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FACTORS AFFECTING GROWTH RATES AND PREFERRED BODY TEMPERATURES IN HATCHLING GOPHER TORTOISES, *Gopherus Polyphemus*: CLUTCH AND SEX

by

MATTHEW CAREY

(Under the Direction of David Rostal)

ABSTRACT

Post hatching growth rates in tortoises can affect size at sexual maturity This study looks at what affect clutch and sex has on hatchling tortoises from these two sites as this data is lacking/insufficient from two populations in Southeast Georgia. Eggs were allowed to incubate in natural nests and then were collected between August 19-21, 2015 and hatched in an incubator in the lab. When they hatched, the tortoises were kept in a single room with a rearing temperature that averaged 26.9° C. Sex was determined using laparoscopy at the end of the study to ensure gonad development and to reduce stress throughout the study. Preferred body temperature was determined using a thermal gradient the first summer after hatching. Clutch had a significant effect on growth rate of mass and straight carapace length ($F_{(1,68)} = 2.9491$, $p < 0.0002$; $F_{(12,57)} = 3.08347$, $p < 0.0021$) while sex did not. Clutch and sex had no effect on preferred T_b in the lab. Preferred body temperature for the tortoises averaged 30.14° C ± 0.6364° The significant maternal effects on growth rates could be an effect on the genotype of these tortoises or an effect caused by egg quality as a result of maternal investment into the egg.

INDEX WORDS: *Gopherus polyphemus*, Hatchling, Growth rates, Clutch, Sex

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by

MATTHEW CAREY

B.A., Georgia Southern University, 2013

A Thesis Submitted to the Graduate Faculty of Georgia Southern University in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

STATESBORO, GEORGIA

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TABLE OF CONTENTS

ACKNOWLEDGMENTS 2
LIST OF TABLES.....4
LIST OF FIGURES 5

CHAPTER

1 FACTORS AFFECTING GROWTH RATES AND PREFERRED BODY TEMPERATURES IN HATCHLING GOPHER TORTOISES, *Gopherus Polyphemus*: CLUTCH AND SEX

Introduction 6
Methods.....8
Results.....11
Discussion.....13
LITERATURE CITED.....19

LIST OF TABLES

Table 1: Clutch size, number of eggs hatched, hatching success, and percent female for all clutches……………………………………...…………………………..……………………………26

Table 2: Number of hatchlings used in the study (N), hatchling measurements, and growth rates, individual clutches. Growth rate is reported as mm/day ± SE and g/day ± SE. Growth rates connected by the same letter are not significantly different.………………………………………………………………..27

LIST OF FIGURES

Figure 1: Tortoise enclosure set up with PVC shelter..28

Figure 2: Thermal gradient set up for preferred T_b trials 29

Figure 3: Graph of temperatures accessible to the tortoise hatchlings in the thermal gradient 30

Figure 4: The linear relationship between mean hatchling mass and mean hatchling straight carapace length (SCL). The regression equation is y =1.2505x + 30.51 (R^2= 0.5459).................31

Figure 5: The relationship between mass at hatching and mass at the end of the study. This relationship is not significant (Pairwise correlation, p = 0.7909; y = -0.2497x + 91.185, R^2 = 0.001)……………………………………………………………………………..…………….32

Figure 6: Preferred body temperatures per clutch. Neither clutch or sex had a significant effect on growth rate in hatchling gopher tortoises. Preferred body temperature averaged 30.14° C ± 0.6364°..33

CHAPTER 1

CLUTCH AND SEX EFFECTS ON THE GROWTH RATE AND PREFERRED BODY TEMPERATURE OF HATCHLING GOPHER TORTOISES (*Gopherus polyphemus*)

INTRODUCTION

Growth is an important and complex biological process that affects the life history of organisms (Stearns, 1992; Brown et al., 2005). In long lived organisms such as turtles, growth occurs in multiple stages depending on reproductive status with the fastest growth occurring in hatchling and juvenile stages and slowing upon sexual maturity (Andrews, 1982; Galbraith et al., 1989; Lagarde et al., 2001). Several factors influence growth in turtles such as maternal effects, habitat quality, temperature, sex, and interactions between these factors (Andrews, 1982; Brooks et al., 1991; Bobyn and Brooks, 1994; Spotila et al., 1994; Steyermark and Spotila, 2001; Tuberville et al., 2014). One of the best studied maternal effects is egg size, which is determined by the amount of yolk, lipids, water, and shell materials the mother invests into the egg (Andrews, 1982; Congdon et al., 1987; Bernardo, 1996). These components are used by the embryo for growth and maintenance, and provide the initial source of energy after the embryo hatches (Andrews, 1982; Bernardo 1996). Though egg size is an important determinate of hatchling size, it has little effect on growth after hatching. Typically, if a turtle hatches larger in size, it remains larger throughout life (Brooks et al., 1991; Bobyn and Brooks, 1994; Spotila et al., 1994; Steyermark and Spotila, 2001).

