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The reproductive cycle of male Eastern Newts (*Notophthalmus viridescens*) from a high-elevation population in North Carolina, U.S.A.

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Abstract. The reproductive cycle of male *Notophthalmus viridescens* from a high-elevation population in North Carolina, U.S.A., was outlined through histological examination of testes, sperm ducts, and secondary sexual characteristics (genial glands and collecting ducts of the kidneys). Similar to other populations of *N. viridescens*, proliferation of spermatogonia was a spring event with the meiotic stages of spermatogenesis restricted to the months of June through August (summer months). Spermiation immediately followed in September with sperm filling the Wolffian ducts. Spermiation continued until the following summer where mature sperm from the previous summer could still be found in cranial portions of testes while the more caudal portions of testes were undergoing meiosis to produce sperm for the subsequent season. This overlap of spermatogenic cycles was not observed in studies of *N. viridescens* from other populations. Sperm were found in Wolffian ducts until the following July, indicating potential for mating from September to July. Secretory granules filled epithelial cells of genial glands and kidney collecting ducts from September to July, providing support for this prolonged potential mating season. In other populations of *N. viridescens*, secondary sexual characteristics were atrophied for a longer window of time, at minimum July through August. In culmination, while the spermatogenic cycle of high-elevation North Carolina populations of *N. viridescens* was found to be similar to that of other populations, the mating season was potentially extended. Considering mating in *N. viridescens* was typically reported as a fall through spring event, the ecological factor most responsible for driving this extended mating window was potentially protracted exposure to cooler temperatures at higher elevations.

Key words. Amphibia, Caudata, newt, Salamandridae, testis, spermatogenesis, spermatozoa, mating.

Introduction

The purpose of the current project is to assess the reproductive cycle of male Eastern Newts (*Notophthalmus viridescens*, Salamandridae, Urodela) from a high-elevation population in North Carolina, U.S.A. Previously, the complete male reproductive cycle of *N. viridescens* was outlined from two other populations in Massachusetts (Adams 1940) and Missouri (Siegel et al. 2012). Whereas numerous studies on salamander reproductive cycles were conducted to better understand their natural history and compare populations inter-specifically, we know of no previous study that compared the reproductive cycles between three distinct populations of the same species group. These types of comparisons are paramount to better understand the variation and drivers of reproductive cycle timing within a species.

The reproductive cycle of male salamanders is composed of two major events: spermatogenesis and mating (e.g., Adams 1940, Siegel et al. 2012). Besides in some tropical lineages (Houck 1977), these two events of the reproductive cycle are typically separated temporally in salamanders, with sperm production occurring prior to mating (Alvino & Siegel 2017, Davenport et al. 2022, Siegel et al. 2012, 2020). During the height of spermatogenic activity, testes of salamanders typically enlarge as spermatogonia proliferate and transition to spermatocytes (spermatocytogenesis), spermatocytes divide meiotically to form spermatids (spermatidogenesis), and spermatids differentiate into mature spermatozoa (spermiogenesis). Because male salamanders often communicate with females during the mating season with pheromones, pheromonal integumentary glands hypertrophy in males during mating activity; e.g., mental glands in plethodontid salamanders (Sever 1975) and genial glands in salamandrids (Siegel et al. 2012). Cyclical changes of primary (testis) and secondary (e.g., pheromone glands) sexual characteristics are pri-

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marily controlled by environment (e.g., temperature) and hormonal (e.g., hypothalamal-pituitary-gonadal axis) interplay (Norris 2024).

In a literature review of all the spermatogenic cycles of plethodontid salamanders, Siegel et al. (2014a) concluded that little variation exists between species across the entirety of North America. With few exceptions (see Davenport et al. 2022, Ireland 1974, 1976), plethodontid salamanders undergo the major meiotic divisions of spermatogenesis in summer. In general, sperm production is a summer event, and mating is a fall through spring event, with females often storing sperm from multiple males from potentially early fall mating to late spring ovulation (Sever 2002). This same pattern was also reported for two populations of a salamandrid species, *Notophthalmus viridescens*, from central Missouri (Siegel et al. 2012) and central Massachusetts (Adams 1940). The similarity of male reproductive cycle events across wide geographic areas between species may be evidence that reproductive cycle timing in salamanders lacks major geographic variation, even in disparate populations within a species.

The current study intends to examine this hypothesis further by outlining the timing of male reproductive cycle events in a population of *Notophthalmus viridescens* from the highest elevation population of newts recorded from the Appalachian Mountains of North Carolina, U.S.A. Mainly, are the timing of reproductive events from this high elevation environment in North Carolina (Ashe County, approximate elevation 1,500 m a.s.l.) different from those of lower elevation populations of Missouri (Crawford County, approximate elevation 230 m a.s.l.) and Massachusetts (South Hadley, approximate elevation 82 m a.s.l.)? To do this, we assessed the testicular cycle to track spermatogenic activity throughout the year, and sperm transport and secondary sexual characteristic atrophy/hypertrophy to outline potential mating and non-mating seasons.

Materials and methods

From March 2019 to February 2020, 23 aquatic adult male *Notophthalmus viridescens* were collected from ponds in Ashe County, North Carolina at Pond Mountain Gamelands. This collection was composed of two adult males per month except for February, where only one adult male was obtained. After capture, all specimens were sacrificed in a lethal dose of MS-222 (Western Chemical, Inc., Ferndale, WA) and fixed in buffered formalin for histological examination. Specimen snout through vent length (SVL) ranged from 47.8 to 58.4 mm (mean = 51.35 mm, SD = 2.47). All specimens possessed easily observable testes, opaque Wolffian ducts, and typical male secondary sexual characteristics (e.g., enlarged, keratinized hind legs).

