

NATURAL HISTORY AND REPRODUCTIVE BIOLOGY
OF A POPULATION OF PRAIRIE RATTLESNAKES (*Crotalus viridis*)

FROM KANSAS

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The primary goal of this study is to determine a relationship between morphological characteristics of Prairie Rattlesnakes, *Crotalus viridis*, and current game management practices in an effort to streamline more efficient use of resources to protect and manage the species.

Using morphological characteristics, I attempted to determine whether there are methods that are less invasive for determining sex of *C. viridis* in the field than those currently in use. Snout-vent length (SVL), tail length, and rattle length of a population collected in southwestern Kansas were highly correlated. The number of subcaudal scales showed a moderate correlation with tail length, a weak correlation with SVL, no correlation with rattle length. Tail length increased more in males than in females as life stages progressed in this population.

C. viridis are generalist predators that tend to utilize the most abundant prey species within an area. The majority of prey items consumed by this population, especially the small mammals, were taken in spring between egress from hibernacula and mating in early summer. The only ontogenic shift in prey preference was related directly

to size. Larger snakes exhibited preferential behavior in favor of larger prey items on occasion.

Within this population of *C. viridis*, 38% of females were gravid. Mean clutch size= 8.3 ± 2.9 (N = 30); mean ova length per clutch= 30.1 ± 7.2 mm (N= 30). Of the 28 male gonads examined, only two exhibited various stages of spermatogenesis. Seven of the 13 females examined exhibited some stage of ovarian follicular development.

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Most especially, Thank you to Dan Fogell for pushing me along that merry path to academic success (or insanity...whichever)! He pointed me in the direction of this project. He gave me the contact information and laid the groundwork for the transfer of the collection to Omaha. He helped me get some of the preservatives that I needed to get started before grant money started coming in. He answered all of my panicked text messages and phone calls, regardless of the time. He believed in me when I had a hard time believing in myself. I absolutely could not have completed this project without his help and support.

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GENERAL INTRODUCTION

As the human population continues to explode and encroach into areas inhabited by predators, the potential for the frequency of human-predator confrontations increases (Gershman, 1999). Many of these predators become regarded as dangerous pests, despite the fact that, historically, they serve a valuable role in keeping other problematic populations in check (Sillero-Zubiri and Laurenson, 2001). Predators, such as mountain lions, wolves and coyotes, keep white-tailed deer populations from exploding in areas where their ranges overlap with that of the deer (Jędrzejewski, et al., 2002). Pit vipers help keep small rodent populations checked (Bouskila, 1995). Yet, when a human encounters one of these animals, one or both often leave the encounter damaged or dead. The primary goal of this study is to determine a relationship between morphological characteristics of Prairie Rattlesnakes, *Crotalus viridis*, and current game management practices in an effort to streamline more efficient use of resources to protect and manage the species. Another goal of this study is to compile diet data for comparison to data sets within the primary literature from populations of *C. viridis* throughout the United States and Canada.

Prairie Rattlesnake ranges overlap those of several rodent species regarded as pests by humans (Fogell, 2010; Reid, 2006; Kays and Wilson, 2009). Rattlesnakes are beneficial to the ecosystem because they exert a measure of population control over pest species such as rats, mice and rabbits, as well as limiting the quantity and scope of diseases spread by these prey species (Bouskila, 1995). Deer mice (*Peromyscus* spp), in particular, are reservoirs for a number of diseases that affect humans such as Hantavirus (Centers for Disease Control (CDC), 2016), Ehrlichiosis and Babesiosis (Cronin, 2014). *Peromyscus*

species are a major part of the diet of many *Crotalus* species, including *C. viridis* (Fitch, 1998). Control of these pest species can be aided by conserving the known predators of the species (Collins, et al., 2010; Fogell, 2010).

The morphological characteristic data examined here, taken individually, are of interest, generally speaking, primarily to herpetologists. When considered as a whole, these data can provide insight to game managers, ecologists, conservation biologists and wildlife educators on how to achieve a better understanding of the species and, perhaps, find ways for humans to better coexist with rattlesnakes.

Education is the key in developing and implementing strategies to manage these dangerous species in such a way that humans can live alongside them with neither species causing undue harm to the other (Treves and Karanth, 2003). Understanding the dynamics of rattlesnake populations in a given area can help us understand how to manage these species by illustrating the where and how of management principles and their applications (Weir, 1992). To that effect, this study aims to examine current management practices centered on *C. viridis* throughout their range in order to determine whether standard field techniques should be altered in any way.

In Hays, Kansas, a preserved collection of approximately 200 *C. viridis* specimens is housed in the Sternberg Museum of Natural History at Fort Hays State University. These specimens were collected over three consecutive years to facilitate a safe working environment for a prairie restoration project. As long distance relocation of crotalids is being identified as detrimental to the snakes (Brown, et al., 2009) and many wild populations are currently at carrying capacity (Brennan and Tischendorf, 2004), these

snakes were euthanized and preserved in a museum collection so they could be used in future studies. (Dan Fogell, pers. comm., 2015).

While data on adult *C. viridis* are abundant and readily available, no other study currently available has examined nearly an entire population (≤ 200 individuals) from a single area collected over a short active period. There are records of many large individuals collected from single populations, as in rattlesnake round-ups (Fitch, 1998; Schmidt, 2002), but there are no collections currently available for study that are quite as comprehensive. This novel collection is particularly valuable from an ecological standpoint in that it represents all the life stages of a single population. Data from such a population can indicate reproduction and survival rates.

In an effort to establish protocols for identifying the sex and age of a snake in the field without subjecting the snake to invasive procedures, morphometric measurements, such as snout-vent length (SVL) and tail length (TL) along with the number of dorsal saddle patterns and subcaudal scales were analyzed to determine sexual dimorphism. Data from this study can be applied to game management strategies for this species throughout their natural range. These analyses will assist in producing life history tables and help determine what, if any, field protocols should be changed by managers of the species.

Several studies have been undertaken using museum specimens of various species of *Crotalus* to determine diet preference (Clark, 2002), reproductive stage (Aldridge, 1979; Aldridge, 2002; Aldridge and Brown, 1995; Schmidt, 2002), and body size (Ashton, 2001; Schmidt, 2002). In addition, all of these studies collected data on life history stages present in the collection. The data sets from the above mentioned studies will be used as

references to compare data collected on a preserved population from Hays, Kansas in order to determine a baseline for any populations repatriating the collection area.

CHAPTER I. MORPHOLOGY

Introduction

Gross morphology of snake species is common with little variation between populations of conspecifics (Gannon and Secoy, 1984). There are certain characteristics which allow anyone with the proper training to tell the difference between two species. For example, a Prairie Rattlesnake only resembles a garter snake in form and propulsion. The differences between the two species are small but important. Prairie Rattlesnakes are pit vipers and have the adaptations common to all pit vipers, such as heat-sensing pits below the eyes, arrowhead-shaped heads, and venom, produced in the venom glands and delivered by two long, needle-like front fangs (Collins, et al., 2010; Fogell, 2010). Identification of species is only the initial step in studying snakes.

Morphological characteristics of snakes are commonly measured using mass, snout-vent length (SVL) and tail length (TL). I wanted to determine whether the TL is a significant measurement that should be added to the standard field protocols. This study also looked for statistically significant differences in dorsal saddle patterns and subcaudal scale counts between the sexes and between life stages within a population.

Current standard field methods for determining sex of a snake in the wild can be invasive and potentially painful for snakes. These methods include cloacal probing for presence/absence of hemipenes (Dellinger and von Hegel, 1990) and/or forceful expulsion of hemipenes by squeezing the tail and cloaca (Gnudi, et al., 2009). A skilled herpetologist can perform the more invasive methods without causing undue harm to the snake while maintaining contact for a minimal amount of time. Unfortunately, not everyone who handles Prairie Rattlesnakes is a skilled herpetologist. This study aimed to

determine whether there are less invasive methods of determining sex in the field using morphological characteristics including SVL, TL, saddle pattern and subcaudal scale counts.

