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Abstract

We examined 91 adult female Ouachita dusky salamanders (*Desmognathus brimleyorum*) to determine the seasonal incidence of sperm within spermathecae. The spermatheca (sperm storage gland) along with supporting tissue was removed from the dorsal cloacal wall of each female and prepared for light microscopy. We recorded the reproductive condition of females (diameter of enlarged ovarian follicles = EOF) and found large aggregates of sperm within the spermathecae during all months, except February (no specimens available). The highest incidence of sperm in spermathecae occurred in July specimens (53%; n = 17). Although the known nesting season runs from July into August in this species, the mating season does not appear to be restricted to spring and summer months. Moreover, females in any month with EOF may or may not possess sperm.

Introduction

The Ouachita dusky salamander, *Desmognathus brimleyorum*, is a large, semi-aquatic plethodontid salamander which ranges throughout the Ouachita Mountains of Arkansas and Oklahoma (Conant and Collins, 1998). Available information on the reproductive biology of this species has been reviewed by several authors (Means, 1999; Petranka, 1998; Trauth et al., 2004), although Petranka (1998) indicated a lack of reproductive information. Both sexes breed annually, but no study has clearly delineated the exact timing and duration of the breeding season (Petranka, 1998). In fact, a combination of several seasonal data sets of information on females as well as on larval size and growth is necessary to clarify this species' breeding phenology. Trauth et al. (1990) reported on the annual oogenic cycle through seasonal sampling of females by examining the total number of ovarian follicles produced, the maximum ovum size, and ovarian clutch size. This kind of data was used in another study (Taylor et al., 1990) to determine a gonosomatic index (GSI) for the species. The GSI peaked during the height of vitellogenesis or growth of ovarian mass in July and, as expected, occurred just prior to ovulation and presumably the onset of oviposition (Taylor et al., 1990; Trauth, 1988). Observations on the presence of nesting females (or egg clutches) and possibly the size of developing embryos have provided an indication of the overall nesting period (Taylor et al., 1990; Trauth, 1988). Herein, we report on the seasonal incidence of sperm within the spermathecae of *D. brimleyorum*. In addition, we include reproductive information on the ovarian cycle of all adult females. These data add critical life-history information that is currently lacking within the reproductive database of this desmognathine salamander.

Materials and Methods

Most of the 91adult female *D. brimleyorum* ($n = 77$) used in this study were collected over a five-year period (1980-1984), and 57% (n = 44) were sampled from May to December, 1980. All specimens were taken from Polk and Montgomery counties and are currently deposited as voucher specimens in the Arkansas State University herpetological collection (ASUMZ). Additional specimens were obtained from the ASUMZ. Salamanders were sacrificed in a dilute chloretone solution within 24-48 hr following capture, fixed in 10% formalin, and preserved in 70% ethanol. The diameter of enlarged (vitellogenic) ovarian follicles (EOF) in each female was also measured to the nearest 0.1 mm with a set of vernier calipers or with the aid of an ocular micrometer. Mean values, when provided, are accompanied by ± 1 standard deviation.

Following preservation, the snout-vent length of each specimen was measured from the tip of the snout to the anterior margin of the vent (range, 62-83 mm; mean = 72.3 ± 5.5). The spermatheca along with supporting tissue was then removed from the cloacal region with a razorblade, and tissue slabs were placed into vials of 70% ethanol. Tissues were dehydrated in a graded series of ethanol, cleared in xylene, embedded in paraffin, sectioned with a rotary microtome into ribbons 8 µm in thickness, stained with Harris hematoxylin, and counterstained with eosin. The descriptive histology of the spermathecal gland of *D.*

brimleyorum has been described elsewhere (Sever and Trauth, 1990) and, thus, is not given herein. All histological slides are deposited in the Arkansas State University Center for Microscopy.

Results

We found large aggregates of sperm (Fig. 1A) within spermathecal sacs of 26 specimens collected from December through August (no February specimens available). Nearly all of these specimens (n = 25) possessed EOF which averaged ≥ 2 mm in diameter (Fig. 2). During the same time period, nearly an equal number of females with EOF ≥ 2 mm in diameter (n = 29) lacked any evidence of sperm within their spermathecae (Fig. 2). We also found 14 females during that time period that exhibited a very small amount (trace) of sperm within their spermathecae (Fig. 1B; 2). Most of these females (n = 8), however, exhibited EOF ≤ 2.0 mm in diameter (Fig. 2). In addition, five females collected from September through November showed a trace of sperm. Number of females with sperm is shown in Fig. 3. For instance, from April through July, the number of females possessing an abundance of sperm generally increased to a peak in July (9/17, 53%), whereas during the same time period the number of females lacking sperm remained greater than the other two categories (except for July values). The percent of total females showing as absence of sperm was also highest from April through July. The number of females possessing sperm decreased sharply following July, but, at the same time, the number of females exhibiting a trace of sperm increased.

