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REPRODUCTION AND LARVAL DEVELOPMENT IN THE MARBLED SALAMANDER, *AMBYSTOMA OPACUM* (CAUDATA: AMBYSTOMATIDAE), FROM ARKANSAS

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ABSTRACT

Field investigations of the reproductive biology and larval growth and development in *Ambystoma opacum* from northeastern Arkansas began in early October, 1987 at the onset of nesting activities, and concluded in early May, 1988 when larval transformation occurred. The onset and timing of clutch deposition were documented. Clutch size averaged 107.1 eggs and was not significantly correlated with snout-vent length (SVL). The incubation period was estimated to be 90 days. Larvae grew at an average rate of 6.3 mm SVL per month. Metamorphosis, in early May, occurred at an average SVL of 30.5 mm. Larval development was recorded by noting the chronological appearance of digits on limb buds as well as noting the change in body color patterns. Premetamorphs characteristically retained a ventrolateral row of white spots throughout development. Postmetamorphs eventually lost these spots and developed a color pattern totally unlike adults.

INTRODUCTION

Numerous studies have documented various aspects of the life history of the marbled salamander, *Ambystoma opacum*, throughout its range in eastern North America (Anderson, 1967). Nesting activities, reproductive potential, and larval growth are among the most common life history traits examined in this species (Petranka and Petranka, 1980). Although photographs of adults or nests with brooding females are depicted in the herpetological literature, authors usually seldom bother with illustrating premetamorphs or postmetamorphs temporally. Moreover, few studies have been conducted on this species west of the Mississippi River, and little information other than distributional records (Black and Dellinger, 1938; Dowling, 1957) is available on this species in Arkansas. This report presents information on the reproductive habits and larval growth of *A. opacum* in northeastern Arkansas.

Table 1. Reproductive parameters of 10 nesting female *Ambystoma opacum* from Craighead County, Arkansas. Ranges are in parentheses.

ASUMZ No.	SVL (mm)	Total Length (mm)	Body Mass (g)	Clutch Size	Clutch Mass (g)	$\bar{X}$ Egg Diameter (mm; n = 10 per clutch)	Nest Volume (cm^3)
9107	68.3	120.6	9.6	131	6.9	5.4 ±0.32 (5.0-6.8)	106.3
9108	61.7	112.7	8.0	87	3.6	5.8 ±0.44 (5.2-7.3)	90.6
9109	66.2	124.5	11.3	100	3.5	5.6 ±0.18 (5.2-6.1)	17.0
9110	62.1	113.0	8.4	124	9.5	5.3 ±0.10 (5.0-5.5)	57.7
9111	59.5	106.7	7.7	93	7.0	5.4 ±0.25 (5.0-6.2)	37.6
9112	66.2	120.7	8.3	117	10.0	5.7 ±0.55 (4.4-7.9)	104.1
9119	61.2	110.5	6.7	97	4.1	5.6 ±0.44 (4.6-7.2)	24.6
9120	62.5	112.5	8.6	107	6.7	5.3 ±0.20 (4.9-6.0)	12.2
9121	65.5	114.3	7.4	108	6.9	5.5 ±0.29 (4.9-6.4)	141.8
9122	60.1	110.5	7.5	107	7.1	5.3 ±0.20 (5.0-5.9)	14.5
Average Totals	63.3 ±1.09	114.6 ±3.51	8.4 ±0.62	107.1 ±8.72	6.5 ±1.40	5.4 ±0.11	60.6 ±29.6

MATERIALS AND METHODS

Field investigations of *A. opacum* were conducted in northern Craighead County, Arkansas, from early October, 1987 through early May, 1988. All adults, eggs, and larvae were collected in or near temporary ponds or depressions which were found in abandoned gravel pits on Crowley's Ridge (a low, narrow, eroded ridge that contrasts sharply with the surrounding alluvial floodplains within the Mississippi River Alluvial Valley). A series of postmetamorphs were also examined from other localities in either Greene or Jackson counties. Ten nests with attending females were studied at one gravel pit site to determine reproductive parameters (Table 1). Adult SVL as well as other adult and larval measurements were rounded to the nearest 0.1 units. Although males (n = 7) and nesting females (n = 10) along with their eggs were removed from the primary study site, many nesting sites were left undisturbed in order to determine time of hatching as well as subsequent larval growth. The ponds were periodically sampled for larvae between late January and early May. All specimens were sacrificed in a 20% chloretone solution, fixed in 10% formalin, and preserved in 70% ethanol. Statistical data are followed by ± 2 SE. Specimens are deposited in the Arkansas State University Museum of Zoology (ASUMZ).

