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EFFECTS OF HABITAT QUALITY ON REPRODUCTION IN TWO GEORGIA POPULATIONS OF *GOPHERUS POLYPHEMUS*

by

Jacqueline Entz

Under the Direction of David C Rostal

ABSTRACT

The purpose of this study was to examine differences in maternal investment by examining variation in the habitat structure and reproductive parameters for two populations of *Gopherus polyphemus* in Southeast GA. Both habitat structure and reproductive parameters for these populations are known from a previous study, thus this study expands upon the previous one and addresses four main questions. (1) Has habitat quality changed in the past ten years within and between population sites? (2) Could a change of habitat have affected female morphology or female reproductive parameters within or between populations? (3) Is female body size shaping egg size and/or clutch size for either population? (4) Are females providing equal resources to hatchlings and is hatchling growth potentially being modified by habitat quality affecting adult size and age to sexual maturity? Tortoises were captured by bucket trapping and hand trapping. Radio-graphs were used to obtain information about female body size, clutch size and egg size. In order to asses the effect of habitat quality on tortoises, hatchlings from both locations were reared under controlled, laboratory conditions. The hatchlings were measured bi-weekly to determine differences in growth. Results indicate that habitat quality for these two populations has not changed in the past fourteen years, indicating that this habitat is slow to change. In response to limited change in habitat quality, female body size and reproductive parameters have not changed. Female tortoises from one population produced significantly larger clutch sizes than the other, even when female body size was accounted for; this trend has been observed in many other Testudine species. When hatchling sizes were compared to other populations of *G. polyphemus*, the hatchling SCL seems consistent while the hatchling mass is variable. Thus, we believe that in *Gopherus polyphemus* female body size plays a small role in clutch size determination and no role on egg size determination. It is believed that *G. polyphemus* is minimizing hatchling size (SCL) in order to maximize clutch size and those females with access to more resources can supply more yolk to developing embryos. When hatchlings from both populations were provided with equal resources they grew at similar rates, indicating that the poorer quality habitat at GLS leads to a slower growth rate. Therefore, habitat quality is playing a major role in tortoise growth rates and reproduction. Based on these results, conservation efforts are needed to maintain healthy tortoise populations need to address habitat quality in development of management plans.

INDEX WORDS: *Gopherus polyphemus***,** Maternal Investment, Clutch Size Determination, Habitat Quality, Environmental Effects

EFFECTS OF HABITAT QUALITY ON REPRODUCTION IN TWO GEORGIA POPULATIONS OF *GOPHERUS POLYPHEMUS*

by

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CHAPTER 1

INTRODUCTION

Variation in reproductive characteristics is a form of maternal investment. Maternal investment is considered to be anything a female does to maximize offspring survivorship (Lacey 1998; Roitberg 1998). In reproduction, tradeoffs are observed between clutch size, offspring size, and clutch frequency (Smith & Fretwell 1974; Brockelman 1974; Congdon et. al 1983; Congdon & Gibbons 1987; Sinvero & Licht 1991; Roosenburg & Dunham 1997). Offspring size is negatively correlated with clutch size in oviparous species (Sinervo & Licht 1991; Shanbhag et al. 2000).

Many factors shape differences in maternal investment. Both abiotic and biotic environmental factors have been documented to cause variation in maternal investment (Clark & Wilson 1981; Sinervo 1990; Moller 1991; Sorci et al. 1996; Miller 1997; Lovich et al. 1999; Sorci & Clobert 1999; Henen 2002). All species of sea turtles are affected by predation so heavily that females multi-clutch and produce many small offspring in an attempt to increase the likelihood offspring survival (Clark & Wilson 1981; Miller 1997). Desert tortoise (*Gopherus agassizii*) reproduction is affected by food availability which is in turn affected by rainfall (Wallis et al.1999; Lovich et al. 1999; Henen 2002). During years of higher rainfall (and thus higher food availability), *G. agassizii* will produce a second or third clutch (Wallis et al.1999; Henen 2002; Lovich et al. 1999). Lizard hatchlings (*Sceloporus occidentalis*) vary in size as an effect of altitude and predation (Sinervo 1990). Female *S. occidentalis* produce larger offspring and smaller clutch sizes at lower altitudes versus female lizards at higher altitudes. The difference in maternal investment is due to high predation levels at low altitudes, and low predation levels at higher altitudes.

Although the environment can have effects on egg size, clutch size, and clutch frequency, female morphology is the ultimate constraint on clutch size and egg size (Shine 1992; Du et al. 2005). Body cavity space is a physical limitation, which has been demonstrated to limit the volume of offspring a female can carry during a gestation period (Shine 1992; Du et al. 2005). In other words, females cannot produce an egg or a clutch size that is physically to big to carry or oviposit (Congdon & Gibbons 1983; Sinervo & Litcht 1991; Nieuwolt-Dacanay 1997; Clarke et. al. 2001). Therefore tradeoffs exist between clutch size, egg size, and clutch frequency in order to maximize offspring survival (Smith and Fretwell 1974; Brockelman 1975).

Many studies have examined the differences in maternal investment among and between populations. Most of these studies were performed on short-lived species (Parker & Begon 1986; Sinervo 1990; Sinervo & Litcht 1991; Sorci et al. 1996; Sorci & Clobert 1999). Studies performed on long-lived species have only examined maternal investment over a relatively small period of time (Congdon & Gibbons 1983; Rowe 1994; Roosenburg & Dunham 1997; Wallis et. al 1999; Wallace et. al 2006). This study is one of few to examine the changes in maternal investment over time for a long-lived species.

Rostal and Jones (2002) examined the population structure, reproductive parameters, and habitat quality for two populations of *Gopherus polyphemus* in Southeast Georgia. Both populations experience similar rainfall and temperatures throughout the year (<http://www.city-data.com/city/Twin-City-Georgia.html>; <http://www.city-

data.com/city/Glennville-Georgia.html>), but have differences in habitat structure. Fort Stewart Army Reservation (FSAR) contains sandhill habitat dominated by Longleaf pine trees and is burned regularly (3-4 years); frequent fires result in a higher quantity and diversity of plants (Landers et al 1981; Diemer 1986; Perdue 2000; Birkhead et al. 2005). Fort Stewart Army Reservation female gopher tortoises reproduce at a younger age and larger body size; they also produce larger egg masses/hatchling masses and larger clutch sizes (Table 1) (Rostal & Jones 2002). In contrast, George L. Smith State Park (GLS) is a sandhill habitat dominated by turkey oak due to the lack of regular controlled burns (last burn in 1984 and 2004). George L. Smith State Park female gopher tortoises first reproduce at an older age and smaller body size; they produce smaller clutch sizes and smaller egg masses/hatchling masses (Table 1) (Rostal & Jones 2002). Rostal and Jones (2002) determined that there were population differences in maternal investment; they believed habitat quality was the cause of different reproductive strategies. They also believed the lack of resources at GLS has lead to a slower growth rate and a smaller body size; which is why females at GLS have a smaller reproductive output. Since Rostal and Jones (2002) there has been one controlled burn (2004) at GLS. This study will directly examine habitat quality as a cause of variation in reproductive parameters.

Like many other studies, Rostal and Jones (2002) established a positive relationship between female body size and clutch size (Congdon et al. 1983; Frazer & Richardson 1986; Congdon & Gibbons 1987; Iverson 1992; Shine 1992; Rowe 1994; Du et al. 2005). However the relationship found in these two populations was very weak. This study expands upon Rostal and Jones (2002) by using additional female measurements and increasing the sample size. Rostal and Jones (2002) only used female straight carapace length (SCL) for comparison to clutch size. In the current study I used multiple measures of female body size (mass, straight carapace length (SCL), shell height (SH), shell width (SW)), and female pelvic aperture for comparison with clutch size and egg size. I compared multiple measurements of female body size to determine the best possible indicator of clutch size and egg size.

