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Reproductive Cycle of Male Snapping Turtles (*Chelydra serpentina*) in Southeastern Virginia

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The snapping turtle, *Chelydra serpentina*, ranges throughout eastern North America from southern Canada to southern Florida and the Gulf of Mexico (Iverson, 1992). In areas of freezing temperatures, snapping turtles are active only during the warm months and typically enter hibernation by late October and emerge March to early May, depending on latitude and temperature (Ernst et al., 1994). In spite of the abundance and widespread occurrence of *C. serpentina*, its reproductive biology is spottily documented, especially for the male gametogenic cycle.

Chelydra serpentina displays a typical North American late spring egg-laying season (Iverson et al., 1997), which requires either early spring courtship and mating or late summer – fall courtship with oviductal sperm retention or fertilization with developmental arrest. Both fall and spring mating have been observed in Virginia (Mitchell, 1994) and elsewhere (Ernst et al., 1994). Studies in Wisconsin (Mahmoud and Cyrus, 1992) and Tennessee (White and Murphy, 1973) show that spermatozoa are stored in the epididymides by September–October, hence sperm is available for fall insemination. Perhaps this is the common pattern for male reproduction in *C. serpentina*.

The better studied female reproductive biology shows geographic variation in clutch and egg size (Iverson et al., 1997). Geographic variation may also occur in the reproductive biology of males. Our study of the spermatogenic cycle in Virginia *C. serpentina* cannot answer the broader geographic question, but it provides an additional geographic and climatic snapshot for male snapping turtle reproduction and allows a comparison of these data to other North American populations of this species and other sympatric turtles.

Methods. — Our sample of male snapping turtles (n = 55) derived largely from the southeastern quarter of Virginia. Most specimens were obtained from the Richmond area and locations south and southeastward to Virginia Beach with one from the central Piedmont. The sample was collected over six years (1979–84) by hand and turtle traps, primarily during March through July. Single specimens collected in February 1988 and August through October 1987 were included to ensure that all seasons were represented. At capture, most turtles were measured (straight midline carapace [CL] and plastron length [PL] to nearest mm) and testes were removed separately and preserved in 10% formalin. Some of the specimens were preserved and retained as museum vouchers; others were necropsied for parasites and discarded.

We used standard paraffin histology with both H&E and Berg's stains to examine the spermatogenic cycle. Several seminiferous tubule cross-sections were examined to determine relative abundance of spermatogonia, primary and secondary spermatocytes, spermatozoa, spermatids, Sertoli cells, and condition of the epididymis. Each testis was assigned a spermatogenic stage in the classification system of Mayhew and Wright (1970). Although this staging classification was developed for lizards, it offers an effective technique for summarizing the progressive differentiation of male sex cells in all reptiles. Our preference for this classification rather than McPherson and Marion (1981) is that the use of the lumen as a landmark allows slightly more precision in stage assignment, rather than trying to assess the relative abundance of different gametogenic cells. The Mayhew-Wright (1970) stages match the McPherson-Marion stages (1981) for turtles in an approximate manner: 2 = 2; 3–4 = 3; 5 = 4–5; 6 = 6; 7 = 7–8; no M-W stage for McP-M 1 (differentiation of McP-M 1 and 8 can be arbitrary). Mensural data (microns) included diameters of seminiferous tubules and epididymal ducts and the epithelial cell heights of the latter ducts. Tubule diameter for each turtle was an average of the diameters of ten tubules from a single testicular cross-section. All statistical analyses were performed in SYSTAT version 9 (SPSS Inc., 1999).

Results and Discussion. — The sample contained males ranging from 150 to 392 mm CL and 121–287 mm PL. The two smallest individuals with stage 2 spermatogenesis (primary spermatocytes at tubule lumen) were 168 and 175 mm CL. There was an 171 mm CL individual at stage 3 and an 170 cm CL turtle at stage 5. Four other small individuals (150, 154, 172, 178 mm CL) with pre-gametogenic testes were collected in June to August. These latter individuals had not reached sexual maturity, suggesting that male snapping turtles in southeastern Virginia attain sexual maturity between 168–178 mm CL.