RESULTS AND DISCUSSION

Reproductive Traits

Female *A. opacum* were observed in gravel pits in early October and had constructed nests prior to the onset of rainy weather. Nests were generally spherical-to-oblong depressions within a red clay substrate with most found beneath trash (old mattresses, boards, rotting logs, etc.). By 15 November, all nests had been submerged by as much as 1.0 m of water. Adult nesting females were larger ($\bar{x}$ = 63.3 ± 1.9 mm SVL; Table 1) than the adult males ($\bar{x}$ = 56.0 ± 4.6 mm SVL; range, 51.2 - 64.8) collected within the immediate vicinity of nesting sites. In two instances, adult males were found together with brooding females, and the SVL of these pairs (male followed by female) and the female's clutch size (in parenthesis) were 64 and 68 (131) and 51 and 61 (97). No spermatophores were observed in or around nesting cavities.

Routine searching of optimal nesting situations revealed that only one egg clutch was laid between 8 and 10 October, and the female had departed from the clutch by 16 October (rainwater partially submerged her nest between 11 and 14 October). We also observed two other females that had abandoned their nests during a period of intermittent rain; nests were found under a mattress on 15 October. This site had been previously searched on 12 October, and no specimens were observed. Both females had left their nests by 27 October, and rainwater eventually inundated the entire site by 1 November.

Female *A. opacum* lay one egg clutch per reproductive season (Noble and Brady, 1933). We found the average clutch size to be 107.1 ± 8.7, and clutch size was not significantly correlated with SVL ($r = 0.2$; $P > 0.05$).

Larval Growth and Development

Growth of larval *A. opacum* from samples taken from a single nesting site (pond) were monitored. Larvae normally hatch between 15 and 19 mm in total length (Brandon, 1964); the incubation period in northeastern Arkansas was estimated to be 90 days. Larvae grew at an average rate of 0.2 mm per day or approximately 6.3 mm per month (Fig. 1). Metamorphosis occurred by early May with an average SVL of 30.5 mm ± 1.4 and an average total length of 49.2 ± 1.7 mm.

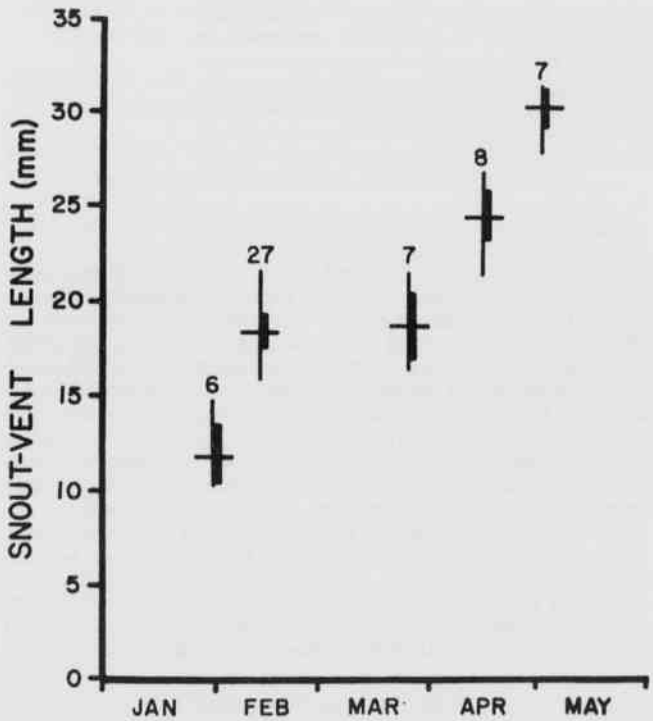

Figure 1. Larval growth in *Ambystoma opacum* from northeastern Arkansas. Numerals = sample sizes; horizontal lines = means; vertical lines = ranges; vertical bars = ± two standard errors.

Larval development was analyzed by noting the chronological appearance of digits on the anterior and posterior limb buds. In late January, most larvae exhibited forelimbs possessing from two to four toes (four the normal complement), whereas the hindlimb varied from an absence of toes to the presence of four or five toe buds (five the normal complement). By mid-March, the forelimbs had four well-developed, elongated toes, but the hindlimbs had only four well-developed toes. All toes on both pairs of limbs were present by mid-April.

Variation in coloration of larvae and postmetamorphs is shown in Fig. 2. Young larvae smaller than 15 mm SVL (Fig. 2A) were uniformly dark dorsally. A series of distinct, circular light spots (associated with the lateral line system) lie on the ventrolateral surfaces between the limbs (one for each costal fold); ventral areas of the head, throat, and belly were mostly transparent. As development proceeds, the dark pigmentation becomes less intense on the entire body and especially on the tail fin of larvae approximately 20 mm SVL (Fig. 2B). A dorsal row of lateral line organs becomes obvious at this size as they are surrounded by circular areas mostly devoid of pigmentation. Ventrolateral white spots remain prominent. By mid-April, larvae have grown to around 25 mm SVL, patches of dark pigmentation are found on the head and face, and melanophores have aggregated on the body to create a mottled appearance (Fig. 2C). Larvae also begin to exhibit varying degrees of a light yellowish-green coloration. The dorsal spots of the lateral line system and the ventrolateral white spots are obvious at this stage. Pigmentation within the tail fin takes on a reticulated appearance. Immediately following transformation in early May, postmetamorphs (Fig. 2D) continue to possess the two linear series of light spotting on the body, and ground color varies from olive green to grayish black. At around 35 mm SVL in mid-May, postmetamorphs (Fig. 2E) have lot all signs of a keeled tail (as seen in Fig. 2B). Ground color of the entire body is now black, and areas of intense white flecking are scattered from snout to tip of tail on the dorsal aspect. This juvenile coloration bears little resemblance to the coloration of the adult marbled salamander (see Conant, 1975; Duellman and Trueb, 1986; Trauth and Richards, 1988). Postmetamorphs may retain the ventrolateral series of white spots for over a week after transformation (Anderson, 1967).

