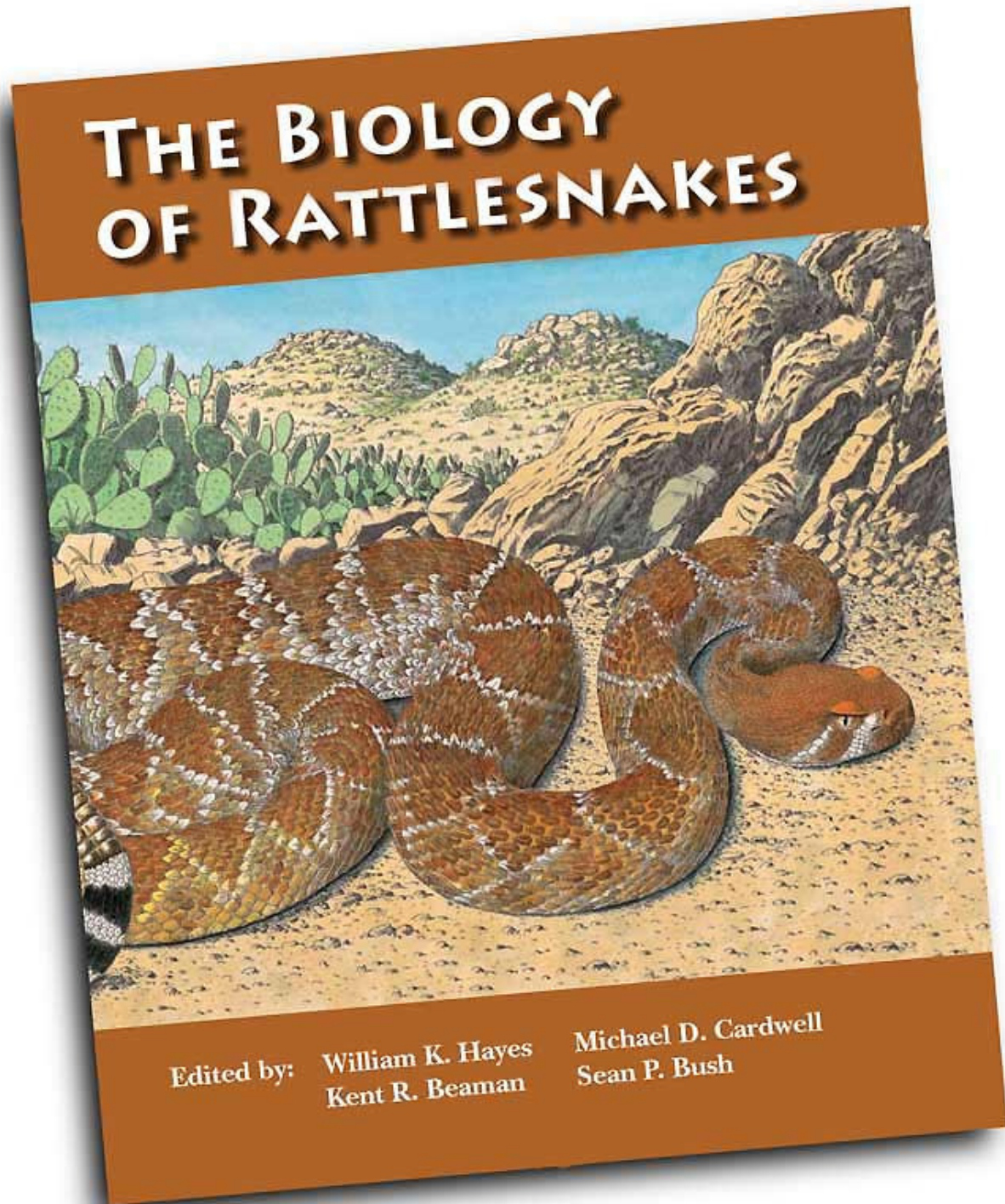


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Reproductive Biology of the Massasauga (*Sistrurus catenatus*) from South-Central Illinois

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ABSTRACT.—This study was conducted at Carlyle Lake, Clinton County, Illinois. Brood size averaged eight and was not significantly correlated with maternal snout-vent length. Based on preserved snakes, females initiate vitellogenesis in the summer/fall. Vitellogenic follicles reach 20 mm in length by late September, overwinter at this size, and resume growth in the spring. Ovulation occurs later in the spring. Spermatogenesis begins in June and peaks in August and September. The diameter of the seminiferous tubules is less than 200 μm in May and reaches a diameter of 300 μm in late July to early September. The seasonal cycle of the sexual segment of the kidney parallels the changes in diameter of the testis. Sexual segment tubules are smallest in the early part of the activity season and peak in diameter and secretory activity from August through September. Mating and male-male combat occur primarily in the summer when the sexual segment of the kidney is hypertrophied. As in other species of snakes, the sexual segment of the kidney never regresses completely, indicating that testosterone levels are elevated throughout the year. The elevated plasma testosterone levels, which may be necessary for long term sperm storage in the vas deferens, may also account for the occurrence of courtship behavior in males in the spring.

