Journal of the Arkansas Academy of Science

Volume 46

Article 8

1992

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John W. Robinette Arkansas State University

Stanley E. Trauth Arkansas State University

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Robinette, John W. and Trauth, Stanley E. (1992) "Reproduction in the Western Mud Snake, Farancia abacura reinwardtii (Serpentes: Colubridae), in Arkansas," *Journal of the Arkansas Academy of Science*: Vol. 46, Article 8. Available at: http://scholarworks.uark.edu/jaas/vol46/iss1/8

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REPRODUCTION IN THE WESTERN MUD SNAKE, FARANCIA ABACURA REINWARDTII (SERPENTES: COLUBRIDAE), IN ARKANSAS

JOHN W. ROBINETTE and STANLEY E. TRAUTH Department of Biological Sciences Arkansas State University State University, AR 72467-0599

ABSTRACT

The reproductive cycle of *Farancia abacura reinwardtii* was studied using samples of snakes collected throughout Arkansas from 1985 to 1991; museum specimens were also examined. The right testis of 22 males was examined by light microscopy. Histological analysis of the testis indicated a postnuptial spermatogenic cycle. Testicular recrudescence begins in late May with sperm production peaking in late summer; sperm overwinter in the ductus deferens. Ovarian follicles of 22 females were measured and counted; two follicular sizes were noted. In those undergoing primary vitellogenesis, a maximum size of 6.5 mm was reached; those exhibiting secondary vitellogenesis ranged in size from 12 to 21 mm. Average clutch size in females with follicles over 10 mm was 14.6 (n=6). Oviductal eggs were observed in a single female in early September. Female *F. abacura* require at least 2.5 years to become sexually mature. Follicular growth is slow over the first two years but increases dramatically during the spring of the third year.

INTRODUCTION

The western mud snake, Farancia abacura reinwardiii, is a largebodied, aquatic colubrid that ranges throughout much of the southeastern and southcentral United States (Conant and Collins, 1991). The mud snake inhabits shallow, soft-bottomed waterways with slow current, favoring cyprus swamps, sloughs, bogs, creeks, and marshes. Amphiumas and sirens are the preferred food of adults, although freshwater eels, frogs, tadpoles, aquatic salamanders, and fish are preyed upon (Ernst and Barbour, 1989).

The reproductive biology of F. a. reinwardtii has received very little attention (McDaniel and Karges, 1982; Mitchell, 1982); most accounts deal with morphological features, feeding habits, distribution, and habitat. The breeding season of this oviparous snake is not well understood with few and varying documented accounts of mating. Fitch (1985) stated that F. abacura is one of the most prolific North American snakes; however, clutch size records are few and not well distributed to show geographic variation. The Arkansas populations of F. a. reinwardtii occupy the northwesternmost extent of the range of the species; thus, data on the reproductive biology in Arkansas can contribute to a better evaluation of the variation in this wide-ranging species.

The present study was undertaken to document the reproductive biology of the species in Arkansas. We specifically investigated the timing of the testicular cycle as well as the type of ovarian cycle in females.

MATERIALS AND METHODS

Mud snakes were collected throughout Arkansas from 1985 to 1991 with additional individuals coming from preserved museum specimens (n = 44). Snakes were collected alive or dead on the road. Live snakes were given a lethal injection of sodium pentobarbital and fixed in 10% formalin. All snakes were sexed, and the SVL and tail length were recorded. Snakes were preserved in 70% ethanol and deposited in the Arkansas State University Museum of Zoology. The right testis and attached epididymis of 22 males were removed and prepared for light microscopy. Tissues were dehydrated in a graded series of ethanol and cleared in xylene. The testes were then embedded in paraffin, sectioned serially at 8 μ m, and stained with Harris' hematoxylin and cosin. Each testis was examined by light microscopy noting testicular stage and presence of sperm in the ductus deferens. Seminiferous tubule diameters were measured using an ocular micrometer. The reproductive tracts of 22 females were examined macroscopically. The diameters of ova were measured to the nearest 0.1 mm with vernier calipers, and the number of variously-sized follicles was counted. Standard statistical data were obtained from all measurements; means are accompanied by ± 2 standard errors.

RESULTS

MALE TESTICULAR CYCLE

The left testis of the mud snake lies about one-third of the body length anterior to the vent. The testis is composed of a coiled mass of seminiferous tubules held together by a thin fibrous tunica albuginea. The intertubular spaces contain many interstitial cells, blood vessels, and some connective tissue cells. The seminiferous tubules contain numerous Sertoli (nurse) cells in variable amounts within a syncytium of germinal cells; germinal cell types fluctuate seasonally during the annual testicular cycle (Fox, 1952).

The ducti deferentia, which consist of tall-to-flat epithelial cells, function in overwinter storage of spermatozoa. Spermatozoa in the ductus deferens were present in large quantities upon emergence from hibernation in the early spring, whereas the testes were completely regressed.

