

The Effects of Climate on the Hypogean Migration of Cave Salamanders, *Eurycea lucifuga* (Rafinesque, 1822)

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ABSTRACT.—Terrestrial migrations of salamanders are influenced by many factors, particularly temperature and moisture availability. Although photoperiod is generally an important migratory cue, it has not been specifically considered for salamanders. We examine the importance of the above abiotic factors at the macro- and microclimatic scales in the subterranean migration of a population of Cave Salamanders (*Eurycea lucifuga*) in Kentucky. This species exhibited a cyclic, seasonal migratory pattern between the twilight and dark zones in a cave. Our regression analysis of macroclimate indicated this migration was influenced largely by photoperiod, but also highly correlated with temperature and relative humidity; principle components analysis confirmed the importance of similar microclimate variables (illumination, temperature, and substrate wetness), explaining 88% of the variability in the data. These environmental variables fluctuate little in the dark zone of caves and thus likely do not provide cues on seasonal conditions to salamanders. However, these cues do vary in the twilight zone and may inform salamanders of epigeal seasonal conditions. This information is critical to these cave-dwelling salamanders, particularly for finding suitable food resources.

Movement of organisms defines the spatial dynamics of an individual's interactions with other organisms and the environment (Brown, 1984; Pittman et al., 2014) and thus is an important determinant of individual fitness. Understanding the factors that drive movement patterns 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. Many biotic and abiotic factors influence animal movements (Begon et al., 2006; Linzey, 2012), and seasonal variation in these factors may lead to pronounced distributional changes in animal populations, such as migrations.

Several abiotic and biotic factors influence localized animal movements, i.e., travel between microhabitats or movements that occur daily. In terrestrial environments, such movements are strongly influenced by temperature and water availability (Begon et al., 2006). Salamanders are especially sensitive to these factors because of their desiccation-prone skin (Spotila, 1972; Feder, 1983), which affects their occupation of terrestrial habitats (e.g., Heatwole, 1962; Wells and Wells, 1976; Milanovich et al., 2006; Peterman and Semlitsch, 2013). These constraints are marked in temperate zones, where temperature and moisture (i.e., precipitation and/or humidity) regimes fluctuate seasonally, and salamanders may further be affected by fine scale temperature and moisture patterns at diel and microhabitat scales (Feder, 1983; Peterman and Semlitsch, 2014). Many other factors can also influence localized terrestrial movements of salamanders, including vegetative (Heatwole, 1962; Pough, 1980) and landscape characteristics (Marsh et al., 2005; Rittenhouse and Semlitsch, 2006), soil pH (Wyman and Hawksley-Lescault, 1987; Sugalski and Claussen, 1997), management activities (Harper and Guynn, 1999 and references cited within), cover availability (Grover, 1998), intraspecific competition (Jaeger, 1979; Whiteman et al., 1994), predator activity (Maerz et al., 2001), and food availability (Whiteman et al., 1994; Harper and Guynn, 1999). Further, photoperiod stimulates metabolic (Whitford and Hutchison, 1965; Wood and Orr, 1969) and

reproductive processes (Werner, 1969) in salamanders, which likely increases directed local movements such as toward food sources or mating areas.