The spermatogenic cycle (Table 1, Fig. 1) begins in April with spermatogonia present and abundant at the perimeter of the tubules (stage 1). The lumen is filled with Sertoli cells and an occasional spermatozoon. Lumens of some tubules are filled primarily with cellular debris. By May, spermatogonia are still actively dividing; a lumen has appeared and primary spermatocytes are the dominant cell type (stage 2). In June, individuals either show a dominance of secondary spermatocytes at the lumen margin (stage 3) or undifferentiated spermatids (stage 4); a few tubules have

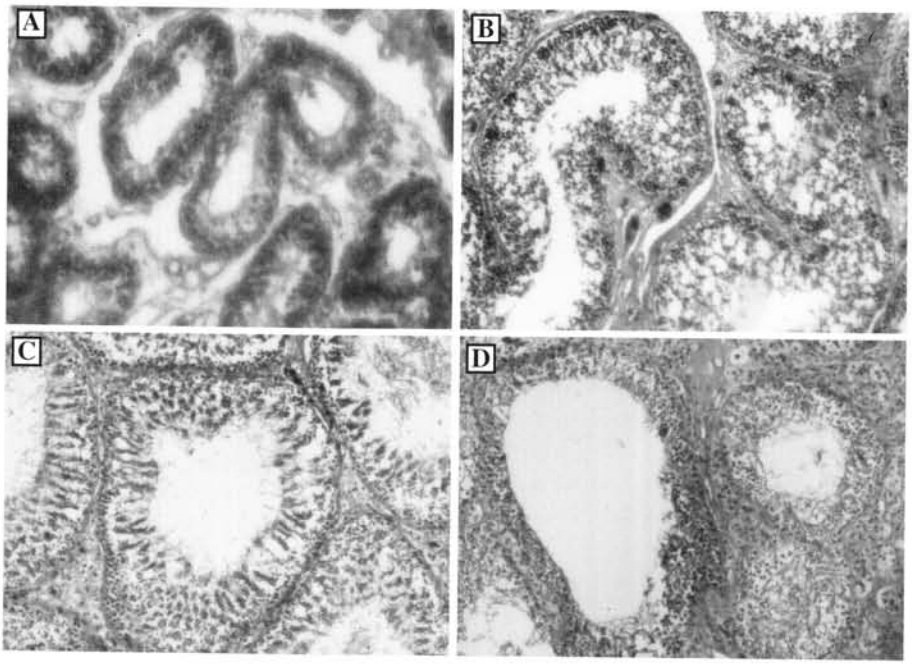

Figure 1. Examples of different spermatogenetic stages in male snapping turtles from southeastern Virginia. **A.** immature or stage 0, lumen developed with only spermatogonia present; **B.** stage 4, abundant spermatids at lumen margin with a few transforming, numerous secondary spermatocytes, about 2X the number of primary spermatocytes; **C.** early stage 6, abundant spermatids with many transformed to spermatozoa, filling the lumen in some tubule sections, primary and secondary spermatocytes still moderately abundant; **D.** stage 7, primary, secondary spermatocytes, and spermatids few, sperm modestly abundant and associated with Sertoli cells; sperm largely transferred to the epididymides.

transforming spermatids (stage 5). Throughout June and July, primary and secondary spermatocytes occur in all mature turtles. Mature spermatozoa begin to appear at the edge of the lumen in some individuals in June, and they dominate the lumen margins in August. By July, some samples already have a few small clusters of free spermatozoa in the lumen (stage 6), but the August and September samples have large bundles of free spermatozoa in the lumen (stage 6), as well as embedded in the margin of the lumen. Both October and November specimens show early phases of regression with the lumen containing small, scattered clusters of sperm, although most of the lumen content is cellular debris (stage 7).

Our sample of epididymides was smaller ($n = 35$) than the testicular sample and the variance of duct diameters within and among monthly or stage-segregated samples was higher than that of the tubule diameters (coefficient of variation = 12–30%, 38–70%, respectively for tubules and ducts) and nonhomogeneous among samples. The seasonal pattern of epididymal duct diameter and epithelial cell heights varies cyclically with spermatogenic activity. For duct diameter, the general pattern is large (median = 762, 794 µm) in stages 1 and 2 decreasing to a low plateau (276, 378, 309 µm) in stages 3 to 5 and enlarging (515 µm) in stage 6. The monthly cycle indicates that the large diameters seen in April decline in size through August, with a slight increase in September. Epididymal cell height matches the pattern of duct-diameter decline and enlargement. In the spring and at early spermatogenic stages, cell height is at its lowest and increases to its maximum in late summer and at stage 6.