Discussion

Long-term sperm storage remains a poorly-studied aspect of the biology of many species of salamanders that exhibit internal fertilization. In a review of urodele courtship and mating glands, Sever (2003) pointed out that very few studies provide a critical analysis of the mating season by noting the presence of sperm within the spermatheca or sperm storage gland(s) found within the roof of the female's cloaca. The timing of mating in plethodontid salamanders can be inferred from a histological examination of the spermathecae (Sever, 2000); the duration of sperm storage can also be derived from an adequate seasonal sample of adults (e.g., Meshaka and Trauth, 1995; Trauth, 1983, 1984). As a general rule, any salamander reproductive researcher attempting to determine the length of sperm retention in females should not only document the duration of the seasonal ovarian cycle, but should also concurrently, examine spermathecal sperm storage (Sever, 2000, 2003).

Trauth (1988) examined the spermathecae of three nesting female *Desmognathus brimleyorum* collected in late July and in mid August and found only traces of residual sperm and the presence of small ovarian follicles (≤ 1.3 mm in mean diameter). We also found females (Fig. 2) exhibitin similar conditions following a probable ovipositional perio (late June – mid July); however, these same morphologica features existed in other females collected during th spring months. Sever (2003) reviewed spermiophagy, a phe nomenon that occurs within the spermathecal epitheliun and the lumina of spermathecal tubules in some species o salamanders. Spermiophagy provides the spermatheca means of removing degenerating sperm prior to the nex mating season (Sever et al., 2001). Our findings suggest, however, that viable sperm may be retained within the spermatheca for a prolonged length of time. This time frame may occur from immediately following oviposition to, and possibly including, the time of sperm transfer from spermatophores during the next mating season (i.e., a time span of approximately one year extending from one ovi-positional period to the next). Tilley and Hausman (1976), using genotypic comparisons of females and their offspring, determined that multiple inseminations occurred at least 7% of the time in a population of a congeneric desmognathine *D. ochrophaeus.* Moreover, Houck and Schwenk (1984) examined the spermathecae of pre- and post-ovipositional *D. ochrophaeus* and, because of the abundance of sperm in their spermathecae, indicated the strong possibility of sperm competition in this species. The presence of residual sperm throughout the year in *D. brimleyorum* (Fig. 2), therefore, suggests long-term sperm storage and the possibility of sperm competition and multiple paternities in this species. Sever and Hamlett (1998) suggested that residual sperm of desmognathine salamanders may become embedded in spermathecal epithelial cells and are eventually degraded. Whether residual sperm in *D. brimleyorum* are actually capable of fertilization must await future investigations.

Taylor et al. (1990) found that the GSI of male *D. brimleyorum* peaks in August, which means that testicular size had reached maximum size and that sperm can begin evacuating the testes to be stored in the vasa deferentia for mating. We found large aggregates of sperm within the spermathecae of several winter specimens (Fig. 2; n = 5). These findings provide credible evidence that post-ovipositional insemination does occur and occurs much earlier than a previously-assumed, spring/early summer mating season.

In conclusion, the present study found support for post-ovipositional, long-term sperm retention in *D. brimleyorum.* Insemination may also occur in some females during the winter months. Females that undergo their oogenic cycle may do so with or without the presence of stored sperm in their spermathecae. Future studies can add greatly to the growing reproductive database for this species by elucidating the span of the nesting season and by determining larval growth increments during all seasons of the year.

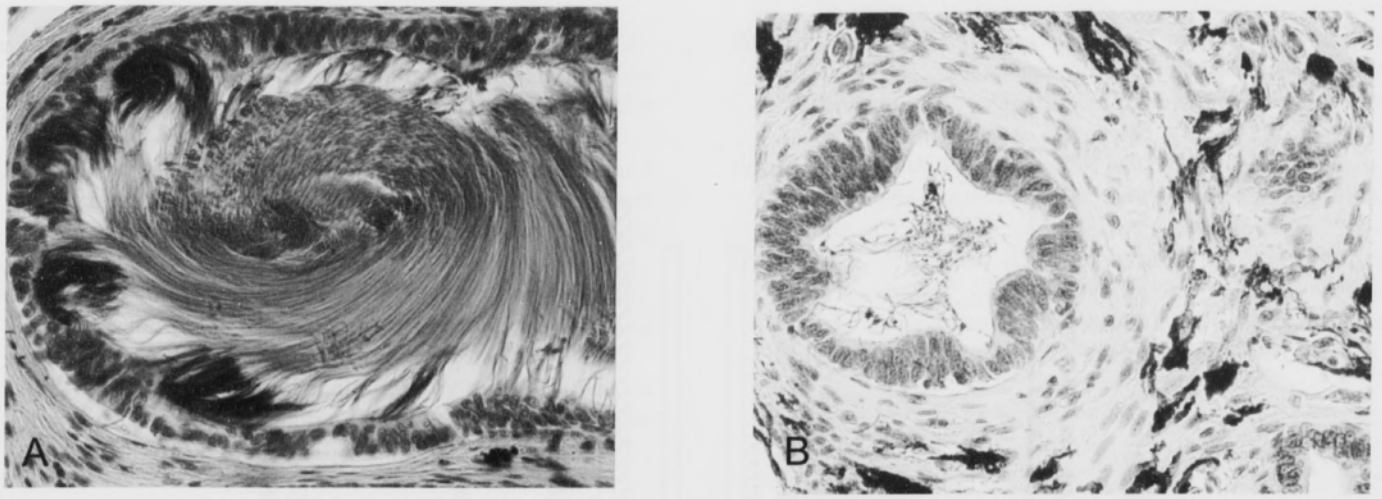

Fig. 1. Spermathecal sacs illustrating an abundance of sperm in a June specimen (A) compared to a trace of sperm in a March specimen (B).