Another maternal effect, though indirect, is the incubation temperature of the nest. Though incubation temperature is an effect that occurs after the female lays her eggs, it is an indirect maternal effect because incubation temperature is determined by nest location which is a result of female choice (Kolbe and Janzen, 2002). Incubation temperature affects embryonic growth (growth in the egg) and post hatching growth (Brooks et al., 1991; Spotila et al., 1994;

Demuth, 2001). Eggs incubated at intermediate temperatures, 25.6° C and 28° C for snapping turtles (*Chelydra serpentina*) and 28° C for gopher tortoises (*Gopherus polyphemus*) have a higher rate of growth than those at more extreme temperatures (Demuth, 2001; Steyermark and Spotila, 2001). Incubation temperature also affects sex determination in turtles (Yntema, 1968; Ewert, 1994; Steyermark and Spotila, 2001; Demuth, 2001).

Sex can affect the growth of organisms that exhibit sexual size dimorphism, SSD (Mushinsky et al., 1994; Lagarde et al., 2001). Growth tends to remain constant throughout hatchling and juvenile stages and slows after turtles reach a sexually mature body size (Galbraith et al., 1989; Mushinsky et al., 1994; Lagarde et al., 2001). This change in growth rate likely represents a shift in the allocation of energy as the turtle shifts energy towards the production of reproductive materials (Lagarde et al., 2001). The larger sex in sexually dimorphic species tend to have a faster growth rate than the smaller. This is can be seen in the gopher tortoise (Demuth, 2001). In gopher tortoises, the female is larger than males and has a faster rate of growth regardless of incubation temperature (Mushinsky et al., 1994; Demuth, 2001; Rostal and Jones, 2002).

Gopher tortoises are a tortoise species that is endemic to the longleaf pine-wiregrass ecosystem of the Coastal Plain region of the Southeastern United States. They have suffered an 80% decline in their population largely due to conversion of their habitat to agriculture and fire suppression (Auffenburg and Franz, 1982, Van Lear et al., 2005). Now, most populations subsist on State/National lands or military installations (Wilson et al., 1997). Gopher tortoises are an important species to study as they are a keystone species for their habitat as they are a primary seed disperser (Dale and Beyeler, 2001; Ashton and Ashton, 2008; Pawelek and Kimball, 2014). Studies have been conducted on populations in Florida, but few have been done on populations in the northern portion of its range (McCoy and Mushinsky, 1994; Mushinsky et al., 1994;

Demuth, 2001; Rostal and Jones, 2002; Tuberville et al., 2005; Entz, 2009; McCoy et al., 2013; Tuberville et al., 2014; Erickson, 2015). Climatic differences can cause shifts in life history patterns such as incubation temperature, environmental temperature, length of growing season for the vegetation it feeds on, and the length of the tortoise's active season (Mushinsky et al., 1994; Rostal and Jones, 2002; Harris et al., 2015). Thus, it is important to study the Gopher tortoise throughout the extent of its range.

This study looks at early hatchling life history characteristics of hatching success, hatchling size, and growth rate of gopher tortoise hatchlings. I used clutch as a measure of maternal effect since clutch can be an indirect way to measure maternal effects as it can be difficult to separate genetic and clutch effects (Steyermark, 2008). I set out to answer the questions: how does the mother (clutch) and sex effect 1) hatchling size, 2) hatchling growth rate, and 3) preferred body temperature (T_b)?

METHODS

Collection sites

The two collection sites used for this project were George L. Smith State Park (GLS) and Fort Stewart (FS), both of which occur in the Southeast Georgia, U. S. A. George L. Smith is located near Twin City, Georgia at approximately N 32.5585600, W 82.1193700 and FS is a military installation that encompasses areas of seven different counties in Georgia. Both sites are ideal gopher tortoise habitats as they are sandhill communities characterized by open pine canopies, dry sandy soils, and an understory consisting of sparse oak. Wiregrass typically makes up the groundcover with interspersed flowering plants and bushes. Though the two are similar in general appearance they differ significantly in habitat management regimes (Rostal and Jones, 2002; Entz, 2009).

Egg collection

Eggs were found by hand probing the apron, the area of loose sand that has been dug out at the entrance of the burrow during the nesting season which occurs from mid to late spring into summer. Gopher tortoises exhibit temperature dependent sex determination (TSD), where the temperature embryos are exposed to during the second quarter of incubation determines the sex of the embryo (Rostal et al, 2002; Rostal and Wibbels, 2014). To ensure TSD was not disturbed, the eggs were collected between August 19-21, 2015 and were transported from the nest to an incubator in the Department of Biology facilities at Georgia Southern University in 32.5cm L x 18cm W x 11cm H Sterilite® plastic container that contained sand collected from the corresponding nest. In the incubator, they were kept at 28.5°C as this temperature is similar to field temperatures that have been observed after Aug. 20th (pers. comm., Rostal).

