

- HUEY, R. B. 1982. Temperature, physiology, and the ecology of reptiles. In C. Gans and F. H. Pough, (eds.), *Biology of the Reptilia* (Physiology C: Physiological Ecology). Vol. 12, pp. 24–91. Academic Press, New York.
- LEMOS-ESPINAL, J. A. AND R. E. BALLINGER. 1995. Comparative thermal ecology of the high altitude lizard *Sceloporus grammicus*. *Can. J. Zool.* 73:2184–2191.
- MARQUET, P. A., J. C. ORTIZ, F. BOZINOVIC, AND F. M. JAKSIC. 1989. Ecological aspects of thermoregulation at high altitudes: the case of Andean *Liolaemus* lizards in northern Chile. *Oecologia* 81:16–20.
- MATHIES, T., AND R. M. ANDREWS. 1995. Thermal and reproductive ecology of high and low elevation populations of the lizard, *Sceloporus scalaris*: implications for the evolution of viviparity. *Oecologia* 104:101–111.
- , AND ———. 1997. Influence of pregnancy on the thermal biology of the lizard, *Sceloporus jarrovi*: why do pregnant females exhibit low body temperatures? *Funct. Ecol.* 11:498–507.
- MCGINNIS, S. M. 1966. *Sceloporus occidentalis*: preferred body temperature of the western fence lizard. *Science* 152:1090–1091.
- MÉNDEZ-DE LA CRUZ, F. R., M. VILLAGRÁN-SANTA CRUZ, AND R. M. ANDREWS. 1998. Evolution of viviparity in the lizard genus *Sceloporus*. *Herpetologica* 54:521–532.
- VAN BERKUM, F. H. 1986. Evolutionary patterns of the thermal sensitivity of sprint speed in *Anolis* lizards. *Evolution* 40:59–604.
- VAN DAMME, R., D. BAUWENS, AND R. F. VERHEYEN. 1986. Selected body temperatures in the lizard *Lacerta vivipara*: variation within and between populations. *J. Thermal Biol.* 11:219–222.

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Reproductive Biology of the Aquatic Salamander *Amphiuma tridactylum* in Louisiana

CLIFFORD L. FONTENOT, JR.

Department of Biological Sciences, Southeastern Louisiana University, Hammond, Louisiana 70402, USA.
E-mail: cfontenot@selu.edu

ABSTRACT.—The timing of reproductive activities in *Amphiuma tridactylum* is described. The vitellogenic process takes almost a full year and female reproduction is biennial or less frequent; only 35% of females were gravid. Clutch size averaged 201, ranging from 44–282, and was significantly related to body size. The relationship between body size and fat body weight was marginally insignificant. The relationship between fat body weight and clutch size was insignificant, although oocyte production significantly reduced fat body weight.

Amphiuma tridactylum is a large, nocturnal, aquatic salamander that inhabits ditches, ponds, and swamps of the southeastern United States (Dundee and Rossman, 1989). It spends most of its time in burrows, from which it emerges at night to forage primarily for crayfish, although a wide variety of invertebrates, vertebrates, and carrion are eaten (Chaney, 1951; Fontenot and Fontenot, 1989). Its reproductive biology is poorly understood, being primarily based on studies from Orleans Parish (Cagle, 1948), Orleans and adjacent Jefferson parishes, Louisiana (Rose, 1966, 1967), and numerous anecdotal observations. Salthe (1973) and Sever et al. (1996) noted that some reports were contradictory, which makes interpretation of reproductive events difficult. A summary of *A. tridactylum* reproductive activities is as follows (but see Sever

et al. [1996] for a more detailed tabulation of literature on reproductive events for *Amphiuma*).