INTRODUCTION

The Massasauga (*Sistrurus catenatus*) is a small rattlesnake (50-70 cm, maximum 100.3 cm) that ranges from Arizona, New Mexico, and Texas to central New York and southern Ontario, Canada (Campbell and Lamar, 2004). Currently, three subspecies are recognized. The focus of this paper is the Eastern Massasauga (*S. c. catenatus*), which occurs from eastern Missouri to the Great Lake states and Ontario, Canada. This subspecies is found in open low-lying areas such as marshes, bogs, and wet prairies (Campbell and Lamar, 2004).

Wright (1941) suggested that the primary reason for the decline of *S. c. catenatus* is human activities, including encroachment on habitats, collecting, and farming. Management efforts require information on the basic biology and activity patterns to design effective conservation policies. It is clear from the literature that much work is needed. For example, Campbell and Lamar (2004) cite Atkinson and Netting (1927) and Johnson (1987) to conclude that mating in *S. c. catenatus* occurs in both the spring and fall. More recent work on our population has shown that mating (Jellen, 2005) and male-male combat (Shepard et al., 2003) occur primarily in the summer/fall.

The purpose of the present study is to describe brood size, offspring size, frequency of reproduction, female reproductive investment, seasonal patterns of vitellogenesis, spermatogenesis, sperm storage in the vas deferens, and development of the sexual segment of the kidney in this species. The seasonal patterns are discussed in relation to the seasonal occurrence of mating and male-male combat behaviors in *S. c. catenatus* in south-central Illinois.

MATERIALS AND METHODS

Study area.—This study was conducted at several sites around the southern periphery of Carlyle Lake, Clinton County, Illinois (38.6°N, 89.3°W). The study site consisted primarily of a fallow grassland community with a patchy forb distribution and adjacent floodplain forest or degraded savanna habitats. Tall Fescue (*Festuca pratensis*) and Tall Goldenrod (*Solidago canadensis*) were the dominant plant species. Burrowing Crayfish (*Fallicambarus fodiens* and *Orconectes immunis*) were present in the more mesic areas of the study site.

Data on live snakes.—From 1999 through 2003, snakes were located during spring egress through random visual searches at potential and known hibernacula. Throughout the active season, we also examined snakes incidentally encountered by us and Illinois Department of Natural Resources and Army Corps of Engineers personnel. Snakes were sexed by cloacal probing for the presence of hemipenial pockets (Schaefer, 1934). Snout-vent length (SVL) was measured to the nearest millimeter with a flexible tape, and mass to the nearest gram with Pesola® pull spring scales or an Ohaus® electronic balance.

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Table 1. Geographic comparisons of mean brood number for *Sistrurus c. catenatus*. Mean Brood Number includes living and dead embryos and unfertilized eggs.

Location	Mean Brood Number	Latitude	N	Authority
Southern Illinois	8.3 (3 – 11)	38.8°	10	Current study
Missouri	6.4 (4 – 10)	40.2°	17	Siegel, 1986
Pennsylvania	7.3 (5 – 9)	41.2°	11	Reinert, 1981*
Northern Illinois	12.0 (8 – 14)	42.3°	6	Wright, 1941
New York	9.3	43.1°	9	Johnson, 1995
Wisconsin	11.1 (6 – 19)	44.4°	58	Keenlyne, 1978
Ontario, Canada	13.3 (9 – 19)	45.2°	15	Parent and Weatherhead, 2000
Overall mean	9.3			

* Includes data from Atkinson and Netting (1927; $N = 3$) and Swanson (1933; $N = 3$, preserved specimens only).

Snakes were individually marked by clipping ventral scales (modified from Brown and Parker, 1976), injecting passive integrated transponder tags (if >20 cm SVL), and painting rattle segments with nail polish. Rattle painting permitted identification of individuals from a distance without disrupting the snake's behavior.

The reproductive condition of females was initially determined by palpation and those that appeared gravid were evaluated using ultrasound to determine reproductive stage and brood size. Six of these females were implanted with radio transmitters, released, and then recaptured and examined using ultrasound approximately twice monthly throughout the gestation period to monitor offspring development. Surgical implantations of transmitters (models SI-2T [8.9 g] and SB-2T [5.2 g], Holohil Systems Ltd., Ontario Canada; and model SM1-H [9.0 g], AVM Instrument Company, Ltd., Colfax, California) were performed by veterinarians at the St. Louis Zoo following the guidelines proposed by Reinert and Cundall (1982). Transmitters were less than or equal to 6% of the snake's body mass. Shortly before the end of the gestation period (as evidenced from the sonogram), 10 gravid females, including the six implanted females, were brought into captivity to give birth to determine brood size and obtain morphological measurements and sex ratio of the offspring. These data were also used to compare the accuracy of estimates of brood size based on sonograms with the actual number of offspring born.

