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Abstract

Post-metamorphic growth and the reproductive cycle of the eastern narrowmouth toad (*Gastrophryne carolinensis*) were studied from 204 individuals collected during the April August 1989 activity season in a two-county area of northeastern Arkansas near the northwestern edge of the species' geographic range. Late summer metamorphs require a full growing season before they can reproduce as they approach their second year of life. The oldest individuals may be at least five years old. By late April, gonadal cycles of adults had commenced; the males were producing sperm, and some of the females were gravid. Fertility of both sexes increased during the season and peaked in June. Males remained fertile through August, but only two gravid females were found after June indicating that adults were physiologically capable of breeding for a period longer than weather conditions were acceptable for oviposition. Neither clutch size nor ovum diameter increased with female body size. Disparity of body size and clutch characteristics throughout the brief breeding season could be explained by deposition of partial clutches. The growth, maturity, and gonadal cycle of this species at the northern edge of its range are similar to findings in southern populations, and climate, not changes in breeding physiology, constrain breeding at this northern site.

Introduction

The eastern narrowmouth toad, *Gastrophryne carolinensis*, is a small, semi-fossorial, microhylid species that attains a maximum body length of 38 mm (Conant and Collins, 1998). This species ranges throughout the southeastern and southcentral United States (from Maryland westward to central Missouri and southward to central Texas). Anderson (1954) provided the most thorough study of the ecology of *G. carolinensis* from populations in Louisiana. Recently, Meshaka and Woolfenden (1999) explained geographic variation in calling and reproductive seasons of *G. carolinensis* to fit within a framework of climatic constraints. Nelson (1972) summarized the literature on this species; however, quantitative life history information on this species is still rare. In the present study, we report on population structure and reproduction from samples collected during one season from sites near the northwestern edge of its geographic range and relate our finding with those of more southerly locations.

Materials and Methods

Post-metamorphic individuals of *G. carolinensis* were collected on 31 separate dates by one of us (RLC) during April-August 1989 from a two-county area (Craighead and Greene) in northeast Arkansas. Individuals were killed in a 20% solution of chloretone and fixed in 10% formalin within 24 hr of capture. Specimens were eventually transferred to 70% ethanol and are currently deposited in the herpetology collection at Arkansas State University (ASUMZ).

Body size of preserved specimens was measured in snout-vent length (SVL) with calipers to the nearest 0.1 mm. Routine histological techniques were used to prepare the testes of 87 males for light microscopy following standard methods (Humason, 1979). Testes were dehydrated in a graded series of ethanol, cleared with xylene, infiltrated and embedded in paraffin, sectioned into serial ribbons (8 µm in thickness), affixed to microscope slides using Haupt's adhesive, stained with Harris hematoxylin followed by eosin counterstaining (H & E), and mounted with coverslips. Maturation of sperm was categorized using the two phase system for *G. carolinensis* of Anderson (1954). Phase I (Ph-1) = recrudescence (i.e., increased mitotic activity) within the primary spermatogonial cysts following the breeding season. Ph-2 = a proliferation of clusters of secondary spermatocytes and spermatids which dominate the germinal epithelium.

After being blotted dry, the ovaries were massed (to the

nearest 0.001g) using an analytical balance; this was followed by the removal of a small subsample of ovarian follicles in specimens containing pigmented ova. The subsamples were also massed using the same method as above. The largest diameters of 10 pigmented ova were measured with an ocular micrometer. Mean values, when given, are accompanied by 1 standard deviation.

Results

Growth and Maturity.--Post-metamorphic individuals were found during late summer of a short active season (Fig. 1). Sexual maturity was reached as individuals entered their second spring of life (Fig. 1) at body sizes smaller in males (21.5 mm SVL) than in females (26.7 mm SVL). Maximum body size of females (36.5 mm SVL also exceeded that of males (33.6 mm SVL), and mean adult body size of females (29.6 ±2.6 mm; range, 24.0 - 36.5; n = 60) was significantly larger (t = -4.44; $P < 0.001$) than that of males (27.6 ± 2.6; range, 24.0 - 36.5); n = 91). Groups of size-classes in males were unclear; however, three groups of size-classes were apparent in sexually mature females (Fig. 1). This being the case, female *G. carolinensis* in northeastern Arkansas could live at least five years. The sex ratio (1:1.52) in favor of males reflected a collecting bias within breeding aggregations ($\chi^2 = 6.36$; df = 1; $P < 0.01$).

