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Abstract

As humans encroach into areas inhabited by predators, the potential of human-predator confrontations increases and the predators become regarded as dangerous pests. Predators exert a measure of population control over pest species such as small rodents, as well as limit the quantity and scope of diseases (e.g. spread of Hantavirus by these prey species). Control of these small rodent pest species can be aided by conserving and managing their predators like rattlesnakes (*Crotalus* spp). Management of any population requires detailed information on population composition and the ability to determine the key information (especially age and sex) for each individual quickly and with high accuracy. To determine the sex of a snake in the field, traditionally, a probe or forceful expulsion of the hemipenes are used. In the hands of a person unskilled in field herpetology, these methods can potentially be painful to the snake, as well as place the observer in unnecessary danger.

The goal of this study was to develop a less invasive field method of determining sex for any life stage in Prairie Rattlesnakes (*C. viridis*) using morphological characteristics that are commonly collected. Snout-vent length (SVL), and absolute and relative measures of tail length (TL, TL/SVL), rattle length (RL, RL/SVL), number of subcaudal scales (SS, SS/SVL), and number of dorsal saddle patterns (DS, DS/SVL) were examined within and across life stages of a *C. viridis* populationnear Ulysses, Kansas, USA, collected from 2012-2015 to facilitate a safe working environment for a prairie restoration project. SVL, , RL, and DS as well ass RL/SVL and DS/SVL did not differ between sexes within and across life stages. TL,SS,TL/SVL and SS/SVL did not differ between male and female neonates and juveniles, but were, on average, larger in adult males than females. Regression tree analysis, however, indicated that TL and SS as well as TL/SVL and SS/SVL are not very reliable for sex determination of adult snakes. Yet, if used in conjunction with other reliable methods, such as palpation of the ventral area of a snake to determine gravidity, both absolute and relative measures of tail length and number of subcaudal scales are viable alternatives to the more invasive methods currently in use.

Keywords: *Crotalus viridis,* morphological traits, prairie rattlesnake, sexual dimorphism, rattle

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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) and many of these predators become regarded as dangerous pests, despite keeping other problematic species such as deer, small rodents, or rabbits in check (Bouskila 1995, Sillero-Zubiri and Laurenson 2001, Jędrzejewski et al. 2002). Rattlesnakes (*Crotalus* spp), for example, exert a measure of population control over pest species like rats, mice and rabbits, as well as limit 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). Control of these small rodent pest species can be aided by conserving and managing their predators (Collins, Collins, and Taggart 2010, Fogell 2010). Management of any population requires detailed information on the composition of the population of interest as well as the ability to determine the key information (especially age and sex) for each individual quickly and with high accuracy, but also with as little disturbance as possible (Koons, Rockwell, and Grand 2006, Sandercock et al. 2008). While current standard field methods for determining the age and overall condition of a snake are based on external morphological measurements (Diller and Wallace 1984), the determination of the sex of a snake, however, can be invasive and potentially painful for snakes. These sex determination 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 these 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 is of particular concern, since over the last decade, an increasing number of volunteers (i.e., citizen scientists) has been recruited to participate in biodiversity assessments and monitoring of reptile populations (Price and Dorcas 2011, Theobald et al. 2015, Kullenberg and Kasperowski 2016).

This study aimed to develop a less invasive, field method of determining sex for any life stage in Prairie Rattlesnakes using morphological characteristics that are commonly collected (Rivas, Ascanio, and Muno 2008) such as snout-vent length (SVL), tail length (TL), saddle pattern, and subcaudal scale counts.

SVL and TL are useful measurements to aid in a visual assessment of sex. Females typically have shorter tails compared to males which tend to have longer tails (Klauber 1997, Shine et al. 1999). It has been suggested that males have longer tails to accommodate larger hemipenes, thereby increasing reproductive success (King 1989). The number of subcaudal scales is another potential indicator of sexual dimorphism because it varies between individuals of different sexes and life stages (Klauber 1997, dos Santos-Costa et al. 2006, López and Giraudo 2008).