Data on preserved snakes.—Specimens in the Illinois Natural History Museum collection (Appendix 1) were used for dissection and histological examination of the reproductive organs.

In females, the SVL and largest ovarian follicles were measured to the nearest millimeter. Due to the trauma resulting from road mortality, the number of follicles per snake could not be determined for many individuals.

In males, the right testis and anterior portion of the kidney, with vas deferens attached, were removed, dehydrated in isopropanol, cleared in toluene, embedded in paraffin,

and sectioned at 7 μ m. Tissues were stained in hematoxylin, Biebrich scarlet, orange G, and fast green. Data taken included: seminiferous tubule diameter (STD), stage of spermatogenesis, diameter of the sexual segment of the kidney (SSK), epithelial height of SSK, and presence of sperm in the vas deferens.

Morphology of the oviduct.—Portions of the oviduct were examined histologically in two snakes (collected October 14 and April 24) to compare with other species. The glandular and furrowed portions were sectioned and stained as described above for male tissue.

Statistical analysis.—Differences between brood size estimates using sonograms were compared with actual brood sizes using a paired t -test. Average brood size versus latitude and the relationship between brood size and mass versus maternal SVL and mass were analyzed by regression. Sex ratio of young was analyzed by Chi-square. For the analysis of male reproductive data, the activity season was divided into two halves: the first half included April-June, and the second half included July-October. These time periods were chosen because the first period represents the time that the testis is normally quiescent and the second period represents a time that the testis is hypertrophied. Because of unequal variances in some measurements, differences in seasonal means for SVL, STD, SSK, and SSK epithelial height were analyzed by Mann-Whitney U tests. For all statistical tests, $\alpha = 0.05$.

RESULTS

Brood size.—Mean (± 1 S.E.) estimated brood size (including embryos and unfertilized eggs) based on the sonograms (8.3 ± 2.2 , $N = 9$) was not significantly different ($t = -0.29$, $df = 16$, $P > 0.77$) from the actual average brood size (8.0 ± 2.6 , $N = 9$). There were, however, substantial differences within individuals. Brood size was overestimated in two cases (by 7 and 2) and underestimated in four cases (by 2, 2, 2, and 6).

Brood size ($y = 0.26x - 6.9$; $r^2 = 0.060$, $F_{1,7} = 0.45$, $P = 0.52$) and brood mass ($y = 0.026x + 25.63$; $r^2 = 0.09$, $F_{1,7} = 26.2$, $P = 0.25$) were not significantly related to maternal SVL.

Combining our data with data from other published accounts on *S. c. catenatus* (with sample sizes >5), brood size (including dead embryos and undeveloped ova) showed a positive relationship with latitude ($y = 0.88x - 27.5$; $r^2 = 0.62$, $F_{1,5} = 8.2$, $P < 0.04$; Table 1, Fig. 1).

Female investment.—Brood size ($y = 0.04x - 4.6$; $r^2 = 0.60$, $F_{1,7} = 22.2$, $P = 0.002$; Fig. 2) and brood mass ($y = 0.66x - 55.9$; $F_{1,7} = 26.2$, $r^2 = 0.79$, $P < 0.002$; Fig. 3) were significantly correlated with maternal prepartum mass. Females lost an average of $43.6 \pm 10\%$ (range = 24.4–55.5%, $N = 8$) of their body mass following parturition.

Offspring size and sex ratio.—Mean neonatal SVL was 19.9 ± 1.4 cm, (range = 16.6–23.2 cm, $N = 59$) and mean mass was 10.7 ± 1.0 g (range = 7.8–13.3 g, $N = 57$). The offspring sex ratio was significantly male-biased (40 males:17 females, $N = 10$ broods; $\chi^2 = 9.28$, $df = 1$, $P = 0.002$; Table 2)

Frequency of reproduction and seasonal vitellogenesis.—Based on road-killed snakes, vitellogenic follicles were present in four of the seven females examined in the summer/fall (September–November) and in four of the 10 females examined in the spring (March–early April). Overall, eight of 17 (47%) females were reproductive, having vitellogenic follicles. Mean follicle lengths in non-vitellogenic snakes were 3.2–3.9 mm. Follicles in vitellogenic snakes reached a mean length of 21–24 mm by October.

Data on preserved male snakes.—The mean SVL of males from April–June (601 ± 93 mm, range = 455–688 mm, $N = 7$) was not significantly different ($Z = -1.4$, $P = 0.15$) from males collected in July–October (529 ± 64 mm, range = 455–701 mm, $N = 13$).