Hatchling Care, Measurement, and Sex Determination

After hatching in an in-lab incubator, internalization of their yolk and becoming mobile, the hatchlings were relocated into the animal facility at Georgia Southern University and marked with a permanent marker for identification purposes. They were held individually in 32.5cm L x 18cm W x 11cm H Sterilite® plastic containers on horse chow bedding (Producer's Pride 12% pellets) and provided small sections of PVC cut in half as shelter (Figure 1). The bedding is digestible if ingested, though this was never observed occurring. The hatchlings were kept at an average of 26.9°C (range 24°-28° C) as a rearing temperature on a 12L: 12D light cycle from 0700 hours to 1900 hours. UV light was provided using a 5.0 Reptisun® Bulb purchased from LLLReptile. They were provided a diet of Mazuri® Small Herbivorous Reptile Feed once a week. Each tortoise was given an average of 12.72 g of food to ensure each tortoise was provided more food than an individual could eat at each feeding. This is an *ad libitum* system of feeding as there was food left over (Brooks et al., 1991). The Mazuri diet was chosen because it has been shown to facilitate growth better than either super market greens or greens supplemented with calcium (Erickson, 2015). The hatchlings were soaked once a week in enough water to cover their plastron and allow them to submerge their heads to rehydrate and void their urates, while ensuring they were not fully submerged if they flipped themselves over. Straight carapace length (SCL) was measured to the nearest 0.01 mm using digital calipers and mass was measured to the nearest 0.01 g using an analytical balance once a week for a period of 245 days from, February 26, 2016 until October 31, 2016. This period corresponds to the active growing season in Georgia. Sex was determined by using laparoscopy (Rostal et al., 1994). The incision site was glued using Surgi-lock 2oc© Instant Tissue Adhesive (Meridian Animal Health, Omaha, NE).

Preferred Body Temperature

A thermal gradient was used to determine the preferred T_b of the hatchlings during the first summer after hatching. The thermal gradient was ~2.4 m L x 0.3 m W x 0.5 m H and was filled with ~2.5cm of sand substrate (Figure 3). Under the sand was an aluminum plate that stretched the entire length of the gradient. Under one end of the aluminum plate was heat tape that stretched for about one-third the length of the gradient. Above the end with the heat tape, a heat lamp (ZooMed San Louis Obispo, CA, U.S.A) provided extra heat. On the opposite end, under the aluminum plate, copper tubes led to an ice bath located outside of the gradient that. A water pump (Aqua Culture, Bentonville, AR, USA) pumped cold water into the thermal gradient. The combination of heat lamp, heat tape, and ice bath provided a linear thermal gradient for the tortoises (Figure 2). When turned on, the thermal gradient was allowed to equilibrate for one hour before placing tortoises in the gradient. A 0.3m section of the warm end of the gradient was blocked off between the heat lamp and nearest end of the gradient because the spacing of the heat tape created an area too warm for the tortoises and could have been fatal had the tortoises had access. Temperatures accessible to the tortoises ranged from 53.9° C – 14.7° C (Figure 3).

After the thermal gradient reached its proper temperature, the tortoises' initial T_b was taken via a cloacal thermometer and then placed in the gradient. Three tortoises were placed in the gradient at a time and any tortoises observed to act aggressively were removed and run by themselves. The tortoises were then allowed access to the gradient for a period of four hours. At the end of the fourth hour, their T_b was again taken via a cloacal thermometer. The thermal gradient runs took place between July 26th and August 14th of 2016 and began at 0700 hours with the last run ending at 1900 hours. Seventy tortoises were run one time through the gradient.

Statistical Analysis

Growth rate was calculated as the slope of a linear regression line fitted to the growth measurements of SCL and mass for the number of days growth was measured (Erickson, 2015). Total number of days growth was measured for was 245. The data was tested for homogeneity of variance using Levene's test and for normality using the Shapiro-Wilk *W* test. If the data did not meet assumptions, the appropriate non-parametric test was used. A one-way ANOVA was used to test for differences in growth rate between clutches for SCL and mass. If there was a significant result, a post-hoc comparison of means was performed using Tukey-Kramer HSD.

Statistics were done using the program JMP (JMP, Pro 12.0.0 SAS Institute Inc., Cary, NC). Results are presented as ± standard error (SE).

RESULTS

Clutch Size, Hatching Success, and Hatchling Sex

A total of 97 eggs from 13 nests were collected for this study. Clutch size averaged 7.38 ± 0.12 eggs per clutch with a range of 5-14 eggs. Of the 97 eggs, 83 eggs hatched for a hatching success of 85.6%. Hatching success ranged from 62.5% to 100%. Seven of the 13 nests had a 100% hatching success rate, one nest had a hatching success of 80% three had hatching success rates ranging from 71.4% to 77.8%, and one nest had a hatching success rate of 62.5%.

Seventy tortoises were sexed at the end of the study. Forty-eight of the tortoises were female and 22 tortoises were male, a 2.18:1 ratio in favor of females. Thirteen tortoises died during the study and gonad type could not be determined by necropsy.

Hatchling Size

Hatchling mass and SCL differed significantly for clutch (mass, $F_{(12,57)} = 6.5671$, $p < 0.0001$; SCL, $F_{(12,57)} = 9.8908$, $p < 0.0001$) but not for sex (mass, $F_{(1,68)} = 0.1551$, $p = 0.6949$; SCL, $F_{(1,68)} = 0.5301$, $p = 0.4691$). Hatchling masses and SCL are reported in Table (1). Female mass and SCL averaged 30.76 g ± 0.42 g and 49.49 mm ± 0.28 mm. Male mass and SCL averaged 31.05 g ± 0.62 g and 49.86 mm ± 0.44 mm. The relationship between hatchling mass and hatchling SCL is positive ($y = 1.2505x + 30.51$, $R^2 = 0.5459$) though weak (Figure 4). Clutch size, hatching success, hatchling size, and percent female per clutch are reported in Table 1.