Scientists and game managers in the field can use gross morphology to identify different individuals of some species. Humans have unique fingerprints. Some species of whale can be identified to individual by the patterns and scars on their fluke (Dufault and Whitehead, 1995). I attempted to determine whether Prairie Rattlesnakes can be identified in the same manner, using dorsal saddle patterns as means of identification. I wanted to determine whether the number of saddle patterns changes across life stages and whether these patterns can be used as a method of identifying individuals.

Materials and Methods

Measurements

Snout-vent length (SVL) and tail length (TL) are commonly collected measurements (Rivas, et al., 2008) and were obtained using a flexible cloth measuring tape, as was rattle length (RL). The condition of the rattle, the number of rattle segments for each snake and the presence/absence of the rattle button were noted. Mass was not recorded due to desiccation during the preservation process.

Snout-to-vent length, a common measurement utilized in snake studies, is used to aid in determination of the overall health and approximate age of a snake. According to Fitch (1998), SVL should correlate directly with the number of rattle segments on the tail.

However, rattles are often fragile and can be damaged or have segments broken off during the course of natural movement through the snake's environment.

Tail length (TL), another common measurement for wild-caught snakes, is a useful measurement to aid in a visual assessment of sex. Females typically have short, fat tails, whereas males have long, slender tails (Shine, et al., 1999). It has been suggested that males have longer tails to accommodate larger hemipenes, thereby increasing reproductive success (King, 1989).

Patterns and Scale Counts

The dorsal pattern of saddles and cross-hatches is unique to *C. viridis* and grows with the snake. I wanted to determine whether the patterning is also unique to individuals, as in human fingerprints, thereby reducing the need for field methods such as scale clipping or Passive Integrated Transponder (PIT) injection. Scale clipping is a temporary identifier because the scales will regrow with each successive ecdysis (Elbin and Burger, 1994). PIT injection is efficient in that it is a "one and done" method and a snake can be identified years later (Elbin and Burger, 1994). However, injection of PITs can potentially cause discomfort for the snake. To that end, I wanted to determine whether the number of dorsal saddles changes across life stages, simplifying field protocols and potentially identifying this as preliminary data for further study.

In an effort to develop protocols where a snake is not subjected to invasive or painful procedures, I wanted to determine whether there is a correlation between the number of saddles in the dorsal pattern and sex of the snake. Digital photos were taken of each

individual prior to measurement using a Fujifilm FinePix S4830 digital camera. Saddle patterns from neck to vent were counted and recorded from these photos.

The number of subcaudal scales varies between individuals. I wanted to determine whether there is evidence of sexual dimorphism in the number of subcaudal scales between males and females of the same life stage. This could be a visible indicator of sex to add to the standard field protocols that can reduce the need for invasive procedures. Subcaudal scales were counted using a dissecting probe to keep accurate count.

Sex and Life History Stage

Life History can be broken down into adult, juvenile and neonate categories. Adult *C. viridis* were classified as in Diller and Wallace (1984) as males >520 mm SVL with 4 or more rattle segments and females >550 mm SVL with 5 or more rattles. Juveniles were defined as <520 mm SVL and/or <4 rattle segments for males and <550 mm SVL and/or <5 rattle segments for females. Neonates were defined as ≤ 260 mm SVL for both sexes with only a button rattle, indicating the individual has yet to shed for the first time. Sex of each individual was verified using anatomical characteristics. Females were further divided into gravid and non-gravid categories.

Statistics

Descriptive statistics (Whitlock and Schluter, 2009) were calculated using Minitab[®] (2010). ANOVA (Whitlock and Schluter, 2009) was used to compare SVL and TL between sexes and life stages. These analyses were carried out using PROC GLM in SAS

(version 9.4 (c) 2002-2012 by SAS Institute Inc., Cary, NC, USA). To compare subcaudal scale number between sexes and life stages, a logistic regression (Whitlock and Schluter, 2009) was carried out using the Genmod procedure in SAS software (version 9.4 (c) 2002-2012 by SAS Institute Inc., Cary, NC, USA). PROC CORR in SAS was used in order to determine the correlations (Whitlock and Schluter, 2009) between SVL length, tail length, subcaudal scale number, and rattle length.

Results

Of the 199 *C. viridis* specimens collected, 16 were omitted from this study due to lack of collection data. Most individuals of the population were adults, almost a third of the individuals were neonates while juveniles were rare (Table 1.1; 1.2, Figure 1.1; 1.2).

Table 1.1. Life stage totals of *C. viridis* population from Ulysses, Kansas.

Life Stage	Male	Female	Total
Adult	52 (28%)	63 (34%) (34 Gravid, 29 Non-Gravid)	115 (63%)
Juvenile	9 (5%)	5 (3%)	14 (8%)
Neonate	34 (19%)	20 (11%)	54 (30%)
Total	95 (52%)	88 (48%)	183 (100%)

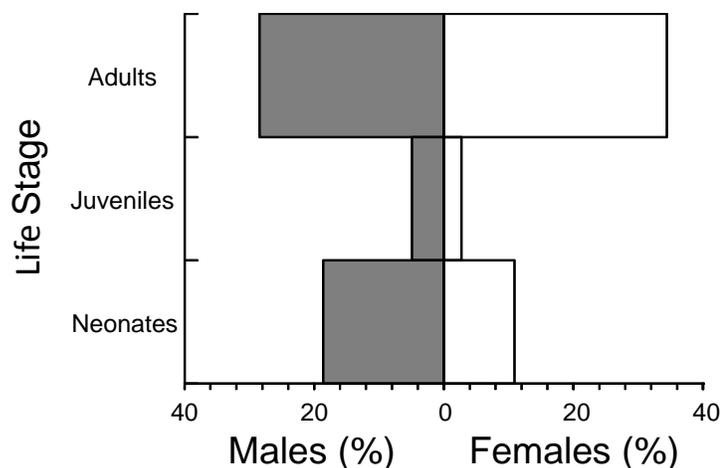


Figure 1.1. Population pyramid for *C. viridis* population from Ulysses, Kansas. N= 183.

SVL, TL, and RL were highly correlated (Table 1.2). Number of subcaudal scales showed a moderate correlation with tail length, a weak correlation with SVL, no correlation with rattle length. Number of dorsal saddles was not correlated with any of the other morphological characteristics.

Table 1.2. Correlations between snout-vent length (SVL), tail length (TL), rattle length (RL), and number of subcaudal scales (SS) and dorsal saddles (DS) in a population of *C. viridis*. Below the diagonal, correlation coefficient and, in parenthesis, P-values are given; above the diagonal sample size is shown.

	SVL	TL	RL	SS	DS
SVL	1	152	136	180	150
TL	0.87 (< 0.0001)	1	136	150	150
RL	0.89 (< 0.0001)	0.75 (< 0.001)	1	134	134
SS	0.17 (0.03)	0.47 (< 0.001)	0.06 (0.48)	1	148
DS	0.03 (0.71)	-0.09 (0.27)	-0.00 (0.99)	-0.14 (0.08)	1

SVL, TL and RL increased as life stages progressed, while the number of subcaudal scales and saddles did not change with life stage (Table 1.3 and 1.4; Figure 1.2). SVL and RL did not differ between males and females for all stages. TL, however, increased more in males than in females, as life stages progressed in *C. viridis* (Table 1.3 and 1.4, Figure 1.2) Neonates all exhibited similar tail length. Juvenile males had slightly larger tail length than juvenile females. In adults, females in this population exhibited shorter, fatter tails ($\approx 7\%$ SVL) and males presented thinner, longer tails ($\approx 10\%$ SVL). The number of subcaudal scales was greater in males than female (Table 1.3 and 1.4, Figure 1.2), but the number of dorsal saddles did not differ between males and females (Table 1.3 and 1.4, Figure 1.2)

Table 1.3. Descriptive statistics (mean, SE, n) of SVL, TL, RL and number of subcaudal scales and dorsal saddles within a population of *C. viridis*.

