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OVIPOSITION, EARLY DEVELOPMENT AND GROWTH OF THE CAVE SALAMANDER, *EURYCEA LUCIFUGA*: SURFACE AND SUBTERRANEAN INFLUENCES ON A TROGLOPHILIC SPECIES

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ABSTRACT: We describe oviposition site, clutch characteristics and breeding phenology of a population of *Eurycea lucifuga*, the cave salamander, from SE Missouri, to understand the impact of biophysical and biotic conditions on a troglophilic species. In the field oviposition occurred in underground rimstone pools over 6 mo, with most reproduction occurring from August to October. Individual females deposited 1–31 eggs per pool, hatching approximately 10–20 d post-oviposition. Larvae remained in the pools as long as 6 mo before moving into the stream. We raised eggs and larvae of *E. lucifuga* in the lab and compared survival, growth, and development under three temperature regimes. Embryonic growth was slowest at cooler temperatures and produced larger larvae, while temperatures typical of summer surface stream temperatures resulted in high mortality. We suggest that the cool, predator free habitats of midwestern caves have allowed for a longer reproductive season in *E. lucifuga*, but that the unpredictable hydrology and limited food supply of these cave environments has expanded the breeding period and slowed embryonic and larval development.

Key words: Caves; Larvae; Plethodontidae; Reproduction; Salamanders; Streams

TEMPERATURE, habitat, and food resources significantly influence early life history and development in amphibians. Although morphological and physiological adaptations to the cave environment (e.g., reduced eyes and pigmentation, elongated limbs and appendages, and increased sensitivity of sensory organs) are best known (Barr, 1968), cave organisms also show modified life history traits, including reduced reproductive seasonality, fewer but larger eggs, increased longevity, and delayed onset of reproduction (Culver, 1982; Hüppop, 2000). Ryan and Bruce (2000) attributed the diversity of life histories seen in hemidactyliine salamanders (family Plethodontidae) to the diversity of environments inhabited. They hypothesized that food resources and the source and frequency of mortality strongly influenced the duration of life stages in these salamanders. Cave streams are probably low in energy, and their temperature and hydrological regimes differ from that of surface streams due to the insulating effects of the earth. Most of the Interior Highlands of the midwestern United States is comprised of porous karst overlying a shallow aquifer, with seasonally variable water flow in surface and cave streams (Taylor et al., 2000). Spring-fed streams may completely dry during summer months. The timing of reproduction in stream-breeding animals should occur such that eggs and larvae avoid exposure to spring floods and summer droughts (Bonett and Chippindale, 2004; Ryan and Bruce, 2000).

In addition to hydrology, temperature also differs between surface and cave streams and greatly influences survival, growth, and development in all ectotherms (Atkinson, 1994; Bernardo, 1994; Roff, 1992; Smith-Gill and Berven, 1979; Stearns, 1992). Cave species may have a longer period for reproduction and growth as compared to surface populations because of protection from seasonal extremes. Many species produce larger young at cooler temperatures, but Bernardo and Reagan-Wallis (2002) found that warmer temperatures produced larger larvae in several stream-dwelling plethodontids, and attributed this effect to energy limitations imposed by low productivity of upland stream environments (Huryn and Wallace, 1987; Minshall, 1967). Like mountain streams, caves are thought to be resource limited (Culver, 1982), and growth and development of cave organisms might be expected to be like that of montane stream organisms. Bernardo and Reagan-Wallis (2002) hypothesized that in cool mountain stream habitats, temperature effects on the biotic and abiotic environment of salamanders (e.g., food resources, predator

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abundance, length of breeding season) were more important in determining larval growth rates than temperature. As a result, carnivorous salamander larvae living in cool mountain streams might compromise how they allocated energy to reproduction, with the result that metamorphs would be smaller under cooler conditions and larger under warmer conditions with more food (Bernardo and Reagan-Wallis, 2002).

