thermoregulatory behavior due to the physical burden of eggs contained within their bodies (Shine 1985; Braña 1993; Andrews et al. 1997). There is a potential fitness cost associated with reproduction because the additional clutch mass may

reduce sprint speed (Sinervo et al.1991), and therefore increase the probability of predation (Shine 1980; Bauwens and Thoen 1981). In some species of lizards and snakes, reproductive females become more secretive, remain relatively hidden, and are generally less active than non-gravid female or male lizards (Shine and Bull 1979; Braña 1993). These behavioral changes associated with reproduction may therefore limit or prevent gravid females from utilizing thermal environments where preferred body temperatures can be attained.

The range of life history characters expressed by a given taxa is influenced both by phylogenetic association as well as "survival costs" imposed by the environment (Dunham and Miles 1985; Schwarzkopf 1994). Species with broad geographic ranges often exhibit considerable variation in life history characters such as growth rate, age of reproductive maturity, and fecundity (Tinkle 1969; Sinervo 1990; Adolph and Porter 1993; Adolph and Porter 1996). Moreover, variation in life history traits within a single species across latitudinal clines may occur due to evolved responses to the environment, phenotypic plasticity, or a combination of both (Sinervo and Adolph 1989; Adolph and Porter 1996). Although the present distribution of viviparous squamate reptiles does not

provide direct evidence for the conditions under which viviparity evolved (Tinkle 1969; Shine 1983), intraspecific variation in reproductive mode or egg retention does provide stronger evidence for the cold climate hypothesis (Shine 1985; Mathies and Andrews 1995). For example, *Lerista bougainvilli* (Scincidae) occupies a wide geographic range over mainland Australia, including south coast islands, and has oviparous and viviparous populations (Qualls and Shine 1998). Consistent with the cold climate model, viviparous populations occupy the coldest habitats with shortest activity seasons, and oviparous populations occupy the warmest habitats. The transitional shift to extended egg retention with decreasing temperature is also observed in the green snake (*Opheodrys vernalis*). Populations in northern Michigan (ca. 45.5° N) retain eggs to an advanced developmental stage, and hatching occurs between 4 and 14 days after oviposition. By contrast, southern populations (ca. 42° N) retain eggs much shorter periods with hatching occurring approximately 30 days after oviposition (Packard et al. 1977). If cold nest temperatures favor extended egg retention, then certain wide ranging squamate taxa might be expected to show intermediate stages of egg retention on a latitudinal or elevational gradient.

Egg Retention and Evolution of
Viviparity in the Lizard
Genus *Sceloporus*

The lizard genus *Sceloporus* (Family Phrynosomatidae) is the largest genus of North American reptiles and occupies a wide geographic range, from southern Canada to Panama (Sites et al. 1995). *Sceloporus* lizards occur in variety of habitats, but are found in the greatest diversity in the southwestern United States and Mexico (Wiens and Reeder 1997). Out of approximately 80 species, roughly 30 are viviparous. The relative number of viviparous species increases with elevation, becoming the dominant mode of reproduction at elevations above 1500 meters (Méndez-De La Cruz et al. 1998). Their abundance, diversity and conspicuous behavior, make *Sceloporus* an excellent model for the study of variation in life history traits and implications for the evolution of viviparity.

Sceloporus occidentalis (western fence lizard) and *Sceloporus graciosus* (sagebrush lizard) are the northernmost members of the genus, with geographic ranges occupying numerous habitats in the western United States (Stebbins 1985). *Sceloporus occidentalis* occurs from arid desert conditions in southern California and northern Baja California to mesic coniferous forest in Washington State and extreme southwestern Canada (Stebbins 1985). Moreover,

S. occidentalis is found across a wide range of elevations, occurring from sea level to approximately 2750 meters. *Sceloporus graciosus* occupies a similar geographic range as *S. occidentalis*, but is generally found at higher elevation between approximately 150-3200 meters (Stebbins 1985). In California, *S. graciosus* is found primarily in the mountains, and is sympatric with *S. occidentalis* at intermediate elevations. In the San Gabriel Mountains of southern California, *S. graciosus* occurs between 1600-3000 meters and *S. occidentalis* from 1200-2300 meters (Adolph and Sinervo 1989). *Sceloporus occidentalis* is the larger of the two species [snout-vent length: 5.5-8.7cm vs. 4.7-6.5cm] (Stebbins 1985) and is more arboreal than *S. graciosus*, although there is considerable overlap in microhabitat use (Adolph 1990; and *personal observation*).

Despite the fact that *S. occidentalis* and *S. graciosus* are the northern most members of the genus *Sceloporus*, both species are oviparous. This is unusual because it violates the trend among many squamates species toward viviparity with increasing latitude and elevation. Interestingly, all viviparous species of *Sceloporus* found in North America occur at relatively high elevation but at low latitudes, as in the mountains of New Mexico and

Arizona (Méndez-De La Cruz et al. 1998). Moreover, *S. malachiticus* one of the two southernmost species is viviparous (Sites et al. 1992). There are several possible explanations as to why viviparity in the genus *Sceloporus* has not extended further into North America, although few have been tested empirically. The explanation receiving the greatest amount of attention posits that the northern oviparous species belong to clades which do not possess the morphological or physiological capability of becoming viviparous (Méndez-De La Cruz et al. 1998; Andrews et al. 1999). In general, members of the *undulatus* species group (including *S. occidentalis*), lay eggs at the approximate mid-point of development, embryonic stage 30 of the Dufaure and Hubert (1961) scale (Shine 1985; DeMarco 1993; Méndez-De La Cruz et al. 1998). *Sceloporus virgatus*, also a member of the *undulatus* species group, has been shown to be capable of facultative egg retention beyond stage 30 in laboratory studies, however embryonic development is retarded and egg mortality increases (Andrews and Rose 1994; Andrews 1997). Andrews et al. (1999) suggests that embryonic developmental rates in *Sceloporus* are lineage-specific, and therefore are not adapted to regional differences in temperature.

Phylogeny undoubtedly places constraints on the evolution of life history characters such as egg retention and the evolution of viviparity. However, both *S. occidentalis* and *S. graciosus* show considerable variation in life history traits among different populations across their geographical ranges (Sinervo 1990; Sinervo et al. 1991; Sinervo and Adolph 1994). Intraspecific comparison of high elevation and low elevation populations of *Sceloporus scalaris* (*Scalaris* group) in southern Arizona have shown that the duration of egg retention increases for high elevation populations compared to low elevation populations (DeMarco 1992). DeMarco (1992) also found increased egg retention and hence shorter incubation periods associated with increasing latitude and elevation for several different species of *Sceloporus* from four separate taxonomic groups.

The geographic range of *S. graciosus* extends to higher elevations than *S. occidentalis*. These high altitude populations are therefore subject to cooler temperatures and shorter activity seasons than populations of *S. occidentalis* at lower elevations (Sinervo and Adolph 1994). As a consequence, high altitude populations of *S. graciosus* may experience constraints on thermoregulatory opportunity and egg incubation

environment. If cold soil temperatures favor extended egg retention, it is possible that *S. graciosus* may exhibit intermediate stages of egg retention, assuming that the species is physiologically capable of doing so.

There are several anatomical and physiological traits that are normally considered prerequisites to the evolution of viviparity. First, the capability must exist for extending the duration of intra-uterine incubation of eggs. Second, it is well established that a tradeoff exists between the total number of eggs produced in a clutch and egg size (Tinkle 1969; Bell 1980; Reznick 1985; Sinervo 1990). Typically, viviparous reptiles produce fewer offspring, but must carry them longer than oviparous reptiles (Shine 1985). There is also a tendency for egg retaining and viviparous species to produce a single clutch or litter respectively, per year. In contrast, oviparous species, especially those inhabiting warm climates may produce multiple clutches in a single year (Tinkle 1969; Shine 1985). Finally, reduced eggshell thickness and increased vascularity of extraembryonic membranes facilitate gas exchange, and hence are viewed as requisites for the evolution of viviparity (Packard et al. 1977; Shine 1985; Mathies and Andrews 1995).

Sceloporus graciosus produces small clutches (2-5 eggs) laid once or twice per season (Stebbins 1985; and *personal observation*). Additionally, under laboratory conditions *S. graciosus* eggs develop approximately 10 percent faster over a range of temperature treatments, and have larger initial hatchling sizes, than *S. occidentalis* (Sinervo and Adolph 1994). The thermal restrictions imposed by the relatively cold, high altitude environment inhabited by *S. graciosus* may therefore influence thermoregulatory behavior of gravid females as well as favor extended egg retention. Body size has also been shown to be an important fitness characteristic in lizards (Sinervo 1990). Earlier hatching and larger initial body size may be especially important characteristics for *S. graciosus* as this permits hatchling lizards to survive, feed, and build fat reserves before cold temperatures prevent activity (Sinervo and Adolph 1989). To date, no study has examined changes in thermoregulatory behavior by gravid females or the possibility of geographic variation in egg retention time for either of these species.

Research Goals

The objectives of this study address three general aspects relating to lizard life history evolution: 1) is

there a difference in thermal biology of gravid female *S. occidentalis* and *S. graciosus* compared to non-gravid (male and female) animals, 2) if differences do exist, are there interspecific as well as intraspecific differences along an elevational gradient, and 3) are there intraspecific and interspecific differences in egg retention time along an elevational gradient. If these lizards are capable of retaining eggs, it is expected, according to the cold-climate hypothesis, that lizards from high elevation populations should retain eggs to a more advanced developmental stage than individuals from low elevations.

During the course of embryonic development, metabolic oxygen consumption increases with embryogenesis (Guillette 1982). It has been proposed that the parchment shelled eggs of most squamates acts as a barrier to gas exchange for the developing embryo (Packard et al. 1977). This observation has been supported because viviparous lizards, or lizard species exhibiting extended egg retention, generally produce thinner eggshells than non-retaining oviparous taxa (Packard et al. 1977; Shine and Bull 1979). It is therefore expected that lizards from high elevation populations will have thinner eggshells than lizards from low elevations.

CHAPTER TWO
THERMAL PHYSIOLOGY

Introduction

The thermal environment exerts a strong influence on many aspects of squamate life history (Porter et al. 1973; Adolph and Porter 1993, 1996). Environmental temperatures constrain daily activity time, seasonal activity time, growth rate (Grant and Dunham 1990; Adolph and Porter 1993, 1996; Smith and Ballinger 1994), and also influences hatchling phenotype (Sinervo and Adolph 1994; Burger 1998; Qualls and Andrews 1999; Andrews et al. 2000).

Heliothermic lizards are capable of maintaining a narrow range of elevated body temperatures corresponding to optimal physiological levels through behavioral thermoregulation (Huey 1974, 1982; Huey and Stevenson 1979; Adolph 1990; Grant 1990; Braña 1993; Andrews et al. 1997; Mathies and Andrews 1997). Optimum or preferred body temperatures in reptiles are often lineage-specific, and generally refer to the temperature at which physiological function is maximal (Braña 1993). However, the preferred body temperature of a given squamate taxon is not necessarily fixed, and optimal temperatures may vary according to different physiological processes, such as

digestion or reproductive condition (pregnancy/gravidity) [Shine 1980; Braña 1993; Andrews et al. 1997; Andrews 1997]. Additionally, the ability to attain and maintain optimal body temperatures is further constrained by ecological characteristics of the thermal environment. Ecological factors such as habitat structure, geographical location, weather, prey availability, and predator avoidance may limit or prevent lizards from thermoregulating at preferred body temperatures (Adolph 1990; Grant 1990; Andrews 1997, 1999).

Shifts in the preferred body temperatures of gravid females have been documented in many different species of squamates (Packard et al. 1977; Shine 1980; Werner 1990; Daut and Andrews 1993; Braña 1993; Andrews 1997). Gravid female lizards may show elevated or reduced body temperatures compared to non-gravid and male lizards. The direction of the temperature shifts varies among lizard taxa suggesting that there is no single underlying causal factor (Andrews 1997). Embryonic development in reptiles is temperature dependent, therefore gravid females may raise body temperature to facilitate more rapid embryonic development (Shine 1985; Daut and Andrews 1993). Conversely, excessively high temperatures, even for a short duration, may be damaging or lethal to developing

embryos (Packard and Packard 1988; Andrews 2000). In the latter situation, gravid females may choose lower body temperatures below the preferred level characteristic of adult females. Collectively, it appears that there may be a number of interrelated physical, physiological, and ecological factors responsible for the shifts in body temperature observed in reproductive female lizards.

The majority of research has evaluated the downward shifts in body temperatures often observed in reproductive females according to two main hypotheses. The encumbrance hypothesis is based upon the observation that reproductive female lizards are physically burdened by the clutch mass or litter mass contained within their bodies (Shine 1985; Andrews 1997). There is a potential fitness cost associated with reproduction in that gravid females have slower sprint speeds and therefore may be more susceptible to predation (Shine 1980; Bauwens and Thoen 1981; Sinervo et al. 1991). As a result, gravid females of some lizard species have been observed to become more secretive and remain closer to refugia than non-gravid and male lizards (Braña 1993). These behavioral changes may therefore limit or prevent reproductive females from basking long enough, or from moving to ideal basking locations, to attain preferred body temperatures.

In contrast, the thermal stress hypothesis asserts that the optimum physiological temperature of the adult female does not coincide with the optimum physiological temperature for embryonic development. As a result, reproductive females must lower their body temperature sufficiently to achieve a "compromise" temperature between the thermal optima for the female and her offspring (Braña 1993).

Andrews (1997) provides a model by which the encumbrance hypothesis and the thermal stress hypothesis may be evaluated. The main purpose of this model is to evaluate whether or not the thermal environment is limiting the ability of gravid females to achieve preferred body temperatures. Under conditions where the thermal environment is not limiting, all individuals can thermoregulate at preferred body temperatures. Under these conditions, according to the thermal stress hypothesis, gravid females should maintain body temperatures lower than non-gravid and male lizards. Alternatively, if the encumbrance hypothesis is true, then gravid females are expected to have body temperatures approximately equal to non-gravid and male individuals.

In the field, obtaining precise measurements of body temperatures between reproductive and non-reproductive

lizards is complicated by such factors as heterogeneity of the thermal environment, geographic location, weather conditions, and capture time. Measuring preferred body temperatures in the laboratory using a thermal gradient eliminates these complications, and lizards can easily thermoregulate by choosing from a variety of temperatures within a relatively short distance along the gradient.

In the present study the lizards *Sceloporus occidentalis* (western fence lizard) and *Sceloporus graciosus* (sagebrush lizard) [Phrynosomatidae] were used to evaluate the influence of reproductive condition on selected body temperature. Despite a wide geographic range encompassing many different environments, the genus *Sceloporus* is thermally conservative with the majority of species maintaining mean body temperatures while active between 34-36°C (Brattstrom 1965; Adolph 1990; Andrews 1998; Andrews et al. 1999). In Southern California, *Sceloporus occidentalis* and *S. graciosus* exist over a range of different elevations and thermal environments. Their abundance, wide distribution, and hypothesized thermal conservatism makes them ideal model organisms for the study of thermal physiology and implications for life history evolution.