	SVL	TL	RL	Number of subcaudal scales	Number of dorsal saddles
<i>Adults</i>					
Males	709.8 \pm 17.6 (52)	61.3 \pm 2.1 (52)	35.5 \pm 1.3 (50)	25.0 \pm 0.4 (52)	42.0 \pm 0.5 (50)
Females	707.2 \pm 8.7 (63)	47.7 \pm 0.8 (63)	34.8 \pm 0.9 (63)	20.8 \pm 0.2 (61)	43.1 \pm 0.4 (63)
<i>Juveniles</i>					
Males	367.8 \pm 38.9 (9)	33.0 \pm 3.3 (9)	14.3 \pm 2.8 (9)	24.1 \pm 0.9 (9)	42.0 \pm 1.5 (9)
Females	358.8 \pm 56.1 (6)	28.2 \pm 4.1 (6)	13.2 \pm 4.4 (5)	21.2 \pm 0.7 (6)	39.5 \pm 1.1 (6)
<i>Neonates</i>					
Males	244.1 \pm 2.0 (33)	22.1 \pm 0.9 (15)	4.9 \pm 0.3 (97)	22.1 \pm 0.6 (34)	42.5 \pm 1.2 (15)
Females	242.7 \pm 2.5 (19)	23.1 \pm 1.8 (7)	5.0 \pm 0.0 (2)	21.3 \pm 0.7 (19)	42.9 \pm 1.3 (7)

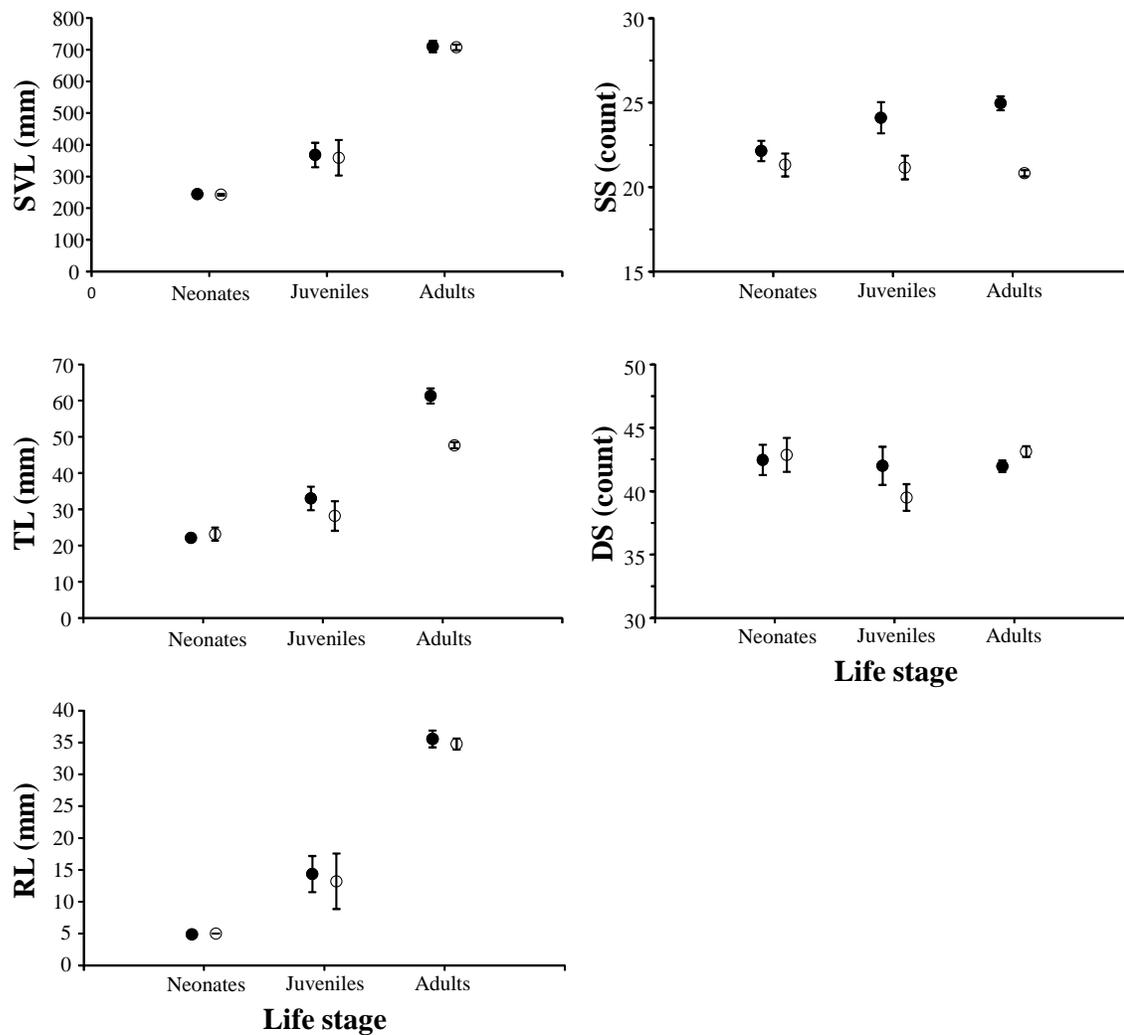


Figure 1.2. Snout-vent length (SVL), tail length (TL), rattle length (RL), and number of subcaudal scales (SS) and dorsal saddles (DS) for each life stage and sex of a population of *C. viridis*. Means and SE are shown. Males are indicated by black circles; females indicated by open circles.

Table 1.4. Effect of sex and life stage on SVL, TL, RL and number of subcaudal scales and dorsal saddles within a population of *C. viridis*.

<i>SVL</i>	<i>d.f.</i>	<i>F</i>	<i>P</i>
Life stage	2, 176	524.58	< 0.0001
Sex	1, 176	0.06	0.81
Life stage × Sex	2, 176	0.01	0.99
 <i>TL</i>	 <i>d.f.</i>	 <i>F</i>	 <i>P</i>
Life stage	2, 146	97.48	< 0.0001
Sex	1, 146	5.26	0.02
Life stage × Sex	2, 146	4.68	0.01
 <i>RL</i>	 <i>d.f.</i>	 <i>F</i>	 <i>P</i>
Life stage	2, 130	78.46	< 0.0001
Sex	1, 130	0.05	0.82
Life stage × Sex	2, 130	0.02	0.99
 <i>Number of subcaudal scales</i>	 <i>d.f.</i>	 χ^2	 <i>P</i>
Life stage	2	1.73	0.42
Sex	1	6.87	0.009
Life stage × Sex	2	3.84	0.15
 <i>Number of dorsal saddles</i>	 <i>d.f.</i>	 χ^2	 <i>P</i>
Life stage	2	1.02	0.60
Sex	1	0.05	0.83
Life stage × Sex	2	1.04	0.60

Discussion

From an ecological perspective, I expected to see more neonates than juveniles and more juveniles than adults. I did not expect such a disparity between the number of juveniles compared to the number of adults. Demographic data are biased, as Fitch (1998) notes, due to the lack of immature juveniles and neonates present in the sample. The data on the population in this study are very similar.

Fitch (1998) observed a population of 70 live Prairie Rattlesnakes from the southwestern region of Kansas, near Sharon Springs, Kansas, though he claims the sample is still distorted due to a lack of immature snakes from the population (29 first and second years). Ulysses, Kansas and Sharon Springs, Kansas are only 150 km away from each other. Spatial autocorrelation suggests the populations will not differ significantly in most aspects. (See Table 1.5)

These results indicate several possibilities. 1.) Juveniles are emigrating from natal areas into other ranges. 2.) Juveniles have a higher mortality rate than in other populations and adults are immigrating from elsewhere. 3.) Juveniles remain undetected due to different microhabitat usage. Without perusing the original collection site, I can only speculate that there is different habitat usage going on in the environment, allowing the surviving juveniles to go undetected and therefore, uncollected.

