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THE POPULATION ECOLOGY AND BEHAVIOR OF THE CAVE SALAMANDER, *EURYCEA LUCIFUGA* (RAFINESQUE, 1822)

By

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B.S., University of Louisville, 2011
M.S., University of Louisville, 2016

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A Dissertation Approved on

July 3, 2018

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James Alexander

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DEDICATION

To Emma, Jameson, and Bailey

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ABSTRACT

THE POPULATION ECOLOGY AND BEHAVIOR OF THE CAVE SALAMANDER, *EURYCEA LUCIFUGA* (RAFINESQUE, 1822)

J. Gavin Bradley

July 3, 2018

The Cave Salamander, *Eurycea lucifuga* (Rafinesque, 1822), is a little-known species, yet a common inhabitant of caves in the eastern United States. Salamanders are often important components of ecological communities and ecosystems, influencing critical processes such as nutrient cycling and community composition through their predation on invertebrates. Cave-dwelling salamanders such as *E. lucifuga* may thus appreciably influence the relatively simple ecosystems and communities of caves. Any such influence may be particularly important because these habitats and the organisms that reside in them are often of conservation concern. I used non-invasive methods to study the demographics, movements, and habitat selection of *E. lucifuga* at Sauerkraut Cave in Louisville, Kentucky. I also conducted an experimental manipulation using clay models to test predation risk to Cave Salamanders in caves and forests in southern Indiana. I discovered that *E. lucifuga* have consistent and distinguishable spot patterns that can be used to identify individuals. Populations of this species may be much larger than previously thought, potentially contributing relatively large and seasonally variable biomass to spring cave systems. Furthermore, this species migrates seasonally within caves using abiotic environmental cues that indicate seasonal change, potentially shuttling acquired energy from forests to deep underground. I also demonstrated that Cave Salamanders likely use caves, and particularly cave walls, as a refuge from greater potential predation risk in forests. This research provides much-needed information on this species and is suggestive that cave-dwelling salamanders may have important ecological roles in subterranean environments.

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CHAPTER I

DISSERTATION INTRODUCTION

The Cave Salamander, *Eurycea lucifuga* (Rafinesque, 1822), is a common inhabitant of caves in the eastern United States. However, little is known regarding the ecology and behavior of this species, leaving its functional relationship to cave ecosystems and communities enigmatic. In this dissertation, I investigate several aspects of the ecology and behavior of *E. lucifuga*. In this first chapter, I provide a comprehensive literature review of this species to encapsulate the present knowledge of *E. lucifuga*, followed by a brief outline of the remaining topics covered in this dissertation.

AMPHIBIA: CAUDATA: PLETHODONTIDAE: HEMIDACTYLIINAE: *EURYCEA LUCIFUGA*

***General*—**The Cave Salamander (Figure 1) is a trogloxenic salamander, i.e., a facultative inhabitant of cave systems that must leave at some point to obtain epigean resources (Trajano and Carvalho, 2017), that is wide ranging in the karst regions associated with the Ozark, Interior Lowland, and Appalachian physiographic regions (Hunt, 1974) of the eastern United States (Figure 2). This species is typified by a bright orange dorsum flecked with black spots, however, piebald (Neff *et al.*, 2015; Smith, 1985) and spotless (Bradley and Eason, 2017a) individuals have been documented. Sexually mature individuals (i.e., adults) of this species are slender and typically between

FIGURE 1. *Eurycea lucifuga* (scale bar = 1 cm). (Photograph by J. Gavin Bradley)

46–62 mm snout-vent length (Carlyle *et al.*, 1998; Hutchison, 1958, 1966; Juterbock, 2005; Williams, 1980) with long tails that are greater than 60% of the salamanders total length (Hutchison, 1966).

Eurycea lucifuga is frequently associated with limestone caves, especially the twilight zone of these environments (Banta and McAtee, 1906; Green *et al.*, 1967; Hutchison, 1958; Mittleman, 1950; Myers, 1958a; Peck, 1974). Preference for this habitat is related to the physical environment of caves: high moisture availability and cool temperatures (Briggler and Prather, 2006; Camp *et al.*, 2014; Camp and Jensen, 2007a; Hutchison, 1958), presence of perennial streams (Briggler and Prather, 2006),

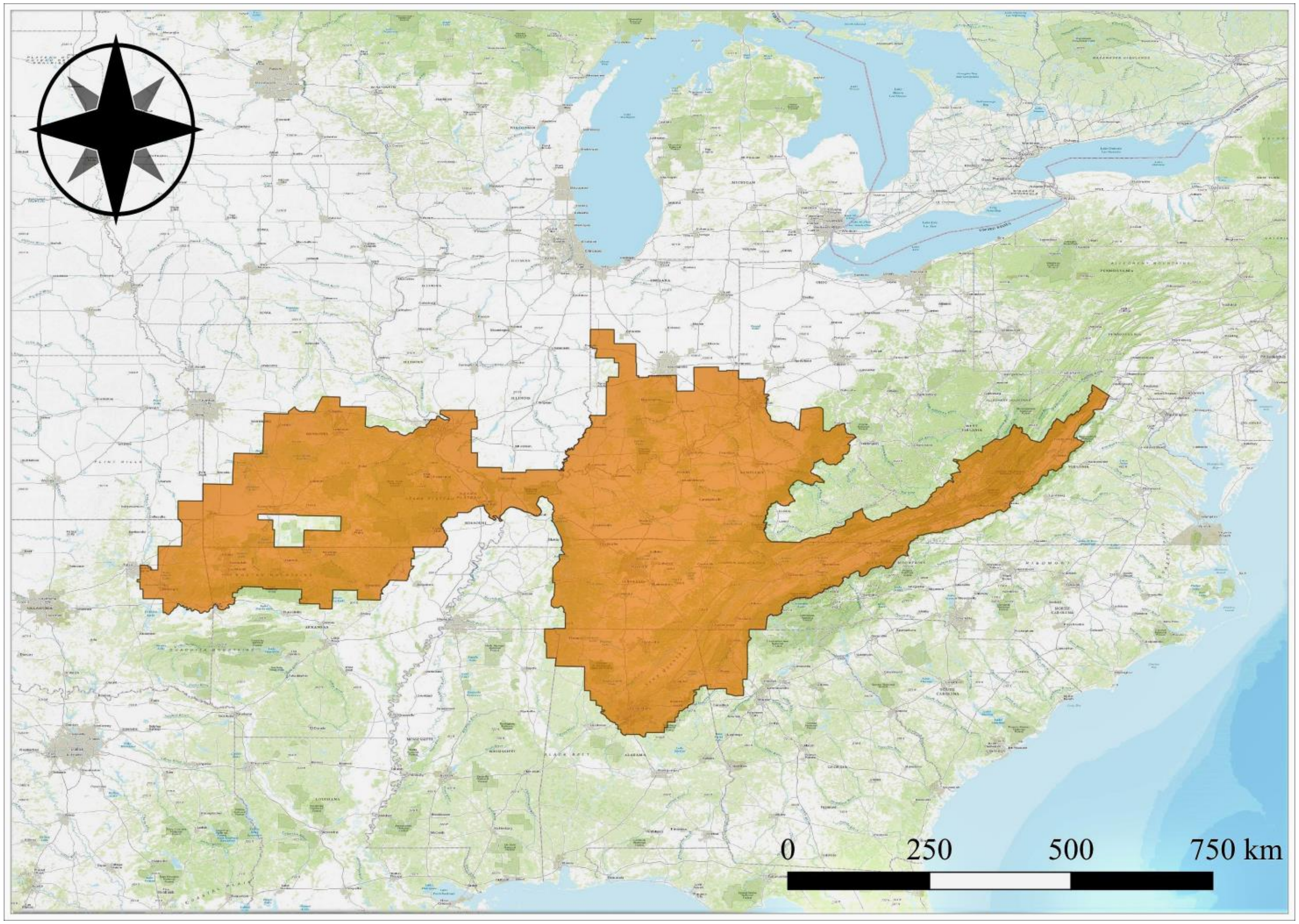


FIGURE 2. Distribution map for *Eurycea lucifuga* in the eastern United States. I acquired the distribution data from IUCN (2014) and I generated the map in QGIS (2017) using an ArcGIS Online basemap.

and available refugia such as wall crevices (Banta and McAtee, 1906; Green *et al.*, 1967; Hutchison, 1958). Mushinsky and Brodie Jr. (1975) report Cave Salamanders' preference for alkaline (pH 7.7) substrates, which is characteristic of limestone, but Hutchison (1958) found no such direct association. Several features of Cave Salamanders make them well suited for the cave environment, including well-developed eyes that improve vision in low light (Hutchison, 1958), long limbs and a prehensile tail that facilitate climbing (Banta and McAtee, 1906; Green *et al.*, 1967; Hutchison, 1958), and an ability to orient to the Earth's magnetic field that permits navigation in darkness (Phillips, 1977).

In temperate regions, environmental conditions of the twilight zone, particularly humidity and temperature, fluctuate seasonally. Generally, during colder months, cold, dry air is sucked into the twilight zone forcing warmer, more humid air to retreat toward the dark zone. As the epigean environment warms, cool, humid air moves toward the twilight zone. Consequently, abundance and distribution of Cave Salamanders in the twilight zone of caves fluctuates seasonally, with higher abundances of salamanders occurring during cool, moist situations and lower abundances during dryer situations. Camp *et al.* (2014) found that *E. lucifuga* prefer microhabitat temperatures of 15–16ºC. Hutchison (1958) demonstrated that as humidity decreased in the twilight zone between late summer through winter, salamander abundance decreased in this area as individuals retreated toward the dark zone of caves. Abundance increased in the twilight zone from late winter through early summer as humidity increased and moist substrates were more available. Williams (1980) noted a similar trend but suggested that courtship behavior may influence influx into the twilight zone from epigean habitats at these times of year. Correspondingly, Cave Salamanders have been found to be most active in the twilight zone of caves in spring, summer, and fall (Briggler and Prather, 2006; Camp *et al.*, 2014; Camp and Jensen, 2007a).

Cave Salamanders exit caves for nocturnal foraging when conditions are cool and moist (pers. obs.). Epigean activities occur in a number of microhabitats, including the ground, rock walls, and various types of vegetation, including trees (Scheffers, 2010).

Taxonomy and genetic history**—***Eurycea lucifuga* forms a clade within *Eurycea* accompanied by Long-tailed Salamanders, *E. longicauda* (Green, 1818), and Three-lined Salamanders, *E. guttolineata* (Holbrook, 1838) (Harlan and Zigler, 2009). The Cave Salamander is phenotypically similar across its range. In an early study, Merkle and Guttman (1977) also found high genetic similarity and low average heterozygosity, and they suggested this low level of divergence was likely due to similar selective pressures acting across this range, as gene flow is constrained by the Mississippi and Ohio Rivers. However, some biochemical divergence has occurred (Merkle, 1975), and recently Edgington *et al.* (2016) defined three major lineages of *E. lucifuga*: eastern (Virginia, West Virginia, and Tennessee), central (north: Indiana; south: Kentucky and Tennessee), and western (Missouri and Oklahoma), with an initial divergence between central and eastern/western clades followed by a split between eastern and western clades (Pleistocene divergences *ca.* two Mya). However, persistent gene flow or incomplete lineage sorting is occurring among regions (Edgington *et al.*, 2016).

Diet**—**Studies of adult food habits highlight the opportunistic nature of Cave Salamanders, with varied, predominantly invertebrate, prey taxa recorded (Crowell, 1981; Cudmore and Rubin, 1982; Hutchison, 1958; Peck, 1974; Peck and Richardson, 1976). Dipterans (flies) consistently compose a large proportion of the prey content in adult Cave Salamander diet (41.3–69.2%). Other numerically important taxa include: Acari (mites and ticks), Araneae (spiders), Lepidoptera (moths and butterflies), Tricoptera (caddisflies), Coleoptera (beetles), Plecoptera (stoneflies), and Isopoda. Other, less common food types include vegetation and shed skin, as well as immature Slimy Salamanders, *Plethodon glutinosus* (Green, 1818), (Peck and Richardson, 1976).

Diet diversity in Cave Salamanders is determined by body size, with larger individuals incorporating more prey items in their diets (Peck, 1974). Cave Salamanders are nonselective foragers, taking invertebrate prey in proportion to their availability in the environment (Peck and Richardson, 1976), and at least at one site no seasonal shift in food habits occurred, as major food types were found in each season (Cudmore and Rubin, 1982). Foraging individuals may move between cave zones and tend to go to areas of greater food abundance, e.g., from the food-poor dark zone to the twilight zone (Peck and Richardson, 1976). However, this species is known to feed in the dark zone of caves (Bradley and Eason, 2017b; Peck and Richardson, 1976) and thus takes food where and when prey are encountered. Camp and Jensen (2007b) determined that stomach content volume was greatest in spring, then summer, with body lipid content highest in summer, followed by spring and fall.

***Ecological interactions*—**Salamanders are prey to a wide array of organisms including mammals, birds, herpetofauna, fish, and crayfish (Hairston Sr., 1987; Petranka, 1998). Hutchison (1958) speculated that several mammal and snake species were predators of Cave Salamanders, and Camp and Jensen (2015) documented one individual in the mouth of a Ring-necked Snake, *Diadophis punctatus* (Linnaeus, 1766), in Georgia. In Rudolph's (1978) assessment of *Eurycea* larval ecology, fish were common predators, and he found evidence of intraguild predation: within the digestive tracts of Grotto Salamanders, *E.* [*Typhlotriton*] *spelaeus* (Stejneger, 1892), were remains of *Eurycea* larvae (*E. longicauda* or *E. lucifuga*). Isopods and amphipods were reported to consume Cave Salamander eggs in Missouri (Ringia and Lips, 2007).

Gastrointestinal parasites have been documented in detail for Cave Salamanders, including Protozoa (*Haptophyra* spp.; *Cepedietta michiganensis*), Nematoda (*Capillaria inequalis* Walton, 1935; *Cosmocercoides dukae* Holl, 1928 and *C. variabilis* Harwood, 1930; *Omeia pappilocauda*; *Oswaldocruzia euryceae* Reiber, Byrd, and Parker, 1940 and *O. pipiens* Walton, 1929; *Rhabdias* sp.; *Thelandros magnavulvaris* Rankin, 1937; *Trichoskrjabinia* sp.), Trematoda (*Brachycoelium* spp.; *Clinostomum complanatum* Rudolphi, 1814; *Cainocreadium pseudotritoni* Rankin, 1937), and Cestoda (*Batrachotaenia cryptobranchi* La Rue, 1914) (Castle *et al.*, 1987; Dyer and Peck, 1975; Hutchison, 1958; McAllister *et al.*, 2007; McAllister and Bursey, 2004).

Cave Salamanders occur in multi-species salamander assemblages in caves (Briggler and Prather, 2006; Camp and Jensen, 2007a; Mittleman, 1950; Myers, 1958a; Rudolph, 1978), suggesting that interspecific competition may occur. Although one study found that food competition might be precluded for *E. lucifuga*, *E. longicauda*, and *P. glutinosus* by utilization of different-sized prey and spatial segregation (Crowell, 1981), other studies have found extensive dietary overlap among these species (Cudmore and Rubin, 1982; Hutchison, 1958; Peck, 1974). *Eurycea lucifuga* may also prefer a different microhabitat temperature than *P. glutinosus* (Camp *et al.*, 2014), possibly reducing competitive interactions. Rudolph (1978) found a lack of spatial segregation between larval *E. lucifuga* and *E. longicauda*, but larval *E. spelaeus* were competitively superior to *E. lucifuga* in cave streams, resulting in spatial segregation. Howard (1985) examined intraspecific territoriality in adult *E. lucifuga* and found that Cave Salamanders are neither territorial nor aggressive during resident-intruder encounters, suggesting that interference competition may not be particularly common. In fact, he often found individuals clustered in groups of 3–10 individuals in the field. No assessment of exploitation competition within Cave Salamanders has been published to my knowledge.

Behavior**—**The behavior of *E. lucifuga* is very little known. Reaction to disturbance (e.g., directing light at, moving toward, or touching an individual) can be inconsistent, with some individuals reacting strongly by jumping and running and others appearing apathetic (Banta and McAtee, 1906). However, individuals in caves on the ground may be more wary and likely to flee than those on cave walls (pers. obs.).

Aggressive behavior, although apparently uncommon toward other salamanders, has been documented twice for *E. lucifuga*, to my knowledge, in occurrence with non-salamander organisms. Smith and Balch (1985) noted a defensive posture, with the head raised exposing the gular region, by one adult individual toward these authors upon release after being contained for several hours. A very similar posture was observed by Bradley and Eason (2017c) during a stand-off encounter between an adult Cave Salamander and a large Nursery Web Spider (*Pisaurina* sp.). This encounter resulted in the salamander aggressively advancing the spider and forcing its retreat, indicating that Cave Salamanders may exhibit aggression toward large carnivorous invertebrates.

Reproduction and development**—**Details of reproduction are largely unknown for *E. lucifuga*. Courtship behavior has not been documented in the wild, but was described from laboratory observations by Organ (1968), who also described the spermatophore.

Secondary sexual characteristics are visible in late summer through early fall in Kentucky (pers. obs.). Description of the reproductive anatomy of males includes the excurrent genital ducts (Siegel *et al.*, 2014; Williams, 1979; Williams *et al.*, 1984), cloacal gland complex (Williams *et al.*, 1985), and caudal courtship glands (Hamlett *et al.*, 1998). Carlyle *et al.* (1998) investigated female anatomy, describing how to estimate timing of oviposition through ova measurements and the condition of oviducts. There has been one account of hybridization between the Dark-sided Salamander, *E. longicauda melanopleura*, and *E. lucifuga* (Smith, 1964).

Development of Cave Salamanders has received more attention. Gravid females have been found in summer through fall (Carlyle *et al.*, 1998; Green *et al.*, 1967; Myers, 1958b). Oviposition predominantly occurs in fall (Carlyle *et al.*, 1998; Green *et al.*, 1967; Niemiller *et al.*, 2009; Ringia and Lips, 2007), but may occur in summer (Ringia and Lips, 2007) or winter (Banta and McAtee, 1906; Myers, 1958b). Eggs are laid singly or in small clusters in pools, streams, and on walls unattached or attached by a pedicel to substrate (Barden and Kezer, 1944; Green *et al.*, 1967; Myers, 1958b). Early larval forms are found in fall (Green *et al.*, 1967) and winter (Banta and McAtee, 1906; Myers, 1958b; Rudolph, 1978) in caves and across many months at rock faces (McDowell, 2010). Yolk absorption is complete *ca.* 100 days post hatching (Ringia and Lips, 2007). Myers (1958b), McDowell (2010), and others document larval growth and the development of orange pigmentation and spot pattern (Banta and McAtee, 1906; Clergue-Gazeau and Thorn, 1976; Green *et al.*, 1967). Timing to metamorphosis varies, with some individuals transitioning their first year while others overwinter in cave streams and pools. Consequently, two size classes of larvae may coincide (Banta and McAtee, 1906; Green *et al.*, 1967; Myers, 1958b). Transformation occurs *ca.* 50 mm total length or 36 mm snout-vent length (Banta and McAtee, 1906; Green *et al.*, 1967).

It is evident from this review that detailed knowledge of the ecology and behavior of this species is lacking. In the following chapters, I present new information on terrestrial (i.e., juvenile and adult) Cave Salamanders. In Chapter 2, I describe non-invasive methods that can be used to work with this species, which previous researchers have suggested to be sensitive to disturbance and handling. In Chapter 3, I report demographics for a population of Cave Salamanders, including the sex ratio, age class proportions, and seasonal estimates of abundance, capture and survival probabilities, and wet-weight biomass and biomass density. I also detail the seasonal migration and associated environmental migratory cues for this population within a cave system in Chapter 4. In Chapter 5, I address cave inhabitance by this species in relation to predation risk in different habitats with a combination of data from an experimental manipulation using clay salamander models and habitat selection by individual Cave Salamanders. Chapter 6 contains concluding remarks for this dissertation.

CHAPTER II

IMAGE-BASED METHODS FOR IDENTIFICATION AND MEASUREMENT OF CAVE SALAMANDERS, *EURYCEA LUCIFUGA* (RAFINESQUE, 1822)

INTRODUCTION

Many ecological wildlife studies require trapping, handling, and/or marking of individuals to estimate various parameters, such as population size, density, demographics, and body size. Unfortunately, adverse reactions (e.g., elevated stress and reduced survival) may be exhibited by wildlife because of these techniques (Bliley and Woodley, 2012; Davis and Ovaska, 2001; Mott *et al.*, 2010; Walker *et al.*, 2012). Alternatively, non-invasive techniques may be used to alleviate some of these problems. Electronic imaging has become increasingly popular in wildlife studies as a non-invasive technique to identify individuals with naturally occurring patterns, such as spots, stripes, or scars (Bolger *et al.*, 2012; Petrovska-Delacretaz *et al.*, 2014), as well as to obtain individual metrics like body length (Abràmoff *et al.*, 2004; Schneider *et al.*, 2012).

Digital images accumulated over time for individual identification may allow investigators to address various individual behaviors and population dynamics. However, sifting through and cross-referencing large numbers of images to detect known individuals is a daunting and time-consuming task. Pattern-recognition software assists in this process because it facilitates quicker, more effective image sorting. This is a successful tool for identification purposes in a variety of taxa, including invertebrates, fish, amphibians, reptiles, and mammals (Sannolo *et al.*, 2016). Similarly, scaling software can be used to obtain measurements, such as body length, from digital images. These techniques allow researchers to study organisms without having to capture and handle them, which may be an important consideration for non-invasive studies. Together, implementation of these methods in wildlife research will allow investigators the opportunity to work closely with behaviorally sensitive species.

The goal of this project was to assess the utility of such non-invasive methods to study the Cave Salamander, *Eurycea lucifuga* (Rafinesque, 1822), particularly concerning the applicability of using spot patterns to identify individuals. This species has been suggested to become increasingly wary during long-term investigation and avoid researchers that use standard capture, handling, and marking techniques (Howard, 1985; Hutchison, 1958; Organ, 1968; Williams, 1980); thus, using non-invasive methods to study *E. lucifuga* is ideal. I used the pattern-recognition software Interactive Individual Identification System (I^3S) (van Tienhoven *et al.*, 2007) and ImageJ (Schneider *et al.*, 2012) to analyze digital images of *E. lucifuga*. Herein, I report the performance of I^3S while using non-invasive methods, in which I do not capture or handle salamanders. I then compare a non-invasive dorsal body length metric of *E. lucifuga* to conventional, more invasive snout-vent length metrics.

METHODS

Study site**—**I monitored a population of terrestrial (adult and juvenile) *E. lucifuga* in the primary channels of the main and side passages of Sauerkraut Cave (Figure 3), a spring cave located in a 222.6 ha urban park, E.P. “Tom” Sawyer State Park, in

FIGURE 3. Sauerkraut Cave in winter. The main passage is the large passage on the right, and the side passage is the smaller hole to the left above the stream (scale bar = 2 m). (Photograph by Julian J. Lewis)

Louisville, Kentucky, USA. Sauerkraut Cave formed in Middle Silurian aged rock of the Louisville Limestone formation on the west side of the anticline known as the Cincinnati Arch (Peterson, 2001). The area surrounding the cave was used as farmland before the development of a mental hospital (known as Central State Hospital or Lakeland Asylum) in the 1800s (Thomas, 2004). The cave was historically used as a springhouse and was modified extensively with brick walls spanning much of the entrances and the construction of troughs (Ford and Ford, 1882), remnants of which still remain (Figure 4). The cave's perennial stream was channelized to the west side of the main passage (Figure 4), which exits the cave and is routed underground in a pipe. Presently, the cave is a

FIGURE 4. Brickwork and passages in Sauerkraut Cave. **A, B,** and **C.** The brick walls, block pile, and brick trough channelizing the stream in the front chamber of the main passage. **D.** Entrance to the side passage. Scale bars = 1 m. (Photographs by J. Gavin Bradley)

popular spot for passersby walking a trail *ca.* 25 m from the entrances and is regularly subject to recreational disturbances.

The main passage of Sauerkraut Cave is *ca.* 130 m long and has a relatively large walk-in entrance (H = 2.20 m, W = 4.56 m) that opens directly to the largest chamber (greatest dimensions: H = 2.23 m, W = 10.04 m, L = 19.97 m) in this system. The north end of this room closes down to a smaller stream crawl-way (H = 1.02 m, W = 1.37 m) that extends the remaining length of this passage. The side passage is *ca.*100 m long and has a smaller entrance (H = 0.50 m, W = 1.41 m) situated just west of the main entrance and stream. This entrance is mostly bricked over (Figure 4), but may be accessed by crawling through the human-sized hole in the brickwork. This is entirely crawl-way passage with at least four persistent pools of variable size. These entrances are situated in a young second-growth forest, with dominant tree species Hackberry, *Celtis occidentalis* (Linnaeus), and Black Maple, *Acer nigrum* (Michaux).

Field data collection**—**This study is associated with a long-term, non-invasive study of the natural history and ecology of *E. lucifuga* spanning March 2015–February 2017 (Chapter 3 and 4). Data collection for this study occurred through two different time periods. From March 2015–December 2016, all data collection was non-invasive; thus, no salamanders were captured or handled. From March–April 2017, some data collection required that salamanders be captured and handled.

I constructed two semi-permanent transects, one 99 m in length in the main passage and the other 82.3 m in length in the side passage, each starting at the drip-line of the cave and following the primary channel of its respective passage; these lengths were used because both passages became too difficult to maneuver and work in beyond these distances. Surveys of these transects were generally conducted weekly, but modified on occasion from March 2015–December 2016 due to flooding or scheduling difficulties. During surveys, I searched the walls, floor, ceiling, and standing water of both transects during March 2015–December 2016, and that of just the main passage from March–April 2017, using red-filtered light during daylight hours (earliest start time was 0920 h, latest start time was 1315 h, typical start time was between 1100–1230 h). Hutchison (1958), Williams (1980), and Briggler and Prather (2006) similarly conducted surveys for this species during daylight hours. I used an Olympus TG-4 digital camera (Olympus America Inc., Center Valley, Pennsylvania, USA) to take pictures of salamanders. From March 2015–December 2016, I photographed the dorsal spot pattern of the head and neck of each salamander *in situ* and in plain sight for individual identification in I^3S-Spot (I^3S-S), an extension of the I^3S system. It was not known previously if the spot patterns of individuals of this species are stable over time, and thus appropriate for identification purposes; however, this technique was found to be successful for Long-tailed Salamanders, *E. longicauda longicauda* (Green, 1818) (Jonas *et al.*, 2011; Nazdrowicz, 2015), a sister species to *E. lucifuga*. Since data collection for individual identification was non-invasive during this time period, I made no attempts to straighten, orient, or otherwise manipulate salamanders during these procedures. From March–April 2017, I photographed adult salamanders in plain sight for both body length measurement and for identification purposes, and then collected these salamanders for physical measurement of snout-vent length (SVL). I photographed the entire dorsal surface of salamanders with the same camera *in situ* and next to a section of metered tape, which was oriented in the same horizontal plane as the salamander's dorsum. To ensure individuals were not used twice in subsequent analyses, I then photographed the dorsal spot pattern of the head and neck, as described above, for individual identification in I^{3}S-S. I identified 10 individuals that were each recaptured once. The data from recapture events were removed; thus, the measurements of 82 individual Cave Salamanders were used in further analyses. To measure SVL, salamanders were directed by hand or an aquarium net into a plastic bag containing a paper towel wetted with cave water, physically straightened by hand, and measured from the tip of the snout to the posterior margin of the vent with vernier calipers. After SVL measurement, salamanders were released at the site of capture. Capture, handling, and SVL measurement followed guidelines from HAAC (2004), Shaffer *et al.* (1994), and Stasiak (2015).