Since female body size and clutch size showed a weak positive relationship (Rostal and Jones 2002), it is possible that egg size or hatchling size determines clutch size (Sinervo & Licht 1991; Shanbhag et al. 2000). Since gopher tortoises have no parental care after egg deposition and do not multi-clutch in any given season, the number of eggs and size of the eggs represent the total maternal investment (Landers et. al 1980; Congdon et. al 1983; Congdon & Gibbons 1985; Congdon & Gibbons 1987; Rostal & Jones 2002; Ashton & Ashton 2008).

Rostal and Jones (2002) believed that habitat quality is affecting the growth rate and reproductive output of *G. polyphemus*. They believed that because tortoises at FSAR had access to more resources than tortoises at GLS, they were able to grow faster. This means they could reach a larger reproductive size quicker and produce a larger size and/or number of offspring (due to larger body size). In order to address the impact of the environment on tortoise growth and reproduction we conducted a laboratory experiment. Hatchlings from both populations were collected and housed under the same conditions. By comparing the growth rates of hatchlings between populations this allowed us to address the extent to which the habitat affects growth rates. If the hatchlings from both populations grow at same rate, then it could provide evidence that environment differences are causing population differences in size and reproduction.

This study expands upon Rostal and Jones (2002) by answering four major questions. (1) Has habitat quality changed in the past ten years within and between population sites? (2) Could a change of habitat have affected female morphology or female reproductive parameters within or between populations? (3) Is female body size shaping egg size and/or clutch size for either population? (4) Are females providing equal resources to hatchlings and is hatchling growth potentially being modified by habitat quality affecting adult size and age to sexual maturity?

CHAPTER 2

METHODS

Study Sites-- Both FSAR and GLS are located in Southeast Georgia. The two sectors of FSAR chosen for Rostal and Jones (2002) were of similar size and topography as the sandhill located at GLS (Jones 1999; Perdue 2000; Rostal and Jones 2002). George L Smith State Park is located at approximately 32^{o}32’38.11” N and 82^{o}07’03.36” W. The two sectors selected for FSAR are located at approximately (Sector E-21: 31^{o}57’04.35” n / 81 51’34.05W and Sector F12: 32^{o}06’19.26”N / 81 46’31.02”W For complete description of the habitats’ size and structure see Rostal and Jones (2002).

Female Subject Sampling -- Data for the current study was conducted in two field seasons (May through August 2007 and 2008) and then combined with previous year’s data (May through August 1994, 1995, 1996, and 1999). In 2007 and 2008, *G. polyphemus* females were captured from the two study sites using hand capture and bucket trapping methods (Rostal & Jones 2002). The tortoises were identified or given an identification number for future recaptures. Further detail on capture methods and tortoise identification can be found in Rostal and Jones (2002).

The following measurements were taken for each tortoise using calipers measured to the nearest millimeter: straight carapace length (SCL), shell height (SH), shell width (SW), straight plastron length (SPL). Female body mass was measured in the field in kilograms with a 10 kg Pesola spring scale. Females were taken to a laboratory where an ultrasound was performed to determine if eggs were present. If shelled eggs were present a radiograph was taken. Radiographs were taken as described in Rostal and Jones (2002). Radiographs from field seasons 2007 and 2008 were used to determine clutch size, egg diameter (smallest and largest). The females' pelvic aperture was also estimated from radiographs based on Congdon and Gibbons (1987). The smallest space between the ilia represents the maximum size egg a female could physically oviposit (Congdon & Gibbons, 1987).

Egg Acquisition--During the nesting season, the aprons of tortoise burrows were searched for nests by hand probing the sand for eggs. The apron of the burrow is a mound of loose sand dug out of the burrow located at the entrance of a gopher tortoise burrow. When a nest was found Hobo ® Data Loggers were placed in the nest at the same depth of the eggs. The Data Logger recorded temperatures every two hours. Vinyl coated metal screens which allow airflow and water to pass through were placed over nests to prevent depredation. The screens were secured with camping stakes. Eight nests from each location were found during 2007. In 2008 six nests from FSAR were found and two nests from GLS were found. During late August, nests were excavated and eggs were transferred to incubators in the lab and maintained at 28.5°C. In 2007, a total of 52 eggs from FSAR and 35 eggs from GLS were excavated from the field. In 2008, 28 eggs and hatchlings from FSAR and 5 eggs from GLS were excavated from the field; some of the eggs hatched before nest excavation and collection due to higher than mean temperatures and low rainfall during the nesting season.

The eggs were incubated together for up to 21 days. Eggs were monitored daily and individually marked for identification as described in Rostal and Jones (2002). When the eggs were collected, temperature dependent sex determination (TSD) had already taken place so removing the eggs did not skew natural sex ratios. The eggs were weighed in grams on a digital scale and egg diameters (smallest and largest) were measured with digital calipers in millimeters to the nearest tenth.

Hatchling Housing—Hatchlings from 2007 were individually housed in the animal facilities at Georgia Southern University in 5.7 L plastic container with horse chow (10% protein) to provide bedding. Horse chow provided affordable bedding which if consumed would be digestible. Hatchlings were not observed feeding on bedding material. To provide hatchlings with warmth, the room temperature was kept at 28.0°C with overhead full spectrum UVA & UVB light fixtures to provide necessary light and additional basking heat. Light fixtures were placed 14 cm above the hatchlings' housing. The photoperiod for the hatchlings was twelve hours of light and twelve hours of dark and was controlled by an electronic timer.

For diet control, all tortoises received the same amount of vegetables and ground Zeģler® iguana food (20% protein). As tortoises grew, the amount of food they were provided was increased; tortoises never consumed all of the food provided. All tortoises were fed *ad libitum* and soaked once per week to rehydrate and void urates. Hatchlings were measured bi-weekly for one year. The following measurements were taken with digital calipers (mm) ± 0.01: SCL, SH, SW, and SPL. Mass (g) was also recorded bi-weekly for one year using a digital scale to the nearest 0.1 gram.

Vegetation Analysis--In order to estimate the resources available, vegetation data was collected. Sampling included fifty randomly selected active burrows from both sites. Burrows were considered active if there was a recent sign of tortoise activity (Rostal and & Jones 2002). Twenty-five random points at GLS and FSAR were picked in the same manner as Gaskell (2007). Vegetation was sampled in the same manner as Rostal and

Jones (2002). The 2007 vegetation data was compared with the data for 1995 reported in Rostal and Jones (2002). Vegetation sampling included the following: percent ground coverage and percent canopy coverage as in Rostal and Jones (2002).

Statistical Analysis—Data from the years 1994, 1995, 1996, 1999, 2007, and 2008 was compiled for analysis. Data from 1999 (Perdue, 2000) was not part of Rostal and Jones (2002) but was added to the data set to increase sample size. All statistics were performed in Jump 4.0. Female body size, clutch size and egg size were mean when females were observed more than once, to prevent pseudo-replication. Female mean body size, clutch size, and egg size were used for all analyses except those determining differences between years. I used one way ANOVAs to determine whether female body size has changed among the years 1994, 1995, 1996, 1999, 2007 and 2008. The same analysis was performed for reproductive variables. Time (year) was declared the independent variable and the various morphometric measurements and reproductive parameters were declared the dependent variables. Each measurement and population was analyzed separately. Linear regression analyses were performed to determine the influence of female body size (SCL, SH, SW, SPL, pelvic aperture and mass) on clutch size and egg size. Linear regression analyses were also performed to determine the effect of egg size on clutch size. In order to determine whether three extreme data points were outliers a leverage test was performed on clutch size versus egg size; in addition, I examined the distribution of the residuals for clutch size versus egg size. The mean daily temperature was taken for each data logger. In order to assess differences in nest temperatures a two way nested ANOVA was performed by nesting data logger within location. An ANCOVA was performed to determine population differences in clutch sizes versus SCL. Multiple t-tests were carried out to determine population differences in egg mass, egg diameter, hatchling SCL, hatchling masses, and vegetation quantity and quality. For all tests performed, alpha is 0.05.