Tail length in this population could act as a proxy for current field methods used to determine sex of adults. Used in conjunction with other reliable methods, such as palpation of the ventral area of a snake to determine gravidity, tail length is a viable alternative to the more invasive methods currently in use.

Fitch (1985) provides a detailed description of the anatomy of the rattle of a rattlesnake, including his own findings that the size of each rattle segment is proportional to the size of the snake at the time of ecdysis. Rattle segments can also be used to determine an approximation of health and resource abundance in a given year in the life of the snake (Martin, 1993). Like tree rings, rattle segments are wide and robust during a growing season in which food is abundant and the snake is healthy. During lean seasons or, in the

case of breeding females, the rings are thin and narrower, indicating a smaller resource allocation for growth and development of the individual (Fitch, 1985).

Conclusions

This study attempted to determine whether or not there are less invasive methods of determining sex in the field using morphological characteristics including SVL, TL, saddle pattern, subcaudal scale counts, rattle length and rattle condition. Taken individually, none of the morphological characteristics examined indicate that any changes in field protocols are needed. Taken collectively, tail length and subcaudal scale counts should be added to the standard field protocols to reduce or eliminate the need for invasive and potentially painful procedures currently in use for determining the sex of an adult *C. viridis*.

While the shape and size of each saddle superficially appears to be unique, there is simply no way this study can determine whether the patterns can be used to identify individuals in the field. I recommend a future study collecting mark-recapture data and photographs of individuals included in the study over several growing seasons to determine the viability of using saddle patterns as a method of identifying unique individuals.

CHAPTER II. DIET

Introduction

Diet composition within populations of *Crotalus viridis* can provide researchers with information on prey preference and relative prey abundance in a study area, as well as identify ontogenic shifts associated with growth and development of individuals.

Comparison of diet composition between populations of conspecifics throughout the range of the species can be accomplished with the use of data reported in the primary herpetological literature. These data comparisons are important to game managers developing and implementing management strategies for populations of *C. viridis* or their prey.

Several studies have looked at diet composition in populations of *C. viridis* and other crotalid species. The majority of diet preferences include small mammals with some geographic variation in composition (Holycross, 1993; Fitch, 1998). There is a possibility that populations in each range have adapted, evolutionarily (Palkovacs and Post, 2008), to prey on more abundant prey items in each range, thus explaining the significant differences of prey items found in each range sample. *C. viridis* are generalist predators that tend to utilize the most abundant prey species within the area (Holycross, 1993).

Dorsal guard hairs are unique to species (Moore, et al., 1974). They are largely indigestible to snakes and can be used as a proxy for determining local prey abundances as well as identify ontogenic shifts in prey preferences (Clark, 2002). A number of characteristics are useful in identifying dorsal guard hair. These include basal configuration; color; color band and location; cortex; medullary configurations; shield

configurations; scale patterns and margins, and hair strictures (Moore, et al., 1974; Holycross, 1993).

I wanted to establish what and when the Ulysses, Kansas population of *C. viridis* was eating in order to compare the data with those previously reported in the primary herpetological literature. I found little overall difference in the diet composition of this population compared to others along similar latitudes. However, the data comparison from other latitudes indicates geographic shifts in prey preference, supporting the idea that *C. viridis* are generalist predators.

Materials and Methods

Dissection

Standard dissection techniques were used (Smith and Schenk, 2014). Liver samples were removed and preserved in 95% ethanol for use in genetic analyses in future studies. The stomach and intestines of each individual were dissected and examined for indigestible mammalian guard hairs and/or larger prey items. The stomach of each snake was dissected to determine presence/absence of prey items. Prey items were either visually identified to lowest taxonomic level possible from the stomach contents or through indigestible guard hairs of prey items collected from intestinal samples (Moore, et al., 1974).

When a whole or nearly whole prey item was found in the stomach of a snake, it was fixed in 10% neutral buffered formalin and stored in 70% isopropanol until it could be identified using body measurements, skull and bone characteristics when available and hair samples. If only guard hairs were found, they were allowed to air dry and were

stored in petri dishes marked with an alpha-numeric code associated with the individual from which the sample was removed for later identification. Location of prey in the digestive tract indicated the timeframe in which the snake had consumed the prey. If prey and guard hairs were found in two different locations in the digestive tract, without continuity, it was assumed to be two different prey items and identified accordingly. Prey items were noted for frequency and abundance by occurrence for comparison to data sets from other studies.

I used the method outlined by Wallace and Diller (1990) to estimate time between feeding and capture of the Ulysses, Kansas population of *C. viridis*. Snakes were estimated to have fed depending on the location of prey remains within the snake. If a prey item was located in the stomach, feeding was estimated to have occurred within one day of capture. If a prey item was located in the small intestine, the snake was estimated to have fed 3 days prior to capture. If it was located in the large intestine, the snake was estimated to have fed 4 days prior. If scat was palpated out in the lab, the snake was estimated to have fed only 7 days prior to capture.

Prey Identification

Often, skull characteristics could not be used in prey identification because the snake had ingested the prey head first and the head was the first part of the body dissolved by the various digestive fluids encountered. In this case, the hind feet and tail were examined for most identifications using current guidebooks on North American mammals (Reid, 2006; Kays and Wilson, 2009). Any remaining prey items were identified using techniques adapted from Moore, et al. (1974).

Hairs were isolated from stomach and intestinal samples dissected from individual snakes and cleared of natural oils and debris in xylene for approximately one hour each. Hairs were placed on a glass slide marked with a number-letter combination unique to each individual and examined at 4x, 10x, and 40x magnification using a light microscope (Leica™). Characteristics visible at 4x magnification were hair strictures and length of hairs. At 10x magnification, color bands and basal configuration could be identified. Medullary configurations and scale patterns were not evident until they were examined under 40x magnification.

If a scale pattern could not be seen clearly and if identification relied solely on the scale pattern, a scale cast was made using techniques modified from the forensic website Identification of Human and Animal Hair (Accessed 3/18/16). Scale casts were created by brushing a thin layer of clear nail polish (Sally Hansen-Hard as Nails™) onto a clean glass slide and placing a hair sample onto the polish. Once the polish was almost dry (tacky), the hair was pulled quickly off the polish, leaving an imprint of the scale pattern which could then be examined using light microscopy.

Results

A timeline for eating was established by Diller and Wallace (1990) and adapted for this study. If food items were found only in the stomach, the snake had eaten within one day of capture. If food items were found in the large intestine, the snake had eaten within 4-6 days of capture. Of the 183 snakes examined, 47 had prey items in the stomach or intestines. Sixteen of the 46 had identifiable animals in the stomach. Four had

feathers in stomach and intestines. The remaining 26 individuals had only hairs and bone fragments in the intestines. (See Appendix Diet Section Results)

Seventeen snakes fed within 24 hours of capture. Seven snakes had eaten within 2-3 days of capture, suggested by presence of hair and no identifiable bones or whole body parts (e.g., tails, feet) in the stomach. The remaining 23 snakes contained only hairs in the small and large intestines, indicating they had eaten within 4-6 days of capture.

Sixteen males, 15 gravid females and 16 non-gravid females had consumed prey items. A single male and a single gravid female had 2 different prey items in different locations within the digestive tract. A single non-gravid female had 3 individual murids in her stomach, suggesting she found a nest and consumed its occupants.

The majority (90.2%) of prey items found in the Ulysses, Kansas population were small mammals; 7.8% were birds and a single lizard accounted for 1.9% of the diet in this population (Table 2.1, Figure 2.1).

The majority of prey items, especially the small mammals, were consumed in spring between egress from hibernacula and mating in early summer (Figure 2.2). Four snakes (8.5%) had eaten in April; 34 snakes (72.3%) had eaten in May and June; 3 snakes (6.4%) had eaten in July; 3 snakes (6.4%) had eaten in August; 1 snake (2.1%) had eaten in September. Two snakes with prey items had no capture date associated with them.

Table 2.1. Identification, frequency and percent occurrence of prey items found during dissection of a population of *C. viridis*.