The entire urogenital tract and integument of the cheek region were excised from every individual, rinsed in deionized water, dehydrated, cleared in toluene, and embedded in paraffin wax. A rotary microtome was used to collect 8 μm frontal sections from testes and their associated Wolffian ducts, and transverse sections from cheek integument/kidneys. Sections were subsequently placed on albumenized slides and then stained with hematoxylin and eosin for general histological examination.

The spermatogenic cycle was assessed qualitatively. The presence/absence of testicular germ cells in different stages of spermatogenesis (following Uribe 2003) and the presence/absence of Wolffian duct sperm were recorded from each individual. These data were aggregated in a qualitative chart to visualize the annual cyclicity of sperm production and transport to compare to the spermatogenic/sperm transport cycles previously reported from *N. viridescens* by Adams (1940) and Siegel et al. (2012).

To assess the seasonality of breeding gland activity, tubular diameters were measured from 10 gland tubules from each excised tissue for every individual, and mean tubular diameter was calculated for every month. Glands included genial glands that produce pheromones (Adams 1940, Siegel et al. 2012) and kidney collecting ducts that currently have an unknown function but have been hypothesized to be involved in mating because secretions from these glandular tubules mix with sperm in the cloaca during spermatophore formation (Alvino & Siegel 2017, Siegel et al. 2012, 2024). The tubular diameter of these glands increases when epithelial cells lining the tubules synthesize products and hypertrophy (Alvino & Siegel 2017). These data were then graphed with month of capture on the x-axis and mean tubular diameter as a ratio to mean SVL on the y-axis to provide a seasonal visualization of hypertrophy and atrophy of glands involved in reproduction. These graphical depictions were then compared with those previously reported for *N. viridescens* by Adams (1940) and Siegel et al. (2012).

Results

Similar to previous reports on *Notophthalmus viridescens* testes (e.g., Humphrey 1926), we found that many of the specimens possessed testes with multiple lobes. Testicular lobes ranged from one to four per individual testis (N = 23, mean = 1.8, SD = 0.9), never varying by more than one between right and left, and testicular lobes of an individual were always in the same stage of spermatogenesis; i.e., the stage of developing sperm cysts in individual compartments of each lobe (lobules) were always represented when comparing between lobes. Nine individuals possessed single-lobed testes, seven possessed two, and two possessed three. Two individuals possessed three lobes of the right testis and two of the left, and two individuals possessed four lobes of the right testis and three of the left. Only one individual with differential testicular lobes between right and left testes possessed more lobes of the left testis than the right, and this individual possessed two lobes of the left testis and one of the right. Testicular lobes were connected by a narrow band of connective tissue. Multiple testicular lobe number was previously linked to age in salamanders (e.g., Humphrey 1926, Sever 1974),