The presence or absence of sperm in the epididymides in the spring samples (April–May) is seemingly contradictory, that is, some individuals have large volumes and a few individuals have none or a very small amount. We believe that reflects whether a male has mated or not. The amount

Table 1. Seasonal distribution of spermatogenic stages in adult *Chelydra serpentina* from southeastern Virginia. Blank entries indicate the absence of specimens for that month and stage; numbers equal the number of individual snapping turtles displaying a specific spermatogenic stage (Mayhew and Wilbur, 1970).

Month	Stage 0	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7	Stage 8
February			1						
April		7							2
May	1	1	8						
June			1	3	4	2			
July			1	1	3	3			1
August						6	1		
September				1			3		
October								1	
November								1	

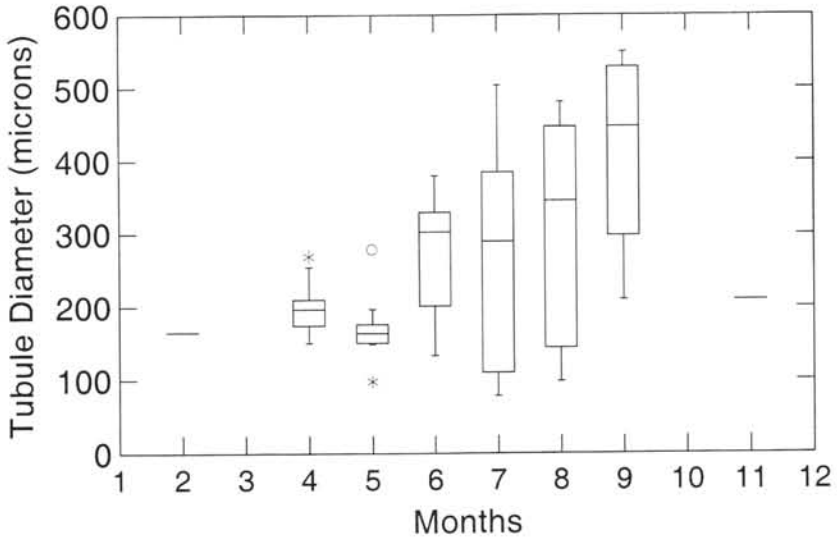

Figure 2. Seasonal changes in seminiferous tubule diameter of male snapping turtles from southeastern Virginia. The boxes in this graph represent the central 50% of the values of each monthly sample, the horizontal bar the median, and the vertical line the range of values except for outliers, asterisk and open circle.

of sperm is low or absent in June through September with only one late September specimen with sperm-packed ducts.

For the spermatogenic cycle, we include a stage 0 in Table 1. This stage is not defined by Mayhew and Wright (1970) and represents a pre-gametogenic stage, i.e., small seminiferous tubules without lumens and no or slight cell division. This stage represents sexually immaturity for most individuals; however, the situation for a 220 mm CL individual collected in May is enigmatic. It is 40 mm larger than the "average" size proposed for the base CL at sexual maturity; however, the simplest interpretation is that this turtle had not attained maturity. Three other outliers or out-of-phase individuals are: 1) stage 2 in February, CL = 235 mm; 2) stage 3 in September, CL = 172 mm; and 3) stage 8 in July, CL = 175 mm (Table 1). No explanation is obvious for the first individual other than it began spermatogenesis much earlier than other Virginia *C. serpentina*. The size of the latter two turtles suggests borderline maturity. Possibly, the stage 8 should be coded as stage 0, and the stage 3 turtle attained sexual maturity in early summer and is out of phase because of its "delayed" maturation during its first season of maturity.