To understand temperature effects on *E. lucifuga*, we studied larval growth and development in the field and in the lab. We describe seasonal and thermal correlates of oviposition, physical characteristics of oviposition sites, and the number and size of eggs per clutch from a 17-mo field study. We compared developmental rates, size and stage at hatching, size at yolk absorption, and survival of *E. lucifuga* eggs and larvae under three temperature regimes in the lab. We hypothesized that timing of oviposition in the wild would occur at times that maximized growth rates, which occur during periods of warm water temperature, low flow conditions, and/or high larval food resources. Additionally, we hypothesized that early life-history traits of *Eurycea lucifuga* would reflect adaptation to both surface and cave environments (e.g., Ryan and Bruce, 2000). Facultative cave inhabitants like the cave salamander should be influenced by the buffered temperature regime of the cave and also by surface temperature and precipitation.

Methods

Study Species

The cave salamander, *Eurycea lucifuga*, is best described as a troglophilic species, one which uses caves regularly but is not restricted to them (Petranka, 1998). *Eurycea lucifuga* is found throughout the east-central United States (Petranka, 1998) and is associated primarily with entrances of limestone caves (Banta and McAtee, 1906), forested areas near damp rock walls, springs, and spring-fed swamps (Hutchinson, 1956).

Early life-history characteristics of *Eurycea lucifuga* are poorly known (Petranka, 1998) although some information exists for terrestrial adults (Banta and McAtee, 1906; Green et al., 1967; Hutchinson, 1956; Myers, 1958; Peck, 1974) and surface stream larvae (Banta and McAtee, 1906; McDowell, 1988; Myers, 1958; Rudolph, 1978; Trauth et al., 1990; Williams, 1980). Myers (1958) reported eight eggs under a rock from a cave stream in Missouri, and Green et al. (1967) found 16 eggs on the bottom of an unspecified number of pools in three caves in West Virginia. Much of what is known about reproductive phenology in *Eurycea* has been inferred from histological sectioning of field-collected animals from Arkansas and Illinois (Ireland, 1976; Williams et al., 1985), or from eggs laid after injection of hormones (e.g., Barden and Kezer, 1944; Trauth et al., 1990). Based on gonadal histology (Williams et al., 1985) and the presence of aquatic larvae in streams (McDowell, 1988) the breeding season in southern Illinois has been reported as October through December. Larval period is estimated as between 6–18 mo based on the rearing of field-collected larvae under unspecified lab conditions (Banta and McAtee, 1906; Rudolph, 1978).

Larval cave salamanders feed on benthic invertebrates, especially ostracods, amphipods and copepods (Petranka, 1998; Rudolph, 1978). Adults eat a diverse array of over 73 species of invertebrates of both surface and cave taxa (Peck, 1974), especially dipterans (Hutchinson, 1956). Potential predators of cave salamander eggs and larvae include fish and several aquatic invertebrates (e.g., crayfish, isopods, amphipods), although we never observed crayfish or fish in our study area.

Field Study

We established a 200-m transect along a passageway of Tom Moore Cave, 4.7 km NW of Perryville, Perry County, Missouri, which is part of the privately owned Moore Cave System. A permanent, shallow, surface-fed stream flowed through the main passageway or beneath adjacent bedrock. Substrate varied along the length of the transect, and included bedrock, silt and boulders. This stream eventually resurfaces in Blue Spring Branch, a tributary of Bois Brule Creek (Burr et al., 2001). Along the transect are numerous rimstone pools, small reservoirs of water located where the passageway meets the walls,

TABLE 1.—Water temperature did not vary between pools used for oviposition and pools not used.

Date	Oviposition temperature (C)	Mean pool temperature (C)	*P*-value
1 Aug.	15.5	15.4	ns
15 Aug.	15.8	15.8	ns
4 Sep.	17.5	16.9	0.06
11 Sep.	16.2	16.1	ns
18 Sep.	16.4	15.6	<0.05
25 Sep.	14.0	14.4	<0.05
2 Oct.	15.4	14.9	0.13
11 Oct.	14.2	14.2	ns
27 Nov.	14.5	13.8	<0.05
4 Dec.	14.5	14.1	<0.05
$\bar{x} \pm$ std	15.63 ± 2.4	15.07 ± 0.97	
Minimum	14.0	13.8	
Maximum	17.5	16.9	

formed by the action of surface water seeping into the cave. We mapped the location of each rimstone pool and measured the length, width, and depth of each pool to estimate maximum water volume. Water level of the rimstone pools varied with surface precipitation, and some pools dried for parts of the year. No floods occurred during our study, but we saw evidence that past floods would have filled the cave passage.

