

**THE EFFECTS OF FOOD AVAILABILITY ON SEASONAL CHANGES
IN REPRODUCTION AND ENERGY ALLOCATION IN
THE EASTERN FENCE LIZARD,
*SCELOPORUS UNDULATUS***

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THE EFFECTS OF FOOD AVAILABILITY ON SEASONAL CHANGES IN REPRODUCTION AND ENERGY ALLOCATION IN THE EASTERN FENCE LIZARD, *SCELOPORUS UNDULATUS*

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Individuals' energy is split between reproductive effort, growth, and maintenance, with the amount beneficial to invest in each depending on food availability, which can change seasonally, between years, and due to climatic events. Energy allocated into reproductive effort is split between offspring, often by a size/number trade-off. This trade-off may shift seasonally from more, smaller offspring early in the season to fewer, larger offspring later. Two hypotheses for this shift are the "parental investment" hypothesis, where females produce larger offspring later to enhance offspring survival in a competitive environment, and the "bet-hedging" hypothesis, whereby females produce fewer late-season offspring due to increased costs of late-season reproduction. My goal was to use Eastern Fence Lizards (*Sceloporus undulatus*) to understand three objectives: which factors influence early-season reproductive traits, how investment differs seasonally between clutches (parental investment vs bet-hedging), and how females invest in maintenance, growth, or reproduction based on food availability. I collected adult lizards, provided them a standard diet until they laid their first clutch, then gave them different treatments to simulate high or low food availability. I measured reproductive traits, growth, and body composition. Body size was critical for reproductive potential, with smaller females investing primarily into growth. Reproductive effort was higher for larger females and for those receiving an increased diet, but a seasonal trade-off in clutch/egg size occurred despite increased food availability, supporting the "parental investment" hypothesis. Body size and food availability affected energy allocation and reproductive effort, with a strong trade-off in growth and reproduction.

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CERTIFICATE OF APPROVAL OF THESIS

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CHAPTER 1: INTRODUCTION

Reproductive effort, the proportion of energy that an organism devotes to reproduction, is a critical component of theories of life history evolution and natural selection (Williams, 1966), as successful reproduction and survival of offspring is what allows traits to persist into future generations. However, an individual must divide its total available energy between reproductive effort, growth, and maintenance (Heino & Kaitala, 1999; Smith & Fretwell, 1974). Often, this results in life-history trade-offs such as a negative relationship between survival and reproduction or between maintenance and growth (Schwarzkopf, 1994). Additionally, an individual's ability to increase or maintain reproductive effort while maintaining body condition is dependent on food resources (Lovern & Adams, 2008; Pianka, 1976), which are subject to change seasonally, between years, and based on environmental conditions (Dunham, 1978; Varpe, 2017). For example, for insectivores, food availability relates to rainfall which fluctuates through time, altering insect abundance (França et al., 2020; Illera & Díaz, 2006; Janzen & Schoener, 1968; Luo et al., 2012) and thus, changing the amount of energy available for growth and reproduction (Ballinger, 1977; Hoddenbach & Turner, 1968; Turner et al., 1973).

For many animals that produce multiple clutches or litters per year, an important example of resource fluctuation is a seasonal fitness decline. Seasonal fitness decline describes the tendency for conditions related to reproduction and offspring survival to deteriorate as the breeding season progresses. Such declines have been found for a variety of species (Angilletta et al., 2001; Dobson & Myers, 1989; Luo et al., 2012; Öberg et al., 2014; Rowe et al., 1994; Williamson & Bull, 1995). For example, Öberg et al. (2014)

found that reproductive traits of the Northern Wheatear (*Oenanthe oenanthe*) including nest success, clutch size, fledging success, and recruitment, declined with lower food availability. Additionally, in some lizards like Brown Anoles (*Anolis sagrei*) and Australian Jacky Dragons (*Amphibolurus muricatus*), the probability of offspring survival declines across the season, so earlier-produced offspring have a survival advantage over those produced later (Pearson & Warner, 2018; Warner & Shine, 2007). This seasonal decline may result from increased competition among reproducing adults late in the year, when resources (e.g. food) are scarce or due to increasing competition among offspring as the current year's offspring enter the environment in growing numbers (Ferguson & Bohlen, 1978; Ferguson et al., 1982). As the habitat degrades and competition increases, resources available to each individual decline, leaving less energy for reproduction, maintenance, and growth which can lead to a seasonal decrease in reproductive effort (Harriman et al., 2017).

In response to the seasonal fitness decline, many species change allocation among the number and size of offspring: early-season clutches consist of many, small offspring and late-season clutches consist of fewer, larger offspring (Heins et al., 2004; Smith & Fretwell, 1974; Williamson & Bull, 1995). This potentially offsets detrimental effects of the seasonal fitness decline by better-provisioning late-produced offspring for a more competitive environment. There are two competing hypotheses which focus on the seasonal fitness decline as it relates to effects on offspring survival versus reproducing adults: “parental investment” vs “bet-hedging,” respectively (Ferguson & Bohlen, 1978; Nussbaum, 1981). The “parental investment” hypothesis states that females produce larger offspring later in the season so offspring will be better competitors and have

greater survival. This is an “adaptive” hypothesis because it posits that natural selection has shaped seasonal shifts in reproduction in response to offspring survival. Thus, observed seasonal changes in offspring size and number should persist regardless of food available late in the season. Alternatively, the “bet-hedging” hypothesis, posits that food for reproducing adults is limited later in the year, and females must ensure that all offspring are at least minimally provisioned. Thus, in response to decreased food supply, females commit to laying fewer eggs, but any additional energy available will be used to increase offspring size. This is an “environmental” hypothesis, because it posits that changes in offspring size and number are driven by the environment and are not endogenous (i.e. genetic or otherwise inevitable). Thus, observed changes in offspring size and number should be most apparent if food is limited late in the season. While a few studies have provided support for parental investment (Ferguson et al., 1982; Mitchell et al., 2018; Sinervo & Doughty, 1996), in most study systems, evidence is mixed because of the difficulty in decoupling the effects of multiple correlated environmental variables in field studies (e.g. parental quality and timing of reproduction; Pärt et al., 2017; Verhulst et al., 1995). Controlled experimental studies are helpful to evaluate these hypotheses.

Lizards make excellent models to understand environmental effects on reproductive effort because most provide no parental care, and therefore, effort can be calculated simply with clutch and egg mass in relation to maternal body mass (Ballinger & Clark, 1973). Moreover, the tendency for lizards to lay several multi-egg clutches per year is helpful to evaluate seasonal changes in offspring size and number. Additionally, many species exhibit seasonal shifts in reproduction by laying many smaller eggs in

early-season clutches and fewer but larger eggs later (Ferguson & Bohlen, 1978; Hall et al., 2020; Nussbaum, 1981; Uller & Olsson, 2010; Warne & Charnov, 2008). In particular, lizards of the genus *Sceloporus* have been an important model for studies of reproduction because they are relatively abundant, easy to maintain in captivity, and lay 1-3 multi-egg clutches per year (Angiletta et al., 2001; DeMarco, 1989; Jones et al., 1987). A few studies demonstrate seasonal changes in clutch or egg size (Du et al., 2014; Ferguson et al., 1980; Ferguson & Snell, 1986). Indeed, Ferguson and Snell (1986) showed that *S. undulatus* in the lab that produced two clutches within a year had consistent clutch mass (i.e. equal amounts of reproductive effort), but effort was divided into fewer, larger eggs in the second clutch. Alternatively, DeMarco (1989) found that seasonal changes for *S. woodi* were not consistent across years indicating a role of year-to-year environmental changes in determining reproductive allocation. To my knowledge, no study has assessed the parental investment and bet-hedging hypotheses using *Sceloporus* lizards in a controlled setting. Thus, my goal is to experimentally evaluate these competing hypotheses by subjugating *S. undulatus* to seasonal changes in food availability in a controlled experiment.

I captured adult Eastern Fence Lizards (*Sceloporus undulatus*) and placed them in breeding pairs under a standard diet. After each female laid a clutch of eggs, those eggs were collected, measured, and then incubated or dissected to evaluate maternal investment per clutch and per offspring. Each mating pair was then placed on a high or low food diet and late-season clutches were likewise collected and analyzed. I aimed to achieve three objectives with this design. My first objective was to determine factors that influence early-season reproduction in the Eastern Fence Lizard. To achieve this, I

analyzed data on reproductive success (1-reproduced, 0 – did not reproduce), initial clutch size, egg size, as well as egg and hatchling morphology for the first clutch of the year. All females were on a standard, control diet during this time. My second objective was to assess the parental investment and bet-hedging hypotheses by comparing early-season and late-season clutches in response to different food treatments. I considered two potential outcomes: first, regardless of high or low food availability late in the season, females may shift reproduction by producing many, small eggs in the first clutch and fewer, larger eggs in the second clutch. This would indicate seasonal trade-offs in offspring size and number are endogenous (i.e. not subject to resources) and support the parental investment hypothesis. Alternatively, females in the low food treatment may exhibit the aforementioned seasonal change, while those in the high treatment may produce large clutches both early and late in the season, supporting the bet-hedging hypothesis (i.e. resource changes drive seasonal shifts in reproduction).

Although my original experimental design assumed all females would reproduce, many did not, leaving me with three categories (i.e. ‘treatments’) of lizards: females that did not reproduce and remained on a standard diet for the entire season, females that did reproduce and subsequently received an enhanced, late-season diet, and females that reproduced and subsequently received a low food, late-season diet. This allowed me to consider a third objective: to determine how females invest in growth, maintenance, or reproduction based on body size and food available across the season. For each of these groups, I analyzed changes in body size, reproductive traits, and final body composition to evaluate whether females invested in growth, maintenance or reproduction (or some combination) based on their “choice” to reproduce or not and their environment. This

allowed me to consider the advantages and disadvantages of reproduction in response to variation in resources.

CHAPTER 2: METHODS

Study Species

The Eastern Fence Lizard (*Sceloporus undulatus*) (Figure 1a) is a widespread, highly fecund lizard found across the southeastern two-thirds of the United States from New Jersey to the Mississippi River and south to central Florida (Powell et al., 2016). *Sceloporus undulatus* are insectivorous, eating a wide variety of insects, with the majority of their diet being ants (Hamilton & Pollack, 1961). They prefer habitats with elevated perches, such as fences, logs, and stumps (Powell et al., 2016) which they use to scan for insects. Males and females are sexually dimorphic: adult males have blue and black coloration on the belly and neck, and females tend to have darker barring across their back (Powell et al., 2016). Both juvenile and adult males possess two enlarged post-anal scales (Parker, 1994). The reproductive season of *S. undulatus* ranges from April to July, with mature females laying two to three clutches per year (Ferguson et al., 1980; Jones et al., 1987; Parker, 1994; Tinkle & Ballinger, 1972; Tinkle & Dunham, 1986). Average clutch size varies from 5.5 to 11.8 eggs and average mass of eggs varies from 0.35 g to 0.38 g across populations, as in Du et al. (2014) and as summarized by Ballinger et al. (1981).