Wilson (1941, 1942) and Cagle (1948) suggested that female reproduction is biennial. Vitellogenesis progresses slowly at least from September through May (Wilson, 1940; Cagle, 1948; Rose, 1967). Peak sperm production occurs from December through March (Wilson, 1940; Rose, 1967). Females with sperm in their cloacae have been collected during this period, suggesting that mating occurs then (Cagle, 1948; Sever et al., 1996). Sperm are retained in female spermathecae for up to seven months until fertilization (Kreeger, 1942), and Sever et al. (1996) reported degradation of sperm in August samples from female cloacae. The nest is attended by an adult (presumably female); although the exact duration of incubation is unknown, it is probably about five months in southern Louisiana

(Salthe, 1973). Hatching perhaps occurs sometime in the fall (Cagle, 1948; Baker, 1962). This paper provides additional data on the reproductive biology of *A. tridactylum*, particularly details on vitellogenesis, and synthesizes a plausible reproductive phenology for southern Louisiana.

MATERIALS AND METHODS

I collected a total of 146 *Amphiuma tridactylum* (77 males, 55 females, 14 juveniles) from Acadia and Evangeline parishes, Louisiana, from January through December, 1989. These were captured primarily at night using a variety of techniques including baited cylindrical funnel traps (85 × 25 cm; 6.4 mm mesh hardware cloth), seining, hook and line, electro-shocking, burrow excavation, artificial shelters, and hand collecting. To ensure access to aerial respiration for captured salamanders, a portion of the trap was kept above the water surface by collecting primarily at the edges of narrow ditches (0.5–2.0 m wide), swamps, and ponds. Most ditches surrounded rice, soybean, and crayfish farms and averaged about 0.5 m in depth, and were subject to drought and flooding. Occasionally, some ditches were completely dry (especially July–October) and sampling was often restricted to excavating burrows in dried ditches or trapping in permanent ponds. Aquatic vegetation in both temporary and permanent water sites was predominantly alligatorweed (*Alternanthera philoxeroides*).

The sex of each adult was determined by inspection of the cloacal walls; brown = female, light gray or pink = male (Kreeger, 1942). Dissection later showed this technique to be 100% accurate; however, some of the largest males had cloacal walls that were partially pigmented near the caudal end, but none was completely pigmented. Cagle (1948) reported the minimum adult size as 33 cm total length (TL); I used 27 cm snout-vent length (SVL) as the minimum adult size (TL = 33 cm) because of potential variation in tail length due to damaged tails (Seigel and Ford, 1988).

Each individual was injected with T-61 euthanasia solution, weighed to the nearest 0.1 g, and SVL and TL measured to the nearest mm. Wet gonads and fat bodies were removed, weighed to the nearest 0.01 g, and the largest size class of vitellogenic follicles in both ovaries counted. A random sample of 30 of those oocytes was measured to the nearest 0.01 mm and the average calculated; for three females a smaller size-class of oocytes was also measured. Because the timing of spermiogenesis was previously identified as occurring during winter (Wilson, 1940, 1941; Rose, 1967), males were sacrificed and dissected only from January through

May. Samples from male cloacae were examined for the presence of sperm with a light microscope.

Data were log-transformed as needed to meet the assumptions of parametric tests (Sokal and Rohlf, 1981). Regression analyses were used to determine relationships between SVL and clutch size, SVL and fat body mass, and between clutch size and fat body mass. Clutch and follicle data were based on the largest size class of yolked oocytes.

RESULTS

Males were in reproductive condition from January through April, possessing enlarged testes and vasa deferentia, and swollen cloacae (some partially everted), which contained motile sperm. No courtship activity was observed, although on several occasions I observed heavily male-biased sex ratios during March and April. The number of individuals by sex and female reproductive condition is shown in Fig. 1. The heavily male-biased sex ratios corresponded to abnormally high water levels and flow rates, which in most cases resulted from heavy rainfall. However, the same water conditions and biased sex ratios were produced by irrigation water pumps intended to flood adjacent rice fields. Only under flooded conditions were individuals (male and female) known to have entered traps during the day.