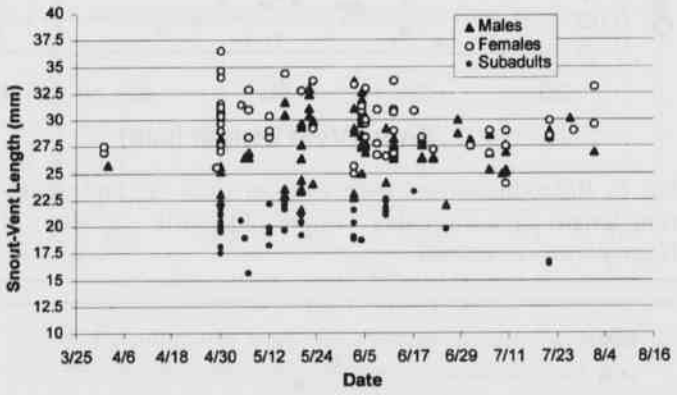

Fig. 1. Seasonal distribution of snout-vent lengths of *Gastrophryne carolinensis* collected in northeastern Arkansas during 1989.

Male Reproductive Cycle.--The testicular structure of *G. carolinensis* consisted of convoluted seminiferous tubule masses surrounded by interstitial cells; the germinal epithelium in each tubule undergoes cystic maturation of sperm (termed cystic spermatogenesis; see Lofts, 1987). Spermatogenic Ph-I and II were observed in the testes of Arkansas specimens of *G. carolinensis* (Figs. 2 and 3). Nearly all males > 21.5 mm SVL exhibited sperm; the smallest male not producing sperm was 22.7 mm SVL collected on 2 June. The percentages of Ph-I males decreased over time: 100% (12 of 12) in April, 74% (17 of 23) in May, and 3% (1 of 33) in June. Testes in smaller males generally remained in Ph-I longer into the breeding season compared to larger males; the latest Ph-I individual was observed on 2 June. Ph-II males were first observed in early May. The greatest concentrations of sperm were observed in Ph-II testes of June individuals. Because the testes of most adult males possessed sperm, males were capable of participating in breeding activities during April - August.