Materials and Methods

Study Sites

Fieldwork was conducted at two locations on the northern slope of the San Gabriel Mountains, in Los Angeles County, and at one location at the desert base of the San Bernardino Mountains, San Bernardino County, California. The low elevation site is located in Summit Valley, on the edge of the Mojave Desert (ca. 1260m), 34°40' N, 117°30' W, approximately 5 km southwest of Hesperia. The plant community at Summit Valley consists of open California Chaparral, and was burned in a wildfire two years prior to the study. The dominant plants of the region consist of *Adenostoma fasciculatum*, *Cercocarpus betuloides*, *Prunus ilicifolia*, *Prunus fasciculata*, *Yucca whipplei* and *Quercus* sp. The site at intermediate elevation is located at Table Mountain (2230m), 34°23' N, 117°40' W, approximately 2 km northwest of Wrightwood. Dominant plants at this location consist of *Pinus jeffreyi*, *Cercocarpus ledifolius*, *Quercus kelloggii*, *Quercus chrysolepis* and *Artemesia* sp. The high elevation site is located at Dawson Saddle (2580m), 34°22' N, 117°48' W, 4km west of Mt. Baden-Powell. The plant community here is largely coniferous forest, consisting of

Pinus ponderosa, *Abies concolor*, *Pinus lambertiana*, and *Eriogonum* sp. *Sceloporus occidentalis* is common at low elevation at the arid base of the San Bernardino Mountains to an upper elevational limit of approximately 2300m (Stebbins 1985). Conversely, *S. graciosus* occurs at Dawson Saddle to a low elevational limit of approximately 1512m on the northeastern slope of the transverse range (Stebbins 1985 and *personal observation*). The two species are sympatric at Table Mountain.

Field Body Temperatures and Thermal Environment

Body temperatures of gravid female, non-gravid female, and male *S. occidentalis* (n = 6 gravid, 10 non-gravid, 18 male) and *S. graciosus* (n = 20 gravid, 35 non-gravid, 15 male) were obtained from May through July, 2000. Active lizards were captured between 1000-1900 hours by noosing, and body temperatures measured immediately using a Sensortek, model BAT-12 thermocouple thermometer with a copper-constantan thermocouple probe coated with epoxy. The probe was inserted 5.0-10.0 mm into the cloaca to obtain body temperatures. If lizards moved from their original microhabitat, temperatures were only recorded for individuals captured within one minute of this change in location. After recording body temperature, I also measured mass and snout-vent length (SVL) for each

individual captured. Ambient air temperatures were measured using a thermocouple probe placed in the shade approximately 30cm above the lizard perch location. The thermocouple thermometer was calibrated using a standard laboratory mercury thermometer.

Laboratory Selected Body Temperatures

To assess the thermoregulatory behavior of lizards without the complications of environmental factors, I measured selected body temperatures of gravid female, non-gravid female, and male *S. occidentalis* (n = 5 gravid, 7 non-gravid, 5 male) and *S. graciosus* (n = 9 gravid, 10 non-gravid, 7 male) in thermal gradients. Each thermal gradient consisted of a rectangular masonite box (154cm x 41cm) with a partition dividing each thermal gradient lengthwise into two equal halves such that a single lizard could be placed in each half. The bottom of the thermal gradients were covered with a thin layer of aquarium gravel to provide the lizards traction and a medium to bury themselves in. A 125-watt incandescent heat lamp was suspended 42 cm above the end of each thermal gradient. The temperatures of the thermal gradient ranged from 60.0° directly under the light, to 26.0°C in the middle of the gradient, and 22.0°C at the opposite (unlighted) end of the gradient. Temperatures were

measured using a lizard model constructed from copper metal piping (65mm x 16mm), and painted with Krylon® "Gray Sandable Primer" (#1318) spray paint. A thermocouple probe was inserted into the model, and the end sealed with a cork. The paint used to coat the model has been shown to closely match the reflectivity of live *Sceloporus* lizards (Adolph *personal communication*). Ambient light was provided by overhead fluorescent lighting as well as natural light from laboratory windows. Lizards were otherwise maintained under a 13:11 light/dark cycle using both incandescent heat and fluorescent lighting.

To facilitate capturing the lizards quickly for temperature measurement, each lizard was tethered to the side of the thermal gradient using a length of fine thread. The tether was tied lightly around the abdomen anterior to the hind limbs, and sufficiently long to allow free movement along the length of the thermal gradient. The behavior of the lizards did not appear to be altered by the presence of the tether. Lizards were fasted for 24 hours and allowed to acclimate overnight in the thermal gradient (under standard 13:11 photoperiod) before temperature readings were taken. All selected body temperature readings were obtained between 1300 and 1700 hours using a Sensortek, model Bat-12 thermocouple

thermometer, and within one week of capture in the field. Three temperature readings were taken at hourly intervals from each lizard during a single day. Each lizard was used only once during each thermal gradient trial.

Statistical Analysis

Statistical analyses were performed using "SPSS for Windows®", version 8.0 statistical software package.

Field body temperature data were analyzed using two-factor analysis of variance (ANOVA), with body temperature as the dependent variable, and species and reproductive condition (levels: gravid, non-gravid, male) as independent variables. Field body temperature data within each species at different elevations were also analyzed using two-factor ANOVAs, with body temperature the dependent variable, reproductive condition and elevation the dependent variables. Laboratory thermal gradient data were analyzed using two-factor repeated measures ANOVA, with body temperature as the dependent variable (three body temperature measurements per individual), and species and reproductive condition the independent variables.

Additionally, differences in selected body temperature relative to reproductive condition within a single species were analyzed using single-factor repeated measures ANOVA. There were no significant differences in selected body

temperature relative to elevation for either species, therefore in each reproductive class (gravid, non-gravid, and male), body temperatures for each species from different elevations were pooled. All assumptions of homogeneity of variance and sphericity were satisfied for these analyses. Post hoc tests for differences in mean body temperatures between the different class variables was analyzed using the Bonferroni test for multiple comparisons. All repeated measure ANOVAs were carried out using the "general linear model-repeated measures" function in SPSS for Windows®. All single factor ANOVAs were conducted using the "general linear model" function in SPSS for Windows®.

Results

Field Body Temperatures

Active *S. graciosus* and *S. occidentalis* in the field maintained a narrow range of body temperatures between 34.0°C and 35.5°C over a range of ambient air temperatures at different elevations (Figure 1a, and 1b, Table 1). Mean field body temperatures of *S. occidentalis* and *S. graciosus* did not vary significantly between elevations (two-way ANOVAs: $F_{1,28} = 0.221$, $P = 0.642$, Appendix A: Table a1, $F_{1,64} = 1.853$, $P = 0.178$, Appendix A: Table a2,

S. occidentalis and *S. graciosus*, respectively). A complete list of tables summarizing all statistical analyses is given in Appendixes A and B. Reproductive condition did not have a significant effect on body temperature for *S. occidentalis* (two-way ANOVA: $F_{2,28} = 1.064$, $P = 0.359$, Appendix A: Table a1), and the reproductive condition x elevation interaction was also not significant ($F_{2,28} = 0.926$, $P = 0.408$). Body temperatures of gravid female *S. occidentalis* were on average approximately 0.9°C warmer than non-gravid females and 0.5°C warmer than male lizards (Figure 2, Table 2).

For *S. graciosus*, the effect of reproductive condition on field body temperature approached significance (two-way ANOVA: $F_{2,64} = 2.714$, $P = 0.074$, Appendix A: Table a2). Gravid, non-gravid, and male sagebrush lizards exhibited the same pattern of field body temperatures at Dawson Saddle and Table Mountain, resulting in non-significant reproductive condition x elevation interaction (two-way ANOVA: $F_{2,64} = 2.133$, $P = 0.127$). Unlike gravid female *S. occidentalis*, mean body temperatures of gravid female *S. graciosus* were lower by an average 0.5°C compared to non-gravid females, and

approximately 1.2°C cooler than the mean body temperature of male lizards (Table 2, Figure 2).

The mean body temperature was approximately 1.0°C higher in gravid female *S. occidentalis* than in gravid female *S. graciosus* (Table 2, Figure 2), however mean field body temperatures did not vary significantly between the two species (two-way ANOVA: $F_{1,98} = 0.142$, $P = 0.707$, Appendix A: Table a3). Although gravid female *S. occidentalis* had higher mean body temperatures than both non-gravid and male *S. occidentalis*, while gravid *S. graciosus* had lower mean body temperatures than non-gravid and male lizards, there was no significant interaction between species and reproductive condition (two-way ANOVA: $F_{2,98} = 1.854$, $P = 0.162$).

Laboratory Selected Body Temperatures

In laboratory thermal gradient trials, there was no significant difference in mean body temperature between *S. occidentalis* and *S. graciosus* (two-way ANOVA: $F_{1,37} = 0.014$, $P = 0.907$, Appendix A: Table a4). Reproductive condition, however had a significant effect on mean selected body temperature (two-way ANOVA: $F_{2,37} = 3.264$, $P = 0.049$), with gravid females maintaining lower mean body temperatures than non-gravid lizards

(Figure 3). Gravid, non-gravid, and male lizards of both species exhibited the same general trend in selected body temperatures, and there was no significant interaction between species and reproductive condition (two-way ANOVA: $F_{2,37} = 1.132$, $P = 0.333$).

For *S. graciosus*, reproductive condition had a significant effect on mean selected body temperature (one-way repeated measures ANOVA: $F_{2,23} = 5.236$, $P = 0.013$, Appendix A: Table a6). Gravid female *S. graciosus* maintained body temperatures an average of 1.5°C lower than non-gravid females, and 1.4°C lower than male lizards ($P = 0.018$ and $P = 0.064$ respectively, independent pairwise comparisons, Bonferroni adjustment for multiple comparisons). By contrast, non-gravid and male sagebrush lizards maintained nearly identical selected body temperatures in the thermal gradient (35.4°C and 35.3°C, non-gravid and male lizards respectively) [Table 2, Figure 3].

The mean selected body temperature of gravid females was lower by an average of 0.8°C compared to non-gravid females (Table 2), though there was no significant difference in selected body temperature observed for *S. occidentalis* relative to reproductive condition

(one-way repeated measures ANOVA: $F_{2,14} = 0.741$, $P = 0.495$, Appendix A: Table a5). Although not significant, gravid female *S. occidentalis* were on average 0.8°C warmer than gravid female *S. graciosus*.

Discussion

In the field, *S. occidentalis* and *S. graciosus* maintain remarkably consistent body temperatures across the 1380m elevational range encompassing the three study areas despite considerable variation in physical and thermal environment. Adolph (1990) found significant variation in mean body temperatures between lizard populations at Dawson Saddle, Table Mountain, and the desert (low elevation), yet these differences were small compared to the large change in mean air temperature from high to low elevation.

Furthermore, despite the relatively high elevations occupied by mountain populations of *S. occidentalis* and particularly *S. graciosus*, altitude per se does not appear to constrain the ability of lizards to obtain preferred body temperatures. The montane environments occupied by these lizards are relatively open, with considerable heterogeneity in habitat structure. Additionally, during late spring and summer, the amount of solar radiation at

this latitude extremely high, exceeding the amount received at the Earth's equator (Andrews 1998). Even at high elevation, intense solar radiation allows lizards to obtain relatively high body temperatures, while complex habitat structure provides numerous microhabitats for thermoregulation (Adolph 1990).

Although mean field body temperatures were not significantly different between the three elevations, these results apply only to the body temperatures of actively thermoregulating lizards. This study does not address the body temperatures of lizards while in their nighttime refugia or during other periods of inactivity. The thermal environment at high elevations likely limits the total amount of time that lizards are able to maintain preferred body temperatures on a daily or seasonal basis (Sinervo and Adolph 1994).

Gravid female *S. graciosus* in the field exhibited a non-significant reduction in body temperature compared to male and non-gravid female lizards. The mean body temperature of gravid females was lower by 1.2°C compared to male lizards, whereas gravid female were cooler by 0.5°C compared to non-gravid females. Likewise, the mean body temperatures of non-gravid female sagebrush lizards was lower by approximately 0.5°C compared to male lizards.

In the laboratory, the reduction was more dramatic; the mean selected body temperature of gravid females was significantly lower by an average of 1.5°C compared to non-gravid females, and 1.4°C lower than males. Moreover, non-gravid females and males had approximately the same selected body temperatures (35.4°C and 35.2°C, respectively).

The fact that gravid female *S. graciosus* maintained significantly lower body temperatures in the thermal gradient suggests that these lizards are actively selecting lower body temperatures rather than passively accepting ambient microhabitat temperatures. These results are also unusual in that most downward shifts in body temperature of reproductive lizards have been recorded in viviparous taxa (Shine 1980; Angilletta et al. 2000). Thermal conditions at the study sites are undoubtedly favorable, with numerous microhabitats for thermal regulation. Therefore, it is unlikely that gravid females would have to move far to locate warm, sunny basking sites. If clutch burden interfered with the ability of gravid females to attain preferred body temperatures, one would expect their mean body temperatures to increase in the thermal gradient compared to the field. Instead, these

data support the view that the normal preferred body temperature for this species may be excessively high for the physiology of the developing embryos.

For gravid lizards, a tradeoff may exist between maintaining optimum temperatures for embryonic development and maintaining optimum physiological temperatures for the female. Maintaining body temperatures that are a compromise between these two factors is further influenced by ecological constraints of the environment (Braña 1993). In the field, there is likely considerable variance in temperature between different microhabitats. To maintain preferred body temperatures, gravid female lizards may have to shuttle between microhabitats that may be excessively hot, or excessively cool. Even at high elevation, ground temperatures in the full sun during late spring and summer may exceed 50°C (*personal observation*). At Dawson Saddle in particular, the difference in ambient air temperatures between sun and shade may be dramatic (Adolph 1990). Additionally, there could be other ecological factors such as prey distribution that limit the ability of gravid *S. graciosus* to maintain low body temperatures in the field. Alternatively, gravid *S. graciosus* may shorten daily activity times as a way of compensating for body temperatures higher than optimum for

embryonic development. Previous studies have shown that hatchling *S. graciosus* tend to be active during only part of the activity day, even when temperatures are high enough to allow extended activity (Sinervo and Adolph 1989, 1994). It is therefore plausible that gravid lizards could modify daily activity periods to ameliorate the effects of constant elevated body temperatures.

Unlike *S. graciosus*, gravid *S. occidentalis* females did not exhibit a significant reduction in body temperatures compared to non-gravid female and male lizards. Although not significant, in the field, gravid *S. occidentalis* exhibited higher body temperatures than non-gravid female and male lizards (Figure 2, Table 2). These values may be biased towards higher than normal temperatures as they were obtained from a sample of only six individuals. In the laboratory thermal gradient, mean body temperatures of gravid female fence lizards were cooler by an average of 0.8°C, compared to non-gravid lizards, while having approximately the same mean body temperatures as male lizards (Figure 3, Table 2).