Prey item	Frequency	
	(count)	(%)
Aves	4	7.8
Reptilia		
<i>Plestiodon obsoletus</i>	1	1.9
Mammalia		
Rodentia		
<i>Microtus</i>	6	11.8
Muridae	3	5.9
<i>Onchomys</i>	1	1.9
<i>Perognathus</i>	3	5.9
<i>Peromyscus</i>	14	27.5
<i>Chaetodipus</i>	1	1.9
<i>Spermophilus</i>	1	1.9
Lagomorpha		
<i>Silvilagus</i>	9	17.6
Insectivora		
Soricidae*	4	7.8

*The Kansas samples were similar to Soricidae found in Wyoming but the medullary configurations and scale patterns were slightly different, with very little crenation.

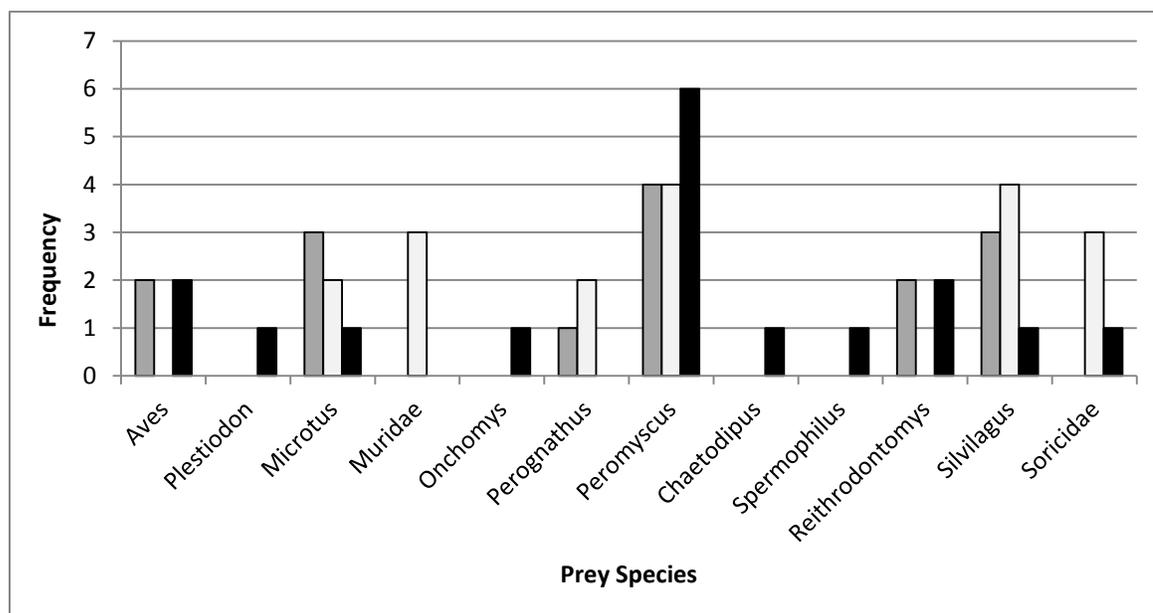


Figure 2.1. Frequency of prey species eaten by gravid females, non-gravid females and males in a population of prairie rattlesnakes (*Crotalus viridis*) near Ulysses, Kansas. Blue: Gravid females, Dark gray: Non-gravid females; Light gray: Males; Black

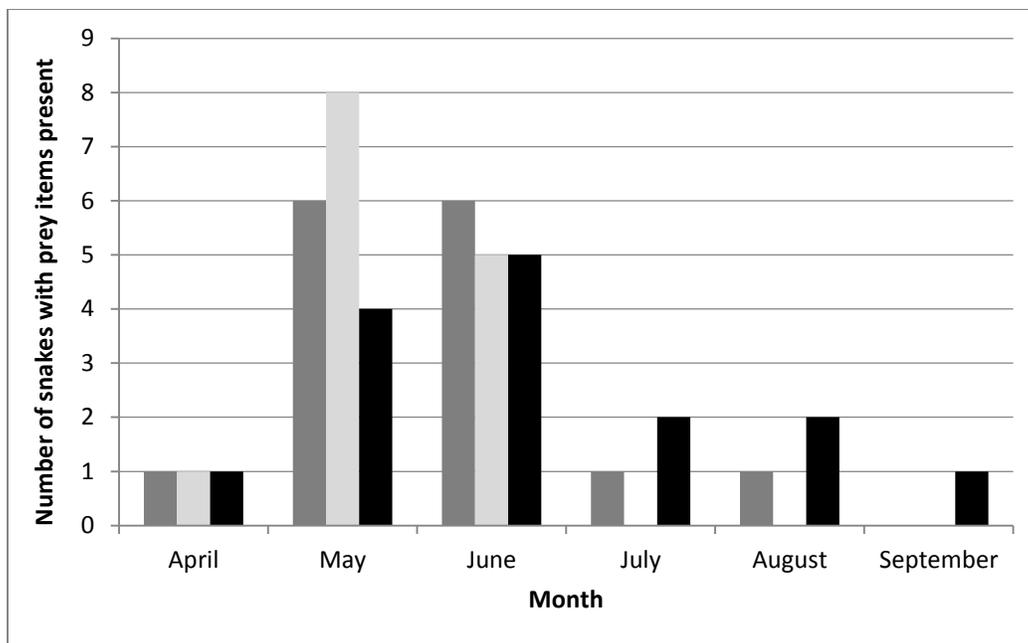


Figure 2.2. Frequency of prey items consumed by month for gravid females, non-gravid females and males in a population of prairie rattlesnakes (*Crotalus viridis*) near Ulysses, Kansas. Dark gray: gravid females; light gray: non-gravid females; black: males.

Discussion

Diet preferences in populations in British Columbia were similar in composition to those of populations in California (MacArtney, 1989; Fitch, 1998). The major difference between the two populations' diets was the presence of lizards in the California population diet (Fitch, 1998). Lizard species distribution at high latitudes such as Idaho or British Columbia are patchy (Stebbins, 2003), therefore they are not prevalent in the diet (Wallace and Diller, 1990). Conversely, the population reported in British Columbia consumed muskrat and several species of sparrow that are not typically found in California (MacArtney, 1989).

Wallace and Diller (1990) examined prey item remains from 104 *Crotalus viridis oregonus* over the course of nine years in northern Idaho. They found that juvenile *C. v.*

oreganus consumed shrews exclusively. Adult diets consisted of larger species of mice, voles, rabbits, and occasionally, a bird or lizard. In more southerly populations, juveniles tend to consume more lizards and an occasional amphibian.

MacArtney (1989) used data obtained from a prior 3-year mark-recapture study of *Crotalus viridis oreganus* in British Columbia to analyze diet preferences. All results for the population sampled in British Columbia were then compared to a population study published by Fitch and Twining (1946). Small mammals and some birds were identified as prey items in MacArtney's (1989) study. Prey items found in adults were more wide-ranging, though species typically consumed by juveniles were still found in great numbers. Larger rodents and birds were also included in adult diets. The only ontogenic shift in prey preference was related directly to size. Larger snakes exhibited preferential behavior in favor of larger prey items on occasion. Similar to the current study, deer mice were the preferred prey item over every age range. Knowing that mice reproduce and mature rapidly suggests that this is an abundant prey species and opportunity to feed on individuals of this species occurs often.

A few members of the family Soricidae are found in Kansas that are not present in Wyoming. Hairs from these species are not part of the key published by Moore, et al. (1974). While members of the family Soricidae are present in Wyoming, the medullary configurations and the scale patterns were different enough from those species present in the Kansas populations that, although I feel confident my samples are shrews of some species, I cannot identify them with any shrew species listed in Moore, et al. (1974). Species presence in Kansas was verified using Mammals of North America field guides (Kays and Wilson, 2009; Reid, 2006)

Prey items from each population of *Crotalus horridus* studied by Clark (2002) varied significantly. As expected, adults ate prey larger and faster than the prey of sub-adults and juveniles. Large snakes did not eliminate small prey from their diets as they grew, but included them along with larger prey items. Graves and Duvall (1993) discussed this with the idea that gravid female *C. viridis* did not cease eating while gravid, they simply captured prey items as opportunities to do so presented themselves, rather than actively foraging. Gravid females in the Ulysses, Kansas population did not cease eating, supporting the suggestion of opportunistic feeding among gravid females in previous studies.