Sceloporus undulatus populations can differ greatly in life history across latitudes, with southern populations growing faster and producing more offspring each year than northern populations (Angilletta, 2001). Southern populations are also active for longer during the day and for a greater portion of the year (March to November vs April to October for northern populations) (Angilletta, 2001). Body size and age at maturity also

Figure 1

Housing conditions of captive Eastern Fence Lizards (Sceloporus undulatus).



(a) An adult lizard basking, (b) cage layout, and (c) arrangement of cages in the aviary.

vary across populations and latitudes. For example, Tinkle and Ballinger (1972) found that females in a South Carolina population initiated reproduction at 9 to 10 months and a minimum of 55 mm SVL, while a population further north in Ohio did not reproduce until 20 months post-hatching at a minimum of 66 mm SVL. Moreover, key reproductive traits like clutch size and mass can vary across latitudes. Du et al. (2014) analyzed reproductive traits for populations from Indiana, Mississippi, and Florida. They found that northern populations produced clutches with greater mass and larger eggs than southern populations. They found that body size increased with latitude and accounted for the trends in reproductive resource allocation. However, southern populations were more likely to lay 2 or 3 clutches per year, due to the longer reproductive season.

Studies of *S. undulatus* life-history and reproduction have been conducted in various regions throughout its range, such as Ohio, South Carolina, New Jersey, Georgia, Indiana, Florida, and Mississippi (Angilletta, 2001; Crenshaw, 1955; Du et al., 2014;

Parker, 1994; Tinkle & Ballinger, 1972). To my knowledge, no research has been conducted on populations in Tennessee or at any location of comparable latitude (i.e. all previous studies either further north or south). Therefore, in addition to addressing my three objectives (outlined above), this thesis fills an important knowledge gap regarding the life history of *S. undulatus* across its range.

Animal Capture and Husbandry

I captured forty-eight Eastern Fence Lizards ($n = 24$ male; $n = 24$ female) from two populations: Edgar Evins State Park, Silver Point, Tennessee and Standing Stone State Park, Hilham, Tennessee (approximately 56 km apart). Both Edgar Evins and Standing Stone are in the Interior Plateau Level III ecoregion of Tennessee, with Edgar Evins being in the Outer Nashville Basin and Standing Stone in the Eastern Highland Rim Level IV ecoregions (Griffith et al., 1997). Tinkle and Ballinger (1972) found the minimum body size of reproductive female *Sceloporus* was between 47- and 66-mm snout-vent length (SVL), depending on the population, with means ranging from 57- to 75-mm; however, the minimum size at reproduction is unknown for populations in Tennessee. Therefore, I attempted to capture animals of reproductive size (females captured ranged from 49- to 80-mm SVL; males from 48- to 73-mm SVL). I aimed to capture lizards as early as possible after they emerged from hibernacula to reduce the impact of field conditions on reproduction; thus, I searched for lizards starting on 16 April and captured lizards from 22 April to 3 June. Captive lizards produced their first clutches on 23 May, and the only lizards captured after that date were males and two gravid females. No other females were gravid at time of capture; therefore, it is unlikely any females laid a clutch prior to capture.

Each lizard was measured for body mass (g), SVL (mm), tail length (mm), and females were palpated for oviductal eggs. I toe-clipped each lizard for identification and housed them in male:female pairs of similar body mass from the same population in screen butterfly cages (Figure 1b,c). However, females can store sperm, and eggs may have been sired by a male prior to capture. Enclosures were 42-x 42-x 76-cm mesh butterfly cages (RESTCLOUD), each with a cement block (41-x 19-x 10-cm) to use as a shelter and for sunning, a box of topsoil for egg laying, and plastic vines for cover (Figure 1b). Cages were housed in the Tennessee Technological University aviary to ensure the lizards experienced the native climate while providing protection from animals that may damage the cages (e.g. raccoons). Importantly, the aviary is 34 km from Standing Stone State Park and 31 km from Edgar Evans; therefore, climate conditions were likely similar to native habitats. Cages were arranged with pairs from different locations and under different treatments dispersed to prevent spatial autocorrelation (Figure 1c). I misted cages with water daily and fed lizards calcium and vitamin dusted crickets per the experimental treatments (see below).

Experimental Design

I initially provided 10 crickets per cage ($n = 5$ per lizard) three times per week until a female laid her first clutch. Then, each pair was randomly assigned one of two feeding treatments for the remainder of the study: a high food treatment, consisting of twenty crickets per cage ($n = 10$ per lizard) given three times per week, or a low food treatment, consisting of six crickets per cage ($n = 3$ per lizard) given three times per week. The pairs from each location were randomly and evenly selected to be distributed between the two feeding treatments ($n = 12$ per treatment). However, there were females

that never reproduced, so I instead had two feeding treatments and one non-reproductive group (n = 8) (Table 1). I recorded the total mass of crickets offered to each cage to determine treatment-specific biomass of food availability. The mean cricket mass and standard deviation for each diet per feeding per lizard were as follows: standard diet, 650 mg (± 70 mg SD), low food diet, 460 mg (± 20 mg SD), and the high food diet was 1,360 mg (± 60 mg SD).

Table 1

Body size of Sceloporus undulatus at time of capture for each study population and each treatment

	N	Location		SVL (mm)				Mass (g)				Tail length (mm)			
		Edgar Evins	Standing Stone	mean	SD	min	max	mean	SD	min	max	mean	SD	min	max
LF males	8	3	5	60.9	5.7	53	69	8.11	2.19	5.19	10.84	72.9	31.9	19	101
HF males	7	3	4	65.6	4.6	60	73	9.69	1.49	7.51	11.97	73.9	30.3	21	101
NR males	11	7	4	57.0	6.0	48	67	6.70	2.47	3.62	11.04	67.9	22.9	17	93
LF fems	8	3	5	68.0	7.9	54	74	13.19	4.37	6.62	17.88	82.0	14.9	61	97
HF fems	8	4	4	67.4	8.9	52	80	12.74	4.74	5.25	19.68	77.6	20.6	46	108
NR fems	8	5	3	53.4	3.1	49	59	5.13	0.82	4.04	6.50	68.4	12.8	38	80

Additional lizards (over the n = 24 males and n = 24 females, were caught to replace ones that died early in the experiment).

Abbreviations: SVL = snout-vent length. SD = standard deviation. LF = low food treatment. HF = high food treatment. NR = non-reproducing. fems = females. Min = minimum value. Max = maximum value. N = sample size

Having all females on the same initial diet and then switching diets after the first clutch allowed me to consider factors that influence reproduction early in the season and then compare that to how energy is allocated among reproduction and growth later when

environments tend to be harsher and reproduction can be more costly, potentially influencing winter survival and/or future reproduction. Moreover, this design allowed me to assess the bet-hedging and parental investment hypotheses in a novel way (i.e. by comparing early- and late-season clutches to each other across treatments). If late season trade-offs in the number and size of offspring are endogenous (i.e. genetic and adaptive), females should exhibit a decrease in clutch size and increase in egg size in the second clutch, despite food resources, though overall reproductive effort would be lower for females in the low food treatment. This supports the parental investment hypothesis. Alternatively, if late-season trade-offs in size and number are dependent on the environment (i.e. bet hedging hypothesis), only females in the low food treatment should exhibit the trade-off or the trade-off should at least be weaker for the high food treatment. Finally, comparing final body measures between the groups allows me to identify the costs of reproduction and growth based on food availability on the individual, as well as identifying how energy was apportioned to reproduction, growth, or maintenance of body condition.

Depending on where the *S. undulatus* invested their energy, they should match certain predictions. Table 2 demonstrates predicted results for a hypothetical female investing in growth, maintenance, or reproduction. Additionally, I made predictions on how growth (Figure 2a), reproduction (Figure 2b), and body condition (Figure 2c) would vary between the treatments. Because some females did not reproduce (i.e. were not mature), I include predictions for these as well.

Table 2

Hypothesized observations given investment in maintenance, growth, or reproduction

Investing in:	Expected responses:
Maintenance only	No reproduction, no growth in SVL, and body mass remains relatively constant.
Growth only	Increase in SVL, but no reproduction, and relative body mass (i.e. body condition) declines.
Reproduction only	Lays at least one clutch of eggs, but there is no growth in SVL, and a decline in body mass.

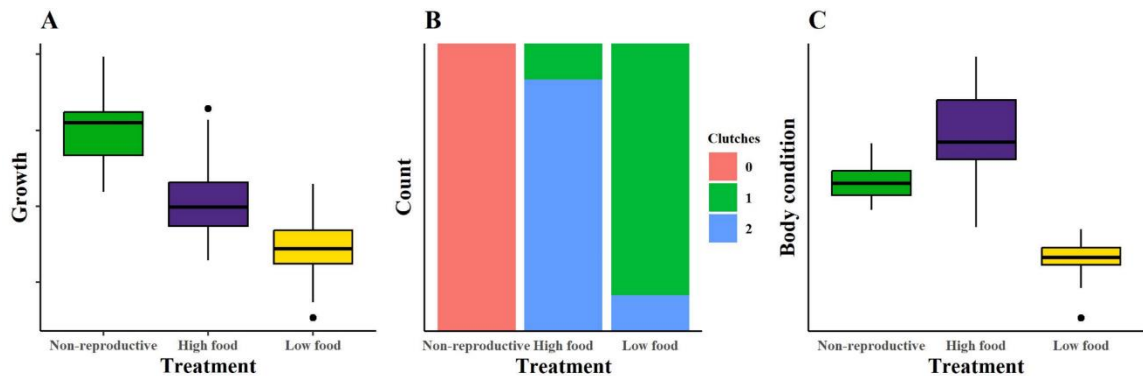
Assumes a minimal investment of energy into maintenance to facilitate survival. Energy

can also be invested into a combination of any of these factors.

Abbreviations: SVL = snout-vent length

Figure 2

Expected patterns between the treatment groups



(a) Boxplot of hypothesized growth trends between the treatments. (b) Bar plot of hypothesized number of clutches laid by individuals in the different treatments. (c) Boxplot of hypothesized trends in final body condition of the individuals in the different treatments.

Egg Incubation and Offspring Phenotypes

I checked nest boxes daily for eggs. When discovered, I removed the eggs and placed them into vermiculite with -150 kPa water potential for transport to the laboratory.