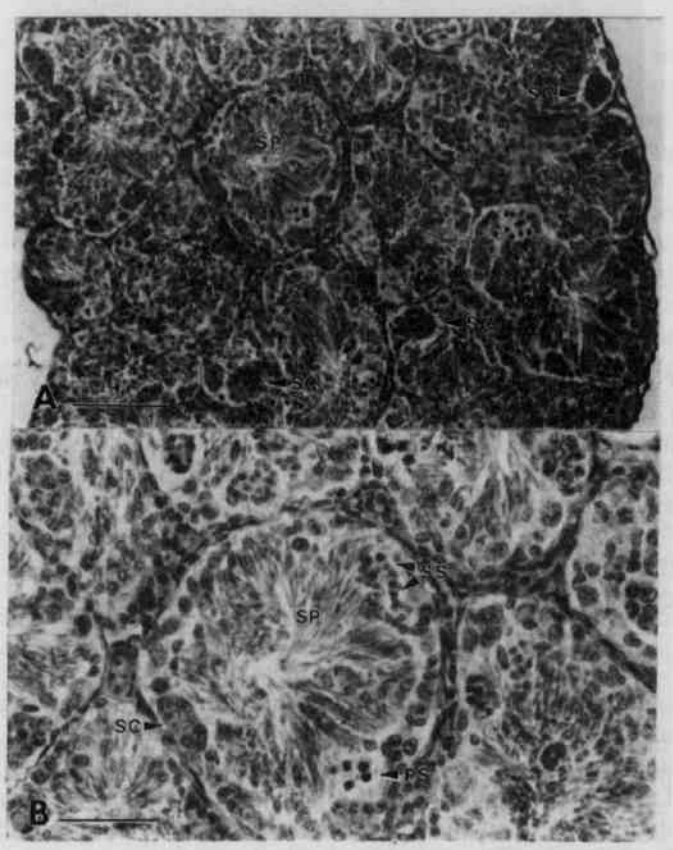

Fig. 2. Light photomicrographs of the testicular histology of *Gastrophryne carolinensis* illustrating Phase I. A. Testis of specimen ASUMZ 13006 (SVL = 30.5 mm) collected on 30 April 1989. Clusters of spermatogonial cells form spermatogonial cysts (SC) in seminiferous tubules; sperm (SP) are numerous within lumina of tubules. Line = 100 µm. B. Magnification of A revealing additional spermatogenic stages (i.e., primary spermatocytes [PS] and secondary spermatocytes [SS]). Line = 50 µm; abbreviations same as in A.

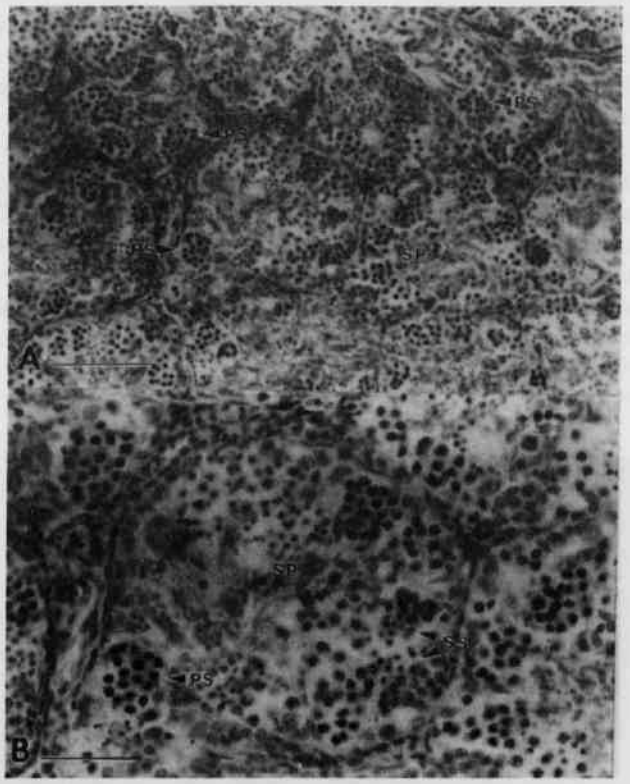

Fig. 3. Light photomicrographs of the testicular histology of *Gastrophryne carolinensis* illustrating Phase II. A. Testis of specimen ASUMZ 13274 (SVL = 30.2 mm) collected on 5 June 1989. Clusters of primary spermatocytes dominate the basal epithelium within seminiferous tubules. Line = 100 m. B. Magnification of A revealing additional spermatogenic stages (i.e., primary spermatocytes [PS] and secondary spermatocytes [SS]). Line = 50 µm; abbreviations same as in Fig. 2.

Female Reproductive Cycle.--Gravid females were present throughout the activity season; however, fertility, as measured by ovarian mass and follicular diameter, increased through the season and peaked in June (Fig. 4). Large females were gravid throughout the season, whereas the ovaries of small females generally did not mature until June (Fig. 5). The range of individual variation among adult females may help explain the seasonal variation observed in clutch size (Fig. 6).

Ovarian mass of all females possessing enlarged, pigmented eggs varied greatly ($\bar{x}$ = 0.4002 ± 0.259 g; range = 0.1021-1.0078; n = 24), and although smaller adult females generally contained fewer pigmented eggs than older, larger females, the largest clutches were not found in the largest females (Fig. 7). Consequently, clutch size ($\bar{x}$ = 673.2 ± 321.0; range = 186 -1459; n = 24) was not significantly correlated (r = 0.262; $P > 0.05$) with body size of the female (Fig. 7).

