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Reproduction in West Virginia Populations of the Southern Two-lined Salamander (*Eurycea cirrigera*)

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Abstract.—We describe reproduction in West Virginia populations of the southern two-lined salamander (*Eurycea cirrigera*) and provide insights into the behavioral ecology of this wide-ranging urodele. The first signs of reproduction are evident well before the arrival of Spring. Sexually mature adults inhabit the cold, rocky streams of southwestern West Virginia in early February. Females are known by the presence of large öocytes visible through the body wall and males by their extremely swollen heads. Breeding occurs in the stream during late March and is marked by the presence of gravid females with sperm caps in their posterior cloacae. Eggs are deposited from mid-March through early April on the underside of flat rocks in cool, shallow, and swiftly flowing streams. Females guard their nests against potential predators, including other two-lined salamanders. Hatchlings emerge as miniature adults with gills after several months of development.

INTRODUCTION

Traditional conservation programs emphasize the protection of species that are imminently threatened by extinction (Dodd and Franz 1993; Lovich and Gibbons 1997). Most funding and research effort is directed at rare species, while the biological status of common species is frequently overlooked (Dodd and Franz 1993). Dramatic and unexpected declines have occurred, however, in many populations of seemingly common amphibian and reptile species, including tortoises (Dodd and Franz 1993), freshwater turtles (Dodd and Franz 1993), and frogs (Blaustein and Wake 1990; Wyman 1990). These examples suggest we can no longer afford to ignore common species. A proactive alternative is the development of monitoring protocols that include rare and common species alike (Dodd and Franz 1993). With such protocols in place, declines in seemingly common species can be identified before the slide towards extinction is irreversible.

Members of the two-lined salamander complex (*Eurycea bislineata*, *E. cirrigera*, and *E. wilderae*) are found throughout eastern North America and are generally perceived as common. Adults are typically found under rocks and logs along streams, springs, and seeps. *Eurycea cirrigera*, the southern two-lined salamander, occurs from southern Virginia, west to eastern Illinois, and south to northern Florida and eastern Louisiana (Mittleman 1966; Conant and Collins 1998; Petranka 1998). In West Virginia, *E. cirrigera* occupies the southwestern two-thirds of the state in the Allegheny Plateau physiographic province (Jacobs 1987; Brophy 1995; Conant and Collins 1998). Because it is such a wide-ranging species, geographic variation in life history traits is common (Petranka 1998). Unfortunately, life histories of many West Virginia salamanders are

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poorly documented. In this paper we describe reproduction in West Virginia populations of *E. cirrigera* (corroborating Green and Pauley 1987) and provide insights into the behavioral ecology of this wide-ranging urodele.

METHODS AND MATERIALS

During 1994 and 1995, various aspects of reproduction were examined in two populations of *E. cirrigera* located at Fitzpatrick's Branch (a tributary to Hisey Fork) in Huntington, Cabell County, West Virginia, and an unnamed tributary to Beech Fork near Bowen, Wayne County, West Virginia. Both are small intermittent streams flowing through *Quercus* dominated, mixed mesophytic forests. In addition to populations of *E. cirrigera*, each supports a population of northern dusky salamanders (*Desmognathus f. fuscus*) and Kentucky spring salamanders (*Gyrinophilus porphyriticus duryi*).

Sexually mature adults and egg masses were located by turning rocks and logs in and along the two streams. Sex and reproductive condition were determined for each adult captured, and snout-vent length (SVL), head width (at broadest point), and head length (tip of snout to gular fold along ventral midline) were measured to the nearest 0.1 mm using dial calipers. Water temperature (°C) was also recorded for each adult captured in the aquatic habitat. In addition, we examined each clutch, counted the number of eggs, and measured the maximum length and width of the egg mass to the nearest 0.1 mm. We also measured the diameter of five eggs (0.1 mm) and recorded the sex and size of adults found near each mass. The following microhabitat characteristics were measured for each nest site and pair of breeding adults: rock size (surface area=SA; thickness=THK in cm); distance to nearest stream edge (cm); water temperature (°C); water depth (cm); and stream flow (m/s).