CHAPTER 3

RESULTS

Habitat Structure— Habitat quality and quantity appears to be similar to observations made by Rostal and Jones (2002) (Table 2). Percent canopy cover and herbaceous groundcover were significantly different between Fort Stewart Army Reservation (FSAR) and George L. Smith State Park (GLS) for the vegetation data collected in 2007 (Table 3). Fort Stewart Army Reservation had significantly less canopy cover than George L. Smith State Park for both random points and burrows. Readings for percent canopy cover at random points on FSAR were 40.25 ± 4.24 and 63.7 ± 5.516 on GLS ($t = -3.904$, $p = 0.0003$, DF = 48); while readings for active burrows on FSAR were 34.81 ± 2.73 and 49.91 ± 2.78 on GLS ($t = -3.86$, DF = 96, P 0.0002) (Table 3). Fort Stewart Army Reservation had significantly more herbaceous groundcover than GLS for both random points and active burrows. Fort Stewart Army Reservation had a mean percent ground cover of 22.84 ± 3.10 at random points and GLS had a mean percent groundcover of 8.723 ± 3.10 ($t = 3.21$, $p = 0.0023$, DF = 48) (Table 3); while mean percent groundcover for active burrows at FSAR were 32.89 ± 2.23 and mean percent groundcover for active burrows at GLS were 12.25 ± 2.78 ($T = 6.47$, DF = 96, $P = < 0.0001$) (Table 3).

Changes in tortoise size and reproductive parameters-- The mean female body size and reproductive parameters were not significantly different from Rostal and Jones (2002). There were no significant differences in female body size (SCL, SH, SW, SPL) nor clutch size throughout years 1994, 1995, 1996, 1999, 2007, and 2008 for both populations (Table 4 & 5). There were no significant differences in hatchling characteristics (hatchling SCL and hatchling mass) for either population throughout the years 1995, 2007, and 2008 (Table 4 & 5). Mean hatching success at FSAR in 2007 and 2008 was 89.4 % while the mean hatching success in 1995 was 81.2 %. However hatching success for GLS was different than Rostal and Jones (2002). Mean hatching success at GLS for 1995 was 87 % while the mean hatching success for 2007 and 2008 was 48.7 %. It should be noted that hatching success in 1995 for both FSAR and GLS is based on incubating the eggs in the lab and not in the field.

Percent hatching success was pooled for 2007 and 2008. There was a significant difference between the sites (t= 5.513, DF = 124 P = <0.001); hatching success at FSAR was 89.4% ± 4.074 and hatching success at GLS was 48.7 % ± 5.86 (Table 8). The observed differences in hatching success were not due to variation in nest temperatures. Nest temperatures were not significantly different between sites; FSAR had a mean nest temperature of 28.41 ± 0.0803 ºC and GLS had an mean nest temperature of 28.41 ± 0.0931 ºC (F = 0.533, DF = 1, 14, p = 0.465) (Fig. 1).

Population Differences (female body size and reproductive parameters) — One hundred three individual female tortoises were captured from FSAR and 69 individual female tortoises were captured from GLS between 1994, 1995, 1996, 1999, 2007, and 2008. Females from FSAR were significantly larger than GLS females for all morphometric measurements (SCL, SH, SW, SPL); mean female SCL at FSAR was 30.56 ± 0.169 cm and mean female size at GLS was 29.16 ± 0.203 cm (t = 5.294, DF = 156, P = <0.0001).

Fort Stewart Army Reservation females produced a mean clutch size of 6.6 ± 0.21 eggs and GLS females produced a mean clutch size of 4.99 ± 0.25 eggs (t= 4.783, DF=

95, P= <0.0001). There was a significant difference in clutch size between populations. Fifty-six female tortoises from FSAR and 41 female tortoises from GLS had shelled eggs present in radiographs (data pooled 1994, 1995, 1996, 1999, 2007, and 2008).

Significant differences in egg masses and hatchling masses were observed between populations (Table 8); data from the years 1995, 2007, and 2008. Fort Stewart Army Reservation had a mean egg mass of 41.63 ± 0.488 g (n= 87) while GLS had a mean egg mass of 36.01 ± 0.610 g (n = 55) (t =7.081, DF=85, P= <0.0001) (Table 8). Fort Stewart Army Reservation had a mean hatchling mass of 33.51 ± 0.44 g (n = 73) and GLS had a mean hatchling mass of 29.70 ± 0.644 g (n = 34) (t = 4.895, DF= 106, P = <0.0001) (Table 8).

No significant differences were observed between the populations for egg diameter and hatchling SCL (Table 8). Fort Stewart Army Reservation females produced a mean egg diameter of 43.75 ± 0.302 mm (n = 28) and GLS females produced a mean egg diameter of 43.25 ± 0.301 mm (n = 19) (pooled data 2007 and 2008). Hatchlings from FSAR had a mean hatchling SCL of 47.59 ± 0.36 mm (n = 73) while hatchlings from GLS had a mean hatchling SCL of 46.65 ± 0.54 mm (n = 34) (t = 1.449, DF = 106, P = 0.1503) (Table 7) (pooled data 1995, 2007, and 2008).

Relationship of female body size to clutch size, egg size, and hatchling size—In order to increase sample size, data was pooled for the years: 1994, 1995, 1996, 1999, 2007 and 2008. All measurements of female body size were positively correlated with clutch size (SCL, SH, SW, SPL and mass) (Table 6) (Fig. 2). Although all of the female morphometric measurements demonstrated a positive relationship with egg size, female SCL was used to represent female body size. Female SCL accounted for the most clutch size variation (Table 6) and is a more reliable measurement than mass; tortoises can urinate and defecate up to 1 Kg when stressed. When analyzed separately, both populations showed a significant positive correlation between SCL and clutch size (GLS: clutch = -4.90 + 0.334 SCL; R^2 =0.267; n = 41) (FSAR: clutch = -7.9711 + 0.473 SCL; R^2 = 0.150; n = 56) (Fig. 2). Analysis of covariance of clutch size versus population differed significantly between FSAR and GLS (F = 12.018, DF = 1, 85, P = <0.0001) (Fig. 2).

When analyzed separately neither population had a significant relationship between female SCL and mean egg diameter nor between female pelvic aperture and mean egg diameter; pooled data from 2007 and 2008 (Table 6) (Fig.3 & 4). The data from both populations were combined to increase sample size, but there was no relationship between female SCL, pelvic aperture and egg size (Table 6) (Fig. 3 & 4).

Data was pooled for years 2007 and 2008 for clutch size and mean egg diameter analyses. There was a significant positive correlation between clutch size and egg size at FSAR (Avg. Egg Dia. = 40.391 + 0.4671 clutch size; R^2 = 0.300) (F = 11.185, DF = 1, 26, P = 0.0025) (Table 7) (Fig. 5). However, there was no significant relationship between clutch size and egg size at GLS (F = 0.006, DF = 1, 17, P = 0.938) (Table 6). When the populations were combined there was a significant positive relationship between mean egg diameter and clutch size (Avg. Egg Dia. = 41.600 + 0.298 clutch size; R^2=0.130) (F = 7.506, DF = 1, 45, P = 0.0088) (Table 6) (Fig. 4). When the distribution of residuals for clutch sizes versus egg sizes and leverage tests were examined, it revealed that three extreme data points were outliers. One female produced a low clutch size of three eggs (mean clutch = 7.43) and of small eggs (mean egg diameter = 40.49

mm) that were 3 standard deviations away from the mean egg diameter (43.75mm). Two other females produced large clutch sizes 9 and 10 eggs (that were very large in diameter (47.46 mm and 48.1 mm respectively); eggs were three standard deviations away from the mean (43.75mm). When the three outliers were removed from FSAR there was no relationship between clutch size and egg size for the FSAR population individually (F = 2.468, DF = 1, 23, P = 0.123) or when GLS and FSAR were combined (F = 1.54, DF = 1, 42, P = 0.221).