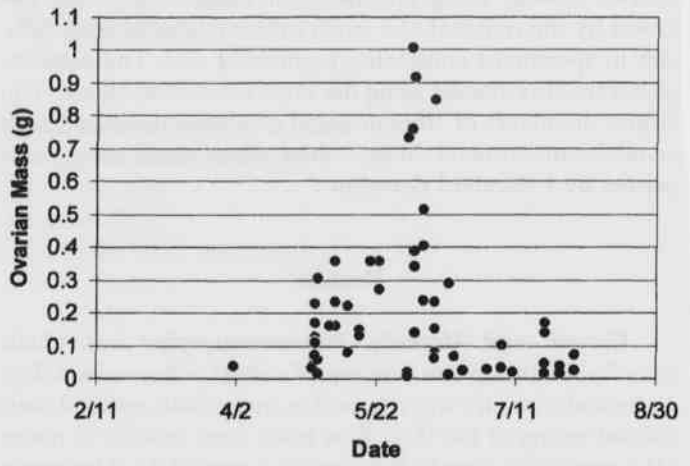

Fig. 4. Relationship between ovarian mass (g) and date of collection in *Gastrophryne carolinensis* in northeastern Arkansas during the 1989 activity season.

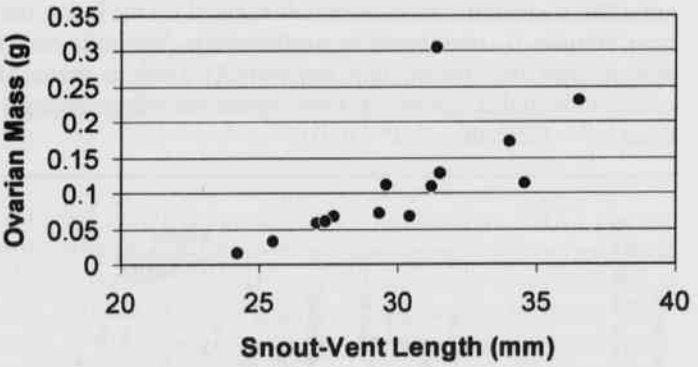

Fig. 5. Relationship between ovarian mass (g) and snout-vent length in a three-day sample (30 April – 2 May) of *Gastrophryne carolinensis*.

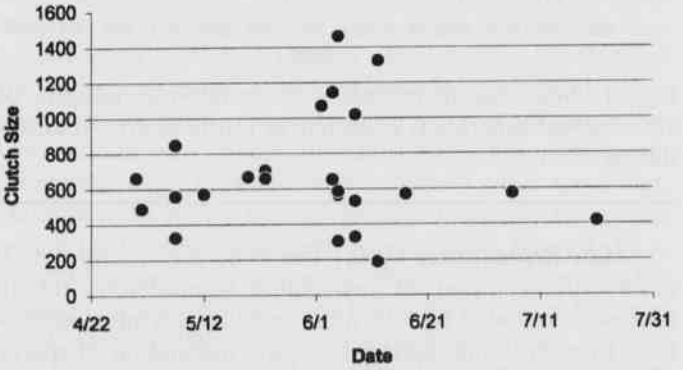

Fig. 6. Relationship between clutch size and date of collection in *Gastrophryne carolinensis*.

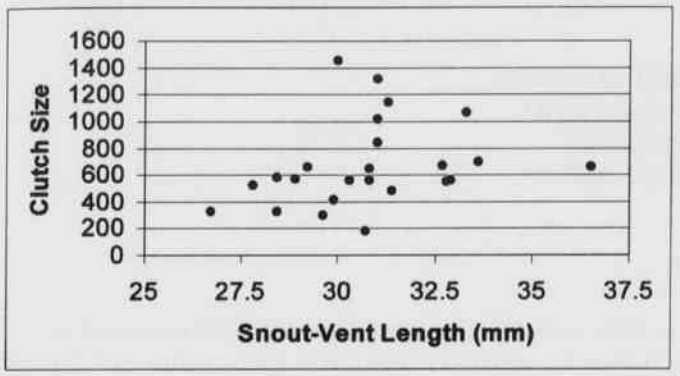

Fig. 7. Clutch size as a function of snout-vent length in *Gastrophryne carolinensis*.