Growth Rate

Clutch significantly affected growth for SCL ($F_{(12,57)} = 3.08347$, $p < 0.0021$) and mass ($F_{(1,68)} = 2.9491$, $p < 0.0002$). A post-hoc comparison of means showed that for mass, one nest from GLS grew faster than seven other nests (Table 2). A post hoc comparison of means for SCL was not able to show differences between clutches, likely due to small amounts of variation in SCL. Sex had no effect on growth rate in either SCL (one-way ANOVA; $F_{(1,68)} = 2.5208$, $p < 0.1170$) or mass (one-way ANOVA; $F_{(1,68)} = 2.9491$, $p < 0.0905$). Individual clutch growth rates are reported in Table 2. Growth rates for SCL for females and males averaged 0.0733mm/day ±

0.0032 mm and 0.0647 mm/day ± 0.0039 mm respectively over the course of the study. Growth rates for mass for females and males averaged 0.2030 g/day ± 0.0117 g and 0.1695 g/day ± 0.0134 g respectively.

Preferred Body Temperature

Preferred T_b was not affected by clutch (one-way ANOVA; $F_{(12,56)} = 1.8269$, $p = 0.0657$) or sex (one-way ANOVA; $F_{(2,66)} = 0.8394$, $p = 0.4365$). Average preferred T_b for gopher tortoise hatchlings was 30.14° C ± 0.6364° (Figure 6).

DISCUSSION

Clutch Size, Hatching Success, and Hatchling Sex

Variation in clutch size could be a result of optimal egg size theory. Optimal egg size theory states that once an optimal egg size is reached, females will vary the number of eggs for optimal reproductive output and larger females will produce a larger number of eggs (Smith and Fretwell, 1974; Brockelman, 1975; Congdon and Gibbons, 1987). A positive relationship, between female size and clutch size has been found in the chicken turtle, the leatherback turtle, and the snapping turtle (Congdon et al, 1983; Congdon and Gibbons 1985; Galbraith et al., 1989; Wallace et al., 2007). Rothermel and Castellón (2014) reported a positive, but weak relationship between body size and egg size in a population of *G. polyphemus* in south-central Florida, with female body size explaining only 21% of variation in clutch size. A weak, positive relationship between egg size and female body size was reported by Rostal and Jones (2002), but there was also a site effect that was independent of female size. A later study showed a slight positive relationship between female body size and clutch size as well as female body size and egg size (Entz, 2009).

Growth Rate

Growth is a complex process that is influenced by more than one factor throughout the life of an organism (Andrews, 1982; Stearns, 1992). In turtles, egg mass and incubation temperature have been shown to have an influence over embryonic growth (Brooks et al., 1991; Roosenburg and Kelley, 1996; Demuth, 2001). Post hatching growth can be affected by habitat quality, food availability, food quality, access to favorable temperatures, sex, and maternal effects (Brooks et al., 1991; Roosenburg and Kelley, 1996; Demuth, 2001; Lagarde et al., 2001; Steyermark and Spotila, 2001; Tuberville et al., 2014; Erickson, 2015).

Clutch, a maternal effect, significantly affected growth rate in my study. This contrasts what has been found in the closely related desert tortoise (Spotila et al., 1994). Spotila et al., (1994) reported no difference in growth among different individuals under different incubation temperatures with larger eggs producing larger hatchlings which remained larger after 40 and 120 days. Clutch has been shown to significantly effect growth in the snapping turtle (Brooks et al., 1991; Steyermark and Spotila, 2001). However, in my study, only one clutch, GLS 3, grew significantly faster than the other clutches (Table 2). This clutch had six eggs, and all eggs hatched as female. The accelerated growth exhibited by this clutch could likely be caused by interactions between incubation temperature and egg quality. This particular mother could have been very healthy and been able to invest more into the eggs, thus laying eggs of a higher quality with more available to the embryo to use for growth in the egg.

In a study on the effects of incubation temperature on hatchling size, growth, and performance, Demuth (2001) found that hatchling gopher tortoises incubated at 28°C grew more rapidly than other incubation temperatures, and therefore masked the differences in growth rates between males and females. However, the same study found that females grew faster than males at incubation temperatures of 26°, 29°, 30°, 31°, and 32°C (Demuth, 2001). Roosenburg and Kelley (1996) similarly found that incubation temperatures influence growth rates in *Malaclemys terrapin*, but this study also observed differences in growth rates between clutches, largely as an effect of egg mass. Brooks et al. (1991) found that snapping turtle eggs incubated at an intermediate temperature, 25.6° C, and wet soils produced larger hatchlings that remained larger after seven months and that post hatching growth rates were unaffected. This was complicated by competitive interactions between the hatchlings (Brooks et al., 1991; Steyermark and Spotila, 2001). In a study that aimed to eliminate the competitive interactions, Steyermark and Spotila (2001), found that interclutch variation and possibly genotype had more influence over post hatching growth than incubation conditions.