Hatchling growth rates – When raised under the same conditions, mean growth rates from both populations appear to be similar. Hatchlings from both populations grew very slowly between August of 2007 and March 2008 (FSAR = 0.378 ± 0.104 grams per 14 days; GLS = 0.312 ± 0.098 grams per 14 days) (Fig. 6). In April 2008, hatchling growth rates from both populations increased fifteen fold (FSAR = 5.194 ± 0.409 grams per 14 days; GLS 5.051 ± 0.436 grams per 14 days) (Fig. 6). Hatchling growth rates began to increase at approximately 250 days of age (April); April is typically when temperatures would naturally heat up in Southeast Georgia.

CHAPTER 4

DISCUSSION

The goal of this study was to examine variation in maternal investment by using two populations of *Gopherus polyphemus* in southeast GA. In both populations, Fort Stewart Army Reservation (FSAR) and George L Smith State Park (GLS), the habitat structure and reproductive parameters were known from a previous study (Rostal and Jones 2002). The results of this study support the conclusion that there have been no major shifts in female body size, female reproductive parameters, or habitat structure (within and between populations) over the past fourteen years at either site. Since both populations from the current study followed the same trends observed in Rostal and Jones (2002), this allowed us to address other questions related to understanding the relationship of female body size to egg size, clutch size and hatchling size. In addition we can determine the extent to which the environment may affect these factors.

When comparing the relationship between female body size and clutch size, all female morphological measurements had a significant positive relationship with clutch size (Table 6). Mass and Straight Carapace Length (SCL) demonstrated the strongest relationship with clutch size when compared with the other female measurements (Table 6) (Fig. 2). Many other studies on Testudines have found the same trend (Congdon et al. 1983; Frazer & Richardson 1986; Congdon & Gibbons 1987; Iverson 1992; Shine 1992; Rowe 1994; Niuwolt-Dacanay 1997; Rostal & Jones 2002; Du et al. 2005). Although both populations demonstrated a positive relationship between SCL and clutch size, FSAR females produced significantly larger clutch sizes than GLS females when body size was controlled for (Fig.2). If female body size (SCL) determined clutch size then we should have observed females from both populations producing clutch sizes proportional to their body size. Since this was not observed, it suggests some other variables are influencing clutch size.

A larger female body size has been found in other Testudine species to influence egg size (larger females can create larger eggs) (Congdon et al. 1983; Frazer & Richardson 1986; Congdon & Gibbons 1987; Iverson 1992; Shine 1992; Rowe 1994; Rostal & Jones 2002; Du et al. 2005). Since egg size can not exceed the size of a female's pelvic aperture, it was tested to see if pelvic aperture was a limiting factor for egg size in *G. polyphemus*. I examined the relationship between female body size (female SCL and pelvic aperture) and egg size and found no relationship for either population; therefore pelvic aperture is not limiting egg size in these two populations of *G. polyphemus*. This is the first species with round hard shell eggs to be investigated. Most species tested have been Emydids which have oblong leathery eggs which are flexible (Gibbons 1982; Congdon et. al 1987; Rowe 1994; Nieuwolt-Dacanay 1997; Roosenburg & Dunham 1997).

Due to limited female body cavity space, egg size and clutch size typically have a negative relationship (Smith & Fretwell 1974; Brockelman 1974; Congdon et. al 1983; Congdon & Gibbons 1987; Ford & Seigel 1989; Sinervo & Licht 1991; Roosenburg & Dunham 1997). However, we found that FSAR females demonstrated a positive relationship between egg size and clutch size, while females from GLS demonstrated no relationship (Fig 5). A positive relationship between egg size and clutch size is highly unlikely and was thought to be driven by three extreme individuals from FSAR and a low sample size. Based on this data, I think that tortoises (on average) from either FSAR or

GLS are not producing a large enough volume of eggs per clutch (egg size and clutch size) to be constrained by female body cavity space. This same trend was also documented by Nieuwolt-Dacanay (1997) in the western box turtle. Galapagos tortoises are also definitely not limited by body size and produce relatively small clutches. In Galapagos tortoises, clutch size appears to be limited more by available nest material depth and not female size (Rostal, personal observation).

Although significant relationships were found between female body size and clutch size (Fig. 2, 3, 4, & 5) (Table 6), like many other studies on Testudines the R^2 value for this relationship was low. On average there is still a large amount of clutch size variation unaccounted for (greater than 50% in most cases). This is true for other Testudine species including: desert tortoises (R2= 0.268) (Wallis et. al. 1999), western box turtles (pearson's correlation = 0.383 (Nieuwolt-Dacanay 1997), the diamond back terrapin (correlation coefficient =0.176(Roosenburg & Dunham 1997)), chicken turtles (R^2 = 0.47) (Congdon et. al 1987), three different populations of red-eared sliders (R^2 = 0.39, R^2 = 0.12 (Gibbons 1982); R^2 = 0.65 (Congdon et. al 1987)) and four different populations of painted turtles (R^2 = 0.43 (Congdon et. al 1987)) (R^2 = 0.30, R^2 = 0.22, R^2 = 0.52, R^2 = 0.47 (Rowe 1994)). Based on the data from this study, female body size for *G. polyphemus* plays a small role in clutch size determination and has no effect on egg diameter. It seems that environmental selection pressures are playing a role in clutch size, egg size, and hatchling size determination. For example, many Testudine species' clutch sizes, egg size, and hatchling sizes are affected by environmental factors such as, predation (all species of sea turtles (Clark & Wilson 1981; Miller 1997)), rainfall and food availability (*Gopherus agassizii* (Wallis et al. 1999) and *Geochelone radiate* (Leuteritz & Ravolanovio 2005)).

As already demonstrated, FSAR has more energy available in the form of food (vegetation) throughout the season than GLS, both quality and quantity (Perdue 2000; Rostal and Jones 2002) (Table 2 & 3). This energy can be used in many different ways including reproduction. There is evidence to suggest that FSAR females are allocating more yolk into individual eggs than GLS females (Table 8). The mean egg diameter was statistically the same for both locations (Table 8); however, mean egg mass was significantly different by location (FSAR produced heavier eggs) (Table 8). Similarly, hatchling SCL did not vary by location (Table 8), but hatchling masses were significantly different by location (FSAR produced heavier hatchlings) (Table 5). Differing ratios of yolk to albumen could account for variation in egg and hatchling mass while maintaining equal hatchling SCL and egg size or diameter.

Eggs contain both yolk and albumen. The yolk contains lipids that are used by the embryo for development during incubation and post hatchling (Romanoff 1960; Nobel & Moore 1964; Congdon et al. 1983; Fischer et al. 1991; Tucker et al. 1998). The albumen (for reptiles) assists in gas exchange and contains a supply of water and antimicrobial properties the developing embryo (Movchan & Gabaeva 1967; Ewert 1979; Tracy & Snell 1985; Palmer & Guillett 1991; Ackerman 1997). Variable ratios of yolk to albumen is not uncommon in Testudines, two other studies on sea turtles found that hatchling and yolk mass remained consistent within and among females, while albumen remains variable as the season progressed (LeBlanc 2004; Wallace et.al 2006; Wallace et. al 2007).