Since there was no significant difference between body temperatures of gravid *S. occidentalis* and the other two reproductive classes either in the field or in the laboratory, it does not appear that clutch burden

interferes with their ability to thermoregulate effectively. Mean selected body temperatures of gravid females in the field were actually slightly higher than mean selected body temperatures in the thermal gradient (Table 2). Additionally, gravid female lizards were often observed perched several meters off the ground in the branches of shrubs, suggesting that clutch mass did not interfere with their mobility (*personal observation*). Sinervo et al. (1991) showed that gravid *S. occidentalis* had reduced sprint speeds compared to non-gravid females; however, speed per se may not constrain the ability of gravid females to obtain basking sites. The lack of significant variation between the body temperatures of gravid female versus non-gravid female and male *S. occidentalis* suggests that the optimum physiological temperature of adult female lizards coincides with the optimum physiological temperature for embryonic development. These observations are consistent with temperature measurements of gravid female, non-gravid female, and male *Sceloporus undulatus*, a close relative of *S. occidentalis* (Angilletta et al. 2000). While gravid female fence lizards did exhibit slightly lower selected body temperature in the thermal gradient, this downward shift was neither significant nor as dramatic as seen in

S. graciosus. At the very least, it appears that the *S. occidentalis* embryos may have higher thermal optimum temperature for development compared to *S. graciosus*.

The egg physiology of *S. occidentalis* and *S. graciosus* differs considerably, and may explain the differences in selected body temperatures of gravid females observed in these two species. In the laboratory, the eggs of *S. graciosus* have a shorter incubation period by approximately 10% over a variety of temperatures compared to the eggs of *S. occidentalis* (Adolph 1990). Additionally, at high incubation temperatures, the eggs of *S. graciosus* experience increased mortality and hatchlings also show a greater degree of developmental abnormalities (Adolph 1990 and *personal communication*). In general, *S. occidentalis* occupies lower elevations and a wider range of habitats compared to *S. graciosus*. Conversely, *S. graciosus* generally occupies higher elevations with presumably lower average soil temperatures compared to *S. occidentalis*. The shift to lower selected body temperatures by gravid female *S. graciosus* may be in response to the lower thermal optima of their eggs during incubation. Faster incubation time and low thermal optima may be favorable traits in the high elevation environments

inhabited by *S. graciosus*. The higher thermal optima of *S. occidentalis* embryos may reflect its occupation of a wider range of environments at generally lower and therefore warmer elevations.

Temperature plays an integral role in molding the life history and ecology of squamates (Adolph 1990; Adolph and Porter 1993, 1996). *Sceloporus graciosus* and *S. occidentalis*, like other members of the genus are thermally conservative, and show little variation in body temperature over a range of altitudes and thermal environments. In addition, these two lizard species share similar thermal physiological requirements such as preferred body temperature and critical thermal maxima and minima (Adolph 1990). Despite these similarities, gravid *S. graciosus* shows a substantial downward shift from its typical preferred body temperature in the laboratory thermal gradient while gravid *S. occidentalis* does not. Previous studies have shown that temperature and moisture requirements of eggs may influence the distribution of some oviparous lizard species (Muth 1980). Adolph (1990) suggests that the egg physiology of *S. occidentalis* and *S. graciosus* may in fact limit the altitudinal distribution of these two species. The variation in the thermal biology of gravid females observed in these two

species may be the result of differences in egg physiology that reflect adaptations to the different environments in which these lizards live.

Table 1. Mean field body temperatures (\pm SE) of active *Sceloporus occidentalis* and *Sceloporus graciosus* from the three study sites

Body Temperature ($^{\circ}$ C)				
Population	Elevation (m)	Gravid Female	Non-gravid Female	Male
<i>S. graciosus</i> , Dawson Saddle	2580	33.8 \pm 0.54 (8)	34.0 \pm 0.44 (12)	35.8 \pm 0.62 (6)
<i>S. graciosus</i> , Table Mountain	2230	34.7 \pm 0.44 (9)	35.3 \pm 0.32 (23)	35.2 \pm 0.50 (9)
<i>S. occidentalis</i> , Table Mountain	2230	36.3 \pm 0.951 (2)	34.3 \pm 0.55 (6)	35.2 \pm 0.44 (9)
<i>S. occidentalis</i> , Summit Valley	1260	35.2 \pm 0.67 (4)	35.0 \pm 0.67 (4)	34.8 \pm 0.45 (9)

Reproductive classes are given, and sample sizes are shown in parentheses beneath mean body temperatures. There are no significant differences between mean body temperatures for either species between elevations.

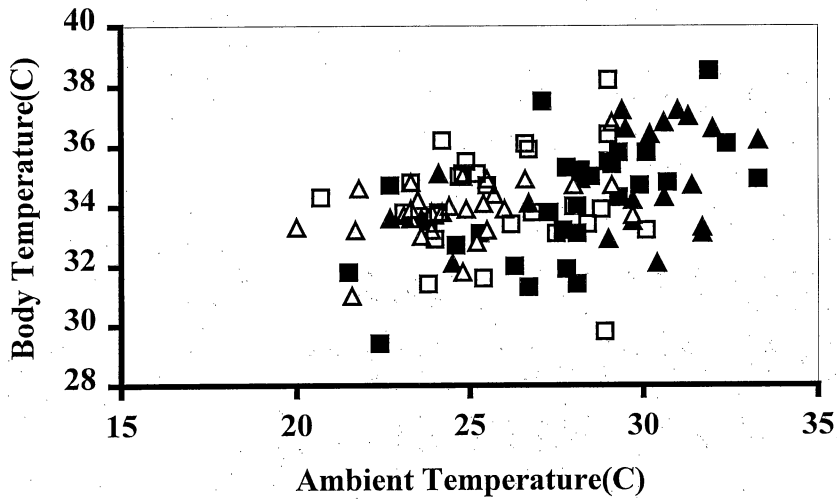
Table 2. Mean body temperatures (\pm SE) of gravid, non-gravid and male, *Sceloporus occidentalis* and *Sceloporus graciosus* in the field and in laboratory thermal gradients

Species				
Reproductive Condition	<i>S. occidentalis</i>		<i>S. graciosus</i>	
	Tb_{field} (°C)	$Tb_{gradient}$ (°C)	Tb_{field} (°C)	$Tb_{gradient}$ (°C)
Gravid	35.5 \pm 0.42 (6)	34.6 \pm 0.6 (5)	34.4 \pm 0.26 (20)	33.8** \pm 0.36 (9)
Non-gravid	34.3 \pm 0.54 (10)	35.4 \pm 0.51 (7)	34.9 \pm 0.27 (35)	35.4** \pm 0.51 (10)
Male	35.0 \pm 0.27 (18)	34.5 \pm 0.6 (5)	35.4 \pm 0.35 (15)	35.2 \pm 0.41 (7)

Sample sizes are shown in parentheses below mean body temperatures. For each species, the body temperatures listed represent the combined population mean body temperature for each of the reproductive classes. In the thermal gradient, *S. graciosus* maintained significantly lower body temperatures than non-gravid female lizards.

(**) = significant at $P = 0.05$

Figure 1a. Body temperature relative to air temperature for male and non-gravid female *S. occidentalis* at Table Mountain (intermediate elevation), and Summit Valley (Low elevation).



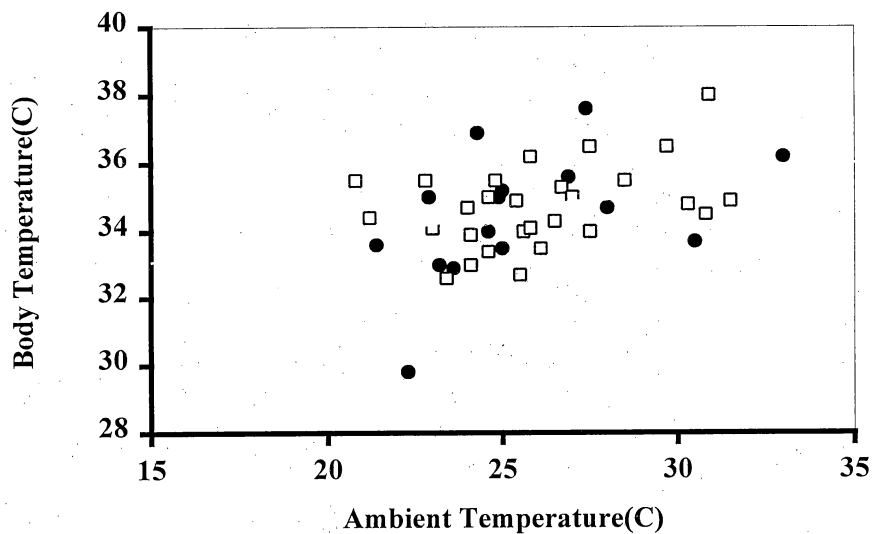
Solid line represents equal body and air temperature.

(▲) = female, low elevation, (■) = male, low elevation,

(△) = female, intermediate elevation, (□) = male,

intermediate elevation.

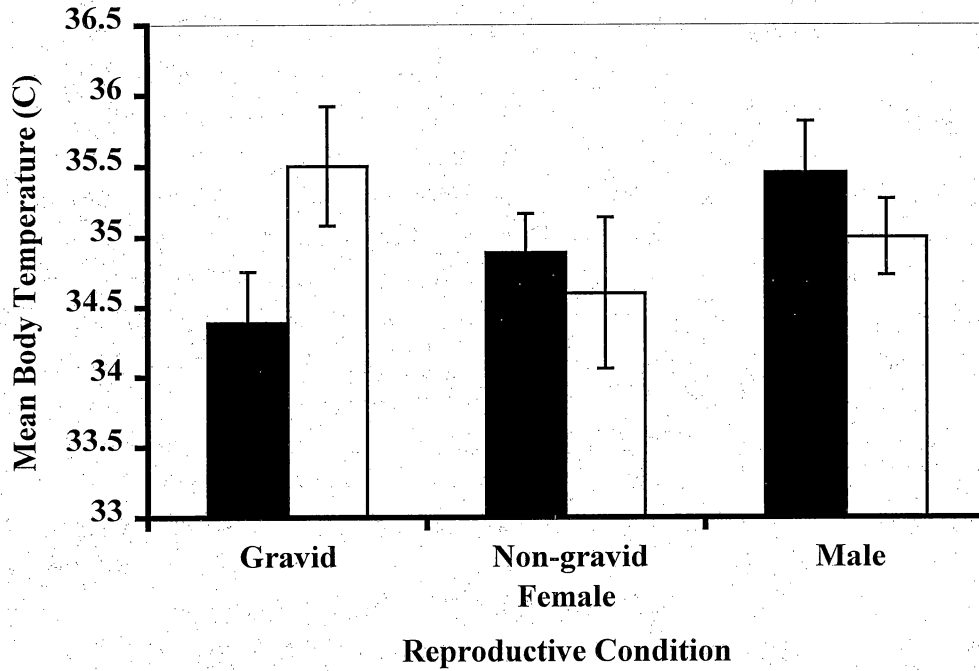
Figure 1b. Body temperature relative to ambient temperature of male and non-gravid female *Sceloporus graciosus* at Table Mountain.



Solid line represents equal body and ambient temperature.

(●) = female, (□) = male

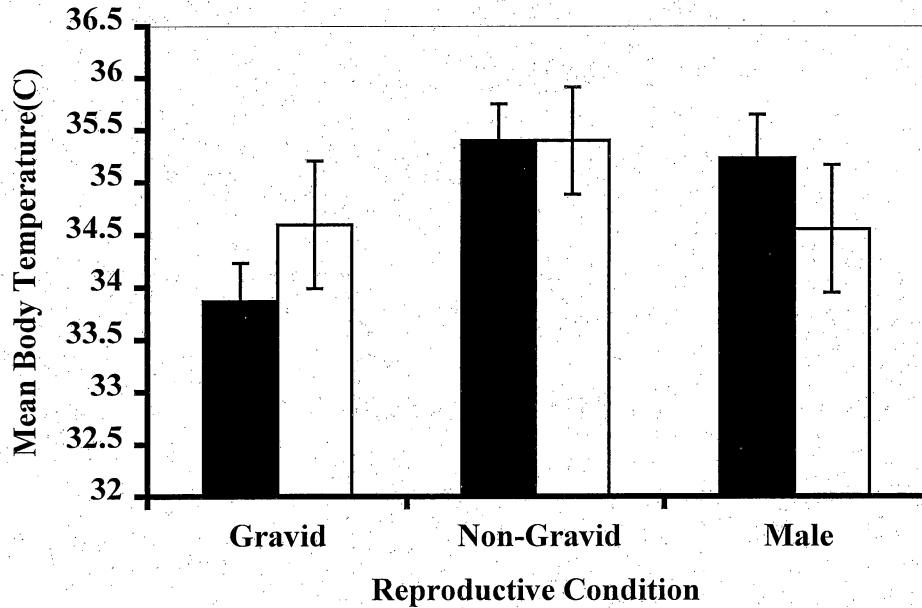
Figure 2. Mean field body temperatures (\pm SE) of gravid, non-gravid, and male *Sceloporus occidentalis* and *Sceloporus graciosus*.



Mean field body temperatures for each species were calculated using the pooled population body temperatures from each of the three reproductive classes.

(■) = *S. graciosus* (□) = *S. occidentalis*

Figure 3. Mean selected thermal gradient body temperatures (\pm SE) for gravid, non-gravid, and Male *Sceloporus occidentalis* and *Sceloporus graciosus*.



Mean selected body temperatures for each species were calculated using the pooled population body temperatures from each of the three reproductive classes. Mean selected body temperatures of gravid *S. graciosus* were significantly lower than non-gravid *S. graciosus*.

(■) = *S. graciosus* (□) = *S. occidentalis*

CHAPTER THREE
REPRODUCTIVE BIOLOGY

Introduction

The thermal environment exerts a strong influence on lizard life histories by constraining such factors as daily and seasonal activity time, fecundity, growth (Sinervo and Adolph 1989; Adolph and Porter 1993, 1996), and embryonic development (Packard et al. 1977; Shine 1985). In squamate reptiles, the rate of embryonic development is temperature dependent, such that developmental rate increases with warmer temperatures (Muth 1980; Shine 1983; Packard and Packard 1988). Cold nest temperatures can slow development of eggs deposited in the soil (Muth 1980), cause developmental abnormalities (Shine 1995; Shine and Harlow 1993; Qualls and Shine 1996; Qualls and Andrews 1998), or result in mortality of embryos (Packard et al. 1977; Andrews 2000). It is widely believed that viviparity in squamate reptiles evolved in response to cold environmental temperatures through a gradual increase in duration of egg retention within the oviduct (Packard et al. 1977; Shine and Bull 1979; Shine 1983, 1985; Mathies and Andrews 1995). Heliothermic reptiles can maintain elevated and relatively constant

body temperatures despite fluctuation in ambient temperature through behavioral thermoregulation (Cowles and Bogert 1944; Shine 1985; Adolph 1990; Hertz et al. 1993). Retaining eggs in the oviduct allows female lizards to maintain a warmer incubation environment for developing embryos than would be possible if the eggs were deposited in the soil (Packard et al. 1977; Shine 1983). According to the "cold climate model," gradual increases in the duration of egg retention within the oviduct results in eggs with embryos being laid at increasingly advanced developmental stages. The final outcome of the evolutionary transition is full intrauterine incubation, resulting in fully developed young at the time of oviposition/parturition (Packard et al. 1977; Shine and Bull 1979).