Consistent with other reports on sexual maturity, all females <27 cm SVL had small, non-yolked follicles; the smallest gravid (vitellogenic) female in this study was 33 cm SVL. Some of the females sampled each month possessed ovarian follicles that showed no vitellogenic activity (Fig. 1). Other females possessed follicles in progressive stages of vitellogenesis (Fig. 2) beginning in July (0.3–0.5 mm) and continuing until ovulation (7 mm) in June–July of the following year. During the development of this group of oocytes, another group of follicles began to accumulate yolk in December such that a second (smaller) size-class of oocytes was developing simultaneously among the larger ones.

The largest size-class of vitellogenic follicles increased in diameter from 0.3 mm in late July to 7 mm by early July of the next year when oviposition occurred (Fig. 2). The group of distinctly smaller oocytes increased in diameter from 0.3 mm to 2.6 mm by the time that oviposition of the group of larger (7 mm) oocytes occurred. A 52 cm SVL female in this condition, collected on 17 July, had apparently recently deposited most of her clutch. Four jelly encapsulated ova (9 mm; ovum only = 7 mm) were found in her coelomic cavity and none was in the enlarged oviducts. The ovaries contained a set of 30 smaller vitellogenic (2.6 mm) follicles.

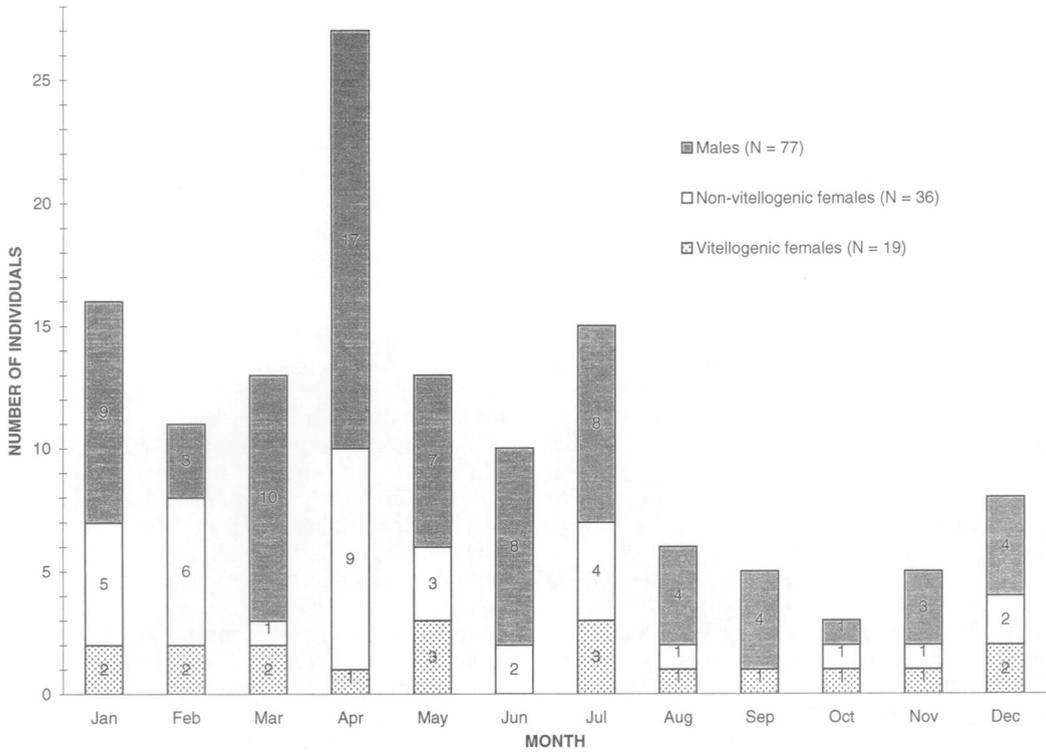


FIG. 1. Number of individual *Amphiuma tridactylum* collected per month, by sex and reproductive condition.