We conducted weekly surveys of 14 rimstone pools, selected due to the presence of eggs, along the 200 m transect from 21 August 1999 to 6 February 2001 and added one more pool to surveys on 3 November 1999, for a total of 1086 pool visits. During weekly visits from February 2000 to February 2001, we measured stream water temperature to the nearest 0.1 C at 0, 100, and 200 m of the transect, as well as in all pools, with a Hanna Instruments H8314 membrane thermometer. We also counted the number of eggs and larvae observed in each pool, scanning crevices and underhangs with the aid of a dentist's mirror. We defined a clutch as the number of eggs found at the same stage in the same pool during the weekly census, although we could not assign eggs to a particular female because we never observed females depositing eggs. We assumed that multiple females did not oviposit in the same pool at the same time. We staged 50–100% of eggs in each pool following Harrison's (1969) staging table for *Ambystoma maculatum*. By noting the approximate number and developmental stage of eggs in a pool, we were able to follow development and survival of eggs and presumed clutches.

We calculated the Pearson product-moment correlation between the number of eggs and clutches per pool and both pool temperature and pool volume. Volumes were log-transformed to achieve normality and homoscedasticity prior to statistical analyses. To determine whether females were using pools of a particular temperature more frequently, we compared the temperatures of pools that contained eggs to those that did not with two-tailed *t*-tests for each census (Table 1).

We conducted a simple experiment to determine whether cave pool invertebrates would feed on salamander eggs by maintaining three amphipods (*Gammarus* spp.), three isopods (*Caecidotea* spp.), and five planarians (*Sphalloplana* spp.) in three individual plastic cups in the lab each with one *Eurycea lucifuga* egg for 72 h.

Lab Study

We collected 95 newly laid eggs representing portions of 18 clutches on 1 and 15 August, 11 and 18 September, and 11 October, 2000. We determined the developmental stage (Harrison, 1969) of 36 of the 67 eggs at collection. We measured yolk diameter of all collected eggs to the nearest 0.01 mm using plastic vernier calipers under a dissecting microscope. We calculated yolk volume for the measured eggs that were between Harrison (1969) stage 1 and 10 using the volume of a sphere ($4/3\pi r^3$). We compared egg volume among temperature treatments (see below) with ANOVA (JMP, 2001) and Tukey-Kramer Honestly Significant Difference (HSD) *post hoc* test.

We randomly assigned one-third of the eggs collected from each clutch to one of three temperature regimes (10, 15 and 21 C), splitting presumed siblings equally among the treatments. Based on field temperatures, we chose 15 C as the average temperature of cave pools during egg development, and bracketed this average temperature with treatments 5 C cooler and 6 C warmer. As many as six eggs per clutch per collection were placed in individual wells (3.5 cm diameter) in plastic Costar® cell culture dishes. Because eggs were maintained separately in individual wells, we considered them independent replicates within each temperature treatment. We drilled holes in the plastic covers to allow for gas exchange, and the dish was submerged in a 40 × 40 × 7.5 cm plastic tub with settled (let stand for 48 h to allow chlorination to dissipate) tap water to minimize temperature variation within treatments. One water bath was placed in each of three 24 h-dark, climate-controlled environmental chambers, for a total of nine dishes with 32 eggs at 10 C, eight dishes with 31 eggs at 15 C, and eight dishes with 32 eggs at 21 C. Larvae were not fed due to the visible availability of yolk sacks though the gut wall.