I recorded clutch size and clutch mass as well as egg mass (mg), egg length (mm), and egg width (mm). Each egg was randomly allocated to be dissected, incubated, or used for another study (results not reported here). To understand the effects of the feeding treatments on embryo development and hatchling morphology, I incubated a subset of eggs ($n = 44$). Each egg was incubated in a glass jar, half-filled with vermiculite with water potential of -150 kPa (Tracy, 1980). I covered the jar with cling film, secured with a rubber band, to reduce evaporation but allow gas exchange, and placed jars in an incubator (GQF 1550 Hatcher) at 28.5°C . I re-weighed the eggs approximately half-way through incubation (i.e. day 26), and three-quarters of the way through incubation (day 39) to calculate water uptake. I estimated these dates using a previous egg incubation study conducted at various incubation temperatures (Andrews et al., 2000). I checked incubators for hatchlings daily.

Maternal food availability can affect hatchling size, growth, and survival (Warner & Lovern, 2014). To determine the effect of the food treatment on the size and morphology of the offspring, I measured each hatchling's SVL (mm), tail length (mm), body mass (mg), and recorded sex by checking for enlarged post-anal scales which are present on males. After measurements were taken, each hatchling was euthanized. These measurements were repeated across clutches to measure how food resource availability affected the change in distribution of reproductive effort and clutch and egg size between clutches.

To understand how the feeding treatments influenced resource allocation among offspring (e.g. water vs yolk vs shell mass), I dissected a subset of eggs ($n = 44$) and desiccated their components to determine a dry mass of the egg yolk and shell. The eggs

were opened, and the yolk and embryo put onto an aluminum foil tray. The empty shell was put onto another tray. I recorded the wet mass of each and then placed them into a drying oven at 65°C. Trays were re-weighed periodically until the mass was constant, at which time the components were assumed devoid of water. Another subset of eggs (n = 43) was placed in the oven whole because females laid them outside of the nest box, and they desiccated prior to collection. Therefore, I dried them in the oven so I could later use coefficients from a regression of wet mass against dry mass of eggs to estimate their original mass at oviposition (see below).

Maternal Reproduction, Growth, and Final Body Composition

I recorded clutch size (i.e. number of eggs) and the mass of individual eggs for each female to assess treatment-specific differences in reproduction. This also allowed me to determine how clutch size and egg mass vary across females and treatments (i.e. trade-off between offspring size and number). I periodically re-massed the adult lizards across the study to assess treatment-specific growth rates. Final measures of body mass (g) and SVL (mm) were taken before euthanasia at the end of the study (23 September). Lizards were euthanized via intraperitoneal injection of neutral buffered MS222 (i.e. overdose of anesthetic). After euthanasia, I removed and weighed each lizards' fat pads and liver, as their mass indicates levels of fat storage in the body (Derickson, 1976; Dessauer, 1955). I also removed the ovaries from each female, recorded their mass and inspected the follicles for yolk. This allowed me to evaluate treatment-specific energy allocation of lizards at the end of the study and confirm that females were no longer reproductive.

Statistical Analyses

I conducted all analyses in R (ver. 4.3.0; R Core Team, 2023) using generalized and general linear mixed effects models for binomial and gaussian data, respectively. Generalized linear models were for analysis of adult survival (0 = died, 1 = survived) and reproduction (0 = did not reproduce, 1 = reproduced). All analyses were conducted using base R for models with no random effects and the ‘lme4’ package (Bates et al., 2009) for mixed effects models. Initial models included two-way interaction terms, but these were dropped from the model in step if not statistically significant ($p > 0.05$). Some interactions were also dropped due to a high degree of autocorrelation with other fixed effects which rendered them uninformative. Statistical assumptions were assessed by visually inspecting model residuals. My statistical approach varied to achieve each of three major objectives. (See Appendix for all initial statistical models).

Objective one was to determine factors that influence early season reproduction and quantify potential trade-offs between offspring size and number during early-season clutches under standard food conditions (i.e. create a baseline to compare late-season clutches). An ancillary sub-objective was to consider the role of maternal body size in such trade-offs given the importance of body size in lizards. To accomplish this, I analyzed the effect of female body size (SVL) on probability of reproduction and the effect of female body size (SVL) and egg mass (mg) on clutch size. To understand factors that influence egg size, egg composition, and hatchling phenotypes I used clutch size, maternal SVL, and their interaction as fixed effects to independently analyze the response variables egg mass (mg), egg density (mg/mm^3), shell mass (mg), yolk mass (mg), water uptake rate (mg/day), hatchling mass (mg), and hatchling SVL (mm). Maternal ID was

included as a random intercept (i.e. for mixed models) to account for similarity between eggs produced by the same female. Sex of hatchlings was also included in the hatchling body size models to determine if there were any sex-specific effects. I did have females that started the season under reproductive size. For the females that started under reproductive size and were able to lay a clutch, I estimated their SVL at time of oviposition using growth rates calculated from initial and final SVL. For females that never laid a clutch, I estimated their SVL on the median date that other females laid their first clutch. To explore the interactions between clutch size and SVL, I divided females into two groups: those larger and those smaller than minimum reproductive size at capture and re-analyzed the data.

Objective two was to consider the effect of food availability on late-season reproduction. In particular, I aimed to consider how food treatments influenced the size and number of eggs in second vs first clutches. To accomplish this, I repeated the analyses for objective one, but I examined only the clutches from females that laid two clutches and used clutch ID (i.e. clutch 1 vs clutch 2) as a fixed effect. Analyses of hatchling body size were not repeated because only two females had eggs that successfully hatched in each clutch (due to desiccation of eggs prior to collection). I also examined the effects of body size and egg size on the size of the second clutch. I estimated each female's SVL at the time she laid her first clutch using growth rates across the study and used this as a fixed effect along with the average egg size in the second clutch.

Objective three was to examine how females invest in maintenance, growth, or reproduction based on food available across the season. For measures of growth, I

analyzed the change in SVL and body mass from the beginning to the end of the study. To consider energy storage (e.g. maintenance), I analyzed final body condition, liver mass, and fat pad mass. To consider any final investment in reproduction, I analyzed ovary mass. Fixed effects for each model were SVL, treatment, and their interaction. Due to autocorrelation between some treatments and SVL (i.e. small females did not reproduce), I first centered the SVL by treatment group. Finally, I also analyzed survival by treatment group to see if the food treatments and/or reproduction had any effect on maternal survival.

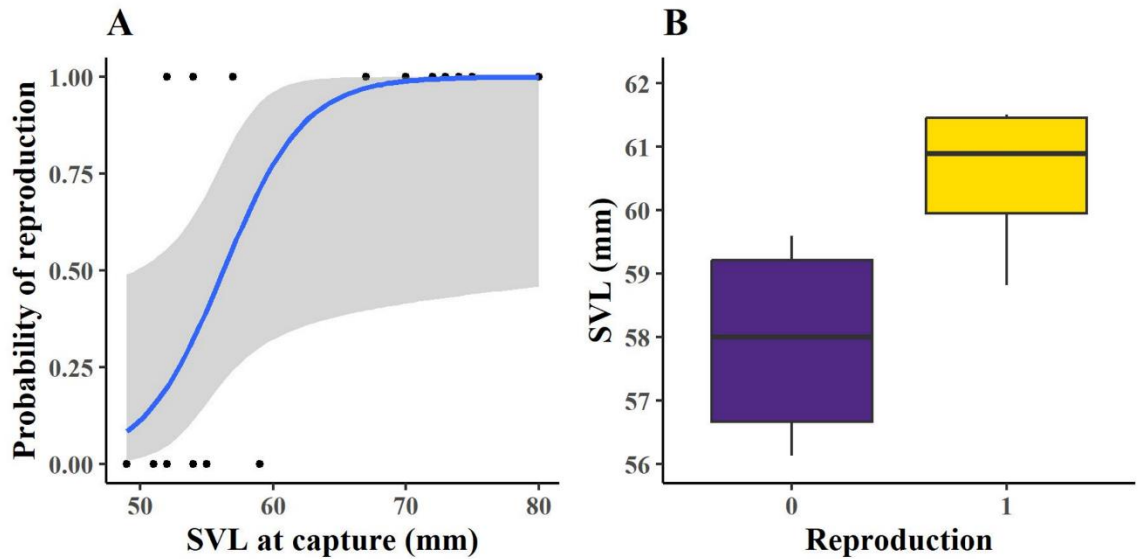
CHAPTER 3: RESULTS

Objective One: Early Season Reproduction

Under a standard diet, the probability of reproduction was correlated with SVL at time of capture ($P = 0.04$), and 60 mm appeared to be a critical minimize size (Figure 3a, Table 3). All females that were ≥ 60 mm SVL at capture laid at least one clutch. However, four females that were smaller than 60 mm at capture ($n = 3$) laid eggs. Three were estimated to be at least 60 mm in size at reproduction, with one being 58.8 mm. For females that never laid a clutch, none of these estimated SVLs were ≥ 60 mm (Figure 3b) at the time the other small females were laying eggs.

Figure 3

(a) Probability of reproduction based on SVL at capture for female *Sceloporus undulatus* and (b) Estimated SVL at time of oviposition for small females



(a) The blue line shows the model estimate of probability of reproduction based on SVL at capture. The black circles are the raw data (1= reproduced; 0 = did not

reproduce). The shaded area represents the 95% confidence interval. (b) Estimated SVL at day of oviposition for females that were under 60 mm at capture (n = 4) and estimated SVL of females that didn't reproduce during the same time interval (n = 8).