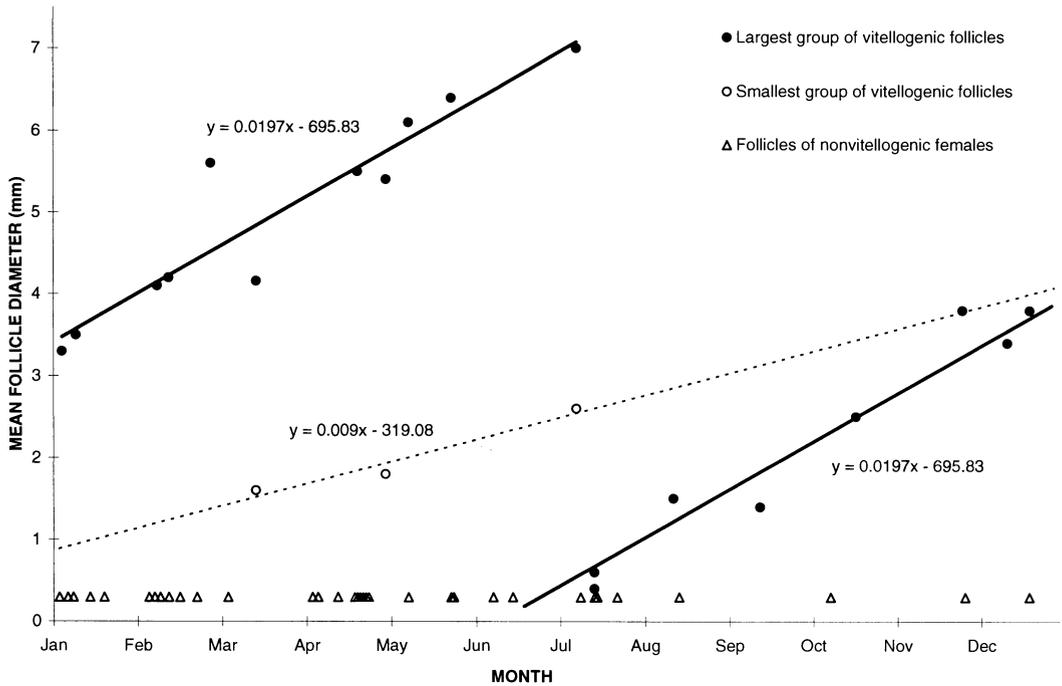


FIG. 2. Vitellogenesis in *Amphiuma tridactylum*. The solid lines represent the seasonal growth pattern of the group of larger oocytes; the dashed line represents a possible growth pattern of the group of smaller oocytes. Each of the open circles is from the females whose respective filled circle is plotted directly above it.