The cold climate model is supported by the observation that the proportion of viviparous squamate taxa increases both at high latitudes and at high elevations (Packard et al. 1977; Shine 1985). However, the conditions which originally led to the evolution of viviparity cannot be explained directly on the basis of the geographic distributions of extant squamate reptiles (Tinkle 1969; Shine 1983). If egg retention confers increased reproductive success in cold environments,

oviparous squamates inhabiting high elevations may exhibit intermediate stages of egg retention compared to oviparous species inhabiting warmer environments at low elevation. Interspecific comparisons of the reproductive life histories of squamates inhabiting different thermal environments lends some evidence in support of the cold climate model (DeMarco 1992). However, the results of these studies may be complicated by patterns that have a phylogenetic rather than adaptive basis (Andrews et al. 1999; review Blackburn 2000). By contrast, intraspecific variation in egg retention time along latitudinal or elevational gradients suggests a potential adaptive response to different thermal environments (Mathies and Andrews 1995).

In the present study, the lizards *Sceloporus occidentalis* (western fence lizard) and *Sceloporus graciosus* (sagebrush lizard) [Phrynosomatidae] were used to evaluate the potential influence of thermal environment on reproductive life history traits. *Sceloporus occidentalis* and *Sceloporus graciosus* have geographic ranges extending farther north than any other member of the genus *Sceloporus*. *Sceloporus occidentalis* occurs in a wide variety of habitats, from arid desert conditions in Southern California to mesic coniferous forest in

Washington State (Stebbins 1985). Throughout much of the Western United States, *Sceloporus graciosus* occurs in mountainous regions at higher elevations than *S. occidentalis* (Stebbins 1985). Despite their northern geographic ranges and occupation of relatively high elevation environments, both of these lizard species are oviparous. This is unusual because it violates the trend toward viviparity with increasing latitude and elevation.

In Southern California, both *S. occidentalis* and *S. graciosus* occur in high abundance over a range of elevations and thermal environments within relatively close geographic proximity. *Sceloporus graciosus* occupies an altitudinal range from approximately 1515m to nearly 3030m, while *S. occidentalis* occurs from sea level to 2300m (Stebbins 1985; and *personal observation*). Cold environmental temperatures may be detrimental to embryonic development by prolonging the incubation period, reducing the quality of offspring, or resulting in mortality of embryos. To evaluate the influence of cold temperatures on egg retention time, I tested the hypothesis that populations of *S. occidentalis* and *S. graciosus* at higher elevations have longer egg retention times than lizards from lower elevations.

There are potential physiological costs associated with increased egg retention time for both the mother and her offspring. For example, the optimum physiological temperature for the adult gravid female may not coincide with the optimum physiological temperature for the developing embryos (Braña 1993). Additionally, an increase in the duration of time spent in the oviduct is generally accompanied by an increase in the metabolic demands of the growing embryos (Packard et al. 1977; Andrews et al. 2000). It is believed that the eggshell limits respiratory gas exchange for the embryo, and many viviparous squamates accordingly have thinner or non-existent eggshells (Packard et al. 1977; Shine 1985; Mathies and Andrews 1995). Therefore, I tested the hypothesis that lizard populations at high elevations have thinner eggshells compared to populations at lower elevations due to selection for enhanced gas exchange with increased egg retention time.

Finally, there is usually a shift to the production of fewer eggs in squamate taxa exhibiting viviparity or extended egg retention (Shine 1980; Shine 1985; Mathies and Andrews 1995). Without a reduction in number of eggs, the increasing mass of the developing offspring associated with extended egg retention could potentially incur a high

survival cost to the female (Shine 1985; Mathies and Andrews 1995). If high elevation populations of *S. graciosus* and *S. occidentalis* exhibit extended egg retention, smaller clutch sizes should be associated with increased retention time in the oviduct (Shine 1985; Mathies and Andrews 1995). I therefore tested the hypothesis that both *S. occidentalis* and *S. graciosus* would have smaller clutch sizes at higher elevations.

Materials and Methods Study Sites

Fieldwork was conducted at two locations on the northern slope of the San Gabriel Mountains, in Los Angeles County, and at one location at the desert base of the San Bernardino Mountains, San Bernardino County, California. The low elevation site is located in Summit Valley, on the edge of the Mojave Desert (1260m), 34°40' N, 117°30' W, approximately 5.0 km west of Hesperia. The plant community at Summit Valley consists of open California chaparral, and was underwent a natural burn two years prior to the study. The dominant plants of the region consist of *Adenostoma fasciculatum*, *Cercocarpus betuloides*, *Prunus ilicifolia*, *Prunus fasciculata*, *Yucca whipplei* and *Quercus sp.* The site at intermediate elevation is located at Table Mountain (2230m), 34°23' N,

117°40' W, approximately 2 km northwest of Wrightwood. Dominant plants at this location consist of *Pinus jeffreyi*, *Cercocarpus ledifolius*, *Quercus kelloggii*, *Quercus chrysolepis* and *Artemesia* sp. The high elevation site is located at Dawson Saddle (2580m), 34°22' N, 117°48' W, 4km west of Mt. Baden-Powell. The plant community here is largely coniferous forest, consisting of *Pinus ponderosa*, *Abies concolor*, *Pinus lambertiana*, and *Eriogonum* sp. *Sceloporus occidentalis* is common at low elevation at the arid base of the San Bernardino Mountains, to an upper elevational limit of approximately 2300m. *Sceloporus graciosus* occurs at Dawson Saddle to a low elevation limit of approximately 1512m on the northeastern slope of the San Gabriel and San Bernardino Mountains (*personal observation*). The two species are sympatric at Table Mountain.

Care of Captive Lizards and Egg Incubation

Gravid female *S. graciosus* were captured in the wild using a lizard noose and brought within 24 hours to the laboratory at California State University San Bernardino, where they were housed individually in 30cm x 20cm plastic tubs. All lizards were provided with a substrate of peat

moss and sand (2:1), with one half of the substrate moistened to prevent the desiccation of eggs. Lizards were maintained under a 13:11 light/dark cycle simulating the natural photoperiod during spring and early summer. Captive lizards were provided with full spectrum heat lamps at one end of their container, and a shaded region at the opposite end. Full spectrum lighting was also provided with fluorescent "Vita-Lights®". Water and food (crickets and mealworms dusted twice weekly with commercial vitamin and calcium supplement) were provided ad libitum.

Containers with gravid females were checked once or twice a day for eggs. Upon oviposition, eggs were weighed individually to the nearest 0.01g using an electronic balance. Eggs to be incubated were placed individually in plastic specimen cups filled with vermiculite, and maintained with a water potential at approximately -200kPa (Tracy et al. 1978). This corresponds to 0.8g water per gram of dry vermiculite. The incubation cups were sealed with laboratory Parafilm®, a single puncture was placed in the upper surface of the Parafilm, and the eggs incubated at a constant temperature of 28°C. The total initial mass of the egg, cup, and incubation media was obtained, and

the specimen cups were weighed weekly, replacing water to maintain initial weight.

Determination of Embryonic
Stage at Oviposition and
Eggshell Thickness

I measured egg retention time for *S. occidentalis* (n = 5 clutches Summit Valley, n = 3 clutches Table Mountain) and *S. graciosus* (n = 8 clutches Dawson Saddle, n = 9 clutches Table Mountain) by determining the embryonic stage at oviposition (ESO) for lizards from the four study populations. I determined the ESO by dissecting two entire clutches for each species from each of the study populations, and thereafter two eggs from each clutch on the day of oviposition. All embryonic stages of development were determined according to the Dufaure and Hubert (1961) scale. The assignment of a given embryonic stage of development required that a number of anatomical characters be present in the embryo (Table 3). This minimizes ambiguity and the possibility of assigning developmental stages based upon subjective criteria.

To compare the rate of embryonic development within and between species, I dissected and staged a single egg from each clutch every seven days (*S. occidentalis*: Summit Valley n = 3 clutches, Table Mountain n = 3 clutches,

S. graciosus: Dawson Saddle, n = 2 clutches, Table Mountain, n = 4 clutches). The developmental rate was determined for only one clutch of *S. occidentalis* over a period of 28 days due to the limited number of eggs and clutches available. Likewise, the developmental rate was determined for only one clutch from *S. graciosus* over a 21-day period for the same reasons.

I measured differences in eggshell thickness using scanning electron microscopy (SEM) for *S. occidentalis* (Table Mountain: n = 6 eggs/3clutches, Summit Valley: n = 6 eggs/3clutches), and *S. graciosus* (Dawson Saddle: n = 13 eggs/8 clutches, Table Mountain: n = 9 eggs/7 clutches). I sampled 1-3 eggshells from each clutch at oviposition and carefully dissected away the extraembryonic membranes from the inner surface of the eggshell. The eggshells were rinsed with distilled water and fixed in a solution of 2.0% formaldehyde and 4.0% glutaraldehyde for 24-48 hours. The shells were then removed and placed in a 1.0% solution of osmium tetroxide for 24 hours. Residual water was removed using an alcohol dehydration series consisting of progressively higher concentrations of ethanol (25, 50, 70, 90, 95, 100% ethanol). The eggshell samples were sequentially placed in each ethanol concentration for 10 minutes, beginning with

the most dilute (25%) concentration. The samples were placed in 100% ethanol solution twice to ensure the removal of any remaining water. After dehydration, the shells were frozen in liquid nitrogen, then fractured (using a frozen razorblade) into three samples per egg. The eggshell samples were placed in glass vials and dried using hexamethyldisilazane (HMDS) to remove ethanol and any residual traces of water. HMDS minimizes shrinkage, resulting in less distortion of the eggshells compared to other drying methods (*personal observation*). The fractured eggshells were mounted on aluminum stubs and coated with gold for 2.0 minutes using a "Hummer VI-A" sputter coater before viewing with the SEM.

I measured eggshell thickness with the measurement function using a Hitachi S-2700 scanning electron microscope with tungsten filament. The microscope was calibrated using a micrometer, and corrections made for proper stigmation and column alignment prior to measuring each sample. It did not appear that specimen preparation altered eggshell thickness or structure, as I did not observe any cracks or other distortions on the surface of the eggshell. I measured eggshell thickness in three randomly chosen locations on each sample for a total of nine measurements per egg. On a small number of eggs

(*S. graciosus* n = 2 eggs, *S. occidentalis* n = 1 egg), I only obtained six measurements per egg due to the poor condition of the samples. All measurements were performed at a magnification of 600X, working distance 25mm, 15kV accelerating voltage, and 40 μ m aperture.

Statistical Analyses

Statistical analyses were performed using "SPSS for Windows®, version 8.0" statistical software package. All statistical analyses using analysis of variance (ANOVA), or analysis of covariance (ANCOVA) were performed using the "general linear model" function in SPSS for Windows. Differences in snout-vent length (SVL) and body mass for male and female *S. occidentalis* and *S. graciosus* at different elevations were analyzed using two-factor ANOVA, with SVL and body mass as the dependent variable, gender and elevation (levels: low, intermediate, high) as the independent variables. Comparisons of SVL and body mass were performed separately for each species. All female lizards measured were reproductively mature; therefore comparisons were made based upon gender instead of reproductive status and gender.

Differences in relative clutch mass (RCM) were analyzed using Student's *t*-test (SPSS for Windows:

"compare means, t-test" function). Relative clutch mass was calculated by dividing the total clutch mass by the female mass (post oviposition), and was transformed using the arcsine-square root transformation prior to statistical analysis. Differences in clutch mass for intermediate and high elevation populations of *S. graciosus* and *S. occidentalis* were analyzed using one-way ANOVA. Analysis of covariance (ANCOVA) was used to test for differences in clutch mass after controlling female SVL (covariate) for intermediate and high elevation populations of *S. graciosus* and intermediate and low elevation populations of *S. occidentalis*. The assumption of homogeneity of slopes was satisfied by testing for significance of the interaction terms SVL * elevation ($P > 0.05$). The individual relationship between SVL and clutch mass for each species at each elevation was analyzed using simple regression (SPSS for Windows: "regression" function).

Differences in egg mass for *S. occidentalis* and *S. graciosus* relative to elevation were analyzed using single-factor nested ANOVA, with the random factor "clutch" nested within elevation. Interspecific differences in egg mass were analyzed using single-factor

ANOVA, with the random factor "clutch" nested within species.

Differences in eggshell thickness for *S. occidentalis* at low and intermediate elevation, and *S. graciosus* at intermediate and high elevation, were analyzed using a hierarchical nested ANOVA. This statistical model utilizes the hierarchical nesting of random factors ("sample", "egg", and "clutch"), nested within fixed factors ("elevation" and "species") such that the specific sources of variation at each level may be identified. There was no significant effect of elevation on eggshell thickness, therefore measurements of eggshell thickness at different elevations were pooled for each species (model: eggshell thickness = species clutch [species] + egg[clutch[species]] + sample[egg[clutch[species]]]). Within species eggshell thickness was analyzed in a similar fashion according to the model: eggshell thickness = elevation clutch [elevation] + egg[clutch[elevation]] + sample[egg[clutch[elevation]]]. The assumption of homogeneity of variance was violated for intraspecific comparisons of eggshell thickness relative to elevation for *S. occidentalis* and *S. graciosus*. However, the sample sizes for each comparison were very

similar, therefore the probability of affecting the accuracy of the P values is minimal (Zar 1999).

Analysis of intraspecific differences in embryonic stage of development at oviposition for *S. graciosus* at intermediate and high elevation was performed using Fisher's Exact Test (1-tailed).

Results

Embryonic Stage of Development and Eggshell Thickness

Embryonic stage at oviposition differed significantly between clutches from high altitude populations ($n = 9$ clutches) and intermediate altitude populations ($n = 8$ clutches) of *S. graciosus* (Fisher's Exact Test (1-tailed): $n = 17$, $P = 0.0401$, Figure 4). At Dawson Saddle, 75 percent of clutches were laid at stage 31, with the remaining 25 percent laid at stage 30. At Table Mountain, only 25 percent of clutches were laid at stage 31, with the remaining 75 percent of clutches laid at stage 30. By contrast, *S. occidentalis* showed no variability in embryonic stage at oviposition; all clutches from both Summit Valley and Table Mountain were laid at stage 30.

Beginning at oviposition, over a 14-day period *S. graciosus* embryos from Dawson Saddle ($n = 2$ clutches) and Table Mountain ($n = 4$ clutches) developed at the same

rate (Figure 5). Likewise, *S. occidentalis* embryos from Summit Valley (n = 3 clutches) and Table Mountain (n = 3 clutches) also showed the same rate of development over a 14-day period beginning at oviposition. During the first seven days after oviposition, embryos of *S. graciosus* developed more rapidly than *S. occidentalis* embryos. However, the rate of embryonic development was the same for both species between 7 to 21 days after oviposition. From 21 to 28 days after oviposition, *S. occidentalis* embryos from a single clutch showed an increase in developmental rate compared to *S. graciosus* embryos. In both species, there was almost no variability in the rate of development between clutches.