A lower hatching success at GLS could be attributed to a lower proportion of yolk allocated to individual eggs (Table 8). It is important to note that nest temperature did not vary between sites and therefore did not affect hatching success, sizes, or incubation rates (Fig 1). Although the *in situ* hatching success at GLS is considerably lower than at FSAR, it was not due to nest temperatures; nest temperatures were statistically the same at both locations. Although Rostal and Jones did not find significant differences in hatching success in their incubator study (GLS = 86.96% and FSAR = 81.22%), hatching success does not infer fertility. Many of the eggs at GLS simply did not develop completely or were infertile (personal observation).

I believe the hatchlings growth rates provide further evidence that environmental factors are influencing growth rates and eventually reproductive output in these two populations. The hatchlings from both populations grow at the same rate under laboratory conditions (Fig 6). Since GLS hatchlings grew at a similar rate to FSAR hatchlings, it is likely that a lack of resources (quantity and/or quality) at GLS could result in a slower growth of tortoises at GLS. A slower growth rate would explain the observed smaller reproductive size of female tortoises at GLS and smaller clutch sizes. This trend has been observed in other laboratory based experiments with other reptiles (European grass snake *Natrix natrix* (Madsen & Shine 1993); the checkered garter snake *Thamnophis marcianus* (Ford & Seigel 1989); and the common lizard *Lacerta vivipara* (Sorci et al. 1996)). These results are consistent with other studies demonstrating resource availability plays a large role in a female's reproductive output (James & Whitford 1994; Nieuwolt-Dacanay 1997; Wallis et. al. 1999; Henen 2002).

It appears that resource availability is affecting clutch size and egg/hatchling mass but not egg size. When comparing GLS and FSAR hatchling sizes with other populations of *G. polyphemus*, hatchling SCL is consistent. Southeastern Mississippi's population has a mean hatchling size (SCL) of 48.3 mm (Epperson & Heise 2003). A population in Merrit Island, FL, has a mean hatchling size (SCL) of 46.9mm (Pike & Seigel 2007). Pike and Seigel (2006) found no statistical difference between hatchlings sizes for three different *G. polyphemus* populations (Central Florida, North Florida, and Mississippi); the mean hatchlings size being 46.9mm. It seems the consistency of hatchling SCL throughout multiple populations is because females are producing a minimum hatchling size to maximize the number of offspring.

Larger females are capable of producing a larger size hatchling SCL but hatchling SCL are similar throughout many populations, indicating that some females are not maximizing hatchling size (Rostal and Jones 2002; Epperson & Heise 2003; Pike & Seigel 2006; Pike & Seigel 2007). Why would it beneficial for female gopher tortoise to produce smaller hatchlings? Hatchling size typically plays a very important role in the first years of survival for many organisms (Janzen 1994; Butler & Sowell 1996; Sinervo 1990; Pike & Siegel 2006). Due to an extremely high mortality rate for *G. polyphemus* during the first two years, a larger body size could be beneficial (Butler & Sowell; Pike & Seigel 2006; Pike & Seigel 2007). Tortoises are heavily preyed upon by coyotes, birds of prey, raccoons, and snakes (Epperson & Heis 2003; Pike & Seigel 2006; Pike & Seigel 2007; Ashton & Ashton 2008). Being preyed upon by large predators seems to have lead to the selection of an optimal hatchling size (Epperson & Heis 2003; Pike & Seigel 2006; Pike & Seigel 2007; Ashton & Ashton 2008).

Theoretically producing a very large offspring would decrease the high predation levels (Janzen 1994; Bodie & Semlitsch 2000). However, for a female *G. polyphemus* to produce a hatchling large enough to deter their natural predators, the egg would be physically too big for the female to oviposit. Pike and Seigel (2007) demonstrated that even larger *G. polyphemus* hatchlings had the same odds of being eaten as the smaller hatchlings. So it could be that natural selection driven by predators has led *G. polyphemus* populations to produce smaller hatchlings in order to produce larger clutches.

In summary, it has been determined that although female body size significantly affects clutch size there is a large amount of unexplained clutch size variation. It seems that environmental factors like resource availability and predation may be influencing clutch size variation, which leads to the different strategies of maternal investment observed in these two populations of *G. polyphemus*. The next logical next step in examining the differences in maternal investment would be a reciprocal transplant study. Individuals from FSAR could be permanently marked and released at GLS, and individuals from GLS could be marked and released at FSAR.

By monitoring their reproduction for multiple years following release, this could help determine weather the observed differences in maternal investment are genetic or habitat related.

CHAPTER 5

CONSERVATION IMPLICATIONS

By better understanding the reproductive adaptations of *Gopherus polyphemus* in different habitats we can design better management plans. The ability to link the differences of *G. polyphemus* reproduction to the habitat quality will further support the practice of controlled burning. A better managed burn regime could possibly help increase the reproduction of females at GLS. Female tortoises might be able to acquire more nutrients and possibly obtain higher fitness. *Gopherus polyphemus* is a keystone species, so if there is a large effect on their reproduction, then many other species are affected. The burrows of *G. polyphemus* are used by many other animals including: insects, mammals, amphibians, reptiles, and other threatened or endangered species like the eastern Indigo (*Drymarchan corais couperi)* and the dusky gopher frog (*Rana capito sevosa*) (Auffenberg 1969; Brode 1959; Guyer & Herman 1997; Hallinan 1923; Hubbard 1893; 1894; Hutt 1967). The large burrows provide shelter, protection from predators, and sources of food for many animals (Breininger et al 1991; Gaskell 2007). Thus, a decline in Gopher tortoises can directly lead to a decline in species diversity in a Longleaf pine ecosystem. More understanding of the reproductive biology of *G. polyphemus* is imperative for its’ conservation and that of many other species.

Chapter 6

OVERALL CONCLUSIONS

(1) Habitat quality for these two populations has not changed in the past fourteen years, indicating that this habitat is slow to change. (2) Most likely as a result of little habitat change, female body size and reproductive parameters also did not change. (3) We combined the data from both populations and seven years of data to determine the effect of female body size on clutch size, egg size and hatchling size. From the data we concluded that in *Gopherus polyphemus* female body size plays a small role in clutch size determination and no role on egg size determination. When comparing hatchling sizes to other populations of *G. polyphemus*, the hatchling SCL seems consistent while the hatchling mass is variable. It is believed that *G. polyphemus* is minimizing hatchling size (SCL) in order to maximize clutch size and those females with access to more resources can supply more yolk to developing embryos. (4) When hatchlings from both populations were provided with equal resources they grew at similar rates, indicating that a poorer quality habitat at GLS may lead to a slower growth rate. With all factors taken into account, it seems that habitat is playing a large role in tortoise growth rates and reproduction.

Table 1. Reproductive parameters of adult females observed in Rostal and Jones (2002) at study sites George L Smith State Park (GLS) and Fort Stewart Army Reservation (FSAR). All reproductive parameters were significantly different between study sites.

	FSAR Means	**GLS Means**
Reproductive Size	306 mm	290 mm
Reproductive Age	18-20 years	20-25 years
Clutch Size	6.52 eggs	4.52 eggs
Egg Mass	42.6 g	40.7 g
Hatchling Mass	32.2 g	29.4 g

Table 2. Comparison of habitat between Rostal and Jones (2002) and the present study (collected in 2007) for active burrow and random burrows.

	Random Points		**Active Burrows**	
Location	**Mean Percent Canopy Cover**	**Mean Percent Ground Cover**	**Mean Percent Canopy Cover**	**Mean Percent Ground Cover**
GLS				
Rostal & Jones (2002)	76.4 ± 1.79	12.2 ± 1.31	26.1 ± 1.75	35.6 ± 2.13
Current Project	63.70 ± 4.25	8.723 ± 3.101	49.91 ± 2.78	12.24 ± 2.32
FSAR				
Rostal & Jones (2002)	40.3 ± 2.51	28.6 ± 1.75	25.8 ± 2.06	40.4 ± 3.03
Current Project	40.25 ± 4.25	22.84 ± 3.101	34.81 ± 2.73	32.8 ± 2.23

Table 3. Comparison of 2007 habitat quality between the study sites for both active burrows and random points (George L Smith State Park (GLS) and Fort Stewart Army Reservation (FSAR).