Typically, male and non-gravid female *C. viridis* migrate greater distances from their hibernacula than gravid females (Graves and Duvall, 1993). Gravid females aggregate in rookeries where they do not engage in the same kinds of feeding behaviors and patterns that males and non-gravid females use. Gravid females are much more sedentary, using energy for the development of offspring, rather than in foraging and mating.

In this study population, a number of rodent species present in the ecosystem are utilized as prey. The majority of prey items were small mammals, though a small percentage of prey items present in this population consisted of birds and a single lizard. The general size of large adults that had consumed *Silvilagus* prey indicated an ontogenic shift toward larger and faster prey, as expected. This result is not unusual when gape limitation is considered. One would not expect a neonate or juvenile with a relatively small gape size to prey on a large rabbit.

The number of gravid females with prey items present in their systems was surprising, given the previous assumptions that they do not actively forage for food. Nearly 45% of

all gravid females had prey items present in their digestive systems. Of these, 20% had *Silvilagus* prey in their systems. These results indicate that gravid females may share microhabitat preferences with an abundance of small and large prey species, thereby maximizing the number of interactions with prey items while minimizing energy consumed in typical foraging behavior.

Conclusions

Game managers, conservation biologists and wildlife educators can utilize the information presented here to preserve the habitat used by gravid female *C. viridis* and their preferred prey items in an effort to stabilize population levels and increase or decrease the carrying capacity of the ecosystem as needed.

The prey most frequently encountered in this population was *Peromyscus*, previously identified as being a reservoir for numerous diseases that can affect humans. *C. viridis* has been identified as a species that can actively assist in controlling the potential spread of disease prevalent in ecosystems with an overabundance of *Peromyscus* species present (Diller and Johnson, 1988). This supports the idea that protection and conservation of local *C. viridis* populations will, in turn, protect local human populations from widespread disease.

CHAPTER III: REPRODUCTIVE BIOLOGY

Introduction

Knowing when and how often venomous species reproduce can help game managers understand what to look for in assuring public safety in places such as state and national parks, where these species are often found. This study attempted to determine when and how often the Ulysses, Kansas population reproduced.

Fitch (1998) determined that female *Crotalus viridis* in latitudes such as those found around Sharon Springs, Kansas and his study in California more often (80% of females in the roundup) exhibited annual reproduction. This was attributed to the size and abundance of available prey items. That study illustrated the differences in the mean number of eggs present in groups categorized by SVL. Studies by Rahn (1942) and MacArtney and Gregory (1988) determined a biennial or triennial reproductive cycle in *C. viridis* populations in northern latitudes. Sharon Springs, Kansas is approximately 150 km from the collection site of the current study near Ulysses, Kansas. Spatial autocorrelation suggests there will be little variation between the two populations in most characteristics.

In gravid female *C. viridis*, fat stores have been noted as essential in whether parturition is reached or the embryos are expelled or resorbed (Graves and Duvall, 1993). Larger, more robust females achieve parturition more often than smaller females (Reading, 2004). Successful spring foraging conditions lead to more successful births. Graves and Duvall (1993) indicate that snakes are incredibly adaptable and respond to environmental stressors quickly, even if that means terminating pregnancy. A female is capable of

resorbing follicular material to gain energy which can be used for another reproductive attempt under more favorable conditions.

Although female *C. viridis* invest a great deal more energy and effort in the production of offspring, the investment of males is also considerable when viewed from morphological and behavioral perspectives (Duvall and Schuett, 1997). Males must have morphological preparations in place before mating can occur (Aldridge, 2002; Aldridge and Brown, 1995), including a ready supply of sperm, likely generated the previous year, stored in the vas deferens (Aldridge, 2002; Diller and Wallace, 1984). The sexual segment of the male kidney secretes the basic material that forms the copulatory plug (Devine, 1975). Adult males can store sperm for long periods in the vas deferens, exhibiting a near continuous production of sperm annually (Almeida-Santos, et al., 2004).

Selective pressures dictate reproduction occurs when it is most advantageous to the survival of the offspring (Vitt and Blackburn, 1983). Both male and female crotalids can store sperm for extended periods (Aldridge, 1979; Vitt and Blackburn, 1983; Gardner-Santana and Beaupre, 2009) therefore; periods of mating cycles are regularly independent of production of sperm and eggs. Mating takes place opportunistically in early summer, though fertilization may not occur until the following spring. Once fertilized, gestation generally lasts around 3 months, ending with the live-birth of several offspring early in the fall (Fogell, 2010).

Materials and Methods

During dissection of each individual, the sex and reproductive stage of the snake were determined using techniques described by Aldridge (1979). Sexual maturity was determined by SVL, number of rattle segments, and follicle size in females as in previous studies (Aldridge, 1979; Aldridge and Brown, 1995).

Testes and oviducts were removed, fixed in 10% neutral-buffered formalin and stored in 70% ethanol for histological analysis. These samples were then delivered to the UNMC-Tissue Sciences Facility for paraffin embedding, cutting and staining using Leica™ Surgipath Select Tech Hematoxylin 560 MX and Leica™ Surgipath Select Tech Alcoholic Eosin Y515. I examined oviducts removed from 13 mature females for yolked eggs. Testes from 28 mature males were also examined to observe the progression of spermatogenesis.

Developing eggs from gravid females were removed during dissection, fixed in 10% neutral buffered formalin and stored in 70% isopropanol until they could be analyzed. The number of eggs per snake was noted to find the mean clutch size within the population. The length of each egg was measured with a flexible cloth measuring tape to determine mean egg length per clutch.

Results

Within this population of *C. viridis*, 18.5% of the population consists of gravid females; of all females present, 38% were gravid. Mean clutch size= 8.3 ± 2.9 (N = 30); mean ova length per clutch= 30.1 ± 7.2 mm (N= 30) (Figure 3.1; 3.2). Mean SVL of all gravid females= 709 ± 70.3 mm (N=34). Because females can store sperm for long periods (Almeida-Santos, et al., 2004), there is no way to determine a timeline for mating in this population with a high degree of certainty. I was also unable to determine whether reproduction is annual, biennial, etc. due to incomplete capture data.

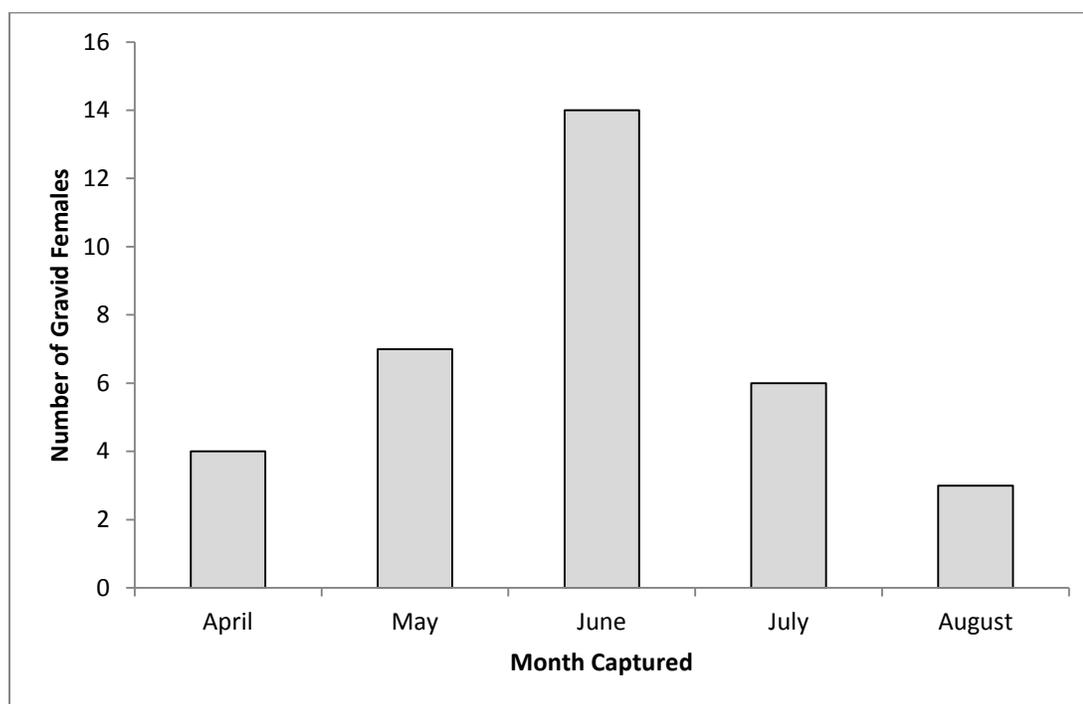


Figure 3.1. Number of gravid females vs. month captured from 2012-2015 in a population of Prairie Rattlesnakes (*Crotalus viridis*) near Ulysses, Kansas.