Materials and Methods

Prairie Rattlesnake Population

For this study, we investigated museum specimens of a population of *C. viridis* originating near Ulysses, Kansas, USA (37.560136, -101.495031). These specimens (N = 199) were collected from April to October over a period of three consecutive years (2012 – 2015) to facilitate a safe working environment for a prairie restoration project after a demolition of a gas plant. As long distance relocation of crotalids is detrimental to the snakes (Brown, Bishop and Brooks 2009) and many wild populations are currently at carrying capacity (Brennan and Tischendorf 2004), these snakes were euthanized and preserved in the museum collection of the Sternberg Museum of Natural History at Fort Hays State University so they could be used in future studies. (Dan Fogell, pers. comm., 2015). Of the 199 *C. viridis* specimens collected, 16 were omitted from this study due to insufficient information regarding location and date of collection.

Morphological Measurements

Snout-vent length (SVL) and tail length (TL) were measured using a flexible cloth measuring tape. The condition of the rattle, and for complete rattles, the number of rattle segments (RL), and the presence/absence of the rattle button were determined. Mass was not recorded due to desiccation during the preservation process. Subcaudal scales were counted with the help of a dissecting probe to keep accurate count.

To determine the dorsal pattern of saddles and number of crosshatches, digital images were taken of each individual snake using a Fujifilm FinePix S4830 digital camera (Appendix 1). Based on these images, saddle patterns and number of crosshatches from neck to vent were determined.

In addition to absolute measures of morphological characteristics, we also assessed whether relative measures were suitable predictors of sex, because relative measures of these characteristics can also differ between sexes. For example, TL relative to SVL (i.e. TL/SVL *100%) in *C. viridis* is larger in males than females (Klauber 1997). We calculated therefore relative measures for TL, RL, SS, and DS in relation to SVL by calculating the ratio of the characteristic to SVL.

Sex and Life Stage

Sex of each snake was determined using presence or absence of hemipenes (Shine et al. 1999, Dellinger and von Hegel 1990, King 1989). Females were further divided into gravid and non-gravid categories based on the presence or absences of follicles

For each sex, life stages were broken down into adult, juvenile, and neonate, following Diller and Wallace (1984). Males were classified as adult if SVL was >520 mm and 4 or more rattle segments were present and as juveniles if SVL was < 520 mm and/or they had less than 4 rattle segments. Females were considered adults if SVL was >550 mm SVL and 5 or more rattles were present and as juveniles if SVL was < 550 mm and/or less than 5 rattle segments were present. Individuals of both sexes were classified as neonates if the SVL was ≤ 260 mm and only a button rattle was present, indicating that the individual had yet to shed for the first time (Diller and Wallace 1984, Fitch 1985).

Statistics

To determine whether the morphological measurements differed between the three life stages and between males and females in this population, we conducted, in the case of SVL, TL and all relative measures, a 3×2 ANOVA using the GLM procedure in SAS software (version 9.4 (c) 2002-2012 by SAS Institute Inc., Cary, NC, USA). For all other morphological measurements (i.e., RL, SS, and DS), we conducted a Poisson regression analysis using the GENMOD procedure in SAS (version

9.4 (c) 2002-2012 by SAS Institute Inc., Cary, NC, USA). If sex or the interaction between sex and life stage was significant, Tukey post-hoc pairwise comparisons were performed to test for differences between males and females at each life stage. Before analysis, SVL, TL and all relative measures were tested for normality using Shapiro-Wilk Test (Whitlock and Schluter 2009) and SVL and TL were log-transformed to achieve normal distribution.

To assess whether morphological traits can be used to determine sex, we also conducted two regression tree analyses in RStudio (2015) version 1.0.136. for each life stage using the package *rpart* (Therneau, Atinson, and Ripley 2015). The first analyses included the absolute measurements of morphological traits (i.e., SVL, TL, RL, SS, and DS) and the relative measures of morphological characteristics (i.e., TL/SVL, RL/SVL, SS/SVL, DS/SVL). The second analysis included only the morphological characteristics which were significantly different between adult males and females using ANOVA or Poisson regression analysis.

