RELATIONSHIP OF DIET TO BODY SIZE IN TEXAS HORNED LIZARDS (*PHRYNOSOMA CORNUTUM*)

by

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RELATIONSHIP OF DIET TO BODY SIZE IN TEXAS HORNED LIZARDS (*PHRYNOSOMA CORNUTUM*)

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Abstract

Age determination is important when monitoring populations of conservation concern. Reptiles often have a positive relationship between age and snout-to-vent (SVL) length and so one can get an estimate of the age distribution of individuals in a population based on their size. Texas horned lizards are a threatened species in the state of Texas yet they are highly cryptic and difficult to find. Horned lizard scat on the other hand is relatively easy to find and recognize. We used the length of scat we found to determine if it was correlated to the SVL of its respective horned lizard. We first genotyped DNA extracted from individual scat to match them to previously captured and measured horned lizards. We found that as feces length increases, the SVL of the same horned lizard also increases. We then used this relationship to reconstruct the size distribution of the population using only feces and compare it to the size distribution of captured lizards. Collecting data on the size of Texas horned lizard feces may provide a relatively easy method to determine the size distribution of horned lizards in a population, especially in areas that need to be quickly or sporadically surveyed.

Introduction

Determining the age of individuals is important for monitoring populations of conservative concern. The distribution of ages in a population can indicate if it is growing or declining in numbers. Unless individuals are marked soon after birth and followed over a period of years it is often difficult to determine the age of individuals (Halliday and Verrell 1988). In some cases age specific coloration can be used, or destructive sampling such as removal of otaliths (fish) and skeletons (reptiles and amphibians) and then counting growth rings using microscopy (Halliday and Verrell 1988, Castanet and Smirina 1990). These destructive sampling methods, however, are not desirable when the species is of conservation concern. Another possibility for some taxa is to use body size as a surrogate for age. Unlike mammals, that undergo determinant growth (individuals stop growing once they reach maturity), reptiles have indeterminate growth and will often continue to get larger as they age. The size of an individual can therefore potentially indicate the age of an individual although other factors such as ambient temperature, food availability, and sex also have an impact on the size of an individual (Halliday and Verrell 1988).

Phrynosoma cornutum, also known as the Texas horned lizard, is a threatened species in the state of Texas. Once a prevalent animal on Texas land, its population has been in major decline in the past few decades (Price 1990, Donaldson et al. 1994, Henke 2003). Due to their status as a threatened species in Texas, and their highly cryptic lifestyle, horned lizards are very difficult to research (Price 1990). Texas horned lizard scat or feces is however, easy to find and identify (Williams pers. comm.). The scat is distinctly cylindrical with a white uric acid cap and contains numerous ant exoskeletons. Horned lizards also often deposit scat on bare ground which they utilize for increasing body temperature and increasing digestion (Sullivan et. al 2014).

These lizards are spikey-bodied reptiles with an average snout-to-vent length (SVL) of sixty-nine millimeters (Zamudio 1998). An odd characteristic of *Phrynosoma cornutum* that distinguishes it from other species is that female horned lizards are larger in size than males (Montgomery et al. 2003). A large female can reach 114 millimeters in SVL, whereas a large male averages 94 millimeters in snout-to-vent (Johnson 2011). The Texas horned lizard is the largest bodied of the 14 horned lizard species in North America. Males and females reach sexual maturity in their second spring. Previous studies have found that this age corresponds to a snout-to-vent length (SVL) of 70 mm or greater in southern populations of Texas horned lizards (Ballinger 1974).

Determination of age (adult versus subadult or juvenile) for Texas horned lizards normally requires the capture and measurement of individuals which is not straightforward due to their cryptic lifestyle. If the size of Texas horned lizards can be determined from their feces then researchers could potentially reconstruct the size distributions of horned lizards in an area from feces which are much easier to find. A previous study on desert (*Phrynosoma platyrhinos*) and regal (*Phrynosoma solare*) horned lizards indicated that feces length was strongly correlated to lizard size although the data were not presented in that paper (Sullivan et al. 2014). In this study I matched individual feces to lizards that had been captured and measured using genetic profiles in order to determine if feces length is correlated to lizard size. I then use this relationship to reconstruct horned lizard sizes from fecal measurements and compare this to known horned lizard sizes on the study area.