Image processing in I^{3}S-S—I identified individuals through annotation and comparison of digital images in I^{3}S-S version 4.0.2 (den Hartog and Reijns, 2014). An individual's spot pattern was annotated on its digital image. First, I manually selected three predetermined, fixed reference points to delineate a reference area that I^{3}S-S could recognize. For *E. lucifuga*, I used the posterior insertions of the left and right forearms (corresponding with the connection point of the posterior side of the scapula and humerus to the coracoid plate) and the snout tip as the three fixed reference points. These reference points were selected based on suggested specifications in the I^{3}S-S Manual (den Hartog and Reijns, 2014): 1) the location of the reference points should be clearly and consistently distinguishable; 2) they should be visible in all images; and 3) the triangle created by these points should have angles as near to 60° as possible. Additionally, it is suggested that the head of animals be used, instead of the entire body, because it is less susceptible to deformation, i.e., more likely to be linear (den Hartog and Reijns, 2014).

Especially for animals such as *E. lucifuga* that are highly flexible in the trunk and tail regions, the head will be much more consistent in shape across images. Second, I manually created ellipses on up to 30 spots within the reference area of an image (Figure 5). I created ellipses starting at the posterior forearm insertions and moved toward the snout tip. One ellipse was used per spot if spots were circular, triangular, or oblong; two or more ellipses were used for amorphous spots.

After annotation of an image, I^{3}S-S creates an accompanying fingerprint (.fgp) file, which is used during the comparison procedure. During comparisons, the new .fgp file is matched against an identification database, which contains the annotated images and associated .fgp files of all previously identified individuals. Then, I^{3}S-S provides results of all comparisons in a ranked list with score values for each comparison. Low scores, those nearest 0.00, indicate a likely match, with a score of 0.00 signifying a perfect match. All newly annotated images were manually compared against the images in the ranked list output. The ranked list is presented as a scroll-down menu in which the observer may advance through multiple 10-image sets to visually inspect comparisons. In general, for each comparison, I searched the ranked list until a positive match was found, indicating a recapture, or until an image was reached with a cutoff score of at least 250.00 (I determined this cutoff score through trial and error testing cutoff scores of 150.00, 200.00, and 250.00 during early image comparisons). Once an image was reached that was at or just beyond this threshold value, the remainder of images in that current 10-image set, if any, were also compared. Thus, the actual cutoff score was usually slightly over 250.00. It was assumed, in most instances, that after this cutoff there was no positive match in the ranked list for the new image being compared. However, if a salamander in

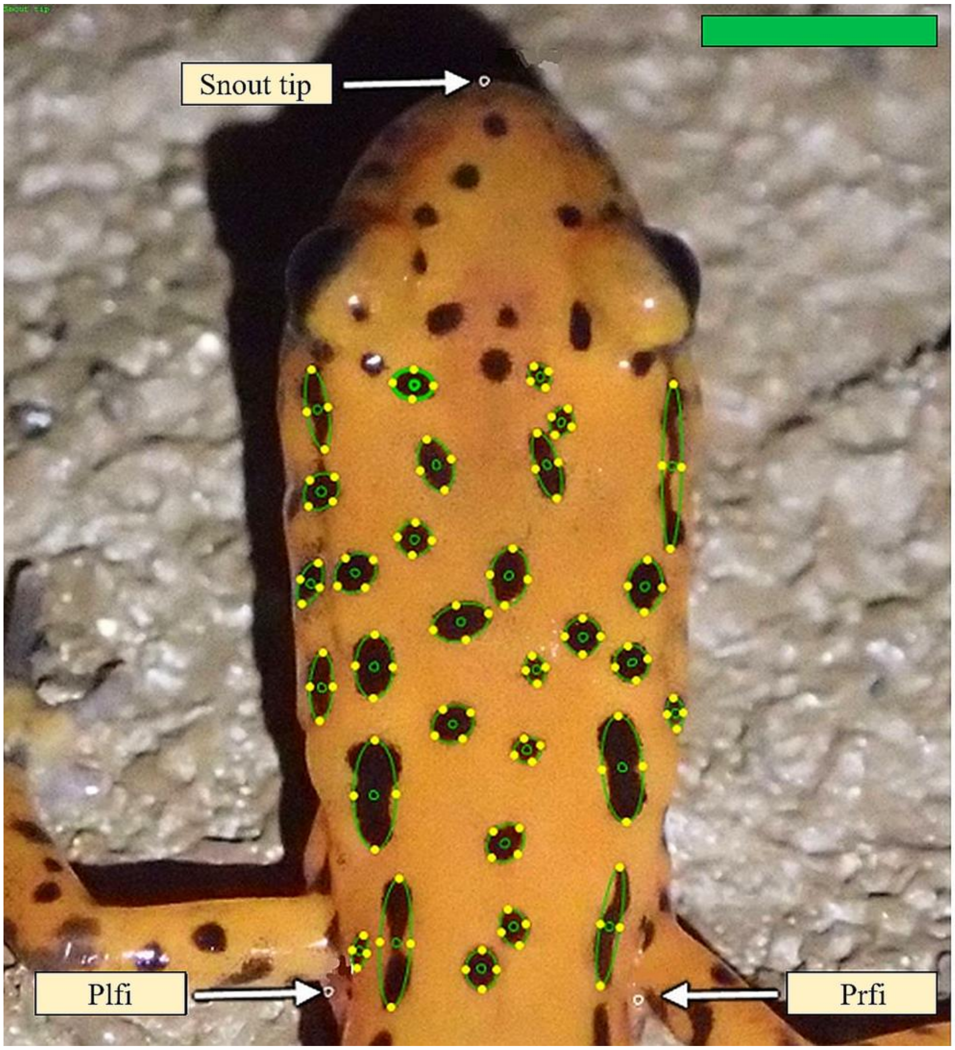


FIGURE 5. Annotation of a digital image in I^{3}S-S. Arrows indicate the three fixed reference points (Plfi = posterior-left-forearm-insertion, Prfi = posterior-right-forearm-insertion, and Snout tip), which delineate the reference area. Created ellipses are shown around the individual's spots (scale bar = 0.5 cm). (Photograph in image by J. Gavin Bradley)

an image had a familiar pattern, or the image was of exceptionally poor quality (e.g., not in sharp focus or taken at an angle from more than *ca.* 30° from the vertical plane), the ranked list was searched until either a positive match was found or the entire ranked list was searched to determine that the image truly had no match. Further, to reduce the likelihood of false-negatives (i.e., finding no match when there was one in the ranked list), every seventh new image in which a positive match was not found by the 250.00 score cutoff was visually compared to the entire ranked list. New images were added to the identification database if a salamander was not a recapture (i.e., new) or if a recaptured individual's new image was of better quality than the original in the database. Only one image per individual was kept in the identification database.

The processing time required for all procedures (annotation, automated comparison, and manual visual inspection) in I^{3}S-S are reported in decimal min. Time tracking began at the start of year two (March 2016), when the identification database consisted of 849 images, and ended in November 2016. Thus, the results are representative of a database with many images; the amount of time needed for manual visual inspection at the beginning of this study was inherently shorter since the identification database contained fewer images. Timing for annotation and automated comparison of the identification database were likely similar for the entire study period. I also report the percent of recaptures per survey because this influenced the total time spent on manual visual inspection for a survey date's digital image set.

***Image processing in ImageJ*—**I measured salamanders from digital images obtained in the field, downloaded to a computer, and opened with ImageJ 1.48v. I calibrated each image to a 1 cm scale using the metered tape that was included in all digital images. I then measured a dorsal body length metric I refer to as snout-furrow length (SFL) by tracing a segmented line along the mid-dorsal surface of the salamander's body (Figure 6). This mid-dorsal line starts at the nose tip and follows a

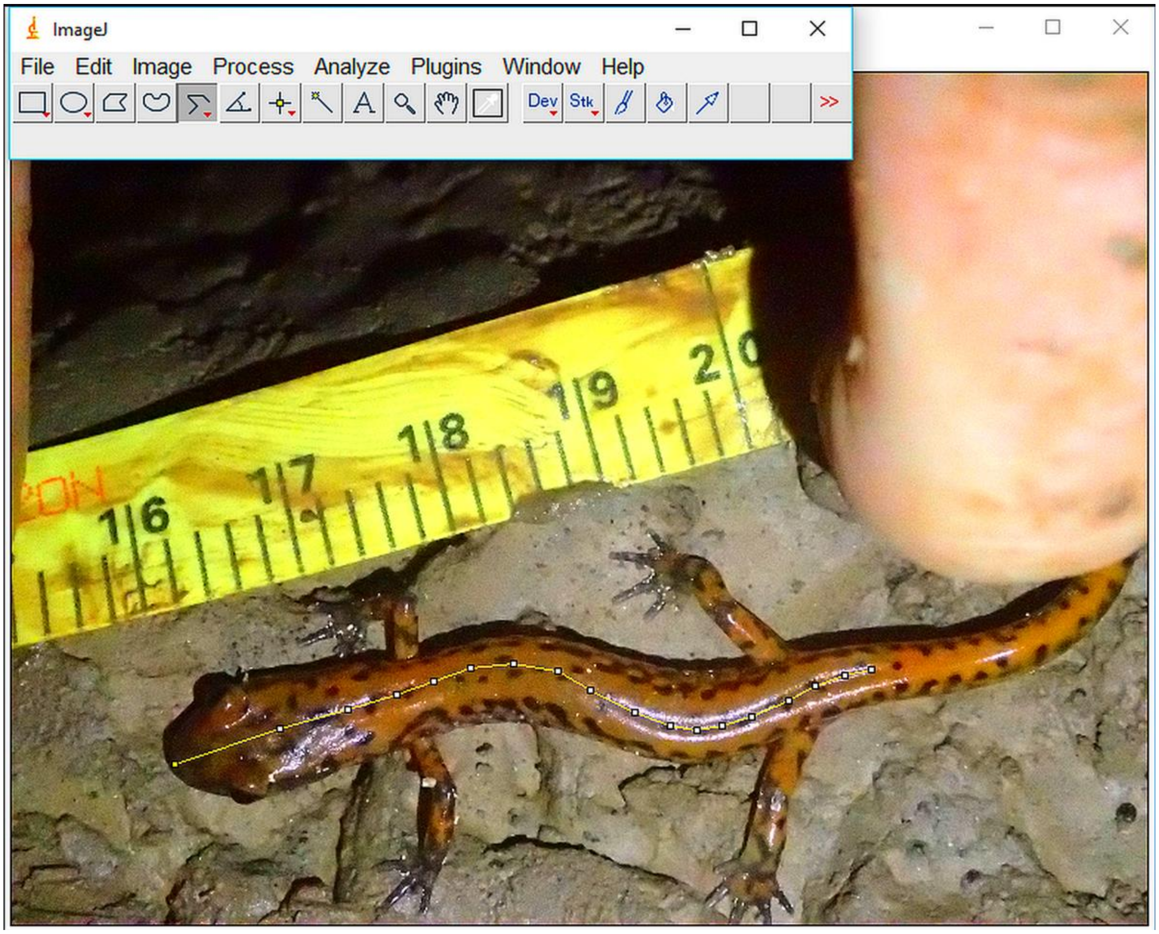


FIGURE 6. The SFL measurement using a scaled image in ImageJ (tape in image is in cm). The line with dots starts at the nose tip of the salamander, tracing the median furrow (which can be seen via glare in the image) to its termination posterior to the hind limbs. (Photograph in image by J. Gavin Bradley)

furrow along the median dorsal surface that terminates just posterior to the hind limbs. The termination point of this median furrow is nearly the same distance from the snout tip as the posterior margin of the vent on the ventral surface of the salamander; thus, SFL measures may be very similar to SVL measures. I confirmed this equivalence by examining preserved specimens of *E. lucifuga* to verify that these two anatomical features corresponded.

Statistical analyses**—**All summary statistics for individual identification and measurement procedures are reported by the mean (± SE). Normality of all body length measurement data was assessed visually and by Shapiro-Wilk tests ($\alpha = 0.05$). I compared SFL to SVL in a pairwise manner by subtracting SFL from SVL for each individual and using a one-sample *t*-test to determine whether the difference between these measures were significantly different from zero ($\alpha = 0.05$). I calculated the coefficients of variation (CV%) (± SE) for SFL and SVL to compare the precision of my measurements to the precision of SVL measurements for Small-mouthed Salamanders, *Ambystoma texanum* (Matthes, 1855), from three different methods reported by Walston and Mullin (2005). I also conducted a regression analysis and developed a regression equation for SFL *vs*. SVL as Bendik (2017) did for the Jollyville Plateau Salamander, *E. tonkawae* (Chippindale, Price, Wiens, and Hillis, 2000), to further address the relationship between these body length metrics. All statistical analyses were carried out in R version 3.4.1 (R Core Team, 2017), and data visualization was accomplished using R and Microsoft Excel 2016.

RESULTS

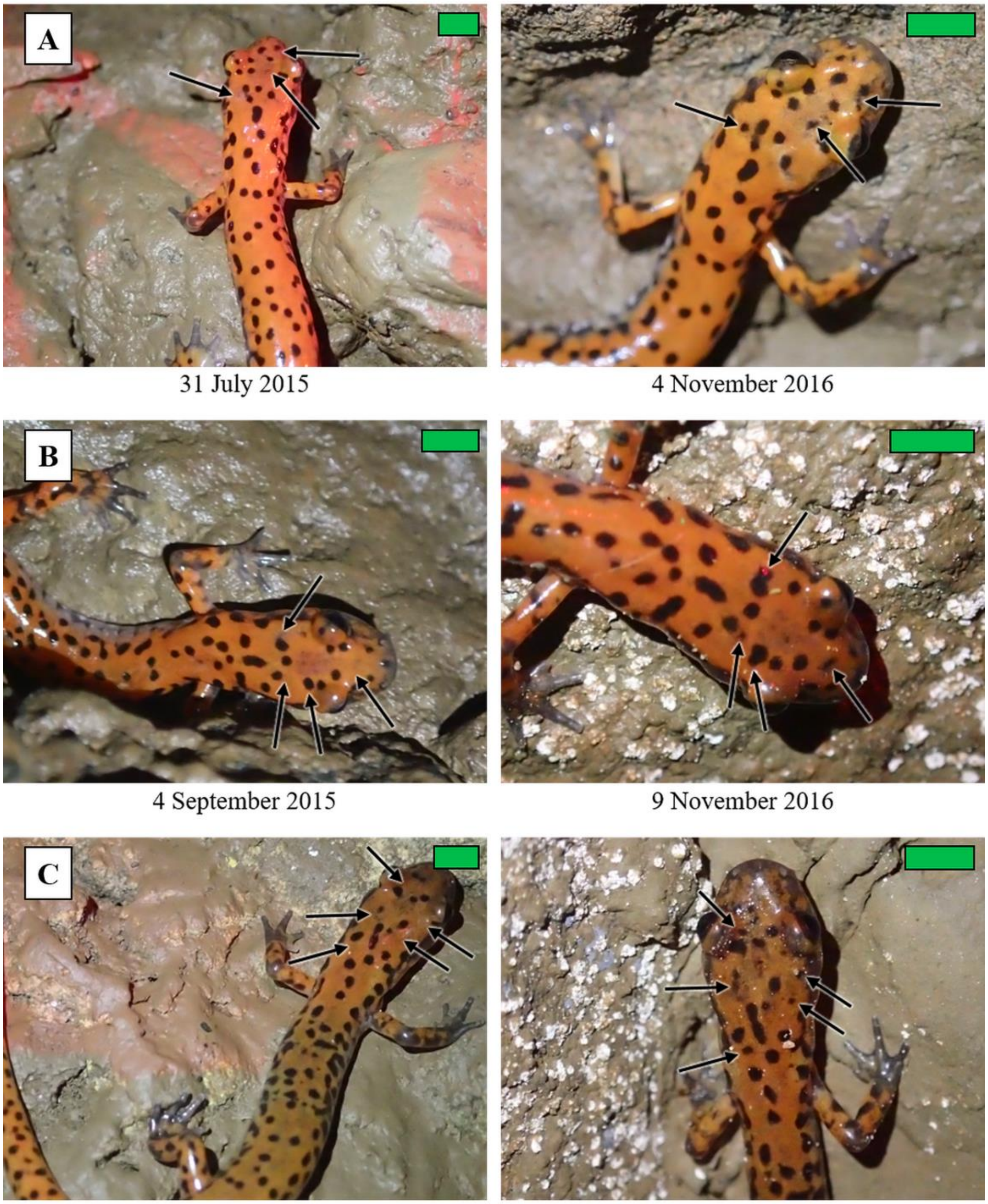
General**—**I conducted 70 surveys from March 2015–December 2016, with 32 surveys in year one (12 months: March 2015–February 2016) and 38 in year two (10 months: March–December 2016). Four surveys were conducted from March–April 2017.

I*^{3}*S-S**—**I identified 1072 individual salamanders in Sauerkraut Cave from March 2015–December 2016, of which 580 were recaptured at least once, 16 of these being captured ≥ 10 times (Table 1). The length of time between recaptures varied, with some individuals being recaptured on consecutive surveys and others being recaptured months to greater than one year apart, with one individual being recaptured after 19 months. The overall spot patterns of individuals were consistent throughout this length of time, with minor changes (addition of smaller spots or slight changes to preexisting spots) occurring in at least 12 individuals (Figure 7).

The I^{3}S-S positively matched 69.9% (N = 736) of new images in 1053 comparisons to images in the identification database (849–1057 total images in the identification database) from March–November 2016. Of the 736 positive matches, 61.3% (N = 451) had a match (i.e., recapture) within < 1% of the inspected ranked list output (Figure 8), with 62.4% (N = 459) having a recapture within the top 10 ranked images, and 53.3% (N = 392) matching the top-ranked image. The mean score value for this < 1% group was 18.58 ± 0.69 (range: 1.75–153.35) (Table 2). I found 10 positive matches for new images (0.95% of all comparisons) that had scores higher than the cutoff score (250.00) and that were beyond the respective 10-image set. I manually searched the

TABLE 1. Number of identified individuals (%) and number of captures per individual salamander.

# times individuals captured	N
1–4	947 (88.3)
5–9	109 (10.2)
≥ 10	16 (1.5)
Total identified	1072



31 July 2015

4 November 2016

4 September 2015

9 November 2016

26 June 2015

18 November 2016

FIGURE 7. Spot change in three individuals (**A**, **B**, and **C**). Images in the left column are from 2015 and on the right from 2016. Arrows indicate the area where spot change, i.e., the addition of a new or change in pre-existing spots, occurred (scale bars = 0.5 cm). (Photographs by J. Gavin Bradley)

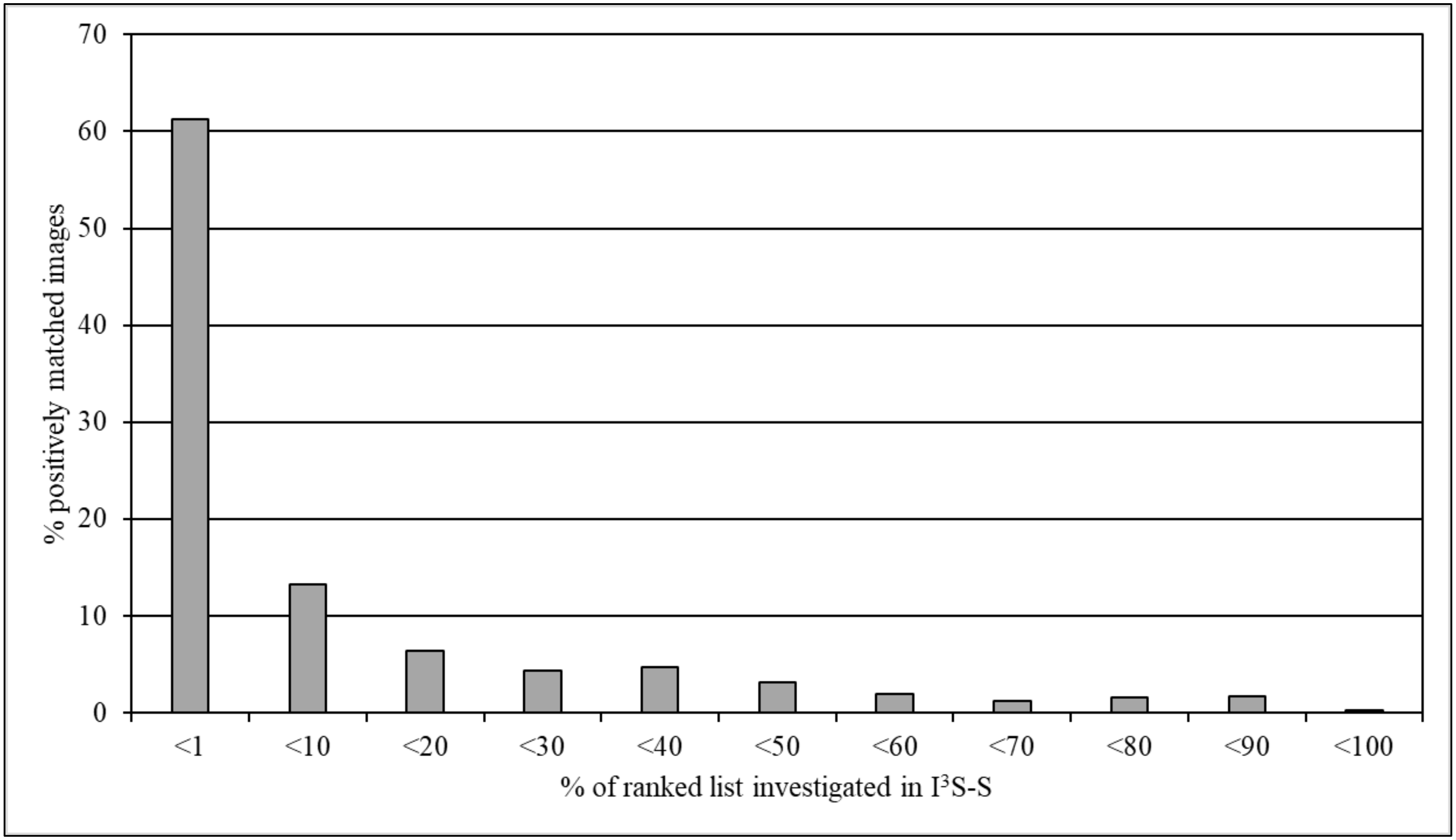


FIGURE 8. I^3S-S comparison success of newly analyzed images in which a positive match was found in the identification database.

TABLE 2. Percentage of ranked list (identified as Groups) visually compared and comparison scores before finding a positive match for newly annotated images.

Group	% images	Mean score (± SE)	Score range	N
<1	61.3	18.58 (0.69)	1.75–153.35	451
<10	13.3	66.40 (2.07)	32.27–135.81	98
<20	6.4	88.43 (3.27)	48.92–150.43	47
<30	4.3	114.59 (5.58)	69.11–192.09	32
<40	4.8	128.72 (4.68)	79.45–211.93	35
<50	3.1	135.10 (6.13)	85.15–202.86	23
<60	1.9	172.04 (13.19)	98.19–263.38	14
<70	1.2	175.56 (14.61)	87.09–229.14	9
<80	1.6	232.17 (40.48)	121.56–604.03	12
<90	1.8	240.70 (10.68)	170.63–289.66	13
<100	0.3	236.68 (20.29)	216.39, 256.97	2

ranked list because individuals exhibited familiar patterns in five of these cases and because images were of poor quality in the other five cases (i.e., photos were taken at oblique angles or were not in focus). Of the additional 46 images that I selected for manual comparison against the entire ranked list, i.e., every seventh image without a recapture at or below the cutoff score, none had a recapture in the ranked list. Image processing times for all procedures in I^{3}S-S are presented in Table 3. Mean percent recapture per survey for the study period was 57.09 ± 2.89% (range: 0.00–90.91%, N = 68), and percent recapture was consistently greater than this mean percent recapture after 32 surveys (Figure 9).

ImageJ—Mean SFL for the 82 adult salamanders was 5.96 ± 0.05 cm (range: 4.94–7.27 cm) and mean SVL was 6.07 ± 0.04 cm (range: 5.18–6.88 cm). The mean

TABLE 3. Image processing times in I^3S-S. Mean values are presented in decimal min.