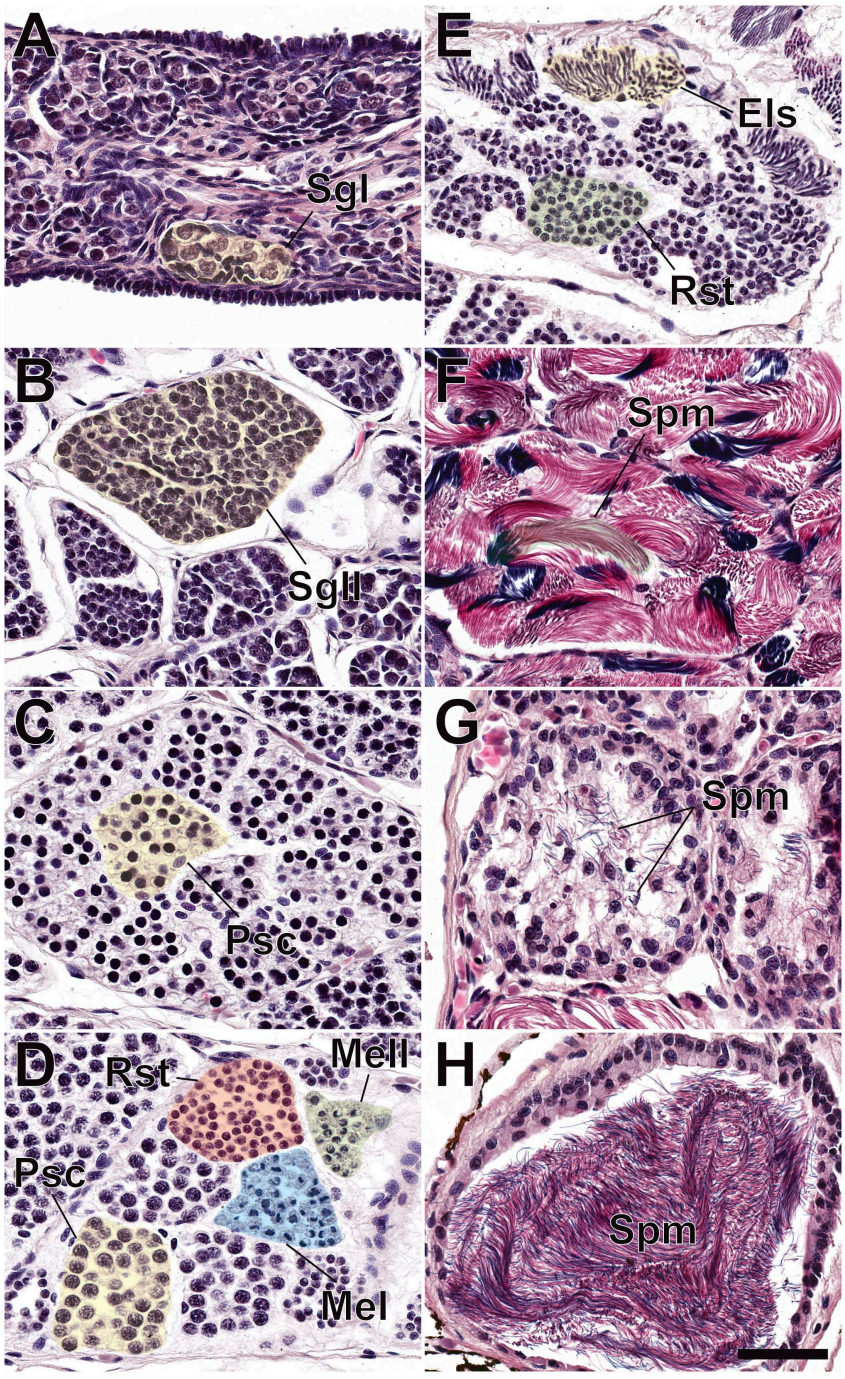

Figure 1. Representative sections detailing cystic stages from the lobules of *Notophthalmus viridescens* testes (hematoxylin and eosin). (A) A lobule is highlighted that consists entirely of primary spermatogonia (SgI) and their associated Sertoli cells (April specimen). (B) A lobule is highlighted that consists entirely of secondary spermatogonia (SgII) and their associated Sertoli cells (July specimen). (C) A lobular cyst is highlighted that consists entirely of primary spermatocytes (Psc) and associated Sertoli cells (July specimen). (D) Four cystic stages are highlighted from the same lobule (July specimen): cysts that consist entirely of primary spermatocytes (Psc) and associated Sertoli cells; primary spermatocytes undergoing meiosis I (MeI) and associated Sertoli cells; secondary spermatocytes undergoing meiosis II (MeII) and associated Sertoli cells; round spermatids (Rst) and associated Sertoli cells. (E) Two cystic stages are highlighted from the same lobule (July specimen): cysts that consist entirely of round spermatids (Rst) and associated Sertoli cells; elongating spermatids (Els) and associated Sertoli cells. (F) A lobular cyst is highlighted that consists entirely of mature sperm (Spm) and associated Sertoli cells (February specimen). (G) A spermiated lobule filled only with Sertoli cells at its border and a few remaining sperm (Spm; January specimen). (H) Sperm (Spm) packed into a cranial portion of a Wolffian duct (January specimen; scale bar = 100 mm, applies to A–H).

and we found a positive direct correlation between SVL and total testicular lobes (R^2 = 0.3069), supporting this previous assertation.

From September to April, clumps of primary spermatogonia (Fig. 1A) and lobules containing cysts of secondary spermatogonia (Fig. 1B) were prominent features of *N. viridescens* testes from our collection, and these cell types were persistent at lower quantities in the testes the rest of the year. Primary spermatocytes (Fig. 1C) were first observable within cysts of testicular lobules of our specimens in April. Actively meiotic cells did not appear until June where primary and secondary spermatocytes progressing through meiosis I and II were first apparent (Fig. 1D). Round spermatids and elongating spermatids (Fig. 1E) were also first observable in June. These four germ cell stages persisted in lobules of testes until August, at which point testicular lobules of specimens from subsequent months were primarily filled with cysts of mature-looking sperm (Fig. 1F). Lobules with mature-looking sperm decreased in number from September to July as the number of spermiated lobules (Fig. 1G) increased over this time. This increase in spermiated lobules coincided with the appearance of sperm in Wolffian ducts (Fig. 1H). During months of spermiation, lobules in the caudal portion of testes contained only primary and/or secondary spermatogonia. Proliferation of spermatognia within lobular cysts occurred from September to April, at which point many germ cells within lobular cysts differentiated into primary spermatocytes to commence meiosis for the subsequent season. A distinct pattern of testicular architecture could be observed: i.e., germ cells in earlier stages of the spermatogenic cycle filled lobules of more caudal regions of testes whereas the more cranial regions of testes possessed germ cells in later stages of spermatogenesis (Fig. 2). The length of the spermatogenic cycle resulted in overlap of successive cycles between one season to the next where mature sperm were still present in the cranial regions of testes while a subsequent wave of meiosis through testes was occurring the following year. In essence, at no time were mature-looking sperm not found in testes of our specimens. This overlap of successive spermatogenic cycles resulted in only August specimens lacking sperm in their Wolffian ducts.

All secondary sexual structures followed a similar pattern of annual hypertrophy/atrophy. Fall, winter, and spring specimens possessed highly hypertrophied breeding glands (genial glands and sexual collecting ducts) where diameter was largest relative to body size. During August, breeding glands were at their lowest activity level with epithelia that stained weakly with eosin and secretory material absence from lumina (Fig. 3A, C). During fall, winter, and spring, products of secretory epithelia of breeding glands were easily observed in their lumina, and epithelia stained more intensely with eosin, often revealing secretory granules (Fig. 3B, D).