Methods

Study Sites

Texas horned lizards were studied in two small Texas towns in Karnes County: Kenedy and Karnes City in the summer of 2015 (Figure 1). These towns are unique because of their recent urbanization, yet a horned lizard population remains. In fact, Karnes County is the Horned Lizard Capital of Texas as declared by the Texas Legislature. The field work consisted of searching public areas (open fields, parks, and schoolyards) and private property areas (residents first gave permission to enter) for Texas horned lizards and their scat.

Figure 1. Karnes County (in red).

Genotyping

We collected fresh fecal pellets of Texas horned lizards (easily recognized by the cylindrical shape of the feces) and preserved the samples in 3.0 mL 8M Urea buffer (10 mM Tris pH 7.5, 125 mM NaCl, 10 mM EDTA, 1% SDS, 8 M urea) (Asahida et al. 1996) until extraction. DNA extraction followed the protocol outlined in the QIAamp DNA Stool Mini-kit (Qiagen Genomics, Valencia, CA). A negative control was made with each round of extraction (8-12 samples) to ensure non-contamination of reagents. Extractions were conducted in an extraction dedicated AirClean® 600 PCR workstation.

We genotyped individuals at 11 microsatellite loci developed using methods described in Williams et al. (2012). Three replicate PCR reactions were initially conducted for each sample in a separate room from DNA extractions in a PCR dedicated AirClean® 600 PCR workstation. Negative controls were used in all PCR reaction batches. Polymerase chain reactions (PCR) (10µL) contained 1 µl DNA, 0.1 µM of each primer, 1X Qiagen Multiplex PCR Master Mix with HotStarTaq, Multiplex PCR buffer with 3mM $MgCl_2$ pH8.7, and dNTPs. Reactions were cycled in an ABI 2720 thermal cycler. The cycling parameters were one cycle at 95°C for 15 min, followed by 40 cycles of 30s at 94°C, 90s at 60°C, 90s at 72°C, and then a final extension at 60°C for 30 minutes. PCR products were then diluted with 50 µl dH_2O and 1 µl was added to 15 µl HIDI formamide and 0.1 µl LIZ-500 size standard. Samples were then electrophoresed on an ABI 3130XL Genetic Analyzer (Life Technologies). Genotypes were scored using GeneMapper 5.0 (Life Technologies).

We then created consensus genotypes for each individual using the comparative method (Frantz et al. 2003). We accepted homozygotes if we observed that genotype at least 3X and a heterozygote if we observed both alleles at least 2X. If a sample did not meet one of these criteria then we amplified it again 3X to determine the consensus genotype. Loci that failed to have a consensus after 6X were scored as missing. Consensus genotypes were constructed manually for each individual. Genotypes were discarded if the consensus had only 7 or fewer loci. We then compared all fecal genotypes to genotypes that were generated from captured and measured horned lizards. Genotypes were considered identical when they matched at all loci or all but one locus. We used the multilocus analysis in GenAlEx v6.5 (Peakall and Smouse 2012) to find genotyping matches.

Statistical Analysis

We regressed the SVL of individual lizards against fecal length using Microsoft Excel to determine if there was a positive relationship between fecal size and horned lizard size. We then regressed fecal length against SVL and used the equation for the best fit line (with the origin set at 0) to back calculate horned lizard sizes from all fecal pellets measured in the field. We then used a Kolmogorov-Smirnov one-sample test to compare this size distribution to the expected distribution generated from the size distribution of horned lizards that were captured and measured. We also compared the proportion of horned lizards with a >69 mm SVL and <69 SVL as calculated from the fecal pellets to horned lizards that were captured and measured with a Fisher's exact test

Results

We captured 221 individual horned lizards during the field work. One-hundred and eleven were female, 90 were male, and the remaining 20 were unknown. The average female weight was 26.40 grams, making them heavier than the males which averaged 22.52 grams in weight (Figure 2). When the snout-to-vent length (SVL) is compared, female horned lizards were once again larger (Figure 2). The SVL of females averages at 68.92 mm, while males had a SVL of 65.41 millimeters.