Some of the aforementioned factors (e.g., temperature, moisture, food availability, and season/photoperiod) are also implicated in the terrestrial migrations of salamanders. Annual migrations are well established for pond-breeding taxa, such as ambystomatids (Petranka, 1998; Lannoo, 2005); adults migrate from overwintering terrestrial sites to and away from breeding ponds during cool, rainy nights (reviewed by Semlitsch, 2008). Terrestrial migrations are less well documented for plethodontids but have been reported for *Plethodon cinereus* (Heatwole, 1962; Woolbright and Martin, 2014), *Plethodon angusticlavius* (Meshaka and Trauth, 1995), *Plethodon websteri* (Mann and Mann, 2017), *Aneides aeneus* (Cupp, 1991), *Eurycea longicauda* (Mohr, 1944; Nazdrowicz, 2015), *Eurycea cirrigera* (Niemiller and Miller, 2007), *Eurycea quadridigitata* (Palis and Aresco, 2007), and *Eurycea wilderae* (Pierson et al., 2019). Some of these species exhibit seasonal migrations between surface and subterranean environments for purposes of finding suitable microhabitat (Heatwole, 1962), breeding (Niemiller and Miller, 2007), and a combination of these factors and foraging (Mohr, 1944; Cupp, 1991; Woolbright and Martin, 2014; Nazdrowicz, 2015; Mann and Mann, 2017). Many plethodontids have known associations with subterranean environments (Petranka, 1998; Lannoo, 2005), but very little is known regarding their hypogean population dynamics.

As a result, we have a poor understanding of the ecology of these animals, which are generally of conservation concern (Collins and Storer, 2003; Davic and Welsh, 2004). Understanding the ecology of salamanders is important because they strongly influence local community structure and function. Salamanders are abundant (Whiles et al., 2006; Semlitsch et al., 2014) keystone predators that regulate nutrient cycling through their strong influence on food webs (reviewed by Davic and Welsh, 2004; Best and Welsh, 2014; Walker et al., 2014) and are a high-energy food for other animals (Semlitsch et al., 2014). Salamanders also couple aquatic and terrestrial ecosystems via resource flow (reviewed by Davic and Welsh, 2004; Regester et al., 2006; Schriever et al., 2014). These ecological characteristics

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and the movements of plethodontids between subterranean and surface habitats suggest salamanders may provide an important link between these environments. Thus, detailing their migratory patterns is essential to fully understanding the ecological roles and habitat associations of these organisms.

Cave Salamanders, *Eurycea lucifuga*, are common plethodontids in the eastern United States. This facultatively cave-dwelling (i.e., nontroglobitic) species is often observed in the twilight zone of caves (i.e., the cave zone dimly lit by sunlight) (Petranka, 1998; Lannoo, 2005). Cave Salamanders can be abundant (Bradley and Eason, 2019), yet little is known about the ecology of this species. One pattern has been described by some authors regarding population distribution changes in caves. Cave Salamanders inhabit the twilight zone from spring through fall when this area is cool and moist, but retreat deeper into caves (toward or into the dark zone) during winter when this area is cold and dry, re-emerging in the twilight zone the following spring (Hutchison, 1958; Williams, 1980). This pattern suggests seasonal migration, but detailed information regarding movements in and between the twilight and dark zones of caves is unavailable for this species. The movements 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.

We examined the movement patterns of a population of *E. lucifuga* in a spring cave. Our goals were to document any seasonal migration between the twilight and dark zones and to explore the environmental 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 (Hutchison, 1958; Williams, 1980; Camp et al., 2014), we hypothesized that the seasonal migration of Cave Salamanders in our study cave was associated with certain abiotic factors known to affect salamanders' inhabitation and activity in terrestrial habitats. Specifically, we predicted that seasonal migrations between the twilight and dark zones of caves occurred in response to changes in temperature and moisture availability as well as changes in photoperiod, which is an important migratory cue for many other animals (Bauer et al., 2011). Although Cave Salamanders inhabiting the dark zone may not sense photoperiod, those individuals in the twilight zone may be able to.

MATERIALS AND METHODS

Study Site.—We monitored a population of *E. lucifuga* during 74 survey dates from March 2015–February 2017 to assess population migration and macroclimate (described below) and once a month from October 2016–April 2017 for our assessment of microclimate (described below). This population was surveyed in the main passage of Sauerkraut Cave, a spring cave in E. P. “Tom” Sawyer State Park in Louisville, Kentucky, USA; further description of this cave can be found in Bradley and Eason (2019). The main passage is ~130 m long with a relatively large walk-in entrance (height [H] = 2.20 m, width [W] = 4.56 m) that opens to the largest chamber in this system (greatest dimensions: H = 2.23 m, W = 10.04 m, L = 19.97 m). The north end of this chamber constricts to a smaller stream crawlway (H = 1.02 m, W = 1.37 m), and the remainder of this passage is stoop-walk or belly-crawl height.