The mean of the STD for April–June (179 ± 15 μ m, range = 165–200 μ m, $N = 4$) was significantly smaller ($Z = -2.9$, $P < 0.004$) than the diameter from July–October (257 ± 26 μ m, range = 200–300 μ m, $N = 12$; Fig. 4). In April and May, the testes contained primarily spermatogonia and Sertoli cells (Fig. 5A). Testicular recrudescence began in June and the first sperm were present in mid June. Maximum spermatogenesis and tubular diameter were reached in July–September (Fig. 5B). Spermiation occurred from late June through October. The vas deferens runs along the medial edge of the kidney (Fig. 5C) and parallels the ureter to the cloaca (Fig. 5D). Hypertrophied SSK tubules were visible on the dorsal surface of the kidney (Fig. 5E). The vas deferens at the level of the kidney contained sperm throughout the active season (Fig. 5F).

The mean diameter of the SSK tubules for April–June (137 ± 40 μ m, range = 90–200 μ m, $N = 5$) was significantly smaller ($Z = -2.4$, $P > 0.016$) compared to males from July–October (201 ± 34 μ m, range = 150–250 μ m, $N = 9$; Fig. 6). The mean height of the SSK epithelium for April–June (46 ± 4 μ m, range = 40–50 μ m, $N = 5$) was significantly smaller ($Z = -2.9$, $P < 0.003$) than males examined from

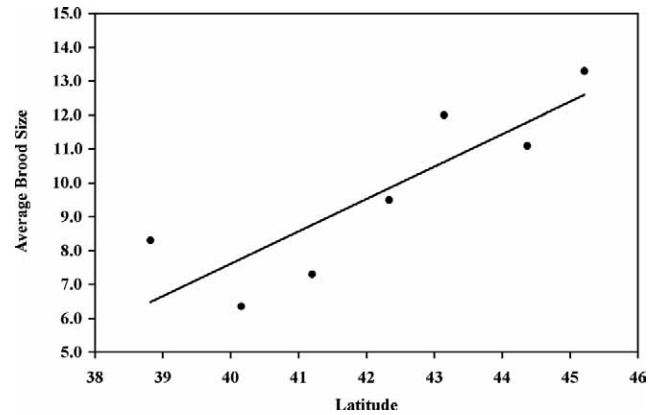


Figure 1. Relationship between brood size and latitude for *Sistrurus c. catenatus*.

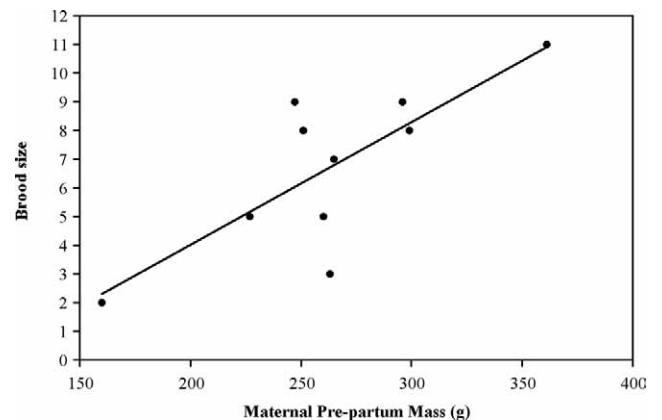


Figure 2. Maternal prepartum mass versus brood size for *Sistrurus c. catenatus* at Carlyle Lake, Clinton County, Illinois.

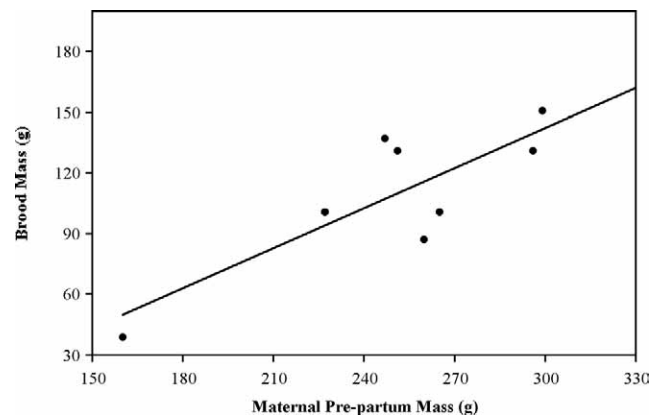


Figure 3. Maternal prepartum mass regressed with brood mass for *Sistrurus c. catenatus* at Carlyle Lake, Clinton County, Illinois.

July–October (77 ± 11 μ m, range = 60–100 μ m, $N = 9$; Fig. 7). Granules were present in the SSK in all snakes examined (Fig. 8A, B).