One egg mass, collected on 31 March 1994 from the tributary to Beech Fork, was taken to the laboratory at Marshall University and maintained in aerated streamwater. Water temperatures ranged from 13-21°C because the egg mass was kept in a poorly heated laboratory within five meters of an old window. The egg mass was monitored and individuals were staged according to Harrison (1969) on four separate dates. Upon hatching, salamanders were killed in chloretone, fixed in 10% buffered formalin, and stored in 70% ethanol. The SVL of hatchlings was measured to the nearest 0.1 mm using a dissecting microscope and ocular micrometer. Data are reported as mean values ± 1 SD (minimum-maximum).

RESULTS AND DISCUSSION

Sexually Mature Adults

Gravid *E. cirrigera* females (N=7) were found in the tributary to Beech Fork between 12 February and 9 April 1994 at water temperatures of 4.0-12.5°C. Females were identified by the presence of large öocytes visible through the body wall (Stewart 1956; Bruce 1988), and the SVL of seven gravid females averaged 43.2 ± 1.9 (40.9-45.8) mm. Published SVL measurements for gravid two-lined salamanders range between 31.0 to 50.0 mm (Wood and McCutcheon 1954; Stewart 1956; Bruce 1988; Bahret 1996).

Sexually mature *E. cirrigera* males (N=30) were found in the tributary to Beech Fork between 25 February and 17 April 1994 at water temperatures of 6.0-14.0°C. They

possessed secondary sexual characteristics including swollen heads (Sever 1979). The SVL of 30 mature males averaged 42.6 ± 2.4 (38.4-49.5) mm and was similar to the published values (30.2-45.3 mm) of Stewart (1956) and Bruce (1988). The average head width to length ratio (HW/HL) for mature males in our study was 0.94 ± 0.05 (n=28), which was significantly greater (t-test, $t = 5.92$, df = 32, $P < 0.001$) than that of mature females (HW/HL = 0.80 ± 0.06, n = 6). Male two-lined salamanders from portions of Ohio, Kentucky, and West Virginia lack cirri but develop an enlarged temporal musculature during the breeding season, representing an extreme case of polymorphism in this species complex (Sever 1972, 1979).

Breeding

On 31 March 1994, a mature male and gravid female *E. cirrigera* were found breeding under a flat rock in the tributary to Beech Fork. The male, 45.1 mm SVL, had an enlarged temporal musculature (HW/HL=1.02) as described above. The gravid female, 43.7 mm SVL, had a sperm cap in her posterior cloaca. The rock (SA=238 cm^2, THK=5 cm) sheltering these salamanders was located in a small riffle (stream flow=0.37 ± 0.04 m/s), 55 cm from the nearest stream edge. Water temperature and depth at the capture point were 7.0°C and 9.0 cm, respectively. Two-lined salamanders breed from September through May (Noble and Weber 1929; Weichert 1945; Stewart 1958; Sever 1979), but patterns in local populations depend on the severity of winter weather and other environmental factors (Petranka 1998).

Egg Masses

Between mid-March and early April 1994 and 1995, six egg masses deposited by *E. cirrigera* (Table 1) were discovered in Fitzpatrick's Branch and the tributary to Beech Fork. All egg masses were oval, attached to the underside of rocks within the streams, and in the early stages of development. Masses were found between 21 March and 8 April in both years. Mean clutch size was 47.2 ± 8.33 and mean egg diameter was 3.11 ±

Table 1. Data for *Eurycea cirrigera* egg masses discovered at the tributary to Beech Fork (BF) and Fitzpatrick's Branch (FB) in southwestern West Virginia. Values for egg diameter are means ± 1 SD and n=5 in all cases.

Mass #	Date	Clutch Size	Mass Size (mm)	Egg Diameter (mm)
BF-1	31 Mar 1994	59	46.4 x 39.1	3.52 ± 0.13
BF-2	6 Apr 1994	42	41.0 x 30.8	3.04 ± 0.22
FB-1	21 Mar 1995	43	40.0 x 35.0	3.00 ± 0.00
FB-2	21 Mar 1995	51	50.0 x 30.0	3.00 ± 0.00
FB-3	8 Apr 1995	36	58.0 x 50.0	3.10 ± 0.22
FB-4	8 Apr 1995	52	50.0 x 30.0	2.90 ± 0.22
Mean ± 1SD		47.2 ± 8.33	47.57 ± 6.67 x 35.82 ± 7.82	3.11 ± 0.27

0.27 mm. Mean length and width of the six masses were 47.57 ± 6.67 mm and 35.82 ± 7.82 mm, respectively. These results correspond well with published reports of egg-laying in the two-lined salamander complex (Table 2).

