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 does not regress 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 hemipenal 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.
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], A VM 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 μm. 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, α = 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).

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

* Includes data from Atkinson and Netting (1927; *N* = 3) and Swanson (1933; *N* = 3, preserved specimens only).
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 \( (y = 0.066x - 55.9; F_{1,7} = 26.2, r^2 = 0.79, P < 0.002; \) Fig. 3) were significantly correlated with maternal prepartum mass. Females lost an average of 43.6 ± 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 μm, range = 165-200 μm, \( N = 4 \)) was significantly smaller \( (Z = -2.9, P < 0.004) \) than the diameter from July-October (257 ± 26 μm, range = 200-300 μm, \( N = 12 \); 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 μm, range = 90-200 μm, \( N = 5 \)) was significantly smaller \( (Z = -2.4, P > 0.016) \) compared to males from July-October (201 ± 34 μm, range = 150-250 μm, \( N = 9 \); Fig. 6). The mean height of the SSK epithelium for April-June (46 ± 4 μm, range = 40-50 μm, \( N = 5 \)) was significantly smaller \( (Z = -2.9, P < 0.003) \) than males examined from July-October (77 ± 11 μm, range = 60-100 μm, \( N = 9 \); 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-
lial lining and submucosa consisted of many folds, approximately 250 μm each, extending into the lumen (Fig. 8D).

**Discussion**

**Brood size.**—In our study, sonogram estimates of brood size taken early in the gestation period were not accurate and both over- and underestimates were common. The extent that implanting a radiotransmitter in the snake affected brood size (i.e., by inducing atresia) could not be determined because of small sample size. Snakes often pass unfertilized ova during parturition (Guthrie, 1927; Farrell et al., 1995) and at least one unfertilized ova was passed in six of 10 litters in this study. Additionally, females might resorb or fail to give birth to all embryos. Three gravid females (two radio-implanted and one non-implanted individual) did not pass all of the developing, seemingly viable, offspring, as evidenced by sonograms and radiographs taken after parturition; and this might account for some of the discrepancy between estimated and actual brood size.

In our study, neither brood size nor brood mass were significantly correlated with maternal SVL. Our results differed from those of Seigel (1986) and Parent and Weatherhead (2000), who reported a significant positive relationship for maternal SVL and brood size in *S. catenatus* in Missouri and Ontario, Canada, respectively.

The comparison of average brood size versus latitude showed a significant positive relationship. This relationship may be attributable to several factors. First, the activity season duration at Carlyle Lake can be four months longer than at the northern limit of the range at Killbear Provincial Park, Ontario, Canada (Parent and Weatherhead, 2000). This allows snakes at Carlyle Lake more time to allocate resources towards growth and reproduction. Consequently, females at Carlyle Lake reach the size of sexual maturity in 2-3 yr (M. J. Dreslik, pers. obs.), compared to the 5-6 yr observed near the northern range limit (Johnson et al., 2000). Klauber (1972) speculated that populations in northern latitudes may compensate for the low frequency of reproduction and delayed maturity in females by increasing brood sizes. Unfortunately, demographic data are not available to compare differences in ages of maturity and frequency of reproduction between populations.

**Female investment.**—In our study, maternal prepartum mass was significantly related to both offspring number and total average offspring mass, suggesting maternal mass, and not SVL, might be a better indicator of fecundity. Females at Carlyle Lake lost approximately 44% of their body mass in 2-3 yr (M. J. Dreslik, pers. obs.), compared to the 5-6 yr observed near the northern range limit (Johnson et al., 2000). Klauber (1972) speculated that populations in northern latitudes may compensate for the low frequency of reproduction and delayed maturity in females by increasing brood sizes. Unfortunately, demographic data are not available to compare differences in ages of maturity and frequency of reproduction between populations.

**Figure 4.** Seminiferous tubule diameter by date for *Sistrurus c. catenatus* at Carlyle Lake, Clinton County, Illinois. Each point represents the mean of 12 measurements per snake.

**Figure 5.** A. Photomicrograph of testis from snake collected 31 May. Seminiferous tubules contain primarily spermatogonia and spermatocytes. Bar represents 40 μm (200x). B. Photomicrograph of testis from snake collected 28 August. Seminiferous tubules contain primarily spermatocytes, spermatids, and spermatozoa. Bar represents 50 μm. C. Ventral view of the left kidney of male snake. Note that kidney consists of lobes approximately 1 cm in length. Arrow denotes vas deferens. D. Ventral view of ducts posterior to the kidney. Vas deferens (arrow) and ureter (right of vas deferens) remain separate and enter cloaca separately. E. Dorsal view of kidney shown in C. Sexual segment of the kidney tubules visible (arrow). F. Photomicrograph of cross section of vas deferens containing spermatozoa(s). Bar represents 30 μm (200x).
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 (¯x = 22.0 ± 1.26 cm) or mass (¯x = 9.7 ± 1.59 g) of the young. Additional data on the total length of newborn *S. catenatus* were presented by Wright (1941; ¯x = 22.4 ± 1.8 cm, N = 6 litters) and Swanson (1933; ¯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 newborn 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

### Table 2

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

**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.
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 pit vipers 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 midto late-summer period occurred during the spring egress.
Aldridge, 1993). This seasonal pattern of spermatogenesis appears to be rare. The only two reports were by Shepard et al. (2003), who observed this behavior in the Carly 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 (estival) spermatogenesis. The relationship between spermatogenesis and mating in *S. c. catenatus* does not conform to the classic definitions of prenuptial and postnuptial spermatogenesis as described by Volsøe (1944). In *S. c. 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. mitchelli*: Goldberg, 2000d; *C. ruber*: Klauber, 1972). Many species of pit vipers 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. c. 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. c. 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 submucosal 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).

ACKNOWLEDGMENTS

Funding for this project was provided through the Illinois Department of Natural Resources (Wildlife Preservation Fund), U.S. Fish and Wildlife Service, U.S. Army Corps of Engineers, Chicago Herpetological Society, and the undergraduate research fund at Saint Louis University (to JMC). All research was conducted in accordance under the approved IACUC protocol #02010 with the University of Illinois and all permits were granted to CAP by the Illinois Department of Natural Resources. We are greatly indebted for all the assistance and volunteer work of R. Junge for surgeries, ultrasounds, x-rays, and general health care of snakes and to J. Martin-de-Camilo for additional ultrasound assistance. We thank the St. Louis Zoo for providing the necessary facilities and equipment for surgical implantations. We thank J. Mui, A. Kuhns, J. Petzing, P. Jellen, M. Redmer, T. Anton, D. Mauger, E. Kershner, J. Walk, D. Olson, D. Jellen, B. Anderson, G. Kruse, S. Ballard, T. Strole, J. Kath, E. Smith, D. Tecic, and B. Heinhold for their assistance in numerous aspects of fieldwork. Finally, we thank G. Tatham, J. Bunnell, J. Birdsell and park maintenance personnel at Eldon Hazlet State Park, J. Smothers, D. Baum, and personnel at the U.S. Army Corps of Engineers, Carlyle Lake facility for aiding in searching and locating snakes and guiding and directing the management of their lands to conserve S. catenatus.

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———, AND A. T. HOLYCROSS. 1999. Reproduction in the


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.