Morphology of the oviduct.—The epithelium of the oviduct consisted of columnar cells; approximately half were ciliated (Fig. 8C). In the glandular portion, many alveolar glands containing granules were present in the submucosa (Fig. 8C). In the furrowed portion of the oviduct, the epithe-

Table 2. Snake number (#), maternal SVL (M – SVL, cm), neonatal SVL (mean ± SD and range, cm), neonatal mass (mean ± SD and range, g), and brood sex ratio for *Sistrurus c. catenatus* at Carlyle Lake, Clinton County, Illinois. Brood number reflects only offspring passed (live and stillborn). M = male, F = female, U = undetermined. Offspring sex was classified as U when the condition of the non-viable offspring could not be determined.

Snake #	M - SVL	Mean SVL	SVL Range	Mean Mass	Mass Range	Sex Ratio
035	58.0	18.7 ± 0.63	18.0 – 19.9	9.9 ± 0.96	7.80 – 10.8	9M, 0F, 0U
113	59.5	18.6 ± 0.40	18.1 – 19.3	9.1 ± 0.31	8.80 – 9.5	6M, 3F, 0U
131	55.8	18.6 ± 2.81	16.6 – 20.6	8.8 ± 0.35	8.85 – 9.0	0M, 1F, 1U
132	62.7	21.1 ± 0.56	20.5 – 22.2	9.7 ± 0.50	8.80 – 10.5	6M, 2F, 0U
162	59.0	20.7 ± 0.50	20.0 – 21.6	10.0 ± 0.44	9.30 – 10.5	4M, 7F, 0U
164	61.7	19.9 ± 0.00	19.9	13.3	13.30	1M, 1F, 0U
174	58.1	22.5 ± 0.52	21.8 – 23.2	11.3 ± 0.25	11.00 – 11.5	3M, 2F, 0U
192	54.6	19.3 ± 0.61	18.5 – 20.1	10.5 ± 0.49	9.50 – 11.0	6M, 1F, 1U
306	57.5	19.8 ± 0.42	19.3 – 20.1	12.0 ± 0.71	11.50 – 12.5	3M, 0F, 0U
335	60.2	19.4 ± 0.28	19.2 – 19.6	9.0 ± 0.35	8.80 – 9.3	2M, 0F, 0U
Overall Mean		19.9 ± 1.4	16.6 – 23.2	10.1 ± 0.80	7.80 – 13.3	40M, 17F, 2U

obs.) and the ability to replenish lost energy reserves often determines whether they will survive hibernation and when they will reproduce next (Brown and Weatherhead, 1997).

Offspring size and sex ratio.—Our mean neonatal SVL (19.9 ± 1.4 cm) was similar to that reported by Seigel (1986) for captive-born *S. catenatus* (18.2 ± 0.71 cm) in Missouri. Seigel (1986) noted that captive-born snakes were smaller than hatchling wild snakes (25.2 ± 2.19 cm), but he could not determine whether the captive-born snakes were stunted or wild snakes had grown prior to collection. In *S. c. catenatus* from Wisconsin, Keenlyne and Beer (1973) found that the length of time that the female was maintained in captivity had no effect on the total length (\bar{x} = 22.0 ± 1.26 cm) or mass (\bar{x} = 9.7 ± 1.59 g) of the young. Additional data on the total length of newborn *S. catenatus* were presented by Wright (1941; \bar{x} = 22.4 ± 1.8 cm, *N* = 6 litters) and Swanson (1933; \bar{x} = 21.6 ± 0.3 cm, *N* = 1 litter). We feel that, because of the small sample sizes and the variation in which the lengths of new born snakes were measured, latitudinal analysis of the data was not possible.

The overall offspring sex ratio in our captive born snakes was strongly male-biased (40 males:17 females). Our results differed from Keenlyne and Beer (1973), who found a nearly-equal sex ratio of 107 males to 100 females in newborns from captive females of *S. c. catenatus* from Wisconsin.

Frequency of reproduction.—We found that approximately 50% of our females were reproductive over the four years of the study. In Wisconsin, Keenlyne (1978) found that 93% (76 of 82) of females three years of age and older were reproductive and concluded that these females reproduced annually. Seigel (1986) found that *S. catenatus* in Missouri had different frequencies of reproduction in different years. He reported that, overall, 62% of the females

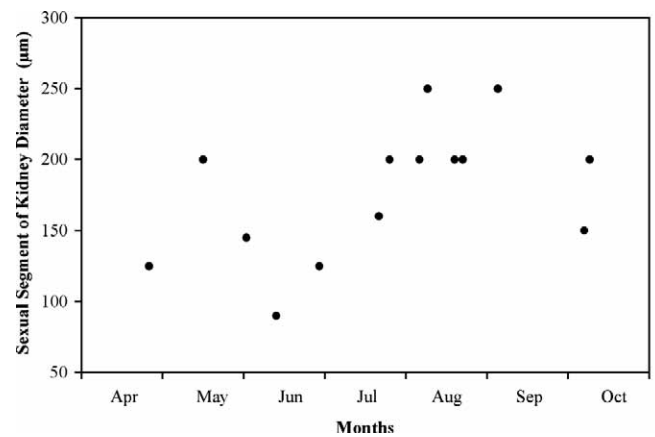


Figure 6. Sexual segment of the kidney diameter by date for *Sistrurus c. catenatus* at Carlyle Lake, Clinton County, Illinois. Each point represents the mean of 12 measurements per snake.