Taken together, the testicular cycle and annual hypertrophy/atrophy of secondary sexual structures indicated that males of *Notophthalmus viridescens* from our collection were in mating condition from September to possibly July (Fig. 4); i.e., sperm were present in their Wolffian ducts, and breeding glands were actively synthesizing and releasing products.

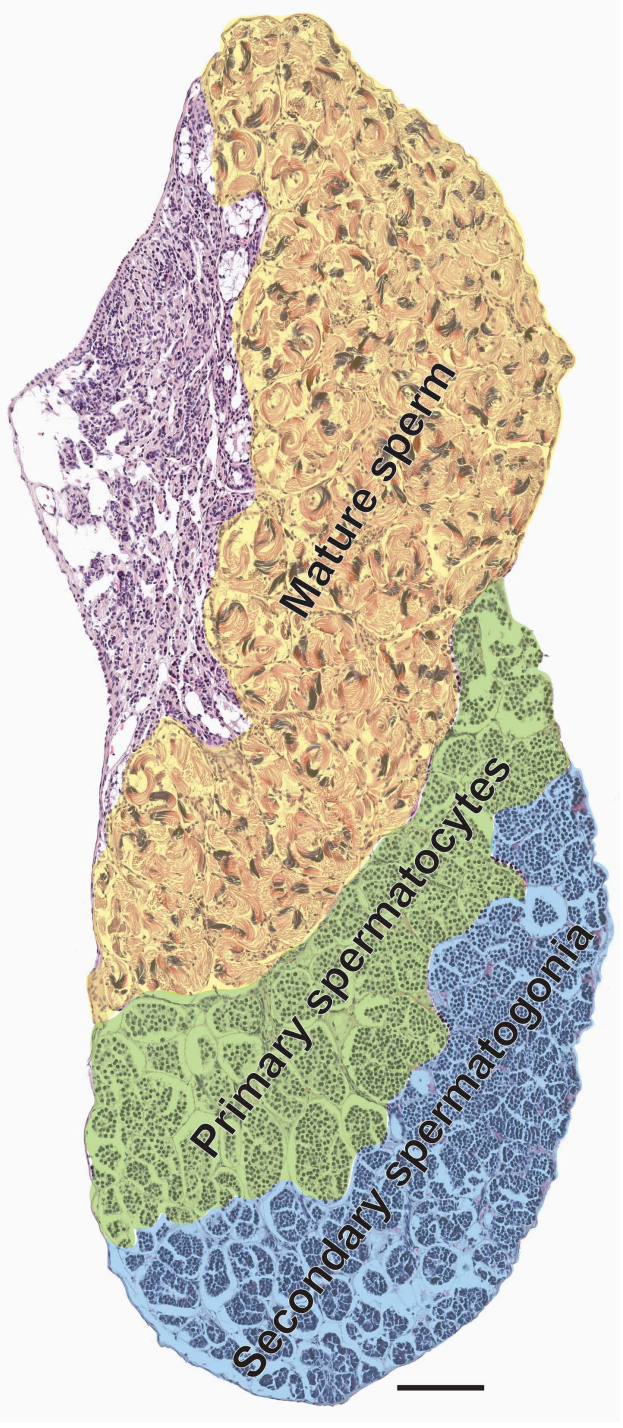

Figure 2. Representative section through the entire testis of *Notophthalmus viridescens* collected in April highlighting the overlap of successive spermatogenic cycles (hematoxylin and eosin). Mature sperm remain in the testis from the previous year's spermatogenic cycle while the current year's spermatogenic cycle has yet to reach meiosis. Scale bar = 300 mm.

Discussion

The testicular cycle of salamandrids is controlled exogenously by temperature and endogenously by hormones. Temperatures above 12 °C were demonstrated to induce spermatocyte formation and temperatures between 21–25 °C stimulated spermiation in *Notophthalmus viridescens* (Ifft 1952). Follicle stimulating hormone (FSH) was shown to increase testicular weight (Tanaka et al. 2004) and stimulate the meiotic stages of spermatogenesis in *Cynops pyrrhogaster* (Abe & Ji 1994) whereas testosterone was shown to have an inhibitory effect on spermatogenesis in *N. viridescens* (Scadding 1978). Luteinizing hormone (LH) stimulated spermiation in *C. pyrrhogaster* (Tanaka et al. 2004). The interplay between temperature and hormone cycles has been studied sparingly in salamandrids (Norris 2024) but it has been demonstrated that FSH loses its effect at temperatures of 8 °C and below, and that the effects of LH were more potent at 8 °C than at higher temperatures (Tanaka et al. 2004). In culmination, sperm development in newts appears to require relatively higher environmental temperature, with lowering temperatures linked to spermiation of sperm into Wolffian ducts.