Category	Mean (± SE)	Range	N
Annotation	2.37 (0.024)	1.35–3.87	256
ID comparison	0.10 (0.001)	0.05–0.23	224
Visual identification:			
Recapture	2.11 (0.26)	0.18–20.50	175
New	19.10 (0.43)	8.20–27.70	81

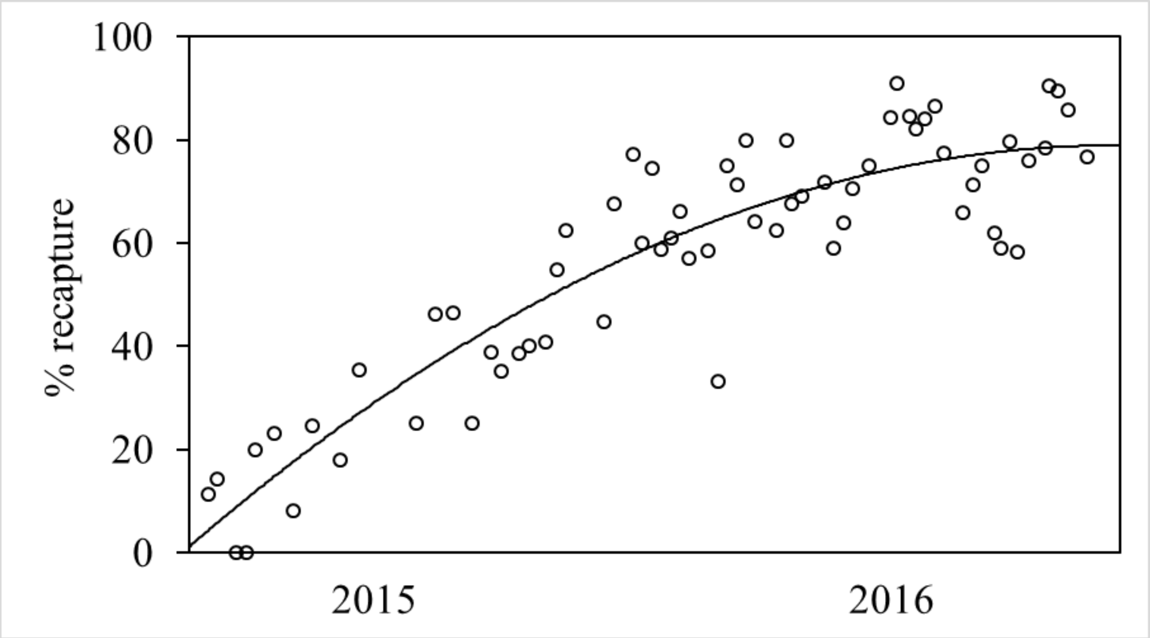


FIGURE 9. Percentage of recaptures per survey for *E. lucifuga* from March 2015–December 2016.

difference between SFL and SVL was -0.11 ± 0.03 cm (range: -0.81–0.47 cm), and in 56 of 82 (68.3%) salamanders measured SFL was smaller than SVL, suggesting a small but consistent difference between the two methods. The Shapiro-Wilk test showed that SFL ($W = 0.990$, $P = 0.810$), SVL ($W = 0.986$, $P = 0.549$), and the values of difference between SFL and SVL ($W = 0.986$, $P = 0.500$) were all normally distributed. The one-sample *t*-test showed that SFL and SVL differed significantly ($t = -3.789$, df = 81, $P < 0.001$). The CV% values for SFL and SVL were 7.65 ± 0.597 and 6.05 ± 0.473, respectively. Regression analysis of SFL *vs*. SVL determined the best fit model to the data was a linear model ($F_{1,80} = 142.9$, $P < 0.0001$; AIC: -10.572) with the regression equation: SVL = 0.646(SFL) + 2.222 ($R^2 = 0.641$) (Figure 10).

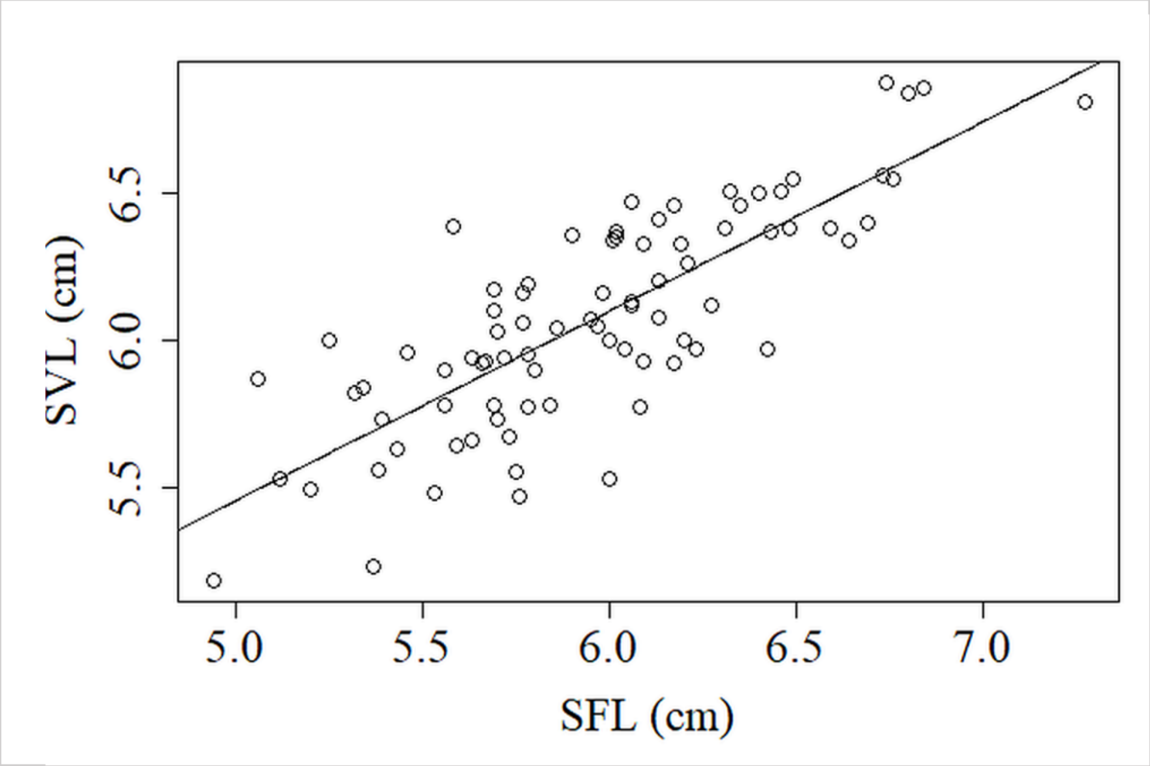


FIGURE 10. Linear regression plot with line of best fit equation: SVL = 0.646(SFL) + 2.222.

DISCUSSION

The use of digital imaging has become increasingly popular in ecological studies, and in conjunction with pattern-recognition and scaling software it provides a useful means of identifying animals that exhibit discernible patterns and for acquiring body length metrics. The degree of success in using these applications is inherently determined by the visual quality of images obtained from photographing individual organisms. Key variables like animal orientation and posture, which can influence the automated matching capabilities of pattern-recognition software like I^{3}S (den Hartog and Reijns, 2014), are not always under the control of the investigator. Such was the case in this study. Manipulation of *E. lucifuga* can change their behavior, thus, I chose to use non-invasive methods as a precautionary measure. Here, I provide insight on the results obtained by these methods, the consequences of this methodology, and the relative success of these non-invasive techniques for studying *E. lucifuga*.

I^{3}S-S—A critical assumption when using pattern-recognition for individual identification is that individuals' patterns will not change greatly over time. Evidence of spot change is documented in salamanders to varying degrees. Waye (2013) found that eight Tiger Salamanders, *A. tigrinum* (Green, 1825), exhibited slight changes in pattern on their dorsal and/or ventral surfaces over one year, while three exhibited drastic changes in pattern. The number of spots on *E. l. longicauda* increases with individual size, suggesting that spot patterns change to some degree over time in this species (Jonas *et al.*, 2011; Nazdrowicz, 2015). However, these authors indicated that the overall, general pattern of individual *E. l. longicauda* did not change, making recognition by spot pattern over time possible. Similarly, some *E. lucifuga* individuals from my study exhibited slight changes in spot pattern within the reference area over time, but retained their overall pattern, which demonstrates use of the spot pattern for recognition is appropriate for this species.

When using patterns for individual identification, the body area of an animal chosen for recognition is also critical. The I^3S-S assumes linearity of the animal in an image, which is why it is important to select a region of the animal that is less susceptible to bending, such as the neck and head of salamanders. Alternatively, there are straightening algorithms that may be applied to straighten an animal in an image. Gamble *et al.* (2008) used images with the whole dorsal surface of Marbled Salamanders, *A. opacum* (Gravenhorst, 1807), for individual recognition. Salamanders were individually placed in a box with a grid background to obtain standardized images. A straightening algorithm was then applied to images before processing with pattern-recognition software. Although these methods were successful, I did not use them because I used only the spot pattern of the head and neck, a relatively linear and short portion of the body. For Cave Salamanders, these areas were sufficient to identify individuals, and spot patterns from the entire dorsal surface were not consistently visible, often because salamanders were found with only their head and necks protruding from holes or physically complex areas of the wall and ground.

The I^3S program has been shown to match images successfully 95% (Rocha *et al.*, 2013) and 83% (Speed *et al.*, 2007) of the time as compared to images matched by-eye, and Rocha *et al.* (2013) found this method to be significantly faster than by visual comparison. These studies controlled for postural positioning and other effects, such as glare, flight behavior, and availability of body surface, which increases the success of I^3S.

These steps could not be taken due to the non-invasive nature of this study, and so the success rates I present are not comparable to these studies. However, I^3S-S successfully matched 61.3% of images within the top 1% of the ranked list despite the absence of controls for postural positioning and other aforementioned effects, and using I^3S-S greatly facilitated the sorting and comparison procedures. Furthermore, there were few instances (0.95% of all image comparisons) when I^3S-S could be deemed unsuccessful under my criteria.

Total image processing time in I^3S-S was primarily dependent upon whether an individual salamander was a recapture or not and the quality of the image. It does not take long for the user to become accustomed to the annotation process, and the speed at which annotation can be completed increases to a certain degree after *ca.* one month. The greatest time impediment is the manual comparison procedure for non-recaptured individuals, which took increasingly longer as the database grew. Over the course of the study, the percentage of recaptures increased, which alleviates this issue somewhat since there are more known individuals in the identification database. However, the number of recaptures per survey varied greatly and completion of individual processing for all salamanders noticeably took a longer total time when the percentage of recaptures was below 60% for a survey date.

***ImageJ*—**Mean SFL and SVL for this population of Cave Salamanders is similar to SVL measures reported by Carlyle *et al.* (1998), Hutchison (1958), Juterbock (2005), and Williams (1980) for other Cave Salamander populations, indicating my measurements are accurate. Although I found that SFL differed significantly from SVL, this was due to a very small and consistent difference between the two measurements, with SFL on average 0.11 (± 0.03) cm less than SVL. My calculated CV% values indicate that SFL measurements were slightly less accurate than my SVL measurements. Some variation around the SFL mean is probably due to measurement error for those salamanders exhibiting dorso-ventral body curvature when resting on uneven substrates in the cave, which reduces the apparent body length of salamanders in two-dimensional digital images. Lateral body curvature, which was common, might also result in slightly inaccurate estimates; however, the segmented line tool in ImageJ accommodates this situation. This is reflective of the minimal disturbance toward Cave Salamanders in this study since I did not manipulate their posture or orientation at all for imaging purposes. Thus, I suggest the difference between my length measurements is due to measurement error caused by body curvature of individuals, and that measures via the anatomical features used for SFL and SVL are fairly similar.

My CV% values are higher, and thus less precise, than those reported for SVL measures using freehand, tube, and stick techniques (CV% ± SE: 4.2 ± 0.4, 4.4 ± 0.4, and 2.7 ± 0.3, respectively) (Walston and Mullin, 2005). Walston and Mullin (2005) measured *A. texanum* using these methods in the highly controlled environment of a laboratory, whereas I was in the confines of a small spring cave, the majority of which is stoop-walking or crawling passage. I was forced into difficult positions to acquire some photographs of salamanders (Figure 11) that undoubtedly reduced the quality of some images, which may have negatively affected those SFL measurements. Likewise, some SVL measures had to be conducted on uneven surfaces, which may have decreased the precision, and thus the accuracy, for some of these measurements. So, it is to be expected that body length measurements will be more precise when measuring conditions are

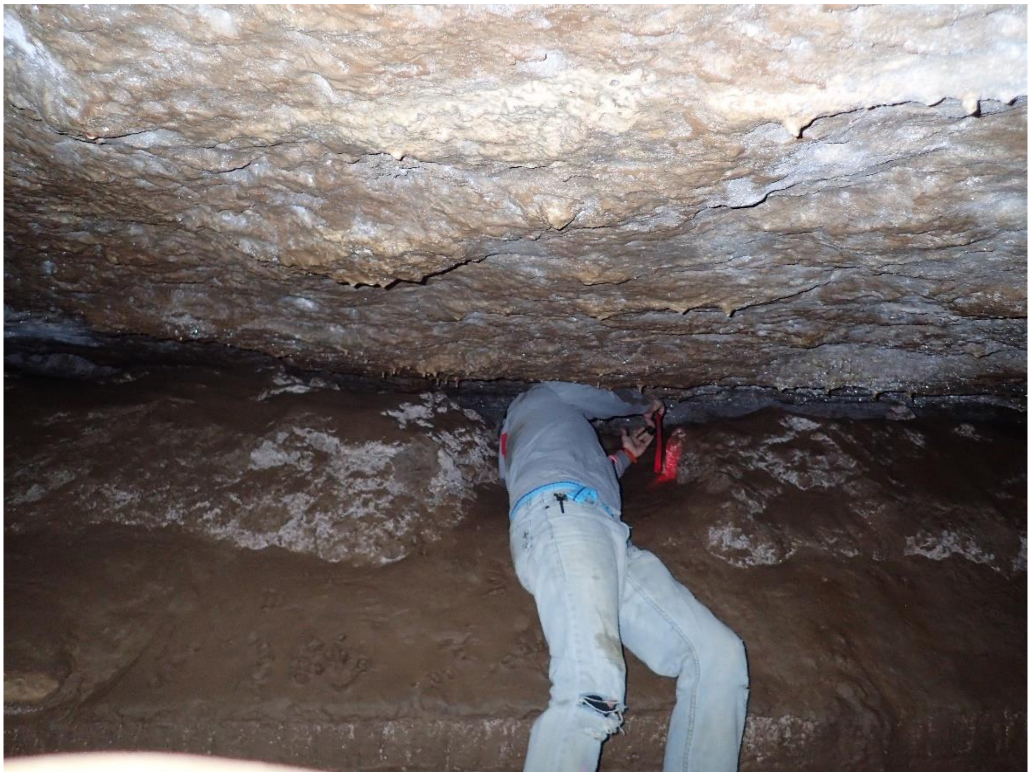


FIGURE 11. J. Gavin Bradley taking a photograph of a Cave Salamander. (Photograph by Paige Wilson)

under greater control. Still, my resulting CV% values are relatively low, indicating a reasonable level of precision across individual measurements.

The regression analysis indicates a linear relationship between SFL and SVL. Bendik (2017) obtained a higher R^2 (0.99) value for his regression of body length (measured from the snout tip to the posterior hindlimb insertion) *vs*. SVL for *E. tonkawae* than I did for my regression of *E. lucifuga* metrics, implying a stronger relationship between his length measures. However, Bendik (2017) captured, anesthetized (as part of a tagging procedure), and photographed salamanders on a standardized grid background, thus minimizing salamander movement and controlling body posture. These factors likely influence user accuracy when measuring digital images in ImageJ because issues such as blur and/or body distortion are minimized. Due to my non-invasive approach, I did not capture, anesthetize, or handle salamanders, and thus could not influence these factors to increase measurement accuracy or precision. It is likely that SFL for Cave Salamanders is more strongly related to SVL than my results suggest because of these factors. However, my results nonetheless indicate a strong relationship between SFL and SVL for Cave Salamanders, which further suggests that SFL is an appropriate body length metric for *E. lucifuga*, especially considering that SFL measures agree with previously reported SVL measures of this species.

***Conclusion*—**The use of digital imaging for individual identification via pattern recognition software and standard measurements provides valuable non-invasive methods for studying *E. lucifuga*. It is clear when certain factors, like animal orientation and posture, are controlled, the matching capabilities of programs such as I^{3}S-S are greatly improved. However, for highly non-invasive studies, pattern-recognition is very useful. Similarly, ImageJ is accessible and provides reasonably accurate and precise non-invasive body length metrics. Thus, I suggest these methods can be of great utility for researchers employing non-invasive techniques to study salamanders, or other organisms that exhibit unique and distinguishable characteristics.

CHAPTER III

OBSERVATIONS ON THE POPULATION ECOLOGY OF THE CAVE SALAMANDER, *EURYCEA LUCIFUGA* (RAFINESQUE, 1822)

INTRODUCTION

Salamanders are generally considered to be important ecological components in part because they are often the most abundant vertebrate taxa in certain systems, such as temperate forests (Burton and Likens, 1975a; Petranka *et al.*, 1993; Semlitsch *et al.*, 2014; Whiles *et al.*, 2006). This, combined with their mid-level trophic position, has important implications at community and ecosystem levels. As keystone predators, salamanders indirectly regulate nutrient cycling through control of food webs (Best and Welsh Jr., 2014; Davic and Welsh, 2004; Walker *et al.*, 2014; Walton, 2013). As prey, they contribute considerable biomass to food webs and are significant energy reservoirs because they efficiently convert assimilated energy to new tissues (Burton and Likens, 1975a, 1975b; Semlitsch *et al.*, 2014). These characteristics are important because most salamander species couple aquatic and terrestrial ecosystems via resource flow due to their biphasic life cycle (Burton and Likens, 1975b; Davic and Welsh, 2004; Regester *et al.*, 2006; Schriever *et al.*, 2014). These factors give credence to the idea that salamanders play integral roles within their ecosystems. Moreover, salamanders are indicators of

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Bradley JG, Eason PK, 2018, Observations on the population ecology of the Cave salamander, *Eurycea lucifuga* (Rafinesque 1822), *J Caves Karst Stud*, (Ms.\@ No.\@ JCKS-S-17-00037).

ecosystem health (Welsh Jr. and Droege, 2001; Davic and Welsh, 2004 and references therein), further substantiating their environmental significance.

These ecological generalizations are primarily based upon epigean salamander species. Knowledge of such relationships for hypogean species is comparatively lacking. It is important to investigate these ecological connections because hypogean environments, such as caves, and their inhabitants are usually of conservation concern (Culver *et al.*, 2000). Many species of salamanders are associated with caves (Lannoo ed., 2005; Petranka, 1998), ranging along a gradient from complete dependence to differing degrees of facultative use of this habitat. Given these associations and the characteristics described above, salamanders likely have a strong influence on cave ecology (Lunghi *et al.*, 2014; Romero, 2009).

The Cave Salamander, *Eurycea lucifuga* (Rafinesque, 1822), is a trogloxenic species, i.e., a facultative cave dweller that must leave the cave to obtain one or more epigean resources (Trajano and Carvalho, 2017), that is native to the eastern United States (Camp *et al.*, 2014; Hutchison, 1958; Petranka, 1998; Williams, 1980). Cave Salamanders are relatively common, but little more than some natural history, morphology, physiology, and basic ecological information exists for this species. However, Cave Salamanders may be important constituents of subterranean ecosystems. To assess this, I collected data on a population of Cave Salamanders from a Kentucky cave to report basic demographic information, open population model parameters, wet-biomass, and wet-biomass and salamander densities. Population modelling and estimates of biomass and density provide important species information, which facilitates a greater understanding of the ecological influence a species has in its ecosystem. The information herein represents the first approximation of these characteristics for Cave Salamanders and will further inform the ecological relationships salamanders have with subterranean ecosystems.

METHODS

***Study site*—**I monitored a population of *E. lucifuga* along two transects, one 99 m in length and the other 83.2 m in length, in the primary channels of the main and side passages, respectively, of Sauerkraut Cave (Chapter 2). I generally conducted surveys of these transects weekly from March 2015–February 2017, but survey dates were modified occasionally due to flooding or scheduling difficulties. In each passage, I searched the walls, floor, ceiling, and standing water for terrestrial (adult and juvenile) salamanders using red-filtered light during daylight hours (typical start time between 1100–1230 h). Hutchison (1958), Williams (1980), and Briggler and Prather (2006) similarly conducted surveys of this species during daylight hours.

***Field data collection*—**I counted all salamanders found and used an Olympus TG–4 digital camera to photograph the dorsal spot pattern of the head and neck of each salamander in plain sight. These images were used for individual identification in I^{3}S-S version 4.0.2 (Chapter 2). I also photographed the entire dorsal surface of individuals in plain sight and next to metered tape to create scaled images from which I measured snout-furrow length (SFL), a similar measure to conventional snout-vent length (SVL) (Chapter 2), in millimeters (± SE) using ImageJ 1.48v. I determined gender by secondary sexual characteristics (males: swollen mental gland and elongated oral cirri; females: ovaries visible through the body wall) (Figures 12 and 13) that were particularly evident

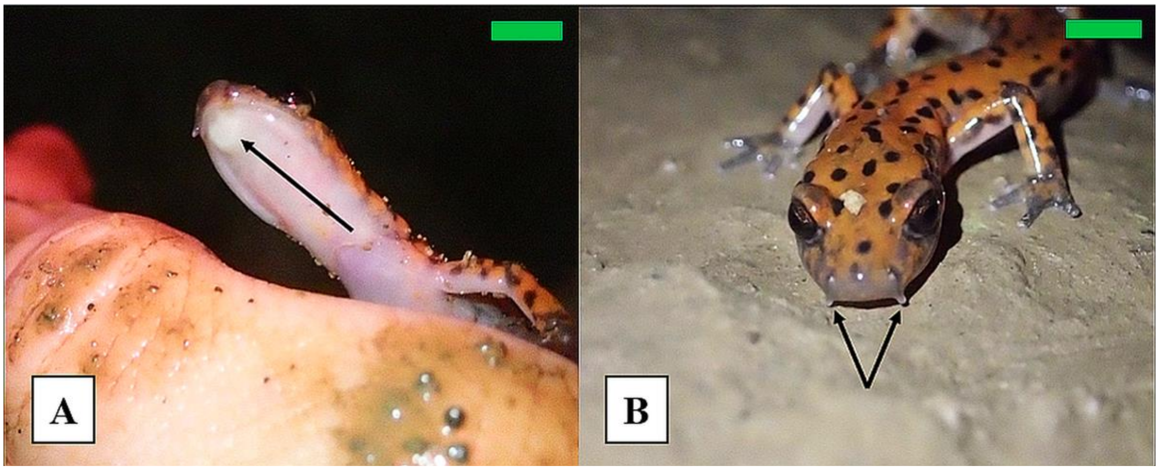

FIGURE 12. Images depicting the swollen mental gland (**A**) and elongated oral cirri (**B**) indicating a male (scale bars = 5 mm). (Photographed by J. Gavin Bradley)

from July–November. I estimated age classes (adult and juvenile) by SFL; referencing measurements of SVL at sexual maturity reported by Carlyle *et al.* (1998) and Hutchison (1966) for Cave Salamanders, I classified individuals ≥ 49 mm SFL as adults.

***Statistical analyses*—**Summary statistics are presented as the mean (± SE). I used a χ^2 goodness-of-fit test to determine if the observed sex ratio of this population differed ($\alpha = 0.05$) from the expected 1:1 ratio. I estimated population parameters (i.e., abundance, capture and survival probabilities) using recapture histories in open (Jolly–Seber) population models. Open population models allow births, deaths, immigration, and emigration to occur and thus are more realistic for long-term studies. The repeated surveys resulted in recapture histories for those individuals that were seen and "marked" (i.e., photographed) more than once. I conducted model estimation by season: spring (March–May), summer (June–August), fall (September–November), and winter (December–February). I assessed model fit with Akaike Information Criterion and by

FIGURE 13. Image depicting ovaries (yellow portion of belly) visible through the body wall indicating a female (scale bar = 5 mm). (Photographed by J. Gavin Bradley)

refitting models with Pearson residuals ≥ 2 (Baillargeon and Rivest, 2007; Beck *et al.*, 2013). Estimates for each sampling period within a season were pooled to provide a single mean (± SE) value of each parameter for each season.

To estimate salamander population biomass, I modified the equation $W = a\mathrm{SVL}^b$ (Huntsman *et al.*, 2011; Salvidio, 1998) by substituting SVL with SFL (i.e., $W = a\mathrm{SFL}^b$), to calculate individual salamander weight (W). To estimate the constants *a* and *b*, I collected, weighed, and measured salamanders from March–April 2017, after the original study period. I measured SFL, as described previously, using ImageJ. I obtained individuals’ W by directing salamanders by hand or an aquarium net into a tared plastic bag containing a paper towel wetted with cave water and then weighed them in grams (± SE) using a Pesola Micro-Line spring scale (Pesola AG, Schindellegi, Switzerland). I then released them at the site of capture. I noted gender during this procedure using similar sexual characteristics as described above; individuals too small to exhibit sexual characteristics were classified as juveniles. Capture and handling followed guidelines by HACC (2004), Shaffer *et al.* (1994), and Stasiak (2015).

I developed regressions (predictor variable = SFL; response variable = W) for males (N = 36), females (N = 20), and juveniles (N = 4) using log-transformed data for preliminary comparisons to determine whether there were differences in regressions between males *vs*. females and adults *vs*. juveniles. I conducted these comparisons using ANCOVA. Since no significant differences in the relationship between SFL and W were detected among groups (see Results), one common regression equation was developed using non-log-transformed data. I used that equation and SFL measurements acquired from March 2015–February 2017 to estimate individuals' W for the study population. I calculated the mean (± SE) individual W and then multiplied the mean W by seasonal abundances to estimate seasonal wet-biomass following Salvidio (1998) and Crawford and Peterman (2013). I estimated mean (± SE) wet-biomass density (g m^{-2}) by first multiplying the mean W by the mean salamander count, then dividing this number by the available surface area of the walls and floor of the cave. I estimated mean (± SE) salamander density (salamanders m^{-2}) by dividing the mean salamander count by the same available cave surface area.

All statistical analyses were conducted using R statistical software 3.4.1 (R Core Team, 2017) and data visualization was accomplished in R or Microsoft Excel 2016. I analyzed population models using Rcapture (Baillargeon and Rivest, 2007) in R.

RESULTS

The mean salamander count per survey was 66.10 ± 4.00 (range: 10–245 salamanders, N = 73) and the mean number of salamanders photographed per survey was 38.40 ± 2.97 (range: 0–172 salamanders, N = 72), with a total of 1127 individual salamanders identified. Mean monthly counts are presented in Figure 14. The numbers of individuals identified as males and females were 97 and 106, respectively. The sex ratio is 1:1.1, which does not deviate significantly from a 1:1 ratio ($\chi^2 = 0.40$, df = 1, P = 0.528). Mean SFL was 55.26 ± 0.41 mm (range: 25.84–74.77 mm, N = 380) in study year one (March 2015–February 2016), with 80.3% of individuals sexually mature (≥ 49 mm SFL). Similarly, mean SFL was 56.82 ± 0.37 mm (range: 35.95–73.91 mm, N = 364) in study year two (March 2016–February 2017), with 81.6% ≥ 49 mm SFL. Open population models revealed that salamander abundance was highest in spring 2016, with the maximum abundance estimated at 492 ± 77.2 individuals (Table 4 and Figure 15). Capture probabilities (Table 4) were correspondingly low in spring 2016 (0.04 ± 0.01 probability); the probability of being captured in the cave was also relatively low in summer (0.13 in 2015 and 2016) but higher in fall and winter (range: 0.20–0.29 probability). Probability of survival was high in spring and summer 2015 and spring 2016 (range: 0.92–0.96 probability) and lowest in fall (0.71 and 0.73 for 2015 and 2016, respectively).

