

**ECOLOGY OF THE EASTERN LONG-TAILED SALAMANDER (*EURYCEA*  
*LONGICAUDA LONGICAUDA*) ASSOCIATED WITH SPRINGHOUSES**

by

Nathan H. Nazdrowicz

A dissertation submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Entomology and Wildlife Ecology

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## TABLE OF CONTENTS

LIST OF TABLES.....	ix
LIST OF FIGURES .....	xii
ABSTRACT.....	xv
1 INTRODUCTION .....	1
Study Areas.....	5
2 LIFE HISTORY AND ECOLOGY OF EASTERN LONG-TAILED SALAMANDERS ( <i>EURYCEA LONGICAUDA LONGICAUDA</i> ) ASSOCIATED WITH SPRINGHOUSES .....	19
Introduction.....	19
Methods.....	21
Site Visits.....	21
Reproductive Data .....	22
Mark-Recapture Data.....	24
Mark-Recapture Analysis .....	28
Results.....	31
Reproduction.....	31
Courtship and Mating .....	31
Reproduction.....	32
Egg Development.....	36
Larval Period.....	37
Activity Patterns.....	39
Daily Activity.....	39
Seasonal Activity .....	40
Mark-Recapture .....	42
Captures .....	43
Age Composition .....	45
Sex Composition.....	45



	Recaptures.....	46
	Population Estimates.....	47
	Discussion.....	48
	Reproduction.....	48
	Larval Period.....	57
	Activity Patterns and Movements.....	59
	Reproduction.....	60
	Overwintering .....	63
	Foraging .....	64
	Population Dynamics.....	66
	Age and Sex Composition .....	68
	Sex Ratios .....	69
	Population Trends .....	72
3	FACTORS INFLUENCING SITE OCCUPANCY OF EASTERN LONG- TAILED SALAMANDERS ( <i>EURYCEA LONGICAUDA LONGICAUDA</i> ) IN AN ANTHROPOGENIC LANDSCAPE .....	104
	Introduction.....	104
	Methods.....	107
	Study Area and Site Selection.....	107
	Multiple Spatial Scales .....	109
	Local-scale Assessment .....	109
	Landscape-scale Assessment .....	111
	Data Analysis.....	112
	Results.....	113
	Discussion.....	113
	REFERENCES .....	123

## LIST OF TABLES

Table 1	Starting and ending dates of capture events ( <i>n</i> ) for the 7 principal study sites in a mark-recapture study of eastern long-tailed salamanders ( <i>Eurycea longicuada longicauda</i> ).....	75
Table 2	Pooled abundance of gravid eastern long-tailed salamanders ( <i>Eurycea longicauda longicauda</i> ) observed by month at 7 springhouses from 2003-2007. Condition was determined by viewing ova through ventral wall of females and was categorized as ‘Complete’: oviducts full of mature ova; ‘Partial’: oviducts partially spent containing less than approximately 50% of mature ova; and ‘Developing’: oviducts with ova small in size. Multiple captures of same individuals per month with same condition were excluded. ....	76
Table 3	Site, date, snout-vent length (SVL), and number of ova from dissected gravid female eastern long-tailed salamanders ( <i>Eurycea longicauda longicauda</i> ) found deceased in springhouses. ....	77
Table 4	Highest count of eggs and larval eastern long-tailed salamanders ( <i>Eurycea longicauda longicauda</i> ) observed inside each springhouse per year. In some years, total larval abundance was not recorded, although presence (P) was observed. ....	78
Table 5	Percent abundance for adult and juvenile eastern long-tailed salamanders ( <i>Eurycea longicauda longicauda</i> ) captured per study site by location (inside vs outside springhouses) and season (breeding season vs non-breeding season).....	79
Table 6	Percent abundance of eastern long-tailed salamanders ( <i>Eurycea longicauda longicauda</i> ) captured per study site by location (inside vs outside springhouses) and season (breeding season vs non-breeding season) for all adult (SVL $\geq 4.5$ cm) male, female, and unknown sex individuals.....	80
Table 7	Chi square statistics comparing age composition and sex ratio of eastern long-tailed salamanders ( <i>Eurycea longicauda longicauda</i> ) among years at each study site. Asterisks denotes significance at $P \leq 0.05$ . ....	81

Table 8	Percent abundance of adult eastern long-tailed salamanders ( <i>Eurycea longicauda longicauda</i> ) captured per study site by sex (male, female, unknown sex) for all individuals captured and all individuals captured inside vs outside springhouses and during the breeding and non-breeding seasons for each study site. ....	82
Table 9	Within year and overall capture sex ratio of eastern long-tailed salamanders ( <i>Eurycea longicauda longicauda</i> ) adults captured during the breeding season (15 July through 30 November) for each study site. Differences from parity were tested using a Chi square analysis at $P \leq 0.05$ .....	84
Table 10	Mean annual recapture rate by sex for adult eastern long-tailed salamanders ( <i>Eurycea longicauda longicauda</i> ) captured per study site for all captures and captures inside springhouses during the breeding season.	86
Table 11	Proportion of eastern long-tailed salamanders ( <i>Eurycea longicauda longicauda</i> ) first captured as juveniles (i.e., $\leq 4.4$ cm SVL; age approximately 1 year) and first captured as adults (i.e., $\geq 4.5$ SVL; unknown age) recaptured at least 2, 3, and 4 years as determined by capture histories at Crum Creek (CC), Darby Creek (DC), Ridley Creek (RC), Pigeon Run (PR), Middle Run (MR), Red Clay Creek (RCC), and White Clay Creek (WCC) springhouses. ....	87
Table 12	Candidate models of the Jolly-Seber model for estimating seasonal combined male and female abundance of eastern long-tailed salamanders ( <i>Eurycea longicauda longicauda</i> ) at Ridley Creek (RC), Pigeon Run (PR), Middle Run (MR), Red Clay Creek (RCC), and White Clay Creek (WCC) springhouses <sup>a</sup> . For each model, I provided AICc values, $\Delta AICc$ (i.e., the difference in AICc between the given model and the best fit model), AICc weight (i.e., the certainty that the given model is the best model for the data), and number of parameters. Subscripts indicate variable over time ( <i>t</i> ) and constant over time ( $\bullet$ ) for the model parameters of survival ( $\phi$ ), recapture probability ( <i>p</i> ), and probability of entrance ( <i>b</i> ). ....	88
Table 13	Local (100 m) and landscape (500 m and 1,000 m) variables used in logistic regression models to predict eastern long-tailed salamander ( <i>Eurycea longicauda longicauda</i> ) occupancy of springhouses located in Chester and Delaware Counties, Pennsylvania, and New Castle County, Delaware. ....	116

Table 14	Pearson's correlation coefficients ( $r$ ) and $P$ -values ( $P$ ) for local scale variables compared between occupied and unoccupied springhouses located in Chester and Delaware Counties, Pennsylvania, and New Castle County, Delaware. Asterisk denotes highly correlated variable with $r \leq -0.70$ and $\geq 0.70$ .....	117
Table 15	Pearson's correlation coefficients ( $r$ ) and $P$ -values ( $P$ ) for landscape scale variables compared between occupied and unoccupied springhouses located in Chester and Delaware Counties, Pennsylvania, and New Castle County, Delaware. Asterisk denotes highly correlated variable with $r \leq -0.70$ and $\geq 0.70$ .....	118
Table 16	Model selection results for occupancy of eastern long-tailed salamanders ( <i>Eurycea longicauda longicauda</i> ) in springhouses located in Chester and Delaware Counties, Pennsylvania, and New Castle County, Delaware.	119
Table 17	Summary statistics and t-test comparison of water chemistry variables measured at occupied and unoccupied springhouses located in Chester and Delaware Counties, Pennsylvania, and New Castle County, Delaware. ....	120
Table 18	Summary statistics and t-test comparison of habitat variables measured from 3 spatial scales at occupied and unoccupied springhouses located in Chester and Delaware Counties, Pennsylvania, and New Castle County, Delaware. Total area for each spatial scale was 3.14 ha, 78.5 ha, and 314.0 ha for 100 m, 500 m, and 1,000 m, respectively.....	121

## LIST OF FIGURES

Figure 1	An example of a typical springhouse structure consisting of a stone foundation and wooden roof enclosing a spring. This springhouse was partially built into the ground, a design common among springhouses. .15
Figure 2	An example of a water compartment containing ground water near the effluence of a spring within a springhouse. ....16
Figure 3	Location of 7 springhouse study sites with population of eastern long-tailed salamanders ( <i>Eurycea longicauda longicauda</i> ) investigated in Chester and Delaware Counties, Pennsylvania, and New Castle County, Delaware and from 2003-2008. ....17
Figure 4	An example of a cement cistern or springbox capping a springhead. ....18
Figure 5	Eastern long-tailed salamander ( <i>Eurycea longicauda longicauda</i> ) spermatophore photographed at on a rock ledge at Red Clay Creek (RCC) Springhouse on 25 September 2005 shortly after deposition in a failed mating attempt. ....89
Figure 6	Earliest (open) and latest (closed) range of dates for eastern long-tailed salamander ( <i>Eurycea longicauda longicauda</i> ) eggs observed (blue), larvae observed inside springhouses (pink), and larvae observed in outside stream (green) for the 7 principal study sites 2003-2007.....90
Figure 7	Total eastern long-tailed salamander ( <i>Eurycea longicauda longicauda</i> ) egg counts by date observed from within Red Clay Creek (RCC) Springhouse from November 2006 through February 2007.....91
Figure 8	Mean snout-vent length ( $\pm$ SE) by month (February=2 to August=8) of eastern long-tailed salamander ( <i>Eurycea longicauda longicuada</i> ) larvae collected inside (blue) and outside (red) springhouses 2004-2006.....92
Figure 9	Partial neotenic eastern long-tailed salamander ( <i>Eurycea longicauda longicauda</i> ) captured at Ridley Creek (RC) Springhouse on 7 April 2004 (a and b) and after metamorphosis on 4 June 2004 (c). ....93

Figure 10	Total eastern long-tailed salamander ( <i>Eurycea longicauda longicauda</i> ) capture abundances for males (blue), females (red) and unknown sex (yellow) inside and outside of springhouses from all study sites ( $n = 7$ ) and years (2003-2007) by snout-vent length (cm) during the reproductive activity period (July 15 – November 31). Dotted line delineates juveniles (SVL <4.5 cm) from adults (SVL $\geq$ 4.5 cm).....	94
Figure 11	Total eastern long-tailed salamander ( <i>Eurycea longicauda longicauda</i> ) capture abundances for males (blue), females (red) and unknown sex (yellow) inside and outside of springhouses from all study sites ( $n = 7$ ) and years (2003-2007) by snout-vent length (cm) during the over-wintering activity period (December 1 – March 31). Dotted line delineates juveniles (SVL <4.5 cm) from adults (SVL $\geq$ 4.5 cm). .....	95
Figure 12	Total eastern long-tailed salamander ( <i>Eurycea longicauda longicauda</i> ) capture abundances for males (blue), females (red) and unknown sex (yellow) inside and outside of springhouses from all study sites ( $n = 7$ ) and years (2003-2007) by snout-vent length (cm) during the foraging activity period (April 1 – July 14). Dotted line delineates juveniles (SVL <4.5 cm) from adults (SVL $\geq$ 4.5 cm) .....	96
Figure 13	Mean snout-vent length (SVL) ( $\pm$ SE) of eastern long-tailed salamanders ( <i>Eurycea longicauda longicauda</i> ) for males (blue) and females (red) captured by month inside springhouses. Means with the same letter do not differ ( $P \leq 0.05$ ). .....	97
Figure 14	Percent abundance of juvenile (blue) and adult (red) eastern long-tailed salamanders ( <i>Eurycea longicauda longicauda</i> ) captured per year at each springhouse. Asterisks denote differences among year determined by a Chi square analysis ( $P \leq 0.05$ ). .....	98
Figure 15	Derived population estimates ( $\pm$ SE) of adult eastern long-tailed salamanders ( <i>Eurycea longicauda longicauda</i> ) for Ridley Creek (RC) Springhouse for the breeding (B) and non-breeding (N) seasons for 2005-2006 using the POPAN extension of the Jolly-Seber model in program MARK.....	99
Figure 16	Derived population estimates ( $\pm$ SE) of adult eastern long-tailed salamanders ( <i>Eurycea longicauda longicauda</i> ) for Pigeon Run (PR) Springhouse for the breeding (B) and non-breeding (N) seasons for 2005-2006 using the POPAN extension of the Jolly-Seber model in program MARK.....	100

Figure 17	Derived population estimates ( $\pm$ SE) of adult eastern long-tailed salamanders ( <i>Eurycea longicauda longicauda</i> ) for Middle Run (MR) Springhouse for the breeding (B) and non-breeding (N) seasons for 2004-2006 using the POPAN extension of the Jolly-Seber model in program MARK.....	101
Figure 18	Derived population estimates ( $\pm$ SE) of adult eastern long-tailed salamanders ( <i>Eurycea longicauda longicauda</i> ) for Red Clay Creek (RCC) Springhouse for the breeding (B) and non-breeding (N) seasons for 2004-2006 using the POPAN extension of the Jolly-Seber model in program MARK. ....	102
Figure 19	Derived population estimates ( $\pm$ SE) of adult eastern long-tailed salamanders ( <i>Eurycea longicauda longicauda</i> ) for White Clay Creek (WCC) Springhouse for the breeding (B) and non-breeding (N) seasons for 2004-2006 using the POPAN extension of the Jolly-Seber model in program MARK. ....	103
Figure 20	Location of occupied (closed circles; $n = 18$ ) and unoccupied (open circles; $n = 18$ ) springhouses distributed among 6 subareas formed by extending a 5,000-m buffer from occupied springhouses in Chester and Delaware Counties, Pennsylvanian, and New Castle County, Delaware.	122

## ABSTRACT

I investigated the natural history, ecology, and distribution of eastern long-tailed salamanders (*Eurycea longicauda longicauda*) because biological information for developing conservation strategies on the species is lacking. On the piedmont of the Delaware Valley in New Castle County, Delaware, and Chester and Delaware Counties, Pennsylvania, populations of eastern long-tailed salamanders may be found associated with springhouses. Eastern long-tailed salamanders utilize the spring within springhouses for reproduction and to overwinter, and their activity cycle occurs as a regular migration between terrestrial and subterranean habitats. Adults begin movements into springhouses in August with males arriving before females, while immature individuals remain active in terrestrial habitats until November. Within springhouses, mating occurs prior to moving deeper within the spring to overwinter. Eggs are laid singly underwater usually in November and females do not exhibit parental care of the eggs. Rather, females scatter eggs and choose oviposition sites to maximize survival. Subsurface spring habitats have few predators and sites are selected that maximize surface area of the egg exposed to water to allow for gas exchange. Adults emerge from overwintering in late April and migrate to surface habitats. Eggs hatch after approximately 7-9 weeks and larvae migrate to surface streams in March. Larvae metamorphose from May to August, although on rare occasions overwintering of larvae occurs. Population estimates per springhouse ranged from 40-49 to 1,216-1,456 adult salamanders per year, with three study sites showing stable populations, one population declining, and one population increasing during the time of the study. Sex ratio was even



to male biased and juveniles comprised 11-35% of the total population per year on average. The majority (90%) of adult males and females in the population were captured inside springhouses and during the breeding season (15 July to 30 November). Recapture rate for males and females  $\geq 4.5$  cm ranged from 0.19-0.48; however, recapture rate for unknown sex of this size was  $\leq 0.10$ , suggesting the dispersal cohort arises from this group. Occupancy of springhouses was related to landscape scale factors such as forest area and total stream length suggesting that habitat quantity and connectivity are important components influencing eastern long-tailed salamander distributions. Species conservation might best be achieved by maintaining forest tract corridors between and near springheads. If springhouses are present, I suggest maintaining or restoring these structures in a manner that minimizes drafts and reduces or eliminates light. If eggs are observed, increasing available surface area may increase population abundance. I suggest that managers or researchers interested in quantifying eastern long-tailed salamander population abundances concentrate capture effort inside springhouses during the breeding season.

## Chapter 1

### INTRODUCTION

The long-tailed salamander (*Eurycea longicauda*) is a medium-sized, slender Plethodontid in the Hemidactyliini Tribe and is currently comprised of two subspecies: the dark-sided salamander (*Eurycea longicauda melanopleura*) and the eastern long-tailed salamander (*Eurycea longicauga longicauda*), which is the focus of this study. The three-lined salamander (*Eurycea guttolineata*) is closely related and was previously assigned subspecies status in the *E. longicauda* complex (Carlin 1997). The eastern long-tailed salamander is distributed throughout most of the Appalachian and Ohio River Valley regions of eastern North America (Petranka 1998), where it occurs sympatrically with several other species of *Eurycea*. Of these, the cave salamander (*Eurycea lucifuga*) is most similar in appearance and life history traits (Petranka 1998).

Eastern long-tailed salamanders have biphasic life cycles, requiring aquatic sites for egg deposition and larval development and suitable terrestrial sites for post-metamorphic stages. Typical habitats include forested areas of shale or limestone deposits, where fissures in the ground surface allow access to underground springs and chambers. Populations are frequently associated with shale banks, caves, mineshafts, springs, and spring-fed ponds (Petranka 1998). Seasonal movements between surface and subsurface habitats occur as an annual migration associated with reproduction and overwintering (Mohr 1944, Petranka 1998). In late summer to fall, eastern long-tailed

salamanders may congregate in large numbers in underground refugia (Mohr 1944), where mating and oviposition occur (Petranka 1998). After a period of overwintering, adults resume surface activity in late winter through spring (Mohr 1944, McDowell and Shepherd 2003, Petranka 1998). Larvae typically appear in surface streams or ponds in early spring (Anderson and Martino 1966, Petranka 1998). Larval period is usually less than 1 year with metamorphosis occurring during summer months (Anderson and Martino 1966, Franz 1967, Petranka 1998), although longer larval periods have been documented (Myers 1958, Rossman 1960, Franz 1967). Sexual maturity is reached 1-2 years following metamorphosis (Petranka 1998).

Current literature on eastern long-tailed salamander life history is limited. Most knowledge is primarily derived from anecdotal accounts and limited observations or a few studies with low sample sizes. Limited access to underground habitats, which eastern long-tailed salamanders utilize for up to 8 months out of the year (Mohr 1944), may in part explain this lack of information. Biological information on reproduction and egg development, which occurs in subsurface stream passages (Petranka 1998), is particularly deficient. There is only one published account of partial courtship observed in the field (Cooper 1960), no published accounts of egg-laying, two published accounts of observations of eggs (Mohr 1943, Franz 1964), and one published observation of incubation period (Mohr 1943). Other studies have examined preserved specimens to estimate timing of reproductive events such as mating and oviposition (Williams et al. 1984, McDowell and Shepherd 2003), and observations of larvae in early development have been used as evidence to infer time of hatching (Anderson and Martino 1966,

McDowell and Shepherd 2003). Aspects of larval period have received the most attention (Mohr 1943, Rossman 1960, Franz and Harris 1965, Anderson and Martino 1966, Franz 1967, McDowell 1988, McDowell and Shepherd 2003), primarily because larvae are more apparent after they migrate from hidden oviposition sites to surface streams.

Evidence suggests that the mating period of the eastern long-tailed salamander occurs primarily in the fall. McDowell and Shepard (2003) suggested mating occurred in October and November in Illinois based on dissected reproductive tracts of adult females, and Williams et al. (1984) determined that peak breeding season in Illinois occurred in October based on characteristics of male genital ducts and presence of sperm. Cooper (1960) provided the only account of mating behavior, which occurred in October from a mineshaft in Maryland. Although complete observations of eastern long-tailed salamander mating have not been reported, courtship behavior is presumed similar to northern two-lined salamanders (*Eurycea bislineata*) (Guttman 1989).

Eggs in early stages of development have been observed in November (Franz 1964) and in early January (Mohr 1943), but no other eggs have been reported. Mohr (1943) found eggs deposited singly and scattered in running water on the top, sides, and edges of rocks and wood, whereas Franz (1964) found eggs clustered together and attached to the ceiling of a cave out of water. Franz (1967) speculated eastern long-tailed salamanders have an extended egg-laying period lasting from September through January, whereas Anderson and Martino (1966) speculated oviposition occurred in January. Reported clutch size from ovarian egg counts of gravid females ranges from 61-

106 eggs (Hutchison 1956, Minton 1972). Only Mohr (1943) has provided data of incubation period based on direct observation. Eggs observed in early development in early January in Pennsylvania had not hatched by mid-March (Mohr 1943). Observations of recently-hatched larvae have been documented as early as December but may extend through May (Franz 1967, McDowell 1988). After hatching, larvae migrate to surface streams or ponds and first appear in March (Anderson and Martino 1966) with metamorphosis typically occurring less than one year after hatching in June or July (Franz and Harris 1965, Anderson and Martino 1966, Franz 1967). Some larvae presumably overwinter and therefore metamorphose at >1 year of age (Rossman 1960, Franz 1967).

Population ecology of eastern long-tailed salamanders has received less attention. Franz (1967) attempted mark-recapture on eastern long-tailed salamanders in Maryland, but abandoned efforts after a return visit yielded few recaptures. Age-class structure and sex ratios were reported from a removal study from 2 study sites in Illinois (McDowell and Shepherd 2003). However, no long-term studies on eastern long-tailed salamanders have been conducted, and population size and survival estimates have not been calculated.

Herein, I present data from a long-term study focused on the natural history, ecology, and distribution of the eastern long-tailed salamander, which represents the most in depth investigation of the species to date. Chapter 2 focuses on natural history and ecology, and I provide descriptive data and anecdotal observations related to reproduction, larval period, movement patterns, and population dynamics. In addition to

the contributions of knowledge and new information on eastern long-tailed salamanders reported, I discuss annual and site-specific variation observed among 7 study sites and compare my data to other published accounts on eastern long-tailed salamanders and related species. Additionally, I speculate on the evolutionary history and adaptive value of life-history traits. In Chapter 3, I investigate factors affecting eastern long-tailed salamander distribution. I use multiple spatial scales to test the effect of land-use and water quality on species distribution.

Unique among any study of salamander ecology, my study sites were centered around springhouses. Springhouses are enclosed stone structures built over springheads (Figure 1) and were common on the piedmont of the mid-Atlantic region during my investigation. Springhouses provided ideal study sites because the structure of springhouses allowed access to the upper subsurface portions of springs that eastern long-tailed salamanders utilized, and I discovered that several springhouses in the region supported relatively large populations of eastern long-tailed salamanders. Since knowledge of eastern long-tailed salamander ecology was limited, these circumstances provided an opportunity to study this species within a context not previously explored.

### **Study Areas**

I investigated aspects of eastern long-tailed salamander ecology associated with springhouses on the piedmont physiographic province of the lower Delaware Valley in an area that encompassed parts of northern New Castle County, Delaware, and adjacent Chester and Delaware County, Pennsylvania from 2002-2008. Springhouses were

originally built as a cold storage area for food prior to the invention of electric refrigeration. Being built over springheads or spring seeps, springhouses enclose the effluence of a spring (Figure 2), creating an artificial environment wherein rock walls insulate the cool water of the spring from outside ambient conditions. The environment inside springhouses is therefore similar to the twilight zone of caves that contain subsurface streams and springs.

I conducted field observations and population analyses (i.e., mark-recapture) at 7 sites, which I named after their closest associated named tributary (Figure 3). Structures covering the spring effluence at these sites ranged in size from a cement cistern capped with a lid (e.g., springbox; Figure 4) to single and multiple room stone springhouses. Herein, I collectively refer to these structures as springhouses. Upwelling groundwater within springhouses remained a relatively constant temperature (7-14 °C) throughout the year. The cool water enclosed by the stone structure of the springhouse regulated the inside ambient conditions, keeping humidity and air temperature within the springhouse more constant than outside conditions.

A description of each of the 7 study sites are as follows:

*Crum Creek, Chester Co., PA (N40.00841 W75.438509, elev. 121 m) – CC*

Springhouse was located on a private residence with a mowed yard and manicured landscaping adjacent to forest. The springhouse walls and foundation were made of stone with the inside covered with deteriorating stucco. The springhouse was built into the ground, so that the ground level met flush with the top of back wall of the springhouse and sloped down the sides so the front of the springhouse was fully exposed at ground

level. A small open window ( $0.12 \text{ m}^2$ ) was positioned on the southwest-facing wall. The interior was approximately  $16.0 \text{ m}^2$  with a maximum ceiling height of 4.0 m. The wooden roof was A-frame and shingled and with no insulation interiorly. The floor was concrete with a water compartment approximately  $13.1 \text{ m}^2$  and a variable substrate of fine silt, gravel, and rocks. Water depth varied throughout the water compartment ranging from 18-52 cm during normal conditions. Periodically water level rose a maximum of 6 cm. Water flowed out of the springhouse through crevices in rocks under the springhouse floor. A small rocky surface stream formed immediately adjacent outside the springhouse and was vegetated with watercress (*Nasturtium officinale*). Water was pumped from CC to supply the associated residence's water usage.

Multiple adults and larvae of northern two-lined salamanders and northern red salamanders (*Pseudotriton ruber ruber*), as well as several pickerel frogs (*Lithobates palustris*), crayfish (unknown species), and 3 small bluegills (*Lepomis macrochirus*) (~3 cm), were observed in CC Springhouse each visit. It was presumed that these fish could not escape, and I suspected they were introduced to the springhouse from a nearby pond. On few occasions, I observed an eastern red-backed salamander (*Plethodon cinereus*), a northern watersnake (*Nerodia sipedon sipedon*), and an eastern gartersnake (*Thamnophis sirtalis sirtalis*) inside CC Springhouse.

*Darby Creek, Chester Co., PA (N40.02387 W75.41947, elev. 101 m) – DC*

Springhouse was a residential house, which contained a spring in the basement. The basement area was approximately 50% below ground with ground level access along two side walls. The house foundation was made of stone and the floor was cement. The



interior was approximately 36.0 m<sup>2</sup> with a maximum ceiling height of 2.7 m. The house contained 3 water compartments (surface area = 10.1 m<sup>2</sup>) which were cement lined and connected via terracotta pipes. The largest compartment averaged 80 cm deep and had a fine silt substrate. Water level remained constant and flowed out of the springhouse through large crevices in a rock wall and formed a surface stream immediately outside. This stream was lined on both sides by stone retaining walls holding soil approximately 1 m above the stream for a length of approximately 7 m. Stream substrate was primarily sand and rock and was vegetated with watercress. Mowed grass and landscaping surrounded the springhouse with the closet forest edge approximately 7 m away.

Species commonly observed in DC Springhouse included a few northern two-lined salamanders and northern red salamander larvae and multiple pickerel frogs. On a few occasions I observed an American bullfrog (*Lithobates catesbeianus*), an eastern red-backed salamander (*Plethodon cinereus*), and an eastern gartersnake (*Thamnophis sirtalis sirtalis*).

*Ridley Creek, Delaware Co., PA (N39.96365 W75.43887, elev. 114 m) – RC*

Springhouse was a large springhouse containing 3 successive rooms with a foundation and most walls constructed of stone and mortar. RC was primarily below ground. The posterior 2 rooms were completely underground with earth covering a cement roof. The entrance (front) room was 75% below ground level, but the front wall was fully exposed lying behind a stone retaining wall approximately 2 m high. Stone steps lead down to the entrance, which consisted of a single wooden door. The interior was approximately 23.4 m<sup>2</sup> with a maximum ceiling height of 3.2 m. The entrance room was the largest and had

a single glass window on opposite walls facing east and west near the ceiling. A small wooden room used for storage was constructed above the front entrance room. The entrance room was divided from the middle room by a stone wall with an open doorway and rotting wood frame. The middle room walls were stone covered with deteriorating plaster, while the back room was constructed of cinderblock. The floor was concrete and was littered with man-made debris, leaves, and flat stones. The water compartment comprised the entire floor area of the middle room and approximately one third the floor area of the entrance room. Water surface area measured approximately 10.2 m<sup>2</sup> with a variable water depth of 13-41 cm. Water level remained relatively constant except in November in 2005, when flow from the springhouse stopped and water level dropped approximately 13 cm. The water compartment contained substrate primarily of fine silt and fibrous plant roots, which grew through the foundation wall in the water. The water flowed out of the springhouse via an opening in the wall high enough to accommodate the water level. Outside of the springhouse water was diverted into a terracotta pipe that ran approximately 7.5 m and emptied into a marshy stream. This area contained some rocks at the outflow source but quickly transitioned to a silty substrate with deep mucky soil. Water flowed from the outflow source for approximately 20 m where it was heavily vegetated with obligate wetland plants including watercress. From here the water emptied into a man-made pond (1300 m<sup>2</sup>) that contained fish (bluegill, *Lepomis macrochirus*; bass, *Micropterus* sp. observed). RC was located on a private farm surrounded primarily by horse pastures. The immediate vicinity was maintained as tall

grass and was only mowed a few times each year. Water was pumped from RC to supply the associated residence's water usage.