Figure 3.2. Full clutch of 11 (4 from the left oviduct; 7 from the right) *Crotalus viridis* embryos surrounded by a translucent membrane (indicated by the red arrows). The female (691mm SVL) carrying this clutch was likely only hours from parturition when captured August 25, 2015.



Figure 3.3. *Crotalus viridis* ova in the process of resorption. Note the hole in the far right ovum indicated by the red arrow.

Of the 28 male gonads examined, only two exhibited various stages of spermatogenesis (Figure 3.5), the rest having suffered severe degradation in the preservation process. Seven of the 13 females examined exhibited some stage of ovarian follicular development (Figure 3.3, 3.4).



Figure 3.4. An example of an immature follicle at 20x magnification.

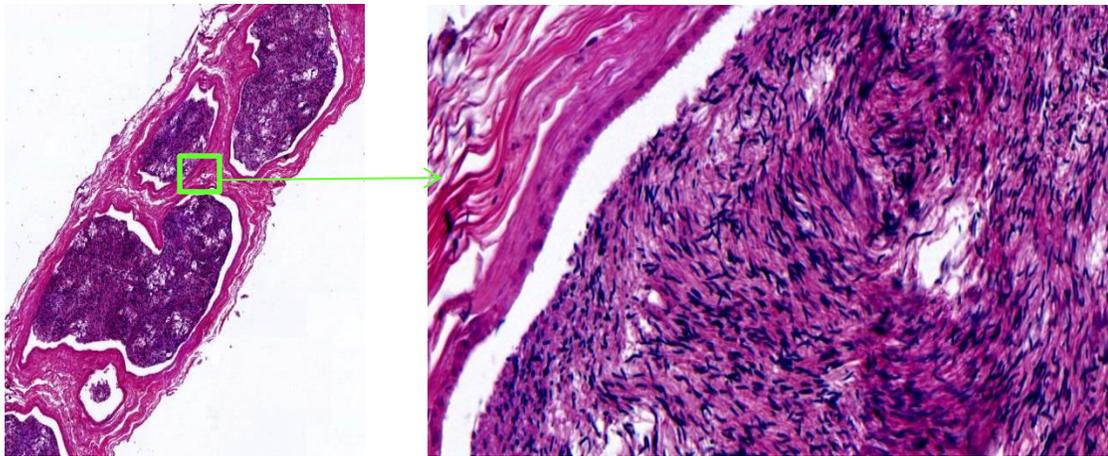


Figure 3.5. (At Left) Longitudinal section of the epididymis at 10x magnification. (At Right) 40x magnification of area indicated by the green box. Note the presence of sperm throughout the tubule.

Discussion

Adult females in this population exhibit a 1.6:1 non-gravid to gravid ratio, indicating an annual to biennial trend in reproduction. By combining these data with data from other studies on similar populations, a distinct latitudinal gradient of reproduction becomes evident. Along the southern latitudes of the species range of *C. viridis*, reproduction occurs annually (Fitch, 1998). As one travels north throughout the range of the species, reproduction becomes more delayed, occurring only bi- or even triennially (MacArtney and Gregory, 1988). This is explained in the primary literature as attributable to shorter photoperiods and active seasons in northern latitudes (MacArtney and Gregory, 1988; MacArtney, et al., 1990).

A certain level of parental care by female *Crotalus* has previously been reported in the primary literature (Holycross, 1993; Greene, et al., 2002), indicating that females remain with the litter until after the offspring complete their first ecdysis. Parental care behaviors have been identified in other reptile species, including the Prairie Skink (*Eumeces septentrionalis*) (Somma and Fawcett, 1989).

It should be noted here that the snakes in this collection were initially preserved by ultra-rapid freezing (-80°F) and were subsequently stored in a standard upright freezer ($\leq 0^{\circ}\text{F}$). Gilkey and Staehelin (1986) discuss ultra-rapid freezing as a more efficient preservation process than traditional freezing methods which can result in cellular degradation associated with ice crystals forming inside the cells of interest, rendering tissues unsuitable for histological examination.

CHAPTER IV: GENERAL DISCUSSION

Species Management Implications

The typical timeline for *C. viridis* reproduction indicates that most snake-human interactions are likely to occur near egress from hibernacula in the spring when snakes are dispersing to find food or mates; or near ingress in the fall at the end of the active season when snakes are returning to hibernacula (Duvall and Schuett, 1997; Brown, et al., 2009). Movement/migration appears to occur in a straight-line fashion, regardless of sex or reproductive condition, but temporally can be significantly different within and between groups from a given hibernaculum (Graves and Duvall, 1993).

While feeding behavior initially appeared to slow or even cease during pregnancy (Gregory, et al., 1999), two observations from within a rookery suggested that this is more from lack of opportunity than from lack of a desire to eat (Graves and Duvall, 1993). Temperature regulation behavior has been observed throughout several studies (Charland and Gregory, 1990; Graves and Duvall, 1993) and results reported suggest a direct correlation with embryogenesis progression. There was a significant difference in mean body temperatures of gravid vs. non-gravid females in a study by Graves and Duvall (1993). Microthermal habitat conditions and associated preferences were cited as reasons for the difference. Gravid females tend to wait for parturition in a relatively small area near the hibernaculum (Graves and Duvall, 1993) while males and non-gravid females disperse and opportunistically locate mates and food (Duvall and Schuett, 1997). Graves and Duvall (1993) observed that use of rookeries can significantly contribute to the overall fitness of the mother and her offspring. Rookeries provide protection from environmental conditions as well as predators. They may be well-suited for successful

parturition and located in an area of abundant prey for neonates. As well as close proximity (<1 km) to hibernacula, mammal burrows and flat rocks on which to warm up in the sun or take shelter under have been identified as key features of rookeries studied in Alberta, Canada (AESRD/ACA, 2012). No such key features exist in the collection area of the Kansas population. Shelter and preferred microhabitat for both snake and prey can be found in the form of a prairie dog town. The topography of the area is relatively flat with little canopy cover. Shelter from predators and environmental conditions is found underground.

In order to increase public safety, those entities charged with educating and protecting the general public must know when and how often mating occurs, as well as how many offspring a female can produce. This particular population mated in the spring, shortly after egress from hibernacula. Those females who encountered prime conditions for reproduction, aggregated in a rookery area and would have likely remained in that area until after parturition in the fall when they would have returned to the hibernacula. Non-gravid females and males would have migrated farther from the hibernacula than gravid females in order to find forage and mates.

It is imperative that education on these life events and when they occur be provided to the general public interacting with the ecosystems in which these snakes are found. By educating people on key time periods to avoid certain habitats, the likelihood of a negative encounter will decrease, resulting in increased protection for the snakes and the public.