Regressions of SFL and W showed that the relationship between length and weight did not differ significantly in either males *vs*. females or adults *vs*. juveniles ($F = 0.37$, df

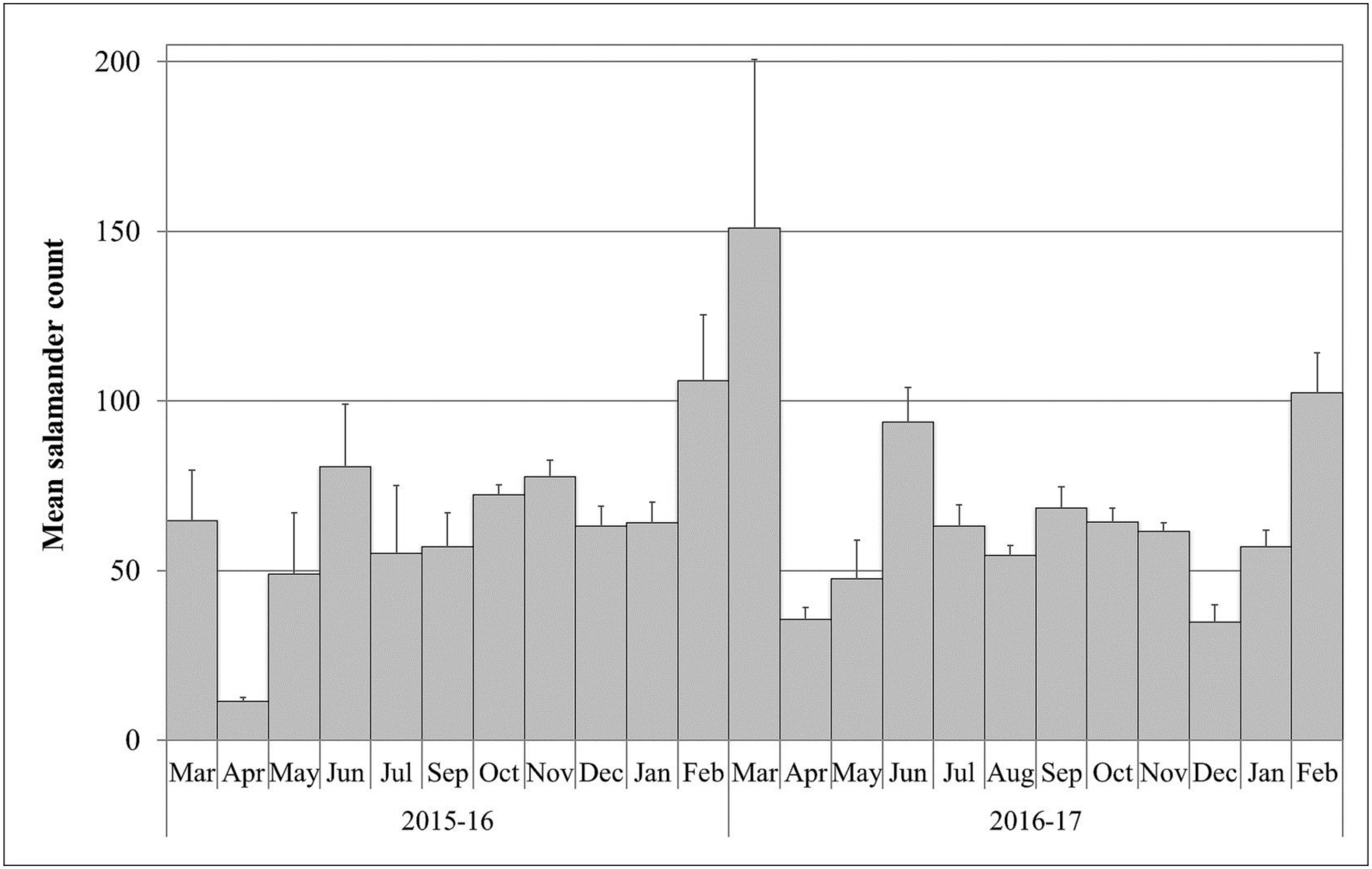

FIGURE 14. Mean monthly Cave Salamander count in Sauerkraut Cave. Error bars are standard error (SE).

TABLE 4. Parameters calculated in open population models in the Rcapture package of R. Values are presented as the mean ± SE.

Season	Year	Abundance	Capture probability	Survival probability
Spring	2015	118 ± 46.5	0.068 ± 0.029	0.923 ± 0.095
Summer	2015	229 ± 109	0.126 ± 0.063	0.961 ± 0.352
Fall	2015	154 ± 31.8	0.204 ± 0.048	0.709 ± 0.063
Winter	2015–16	158 ± 22.6	0.227 ± 0.032	0.858 ± 0.043
Spring	2016	492 ± 77.2	0.038 ± 0.010	0.930 ± 0.024
Summer	2016	204 ± 31.1	0.127 ± 0.024	0.798 ± 0.040
Fall	2016	123 ± 11.8	0.292 ± 0.038	0.730 ± 0.037
Winter	2016–17	134 ± 17.8	0.208 ± 0.029	0.850 ± 0.026

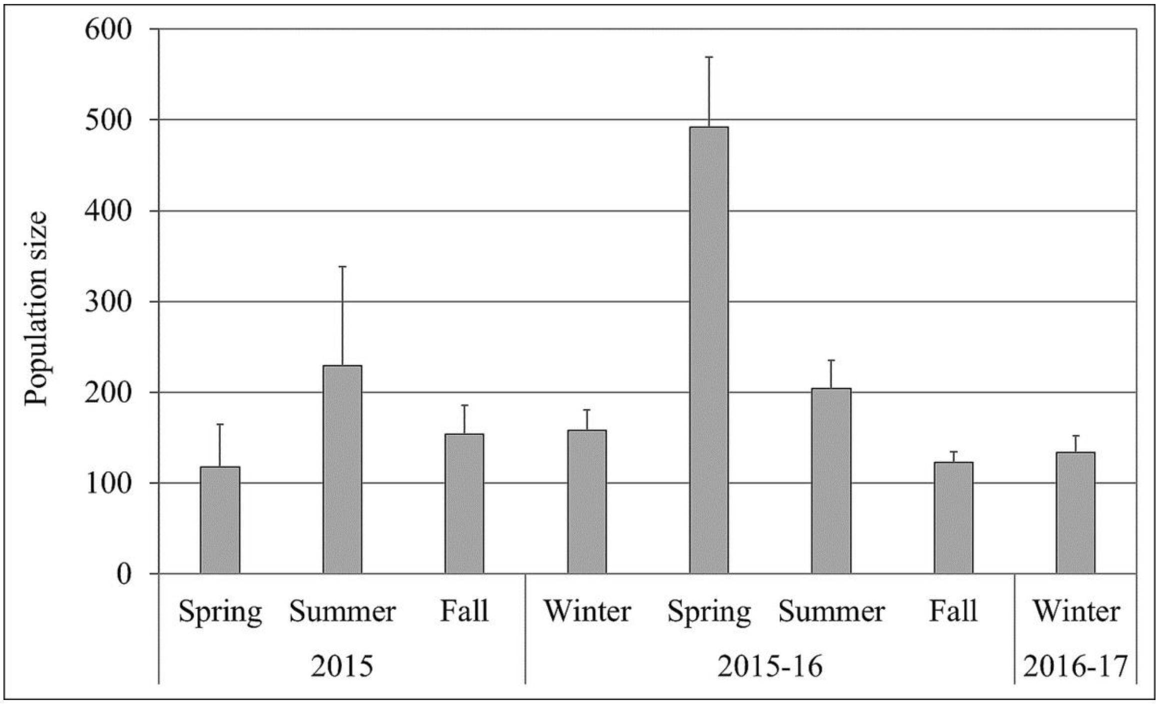

FIGURE 15. Seasonal estimates of population size of Cave Salamanders in Sauerkraut Cave. Error bars are standard error (SE).

= 1, P = 0.55 for males *vs*. females; *F* = 1.58, df = 1, P = 0.21 for adults *vs*. juveniles). Thus, I used a common regression line for all individuals, $W = 0.0155(SFL)^{3.0042}$ (Figure 16). Mean calculated W for salamanders with measured SFL from March 2015–February 2017 was 2.90 ± 0.042 g (range: 0.27–6.31 g, N = 649). Estimates of seasonal wet-biomass (Table 5) showed high biomass in spring 2016 and relatively low biomass in fall and winter, as would be expected given the numbers of salamanders seen during respective surveys. The total available surface area of walls and floor in Sauerkraut Cave

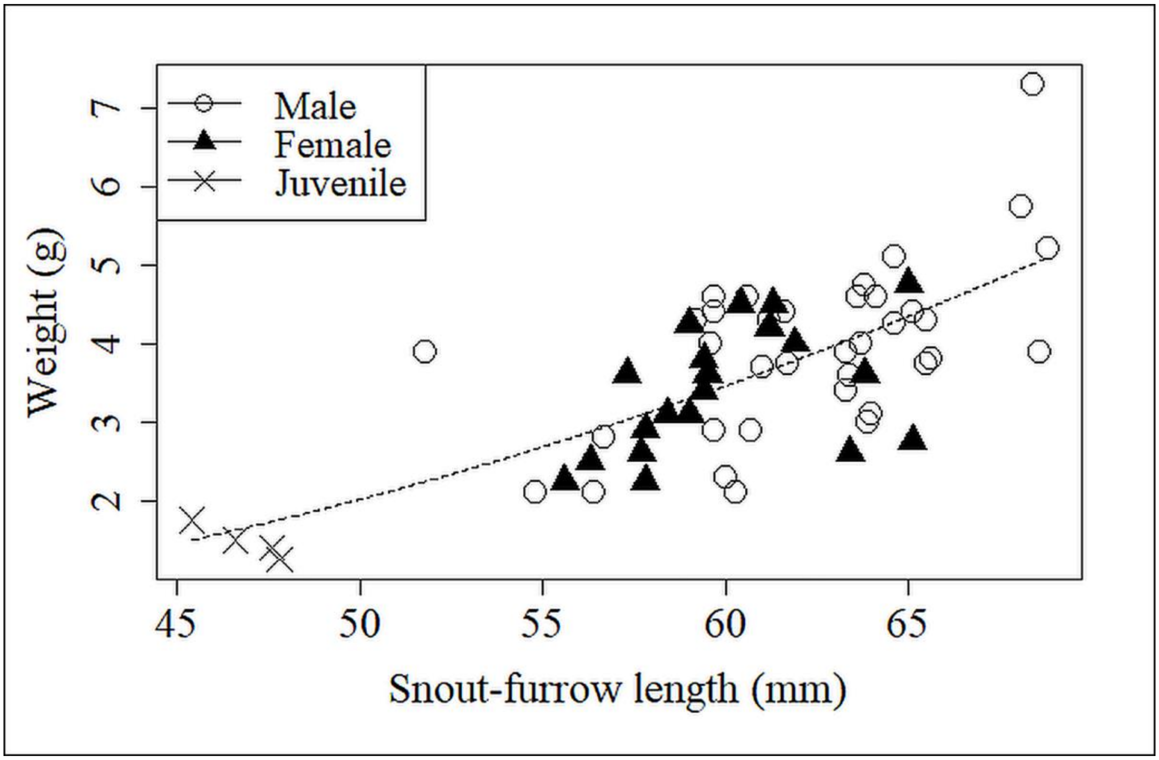

FIGURE 16. Scatterplot and power trendline, $W = 0.0155(SFL)^{3.0042}$, for the relationship between SFL and W for male, female, and juvenile Cave Salamanders.

TABLE 5. Estimates of seasonal population wet-weight biomass for Cave Salamanders at Sauerkraut Cave.

Season	Year	Wet-weight biomass (g)
Spring	2015	342.2
Summer	2015	664.1
Fall	2015	446.6
Winter	2015 – 16	458.2
Spring	2016	1426.8
Summer	2016	591.6
Fall	2016	356.7
Winter	2016 – 17	388.6

TABLE 6. Wet-weight biomass density and salamander density of Cave Salamanders in Sauerkraut Cave.

		Wet-weight Biomass Density (g m^{-2})			Salamander Density (individuals m^{-2})		
Passage	N	Mean	SE	Range	Mean	SE	Range
Main	76	0.34	0.022	0.04 – 1.37	0.12	0.007	0.01 – 0.47
Side	73	0.09	0.007	0.00 – 0.38	0.03	0.003	0.00 – 0.13
Total	73	0.22	0.014	0.03 – 0.83	0.08	0.005	0.01 – 0.29

for this study is 853.4 m^2 (main passage: 468.8 m^2; side passage: 384.6 m^2). Mean wet-biomass density was 0.22 ± 0.014 g m^{-2} (range: 0.03–0.83 g m^{-2}, N = 73). Mean salamander density was 0.08 ± 0.005 salamanders m^{-2} (range: 0.01–0.29 m^{-2}, N = 73). Mean wet-biomass density and salamander density for each passage separately is shown in Table 6.

DISCUSSION

The sex ratio is near the expected 1:1, which is similar to the findings of Williams (1980) for another population of this species at 1.125:1 (male:female). Hutchison (1958) found that males were more dominant in his study with a ratio of 1.6:1, mentioning there was no apparent reason for this deviation. He suggested that gravid females may become less active and/or more secluded than males during the breeding season to explain part of this bias. However, Hutchison (1958) sampled 11 months out of one year and consistently found more males than females regardless of season. No evidence was found in this study to support Hutchison’s suggestion. Mean snout-furrow length (SFL) for this population of Cave Salamanders is similar to snout-vent length measures reported by Carlyle *et al.* (1998), Juterbock (2005), Hutchison (1958), and Williams (1980) for other Cave Salamander populations. Mean SFL and the proportion of salamanders ≥ 49 mm SFL indicates adults are numerically dominant in this population with a relatively low presence of juveniles (19.7% in study year one and 18.4% in study year two). Juveniles composed 11.35% of marked individuals in Hutchison (1958) and Nazdrowicz (2015) found that juveniles of closely related Long-tailed Salamanders, *E. longicauda longicauda* (Green, 1818), comprised 11–35% of total populations. This pattern is characteristic of other plethodontid salamanders as well (Hairston Sr., 1987).

To my knowledge, this study provides the first estimates of abundance using open population models for *E. lucifuga*, and the first account of capture and survival probabilities for this species. Previously, Hutchison (1958) provided estimates of population size for *E. lucifuga* using a closed (Lincoln–Peterson) population model. His estimates of population size (36–63 individuals each in four caves in Virginia, USA) are low compared to mine, likely because he only sampled from the twilight zone of his study caves. *Eurycea lucifuga* may inhabit both the dark and twilight zones of caves (Green *et al.*, 1967), and population distribution patterns change seasonally within these zones (Camp *et al.* 2014; Hutchison 1958; Williams 1980; see Chapter 4). My observations in the dark zone of Sauerkraut Cave indicate that a substantial proportion of the population occurs there depending upon the time of year. Hutchison (1958) also pooled counts and recaptures for his entire study year, which violates the assumption of closure and thus results in poor model estimation; he acknowledged the inadequacies of using this model, suggesting rough estimates at best. I have found that population size estimates for cave-inhabiting *E. lucifuga* may be much larger, and probably more accurate, when individuals from both the dark and twilight zones are included in open population modelling. I believe my estimates are reasonable because they are within range of estimates for other species of salamanders occupying caves (Fenolio *et al.*, 2014; Huntsman *et al.*, 2011; Taylor *et al.*, 2015) or similar habitats, i.e., springhouses (Nazdrowicz, 2015) and wet rock faces (Crawford and Peterman, 2013; Salvidio, 1998) (Table 7).

TABLE 7. Population estimates and densities of salamander species that inhabit caves or similar habitat (i.e., springhouses and rock faces).

Source	Species	Population Estimate	Density (salamanders m^{-2})
Salvidio (1998)	*Hydromantes* [*Speleomantes*] *strinatii* (Aellen, 1958)	155	0.8
Huntsman *et al.* (2011)	*Gyrinophilus palleucus* (McCrady, 1954)	109, 215	0.03 & 0.10
Crawford and Peterman (2013)	*Desmognathus* spp.	496	14.69
Fenolio *et al.* (2014)	*Eurycea spelaea* (Stejneger, 1892)	342, 507	0.04 & 0.12 (larvae & adults)
Nazdrowicz (2015)	*Eurycea l. longicauda* (Green, 1818)	29–1410	—
Taylor *et al.* (2015)	*Plethodon albagula* (Grobman, 1944)	157	0.61–1.14

My highest seasonal abundance estimates, which occurred in spring 2016, followed by summer of both years, generally reflect trends that others have found for *E. lucifuga*, with monthly (May–June: Hutchison, 1958; May: Williams, 1980) or seasonal (spring: Camp *et al.*, 2014; summer: Briggler and Prather, 2006) highest counts and average counts similarly reported at these times. These earlier authors studied Cave Salamanders in the twilight zone of their study caves, and given that Cave Salamanders predominantly reside in this area of caves in spring and summer (Hutchison, 1958; Williams, 1980), it is unsurprising that their studies found highest abundance then.

In my study, the lowest seasonal abundance occurred in spring 2015, which is probably atypical for the spring season. Two factors, flooding and temperature, may account for this discrepancy. A substantial flood (*ca.* 17.3 cm rainfall in two days; Figure 17) occurred in April 2015 that likely explains the low counts of salamanders in subsequent surveys that month (Figure 18) because salamanders probably retreated to other areas of the cave or were flushed out; this undoubtedly affected abundance estimates for spring 2015. Furthermore, average temperature in February and March of 2015 (-3.2 and 6.9ºC, respectively) for Louisville, Kentucky was lower than the central state average (3.4 and 8.2ºC, respectively), and lower that year than in 2016 (3.9 and 11ºC, respectively) (UKAWC, 2017). This may have influenced the distribution and activity of Cave Salamanders within the cave, especially in the twilight zone, where surface environmental fluctuations are most apparent. My next lowest abundance estimate was in fall 2016. In two studies that focused on the twilight zone, the lowest counts for this species were reported in winter (Camp *et al.*, 2014; Hutchison, 1958); Cave Salamanders are rarely found in the twilight zone of caves during winter because

FIGURE 17. Major flood event at Sauerkraut Cave in April 2015 (scale bar = 2 m). The water level is *ca*. 0.7 m. (Photographed by J. Gavin Bradley)

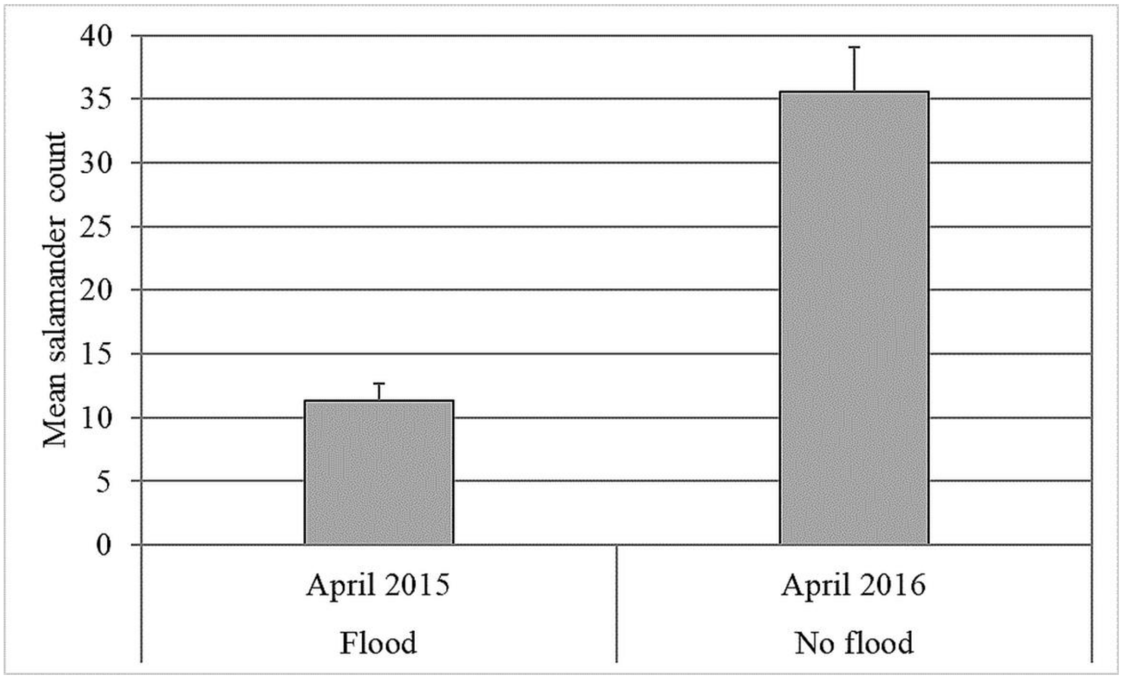

FIGURE 18. Mean counts of Cave Salamanders in Sauerkraut Cave in April 2015 and 2016. Error bars are standard error (SE).

populations move deeper into caves to escape the inhospitable (i.e., cold and dry) environment near cave entrances (Hutchison, 1958; Williams, 1980; Chapter 4). In a third study that also found the lowest counts in winter, Briggler and Prather (2006) likely surveyed the dark zone of some study caves, but it is unclear how many of their study caves included the dark zone or how far they penetrated. It is uncertain why low abundance occurred in fall in my study, but this result may be associated with low survival probabilities (discussed below).

Capture probabilities for salamanders are generally low due to reduced levels of detectability (O'Donnell and Semlitsch, 2015) and have been shown to vary temporally for both epigean (Bailey *et al.*, 2004; Muncy *et al.*, 2014; Price *et al.*, 2012) and hypogean (Fenolio *et al.*, 2014) species. As expected, capture probabilities in my study were also low and variable, and they were lowest in spring 2015 and 2016. Cave Salamanders may leave caves in spring to explore adjacent epigean habitats for foraging or to emigrate (Petranka, 1998), thus being unavailable for capture in the cave. High capture probability occurred in fall and winter of 2015 and was highest in fall 2016. Cave Salamanders reinvade the cave for courtship and/or to escape approaching inhospitable climate at these times of year (Hutchison, 1958; Petranka, 1998; Williams, 1980), making them relatively accessible for capture in the cave.

My estimates of survival probability are generally similar to those reported for other plethodontids (Fenolio *et al.*, 2014; Muncy *et al.*, 2014; Price *et al.*, 2012). The low probabilities of survival I observed during the fall of both study years may be due to decreasing food availability as salamanders move farther back into the dark zone and to movement of potential predators into the cave for eventual torpor (e.g., Black Rat Snakes, *Pantherophis* [*Elaphe*] *obsoletus* (Say, 1823), and Green Frogs, *Rana* [*Aquarana*] *clamitans* (Latreille, 1801), were seen in Sauerkraut Cave in fall). I observed an anecdotal increase in individual tail loss in this season, which may have been a result of interactions with these potential predators. Highest survival probability occurred in spring and summer 2015 and in spring 2016, and thus in seasons when epigean and twilight zone climates are equitable and food is readily available.

Total biomass and biomass density of cave-inhabiting salamanders have seldom been reported. Huntsman *et al.* (2011) reported biomass density for a troglobitic species as 0.18 g m^{-2} and 0.03 g m^{-2} ash-free dry mass in two caves in Alabama, USA. Salvidio (1998) reports average total wet-biomass and wet-biomass density for a troglophile in Liguria, Italy as 238.48 g and 1.25 g m^{-2}, respectively. An assemblage of *Desmognathus* spp. at a wet rock face, a similar habitat to caves albeit likely having higher food densities, had estimated wet-biomass of 916.56 g and wet-biomass density of 27.16 g m^{-2} (Crawford and Peterman, 2013) in North Carolina, USA. My study indicates seasonally variable wet-biomass in this population of Cave Salamanders, which likely has important implications for energy distribution within the cave. Dead individuals were rarely observed (nine total for the study period), but those that were seen were occupied by invertebrates, and all but vertebrae were gone the following surveys one week later. Given this short amount of time dead individuals may be visible before consumed, and that I could not survey the entire cave, it is likely that more individuals died in the cave without being noticed. This may represent an important pulse nutrient resource for cave-inhabiting organisms. Salamanders may also supply energy through fecal deposition (Bohonak and Whiteman, 1999; Lilleskov and Bruns, 2005) in caves, and I regularly observed salamander feces in Sauerkraut Cave in spring. This contribution may be substantial with greater levels of population biomass.

Salamander densities have been reported for troglobites (Fenolio *et al.*, 2014; Huntsman *et al.*, 2011), troglophiles and trogloxenes (Salvidio, 1998; Taylor *et al.*, 2015), and potential trogloxenes (Crawford and Peterman, 2013) (Table 7). My density estimates are most similar to densities reported for troglobitic species (Fenolio *et al.*, 2014; Huntsman *et al.*, 2011), likely because my study took place exclusively within a cave system. Although Taylor *et al.* (2015) similarly investigated a population of trogloxenic salamanders in a cave system, they mention that individuals often congregated in a pit, which may have inflated density estimates; my highest density estimate (0.47 m^{-2}) approaches their lowest (0.61 m^{-2}). Densities of those populations studied at rock faces (Crawford and Peterman, 2013; Salvidio, 1998) were higher than my estimates, which may be due to the limited surface area and probable higher food abundance of this habitat compared to a cave system. Rock outcrops are important habitat for many fauna (Fitzsimons and Michael, 2017) and may provide an accessible, safer refuge than what can be found on the ground, making this ideal habitat for salamanders. Likewise, many individuals may utilize this habitat, trading off space for safety, which may explain increased densities at rock faces.

Conclusion**—**There is much yet to be learned about the natural history and ecology of *Eurycea lucifuga*, and trogloxenic salamanders in general. It is likely, though not well established, that these facultative cave inhabitants play important ecological roles in cave ecosystems. This is an important consideration as caves, and the organisms restricted to them, are often of conservation concern. Unraveling the ecological dynamics of trogloxenic salamanders will surely provide valuable information that can be used to manage and conserve these fascinating ecosystems.

CHAPTER IV

THE EFFECTS OF CLIMATE AND BEHAVIOR ON MIGRATORY MOVEMENTS OF CAVE SALAMANDERS, *EURYCEA LUCIFUGA* (RAFINESQUE, 1822)

INTRODUCTION

Movement is a fundamental property of organisms and a primary determinant of both individual fitness and the ecology of a species. Movement defines the spatial dynamics of an individual's interactions with other organisms and with the environment (Brown, 1984; Pittman *et al.*, 2014), and when we consider movement patterns in the context of environmental variation, we can begin to discern the factors that drive such patterns. This information helps to explain how organisms use and interact with their environment, thereby informing the ecology, life history, behaviors, and conservation (Rubenstein and Hobson, 2004) of a species. Animal movements are influenced by a multitude of biotic and abiotic factors (Begon *et al.*, 2006; Linzey, 2012). Seasonal variation in these factors, if extreme enough, may lead to pronounced distributional changes in animal populations. Such changes occur during migrations, the periodic movements of a population, in whole or in part, away from an area with subsequent return to that area, which may happen over variable lengths of time (e.g., daily, seasonal, or irregular) and different spatial scales (e.g., micro- or macrogeographic) depending upon the species and reasons for migrating (Linzey, 2012).