Northern two-lined salamander larvae and pickerel frogs were commonly observed in RC Springhouse. Occasionally, I observed eastern red-backed salamanders, green frogs (*Lithobates clamitans*) and eastern American toads (*Anaxyrus americanus americanus*).

*Pigeon Run, Chester Co., PA (N40.09492 W75.58599, elev. 84 m) – PR*

Springhouse was located on a private residence along the edge of yard and forest. The springhouse walls and foundation were made of stone with the inside covered with deteriorating stucco. The springhouse was partially below ground, with 2 walls fully exposed at ground level. The interior was approximately 23.8 m<sup>2</sup> with a ceiling height of 2.5 m. A large storage room constructed from wood was built above the springhouse. The springhouse floor was concrete with a large water compartment approximately 10.7 m<sup>2</sup> and 24-47 cm deep with a substrate of fine silt, sand, and pebbles. Water level remained constant and water flowed out of the springhouse via a pipe and emptied into an unvegetated, first order stream with a substrate of sand, silt, and clay approximately 20 m away. A stone retaining wall, approximately 1 m high, extended approximately 54 m from the southeast corner of the springhouse. Water was pumped from PR to supply water to the associated residence.

Crayfish (unknown species) and pickerel frogs were commonly observed in PR Springhouse along with an occasional eastern red-backed salamander.

*Red Clay Creek, New Castle Co., DE (N39.79879 W75.66936, elev. 63 m) – RCC*

Springhouse was a medium sized springhouse in a manicured yard setting. The foundation and walls surrounding the water compartment were constructed of stone while the remaining portion was constructed of cinderblock. RCC was built approximately 50% below ground level; only the front wall was fully exposed above ground, which contained a single wooden door for entry, and was accessed via concrete steps between two 1 m high stone retaining walls. The interior was approximately 7.0 m<sup>2</sup> with a maximum ceiling height of 2.75 m. The wooden roof was A-framed with tar shingles and no interior insulation. The floor was concrete and free of debris. The water compartment had a surface area of approximately 2.9 m<sup>2</sup> and a variable depth of 2-34 cm. Water level remained constant. The substrate was primarily fine silt and contained fibrous plant roots growing through the foundation wall in the water. Water flowed out of the springhouse via an iron pipe and emptied into a small fishless pond (10.8 m<sup>2</sup>) approximately 20 m away. RCC was located on a private residence and abutted a paved driveway. RCC was connected structurally to a residential house via a low stone retaining wall approximately 12.5 m long and 0.3 m high, which separated the driveway from an elevated yard. Mowed grass and landscaping surrounded the springhouse. Water was pumped directly from the water compartment to supply water to the associated residence.

Pickerel frogs were common in RCC Springhouse. Occasionally I observed eastern red-backed salamanders and a short-tailed shrew (*Blarina brevicauda*).

*Middle Run, New Castle Co., DE, (N39.71378 W75.72810, elev. 61 m) – MR*

Springhouse was a medium sized springhouse with walls and foundation constructed of stone and mortar. MR was built into a steep south-facing forested hillside, so that the ground level met flush with the top of the back wall and sloped down the side walls with the front wall of the springhouse fully exposed. A single wooden door allowed entry inside. The interior was approximately 8.9 m<sup>2</sup> with a maximum ceiling height of 2.28 m. The roof was sloped and constructed of wood and tar shingles. The roof was lined interiorly with polyisocyanurate insulation. The floor was concrete with small piles of man-made debris. The water compartment was large with a surface area of approximately 3.7 m<sup>2</sup> and an average depth of 91 cm. Water level fluctuated up to approximately 15 cm above and below the normal level. The substrate consisted of fine silt and some woody debris. Water flowed out of the springhouse through fissures in the ground and surfaced at a spring head approximately 1 m away. Surface water formed a small stream and flowed approximately 3 m before joining with a first order stream. Both surface streams contained substrates of sand and rocks and no vegetation. MR was surrounded entirely by mature deciduous forest and was located within Middle Run Valley Natural Area.

Other species observed in MR Springhouse included pickerel frogs and northern red salamander larvae and adults. Less frequently I observed green frogs, eastern red-backed salamanders, eastern gartersnakes, and eastern ratsnakes (*Pantherophis alleghaniensis*).

*White Clay Creek, New Castle Co., DE, (N39.74490 W75.75607, elev. 79 m) –*

WCC was a springbox consisting of a concrete circular cistern measuring 91 cm in diameter and lying nearly flush with the ground. The cistern was capped with a fitted cement lid that fit imperfectly creating small gaps large enough for eastern long-tailed salamanders to crawl through. An inner concentric concrete cylinder 51 cm in diameter partitioned the water column of the contained spring water into inner and outer compartments. The top edge of the inner cylinder was approximately flush to a few centimeters below the average water surface level and was 30 cm below the top edge of the cistern. The cylinder also divided the water depth with the inner compartment averaging approximately 40 cm deep and the outer compartment approximately 15 cm deep. Water level fluctuated slightly by about 5 cm above and below the normal level. Substrate within the inner compartment was comprised of fine mud and silt whereas the outer compartment contained larger particles of sand from which water welled up through the substrate. Water exited the cistern through a 2.5 cm diameter metal pipe, approximately 2 m long, which emptied into a porcelain bathtub and overflowed into a first order stream. Surrounding the cistern, water seeped from the ground forming a muddy rivulet. Approximately 12.5 m away, a second uncapped springhead formed the headwaters of the first order stream that flowed past the bathtub. Substrate of the associated stream was primarily clay and lacked rocks, coarse woody debris, and aquatic vegetation. To increase cover at this site, I placed a few medium, flat rocks and boards (400 cm<sup>2</sup>) on and adjacent to the uncapped springhead and around the cistern. WCC was located in White Clay Creek State Park and was surrounded by scrub/shrub habitat

dominated by invasive plant species such as multi-flora rose (*Rosa multiflora*) and autumn olive (*Elaeagnus umbellata*).

Pickerel frogs were most commonly observed in WCC, but less frequently I observed northern two-lined salamanders, northern dusky salamanders (*Desmognathus fuscus*), eastern red-backed salamanders, and green frogs.



Figure 1      An example of a typical springhouse structure consisting of a stone foundation and wooden roof enclosing a spring. This springhouse was partially built into the ground, a design common among springhouses.





Figure 2      An example of a water compartment containing ground water near the effluence of a spring within a springhouse.

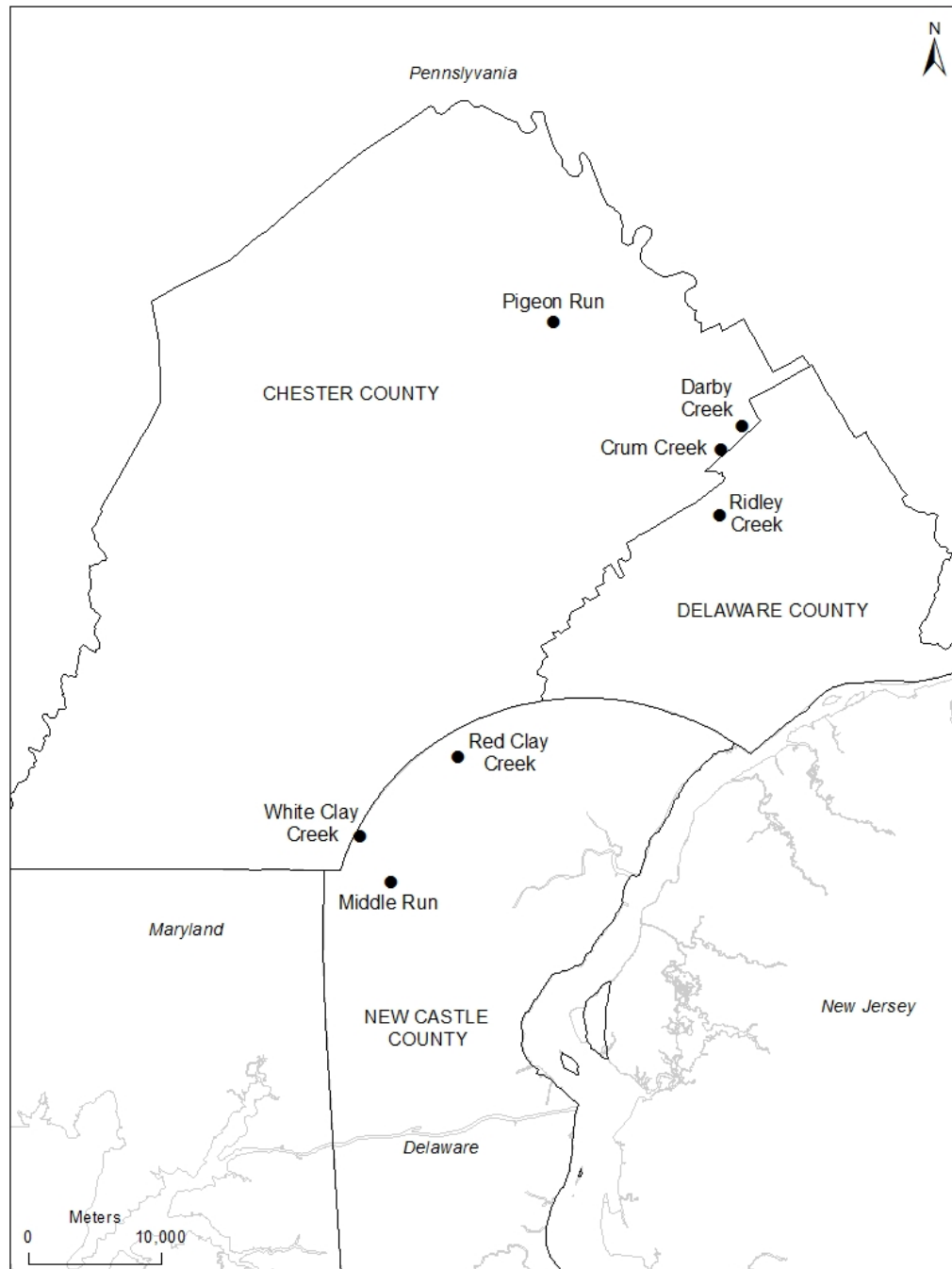


Figure 3 Location of 7 springhouse study sites with population of eastern long-tailed salamanders (*Eurycea longicauda longicauda*) investigated in Chester and Delaware Counties, Pennsylvania, and New Castle County, Delaware and from 2003-2008.



Figure 4      An example of a cement cistern or springbox capping a springhead.

## **Chapter 2**

### **LIFE HISTORY AND ECOLOGY OF EASTERN LONG-TAILED SALAMANDERS (*EURYCEA LONGICAUDA LONGICAUDA*) ASSOCIATED WITH SPRINGHOUSES**

#### **Introduction**

Key to the development of conservation and management strategies of a species is an understanding of biological information pertaining to its life history and ecology, as well as the ability to assess its population status. With recent evidence of global amphibian declines (Stuart et al. 2004), the need for this information becomes more imperative. However, even from the well-studied eastern United States, many species of amphibians remain poorly understood. One reason for such deficiencies might be that the habits of some species present logistical challenges for field investigation.

The eastern long-tailed salamander is a common species of the Appalachian and Ohio Valley regions of eastern North America, yet knowledge of its life-history traits remains incomplete. This lack of information is due in part to the eastern long-tailed salamander's secretive lifestyle. Behaviors associated with reproduction, such as mating and oviposition likely occur underground in stream passages and rock fissures within springs (Petranka 1998): a habitat that is primarily inaccessible to researchers. Behaviors associated with mating have rarely been observed (Cooper 1960) and egg deposition has not been documented. Few eggs of eastern long-tailed salamanders have been observed (Mohr 1943, Franz 1964); therefore, information on eggs and their development remains deficient. Egg laying period, incubation time, and hatching has been pieced together from anecdotal accounts of eggs discovered in early stages of development (Mohr 1943,

Franz 1964) and from observations of larvae (Anderson and Martino 1966, McDowell and Shepherd 2003), and has been inferred through dissection of reproductive tracts of adult females (McDowell and Shepherd 2003). Aspects of larval development have been described by Anderson and Martino (1966) and McDowell and Shepherd (2003), and Franz (1967) has suggested overwintering of larvae may occur, but the extent to which this occurs is unknown. Population dynamics have not been assessed in eastern long-tailed salamanders.

The purpose of my investigation was to provide additional knowledge on the natural history of eastern long-tailed salamanders and to assess the dynamics of populations associated with springhouses. I focused my study sites around springhouses (stone structures built over springheads), because springhouses remain common structures on the piedmont of the mid-Atlantic region, and the contained springs of springhouses are utilized by eastern long-tailed salamanders for reproduction (N. Nazdrowicz, personal observation). Springhouses provide access to the upper portion of the spring habitat that is otherwise inaccessible to researchers. Additionally, I address geographic variation by comparing my results to previous studies, and I investigate the influence of environment on local variation. I provide several new observations of eastern long-tailed salamander reproduction and larval ecology, compare my data with previously published data, and speculate on the evolutionary history and adaptations of observations and behaviors.

## Methods

### Site Visits

I investigated reproduction, larval period, activity patterns, and population dynamics of eastern long-tailed salamanders from 2003-2008 at 7 study areas associated with springhouses on the piedmont physiographic province in Chester and Delaware County, Pennsylvania, and New Castle County, Delaware. See Chapter 1 for study site descriptions: Crum Creek (CC), Darby Creek (DC), Middle Run (MR), Pigeon Run (PR), Ridley Creek (RC), Red Clay Creek (RCC), and White Clay Creek (WCC). Since field investigations commenced upon discovery, duration was variable among springhouses, lasting approximately 2.5-4.5 years. I visited each study site approximately every 2-4 weeks. Time between visits varied seasonally depending on activity of eastern long-tailed salamanders. During the reproductive period (mid-July – mid-December) when eastern long-tailed salamander abundance within springhouses was greatest, I visited springhouses approximately every 2 weeks (12-16 days). During the hatching and early larval period and when eastern long-tailed salamanders typically retreated deeper within the spring and were inactive, I visited springhouses monthly (January – March). When larvae and adults were active and migrating to surface habitats (late March – May), I increased visits to every 2 weeks (12-16 days). During the summer (June – July) when most eastern long-tailed salamander larvae and adults had moved out of springhouses, I visited springhouses approximately every 3 weeks (19-26 days).

I usually visited springhouses around dusk or after dark but occasional visits were made during daylight hours. Length of each visit ranged from 0.25-24 hours and was not standardized but rather was dependent on abundance and activity of eastern long-tailed salamanders at each site. During visits, I conducted area-constrained visual searches for eastern long-tailed salamander adults, juveniles, larvae, and eggs within and outside

springhouses up to approximately 50 m from the springhouse with the aid of a flashlight. I searched for adults and juveniles inside springhouses during each visit and outside springhouses after dusk from April through November by turning debris and cover, looking in cracks and crevices, and visually searching the ground, vertical surfaces, and among vegetation. I focused my efforts in areas with complex structure and potential access to underground retreats (e.g., logs, rocks, stone walls, etc.). To search for larvae and eggs, I visually searched within the water compartment inside springhouses November through July. I began checking associated outside surface streams for larvae in late February and continued searching for larvae in surface streams each year during each visit until no more larvae were observed. I searched for larvae in surface streams after dusk by visual searches with a flashlight and by random dip netting up to approximately 50 m from the springhouse.

#### Reproductive Data

When eggs and larvae were observed, I noted location and development, and counted abundance or noted presence at each springhouse. I used the Table of Normal Development for spotted salamanders (*Ambystoma maculatum*) to estimate developmental stage of eggs (Harrison 1969).

I determined clutch size from deceased gravid females. I collected and preserved all dead females found on site in 10% formalin. Prior to preservation, I recorded snout-vent length (SVL) by measuring from the tip of the snout to the posterior end of the cloaca. I dissected oviducts of preserved gravid females and counted number of ova. To investigate the relationship between clutch size and SVL, I regressed number of ova to SVL. I excluded any females with partially spent oviducts from the analysis.



RCC Springhouse represented a unique opportunity to investigate oviposition and egg development because this was the only site where a large number of eggs (e.g.,  $n > 1,000$  per year) were laid in visibly accessible areas. Due to the high abundance of eggs at this site, I set up egg-laying “fences” in order to increase available surface area for oviposition, making eggs easier to observe, count, and manipulate. I constructed rectangular fences from fiberglass screen cloth that measured approximately 25 cm long by 15 cm tall. Each year prior to oviposition in late October, I submerged 8-12 fences staked with wood skewers vertically in the water column of the spring compartment. At RCC, I increased site visits to every 1-7 days during oviposition in order to count egg abundance.

In November 2008, I visited RCC daily during the active oviposition period to observe oviposition behavior and to document egg development. I observed individual females as they oviposited eggs and recorded initial and end time of oviposition, time and distance between oviposition, and female behavior. I collected and isolated 98 eggs with known time of oviposition to investigate egg development. After an egg was laid, I removed the egg by cutting an approximate 2 X 2-cm section of the fiberglass screen cloth from which the egg was attached or by gently scraping it from other substrates with forceps. I placed eggs individually in a 29.6-ml translucent plastic Solo® soufflé cup with 2 holes (6 mm in diameter) punched into the sides and 2 holes punched into the lid, which I then submerged into the water compartment of the springhouse. I tracked age of each egg and measured embryo length, inner envelope diameter, and egg diameter a maximum of 5 times per egg throughout early development. Each egg was measured at time of collection and then measurements were staggered so that at least one measurement was record for all consecutive day ages, up to 34 days old. This age ensured that I collected measurements on eggs through early embryo development and



differentiation (Harrison 1969). I compared egg and embryo sizes among developmental stages grouped by similarity in differentiation and appearance: cleavage (stages 1-7), blastulation (stages 8-9), gastrulation (stages 10-12), neurulation and development of tail bud (stages 13-21), operculum development (stages 22-25), and hind limb development (stages 26-29) (Duellman and Treub 1994). I compared stage groupings successively using a one-tailed t-test, and when values did not differ, I pooled data for reported values.

I collected larval body measurements from all springhouses except CC. In 2004-2006, I collected approximately 20 larvae from inside springhouses and 20 larvae from surface streams during each visit at DC, RC, MR, and RCC springhouses. I captured larvae via random dip netting and measured SVL and tail length to the nearest mm by placing larvae upside-down in a clear plastic bag. I excluded measurements of broken tails from all data summaries and analyses. I categorized observed larvae into 3 types (newly hatched, developing, and developed) based on development. Newly hatched larvae were distinguished by the presence of erupted front leg buds, no hind legs, a visible yolk reserve, and lying on their sides on the bottom substrate. Developing larvae were fully mobile, but lacked fully developed digits on front feet or fully erupted back legs with digits, whereas developed larvae had fully erupted front and back legs with fully developed digits.

#### Mark-Recapture Data

I conducted mark-recapture of eastern long-tailed salamanders at each study site. Starting date for each study site varied, as I initiated mark-recapture upon discovery of a new population and continued through mid-July 2007 (Table 1). I attempted to capture all juvenile and adult (i.e., non-larval) salamanders at each site, each visit. For capture of eastern long-tailed salamanders, I employed aquarium nets or captured salamanders by

hand. Upon capture, I placed salamanders into 25 X 25-cm re-sealable plastic bags containing paper towel moistened with water from the springhouse with a maximum of 20 salamanders per bag. I placed any salamanders that appeared to be sick or injured in separate bags. Salamanders collected from discrete areas outside of springhouses (approximately 1-3-m<sup>2</sup> area) or from discrete rooms or water compartments inside springhouses were kept separate to insure release of individuals to approximate place of capture.

After no more salamanders could be found at a site, I began data collection of each captured individual. Usually, I processed salamanders inside the springhouse. However, when conditions did not allow for processing on site, I transported salamanders to the closest available site for processing (e.g., private residence). During data collection of individuals, I stored salamanders within collection bags in a cooler. After processing all captured salamanders, I conducted a second search for eastern long-tailed salamanders and captured and processed any additional salamanders. I repeated this process until no additional salamanders could be found. I recorded data collected from successive salamander searches separately. When salamander processing was completed, I released all captured salamanders back to the general locality from which they were captured.

I measured, weighed, and sexed each salamander captured. To measure salamanders, I placed each individual into a 15 X 15-cm re-sealable plastic bag. The bag allowed me to easily straighten the salamander along the bottom and turn it upside-down without injury to accurately record snout-vent length (SVL) and tail length to the nearest 1 mm. I recorded SVL by measuring from the tip of the snout to the posterior end of the cloaca. I checked the body and noted any injuries or disease. I recorded tail length by measuring from the posterior end of the cloaca to the tip of the tail and noted the caudal

condition (e.g., broken, regenerating). I weighed each salamander to the nearest 0.01 gram using a digital balance. I considered all individuals  $\leq 4.4$  cm SVL juveniles and individuals  $\geq 4.5$  cm SVL adults (Anderson and Martino 1966). For each adult, I assessed reproductive condition to determine sex. Presence of a mental gland on the chin of males and developing ova seen through the ventral skin in females were the most reliable characters for sexing individuals. However, these characteristics were not always present year round or on non-breeding individuals, and therefore, I could not accurately determine sex on all individuals categorized as adults. I recorded all adult salamanders lacking secondary sex characteristics as unknown sex.

Finally, I used a digital camera (Nikon Coolpix 4500) to photograph each salamander from above in approximately the same orientation (i.e., body horizontal relative to the camera lens and head to the right) to “mark” and later identify individuals. I used patterning on the top of the head and other distinguishing body characteristics (e.g., unique body marks and patterns, abnormalities, sex, etc.), when necessary, to identify individuals. Number of head spots of eastern long-tailed salamanders was correlated to size suggesting number of spots increased as individuals grew and matured (Jonas et al. 2011); however, the underlying general pattern was unique and did not change over time, making identification via natural marks feasible. For approximately the first 50% of captures ( $n = 7,729$ ), I distinguished individuals by visually comparing photographs from each visit to a photo-library of all individuals identified per study site. However, for the remaining 50% of captures ( $n = 7,898$ ), I used Interactive Individual Identification System (I<sup>3</sup>S) software (Ver 2.0, July 2007; Van Tienhoven et al. 2007) to assist with identification. With this program, I marked 3 reference points (i.e., the highest point of each eye and the suprascapular region along the vertebrae) and all natural marks (i.e., spots) on the dorsal surface anterior to the suprascapular region (i.e., head and

neck) to create a “fingerprint” for each individual (Van Tienhoven et al. 2007). I delineated the natural spot pattern by placing a single point centrally on each spot and used multiple points to mark irregularly shaped markings. The I<sup>3</sup>S program compared spot pattern fingerprints of unknown individuals to a database of known individuals (Van Tienhoven et al. 2007) and returned photographs of the 50 most likely matches. The database of known individuals consisted of one fingerprint per individual. I continuously updated this database by replacing previous fingerprints with more current fingerprints of same individuals. I visually compared each potential match of known individuals to the unknown individual to determine its identity. If none of the 50 most likely returned matches matched the unknown individual, I assumed the individual in question was a new capture, and I added it to the database of known individuals. Additionally, to check the accuracy of the visual comparison method I used for the first 50% percent of captures, I used the I<sup>3</sup>S program to compare all individuals from within my photo-library of identified individuals amongst each other.

I used mark-recapture data to summarize demographic statistics for each study site. Since eastern long-tailed salamanders migrate seasonally among reproduction, hibernation, and foraging sites, the distribution and abundance of sex and age-class likely varied throughout the year. Therefore, I excluded captures from the initial year of data collection of each study site from data summaries because these represented a partial set of annual data. I determined percent abundance, age composition, sex composition and ratio, and recapture rates for each springhouse. I pooled data for each study site within the eastern long-tailed salamander annual cycle (commencing on 15 July), by location (inside vs. outside springhouses), by season (breeding = 15 July to 30 November; non-breeding = 1 December to 14 July), and by springhouse for data summaries. I compared age composition and adult sex composition among years and springhouses and tested for

parity of sex ratios within study sites using a chi-squared analysis. Since sex could not be adequately determined year round, I limited analyses on sex ratio to the breeding season.

### Mark-Recapture Analysis

I used the POPAN extension of the Jolly-Seber (JS) model in program MARK (version 7.1) to estimate seasonal population size ( $N$ ) for each study site (White and Burnham 1999). I choose an open population model to estimate population abundances because births and deaths occurred during the sampling period. I pooled capture histories based on knowledge of eastern long-tailed salamander ecology to satisfy assumptions of the Jolly-Seber model. The JS model is based on the following 4 assumptions: 1) each marked and unmarked salamander has the same probability of recapture, 2) each marked salamander has the same probability of surviving to the next sampling period, 3) marks are not lost or missed, and 4) all samples are instantaneous and each salamander is released immediately after each capture. I met assumption 3 and 4 based on sampling design. However, the full capture histories for each study site likely violated assumption 1 and 2 of the JS model, and therefore, I reduced capture histories to reduce potential biases.

To minimize potential violations of assumption 1 and 2, I pooled capture events by season and reduced the dataset to include only males and females captured inside springhouses. Eastern long-tailed salamanders follow a general movement pattern between above and belowground habitats on an annual cycle (Mohr 1943). At my study sites, the springhouse represented the intermediary habitat as salamanders moved from aboveground to belowground sites in the fall and returned to aboveground sites in the spring. This movement into and out of springhouses was gradual with respect to the population. During any one capture event, individuals may have been outside

springhouses, inside springhouses, or deeper within the spring. Thus, all individuals of the population were not available for capture within the same habitat at the same time representing a likely violation of assumptions of the JS model. Since capture probabilities were more likely to differ among habitats than within habitats, I reduced capture histories to include only captures inside springhouses, which was the primary location of captures. Additionally, activity within springhouses coincided with the breeding (15 July through 30 November) and non-breeding (1 December through 14 July) seasons. Since survival probability potentially differed among seasons and habitats occupied, I pooled data by season (breeding, non-breeding). Pooling mark-recapture data reduced the number of capture events at CC to too few estimable parameters to show trends in the data, and therefore this study site was excluded from the analyses.

Capture and survival probabilities also potentially differed between sexes and among age-class. Juvenile capture and survival rates were unknown, but unknown sex had very low annual recapture rates. Therefore, I restricted capture histories to include only adult male and female captures to satisfy assumption 1 and 2 of the JS model. However, I did not distinguish between males and females in the mark-recapture datasets because I was interested in estimating total abundance within springhouses.

After constructing capture histories to minimize potential violations of assumption 1 and 2 of the JS model, I tested goodness-of-fit of each dataset by using the median  $\hat{c}$ -hat method of the Cormack-Jolly-Seber (CJS) model in program Mark. The assumptions for the CJS model were similar to the JS model except for assumption 1, which only applied to marked individuals of the population. I used the median  $\hat{c}$ -hat method to derive an estimate of the dispersion parameter ( $\hat{c}$ ) for the most saturated model ( $\hat{c} = 1$  means perfect fit,  $> 1$  means over-dispersion,  $< 1$  means under dispersion; Burnham and Anderson 1998). I considered all sites with a  $\hat{c} \leq 3.0$  as having adequate

model fit (Lebreton et al. 1992) and did not calculate population estimates for sites with poor model fit.

The POPAN extension of the JS model incorporated 4 variables in model estimates: survival rate ( $\phi$ ), recapture rate ( $p$ ), probability of entrance ( $b$ ), and initial population size ( $N$ ). Since I assumed no salamanders entered the population (i.e., no birth or immigration) while the population occupied subterranean habitats (i.e., between breeding and non-breeding seasons), I fixed  $b$  to equal zero for the corresponding parameters in each model.