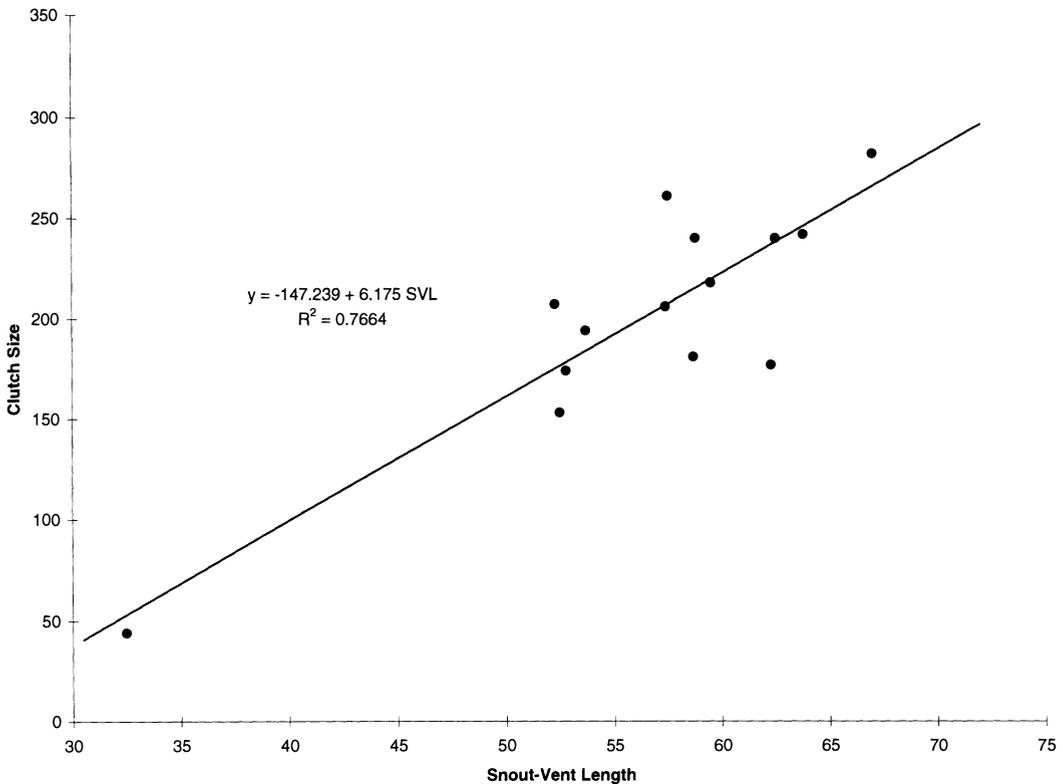


FIG. 3. The relationship between SVL and clutch size in *Amphiuma tridactylum*.

The function and fate of this smaller group of oocytes is not known; unfortunately, these were only measured for three females. The mean diameters of the smaller follicle group for these are shown as open circles (Fig. 2); the dashed line indicates a potential growth pattern (discussed below).

Only (35%) of adult females possessed vitellogenic follicles. After mid-July none with mature ova (>6.5 mm) or enlarged oviducts was collected, suggesting that oviposition had occurred and/or that females modified their behavior (e.g., nest attendance) and were not susceptible to sampling methods. Whether females feed during nest attendance is not known, although food is likely available and probably concentrated in the underground burrows during the dry summer months of incubation. Some of the burrows from which I collected aestivating (non-nesting) *Amphiuma* also contained live crayfish (*Procambarus clarkii*), their most commonly consumed food item. Why these prey were not eaten is unclear; perhaps feeding and digestion is physiologically impeded during aestivation.

Gravid females had a mean clutch size of 201.4 ($N = 14$, $SE = 15.54$, range = 44–282).

Using SVL as the independent variable, regression analyses indicated a significant positive relationship (Fig. 3) between SVL and clutch size ($y = -147.239 + 6.175 \text{ SVL}$, $N = 14$, $r = 0.876$, $P < 0.001$). The relationship between SVL and fat body mass was marginally insignificant in non-vitellogenic ($y = -2.477 + 0.030 \text{ SVL}$, $N = 32$, $r = 0.341$, $P = 0.056$) and vitellogenic ($y = -3.071 + 0.092 \text{ SVL}$, $N = 14$, $r = 0.488$, $P = 0.077$) females. To remove the effect of SVL, the residuals from the latter regression were saved and used as the dependent variable in the following regression analyses. These indicated no significant relationship between clutch size (independent variable) and fat body mass ($y = 0.396 - 0.002 \text{ clutch Size}$, $N = 14$, $r = 0.091$, $P = 0.758$). However, with the effect of body size (SVL) removed, fat body mass decreased significantly as oocyte diameter (independent variable) increased ($y = 1.039 - 0.254 \text{ oocyte diameter}$, $N = 14$, $r = -0.636$, $P = 0.015$).

DISCUSSION

Copulation in *Amphiuma* has been observed only once by Baker et al. (1947) during July in Tennessee, and was described as an eight-day event whereby a male *A. tridactylum* chose be-

tween two females and sperm transfer subsequently occurred via cloacal apposition. However, based on the reproductive condition of males in Louisiana from this study, Wilson (1940, 1941), Cagle (1948), Rose (1967), and Sever et al., (1996), breeding probably occurs in Louisiana from January through April. Sperm are then retained by females in spermathecae until ovulation (Kreeger, 1942).