In terrestrial environments, animal movements are strongly influenced by temperature and water availability (Begon *et al.*, 2006). Salamanders are especially sensitive to these factors because of their thin, desiccation-prone skin (Feder, 1983; Spotila, 1972) that governs their occupation of terrestrial habitats (e.g., Heatwole, 1962; Milanovich *et al.*, 2006 and references cited within; Peterman and Semlitsch, 2013; Wells and Wells, 1976). This constraint is particularly marked in temperate zones, where temperature and moisture (i.e., precipitation and/or humidity) regimes fluctuate seasonally, and it is further affected by finer scale temperature and moisture patterns at diel and microhabitat levels (Feder, 1983; Peterman and Semlitsch, 2014). Many other environmental factors also influence the terrestrial movements of salamanders, including vegetative (Heatwole, 1962; Pough, 1980) and landscape characteristics (Marsh *et al.*, 2005; Rittenhouse and Semlitsch, 2006), soil pH (Sugalski and Claussen, 1997; Wyman and Hawksley-Lescault, 1987), management activities (Harper and Guynn Jr., 1999 and references cited within), available cover objects (Grover, 1998), intraspecific competition (Jaeger, 1979; Whiteman *et al.*, 1994), predator activity (Maerz *et al.*, 2001), and food availability (Harper and Guynn Jr., 1999; Whiteman *et al.*, 1994). Further, although not tested directly, photoperiod stimulates metabolic (Whitford and Hutchison, 1965; Wood and Orr, 1969) and reproductive processes (Werner, 1969) in salamanders, which likely increases directed movement, such as toward food sources or mating areas.

Some of these factors are also implicated in the terrestrial migratory movements of salamanders. Annual microgeographic migrations have been well established for pond-breeding species, such as ambystomid salamanders (Lannoo ed., 2005; Petranka, 1998); adults make migratory movements through preferred habitat (e.g., forest) from overwintering terrestrial sites to and away from breeding ponds during cool, rainy nights, with secondary migrations between summer retreats and foraging habitats (reviewed by Semlitsch, 2008). Migratory movements have been documented to a lesser extent in plethodontids. Heatwole (1962) described horizontal and vertical summer migrations by Red-backed Salamanders, *Plethodon cinereus* (Green, 1818), amongst forest floor and soil microhabitats in search of favorable temperature and moisture conditions in response to changing surface conditions. Woolbright and Martin (2014) described a seasonal migration for this species, with individuals entering rocky outcrops in the fall, where it is warm enough to remain active and mate through the winter, then exiting to return to wet areas in spring to breed. Meshaka Jr. and Trauth (1995) suggested Ozark Zig-zag Salamanders, *P. angusticlavius* (Grobman, 1944), migrate to cool, moist glades of low predator presence for courtship purposes, then to leaf litter at bluff habitat when the glades become hot and dry. Mann and Mann (2017) documented annual migration for Webster's Salamanders, *P. websteri* (Highton, 1979), with individuals in one population entering a limestone outcrop in spring for suitable refuge and oviposition sites away from summer heat, and then exiting this outcrop in the fall to return to adjacent forested areas. Cupp Jr. (1991) discussed the annual movement pattern of the Green Salamander, *Aneides aeneus* (Cope and Packard, 1881), in which some individuals likely migrated in early spring from hibernacula at sandstone crevices to adjacent areas, returning to breed at these crevices in late spring. Mohr (1944) briefly detailed a migration of Long-tailed Salamanders, *E. longicauda longicauda* (Green, 1818), from a mine in mid-spring, likely for foraging purposes, returning to the mine in mid-summer to remain through the next winter while eggs were laid.

It appears that terrestrial migrations of plethodontids occur for purposes of breeding, foraging, and finding suitable climatic conditions, often resulting in movements between surface and subterranean habitats. Although many plethodontids have known associations with subterranean environments, especially caves (Lannoo ed., 2005; Petranka, 1998), little is known regarding the hypogean dynamics of their populations because salamanders are notoriously difficult to work with due to their small size and secretive habits (Muñoz *et al.*, 2016; Woolbright and Martin, 2014), and because subterranean habitats are often inaccessible. This creates a void in our understanding of the ecology of these animals, which are generally of conservation concern (Collins and Storfer, 2003; Davic and Welsh, 2004). Furthermore, understanding the ecology of salamanders is important because they influence local community structure and function. Salamanders are often abundant (Semlitsch *et al.*, 2014; Whiles *et al.*, 2006), are keystone predators that regulate nutrient cycling through control of invertebrate and fungal food webs (Best and Welsh Jr., 2014; Walker *et al.*, 2014), and are a high-energy food source for other predators and scavengers (Semlitsch *et al.*, 2014). Salamanders also couple aquatic and terrestrial ecosystems, for example, via resource flow (Regester *et al.*, 2006; Schriever *et al.*, 2014) (reviewed by Davic and Welsh, 2004). These important ecological characteristics of salamanders and the movements of plethodontids between subterranean and surface habitats suggest an important link between these environments. Thus, detailing these movement patterns is essential to further understand the ecological roles and habitat associations of these organisms.

A common plethodontid salamander in the eastern United States is the Cave Salamander, *Eurycea lucifuga* (Rafinesque, 1822) (Chapter 1). This is a trogloxenic (i.e., facultative cave-dwelling) salamander and is often observed in the twilight zone of caves. Populations of this species may be locally abundant (Chapter 3), yet little is known regarding the ecology or behavior of this species. One emergent pattern has been described by some authors concerning a change in seasonal population distribution in caves; Cave Salamanders generally inhabit the twilight zone of caves from spring through fall when this area is cool and moist, but retreat to the dark zone of caves during winter when this area is cold and dry, only to re-emerge in the twilight zone at the onset of the following spring (Hutchison, 1958; Williams, 1980). This pattern is indicative of seasonal microgeographic migration, but detailed information regarding movements in and between the twilight and dark zones of caves is unavailable for this species. The movement dynamics of salamanders in caves undoubtedly affect other cave-dwelling organisms. Therefore, studying the details of how salamanders move in caves will further inform the management and conservation of these systems and the unique organisms that reside within them.

The purpose of this study is to explore the movement patterns of a population of *E. lucifuga* in a spring cave in Kentucky. My primary goal is to document precisely any seasonal migration between the twilight and dark zones, and to explore the environmental and behavioral factors influencing such movements. Because the abundance of this species varies seasonally in the twilight zone and is related to changing temperature and moisture conditions, I hypothesize that seasonal migrations of Cave Salamanders in caves are associated with certain abiotic factors known to affect salamanders' occupation and activity in terrestrial habitats. Specifically, I predict seasonal migrations between the twilight and dark zones of caves to be in response to changes in temperature and moisture availability in this habitat, as well as photoperiod since this is an important migratory cue for many other animals (Bauer *et al.*, 2011) and may be detectable to Cave Salamanders while inhabiting the twilight zone. With this information, we will gain a greater understanding of the ecology of this species and begin to recognize how Cave Salamanders, and trogloxenic salamanders in general, influence cave ecosystems.

METHODS

***Study site*—**I monitored a population of *E. lucifuga* during 74 survey dates from March 2015–February 2017 in two passages of Sauerkraut Cave in Louisville, Kentucky (Chapters 2 and 3).

DATA COLLECTION

Salamanders—I constructed two semi-permanent transects, one each in the main and side passages of Sauerkraut Cave (Chapters 2 and 3). Each transect started at the drip-line and followed the primary channel of its respective passage. Surveys of these transects generally were conducted weekly. During surveys, I searched the walls, floor, ceiling, and standing water of each transect for salamanders using red-filtered light during daylight hours (Chapters 2 and 3). When a salamander was located, I recorded the distance into the cave from the drip-line that it was found, and I took pictures of the dorsal spot pattern of the head and neck of each salamander in plain sight with an Olympus TG-4 digital camera for individual identification in I^{3}S-Spot (Chapter 2). I did not capture or handle any salamanders for this study.

Macroclimate—I measured air temperature (°C), relative humidity (%RH) (model 625 thermo-hygrometer, B&K Precision Corporation, California, USA), and water depth (cm) of the stream and/or largest pool at six set stations along each transect. Three of these stations were located at different distances in the twilight zone and the other three set at different distances in the dark zone in each passage; these environmental measurements are referred to as "Cave". The level of the streambed in the main passage was inconsistent and caused some water depth measurements to be very different at certain stations, so for each survey date I calculated the change in water depth (Δ H_2O) from that measured on the previous survey date for each station. I also measured these variables at one spot in the epigean environment just outside the cave entrances; these measurements are referred to as "Epigean". I obtained mean temperature (°C), precipitation (cm), maximum %RH (UKAWC, 2017), and photoperiod (decimal hrs.) (USNO, 2017) for Louisville, Kentucky for each survey date; these measurements are referred to as "Louisville". The precipitation data contained zeros for 67.6% of my survey dates (N = 50), however precipitation levels were often reported on days prior to my surveys. Accordingly, to better represent precipitation regimes, I summed precipitation measures across a five-day period that included precipitation on my survey date and the previous four days.

Microclimate—I measured microclimate variables, including temperature (°C) (RT-1 REPTITEMP® digital infrared thermometer, Zoo Med Laboratories Inc., California, USA), illuminance (Lux) (EXTECH™ EA30 EasyView digital light meter, FLIR Commercial System Inc., New Hampshire, USA), and wetness (% Dry or Damp), in the front chamber (0–20 m) of the main passage (Figure 19) once per month from October 2016–April 2017 during daylight hours. The climate of the twilight zone near the cave entrance is highly variable throughout the year (see Results) and dependent upon

FIGURE 19. The front chamber (0–20 m) of the main passage at Sauerkraut Cave.

immediate epigean conditions. During winter, this zone is colder and often dryer than in other seasons, and thus is relatively inhospitable toward salamanders. However, my observations (see Discussion) unexpectedly showed that some salamanders still inhabited this front chamber during this season, warranting investigation of microclimate in this area. I chose this six-month period to investigate the available microclimates in this area of the twilight zone before, during, and after winter.

During microclimate surveys, I recorded the locations of salamanders in this front chamber, noting the side of the passage (left or right) and area (i.e., wall or floor) of these "Salamander" microsites. I measured microclimate variables immediately adjacent to each salamander location. Then, I measured microclimate variables at paired "Open" and

“Refuge” microsites for comparison with the Salamander microsites. Open microsites were not covered in any way and were thus “out in the open”, whereas Refuge microsites were locations that could conceivably provide cover (e.g., under a rock or in a hole) for a salamander and that were located within 1 m of its paired Open site. Before each survey, to determine the distance into the cave at which I would sample Open and Refuge microsites, I staked out a 20 m line running from the mouth of the cave to the back of the front chamber and randomly selected four whole-number distances between 0–20 m. I measured microsites on the left side of the midline when the selected distance was even, and on the right side when the selected distance was odd. From each selected distance, I walked at a 90° angle from the midline of the passage and measured microsites on the floor area near ($\bar{x}$ ± SD: 0.33 ± 0.24 m, N = 56), midway ($\bar{x}$ ± SD: 2.40 ± 0.73 m, N = 56), and far ($\bar{x}$ ± SD: 4.00 ± 1.03 m, N = 56) from the midline. Then, when I reached the cave wall I measured microsites on the wall area at low ($\bar{x}$ ± SD: 0.52 ± 0.66 m, N = 56), midway ($\bar{x}$ ± SD: 1.26 ± 0.22 m, N = 56), and high ($\bar{x}$ ± SD: 1.74 ± 0.27 m, N = 56) elevations.

STATISTICAL ANALYSES

General—Population analyses (described below) only include data collected from the main passage of Sauerkraut Cave because sample sizes of salamanders from the side passage were inconsistent, frequently small ($\bar{x}$ ± SD: 11.10 ± 8.20 salamanders, range: 0–50 salamanders, N = 71), and not comparable to those of the main passage, even though these passages are relatively similar in length and available surface area (Chapter 3). Likewise, I only consider Cave environmental variables from the main passage in subsequent population analyses and summarizations. The reason for this discrepancy in salamander numbers between the passages of this cave is unknown. I do, however, include data from salamanders found in the side passage for individual movement analyses in order to enhance the sample size of this measure (described below).

I calculated mean (± SD) Cave Salamander population distance into the cave, referred to as "Salamander Distance", and the proportions of total salamanders seen in the twilight and dark zones for each survey date. I used patterns evident in Salamander Distance and information on life history characteristics for this species (Chapter 1) to define "Salamander Seasons": Pre-foraging (January–March), Foraging (April–June), Early Breeding (July–September), and Late Breeding (October–December); my use of the term season going forward is in reference to these Salamander Seasons. I pooled Cave stations by zone (i.e., twilight and dark zones) and calculated the mean (± SD) of each Cave macroclimate variable for each survey date; I also calculated seasonal mean (± SD) temperature, %RH, and Δ H_2O for each twilight and dark zone station separately. I pooled each macroclimate variable by season and calculated the means (± SD).

All statistical analyses were run in R version 3.4.1 (R Core Team, 2017) and data visualization was accomplished using R and Microsoft Excel 2016.

Population movement—I conducted multiple linear regression analysis using selected explanatory variables from Cave, Epigean, and Louisville macroclimate measurements, with Salamander Distance as the response variable. For Cave variables, I used mean temperature and %RH from the twilight zone of the main passage, but not from the dark zone of this passage because temperature and humidity from the dark zone had low variability (see Results), and thus little explanatory power; for consistency, I only used mean Δ H_2O from the twilight zone.

In multiple linear regression, explanatory variables are assumed to be correlated with the response variable and cannot be collinear with other explanatory variables (Diez *et al.*, 2012; Zar, 1999). To conform to these model assumptions, I used Pearson's correlation tests for explanatory variable selection prior to regression analysis. Correlation analyses were conducted using "Full" and "Reduced" datasets because some macroclimate variables had missing values; I removed survey dates with incomplete rows of data from the Full dataset to create the Reduced dataset. Since some explanatory variables were similar in type (e.g., three temperature variables including Cave, Epigean, and Louisville), I first determined which of the similar variables was most highly correlated with the response variable and selected that particular variable for further analysis. Then, the remaining explanatory variables were correlated with the response variable. Those explanatory variables that were significantly correlated ($\alpha = 0.05$) with the response variable were then tested for significant ($\alpha = 0.05$) collinearities. If explanatory variables were collinear, the variable that was most significantly correlated to the response variable was selected for regression analysis, as were those explanatory variables that did not exhibit collinearity. I constructed multiple linear regression models for the Full and Reduced datasets and conducted two-way stepwise Akaike Information Criterion (AIC) model selection for each. I then checked the residuals of the final models for normality using Shapiro-Wilk tests ($\alpha = 0.05$).

Individual movement—I calculated greatest weekly distance moved (m week^{-1}), referred to as "Weekly Distance", for individuals I observed on two consecutive survey dates, i.e., individuals with two measured distances into the cave separated by one week's time. I obtained an individual's Weekly Distance by calculating the difference between their consecutive measured distances. I only used one set of consecutive measurements per individual per season to reduce individual bias, such that if an individual had more than one set of consecutive measurements in a season, only the set with the greatest difference between consecutive measurements was used. I pooled data by season and across years to enhance the sample size of each season and calculated the means (± SD) and medians. Because the raw and transformed data were non-normal and there were large discrepancies in sample sizes among seasons, I analyzed Weekly Distance using a Kruskal-Wallis rank sum test ($\alpha = 0.05$), followed by Bonferroni-corrected post-hoc Dunn's tests for multiple comparisons ($\alpha = 0.05$).

Microclimate—I pooled microclimate variables across months and by microsite type (i.e., Salamander, Open, or Refuge) and calculated the means (± SD). I then separated each microsite type by area (i.e., wall or floor) to create six microsite groups: Salamander wall, Salamander floor, Open wall, Open floor, Refuge wall, and Refuge floor. I calculated mean (± SD) temperature and Lux, and the percent of wet observations (%Wet) for each group and analyzed these data using principle components analysis (PCA).

RESULTS

Salamanders—On average there were 56.0 ± 30.3 salamanders (range: 7–222 salamanders, N = 74,) in the main passage on each survey date. Salamander Distance ranged from 6.41 ± 8.40 m in May to 77.97 ± 15.05 m in January (N = 74) (Figure 20), and the proportions of the total salamander population seen in the twilight *vs*. dark zones exhibited a cyclic pattern over time (Figure 21).

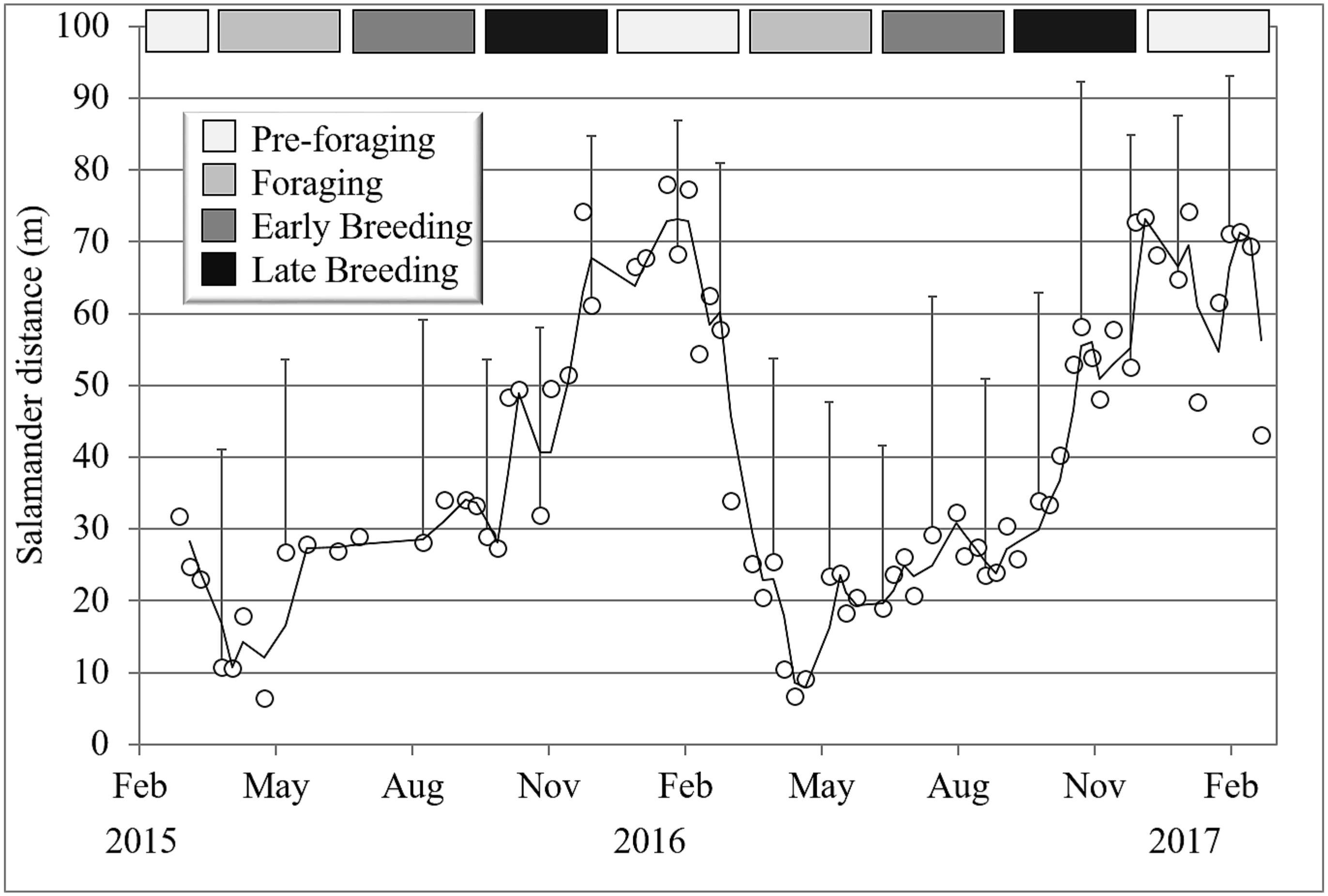

FIGURE 20. Mean (± SD) Cave Salamander population distance (i.e. "Salamander Distance") into the main passage of Sauerkraut Cave. Only selected error bars are represented to maintain clarity of the figure. Salamander Seasons are indicated at the top of the graph, corresponding with certain ranges of months on the x-axis.

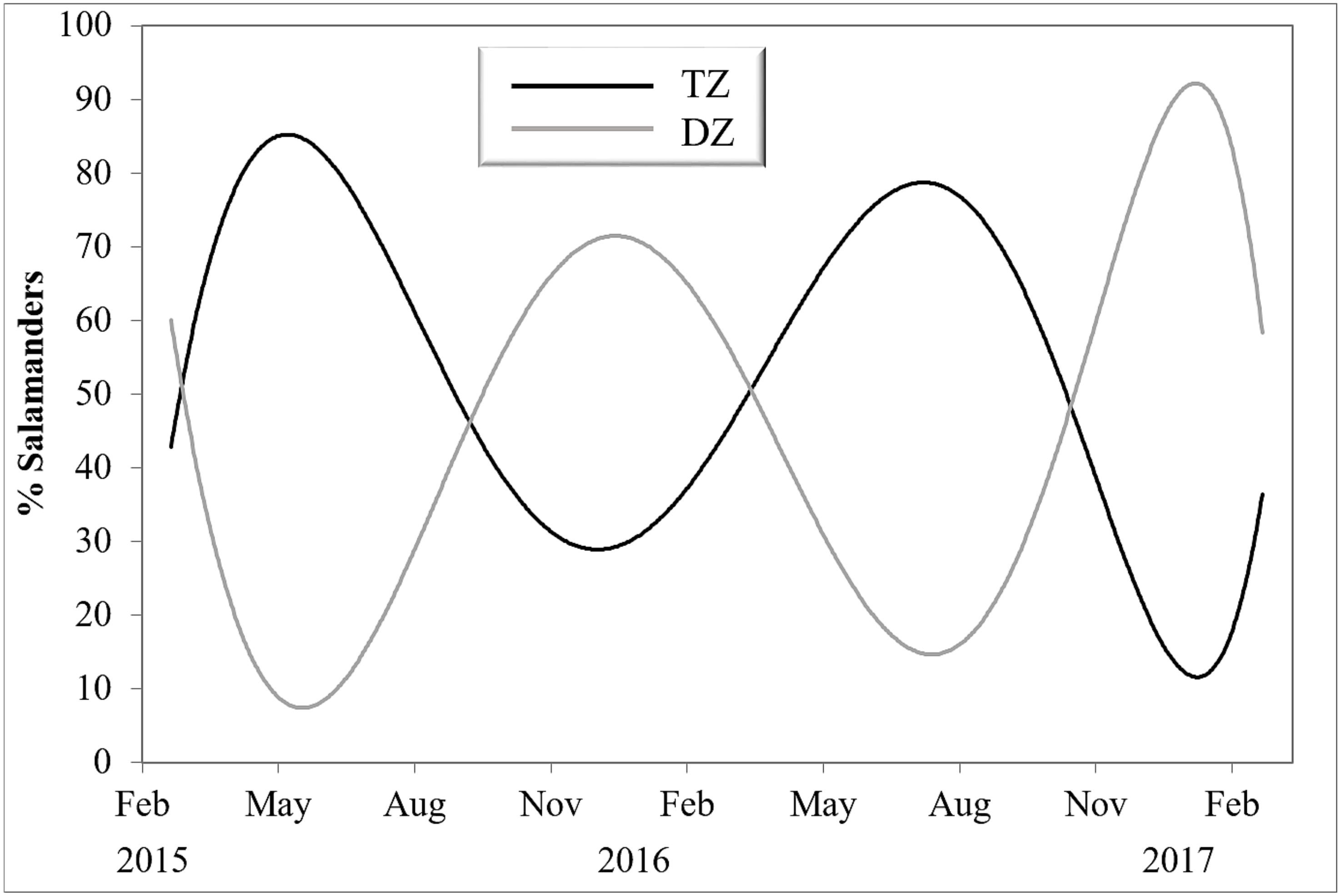

FIGURE 21. The proportion of the Cave Salamander population seen in the twilight and dark zones on survey dates from the main passage of Sauerkraut Cave. TZ = twilight zone; DZ = dark zone.

Macroclimate and population movement—Seasonal summary statistics for all macroclimate variables are given by area in the Appendix to this chapter, and by each Cave station in Table 8. Macroclimate was more variable in the twilight zone in general, and increasingly variable from the back of the twilight zone toward the front near the cave entrance. The Full and Reduced datasets contained 74 and 58 rows of data, respectively, as 16 of the 74 rows in the Full dataset were incomplete, missing one to six values for Cave and/or Epigean macroclimate variables. Pearson correlations (Table 9) within similar types of explanatory variables (i.e., temperature, %RH, and Δ H_2O) indicated that Louisville temperature (Full: $R = -0.68$, $P < 0.0001$; Reduced: $R = -0.73$, $P < 0.0001$), Epigean %RH (Full: $R = -0.36$, $P = 0.006$; Reduced: $R = -0.36$, $P = 0.006$), and Cave Δ H_2O (Full: $R = -0.25$, $P = 0.052$; Reduced: $R = -0.26$, $p = 0.049$) were most highly correlated with Salamander Distance in each dataset.

Significant collinearity was found amongst Louisville temperature and Epigean %RH with Louisville photoperiod for both datasets (Table 9), and of these variables Louisville photoperiod had the highest correlation with Salamander Distance (Full: $R = -0.83$, $P < 0.0001$; Reduced: $R = -0.85$, $P < 0.0001$); thus, Louisville temperature and Epigean %RH were dropped from further analysis and Louisville photoperiod was kept. Significant collinearity was also found between Cave Δ H_2O and Louisville precipitation in both datasets (Table 9); Cave Δ H_2O was most significantly correlated with the response variable in the Full dataset (see above) and Louisville precipitation was most correlated with the response variable in the Reduced dataset ($R = -0.26$, $P = 0.045$); thus, Louisville precipitation was dropped and Cave Δ H_2O was kept for further analysis of the Full dataset, and *vice versa* for the Reduced dataset. The

TABLE 8. Seasonal means (± SD) of Cave environmental variables for each station.