Considering environmental factors (temperature) are involved in coordinating the testicular cycle of salamandrids, it is easy to predict that variation would be observed across the geographic range of a wide-ranging species; however, very little variation has been observed. *Notophthalmus viridescens* from Massachusetts (Adams 1940), Missouri (Siegel et al. 2012), North Carolina (this study),

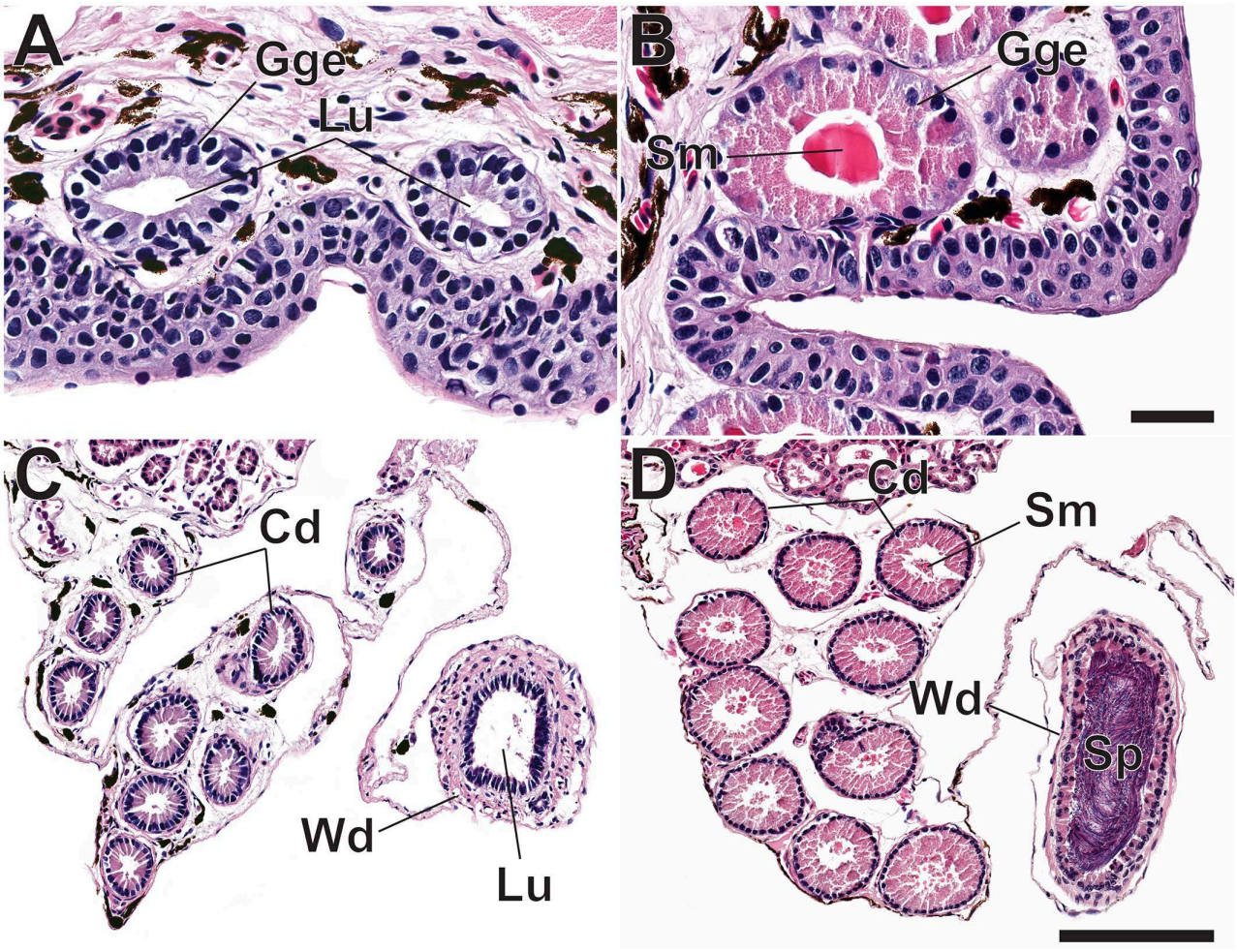

Figure 3. Representative sections through secondary sexual structures of male *Notophthalmus viridescens* (hematoxylin and eosin). (A) Genial gland tubules from an August specimen exhibiting no secretory activity of the genial gland epithelium (Gge) and no secretory material in the lumina (Lu) of the tubules. (B) Genial gland tubules from a December specimen exhibiting a highly secretory genial gland epithelium (Gge) and secretory material (Sm) in the lumina of the tubules (scale bar = 62 mm for both A and B). (C) Collecting duct (CD) tubules from an August specimen exhibiting relatively low epithelial heights and tubule diameters, and no sperm in the lumen (Lu) of the Wolffian duct (Wd). (D) Collecting duct (CD) tubules from a December specimen with relatively high epithelial heights and tubule diameters, secretory material (Sm) in the collecting duct lumina, and abundant sperm in the lumen of the Wolffian duct (Wd; scale bar = 300 mm for both C and D).

and apparently Connecticut (Ifft 1942; although no data were provided) all possess spermatogenic cycles that are practically identical: proliferation of spermatogonia and transition to spermatocytes in the spring, the meiotic stages of spermatogenesis in the summer (specifically June, July, and August), and onset of spermiation in early fall (September). Whether this similarity is because of differential interplay between temperature and hormones in different environments or lack of resolution when sampling monthly is unknown; however, it is apparent that salamandrids, and many other North American salamanders (Siegel et al. 2014a), are constrained to produce sperm during the summer.