The long slow progression of vitellogenesis (Fig. 2) would likely prevent non-vitellogenic females from reproducing during the upcoming season whereas those with vitellogenic follicles would reproduce. The smaller set of oocytes may be a part of the current year's reproductive effort that lacked sufficient energy to attain mature size for that season and may become atretic. Alternatively, these may represent the beginning of the next season's reproductive effort. If the smaller set of follicles grew at the same rate (slope) as the larger set, these would reach mature size during January, which is not the case. However, if the group of smaller oocytes initially continued to increase in size at a different rate than the group of larger oocytes, these may become synchronous with the growth pattern of the larger oocytes of other individuals during December (Fig. 2, dashed line). If this scenario is true, this smaller group of oocytes must recruit additional follicles to achieve an average clutch size. The SVL-clutch size relationship estimates that the clutch size of the 52 cm female of 17 July (that contained mature ova in her coelomic cavity) was 170. Given that the smaller set contained only 30 oocytes, she would have to recruit and develop an additional 140 oocytes to "catch up" with the rest of the vitellogenic female population that would reproduce the following season. Whether this scenario is true might be determined by a more complete data set on the smaller oocytes, particularly for July through November. However, data during that period are lacking presumably because those females are attending nests in burrows or under cover, and are not susceptible to collecting. Albeit a hypothesis based on a very small sample, the potential incorporation of the smaller size-class of oocytes into a future clutch is worth considering in future studies.

Whatever the fate of the smaller oocytes, vitellogenesis of the larger group of oocytes continues into late June or early July when egg deposition occurs. Other investigators have suggested that based on oocyte diameter and ovary weight, vitellogenesis occurs from September to sometime between March and July (Wilson, 1940; Cagle, 1948; Rose, 1967; Fontenot, 1990). However, Fontenot (1990) did not initially recognize that the very small (0.3 mm) follicles just beginning to show yolk in late July represented

vitellogenesis. With individuals in this condition recategorized as vitellogenic in this paper and plotted as such (Fig. 2), these represent the very beginning of vitellogenesis, suggesting that the process may take almost a full year.

Females presumably attend the nest throughout the incubation period until the eggs hatch in November. This hypothesis is supported by reports of nests with eggs in progressive stages of development from July through November in Louisiana (Hay, 1888; Parker, 1937; Baker, 1937; Bishop, 1943; Baker et al., 1947) and Alabama (Ultsch and Arceneaux, 1988). Cagle (1948) reported a female with "eggs projecting from the cloaca" collected on 25 June, 1946 in New Orleans; and Bishop (1943) reported a nest found in Baton Rouge on 16 November, 1941 with eggs that hatched the next day. In addition, Chaney and Spauschus collected 18 "hatchlings" on 11 November, 1947; these were the same individuals referred to in Cagle's (1948) paper, although only 11 of the 18 actually possessed gill vestiges [Tulane cat. no. 2331 (pers. comm. H. Dundee)]. The presence of gill vestiges indicates that these had recently hatched (Ultsch and Arceneaux, 1988).

Published reports on percentages of gravid females indicate 35% (this study), 42% (Cagle, 1948), 43% and 48% (Wilson, 1942), and 60% (Sever et al., 1996) of females are reproductively active each year. Because most of these reports are less than 50%, a tempting suggestion is that a biennial or even triennial reproductive cycle is indicated. However, size distribution of follicles does not necessarily translate into patterns of oviposition (Feder and Burggren, 1992), and caution should be exercised, especially in this case, because of the extended period of nest attendance. Samples taken during the incubation period would likely be biased if nesting females are otherwise inactive and not susceptible to collecting methods. Also, female reproductive frequency is likely a phenotypically plastic trait, directly affected by habitat quality. For example, a female living in a perennial pond with an abundant food supply could presumably devote more energy toward reproduction than the same female living in a ditch with a poor food supply that is subject to drought. Given the substantial annual variation incurred within and among habitats (and locations), these females may not have a standard reproductive frequency. Females probably have the potential to reproduce biennially, although individuals (or populations) experiencing more limited resources may reproduce less frequently. However, an accurate assessment of reproductive frequency in *A. tridactylum* may not be determinable without monitoring individual females for at least three consecutive years.