Table 3

Results of general and generalized linear models for objective one: statistical analyses of early season reproduction

Response variable	Fixed effect	Estimate	Std. Error	df	t	P
Reproduced (reproduced = 1; did not = 0)	(Intercept)	-18.50	8.97	23	-2.06*	0.04
	SVL (mm)	0.33	0.16		2.03*	0.04
Clutch size	(Intercept)	54.78	14.22	13	3.85	0.002
	SVL (mm)	-0.73	0.22		-3.35	0.01
	Mean egg mass (mg)	-0.15	0.037		-4.00	0.002
	SVL (mm):Mean egg mass (mg)	0.002	0.001		4.20	0.001
Egg mass (mg)	(Intercept)	1529.06	472.71	13.57	3.24	0.01
	SVL (mm)	-17.23	6.90	13.51	-2.50	0.03
	Clutch size	-135.78	51.71	13.42	-2.63	0.02
	SVL (mm):Clutch size	2.01	0.72	13.38	2.77	0.02
Egg mass (mg) (Larger females)	(Intercept)	222.00	64.34	11.30	3.45	0.01
	Clutch size	16.26	5.89	11.15	2.76	0.02
Egg mass (mg) (Smaller females)	(Intercept)	723.74	95.51	2.00	7.58	0.01
	Clutch size	-45.06	11.68	1.98	-3.86	0.06
Egg density	(Intercept)	1.18	0.41	14.09	2.86	0.01
	Clutch size	0.01	0.03	14.00	0.51	0.62
	SVL (mm)	-0.01	0.01	14.06	-0.71	0.49
	Sex	8.99	7.91	21.70	1.14	0.27
	Clutch size	8.61	7.66	8.49	1.13	0.29
	SVL (mm)	-2.72	2.10	8.90	-1.29	0.23
	Egg mass (mg)	0.50	0.13	31.45	3.72	0.001

Table 3(continued)

Response variable	Fixed effect	Estimate	Std. Error	<i>df</i>	<i>t</i>	<i>P</i>
	Sex	0.44	0.32	25.02	1.40	0.17
	Clutch size	0.20	0.17	9.29	1.23	0.25
	SVL (mm)	-0.08	0.05	10.52	-1.63	0.13
	Egg mass (mg)	0.01	0.004	16.84	2.24	0.04
Shell mass (mg)	(Intercept)	20.72	21.59	9.64	0.96	0.36
	SVL (mm)	0.30	0.41	9.75	0.74	0.48
	Clutch size	-1.22	1.47	9.26	-0.83	0.43
Yolk mass (mg)	Egg mass (mg)	0.03	0.03	26.55	1.20	0.24
	(Intercept)	66.56	40.07	4.17	1.66	0.17
	SVL (mm)	0.38	0.77	4.08	0.49	0.65
	Clutch size	0.83	2.76	3.99	0.30	0.78
Water uptake rate (mg/day)	Egg mass (mg)	0.03	0.04	33.97	0.86	0.39
	(Intercept)	20.58	7.27	9.54	2.83	0.02
	Egg mass (mg)	-0.01	0.01	30.78	-0.96	0.34
	SVL (mm)	0.06	0.15	8.88	0.43	0.68
	Clutch size	0.27	0.51	8.45	0.52	0.62

Bold type represents statistical significance. Asterisk denotes *z*-value statistic rather than *t*-value.

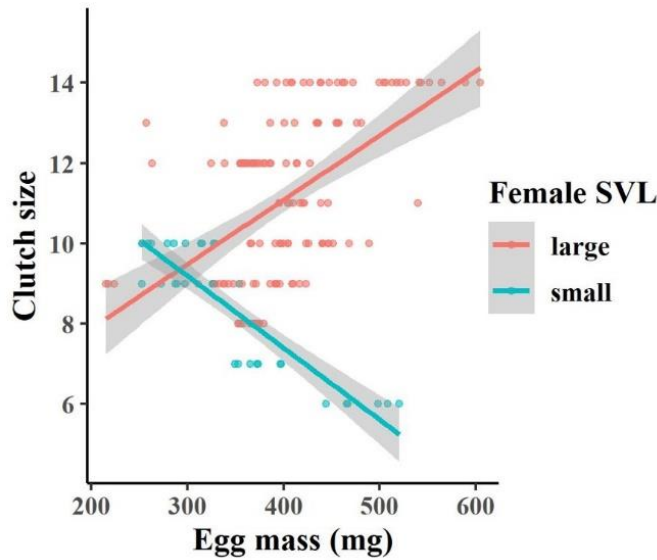
Abbreviations: SVL = snout-vent length. *df* = degrees of freedom. *t* = *t*-value. *P* = *p*-value.

Egg size and clutch size correlated with each other as well as with maternal body size (Table 3). Additionally, clutch size was influenced by the interaction between egg mass and SVL ($P = 0.001$; Table 3), and egg mass was affected by the interaction between clutch size and SVL ($P = 0.02$; Table 3). Analyzing the data grouped by those larger and those smaller than 60 mm SVL revealed that larger females had a positive

relationship between egg mass and clutch size; for every additional egg in the clutch, egg mass increased by 16.26 mg (\pm 5.89 SE) (Table 3; Figure 4). Smaller females, however, exhibited a trade-off between clutch size and egg mass; for every additional egg in the clutch the egg mass decreased by 45.06 mg (\pm 11.68 SE) (Table 3; Figure 4).

Figure 4

*Interaction between female body size of *Sceloporus undulatus* and clutch and egg mass*



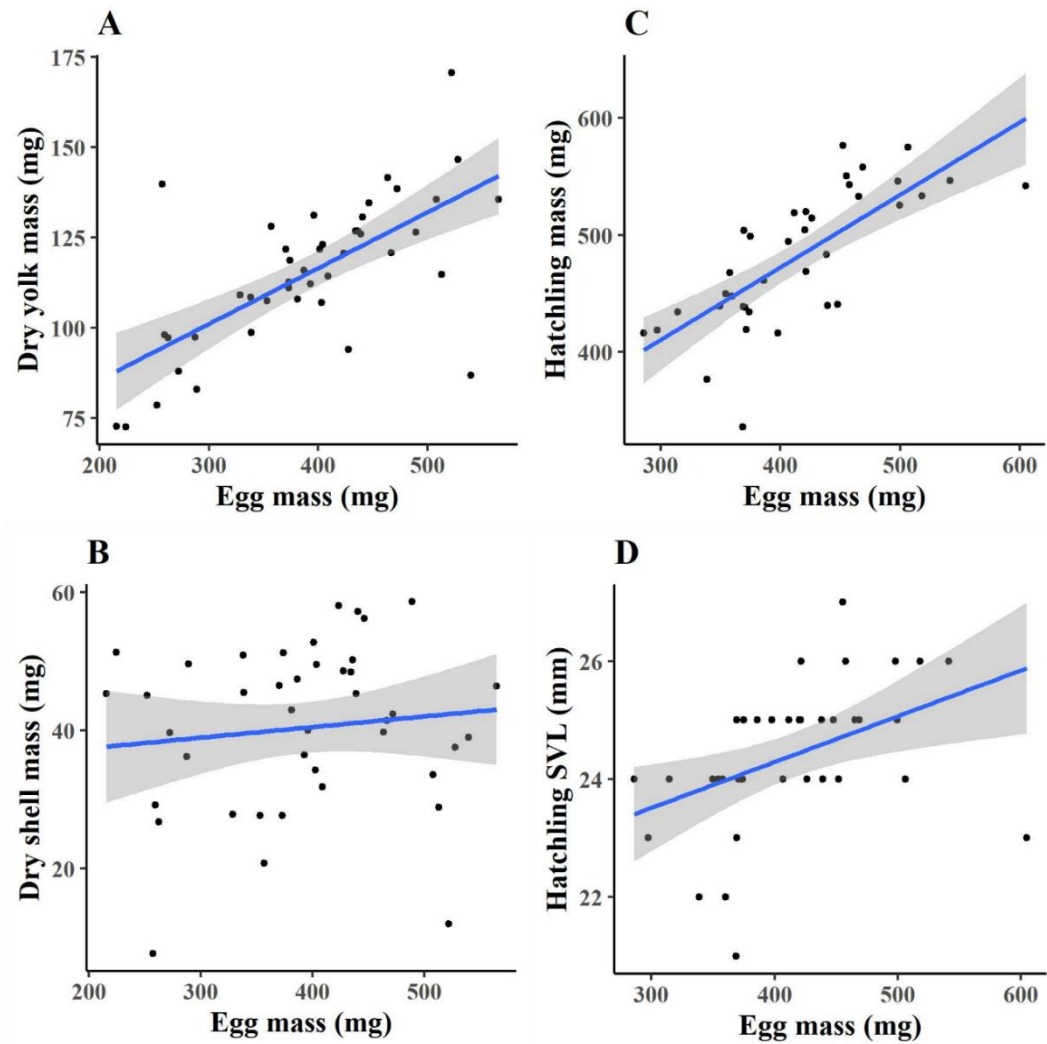
Red line represents the trend for larger females (> 60 mm SVL upon capture). Blue line represents the trend between clutch size and egg size for smaller females (≤ 60 mm SVL upon capture). Shaded areas represent a 95% confidence interval. Closed circles are the raw data.

In general, maternal body size and clutch size had little effect on egg components and hatchling phenotypes when using egg mass as a covariate (Table 3). Yolk mass did not correlate with SVL or clutch size but, while not statistically significant, did show an increase with egg mass (Table 3; Figure 5a). Eggshell mass did not correlate with maternal SVL, clutch size, or egg mass (Table 3; Figure 5b). Hatchling mass and SVL

increased with egg mass ($P = 0.0008$, $P = 0.02$, respectively; Table 3). For every 1 mg increase in egg mass, hatchling mass increased by 0.50 mg (± 0.13 SE) (Figure 5c) and SVL increased by 0.01 mm (± 0.004 SE) (Figure 5d). However, the mass and SVL of hatchlings were not affected by maternal SVL nor did they differ between hatchling sexes (Table 3).

Figure 5

Correlations between egg mass of Sceloporus undulatus on egg components and hatchling body size



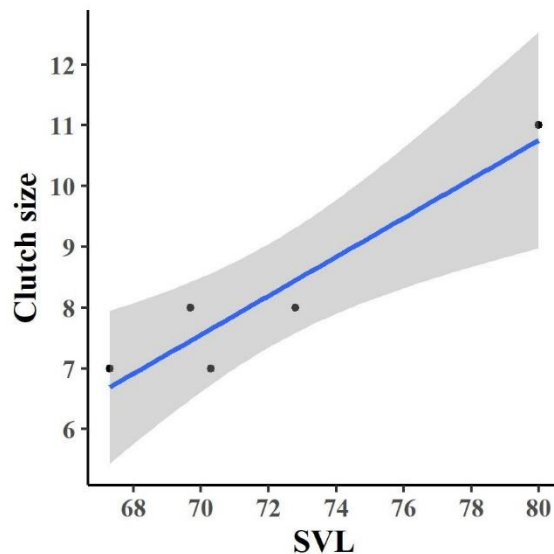
Closed circles represent individual data points, blue line represents trendline, and shaded area is the 95% confidence interval. (a) Egg mass vs dry mass of the yolk. (b) Egg mass vs dry mass of the shell. (c) Egg mass vs hatchling mass. (d) Egg mass vs hatchling SVL.

Objective Two: Early versus Late Season Reproduction

Only females on the high food treatment reproduced twice, with five of the eight laying a second clutch. For the three that did not lay a second clutch, two were under 60 mm SVL at capture and one died. The size of the second clutch correlated with SVL (Table 4): for every 1 mm increase in SVL, clutch size increased by 0.26 eggs (± 0.04 SE) (Figure 6). Clutch size was negatively correlated with egg size, but this was not statistically significant ($P = 0.09$; Table 4).