	Cave environmental stations					
	Twilight zone			Dark zone		
Variable	1	2	3	4	5	6
Temperature (°C)						
Pre-foraging	7.74 (3.73)	10.77 (1.77)	12.70 (1.25)	13.78 (0.92)	15.32 (0.55)	15.72 (0.31)
Foraging	16.62 (3.24)	15.58 (2.22)	15.24 (0.90)	15.06 (0.93)	15.53 (0.85)	15.78 (1.11)
Early Breeding	20.41 (1.85)	18.44 (1.04)	16.70 (0.69)	15.93 (0.68)	15.59 (0.48)	15.65 (0.31)
Late Breeding	13.53 (4.32)	14.39 (2.35)	15.13 (1.61)	15.30 (1.09)	15.66 (0.41)	15.78 (0.30)
Relative humidity (%RH)						
Pre-foraging	81.09 (11.27)	93.88 (4.65)	96.74 (2.24)	97.41 (1.56)	97.19 (1.15)	96.86 (1.89)
Foraging	83.96 (8.77)	94.38 (2.46)	96.63 (2.34)	97.32 (2.13)	97.64 (1.62)	98.05 (0.91)
Early Breeding	91.57 (5.54)	94.95 (3.22)	95.49 (2.46)	95.85 (2.25)	96.10 (2.50)	95.41 (3.72)
Late Breeding	82.74 (8.93)	93.00 (4.53)	95.32 (2.42)	95.93 (1.99)	96.11 (1.61)	95.60 (2.02)
Water depth change (Δ H2O)						
Pre-foraging	3.88 (3.46)	3.50 (3.35)	3.63 (3.53)	3.06 (2.74)	2.94 (2.41)	2.75 (2.02)
Foraging	3.91 (3.75)	2.73 (3.80)	3.55 (3.83)	2.64 (3.80)	2.73 (4.41)	1.91 (2.34)
Early Breeding	4.47 (5.11)	4.27 (3.71)	4.07 (4.17)	2.27 (4.17)	2.73 (3.24)	2.00 (2.24)
Late Breeding	2.19 (2.60)	1.86 (2.35)	1.67 (2.11)	0.57 (1.50)	1.10 (1.26)	1.24 (1.22)

TABLE 9. Pearson's correlations and collinearities for explanatory variables used in regression analysis. *R* and P values for the Full and Reduced datasets represent the correlation between an explanatory variable and Salamander Distance. *R* and P values for Collinearities identify a dropped explanatory variable that was correlated to another explanatory variable, which is labelled by [a, b, c, d, e] or [f].

	Full dataset		Collinearity	Reduced dataset		Collinearity
Variable	*R*	P	*R*, P	*R*	P	*R*, P
Temperature (°C)						
Cave	-0.67	< 0.001	—	-0.71	< 0.001	—
Epigean	-0.65	< 0.001	—	-0.70	< 0.001	—
Louisville	-0.68	< 0.001	[a] 0.70, < 0.001	-0.73	< 0.001	[d] 0.71, < 0.001
Relative humidity (%RH)						
Cave	-0.32	0.015	—	-0.32	0.016	—
Epigean	-0.36	0.006	[b] 0.28, 0.035	-0.36	0.006	[e] 0.28, 0.035
Louisville	-0.28	0.014	—	-0.25	0.063	—
Water depth change (Δ H_2O)						
Cave [c]	-0.25	0.052	—	-0.26	0.049	[f] 0.32, 0.013
Epigean	-0.24	0.057	—	-0.25	0.059	—
Precipitation (cm) [f]	-0.21	0.074	[c] 0.37, 0.003	-0.26	0.045	—
Photoperiod (decimal hrs.) [a, b, d, e]	-0.83	< 0.001	—	-0.85[c]	< 0.001	—

remaining explanatory variables, Cave Δ H_2O/Louisville precipitation and Louisville photoperiod, were used for construction of initial regression models; although Cave Δ H_2O was not significantly correlated to the response variable at $\alpha = 0.05$ for the Full dataset, it was marginally non-significant, suggesting it could still be important to the Full regression model. Summary statistics for variables from the Full and Reduced datasets can be seen in Table 10.

The initial regression models were Salamander Distance ~ Cave Δ H_2O + Louisville photoperiod for the Full dataset, and Salamander Distance ~ Louisville precipitation + Louisville photoperiod for the Reduced dataset. The outcome of stepwise AIC model selection was similar for both datasets, such that the final model was Salamander Distance ~ Louisville photoperiod; model diagnostics are presented in Table 11. The final model residuals for both datasets were normally distributed (Full: $W = 0.98$, P = 0.197; Reduced: $W = 0.98$, P = 0.675).

Individual movement—Weekly Distance varied by season (Table 12), ranging from a mean of 8.74 (± 10.04) m week^{-1} in Foraging to 13.60 (± 15.04) m week^{-1} in Pre-foraging, or a median of 2.25 (± 16.78) m week^{-1} in Early Breeding to 9.20 (± 17.45) m week^{-1} in Late Breeding. Results of the Kruskal-Wallis test were significant ($H = 13.604$, P = 0.003); post-hoc Dunn's tests indicated that movement in Early Breeding was significantly less than movement in both Pre-foraging ($Z = 2.82$, P = 0.005, adjusted P = 0.029) and Late Breeding seasons ($Z = 3.45$, P < 0.001, adjusted P = 0.003) (Table 13).

***Microclimate*—**Summary statistics for each microsite type can be seen in Table 14. Salamanders were found in a much narrower range of temperature and Lux compared to that available in their environment, and were most often found at wet microsites (high

Table 10. Summary statistics for variables used in procedures to construct multiple linear regression models.

	Full dataset		Reduced dataset			
Variable	Mean (± SD)	N	Mean (± SD)	N	Range	
Salamander Distance (m)	39.89 (20.44)	74	43.52 (20.73)	58	6.41 (F), 6.77 (R)	77.97
Temperature (°C)						
Cave	14.63 (3.50)	73	14.32 (3.65)	58	5.53	20.23
Epigean	15.78 (7.39)	71	15.13 (7.64)	58	-2.10	27.60
Louisville	15.08 (9.08)	74	14.20 (9.26)	58	-10.00	29.44
Relative humidity (%RH)						
Cave	91.33 (4.62)	59	91.35 (4.65)	58	79.70	98.87
Epigean	71.56 (18.47)	58	71.56 (18.47)	58	29.10	100.00
Louisville	80.74 (12.13)	74	79.55 (13.08)	58	47.00	96.00
Water depth change (Δ H_2O)						
Cave	3.17 (3.30)	63	2.91 (3.10)	58	0.00	12.00
Precipitation (cm)	1.59 (1.72)	74	1.45 (1.52)	58	0.00	6.12
Photoperiod (decimal hrs.)	12.02 (1.76)	74	11.69 (1.78)	58	9.52	14.82

TABLE 11. Results of the stepwise AIC model selection for the Full and Reduced datasets.

Model	AIC	Adj. R^2	*F*	P
Initial				
Full dataset	304.65	0.71	75.91	< 0.001
Reduced dataset	281.67	0.72	72.65	< 0.001
Final				
Full dataset	303.46	0.69	163.9	< 0.001
Reduced dataset	281.05	0.71	143.1	< 0.001

TABLE 12. Summary statistics for seasonal weekly distance moved, i.e., Weekly Distance.

	Weekly Distance (m week^{-1})			
Season	Mean (± SD)	Median	Range	N
Pre-foraging	13.60 (15.04)	7.21	0.06–65.59	144
Foraging	8.74 (10.04)	6.43	0.03–32.88	19
Early Breeding	8.90 (16.78)	2.25	0.00–85.09	47
Late Breeding	15.93 (17.45)	9.20	0.04–66.76	105

TABLE 13. Results of the post-hoc Dunn's test with adjusted P-values (Adj. P) for multiple comparisons following a significant Kruskal-Wallis test (H = 13.604, P = 0.003) for seasonal Weekly Distance. Significant difference indicated by *.

Seasons compared	Z	P	Adj. P
Late Breeding–Foraging	1.79	0.073	0.439
Late Breeding–Pre-foraging	1.02	0.307	1.000
Foraging–Pre-foraging	-1.29	0.196	1.000
Late Breeding–Early Breeding *	3.45	0.0006	0.003
Foraging–Early Breeding	0.58	0.560	1.000
Pre-foraging–Early Breeding *	2.82	0.005	0.029

%Wet). The PCA explained 88.00% of the variation in microsite groups and demonstrated that microsite groups loaded differently. Principal component one (PC1) is strongly correlated with Lux (0.73) and temperature (-0.67), and explains 50.32% of the variation in microsite groups; principal component two (PC2) is strongly correlated with %Wet (0.90) and explains 37.68% of the variation in microsite groups (Table 15). Salamander microsites load negatively on PC1 and positively of PC2, indicating salamanders are selecting increased temperature and wetness, while avoiding light (Figure 22). The Floor microsites load positively on PC1 and PC2, indicating Floor microsites have more light, lower temperatures, and high wetness. Wall microsites load slightly negatively on PC1 and negatively on PC2, indicating that Wall microsites are typically dryer, but maintain slightly warmer temperatures and lower light than floor microsites. For both Floor and Wall sites, Open microsites have higher loadings on the principal components than do Refuge microsites, indicating that Refuge sites are closer to each other in multivariate space, and thus are more similar in measured variables than Open sites are to each other.

TABLE 14. Summary statistics for microclimate variables by microsite type.

Variable	Mean (± SD)	Range	N
Temperature (°C)			
Salamander	13.9 (1.33)	8.0–16.4	91
Open	11.2 (3.75)	1.6–16.4	158
Refuge	11.0 (3.65)	0.6–16.2	158
Illuminance (Lux)			
Salamander	0.63 (1.70)	0.0–12.4	109
Open	40.95 (193.29)	0.0–1755.0	158
Refuge	5.57 (50.67)	0.0–629.0	158
	Dry	Wet	N
Wetness (%Wet)			
Salamander	3.7	96.3	109
Open	25.9	74.1	158
Refuge	10.1	89.9	158

Table 15. Eigenvalues from the PCA of microclimate for microsite groups.

Variable	PC1	PC2
Temperature (°C)	-0.67	0.40
Illuminance (Lux)	0.73	0.20
Wetness (%Wet)	0.14	0.90

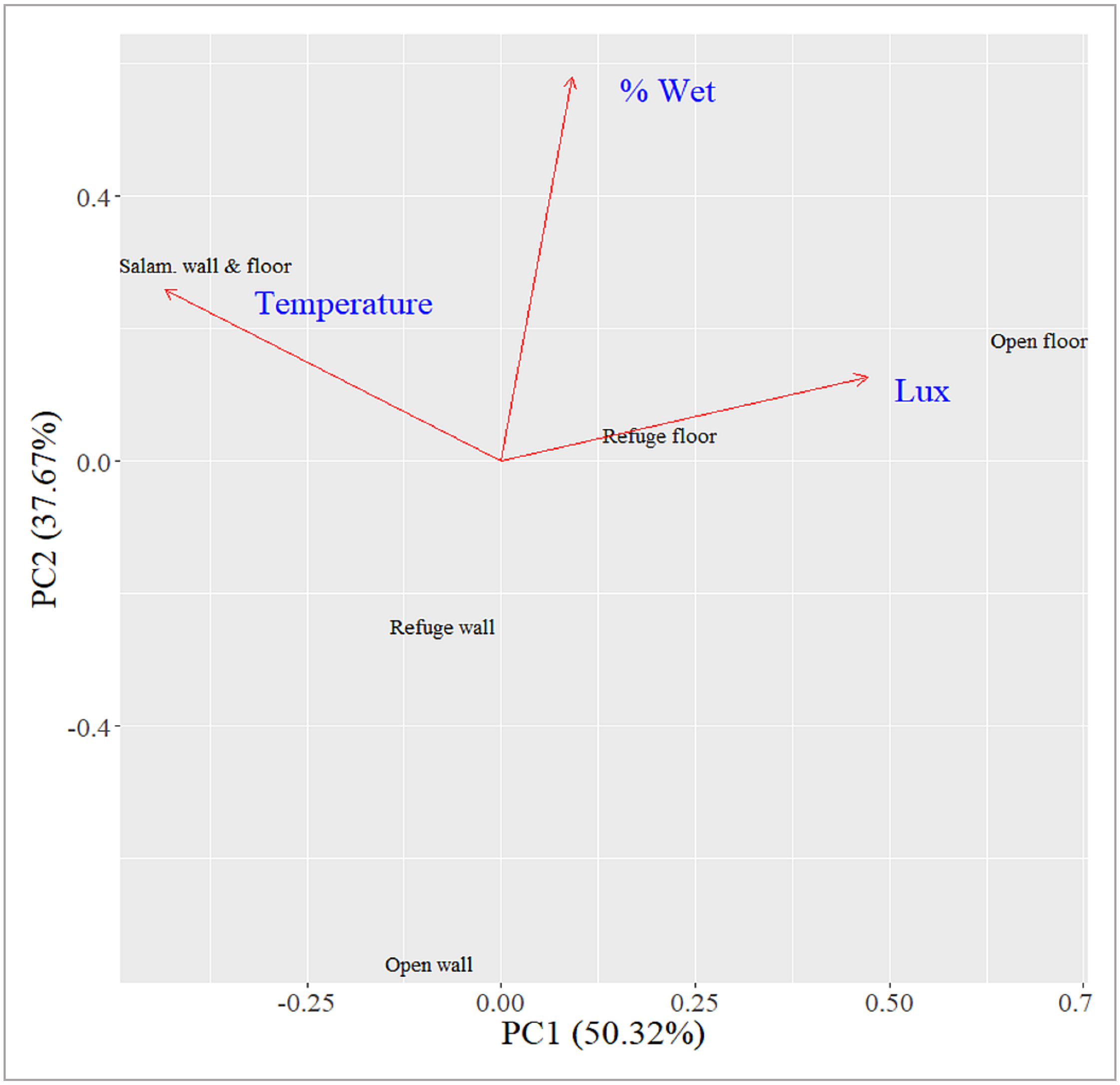

FIGURE 22. Biplot from PCA of microhabitat variables for microsite groups.

DISCUSSION

Animal movements are influenced by many factors, and pronounced changes in certain factors may induce the movements of animal populations between different habitats, such as during migrations. Here, I have demonstrated the influence of certain abiotic factors on the seasonal migratory patterns of a cave-dwelling salamander. These movements likely impact cave ecosystems because salamanders are generally considered to influence community and ecosystem dynamics. This information will not only contribute to our understanding of the ecology of this species and other plethodontids, but also to our understanding of cave ecosystems and their conservation.

Louisville photoperiod was the most significant predictor of Salamander Distance and the only variable in final regression models, and thus is important in explaining movement patterns of this population of Cave Salamanders. Photoperiod has not received much attention regarding the migratory patterns of salamanders, even though it is generally considered an important migratory cue in many other animals (Bauer *et al.*, 2011). This may be because salamanders are typically nocturnal and hide in dark areas, such as under rocks or leaf litter, during the day and are not exposed to light a great deal; however, it appears that photoperiod is an important indicator of seasonal change for Cave Salamanders, and perhaps other plethodontid species as well. This may also be because salamanders' activities are intimately associated with temperature and moisture conditions. Accordingly, Louisville temperature and Epigean %RH were highly correlated with Salamander Distance and were only excluded from final regression analyses due to significant collinearity with Louisville photoperiod; therefore, these are still key factors to consider in the movement of Cave Salamanders at Sauerkraut Cave. This has been the case in other studies; Camp *et al.* (2014) demonstrated that temperature significantly influenced the distribution of Cave Salamanders at caves in Georgia, and Hutchison (1958) and Williams (1980) attributed importance to moisture availability in regulating this species' local distribution at caves in Virginia and Illinois, respectively. Louisville precipitation and Cave Δ H_2O were also correlated with Salamander Distance (and with each other) and included in respective initial regression models. Although Louisville precipitation was dropped from the final Reduced model, it may affect Cave Salamander movement because precipitation is generally important for terrestrial salamander physioecology (Feder, 1983; Spotila, 1972), may influence fecundity, such as in *P. albagula* (Milanovich *et al.*, 2006), and stimulates migratory movements in other salamander species (Semlitsch, 2008). Cave Δ H_2O was dropped from the final Full model, but changes in water depth may indicate seasonal change; flooding alters depth, flow rate, and temperature of water in caves, and is an indicator of seasonal change for some cave-dwelling organisms (Barr Jr., 1968). It is possible that Cave Salamanders may perceive changes in water depth, or some related water parameter (e.g., temperature or conductivity), due to precipitation cycles and seasonal flooding; however, my data do not strongly support water depth as an important variable for the population at Sauerkraut Cave.

Temperature and relative humidity fluctuated very little, and photoperiod was absent in the dark zone of Sauerkraut Cave; thus, these environmental cues are lacking in this zone. However, temperature and relative humidity were variable, and photoperiod is detectable in the twilight zone, and these factors likely act in parallel as important environmental cues for the migratory movements of Cave Salamanders when they inhabit this zone. Salamanders are particularly sensitive to changes in temperature and moisture availability (e.g., Heatwole, 1962; Milanovich *et al.*, 2006 and references cited within; Peterman and Semlitsch, 2013; Wells and Wells, 1976), and photoperiod stimulates endogenous physiological processes in some species (Werner, 1969; Whitford and Hutchison, 1965; Wood and Orr, 1969), so it is likely these variables are perceivable to

Cave Salamanders. Although light intensity is relatively low in the twilight zone of caves, this species is likely adept at perceiving even low levels of light given their relatively large eyes (Hutchison, 1958), and can probably sense changes in photoperiod when inhabiting the twilight zone. It is initially unclear, though, how Cave Salamanders perceive seasonal change while inhabiting the dark zone. In the following discussion, I explain how microclimate and individual movement may inform Cave Salamanders of seasonal change while restricted to deeper recesses of Sauerkraut Cave.

Microclimate surveys in the front chamber of the twilight zone at Sauerkraut Cave indicate that Cave Salamanders preferentially select microhabitat of the cave wall and/or floor that is relatively warm, has low light, and is wet, and that these microclimates exist in the twilight zone during harsh seasons when this area is expected to be inhospitable (i.e., colder and dryer) to salamanders. Although microclimate was not measured in the deeper portion of the twilight zone (20–33 m), the macroclimate here was typically less harsh during Late Breeding and Pre-foraging seasons (late fall–early spring) than that of the front chamber, so it can be assumed suitable microclimate sometimes existed in this area as well. Movement analysis indicated that individuals moved greater Weekly Distances during Late Breeding and Pre-foraging seasons, especially compared to the Early Breeding season (mid-summer–fall). Some of this is attributable to the gradual movement of the population into the dark zone during the Late Breeding season and the seemingly abrupt movement of the population from the dark zone to the twilight zone in the Pre-foraging season. However, upon closer inspection of the individual movement data, I made an unexpected observation; individuals moved between the dark zone and areas of the twilight zone, even the front chamber (Figure 23), between Late Breeding

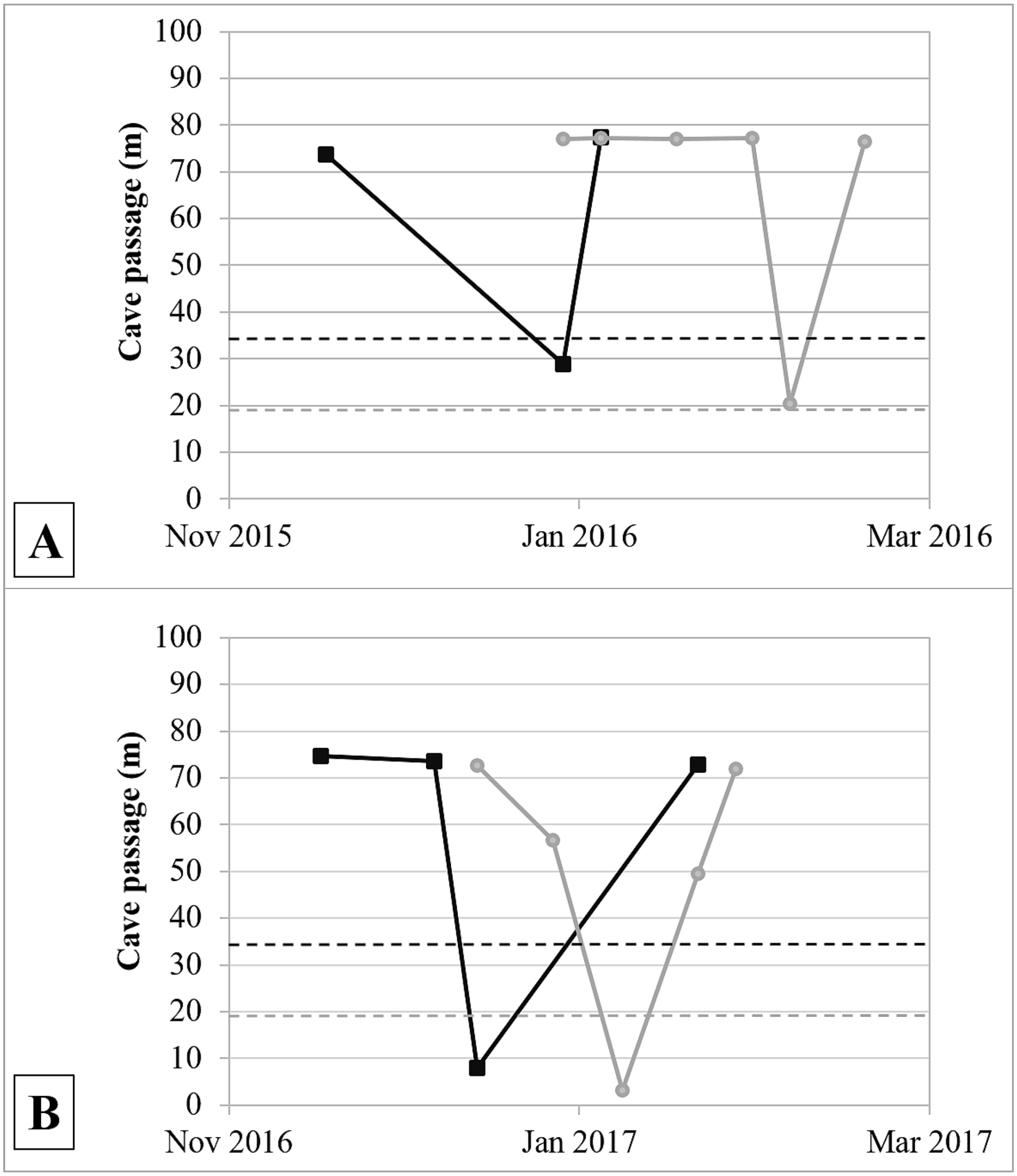

FIGURE 23. Movement graphs of four individual Cave Salamanders in the main passage of Sauerkraut Cave between Late Breeding and Pre-foraging seasons; two different individuals are represented per graph. Markers indicate a visual encounter of an individual on a survey date. The black hashed line represents the transition from the twilight zone to the dark zone at 33 m into the passage; the gray hashed line indicates the transition from the front chamber to the smaller corridor of the twilight zone.

and mid Pre-foraging seasons (December–February) when macroclimatic conditions were generally unsuitable for Cave Salamanders. However, equitable microclimate sometimes existed in the twilight zone during these seasons, so Cave Salamanders could inhabit some areas of the twilight zone at these times. Consequently, salamanders could prospect the environment of the twilight zone and sense variability in photoperiod, temperature, and moisture availability. Exposure to such environmental cues, or Zeitgebers, have been shown to influence circadian rhythms, and thus physiological cycles in salamanders (Hervant *et al*., 2000 and references cited within). Therefore, Cave Salamanders may be able to detect seasonality and adjust endogenous circadian clocks by inhabiting the twilight zone at these times of year.

The migratory scenario I suggest for this population in Sauerkraut Cave is as follows: Cave Salamanders predominantly inhabit the twilight zone during Foraging and Early Breeding seasons (late March–late September), when this area is typically cool and wet, photoperiod is longer, and an abundant food source (i.e., invertebrates) is available in the forest just outside the cave. Secondary sexual characteristics become evident near the end of the Foraging season (Chapter 3), indicating courtship activities are occurring. As climatic conditions decline toward Late Breeding season, Cave Salamanders progressively move into the dark zone where breeding activities continue, including oviposition in the deeper portions of the cave (Chapter 1). After breeding activities, individuals begin to move between the dark and twilight zones in search of detectable environmental cues (i.e., photoperiod, temperature, and moisture availability) nearer the entrance of the cave. This is accomplished by seeking and selecting favorable microclimate in the predominantly inhospitable environment of the twilight zone during this season. As conditions improve, the population increasingly inhabits the twilight zone, waiting for favorable epigean conditions to commence nocturnal foraging in the forest after months with little to no available food.

This migratory pattern is generally similar to those found for other plethodontid salamanders and seems to occur for similar reasons. Other species migrate between surface and subterranean habitat to escape environmental extremes, where they may remain active and continue breeding activities, emerging to be surface active when climatic conditions permit and where abundant food (i.e., invertebrates) is available (Cupp Jr., 1991; Heatwole, 1962; Mann and Mann, 2017; Meshaka Jr. and Trauth, 1995; Mohr, 1944; Woolbright and Martin, 2014). However, some Cave Salamanders inhabited the cave year-round in my study, i.e., the population did not entirely leave the subterranean environment. Many individuals exited the cave for nocturnal foraging (pers. obs.), but some of these individuals were found back in the cave the following day during a regular survey. It is likely that some Cave Salamanders left the cave to inhabit the limestone walls and other adjacent areas just outside the entrances for some length of time, given the relatively small number of individuals found in the cave during surveys in the early Foraging season (April–May) (Chapter 3) and the observations of salamanders in these epigean areas during some evening outings. Whether individuals emigrated from this cave is unknown; however, Hutchison (1958) concluded that Cave Salamanders do not migrate away from caves in Virginia. A population of *E. longicauda*, a sister species to *E. lucifuga*, was described to exhibit similar migratory movements in and out of subterranean habitat (i.e., an abandoned mine); however, the entire population exited the mine for two to four months before returning (Mohr, 1944). Thus, the migratory pattern of this population of Cave Salamanders is somewhat unique in that it takes place mainly within a subterranean system.

The persistence and movement of Cave Salamanders in caves likely has important implications for the co-occurring subterranean community. Population abundance and biomass of salamanders tend to be large, and salamanders are a rich food source (Semlitsch *et al.*, 2014; Whiles *et al.*, 2006). The population of Cave Salamanders at Sauerkraut Cave has been found to be more abundant than what has previously been reported for this species (Chapter 3) and may contribute a substantial proportion of biomass to this spring cave system. Furthermore, my observations indicate that Cave Salamanders are the most abundant animals in Sauerkraut Cave, likely influencing the ecosystem of this cave a great deal. This population forages on the abundant resources in the epigean forest surrounding the cave during Foraging and Early Breeding seasons (April–September). As the population moves to inhabit the dark zone, individuals shuttle acquired epigean nutrients deep into the cave system, where they defecate and sometimes die (pers. obs.). This organic input to the cave may represent an important pulse nutrient resource for cave-dwelling organisms; in fact, dead salamanders were observed in the cave and were occupied by foraging invertebrates (Chapter 3). Furthermore, salamanders are important predators of invertebrates (Best and Welsh Jr., 2014; Walker *et al.*, 2014). Cave Salamanders will take prey when encountered (pers. obs.), and although few prey are available in the dark zone of caves, the increased abundance of salamanders in this zone in the Late Breeding and Pre-foraging seasons will lead to increased encounters and possibly consumption (Bradley and Eason, 2017b) of these organisms. Therefore, these dynamics may be important for understanding cave communities, and provide critical information for cave-associated species of conservation concern, such as troglobites (Culver *et al.*, 2000).