One area of variation in the testicular cycle between North Carolina *N. viridescens* and the other geographic areas is the presence of mature-looking sperm in the testis throughout the year. While new sperm are being produced in the summertime, sperm from the previous season are still found in the most cranial testicular regions. Whether or not this has any functional affect is not clear but may help with a potentially prolonged mating season of North Carolina *N. viridescens*. Takahashi & Parris (2008) demonstrated that sperm quality and quantity decreases with every spermatophore deposited by male *N. viridescens* within a mating bout. Persistent maintenance of sperm in the testes may indicate that more sperm are produced to counter

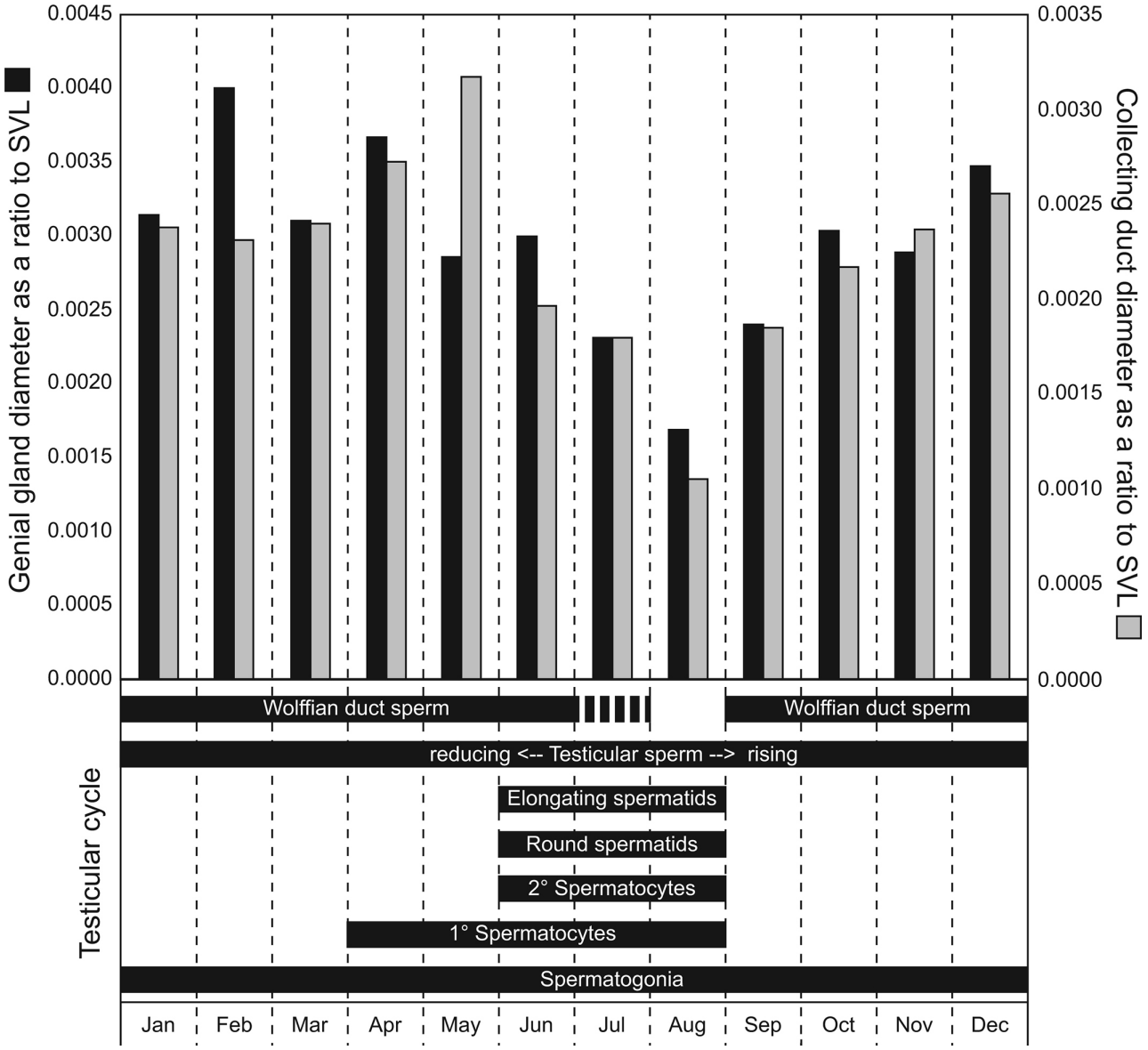

Figure 4. Seasonal summary of testicular cycle and secondary sexual structure hypertrophy/atrophy through histological examination of testes and breeding glands (genial glands, sexual collecting ducts of the kidneys, and cloacal glands) of male *Notophthalmus viridescens* respectively. Dashed lines in the testicular cycle portion of the summary for Wolffian duct sperm indicate that one specimen possessed sperm in the Wolffian ducts and one did not.

depletion, or potentially to maintain more viable sperm for longer periods of time, as sperm activation appears to occur in the Wolffian ducts (Siegel et al. 2014b). At present, it is not clear if North Carolina *N. viridescens* possess an extended mating season compared to other populations but sperm presence in Wolffian ducts from September to July in North Carolina versus September to May/June in Massachusetts/Missouri (Adams 1940, Siegel et al. 2012) provide support for this hypothesis.