Figure 6

Relation between size of second clutch of Sceloporus undulatus and maternal SVL



The blue line represents the trendline, closed circles are raw data, and the shaded area is the 95% confidence interval.

Table 4

Results of general linear models for objective two: statistical analyses of early versus late season reproduction

Response variable	Fixed effect	Estimate	Std. Error	df	t	P
Clutch size (only clutch two)	(Intercept)	-3.57	4.27	2	-0.84	0.49
	Mean egg mass (mg)	-0.02	0.01		-3.11	0.09
Clutch size	(Intercept)	-21.64	5.10	3.51	-4.25	0.02
	Mean egg mass (mg)	0.04	0.10	3.20	3.86	0.03
	Clutch ID	20.41	5.73	2.94	3.56	0.04
	SVL (mm)	0.26	0.07	3.19	3.80	0.03
	Mean egg mass (mg):Clutch ID	-0.06	0.01	2.94	-4.09	0.03
Egg mass (mg)	(Intercept)	364.57	342.64	3.38	1.06	0.36
	Clutch ID	178.34	21.17	89.96	8.42	5.47E-13
	Clutch size	35.35	6.91	90.53	5.12	1.72E-06
	SVL (mm)	-7.98	5.24	4.94	-1.52	0.19
Egg density	(Intercept)	-1.13	1.02	3.14	-1.10	0.35
	SVL (mm)	0.03	0.01	3.13	2.02	0.13
	Clutch ID	3.51	0.39	88.02	9.04	3.37E-14
	SVL (mm):Clutch ID	-0.05	0.005	88.02	-8.56	3.38E-13
Shell mass (mg)	(Intercept)	157.89	102.98	3.07	1.53	0.22
	SVL (mm)	-1.39	1.40	2.84	-0.99	0.40
	Clutch ID	8.09	2.92	20.27	2.77	0.01
	Egg mass (mg)	-0.03	0.03	19.94	-1.08	0.29
Yolk mass (mg)	(Intercept)	99.91	101.50	3.35	0.98	0.39
	SVL (mm)	0.15	1.33	2.67	0.11	0.92
	Clutch ID	15.44	4.81	21.01	3.21	0.004
Water uptake rate (mg/day)	Egg mass (mg)	-0.01	0.05	20.03	-0.16	0.87
	(Intercept)	37.18	19.41	2.38	1.92	0.18

Table 4(continued)

Response variable	Fixed effect	Estimate	Std. Error	df	t	P
	Clutch ID	2.99	0.87	15.35	3.41	0.004
	SVL (mm)	-0.15	0.25	1.94	-0.59	0.62

Bold type represents statistical significance

Abbreviations: SVL = snout-vent length. *df* = degrees of freedom. *t* = *t*-value. *P* = *p*-value.

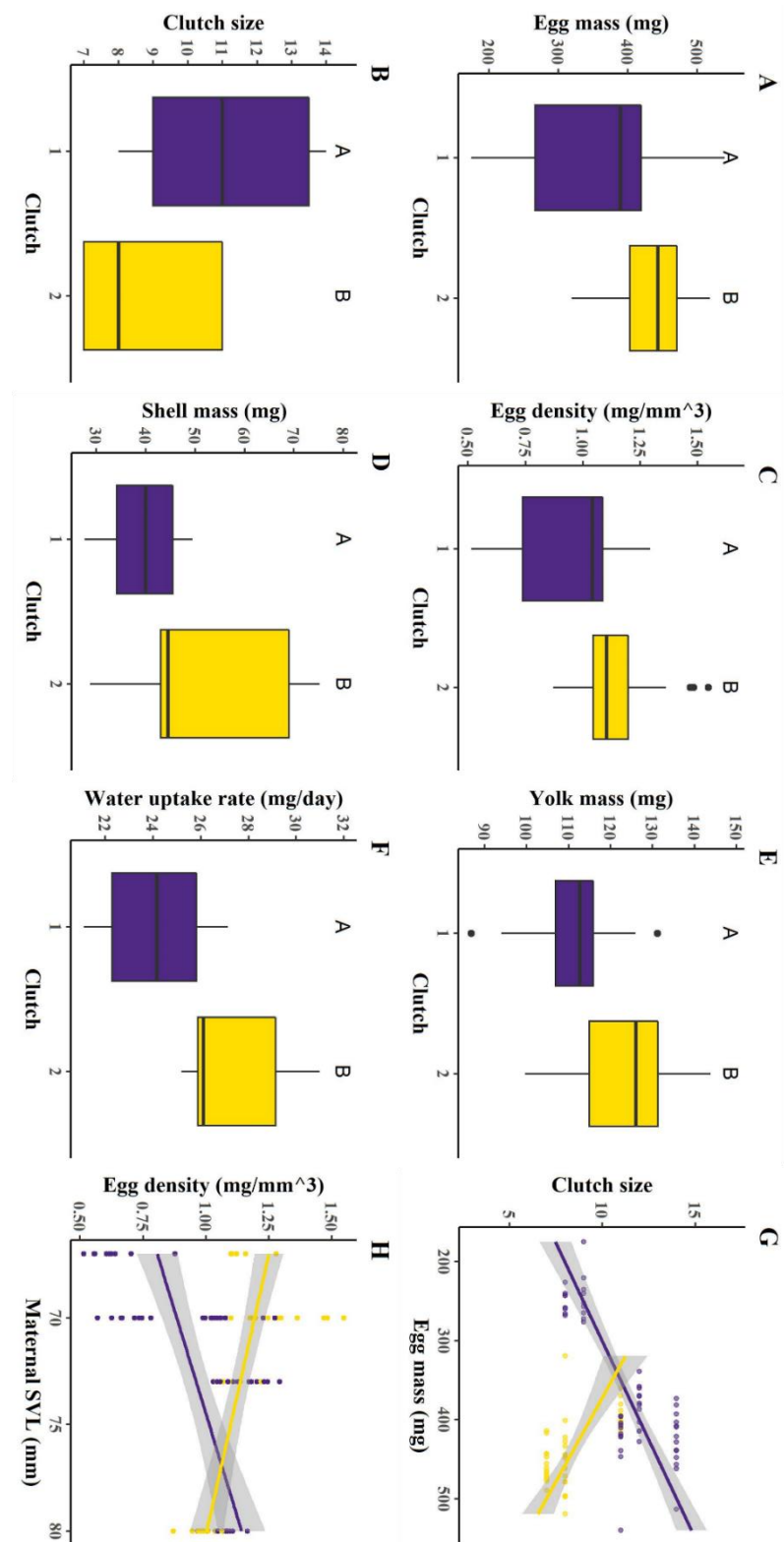
Second clutches differed from the first clutches in several ways. Second clutches contained fewer ($P = 0.04$) but larger ($P = 5.47E-13$) eggs (Table 4; Figure 7a,b) and these eggs were more dense ($P = 3.37E-14$) with greater shell mass ($P = 0.01$) and yolk mass ($P = 0.004$) (Table 4; Figure 7c,d,e). Additionally, eggs from the second clutch absorbed more water during incubation (Table 4; Figure 7f). There was also an interaction between egg mass and clutch ID (i.e. first vs second clutch; $P = 0.03$), such that the second clutch exhibited a trade-off between egg mass and clutch size, having to choose between either smaller eggs or larger eggs, but clutch one did not (Figure 7g, Table 4). Smaller females produced denser eggs in their second clutch, but egg density was similar between clutches for larger females ($P = 3.38E-13$) (Figure 7h; Table 4).

Objective Three: Energy Investment

In general, females on the high food diet had greater liver, ovary, and fat pad mass and higher body condition than non-reproducing females and females on the low food diet (Figure 8); however, not all trends were statistically significant. Specifically, females on the high food diet had greater liver ($P = 0.01$; Figure 8a) and ovary mass ($P = 0.02$; Figure 8b) than non-reproducing females and higher fat mass than both non-reproducing females and those on the low food treatment ($P = 0.003$; $P = 0.008$, respectively; Figure

Figure 7

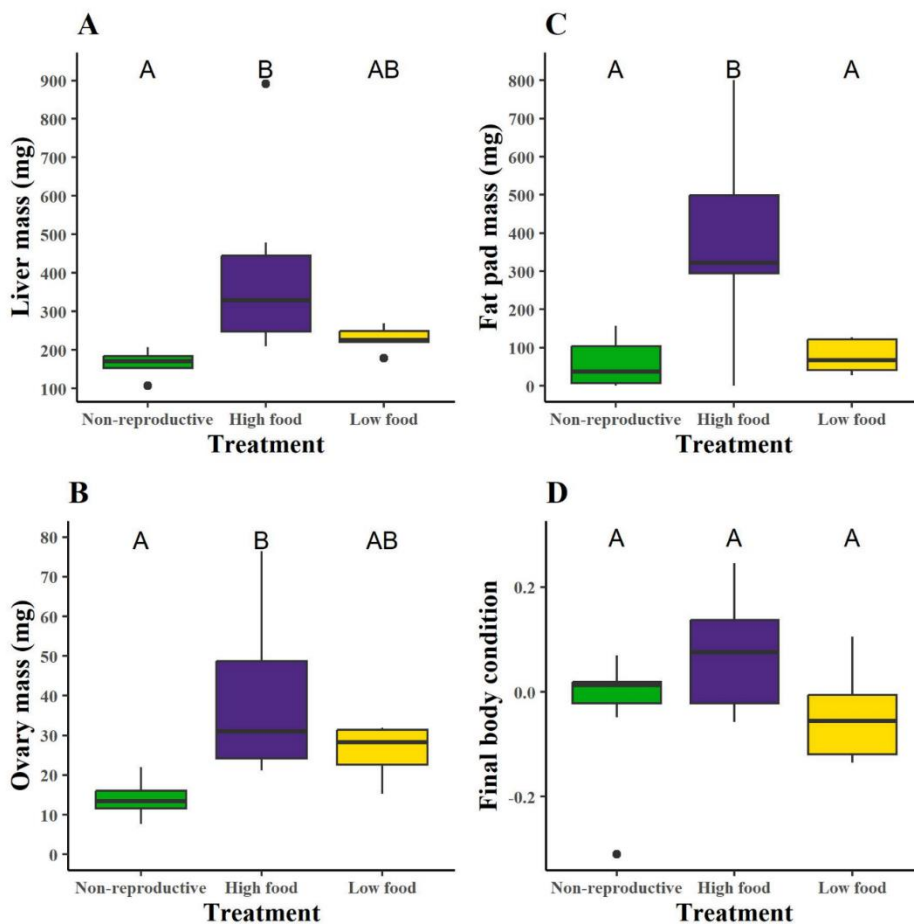
Differences between first and second clutches of female Sceloporus undulatus



Purple represents clutch one and yellow is clutch two. Letters denote statistical significance ($p < 0.05$) among groups. Dots denote outliers, which are calculated as observations 1.5 times the interquartile range less than the first quartile or 1.5 times the interquartile range greater than the third quartile. (a) Boxplot of egg masses by clutch. (b) Boxplot of clutch sizes by clutch. (c) Boxplot of egg density by clutch. (d) Boxplot of dried shell mass by clutch. (e) Boxplot of dried yolk mass by clutch. (f) Boxplot of daily water uptake of eggs by clutch. (g) Clutch size vs individual egg mass by clutch. (h) Egg density vs maternal capture SVL by clutch.