		FSAR Mean	**GLS Mean**	**T value**	**P value**	**DF**
Random Point	**Percent Canopy Cover**	40.25 ± 4.24	63.7 ± 5.51	-3.904	0.0003	48
	Percent Herbaceous Ground cover	22.84 ± 3.10	8.723 ± 3.01	3.21	0.0023	48
Active Burrow	**Percent Canopy Cover**	34.81 ± 2.73	49.91 ± 2.78	-3.86	0.0002	96
	Percent Herbaceous Ground cover	32.86 ± 2.23	12.25 ± 2.78	6.47	<0.0001	96

Table 4. Summary of ANOVAs for Fort Stewart Army Reservation (FSAR) demonstrating no differences in female morphology or reproductive parameters over time. The years 1994, 1995, 1996, 1999, 2007 and 2008 were included for these analyses.

Measurement	**F-value**	**DF**	**P value**
Female Straight Carapace Length (cm)	2.13	5, 134	0.065
Female Shell Height (cm)	1.62	5, 133	0.2844
Female Shell Width (cm)	1.474	5, 134	0.128
Female Straight Plastron Length (cm)	2.24	5, 133	0.0536
Clutch size	1.284	5, 60	0.298
Hatchling Straight Carapace Length (mm)	1.549	2, 71	0.2184
Hatchling Mass (g)	2.194	2, 71	0.1190

Table 5. Summary of ANOVAs for George L Smith State Park (GLS) demonstrating no differences in female morphology or reproductive parameters over time. The years 1994, 1995, 1996, 1999, 2007 and 2008 were included for these analyses.

Measurement	**F-value**	**DF**	**P value**
Female Straight Carapace Length (cm)	1.52	5, 101	0.191
Female Shell Height (cm)	0.9208	5, 101	0.312
Female Shell Width (cm)	2.13	5, 101	0.0861
Female Straight Plastron Length (cm)	2.13	5, 101	0.0861
Clutch size	1.807	4, 49	0.312
Hatchling Straight Carapace Length (mm)	0.7602	1, 32	0.398
Hatchling Mass (g)	0.5615	1,32	0.561

Table 6. Relationship between female morphology and clutch size from combined data of two populations of *Gopherus polyphemus*, George L Smith State park (GLS) and Fort Stewart Army Reservation (FSAR); pooled data from 1994, 1995, 1996, 1999, 2007 and 2008.

Measurement	**F value**	**DF**	**p-value**	**R^2**
Mass (Kg)	16.89	1,70	<0.0001	0.19
Straight Carapace Length (cm)	27.27	1,115	<0.0001	0.192
Shell Width (cm)	4.56	1,115	<0.0036	0.03
Shell Height (cm)	25.4	1,114	<0.0001	0.183
Straight Plastron Length (cm)	24.14	1, 115	<0.0001	0.173

Table 7. Demonstrating the relationship between egg diameter and female straight carapace length, egg diameter and pelvic aperture, and egg diameter and clutch size. Populations were examined independently and combined; pooled data from 1994, 1995, 1996, 1999, 2007 and 2008.

	P –value	**DF**	**F value**	**R^2**
Female Straight Carapace Length (cm)				
GLS	0.346	1,16	0.9403	0.056
FSAR	0.616	1,22	0.0062	0.0116
Combined	0.3760	1,40	0.8016	0.019
Female Pelvic Aperture (mm)				
GLS	0.3208	1,9	1.104	0.109
FSAR	0.616	1,21	0.258	0.012
Combined	0.3290	1, 32	0.9825	0.029
Clutch Size				
GLS	0.9379	1, 17	0.0062	0.000367
FSAR	0.0025	1, 26	11.185	0.300
Combined	0.0088	1, 45	7.51	0.130

Table 8. Egg and hatchling characteristics differences between two populations of *Gopherus polyphemus*, George L Smith State park and Fort Stewart Army Reservation. Data from 1995, 2007 and 2008 was used for these analyses.

	FSAR	**GLS**	**t- value**	**DF**	**p-value**
Mean Egg Diameter (mm)	43.76 ± 0.302	42.25 ± 0.301	1.07	45	0.298
Egg Mass (g)	42.2 ± 0.506	37.6 ± 0.635	5.56	140	<0.001
Hatchling SCL (mm)	47.59 ± 0.365	46.65 ± 0.539	1.45	140	0.15
Hatchling Mass (g)	33.51 ± 0.436	29.70 ± 0.644	4.99	106	<0.001
Mean Hatching Success	89.4 ± 4.074	48.7 ± 5.86	4.783	95	<0.0001

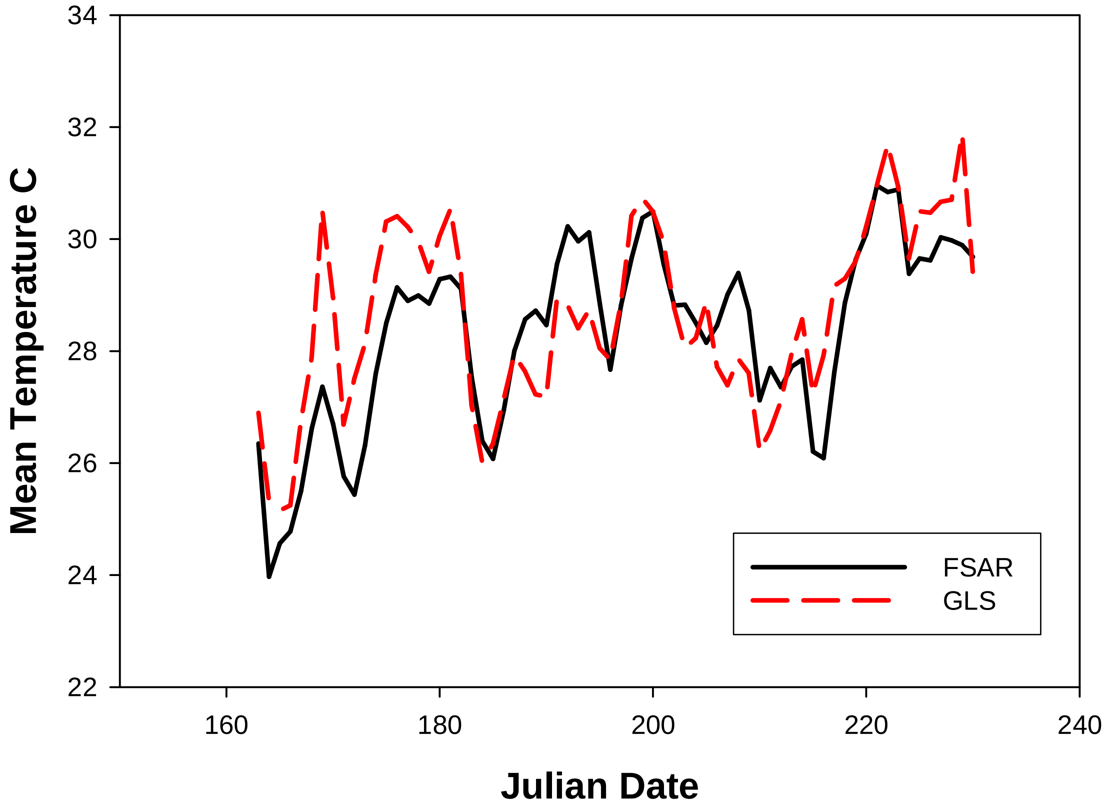

Fig 1. Nesting temperature from 2007 recorded by HOBO® dataloggers for two populations of tortoises. George L. Smith State Park (GLS) and Fort Stewart Army Reservation (FSAR). No significant differences in nesting temperatures were observed between populations.