I compared the full time-dependent model for survival, recapture rate, and probability of entrance ( $\phi_{(t)}$   $p_{(t)}$   $b_{(t)}$ ) with a subset model with constant survival ( $\phi_{(\bullet)}$   $p_{(t)}$   $b_{(t)}$ ). I did not consider any subset models with reduced parameters of recapture rate because the seasonal movement patterns of eastern long-tailed salamanders through springhouses made it unlikely that recapture rates were constant over time. I determined the best model by comparing the Akaike information criterion corrected for small sample size (AICc), and I ranked models according to their AICc values (Anderson and Burnham 1999). To estimate the relative likelihood of each model, I calculated Akaike weights (Burnham and Anderson 1998). The greatest support was given to the model with the lowest AICc value. I reported the derived estimates of population size ( $N$ -hat) for each season from the best model. However, since I used models with full time dependence for recapture probability, the first and last population estimates were not reported because they were confounded and non-identifiable (Schwarz et al. 1993).

## **Results**

### **Reproduction**

#### **Courtship and Mating**

I observed 4 instances of partial courtship within springhouses occurring in September. The most extensive observation of courtship occurred on 14 September 2003 on a rock wall approximately 1 m above the water surface at MR Springhouse. At approximately 1950 hours on a vertical rock surface, I observed a male aligned adjacent to a female but in opposite orientation with his head positioned near the female's hind limbs. (I observed males and females in this orientation on several other occasions, but in each case they remained stationary and no interactions were observed.) In this case, the female was partially emerged from a crevice in the wall with most her of tail extending back into the crevice. While remaining in this orientation, the male moved his head over the dorsal surface of the female's pelvic region and stroked his chin and throat across the female's back towards her head several times in a slow and deliberate fashion. This behavior was accompanied by occasional nudging and rubbing of his snout along the female's venter; repetitive, posteriolateral head jerks; and stroking of the females back with his left and right forelimbs. This behavior ensued for approximately 13 minutes while the female remained motionless. The female then moved forward approximately 2 cm and the male turned 180 degrees to orientate himself adjacent to the female so both were facing the same direction with his head aligned with hers. The male turned and positioned his head over the female and began stroking his chin anteriorly over the dorsal surface of her head, again in a slow and deliberate manner. After approximately 12 minutes, the female turned around and crawled back into the crevice and the male wandered off. Two other observations of courtship involved similar



behaviors, but interactions were brief as the salamanders may have been disturbed by my presence.

On 25 September 2005, I observed a female engaged in a tail straddle with a male on a rock ledge approximately 30 cm from the water surface at RCC Springhouse. The female was straddling the male's tail with her head over the male's pelvic region. The male began undulating his tail with waves originating from the pelvic region and dampening towards the tail tip. After several seconds of undulations, the male lifted the base of his tail and deposited a spermatophore on the rock surface. The male then crawled forward; however, the female did not follow, and the spermatophore transfer was unsuccessful, possibly due to my presence. The spermatophore measured approximately 2.5 mm high by 2.0 mm wide, with a sticky, clear base and white sperm cap (Figure 5).

## **Reproduction**

I observed gravid females with mature ova most frequently during August through November (93%) with peak abundance observed in September (39%) (Table 2). Few gravid individuals with mature ova were observed during other months. However, at WCC in 2005, I observed gravid individuals with mature ova for an extended period of August through May. Mark-recapture revealed that these latter observations were from the same gravid individuals captured August through November. I observed few females with partially spent (i.e., <50% ova remaining) oviducts (Table 2). Females with oviducts approximately 50% spent were observed in November ( $n = 4$ ) and December ( $n = 3$ ). Additional observations of partially spent oviducts were of females with oviducts almost completely spent with only a few eggs ( $\leq 5$  eggs) remaining in the reproductive tract (Table 2). I observed this condition in females October through February with a single observation in May. I observed developing ova (as defined by smaller sized ova

observed through the female ventral wall) infrequently. The earliest observation of individuals with developing ova was in March ( $n = 1$ ) and the latest observations were in September ( $n = 5$ ), with the most observations in July ( $n = 14$ ) (Table 2).

Ovarian eggs counted from dissected females ranged from 57-120 mature ova ( $\bar{x} = 75.8$ ;  $SE = 7.85$ ) and was related to SVL ( $\bar{x} = 5.8$  cm;  $SE = 0.16$ ;  $n = 8$ ;  $r = 0.900$ ; Table 3). Although collection dates varied, most examined females appeared to have full oviducts. One gravid female (SVL = 5.8) collected on 7 November had partially spent oviducts and contained only 29 eggs (Table 3).

I observed eggs in the contained spring compartment of all sites except CC. Reproduction occurred in all years at all springhouses with the exception of WCC in 2005, where I observed no eggs or larvae despite repeated searches throughout the summer both in the spring and surface stream. I observed newly deposited eggs (i.e., cleavage stage) as early as 24 October at WCC and as late as 21 February at RCC (Figure 6). Developed eggs (i.e., near hatching) were observed as late as 2 April at RCC (Figure 6). However, the majority of eggs were often deposited within a 2-4 week period occurring in November (Figure 7). In CC, PR, and MR springhouses, I observed few to no eggs (Table 4). A single egg observed at PR on 30 October 2003 had disappeared by a subsequent visit on 19 November 2003. At DC, RC, and WCC springhouses, I observed fewer than 365 eggs per year (Table 4). However at DC, I observed 3 to 29 times as many larvae as eggs and at DC and RC springhouses egg count was disproportionately less than the number of gravid females observed. At RCC, I observed greater than 1,500 eggs within the water compartment of the springhouse (Table 4). At both WCC and RCC springhouses, I observed greater than twice as many eggs as larvae and number of gravid females observed was congruent with the number of eggs observed.

I observed females ovipositing only at RCC Springhouse. Most females oviposited while submerged at various depths from a few centimeters below the water surface to a few centimeters above the bottom substrate. I observed one female ovipositing while partially emerged with her head and approximately 50% of her trunk out of water. On few occasions, I observed eggs that were deposited within a few centimeters below the water's surface become exposed as water level decreased in the springhouse in subsequent visits. Eggs were deposited on rocks, brick, cement, iron pipes, fibrous tree roots, wood, and plastics. Oviposition occurred primarily on vertical and ceiling surfaces, particularly along ridges, edges, or convex surfaces that were exposed to open water; rarely were eggs deposited within narrow passages between objects and were not found under rocks or debris lying in the substrate. Upward facing surfaces were avoided, and I only observed oviposition on upward facing surfaces when laid among fibrous roots. Females readily oviposited on the fiberglass screen cloth (i.e., "fences") I added as oviposition sites at RCC. The screening was flexible and thus formed a consortium of orientations available for oviposition when suspended in the water. Females rarely oviposited on fence surfaces that angled upward (i.e., >90 degrees from horizontal), but I did observe some eggs lying loose on the bottom substrate.

To oviposit, females crawled along surfaces underwater and pressed their cloaca against the oviposition substrate. Oviposition was often preceded by bending and wriggling of the vertebral column presumably to push eggs through the reproductive tract to the cloaca. Oviposition time (i.e., time from when cloaca was pressed against substrate until the female moved off the egg) lasted <1-24 minutes ( $\bar{x} = 8$  mins,  $n = 63$ ). I observed 24 females lay 2 or more (max = 6) consecutive eggs. Females oviposited one to a few eggs within close proximity before swimming away to seek other sites for oviposition. Distance between observed consecutively oviposited eggs from same

females ranged from immediately adjacent to 25 cm ( $\bar{x} = 5.5$  cm;  $n = 40$ ). Time between oviposition ranged from <1-84 minutes ( $\bar{x} = 14$  min;  $n = 35$ ).

A curious example of oviposition involved a female from RCC Springhouse, which I observed on 18 November 2008. I observed the female crawling around the spring compartment with a chain of 4 eggs trailing from her cloaca. Three of the eggs were covered with fine sediment, but the first egg was not, indicating it had been laid very recently. At 1000 hrs she attempted to oviposit on a rock, but when she moved away from the newly laid egg, the egg did not adhere to the rock surface but instead stuck to the pervious egg protruding from her cloacal. Seven minutes later she laid a 6<sup>th</sup> egg on the fiberglass screen cloth, which again stuck to her and the previous egg in the egg chain. The female was again observed at 1850 hrs and 2120 hrs later that day, and 945 hrs the next day with 4, 2, and 8 eggs trailing from her respectively. In each observation, newly laid eggs could be identified by a lack of sediment covering the egg envelop indicating that she continued to lay new eggs between observations, while older eggs eventually detached from the egg chain.

On several occasions, I documented oophagy among females that were actively ovipositing. Most observations involved the females consuming ( $n = 3$ ) or attempting to consume ( $n = 2$ ) one egg before swimming away, but for one occasion I observed a female consume an egg and then attempt to consume a second egg, although unsuccessfully. Consumed eggs had fully expanded jelly envelopes and were in early stages of development (i.e., cleavage). Before consuming an egg, females would press their snout against the egg or rub their snout side to side along the egg. The egg was then engulfed via a strong suction feeding process.

## Egg Development

Embryo and egg diameter measured 2.1-2.9 mm ( $\bar{x} = 2.4$  mm; SE = 0.02) and 3.0-4.2 mm ( $\bar{x} = 3.5$  mm; SE = 0.04), respectively, within 0-3 hours of oviposition ( $n = 77$ ). The inner and outer egg envelopes were fully expanded by day five, although inner envelope boundary could not be seen in some eggs. I could not distinguish individual stages of eggs in cleavage (stages 1-7) and blastulation (stages 8-9) in the field. The beginning of gastrulation (stages 10-12) was discernable at approximately 9-14 days old. Embryo diameter ( $P = 0.168$ ), inner envelope diameter ( $P = 0.461$ ), and egg diameter ( $P = 0.422$ ) did not differ between gastrulation (stages 10-12) and neurulation and tail development (stages 13-21). Embryos were larger for stages 22-25 ( $P < 0.001$ ) and stages 26-29 ( $P = 0.020$ ); whereas, inner envelope diameter and egg diameter were larger for stages 22-25 ( $P = 0.008$ ,  $P = 0.002$ , respectively), but were not larger for stages 26-29 ( $P = 0.290$ ,  $P = 0.108$ , respectively). For eggs in developmental stage 10-21, embryo diameter measured 2.5-3.2 mm ( $\bar{x} = 2.8$  mm; SE = 0.03;  $n = 40$ ), inner envelope measured 5.7-7.5 mm ( $\bar{x} = 6.6$  mm; SE = 0.08;  $n = 33$ ), and egg diameter measured 8.5-10.5 mm ( $\bar{x} = 9.3$  mm; SE = 0.09;  $n = 36$ ). Mean embryo length in stages 22-25 was 3.3 mm (SE = 0.05,  $n = 21$ ) and in stages 26-29 was 3.4 mm (SE = 0.34,  $n = 28$ ). For stages 22-29, inner envelope diameter measured 6.0-8.5 mm ( $\bar{x} = 7.1$  mm; SE = 0.12;  $n = 32$ ) and egg diameter measured 8.5-12.0 mm ( $\bar{x} = 9.7$  mm; SE = 0.09;  $n = 49$ ).

At RCC Springhouse, I observed that approximately 3-12% of eggs did not develop and were likely infertile. These eggs develop a white, hair-like fungus. I observed that apparently healthy, developing eggs (e.g., post blastula stage) adjacent to eggs with fungus may become covered with the fungus and die. Of 98 eggs isolated and monitored in 2008, I determined 5 eggs did not undergo cleavage suggesting they were infertile. Two additional eggs died due to irregular division of the blastocyte. Hatching

occurred approximately 7-9 weeks after oviposition. Water temperature during egg development at all springhouses with eggs ranged from 5-14 °C.

### Larval Period

I observed larvae in water compartments within springhouses from December through June (Figure 6). The earliest observation of larvae was 7 December at RCC and the latest observation of larvae was on 28 June at DC. The highest densities of larvae were observed February through March. I observed newly hatched larvae only at RCC and WCC springhouses. Developing larvae measured between 0.8-1.2 cm SVL ( $\bar{x} = 1.1$  cm; SE = 0.01;  $n = 140$ ) with a mean tail length of 0.7 cm (SE = 0.01,  $n = 136$ ), whereas developed larvae inside springhouses measured 1.0-1.5 cm SVL ( $\bar{x} = 1.2$  cm; SE < 0.01;  $n = 773$ ) with a mean tail length of 0.8 cm (SE < 0.01;  $n = 763$ ). Trends in larvae size inside springhouse by month showed no increase in size (Figure 8).

Two larvae in DC Springhouse were larger than expected for the time and location of their capture. On 6 December 2005, I captured one larva that measured 1.6 cm SVL and 1.0 cm tail (broken). This larva was observed approximately 2 months before the appearance of recently hatched larvae in the springhouse. On 16 March 2005, I captured one larva that measured 2.2 cm SVL and 2.0 cm tail length. All other larvae captured that day inside the springhouse measured 1.1-1.3 cm SVL and 0.8-0.9 tail length ( $n = 20$ ). In RC Springhouse on 7 April 2004, I captured a larval individual with adult markings and coloration (Figure 9). This individual measured 3.3 cm SVL and 3.5 cm tail length. On 4 June 2004, I recaptured this individual on the wall inside the springhouse, and it had fully metamorphosed (Figure 9).

I first observed larvae appearing in surface streams outside springhouses in the beginning of March in most years (Figure 6). The earliest observation of larvae outside

springhouses was 29 February at DC Springhouse. I estimated that the majority of larvae had migrated to exposed surface streams by mid-April (Figure 6). The latest observation of larvae in surface streams was on 16 August at WCC. Observations from searching for larvae at night revealed larvae were most commonly encountered in slow moving sections of streams and along pond margins in shallow water. Larvae remained abundant within the first 25 m of the spring effluence until metamorphosis.

Outside springhouses the greatest increase in larval body size occurred between April and June (Figure 8). Mean larval SVL and tail length pooled among study sites, years, and within months was similar from March (SVL = 1.3 cm; SE = 0.01;  $n = 66$  and tail = 0.9 cm; SE = 0.02;  $n = 66$ ) to April (SVL = 1.3 cm; SE = 0.01;  $n = 525$  and tail = 0.9 cm; SE = 0.01;  $n = 513$ ). The greatest increase occurred from April to June (SVL = 2.0 cm; SE = 0.01;  $n = 538$  and tail = 1.5 cm; SE = 0.02;  $n = 514$ ). Larval sizes were similar from June to July (SVL = 2.1 cm; SE = 0.02;  $n = 163$  and tail = 1.6 cm; SE = 0.02;  $n = 148$ ) and July to August (SVL = 2.0 cm; SE = 0.03;  $n = 36$  and tail = 1.6 cm; SE = 0.04;  $n = 34$ ). Larvae collected on the last observed occasions at DC, RC, MR, RCC, and WCC springhouses averaged 2.2 cm SVL (SE = 0.01;  $n = 226$ ) and 1.8 cm tail length (SE = 0.02;  $n = 209$ ).

Collectively, metamorphosis occurred from the end of May through mid-August, although few metamorphosing individuals were visually observed. At most study sites, metamorphosis occurred in July. However, I did document annual variation in timing of metamorphosis among years at some study sites. At RC, I documented metamorphosis occurring at the end of May in 2005. On a site visit on 9 May 2005, I observed numerous larvae in surface waters; however, on 28 May, I could only find 10 larvae and several showed signs of nearing metamorphosis including reduction of external gills. On the

subsequent visit on 14 June, no larvae were found. However, in other years at RC, I documented metamorphosis occurring in late June and July.

## Activity Patterns

### **Daily Activity**

I observed little temporal difference in eastern long-tailed salamander circadian activity inside springhouses. Most eastern long-tailed salamanders I observed inside springhouses were at rest or intermittently crawled slowly along surfaces kept moist from inside humidity. Dry surfaces were avoided. Wall surfaces above the water compartment, as well as associated cracks and crevices, were the most commonly occupied spaces. Typically, salamanders positioned themselves on vertical and climbing surfaces or along the edge of ledges directly above the contained spring compartment. Salamanders periodically moved about these surfaces and within cracks and crevices of the stone walls. Additionally, salamanders could also be found under and within natural and man-made debris scattered on the springhouse floor.

Outside springhouses, I observed eastern long-tailed salamanders active at dusk and after dark through the early hours of the morning. During daylight hours, I found relatively few to no eastern long-tailed salamanders by turning rocks, logs, or other movable cover. At some springhouses, I documented large numbers eastern long-tailed salamanders utilizing stone retaining walls as daytime retreats. As dusk approached, eastern long-tailed salamanders could be seen congregating within cracks and crevices of these walls, and as darkness fell, they would make their way out into the surrounding habitats en masse. Nighttime encounters of eastern long-tailed salamanders were more often associated with climbing than terrestrial movement. Eastern long-tailed



salamanders could be found crawling up and resting on herbaceous vegetation such as stems, leaves, or blades of grass, vertical surfaces of retaining walls, or among rock piles.

### **Seasonal Activity**

The circannual pattern of eastern long-tailed salamander movements was defined by a migration between terrestrial and subterranean habitats. Beginning as early as mid-July, eastern long-tailed salamanders began appearing inside springhouses. Abundance generally peaked in September and diminished considerably by December (Figure 10). On 28 September 2004, I captured 321 salamanders inside RC Springhouse and observed an additional 20 salamanders before leaving the site, which was the highest abundance observed.

I observed differences in timing of movements into springhouses between sexes and age class. Sexually active males typically arrived inside springhouses first followed by gravid females several weeks later (Figure 10). For example, from 15 July to 31 July, I captured 84 males inside springhouses compared to only 4 females over the same time period. Larger males and females also preceded smaller individuals. Mean SVL of sexually mature males found inside springhouses between 15 July and 31 July (SVL = 5.6 cm; SE = 0.03;  $n = 84$ ) was greater than sexually mature males found outside springhouses during the same time period (SVL = 5.4 cm; SE = 0.03;  $n = 107$ ;  $P < 0.001$ ). Likewise mean SVL decreased monthly for both males and females from July through October (Figure 13). Non-sexual adults (unknown sex) typically did not enter springhouses until September (Figure 10). Juvenile eastern long-tailed salamanders also delayed movement into springhouses until October with some individuals remaining active outside springhouses until November (Figure 10).

From December through March, eastern long-tailed salamander abundance was low inside springhouses (Figure 11). In most springhouses in most years, eastern long-tailed salamanders became sparse to entirely absent during this time. The few individuals that remained near the spring's surface inside springhouses typically resided within narrow cracks and crevices and were not active on wall surfaces; however, annual variation did exist. At MR during winter of 2006, many salamanders failed to retreat to deeper subterranean chambers. As humidity levels dropped within the springhouse, salamanders receded to just above the water line along the walls of the contained spring compartment, where humidity levels were highest and the wall was still damp. I recorded a mean of 60 captures per visit in winter 2006 at MR, compared to 16 captures per visit in 2003, and <2 captures per visit in 2004, 2005, and 2007.

Salamander abundance increased within springhouses during April and May as salamanders made their way from underground retreats to outside terrestrial habitats. During this time, daily abundances inside springhouses did not peak as high as in the fall (Figure 12). Additionally, I observed no temporal trends associated with abundances of sex or age class: all salamanders dispersed from springhouses at the same time.

Dispersal from springhouses occurred in all directions. Salamanders typically dispersed directly via a combination of terrestrial and subterranean routes from the springhouse as opposed to following the surface stream. I observed eastern long-tailed salamanders dispersing from under the springhouse door and through cracks and crevices of the outside walls of springhouses. If stone retaining walls were within the vicinity or structurally connected to springhouses, eastern long-tailed salamanders appeared to utilize these structures as subterranean corridors for dispersal.

From June through mid-July, eastern long-tailed salamanders were either nearly or entirely absent from within springhouses (Figure 12). Outside springhouses, I

observed eastern long-tailed salamanders first appearing in late April. By mid-May, most of the population had dispersed to terrestrial habitats, but occasionally a few eastern long-tailed salamanders lingered inside springhouses into June.

At some sites, I observed eastern long-tailed salamanders within the vicinity ( $\leq 50$  m) of springhouses throughout the summer. Most often I observed eastern long-tailed salamanders associated with microhabitats that provided subterranean daytime retreats, such as the springhouse foundation, retaining walls, rock piles, and at the effluence of buried drain pipes throughout the summer. At sites where these structures were absent, I found very few eastern long-tailed salamanders outside springhouses. Rather, eastern long-tailed salamanders could be found beneath rocks and logs in May when salamanders were actively dispersing from the springhouse, but later in the season, eastern long-tailed salamanders did not utilize these same cover objects, and I failed to locate any surface activity. I also observed greater abundances of eastern long-tailed salamanders outside springhouse on rainy and humid nights.

#### Mark-Recapture

Using visual methods comparing digital photographs, I identified 3,618 individuals among 7,729 captures. Of these only PR, MR, and WCC included more than 1,000 captures. For these sites, I made a total of 18 matching errors (as determined by quality control with I<sup>3</sup>S software), meaning I identified an individual as new when in fact the individual was previously captured. Error rate was  $<0.3\%$  for each site. I used I<sup>3</sup>S software for identification of the remaining captures ( $n = 7,898$ ). For recaptured individuals, 95% were matched with in the first 4 mostly likely individuals, and 99% were matched to within the first 19 returned matches.

## Captures

From December 2002 through July 2007, I captured 8,666 eastern long-tailed salamanders 15,573 times at the 7 study sites. At each study site, mark-recapture of individuals commenced mid-year of the eastern long-tailed salamander circannual cycle, so I excluded these partial year data from capture summaries. The number of complete years studied varied among study sites ranging from 2 years at CC, 3 years at DC, RC, and PR, and 4 years at MR, RCC, and WCC springhouses. Number of capture events per year among study sites did not differ ( $\bar{x} = 20.3$ ;  $P = 0.807$ ).

Excluding partial year data, I captured 7,899 eastern long-tailed salamanders among all study sites, including captures of 2,181 juveniles ( $SVL \leq 4.4$  cm) and 5,795 adults ( $SVL \geq 4.5$  cm). I captured the most individuals per year at RC ( $\bar{x} = 1,026$ ) and the fewest individuals per year at WCC ( $\bar{x} = 100$ ). At all other springhouses, I captured an average of 211 to 554 eastern long-tailed salamanders per year. For all study sites combined, I captured 81% ( $n = 6,379$ ) of all individuals inside springhouses compared to only 26% ( $n = 2,065$ ) of individuals outside springhouses. Likewise, 77% ( $n = 6,070$ ) of individuals were captured during the breeding season compared to 38% ( $n = 3,019$ ) of individuals captured during other times of the year.

Most captures of adults occurred inside springhouses and during the breeding season (Table 5). Percentage of adults captured ranged from 68% at DC to 99% at MR inside springhouses and 4% at MR to 39% at DC outside springhouses. Combined, I captured 4,969 (86%) adults inside springhouses and 1,219 (21%) adults outside springhouses. In most springhouses, I captured at least 75% of adults during the breeding season, and at PR, MR, and WCC, I captured >92% of adults during the breeding season (Table 5). CC had a more even distribution with 61% of adults captured during the breeding season and 52% captured other times of the year. Combined, I captured 4,791

(83%) adults during the breeding season, and 1,903 (33%) adults during other times of the year.

For most springhouses, I captured the majority of individual juveniles inside springhouses (Table 5), which ranged from 66% at RC to 93% of juveniles captured at WCC. However, at DC and RCC, I captured only 33% and 17% of individuals inside springhouses, respectively. Combined, I captured 1,375 (63%) juveniles inside springhouses and 896 (41%) juveniles outside springhouses. Time of year juveniles were captured was variable among springhouses (Table 5). At CC, DC, and RCC springhouses number of juveniles captured during the breeding season and number captured the remainder of the year was similar. I captured 49%, 54%, and 58% of juveniles during the breeding season and 55%, 51%, and 46% outside the breeding season at CC, DC, RCC, respectively. For RC Springhouse, I captured approximately one-third (36%) of juveniles during the breeding season, whereas at PR and WCC, I captured 72% and 96% of juveniles during the breeding season. Overall, these data accounted for 54% ( $n = 1,181$ ) of juveniles captured during the breeding season compared to 54% ( $n = 1,183$ ) captured outside of the breeding season.

Most males (>80%) were captured inside springhouses and during the breeding season (Table 6). For females, I captured >90% inside springhouses at most study sites, excluding DC where I captured 76% of females, and CC where I only captured 52% females (Table 6). At least 95% of females were captured during the breeding season at all study sites excluding CC, where only 62% of females were captured. Captures of unknown sex individuals were variable among study sites ranging from 47-97% inside springhouses and 30-81% captured during the breeding season (Table 6). Combined, I captured 92% ( $n = 2,883$ ) of males, 91% ( $n = 990$ ) of females, and 70% ( $n = 1,095$ ) of unknown sex eastern long-tailed salamanders inside springhouses, whereas outside

springhouses, I captured 17% ( $n = 520$ ) of males, 17% ( $n = 185$ ) of females, and 33% ( $n = 517$ ) of unknown sex. I captured most males (92%;  $n = 2,889$ ) and most females (97%;  $n = 1,064$ ) during the breeding season, with only 26% ( $n = 820$ ) of males and 23% ( $n = 253$ ) females captured during the non-breeding season. Overall, captures of unknown sex were more evenly distributed between the breeding (54%;  $n = 844$ ) and non-breeding (53%;  $n = 831$ ) seasons.

### **Age Composition**

Age composition differed among years for all springhouses (Figure 14, Table 7). Juveniles ( $SVL \leq 4.4$  cm) comprised an average of 11-35% of the total population per year. WCC had the most variable range of age composition with 72% ( $n = 162$ ) of the population juveniles in 2005 and only 4% ( $n = 3$ ) of the population juveniles in 2006. PR and MR also had less than 6% juveniles in one year; however, no other study site had greater than 44% juveniles in any year (Figure 14).

### **Sex Composition**

In total, I captured 3,145 males, 1,096 females, and 1,566 unknown sex eastern long-tailed salamanders with a  $SVL \geq 4.5$  cm representing 54%, 19%, and 27% of the total adults captured, respectively. At most study sites, the composition of males in the adult population ranged from 45-58% (Table 8). WCC had the lowest proportion of males comprising approximately one third of all adult captures. At most study sites, percent females ranged from 12-28% of all captures. CC had the lowest proportions of females (7%), and WCC had the highest proportion of females (38%). Proportions of males and females were generally highest during the breeding season relative to other comparisons; however, at some study sites captures inside springhouses yielded the highest or similar proportions of males or females (Table 8). Conversely, unknown sex

comprised 11-34% of the adult population for captures during the breeding season which represented the lowest proportions of captures by season or location at all study sites except for DC Springhouse (Table 8).

For adults captured during the breeding season, within-year sex ratio did not differ among years at most springhouses; however, MR ( $P < 0.001$ ) and RCC ( $P = 0.019$ ) springhouses had sex ratios that varied among years (Table 7). Most springhouses also had a male biased sex ratio for within-year captures and overall captures (Table 9). CC had the highest male biased sex ratio of 10.0 males per female. I documented even sex ratios at RCC during 2003-04 and 2004-05, (although the overall sex ratio was male biased) and at WCC (Table 9).

### **Recaptures**

Mean annual recapture rate for adult eastern long-tailed salamanders ranged from 0.20-0.36 at all springhouses except at DC (0.12; Table 10). These rates were similar to slightly lower than recaptures during the breeding season inside springhouses. Mean annual recapture rates for adult male and female eastern long-tailed salamanders were variable. Males and females were recaptured annually at similar rates at DC, PR, and MR springhouses, males more frequently than females at CC, and females more frequently than males at RCC and WCC (Table 10). However, during the breeding season inside springhouses, males were usually recaptured at higher rates than females except at RCC, where males and females were recaptured at similar rates, and WCC, where females were recaptured nearly twice as frequently as males. Unknown sex had a very low annual recapture rate, which was less than 0.10 at all study sites (Table 10).

Individuals first captured as juveniles ( $SVL \leq 4.4$  cm) had a 2-year recapture rate of 0.13-0.28 among study sites and a 3-year recapture rate of 0.02-0.07 (Table 11).

Individuals first captured as adults (SVL  $\geq 4.5$  cm) had a 2-year recapture rate of 0.15-0.44 among study sites and a 3-year recapture rate of 0.01 to 0.15. Both adults and juveniles had a 4-year recapture rate of  $\leq 0.03$ . Two- and 3-year recapture rates were similar for individuals first captured as juveniles and individuals first captured as adults for CC, DC, RC, and PR springhouses, but at MR, RCC, and WCC adult recapture rates were approximately 2 times higher (Table 11).