Data Collection.—We constructed a survey transect starting at the drip line of the entrance and following the length of the main

passage to 99 m deep. Surveys generally were conducted weekly, but the schedule was modified occasionally because of flooding or other logistical constraints. During surveys, we searched the walls, floor, ceiling, and standing water of the transect for Cave Salamanders during daylight hours using red filtered light. Hutchison (1958), Williams (1980), and Briggler and Prather (2006) similarly conducted surveys for this species during daylight hours. When a salamander was located, we recorded its distance from the drip line and 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 (Olympus America Inc., Pennsylvania, USA) for individual identification in I³S-Spot (Bradley and Eason, 2018). We used these pictures to track individual movements in the cave passage over time and to reduce the likelihood of pseudoreplication during transect searches on survey dates. We did not capture or handle any salamanders during this study.

Macroclimate.—We measured cave air temperature (°C), relative humidity (% RH; 625 thermo-hygrometer, B&K Precision Corporation, California, USA), and the weekly change in water depth (cm; ΔH_2O) of the stream or largest pool at six fixed stations along the transect. Three of these stations were located at different distances in the twilight zone (0–33 m) and the other three set at different distances in the dark zone (33–99 m). We measured similar epigeal variables at one spot just outside the cave entrance. We obtained Louisville mean temperature (°C), precipitation (cm), maximum % RH (University of Kentucky Agricultural Weather Center [UKAWC], 2017), and photoperiod (decimal hour) (United States Naval Observatory [USNO], 2017) for Louisville, Kentucky for each survey date. We summed precipitation measures across a 5-day period that included precipitation on our survey date and the previous 4 days to better represent precipitation regimes.

Microclimate.—We measured microclimates in the front chamber (0–20 m) of the main passage once per month during daylight hours. We chose the 6-mo period described previously (October–April) to investigate the available microclimates in this area before, during, and after winter because we unexpectedly observed salamanders in this inhospitable (i.e., cold and dry) environment during winter. We recorded “salamander” microsite locations (i.e., side of the passage (left or right) and area (wall or floor)) where salamanders were found and measured microclimate variables immediately adjacent to each salamander. These variables included temperature (°C) (RT-1 REPTITEMP[®] infrared thermometer, Zoo Med Laboratories Inc., California, USA), illuminance (Lux) (EXTECH[™] EA30 EasyView light meter, FLIR Commercial System Inc., New Hampshire, USA), and wetness (dry or wet to touch). We also measured these microclimate variables at paired “open” and “refuge” microsites for comparison with salamander microsites. Open microsites lacked available cover, whereas refuge microsites were locations that could conceivably provide cover (e.g., under a rock or in a hole) for a salamander and were within 1 m of a paired open microsite. To choose these sites, we randomly selected four whole-number locations along a 20-m midline to determine the distances into the front chamber within which we would sample open and refuge microsites. We measured microsites on the left side of the midline when the whole-number distance was even and on the right side when the whole-number distance was odd. From each selected distance, we walked from the midline at 90° and measured microsites on the floor near ($X \pm SD$: 0.33 ± 0.24 m, $n = 56$), midway ($X \pm SD$: 2.40 ± 0.73 m, $n = 56$), and far ($X \pm SD$: 4.00 ± 1.03 m, $n = 56$) from the midline. We also measured microsites on