Sexual size dimorphism, SSD, is another factor that can complicate growth in long lived individuals (Lagarde et al., 2001). As tortoises approach sexual maturity, there is a shift in energy allocation to reproduction as they reach a sexually mature body size (Andrews, 1982; Lagarde et al., 2001). Lagarde et al., (2001) reported different growth rates for male and female steppe tortoises, but these differences appeared once signs of sexual maturity were starting to show at around 8 years old. Sexual maturity in gopher tortoises is a product of body size and changes throughout its range. They also exhibit sexual size dimorphism with females being larger than males (Mushinsky et al., 1994; Rostal and Jones, 2002). Demuth (2001) did not report SSD in a population from the Kennedy Space Center and Mushinsky et al. (1994) reported low degrees of SSD in their population from The University of South Florida’s Ecological Research Area in Tampa. The two populations in this study have not received much study, though Rostal and Jones (2002) reported high degrees of SSD in populations on FS and GLS in Georgia. Females from FS are larger than males from both FS and GLS as well as females from GLS. Females from GLS are larger than males from both sites and there is no difference between males from both sites (Rostal and Jones, 2002). While previous studies looking at the influence of sex have shown differences, I likely did not see any due to the short-term nature of this study and the confounding nature of clutch.

Egg sizes were not collected for this study, though it has been shown that egg size is not a good predictor of post hatching growth, instead it influences hatchling size (Brooks et al., 1991; Spotila et al., 1994; Steyermark and Spotila, 2001). Hatchling size also does not influence post hatching growth. Typically, hatchlings that start out larger, end larger (Spotila et al., 1994; Steyermark and Spotila, 2001; Entz, 2009). I did not see this however in this study. The relationship between hatchling size and the size of the hatchlings at the end of the study is not significant (Figure 5). This could be due to variation caused by incubation conditions, sex, or early growth during a time when growth was unexpected.

Preferred Body Temperature

Body temperature in ectotherms can have important effects on their fitness and performance (Huey and Kingsolver, 1993). Physiological processes, such as growth, tend to increase quickly over a range of body temperatures (the optimum temperature range), but are slowed at temperatures on the ends of this range (Huey and Stevenson, 1979). Being able to maintain a preferred body temperature for longer can allow an ectotherm to assimilate more energy from its food (Angiletta Jr., 2001). This can be vital to an herbivore, as herbivorous diets tend to provide less energy than carnivorous or insectivorous diets (Zimmerman and Tracy, 1989).

In this study, the gopher tortoises were held under constant temperature conditions and not allowed to thermoregulate. When placed in the thermal gradient, however, they selected temperatures higher than those they were kept at, but lower than that preferred by adult gopher tortoises, around 32° C (Rostal, unpublished data). Radzio and O'Connor, (2017), report growth rates are highest for hatchling/juvenile gopher tortoises at ~33° C. This temperature not only allows for optimal growth, but also allows for reduced physiological cost of predator avoidance. (Radzio and O'Connor, 2017). The lower temperatures reported in this study could be due to the energy density of the food, lack of predation risk, or experimenter error as each hatchling was only placed in the thermal gradient once.

Conclusion and Future Direction

Growth among gopher tortoises is variable though there is a maternal influence on post hatching growth. Whether this is an effect by the mother on the genotype of the egg, or a result of the quality of the egg (based off the amount of components the mother has invested into the egg) remains to be tested. Testing this variation of investment into the eggs is necessary, however it can be difficult due to the low number of eggs a single female lays and the conservation status of the tortoise.

Interaction between competition and thermoregulation also need to be investigated. These are well known to have an effect on post hatching growth of common snapping turtles (Brooks et al., 1991; Steyermark and Spotila, 2001). Larger snapping turtle hatchlings tend to have a competitive advantage over their smaller conspecifics when it comes to feeding and basking opportunities and I expect that larger gopher tortoises will also have this advantage. A competitive advantage for food could lead to a tortoise ingesting more food than another and thus, having more energy to use for growth and maintenance while a competitive advantage for a thermoregulatory opportunity can lead to longer time at optimal temperatures for faster growth to occur. This study is important not only because it provides information on post hatching growth in gopher tortoises in comparatively unstudied populations, it also highlights the need to look at multiple facets of growth in these turtles as there are many factors that interact to influence growth in populations.

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TABLES

Table 1: Clutch size, number of eggs hatched, hatching success, and percent female for all clutches.

Clutch	Clutch Size	Number of eggs hatch	Hatching Success (%)	Percent Female
FSAR10	8	8	100	87.5
FSAR 9	14	10	71.4	55.6
FSAR8	7	5	71.4	20
FSAR 7	5	4	80	75
FSAR 6	5	5	100	100
FSAR 5	8	7	77.8	100
FSAR 3	8	5	62.5	50
FSAR 2	8	8	100	37.5
GLS 5	7	7	100	100
GLS 4	7	5	71.4	0
GLS 3	6	6	100	100
GLS 2	6	6	100	80
GLS 1	7	7	100	100

Table 2: Number of hatchlings used in the study (N), hatchling measurements, and growth rates, individual clutches. Growth rate is reported as mm/day ± SE and g/day ± SE. Growth rates connected by the same letter are not significantly different.