### **Population Estimates**

The mark-recapture dataset for RC ( $\hat{c} = 0.955$ ), MR ( $\hat{c} = 1.226$ ) and RCC ( $\hat{c} = 0.995$ ) springhouses showed good model fit. The WCC ( $\hat{c} = 2.294$ ) dataset was over-dispersed but was considered adequate because  $\hat{c} < 3.0$  (Lebreton et al. 1992), whereas data for PR springhouse was under-dispersed ( $\hat{c} = 0.125$ ). DC failed to converge due to insufficient data in goodness-of-fit testing and therefore was excluded from the analyses. The best supported model for PR, MR, and RCC allowed for seasonal variation of survival ( $\phi_{(t)}$ ), whereas the best model for PR and RCC had constant survivorship ( $\phi_{(\cdot)}$ ; Table 12). For each springhouse, the best model was well supported with a  $\Delta AIC_c > 2.0$  compared to the other model tested (Table 12).

Overall trends in population estimates showed lower population abundances during the non-breeding season compared to the breeding season with stable abundances over time at most study sites (Figure 15, 16, & 19). MR showed a decrease over time in abundance (Figure 17), and the population at RCC increased (Figure 18). Population estimates for the breeding season at MR decreased from 770 males and females (95% CI = 711-833; SE = 31.2) in 2003 to only 418 males and females (95% CI = 321-544; SE = 56.5) in 2006. Abundances increased at RCC from 100 males and females (95% CI = 79-126; SE = 11.9) in the 2003 breeding season to an estimated 266 males and females (95%



CI = 196-361; SE = 41.9) in 2006 breeding season. Population estimates did not differ among years at RC, PR, and WCC springhouses with breeding season abundances of males and females ranging from 1216 (95% CI = 1014-1459; SE = 113.0) to 1456 (95% CI = 1199-1770; SE = 145.1) between 2004 to 2006; 343 (95% CI = 255-463; SE = 52.8) to 392 (95% CI = 293-526; SE = 59.1) between 2004 to 2006; and from 40 (95% CI = 23-69; SE = 11.3) to 49 (95% CI = 50-60; SE = 5.0) from 2003 to 2006, respectively.

## **Discussion**

### **Reproduction**

Previously very little information was available on the reproductive ecology of eastern long-tailed salamanders, particularly pertaining to oviposition and eggs. This was likely due to the subterranean lifestyle of eastern long-tailed salamanders during the breeding season, which is associated with subsurface springs—a habitat that is not readily accessible to observation. The exception, mineshafts and caves, have been investigated by a few researchers (Mohr 1944, Franz 1967, Taylor and Mays 2006). Yet, very few direct observations associated with reproduction have been reported (Mohr 1943, Franz 1964, Cooper 1960). Similarly, some of my study sites did not provide a complete opportunity to study eastern long-tailed salamander reproduction, as this species did not fully utilize the visually accessible areas of the springhouse for reproduction. Still, I documented more observations of reproduction than previously reported in the literature and discovered new information on eastern long-tailed salamander reproductive ecology.

My observations suggest that courtship likely commences in late-summer when females enter subterranean passages. Here, males and females may congregate in large numbers, and this aggregation of sexes likely provides the best opportunity for mating encounters. My observations of courtship occurring in September and an observation by

Cooper (1960) in October coincide with peak abundances of females observed inside springhouses. At my study sites, salamander abundances began decreasing in November, likely signaling the end of the mating season as males and females retreated deeper underground presumably to seek out oviposition and overwintering sites. Petranka (1998) suggested mating occurring in the fall and early winter with evidence derived from examination of mature adult reproductive tracts (McDowell and Shepard 2003), observations of courtship in the field (18 October, Cooper 1960), and observations of eggs found in early stages of development (23 November, Franz 1964; 2 January, Mohr 1943). My observations suggest an earlier time period for mating lasting about 2-3 months and occurring prior to initiation of ovipositing. Since mating appears to be associated with movement to subsurface habitats, the time period for mating may vary geographically.

Although none of my observations provided a complete sequence of courtship, the behaviors I observed associated with mating provide a more complete picture of eastern long-tailed salamander courtship. Previously, Cooper (1960) provided the only published account of eastern long-tailed salamander mating and his observation was of a partial courtship attempt. Cooper (1960) observed a male in pursuit of a female that moved about in an erratic and “spastic” manner as the male chased a female in an attempt to head her off. The male also rubbed its snout around the cloacal region of the female (Cooper 1960). In contrast, I did not observe any chasing behavior. Rather, male movements associated with female interactions were slow and methodical. In general, I did not observe erratic movements by eastern long-tailed salamanders except when I disturbed individuals. I also observed males nudging and rubbing their snout along females’ venter. Nudging in the ventral and cloacal region has also been reported in

northern two-lined salamanders (Nobel 1929), and may provide cues of a female's reproductive condition or may be an attempt to stimulate the female (Cooper 1960).

Other behaviors I documented associated with mating represent new observations for eastern long-tailed salamanders but are common among Plethodontids (Petranka 1998) including pheromone stimulation, spermatophore deposition, and evidence of tail straddle walk. I observed a male rubbing its mental gland (located on the chin) across the dorsum and head of a female as well as the male stroking the female's dorsum with his forelimbs. The mental gland, which is a unique structure among Plethodontids, secretes pheromones to increase the female's receptivity to mating (Houck et al. 2008). The forelimb stroking behavior I observed may be associated with spreading these pheromones on the female. Pulling the chin slowly across the female has been reported in two-lined salamanders, arboreal salamanders (*Aneides lugubris*), and Mt. Lyell salamanders (*Hydromantes platycephalus*) (Duellman and Trueb 1994), however stroking with the legs has not been reported, based on an extensive review by Petranka (1998).

I documented newly laid eggs occurring as early as late October and as late as February indicating an extended egg-laying period. Observations reported in the literature of eggs and recently hatched larvae also suggested an extended egg-laying period for eastern long-tailed salamanders lasting from late fall through early spring (Petranka 1998). Newly laid eggs have been found on 23 November in Maryland (Franz 1964) and on 2 January in Pennsylvania (Mohr 1943) although not in great abundance. Additionally, Franz (1967) suggested an egg-laying period of September through January based on observations of recently hatched larvae found December through March in Maryland, and Anderson and Martino (1966) estimated oviposition occurred in January in New Jersey. Despite my observations of newly laid eggs occurring over an extended period, observations from RCC Springhouse suggest that the majority of females laid

their eggs in a much shorter time period lasting about 2-4 weeks in late October to early December. Eggs laid later in January and February seemed to be isolated occurrences. Although oviposition was not directly observed for eggs laid later in the season, these represented smaller clutches than observed earlier in the season. Observations of females with partially spent oviducts after December suggests that some females retain some eggs for laying at a later time. A similar reproductive cycle has been documented in cave salamanders, in which oviposition has been documented over an extended period, with a large peak in oviposition occurring early in the season followed by a smaller peak later in the breeding season (Ringia and Lips 2007).

Location of oviposition sites among substrates suggests that females selected specific microtopographic features for egg deposition. Specifically, eggs were typically laid on vertical and ceiling surfaces, particularly along ridges, edges, or convex surfaces, that were exposed to open water; rarely were eggs deposited within narrow passages between objects. Upward facing surfaces were avoided, and I documented no eggs in excavations under objects lying on the substrate. Eggs lying unattached on the substrate were likely knocked loose or did not adhere during oviposition. I hypothesize that females oviposited to maximize surface area of the eggs exposed to water. Since eastern long-tailed salamander eggs have a relatively large jelly envelop around the embryo, diffusion of oxygen is likely a limiting factor for development. Placing eggs suspended in water would maximize surface area available for oxygen diffusion. This might explain why upward facing surfaces were avoided, as the weight of the egg would press the bottom surface of the egg against the substrate reducing the surface area exposed to water, while exposing the upper surface of the egg to settling silt and debris from the water column. Oviposition of eggs in open areas as opposed to concealed in crevices or hidden under objects would allow water flow to remove respired carbon dioxide and

replenish oxygen to the egg surface, and I speculate that females seek out oviposition sites based on presence of flowing water. For example, at WCC, I observed eggs were more concentrated along the cistern wall above an upwelling of spring water from the substrate than at more distant locations. Based on these observations, dissolved oxygen may play an important role in egg development and survival, and the sensitivity of embryos to changes in oxygen concentration warrants further investigation.

During one year at WCC, I failed to document evidence of successful reproduction within the spring, even though I documented gravid females present during the fall and throughout the winter. Mark-recapture revealed that these gravid females forewent oviposition and were still gravid when they emerged from the springhouse in spring. While it is possible that I did not capture all gravid females and some reproduction did occur, I did not observe any eggs nor did I document any larvae. Additionally, I did not observe evidence of gravid females foregoing oviposition at any other site. This suggests that specific environmental conditions are required for females to oviposit and in the absence of these conditions females choose to forego reproduction to conserve reproductive energy.

Other factors may play a role in site selection for oviposition. In most springhouses, I observed many fewer eggs compared to the number of gravid females and number of larvae present. These observations suggest that females were selecting ovipositing sites that were more secluded than the exposed water compartment of the springhouse and therefore deeper within the spring. Ovipositing in deeper sites within the spring may be advantageous because deeper sites may contain fewer predators. Additionally, water temperature and water levels are likely more stable in deeper sites. Water deeper underground maintains a relatively constant temperature due to the insulating factors of the earth, whereas water temperature close to the surface may be

more variable due to the influence of ambient surface temperatures. Likewise, fluctuations in water level are likely less variable in deeper sites than sites closer to the surface because deeper sites are closer to the water table. Also, should water level drop in deeper or concealed sites exposing eggs, the chambers may possibly retain high humidity preventing desiccation (Franz 1967). Laying eggs in deeper, subsurface sites might be an adaptation to maximize reproductive success by avoiding predation, temperature extremes, and desiccation.

Since I did not have access to these secluded sites, I could not determine how much deeper within the spring oviposition occurred or how conditions of these sites compared to the conditions in the water compartment where at least some eggs were laid in most springhouses. However, I did observe a possible trend based on presence of predators and number eggs observed in springhouses. In 4 springhouses, I documented presence of crayfish and/or northern red salamanders, which are both potential egg predators. At some of these sites, I noticed some eggs disappeared prior to hatching suggesting predation had occurred. At 3 of these 4 sites, very few eggs were observed suggesting that presence of predators may influence site selection for egg deposition.

I did not detect any trends in changes in water temperature or water level with abundance of eggs. Overall variability in water temperature was not extreme, with the greatest change in water temperature over the egg laying period being  $-6^{\circ}\text{C}$ . The temperature of water within springhouses at my study sites was influenced by multiple factors including the outside ambient temperature, the insulating ability of the springhouse, and the flow rate of ground water exiting the spring. Springhouses, being built for the purpose of insulating the spring from ambient conditions, might provide better insulation from wind drafts than twilight zones in large caves. This might explain why I observed more eggs at more sites than any previous study. Likewise, I did not

document any rapid or extreme fluctuations in water depth in the water compartments at any springhouse. Overall most springhouses maintained a relatively constant water level with the greatest change in water level being approximately  $\pm 15$  cm. Although I did not document significant variation in water temperature or water level at my sites, variability might be more extreme in other parts of the range of eastern long-tailed salamanders particular in montane regions. The tendency of eastern long-tailed salamanders to oviposit deeper within the spring may have evolved as an adaptation to predator avoidance or may reflect an evolutionary history of living in an environment with more variable subsurface conditions.

RCC and WCC springhouses differed from other study sites in that the number of eggs observed corresponded with the total reproductive potential based on the number of gravid females observed, indicating that secluded sites were utilized infrequently or not at all at these sites. Possible explanations for this observation could be that deeper, secluded sites did not exist at these springhouses, were not accessible to eastern long-tailed salamanders, or they did not exhibit suitable conditions for oviposition. These observations and the previously discussed data suggest that multiple factors likely influence oviposition site selection among populations. While eastern long-tailed salamanders appear to have a tendency to oviposit deeper within springs, populations may exhibit plasticity in this trait depending on local conditions.

Previous evidence suggested that eastern long-tailed salamanders may deposit eggs out of water under special conditions such as high humidity. This suggestion was based on an observation by Franz (1964), who found a cluster of 5 newly deposited eggs approximately 60 cm above the water surface on the ceiling of a cave in Maryland. I did not observe any eggs oviposited out of water at my study sites, and I suspect that non-aquatic egg deposition does not occur in eastern long-tailed salamanders. On few

occasions, I observed eggs, which were deposited near the water's surface, became exposed after water level decreased in the springhouse. Considering my observations, it is plausible that the eggs observed by Franz (1964) were laid underwater, and were later discovered after water level receded. Supporting this hypothesis, Franz (personal communication) noted that water fluctuation within this cave system occurred regularly. Since eggs are large and the outer jelly envelope thin, there is little support to maintain the shape of the egg when out of water. Aquatically deposited eggs that I observed out of water lost their shape and drooped from the weight of the egg, and Franz (1967) observed a similar situation.

Observations of oviposition revealed that females scattered eggs within the spring, and oviposition time and time between oviposition suggests that it may take females up to a week or longer to lay all of their eggs. Additionally, observations of gravid females with partially full ovaries and low abundances of newly laid eggs late in the egg laying season (e.g., January and February) suggest that some females may suspend oviposition after laying some of their eggs early in the egg laying period and lay the remainder of their eggs later in the season. This mode of egg-laying precludes parental care in eastern long-tailed salamanders. Dispersed egg-laying is a reproductive strategy of not putting all your eggs in one basket and provides a benefit by not exposing all of the eggs to the same conditions or predators. The length of time it takes for a female to oviposit her entire clutch suggest the female puts considerable investment in site selection.

On a few occasions, I documented oöphagy among females that were actively ovipositing. Before consuming an egg, females would press their snout against the egg or rub their snout side to side along the egg. This behavior strongly suggested that females were assessing chemical signals from the egg prior to consumption. Consuming infertile eggs might provide an advantage by reducing the spread of fungal infections. For



example, at RCC, I documented that a small percentage of eggs laid were likely infertile and developed a fungus as they decayed. When these eggs were in contact with living eggs, the fungus spread to these eggs and killed them. Another advantage to oöphagy would be to reduce competition from conspecifics. In this case females would need to be able to recognize their own eggs from the eggs of others, since accidentally consuming their own eggs would reduce a female's fitness. However, since I could not determine condition or relatedness of the eggs consumed, further investigations are required to determine the reason for oöphagy in eastern long-tailed salamanders.

I documented hatching of eastern long-tailed salamander eggs occurred approximately 7-9 weeks after oviposition. This estimate is shorter than 10 weeks reported by Mohr (1943). Temperature may have played a role in this difference in incubation period. Ringia and Lips (2007) determined that eggs of cave salamanders, which have similar reproductive and life history traits as eastern long-tailed salamanders, incubated at cooler temperatures took longer to develop. Eggs incubated at 10°C had a mean incubation period of 73 days whereas eggs incubated at 15°C hatched in approximately 25 days (Ringia and Lips 2007). Mohr's (1943) observation came from a mineshaft in Pennsylvania, from which he reported a near constant water temperature of approximately 13°C. I documented water temperature during the incubation period at RCC ranged from 14 to 8°C. While our reported temperatures seem similar, data from Ringia and Lips (2007) suggests that development may be extremely sensitive to small temperature changes. Since I did not track individual eggs, my estimate is derived from first observations of eggs to first observations of larvae, and the early eggs were exposed to the warmest water temperatures of the egg laying period.

## Larval Period

The first observed larvae began appearing in water compartments within springhouses from the beginning of December to beginning of March. Larvae were observed in all springhouses, even where no eggs were observed, suggesting a movement from deeper within the spring. Ringia and Lipps (2007) suggested that timing of larval movements of cave salamanders from cave pools to surface streams was associated with periods of increased water flow December through May. However, water flow was not strong in springhouses, and I speculate larvae detect and follow water currents to migrate to surface streams, with timing of larval movement from subsurface to surface sites dependent on time of hatching and location of eggs. In some springhouses, larvae may face challenges leaving the spring, as some water is piped to outside the house, whereas in other springhouses water flows as a continuous stream. For example, nearly all larvae had exited springhouses by the end of April in most years, but at DC Springhouse larvae lingered until as late as June. DC Springhouse differed from other springhouses in structure in that the contained water compartment was large and deep, and water flowed out of this compartment via a 10 cm pipe at the water's surface. Therefore timing of movements to surface streams may also be influenced by spring structure.

Little growth of larvae occurred within springhouses. This was evident at DC Springhouse, where larvae remained inside springhouses for a longer period of time than at most other sites, but remained about the same size during this time period. Both the cool temperature of springs, as well as the limited food resources available are likely responsible for lack of growth during this time period (Culver 1982, Atkinson 1994).

In March, larvae began appearing in surface streams outside the springhouses, and all larvae have typically moved out of the springhouse to exposed surface streams by late May, approximately 2-4 months after hatching. Anderson and Martino (1966) also

observed eastern long-tailed salamander larvae appearing in surface waters in early spring, with the first observations made on 28 March and on 13 April. After entering the surface streams, larval drift or dispersal downstream does not appear to be significant; larvae remained abundant within the first 25 m.

I documented that larvae grew fastest during April through June at a rate of approximately 3-4 mm per month. Similarly, larvae studied in limestone ponds in New Jersey grew approximately 4-5 mm SVL per month during this time period (Anderson and Martino 1966). Size at metamorphosis in my study was similar to Anderson and Martino (1966), who documented average size at metamorphosis between 20-21 mm SVL in New Jersey, and Franz and Harris (1965), who observed eastern long-tailed larvae metamorphosed at 18-21 mm SVL in Maryland.

The timing of metamorphosis varied among study sites and among years occurring from May through mid-August with a peak in July. This suggests a larval period of approximately 5-8 months. Similarly, Franz and Harris (1965) observed a mass transformation on 5 July in Maryland and Anderson and Martino (1966) documented the earliest date of metamorphosis occurred on 15 June and continued through July in New Jersey. This short larval period suggests eastern long-tailed salamanders are adapted to ephemeral habitats, as Anderson and Martino (1966) noted that larvae began metamorphosing before drying of the temporary limestone ponds they inhabited in New Jersey. Freeman and Bruce (2001) suggested that three-lined salamanders inhabiting permanent habitats were more likely to prolong larval development into a second year, and therefore attain a larger size at metamorphosis. This strategy, however, does not appear to be typical of eastern long-tailed salamanders, as all populations I studied inhabited permanent springs, although populations of eastern long-tailed salamanders

with >1 year larval period have been documented in the western part of their range (Illinois; Rossman 1960).

On three instances, I found larvae inside springhouses much larger than the typical size I had documented for the time of the observations, suggesting that these larvae over-wintered in the spring and metamorphosed at >1 year of age. Similarly, Franz (1967) found a single large larval eastern long-tailed salamander (TL = 51 mm) among many small larvae (TL = 19-24 mm) in a cave in West Virginia. This evidence suggests that overwintering is a rare event for eastern long-tailed salamanders. However, for the dark sided salamander, overwintering in caves of at least some larvae may be more common (Rudolph 1978). Although Rudolph (1978) suggested larval period lasting into the second year was due to low food availability within caves, I hypothesize that the three larvae in my study were trapped within the spring and unable to migrate to the surface streams, prolonging their larval period. This plasticity in the timing of metamorphosis may be an adaptation to spring habitats with an unpredictable water source. Should water flow diminish in one year and not flow to the surface trapping larvae underground, they could delay metamorphosis until water flow increases or possibly metamorphose underground allowing them to crawl to the surface.

#### Activity Patterns and Movements

The annual activity cycle of the eastern long-tailed salamander can be summarized by three primary activity periods: 1) reproduction, 2) overwintering, and 3) foraging, with each characterized by a directed migration or movement. Mohr (1944) first described this annual movement pattern when he documented abundances of eastern long-tailed salamanders in a mineshaft. Plotting the distribution of salamanders along the mineshaft walls over time revealed the salamander's movement into and out of the mine

(Mohr 1944). Mohr (1944) documented reduced abundances in the mine during winter months, which were attributed to salamanders crawling deeper into crevices, while salamanders were completely absent from the mine in June and July. Franz (1967) suggested a similar annual cycle of eastern long-tailed salamanders in Maryland.

My observations of movement patterns revealed that eastern long-tailed salamander activity was primarily subterranean and associated with springs during 7-9 months out of the year. A regular migration occurred in late summer/early fall as eastern long-tailed salamanders moved from terrestrial to subterranean sites associated with springs, where mating occurred. Following mating, eastern long-tailed salamanders moved deeper into the spring to overwinter, with oviposition occurring before, during, and in some cases after this movement. In the spring, salamanders migrated out of the spring to terrestrial habitats to forage during the summer. I documented differences in activity patterns between sexes and age class with respect to these activity periods, which had not previously been described.

## **Reproduction**

The reproductive activity period, which was preceded by a migration to underground retreats associated with springs beginning in mid-summer and extending through fall, encompassed mating and oviposition. Migrating, sexually active salamanders formed breeding aggregations inside springhouses, which typically peaked in abundance during September. Similar aggregations have been documented inside mineshafts and caves. Mohr (1944) observed high abundance of long tailed salamanders beginning in August ( $n = 51$ ) inside a mineshaft in Pennsylvania, which peaked in mid-October at approximately 250 individuals. Franz (1967) made similar observations inside a cave in Maryland. Eastern long-tailed salamanders have also been found in association

with springs that lacked well-defined subterranean entrances (Anderson and Martino 1966). Presumably, in these circumstances, eastern long-tailed salamanders form aggregations at or near the surface of springs hiding under rocks or debris or in small subterranean chambers or crevices that are inaccessible to researchers. WCC provided one such example. WCC had two spring effluences: the main effluence was capped by a cistern, while the second effluence lacked a constructed structure but rather contained several movable flat rocks. Eastern long-tailed salamanders gathered under these rocks at the spring effluence beginning in July. Abundance of adults was highest August through November and then decreased to only a few individuals in December. Likewise, Anderson and Martino (1966) documented a movement pattern along the margin of ponds in New Jersey, presumably near their underground retreat. At this site, eastern long-tailed salamanders increased in abundance during September followed by a slow decrease in abundance and disappearance from the surface by the end of October (Anderson and Martino 1966).

I documented differences in timing of movements into springhouses between sexes. Sexually active males were the first individuals to arrive inside springhouses followed by gravid females about two to three weeks later. The strategy of males arriving at breeding sites before females is well documented among pond-breeding salamanders that migrate to breeding sites (*Ambystoma jeffersonianum*– Douglas 1979, Douglas and Monroe 1981, Downs 1989; *A. laterale*– Uzzell 1969, Downs 1989, Lowcock et al. 1991; *A. maculatum*– Blanchard 1930, Peckham and Dineen 1954, Shoop 1974, Hillis 1977, Downs 1989, Sexton et al. 1990; *A. talpoidium*– Semlitsch 1985; *A. tigrinum*– Semlitsch 1983). The advantage to early arriving males may be an increased potential to mate. However, for eastern long-tailed salamanders moving to subterranean habitats prior to the end of the foraging season represents a trade-off with feeding. I

observed very few prey items inside springhouses and most likely very little, if any, feeding occurs. Males that arrive earlier will experience an extended time without food, since the mating period occurs at the end of the active season and is followed by overwintering. Due to this extended period, early arriving males should be more fit than later arriving males, and this hypothesis is supported by the fact that the earlier males are larger than the males that arrive later.

I also documented differences in timing of movements into springhouses between age classes. Non-reproductive adults and juvenile eastern long-tailed salamanders delayed movement into springhouses until September and October. These differences in movement are likely associated with reproduction. Movement to springs for overwintering brings males and females within close proximity and is, therefore, a convenient opportunity for mating encounters. Since immature individuals do not take part in reproduction, it would be advantageous to delay movement to overwintering sites until prey items are no longer available in order to build up energy reserves for overwintering. Non-reproductive adults should employ the same strategy.

My observations suggest that the mating period and egg-laying period are distinct and separate events, with oviposition occurring as females and males begin movements deeper into the spring. From the peak of male abundance within springhouses in September to the beginning of oviposition in November, male abundance saw an 88% decrease, whereas females only decreased 39% from peak abundance. These data suggests that during this time period, males made a directed movement to overwintering sites, whereas females lingered as they searched for oviposition sites. The oviposition sites varied, however, among springhouses. In some springhouses, oviposition was at the spring effluence in visible localities, whereas in other springhouse oviposition occurred out of sight presumably closer to where females overwintered. Movement of females

associated with oviposition was best supported by observations in RCC Springhouse. At this springhouse, most females oviposited in plain sight, and therefore I was able to document behaviors leading up to oviposition. In mid to late October, activity and abundance of males within springhouses decreased suggesting an end to the mating period. However, for females, activity shifted from the walls inside the springhouse to the water compartment. During this time, multiple gravid females were observed actively swimming in and out of gaps between rocks of walls of the water compartment and crawling among submerged debris, presumably actively searching for oviposition sites. This behavior was initiated 1-2 weeks before the first eggs were laid. This shift in activity of females to aquatic sites was also observed in other springhouses.

### **Overwintering**

During the winter months (December through March), eastern long-tailed salamanders typically moved deeper within the spring to overwinter. In most springhouses in most years, eastern long-tailed salamanders became sparse to entirely absent during this time. The few individuals that remained near the spring surface inside springhouses typically resided deep within narrow cracks and crevices and were typically not active on wall surfaces or in the water. Although activity was reduced during this time, some activity did occur such as moving around and oviposition. In MR Springhouse during winter of 2006, many salamanders failed to retreat to deeper terrestrial chambers. As humidity levels dropped within the springhouse, salamanders receded to just above the water line along the walls of the contained spring compartment, where humidity levels were high enough to keep the walls moist. This evidence suggests that overwintering sites deeper within the spring were non-submerged subterranean chambers and crevices with high humidity. One possibility for salamanders failing to



move to deeper subterranean sites at MR could be that chambers typically utilized deeper underground were flooded or inaccessible that year. Presence and accessibility of non-flooded portions of the spring suitable for overwintering is likely a determining factor in long tailed salamander distributions.

### **Foraging**

I documented an increase in salamander abundance inside springhouses during April and May as salamanders moved from underground retreats to outside terrestrial habitats. I observed no differences in movement between sexes or age class during this period and abundances did not peak as high as in the fall. This suggests that most salamanders made faster and directed movements through the springhouse compared to the fall. Also individuals may have exited underground retreats via fossorial routes that bypassed the spring surface within the springhouse and therefore were not available for capture. By June, eastern long-tailed salamanders were nearly to entirely absent from within springhouses. Those that were captured inside springhouses during this time may have been temporarily trapped in their underground retreat, or may have gotten lost on the way out.

I observed eastern long-tailed salamanders first appearing in terrestrial habitats outside springhouses in late April, which was similar to first emergence of eastern long-tailed salamanders observed in New Jersey (Anderson and Martino 1966). However, in Illinois, McDowell and Shepard (2003) documented a longer seasonal surface activity beginning late February. At WCC in some years, I documented eastern long-tailed salamanders under rocks at the spring effluence throughout the winter.

Dispersal from springhouses occurred in all directions. Salamanders typically dispersed via a combination of terrestrial and subterranean routes as opposed to

swimming down or following the surface stream. I have observed eastern long-tailed salamanders dispersing from under the springhouse door and through cracks and crevices of the outside walls of springhouses. If stone retaining walls were within the vicinity or structurally connected to springhouses, eastern long-tailed salamanders utilize these structures as subterranean corridors for dispersal. Interestingly, Anderson and Martino (1966) noted that during spring emergence in April, eastern long-tailed salamanders were found farther away from the water than any other time of the year, also suggesting subterranean routes for emergence.

Although I did not track terrestrial movements, observations and mark-recapture data suggests that adult eastern long-tailed salamanders remain within the vicinity (<50 m) of springhouses throughout the summer. I did document a decrease in abundance at some spring houses as the season progressed. This decrease may be related to individuals dispersing farther away from the springhouse, or becoming less active due to drier terrestrial conditions. Most often I found eastern long-tailed salamanders associated with microhabitats that provided subterranean daytime retreats, such as the springhouse foundation, retaining walls, large rock piles, and at the effluence of buried drain pipes throughout the summer. At sites where these structures were absent, I found very few eastern long-tailed salamanders. At these sites, eastern long-tailed salamanders could be found beneath rocks and logs in May when salamanders were actively dispersing from the springhouse. However, in later parts of the season, eastern long-tailed salamanders did not utilize these same cover objects, and I failed to locate any surface activity. These observations differed from Anderson and Martino (1966) who documented a movement towards and an association with the water's edge. Anderson and Martino (1966) also frequently found eastern long-tailed salamander adults throughout the summer. The unique habitat of their study sites may explain these differences.