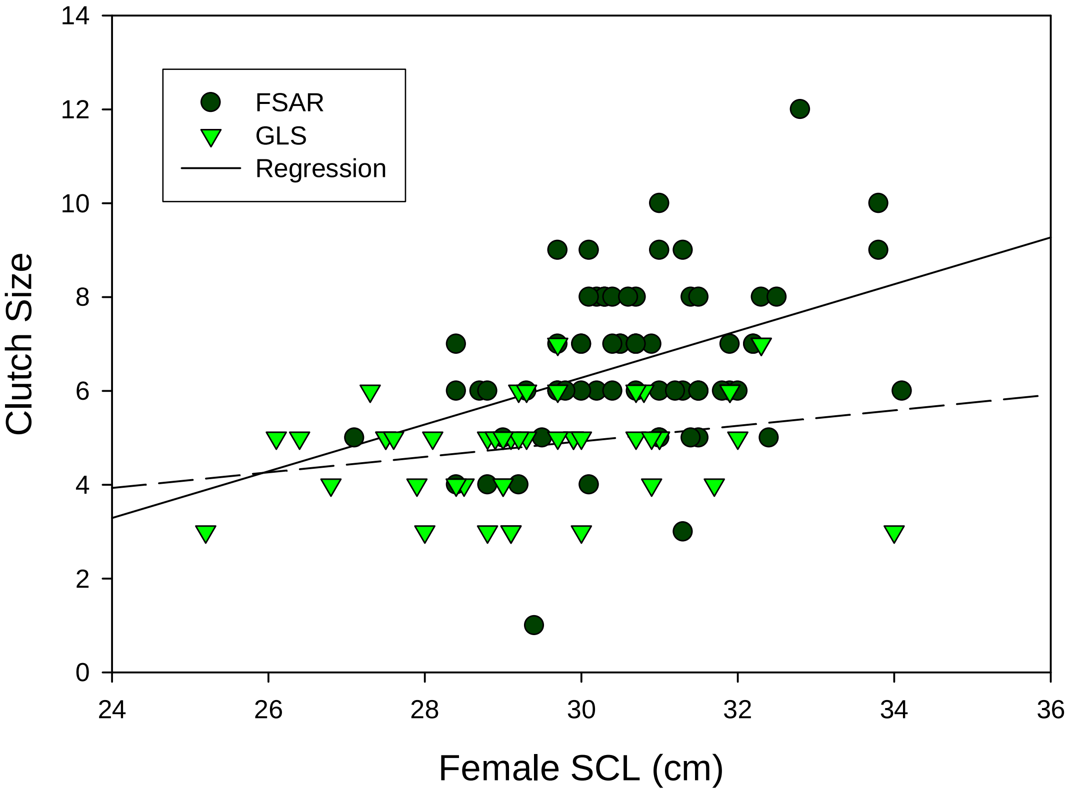

Fig 2. Clutch size vs. female straight carapace length (SCL) for gopher tortoises (*Gopherus polyphemus*) studied at George L Smith State Park (GLS) and Fort Stewart Army Reservation (FSAR). Populations produced significantly different clutch size when body size is accounted for (ANCOVA: F = 12.018, DF = 1, 85, P = < 0.0001).

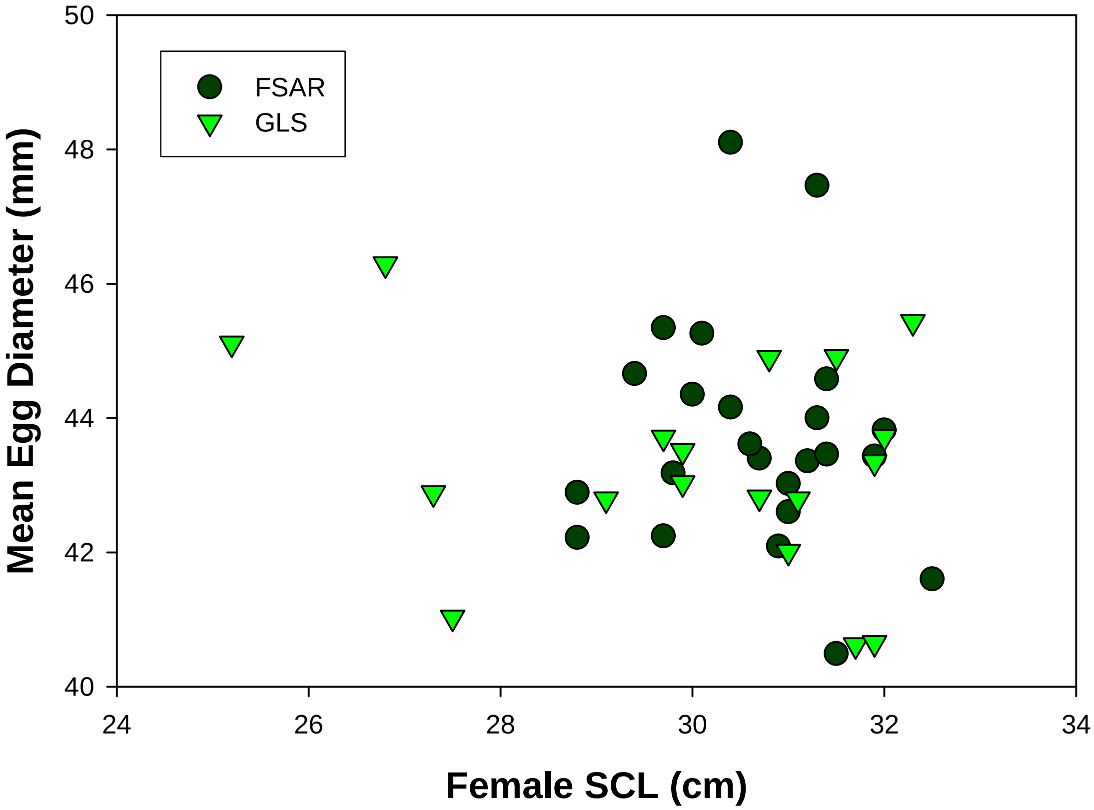

Fig 3. Female straight carapace length vs. mean egg diameter for gopher tortoises at study sites George L Smith State Park (GLS) and Fort Stewart Army Reservation (FSAR). There was no relationship between female SCL and egg diameter when populations were examined independently or combined.

