Kw: amphib, herp, Reproduction

839

BODY LENGTH OF MALE *CRYPTOBRANCHUS ALLEGANIENSIS* AT SEXUAL MATURITY

The size of male *Cryptobranchus alleganiensis* at sexual maturity has not been accurately documented. Nickerson and Mays (1972) indicated it to be approximately 340 mm (probably total) length but expressed a degree of uncertainty.

As in other urodeles, the testes of *Cryptobranchus* undergo an annual maturational cycle (Burger, 1937; Humphrey, 1921). According to Humphrey (1921), primary spermatogonia are surrounded by epithelial cells comparable in function to mammalian Sertoli cells. Division of spermatogonia and epithelial cells initiates formation of lobular lumina, after which the full sequence of germinal cell maturation proceeds as a cephalocaudal wave. This progression is rapid and difficult to observe (Humphrey, 1921) but McGregor (1899) considered sperm maturation in *Cryptobranchus* to occur immediately prior to fertilization without prolonged storage in the testes. Maturation divisions occur between June and August (Humphrey, 1921) and spawning is in October (Ratcliff, 1965).

We have determined by light and scanning electron microscopy the body size at which male *Cryptobranchus alleganiensis* become gametogenic.

Thirty-one *Cryptobranchus alleganiensis* were hand-caught in the Allegheny River 3 km southwest of Tionesta, Pennsylvania, on May 29 (7 animals) and on 31 August, 1980 (24 animals). They were killed with ether and measured (standard snout-vent length) dead. Cranial, middle and caudal portions of both testes of each animal were fixed in Karnovsky's (1961) fixative or in alcohol-formol-acetic acid (AFA) for electron and light microscopy, respectively. The presence of sperm was also confirmed by microscopic examination of fresh testicular exudate of the larger salamanders. Fixed exudate of mature animals was filtered through Whatmans No. 1 paper which was then trimmed, folded, and stapled. The resulting packet was dehydrated through a series of ethyl alcohols and critical-point dried. A small piece of double-stick tape was used to transfer clumps of sperm to the specimen stub. Sperm were coated with gold approximately 45 nm thick and observed with a Coates and Welter (Cwikscan 106A) field emission scanning electron microscope. AFA-fixed samples were dehydrated through a series of ethanols and embedded in Paraplast. Sections 6 and 8 mμ thick were stained with hematoxylin and fast green.

The smallest specimen with testicular sperm was 200 mm snout-vent length (S-V-L). All longer animals were also spermatogenic (Figure 1). The testes of a 187 mm S-V-L salamander in August contained spermatids and some secondary spermatocytes. The next four smaller animals with S-V-L of 184-, 178-, 166- and 154 mm were collected in May. Since *Cryptobranchus* testes being their recrudescence in June (Humphrey, 1921) no sperm were expected. However, spermatids were present in the 184- and 166 mm animals. Secondary