Conclusion**—**It is evident there is much to be learned regarding the migration dynamics of plethodontid salamanders. This research allowed a unique perspective of a species that commonly inhabits caves and suggests the influence of certain abiotic factors on population migratory movements and individual movement behaviors of salamanders in a cave system. To gain a clearer understanding of how these mechanisms drive salamander migrations, carefully controlled experiments must be conducted to clarify the significance of individual environmental factors to salamander movement patterns. It is also necessary to assess these dynamics in multiple populations of Cave Salamanders in order to establish generalizable trends. The results of this study were obtained from only one population of Cave Salamanders, and thus must be interpreted cautiously. However, this research was a necessary first step to begin understanding the influence of Cave Salamanders, and trogloxenic salamanders in general, on cave ecosystems. Additionally, this represents the first approximations of this dynamic for this species that I am aware of, and the first detailed account of migration of a terrestrial salamander in a cave, providing important species and organismal information. The potential ecological implications of subterranean migratory movements of trogloxenic salamanders suggest an important link between surface and subterranean environments. Understanding these processes in greater detail will further inform our understanding of cave ecosystems and their conservation.

CHAPTER IV APPENDIX. Seasonal **s**ummary statistics for all measured macroclimatic variables by area.

Area		Season	Mean (± SD)	N	Range
Cave twilight zone					
	Temperature (°C)	Pre-foraging	10.73 (2.50)	19	5.53–16.60
		Foraging	15.81 (2.01)	18	11.50–19.23
		Early Breeding	18.52 (0.98)	15	16.80–20.23
		Late Breeding	14.35 (2.70)	21	7.87–18.30
	Relative humidity (%RH)	Pre-foraging	90.57 (5.36)	16	79.70–97.70
		Foraging	91.66 (3.42)	11	83.53–94.97
		Early Breeding	93.99 (3.71)	11	88.90–98.87
		Late Breeding	90.35 (4.70)	21	81.10–97.60
	Water depth change (Δ H_2O)	Pre-foraging	3.67 (3.13)	16	0.33–10.00
		Foraging	3.39 (3.58)	11	0.33–10.67
		Early Breeding	4.27 (4.18)	15	0.00–12.00
		Late Breeding	1.90 (2.26)	21	0.33–9.00
Cave dark zone					
	Temperature (°C)	Pre-foraging	15.03 (0.64)	19	14.03–16.60
		Foraging	15.46 (0.92)	18	14.50–17.50
		Early Breeding	15.72 (0.43)	15	14.90–16.40
		Late Breeding	15.58 (0.51)	21	14.37–16.50
	Relative humidity (%RH)	Pre-foraging	97.15 (1.39)	16	94.60–98.87
		Foraging	97.67 (1.52)	11	94.13–99.57
		Early Breeding	97.07 (1.62)	11	93.83–98.97
		Late Breeding	95.89 (1.60)	21	93.00–99.00
	Water depth change (Δ H_2O)	Pre-foraging	2.92 (2.23)	16	0.33–7.33
		Foraging	2.42 (3.46)	11	0.00–9.33
		Early Breeding	2.33 (3.04)	15	0.00–9.33
		Late Breeding	0.97 (1.20)	21	0.00–5.00

Epigean				
Temperature (°C)	Pre-foraging	7.22 (5.13)	17	-2.10–17.90
	Foraging	19.53 (4.16)	18	10.00–27.60
	Early Breeding	22.93 (3.06)	15	16.30–26.90
	Late Breeding	14.39 (5.78)	21	-1.70–21.40
Relative humidity (%RH)	Pre-foraging	65.23 (20.18)	15	29.10–100.00
	Foraging	67.59 (15.88)	11	40.40–96.00
	Early Breeding	86.76 (9.10)	11	73.40–100.00
	Late Breeding	70.19 (18.75)	21	32.20–100.00
Water depth change (Δ H_2O)	Pre-foraging	4.25 (4.09)	16	0.00–11.00
	Foraging	4.18 (4.81)	11	0.00–14.00
	Early Breeding	4.20 (5.27)	15	0.00–15.00
	Late Breeding	1.90 (2.36)	21	0.00–10.00
Louisville				
Temperature (°C)	Pre-foraging	5.38 (6.01)	19	-10.00–15.00
	Foraging	19.38 (5.82)	18	6.11–28.33
	Early Breeding	25.11 (3.80)	15	14.44–29.44
	Late Breeding	12.70 (6.33)	21	-3.89–22.78
Relative humidity (%RH)	Pre-foraging	77.32 (14.21)	19	47.00–93.00
	Foraging	81.67 (10.51)	18	50.00–93.00
	Early Breeding	85.80 (5.19)	15	78.00–93.00
	Late Breeding	79.14 (14.39)	21	47.00–96.00
Precipitation (cm)	Pre-foraging	1.62 (1.76)	19	0.00–5.97
	Foraging	2.17 (1.86)	18	0.00–5.44
	Early Breeding	1.26 (1.77)	15	0.00–6.12
	Late Breeding	1.17 (1.32)	21	0.00–5.13
Photoperiod (decimal hrs.)	Pre-foraging	10.86 (0.93)	19	9.65–12.42
	Foraging	13.97 (0.74)	18	12.67–14.82
	Early Breeding	13.41 (0.93)	15	11.82–14.75
	Late Breeding	10.39 (0.75)	21	9.52–11.77

CHAPTER V

REFUGE FROM DANGER: HABITAT SELECTION BY CAVE SALAMANDERS, *EURYCEA LUCIFUGA* (RAFINESQUE, 1822)

INTRODUCTION

Habitat selection, the process in which individuals use or occupy a non-random set of available habitats (Morris, 2003), is a central characteristic of all animals (Andrewartha and Birch, 1954; Huey, 1991). When selecting habitat, individuals must consider multiple factors, including the availability of necessary resources (e.g., food, space, and mates) and refuge from adverse environmental conditions and interactions (e.g., predation and competition) (Huey, 1991; Morris, 2003). Because no one location is likely to be the best for all the factors that drive habitat selection, individuals are subject to trade-offs when choosing suitable habitat (e.g., Abrahams, 1993; Godvik *et al.*, 2009; Krebs, 1980; Pallini *et al.*, 1998; Pawlik *et al.*, 2008).

Habitats that provide a clear example of such trade-offs are caves in temperate zones. These systems are energy deprived due to the lack of light and primary production in this environment (Barr Jr., 1967; Romero, 2009; Venarsky *et al.*, 2014), but otherwise offer suitable and even necessary habitat for many organisms, such as cave-restricted (i.e., troglobitic) animals. However, some animals (i.e., troglophiles and trogloxenes), including many salamander species (Lannoo ed., 2005; Petranka, 1998), show predilection for this habitat, yet do not technically require cave environments (Lunghi *et al.*, 2014). In the eastern United States, the Cave Salamander, *Eurycea lucifuga* (Rafinesque, 1822), is a well-known example of a facultative cave-dwelling salamander. This species is best described as trogloxenic (Trajano and Carvalho, 2017). Specifically, it inhabits caves but requires certain epigean resources to complete its life cycle and thus must leave the cave for some duration of time each year. However, some individuals of this species may be active in caves year-round, leaving to forage nocturnally and returning the next day (Chapters 3 and 4). The intimate association between *E. lucifuga* and caves has typically been linked to the wet and cool climatic conditions of this environment (Briggler and Prather, 2006; Camp *et al.*, 2014; Hutchison, 1958; Williams, 1980) and to a suite of Cave Salamander characteristics that allow it to exploit this habitat, i.e., long limbs and a prehensile tail for climbing walls (Banta and McAtee, 1906; Green *et al.*, 1967; Hutchison, 1958), large eyes to operate in low light in cave twilight zones (Hutchison, 1958), and orientation to Earth's magnetic field for navigation in darkness (Phillips, 1977). In addition, to a lesser extent, Cave Salamander use of caves has been ascribed to the availability of refuges such as the crevices and holes of cave walls (Banta and McAtee, 1906; Green *et al.*, 1967; Hutchison, 1958). However, these types of physical habitat features exist outside caves, as does an abundant invertebrate food source. Consequently, Cave Salamanders must leave caves to find sufficient amounts of food, suggesting a trade-off between food availability and suitable climatic conditions for this species.

Temperate caves may also provide protection to Cave Salamanders from adverse biotic interactions, such as predation. This is implicated in the colonization of subterranean environment by the Mexican Tetra, *Astyanax fasciatus mexicanus* (de Filippi, 1853), avoiding fish-eating bats (Romero, 1985), and in the choice of breeding habitat by the Fire Salamander, *Salamandra salamandra* (Linnaeus, 1758), in predator-free cave streams (Manenti *et al.*, 2016). Salvidio *et al.* (2017) addressed the concept that cave inhabitance by the European Cave Salamander, *Hydromantes* [*Speleomantes*] *strinatii* (Aellen, 1958), is partly due to the safety caves provide. Specifically, they suggested higher levels of predation risk in forests resulted in the colonization of caves by this troglophilic species.

The purpose of this study is to assess predation risk for *E. lucifuga* in and near caves. I hypothesize that caves provide Cave Salamanders with refuge from predators. To address this hypothesis, I use a combination of two methods: 1) an experimental manipulation testing for predation risk in different habitats with clay Cave Salamander models; and 2) field data on substrate selection by Cave Salamanders from previous studies of a population of this species (Chapters 3 and 4). I address three predictions: 1) *E. lucifuga* clay models are more likely to be damaged outside *vs.* inside caves; 2) clay models are more likely to be damaged when on the ground *vs.* in higher areas (e.g., walls); and 3) *E. lucifuga* preferentially select vertical substrate (i.e., walls) over non-vertical substrate (e.g., the ground) in caves.

METHODS

DATA COLLECTION

Predation risk—Study site selection for placement of clay models was based on five criteria: 1) caves must be “walk-in”, not sinkhole caves, 2) Cave Salamanders must be present in the cave, 3) the cave passage must be at least 30 m long and 1 m tall, 4) “ramp” access, (i.e., connectivity from the ground to the cave wall) must be limited in order to define distinct wall and ground areas, and 5) the cave must be surrounded by forest. Five accessible caves meeting these criteria were found in southern Indiana; Mill, Middle, and Waterfall Caves are located at Twin Creek Valley and Henderson Park (property of The Nature Conservancy and the City of Salem) in Washington County, Buddha Cave is located at the Buddha Karst Preserve (property of the Indiana Karst Conservancy) in Lawrence County, and Klinstiver Spring Cave (privately owned) is located in Harrison County. Specific locations are withheld to reduce potential disturbance to these caves, but may be acquired by contacting The Nature Conservancy or the Indiana Karst Conservancy.

Salamander models were made in the laboratory using white Van Aken Plastalina® clay (Van Aken International, South Carolina, USA), an oil-based modeling clay that does not harden, yet retains its molded shape. For standardization purposes, preliminary models were made to be about the shape and total length of adult Cave Salamanders (11–18 cm total length as reported by Hutchison (1958) and Williams (1980)) in order to establish a weight range of clay to use for individual experimental models. This resulted in individual experimental models being made to weigh between 9.0–9.9 g. I first weighed an appropriate amount of clay, then rolled the head, body, and tail from this piece of clay. The model was pinched to create a neck region and the head flattened. The tail was rolled from its base to the tip to be progressively smaller in diameter than the torso, and then the tail was curved. Two small sections (*ca.* 5 cm each) of rolled clay were used for the front and hind limbs and were bent into a natural-looking orientation (Figure 24). Models were painted on their dorso-lateral surfaces using Anita's™ #11312 Orange All Purpose Acrylic paint (Rust-Oleum Corporation, Illinois, USA), allowed to dry, and then manually and randomly spotted with Americana® Lamp Black/Ebony Acrylic paint (DecoArt, Kentucky, USA) using a blunt probe (Figure 24). Mean (± SD) body weight of models was 9.5 ± 0.23 g (range: 9.0–9.9 g) and mean (± SD) total length of models was 13.2 ± 0.70 cm (range: 11.5–14.6 cm).

Three hundred clay models were deployed at sites between July–August 2017. At each site, 30 models were placed inside the cave, 15 high (on the cave wall) and 15 low (on the ground). An additional 30 models were placed in the forest just outside the cave, again with 15 high (on a rock outcrop and/or vegetation) and 15 low (on the ground). Model placement was determined in a stratified random manner. Models were placed in *ca.* 2 m intervals in low and high positions, using a stretched meter tape starting near the mouth of the cave and going either deeper into the cave or into the forest. A buffer zone of 3–4 m (1–2 m on either side of the cave mouth) with no models was left at the entrance of the cave to separate cave and forest models, and high models were placed at least 0.8 m from the ground (range: 0.8–2 m high). Models were placed along one 30 m transect in the cave and two transects, one 14 m and one 16 m, in the forest. Two transects were used in the forest to utilize as much rock wall outcrop (typical Cave Salamander habitat) near and on either side of the cave entrance for high placement of models. The sides of the cave entrance designated as the start of the 14 or 16 m transects were determined randomly for each site. Placement on either side of the meter tape (i.e., left or right) for low heights and placement for high heights (i.e., relatively high or low) was determined randomly for each clay model. Models placed at high heights in the cave

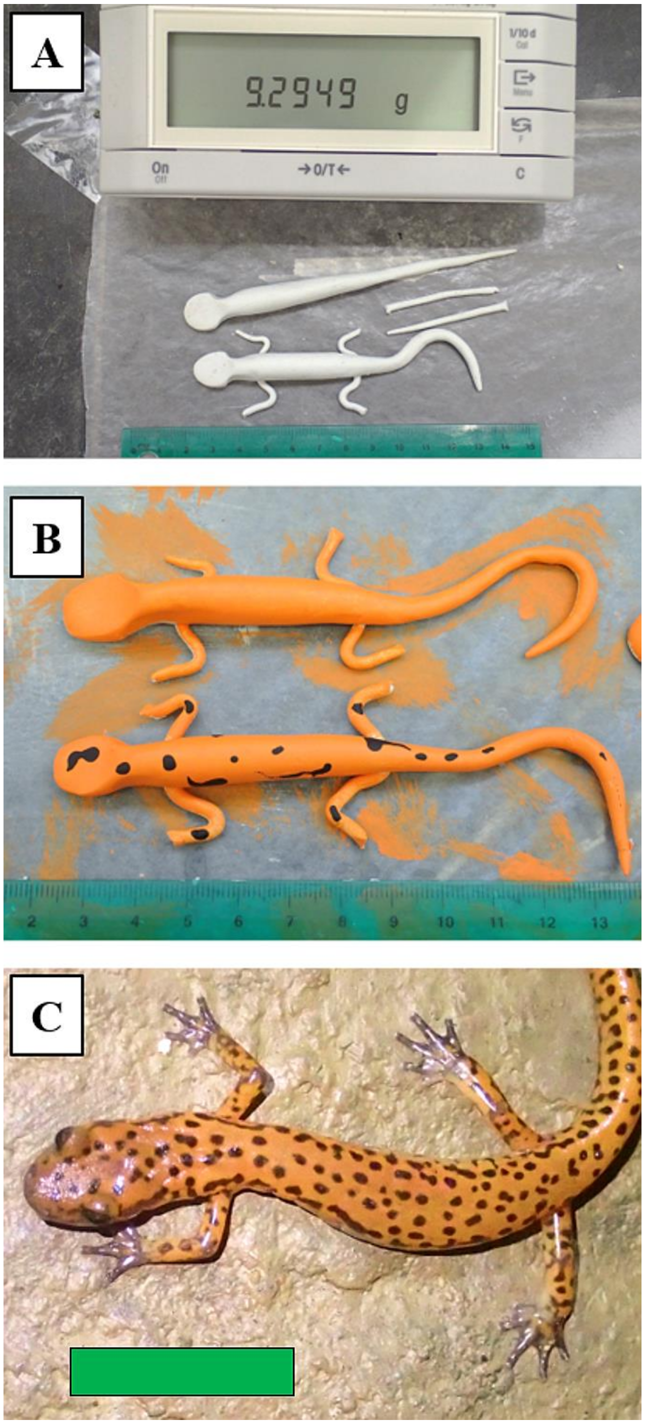

FIGURE 24. Clay models of Cave Salamanders. **A**. One piece of clay was weighed within 9.0–9.9 g, shaped into a salamander body, then legs were added. **B**. The dorso-lateral surfaces of clay models were painted orange, then spotted with black. **C**. Cave Salamander (scale bar = 2 cm).

were only placed on one cave wall (left or right) to maintain spatial distances between model salamanders that were similar to those in other areas. The cave wall to be used for placement of models was determined randomly for each cave. Models were secured to substrate using white, low odor Gorilla® Heavy Duty Construction Adhesive glue (The Gorilla Glue Co., Ohio, USA); substrate was wiped of loose dirt and debris to increase contact between the glue and substrate. Clay models were left in the field for seven days. Upon collection, data were recorded for the Site (cave name), Area (cave or forest), Height (high or low, and detailed substrate type), Distance (m) from the cave mouth, and condition of models (Damaged or not). Each model was labelled sequentially with a number using a permanent marker. Models were organized and stored in plastic tubs for transport and later inspection.

I used a key (Low *et al.*, 2014) to identify organisms that damaged the models. I only considered models to be damaged if they had been attacked by an organism that might conceivably harm a living salamander (e.g., non-human vertebrates equal or larger in size to salamanders, and carnivorous invertebrates such as centipedes, spiders, and wasps). Models were not considered damaged if all damage was done by non-threatening invertebrates, such as isopods, crickets, and ants, which are more likely to be considered prey items of salamanders than an imminent threat. I classified damage regarding the type of organism that caused it (e.g., mammal or reptile), however I used more detailed classification if possible (e.g., rodent or lizard). I also categorized damage as fatal or non-fatal. Damage was considered fatal if the head, neck, or spine area were heavily damaged, or if the model was highly deformed or destroyed. Models that were lost were not included in analyses.

Substrate selection by Cave Salamanders—Estimation of substrate selection was completed using positional data for individual Cave Salamanders and habitat surface area estimates collected from March 2015–February 2017 during previous studies of this species in Sauerkraut Cave (Chapters 3 and 4). Positional data were recorded for each salamander observed during each survey date. Focal variables used to define the location of individuals included cave passage side (left or right), substrate type and position (e.g., floor under block, hole in wall, or mud bank), distance (m) into the cave from the cave entrance, and height (m) from the base level floor or stream bed. The cave was divided into vertical and non-vertical surfaces. Substrate was considered vertical if most of its structure was approximately 90° from the horizontal plane, i.e., a brick/rock/mud wall; all other surfaces were considered non-vertical, including the floor, bricks/blocks/rocks on the floor, mud embankments, mud mounds, shelf (i.e., extensive elevated horizontal areas not part of walls), and debris piles. Using these criteria and the positional information gathered for individuals, all observed salamanders were assigned to vertical or non-vertical substrate observation categories. To reduce individual bias in substrate selection, only the first observation was used for individuals that had known multiple observations, i.e., recaptured individuals (Chapter 3).

STATISTICAL ANALYSES

Predation risk—I calculated mean (± SD) percentage of damaged models for cave and forest areas (variable: Area), and for high and low heights (variable: Height) in both habitats for the five caves (variable: Site). I calculated the percentage of models damaged by organism type, Area, and Height, including the percentage of fatally damaged models by organism type.

I conducted multiple logistic regression using Site, Area, Height, Distance, and the interaction term Area:Height as explanatory variables, and the condition Damaged as a binary response variable. I selected this interaction term for inclusion in the initial model because I suspected damage to high-positioned models might be different depending on whether they were in forests or caves. I used two-way stepwise Akaike Information Criterion (AIC) model selection to identify the best model, and individual coefficients were analyzed for overall significance using Wald tests ($\alpha = 0.05$). I assessed the explanatory value of the final model by comparing it to a null model (Damage ~ 1) using a Likelihood Ratio test ($\alpha = 0.05$) and calculating the AIC value for the null model.

Substrate selection by Cave Salamanders—I counted the number of vertical and non-vertical substrate observations of individual Cave Salamanders and calculated the ratio of vertical to non-vertical surfaces in Sauerkraut Cave using the surface area calculated previously for this cave (Chapter 3). I then applied this ratio to the total number of salamander observations to calculate expected numbers of vertical and non-vertical substrate observations, assuming salamanders were choosing substrate types in proportion to their availability. I analyzed observed *vs.* expected vertical and non-vertical substrate observations using a χ^2 goodness of fit test ($\alpha = 0.05$).

All statistical analyses were run in R version 3.4.1 (R Core Team, 2017) and data visualization was accomplished using R and Microsoft Excel 2016.

RESULTS

Predation risk—At least 95% (N = 57) of clay models were retrieved from each site (Table 16), with the remainder of the models lost due to unknown causes. The total

TABLE 16. The percent of clay Cave Salamander models retrieved from the field, and the percent damaged, by Area and Site. Raw counts are given in parentheses with each respective percentage.

				% Damaged						
	% Retrieved			Cave			Forest			Site
Site	Cave	Forest	Total	High	Low	Total	High	Low	Total	Total
Buddha	96.7 (29)	100 (30)	98.3 (59)	0	13.6 (8)	27.6 (8)	8.6 (5)	10.2 (6)	36.7 (11)	32.2 (19)
Klinstiver	100 (30)	100 (30)	100 (60)	3.3 (2)	6.7 (4)	20.0 (6)	3.3 (2)	5.0 (3)	16.7 (5)	18.3 (11)
Middle	90.0 (27)	100 (30)	95.0 (57)	0	0	0	10.5 (6)	12.3 (7)	43.3 (13)	22.8 (13)
Mill	96.7 (29)	93.3 (28)	95.0 (57)	0	1.8 (1)	3.4 (1)	5.3 (3)	3.5 (2)	17.9 (5)	10.5 (6)
Waterfall	100 (30)	93.3 (28)	96.7 (58)	1.7 (1)	0	3.3 (1)	8.6 (5)	3.5 (2)	25.0 (7)	13.8 (8)
Mean [± SD]	96.7 [4.08]	97.3 [3.65]	97.0 [2.17]	1.0 [1.50]	4.4 [5.81]	10.9 [12.17]	7.2 [2.89]	6.9 [4.08]	27.9 [11.73]	19.5 [8.47]

number of damaged models for all sites was 57 (19.6% of 291 retrieved models). The final regression model was Damaged ~ Site + Area + Height + Area:Height (AIC = 272.68), and the Likelihood Ratio test indicated the final model was significantly different (χ^2 = 31.2, P < 0.0001) from the null model (AIC = 289.88). Logistic regression analysis showed that distance from the cave mouth did not significantly affect the probability of model damage (Wald χ^2 = 1.3, df = 1, P = 0.25), and that variable was dropped from the final regression model. Site had a significant effect on damage (Table 17) with the percentage of damaged models varying widely across sites (range: 10.5–32.2%) (Table 16). The main effect of area was also significant, and in this case meaningful even though there was also a significant area:height interaction (Table 17). Overall, models were less likely to be damaged in caves than forests. At the five study sites, 10.9 ± 12.17% of models in caves were damaged *vs.* 27.9 ± 11.73% in forests. Height and an area:height interaction also had significant effects on the probability of model damage. In caves, models placed on cave walls were much less likely to be damaged than models on the floor; in forests, however, model height did not affect damage probability (Table 16; Figure 25).

Identifiable organisms that caused damage included rodents and an additional mammal that could not be identified more narrowly, birds, lizards, and one unknown vertebrate (Table 18; Figure 26). Seven models were classified as damaged from unknown animals because I could not determine whether it was from carnivorous invertebrates or small vertebrates. Fatal damage occurred for 14 (24.6%) models (Table 18; Figure 26).

TABLE 17. Summary of the multiple logistic regression for damaged clay models.

Factor	Coefficient	SE	OR	df	χ^2	P
Intercept	-2.426	0.633	-	1	14.7	<0.001
Site (overall)	-	-	-	4	10.2	0.037
Klinstiver	-0.809	0.452	0.445	-	-	-
Middle	-0.540	0.440	0.583	-	-	-
Mill	-1.469	0.529	0.230	-	-	-
Waterfall	-1.132	0.489	0.322	-	-	-
Area	2.218	0.651	9.184	1	11.6	< 0.001
Height	1.575	0.670	4.833	1	5.5	0.019
Area:Height	-1.595	0.770	0.203	1	4.3	0.038

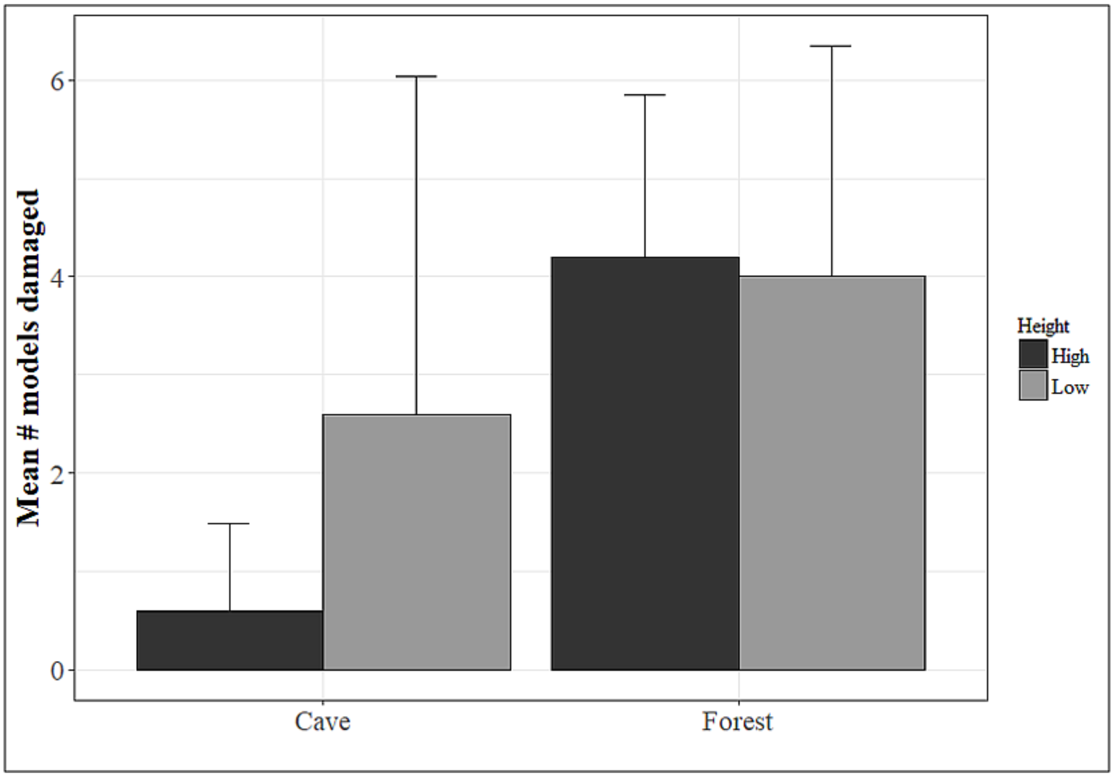

FIGURE 25. The mean number of salamander models damaged in caves and forests. Error bars represent the standard deviation.