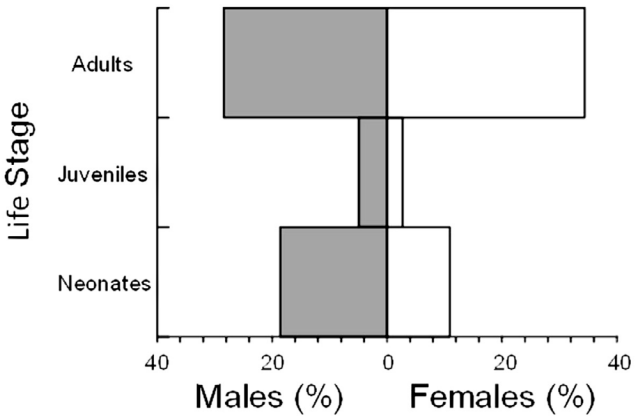

Figure 1. Population pyramid for Prairie Rattlesnake (*Crotalus viridis*) population near Ulysses, Kansas, USA.

Results

Composition of C. viridis Population

Almost two-thirds of the individuals within the population were adults, 29% neonates, and only 8% juveniles (Table 1; Figure 1).The number of males and females within each life stage was not significantly different (χ^2 = 5.57; *d.f.* = 2, *P* = 0.06; Table 1; Figure 1).

Within this population of *C. viridis*, 18.5% of the population (N = 183) consisted of gravid females; of all females present (N = 63), 38% were gravid (Table 1). Gravid females were present April through August; however, the majority of gravid females was caught in June (Figure 2).

Morphological Traits as Indicators of Sexual Dimorphism

SVL did not differ between males and females nor was there an interaction between sex and life stage (Table 2 and 4; Figure 3). In contrast, TL was significantly affected by sex and the interaction between sex and life stage (Table 2 and 4; Figure 3). The tail of females grew slower than that of males and was at the adult stage significantly shorter (≈7% SVL) than in males (≈10% SVL) (Table 2; Figure 3). Yet, the distribution of tail length for males was about twice as wide as for females and included the full range of female tail length (Figure 4A). Relative tail length (TL/SVL) showed a pattern similar to TL: both sex and the interaction between sex and life stage were significant, with males and females being significantly different only at the adult stage (Table 3 and 5; Figure 3). For both measures of RL, absolute and relative, there was no difference between males and females and the interaction between sex and life stage was not significant (Tables 2 through 5; Figure 3). SS and SS relative to SVL (SS/SVL) were different between males and females, with adult males having a greater absolute and relative number of subcaudal scales than adult females (Tables 2 and 3; Figure 3). As for TL, the distribution of adult male SS was twice as wide as for adult females and included the full range of adult female SS (Figure 4B). DS and relative DS (DS/SVL) were neither affected by sex nor the interaction between sex and life stage (Tables 2 through 5; Figure 3).

Regression trees constructed separately for each life stage using all absolute and relative measures of morphological characteristics resulted in decision trees with misclassification probabilities between males and females

Table 1. Distribution of sex and life stages within a Prairie Rattle snake (*C. viridis* population near Ulysses, Kansas. Sample size and, in parentheses, population percentage are shown.

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%)