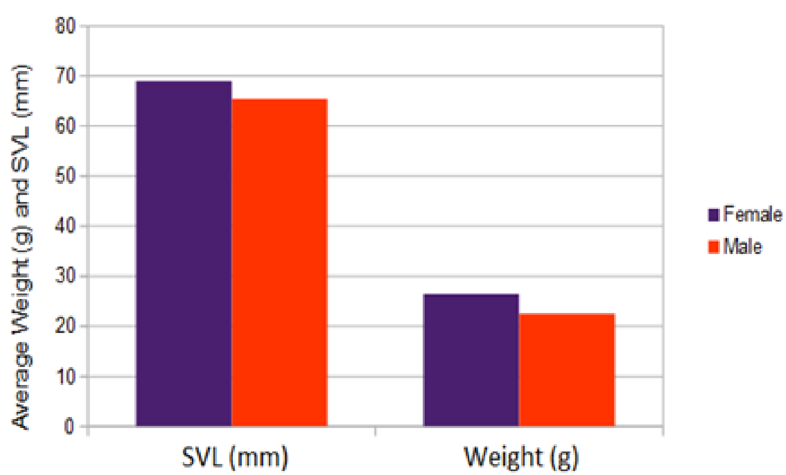

Figure 2. Average weight and snout-to-vent length of male and female horned lizards in Kenedy and Karnes County.

The horned lizards had an average snout to vent length of 64.10 mm (range20-91 mm) (male and female combined) (Figure 3).

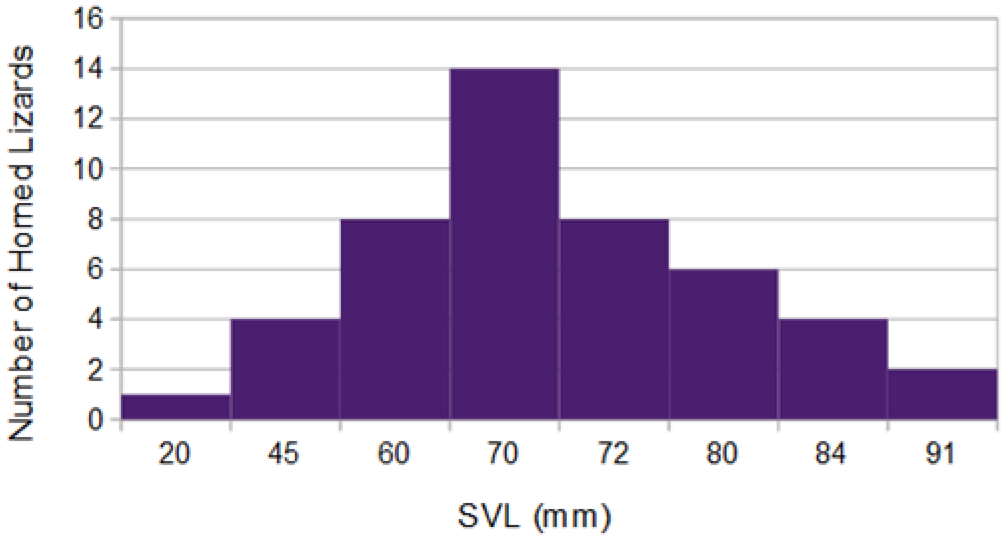

Figure 3. Snout to vent length (millimeters) of Texas horned lizards found in South Texas.

The distribution of horned lizard feces follows a similar trajectory as the SVL did. Most feces had an average length of 2 centimeters with a range of 1-4 cm (Figure 4).