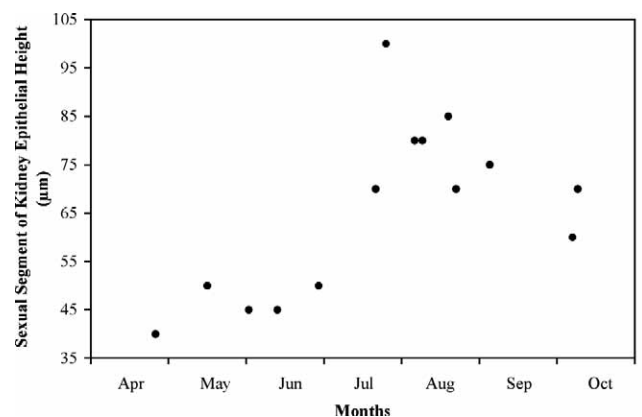


Figure 7. Sexual segment of the kidney epithelial height by date for *Sistrurus c. catenatus* at Carlyle Lake, Clinton County, Illinois. Each point represents the mean of 12 measurements per snake.

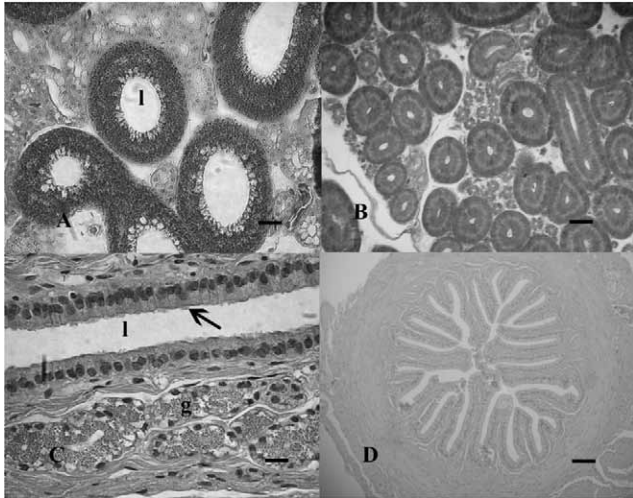


Figure 8. **A.** Photomicrograph of kidney from snake collected 23 April. Enlarged tubules with granules present represent the sexual segment of the nephron. Note lumen (l) is large. Surrounding tubules are non-sexual portions of the nephron. Bar represents 35 μm (200x). **B.** Photomicrograph of kidney from snake collected 16 August. Sexual segment tubules are larger than April specimen and epithelium is higher. Bar represents 100 μm (100x). **C.** Photomicrograph of the glandular portion of the oviduct (longitudinal section) from a vitellogenic snake collected 14 October. The lumen (l) is lined with columnar epithelial cells. Approximately half of the cells are ciliated (arrow). Uterine glands containing granules (g) are present in the connective tissue. Bar represents 10 μm (400x). **D.** Photomicrograph of the furrowed portion (cross section) of the oviduct from the same snake. Bar represents 100 μm (100x).

reproduced in the five years of the study; however, the annual frequency varied from 33-71%. Seigel et al. (1998) re-examined his population following an extensive flood and found that only 22.7% were reproductive. They attributed the low frequency to reduced prey availability caused by the flooding.

Seasonal vitellogenesis.—Vitellogenesis in *S. catenatus* begins in the summer/fall (i.e., following parturition in those individuals with sufficient energy to reproduce annually). The vitellogenic follicles overwinter in the ovary, resume growth in the spring, and are ovulated later in the spring (Wright, 1941; Jellen, 2005). This seasonal pattern of vitellogenesis is similar to the majority of temperate zone North American pit vipers (Aldridge and Duvall, 2002). Keenlyne (1978) found a vitellogenic female *S. catenatus* in the late summer in his study; however, because he examined very few females in the summer/fall and early spring, he assumed that this individual was not representative of most females. He assumed that most females initiated vitellogenesis in the spring.

Aldridge and Duvall (2002) discussed the 10 species of North American pitvipers in which vitellogenesis has been described and all had the pattern described for *S. catenatus*. Recent work, however, by Schuett et al. (2004), Taylor et al. (2004), and Taylor and DeNardo (2005), has suggested

that vitellogenesis is restricted to the spring in populations of *C. atrox* in low elevation regions of southern Arizona. Taylor et al. (2004) and Taylor and DeNardo (2005) based their conclusion on the lack of identifiable vitellogenic follicles using ultrasound and palpation and also the lack of elevated plasma levels of estrogen in summer/fall females. Schuett et al. (2004) based their conclusion on unpublished data and added that the observations made by Fitch and Pisani (1993) on *C. atrox* in Oklahoma were similar to their findings. Fitch and Pisani (1993), however, did not mention when vitellogenesis began, but rather suggested that the vitellogenic follicles of *C. atrox* were smaller in the spring compared to *C. viridis* and *C. horridus*.