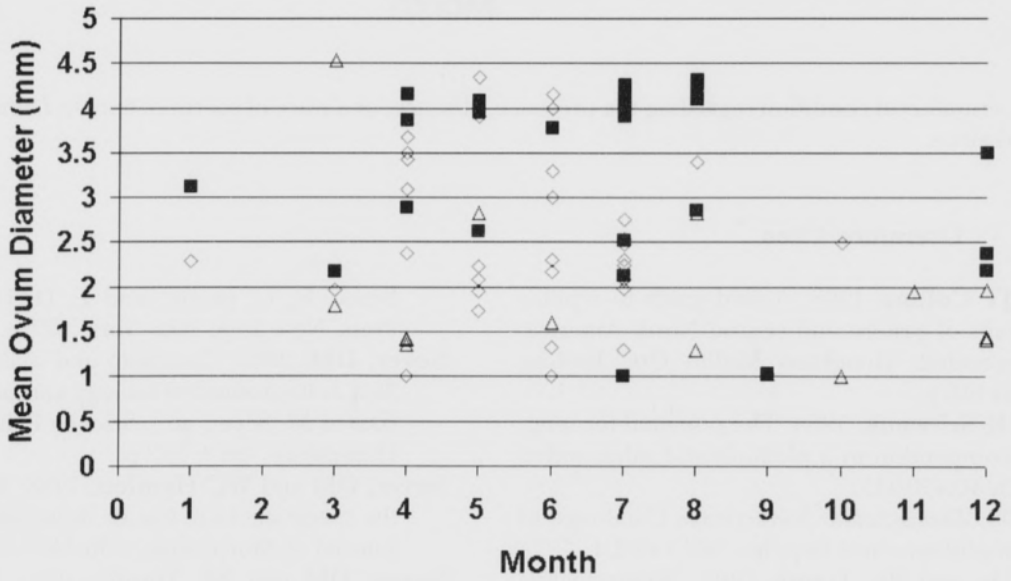

Fig. 2. Presence of sperm (solid squares), trace of sperm (triangles), and absence of sperm (diamonds) within spermatheca vs. mean ovum diameter (mm) in monthly samples of *Desmognathus brimleyorum* from Arkansas. (Some ovarian data were extracted from Trauth et al. [1990]).

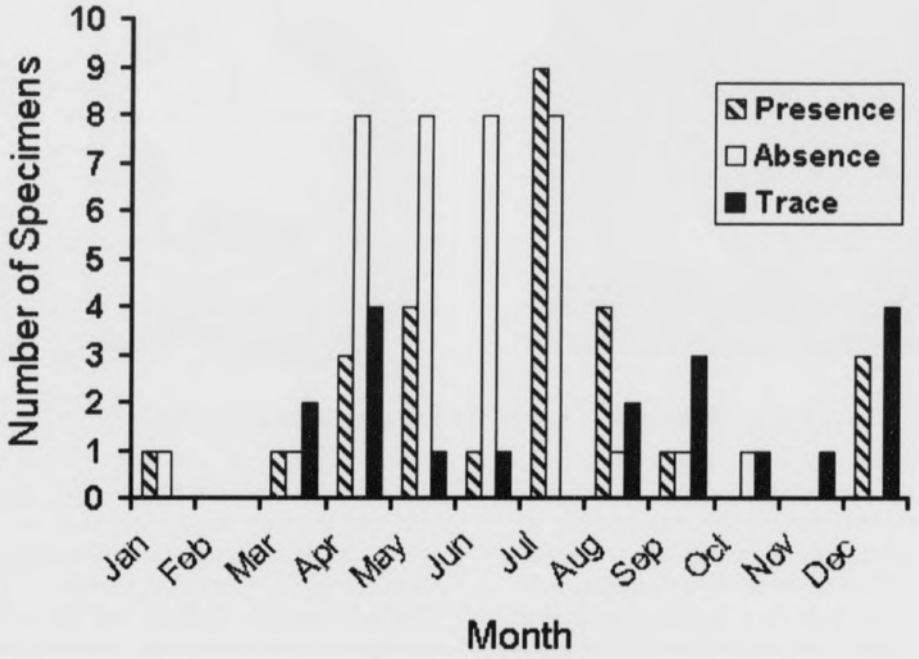

Fig. 3. Monthly spermathecal condition regarding the presence, absence, or a trace of sperm of female *Desmognathus brimleyorum* collected from Arkansas.

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