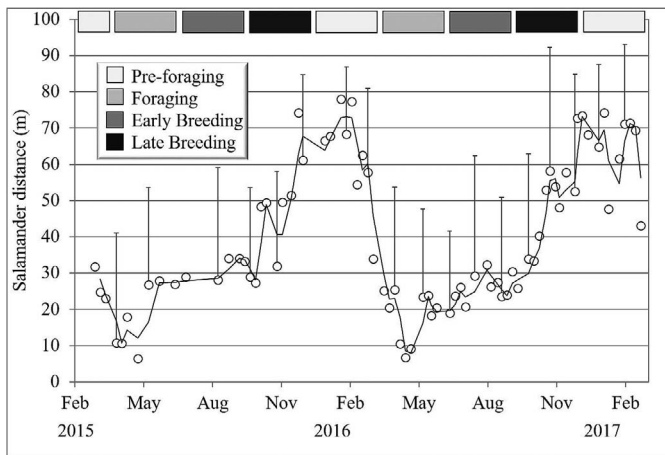


FIG. 1. Mean (\pm SD) population distance (i.e., salamander distance) into the main passage of Sauerkraut Cave. The trendline is a moving average (period = 2) set by Excel to provide visual clarity of the data. Error bars are shown only for every fourth survey to maintain clarity of the figure. Salamander seasons are indicated at the top of the graph and correspond with certain ranges of months on the x-axis.

the wall at those distances and at low ($X \pm$ SD: 0.52 ± 0.66 m, $n = 56$), medium ($X \pm$ SD: 1.26 ± 0.22 m, $n = 56$), and high ($X \pm$ SD: 1.74 ± 0.27 m, $n = 56$) elevations. There were thus six sets of open and refuge microsites at each of four distances into the cave, for a total of 24 microsites assessed on each sampling day.

Data Summarization.—We calculated mean population distance into the cave, i.e., “salamander distance,” and the proportions of total salamanders seen in the twilight and dark zones for each survey date. We defined “salamander seasons” using patterns evident in salamander distance and life history information for this species (Bradley, 2018): preforaging (January–March), foraging (April–June), early breeding (July–September), and late breeding (October–December). To compare macroclimates between cave zones and by seasons, we pooled cave stations by zone (i.e., twilight and dark zones) and calculated the mean of each cave macroclimate variable for each survey date, and we calculated seasonal mean temperature, %RH, and Δ H₂O for each cave station separately. We also pooled each macroclimate variable by season and calculated the means.

Macroclimate and Population Migration Analysis.—To determine which factors were associated with salamander migration within the cave, we conducted multiple linear regression using selected explanatory variables from cave, epigeal, and Louisville macroclimate measurements, with salamander distance as the response variable. For cave variables, we used mean temperature and %RH from the twilight zone, but not from the dark zone because temperature and humidity had low variability there (see Results) and thus little explanatory power; for consistency, we used mean Δ H₂O only from the twilight zone. We used Pearson’s correlation tests for explanatory variable selection prior to regression analysis. Because some explanatory variables were similar in type (e.g., cave, epigeal, and Louisville temperatures), we first determined which of these similar variables was most correlated with the response variable and selected it for further analysis. We then tested for significant ($\alpha = 0.05$) collinearities among explanatory variables that were significantly ($\alpha = 0.05$) or nearly significantly ($\alpha < 0.10$) correlated with the response variable. For the regression model, we included all explanatory variables that were not collinear, and from each group of collinear variables we included the variable that was most correlated to the response

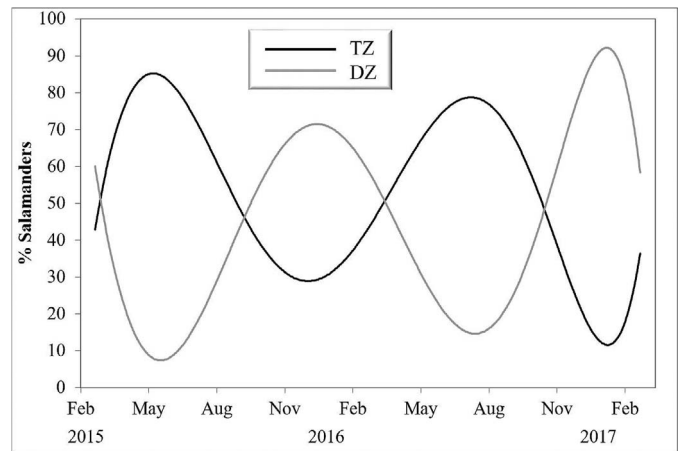


FIG. 2. The proportion of the Cave Salamander population seen in the twilight and dark zones on survey dates from the main passage of Sauerkraut Cave. Data points are removed and trendlines are fit using a sixth-order polynomial in Excel to provide visual clarity. TZ = twilight zone; DZ = dark zone.