A single adult was found near four of the six egg masses. Two were spent females (BF-1 and BF-2; SVL of BF-2 female=41.9 mm), one a gravid female (FB-2; SVL=45.0 mm), and one a mature male (FB-1). The spent females were probably guarding their nests, which is a common practice in this species complex (Wood 1949, 1953; Richmond 1945; Stewart 1956; Baumann and Huels 1982; Bruce 1982; Marshall 1996). Both streams in our study contain numerous potential egg predators including crayfish (*Cambarus* sp.), dusky salamanders (*D. f. fuscus*), and spring salamanders (*G. porphyriticus duryi*). The presence of *E. cirrigera* females may deter some of these predators (Stewart 1956; Baumann and Huels 1982). The gravid female with egg mass FB-2 (Table 1) may have been attempting to oviposit on that same rock (Table 3), as the complements of several females are frequently found in the same nest (Bishop 1941; Weber 1928; Wood 1953; Wood and McCutcheon 1954; Baumann and Huels 1982; Green and Pauley 1987). Alternatively, both the gravid female and mature male may have been attempting öophagy (D. C. Forester, pers. comm.).

Nest Site Microhabitat

Microhabitat characteristics for each nest site are presented in Table 3. Nests were found in cool, shallow, swiftly flowing stream riffles. Mean water temperature was 9.4 ± 2.1° C. Mean water depth was 6.36 ± 3.73 cm. Mean stream flow was 0.24 ± 0.08 m/s. Nests were found at both the edge and center of the streams. Mean distance to the nearest stream edge was 28.83 ± 19.58 cm. Two-lined salamanders prefer the underside of flat rocks for nesting (Wilder 1899; Noble and Richards 1932; Baumann and Huels 1982), and these are often a limiting factor in reproduction (Stewart 1968). Mean surface area and thickness of the rocks utilized in our study were 173.08 ± 33.66 cm^2 and 6.38 ± 4.82 cm, respectively. Our data on nest site microhabitats corroborates the information presented by other authors (Table 4).

Development and Hatching

The egg mass reared in the laboratory (BF-1) was deposited on 29 March 1994 and completed development on 25 April 1994. On 1 April 1994, three days from deposition, the eggs were between Harrison (1969) stages 1 and 12. On 12 April 1994, 14 days from deposition, the eggs were between Harrison (1969) stages 29 and 30. On 21 April 1994, 23 days from deposition, the eggs were between Harrison (1969) stages 42 and 45. At this point, some of the eggs had hatched and by 25 April 1994 all eggs were hatched. All eggs hatched between Harrison (1969) stages 42 and 45. The development period for two-lined salamanders ranges from 1-3 months in nature (Wilder 1924; Bishop 1941; Duellman and Wood 1954; Johnson and Goldberg 1975; Green and Pauley 1987; Bruce 1982, 1985, 1988). The egg mass in our study completed development in 27 days at water temperatures of 13-21° C. Stream temperatures were much lower during this period (7-15°C), so development in the field would likely have taken longer (Bishop 1941; Petranka 1998).

Forty-nine (83%) of the original 59 laboratory reared eggs reached the hatching stage. The condition of two of the 49 hatchlings did not allow measurement of SVL; the

Table 2. Published reports of oviposition dates and egg mass characteristics for members of the *Eurycea bislineata* (Two-lined Salamander) complex. Numbers are means unless otherwise indicated. Single observations are marked with an asterisks and numbers in parentheses are ranges. A dash indicates no data reported.