Eggshells of *S. occidentalis* (n = 12 eggs) and *S. graciosus* (n = 22 eggs) were unlike in general morphology. The surface of *S. occidentalis* eggshells was more convoluted, and appeared to have a thicker calcareous layer than *S. graciosus*. Mean eggshell thickness did not vary significantly between elevations for either species (nested ANOVA: *S. occidentalis*; $F_{1,4} = 4.621$, $P = 0.098$, Appendix B: Table b1, and *S. graciosus*; $F_{1,13} = 0.596$, $P = 0.454$, Appendix B: Table b2). Mean clutch eggshell thickness, was however, significantly different between species (nested ANOVA: $F_{1,17} = 47.127$, $P < 0.0001$, Table 4,

Appendix B: Table b3). Eggshells of *S. graciosus* were thinner on average than *S. occidentalis* eggshells (31.30 μ m versus 49.28 μ m *S. graciosus* and *S. occidentalis*, respectively). Within each species, there was a general increase in mean eggshell thickness with mean egg mass (Figure 6). Additionally, for *S. graciosus* there was no significant difference in mean eggshell thickness between clutches within elevations (nested ANOVA: $F_{13,6} = 0.831$, $P = 0.636$). However, there was significant variation in mean eggshell thickness between eggs within clutches within elevation (nested ANOVA: $F_{6,34} = 5.255$, $P = 0.001$), and between samples within individual eggs (nested ANOVA: $F_{34,110} = 3.357$, $P < 0.0001$). Conversely, for *S. occidentalis*, mean eggshell thickness within elevation was significantly different between clutches (nested ANOVA: $F_{4,6} = 7.566$, $P = 0.016$). There was also no significant difference in mean eggshell thickness between eggs (nested ANOVA, $F_{6,21} = 1.954$, $P = 0.119$), however variation between samples approached significance (nested ANOVA: $F_{21,66} = 1.585$, $P = 0.08$).

Female Size, Egg Mass, and Clutch Mass

There was a significant positive relationship between clutch mass and SVL for *S. graciosus* at Table Mountain, ($R = 0.735$, adjusted $R^2 = 0.474$, $F_{1,7} = 8.221$, $P = 0.024$,

Appendix B: Table b4), and a marginally significant relationship at Dawson Saddle ($R = 0.705$, adjusted $R^2 = 0.414$, $F_{1,6} = 5.943$, $P=0.050$, Figure 7, Appendix B: Table b5). Mean clutch masses were larger at Dawson Saddle compared to Table Mountain (Figure 9), and the difference between these values approached significance (one-way ANOVA: $F_{1,15} = 4.266$, $P = 0.057$, Appendix B: Table b6). The mean clutch mass produced by high elevation gravid females was 2.23g compared to 1.67g produced by gravid females at intermediate elevation (Table 5). However after adjusting for SVL, the difference in mean clutch mass between the two populations was not significant (ANCOVA: $F_{1,14} = 0.713$, $P = 0.413$, SVL used as the covariate, Appendix B: Table b7).

Mean egg mass of *S. graciosus* females did not vary significantly between elevations, (nested ANOVA: $F_{1,15} = 1.919$, $P = 0.185$, Appendix B: Table b8) The mean egg mass was 0.60g at Dawson Saddle compared to 0.54g at Table Mountain (Table 5 and Figure 11). Within population however, there was significant variation in egg mass among clutches (nested ANOVA: $F_{15,41} = 3.833$, $P < 0.0001$). There was also no significant difference in clutch size of high elevation females compared to low elevation females

(one-way ANOVA: $F_{1,15} = 1.802$, $P = 0.199$, Table 5, Appendix B: Table b9).

Overall, reproductively mature male sagebrush lizards had significantly larger body masses (two-way ANOVA: $F_{1,60} = 29.104$, $P < 0.0001$, Appendix B: Table b10) and SVL (two-way ANOVA: $F_{1,61} = 16.012$, $P < 0.0001$, Appendix B: Table b11) compared to female lizards (Table 5). Furthermore, reproductively mature male and female *S. graciosus* from high elevation had significantly larger masses (two-way ANOVA: $F_{1,60} = 11.661$, $P = 0.001$) and SVL (two-way ANOVA: $F_{1,61} = 12.038$, $P = 0.001$) compared to reproductively mature males and females from intermediate elevation. At Dawson Saddle, the mean SVL and body mass of males were 63.4mm (SVL), and 9.3g (body mass), compared to 57.8mm (SVL), and 6.93g (body mass) for females. At Table Mountain, the mean SVL and body mass for males were 63.4mm (SVL) and 7.71g (body mass), compared to 55.7mm (SVL), and 5.8g (body mass) for females. There was no significant interaction between gender and elevation for either SVL (two-way ANOVA: $F_{1,61} = 1.666$, $P = 0.202$) or body mass (two-way ANOVA: $F_{1,60} = 0.305$, $P = 0.583$).

There was a significant positive relationship between SVL and clutch mass for gravid female *S. occidentalis* from Summit Valley (regression: $R = 0.882$, adjusted $R^2 = 0.705$,

$F_{1,3} = 10.599$, $P = 0.047$, Figure 8, Appendix B: Table b12). Only three gravid females were obtained from Table Mountain, and the relationship between SVL and clutch mass was not significant (regression: $R = -0.124$, adjusted $R^2 = 0.015$, $F = 0.0156$, $P = 0.926$, Appendix B: Table b13). The mean clutch mass at intermediate elevation was 3.65g compared to 3.01g at low elevation (one-way ANOVA: $F_{1,6} = 1.641$, $P = 0.248$, Table 5, Figure 10, Appendix B: Table 14b). There was also no significant difference in mean clutch mass between elevations after adjusting for SVL (ANCOVA: $F_{1,5} = 0.808$, $P = 0.223$, SVL as the covariate, Appendix B: Table b15). Mean number of eggs per clutch were similar between the two elevations (t -test: $df = 6$, $t = 0.417$, $P = 0.690$, Figure 10 and Table 5, Appendix B: Table b16). The mean number of eggs per clutch was 7.6 at Summit Valley, compared to a mean of 7 eggs per clutch at Table Mountain. The mean egg mass of female fence lizards at Table Mountain (0.567g) was slightly larger than females from Summit Valley (0.396g) [Figure 11], but the difference was not significant (nested ANOVA: $F_{1,6} = 4.336$, $P = 0.082$, Appendix B: Table b17). As observed in *S. graciosus*, there was significant variation in mean egg mass between clutches within elevation (nested ANOVA: $F_{6,51} = 78.680$, $P < 0.0001$)

Unlike *S. graciosus*, there was no significant difference in the mean SVL of reproductively mature male and female *S. occidentalis* (two-way ANOVA: $F_{1, 160} = 0.001$, $P = 0.976$, Table 5, Appendix B: Table 18b). Male and female lizards from intermediate and low elevation also had similar SVL (two-way ANOVA: $F_{1,160} = 0.584$, $P = 0.446$). The mean SVL for male and female lizards at Summit Valley was 67.9mm for males and 68.6mm for females, compared to 67.4mm male and 66.8mm for females at Table Mountain. The mean body masses of male and female fence lizards from intermediate and low elevation were not significantly different (two-way ANOVA: $F_{1,160} = 0.324$, $P = 0.570$, Table 5, Appendix B: Table 19b), and the interaction of gender and elevation was also non-significant ($F_{1,160} = 0.103$, $P = 0.748$). Reproductively mature male and female lizards from Summit Valley had mean body masses of 12.6g and 12.5g, respectively, compared to 11.9g and 11.4 g, at Table Mountain (Table 5).

Despite the large size difference between *S. occidentalis* and *S. graciosus*, the pooled relative clutch masses (RCM) for each species from the three populations were similar (t -test: $df = 23$, $t = 0.4664$, $P = 0.6453$, Appendix B: Table 20b). The mean RCM for gravid female *S. occidentalis* ($n = 8$) was 0.33, compared

to 0.34 for gravid female *S. graciosus* (n = 17).

Additionally, the mean egg mass for *S. graciosus* (0.570g, n = 17 clutches) was significantly larger (one-way ANOVA: $F_{1,22} = 5.903$, $P = 0.023$, Appendix B: Table b21) than the mean egg mass of *S. occidentalis* (0.460g, n = 8 clutches).

Discussion

Comparison of Egg Retention

The results of this study are consistent with the prediction that extended egg retention is a favorable trait for lizards inhabiting cold climates at high elevations. Of particular interest is that significant differences in egg retention time were observed between populations of *Sceloporus graciosus* inhabiting different thermal environments. Gravid female *S. graciosus* at high elevation laid a significantly greater number of clutches at a more advanced developmental stage compared to gravid females at intermediate elevation (Figure 4). Although *Sceloporus graciosus* does not appear capable of the extremely prolonged egg retention that is observed in some other members of the genus, it was previously believed that *S. graciosus* was incapable of egg retention beyond stage 30 (Andrews and Mathies 2000). Assuming a constant temperature of 28°C, eggs laid with embryos at stage 31

would hatch approximately 7 days earlier than eggs laid with embryos at stage 30. Shortening the incubation period even by as little as 7 days may be an advantage in that hatchling lizards have additional time to feed and grow before cold temperatures restrict or preclude activity. The construction of nests at a shallower depth below the surface of the soil at high elevation could provide warmer conditions for egg incubation; however such a shift would also expose eggs to excessively high and low temperatures in addition to increasing the threat of predation (Andrews 2000). By retaining eggs to stage 31, female *S. graciosus* could potentially shorten the incubation period by providing more favorable thermal conditions for embryonic development.

In contrast, *S. occidentalis* from low and intermediate elevations showed no variability in embryonic stage at oviposition. These results support the hypothesis that for *S. occidentalis*, the ability to extend egg retention is phylogenetically constrained (Andrews 1999; Mathies and Andrews 2000). In other laboratory studies, gravid female *S. occidentalis* oviposited eggs at stages 28-30 even when not provided with suitable substrate for nesting (Mathies and Andrews 2000). The inability to retain eggs beyond stage 30 appears to be a characteristic

shared by most members of the undulatus species group with the exception of *Sceloporus virgatus*, which normally oviposits between stages 31-33 (Mathies and Andrews 2000).

There is some evidence suggesting that embryonic physiology limits the altitudinal range of *S. occidentalis* (Adolph 1990). In the laboratory, eggs of *S. graciosus* have higher mortality at elevated incubation temperatures, a shorter incubation period over a variety of incubation temperatures, and hatchlings have a larger initial body size compared to *S. occidentalis* (Adolph 1990). Additionally, hatchling *S. graciosus* exhibit a higher growth rate compared to *S. occidentalis* when reared under limited (6hr) access to radiant heat (Sinervo and Adolph 1994). Collectively, the ability to retain eggs past stage 30, lower thermal optimum of embryos, larger initial hatchling size, and higher initial hatchling growth rate under shorter periods of thermally favorable conditions, may allow *S. graciosus* to occupy higher and colder elevations than *S. occidentalis*.

Despite an elevation of 2230m, the thermal environment at Table Mountain may not restrict lizard activity to the same extent as Dawson Saddle. At an elevation of 2580m, portions of Dawson Saddle may be covered with snow until early May (personal observation).

Moreover, the fact that *S. graciosus* appears to be the only species of reptile present at Dawson Saddle (personal observation), suggests that the thermal environment may be prohibitive to occupation by other squamate taxa. Adolph and Sinervo report finding a single male *Phrynosoma coronatum*, at Dawson Saddle although it is likely that this individual strayed from lower elevations (Adolph personal communication). By contrast, at Table Mountain, at least six species of lizards and four species of snakes are present (personal observation), suggesting that thermal conditions are favorable to the reproductive biology of a variety of squamate species. Therefore, at intermediate elevation, egg retention may not be as important, and could pose a liability to gravid females compared to Dawson Saddle. The burden of eggs carried by the gravid female represents a potential reproductive cost in that sprint speeds are reduced (Sinervo et al. 1991) and there may be increased risk of predation (Shine 1980). If the advantage associated with retaining eggs is minimal compared to the potential survival cost to the female, then oviposition at stage 30 rather than retaining eggs to stage 31 or beyond, may increase fitness at intermediate elevation.

Alternatively, temperature may not be the selective agent for the increase in egg retention time observed in the high elevation population of *S. graciosus*. Instead, egg retention may be influenced by density dependent factors, and indirectly constrained by the thermal environment (Sinervo, personal communication). At high elevation, the activity season for lizards is reduced compared to lower elevations and therefore warmer environments (Sinervo and Adolph 1994). Moreover, large body size is an advantageous trait throughout the entire life history of most reptiles (Sinervo 1990). Shortening the incubation period by extending egg retention would promote earlier hatching and allow hatchlings from retained clutches to exploit food resources and therefore increase in growth compared to hatchlings from non-retained clutches that emerge later in the season.

Eggshell Thickness and Embryonic Stage at Oviposition

The prediction that there should be a reduction in eggshell thickness in high elevation lizard populations exhibiting egg retention was not supported. Mean eggshell thickness from populations of *S. graciosus* at high and intermediate elevations were nearly identical (Table 4). The mean eggshell thickness for populations of *S. occidentalis* at low and intermediate elevation varied

by an average of approximately 11.0 μ m, but were not significantly different from each other (Table 4). The comparison of eggshell thickness between populations of *S. occidentalis* may not be a reliable estimate of the true population mean eggshell thickness because of the small number of clutches sampled from each population.

Overall, eggshells of *S. graciosus* were thinner by an average of 18 μ m compared to *S. occidentalis*, however this difference may be related to phylogenetic factors rather than the capacity to support embryogenesis. These results are consistent with recent work conducted by Mathies and Andrews (2000), which suggests that vascularization of the oviduct and extraembryonic membranes are more critical components for embryonic gas exchange than eggshell thickness. Instead, eggshell thickness most likely functions as a structural component that is positively related to egg size (Mathies and Andrews 2000). It is also possible that embryos retained to stage 31 do not require thinner eggshells to support embryogenesis.

The mean egg mass of *S. graciosus* is larger than *S. occidentalis*, however eggshells are thinner in *S. graciosus* (Table 4). Interestingly however, the positive relationship between egg mass and eggshell thickness holds true between populations within each

species. Gravid female *S. graciosus* from Dawson Saddle have larger mean egg mass and slightly greater mean eggshell thickness than gravid females from Table Mountain. Likewise, gravid female *S. occidentalis* from Table Mountain have larger mean egg mass and larger mean eggshell thickness compared to gravid females from low elevation at Summit Valley. Furthermore, the increase in egg mass between populations of *S. occidentalis* was accompanied by a greater increase in eggshell thickness relative to that observed in *S. graciosus* where the variation between population mean egg masses was not as large (Figure 6).

Presently, little is known concerning the possible relationship between eggshell morphology and function in lizard species. There was considerable variation in the thickness of samples within individual eggs for *S. graciosus* and to a lesser extent *S. occidentalis*. These data indicate that in individual eggs, eggshell thickness is not uniform, and therefore different regions of the eggshell may be more permeable to gas exchange or influx of water compared to other regions. However, variation in structural morphology between the eggshells of *S. graciosus* and *S. occidentalis* may be phylogenetic in origin, and not directly related to variation in embryonic

physiology between the two lizard species (Garland and Adolph 1994).