The most convincing evidence presented by Taylor and DeNardo (2005) is their data on a female examined on 14 April with previtellogenic follicles and, again, on 17 May with vitellogenic follicles (35-37 mm diameter). In addition, females in which they found only previtellogenic follicles in the summer/fall ($N = 3$) and early spring ($N = 3$) did not reproduce. Rosen and Goldberg (2002) studied preserved *C. atrox* from southern Arizona in areas that overlapped the study areas of Taylor and DeNardo (2005) and of Schuett et al. (2004). They found that six of the 14 females examined had vitellogenic follicles in the fall (September-November). This pattern of summer/fall vitellogenesis was also observed by Tinkle (1962) for *C. atrox* from northwestern Texas. However, because the studies by Rosen and Goldberg (2002) and Tinkle (1962) were based on examination of preserved specimens, some females could have initiated vitellogenesis in the spring. Perhaps the lack of summer/fall vitellogenesis observed by Taylor et al. (2004) was due to the lack of females examined in October and November or that some females occasionally begin vitellogenesis in the spring.

Seasonality of mating and male-male combat.—The mating season of *S. catenatus* is often reported as occurring in the spring and summer/fall (Aldridge and Duvall, 2002; Campbell and Lamar, 2004). These conclusions are based on a captive courting observation (Guthrie, 1927) and the speculation by Crawford (1936). Guthrie (1927) described a mating attempt in a recently-caught pair (apparently 5 May, 1926) of *S. c. catenatus*. Guthrie (1927) reported that, on May 7, the male made three separate attempts to mate with the female. Guthrie (1927) reported that the female was passive. The data from our work and those of Reinert (1981) clearly suggest that summer/fall is the primary mating period in *S. c. catenatus*. The presence of a summer-only mating season requires that sperm must survive a minimum of ca. eight months in the oviduct prior to fertilization.

The limited number of courtships, matings, and male-male combat behaviors described in wild *S. catenatus* suggest a summer-only mating season. For the Carlyle Lake population, all courtships ($N = 7$) and copulations ($N = 11$) occurred between 24 July and 22 September (Jellen, 2005). The only male-female pairings reported outside the mid-to late-summer period occurred during the spring egress (7

April 1999 and 16 April 2002, Jellen, 2005; 9 April 2005, Dreslik, unpubl. data), when male-female pairs were observed coiled together. None of these instances lasted longer than 1 d and copulation was not observed (Jellen, 2005; Dreslik, unpubl. data). Crawford (1936) also observed bisexual pairs in the spring in an Ohio population which led him to the conclusion that mating occurred in the spring. Crawford (1936) added however, that the absence of observed matings was not due to the lack of observation in the spring.

In a Pennsylvania population of *S. c. catenatus*, Reinert (1981) reported that mating occurred in the summer (28 July and 6 August, 1977) and also that all male-female pairs were also observed in August ($N = 5$).

Male-male combat behaviors in wild *S. catenatus* appear to be rare. The only two reports were by Shepard et al. (2003), who observed this behavior in the Carlyle Lake population on 20 August, and by Vandewalle (2005), who observed this behavior in an Iowa population on 22 August (2003). In both instances, these authors described the behavior as occurring in dense vegetation and involving radio-tracked snakes.

In the congeneric *S. miliarius*, data on mating and male-male combat behavior also suggest a summer/fall mating period. In central Florida, Farrell et al. (1995) reported that mating (3 observations, September and October) and pairing (10 observations, September-December) occurred in the summer/fall. In western Georgia, Hamilton and Pollack (1955) reported a mating pair in the wild on 8 September. In southeastern North Carolina, Palmer and Williamson (1971) reported that *S. miliarius* captured on 4 October displayed male-male combat 8 d later.

Indirect evidence of a summer/fall mating season is presented by Dalrymple et al. (1991). They examined the frequency of adult male and female *S. miliarius* encountered during the year in Long Pine Key, Everglades National Park, Florida. They found a large increase in the proportion of males in the sample during October-December. In these months, males comprised 84% of the 43 snakes, compared to 54% of the 35 snakes collected in all other months. Overall, 65% of the adult males ($N = 55$) were encountered in October-December. Since males typically search for females during the mating season, the mating season probably occurs during these months.

Thus, both species within the genus *Sistrurus* appear to have primarily a summer mating season, with southern populations extending the mating season into late fall.