We examined eggs and larvae daily between 1 August 2000 and 19 March 2001 to quantify survival and developmental rate. Development of cave salamanders is similar to, but does not exactly follow, development of *Ambystoma maculatum* (Harrison, 1969). We noted slight differences in the timing of particular events, and the appearance of certain stages, so we photographed five *E. lucifuga* eggs during development with a Sony MAVICA MVC-FD91 digital camera. Brandon (1964) illustrated some of the larval stages of *E. lucifuga*, but did not provide a complete staging table. We used our photographs and Brandon's (1964) illustrations to modify Harrison's staging table for *A. maculatum* for use with *E. lucifuga* (Appendix 1). We described development with this modified staging table, hereafter referred to as modified-Harrison, and used it to calculate embryonic growth rate (increase in mm total length (= TL/d); Walls and Altig, 1986). We used a χ^2 test to compare the proportion of surviving eggs among temperature treatments. We used ANOVA and Tukey-Kramer HSD post-hoc tests to compare mean hatching sizes, hatching stages, and embryonic growth rates. Most (93%) eggs died in the 21 C treatment, so we used a Welch ANOVA for unequal variances to compare development among the three regimes, then used a *t*-test to compare the 10 and 15 C treatments separately.

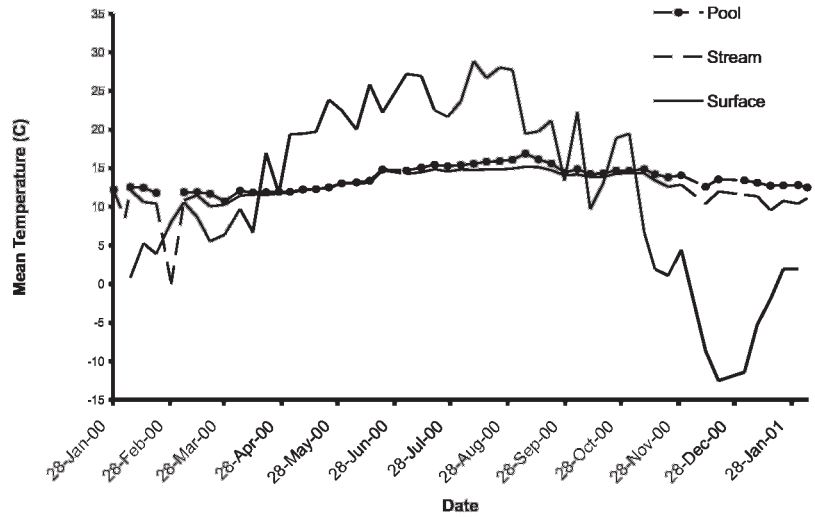
We measured larval growth rate (mm increase in TL/d) between hatching and complete yolk absorption (Walls and Altig, 1986), and compared the proportion of larvae surviving to yolk absorption with a χ^2 test. We used ANOVA and Tukey-Kramer HSD post-hoc tests to compare mean larval size and stage at yolk absorption, and embryonic growth rates among treatments. All statistics were computed using JMP IN statistical software, with $\alpha = 0.05$; results are presented as mean ± SD unless otherwise indicated.

Results

Field Results

Between January 2000 and January 2001, the warmest daily surface air temperature occurred in August 2000 (28.9 C) and the coolest in December 2000 (−17.2 C), as recorded by the National Data Center at Carbondale Airport, Carbondale, IL (http://nndc.noaa.gov; Fig. 1A), 57 km E of Tom Moore Cave. Average pool temperature was 14.0 ± 1.7 C, and varied from 8.4–18.1 C (Fig. 1B) on 15 dates taken at approximately monthly intervals over the same period.

The 4-yr average (1998–2002) stream flow for the South Fork of Saline Creek, located about 4 km from Tom Moore Cave (http://waterdata.usgs.gov/mo/nwis/rt), shows seasonal fluctuation, with low monthly flows of 0.340–0.793 $m^3\ s^{-1}$ between June and November, and high flows of 1.416–3.993 $m^3\ s^{-1}$ between December and May. No flood events were recorded during the study period at this gauging station, and we saw no signs of cave flooding during the study. Water flow into and out of the rimstone pools varied with substrate and location relative to the stream and surface seeps. During our study, one pool dried completely both years, three pools dried in one year, and water level in the others varied weekly. Drying was not related to pool size;