Figure 8

Final measures of body composition and condition for female Sceloporus undulatus



Green represents the non-reproducing group, purple represents the high food treatment, and yellow represents the low food treatment. Letters denote statistical significance ($p < 0.05$) among groups. Dots denote outliers, which are calculated as observations 1.5 times the interquartile range less than the first quartile or 1.5 times the interquartile range greater than the third quartile. (a) Liver mass vs treatment boxplot. (b) Ovary follicle mass vs treatment boxplot. (c) Fat pad mass vs treatment boxplot. (d) Final body condition by treatment group. Body condition = residuals(lm(log(FinalBodyMass) ~ log(FinalSVL))).

8c) (Table 5). Group-specific differences in body condition were not statistically significant (Figure 8d, Table 5). Moreover, differences in liver, ovary, and fat pad mass between non-reproducing females and females on the low food diet were not statistically significant (Table 5).

Table 5

Results of general and generalized linear models for objective three: statistical analyses of energy investment

Response variable	Fixed effect	Estimate	Std. Error	df	t	P
Liver mass (mg)	(Intercept)	166.08	56.91	14	2.92	0.01
	Centered SVL (mm)	9.08	10.45		0.87	0.40
	TreatHIGH	245.05	83.76		2.93	0.01
	TreatLOW	62.95	88.16		0.71	0.49
Ovary mass (mg)	(Intercept)	0.02	0.01	11	2.18	0.05
	Centered SVL (mm)	0.002	0.001		1.64	0.13
	TreatHIGH	0.02	0.01		2.70	0.02
	TreatLOW	0.01	0.01		1.15	0.28

Table 5(continued)

Response variable	Fixed effect	Estimate	Std. Error	df	t	P
Final body condition	Centered SVL (mm)	12.58	11.31		1.11	0.28
	TreatHIGH	322.13	90.60		3.56	0.03
	TreatLOW	16.77	95.36		0.18	0.86
	(Intercept)	-0.03	0.04	15	-0.76	0.46
	TreatHIGH	0.11	0.07		1.66	0.12
	TreatLOW	-0.01	0.07		-0.13	0.90
SVL growth rate (mm/day)	(Intercept)	0.07	0.02	15	4.39	0.001
	TreatHIGH	-0.03	0.02		-1.28	0.22
	TreatLOW	-0.05	0.03		-2.13	0.051
	Centered SVL (mm)	0.01	0.01		1.72	0.11
Survival	TreatHIGH	-0.01	0.01		-0.97	0.35
	TreatLOW	-0.04	0.01		-3.38	0.01
	Centered SVL (mm):TreatLOW	-0.01	0.01		-2.70	0.02
	(Intercept)	1.95	1.07	20	1.82*	0.07
	TreatHIGH	-0.85	1.35		-0.63*	0.53
	TreatLOW	-1.03	1.36		-0.76*	0.45

Bold type represents statistical significance. Asterisk denotes z -value rather than t -value.

For treatments, the reference group is the non-reproducing females

Abbreviations: Centered SVL = snout-vent length centered per treatment group.

TreatHigh = high food treatment group. TreatLow = low food treatment group. df = degrees of freedom. t = t -value. P = p -value.

Growth varied among treatments and, for growth in SVL, was affected by the number of clutches laid (Figure 9). Non-reproducing females overall exhibited the highest growth in SVL and body mass (Figure 9a,b); however, few differences were statistically significant (Table 5). The difference in growth of SVL was marginally

statistically significant between the non-reproductive and low food groups ($P = 0.051$) (Figure 9a, Table 5) with the non-reproductive individuals having the most growth and the low food individuals having the least. Growth in body mass was lower for females on the low food treatment than both the high food ($P = 0.04$) and non-reproducing females ($P = 0.005$) (Figure 9b). While statistical significance couldn't be determined due to singularities in the model, there was an observable effect of number of clutches laid on growth in SVL, particularly in the high food treatment, with females that laid only a single clutch having more growth than the females that laid two clutches (Figure 9c). There was an interaction between treatment group and SVL on growth of body mass such that larger females experienced more growth than small females if they did not reproduce, but the opposite was true for females on the low ($P = 0.02$) and high food ($P = 0.02$) treatments (Figure 9d; Table 5).

Figure 9

Growth of Sceloporus undulatus females

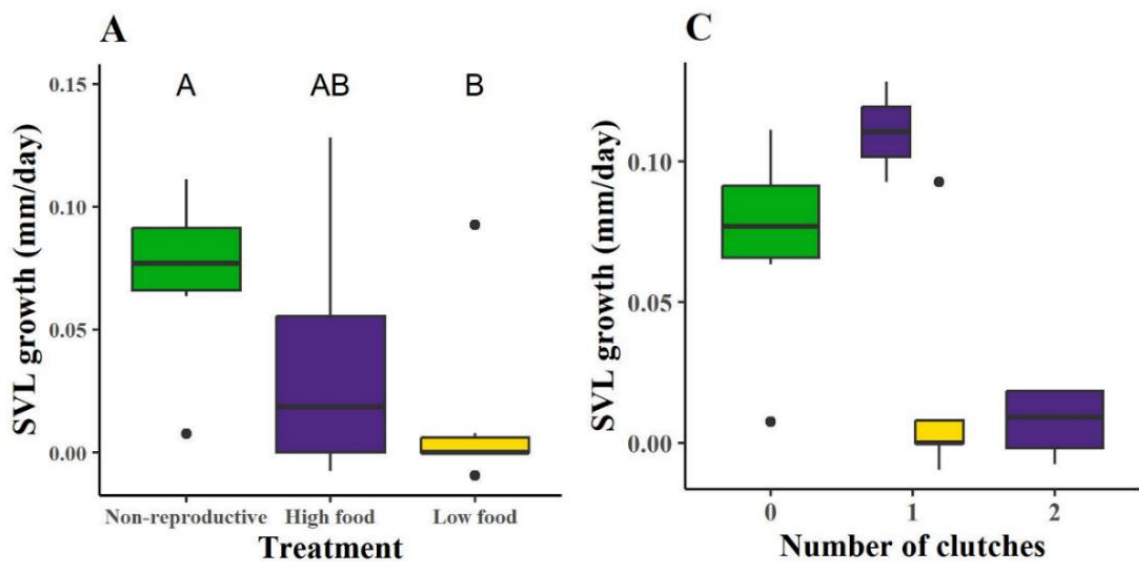
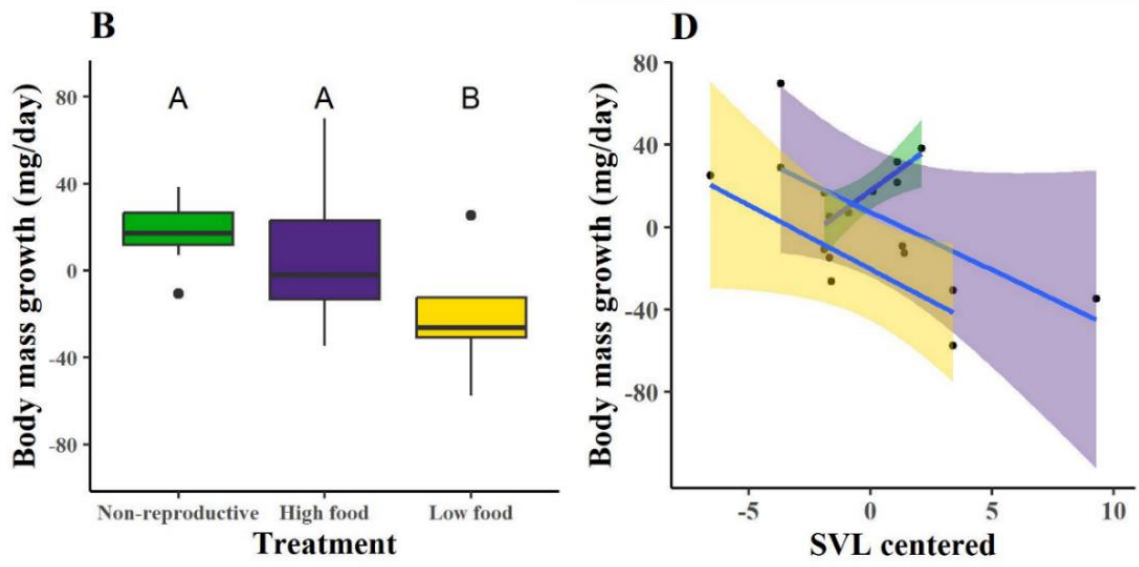


Figure 9(continued)

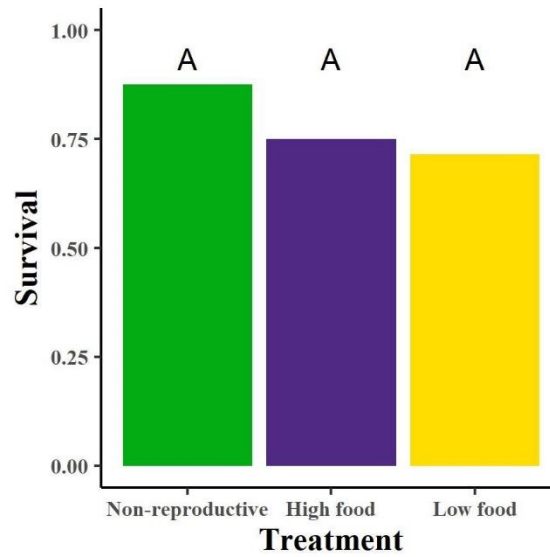


Green represents the non-reproducing group, purple represents the high food treatment, and yellow represents the low food treatment. Letters denote statistical significance ($p < 0.05$) among groups. Dots denote outliers, which are calculated as observations 1.5 times the interquartile range less than the first quartile or 1.5 times the interquartile range greater than the third quartile. (a) SVL growth by treatment group boxplot based on initial and final body measurements. (b) SVL growth by number of clutches laid and treatment group. (c) Mass growth by treatment group boxplot based on initial and final body measurements. (d) Mass growth vs the SVLs centered per treatment group.