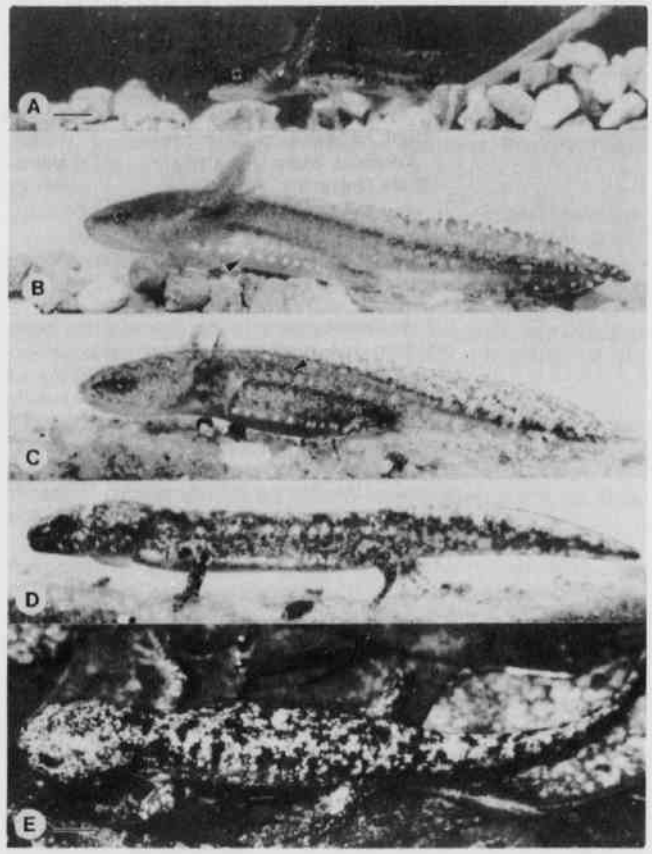

Figure 2. External appearance of larval (A, B, and C) and postmetamorphic (D and E) *Ambystoma opacum* from northeastern Arkansas. Pointer in B denotes ventrolateral series of white spots and in C, the dorsolateral spots associated with the lateral line organs. See text for further explanations. Line in A = 3 mm for A – D; line in E = 4 mm.

A comparison of the reproductive traits and larval development of *A. opacum* from northeastern Arkansas with previous accounts on the species throughout its range (see reviews in Anderson, 1967; Petranka and Petranka, 1980) revealed no major variations. For example, average clutch size and female SVL in Mississippi ($\bar{x}$ = 107.3 and 62.5, respectively) were essentially the same as our findings (Walls and Altig, 1986). Size at metamorphosis in Alabama (32.5 mm SVL; Petranka and Petranka, 1980), Kentucky (33.8 mm SVL; Keen, 1975), and Maryland

(34.2 mm SVL; Worthington, 1968) was similar to our results. In addition, time of metamorphosis was March to April, May, and May, respectively, in these studies and did not differ from our results.

Larval growth rates in *A. opacum* are variable under natural conditions as low temperatures during winter months may inhibit growth (Hassinger *et al.*, 1970). We noted that larval growth at our primary study site slowed dramatically between mid-February and mid-March. Also, our observations on the development of the limbs and toes were similar to data presented by Hassinger *et al.* (1970) for New Jersey populations, although larvae from Arkansas had their full complement of toes slightly earlier.

Our presentation of variation in larval external morphology via photographs clearly illustrates the chronological changes in color pattern that take place during ontogenetic development in *A. opacum*. Viosca (1924) and Anderson (1967) described color patterns of pre- and postmetamorphic individuals, but they provided no photographs or illustrations of these stages. Bishop (1943) pictured both phases; however, his larval photograph presented no diagnostic features. Duellman and Trueb (1986) showed a premetamorph (p. 157) and an adult and postmetamorph (p. 185). Our series of photographs are the only published data to summarize external coloration and diagnostic features of larval and postmetamorphic specimens of *A. opacum*. The presence of the ventrolateral series of white spots has been used as an identification aid in this species (Brandon, 1964; Anderson, 1967, Altig and Ireland, 1984). We suggest that since other *Ambystoma* larvae (e.g., *A. annulatum, A. maculatum,* and *A. talpoideum*) may also exhibit a similar spotting pattern (at least in young larvae), some caution be exercised when making field identification of larvae in sympatric situations.

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