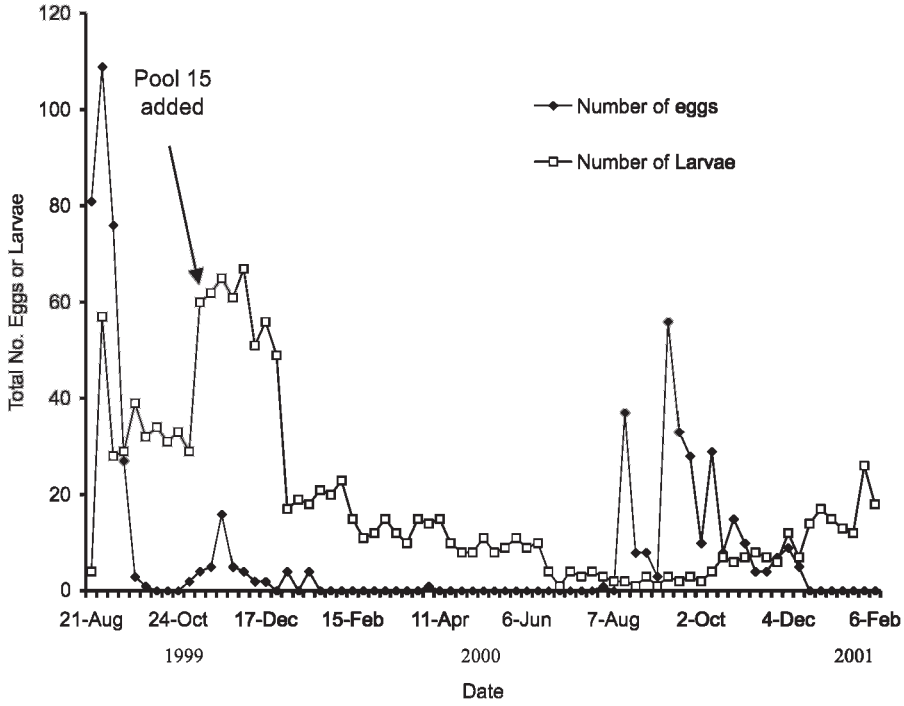


FIG. 1.—(A) Mean weekly temperature of surface air temperature and water temperature for cave stream and rimstone pools for the duration of the study. Measurements were taken approximately every 7 days. (B) Reproductive phenology and abundance of eggs and larvae in rimstone pools in Tom Moore Cave, Missouri, U.S.A. based on combined counts of all pools surveyed weekly.

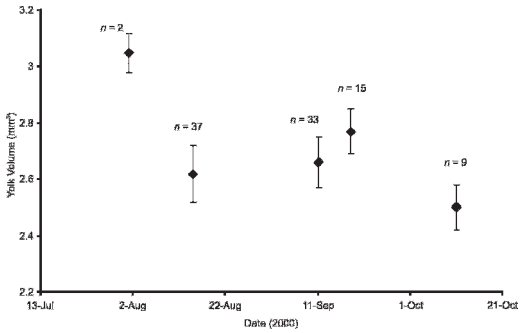


FIG. 2.—Variation in mean (± SD) egg volume (mm^3) for *Eurycea lucifuga* in Tom Moore Cave, MO.

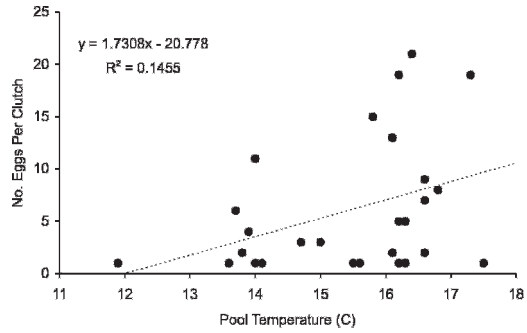


FIG. 3.—Correlation between egg number and pool temperature.

pools that dried ranged from 369 to 26,498 cm^3, while permanent pools ranged from 160 to 679,604 cm^3 (logistic regression: $r^2 = 0.076$, $P < 0.249$).