## Population Dynamics

Trends in the capture data indicate that eastern long-tailed salamanders are most often encountered inside springhouses and during the breeding season, which accounted for nearly 80% of the adults captured at each springhouse. Captures outside of springhouses and during the non-breeding season were potentially biased because I visited springhouses less frequently in the summer when salamanders were primarily outside springhouses. However, this time period was short and only represented a decrease in capture effort from every two weeks to every three weeks. Had I maintained a capture effort of every two weeks during this time period, I would have added one additional capture event, thus this difference most likely would have had little effect on the observed trend.

Abundance of males and females inside springhouses and during the breeding season accounted for nearly 90% of the captured population at most springhouses, whereas abundance of juveniles and individuals of unknown sex was lower inside springhouses and more evenly distributed between the breeding and non-breeding seasons. These differences reflect the differences in movement patterns observed. For example, the high percent abundance of males and females observed supports the observation that the majority of the sexually active individuals congregated inside springhouses for reproduction. The lower percent abundance inside springhouses and even distribution between seasons for juveniles and unknown sex individuals supports the observation that these groups remained outside springhouses longer and made more directed movements through the springhouses to overwintering sites. Differences among study sites within sex and age class were likely related to habitat structure both inside and outside springhouses thus affecting movements and habitat selection. For example, at CC abundance inside the springhouse accounted for only 52% of females captured. CC was

the only springhouse that had a non-insulated roof and an open window. These characteristics may have created less favorable conditions inside the springhouse relative to other springhouses causing salamanders to move deeper within the springhouse wall where they were unavailable for capture. In MR Springhouse, 90-100% of males, females, unknown sex, and juveniles were captured inside. One hypothesis for this large percentage of individuals captured inside springhouses may be that the cracks and crevices in the springhouse wall were not as deep as in other houses, keeping salamanders closer to the exposed surfaces. Likewise deeper portions of the spring may have been difficult for salamanders to access, resulting in salamanders remaining in the upper portions of the spring longer. This hypothesis was also supported by observations in 2006 in MR Springhouse when some salamanders overwintered in the springhouse at the surface of the water compartment rather than moving deeper underground. MR also had low abundance of captures outside springhouses, with only 4% of adults and 12% of juveniles found. Exact reasons for this apparent disappearance are unclear. MR was the only springhouse that was primarily surrounded by mature forest. Although I turned rocks, logs, and debris and searched the forest floor during humid nights within the vicinity of the springhouse, I was only able to locate a few individuals early in the spring. Perhaps salamanders dispersed farther away from this springhouse than the area that I searched, or salamanders took refuge under the leaf litter rather than the larger debris. Finally, low abundance of juveniles was documented at DC and RCC inside springhouses. Perhaps at these sites the majority of juveniles accessed overwintering sites deeper in the spring by bypassing the springhouse.

## **Age and Sex Composition**

Age composition among study sites and years was variable with mean percent juveniles ranging from 11-35% per study site. Differences in age composition among years likely resulted from changes in population size and reproductive success. For example, at the extreme, WCC had 72% juveniles in 2005-2006 but only 4% juveniles the following year. This swing in age composition was compounded by an increase in the number of young adults in 2006-2007 due to the high juvenile abundance in 2005-2006 and no observed reproduction in 2006. Of the three juveniles captured in 2006-2007, two measured 4.3 and 4.4 cm SVL, which suggests they may have been second year individuals. The third juvenile measured only 3.3 cm SVL, a size that indicates it was less than 1 year old. This lone individual suggests that at least some eggs were laid the previous year even though no eggs or larvae could be found. However, it is possible that this individual originated from eggs laid in a spring farther downstream. Yet another possibility is that this individual may have overwintered as a larva.

Sex composition varied among seasons and location. Overall, percent abundance of captures during the breeding season and captures inside springhouses had the highest proportion of males and females and lowest proportion of unknown sex compared to captures during the non-breeding season and captures outside springhouse. This trend reflects the reproductive cycle of eastern long-tailed salamanders, which are sexually active during the fall and congregate inside springhouses. It is during the breeding season that secondary sexual characteristics are most pronounced, and therefore the easiest time of year to sex individuals. It should be noted, that sex determination for these compiled data are not independent by season or location, meaning if an individual lacking secondary sexual characteristics was captured on a previous or subsequent capture event when sex could be determined, that individual was treated as a known sex

during data analysis. This method provided the most accurate estimate of sex composition by reducing the proportion of unknown sex even though sex may have been unknown for an individual at time of capture.

Among breeding season captures, unknown sex represented between 10-34 % of the adult ( $SVL \geq 4.5$  cm) population. Although some of these individuals may have been mature males or females that I did not accurately sex during time of capture, the majority of the unknown sex group was likely comprised of immature males and females that were larger than the minimum body size I used to designate adult individuals. This may have been due in part to inaccurate measurements of SVL but could also be a result of variable growth and age at maturity. For example, while I estimated that most individuals took two years to reach an  $SVL \geq 4.5$  cm, some individuals may have reached this length within their first year of life. Eastern long-tailed salamanders also exhibit sexual size dimorphism with females being the larger sex; therefore, females may not reach sexual maturity until a larger body size. Since unknown sex individuals were never recaptured as a known sex, the sex composition of this group is unclear. Interestingly, however, unknown sex individuals had a very low annual recapture rate ranging from 0.02-0.10 per study sites. It seems unlikely that differential mortality can explain the observed low recapture rate because the recapture rate for individuals first captured as juveniles regardless of sex ranged from 0.13-0.28. Alternatively, the low recapture rate for unknown sex may be evidence for dispersal.

### **Sex Ratios**

Sex ratio was variable among study sites and in most cases was male biased during the breeding season. Among salamanders, male biased sex ratios have been documented at breeding sites of aquatic breeding species (Semlitsch 1985, Sexton et al.

1990) as well as in surface populations of terrestrial species during the breeding season (Sayler 1966, Semlitsch 1980, Petranka 1998). Breeding-site male biases may be due to differential movement between sexes or reproductive output of females. For example, in pond breeding salamanders, males congregate at breeding sites and remain during the entire breeding period whereas females spend a shorter period time at breeding sites and leave after mating and oviposition, which may skew breeding period sex ratios to male bias (Semlitsch 1985, Sexton et al. 1990). In some species, the energy required for reproductive output in females leads to hyper-annual reproductive cycles, in which females do not breed every year. Since not all of the females are present at the breeding site in any one year, sex ratios may appear male biased. Among terrestrial species, male biased sex ratios have also resulted from differential movement between sexes and reproductive output of females. For example, in red-backed salamanders, females produce clutches every 2-3 years and lay their eggs in subterranean sites (Sayler 1966). Since red-backed salamanders employ parental care and females remain with the eggs throughout the incubation period, approximately 33-50% of the female population may be underground during a typical year and unavailable for capture, thus resulting in male biased sex ratios (Sayler 1966).

Differential movements to breeding sites and reproductive output of females were likely not major factor influencing sex ratios at most of my study sites. Although I limited calculations of sex ratio to the breeding season, these data were not limited to the breeding site (i.e., inside springhouses), but rather included captures inside and outside of springhouses, therefore reducing any bias associated with differential movements associated with reproduction. Additionally, the entry of eastern long-tailed salamanders into springhouses was not only for breeding, but movements were also associated with overwintering. Although it is unknown whether eastern long-tailed salamanders exhibit

hyper-annual cycles, capture data suggests it is uncommon. Also, considering I captured >95% of the total female population during the breeding season for all springhouses except at CC, and >95% of the male population during the breeding season for all springhouses except CC, RC, and RCC over the course of the study, biases related to differential movement or capture between sexes seems unlikely. At RC and RCC, I captured fewer males (~85%) than females during the breeding season, so sex ratio calculations for these sites were likely conservative estimates. At CC, however, I only captured 62% of females during the breeding season and 80% of males. These differences in capture between sexes suggest that the highly skewed sex ratio of 10 males per female at this springhouse was over-estimated.

Other factors influencing sex ratios within populations may be related to differential survival and differential age of maturity between sexes. For example, increased movement rates associated with acquiring energy for reproduction in females can lead to lower survival rates and male biased sex ratios especially in habitats with anthropogenic land-use. Differential age at maturity can also affect sex ratios with bias associated with the earlier maturing sex. Anderson and Martino (1966) suggested sexual maturity was reached for male eastern long-tailed salamanders at a SVL >4.3 cm and for females at a SVL >4.6 cm at an age of 2 years. However, I documented most males showed secondary sexual characteristics at approximately >4.4 cm SVL, whereas gravid females measured >5.0 cm. This discrepancy in size at maturity may suggest that most females matured at 3 years of age at my study sites, assuming growth rates are equal between sexes.

Therefore, differential survival or age at maturity between sexes may be an important factor influencing sex ratios at my study sites. Although I did not estimate survival rates, mean annual recapture rates suggested differential survival occurred at



some study sites. Mean annual recapture rates did not differ between males and females at DC, PR, and MR. This suggests that differential age at maturity may have had a greater influence on the male biased sex ratios at these sites, whereas at RC mean annual recapture rates for males were two times greater than females, suggesting differential survival may have also played a role in the male biased sex ratio. However, inferences as to why these recaptures rates differed between sexes at this study site are speculative. Habitat within a 100-m radius of the springhouse for the most part experienced low anthropogenic disturbance; however, the majority of the habitat consisted of tall grasses with forest comprising only about 4% of the total area. The lack of forest may have resulted in reduced activity due to low soil moisture retention. If females had higher energy demands associated with reproduction than males, a decrease in soil moisture have had had a greater effect on females leading to differential survival at this site.

I documented an even sex ratio at WCC in all seasons and at RCC during two seasons. Interestingly, at RCC and WCC, females had a higher mean annual recapture rate compared to males suggesting males had a lower survival rate than females or males had a higher emigration rate than females. However, I could not detect any trends in the data to suggest why males might have a lower annual recapture rate than females for these study sites.

### **Population Trends**

Overall trends in population estimates showed lower abundances of males and females during the non-breeding season compared to the breeding season, and these estimates reflected my observations associated with seasonal movements. Eastern long-tailed salamanders had higher abundance inside springhouses during the breeding season because they congregated inside springhouses for reproduction, whereas during the non-

breeding season salamanders made faster movements through springhouses to terrestrial sites. Therefore, non-breeding season abundances likely do not reflect a decrease in population size due to overwintering mortality, but more likely was associated with reduced capture probability due to sampling design (i.e., capture events were not frequent enough to adequately sample the population during this time period).

I estimated stable population trends at RC, PR, and WCC, which suggests that survival rates were stable during this time period. This also suggests eastern long-tailed salamanders may exhibit fidelity to their breeding sites. However, I did not document occupied springs adjacent to any of my study sites, and if eastern long-tailed salamanders do not disperse far from springs while foraging during summer months, returning to the same site may be a result of need rather than choice.

I documented an approximate 50% decline in abundance at MR over a 4-year period. Reasons for this decline are unclear. During the non-breeding season in 2006 a change within the spring caused salamanders to alter their overwintering behavior and overwinter within the springhouse compartment. I observed a decline in health in salamanders during this time period with many salamanders becoming emaciated, but no deaths were observed. Although this may have been a factor in the decrease in abundance the following fall, the population decline was detected each year of the study. Additionally, I documented variable water flow at other springhouses with no detectible decrease in abundance at other springhouses. For example, at WCC, water level was reduced and water flow from the adjacent connected springhead stopped during the 2004-2005 activity cycle, but no negative effects were observed.

At RCC, I documented a 265% increase in population abundance of male and female eastern long-tailed salamanders over 4 years, with the most marked increases occurring in 2005 and 2006. I hypothesize that this increase is a result of the egg laying

“fences” I placed inside the water compartment of the springhouse during oviposition. I specifically placed these fences to increase the surface area for oviposition. The increased surface area likely resulted in eggs being laid more spaced out than in previous years. Considering I documented fungus on unfertilized eggs could infect and kill adjacent developing eggs, and eastern long-tailed salamanders appeared to lay eggs in orientations that maximize oxygen availability, the effect of the oviposition fences may have resulted in an increase in egg survival. The increase in population size occurring 2 years after I added the egg-laying fences further supports my hypothesis.

Considering the above data documenting eastern long-tailed salamander activity patterns, I suggest future studies interested in investigating eastern long-tailed salamander demographics focus capture efforts during the breeding season inside springhouses. Captures outside springhouses were too infrequent and would require a very intensive effort in the absence of springhouses. Likewise movements of eastern long-tailed salamanders during the non-breeding season affected capture probability as salamanders were not present inside springhouses for long during this period. Employing Pollock’s robust design may be the best approach for estimating population abundances of eastern long-tailed salamanders inside springhouses during the breeding season because it provides more flexibility over open population models (Bailey et al. 2004).

Table 1 Starting and ending dates of capture events (*n*) for the 7 principal study sites in a mark-recapture study of eastern long-tailed salamanders (*Eurycea longicuada longicauda*).

Study Site	1 <sup>st</sup> event	last event	<i>n</i>
Crum Creek	3 Apr 2005	9 Jul 2007	48
Darby Creek	30 Oct 2003	10 Jul 2007	75
Ridley Creek	29 Dec 2003	10 Jul 2007	72
Pigeon Run	30 Oct 2003	9 Jul 2007	74
Middle Run	5 Jan 2003	8 Jul 2007	97
Red Clay Creek	4 Nov 2002	8 Jul 2007	100
White Clay Creek	13 Dec 2002	8 Jul 2007	89

Table 2 Pooled abundance of gravid eastern long-tailed salamanders (*Eurycea longicauda longicauda*) observed by month at 7 springhouses from 2003-2007. Condition was determined by viewing ova through ventral wall of females and was categorized as ‘Complete’: oviducts full of mature ova; ‘Partial’: oviducts partially spent containing less than approximately 50% of mature ova; and ‘Developing’: oviducts with ova small in size. Multiple captures of same individuals per month with same condition were excluded.

Condition	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
Complete	201	542	398	157	30	13	8	10	14	1	1	15
Partial	0	0	4	31	10	3	3	0	0	1	0	0
Developing	2	5	0	0	0	0	0	1	3	2	0	14

Table 3 Site, date, snout-vent length (SVL), and number of ova from dissected gravid female eastern long-tailed salamanders (*Eurycea longicauda longicauda*) found deceased in springhouses.

Site	Date	SVL (cm)	Ova Count
Crum Creek	12 Oct 2006	5.2	57
Ridley Creek	16 Jan 2004	5.3	69
Ridley Creek	6 Feb 2004	5.4	66
Red Clay Creek	25 Sep 2006	5.4	57
Middle Run	7 Nov 2005	5.5	59
Middle Run	7 Nov 2005	5.8	29 <sup>a</sup>
Red Clay Creek	unknown	6.0	86
Ridley Creek	16 Jan 2004	6.3	120
Unknown	unknown	6.3	92

<sup>a</sup>Some eggs already laid.

Table 4      Highest count of eggs and larval eastern long-tailed salamanders (*Eurycea longicauda longicauda*) observed inside each springhouse per year. In some years, total larval abundance was not recorded, although presence (P) was observed.

Site	Eggs/Larvae			
	2003-04	2004-05	2005-06	2006-07
Crum Creek	--	--	0/32	0/P
Darby Creek	73/250	12/352	60/P	136/P
Ridley Creek	54/P	2/P	365/P	158/P
Pigeon Run	1/153	0/64	0/143	0/238
Middle Run	0/P	0/P	0/P	0/P
Red Clay Creek	1599/P	1732/270	1673/P	2103/654
White Clay Creek	166/9	82/35	0/0	346/19

Table 5      Percent abundance for adult and juvenile eastern long-tailed salamanders (*Eurycea longicauda longicauda*) captured per study site by location (inside vs outside springhouses) and season (breeding season vs non-breeding season).

Age/Springhouse	Location		Season	
	In	Out	Breeding <sup>a</sup>	Non-breeding <sup>b</sup>
Adults (SVL $\geq 4.5$ cm)				
Crum Creek	77.5	30.6	60.6	52.2
Darby Creek	68.4	38.7	83.3	24.3
Ridley Creek	82.9	24.5	74.9	38.3
Pigeon Run	91.2	14.4	94.7	11.6
Middle Run	99.3	3.7	94.3	32.5
Red Clay Creek	78.4	31.6	83.1	35.5
White Clay Creek	92.1	30.7	92.1	47.1
Juveniles (SVL $\leq 4.4$ cm)				
Crum Creek	73.8	28.7	49.2	55.4
Darby Creek	32.7	69.1	54.1	50.5
Ridley Creek	65.9	38.3	35.9	69.2
Pigeon Run	68.5	34.3	72.0	35.7
Middle Run	89.4	12.0	60.9	45.9
Red Clay Creek	16.7	85.5	57.5	46.1
White Clay Creek	92.5	25.1	96.0	43.2

<sup>a</sup>15 July through 30 November

<sup>b</sup>1 December through 14 July



Table 6 Percent abundance of eastern long-tailed salamanders (*Eurycea longicauda longicauda*) captured per study site by location (inside vs outside springhouses) and season (breeding season vs non-breeding season) for all adult (SVL  $\geq 4.5$  cm) male, female, and unknown sex individuals.

Sex/Springhouse	Location		Season	
	In	Out	Breeding <sup>a</sup>	Non-breeding <sup>b</sup>
Males				
Crum Creek	88.6	21.9	80.2	38.0
Darby Creek	86.7	22.2	95.2	14.9
Ridley Creek	89.1	20.8	88.6	28.7
Pigeon Run	95.2	11.7	97.9	9.2
Middle Run	99.7	4.0	97.9	29.7
Red Clay Creek	80.2	30.4	85.7	34.6
White Clay Creek	87.5	33.3	97.9	35.4
Females				
Crum Creek	52.4	54.8	61.9	45.2
Darby Creek	75.7	35.7	95.7	11.4
Ridley Creek	89.8	16.8	98.7	15.5
Pigeon Run	92.3	14.4	100.0	8.1
Middle Run	100.0	1.8	98.5	34.5
Red Clay Creek	92.3	20.8	96.9	29.2
White Clay Creek	96.2	41.5	98.1	52.8
Unknown Sex				
Crum Creek	64.7	38.6	29.8	74.4
Darby Creek	46.6	58.1	67.1	37.6
Ridley Creek	65.0	37.5	30.5	73.3
Pigeon Run	80.0	21.0	81.0	21.5
Middle Run	97.4	4.9	79.2	37.7
Red Clay Creek	59.5	45.7	56.9	50.0
White Clay Creek	92.5	12.5	77.5	52.2

<sup>a</sup>15 July through 30 November

<sup>b</sup>1 December through 14 July

Table 7 Chi square statistics comparing age composition and sex ratio of eastern long-tailed salamanders (*Eurycea longicauda longicauda*) among years at each study site. Asterisks denotes significance at  $P \leq 0.05$ .

	df	$\chi^2$	P-value
Age Composition			
Crum Creek	1	47.68	<0.001*
Darby Creek	2	12.19	0.002*
Ridley Creek	2	217.00	<0.001*
Pigeon Run	2	28.49	<0.001*
Middle Run	3	92.72	<0.001*
Red Clay Creek	3	10.33	0.016*
White Clay Creek	3	117.47	<0.001*
Sex Ratio			
Crum Creek	1	<0.01	0.951
Darby Creek	2	1.95	0.377
Ridley Creek	2	4.87	0.087
Pigeon Run	2	3.59	0.166
Middle Run	3	45.20	<0.001*
Red Clay Creek	3	9.91	0.019*
White Clay Creek	3	4.35	0.226

Table 8      Percent abundance of adult eastern long-tailed salamanders (*Eurycea longicauda longicauda*) captured per study site by sex (male, female, unknown sex) for all individuals captured and all individuals captured inside vs outsides springhouses and during the breeding and non-breeding seasons for each study site.

Loc/Springhouse	M	F	Unknown
All Captures			
Crum Creek	56.1	7.3	37.2
Darby Creek	45.0	12.7	42.5
Ridley Creek	57.3	16.3	26.4
Pigeon Run	54.9	23.4	21.6
Middle Run	57.5	21.9	21.1
Red Clay Creek	47.0	28.1	25.1
White Clay Creek	34.3	37.9	28.6
Inside Captures			
Crum Creek	64.1	4.9	31.0
Darby Creek	57.0	14.1	28.9
Ridley Creek	61.6	17.7	20.7
Pigeon Run	57.3	23.7	19.0
Middle Run	57.7	22.1	20.7
Red Clay Creek	48.1	33.1	19.1
White Clay Creek	32.6	39.5	28.7
Outside Captures			
Crum Creek	40.1	13.0	46.9
Darby Creek	25.8	11.7	63.8
Ridley Creek	48.6	11.2	40.3
Pigeon Run	44.9	23.5	31.6
Middle Run	61.7	10.6	27.7
Red Clay Creek	45.2	18.5	36.3
White Clay Creek	37.2	51.2	11.6

Table 8. Cont.

Loc/Springhouse	M	F	Unknown
Breeding <sup>a</sup> Captures			
Crum Creek	74.3	7.4	18.3
Darby Creek	51.4	14.6	34.3
Ridley Creek	67.7	21.5	10.8
Pigeon Run	56.7	24.7	18.5
Middle Run	59.7	22.9	17.8
Red Clay Creek	48.4	32.8	17.2
White Clay Creek	36.4	40.3	24.0
Non-breeding <sup>b</sup> Captures			
Crum Creek	40.7	6.3	53.0
Darby Creek	27.8	6.0	66.2
Ridley Creek	42.9	6.6	50.5
Pigeon Run	43.6	16.4	40.0
Middle Run	52.5	23.3	24.5
Red Clay Creek	45.7	23.2	35.4
White Clay Creek	25.8	42.4	31.8

<sup>a</sup>15 July through 30 November

<sup>b</sup>1 December through 14 July

Table 9 Within year and overall capture sex ratio of eastern long-tailed salamanders (*Eurycea longicauda longicauda*) adults captured during the breeding season (15 July through 30 November) for each study site. Differences from parity were testing using a Chi square analysis at  $P \leq 0.05$ .

	M:F Ratio	df	$\chi^2$	P-value
Crum Creek				
2005-06	10.4	1	100.57	<0.001
2006-07	10.6	1	111.81	<0.001
Overall	10.0	1	191.46	<0.001
Darby Creek				
2004-05	4.9	1	31.11	<0.001
2005-06	2.9	1	30.51	<0.001
2006-07	3.2	1	39.34	<0.001
Overall	3.2	2	86.32	<0.001
Ridley Creek				
2004-05	4.2	1	205.92	<0.001
2005-06	3.2	1	135.43	<0.001
2006-07	3.1	1	93.53	<0.001
Overall	3.2	2	333.70	<0.001
Pigeon Run				
2004-05	2.0	1	30.12	<0.001
2005-06	2.9	1	72.85	<0.001
2006-07	2.4	1	51.60	<0.001
Overall	2.3	2	112.05	<0.001
Middle Run				
2003-04	2.0	1	30.68	<0.001
2004-05	3.9	1	149.90	<0.001
2005-06	2.0	1	48.82	<0.001
2006-07	5.5	1	135.32	<0.001
Overall	2.6 <sup>†</sup>	3	190.22	<0.001

Table 9. Cont.

	M:F Ratio	df	$\chi^2$	<i>P</i> -value
Red Clay Creek				
2003-04	1.1	1	0.33	0.564
2004-05	0.9	1	0.17	0.680
2005-06	1.6	1	4.26	0.039
2006-07	1.7	1	12.62	<0.001
Overall	1.5 <sup>a</sup>	3	11.19	<0.001
White Clay Creek				
2003-04	0.6	1	1.14	0.285
2004-05	0.6	1	2.13	0.144
2005-06	0.6	1	3.34	0.069
2006-07	1.3	1	0.61	0.435
Overall	0.9	3	0.51	0.477

<sup>a</sup>Annual sex ratios differed among years determined by a Chi-squared analysis at  $P \leq 0.05$ .

Table 10 Mean annual recapture rate by sex for adult eastern long-tailed salamanders (*Eurycea longicauda longicauda*) captured per study site for all captures and captures inside springhouses during the breeding season.

Springhouse	Adults	Male	Female	Unknown
All Captures				
Crum Creek	0.22	0.27	0.19	0.10
Darby Creek	0.12	0.20	0.19	0.02
Ridley Creek	0.27	0.35	0.18	0.08
Pigeon Run	0.22	0.27	0.25	0.04
Middle Run	0.36	0.42	0.43	0.09
Red Clay Creek	0.31	0.33	0.44	0.03
White Clay Creek	0.32	0.34	0.48	0.09
Inside & Breeding Season <sup>a</sup>				
Crum Creek	0.14	0.16	0.07	0.00
Darby Creek	0.09	0.14	0.09	0.00
Ridley Creek	0.21	0.27	0.06	0.04
Pigeon Run	0.20	0.25	0.18	0.02
Middle Run	0.34	0.41	0.36	0.06
Red Clay Creek	0.36	0.39	0.41	0.01
White Clay Creek	0.23	0.21	0.39	0.00

<sup>a</sup>15 July through 30 November

Table 11 Proportion of eastern long-tailed salamanders (*Eurycea longicauda longicauda*) first captured as juveniles (i.e.,  $\leq 4.4$  cm SVL; age approximately 1 year) and first captured as adults (i.e.,  $\geq 4.5$  SVL; unknown age) recaptured at least 2, 3, and 4 years as determined by capture histories at Crum Creek (CC), Darby Creek (DC), Ridley Creek (RC), Pigeon Run (PR), Middle Run (MR), Red Clay Creek (RCC), and White Clay Creek (WCC) springhouses.

	CC	DC	RC	PR	MR	RCC	WCC
<u>Individuals first captured as juveniles</u>							
2 years	0.18	0.13	0.15	0.28	0.28	0.20	0.18
3 years	-	0.02	0.06	0.03	0.07	0.05	0.06
4 years	-	-	-	-	0.02	0.00	0.00
<u>Individuals first captured as adults</u>							
2 years	0.20	0.15	0.17	0.22	0.44	0.41	0.40
3 years	-	0.01	0.05	0.04	0.14	0.15	0.10
4 years	-	-	-	-	0.02	0.03	0.00



Table 12 Candidate models of the Jolly-Seber model for estimating seasonal combined male and female abundance of eastern long-tailed salamanders (*Eurycea longicauda longicauda*) at Ridley Creek (RC), Pigeon Run (PR), Middle Run (MR), Red Clay Creek (RCC), and White Clay Creek (WCC) springhouses<sup>a</sup>. For each model, I provided AICc values,  $\Delta$ AICc (i.e., the difference in AICc between the given model and the best fit model), AICc weight (i.e., the certainty that the given model is the best model for the data), and number of parameters. Subscripts indicate variable over time ( $t$ ) and constant over time ( $\bullet$ ) for the model parameters of survival ( $\phi$ ), recapture probability ( $p$ ), and probability of entrance ( $b$ ).

Site & Model	AICc	$\Delta$ AICc	Weight	Parameters
RC				
$\phi(\bullet) p(t) b(t)$	2144.40	0.00	1.000	11
$\phi(t) p(t) b(t)$	2179.96	35.56	0.000	12
PR				
$\phi(t) p(t) b(t)$	925.56	0.00	0.828	14
$\phi(\bullet) p(t) b(t)$	928.71	3.15	0.172	11
MR				
$\phi(t) p(t) b(t)$	3255.31	0.00	0.833	19
$\phi(\bullet) p(t) b(t)$	3258.52	3.21	0.167	14
RCC				
$\phi(t) p(t) b(t)$	912.29	0.00	0.923	18
$\phi(\bullet) p(t) b(t)$	917.25	4.96	0.077	14
WCC				
$\phi(\bullet) p(t) b(t)$	210.44	0.00	0.988	13
$\phi(t) p(t) b(t)$	219.23	8.79	0.012	19

<sup>a</sup>Crum Creek Springhouse was excluded due to too few capture events, and Darby Creek Springhouse was excluded due to poor fit of the model.



Figure 5 Eastern long-tailed salamander (*Eurycea longicauda longicauda*) spermatophore photographed at on a rock ledge at Red Clay Creek (RCC) Springhouse on 25 September 2005 shortly after deposition in a failed mating attempt.