variable. We then constructed a multiple linear regression model and conducted two-way stepwise Akaike information criterion (AIC) model selection. We checked the final model residuals for normality using a Shapiro-Wilk test ($\alpha = 0.05$).

Microclimate Analysis.—We pooled microclimate variables across months and by microsite type (i.e., salamander, open, or refuge) and calculated the means. We separated each microsite type by area (i.e., wall or floor), creating six microsite groups: salamander wall, salamander floor, open wall, open floor, refuge wall, and refuge floor. We calculated mean temperature and Lux, and the percent of wet observations (%Wet) for each group and analyzed these data using principle components analysis (PCA).

All statistical analyses were run in R version 3.4.1 (R Core Team, 2017), and all means are given \pm SD.

RESULTS

Salamanders.—On average we found 56.0 ± 30.3 salamanders (range: 7–222, $n = 74$) in the main passage on each survey date. Mean salamander distance ranged from 6.41 ± 8.40 m in May to 77.97 ± 15.05 m in January ($n = 74$) (Fig. 1), and the proportions of the salamander population seen in the twilight vs. dark zones exhibited a cyclic pattern over time (Fig. 2).

Macroclimate and Population Migration.—Macroclimate was generally more variable in the twilight zone (Appendix 1) and increasingly variable from the back of the twilight zone toward the cave entrance (Table 1). Pearson correlations (Table 2) within similar types of explanatory variables (i.e., temperature, % RH, and Δ H₂O) indicated Louisville temperature ($R = -0.68$, $P = 1.76e^{-11}$), Epigeal % RH ($R = -0.36$, $P = 0.006$), and Cave Δ H₂O ($R = -0.25$, $P = 0.052$) were most correlated with salamander distance. Significant collinearity was found amongst Louisville temperature, epigeal % RH, and Louisville photoperiod (Table 2); Louisville photoperiod was most correlated with salamander distance ($R = -0.83$, $P = 2.20e^{-16}$). Significant collinearity was found between cave Δ H₂O and Louisville precipitation (Table 2); cave Δ H₂O was more correlated (see above) with the response variable.

The initial regression model was salamander distance \sim cave Δ H₂O + Louisville photoperiod. Stepwise AIC model selection produced salamander distance \sim Louisville photoperiod as the

TABLE 1. Seasonal means (\pm SD) of cave environmental variables for each station.

Variable	Cave environmental stations					
	Twilight zone			Dark zone		
	1	2	3	4	5	6
Temperature ($^{\circ}$ C)						
Preforaging	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)						
Preforaging	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 H ₂ O)						
Preforaging	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)

final model (Table 3). However, the AIC values for both models were similar, suggesting that these models may be equivalent in their ability to predict salamander distance. Final model residuals were normally distributed ($W = 0.98$, $P = 0.197$).

Microclimate.—Salamanders were found in a narrower range of temperature and Lux compared to the range available in their environment, and they were most often found at wet microsites (high % wet; Table 4). The PCA explained 88.00% of the variation in microsite groups. Principal component one (PC1) is strongly correlated with Lux (0.73) and temperature (-0.67) and explained 50.32% of the variation in microsite groups; principal component two (PC2) is strongly correlated with % wet (0.90) and explained 37.68% of the variation in microsite groups (Table 5). Salamander microsites loaded negatively on PC1 and positively on PC2, indicating that salamanders selected increased temperature and wetness, but avoided light (Fig. 3). Floor microsites loaded positively on PC1 and PC2, indicating that randomly selected floor microsites have more light, lower temperatures, and high wetness. Wall microsites loaded negatively on PC1 and PC2,

TABLE 2. Pearson's correlations and collinearities for explanatory variables used in regression analysis. The R and P values are presented for correlations between an explanatory variable and the response variable (salamander distance). The R and P values for collinearities among explanatory variables identify a dropped explanatory variable that was correlated to another explanatory variable that is labelled by a or b .