Interspecific and Intraspecific Variation in Reproductive Biology

According to life history theory, a trade-off exists between clutch size and egg size, such that larger clutches usually consist of smaller eggs (Sinervo 1990). Lizards exhibiting extended egg retention typically show a reduction in number of eggs per clutch presumably because the reproductive burden imposed by the developing offspring increases dramatically throughout incubation/gestation (Shine 1985; Mathies and Andrews 1995). Without a reduction in clutch size, reproductive burden could potentially result in increased female mortality due to predation (Shine 1980; Mathies and Andrews 1995). The majority of gravid female *S. graciosus* at Dawson Saddle lay eggs at a more advanced embryonic developmental stage and have both larger mean egg masses and mean clutch masses compared to gravid females from Table Mountain. However, most of the variation in clutch mass is attributable to female size, and after adjusting for SVL, there was no significant difference in clutch mass between populations. Therefore the larger clutch masses at high elevation are most likely due to the larger

average size of high elevation females compared to females at intermediate elevation (Figure 7 and Table 5). Reproductively mature male and female *S. graciosus* from Dawson Saddle are larger both in mass and SVL compared to reproductively mature males and females at Table Mountain. The larger size of lizards at Dawson Saddle may be a result of character release due to the lack of competition with other lizards and absence of predators such as whip snakes (*Masticophis lateralis*) at high elevation (Adolph and Sinervo, personal communication). Additionally, given that retention of eggs to stage 31 does not represent extremely prolonged development in the oviduct, a reduction in clutch size may not be necessary.

The opposite trend is observed between gravid female *S. occidentalis* at Table Mountain, and Summit Valley at low elevation. Although not significantly different, gravid females at Summit Valley had slightly larger mean body mass and SVL compared to gravid females at Table Mountain (Table 5). Accordingly, low elevation gravid females on average had a larger number of smaller eggs per clutch than gravid females from intermediate elevation. This trend is not consistent with previous research by Sinervo (1990), who found that gravid female *S. occidentalis* from Table Mountain had larger clutches of

smaller eggs than gravid female from the low elevation site on the edge of the Mojave Desert. The results of the present study may be biased due to the small number of clutches obtained from Table Mountain and Summit Valley, and therefore might not represent the true relationship between clutch size and egg size in these populations.

Implications for the Evolution of Viviparity

The greater tendency of high elevation populations of *S. graciosus* to retain clutches to embryonic stage 31 compared to populations at intermediate elevation is consistent with the predictions of the cold climate model. Furthermore, the fact that *S. graciosus* shows the capability of short-term egg retention, while *S. occidentalis* shows virtually no capability of egg retention, suggests that for *S. graciosus*, there is phenotypic variability for selection to act upon. For *S. graciosus*, the higher frequency of embryos laid at stage 31 at Dawson Saddle appears to be adaptive, whether due to phenotypic plasticity or selection. It is unclear whether the observed increase in egg retention by high elevation females is due to cold environmental temperatures, or some other factor. It is not known whether the embryonic stage of development at oviposition is temporally variable between or within activity seasons.

The lizard *Uta stansburiana* exhibits a shift to larger egg size for late season clutches when the density of hatchling lizards is high (Svensson and Sinervo 2000). In *Uta*, particular adaptive characters may vary with changing ecological factors in the environment (Svensson and Sinervo 2000). Likewise, in *S. graciosus*, the embryonic stage of development at oviposition may vary for early versus late clutches, or according to changes in population density from season to season.

Research conducted by Andrews (1994) on *Sceloporus virgatus* demonstrated that mean temperatures of gravid females were similar to mean nest temperatures, suggesting that egg retention did not change the embryonic incubation environment. It is believed that for *S. virgatus*, the ability to retain eggs for short periods of time allows gravid females to oviposit their eggs in synchrony with summer monsoonal precipitation. However, in the San Gabriel Mountains of Southern California, summer precipitation is generally low and unpredictable. It therefore seems unlikely that egg retention in Southern California populations of sagebrush lizards is related to seasonal moisture. The high altitude populations of *S. graciosus* at Dawson Saddle are subject to a relatively short activity season (Sinervo and Adolph 1994) and widely

fluctuating diel temperature ranges (personal observation). Under these conditions, egg retention may provide a more thermally stable incubation environment. Moreover, if even slightly earlier hatching avoids periods of lethally cold temperatures late in the activity season, then there may be selection for egg retention at high elevation.

Finally, the results of this study add to the increasing body of data suggesting that the evolution of life history characters such as viviparity may involve the interaction of several factors, including phylogenetic history and selective pressures of the environment. Recent research suggests that the evolution of viviparity requires the simultaneous development of increased vascularization of the oviduct and extraembryonic membranes, along with extended egg retention (Mathies and Andrews 2000; Blackburn 2000). The transition to viviparity therefore requires that selection operate on a suite of complex physiological and anatomical characters to accommodate intrauterine incubation of embryos. Given these circumstances, it is reasonable that within the genus *Sceloporus*, certain lineages may be more likely to evolve viviparity compared to others (Menendez-de la Cruz et al. 1998; Andrews and Mathies 2000). Although

intraspecific increases egg retention may be correlated with lizard populations at high elevation, further research is necessary to determine the extent to which thermal environment influences this life history character.

Table 3. Summary of anatomical characters used to determine embryonic stage of development for lizard embryos

Character	Developmental Stage		
	29	30	31
Somites (pairs)	40	50	Numerous
Branchial Slits	1,2,3	1,2,3,4,5	1,2,3
Limbs	Stubs	Flattened Paddles	Less flattened, autopodia visible
Eyes	Little or no pigmentation, choroid fissure not joined	Pigmented, lips of choroid fissure in contact	Heavily pigmented, lips of choroid fissure joined
Penes	Not visible	Not visible	Visible

Designation of a particular embryonic stage of development required all characters listed in that stage to be visible in the embryo. (Dufaure and Hubert 1961)

Table 4. Mean clutch eggshell thickness (\pm SE) for *Sceloporus occidentalis* and *Sceloporus graciosus*

Mean Eggshell Thickness (μm) per Clutch							
<i>S. occidentalis</i>				<i>S. graciosus</i>			
Summit Valley		Table Mountain		Dawson Saddle		Table Mountain	
Mean	SE	Mean	SE	Mean	SE	Mean	SE
46.74	1.43	38.10	1.05	35.50	0.88	31.95	0.95
61.37	1.53	45.76	1.13	22.79	0.43	26.90	2.01
54.66	1.67	46.09	1.48	29.14	1.68	33.33	1.43
--	--	--	--	33.82	0.85	27.46	0.87
--	--	--	--	29.97	0.72	29.79	2.03
--	--	--	--	28.37	1.11	26.90	2.01
--	--	--	--	30.17	2.68	34.77	0.85
--	--	--	--	34.87	0.91	--	--

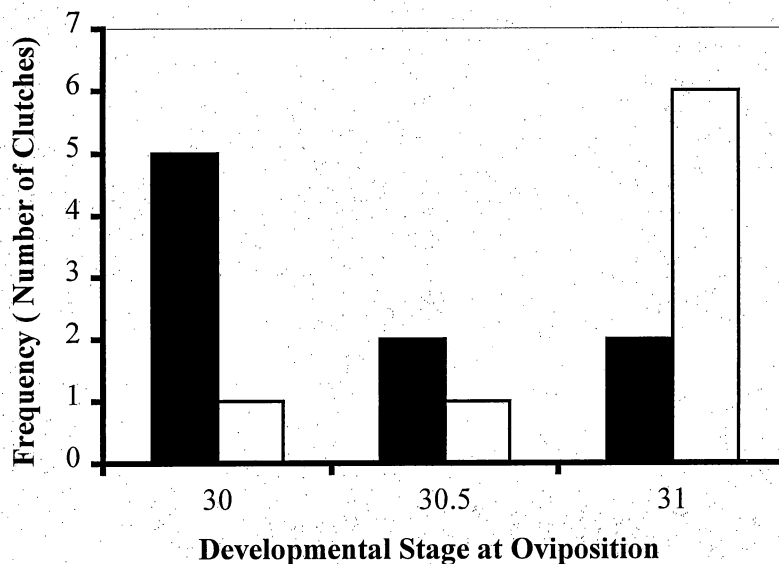
Mean values represent the mean eggshell thickness per clutch for each species at each of the four study populations.

Table 5. Summary of mean number of eggs, clutch mass, egg mass, Snout-vent length, and body mass for *Sceloporus occidentalis* and *Sceloporus graciosus*

Population	Elevation (m)	Number of Eggs	Clutch Mass (g)	Egg Mass (g)	Snout-Vent Length (mm)	Body Mass (g)
<i>S. graciosus</i> , Dawson	2580	3.7 ± 0.35 (8)	2.23 ± 0.17 (8)	0.60 ± 0.03 (8)	57.9 ± 0.71 (21)	6.9 ± 0.26 (21)
<i>S. graciosus</i> , Table Mountain	2230	3.1 ± 0.31 (9)	1.67 ± 0.21 (9)	0.54 ± 0.01 (9)	55.7 ± 0.58 (31)	5.8 ± 0.21 (31)
<i>S. occidentalis</i> Table Mountain	2230	7.0 ± 1.5 (3)	3.65 ± 0.29 (3)	0.567 ± 0.08 (3)	66.8 ± 1.4 (46)	11.4 ± 0.59 (46)
<i>S. occidentalis</i> Summit Valley	1260	7.6 ± 0.68 (5)	3.0 ± 0.34 (5)	0.396 ± 0.02 (5)	68.5 ± 1.5 (37)	12.5 ± 0.65 (37)

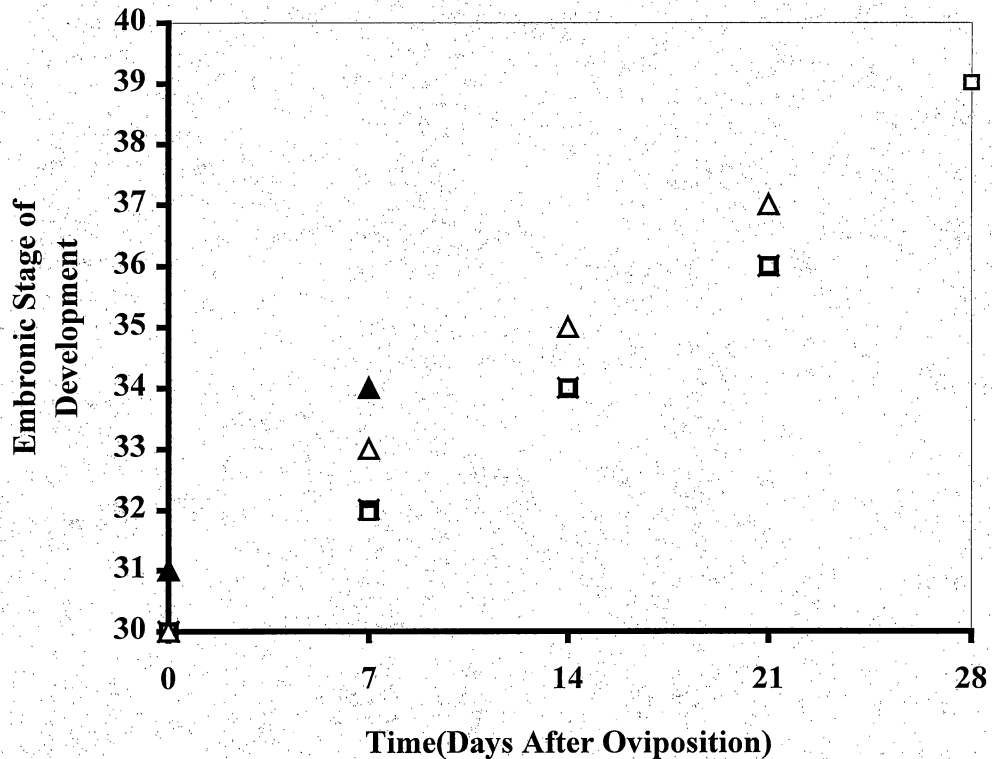
Lizard species are listed including populations, and mean values (\pm SE). Sample sizes are included in parentheses beneath. Sample sizes of all egg data refer to the number of clutches. Sample sizes for SVL and body mass data refer to number of females measured.

Figure 4. Number of clutches and embryonic stage of development at oviposition for *Sceloporus graciosus* at Dawson Saddle and Table Mountain.



Dawson Saddle (□ = high elevation) and Table Mountain (■ = intermediate elevation). Stage 30 represents the least advanced embryonic stage, 31 represents the most advanced embryonic stage of development and, stage 30.5 represents intermediate stage of development between 30 and 31.

Figure 5. Rate of embryonic development for *Sceloporus occidentalis* from intermediate and low elevation and *Sceloporus graciosus* from intermediate and high elevation.



Sceloporus occidentalis [intermediate elevation]

(□): $n = 3$ clutches, low elevation (■): $n = 3$ clutches and

Sceloporus graciosus [intermediate elevation] (△): $n = 4$

clutches, high elevation (▲): $n = 2$ clutches embryos

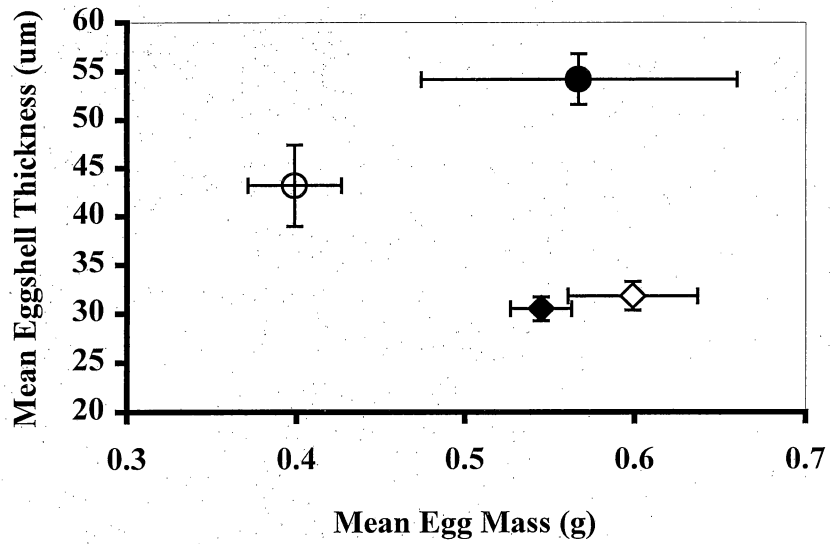
incubated at a constant temperature of 28.0° C. The

developmental rate was identical for *S. occidentalis*

embryos from intermediate and low elevation females,

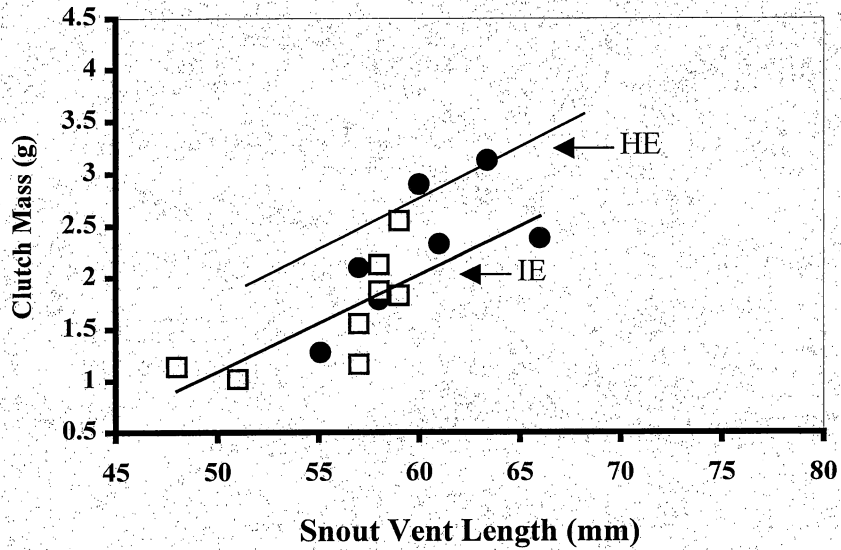
therefore these data points overlap in the figure.