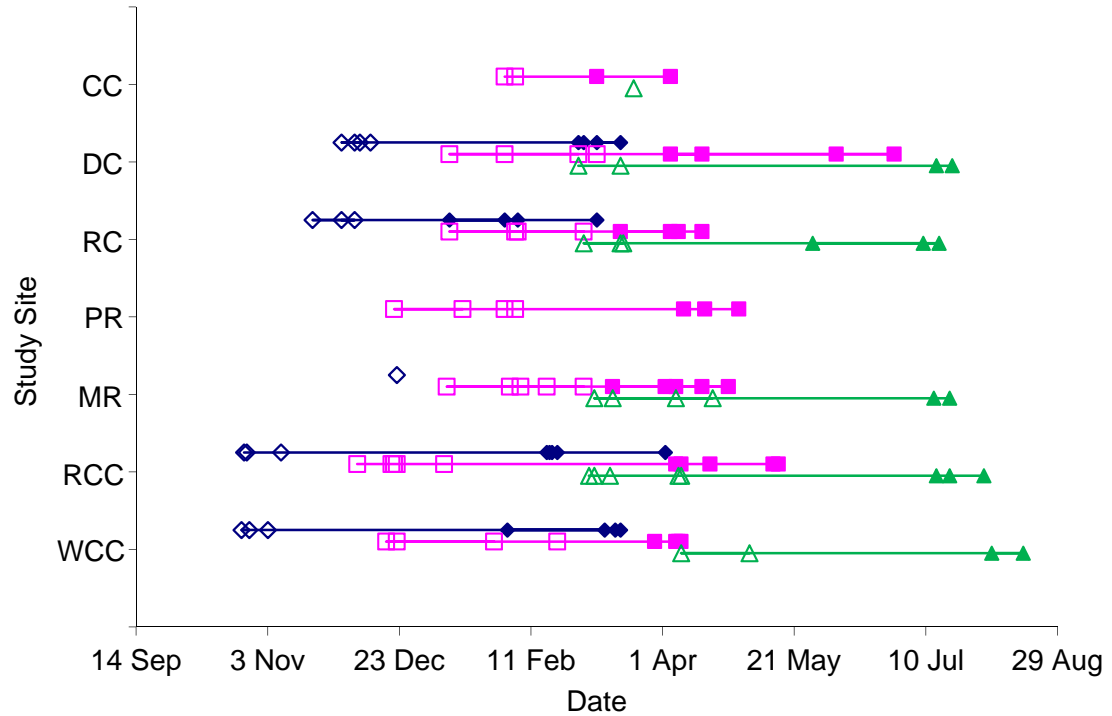


Figure 6      Earliest (open) and latest (closed) range of dates for eastern long-tailed salamander (*Eurycea longicauda longicauda*) eggs observed (blue), larvae observed inside springhouses (pink), and larvae observed in outside stream (green) for the 7 principal study sites 2003-2007.

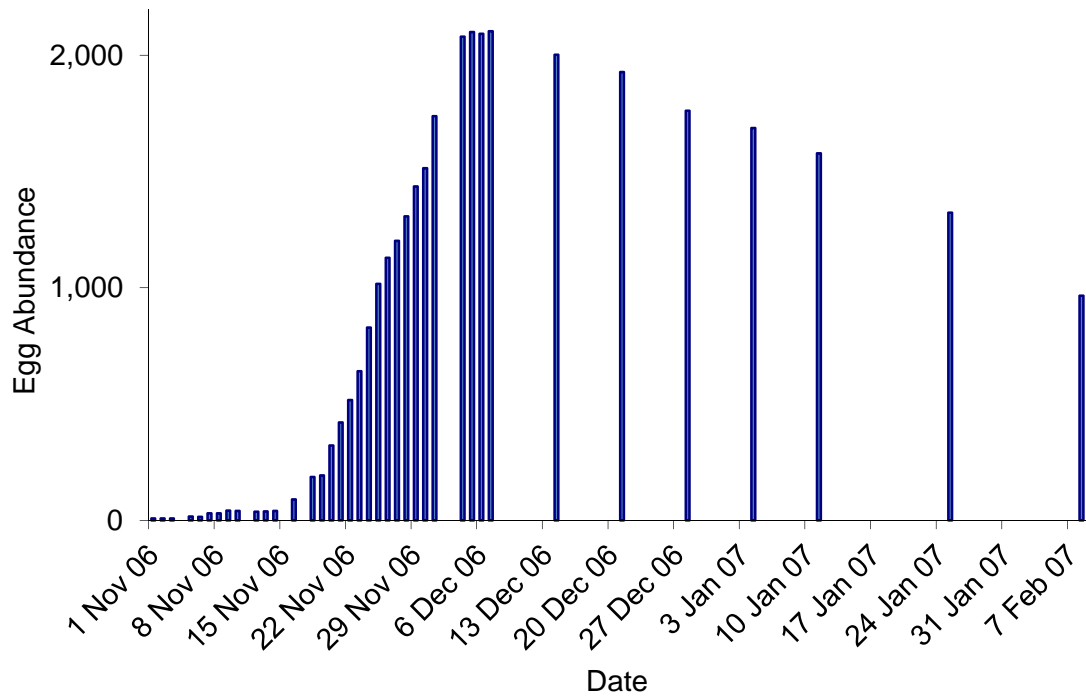


Figure 7 Total eastern long-tailed salamander (*Eurycea longicauda longicauda*) egg counts by date observed from within Red Clay Creek (RCC) Springhouse from November 2006 through February 2007.

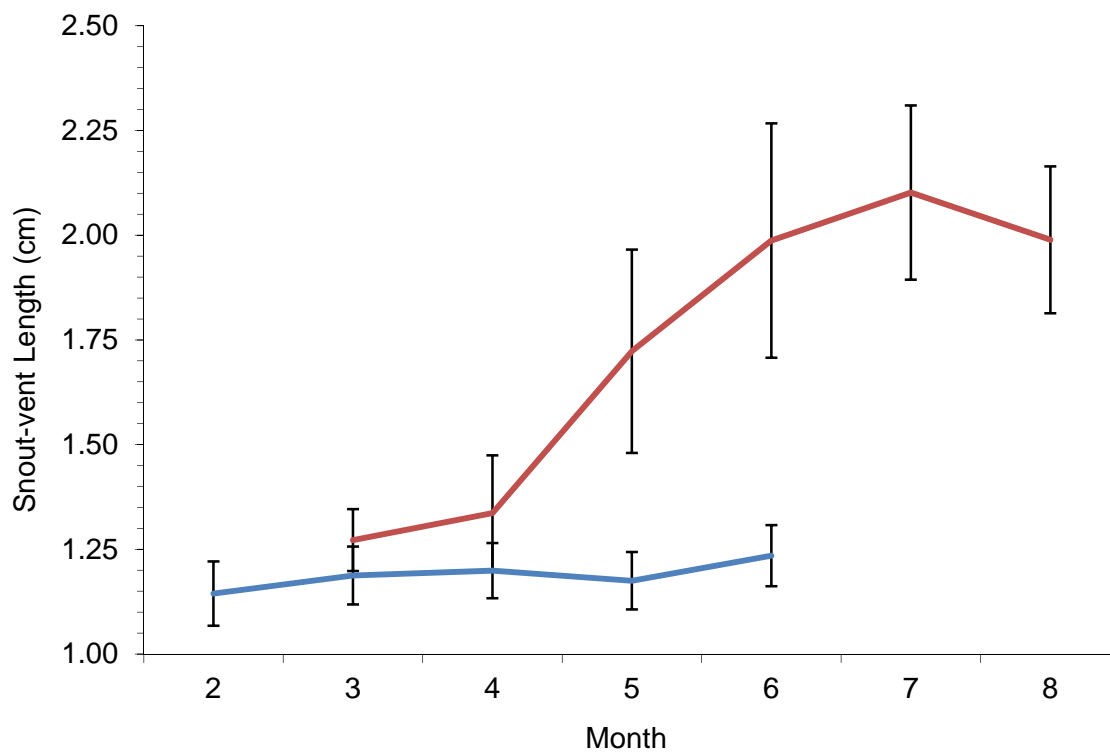


Figure 8 Mean snout-vent length ( $\pm$ SE) by month (February=2 to August=8) of eastern long-tailed salamander (*Eurycea longicauda longicauda*) larvae collected inside (blue) and outside (red) springhouses 2004-2006.



Figure 9 Partial neotenic eastern long-tailed salamander (*Eurycea longicauda longicauda*) captured at Ridley Creek (RC) Springhouse on 7 April 2004 (a and b) and after metamorphosis on 4 June 2004 (c).

## Captures Inside Springhouses

## Captures Outside Springhouses

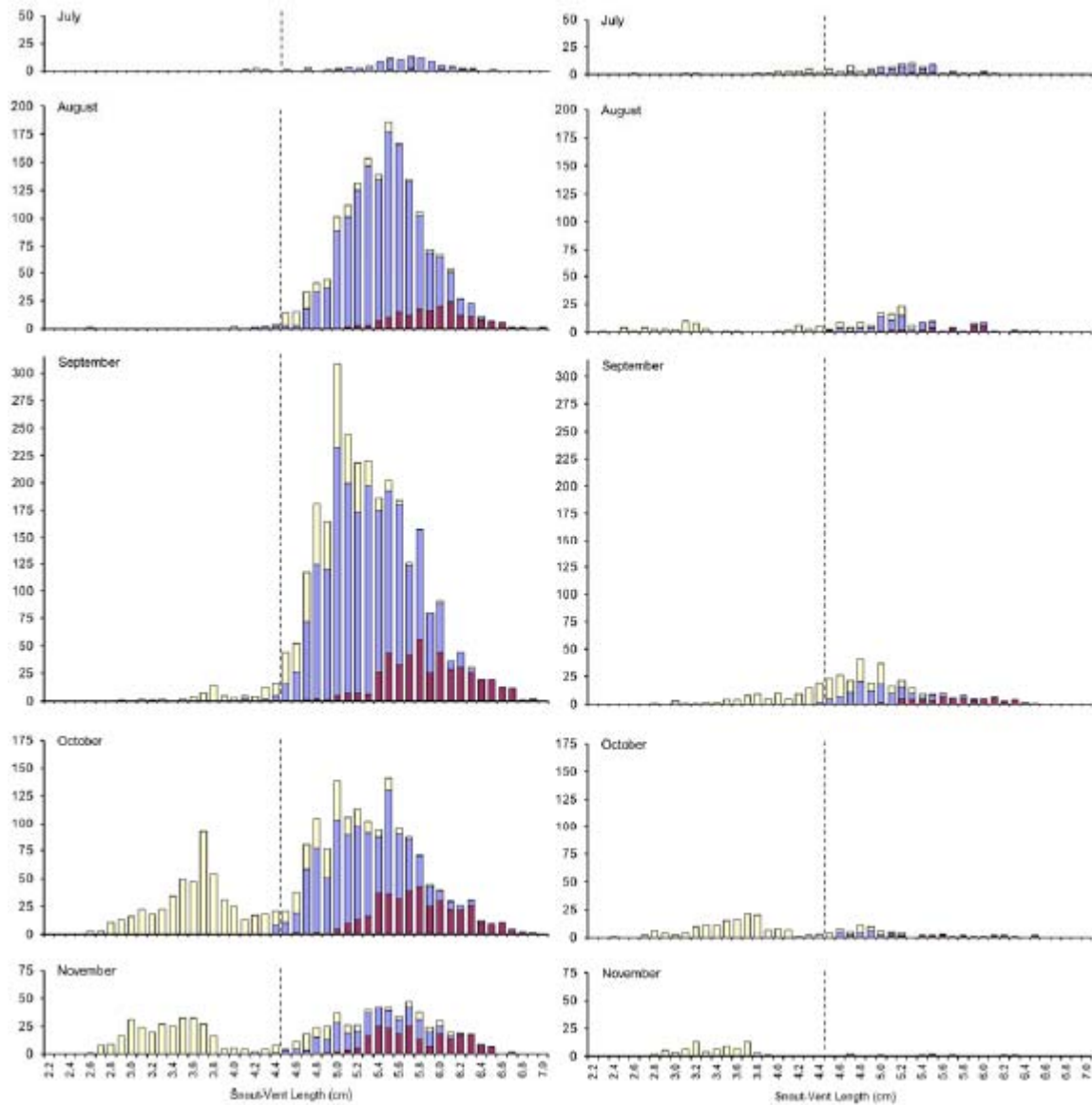


Figure 10 Total eastern long-tailed salamander (*Eurycea longicauda longicauda*) capture abundances for males (blue), females (red) and unknown sex (yellow) inside and outside of springhouses from all study sites ( $n = 7$ ) and years (2003-2007) by snout-vent length (cm) during the reproductive activity period (July 15 – November 31). Dotted line delineates juveniles (SVL < 4.5 cm) from adults (SVL  $\geq$  4.5 cm)

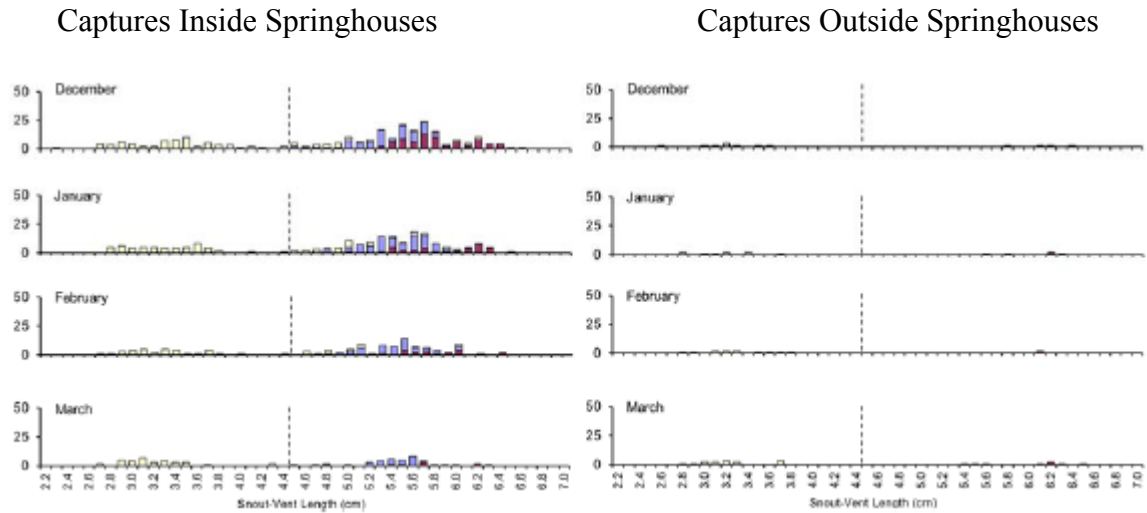


Figure 11 Total eastern long-tailed salamander (*Eurycea longicauda longicauda*) capture abundances for males (blue), females (red) and unknown sex (yellow) inside and outside of springhouses from all study sites ( $n = 7$ ) and years (2003-2007) by snout-vent length (cm) during the over-wintering activity period (December 1 – March 31). Dotted line delineates juveniles (SVL < 4.5 cm) from adults (SVL  $\geq$  4.5 cm).



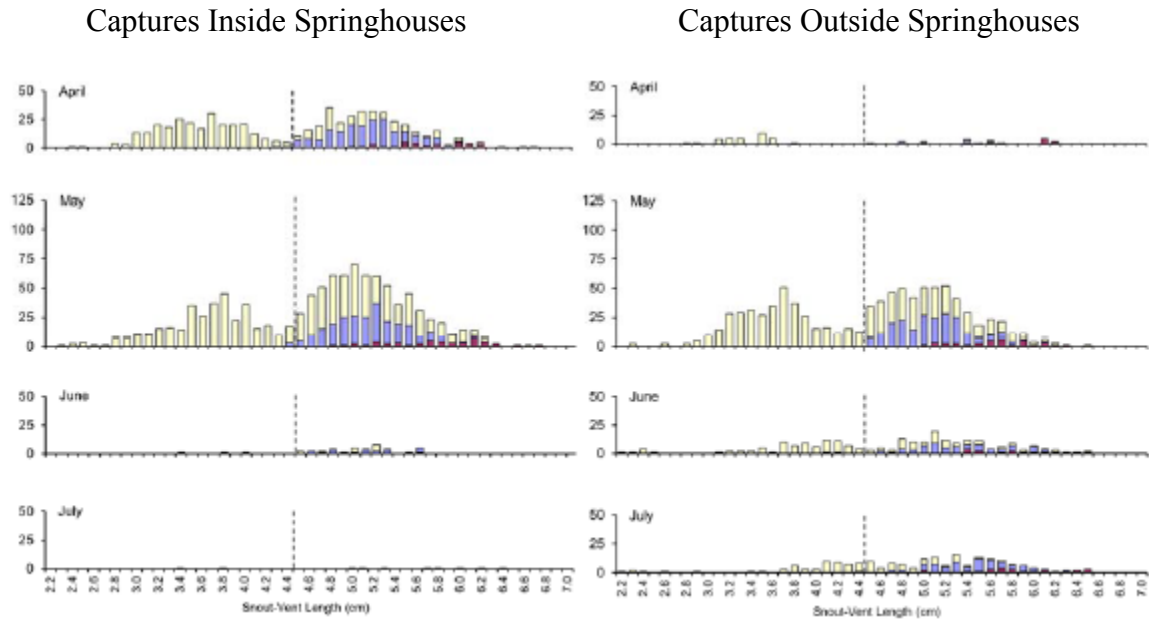


Figure 12 Total eastern long-tailed salamander (*Eurycea longicauda longicauda*) capture abundances for males (blue), females (red) and unknown sex (yellow) inside and outside of springhouses from all study sites ( $n = 7$ ) and years (2003-2007) by snout-vent length (cm) during the foraging activity period (April 1 – July 14). Dotted line delineates juveniles (SVL < 4.5 cm) from adults (SVL  $\geq$  4.5 cm)

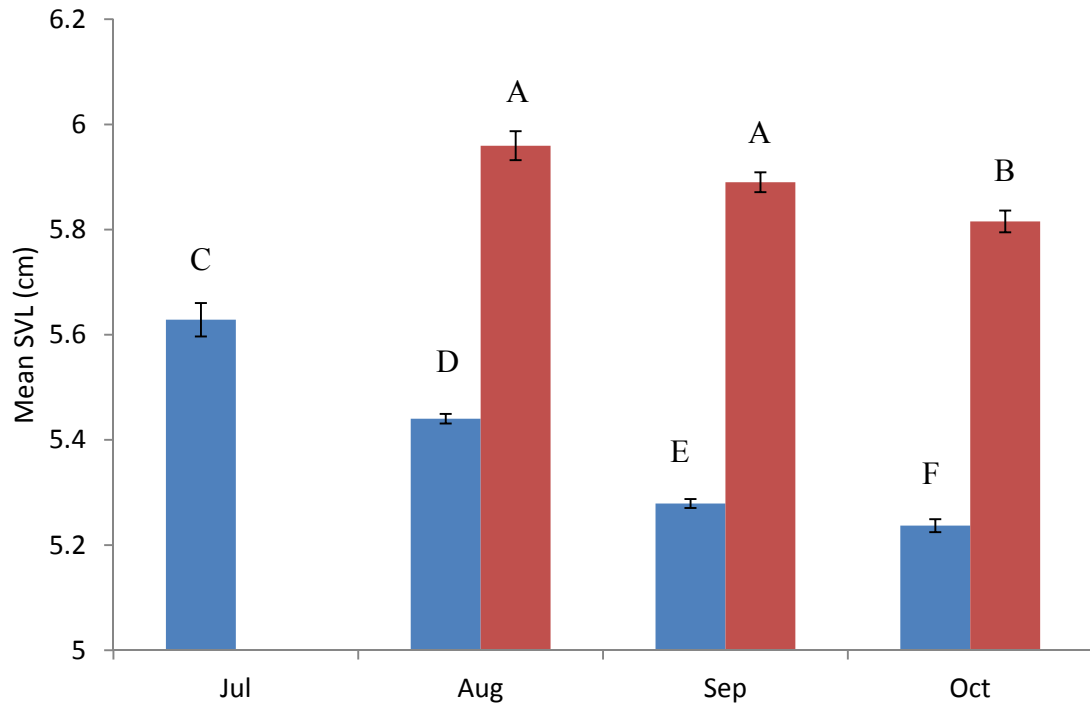


Figure 13 Mean snout-vent length (SVL) ( $\pm$ SE) of eastern long-tailed salamanders (*Eurycea longicauda longicauda*) for males (blue) and females (red) captured by month inside springhouses. Means with the same letter do not differ ( $P \leq 0.05$ ).

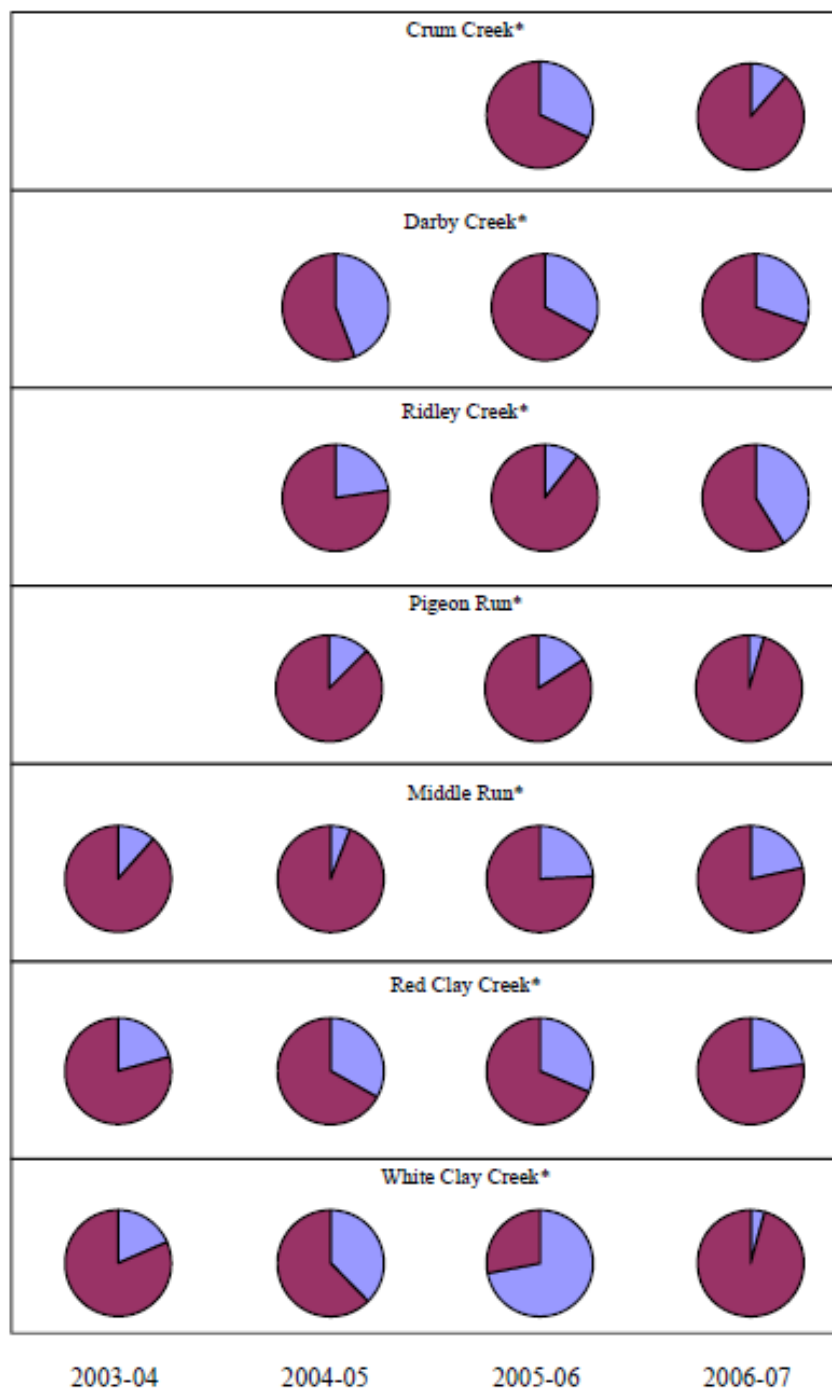


Figure 14 Percent abundance of juvenile (blue) and adult (red) eastern long-tailed salamanders (*Eurycea longicauda longicauda*) captured per year at each springhouse. Asterisks denote differences among year determined by a Chi square analysis ( $P \leq 0.05$ ).

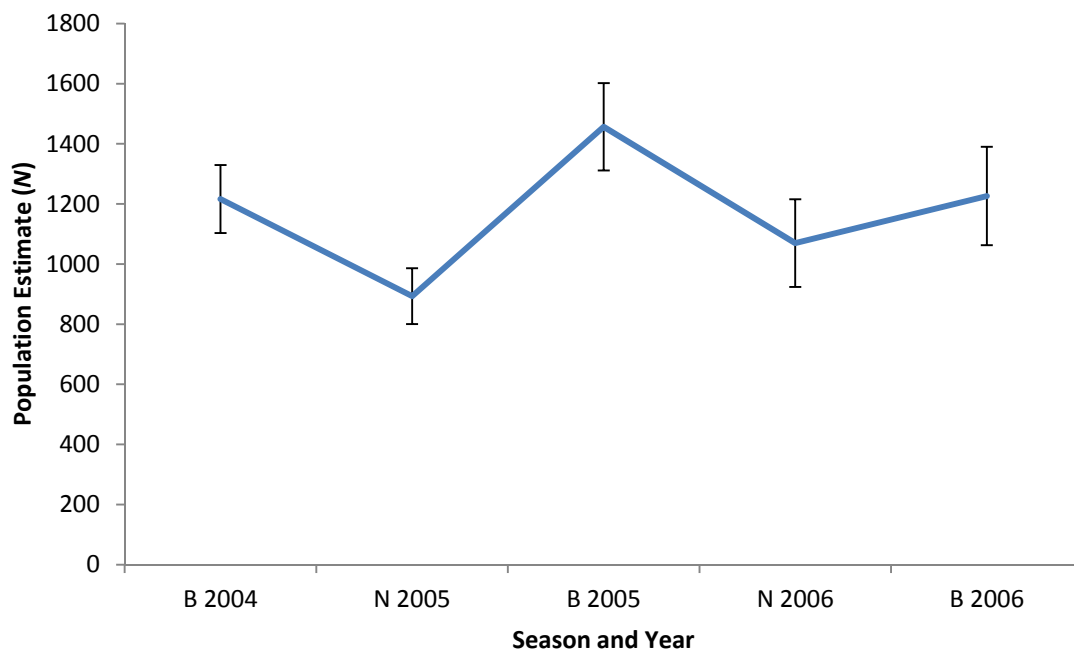


Figure 15 Derived population estimates ( $\pm$ SE) of adult eastern long-tailed salamanders (*Eurycea longicauda longicauda*) for Ridley Creek (RC) Springhouse for the breeding (B) and non-breeding (N) seasons for 2005-2006 using the POPAN extension of the Jolly-Seber model in program MARK.

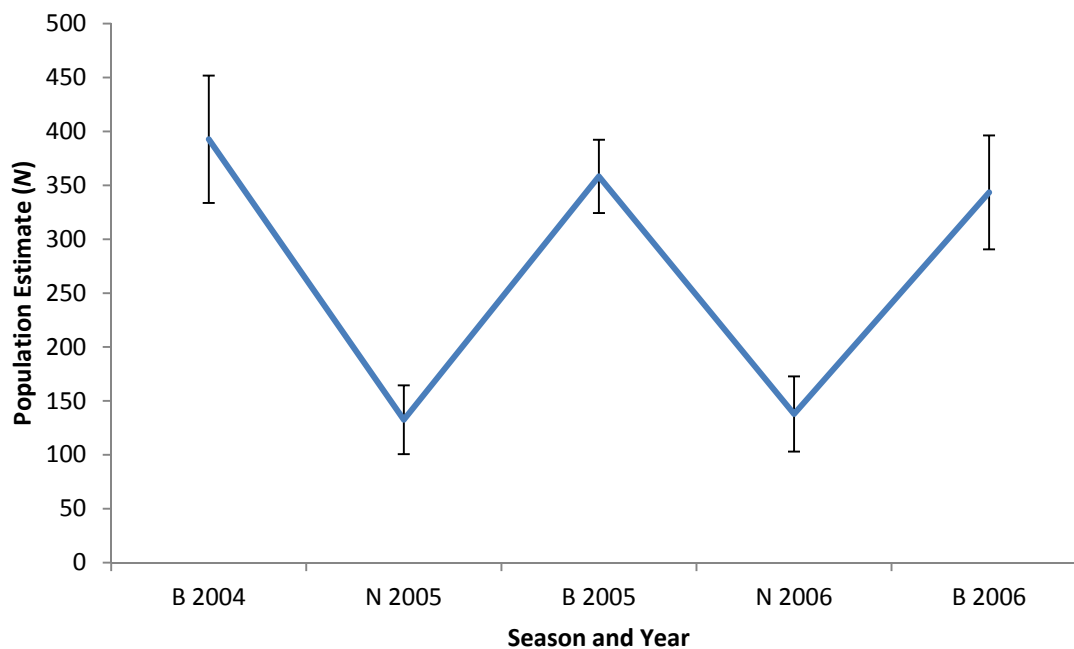


Figure 16 Derived population estimates ( $\pm$ SE) of adult eastern long-tailed salamanders (*Eurycea longicauda longicauda*) for Pigeon Run (PR) Springhouse for the breeding (B) and non-breeding (N) seasons for 2005-2006 using the POPAN extension of the Jolly-Seber model in program MARK.