Clutch	N	Hatchling SCL (mm)	Ending SCL (mm)	Hatchling Mass (g)	Ending Mass (g)	Growth rate (mm/day)	Growth rate (g/day)
FS10	8	51.96 ± 0.30 ^{A}	70.21 ± 1.79	32.05 ± 0.70 ^{ABC}	84.86 ± 6.32	0.0646 ± 0.0057	0.1728 ± 0.0264 ^{B}
FS9	10	50.22 ± 0.39 ^{BC}	70.18 ± 2.37	32.52 ± 0.57 ^{AB}	83.17 ± 7.41	0.0678 ± 0.0082	0.1796 ± 0.0262 ^{B}
FS8	5	50.63 ± 0.68 ^{ABC}	67.50 ± 2.75	31.06 ± 1.41 ^{ABC}	75.62 ± 7.86	0.0510 ± 0.0096	0.1588 ± 0.0275 ^{B}
FS7	4	48.44 ± 0.52 ^{BCD}	70.27 ± 1.64	27.97 ± 0.56 ^{CD}	79.56 ± 4.44	0.0873 ± 0.0044	0.2120 ± 0.0129^{AB}
FS6	5	47.96 ± 0.45 ^{CD}	66.37 ± 2.85	29.96 ± 0.90 ^{BCD}	70.70 ± 7.73	0.0648 ± 0.0084	0.1628 ± 0.0264 ^{B}
FS5	2	50.36 ± 0.36 ^{ABC}	66.39 ± 2.22	32.70 ± 1.26 ^{ABC}	67.13 ± 3.59	0.0729 ± 0.0085	0.1614 ± 0.0136^{AB}
FS3	5	49.90 ± 0.58 ^{AB}	66.06 ± 1.90	33.20 ± 0.37 ^{AB}	69.90 ± 5.68	0.0567 ± 0.0060	0.1380 ± 0.0162 ^{B}
FS2	7	51.09 ± 0.36 ^{AB}	66.54 ± 1.70	33.16 ± 0.28 ^{ABC}	71.83 ± 4.72	0.0568 ± 0.0070	0.1484 ± 0.0183 ^{B}
GLS5	5	50.45 ± .074 ^{ABC}	73.58 ± 0.99	34.75 ± 0.04 ^{A}	101.02 ± 11.60	0.0902 ± 0.0027	0.2641 ± 0.0138^{AB}
GLS4	5	46.27 ± 0.37 ^{D}	67.57 ± 2.99	27.39 ± 0.69 ^{CD}	78.01 ± 10.05	0.0710 ± 0.0083	0.1820 ± 0.0317^{AB}
GLS3	6	49.53 ± 0.69 ^{BC}	79.57 ± 2.24	30.37 ± 1.06 ^{BCD}	124.24 ± 8.87	0.0904 ± 0.0059	0.3133 ± 0.0283 ^{A}
GLS2	6	49.52 ± 0.38 ^{BC}	65.20 ± 2.77	30.69 ± 0.62 ^{ABCD}	73.08 ± 8.42	0.0534 ± 0.0106	0.1452 ± 0.0375 ^{B}
GLS1	7	46.76 ± 0.64 ^{D}	73.73 ± 2.04	26.73 ± 0.86 ^{D}	95.89 ± 6.20	0.0905 ± 0.0049	0.2461 ± 0.0217^{AB}

FIGURES

Figure 1: Tortoise enclosure set up with PVC shelter

Figure 2: Thermal gradient set up for preferred T_b trials

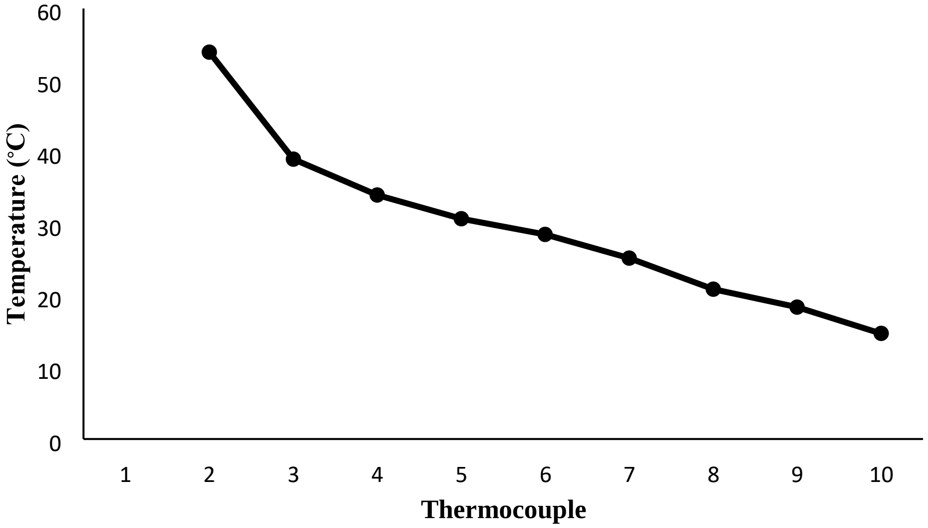

Figure 3: Graph of temperatures accessible to the tortoise hatchlings in the thermal gradient