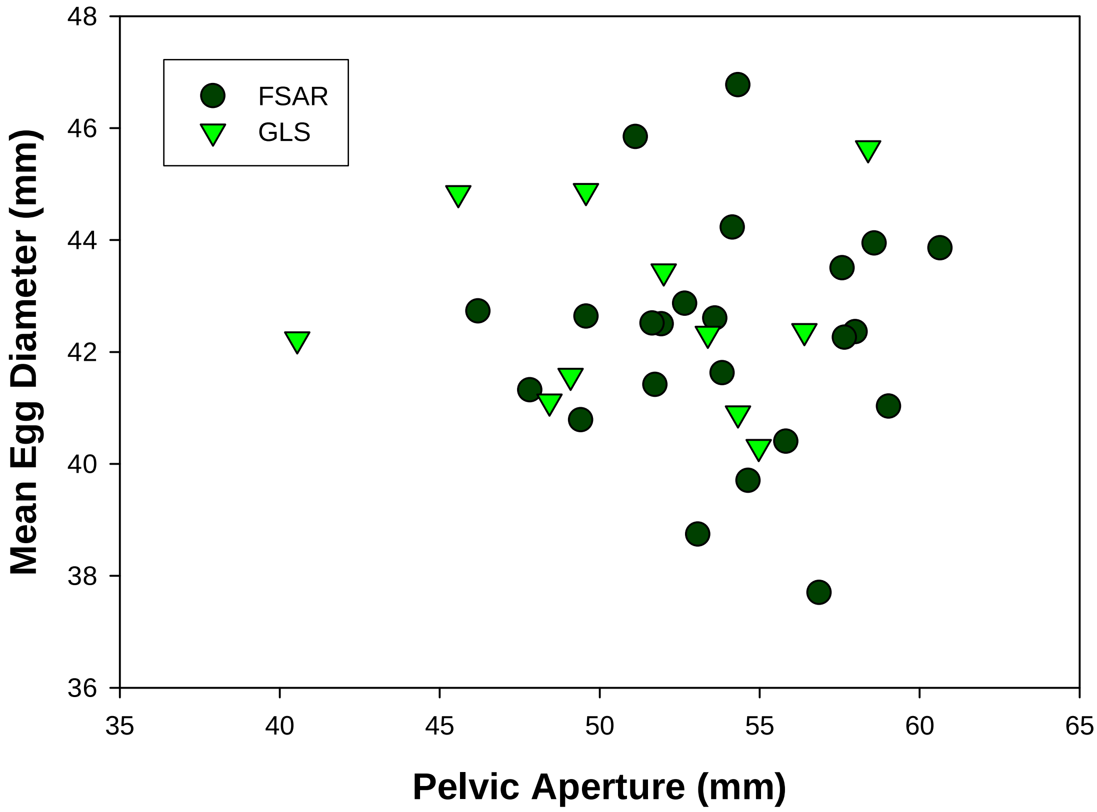

Fig. 4. Pelvic size vs. mean egg diameter for gopher tortoises (*Gopherus polyphemus*) studied at George L Smith State Park (GLS) and Fort Stewart Army Reservation (FSAR). There was no relationship found between pelvic size and egg diameter for either population independently or when combined.

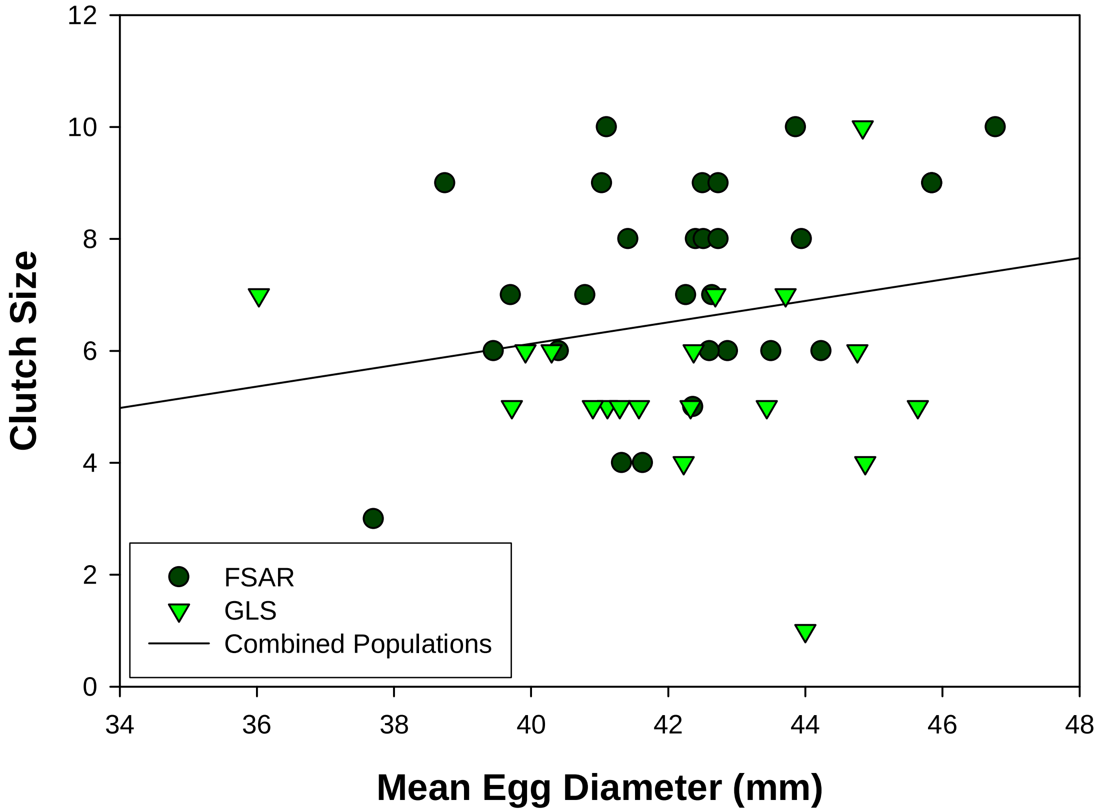

Fig 5. Clutch size vs. mean egg diameter for gopher tortoises (*Gopherus polyphemus*) studied at George L Smith State Park (GLS) and Fort Stewart Army Reservation (FSAR). There was a significant positive relationship at FSAR (Avg. Egg 40.39 + 0.461 clutch size; $R^2 = 0.300$) (F = 11.185, DF = 1, 26, P = 0.0025); there was no relationship found at GLS. There was a significant positive relationship observed when both populations were combined (Avg. Egg Dia.=41.600 +0.298 clutch size; $R^2=0.130$) (F = 7.506, DF = 1, 45, P = 0.0088).

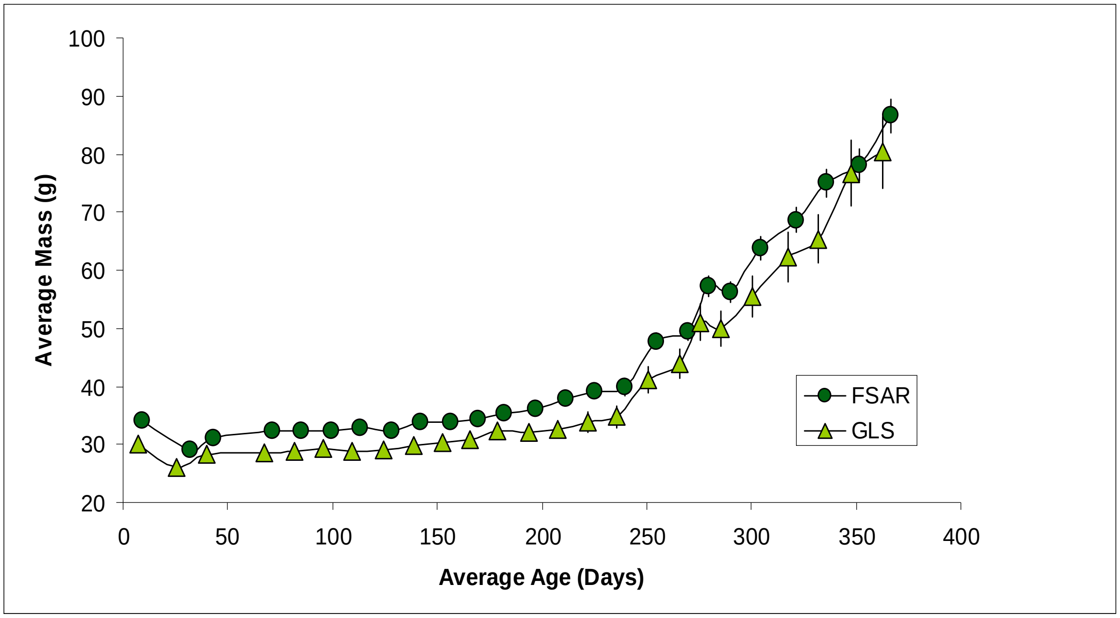

Fig 6. The mean growth rates of gopher tortoise hatchlings from FSAR (n = 48) and GLS (n=14).Tortoises appear to grow at the same rate.

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