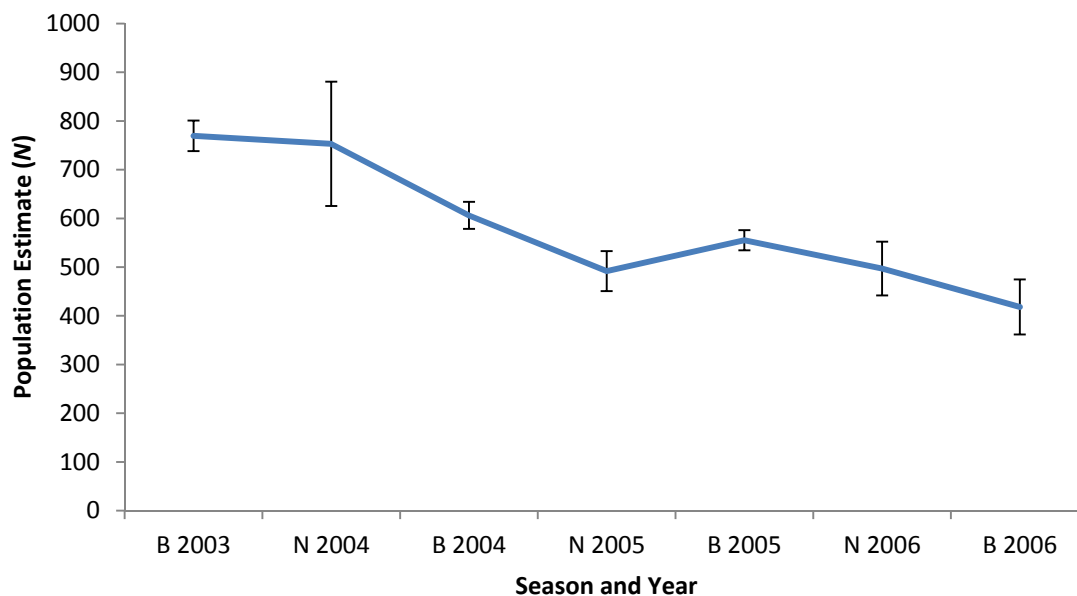


Figure 17 Derived population estimates ( $\pm$ SE) of adult eastern long-tailed salamanders (*Eurycea longicauda longicauda*) for Middle Run (MR) Springhouse for the breeding (B) and non-breeding (N) seasons for 2004-2006 using the POPAN extension of the Jolly-Seber model in program MARK.

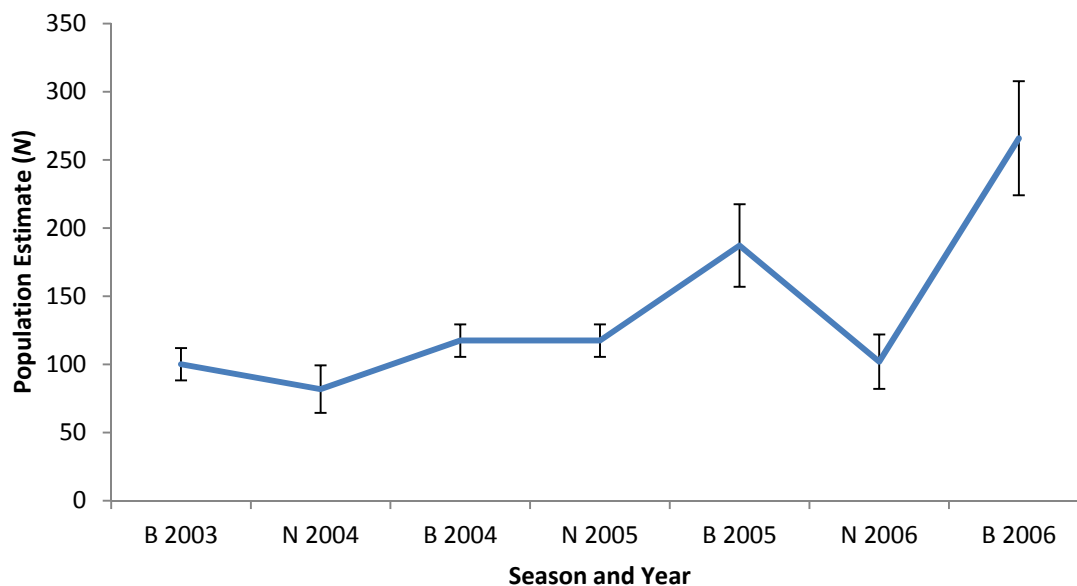


Figure 18 Derived population estimates ( $\pm$ SE) of adult eastern long-tailed salamanders (*Eurycea longicauda longicauda*) for Red Clay Creek (RCC) Springhouse for the breeding (B) and non-breeding (N) seasons for 2004-2006 using the POPAN extension of the Jolly-Seber model in program MARK.

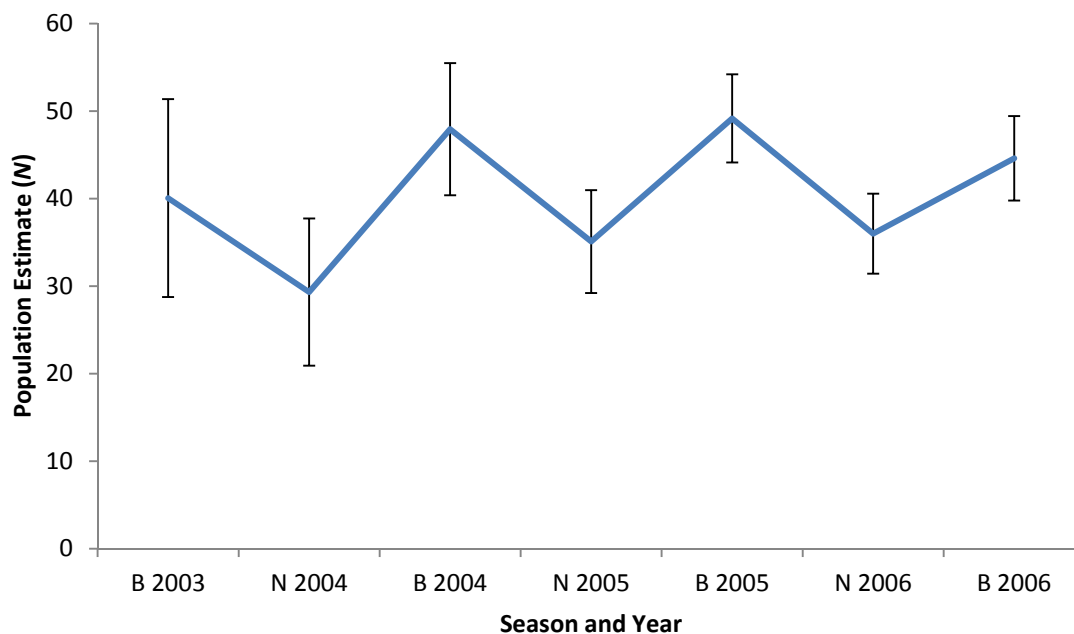


Figure 19 Derived population estimates ( $\pm$ SE) of adult eastern long-tailed salamanders (*Eurycea longicauda longicauda*) for White Clay Creek (WCC) Springhouse for the breeding (B) and non-breeding (N) seasons for 2004-2006 using the POPAN extension of the Jolly-Seber model in program MARK.



### **Chapter 3**

## **FACTORS INFLUENCING SITE OCCUPANCY OF EASTERN LONG-TAILED SALAMANDERS (*EURYCEA LONGICAUDA LONGICAUDA*) IN AN ANTHROPOGENIC LANDSCAPE**

### **Introduction**

Species distributions are influenced by a multitude of biotic and abiotic factors that interact on the landscape and local scales (Ricklefs 1987). Landscape factors are related to the quantity and connectivity of habitats. These factors primarily affect dispersal (Saunders et al. 1991) and can influence metapopulation structure (Levins 1969). Local factors are related to the quality of habitats and affect the reproduction and survival of populations (Pulliam 1988). Understanding how these factors interact across multiple spatial scales to affect distribution patterns is an important component to species conservation (Cushman 2006, Marsh and Trenham 2001).

Numerous studies have investigated how quantity and connectivity of habitats influence amphibian distributions at the landscape scale. Site occupancy has been positively correlated with the amount of forest cover (Dupuis and Steventon 1999, Knutson et al. 1999, Houlahan et al. 2000, Guerry and Hunter 2002, Herrmann et al. 2005, Trenham and Shaffer 2005) and number of breeding sites present (Joly et al. 2001) and negatively correlated with urban development (Delis et al. 1996, Knutson et al. 1999) and road density (Carr and Fahrig 2000). These factors may influence metapopulation structure through fragmentation and isolation by impacting immigration and emigration rates among populations. For example, vagile species, which have high dispersal rates, may be most susceptible to negative landscape scale effects (Carr and Fahrig 2000).

Local scale factors may play a more important role in determining site occupancy (Marsh and Trenham 2001). Site occupancy has been correlated to microhabitat conditions of terrestrial habitats (deMaynadier and Hunter 1995, Dillard et al. 2008), juxtaposition and distance of upland habitat to breeding sites (Lann and Verboon 1990, Porej et al. 2004, Guerry and Hunter 2002), and water quality of breeding sites (Sanzo and Hecnar 2006). Thus, for less vagile amphibians, such as salamanders, degradation of local environments may have a stronger influence on population dynamics due to direct impacts on survival and reproduction as opposed to movements among populations.

Anthropogenic changes to the landscape through land-use can negatively impact amphibians at local and landscape scales by reducing the quality, quantity, and ecological connectivity of habitats. Land-use alters natural habitats and leads to fragmentation and isolation of terrestrial habitat patches and may affect quality of patches or aquatic breeding sites. For example, agricultural lands and urban development result in increased runoff, which can alter hydrology and water chemistry. Aquatic habitats in agricultural settings are subject to increases in water temperature, sediment loads, and nutrient inputs due to runoff (Schoonover et al. 2006). Increases in impervious surface cover due to urbanization leads to elevated storm flow intensity, which increases erosion and contributes non-point source nutrient loads and chemical pollutants to aquatic habitats (Paul and Meyer 2001, Davis et al. 2003, Tufford et al. 2003).

Amphibians that are particularly susceptible to the effects of land use are species that occupy multiple habitat types to complete their life cycle (e.g., pond breeding amphibians). Such species regularly move between non-breeding terrestrial habitats and aquatic breeding sites, therefore making them susceptible to more environmental impacts than species occupying a single habitat (e.g., terrestrial breeding species). While trends in distribution of amphibians may be correlated with multiple factors of landscape including

amount, type, and arrangement of surrounding habitats, as well as amount, degree, age, and duration of urbanization and anthropogenic disturbances (Rinehart et al. 2009), interactions of these variables occur across multiple scales and are species-specific (Cushman 2006). Likewise, species-environment relationships may differ across spatial scales: a relationship important at one scale may not be important at a smaller or larger scale (Cushman 2006). Additionally species-environment relationships differ among species. For example, Guerry and Hunter (2002) investigated pond occupancy in relation to forest distance in 9 species of amphibians and found that 5 species showed a positive association whereas 2 species showed a negative association with forest and pond occupancy. Therefore, in order to obtain species specific inferences and apply appropriate conservation strategies, researchers must adopt multi-scale approaches that are biologically relevant to the species investigated (Cushman 2006).

The goal of this study was to assess the relative importance of landscape and local factors on site occupancy of eastern long-tailed salamander (*Eurycea longicauda longicauda*) populations. Eastern long-tailed salamanders are biphasic and require two discrete habitats to complete their life cycle: they occupy spring seeps and associated underground passages for reproduction and overwintering, and during the summer months, they occupy upland terrestrial sites. Dispersal and movement within and among terrestrial habitats is poorly understood. I developed models related to quality, quantity, and connectivity of habitats based on landscape and local factors that influence amphibian occurrence and the biology of eastern long-tailed salamanders. Since, on the piedmont of the mid-Atlantic region, springhouses have been constructed over many springheads that eastern long-tailed salamanders potentially occupy (N. Nazdrowicz, personal observation), I focused on springhouses as study sites. I compared springhouses with eastern long-tailed salamander populations (hereafter referred to as occupied

springhouses) to springhouses where eastern long-tailed salamanders were absent (hereafter referred to as unoccupied springhouses) to elucidate local and landscape factors that might affect this species' distribution.

## **Methods**

### **Study Area and Site Selection**

I investigated site occupancy of eastern long-tailed salamanders in springhouses in northern New Castle County, Delaware, and eastern Chester County and western Delaware County, Pennsylvania. This region lied within the piedmont physiographic province, which was underlain mostly by dense, impermeable bedrock. The lithology was primarily composed of metamorphic rock and all springhouses occurred upon schist or gneiss rocks. The hydrology was driven by crystalline-rock aquifer systems recharged through precipitation, which yielded water primarily through fractures in the bedrock (Trapp and Horn 1997). The landscape in the region was dominated by a mix of low to medium density residential land use, agriculture, and forest.

Prior to this investigation, I had identified 11 occupied springhouses. These springhouses served as the basis for study area formation and selection of unoccupied springhouses. I defined my study area as the area formed by a 5-km radius extended around each occupied springhouse, which formed 6 discontinuous or nearly discontinuous subareas (Figure 20). From within these subareas, I conducted a thorough search of springhouses in order to randomly select unoccupied springhouses. During this process (described below), I identified an additional 7 occupied springhouses, from which I redefined my study area boundary.

Within the defined study area, I searched for springhouses primarily by visual surveys from 2006-2008. To locate springhouses visually, I surveyed from roadsides,

while driving during winter months (November-March). Winter was ideal for visual searches because leaves were absent from trees and visibility of the landscape from the road was greatest. I conducted roadside searches in a systematic process to ensure all roads within the study area were surveyed. When a potential springhouse was observed, I marked an approximate location from the road with a GPS and noted direction from the road to the springhouse. Later, I adjusted the marked locations to that of the springhouse using aerial photographs. Since not all springhouses were viewable from the road due to topography and visual obstructions, I contacted park managers and private property owners of large land tracts to find additional springhouse locations. For northern New Castle Co., Delaware, I also referenced Cupp (2007), which mapped 120 springhouse locations.

After I surveyed for springhouses within the study area, I randomly sorted all identified springhouses ( $n = 651$ ) by subarea for field investigation and site selection in 2008. I visited the first randomly selected springhouses within each subarea to identify unoccupied springhouses that were similar in structure to occupied springhouses. While occupied springhouses were variable in structure, the basic criteria included a perennial spring contained in a water compartment and enclosed on all sides by stone or cement walls and a complete roof with flow to a surface stream. The other criteria for an unoccupied springhouse was no evidence of eastern long-tailed salamander reproduction. I used presence of larvae as a determination of reproduction, since eggs were usually laid out of sight and not usually observed in springhouses (N. Nazdrowicz, personal observation). I considered a springhouse unoccupied if larvae were not observed during February or March (i.e., after eggs typically hatch) after a minimum of 2 visits. Springhouses in which I observed adult eastern long-tailed salamanders but no evidence of reproduction were excluded. If I could not gain access to a springhouse (e.g., property

owner not home, permission denied) or if the springhouse did not meet the criteria, I visited the next selected springhouse on the randomly generated list until I identified approximately equal number of unoccupied to occupied springhouses per subarea (Figure 20). I excluded springhouses that fell within 1 km of occupied springhouses to eliminate overlap of landscape attributes.

### Multiple Spatial Scales

I used a multi-scale approach to investigate the influence of surrounding landscape on site occupancy. For the local scale, I selected 100-m radius from the springhouses center to assess habitat area attributes. This distance was biologically significant because it incorporated the core habitat use of adults (Anderson and Martino 1966) and was twice the mean distance larvae have been observed within surface streams from spring effluences (Rudolf 1978). For the landscape scale, I assessed habitat variables at 500 m and 1,000 m from springhouse centers. These distances were chosen to allow for comparisons among similar studies.

### Local-scale Assessment

At the local scale, I measured abiotic variables of habitat and water chemistry that could potentially impact survival and reproduction of eastern long-tailed salamanders. Since eastern long-tailed salamanders reproduce within springs, I limited my investigation of water quality to groundwater constituents. I assessed water chemistry during hatching and early larval development periods of eastern long-tailed salamanders, which occurred during two 3-week periods from 30 January-17 February and 12 March-1 April in 2009. For determination of water chemistry, I used an ExStik II EC500 pH/Conductivity Meter (Extech Instruments Corp., Waltham, Mass, USA) to record pH, salinity (ppm), total dissolved solids (mg/L), and specific conductance (conductivity,

$\mu\text{S}/\text{cm}$ ) within each springhouse in water collected into a sample cup. Each week before use, I calibrated the pH/conductivity meter for pH with a 7.00-pH standard buffer solution and for conductivity with a 1,413- $\mu\text{S}$  conductivity calibration solution. I used a Traceable Digital Oxygen Meter (Model 06-662-66; Control Company, Friendswood, TX, USA) to record dissolved oxygen ( $\text{mg}/\text{L}$ ) by placing a probe in the water inside each springhouse. I calibrated the DO meter to oxygen percentage of the air prior to each use. I also collected sample water from within each springhouse to test for nitrates ( $\text{NO}_3$ ), phosphates ( $\text{PO}_4$ ), iron, and coliform bacteria in the lab. I stored collected water in a cooler and refrigerator prior to analysis, which occurred within 24 hrs of collection. I used LaMotte Test Kits (LaMotte Company, Chestertown, MD, USA) and provided calculations to determine ppm nitrates (Model NCR, Code 3110), ppm phosphate (Model VM-12, Code 4408), ppm iron (Model P-61, Code 4447), and presence/absence of coliform bacteria (Code 5850). Since I did not detect the presence of ppm phosphate or ppm iron during the first period of sampling, I did not collect data for phosphate or iron in the spring sample and excluded these variables from the analysis. I averaged water chemistry data for the first and second sampling. Due to accessibility issues, I did not collect water chemistry data from 2 occupied and 2 unoccupied springhouses during the spring sampling period.

For habitat variables, I used ArcMap 10 (Esri Inc., Redlands, CA, USA) to calculate total area of low disturbance habitat and forest within a 100-m radius from the springhouse center, and measured distance to nearest road and distance to nearest forest from each springhouse. I considered habitat where vegetation was permitted to grow without disturbance (i.e., mowing, plowing, grazing) throughout the season as low disturbance habitat. These areas included forest cover, scrub/shrub, old-field, and open canopy freshwater habitats. High disturbance areas included agricultural fields,

rangeland, recreational fields and golf courses, residential and urbanized areas, highways, and industrialized lands. I digitized these habitat categories using 2002 digital orthoimagery for Delaware (pixel resolution = 0.09 m<sup>2</sup>; Delaware Office of State Planning Coordination and Office of Management and Budget 2003) and 2005 digital orthoimagery for Pennsylvania (pixel resolution = 0.09 m<sup>2</sup>; Delaware Valley Regional Planning Commission 2007).

### **Landscape-scale Assessment**

At the landscape scale, I investigated variables related to quantity and connectivity of habitats at 500-m and 1,000-m radii distances from the springhouse center. For habitat quantity, I digitized low disturbance habitat and forest habitat (see Local-scale Assessment). For connectivity of habitats, I quantified total road length and stream length at each spatial scale. Roads might affect occurrence because roads fragment habitats and are a source of direct mortality of amphibians (Hels and Buchwald 2001) or may be barriers to salamander movement (Marsh et al. 2004). Streams may play a role in metapopulation functioning because streams could be potential dispersal corridors and associated spring seeps represent potential breeding sites of eastern long-tailed salamanders. I calculated total road length using the “Delaware Roads” dataset (Tele Atlas®, July 2007, ed. 9.2) and the “Local Roads” and “State Roads” datasets for Pennsylvania (Pennsylvania Department of Transportation 2010, Harrisburg PA ed. 201001). I modified these datasets to exclude driveways of 3 or fewer residences and historic roads no longer in use. I calculated stream length using the National Hydrography Dataset (U.S. Geological Survey et al., 2005) for Delaware and Pennsylvania. I modified the stream layers to include visible first order streams on the orthoimagery layers excluded from the datasets.



## Data Analysis

I derived a set of candidate models of local and landscape-scale variables (Table 13) to evaluate occupancy of springhouses. I compared among four main models including a water chemistry model and a habitat model for each spatial scale (i.e., 100 m, 500 m, 1,000 m), as well as subsets of these models consisting of all possible combinations of variables. Prior to model testing, I used Pearson's correlation coefficient test to identify redundancy among variables within models, and I excluded variables with a Pearson's  $r \geq 0.70$  or  $\leq -0.70$ . I found total dissolved solids, conductivity, and salinity to be highly correlated (Table 2). Therefore, I only included salinity in the models because several studies have demonstrated a negative effect of salinity on amphibian survival (Turtle 2000, Christy and Dickman 2002, Chinathamby et al. 2006, Karraker and Gibbs 2011). I also found low disturbance area highly correlated with forest area at the local and landscape scales (Table 14 and Table 15). Since eastern long-tailed salamanders are typically associated with forest habitats (Petranka 1998), I retained forest area in the models. Reduction of variables due to correlation resulted in a set of 36 candidate models for analysis. The water chemistry models included pH, salinity, dissolved oxygen, and nitrates. The habitat models for the local scale included forest area, distance to nearest forest, and distance to nearest road and for the landscape scales the models included forest area, road length, and stream length.

I used logistic regression to determine the best model for predicting probability of occurrence of eastern long-tailed salamanders. I used Akaike's Information Criterion corrected for small sample size (AICc) to evaluate strength of support for each model (Burnham and Anderson 1998). I ranked models according to their AICc values, and the greatest support was given to the model with the lowest AICc value, although models with  $\Delta\text{AICc}$  values  $\leq 2$  had similar support (Burnham and Anderson 1998). To estimate

the relative importance of each individual parameter, I calculated Akaike weights ( $w$ ) and summed weights of parameters within models to determine the weight of evidence in favor of each model (Burnham and Anderson 1998). I used a t-test to compare mean values of individual parameters between occupied and unoccupied springhouses.

## **Results**

No models were strongly supported for predicting occupancy of eastern long-tailed salamanders (Table 16). Landscape level variables of forest area (FA) and stream length (SL) were generally associated with the models with the greatest support (Table 16). Overall, local scale models were not well supported (Table 16). The only local scale model with  $\Delta AICc < 2$  was forest distance (FD).

I found no difference in individual parameters of water chemistry or habitat variables at each spatial scale between occupied and unoccupied springhouses (Table 17 and Table 18). Forest area of occupied sites comprised as little as 0%, 17.7%, and 16.2% for 100-m, 500-m, and 1,000-m spatial scales, respectively.

## **Discussion**

Generally, models with forest area and stream length at the 500-m and 1,000-m spatial scales and forest distance at the 100-m spatial scale provided the best support for eastern long-tailed salamander occurrence. Road length when interacting with forest area and stream length was also supported. This suggests that aspects of habitat quantity and connectivity are an important component influencing eastern long-tailed salamander distributions. While I did not directly measure forest habitat isolation or connectivity, streams could be potential dispersal corridors for eastern long-tailed salamanders at the landscape scale. Additionally, while streams are not important habitats utilized by eastern long-tailed salamanders, stream length may be positively correlated with abundance of

springs. If so, a high abundance of springs, a habitat important to the life history of eastern long-tailed salamanders in the landscape, associated with site occupancy might implicate the potential for metapopulation structure in eastern long-tailed salamanders. Likewise forest habitat, forest distance, and road length may play a role in connectivity and metapopulation functioning because forest habitats are least impacted by anthropogenic land-use and therefore provide the safest habitat for dispersal among springs, whereas roads are a potential threat to increased mortality.

Forest area at the local scale, however, was not well supported in predicting occupancy. One occupied site contained no forest habitat within the 100-m scale and 7 occupied sites contained less than 20% forest area at the 100-m scale. This suggests that forest is not an important habitat requirement for the terrestrial phase of the eastern long-tailed salamander annual cycle. Rather, I noted eastern long-tailed salamanders in non-forested areas were associated with microhabitats that provided subterranean daytime retreats, such as the springhouse foundation, retaining walls, and rock piles. These habitat variables were not quantified in this study, but future investigations may focus on the importance of these microhabitats at the local scale.

I found no evidence among water chemistry models to explain eastern long-tailed salamander occurrence. This is not surprising, given the values for water quality constituents overlapped considerably between occupied and unoccupied springhouses. Springhouses were located in a region with similar rock types that have produced generally good quality ground water (Ludlow and Loper 2004). Although poor water quality has been shown to negatively affect site occupancy for amphibians in urban landscapes (Clinton and Vose 2006), my study sites were comprised mostly of suburban and rural landscape. Additionally, I measured ground water chemistry as opposed to surface water chemistry which can be influenced much more by non-point source nutrient

loads and chemical pollutants from the surrounding landscape (Paul and Meyer 2001, Davis et al. 2003, Tufford et al. 2003).

Based on observations from within springhouses, I hypothesize that at the local scale occurrence is likely related to habitat features that may be logistically difficult to quantify, specifically the subterranean structure of the spring. Eastern long-tailed salamanders occupy springs for greater than 50% of the year and utilize springs for reproduction and overwintering. Observations from within springhouses suggest that overwintering sites consist of subterranean non-flood chambers within the spring or near the spring effluence. If springs lack appropriate overwintering sites, eastern long-tailed salamanders likely cannot survive the winter months, and what may appear to be suitable habitat on the surface may not be suitable habitat below the surface. Not being able to quantify key habitat requirements at the local scale severely limits the implications of landscape level site occupancy from a conservation perspective.

Table 13 Local (100 m) and landscape (500 m and 1,000 m) variables used in logistic regression models to predict eastern long-tailed salamander (*Eurycea longicauda longicauda*) occupancy of springhouses located in Chester and Delaware Counties, Pennsylvania, and New Castle County, Delaware.

Variable	Abbreviation	Definition
<i>Local (100 m)</i>		
pH	pH	pH
Dissolved oxygen	DO	mg/L
Salinity	S	ppm
Total dissolved solids	TDS	mg/L
Conductivity	C	μS/cm
Nitrates	N	ppm
Forest distance	FD	distance (m) to nearest forest edge
Road distance	RD	distance (m) to nearest road
Low disturbance area	LA	total area (ha) of low disturbance habitat within 100-m buffer (including forest cover, scrub/shrub and early successional areas, and open canopy freshwater habitats)
Forest area	FA	total area (ha) of forested habitats
<i>Landscape (500 m &amp; 1,000 m)</i>		
Road length	RL	length (m) of roads within 500-m & 1,000-m buffers
Stream length	SL	length (m) of streams within 500-m & 1,000-m buffers
Low disturbance area	LA	total area (ha) within 500-m & 1,000-m buffers of forest cover, scrub/shrub and early successional areas, and open canopy freshwater habitats
Forest area	FA	total area (ha) of forested habitats within 500-m & 1,000-m buffers

Table 14 Pearson's correlation coefficients ( $r$ ) and  $P$ -values ( $P$ ) for local scale variables compared between occupied and unoccupied springhouses located in Chester and Delaware Counties, Pennsylvania, and New Castle County, Delaware. Asterisk denotes highly correlated variable with  $r \leq -0.70$  and  $\geq 0.70$ .