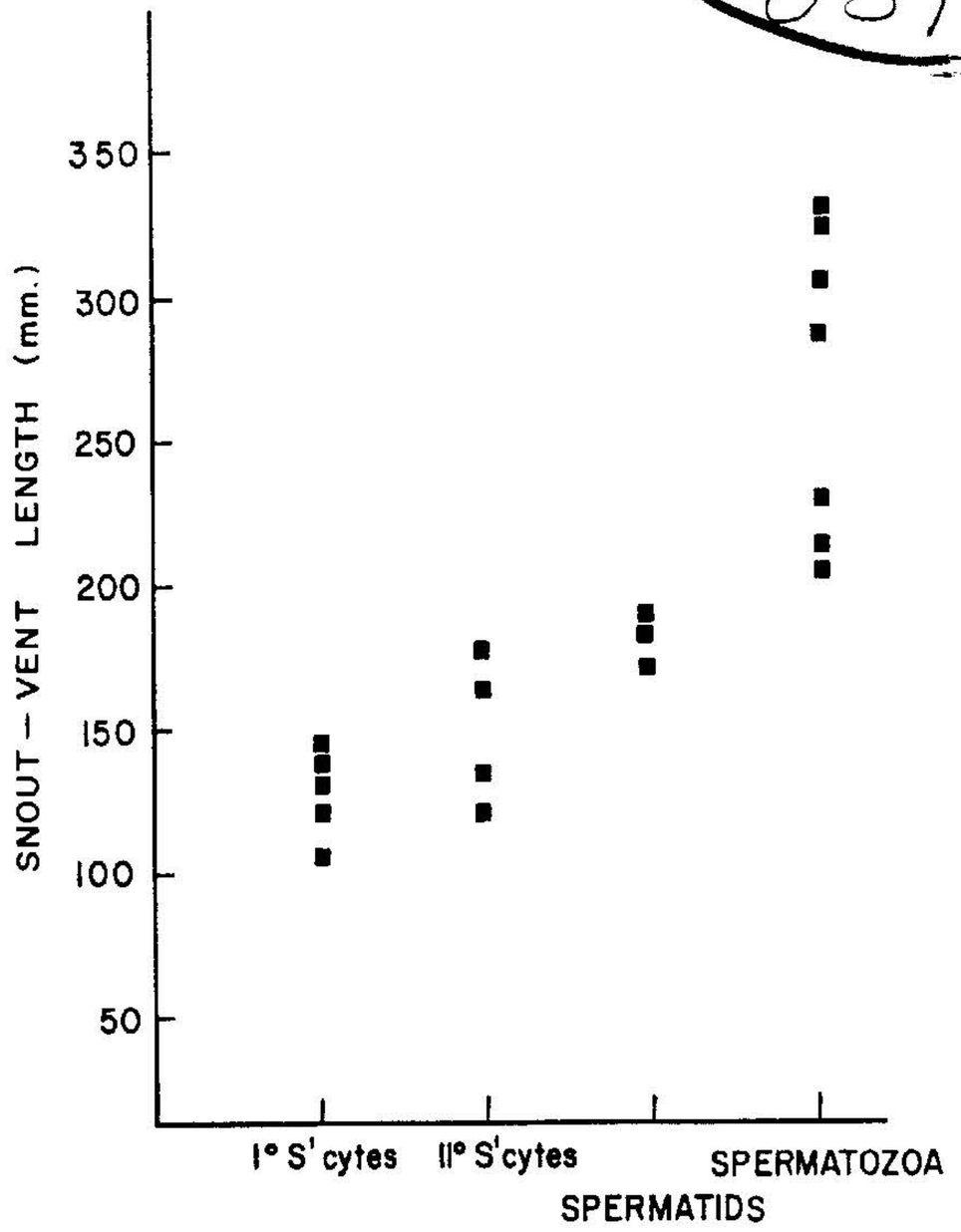

Figure 1. Correlation of most-differentiated germinal epithelium type with snout-vent length of 19 *Cryptobranchus*. All longer animals examined bore spermatozoa.

spermatocytes characterized the germinal epithelium of the smaller animals. Since gametes in immature *Plethodon* develop until the spermatocyte stage before degenerating (Burger, 1937) and during May immature *Necturus* testicular lobules consist of spermatogonia with no indication of spermatocytes (Humphrey, 1921) it is possible that the 178 mm animal would have shortly experienced gametogenesis. His testes were more distended than those of smaller, immature animals. Similarly, the presence of spermatids in the May animals measuring 184 mm and 166 mm S-V-L also suggest sexual maturity with the possibility of spermiogenesis during the current summer.

The presence of spermatids in two animals collected in May advance the date of gametogenesis relative to that (June) cited by Humphrey (1921). Spermatozoa in the seven animals collected in August and spermatids in another smaller animal (Figure 1) indicate spermiogenesis and a breeding season beginning in mid to late August since the duration of testicular sperm storage is minimal (McGregor, 1899). Two immature salamanders taken in August contained primary and secondary spermatocytes. Testes of the other animals (137-, 136-, 132-, 122-, and 102 mm S-V-L) had no germ cells advanced beyond primary spermatocyte level. Their testicular lobules were fewer in number and the testis was thread-like.

These observations show that male *Cryptobranchus* are gametogenic at 166 mm S-V-L. From comparable observations of gametogenesis in other salamanders, it is assumed but not known that *Cryptobranchus* of this size could become active breeders in the streams from which they were taken.

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—Eric D. Wikramanayake and G. L. Dryden, *Department of Wildlife and Fisheries Biology, University of California, Davis, California 95616*, and *Biology Department, Slippery Rock University, Slippery Rock, Pennsylvania 16057*.

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