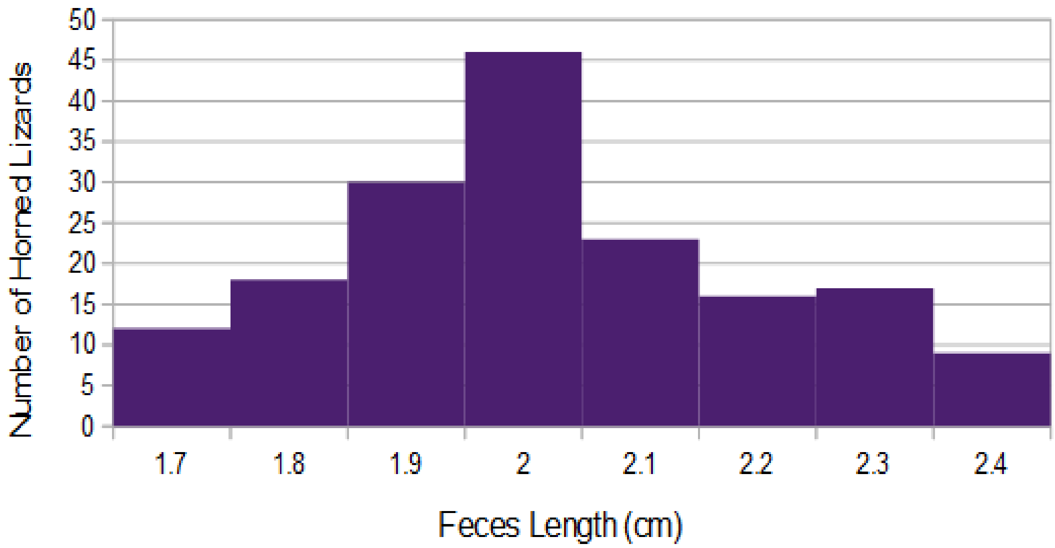

Figure 4. Feces length (cm) distribution of Texas horned lizards.

I was able to match feces to 25 different lizards. There was a positive relationship between SVL and feces length ($F_{1,24}$ = 17.05, R^2 = 0.42, P = 0.0004, y = 0.026x + 0.477) (Figure 5). I then performed a regression on feces length versus SVL and used the resulting equation for the best fit line (with origin set at zero) to calculate the predicted SVLs of the lizards that were responsible for all measured feces (y = 29.53X) (Figure 6).

The distribution of SVL for captured lizards was different than the SVL that was back-calculated from all the feces collected (Kolmogorov-Smirnov one-sample test, P = 0.052). When only using feces from a single sampling period, however, the distributions were the same (Kolmogorov-Smirnov one-sample test, P = 0.15) (Figure 7). Similarly the proportion of lizards captured that were > or < than 69mm was significantly different than the proportion back-calculated from all the feces (Fisher's exact test, P = 0.007) but was not different than the proportion back-calculated from the feces collected on a single trip (Fisher's exact test, P = 0.15) (Figure 8).