Seminiferous tubules exhibited mainly Sertoli cells and spermatogonia in April. Lumina of seminiferous tubules were occluded by the spermatogonia/Sertoli cell syncytium and large amounts of lipid droplets (Fig. 1). The ductus deferens were completely packed with sperm at this time (Fig. 1). This condition remained throughout April. Spermatogenesis began in May (Fig 2). The seminiferous tubules exhibited primary spermatocytes as the dominant cell type. By mid-May one individual had a few secondary spermatocytes, and another individual had started producing secondary spermatocytes by the end of May. While the ductus deferens remained packed with sperm, the amount of lipid material within the tubules gradually disappears. In June secondary spermatocytes were the dominant cell type within the lumina of the seminiferous tubules (Fig. 2). Lipid droplets had become very scarce, and the ductus deferens, which had been packed with sperm until this time, had few or no sperm present. Only one individual was examined for the month of July. It possessed transforming spermatids, no lipid droplets, and scattered cellular debris in the ductus deferens (but no sperm). For the month of August there again was only one individual. In this male, mature sperm were present within



Figure 1. Photomicrographs of testis and epididymis of Farancia abacura reinwardtii from Arkansas. A. Seminiferous tubules of adult male collected in early April. Seminiferous tubules interspersed with lipid droplets (see arrowheads); germinal epithelium in regressed stage. B. Epididymis of male in early April. Ductus deferens packed with sperm.



Figure 2. Photomicrographs of seminiferous tubules of Farancia abacura reinwardtii from Arkansas. A. June specimen showing secondary spermatocyte (SS) at luminal margin; PS = primary spermatocyte. B. May specimen showing secondary spematocyte surrounded by evacuated lipoidal droplets (LD). C. July specimen in late stages of spermatogenesis exhibiting transforming spematids (SP). D. Postspermiogenic male collected in late summer; lumen present and germinal Sertoli cell syncytium reduced to several cell layers in thickness.

the lumina of seminiferous tubules, (no lipids present), and once again there were sperm in the ductus deferens. Because of our small sample size for the late summer and autumn months, it is difficult to ascertain exactly when spermatogenesis peaks; however, we believe peak spermatogenesis occurs in late July and August (see Discussion). No individuals were collected throughout the remainder of the year. Testicular regression is assumed to be completed in the fall.

The diameter of 30 mostly-circular seminiferous tubules per testis were measured. Each individual was categorized according to the cell type at the luminal margin. The mean tubule diameter with spermatogonia as the dominant cell type was $0.112 \text{ mm} \pm 0.01 (0.101 - 0.142)$. In tubules with primary spermatocytes as the dominant cell type we found a mean diameter of $0.125 \text{ mm} \pm 0.02 (0.086 - 0.197)$. Tubules with secondary spermatocytes present had a mean diameter of $0.116 \text{ mm} \pm 0.01 (.100 - 0.143)$. One individual with transforming spermatids present had a tubule diameter of 0.111 mm. The one individual with mature sperm present exhibited a tubule diameter of 0.173 mm. No obvious difference was observed between tubule diameters during the progressive stages of spermatogenesis.

FEMALE OVARIAN CYCLE

There are four basic size groups of ova in female F. a. reinwardtii. The first, group I, included follicles less than one mm in diameter (and were not counted or measured). Group II ova had a mean diameter of 4.19 mm \pm 0.30 (range, 1.52 - 6.12). Group III ova had a mean diameter of 16.57 mm \pm 1.32 (12.90 - 20.58). One female contained oviductal eggs, or group IV ova. We observed that older females (those with a greater SVL) underwent secondary vitellogenesis earlier in the year (Figs. 3 and 4) than less mature individuals (those with a smaller SVL).









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We also observed individuals with enlarged follicles from mid-May to mid-July with one individual with oviductal eggs in early September (Fig. 3).

Mean clutch size using ova greater than 8 mm (group III ova) was 14.67 ± 1.94 (range, 8 - 20). This is approximately half the average number of group II ova present in each female. The mean clutch size of ova under 8 mm (group II ova) was 33.5 ± 3.44 (21 - 59).