Figure 6. Relationship between mean population eggshell thickness and mean clutch egg mass for *Sceloporus occidentalis* at intermediate and low elevation, and *Sceloporus graciosus* at intermediates and high elevation.



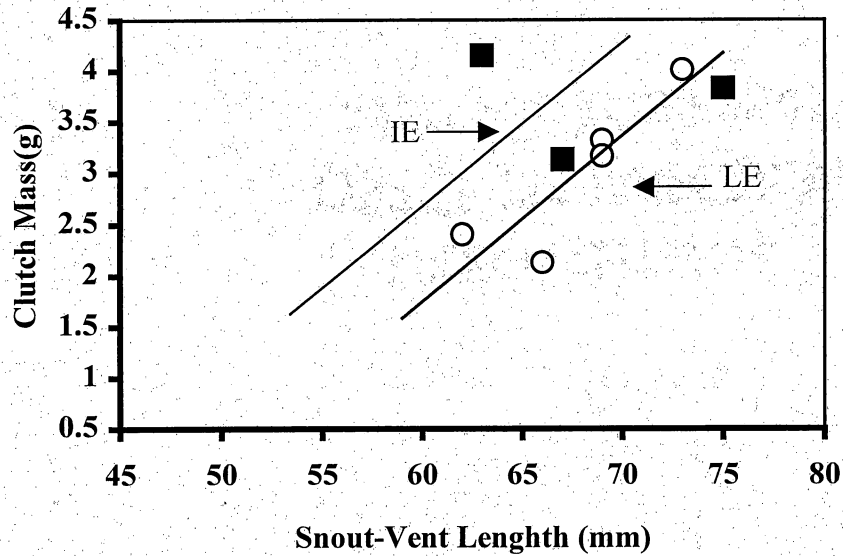
Sceloporus occidentalis at intermediate (●) and low (○) elevation, and *Sceloporus graciosus* at intermediates (◆) and high (◇) elevation.

Figure 7. Relationship between clutch mass and snout-vent length for *Sceloporus graciosus* from high elevation and intermediate elevation.



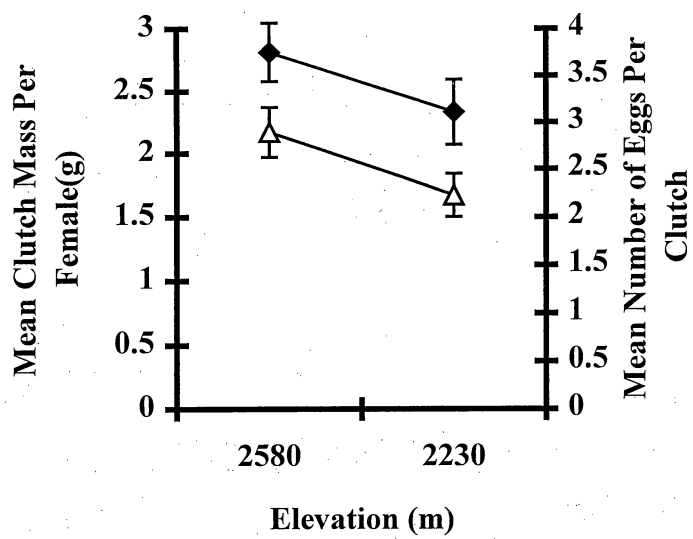
Sceloporus graciosus from high elevation [HE] (●), and intermediate elevation [IE] (○). The slopes of the individual population regressions are not significantly different (ANOVA: population * SVL, $P > 0.05$), therefore regression lines are plotted showing common slope.

Figure 8. Relationship between clutch mass and snout-vent length for *Sceloporus graciosus* from intermediate and low elevation.



Sceloporus graciosus from intermediate (o) and low elevation [LE] (♦). The slopes of the individual population regressions are not significantly different (ANOVA: population * SVL, $P > 0.05$), therefore regression lines are plotted showing common slope.

Figure 9. Mean clutch mass and mean number of eggs per female for *Sceloporus graciosus* at high elevation and intermediate evaluation.



Dawson Saddle (2580 m) and Table Mountain [2230 m]

(♦) = mean number of eggs per clutch, (Δ) = mean clutch mass per female.

Figure 10. Mean clutch mass and mean number of eggs per female for *Sceloporus occidentalis* at intermediate elevation and low evaluation.

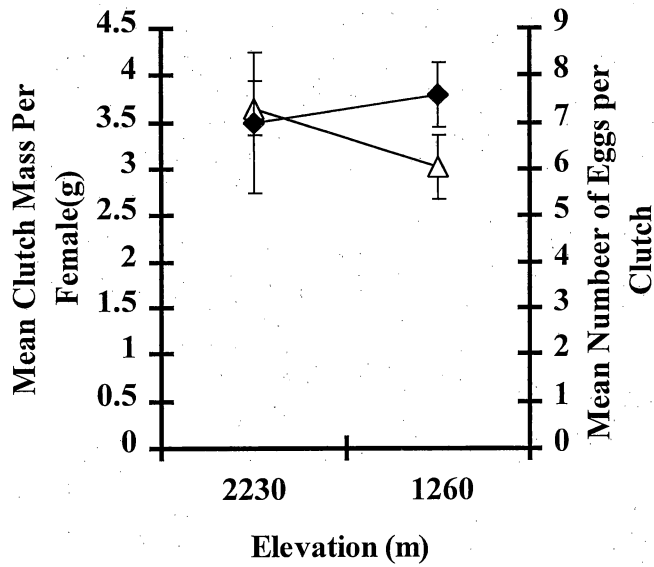
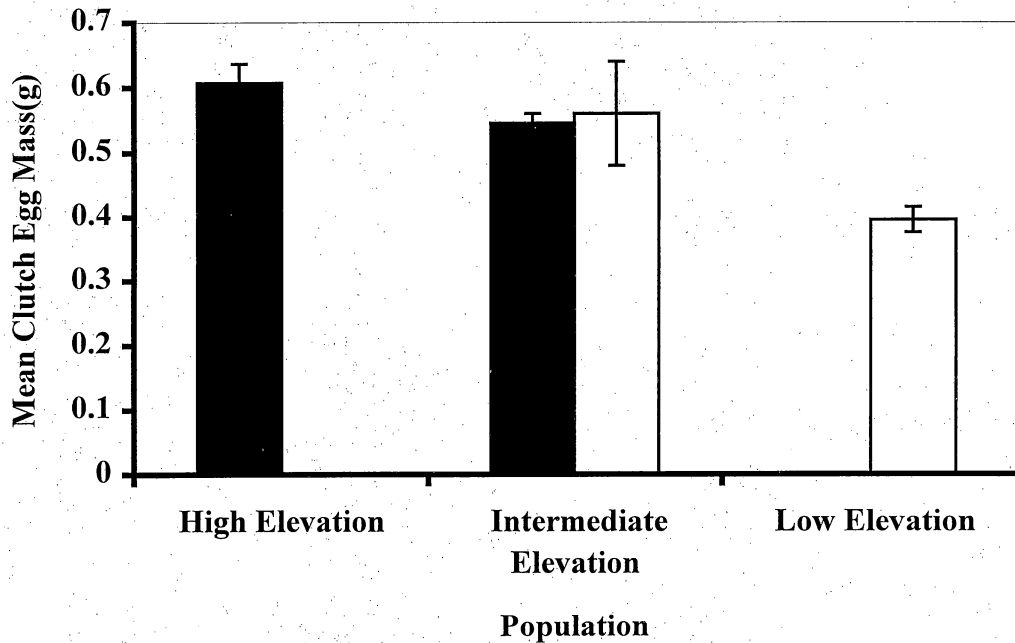


Table Mountain and Summit Valley (1260 m)

(♦) = mean number of eggs per clutch, (Δ) = mean clutch mass per female.

Figure 11. Mean clutch egg mass for *Sceloporus occidentalis* from intermediate elevation and low elevation, and *Sceloporus graciosus* at intermediate and high elevation.



Dark fill refers to *S. graciosus*, and light fill refers to *S. occidentalis* (□), at intermediate elevation (Table Mountain), and low elevation (Summit Valley), and (■), at high elevation (Dawson Saddle), and intermediate elevation. The mean clutch egg mass represents the average of the mean clutch egg masses for each species in each population.

CHAPTER FOUR

SUMMARY

The differences in thermal and reproductive biology of *Sceloporus occidentalis* and *Sceloporus graciosus* may partly explain the altitudinal and geographical distributions of these two species. The results of this study show that gravid female *S. graciosus* maintain lower selected body temperatures in the thermal gradient compared non-gravid female and male *S. graciosus* (Table 2, Figure 3). Furthermore, it is not clutch burden that prevents gravid females from maintaining preferred body temperatures; instead reproductive females appear to be actively selecting lower body temperatures possibly reduce thermal stress to developing embryos. In contrast, gravid female *S. occidentalis* did not choose lower body temperatures in the thermal gradient, and mean selected body temperatures were similar to male and non-gravid female lizards (Table 2 and Figure 3). This suggests that the optimum temperature for embryonic development may coincide with the optimum physiological temperature of adult female *S. occidentalis*. Additionally, gravid *S. graciosus* females have the capability of short term egg retention, a trait not observed in intermediate and low

elevation populations of *S. occidentalis*. Previous research by Adolph (1990; and *personal communication*) demonstrated that *S. graciosus* eggs have increased mortality when incubated at temperatures above 30.0° C, have a shorter incubation period over a variety of temperatures, and larger initial hatchling size compared to *S. occidentalis*. Collectively, these characteristics may be advantageous in cold, high elevation environments. The higher thermal optima of *S. occidentalis* embryos to higher incubation temperatures, longer incubation period, and smaller initial hatchling size may reflect its occupation of a wide range of warmer environments at lower elevations.

Despite differences in the physical and thermal environment at the three elevations, mean field body temperatures of active gravid, non-gravid and male *S. occidentalis* and *S. graciosus* were not significantly different between elevations (Table 1). Thus, even at high elevations, lizards are able to obtain relatively high body temperatures. Likewise, within each species, the rate of embryonic development did not vary between populations occurring at different elevations. These results are consistent with previous research suggesting that optimum physiological temperatures and embryonic developmental

rates in the genus *Sceloporus* are lineage specific, and therefore attributable to phylogenetic factors (Andrews et al. 1999).

Intraspecific variation in life history characters between elevations is more suggestive of an adaptive response to local environmental conditions (Andrews 1995). High elevation *S. graciosus* females laid eggs at a significantly more advanced developmental stage compared to female *S. graciosus* at intermediate elevation (Figure 4). This response appears to be adaptive, whether due to phenotypic plasticity or genetic differences between populations. In the laboratory, at a constant incubation temperature, the rate of embryonic development was identical between populations at high and intermediate elevation. Assuming a constant incubation temperature of 28°C, eggs laid with embryos at stage 31 would hatch approximately 7 days earlier than eggs laid with embryos at stage 30.

It remains unclear whether cold environmental temperatures or other factors are the primary selective agents responsible for the observed difference in embryonic stage of development between elevations. *Sceloporus graciosus* is the only reptile species present at Dawson Saddle, and in the absence of interspecific

competition and reduced predation, the cost of retaining eggs may be minimized compared to Table Mountain at intermediate elevation. Alternatively, the retention of eggs to stage 31 may be temporally variable and occur in response to changes in hatchling density whereby larger offspring have a competitive advantage relative to smaller hatchlings.

Sceloporus graciosus females from high elevation had larger clutch masses than females from intermediate elevation (Table 3 and Figure 9). In contrast, clutch mass did not differ between intermediate and low elevation populations of *S. occidentalis* (Figure 10). In other lizard species, decrease in clutch size typically occurs with prolonged egg retention because of the reproductive cost associated with the increase in mass of developing embryos. High elevation populations of *S. graciosus* have both increased egg retention and larger clutch masses. However, the larger clutch masses observed in high elevation female *S. graciosus* was due to their larger size rather than an overall increase in reproductive investment.

The mean clutch eggshell thickness did not differ between populations for either species (Table 4). Moreover, the mean clutch eggshell thickness for

S. graciosus at Dawson Saddle and Table Mountain were almost identical, even though a higher frequency of clutches were laid at stage 31 at high elevation. The mean clutch eggshell thickness was significantly thinner for *S. graciosus* compared to *S. occidentalis*, however it is presently unknown whether these differences are related to the capacity to support embryogenesis. The variability in eggshell thickness within clutches was not uniform for both *S. graciosus*, or *S. occidentalis*. Different regions of the eggshell may therefore be more permeable to gas and water flux compared to other regions. The results of this study are consistent with recent research by Mathies and Andrews (2000), suggesting that eggshell thickness functions as a structural component and is positively related to egg size rather than the duration of time spent in the oviduct. The mean egg mass is smaller, and the mean eggshell thickness is larger for *S. occidentalis* compared to *S. graciosus* (Table 3 and Table 4). However, within each species, the mean eggshell thickness increases with mean egg mass (Figure 6).

Reproductive Biology and
Implications for the
Cold Climate
Hypothesis

The observation that high elevation female *S. graciosus* tend to lay eggs at a more advanced developmental stage is consistent with the predictions of the cold climate hypothesis (Figure 4). Gravid female *Sceloporus occidentalis* from intermediate and low elevation showed no variability in embryonic stage of development at oviposition. This supports the view proposed by Andrews (2000), that members of the *undulatus* species group (with the exception of *S. virgatus*) are physiologically incapable of retaining eggs past stage 30. However, although *S. graciosus* is capable of retaining eggs beyond stage 30, it is unclear whether this species has the capacity to support embryogenesis for extended periods beyond stage 31.

The viviparous species of *Sceloporus* occur at relatively high elevations but at low latitudes (Méndez-De La Cruz et al. 1998; Sites et al. 1995; Table 6). High elevations in tropical latitudes are relatively mild during the winter compared to similar elevations at high latitudes (Méndez-De La Cruz et al. 1998). The gestation period for these tropical, high elevation viviparous

species of *Sceloporus* occurs over the winter, and birth occurs during the spring (Méndez-De La Cruz et al. 1998). By contrast nearly all temperate zone lizards mate in the spring and lay eggs or give birth in late summer or early autumn. The extremely cold temperatures at high elevations in northern latitudes would thus be lethal to embryos incubated over the winter during the female's hibernation. It is therefore believed that the evolution of viviparity in the genus *Sceloporus* required a shift to reproduction early in the season, birth in the spring, and alteration of the synchrony of male and female reproductive cycles (Méndez-De La Cruz et al. 1998). For these reasons, it appears that in the genus *Sceloporus* the transition from oviparity to viviparity occurs more readily in some lineages than in others (Méndez-De La Cruz et al. 1998; Andrews et al. 2000).