Variable	Response variable correlation		Collinearity	
	R	P	R	P
Temperature ($^{\circ}$ C)				
Cave	-0.67	$9.91e^{-11}$	—	—
Epigeian	-0.65	$1.16e^{-09}$	—	—
Louisville	-0.68	$1.76e^{-11}$	0.70^a	$3.95e^{-12}$
Relative humidity (% RH)				
Cave	-0.32	0.015	—	—
Epigeian	-0.36	0.006	0.28^a	0.035
Louisville	-0.28	0.014	—	—
Water depth change (Δ H ₂ O)				
Cave ^b	-0.25	0.052	—	—
Epigeian	-0.24	0.057	—	—
Precipitation (cm)	-0.21	0.074	0.37^b	0.003
Photoperiod (decimal hrs.) ^a	-0.83	$2.20e^{-16}$	—	—

indicating that wall microsites are dryer, but maintain warmer temperatures and lower light than do floor microsites.

DISCUSSION

Our results indicate that migrations of Cave Salamanders between the twilight and dark zones of a cave are related to seasonal fluctuations in photoperiod, temperature, and relative humidity, and possibly to changes in water depth within the cave. One of these factors, photoperiod, has not received much attention as a potential trigger for salamander migrations (Bradshaw and Holzapfel, 2007; but see Mettouris et al., 2018). Photoperiod may be generally ignored because salamanders are not often exposed to light because they are typically nocturnal and hide in dark areas during the day. However, photoperiod is an important migratory cue in many animals (Bauer et al., 2011); furthermore, it was the most significant predictor of salamander distance. This suggests that photoperiod may be an important indicator of seasonal change for Cave Salamanders, although more studies are needed to address this phenomenon.

Temperature and % RH were also highly correlated with salamander distance although they were excluded from final regression analysis because of collinearity with photoperiod; these thus are likely key factors in the movements of Cave Salamanders, as previous researchers suggested. Temperature significantly influenced the distribution of Cave Salamanders at Georgia caves (Camp et al., 2014), and moisture availability was an important factor in regulating this species' distribution at Virginia (Hutchison, 1958) and Illinois (Williams, 1980) caves. In this study, precipitation was also correlated with salamander distance. Although precipitation was not included in the regression analysis because of collinearity with Δ H₂O, it may affect the movement of Cave Salamanders because it is

TABLE 3. Results of the stepwise AIC model selection.

Model	AIC	Δ AIC	Adj. R^2	F	P
Initial					
Cave Δ H ₂ O + Louisville photoperiod	304.65	—	0.71	75.91	$2.20e^{-16}$
Final					
Louisville photoperiod	303.46	1.19	0.69	163.9	$2.20e^{-16}$

TABLE 4. Summary statistics for microclimate variables by microsite type.

Variable	Mean (\pm SD)	Range	<i>n</i>
Temperature ($^{\circ}$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
Wetness (% Wet)			
	Dry	Wet	<i>n</i>
Salamander	3.7	96.3	109
Open	25.9	74.1	158
Refuge	10.1	89.9	158

generally important for terrestrial salamander physioecology (Spotila, 1972; Feder, 1983), may influence fecundity (Milanovich et al., 2006), and stimulates migrations in other salamanders (Semlitsch, 2008).