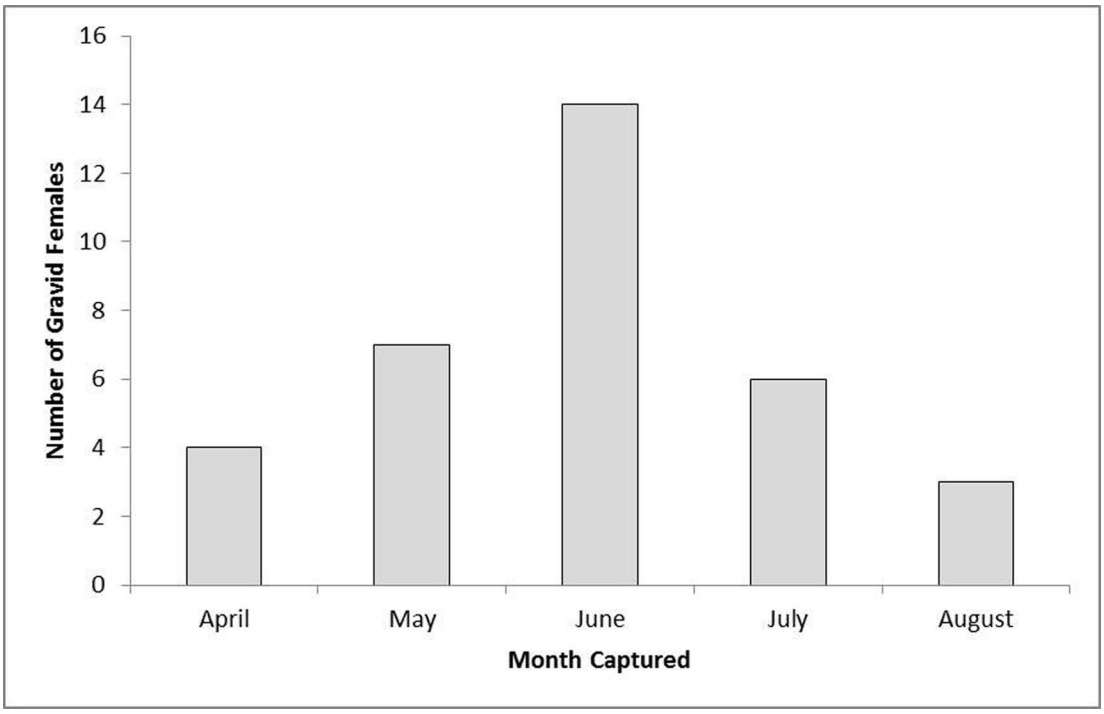

Figure 2. Number of gravid females captured April through August in 2012-2015 in a population of Prairie Rattlesnakes (*Crotalus viridis)* near Ulysses, Kansas, USA.

Table 2. Descriptive statistics (mean ± SEM, (sample size)) of absolute measures of morphological characteristics: Snout-vent length (SVL), tail length (TL), rattle length (RL), and number (n) of subcaudal scales (SS) and dorsal saddles (DS).

	SVL (mm)	TL (mm)	RL (mm)	SS (n)	DS (n)
Adults					
Males	709.8 ± 17.6 (52)	61.3 ± 2.1 (52)	35.5 ± 1.3 (50)	25.0 ± 0.4 (52)	42.0 ± 0.5 (50)
Females	707.2 ± 8.7 (63)	47.7 ± 0.9 (63)	34.8 ± 0.9 (63)	20.8 ± 0.2 (61)	43.1 ± 0.4 (63)
Juveniles					
Males	367.8 ± 38.9 (9)	33.0 ± 3.3 (9)	14.3 ± 2.8 (9)	24.1 ± 0.9 (9)	42.0 ± 1.5 (9)
Females	358.8 ± 56.1 (6)	28.2 ± 4.1 (6)	13.2 ± 4.4 (5)	21.2 ± 0.7 (6)	39.5 ± 1.1 (6)
Neonates					
Males	244.1 ± 2.0 (33)	22.1 ± 0.9 (15)	4.9 ± 0.3 (7)	22.1 ± 0.6 (34)	42.5 ± 1.2 (15)
Females	242.7 ± 2.5 (19)	23.1 ± 1.8 (7)	5.0 ± 0.0 (2)	21.3 ± 0.7 (19)	42.9 ± 1.3 (7)