Alternatively, the viviparous lizards *Phrynosoma douglassii* (short-horned lizard) and *Elgaria coeruleus* (northern alligator lizard) have geographic ranges extending into southern Canada and Washington State respectively (Stebbins 1985). Both species have reproductive cycles typical of most temperate zone lizards which mate in the spring and give birth in the fall. The fact that *S. graciosus* inhabits among the coldest habitats

of any members of the genus yet retains oviparity suggests that a variety of selective agents, including temperature, may be responsible for the evolution of squamate reproductive life history traits.

Table 6. Representative summary of elevation, latitude, and reproductive mode (oviparity/vivparity), for members of the lizard genus *Sceloporus* (Phrynosomatidae)

Species	Elevation (m)	Latitude (°N)	Reproductive Mode
<i>S. malachiticus</i> ^a	1600-2500	14	V
<i>S. squamosus</i> ^b	800	14	O
<i>S. aeneus</i> ^e	2800	19	O/R
<i>S. bicanthalis</i> ^e	3200-4000	19	V
<i>S. grammicus</i> ^d	1100-4400	19	V
<i>S. variabilis</i> ^c	45-1000	19	O
<i>S. woodi</i> ⁱ	60	27	O
<i>S. merriami</i> ^f	560-1610	29	O
<i>S. scalaris</i> ^e	2550	32	O/R
<i>S. consobrinus</i> ⁱ	1300-2290	32	O
<i>S. virgatus</i> ⁱ	1725	32	O/R
<i>S. clarkii</i> ⁱ	1300	33	O
<i>S. jarrovi</i> ^g	1650-2250	33	V
<i>S. poinsettii</i> ^g	1100	33	V
<i>S. olivaceus</i> ^h	190-350	33	O
<i>S. graciosus</i> ^j	2200-2500	34	O
<i>S. orcutti</i> ^k	260	34	O
<i>S. magister</i> ^k	1200	34	O
<i>S. occidentalis</i> ⁱ	0-2230	34	O
<i>S. hyacinthinus</i> ⁱ	180-750	37	O

Subscripts refer to taxonomic species group.

^a *formosus*, ^b *siniferus*, ^c *varabilis*, ^d *grammicus*,

^e *scalaris*, ^f *merriami*, ^g *torquatus*, ^h *olivaceus*,

ⁱ *undulatus*, ^j *graciosus*, ^k *magister*, ^l *clarkii*.

O = oviparous, O/R = oviparous with capability of egg retention, and V=viviparous. Data from Méndez-De La Cruz et al. 1998; Andrews 1998; Andrews et al. 1999

APPENDIX A
THERMAL STATISTICAL ANALYSES

Table a1. Two-way ANOVA testing for difference in mean field body temperature between elevation (high elevation, intermediate elevation), and reproductive condition (gravid, non-gravid, and male) *Sceloporus occidentalis*.

Source	Sum of Squares	DF	MS	F	Significance
Elevation	0.399	1	0.399	0.221	0.642
Reproductive Condition	3.846	2	1.923	1.064	0.359
Elevation * Reproductive Condition	3.347	2	1.674	0.926	0.408
Error	50.617	28	1.808		

Table a2. Two-way ANOVA testing for difference in mean field body temperature between elevation (intermediate and low elevation), and reproductive condition (gravid, non-gravid, and male) *Sceloporus graciosus*.

Source	Sum of Squares	DF	Mean Square	F	Significance
Elevation	4.276	1	4.276	1.853	0.178
Reproductive Condition	12.524	2	6.262	2.714	0.074
Elevation * Reproductive Condition	9.842	2	4.921	2.133	0.127
Error	147.661	64	2.307		

Table a3. Two-way ANOVA testing for difference in mean field body temperature between gravid female, non-gravid female, and male *Sceloporus occidentalis* and *Sceloporus graciosus*.

Source	Sum of Squares	DF	MS	F	Significance
Species	0.320	1	0.320	0.142	0.707
Reproductive Condition	3.921	2	1.960	0.867	0.424
Species * Reproductive Condition	8.384	2	4.192	1.854	0.162
Error	221.650	98	2.262		

Table a4. Two-way repeated measures ANOVA testing for difference between mean selected body temperature (thermal gradient) between gravid, non-gravid, and male *Sceloporus occidentalis* and *Sceloporus graciosus*.

Source	Sum of Squares	DF	MS	F	Significance
Species	0.0603	1	0.0603	0.014	0.907
Reproductive Condition	28.459	2	14.229	3.254	0.049
Species * Reproductive Condition	9.873	2	4.936	1.132	0.333
Error	161.281	37	4.359		

Table a5. One-way repeated measures ANOVA testing for difference in mean selected body temperature (thermal gradient) between gravid, non-gravid and male *Sceloporus occidentalis*.

Source	Sum of Squares	DF	MS	F	Significance
Reproductive Condition	8.218	2	4.109	0.741	0.495
Error	77.666	14	5.548		

Table a6. One-way repeated measures ANOVA testing for difference in mean selected body temperature (thermal gradient) between gravid, non-gravid and male *Sceloporus graciosus*.

Source	Sum of Squares	DF	MS	F	Significance
Reproductive Condition	38.070	1	19.035	5.236	0.013
Error	83.615	23	3.635		

APPENDIX B
REPRODUCTIVE STATISTICAL
ANALYSES

Table b1. Nested ANOVA testing for difference in mean eggshell thickness between intermediate and low elevation populations of *Sceloporus occidentalis*.

Source		Sum of Squares	DF	MS	F	Significance
Elevation	Hypothesis	2908.642	1	2908.642	4.621	0.098
	Error	2521.414	4.006	629.464		
Clutch (Elevation)	Hypothesis	2542.058	4	635.515	7.566	0.016
	Error	498.535	5.935	83.996		
Egg (Clutch (Elevation))	Hypothesis	501.361	6	83.560	1.954	0.119
	Error	897.934	21	42.759		
Sample (Egg (Clutch (Elevation)))	Hypothesis	897.934	21	42.759	1.585	0.080
	Error	1780.219	66	26.973		

Table b2. Nested ANOVA testing for difference in mean eggshell thickness between high elevation and intermediate elevation populations of *Sceloporus graciosus*.

Source		Sum of Squares	DF	MS	F	Significance
Elevation	Hypothesis	74.964	1	74.964	0.596	0.596
	Error	1647.316	13.100	125.751		
Clutch (Elevation)	Hypothesis	1677.816	13	129.063	0.831	0.636
	Error	939.310	6.048	155.320		
Egg (Clutch (Elevation))	Hypothesis	947.554	6	157.926	5.255	0.001
	Error	1021.827	34	30.054		
Sample (Egg(Clutch (Elevation)))	Hypothesis	1021.827	34	30.357	3.357	0.00001
	Error	984.751	110	8.952		

Table b3. Nested ANOVA testing for difference in mean eggshell thickness between *Sceloporus occidentalis* and *Sceloporus graciosus*.

Source		Sum of Squares	DF	MS	F	Significance
Species	Hypothesis	19656.218	1	19656.218	47.172	0.00001
	Error	7293.940	17.504	416.691		
Clutch (Species)	Hypothesis	7185.390	19	378.178	3.168	0.022
	Error	1445.897	12.114	119.356		
Egg (Clutch (Species))	Hypothesis	1448.915	12	120.743	3.459	0.001
	Error	1919.760	55	34.905		
Sample (Egg (Clutch (Species)))	Hypothesis	1919.760	55	34.905	2.222	0.0001
	Error	2764.970	176	15.710		

Table b4. Regression analysis of the relationship between clutch mass (dependent variable), and snout-vent length (independent variable), for female *Sceloporus graciosus* from intermediate elevation.

Model	Sum of Squares	DF	MS	<i>F</i>	Significance
Regression	1.106	1	1.106	8.221	0.024
Residual	0.942	7	0.135		
Total	2.048	8			

Table b5. Regression analysis of the relationship between clutch mass (dependent variable), and snout-vent length (independent variable), for female *Sceloporus graciosus* from high elevation.

Model	Sum of Squares	DF	MS	<i>F</i>	Significance
Regression	1.295	1	1.295	5.943	0.051
Residual	1.308	6	0.218		
Total	2.603	7			

Table b6. One-way ANOVA testing for difference in mean clutch mass for *Sceloporus graciosus* females between high elevation and intermediate elevation.

Source	Sum of Squares	DF	MS	<i>F</i>	Significance
Elevation	1.323	1	1.323	4.266	0.057
Error	4.651	15	0.310		

Table b7. ANCOVA testing for difference in mean clutch mass between high elevation and intermediate elevation populations of *Sceloporus graciosus* using snout-vent length (SVL) as the covariate.

Source	Sum of Squares	DF	MS	F	Significance
SVL	2.360	1	2.360	14.423	0.002
Elevation	0.117	1	0.117	0.713	0.413
Error	2.291	14	0.164		

Table b8. Nested ANOVA testing for difference in mean clutch egg mass between high elevation and intermediate elevation populations of *Sceloporus graciosus*.

Source		Sum of Squares	DF	MS	F	Significance
Elevation	Hypothesis	0.0396	1	0.0396	1.919	0.185
	Error	0.323	15.650	.0206		
Clutch (Elevation)	Hypothesis	0.328	15	0.0219	3.866	0.0001
	Error	0.232	41	0.00566		

Table b9. One-way ANOVA testing for differences in the mean number of eggs per clutch for high elevation and intermediate elevation populations of *Sceloporus graciosus*.

Source	Sum of Squares	DF	MS	<i>F</i>	Significance
Elevation	1.729	1	1.729	1.802	0.199
Error	14.389	15	0.959		

Table b10. Two-way ANOVA testing for difference in mean body mass between male and female *Sceloporus graciosus* from high elevation and intermediate elevation.

Source	Sum of Squares	DF	MS	<i>F</i>	Significance
Gender	42.893	1	42.893	29.104	0.00001
Elevation	17.185	1	17.185	11.661	0.001
Gender * Elevation	0.450	1	0.450	0.305	0.583
Error	88.426	60	1.474		

Table b11. Two-way ANOVA testing for difference in mean snout-vent length between male and female *Sceloporus graciosus* from high elevation and intermediate elevation.

Source	Sum of Squares	DF	MS	F	Significance
Elevation	126.00	1	126.00	12.038	0.001
Gender	167.616	1	167.616	16.012	0.0001
Elevation * Gender	14.440	1	17.440	1.666	0.202
Error	638.539	61	10.468		

Table b12 Regression analysis of the relationship between clutch mass (dependent variable) and snout-vent length (SVL) [independent variable] for female *Sceloporus occidentalis* from low elevation.

Model	Sum of Squares	DF	MS	F	Significance
Regression	1.757	1	1.757	10.599	0.047
Residual	0.497	3	0.16577		
Total	4	2.254			

Table b13. Regression analysis of the relationship between clutch mass (dependent variable) and snout-vent length (independent variable) for female *Sceloporus occidentalis* from intermediate elevation.

Model	Sum of Squares	DF	MS	<i>F</i>	Significance
Regression	0.00845	1	0.000845	0.0156	0.920
Residual	0.539	1	0.539		
Total	0.547	2			

Table b14. One-way ANOVA testing for difference in mean clutch mass between female *Sceloporus occidentalis* from intermediate and low elevation.

Source	Sum of Squares	DF	MS	<i>F</i>	Significance
Elevation	0.784	1	0.784	1.641	0.248
Error	2.869	6	0.478		

Table b15. ANCOVA testing for difference in mean clutch mass for female

Sceloporus occidentalis from intermediate and low elevation using snout-vent length

as the covariate.

Source	Sum of Squares	DF	MS	F	Significance
Snout-vent Length	0.712	1	0.712	1.705	0.249
Elevation	0.808	1	0.808	1.934	0.223
Error	2.090	5	0.418		

Table b16. Student's *t*-Test analyzing difference in mean number of eggs per clutch

for female *Sceloporus occidentalis* from intermediate and low elevation.

Source	DF	<i>t</i> -statistic	<i>t</i> -critical	Significance
Elevation	6	0.417	2.446	0.690

Table b17. Nested ANOVA analyzing difference in mean clutch egg mass for female *Sceloporus occidentalis* from intermediate and high elevation.

Source		Sum of Squares	DF	MS	F	Significance
Elevation	Hypothesis	0.370	1	0.370	4.339	0.082
	Error	0.513	6.011	0.0853		
Clutch (Elevation)	Hypothesis	0.550	6	0.0916	78.680	0.00001
	Error	0.0594	51	0.00165		

Table b18 Two-way ANOVA analyzing difference in mean snout-vent length (SVL) for male and female *Sceloporus occidentalis* from intermediate and low elevation.

Source	Sum of Squares	DF	MS	F	Significance
Elevation	53.582	1	53.582	0.584	0.446
Gender	0.0843	1	0.0843	0.001	0.976
Elevation * Gender	15.320	1	15.320	0.167	0.683
Error	14677.908	160	91.737		

Table b19. Two-way ANOVA analyzing difference between mean body mass of male and female *Sceloporus occidentalis* at intermediate and low elevation.

Source	Sum of Squares	DF	MS	F	Significance
Elevation	34.578	1	34.578	2.183	0.142
Gender	5.131	1	5.131	0.324	0.570
Gender * Elevation	1.638	1	1.683	0.103	0.748
Error	2534.598	160	15.841		

Table b20. Student's *t*-test analyzing difference in relative clutch mass between *Sceloporus occidentalis* and *Sceloporus graciosus* (pooled populations).

Source	DF	<i>t</i> -statistic	<i>t</i> -critical	Significance
Species	23	0.466	2.068	0.645

Table b21. One-way nested ANOVA testing for difference in mean egg mass between female *Sceloporus occidentalis* and *Sceloporus graciosus* (populations pooled).

Source		Sum of Squares	DF	MS	<i>F</i>	Significance
Species	Hypothesis	0.328	1	0.328	5.903	0.023
	Error	1.265	22.787	0.0555		
Clutch (Species)	Hypothesis	1.186	23	0.0515	16.266	0.0001
	Error	0.292	92	0.0317		

APPENDIX C
USE OF ANIMALS IN
RESEARCH APPROVAL

State of California

Division of Graduate Studies

California State University, San Bernardino
5500 University Parkway
San Bernardino, CA 92407-2397
(909) 880 - 5058

MEMORANDUM

Date : April 23, 1999
To : Scott Parker
Biology Department
From : Julius D. Kaplan, Dean
IACUC, Chair
Subject: Protocol Approval



At the April 23, 1999 meeting the Institutional Animal Care and Use Committee at CSUSB approved your Protocol #98-008 "Differential Microhabitat Use, and Egg Retention Times Between the Lizards *Sceloporus occidentalis*, and *Sceloporus graciosus*." Your protocol has been approved for the dates of May 1999 through May 2000.

If any changes are made in your protocol, such as an increase in the number of animals required or the anesthetic agent used, an amendment must be submitted to and approved by the IACUC before implementation.

JK/ml

cc: Cindy Chrisler
David Polcyn

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