The data found in this thesis can be used to review and modify field protocols for handling of this species by adding TL and subcaudal scale counts to the standard data

gathered. Game managers can use these data to determine whether there is a need for population control of the snakes or their primary prey items. Wildlife educators can use these data to implement an education program for the general public to reduce or eliminate negative accidental rattlesnake-human interactions in their area.

Rattlesnake Roundup Management Suggestions

In the case of rattlesnake roundup events, which are not condoned here, this study supports the findings of Fitch (1998) and Schmidt (2002). While rattlesnake roundup festivals are prime opportunities to boost the economy of the host city and increase the exposure of these animals to further education of the populace about them, there is an ethical need for more stringent guidelines for collection limits (Fitch, 1998; Weir, 1992). The public education opportunities traditionally available at these festivals are considered substandard at best (Schmidt, 2002) and an effort should be made to recruit experienced herpetologists to produce quality education and materials for festival attendees. Collection areas need to be strictly enforced around the community in which the festival is held to protect populations outside the collection zone. All gravid females should be released near the areas they are found in order to preserve the population from which they were collected so the festival can continue as a future source of income for the host city. Field data should be collected in a standardized format that can be later analyzed by managing entities and a biologist should be present at all festivals to record any data that becomes available in order to assist with developing and revising existing management practices.

Suggestions for Further Research

- 1.) A mark-recapture study of a population of *C. viridis* to determine whether dorsal saddle patterns change across life stages and if the patterns can be used to identify individuals.
- 2.) An ecological study determining immigration/emigration of a population with a tie-in to repatriation of a species formerly removed from an area.
- 3.) Publication of an updated guidebook on mammal dorsal guard hairs and techniques for identifying them for the region.
- 4.) A study comparing the effects of ultra-rapid freezing on tissues slated for histological examination and how they are affected by subsequent storage at temperatures $\leq 0^{\circ}\text{F}$.

Table 1.5. Adapted from Fitch (1998). Geographic variation in demographic traits of *Crotalus viridis*

Authority	Origin of Sample	Mean adult size (SVL, mm)	Neonate size (SVL, mm)	Mean litter	Frequency of breeding	Main prey
Present study	SW Kansas	♂ 709.83(524-1032) ♀ 707.22(552-906)	243.58	8.3	Mostly annual- Some biennial	<i>Peromyscus</i> , <i>Silvilagus</i>
Fitch, 1998	W Kansas	♂ 890(750-1208) ♀ 850(730-987)	266	10.7	Mostly annual	Ground squirrel, wood-rat
Klauber, 1956	Platteville, Boulder Co., N central Colorado	♂ 776(675-1025) ♀ 735(575-863)	250-299	11.85±0.18	Mostly annual	Ad: deer mouse Yg: lizard (<i>Holbrookia</i>)
Rahn, 1942	Horse Creek, Laramie Co., SE Wyoming	♀ 874(705-1010)			biennial	
Duvall, King, and Gutzwiller, 1985	Carbon Co., S-central Wyoming, 6900'	♂ 821 ♀ 733	250		biennial	Deer mouse
Klauber, 1936	Pierre, Hughes Co., central South Dakota	♂ 862 ♀ 822(683-965)	255	10.76±0.21	biennial	Vole, lark bunting, deer mouse
Gannon and Secoy, 1984	Leader, SW Saskatchewan	♂ 975(768-1150) ♀ 940	245±8.0	10.2	biennial	Ground squirrel, cottontail

Diller and Wallace, 1984; Wallace and Diller, 1990	Nez Perce Co. and Latah Co., N Idaho	♂ 706(460-930) ♀ 650(570-780)		5.5		Ad: vole, deer mouse Yg: shrew
McCartney and Gregory, 1988; McCartney, 1989	Okanagan Valley, S British Columbia	♀ >800	268	4.6	Biennial and triennial	Ad: vole, gopher, mouse Yg: vole, shrew
Fitch, 1949	Madera Co., central California	♂ 959(755-1250) ♀ 820(775-1100)	251-300	7.6	Annual	Ad: ground squirrel Yg: pocket mouse, spadefoot toad
Heyrend and Call, 1951; Glissmeyer, 1951	Grantville, Tooele Co., NW Utah	♂ 731(635-965) ♀ 680(635-790)	294	5.5	biennial	Ad: small mammals Yg: lizards
Klauber, 1956	South Coronados Is., Baja California	♂ 650 ♀ 528	190	2.6		Lizards: <i>Uta</i> , <i>Eumeces</i> , <i>Elgaria</i>

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APPENDIX

Appendix Diet section (Chapter II.) results from a population of *C. viridis* collected near Ulysses, Kansas

Sex	Gravid	SVL	Month	Stomach or Intestine?	Prey ID	Timeline for eating (days)
M	NA	650	April	Stomach/Intestine	<i>Peromyscus</i>	2-3
F	N	632	April	Stomach	<i>Peromyscus</i>	1
F	Y	687	April	Stomach	<i>Peromyscus</i>	1
F	N	906	April?	Intestine	<i>Peromyscus</i>	4-6
F	Y	697	May	Intestine	<i>Silvilagus</i>	4-6
M	NA	689	May	Intestine	<i>Reithrodontomys</i>	4-6
F	N	766	May	Intestine	<i>Silvilagus</i>	4-6
F	Y	682	May	Intestine	Aves	4-6
F	N	763	May	Intestine	Soricidae	4-6
M	NA	1032	May	Intestine	<i>Peromyscus</i>	4-6
M	NA	744	May	Stomach	Aves	2-3
M	NA	650	May	Stomach	Aves	2-3
F	N	745	May	Stomach	<i>Silvilagus</i>	1
F	N	749	May	Stomach	<i>Perognathus</i>	1
F	Y	810	May	Stomach	<i>Microtus</i>	2-3
F	N	730	May	Intestine	<i>Silvilagus</i>	4-6
F	Y	739	May	Intestine	<i>Peromyscus</i>	4-6
F	Y	727	May	Intestine	<i>Silvilagus</i>	4-6
F	N	720	May	Intestine	<i>Perognathus</i>	4-6
F	Y	840	May	Intestine	<i>Perognathus</i>	4-6
F	N	709	May	Intestine	Soricidae	4-6
F	N	673	May	Intestine	<i>Silvilagus</i>	4-6
M	NA	694	June	Intestine	<i>Peromyscus</i>	4-6
F	Y	687	June	Intestine	<i>Silvilagus</i>	4-6
F	N	742	June	Stomach	3-Muridae	1
F	Y	705	June	Stomach	<i>Microtus</i>	1
F	N	685	June	Stomach	<i>Peromyscus</i>	1
F	Y	724	June	Stomach	<i>Reithrodontomys</i>	1
M	NA	490	June	Intestine	<i>Onchomys</i>	4-6
F	N	713	June	Intestine	Soricidae	4-6
F	Y	715	June	Stomach AND Intestine	<i>Reithrodontomys</i> hair in stomach. <i>Silvilagus</i> hair in intestine	R-2-3; S-4-6

M	NA	903	June	Stomach	<i>Plestiodon</i> <i>obsoletus</i>	1
F	Y	777	June	Stomach	<i>Microtus</i>	1
M	NA	904	June	Stomach	<i>Spermophilus</i>	2-3
F	N	696	June	Stomach	<i>Peromyscus</i>	1
F	Y	697	June	Stomach	Aves	1
F	N	570	June	Intestine	<i>Microtus</i>	4-6
M	NA	540	June	Stomach	<i>Peromyscus</i>	1
F	Y	685	July	Stomach	<i>Peromyscus</i>	2-3
M	NA	725	July	Intestine	Soricidae	4-6
M	NA	664	July	Animal in stomach Hair in Intestine	<i>Peromyscus</i> in stomach <i>Chaetodipus</i> hair in intestine	P-1; C-4-6
F	Y	658	August	Stomach	<i>Peromyscus</i>	1
M	NA	550	August	Stomach	<i>Peromyscus</i>	1
M	NA	707	August	Stomach	<i>Reithrodontomys</i>	1
M	NA	562	September	Intestine	<i>Silvilagus</i>	4-6