Evidence from secondary sexual structures also indicate that the North Carolina *N. viridescens* studied herein have an extended mating season compared to Massachusetts and Missouri populations studied previously. Although originally reported to be spring breeders with a "false" breeding season in the fall (for review see Sever 2006), it is clear now that *N. viridescens* mate from fall, through winter, and until spring, in at least some populations. The "false" fall breeding season was inaccurately described because of the lack of understanding of female sperm storage until ovulation (Sever 2006), and *N. viridescens* have been reported to mate during winter in South Carolina (e.g., Sever et al. 1996) with adults captured in amplexus under 6+ inches of ice in January in Missouri (Siegel & True pers. comm.). Only in August were epithelial cells of genial glands that are involved in pheromone transfer to females during amplexus (Verrell 1982) devoid of secretory granules in our North Carolina sample. Collecting duct epithelial cells were never devoid of secretory granules. In Missouri, the lack of secretory granules in the genial gland epithelia was observed in July and August (Siegel et al. 2012) and June through August for collecting ducts in both Massachusetts and Missouri populations (Adams 1940, Siegel et al. 2012). The function of collecting duct secretions is currently unknown but it is obvious that they serve in some role as a secondary sexual characteristic in many salamanders based on their hypertrophy during the mating season (Alvino & Siegel 2017, Aron 1924, Siegel et al. 2010, 2012) and mixing with sperm during spermatophore formation (Siegel et al. 2024). Development of secondary sexual characteristics in salamandrids has been linked to testosterone and dihydrotestosterone (Specker & Moore 1980), and high temperatures over 29 °C were previously shown to cause atrophy of secondary sexual characteristics (McReynolds 1968); thus, dissimilar to spermatogenesis, high environmental temperature is not as suitable for mating.

In at least two of the main studies compared herein (Siegel et al. 2012; this study), adult *N. viridescens* were easily captured in ponds throughout the year. Although not mentioned, we presume that this was also the case for the Massachusetts collection by Adams (1940) because Healy (1974, 1975) noted that adult *N. viridescens* do not leave ponds in Massachusetts. This is not consistent across the entire geographic range of *N. viridescens* as some adults leave ponds during the summer, doubtlessly when the testes of adult males would be undergoing the meiotic stages of spermatogenesis. In at least populations in Arkansas (Trauth & Siegel pers. comm.), South Carolina (Sever et al. 1996), and Virginia (Gill 1978, Massey 1990), adult *N. viridescens* leave ponds in the summer and sometimes do not return to ponds until the spring. Hurlbert (1969) noted that some *N. viridescens* even possess wandering behavior and move from aquatic to terrestrial habitats frequently, sometimes changing ponds altogether. It is currently unknown how this geographic variation in life history affects the cyclicity of sperm development, hypertrophy of secondary sexual characteristics, or mating season.

Obviously, the current study and Siegel et al. (2012) are male centric. This was mainly a product of collection limitation by state agencies for the Missouri (20 individual per year permitted) and North Carolina (no females permitted) collections. Of the three studies that completely outline the reproductive cycles of male *N. viridescens* in detail, only Adams (1940) provided data on the female reproductive cycle. Sever et al. (1996) also provided extensive data from females from October to June in a South Carolina population. These data indicated that vitellogenesis commenced in fall and ovulation occurred as early as April, continuing until June. Sperm in spermathecae were reported as "especially abundant" in the fall and spring, which coincides with fall and spring mating seasons (Sever 2006), and the hypertrophy of male secondary sexual characteristics (Adams 1940, Siegel et al. 2012, this study). Variation in female reproductive cycle events between populations is reasonable, considering variation was found between populations in males.

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References

Abe, S. I. & Z. S. Ji (1994): Initiation and stimulation of spermatogenesis in vitro by mammalian follicle-stimulating hormone in the Japanese newt, *Cynops pyrrhogaster*. – International Journal of Developmental Biology, **38**: 201–208.

Adams, A. E. (1940): Sexual conditions in *Triturus viridescens*. III. The reproductive cycle of the adult aquatic form of both sexes. – The American Journal of Anatomy, **66**: 235–275.

Alvino, S. & D. S. Siegel (2017): Sexual kidneys in a plethodontid salamander. – Journal of Herpetology, **51**: 202–208.

Aron, M (1924): Recherches morphologiques et expérimentales sur le déterminisme des caracateres sexuels males chez les urodeles. – Archives de Biologie, **34**: 1–166.

Davenport, J. M., D. A. Beamer, C. L. Long, J. T. Waltz, S. Wren & D. S. Siegel (2022): Reproductive biology and population structure of *Eurycea chamberlaini* in North Carolina. – Ichthyology and Herpetology, **110**: 728–736.

Gill, D. E. (1978): The metapopulation ecology of the red-spotted newt, *Notophthalmus viridescens* (Rafinesque). – Ecological Monographs, **48**: 145–166.

Healy, W. R. (1974): Population consequences of alternative life histories in *Notophthalmus v. viridescens*. – Copeia, **1974**: 221–229.

Healy, W. R. (1975): Breeding and postlarval migrations of the red-spotted newt, *Notophthalmus viridescens*, in Massachusetts. – Ecology, **56**: 673–680.

Houck, L. D. (1977): Reproductive biology of a neotropical salamander, *Bolitoglossa rostrata*. – Copeia, **1977**: 70–83.

Humphrey, R. R. (1926): The multiple testis in *Diemyctylus*. – Journal of Morphology, **41**: 283–309.

Hurlbert, S. H. (1969): The breeding migrations and interhabitat wandering of the vermilion-spotted newt *Notophthalmus viridescens* (Rafinesque). – Ecological Monographs, **39**: 465–488.