Salamanders deposited eggs in all 15 rimstone pools during our study. We found eggs during 22 of the 59 censuses conducted between August 1999 and February 2001, with the greatest number of new eggs deposited in August, and a second smaller peak in November (Fig. 1B). In 1999, 169 of 187 eggs (91%) representing 25 of 32 clutches (78%) were laid from mid-August through mid-October. In 2000, 110 of the 119 eggs (92%) from 24 of 27 clutches were laid during this same period (Fig. 1B). We found eggs in pools with water temperatures ranging from 14–17.5 C (15.63 ± 2.4 C), but because of annual temperature variation, or the lack thereof we found no significant relationship (2-tailed *t*-test, $t = 0.58$, $P = 0.56$) between the presence of eggs and pool temperature. However, pools with eggs were significantly warmer than pools without eggs on five survey dates, were cooler on one date, and were equal on the remaining four dates (Table 1).

In both years, eggs were laid singly or in small clusters of up to 31 eggs ($n = 59$; 5.52 ± 6.20 eggs per clutch). Some eggs were attached to the substrate by a pedicel, while other eggs were lying unattached on the pool bottom, as described by Green et al. (1967). The number of eggs observed in each pool varied from 1–81 per pool ($n = 309$; 14.71 ± 21.73), and the number of clutches per pool varied from 1–9 throughout the year ($n = 59$; 2.90 ± 2.68). More clutches were found in 1999 ($n = 33$; 3.30 ± 3.02) than in 2000 ($n = 28$; 2.55 ± 2.42), and eggs were also more abundant in 1999 ($n = 186$; 16.80 ± 22.45) than in 2000 ($n = 123$; 9.67 ± 15.25).

The mean yolk volume was 2.66 ± 0.13 mm^3 (range: 2.37–3.1 mm^3, $n = 103$ eggs) in 2000. There was no correlation between yolk volume and clutch size for the 10 clutches measured ($r^2 = 0.03$, $F_{1, 9} = 0.28$, $P > 0.61$), although larger eggs were deposited earlier (1 August 2000, $\bar{x} = 3.05$ mm^3), rather than later in the breeding season (11 October 2000, $\bar{x} = 2.47$ mm^3; ANOVA, $F_{5, 102} = 21.68$, $P < 0.0001$; Fig. 2).

Pool temperature was significantly correlated with the number of eggs per clutch ($r^2 = 0.11$, $F_{1, 27} = 4.43$, $P < 0.05$; Fig. 3) but not egg size ($r^2 = 0.02$, $F_{1, 102} = 2.86$, $P > 0.09$). We found no correlation between pool volume and either number of eggs ($r^2 = 0.118$, $F_{1, 14} = 1.74$, $P < 0.21$) or clutches ($r^2 = 0.043$, $F_{1, 14} = 0.58$, $P < 0.46$) for any years.

We only could verify hatching success for about half of all eggs: 103 of 187 (55%) eggs in 1999, 38 of 119 (32%) in 2000, and 141 of 306 (46%) for the entire study. We assumed that all remaining eggs hatched, but because we only surveyed weekly, many larvae may have left the pools and moved into the stream between censuses. We could not determine whether all eggs hatched or if some were eaten by predators between surveys, but the only evidence of predation we saw in 17 mo was one partially eaten egg. We observed mortality in eight eggs with infection by an unknown white, filamentous fungus. Larvae

TABLE 2.—Effects of temperature on development of *Eurycea lucifuga*. Different letters indicate a significant difference among columns ($P < 0.05$) following analysis by ANOVA. Due to small sample size, the 21 C treatment was removed from the analysis for body size at yolk absorption and larval development rate. Values are $\bar{x} \pm$ SD. Embryonic growth rate describes the period from oviposition to hatching, while larval growth rate describes the period from hatching to yolk absorption.