Another facet of the spermatogenic cycle is the concordant cyclic increase in seminiferous tubule size and mass of the testes. Although some mass data were gathered, they are too preliminary and are not presented here. Seminiferous tubule diameter data (Fig. 2) adequately demonstrates increasing tubule diameter with gametogenesis through spermiogenesis (stage 6) and its rapid reduction thereafter. An ANOVA of the entire data set ($n = 55$) showed that the size changes were significant ($F = 10.398$, df 8, 46, $p < 0.001$). This change in diameters between individual stages, however, was not significant as revealed by a series of pairwise ANOVA tests for diameters of adjacent stages for the series from stage 1 through 6. Tubule diameter does change significantly from the immature tubule (stage 0) to the mature one (stage 1, $F = 36.471$, df 1, 10, $p < 0.001$). Early spermatogenic regression (stage 6 to 7) produces a rapid decrease in diameter (Fig. 1, $F = 10.074$, df 1, 4, $p = 0.034$). Adjusting the analysis for body size (ANCOVA model: Tubule diameter = constant + stage + CL) revealed that the change in tubule diameters between stages was statistically significant ($p \leq 0.05$) between most adjacent stages. Statistical differences in diameter existed between pairs 0-1, 2-3, 3-4, 4-5, 5-6, and marginally so for pair 6-7. The comparison of 1-2 and 7-8 were not statistically significant.

Male snapping turtles in southeastern Virginia reach maturity at 168–178 mm CL and 121–138 mm PL. Comparison among different North American populations (Table 2) suggests a trend of increasing size at maturity with higher latitudes. We hypothesize that this trend would appear more

Table 2. Carapace and plastron lengths (mm) of male *Chelydra serpentina* at sexual maturity. The italicized length measurements are converted values based on the 132% CL:PL relationship derived from Fig. 4 of Mosimann and Bider (1960).

Locality	Latitude	CL	PL	Source
Tennessee	36°	*191*	145	White and Murphy, 1973
Tenn. subadults	36°	*162-178*	123-135	White and Murphy, 1973
Virginia	37°	168-178	121-133	present study
Iowa	41°	*197-205*	149-155	Christiansen and Burken, 1979
Wisconsin	44°	*211-231*	160-175	Mahmoud and Cyrus, 1992
Quebec	45°	200-210	*152-169*	Mosimann and Bider, 1960

robust if matched to the duration of the average growing season. We also note that the criteria for maturity differ among the various studies. We assumed that early stages of spermatogenesis denote maturity; the other studies used either sperm in testes or epididymal ducts. That this different criterion yields a different maturity prediction is evident in the Tennessee sample (Table 2); the Tennessee subadults match better the Virginia adults than the Tennessee adults.

Only two other studies document the spermatogenic cycle of *C. serpentina*; however, both (Tennessee [White and Murphy, 1973]; Wisconsin [Mahmoud and Cyrus, 1992]) used a narrative approach to describing the annual cycle. Our stage-classification does not permit a precise comparison, so any differences that we note among the three populations may not be as distinct or even different. In general, the spermatogenic cycles in Tennessee and Virginia populations are identical, and the Wisconsin population begins spermatogenesis several weeks to a month later. By midsummer (July), however, the three populations are roughly synchronized. The delay probably arises from different hibernation emergence times (late March or early April in Virginia [Mitchell, 1994] and Tennessee and mid to late April [Vogt, 1981] in Wisconsin).

Spermiogenesis in *C. serpentina* begins in Virginia and Tennessee by late June and occurs in July into September in all populations. Males in the Tennessee population transfer sperm to the epididymides in late August to early September. In Wisconsin, sperm is most abundant in the epididymides from November onward. The limited Virginia sample suggests that sperm transfer occurs in mid-September. We are uncertain that these reported difference are actual differences in the cycle or difference in sampling and interpretation of histological sections.

We conclude that overall spermatogenesis in the northern half of *C. serpentina* range is a spring through summer process and that emergence from hibernation is closely linked to its initiation. Fall mating may result in successful insemination of females, although it seems likely that spring mating is more successful in fertilization.

Comparisons of the seasonal timing of spermatogenic events in *C. serpentina* with that of *Chrysemys picta* and *Sternotherus odoratus* from Virginia (Mitchell, 1985a, 1985b) show concordance in most respects. The male reproductive cycle in these turtles conforms to the typical postnuptial pattern of spermatogenesis. Seasonal changes can be described as follows: initiation of the spermatogenic cycle in April to early May with testicular enlargement and production of spermatogonia and primary spermatocytes, maximum testis size and peak production of mature sperm and its presence in testicular lumen in late July through September, and regression in late September and October with few spermatogonia and no spermatids. Seminiferous tubule diameters reach peak size in August in all three species. Epididymides contain mature sperm in fall months. Slight differences in timing among species can be attributed to individual variation and possibly that *C. serpentina* samples were obtained over several years.

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