Survival was relatively high for all groups (0.88 for non-reproductive, 0.75 for high food, and 0.71 for low food); thus, there was no statistical difference in survival among groups (Figure 10, Table 5).

Figure 10

*Percent survival of female *Sceloporus undulatus* in each treatment*



Green represents the non-reproducing group, purple represents the high food treatment, and yellow represents the low food treatment. Letters denote statistical significance among groups.

CHAPTER 4: DISCUSSION

Organisms have a finite amount of energy to apportion to growth, maintenance, and reproduction, and this is limited by energy available in the environment. Often this results in a trade-off between size and number of offspring produced (e.g., mammals, Charnov & Ernest, 2006; amphibians, Gould et al., 2022; birds, Martin et al., 2006; fish, Morrongiello et al., 2012). Animals that produce multiple times per year may decrease litter or clutch size later in the reproductive season as the quality of the environment for parents and/or offspring declines (Angilletta et al., 2001; Dobson & Myers, 1989; Nussbaum, 1981; Rowe et al., 1994; Uller & Olsson, 2010; Warne & Charnov, 2008). This trend may result from the seasonal decline of the environment, requiring them to either limit the amount of energy they expend towards reproduction to enhance their own survival (bet-hedging hypothesis) or to increase the amount of reproductive effort toward individual offspring to improve offspring survival (parental investment hypothesis) (Nussbaum, 1981). I found body size at the start of the season to be critical for the amount that females invested in reproductive effort, there is a seasonal trade-off in size and number of eggs even in high food environments (i.e. support for parental investment), and body size and food availability are significant factors in how females allocated energy, with a trade-off between reproduction and growth.

Objective One: Early Season Reproduction

My study provides additional support for latitudinal trends in body size and reproduction observed for *S. undulatus* in previous studies. For my study populations, the minimum reproductive size of female *Sceloporus undulatus* is about 60 mm SVL, which fits into the existing latitudinal trend: minimum size at reproduction for *S. undulatus* is 55

mm for South Carolina and 66 mm for Ohio, as reported in Tinkle and Ballinger (1972), with Tennessee in between. Moreover, the clutch and egg sizes I observed fit with the latitudinal trends seen in *S. undulatus*. For clutch one, mean clutch size was 10.1 (± 2.3 SD) which is intermediate between those observed for populations more northern and southern than Tennessee. In northern latitudes (e.g. Ohio and Missouri) the average clutch size was 11.8 and 11, respectively, while further south (e.g. South Carolina and Georgia) the average clutch size was 7.4 and 7.6, respectively (Ballinger et al., 1981). Du et al. (2014) additionally found the average clutch size for two Florida populations were 5.5 and 6.8. My results support the expected trend of lizards at higher latitudes producing larger clutches (Deme et al., 2023; Du et al., 2014) likely as an adaptation to a shorter reproductive season. Having a shorter reproductive season means they have less time to lay eggs and their reproductive effort is split between fewer clutches.

The average egg mass I observed ($0.39 \text{ g} \pm 0.06$) was similar to those reported in Ballinger et al. (1981) for various populations: Ohio- 0.35 g, Missouri- 0.38 g, South Carolina- 0.33 g and in Du et al. (2014) for Florida- 0.35 to 0.40 g. Therefore, there does not appear to be a latitudinal trend in egg size.

My study highlights that body size at the start of the reproductive season is particularly important in determining reproductive success. Larger females reproduced earlier, produced more and larger eggs, and produced more clutches across the breeding season. Females that were initially too small to reproduce but achieved reproductive size during the summer had lower reproductive potential, producing only one clutch of either many, small eggs or a few, large eggs. Conversely, larger females were able to reproduce early and maximized both the size and number of eggs in the first clutch. These results

may be associated with physical constraints of body size on egg production with smaller females having limited abdominal space for eggs and a smaller pelvic aperture which constrains egg size (Du & Lü, 2010; Luo et al., 2012). Small females also may be balancing energy allocation between reproduction and growth, while larger females were able to invest energy mainly to reproduction. These trends have been observed in other lizards (e.g. *Lacerta vivipara*, *Phymaturus* spp., *Anolis sagrei*) where females that invest less energy in growth have more available for reproduction and/or fat storage or, vice versa (Bauwens & Verheyen, 1985; Boretto et al., 2018; Cox et al., 2010).

Because larger eggs contained greater yolk mass and resulted in larger hatchlings, a fitness benefit of greater body size should be enhanced offspring survival if hatchling body size increases survival probability (Sinervo et al., 1992; Uller & Olsson, 2010). Across lizard species, larger females tend to produce larger eggs, resulting in larger offspring (Deme et al., 2022; Du et al., 2014; Hall et al., 2020; Ferguson et al., 1980). However, timing of reproduction is also important, because those that hatch earlier, as well as larger hatchlings, have enhanced survival (Civantos et al., 1999; Ferguson et al., 1982; Ferguson & Fox, 1984; Olsson & Shine, 1997; Uller & Olsson, 2010; Warner & Shine, 2007), especially in the competitive late season environment (Ferguson & Bohlen, 1978; Pianka, 1974). Thus, larger females experience a two-fold benefit since they reproduce earlier and produce larger offspring.

Females that were initially below reproductive size may have hatched during the previous year, while larger females may have been more than one year old (i.e. on their second breeding season) considering that *S. undulatus* from southern latitudes may reproduce in their first year (Tinkle & Ballinger, 1972). Additionally, lizards that hatch

earlier in the season are likely larger at the start of the next season (Uller & Olsson, 2010; Warner & Shine, 2007) which indicates a form of positive feedback for body size, reproduction, and offspring survival: offspring produced earlier are more likely to survive and are large enough to reproduce early in the season, which perpetuates a body size and seasonal timing advantage into the next generation.

Finally, it is often assumed there is a necessary trade-off between the size and number of offspring during any given reproductive bout (e.g., mammals, Charnov & Ernest, 2006; amphibians, Gould et al., 2022; birds, Martin et al., 2006; fish, Morrongiello et al., 2012). This trade-off tends to favor an increased number of offspring, unless increasing the size of the offspring concurs a significant survival advantage (Lack, 1954; Sinervo & DeNardo, 1996), since individuals leaving the most descendants are favored by natural selection (Shine & Schwarzkopf, 1992). This trade-off is necessary due to the limited amount of reproductive effort that must be divided between offspring (Williams, 1966). However, I observed that larger females were seemingly able to maximize both the size and number of eggs in the first clutch (Figure 4). This is likely an artifact of my inability to evaluate egg size and number trade-offs within vs among individuals. Due to large body size and greater energy reserves, larger females can produce a relatively large number of big eggs, but they are likely still subject to number and size trade-offs that I could not observe (i.e. “big houses, big cars” concept; Careau & Wilson, 2017; Reznick et al., 2000).

Objective Two: Early versus Late Season Reproduction

Despite the additional food available, not all females on the high food diet produced a second clutch. Those that did, produced fewer, higher quality eggs in their

second clutch. Eggs in the second clutch had greater mass, resulting in denser eggs with greater yolk content. These eggs also benefited from greater water absorption, which is vital to *Sceloporus* egg survival given that parchment-shelled eggs must absorb water from the nest to successfully develop (Tracy, 1980). Instead of increasing egg size and quality, these females could have, maximized the number of eggs late in the season, but did not. Thus, my results provide evidence for the “parental investment” hypothesis, whereby females produce fewer, better provisioned offspring, to enhance offspring survival in a competitive, late-season environment (Nussbaum, 1981).

Ferguson et al. (1982) found that, while their experimental design (with *S. undulatus* and *Uta stansburiana*) could not determine the extent to which modes of selection occur for bet-hedging or parental investment, there is evidence for the selective advantage of parental investment in terms of enhanced fitness of larger hatchlings resulting from larger eggs. Sinervo and Doughty (1996) supported this with evidence that, in *Uta stansburiana*, egg and clutch size were heritable, and therefore intrinsic traits. Mitchell et al. (2018) and Hall et al. (2020) also provided evidence for the parental investment hypothesis by showing that, in Brown Anoles (*Anolis sagrei*), females still exhibited the seasonal shift in egg size and number, despite being in a controlled environment and therefore not experiencing any form of seasonal decline in the environment or change in food availability. My study agrees with these conclusions in showing that the seasonal trade-off in clutch and egg size persisted in a setting where, not only did food availability not decline, but was instead increased late in the year.

However, several of my results could provide evidence for the “bet-hedging” hypothesis. The fact that some of the high food females did not produce a second clutch

in favor of growth and the fact that none of the low food females reproduced, instead allocating their energy to maintenance and surviving to another potential breeding season, provide support for the “bet-hedging” hypothesis. There is a trade-off between energy expenditure and survival, and for species, like *Sceloporus undulatus*, that reproduce over several years, reproduce multiple times in a year, and/or have increased fecundity with increased body size, decreasing reproductive effort in favor of survival can be beneficial (Shine & Schwarzkopf, 1992). My results showed that in an environment with low enough food availability, females are decreasing reproductive effort in favor of their own survival, but in a high food environment they will still favor egg quality over quantity. This indicates that these hypotheses likely exist on a spectrum. Ideally, some females in the low food treatment would have reproduced, allowing me to compare second clutches between the treatments. I would have been able to see more clearly if the increased size or decreased number of eggs was more affected and been able to better disentangle the two hypotheses.

Objective Three: Energy Investment

Several differences in the final body size and condition across the treatment groups were not statistically significant; however, the observed trends aligned with my hypotheses that females on the high food treatment would increase in body condition and composition (i.e. energy reserves) relative to other treatments and the females on the low food diet would exhibit a decrease in body condition and fat reserves. However, non-reproducing females generally experienced the most growth and had similar body condition and fat reserves as larger females on the low-food diet. The observed trend in body mass, with the smaller females increasing mass at a higher rate than large females

(Figure 9d), matches the expected growth curve, with growth increasing rapidly and then slowing down as individuals reach terminal body size (Zúñiga-Vega et al., 2005).

Food availability had a significant effect on energy storage, with the females in the high-food treatment having greater fat, liver, and ovary mass at the end of the season. This provides an advantage to females that have greater food supply for surviving the winter and for the following reproductive season, as they can utilize energy stores in production of their first clutch. This would allow females that experienced high food availability and were able to store more lipids to reproduce earlier in the following year, somewhat independent of food availability at that time (Bauwens & Verheyen, 1985; Derickson, 1976; Telford, 1970).