DISCUSSION

Because of our lack of specimens during certain times of the year (i.e., late summer and autumn), we were unable to fully document the complete reproductive cycle. To better understand what course of events the testicular cycle of F. a. reinwardtii undergoes, we relied on the literature of previously described snake reproductive cycles. Our study indicated the following: 1) the testes are completely regressed upon emergence from hibernation in April with the ducti deferentia packed with sperm, 2) primary spermatocytes are produced during May and secondary spermatocytes appear by early June with few to no sperm in the ductus deferens, 3) in July transforming spermatids were observed, and 4) by early August the testes had mature sperm present in the lumen and showed early signs of regression. With this in mind, the known part of the testicular cycle of F. a. reinwardtii closely parallels that of a number of other snakes. The ringneck snake, Diadophis punctatus, begins its annual testicular cycle in March which is earlier than that of Farancia, and regression begins in late autumn (Myers, 1965). This same pattern was observed in the prairie rattlesnake, Crotalus viridis, (Aldridge, 1979a). The striped racer, Masticophis lateralis, has a testicular cycle in which recrudescence (proliferation of spermatogonia) begins later than that of Farancia; i.e. recrudescence begins in late June with spermatogenesis ending before hibernation and sperm overwintering in the ductus deferens (Goldberg, 1975). Both the whipsnake, Masticophis taeniatus, and the bullsnake, Pituophis catenifer (syn. P. melanoleucus), have testicular cycles that also closely resemble that of F. a. reinwardtii. There is an early summer recrudescence and late summer and autumn regression with sperm overwintering in the ductus deferens (Goldberg and Parker, 1975); other similar colubrid snakes include the worm snake, Carphophis vermis (Aldridge and Meter, 1973), the Pacific Coast garter snake, Thamnophis elegans (Fox, 1952), and the glossy snake, Arizona elegans (Aldridge, 1979a). The cottonmouth, Agkistrodon piscivorus, (a viper) also has this same cycle (Johnson et al., 1982). A reproductive cycle of the aestival type is very common among the Colubridae and is probably the most prevalent kind in temperate regions (Saint Girons, 1982). Farancia does not fit the pattern of the coral snake, Micrurus fulvius. In this species recrudescence begins in early summer, but spermatogenesis is interrupted by hibernation causing sperm to mature in the early spring before copulation (Quinn, 1979). By using comparisons with these testicular cycles, F. a. reinwardtii probably undergoes peak spermatogenesis in mid-to-late summer and is followed by an autumn regression.

We observed that F. a. reinwardtii testes in their regressed state (upon emergence from hibernation) contained large numbers of lipid droplets in the lumina of the seminiferous tubules. These lipid droplets began to decrease in size and number as spermatogenesls proceeds. By June nearly all of the lipid material was depleted. This lipid cycle is inversely related to the spermatogenic cycle in that lipid concentration is highest during times of no spermatogenic activity, and in lowest concentration during maximum sperm production. This lipid cycle has also been observed in the checkered water snake, Natrix piscator (Sfivastava and Thapliyal, 1965); the striped racer, Elaphe taeniura (Chu and Wang, 1974); and the cobra, Naja naja (Lofts, 1966). The lipid material in Naja naja starts to appear as soon as testicular regression begins. Lipids reach their maximum concentration (size and number of droplets) just before testicular recrudescence. The last of the lipid droplets are then sloughed during the maturation of spermatids into spermatozoa (Lofts, 1966). Chu and Wang (1974) and Lofts (1966) indicated that lipid depletion was an indication of androgen production and release.

In female F. a. reinwardtii we observed the four basic size groups of

ova. Betz (1963) provided a description of these size groups that he observed in the diamondbacked water snake, Nerodia rhombifer. In N. rhombifer group I follicles measured 0.1 mm in diameter (as we observed in F. a. reinwardtii). Betz (1963) also described group II follicles as ranging in size from 5 to 10 mm in Nerodia; we also observed a range of 1.52 - 6.12 mm in F. a. reinwardtii. Group III ranged from 10 - 20 mm in Nerodia and ranged from 12.90 -20.58 mm in F. a. reinwardtii. At the end of a females first full year one would expect to find group I and II follicles present. At the end of the second year, group I, II, and III follicles are present. During the early summer of the third year (2.5 actual years), the group III follicles rapidly enlarge and become group IV, (i.e., oviductal eggs). Each year a new crop of group I follicles emerge from the germinal epithelium. The group I follicles from the previous year will then enlarge to become group II follicles and so on. Betz noted a decrease in the number of follicles each time follicles advanced to the next group. This was also observed in F. a. reinwardtii. He contributed this loss to atresia. There are approximately three times as many previtellogenic follicles as oviductal eggs in Nerodia sipedon (Aldridge, 1982). Farancia a. reinwardtii falls into Aldridge's (1979b) type I ovarian

Farancia a. reinwardtii falls into Aldridge's (1979b) type I ovarian cycle. In this cycle yolk deposition occurs in two stages. Primary vitellogenesis occurs in follicles of group I causing them to advance to group II. Secondary vitellogenesis occurs in group II causing them to advance to group III; in this stage they will mature in time to form oviductal eggs or group IV follicles. Type I ovarian cycles have been described in Arizona elegans, Diadophis punctatus, Pseudonaja nuchalis, Thamnophis proximus, T. radix, Nerodia sipedon, N. rhombifer, and Pseudechis porphyriacus (Aldridge, 1979b).

ACKNOWLEDGMENT

We thank the Milwaukee Public Museum for kindly loaning us specimens and the Arkansas Game and Fish Commission for financial support during this study. Mr. Charles Robinette is thanked for his field assistance.

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