The inclusion of ΔH_2O in the initial regression model suggests this may be an important predictor of salamander distance. Generally, flooding alters depth, flow rate, and temperature of water in caves and is an indicator of seasonal change for some cave-dwelling organisms (Barr, 1968). Furthermore, the reproductive cycle of Cave Salamanders may be linked to changes in water flow (Ringia and Lips, 2007) and thus changes in water depth. However, the final regression model did not include ΔH_2O , yet AIC values for both models were similar ($\Delta AIC = 1.19$). It is common practice to accept competing models as equivalent if $\Delta AIC < 2$; however, following this general rule may not always result in best model selection, and favoring a competing, more parsimonious model with a lower AIC may also be acceptable (Richards, 2008; Symonds and Moussalli, 2011). As a result, further studies need to be conducted to determine the importance of ΔH_2O as an indicator of seasonal change for Cave Salamanders.

Temperature and relative humidity fluctuated little, and photoperiod was absent in the dark zone of Sauerkraut Cave; thus, these environmental cues are lacking for Cave Salamanders while inhabiting this zone. However, temperature and relative humidity were variable in the twilight zone, where photoperiod is detectable, and these factors likely act in parallel as important environmental cues for migrations of Cave Salamanders. In general, salamanders are sensitive to changes in temperature and moisture availability (e.g., Heatwole, 1962; Wells and Wells, 1976; Milanovich et al., 2006; Peterman and Semlitsch, 2013), and photoperiod stimulates endogenous physiological processes in some salamanders (Whitford and Hutchison, 1965; Werner, 1969; Wood and Orr, 1969), so these variables are likely perceptible to Cave Salamanders. Although light intensity is low in the twilight zone of caves, this species

TABLE 5. Eigen values from the PCA of microclimate for microsite groups.

Variable	PC1	PC2
Temperature ($^{\circ}$ C)	-0.67	0.40
Illuminance (Lux)	0.73	0.20
Wetness (% Wet)	0.14	0.90

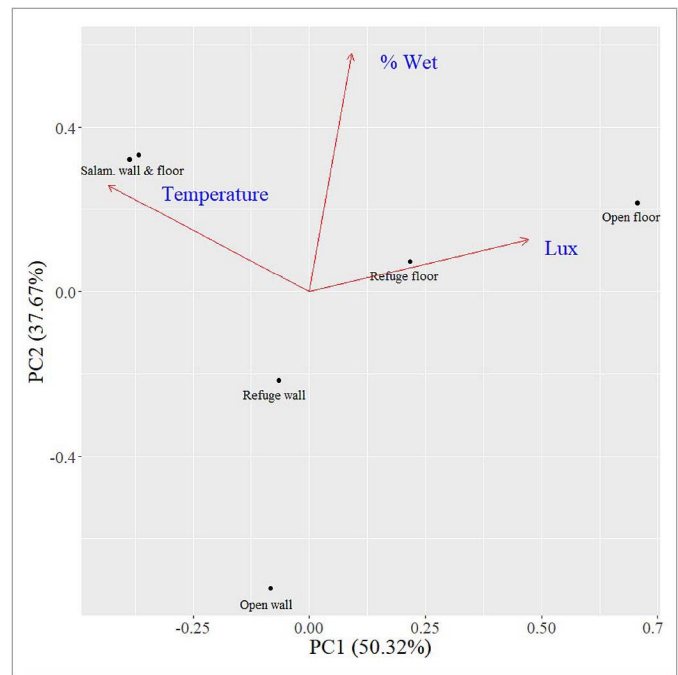


FIG. 3. Biplot from PCA of microhabitat variables for microsite groups. Sites selected by salamanders are wetter, warmer, and darker than randomly selected refuge and open sites in the outermost chamber of the study cave.

likely perceives even low levels of light, given their relatively large eyes (Hutchison, 1958), and thus may sense changes in photoperiod when inhabiting this zone. It is unclear, however, how Cave Salamanders perceive seasonal change while in the dark zone.