Ifft, J. D. (1942): The effect of environmental factors on the sperm cycle of *Triturus viridescens*. – Biological Bulletin, **83**: 111–128.

Ireland, P. H. (1974): Reproduction and larval development of the dark-sided salamander, *Eurycea longicauda melanopleura* (Green). – Herpetologica, **30**: 338–343.

Ireland, P. H. (1976): Reproduction and larval development of the gray-bellied salamander *Eurycea multiplicate griseogaster*. – Herpetologica, **32**: 233–238.

Massey, A. (1990): Notes on the reproductive ecology of red-spotted newts (*Notophthalmus viridescens*). – Journal of Herpetology, **24**: 106–107.

McReynolds, S. B. (1968): Effects of temperature on secondary sexual characteristics of the newts *Notophthalmus viridescens* and *Notophthalmus meridionalis*. – Unpubl. MS Thesis, Texas Technical College.

Norris, D. O. (2024): Hormones and reproductive cycles in urodele and gymnophone amphibians. – pp. 235–255 in: Norris, D. O. & K. H. Lopez (eds): Hormones and Reproduction of Vertebrates: Amphibians Volume 3, 2nd edition, – Academic Press, Cambridge, Massachusetts.

Scadding, S. R. (1978): Testosterone and 5a-dihydrotestosterone inhibit both growth and spermatogenesis in the remaining testis in partially castrated adult newts, *Notophthalmus viridescens*. – Journal of Experimental Zoology, **205**: 403–432.

Sever, D. M. (1974): The occurrence of multiple testes in the genus *Eurycea* (Amphibia: Plethodontidae). – Herpetologica, **30**: 187–193.

Sever, D. M. (1975): Morphology and seasonal variation of the mental hedonic glands of the dwarf salamander, *Eurycea quadridigitata* (Holbrook). – Herpetologica, **31**: 241–251.

Sever, D. M. (2002): Female sperm storage in amphibians. Journal of Experimental Zoology, **292**: 165–179.

Sever, D. M. (2006): The "false breeding season" of the eastern newt, *Notophthalmus viridescens*. – Bulletin of the Chicago Herpetological Society, **41**: 149–153.

Sever, D. M. & L. D. Houck (1985): Spermatophore formation in *Desmognathus ochrophaeus* (Amphibia: Plethodontidae). – Copeia, **1985**: 394–402.

Sever, D. M., L. C. Rania & J. D. Krenz (1996): Annual cycle of sperm storage in spermathecae of the red-spotted newt, *Notophthalmus viridescens* (Amphibia: Salamandridae). – Journal of Morphology, **227**: 155–170.

Siegel, D. S., D. M. Sever & R. D. Aldridge (2010): The pelvic kidney of male *Ambystoma maculatum* (Amphibia, Urodela, Ambystomatidae) with special reference to the sexual collecting ducts. – Journal of Morphology, **27**: 1422–1439.

Siegel, D. S., C. M. Murray, E. R. Wilson, E. R. Puleo, K. A. True & R. A. Aldridge (2012): Sexual kidneys in the red-spotted newt (*Notophthalmus viridescens*). – Journal of Herpetology, **46**: 136–144.

Siegel, D. S., S. Alvino, S. E. Trauth, D. M. Sever & K. M. Gribbins (2014a): Spermatogenic cycle of a plethodontid salamander, *Eurycea longicauda* (Amphibia, Urodela). – Spermatogenesis, **4**: 3, e982420.

Siegel, D. S., K. M. Gribbins, J. L. Rheubert & R. D. Aldridge (2014b): *Notophthalmus viridescens*. Sperm motility. – Herpetological Review, **45**: 474–475.

Siegel, D. S., C. L. Long, J. T. Waltz, S. A. Wren, K. E. Pereira, S. J. McClelland, C. M. Murray & D. M. Sever (2020): Sexually dimorphic heads of *Eurycea bislineata*. – Copeia, **3**: 578–592.

Siegel, D. S., M. A. Herrboldt & N. L. Staub (2024): Hormones, sex accessory structures, and secondary sexual characteristics in amphibians: a focus on breeding glands. – pp. 105–119 in: Norris D. O. & K. H. Lopez (eds): Hormones and Reproduction of Vertebrates: Amphibians Volume 3, 2nd edition. – Academic Press, Cambridge, Massachusetts.

Specker, J. L. & F. L. Moore (1980): Annual cycle of plasma androgens and testicular composition in the rough-skinned newt, *Taricha granulosa*. – General and Comparative Endocrinology, **42**: 297–303.

Takahashi, M. K. & M. J. Parris (2008): When do the costs of spermatogenesis constrain sperm expenditure? Remarks on the pattern of the spermatogenic cycle. – Ethology, **115**: 96–104.

Tanaka, S., M. Sakai, M. Hattori, S. Kikuyama, K. Wakabayashi & Y. Hanaoka (2004): Effect of bullfrog LH and FSH on newt testes under different temperatures. – General and Comparative Endocrinology, **138**: 1–7.

Uribe, M. C. A. (2003): The testes, spermatogenesis and male reproductive ducts. – pp. 183–202 in: Sever, D. M. (ed.): Reproductive Biology and Phylogeny of Urodela. – Science Publishers Inc., Enfield, New Hampshire.

Verrell P. (1982): The sexual behavior of the red-spotted newt, *Notophthalmus viridescens* (Amphibia : Urodela : Salamandridae). – Animal Behavior, **30**: 1224–1236.