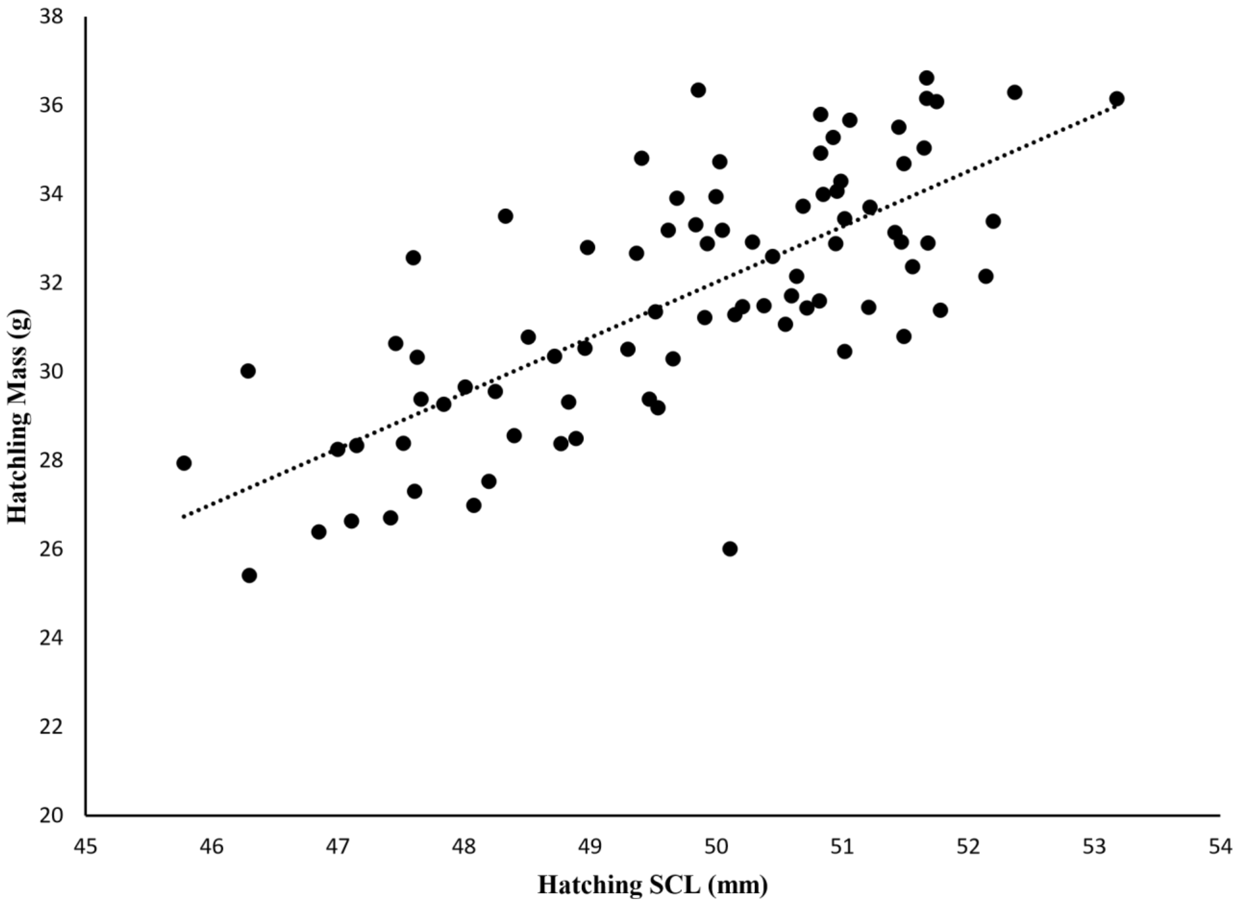

Figure 4: The linear relationship between mean hatchling mass and mean hatchling straight carapace length (SCL). The regression equation is y =1.2505x + 30.51 (R^2= 0.5459)