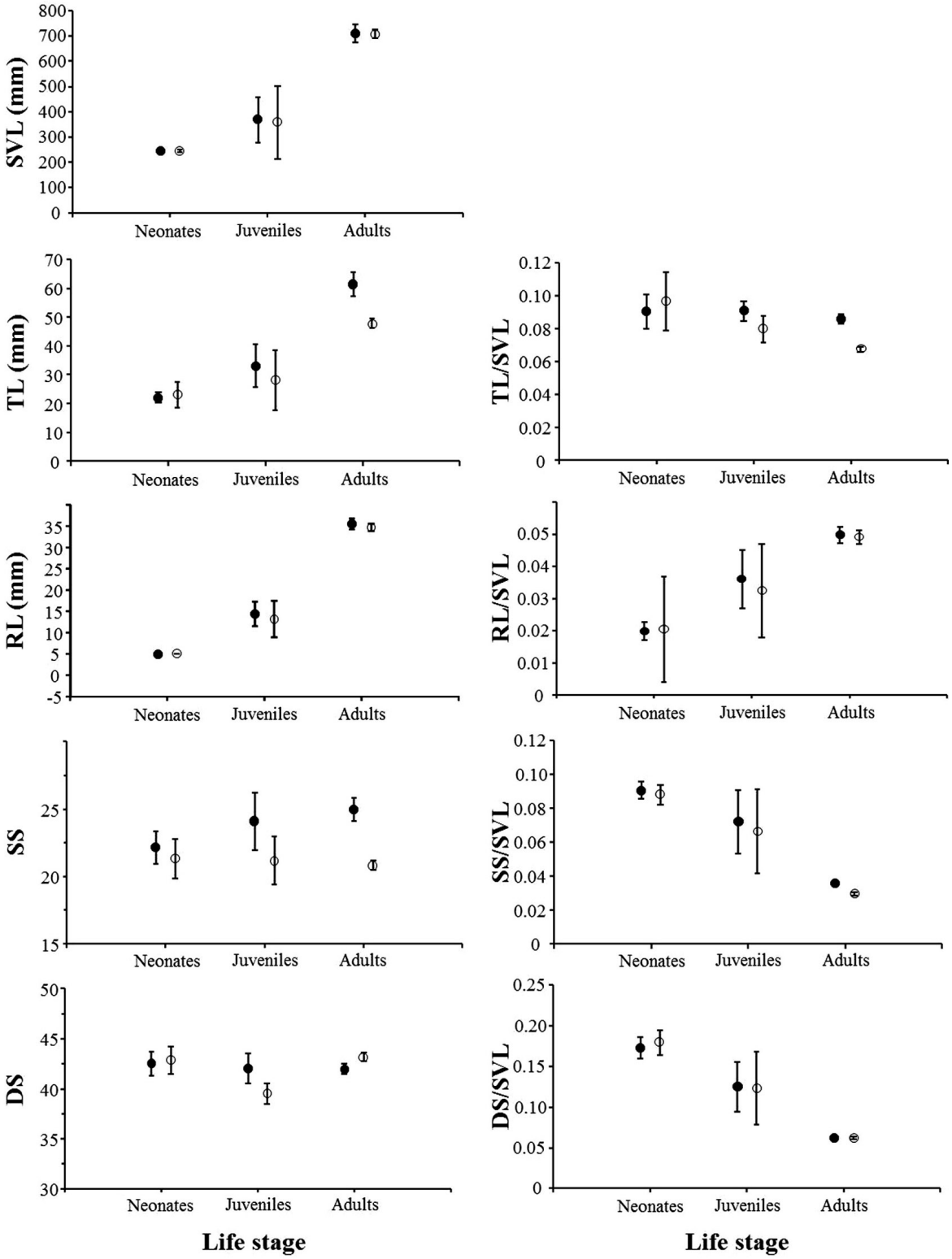

Figure 3. Morphological characteristics for male and females at each life stage. Absolute measures of morphological characteristics: Snout-vent length (SVL), tail length (TL), rattle length (RL), and number (n) of subcaudal scales (SS) and dorsal saddles (DS). Morphological characteristics relative to SVL: relative tail length (TL/SVL), relative rattle length (RL/SVL), and relative number (n) of subcaudal scales (SS/SVL) and dorsal saddles (DS/SVL).Black circles indicate males; open circles females. Means and 95%-confidence intervals are shown.

Table 3. Descriptive statistics (mean ± SEM (sample size)) of morphological characteristics relative to snout-vent length (SVL): relative tail length (TL/SVL), relative rattle length (RL/SVL), and relative number of subcaudal scales (SS/SVL) and dorsal saddles (DS/SVL).

	TL/SVL	RL/SVL	SS/SVL	DS/SVL
Adults				
Males	0.086 ± 0.091 (52)	0.050 ± 0.036 (50)	0.036 ± 0.072 (52)	0.061 ± 125 (50)
Females	0.067 ± 0.001 (63)	0.049 ± 0.001 (63)	0.030 ± 0.000 (61)	0.062 ± 0.001 (63)
Juveniles				
Males	0.091 ± 0.003 (9)	0.036 ± 0.004 (9)	0.072 ± 0.008 (9)	0.125 ± 0.013 (9)
Females	0.080 ± 0.003 (6)	0.032 ± 0.005 (5)	0.066 ± 0.010 (6)	0.123 ± 0.018 (6)
Neonates				
Males	0.090 ± 0.005 (15)	0.020 ± 0.001 (7)	0.090 ± 0.002 (33)	0.172 ± 0.006 (15)
Females	0.097 ± 0.007 (7)	0.020 ± 0.001 (2)	0.088 ± 0.003 (19)	0.179 ± 0.006 (7)

Table 4. Results of general linear model analyses for effect of sex and life stage and their interaction on absolute measures of morphological characteristics: snout-vent length (SVL), tail length (TL), rattle length (i.e., number of rattle segments; RL), and number of subcaudal scales (SS) and dorsal saddles (DS).