Male reproductive cycle.—The seasonal cycle of spermatogenesis in *S. c. catenatus* is similar to that reported for the western subspecies, *S. c. edwardsii* (Goldberg and Holycross, 1999), in Arizona and Colorado and all other New World crotalids inhabiting the temperate zone (*Agkistrodon piscivorus*: Johnson et al., 1982; *C. lepidus*: Goldberg, 2000a; *C. molossus*: Goldberg, 1999a; *C. pricei*: Goldberg, 2000b; *C. ruber*: Goldberg, 1999b; *C. scutulatus*: Goldberg and Rosen, 2000; *C. tigris*: Goldberg, 2000c; and *C. viridis*: Aldridge, 1993). This seasonal pattern of spermatogenesis

is described by Saint Girons (1982) as summer (aestival) spermatogenesis. The relationship between spermatogenesis and mating in *S. catenatus* does not conform to the classic definitions of prenuptial and postnuptial spermatogenesis described by Volsøe (1944). In *S. catenatus*, spermatogenesis occurs in the summer and fertilization in the spring, consistent with the pattern described as postnuptial spermatogenesis. Mating, however, occurs in the summer/fall and sperm are stored in the oviduct for fertilization in the spring. This pattern is similar to *S. miliarius* (Farrell et al., 1995), *C. horridus* (Martin, 1992; Aldridge and Brown, 1995), and *C. viridis* (Aldridge, 1993). Thus, the age of the sperm at fertilization is the same as in species with postnuptial spermatogenesis which mate only in the spring (*C. mitchellii*: Goldberg, 2000d; *C. ruber*: Klauber, 1972). Many species of pitvipers mate in both the summer/fall and the following spring (see Aldridge and Duvall, 2002, and Dugan et al., this volume).

The SSK of squamates is an androgen-sensitive portion of the nephron (Bishop, 1959) that secretes material which is transferred to the female's cloaca/oviduct during copulation and apparently forms the copulatory plug (Devine, 1977). In all snakes studied, the SSK is hypertrophied during the mating season (Saint Girons, 1982). The SSK of *S. c. catenatus* (this study) and *S. c. edwardsii* (Goldberg and Holycross, 1999) contained granules throughout the year. In the present study, however, the SSK diameter and epithelial height were significantly larger during the summer/fall, corresponding to the mating season.

If there is a metabolic cost for maintaining sperm in the vas deferens and the development of the SSK during the winter and spring, it is surprising that male *S. catenatus* and other species (*C. viridis*, *C. tigris*; see Aldridge and Duvall, 2002) do not shut down the production of androgens at the conclusion of the summer/fall mating season. Complete involution of the SSK during the non-mating season is seen in many species of lizards (Fox, 1977). In *S. catenatus*, spermatogenesis is initiated early enough in the summer to supply sperm for a late July-August mating season. We propose three hypotheses to explain the continued hypertrophy of the SSK (and elevated plasma testosterone levels): 1) elevated testosterone levels may be necessary for the maintenance of sperm in the vas deferens, 2) perhaps a few females may mate in the spring (as seen in some captive snakes; Guthrie, 1927), or 3) the continuous hypertrophy may be the result of phylogenetic inertia (many species of pit vipers in North America have both summer/fall and spring mating seasons; Aldridge and Duvall, 2002). Whatever the reason for the continued development of the SSK, the elevated plasma testosterone levels associated with this may account for the spring mating behaviors seen in species possessing a predominately summer/fall mating season.

Morphology of the oviduct.—The epithelium of the uterine portion of the oviduct of *S. catenatus* consists of ciliated and non-ciliated columnar cells. The uterine portion also has many alveolar glands, containing granules, in

the submucosal layer. The morphology of this portion of the oviduct is similar to other viviparous snakes (*Nerodia* and *Thamnophis*: Blackburn, 1998; *Seminatrix pygaea*: Sever et al., 2000). Blackburn (1998) reported that the uterine glands in viviparous species secrete a "vestigial shell membrane." The furrowed portion of the oviduct consists of a series of longitudinal folds of the epithelial and submusosal layers, which function to expand the lumen for pregnancy and birth. The furrowed portion lacks multicellular glands. The morphology of the furrowed portion of the oviduct appears to be typical for the majority of snakes examined (Blackburn, 1998).

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APPENDIX I

ILLINOIS NATURAL HISTORY SURVEY MUSEUM NUMBERS INCLUDED IN STUDY

Males: INHS 1542, 1543, 8485, 15985, 16262-16264, 16395, 16420, 16422, 16424, 16672, 16827, 17345, 17444, 17705, 18202, 18768, 18770, 18778, 19334. Females: INHS 11184, 15986, 16667, 16668, 16670, 16671, 17347, 17348, 17349, 17422, 17423, 17702, 18451, 18452, 18466, 18595, 18596, 18771, 18774, 19333, 19335, 19473, 19505.