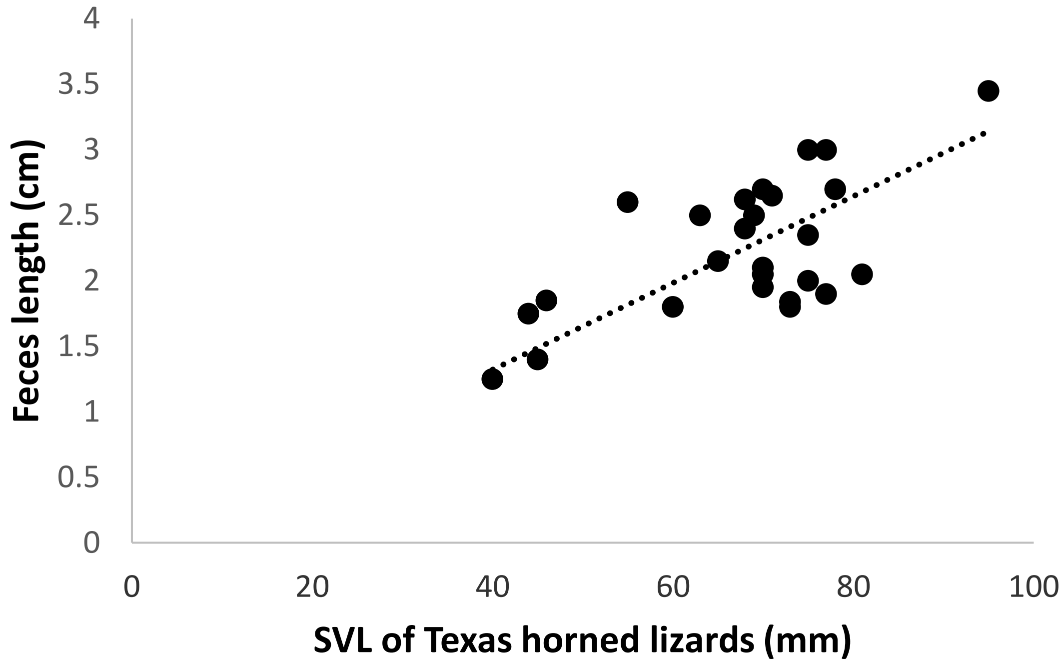

Figure 5. The relationship of feces length (cm) to horned lizard SVL (mm).

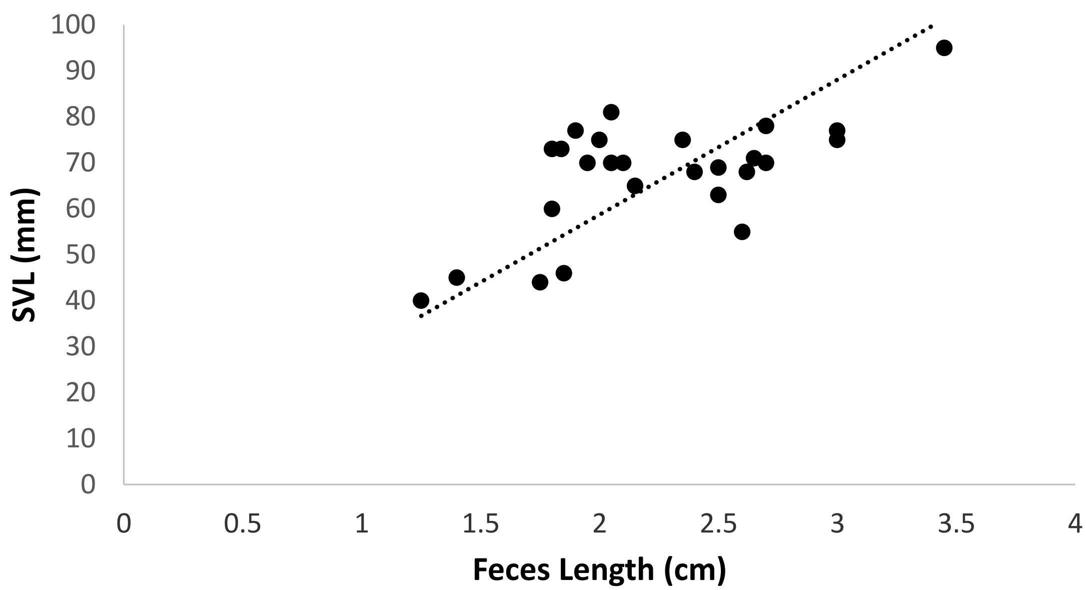

Figure 6. An estimate of horned lizard SVL (mm) is calculated according to the feces length (cm).

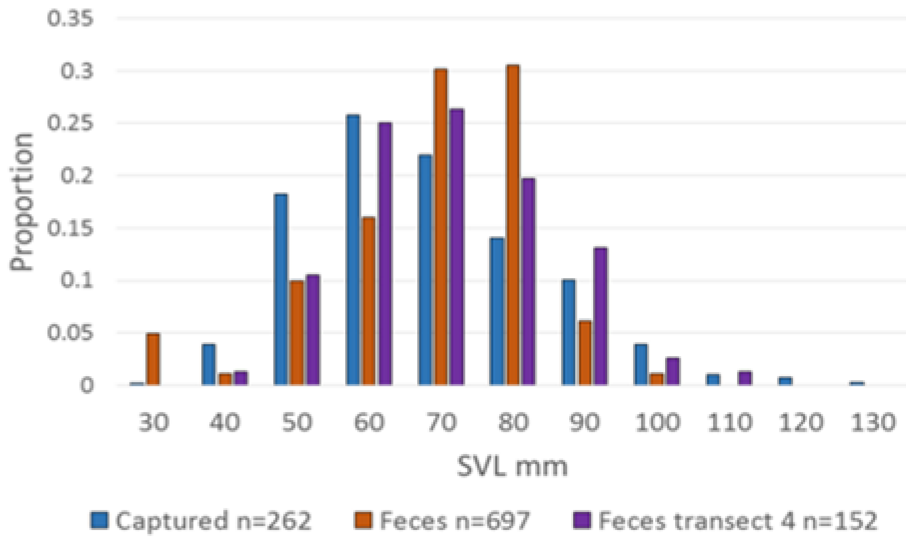

Figure 7. Proportion of SVL for captured horned lizards (blue), back-calculated SVL from total number of feces (orange), and the back calculated SVL from feces in a single trip (purple).