Ovum diameter, likewise, varied greatly ($\bar{x}$ = 1.0448 ± 0.144 mm; range = 0.7312 -1.2870) among females. Like the seasonal shifts in frequency of gravid females, average ovum diameter gradually increased as the season progressed (Fig. 8); the largest diameters (> 1.10 mm) were recorded in June. No significant correlation (r = -0.2195; $P > 0.05$) was found between ovum diameter and body size of the female.

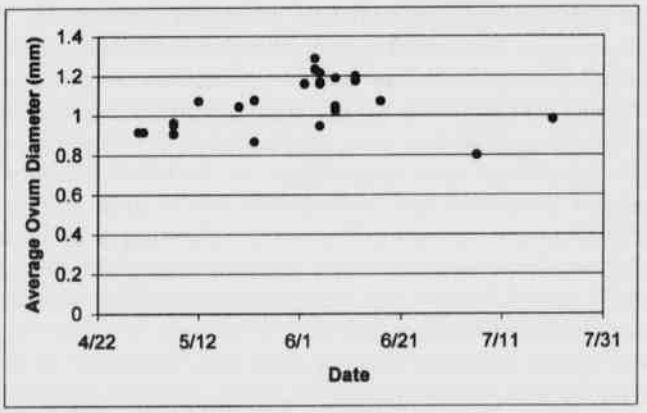

Fig. 8. Relationship between average ovum diameter and date of collection in gravid *Gastrophryne carolinensis*.

Discussion

Despite a shorter active season in northeastern Arkansas, post-metamorphic growth and reproduction of *G. carolinensis* from northeastern Arkansas were similar in many respects to findings from southern populations. Sexual maturity appeared to occur at similar ages as in a Louisiana population (Anderson, 1954). Although body size at maturity was slightly larger in Arkansas populations, body size/age cohorts among Arkansas females were similar to those in Louisiana (Anderson, 1954). For example, in Louisiana body size of individuals ranged 13.0 - 19.0 mm SVL in the first spring of life, 21.0 - 24.0 mm SVL in the second year, 24.5 - 26.9 mm SVL in the third year, and 27.0 - 35.0 mm SVL in succeeding year samples. Using our late April sample, there appeared to be size gaps in the frequency in adult females between 25.5 -27.1 mm, 31.5 -34.0 mm, and 34.6 -36.5 mm in body size. This suggests the possibility of at least five different age classes using female size as noted by Anderson (1954). Like south-central Florida (Meshaka and Woolfenden, 1999) and Louisiana (Anderson, 1954) populations, mean adult body sizes were similar between the sexes, and the sex ratio did not differ from unity.

The gonadal cycle of Arkansas males and females were in synchrony. Although both sexes mere capable of reproduction from emergence in the spring until the end of their active season, frequency of fertile individuals peaked strongly in June. For males, this was evidenced by few Ph-I and maximal number of Ph-II testes in June. High midsummer fertility in females was disclosed by the high frequency of gravid individuals and large ova at the same time. Louisiana *G. carolinensis* captured over a longer period than those from our study revealed a similar pattern and synchrony to the gonadal cycle during April - August (Anderson, 1954). In this connection, throughout its geographic range, mid summer is the peak in calling and presumably oviposition in *G. carolinensis* (Meshaka and Woolfenden, 1999).

Anderson (1954) observed that some females oviposited early in the season, while others oviposited later in the season, but was unsure why this occurred. In Arkansas, smaller females were generally ready to oviposit later in the season than larger-bodied females, perhaps because of recent attainment of sexual maturity. We also observed that among size-classes of females, clutches ranged from small to very large. Although energetics could be responsible for this phenomenon, partial deposition of clutches, suspected in other Arkansan anurans (Trauth et al., 1990), might better explain the wide range in clutch characteristics within size-classes in light of the shortend available months for oviposition.

Meshaka and Woolfenden (1999) identified temperature and rainfall thresholds that constrain calling and reproductive-related movements in this species. Our findings indicate that although climate severely constrains the breeding season in northeastern Arkansas, physiologically, *G. carolinensis* at the northwestern edge of its geographic range is no less capable of breeding over a longer season than southerly populations, and adheres to the mid-summer peak in breeding found throughout its range.

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