	Temperature		
	10 C	15 C	21 C
n	32	31	32
Hatching success	75%	74%	69%
Developmental abnormalities	0%	6%	6%
No. days to hatching	72.79 ± 10.15[a]	24.57 ± 5.44[b]	17.95 ± 2.59[c]
Embryonic growth rate (mm TL/d)	0.17 ± 0.02[a]	0.47 ± 0.09[b]	0.66 ± 0.08[c]
Hatching size (mm TL)	12.62 ± 0.93[a]	11.27 ± 1.11[b]	11.74 ± 1.10[b]
Hatching stage (modified Harrison)	39.63 ± 5.14[a]	34.20 ± 4.61[b]	39.14 ± 6.04[a,b]
Survival to yolk absorption	100%[a]	73%[a]	8.3%[b]
Size (mm TL) at yolk absorption	18.21 ± 0.36	18.17 ± 0.39	17.20 ± 1.14
No. days to yolk absorption (from oviposition)	106.77 ± 15.12	100.82 ± 12.84	74.00 ± 5.66
Larval growth rate (mm TL/d)	0.05 ± 0.01[a]	0.07 ± 0.01[b]	0.09 ± 0.02

remained in their natal pools from less than seven days to six months after hatching, although there were no noticeable differences in pools or triggers to migration to account for this variability. By mid-June few larvae remained in pools, but many were seen in the stream.

Laboratory Results

Yolk volume did not differ among temperature treatments ($F_{2, 66} = 0.76$, $P > 0.48$) and was normally distributed in each treatment (Shapiro-Wilks; $W_{10} = 0.914$, $P < 0.054$; $W_{15} = 0.947$, $P < 0.289$; $W_{21} = 0.954$, $P < 0.370$).

Temperature affected embryonic growth rate, and size and stage at hatching. Eggs took longer to hatch at 10 C ($F_{2, 66} = 428.01$, $P < 0.0001$; Table 2), and resulted in larger hatchlings than at 15 and 21 C ($F_{2, 66} = 9.74$, $P < 0.0002$, Table 2). Embryos raised at 10 C grew more slowly than those at warmer temperatures ($F_{2, 66} = 276.90$, $P < 0.0001$; Table 2), and hatched at more advanced stages than those at 15 C, although embryos at 21 C were intermediate to those raised at 10 or 15 C ($F_{2, 66} = 4.22$, $P < 0.04$, Table 2). Stage at hatching was variable within and among treatments, with similar ranges of modified-Harrison stage 34–45 for both the 10 and 21 C treatments, but a lower range of modified-Harrison stage 29–38 for the intermediate temperature (Table 2).

Temperature did not affect embryonic survival ($\chi^2 = 3.73$, $P > 0.05$; Table 2), although larval survival was reduced from 75% at 10 C to 6% at 21 C. All embryos that died were infected by a white filamentous fungus that was similar to that found on the dead eggs in the cave.

Larvae raised at 21 C completed yolk absorption faster than those raised at 10 or 15 C ($F_{2, 39} = 7.04$, $P < 0.0046$), although there was no difference between 10 and 15 C ($F_{1, 37} = 1.69$, $P > 0.20$). Temperature had no effect on larval growth rate ($F_{2, 39} = 8.77$, $P > 0.07$; Table 2), although when we excluded the 21 C treatment from the analysis growth rate was slower at 10 C than at 15 C treatments ($F_{1, 37} = 17.54$, $P < 0.0002$). Temperature had no effect on size at yolk absorption when either two ($F_{1, 37} = 0.14$, $P > 0.708$) or three ($F_{2, 39} = 0.67$, $P > 0.58$) treatments were compared. There was no difference in larval survival among treatments until week 5 when all but two larvae in the 21 C treatment died ($\chi^2 = 16.74$, $P < 0.05$).

Both isopods and amphipods (but not planarians) consumed salamander eggs in our predation test.

DISCUSSION

Breeding of *E. lucifuga* followed seasonal temperature cycles; oviposition peaked in late summer and early fall when surface tempera-

tures were highest but cave temperatures were moderate with low flow. Females tended to oviposit in warmer pools, especially as temperatures dropped throughout the fall. However, the small temperature differences found in the field may not be biologically significant. In laboratory simulated temperatures typical of midwestern surface streams in the summer however, we found reduced larval survival (Fig. 1A). Embryonic growth and development was faster at warmer temperatures in the laboratory, although all larvae were of equal size by the time of yolk absorption, which suggests little size penalty for increased growth rate.