Even when food was severely limited, larger females maintained reasonable levels of liver, fat, and ovary mass. Indeed, for the females on the low-food diet, fat mass was like that of the non-reproducing females and body condition overlapped somewhat with that of females on the high food treatment. Therefore, it is possible to assume that these females could have allocated additional energy toward reproduction. Given that *Sceloporus* commonly reproduce across multiple seasons (Ferguson et al., 1980; Tinkle & Ballinger, 1972), these females were probably investing in maintenance under low food conditions to enhance over winter survival and potentially invest in future reproduction, when conditions may be more favorable, rather than expend energy towards a “terminal investment” in reproduction (Ma et al., 2019).

Individuals must balance the trade-offs between current reproduction and future reproduction, with future reproduction being highly dependent on survival (Heino & Kaitala, 1999; Pianka, 1976; Williams, 1966), particularly in squamates (Shine &

Schwarzkopf, 1992). For the females on the high food treatment, some didn't produce a second clutch, and none produced a third, despite the abundance of available energy. Those in the high treatment that did not lay a second clutch exhibited relatively high growth rates, compared with those that did (Figure 9c). Females that had two clutches had growth rates comparable to females on the low diet, whereas high diet females that did not have two clutches had the highest growth rate, illustrating the trade-off between reproduction and growth. Additionally, reproducing late in the season has an added cost of utilizing energy reserves that may be needed for over winter survival (Goldberg, 1972) or that could be used to produce a clutch early next year when the likelihood of offspring survival is greater. Many lizards, including *S. undulatus*, utilize stored lipids to produce their first clutch (i.e. exhibit "capital" reproduction; Derickson, 1976; Guillette & Sullivan, 1985; Telford, 1970). No females in the study produced a third clutch, regardless of food availability or fat stores. The relatively low survival of late-season offspring may reduce the likelihood of late-season reproduction even when food is abundant, but this is likely related to correlations between latitude and season length (Du et al., 2014). Thus, it can be more beneficial for females to forgo late-season reproduction in favor of earlier-produced, better provisioned clutches in the next year when competition is lower, and resources are more abundant.

Broader Implications and Conclusions

Body size and food availability are important determinants of energy allocation to growth, maintenance, and reproduction in *Sceloporus undulatus*. Larger females invest less energy in growth and more in reproduction, while smaller females tend to invest mostly in growth. However, larger females often opt to maintain body condition at the

expense of growth and reproduction when food is scarce. In Tennessee, the reproductive threshold for female *S. undulatus* is 60 mm SVL, and clutch sizes fall within the expected range given latitudinal trends in reproduction. Females that start the breeding season under reproductive size primarily invest energy in growth to reach that size as soon as possible, either not reproducing or producing a single clutch during that year. Females starting the breeding season at a larger size may produce two clutches if there is enough food available in the environment; however, it is unlikely at this latitude that a third clutch is possible (Du et al., 2014). For those that produce a second clutch, there is a seasonal shift to fewer, larger eggs in the second clutch. The presence of this seasonal shift despite an increase in food availability provides support for the “parental investment” hypothesis.

Climatic factors can influence food availability in ways that affect fitness. For insectivores like lizards, the abundance and density of prey is heavily influenced by rainfall (França et al., 2020; Illera & Díaz, 2006; Janzen & Schoener, 1968; Turner et al., 1973; Zúñiga-Vega et al., 2005). Due to climate change, seasonal patterns of precipitation are progressing towards greater extremes with wet seasons getting wetter, dry seasons getting drier, and floods and droughts becoming more common (Konapala et al., 2020; Kumar et al., 2014; Lee & Kim, 2013). This shift towards greater extremes could lead to an environment that is less predictable in food resources. My treatments replicated such boom-and-bust cycles which greatly affect the costs of growth and reproduction.

DeMarco (1989) found that *Sceloporus woodi* laid fewer, larger eggs in later clutches in years with below normal rainfall but in years with above average rainfall, all clutch and egg sizes were approximately identical across clutches. Dunham (1978) also found that

less rainfall resulted in lower growth, fecundity, and fat storage in *Sceloporus merriami*.

Understanding how females allocate resources among reproduction, growth, and maintenance will be critical in the future to understand species' responses to climate change. Reduced food led to a complete cessation of reproduction in my study population; however, abundant food only enhanced immediate reproductive output in some females. My data indicates that boom- and -bust cycles may not be zero sum in the future. If increased food availability in the early season is not able to increase reproductive effort enough to offset the decrease in reproduction later in the season and the cost of reproduction in extreme environments is exceedingly great, reproductive effort will decrease, potentially leading to population declines in *S. undulatus* and similar species. This may alter selection pressures, changing seasonal patterns of reproduction for populations in the future.

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APPENDIX A

Initial Statistical Models

response variable ~ fixed effects + (1| random effect)

Objective 1

Reproduction (0,1) ~ maternal SVL

Clutch size ~ maternal SVL + egg size + egg size:maternal SVL + (1|maternal ID)

Egg size ~ maternal SVL + clutch size + clutch size:SVL + (1|maternal ID)

Hatchling SVL ~ offspring sex + clutch size + egg mass + maternal SVL + clutch size:egg mass + clutch size:maternal SVL + egg mass:maternal SVL + (1|maternal ID)

Hatchling mass ~ offspring sex + clutch size + egg mass + maternal SVL + clutch size:egg mass + clutch size:maternal SVL + egg mass:maternal SVL + (1|maternal ID)

Egg density ~ maternal SVL + clutch size + maternal SVL:clutch size + (1|maternal ID)

Shell mass ~ maternal SVL + clutch size + egg mass + clutch size:egg mass + clutch size:maternal SVL + egg mass:maternal SVL + (1|maternal ID)

Yolk mass ~ maternal SVL + clutch size + egg mass + clutch size:egg mass + clutch size:maternal SVL + egg mass:maternal SVL + (1|maternal ID)

Water uptake rate ~ egg mass + clutch size + maternal SVL + egg mass:clutch size + egg mass:maternal SVL + clutch size:maternal SVL + (1|maternal ID)

Objective 2

Clutch 2 size ~ maternal SVL + egg mass + maternal SVL:egg mass + (1|maternal ID)

Clutch size ~ maternal SVL + egg mass + clutch ID + maternal SVL:egg mass + maternal SVL:clutch ID + clutch ID:egg mass + (1|maternal ID)

Egg size ~ maternal SVL + clutch size + clutch ID + maternal SVL:clutch size + maternal SVL:clutch ID + clutch size:clutch ID + (1|maternal ID)

EggDensity ~ maternal SVL + clutch ID + maternal SVL:clutch ID + (1|maternal ID)

Shell mass ~ maternal SVL + clutch size + egg mass + clutch size:egg mass + clutch size:maternal SVL + egg mass:maternal SVL + (1|maternal ID)

Yolk mass ~ maternal SVL + clutch size + egg mass + clutch size:egg mass + clutch size:maternal SVL + egg mass:maternal SVL + (1|maternal ID)

Water uptake rate ~ egg mass + clutch ID

Objective 3

SVL growth rate ~ centered SVL + treatment + centered SVL:treatment

Mass growth rate ~ centered SVL + treatment + centered SVL:treatment

Final body condition ~ centered SVL + treatment + centered SVL:treatment

Liver mass ~ centered SVL + treatment + centered SVL:treatment

Ovary follicle mass ~ centered SVL + treatment + centered SVL:treatment

Fat pad mass ~ centered SVL + treatment + centered SVL:treatment

Final body condition ~ centered SVL + treatment + centered SVL:treatment

Table A.1

Final measurements of Sceloporus undulatus from each population and treatment

	N	Location		SVL (mm)				Mass (g)			
		Edgar Evins	Standing Stone	mean	SD	min	max	mean	SD	min	max
LF males	2	1	1	65.0	7.1	60	70	8.14	2.16	6.61	9.67

Table A.1(continued)

	N	Location		SVL (mm)				Mass (g)			
		Edgar Evins	Standing Stone	mean	SD	min	max	mean	SD	min	max
NR males	6	3	3	61.7	2.5	59	66	7.41	1.04	5.43	8.19
LF fems	5	1	4	70.6	4.2	64	74	10.75	0.88	9.35	11.45
HF fems	6	3	3	70.7	4.9	67	80	12.23	2.25	10.03	15.32
NR fems	7	5	2	61.9	1.6	60	64	7.44	1.16	5.10	8.61

Lizards that survived to the end of the study and were euthanized.

Abbreviations: SVL = snout-vent length. SD = standard deviation. LF = low food treatment. HF = high food treatment. NR = non-reproducing. fems = females. Min = minimum value. Max = maximum value. N = sample size

Table A.2

*Final body composition of *Sceloporus undulatus* from each experimental treatment*

	N	Liver mass (g)				Fat pad mass (g)				Follicle/testes mass (g)			
		mean	SD	min	max	mean	SD	min	max	mean	SD	min	max
LF males	2	0.1422	0.0658	0.0956	0.1887	0.0000	0.0000	0.0000	0.0000	0.1190	0.0925	0.0536	0.1844
HF males	7	0.2312	0.0359	0.1881	0.2685	0.1096	0.1043	0.0183	0.3279	0.1620	0.0638	0.0946	0.2471
NR males	6	0.1443	0.0313	0.0983	0.1919	0.0245	0.0500	0.0000	0.1249	0.0489	0.0242	0.0096	0.0722
LF fems	5	0.2290	0.0342	0.1787	0.2697	0.0768	0.0457	0.2800	0.1275	0.0259	0.0070	0.0153	0.0319
HF fems	6	0.4108	0.2543	0.2094	0.8909	0.3817	0.2717	0.0000	0.8009	0.0394	0.0216	0.0212	0.0764
NR fems	7	0.1657	0.0321	0.1070	0.2072	0.0595	0.0668	0.0000	0.1575	0.1535	0.2290	0.0077	0.6356

Lizards that survived to the end of the study and were euthanized.

Abbreviations: SD = standard deviation. LF = low food treatment. HF = high food treatment. NR = non-reproducing. fems = females. Min = minimum value. Max = maximum value. N = sample size

Table A.3

Reproductive traits of Sceloporus undulatus for first and second clutches

	Clutch Size				Egg Wet Mass (mg)				Egg Dry Mass (mg)			
	mean	SD	min	max	mean	SD	min	max	mean	SD	min	max
Clutch 1	10.1	2.3	6	14	388.2	62.0	292.4	529.2	150.5	18.6	124.0	185.6
Clutch 2	8.2	1.6	7	11	442.2	36.1	392.6	482.7	174.9	12.3	158.1	192.7

Abbreviations: SD = standard deviation. Min = minimum value. Max = maximum value.