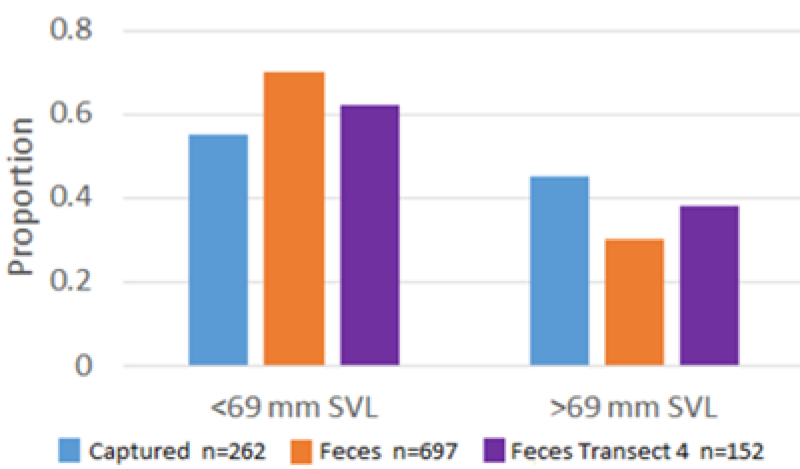

Figure 8. Proportion of SVL for horned lizards above (adult) or below (subadult) 69 mm. Proportion of SVL for captured horned lizards (blue), back-calculated SVL from total number of feces (orange), and the back calculated SVL from feces in a single trip (purple).

Discussion

We evaluated scat of Texas horned lizards in the southern area of Texas around Kenedy and Karnes City. First, *Phrynosoma cornutum* was tracked and traced in order to retrieve their scat. Measurements of the horned lizards' SVL and weight was also taken. Second, genetic analysis through genotyping and sequencing allowed insight into the individual horned lizard's phenotype.

Statistical analysis of the horned lizards and their scat supports Zamudio (1998). Female Texas horned lizards do indeed have a larger snout to vent length when compared to the males. Not only is SVL data correctly correlated with previously published data, but the weight also aligns with prior information.

Sullivan et al. (2014) discovered that there was a correlation of horned lizard size when measuring lizard scat. This correlation is further supported through our findings in Karnes County. The horned lizards that had the bigger snout to vent length also had the larger feces length. The same is represented with the smaller horned lizards. A small SVL directly correlates with a small feces length.

With the information gathered, a distribution of sizes in the population was constructed. We used feces data from a single sampling period because this was more accurate than using all of the feces data from the entire trip. The SVLs ranged from 30 mm to 130 mm, with the majority being in the 60-80 spectrum. When looking at the proportion of adults and subadults (above or below 69 mm, respectively), there is a greater proportion of young individuals compared to old individuals. This indicates that the population is likely growing, or at least stable.

Conclusion

Through the process of collecting, genotyping, and analyzing the fecal DNA of these lizards, we now understand more about the qualities of Texas horned lizards. A lizard's snout to vent length has been shown to have a direct correlation to its feces size. Not only that, but age structure can also be determined through the analysis of the SVL. Interestingly, measuring feces on a single trip is very quick and a more cost effective means to determine the size distributions of lizards in an area. However, this should be tested in other habitats and areas because the size distribution of lizards may be different. Research efforts should continue to focus on monitoring the age and size structure of these populations. The horned lizards in Kenedy and Karnes County represent a healthy population because the sizes of the scat found were on both extremes. This symbolizes newborns being present and a healthy population that will continue to grow. Future research should be based on preserving this healthy distribution of sizes.

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