Var.	Stat.	pH	S	TDS	C	DO	N	FD	RD	FA	LA
pH	$r$	1.000	-0.136	-0.129	-0.128	0.157	-0.317	0.290	-0.371	-0.299	-0.264
	$P$		0.429	0.454	0.457	0.361	0.060	0.086	0.026	0.077	0.120
S <sup>a</sup>	$r$		1.000	0.998*	0.998*	-0.365	0.228	0.034	-0.055	0.020	-0.050
	$P$			<0.001	<0.001	0.029	0.180	0.843	0.751	0.910	0.771
TDS <sup>b</sup>	$r$			1.000	0.999*	-0.357	0.232	0.023	-0.050	0.038	-0.035
	$P$				<0.001	0.033	0.174	0.894	0.776	0.829	0.841
C <sup>c</sup>	$r$				1.000	-0.357	0.229	0.024	-0.051	0.039	-0.034
	$P$					0.033	0.180	0.892	0.768	0.824	0.845
DO <sup>d</sup>	$r$					1.000	0.141	0.250	-0.116	-0.068	-0.107
	$P$						0.414	0.142	0.502	0.694	0.537
N <sup>e</sup>	$r$						1.000	0.156	0.052	-0.006	-0.141
	$P$							0.364	0.764	0.972	0.413
FD <sup>f</sup>	$r$							1.000	0.023	-0.664	-0.646
	$P$								0.893	<0.001	<0.001
RD <sup>g</sup>	$r$								1.000	0.187	0.262
	$P$									0.276	0.122
FA <sup>h</sup>	$r$									1.000	0.904*
	$P$										<0.001
LA <sup>i</sup>	$r$										1.000

<sup>a</sup>Dissolved oxygen; <sup>b</sup>salinity; <sup>c</sup>total dissolved solids; <sup>d</sup>conductivity; <sup>e</sup>nitrate; <sup>f</sup>forest distance; <sup>g</sup>road distance; <sup>h</sup>forest area; <sup>i</sup>low disturbance area.

Table 15 Pearson's correlation coefficients ( $r$ ) and  $P$ -values ( $P$ ) for landscape scale variables compared between occupied and unoccupied springhouses located in Chester and Delaware Counties, Pennsylvania, and New Castle County, Delaware. Asterisk denotes highly correlated variable with  $r \leq -0.70$  and  $\geq 0.70$ .

Var.	Stat.	500 m				1,000 m			
		FA	LA	RL	SL	FA	LA	RL	SL
FA <sup>a</sup>	$r$	1.000	0.923*	-0.259	-0.129	1.000	0.949*	-0.347	0.183
	$P$		<0.001	0.127	0.455		<0.001	0.038	0.288
LA <sup>b</sup>	$r$		1.000	-0.259	-0.122		1.000	-0.385	0.247
	$P$			0.127	0.478			0.020	0.146
RL <sup>c</sup>	$r$			1.000	-0.200			1.000	-0.401
	$P$				0.241				0.015
SL <sup>d</sup>	$r$				1.000				1.000

<sup>a</sup>forest area; <sup>b</sup>low disturbance area; <sup>c</sup>road length; <sup>d</sup>stream length.

Table 16 Model selection results for occupancy of eastern long-tailed salamanders (*Eurycea longicauda longicauda*) in springhouses located in Chester and Delaware Counties, Pennsylvania, and New Castle County, Delaware.

Scale-Model <sup>a</sup>	AICc	ΔAICc	likelihood	weight
500-FA_SL	49.795	0.000	1.0000	0.109
1000-FA_SL	49.924	0.129	0.9375	0.103
1000-FA	50.300	0.505	0.7768	0.085
1000-SL	50.700	0.905	0.6360	0.070
500-FA_RL_SL	51.023	1.228	0.5411	0.059
500-FA	51.265	1.470	0.4795	0.052
100-FD	51.294	1.499	0.4726	0.052
500-SL	51.425	1.630	0.4426	0.048
1000-FA_RL_SL	51.743	1.948	0.3775	0.041
100-Do	52.195	2.400	0.3011	0.033
1000-FA_RL	52.284	2.489	0.2880	0.031
1000-RL_SL	52.694	2.899	0.2346	0.026
100-FA	53.148	3.353	0.1870	0.020
100-FD_FA	53.149	3.354	0.1869	0.020
500-FA_RL	53.157	3.362	0.1861	0.020
100-FD_RD	53.267	3.472	0.1762	0.019
1000-RL	53.323	3.528	0.1713	0.019
500-RL_SL	53.368	3.573	0.1675	0.018
100-S	53.670	3.875	0.1440	0.016
100-pH	53.687	3.892	0.1428	0.016
100-pH_Do	53.709	3.914	0.1412	0.015
100-N	53.856	4.061	0.1312	0.014
100-RD	53.895	4.100	0.1287	0.014
500-RL	53.895	4.100	0.1287	0.014
100-Do_N	54.017	4.222	0.1211	0.013
100-S_Do	54.195	4.400	0.1108	0.012
100-RD_FA	55.071	5.276	0.0715	0.008
100-FD_RD_FA	55.141	5.346	0.0690	0.008
100-pH_Do_N	55.172	5.377	0.0679	0.007
100-pH_S	55.377	5.582	0.0613	0.007
100-pH_N	55.532	5.737	0.0567	0.006
100-S_N	55.656	5.861	0.0533	0.006
100-pH_S_Do	55.700	5.905	0.0522	0.006
100-S_Do_N	56.006	6.211	0.0448	0.005
100-pH_S_Do_N	57.163	7.368	0.0251	0.003
100-pH_S_N	57.291	7.496	0.0235	0.003

<sup>a</sup>Model abbreviations: DO=dissolved oxygen; S=salinity; N=nitrates; FD=forest distance; RD=road distance; FA=forest area; RL=road length; SL=stream length.



Table 17 Summary statistics and t-test comparison of water chemistry variables measured at occupied and unoccupied springhouses located in Chester and Delaware Counties, Pennsylvania, and New Castle County, Delaware.

	pH	S <sup>a</sup>	TDS <sup>b</sup>	C <sup>c</sup>	DO <sup>d</sup>	N <sup>e</sup>
Occupied						
Mean	6.03	120.32	169.18	243.38	7.42	14.85
SE	--	8.156	11.565	16.360	0.225	1.840
Minimum	5.60	66.20	95.40	138.65	5.80	2.20
Maximum	6.57	200.50	289.50	415.50	9.75	26.40
Unoccupied						
Mean	6.03	128.50	181.14	259.27	6.98	15.40
SE	--	15.246	21.640	31.045	0.262	1.708
Minimum	5.52	51.15	71.60	103.30	5.10	1.10
Maximum	6.55	277.00	388.00	555.00	9.55	26.40
<i>P</i> -value	0.998	0.640	0.630	0.654	0.207	0.828

<sup>a</sup>Dissolved oxygen (mg/L); <sup>b</sup>salinity (ppm); <sup>c</sup>total dissolved solids (mg/L); <sup>d</sup>conductivity (μS/cm); <sup>e</sup>nitrate (ppm).

Table 18 Summary statistics and t-test comparison of habitat variables measured from 3 spatial scales at occupied and unoccupied springhouses located in Chester and Delaware Counties, Pennsylvania, and New Castle County, Delaware. Total area for each spatial scale was 3.14 ha, 78.5 ha, and 314.0 ha for 100 m, 500 m, and 1,000 m, respectively.

	100 m			500 m			1,000 m					
	FD <sup>a</sup>	RD <sup>b</sup>	FA <sup>c</sup>	LA <sup>d</sup>	FA	LA	RL <sup>e</sup>	SL <sup>f</sup>	FA	LA	RL	SL
Occupied												
Mean	28.06	171.61	1.28	1.62	32.27	40.14	2110.21	1742.98	127.04	153.75	8638.78	6910.72
SE	7.259	45.287	0.238	0.204	3.192	3.069	226.815	135.913	11.233	10.081	662.614	349.981
Minimum	0.00	2.00	0.00	0.34	13.89	23.25	947.10	618.57	50.75	87.16	3253.79	4738.35
Maximum	104.00	697.00	3.14	3.14	56.19	62.43	4321.81	3316.71	240.25	243.69	15173.02	9407.11
Unoccupied												
Mean	55.28	178.28	1.00	1.44	25.42	35.32	2163.90	1506.90	100.67	134.76	9822.69	5903.93
SE	16.054	43.354	0.220	0.182	2.782	3.487	465.037	73.200	8.323	9.389	1455.546	442.145
Minimum	0.00	24.00	0.00	0.20	6.92	16.35	0.00	770.51	55.74	71.91	3373.42	2866.04
Maximum	261.00	590.00	2.35	2.56	49.14	65.99	8023.70	2036.60	163.79	217.70	26506.28	9164.15
<i>P</i> -value	0.135	0.916	0.400	0.508	0.115	0.307	0.918	0.138	0.069	0.177	0.466	0.084

<sup>a</sup>Forest distance (m); <sup>b</sup>road distance (m); <sup>c</sup>forest area (ha); <sup>d</sup>low disturbance area (ha); <sup>e</sup>road length (m); <sup>f</sup>stream length (m).

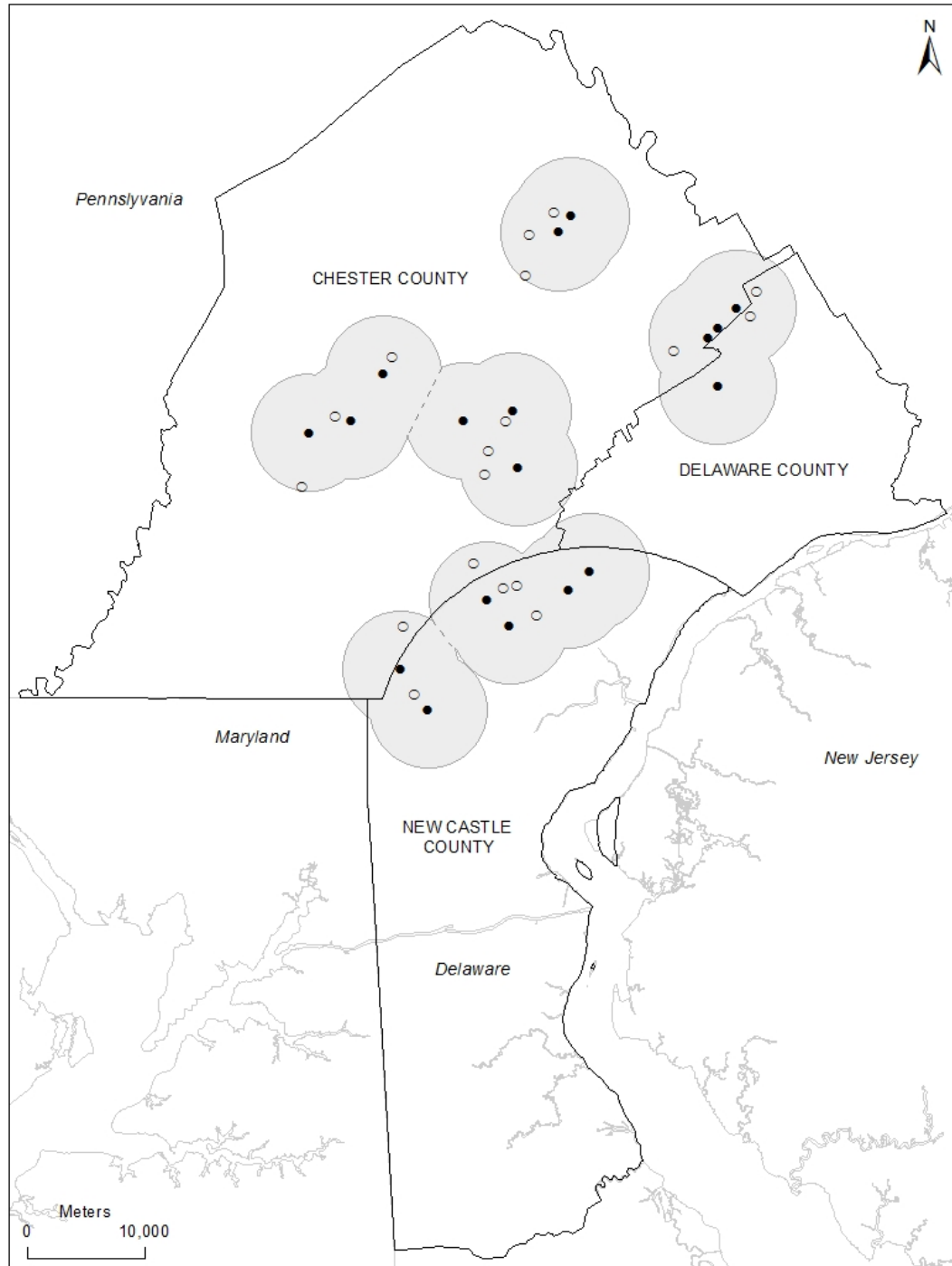


Figure 20 Location of occupied (closed circles;  $n = 18$ ) and unoccupied (open circles;  $n = 18$ ) springhouses distributed among 6 subareas formed by extending a 5,000-m buffer from occupied springhouses in Chester and Delaware Counties, Pennsylvania, and New Castle County, Delaware.

## REFERENCES

- Anderson, J. D., and P. J. Martino. 1966. The life history of *Eurycea l. longicauda* associated with ponds. *American Midland Naturalist* 75:257-279.
- Anderson, D. R., and K. P. Burnham. 1999. Understanding information criterion for selection among capture-recapture or ring recovery models. *Bird Study* 46:S14-S21.
- Atkinson, D. 1994. Temperature and organism size – a biological law for ectotherms? *Advances in Ecological Research* 25:1-58.
- Bailey, L. L., T. R. Simons, and K. H. Pollock. 2004. Estimating site occupancy and species detection probability parameters for terrestrial salamanders. *Ecological Applications* 14:692-702.
- Blanchard, F. N. 1930. The stimulus to the breeding migration of the spotted salamander, *Ambystoma maculatum* (Shaw). *The American Naturalist* 64:154-167.
- Burnham, K. P. and D. R. Anderson. 1998. Model selection and inference: a practical information-theoretic approach. Springer, New York, USA.
- Carlin, J. L. 1997. Genetic and morphological differentiation between *Eurycea longicauda longicauda* and *E. guttolineata* (Caudata: Plethodontidae). *Herpetologica* 53:206-217.
- Carr, L. W., and L. Fahrig. 2001. Effect of road traffic on two amphibian species of differing vagility. *Conservation Biology* 15:1071-1078.
- Christy, M. T., and C. R. Dickman. 2002. Effects of salinity on tadpoles of the green and golden bell frog (*Litoria aurea*). *Amphibia-Reptilia* 23:1-11.
- Chinathamby, K., R. D. Reina, P. C. E. Bailey, and B. K. Lees. 2006. Effects of salinity on the survival, growth and development of tadpoles of the brown tree frog, *Litoria ewingii*. *Australian Journal of Zoology* 54:97–105.
- Clinton, B. D., and J. M. Vose. 2006. Variation in streamwater quality in an urban headwater stream in the southern Appalachians. *Water, Air, and Soil Pollution* 169:331-353.

- Cooper, J. E. 1960. The mating antic of the long-tailed salamander. *Maryland Naturalist* 30:17-18.
- Culver, D. C. 1982. *Cave life: evolution and ecology*. Harvard University Press, Cambridge, Massachusetts, USA.
- Cupp, H. S. 2005. *Springhouses of Delaware*. Samuel B. and Helen S. Cupp, Wilmington, Delaware, USA.
- Cushman, S. A. 2006. Effects of habitat loss and fragmentation on amphibians: a review and prospectus. *Biological Conservation* 128:231-240.
- Davis, N. M., V. Weaver, K. Parks, and M. J. Lydy. 2003. An assessment of water quality, physical habitat, and biological integrity of an urban stream in Wichita, Kansas, prior to restoration improvements (Phase I). *Archives of Environmental Contamination and Toxicology* 44:351-359.
- Delis, P. R., H. R. Mushinsky, E. D. McCoy. 1996. Decline of some west-central Florida anuran population in response to habitat degradation. *Biodiversity and Conservation* 5:1579-1595.
- deMaynadier, P. G., and M. L. Hunter, Jr. 1995. The relationship between forest management and amphibian ecology: a review of the North American literature. *Environmental Review* 3:230-261.
- Dillard, L. O., K. R. Russell, and W. M. Ford. 2008. Site-level habitat models for the endemic, threatened Cheat Mountain salamander (*Plethodon nettingi*): the importance of geophysical and biotic attributes for predicting occurrence. *Biodiversity Conservation* 17:1475–1492.
- Douglas, M. E. 1979. Migration and sexual selection in *Ambystoma jeffersoninum*. *Canadian Journal of Zoology* 57:2303-2310.
- Douglas, M. E., and B. L. Monroe, Jr. 1981. A comparative study of topographical orientation in *Ambystoma* (Amphibia: Caudata). *Copeia* 1981:460-463.
- Duellman, W. E., and L. Trueb. 1994. *Biology of amphibians*. Johns Hopkins University Press, Baltimore, Maryland, USA.
- Dupuis, L. A., and D. Steventon. 1999. Riparian management and the tailed frog in northern coastal forests. *Forest Ecology and Management* 124:35-43.

- Downs, F. L. 1989. Family Ambystomatidae. In R. A. Pfingsten and F. L. Downs, editors. Salamanders of Ohio. Ohio Biological Survey Bulletin, New Series 7:87-172.
- Franz, R. 1964. Eggs of the long-tailed salamander from a Maryland cave. *Herpetologica* 20:216.
- Franz, R. 1967. Notes on the long-tailed salamander, *Eurycea longicauda* (Green), in Maryland. *Bulletin of the Maryland Herpetological Society* 3:1-6.
- Franz, R., and H. Harris. 1965. Mass transformation and movement of larval long-tailed salamanders, *Eurycea longicauda longicauda* (Holbrook). *Journal of the Ohio Herpetological Society* 5:32.
- Freeman, S. L., and R. C. Bruce. 2001. Larval period and metamorphosis of the three-lined salamander, *Eurycea guttolineata* (Amphibia: Plethodontidae), in the Chattooga River watershed. *American Midland Naturalist* 145:194–200.
- Guerrey, A. D., and M. L. Hunter, Jr. 2002. Amphibian distributions in a landscape of forests and agriculture: an examination of landscape composition and configuration. *Conservation Biology* 16:745-754.
- Guttman, S. I. 1989. *Eurycea longicauda*. In R. A. Pfingsten and F. L. Downs, editors. Salamanders of Ohio. Ohio Biological Survey Bulletin, New Series 7:204-209.
- Harrison, R. G. 1969. Harrison stages and description of the normal development of the spotted salamander, *Ambystoma punctatum* (Linn.). In R. G. Harrison, editor. Organization and development of the embryo. Yale University Press, New Haven, Connecticut, USA.
- Hels, T., and E. Buchwald. 2001. The effect of road kills on amphibian populations. *Biological Conservation* 99:331-340.
- Herrmann, H. L., K. J. Babbitt, M. J. Baber, and R.G. Congalton. 2005. Effects of landscape characteristics on amphibian distribution in a forest-dominated landscape. *Biological Conservation* 123:139-149.
- Hillis, D. M. 1977. Sex ratio, mortality rate, and breeding stimulus in a Maryland population of *Ambystoma maculatum*. *Bulletin of the Maryland Herpetological Society* 13:84-91.
- Houck, L. D., R. A. Watts, S. J. Arnold, K. E. Bowen, K. M. Kiemnec1, H. A. Godwin, P. W. Feldhoff, and R. C. Feldhoff. 2008. A recombinant courtship pheromone

- affects sexual receptivity in a Plethodontid salamander. *Chemical Senses* 33:623-631.
- Houlahan, J. E., C. S. Findlay, B. R. Schmidt, A. H. Meyer, and S. L. Kuzmin. 2000. Quantitative evidence for global amphibian population declines. *Nature* 404:752-755.
- Hutchison, V. H. 1956. Notes on the Plethodontid salamanders *Eurycea lucifuga* (Rafinesque) and *Eurycea longicauda longicauda* (Green). *Occasional Papers of the National Speleological Society* 3:1-24.
- Joly, P., C. Miaud, S. Lehmann, and O. Grolet. 2001. Habitat matrix effects on pond occupancy in newts. *Conservation Biology* 15:239-248.
- Jonas, M., J. L. Bowman, and N. H. Nazdrowicz. 2011. Using spot pattern to identify individual long-tailed salamanders. *Herpetological Review* 42:520–522.
- Karraker, N. E., and J. P. Gibbs. 2011. Road deicing salt irreversibly disrupts osmoregulation of salamander egg clutches. *Environmental Pollution* 159:833-835.
- Knutson, M. G., J. R. Sauer, D. A. Olsen, M. J. Mossman, L. M. Hemesath, M. J. Lanoo. 1999. Effects of landscape composition and wetland fragmentation on frog and toad abundance and species richness in Iowa and Wisconsin, U.S.A. *Conservation Biology* 13:1437-1446.
- Laan, R., and B. Verboon. 1990. Effects of pool size and isolation on amphibian communities. *Biological Conservation* 54:251-262.
- Lebreton, J., K. P. Burnham, J. Clobert, and D. R. Anderson. 1992. Modeling survival and testing biological hypotheses using marked animals: a unified approach with case studies. *Ecological Monographs* 62:67-118.
- Levins, R. A. 1969. Some demographic and evolutionary consequences of environmental heterogeneity for biological control. *Bulletin of the Entomological Society of America* 15:237-240.
- Lowcock, L. A., H. Griffith, and R. W. Murphy. 1991. The *Ambystoma laterale-jeffersonianum* complex in central Ontario: ploidy structure, sex ratio and breeding dynamics in bisexual-unisexual communities. *Copeia* 1991:87-105.
- Ludlow, R. A. and C. A. Loper. 2004. Chester County Ground-Water Atlas, Chester County, Pennsylvania. U.S. Department of the Interior, USGS 03-442, New Cumberland, Pennsylvania, USA.

- Marsh, D. M., and P. C. Trenham. 2001. Metapopulation dynamics and amphibian conservation. *Conservation Biology* 15:40-49.
- Marsh, D. M., K. A. Thakur, K. C. Bulka, and L. B. Clarke. 2004. Dispersal and colonization through open fields by a terrestrial, woodland salamander. *Ecology* 85:3396-3405.
- McDowell, W. T. 1988. Egg hatching season of *Eurycea longicauda longicauda* and *Eurycea lucifuga* (Caudata: Plethodontidae) in southern Illinois. *Bulletin of the Chicago Herpetological Society* 23:145.
- McDowell, W.T., and B. A. Shepherd. 2003. Reproductive biology and population structure of *Eurycea longicauda longicauda*. *Bulletin of the Maryland Herpetological Society* 39:52-61.
- Minton, S. A., Jr. 1972. Amphibians and reptiles of Indiana. *Indiana Academy of Science Monographs* 3:1-346.
- Mohr, C. E. 1943. The eggs of the Long-tailed Salamander, *Eurycea longicauda* (Green). *Proceeding of the Pennsylvania Academy of Science* 17:86.
- Mohr, C. E. 1944. A remarkable salamander migration. *Proceedings of the Pennsylvania Academy of Science* 18:51-54.
- Myers, C. W. 1958. Amphibia in Missouri caves. *Herpetologica* 14:35-36.
- Noble, G. K. 1929. The relationship of courtship to the secondary sexual characteristics of the two-lined salamander, *Eurycea bislineata* (Green). *American Museum Novitates* 362:1-5.
- Paul, M. J., and J. L. Meyer. 2001. Streams in the urban landscape. *Annual Review of Ecology and Systematics* 32:333-365.
- Peckham, R. S., and C. F. Dineen. 1954. Spring migrations of salamanders. *Proceedings of the Indiana Academy of Science* 64:278-280.
- Petranka, J. W. 1998. *Salamanders of the United States and Canada*. Smithsonian Institution Press, Washington, D. C., USA.
- Porej, D., M. Micacchion, and T. E. Hetherington. 2004. Core terrestrial habitat for conservation of local populations of salamanders and wood frogs in agricultural landscapes. *Biological Conservation* 120:399-409.



- Ricklefs, R. E. 1987. Community diversity: relative roles of local and regional processes. *Science* 235:167.
- Rinehart, K. A., T. M. Donovan, B. R. Mitchell, and R. A. Long. 2009. Factors influencing occupancy patterns of eastern newts across Vermont. *Journal of Herpetology* 43:521-531.
- Ringia, A. M., and K. R. Lips. 2007. Oviposition, early development and growth of the Cave Salamander, *Eurycea lucifuga*: Surface and subterranean influences on a troglomorphic species. *Herpetologica*, 63:258-268.
- Rossman, D. A. 1960. Herpetofaunal survey of the Pine Hills area of southern Illinois. *Journal of the Florida Academy of Science* 22:207-225.
- Rudolph, D. C. 1978. Aspects of the larval ecology of five plethodontid salamanders of the western Ozarks. *American Midland Naturalist* 100:141-159.
- Sanzo, D. and S. J. Hecnar. 2006. Effects of road de-icing salt (NaCl) on larval wood frogs (*Rana sylvatica*). *Environmental Pollution* 140:247-256.
- Saunders, D. A., R. J. Hobbs, and C. R. Margules. 1991. Biological consequences of ecosystem fragmentation: a review. *Conservation Biology* 5:18-32.
- Sayler, A. 1966. The reproductive ecology of the red-backed salamander, *Plethodon cinereus*, in Maryland. *Copeia* 1966:183-193.
- Schwarz, C. J., R. E. Bailey, J.R. Irvine, and F. C. Dalziel. 1993. Estimating salmon spawning escapement using capture-recapture methods. *Canadian Journal of Fisheries and Aquatic Sciences* 50:1181-1191.
- Semlitsch, R. D. 1980. Geographic and local variation in population parameters of the slimy salamander *Plethodon glutinosus*. *Herpetologica* 36:6-16.
- Semlitsch, R. D. 1983. Structure and dynamics of two breeding populations of the eastern tiger salamander, *Ambystoma tigrinum*. *Copeia* 1983:608-616.
- Semlitsch, R. D. 1985. Analysis of climatic factors influencing migrations of the salamander *Ambystoma talpoideum*. *Copeia* 1985:477-489.
- Sexton, J. O., C. Phillips, and J. E. Bramble. 1990. The effects of temperature and precipitation on the breeding migration of the spotted salamander (*Ambystoma maculatum*). *Copeia* 1990:781-787.

- Shoop, C. R. 1974. Yearly variation in larval survival of *Ambystoma maculatum*. Ecology 55:440-444.
- Stuart, S. N., J. S. Chanson, N. A. Cox, B. E. Young, A. S. L. Rodrigues, D. L. Fischman, and R. W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306:1783-1786.
- Taylor, N., and J. Mays. 2006. The salamanders *Eurycea longicauda* and *Plethodon glutinosus* in Gregorys Cave, TN: monitoring and observations on ecology and natural history. Southeastern Naturalist 5:435-442.
- Trapp, H. Jr., and M. A. Horn. 1997. Ground water atlas of the United States: Delaware, Maryland, New Jersey, North Carolina, Pennsylvania, Virginia, West Virginia. Hydraulic Atlas 730-L. U. S. Geological Survey, Reston, Virginia, USA.
- Trenham, P. C., and H. B. Shaffer. 2005. Amphibian upland habitat use and its consequences for population viability. Ecological Applications 15:1158-1168.
- Tufford, D. L., C. L. Samarghitan, H. N. McKellar, Jr., D. E. Porter, and J. R. Hussey. 2003. Impacts of urbanization on nutrient concentrations in small southeastern coastal streams. Journal of the American Water Resources Association 39:301-312.
- Turtle, S. L. 2000. Embryonic survivorship of the spotted salamander (*Ambystoma maculatum*) in roadside and woodland vernal pools in southeastern New Hampshire. Journal of Herpetology 34:60-67.
- Uzzell, T. M., Jr. 1969. Notes on spermatophore production by salamanders of the *Ambystoma jeffersonianum* complex. Copeia 1969:602-612.
- Van Tienhoven, A. M., J. E. Den Hartog, R. Reijns, and V. M. Peddemors. 2007. A computer-aided program for pattern-matching of natural marks of the spotted raggedtooth shark *Carcharias taurus*. Journal of Applied Ecology 44:273-280.
- White, G. C., and K. P. Burnham. 1999. Program MARK: survival estimation from populations of marked animals. Bird Study 46:S120-S139.
- Williams, A. A., R. A. Brandon, and J. Martan. 1984. Male genital ducts in the salamander *Eurycea lucifuga* and *Eurycea longicauda*. Herpetologica 40:322-330.