SVL	*d.f.*	*F*	*P*
Sex	1, 176	0.14	0.71
Life stage	2, 176	938.0	**< 0.0001**
Life stage × Sex	2, 176	0.15	0.86
TL	*d.f.*	*F*	*P*
Sex	1, 146	5.73	**0.02**
Life stage	2, 146	178.19	**< 0.0001**
Life stage × Sex	2, 146	3.43	**0.04**
RL	*d.f.*	χ^2	*P*
Sex	1	0.04	0.85
Life stage	2	260.81	**< 0.0001**
Life stage × Sex	2	0.17	0.92
SS	*d.f.*	χ^2	*P*
Sex	1	6.87	**0.009**
Life stage	2	1.73	0.42
Life stage × Sex	2	3.84	0.15
DS	*d.f.*	χ^2	*P*
Sex	1	0.05	0.83
Life stage	2	1.02	0.60
Life stage × Sex	2	1.04	0.60

Table 5. Results of general linear model analyses for effect of sex and life stage and their interaction on morphological characteristics relative to snout-vent length (SVL): relative tail length (TL/SVL), relative rattle length (RL/SVL), and relative number (n) of subcaudal scales (SS/SVL) and dorsal saddles (DS/SVL).

TL/SVL	*d.f.*	*F*	*P*
Sex	1, 146	8.81	**0.004**
Life stage	2, 146	22.35	**<0.0001**
Life stage × Sex	2, 146	11.35	**<0.0001**
RL/SVL	*d.f.*	*F*	*P*
Sex	1, 130	0.19	0.67
Life stage	2, 130	49.37	**<0.0001**
Life stage × Sex	2, 130	0.20	0.82
SS/SVL	*d.f.*	*F*	*P*
Sex	1, 174	4.75	**0.04**
Life stage	2, 174	505.27	**< 0.0001**
Life stage × Sex	2, 174	0.52	0.60
DS/SVL	*d.f.*	*F*	*P*
Sex	1,144	0.21	0.65
Life stage	2, 144	410.65	**<0.0001**
Life stage × Sex	2, 144	0.33	0.72

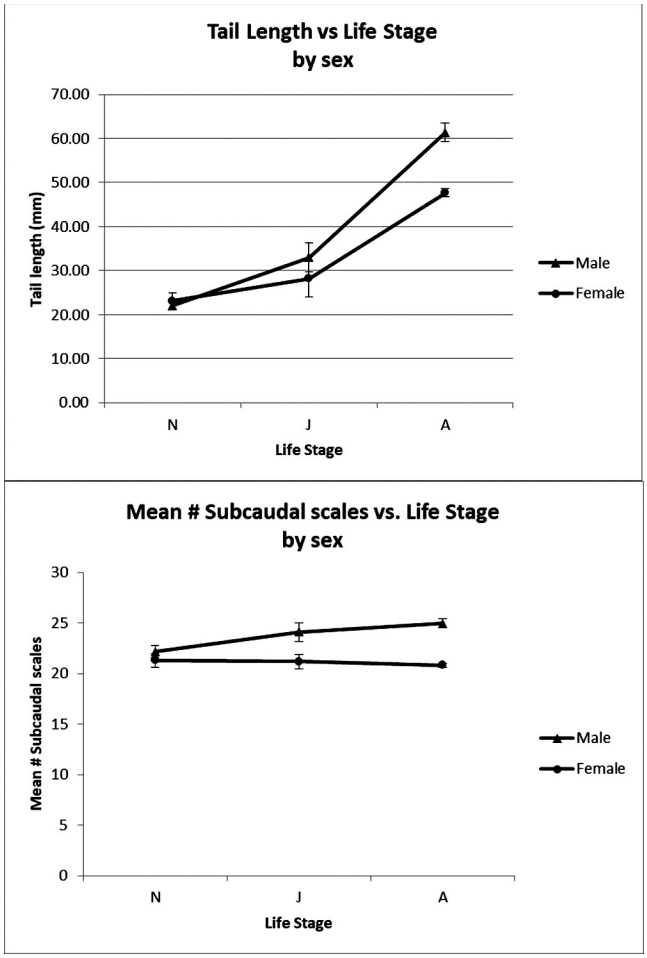

Figure 4. Distribution of tail length (Figure 4A) and number of subcaudal scales (Figure 4B) for adult males (black columns) and females (white columns).

of 0.45 and 0.36 for adults and neonates, respectively. Using DS/SVL and TL/SVL, adult males and females of known sex were misclassified with a 45% chance (N = 115). Neonates were misclassified with a 36% (N = 54) chance using SS/SVL, SVL, and TL/SVL. For juveniles, none of the morphological traits used in the regression tree analysis was suited to distinguish between males and females (N = 15).