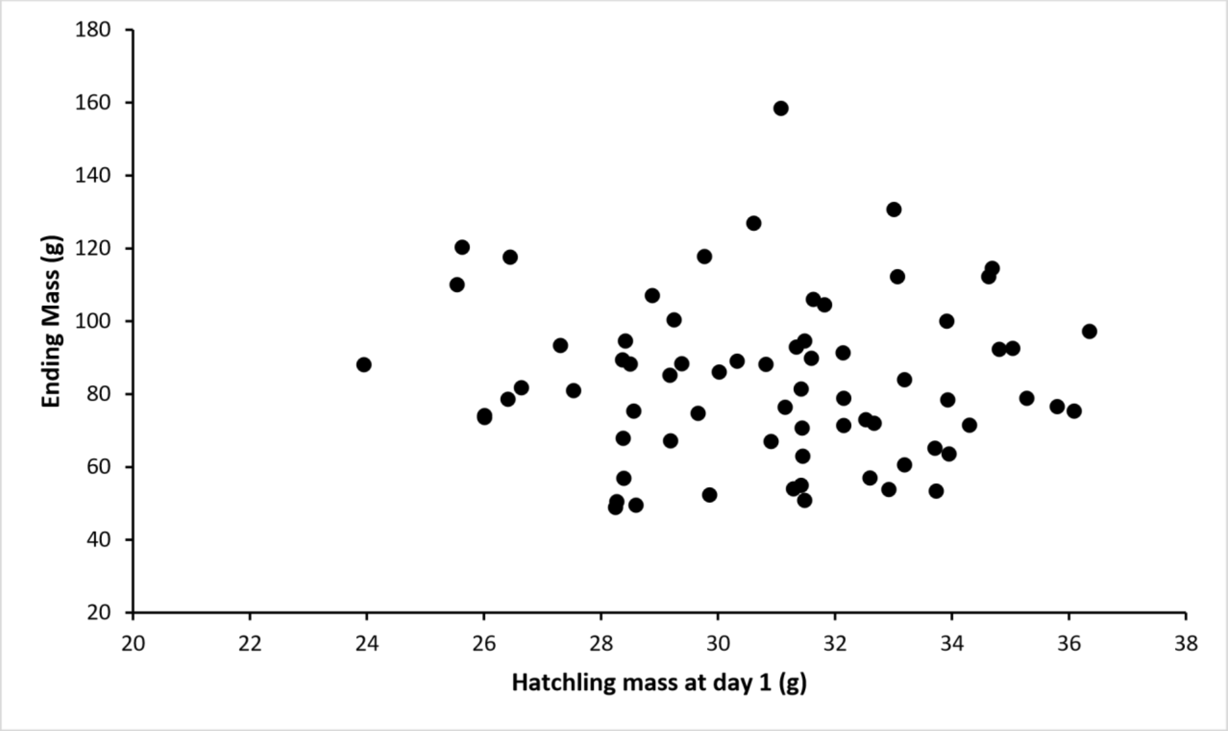

Figure 5: The relationship between mass at hatching and mass at the end of the study. This relationship is not significant (Pairwise correlation, *p* = 0.7909; $y = -0.2497x + 91.185$, $R^2 = 0.001$)

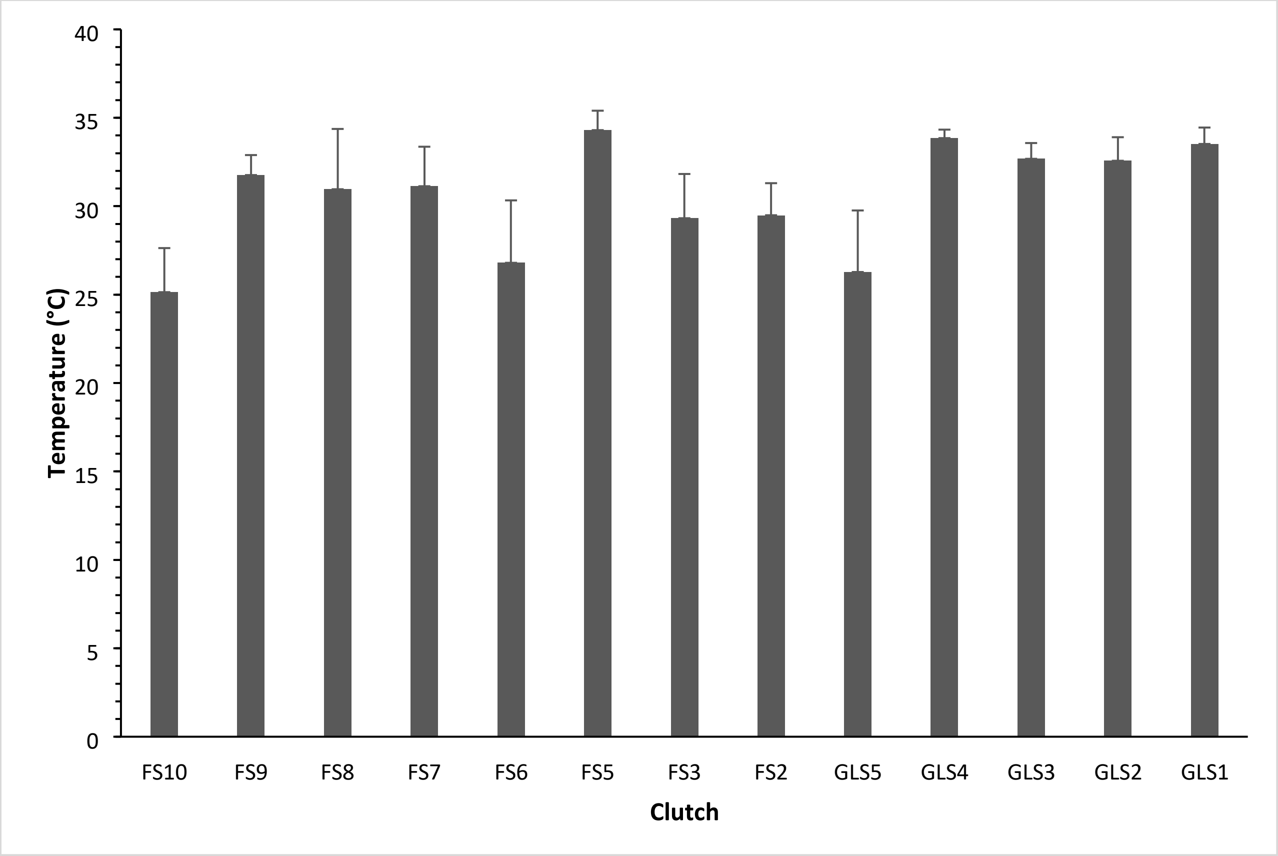

Figure 6: Preferred body temperatures per clutch. Neither clutch or sex had a significant effect on growth rate in hatchling gopher tortoises. Preferred body temperature was 30.14° C ± 0.6364°.