TABLE 18. Types of animals that damaged clay salamander models. Raw counts are given in parentheses.

Site	% Organism damage area^{-1}		% Fatal organism damage area $^{-1}$	
	Cave	Forest	Cave	Forest
Buddha Cave				
Rodent	100 (8)	45.5 (5)	—	—
Bird	—	27.3 (3)	—	—
Unknown:				
Animal	—	9.1 (1)	—	—
Vertebrate	—	9.1 (1)	—	50.0 (1)
Mammal	—	9.1 (1)	—	50.0 (1)
Klinstiver Cave				
Rodent	100 (6)	40.0 (2)	—	—
Bird	—	60.0 (3)	—	100 (2)
Middle Cave				
Rodent	—	61.5 (8)	—	50.0 (2)
Bird	—	15.4 (2)	—	50.0 (2)
Unknown:				
Animal	—	23.1 (3)	—	—
Mill Cave				
Rodent	100 (1)	40.0 (2)	100 (1)	—
Lizard	—	40.0 (2)	—	100 (2)
Unknown:				
Animal	—	20.0 (1)	—	—
Waterfall Cave				
Rodent	100 (1)	71.4 (5)	—	100 (3)
Unknown:				
Animal	—	28.6 (2)	—	—
Total				
Rodent	100 (16)	53.6 (22)	100 (1)	38.4 (5)
Bird	—	19.5 (8)	—	30.8 (4)
Lizard	—	4.9 (2)	—	15.4 (2)
Unknown	—	22.0 (9)	—	15.4 (2)

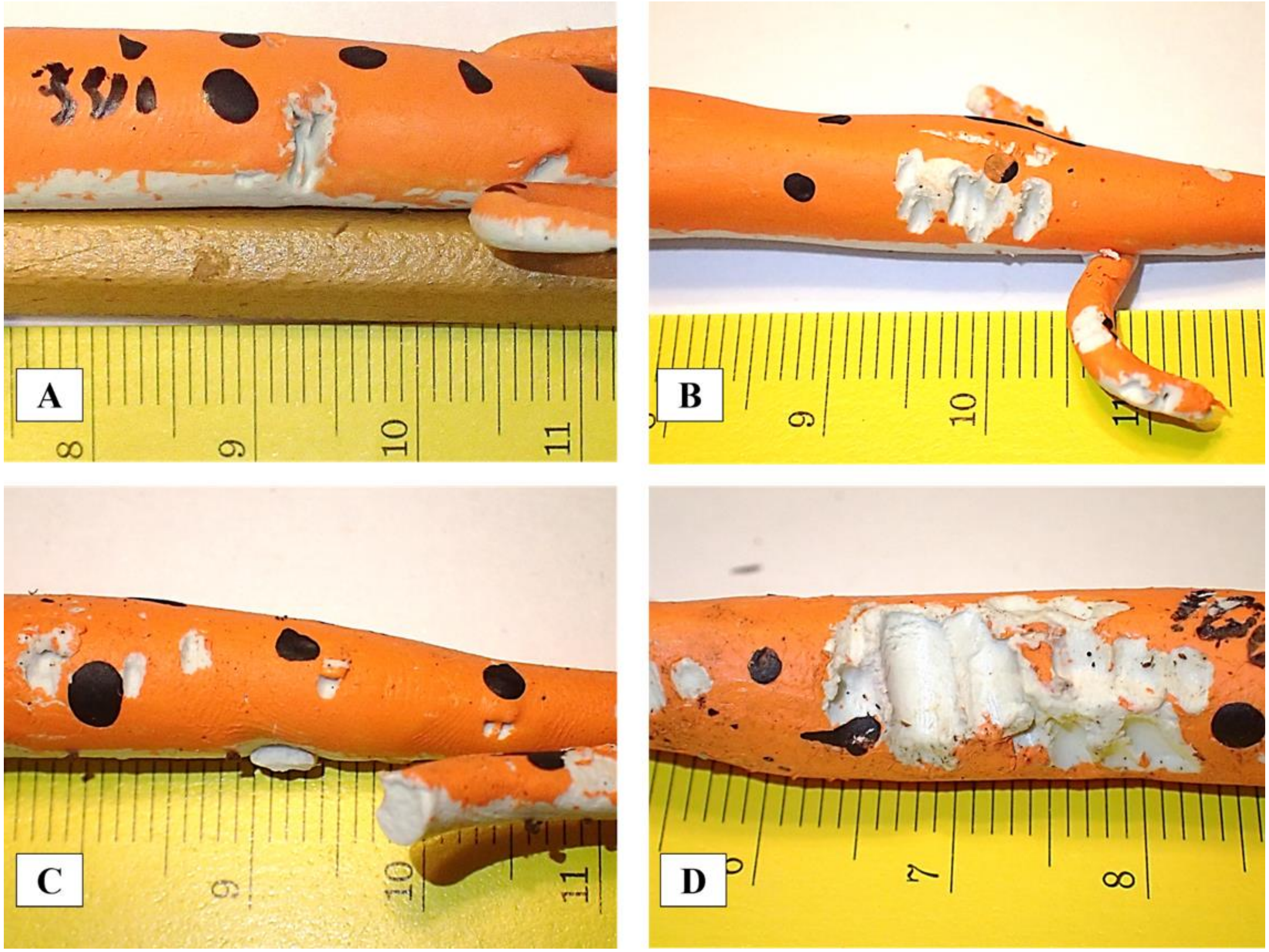

FIGURE 26. Damaged clay models. **A, B,** and **C.** Paired dorsal and ventral incisor marks from rodents (likely *Peromyscus* spp.) in the mid-body area. **D.** Dorsal and ventral, flat teeth marks (probably incisors) of an unknown mammal in the mid-body area.

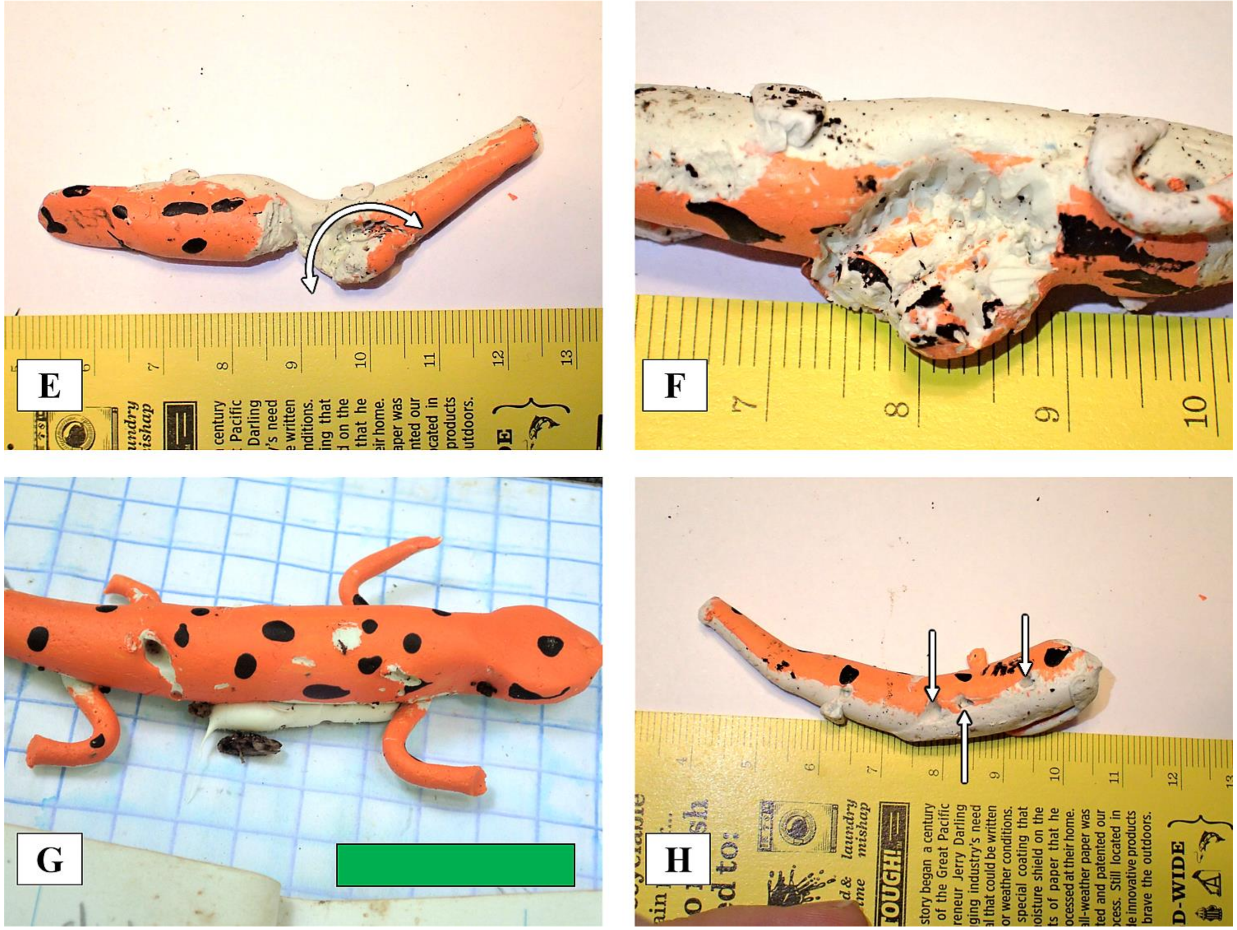

FIGURE 26 *continued*. **E** and **F.** Lizard damage to the mid-body, indicated by the impression left by an arc of homodont teeth (represented by the arced arrow in E); damage to these models was considered fatal. **G** and **H.** Bird damage in multiple areas of the body (beak punctures indicated by arrows in H); damage to the model in H was considered fatal (scale bar = 2.54 cm).

Substrate selection by Cave Salamanders—I made a total of 5039 substrate observations of Cave Salamanders in Sauerkraut Cave. After removing all but the first observation of known individuals, there were 2720 substrate observations. The area of vertical surfaces in Sauerkraut Cave was 364.4 m^2, and for non-vertical surfaces 489.0 m^2; the ratio is 1:1.3 vertical:non-vertical surface area. The expected number of vertical and non-vertical substrate observations are 1183 and 1537, respectively. The observed vertical and non-vertical substrate observations were 1912 and 808, respectively. The χ^2 goodness of fit test for expected *vs.* observed substrate observations was highly significant ($\chi^2 = 794.53$, df = 1, $P < 0.0001$).

DISCUSSION

Taken together, the damage to clay models that I observed and salamanders' substrate selection strongly suggest that caves, and in particular cave walls, provide important refugia from interactions with potential predators. As I predicted, models in caves were less likely to be damaged than models in forests outside of caves, with a mean of 10.9% damaged in caves *vs.* 27.9% in forests. Further, models in high positions were less likely to be damaged than models on the ground, but only in caves. Lastly, individual Cave Salamanders were more likely to be found on vertical substrates (i.e., walls) compared to non-vertical substrates (e.g., the ground) in Sauerkraut Cave.

The higher probability of damage for clay models in forests could be a result of more potential predators foraging there compared to inside caves. The results from this study should be interpreted cautiously, though, because the clay models were left in the open during day and night in the forest for one week before collection. This may have resulted in artificially higher damage rates, given that Cave Salamanders are more often nocturnal outside caves, although they do sometimes move in epigean environments diurnally (pers. obs.). However, Salvidio *et al.* (2017) similarly reported caves to be safer habitats than surrounding forests for the troglophilic salamander *H. strinatii*. Specifically, predation risk, as determined by damage to clay models, was about four times greater in forested areas compared to caves (Salvidio *et al.*, 2017); in my study, damage to clay models was 2.6 times greater in forests than in caves. Further, just over half of the damage (53.6 %) to models in forests was from rodents, some of which are nocturnal and are likely to interact with Cave Salamanders both inside and outside caves; in fact, damage from rodents was the most prevalent damage type, occurring in both caves and forests. Conversely, the birds and lizards that damaged forest models are probably less likely to interact with Cave Salamanders because these two groups are typically diurnal in forests. A future study could cover models in the forests during the day and reveal them in the evening when Cave Salamanders are more likely to become surface active. Logistical constraints prevented such an experiment in this study. Still, these results suggest that predation risk is greater outside of caves for *E. lucifuga*. Furthermore, fatal damage occurred to only one clay model (7.7% of 14 fatally damaged models) inside of caves, with the remaining found in the forests. Fatal damage was usually the result of punctures in critical areas of clay models by rodents and birds, but all lizard damage was fatal and damages from an unknown mammal and from an unknown vertebrate were also fatal. Although the interactions between birds or lizards and salamanders is less likely, they are still possible, and at least six of these fatal damages were from organisms that likely interact with salamanders (i.e., rodents and other mammals). This further signifies the greater risk of detrimental interactions in forests compared to caves.

The tendency for low models to have higher attack rates within caves was the result of rodent interactions, probably *Peromyscus* spp. given that these mice commonly inhabit southern Indiana caves (Julian J. Lewis, pers. comm.). These results suggest that height above the ground reduces potential agonistic biotic interactions in caves. Because I controlled for connectivity by selecting caves with little access via ramps or shelves along walls, very few high-positioned models were damaged by rodents in this habitat. This is further supported by substrate selection of Cave Salamanders in Sauerkraut Cave, which indicated a strong preference for vertical surfaces in caves by this species. *Eurycea lucifuga* is adept at climbing and can move from horizontal surfaces, such as the ground, to vertical surfaces at *ca.* 90° with relative ease (pers. obs.). Cave Salamanders can also cling to surfaces on which their final orientation is completely upside down (Mary Kate O'Donnell, pers. comm.). This ability allows them to utilize cave walls better than many other organisms of similar or greater size, which greatly reduces the likelihood of adverse interactions between Cave Salamanders and potential predators. Furthermore, the relatively small size and slender body of Cave Salamanders allows them to access thin crevices and holes in cave walls, providing enhanced refuge and safety. Conversely, height was less important outside of caves because birds could access clay models in high positions, and some rodents also reached these high positioned models via the increased connectivity between low and high substrates in the forest. *Peromyscus* spp. exhibit arboreal behavior (e.g., Persons and Eason, 2017), indicating they can climb to higher habitat if sufficient connectivity exists between lower and higher positions. The physical complexity of many caves may result in increased connectivity between low and high surfaces, especially in sinkhole caves. It would be interesting to run a similar experiment comparing levels of connectivity in different caves to see how this might affect interactions between Cave Salamanders and rodents, and perhaps other potentially dangerous organisms.

Distance from the cave mouth did not affect the probability of damage in this study, but sites did, possibly in part due to the distinctiveness of Buddha Cave, which had a larger entrance, a more expansive floor area, and extensive fragmented boulders and rocks on the ground compared to all other caves. These traits may have contributed to a greater presence of rodents in Buddha Cave and thus increased damage to cave clay models. Distance was considered in this study because more damage might occur to cave clay models nearer cave entrances, since potentially predatory animals are more likely to see models in the low light of the twilight zone than in the dark zone during the day. However, this was not the case because rodents were active potential predators up to 30 m deep into these caves, a length that incorporated the twilight and dark zones of each study cave.

I could not definitively attribute any damage to carnivorous invertebrates such as spiders, centipedes, or wasps. However, Cave Salamanders (Bradley and Eason, 2017c) and other salamander species (Crane and Mathis, 2015; Ficetola *et al.*, 2013; Hickerson *et al.*, 2004, 2017; Rubbo *et al.*, 2003) interact with these invertebrate taxa, which could lead to agonistic encounters resulting in injury and perhaps death to salamanders (Crane and Mathis, 2015; Rubbo *et al.*, 2003).

Conclusion**—**Caves provide favorable climatic conditions (Briggler and Prather, 2006; Camp *et al.*, 2014; Hutchison, 1958; Williams, 1980) and refugia for Cave Salamanders. Salamanders selecting for these benefits face a tradeoff with low food abundance and the cost of commuting between cave and forest habitat to find food, but apparently, it is worth this cost. This study thus highlights another benefit of the cave environment for *E. lucifuga*, which may be extrapolated to other trogloxenic salamanders. Understanding this driver of habitat use by trogloxenic salamanders provides important species information, and ultimately contributes to a greater understanding of the unique cave environment and its influence on the ecological relationships and behaviors of cave-inhabiting animals.

CHAPTER VI

DISSERTATION CONCLUSION

This study has uncovered important ecological and behavioral relationships of the Cave Salamander that were previously little-known for this species. Past researchers suggested Cave Salamanders were difficult to work with because they became increasingly wary during long-term investigation and avoided researchers using standard capture, handling, and marking techniques (Howard, 1985; Hutchison, 1958; Organ, 1968; Williams, 1980). Whether this species is truly more wary and avoidant relative to other species of salamanders is inconclusive. It is evident from the “mark”-recapture procedure of this study that individuals commonly disappear from view for variable lengths of time, and perhaps this is a normal activity pattern. Also, depending upon the time of year, Cave Salamander populations shift their distribution between the twilight and dark zones of caves, and past research has typically focused on salamanders in the twilight zone. Additionally, caves are physically complex and often inaccessible to humans, providing enhanced refuge to small animals such as salamanders. With these factors considered together, it is not difficult to imagine why some considered this species to be more behaviorally sensitive to the presence of researchers. However, out of caution, I employed non-invasive techniques and successfully studied this species at a higher intensity (i.e., *ca.* weekly) and for a longer duration of time than has been achieved by past researchers. It is possible the degree of non-invasiveness I used in this study was not necessary, and perhaps a mixture of these and slightly more-invasive techniques could be used, such as handling salamanders while using mild anesthetics. Using this method, for example, would likely greatly improve accuracy of pattern-recognition software, and thus the time spent analyzing digital images, because position and orientation of individuals could be under greater control.

It is evident that more long-term studies of *E. lucifuga* and a larger sample size of cave sites is necessary to formulate general ecological patterns of the demographics and movements of this species. I only used one cave site for the majority of this study. Due to the unexpected high number of individuals at Sauerkraut Cave and the methodology used to identify individuals, another population could not be considered for this study because of time and logistical constraints. It will be important in future research of this species to hone methods and carefully coordinate and implement field data collection to expedite data processing from multiple populations.

Perhaps the most alluring and lingering question to come from this research concerns the potential ecological impacts of trogloxenic salamanders to cave ecosystems. Given the important general ecological characteristics of salamanders (e.g., salamanders are important invertebrate predators, a high-energy food source as prey, and couple ecosystems via life history stages (e.g., Davic and Welsh, 2004; Schriever *et al.*, 2014; Semlitsch *et al.*, 2014), and the potential for trogloxenic salamander populations to be larger than previously expected in some caves, these organisms may have profound impacts on subterranean environments. This is important because caves and many of the troglobitic species restricted to them are of great conservation concern. In order to properly inform cave conservation and management, it may be necessary to understand the dynamics of trogloxenic salamanders both inside and outside of these habitats. Other trogloxenic species, such as bats and cave crickets, are known to greatly influence cave ecosystems (Barr Jr., 1968). Perhaps salamanders should be added to that list as well?

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APPENDIX

NATIONAL
SPELEOLOGICAL
SOCIETY

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DEDICATED TO THE EXPLORATION, STUDY, AND CONSERVATION OF CAVES

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April 2, 2018

J. Gavin Bradley
University of Louisville
139 Life Sciences Bldg.
Louisville, Kentucky 40292

Dear Mr. Bradley,

As Editor-in-Chief of the *Journal of Cave Karst Studies* one of my responsibilities is the granting of permission f or the reprinting of articles, figures, photos, etc., originally published in the *Journal of Cave Karst Studies* and/or *Bulletin of the National Speleological Society*. This letter is my official approval to grant you permission to reproduce the draft manuscript

Bradley, J.G., and Eason, P.K., 2018, Observations on the population ecology of the cave salamander, Eurycea *lucifuga* (Rafinesque 1822), Journal of Cave and Karst Studies, (Ms.\@ No.\@ JCKS-S-17-00037). Currently under review for consideration for publication in your students Ph.D.\@ thesis.

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Sincerely yours,

Malcolm S. Field

Malcolm Field, Editor-in-Chief
Journal of Cave and Karst Studies

cc: Curt Harler

CURRICULUM VITAE

NAME	Joseph Gavin Bradley
ADDRESS	Department of Biology 139 Life Sciences Building University of Louisville Louisville, KY 40292
DOB	Louisville, Kentucky – May 16, 1985

EDUCATION

Doctor of Philosophy, Biological Sciences University of Louisville, Louisville, KY Ecology and behavior of the Cave Salamander, *Eurycea lucifuga*	Aug. 2018
Master of Science, Biological Sciences University of Louisville	Dec. 2016
Bachelor of Science, Biological Sciences University of Louisville	Dec. 2011

TEACHING EXPERIENCE

Graduate Teaching Assistant
University of Louisville, Jan. 2013–Aug. 2017, Jan.–Aug. 2018
Introductory Biology Laboratory for Majors (Bio. 244) and Non-majors (Bio. 104), Vertebrate Zoology Lab, Comparative Vertebrate Anatomy Lab, Human Anatomy and Physiology Lab

Undergraduate Mentorship
University of Louisville, Mar. 2015–May 2017
Guiding undergraduate students in a research program concerning the ecology and behavior of Cave Salamanders to provide experience in ecological fieldwork, data processing and analysis, and scientific writing
Paige Wilson (Honor's thesis: Movement patterns of the Cave salamander (*Eurycea lucifuga*) in Sauerkraut Cave, KY), Danica Shepherd (Independent research course

credit), Faith Bowers, Nick Callahan, Will Seibt, Logan Stone, Emily Petri, Isaac Kaufman

Graduate Teaching Assistant Academy
University of Louisville, Sep. 2015–Apr. 2016
Program for graduate students to develop pedagogical skills and knowledge through interactions with faculty, group projects, and informative lectures and readings

Tutoring
University of Louisville, Nov.–Dec. 2012
Biology tutor for the Athletic Department guiding athletes in one-on-one and small group study sessions covering core concepts and principles of general biology

Service
Farmer Elementary, Louisville, KY, Dec. 2016
Presented for elementary school students during their 'Career Day' about working as a teacher and a scientific researcher

Jefferson Memorial Forest and Jeff. Co. Public Schools, Louisville, KY, Aug. 2012–May 2013
Volunteered for the Engaging Children Outdoors (ECHO) program teaching mammalian biology and ecology to local elementary school students

Invited Talks
Bradley JG, 2016, Non-invasive mark-recapture methods for salamanders, Karst Field Studies: Experiential Ecology, Mammoth Cave National Park, KY

Bradley JG, 2016, Natural history and ecology of the Cave Salamander (*Eurycea lucifuga*), Kentucky Society of Natural History, Louisville Nature Center, Louisville, KY

Guest Lectures
Bradley JG, 2018, Cladograms and evolutionary relatedness, Comparative Vertebrate Anatomy course, University of Louisville

Bradley JG, 2015, Cave vertebrate ecology, Vertebrate Zoology course, University of Louisville

RESEARCH

Projects
Population ecology and behavior of Cave Salamanders (*Eurycea lucifuga*)
University of Louisville, Jan. 2015–Aug. 2018
Doctoral research investigating non-invasive methods for individual identification, demographics, population migration, individual movements, and habitat selection of Cave Salamanders

Amphibian colonization of new habitat
University of Louisville and Bernheim Arboretum and Research Forest, Feb.–Jul. 2016
Master's project assessing colonization and long-term persistence of amphibians in newly created ponds

Soil erosion and invasive species impact on plant communities
University of Louisville and Olmsted Parks Conservancy, May 2010–May 2012
Team leader on an EPA funded project concerning the effects of invasive plant removal in urban parks

Population ecology and behavior of urban mammals
University of Louisville, Aug. 2010–Aug. 2011
Undergraduate research including live trap and release of mammals and telemetry systems in urban settings

Presentations

Bradley JG, 2017, Individual identification of salamanders using non-invasive methods Biology Awards Day, University of Louisville

Bradley JG and Eason PK, 2017, The natural history and ecology of the Cave Salamander (*Eurycea lucifuga*), 40th Annual Herpetology Conference, Florida Museum of Natural History and Reptile and Amphibian Conservation Corps, Gainesville, FL

Bradley JG and Eason PK, 2015, A hands-off approach to monitoring Cave Salamanders (*Eurycea lucifuga*), Kentucky Academy of Sciences Conference, Northern Kentucky University, Newport, KY

Publications

Bradley JG and Eason PK, 2017, Novel interaction between a pisaurid spider (Araneae: Pisauridae) and an adult *Eurycea lucifuga* (Caudata: Plethodontidae), Phyllomedusa 16(2): 279–282

Bradley JG and Eason PK, 2017, *Eurycea lucifuga* (Cave Salamander) Coloration, Herpetological Review 48(4): 827

Bradley JG and Eason PK, 2017, *Eurycea lucifuga* (Cave Salamander) Diet, Herpetological Review 48(1): 155

Accepted:

Bradley JG and Eason PK, 2018, Predation risk and microhabitat selection of cave salamanders, *Eurycea lucifuga* (Rafinesque, 1822), Behaviour

Bradley JG and Eason PK, 2018, *Eurycea lucifuga* (Cave Salamander) Vocalization, Herpetological Review

Revised manuscript submitted:
Bradley JG and Eason PK, Observations on the population ecology of the Cave salamander, *Eurycea lucifuga* (Rafinesque, 1822), Journal of Cave and Karst Studies

Assistantships

University of Louisville, Aug.–Dec. 2017
Maintaining the vertebrate collection for the Biology Department, including preserved specimens and display units

Grants and Awards

Graduate Student Council Travel Award
University of Louisville, 2017
Award used for travel to the 40th Annual Herpetology Conference in Gainesville, FL

Biology Graduate Student Association Grant
University of Louisville, 2014
A research grant used for the exploration of Central American anteaters as study organisms for my dissertation research

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AFFILIATIONS

National Speleological Society	2016–present
Kentucky Academy of Science	2015–present