Including in the regression tree analysis only those morphological characteristics that were significantly different between adult males and females (Table 4 and 5: TL, TL/SVL, SS, and SS/SVL; Figure 3), the regression tree analysis used only TL and TL/SVL in the construction the final decision tree; but the chance of misclassifying an individual of known sex was remained unchanged (i.e., 45%).

Discussion

Composition of C. viridis Population

The observed life stage composition of the *C.viridis* population near Ulysses, Kansas, with a heavy bias towards adults (63%), fewer neonates (29%) and very few juveniles (8%) is similar to population compositions reported in the literature for *Crotalus* spp. (Diller and Wallace 1984; Martins, Arnaud, and Ávila-Villegas 2012). Reports on population compositions from rattlesnake roundups at Sharon Springs, Kansas, are difficult to compare with our sample because the snakes were collected from different populations and the origin of individual snakes is unknown (Fitch 1998, Schmidt 2002). Further, rattlesnake roundup samples are biased towards adult snake as snakes smaller than 382mm were prohibited from being collected (Fitch 1998, Schmidt 2002)

The disparity seen in the population structure of this *C. viridis* population between the number of juveniles compared to the number of adults and neonates is difficult to explain. Without repeated observations of individual snakes, essential life table data are unavailable; thus mortality rates, growth rates, and duration within each life stages cannot be estimated and evaluated. A mark-and recapture study on *C. viridis oreganus* showed that adult snakes have the highest survival rates while the neonates have the lowest and juveniles intermediate survival rates (Diller and Wallace 2002). Further, the same study found that the capture probability of juveniles were the lowest for all three life stages which was attributed to different movement pattern (Diller and Wallace 2002). Due to the similarity of the population structure with the one studied by Diller and Wallace (1984, 2002), the underlying mortality rates in our population may be similar. In addition, the juveniles may have been overlooked more often, because they coil individually and are thus less likely to be detected than the much larger adult snakes or neonates that aggregate in small piles (Curtis J. Schmidt, personal communication).

Morphological Traits as Indicators of Sexual Dimorphism

None of the morphological traits studied was suited to indicate males and females across all life stages. This result was supported by both analysis approaches: the ANOVA/logistic regression analyses and the regression tree analysis. This may be primarily due to the fact that none of the morphological traits showed a significant sex effect in neonates and juveniles. In many species of venomous snakes, including *C. viridis*, males tend to have a prolonged growth period after reaching sexual maturity,

which has been correlated with male-male combat (Shine 1994). Males also tend to reach sexual maturity earlier than females (MacArtney, Gregory, and Charland 1990), resulting in the sample size being skewed toward adults. The lack of sex effect in juveniles may also be due to the small sample size which results in low statistical power to distinguish differences between means (Whitlock and Schluter 2009).

As indicators of sexual dimorphism in *C.viridis* adults, tail length and number of subcaudal scales– absolute and relative measures: TL, TL/SVL, SS, and SS/SVL – showed the greatest potential. Tail length and number of subcaudal scales were both significantly larger in adult males than females. However, the distributions of male tail length and number of subcaudal scales included both the female distribution of tail length and number of subcaudal scales, respectively. Further, regression tree analysis for adult snakes using only TL and SS or TL/SVL and SS/SVL, respectively, as morphological traits, resulted in a decision tree with a 45% chance of misclassifying a snake of known sex. This suggests that tail length and number of subcaudal scales are not very reliable for sex determination. However, if used in conjunction with other reliable methods, such as palpation of the ventral area of a snake to determine gravidity, absolute or relative measures of tail length and number of subcaudal scale are viable alternatives to the invasive methods currently in use. We suggest therefore, that tail length and subcaudal scale counts should be collected during 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*.

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