Species	Locality	Oviposition Period	Clutch Size	Mass Size (mm)	Egg Diameter (mm)	Reference
cirrigera	southern WV	mid Mar-mid Apr	40*	–	–	Green and Pauley 1987
	SE OH	3-17 May	39 (15-110)	50 x 32	2.5	Baumann and Huels 1982
	SE OH	–	36*	–	–	Siebert and Brandon 1960
	Cincinatti, OH	late Mar-mid Apr	–	–	–	Weichert 1945
	James City Co., VA	23 Jan-16 Apr	52 (18-96)	–	–	Wood and McCutcheon 1954
	New Kent Co., VA	21 Apr	(42-45)	–	2	Richmond 1945
	Lafayette Co., MS	Apr-May	53	–	–	Marshall 1996
	Athens, GA	–	36*	–	–	Martof 1955
wilderae	SW NC	Feb-May	23*	–	–	Bruce 1982, 1985, 1988
	Mt. Mitchell, NC	4 May	87*	–	2.5	Wood 1949
bislineata	Tucker Co., WV	early Apr	–	–	–	Marcum 1994
	NY	–	25 (3-41)	–	2	Noble and Richards 1932
	NY	11 Apr-25 Jun	(18-68)	(76 x 152)	(2.5-3.0)	Bishop 1941
	NY	20 Apr-21 May	34 (12-60)	–	(1.9-2.1)	Stewart 1956, 1968
	Shawangunk Mts., NY	–	15 (5-28)	–	–	Bahret 1996
	western MA	27 May-12 Jun	(30-50)	–	–	Wilder 1899
	Sunderland, MA	May-early Jun	18 (12-36)	–	–	Wilder 1924
	ME	20 May	40*	–	–	Verill 1863

Table 3. Microhabitat characteristics of *Eurycea cirrigera* nest sites from southwestern West Virginia. Values for water depth and stream flow are means ± 1 SD (n=3).

Mass #	Rock SA (cm^2)	Rock THK (cm)	Distance to Edge (cm)	Water Depth (cm)	Water Temp (°C)	Stream Flow (m/s)
BF-1	144.5	16.0	56.0	5.0 ± 0.00	7.0	0.37 ± 0.01
BF-2	165.0	6.0	50.0	13.0 ± 1.73	10.0	0.17 ± 0.02
FB-1	209.0	5.0	10.0	5.33 ± 2.31	8.0	0.22 ± 0.04
FB-2	156.0	4.0	20.0	6.0 ± 0.87	8.0	0.26 ± 0.06
FB-3	221.0	3.0	12.0	2.33 ± 0.29	12.5	0.23 ± 0.00
FB-4	143.0	4.2	25.0	5.58 ± 0.95	11.0	0.16 ± 0.03
Mean ± 1 SD	173.08 ± 33.66	6.38 ± 4.82	28.83 ± 19.58	6.36 ± 3.73	9.42 ± 2.11	0.24 ± 0.08

Table 4. Published reports of nest site microhabitat characteristics for members of the *Eurycea bislineata* (Two-lined Salamander) complex. Numbers are means unless otherwise indicated. Single observations are marked with an asterisks and numbers in parentheses are ranges. A dash indicates no data reported.

Species	Locality	Rock Size (cm^2) x Thickness (cm)	Distance to Edge of stream (m)	Water Depth (cm)	Water Temperature (°C)	Stream Flow (m/s)	Reference
cirrigera	SE OH	(132-1260)	–	–	–	–	Baumann and Huels 1982
	SE OH	929*	–	–	–	–	Siebert and Brandon 1960
	New Kent Co., VA	–	–	(2.5-7.6)	–	–	Richmond 1945
	Lafayette Co., MS	–	–	20*	(15-18)	(2.4-16.8)	Marshall 1996
wilderae	SW NC	2100x30*	4*	24*	–	–	Bruce 1982
bislineata	Essex Co., NY	329x10*	–	–	–	–	Weber 1928
	NY	–	–	(20.3-30.5)	–	–	Bishop 1941
	Ithaca, NY	330x9	(0.3-1.5)	(5.1-25.4)	(9-26)	–	Stewart 1956
	Shawangunk Mts., NY	–	32 (21-50)	1130 (900-1350)	–	–	Bahret 1996

remaining 47 hatchlings averaged 7.73 ± 0.23 (7.1-8.2) mm. Hatchling two-lined salamanders from other portions of the species range measure 6-11 mm SVL (Duellman and Wood 1954; Johnson and Goldberg 1975; Bruce 1982; Marcum 1994; Bahret 1996).

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