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LITERATURE CITED

- BAKER, C. L., L. C. BAKER, AND M. F. CALDWELL. 1947. Observation of copulation in *Amphiuma tridactylum*. J. Tennessee Acad. Sci. 22:87–88.
- BAKER, L. C. 1937. Mating habits and life history of *Amphiuma tridactylum* Cuvier and effect of pituitary injections. J. Tennessee Acad. Sci. 12:206–218.
- . 1962. Spermatozoa of Amphiumae: Spermateliosis, helical motility and reversibility. J. Tennessee Acad. Sci. 37:23–37.
- BISHOP, S. C. 1943. Handbook of Salamanders. The Salamanders of the United States, and of Lower California. Comstock Publ. Co., Inc., Ithaca, New York.
- CAGLE, F. G. 1948. Observations on a population of the salamander, *Amphiuma tridactylum* Cuvier. Ecology 29:479–491.
- CHANEY, A. H. 1951. The food habits of the salamander *Amphiuma tridactylum*. Copeia 1951:45–49.
- DUNDEE, H. A., AND D. A. ROSSMAN. 1989. The Amphibians and Reptiles of Louisiana. Louisiana State Univ. Press, Baton Rouge.
- FEDER, M. E., AND W. W. BURGGREN (EDS.). 1992. Environmental Physiology of the Amphibians. Univ. Chicago Press, Chicago.
- FONTENOT, C. L., JR. 1990. Sexual dimorphism and reproductive cycles in the aquatic salamander *Amphiuma tridactylum*, in central Louisiana. Unpubl. M.S. Thesis, Southeastern Louisiana Univ., Hammond.
- , AND L. W. FONTENOT. 1989. *Amphiuma tridactylum* (Three Toed *Amphiuma*) Feeding. Herpetol. Rev. 20:48.
- HAY, O. P. 1888. Observations on *Amphiuma* and its young. Amer. Natur. 315–321.
- KREEGER, F. B. 1942. The cloaca of the female *Amphiuma tridactylum*. Copeia 1942:240–245.
- PARKER, M. V. 1937. Some amphibians and reptiles from Reelfoot Lake. J. Tennessee Acad. Sci. 12:60–86.
- ROSE, F. L. 1966. Reproductive potential of *Amphiuma means*. Copeia 1966:598–599.
- . 1967. Seasonal changes in lipid levels of the salamander *Amphiuma means*. Copeia 1967:662–666.
- SALTHER, S. N. 1973. *Amphiuma tridactylum*. Cat. Amer. Amphib. Rept. 149:1–149:3.
- SEIGEL, R. A., AND N. B. FORD. 1988. A plea for standardization of body size measurements in studies of snake ecology. Herpetol. Rev. 19:9–10.
- SEVER, D. M., J. S. DOODY, C. A. REDDISH, M. M. WENNER, AND D. R. CHURCH. 1996. Sperm storage in spermathecae of the great lamprey eel, *Amphiuma tridactylum* (Caudata: Amphiumidae). J. Morphol. 230:79–97.
- SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry: The Principles and Practice of Statistics in Biological Research, 2nd ed. W. H. Freeman, San Francisco.
- ULTSCH, G. R., AND S. J. ARCENEUX. 1988. Gill loss in larval *Amphiuma tridactylum*. J. Herpetol. 22:347–348.
- WILSON, F. H. 1940. The life cycle of *Amphiuma* in the vicinity of New Orleans based on a study of the gonads and gonoducts. Anat. Rec. 78 Suppl.:104 (abstr.).
- . 1941. The cloaca in the male *Amphiuma tridactylum*. Anat. Rec. 81 Suppl.:63 (abstr.).
- . 1942. The cycle of egg and sperm production in *Amphiuma tridactylum* Cuvier. Anat. Rec. 84 Suppl.:532 (abstr.).

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