Eurycea lucifuga conformed to the general patterns of slower development and growth and larger body size at lower temperatures (Bernardo, 1994; Bernardo and Reagan-Wallis, 2002). We predicted that developmental stage of *Eurycea lucifuga* also would increase with temperature, as seen in three *Triturus* species (Griffiths and De Wijer, 1994), although we found no relationship between the stage at hatching and developmental temperature. Instead, stage at hatching was highly variable at all temperatures, perhaps indicating a mechanism to avoid unpredictable threats like flooding or predators (e.g., Warkentin, 1995). Bernardo and Reagan-Wallis (2002) found some lotic hemidactyline salamanders metamorphosed at smaller sizes at cooler temperatures and hypothesized that carnivorous larvae living in low productivity environments might trade large size for more rapid development to escape these food-poor habitats.

The reproductive cycle in *E. lucifuga* appears to be linked to water flow and may be related to variation in larval food supply as well. Oviposition occurred between June and November during periods of low stream flow. Larvae left pools and moved into the stream following increased flow between December and May; it is during this time when larvae are seen in resurgence springs (A. M. Ringia, personal observation), and this indicates the potential role of larval drift in these populations (Adams et al., 2001). This population of *E. lucifuga* had an extended breeding season of 5–8 mo, with the largest peak early and a smaller peak later in the breeding season. Oviducal egg counts of females (Carlyle et al., 1998; Hutchison, 1956; Trauth et al., 1990) were two to three times larger than our field clutches, and we hypothesize that female *Eurycea lucifuga* in this population laid multiple clutches. Production of multiple clutches has been documented for females of the closely related *E. longicauda* (Petranka, 1998), and often is thought to be an adaptation to survival and reproduction in unpredictable environments (Roff, 1992).

Based on our laboratory observations, wild larvae should consume yolk ~100 d post-hatching, which would correspond to November–December, a period characterized by heavy precipitation (Midwestern Climate Center, 2002). These heavy rains increased water flow and nutrients in cave streams (Poulson and White, 1969) and should provide a large food base for larval salamanders (Taylor et al., 2000). If food is limiting in caves, then selection should favor rapid larval development, as seen within other species in this group (e.g., Ryan and Bruce, 2000). However, we found no data on the productivity of midwestern caves and little on the diet of larval salamanders in these habitats, limiting our conclusions. Additional studies that quantify these factors are needed to understand selective pressures imposed by these habitats on salamander larvae.

Patterns of larval survival likely have influenced the reproductive biology of *Eurycea lucifuga*. Benefits of subterranean breeding include a prolonged reproductive season and limited predation, but these habitats also have drawbacks, including flooding and limited food supply. This population of cave salamanders exhibited rapid larval development, and variation of hatching stage all of which may be adaptive to unpredictable environments and are similar to those found in other cave organisms (Culver, 1982; Hüppop, 2000). We predict that flooding or limited larval food resources might be responsible for these patterns.

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Appendix 1

Staging table of embryonic and larval traits during development of *Eurycea lucifuga* used in this study (modified from Harrison, 1969).

Description	Harrison (1969)	This study
Sphere	1–10	1–9
Shallow circular concavity	NA	10
Narrow vertical invagination	13	13
Neural Plate begins to form	14	14
Neural fold surrounds neural plate	15	15
Raised lip vertical invagination	18–19	18
Wide, blunt head with two distinct parts; tail bud visible	20	20
Head differentiated	21	21
Gill region differentiated from head	22	22
Tail fold	26	22
Gill bud visible	23	24
Branchial groove evident	26	25
Tail flattening, fin development; yolk sac L = 2 × W; hatching begins	30	29
Balancer organ visible	35–36	30
Gill folds, trunk straightening	35	31
Body lengthening	35	31
Optical vesicle visible	22	32
Upright body positioning	NA	33
Distinct forelimb bud	37	37
Eye pigmentation begins	32	38
Gills stand out from body	36	39
Eye darkly pigmented	34	40
Dorsal pigmentation	NA	41
Hind limb bud visible	>43	42
Hind limb bud distinct	43	43
Head pigmentation distinct from body	NA	44
Gills well developed	38	45
Forelimbs well developed, distinct hind limbs, increased body pigmentation	NA	46