Microclimate surveys in the twilight zone indicate Cave Salamanders preferentially select microhabitat of the cave wall and/or floor that is relatively warm, dimly lit, and wet. These surveys further show that such microclimates exist in the twilight zone even during seasons when this area is expected to be inhospitable (i.e., colder and dryer) to salamanders. Unexpectedly, we observed that individuals moved between the dark and twilight zones (Fig. 4) between late breeding and mid preforaging seasons when the overall macroclimatic conditions were generally unsuitable in the twilight zone. They were able to do so because Cave Salamanders could inhabit areas of the twilight zone where suitable microclimate existed even during these harsher seasons. Consequently, salamanders could explore the environment of the twilight zone and sense variability in photoperiod, temperature, and moisture availability. Exposure to such environmental cues, or Zeitgebers, influences 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 visiting the twilight zone at these adverse times of year.

The migratory scenario we suggest for this population is as follows: Cave Salamanders predominantly inhabit the twilight zone during foraging and early breeding seasons, when this area is typically cool and wet, photoperiod is longer, and abundant food (i.e., invertebrates) is available in the forest outside the cave. Secondary sexual characteristics become evident near the end of foraging season (Bradley and Eason, 2019), indicating that courtship activities are occurring. As climatic conditions deteriorate toward late breeding season,

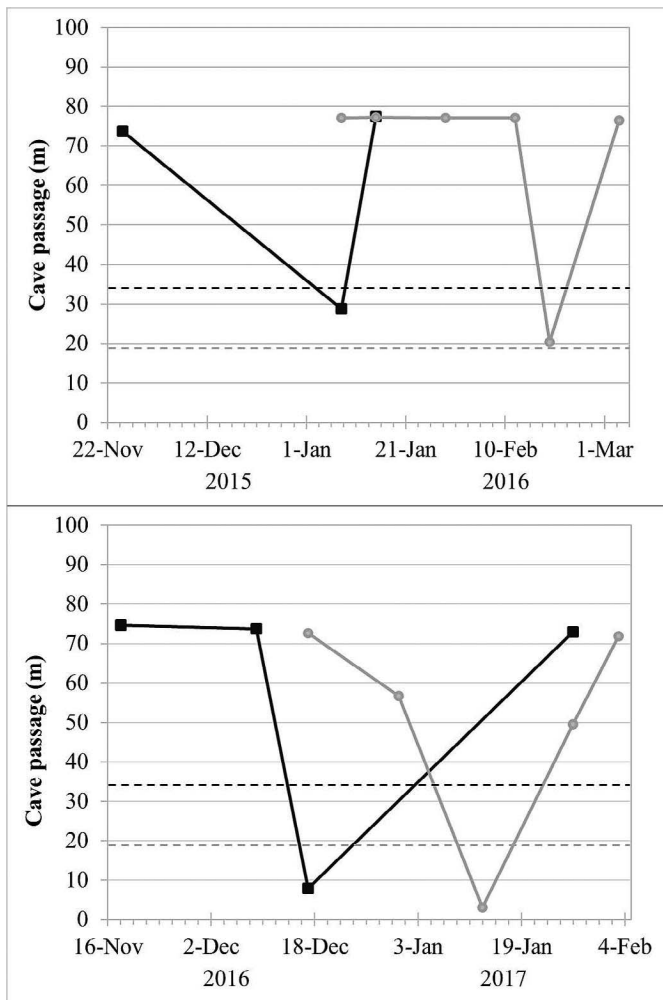


FIG. 4. Movement graphs of four individual Cave Salamanders in the main passage of Sauerkraut Cave between late breeding and preforaging seasons; two different individuals are represented per graph. Markers indicate a sighting of an individual on a survey date. The black hashed line represents the transition 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.

Cave Salamanders progressively move into the dark zone where breeding activities continue. After breeding, individuals begin to move between the dark and twilight zones, likely searching for food and detecting environmental cues (i.e., photoperiod, temperature, and moisture availability) nearer the entrance. This is accomplished by seeking and selecting favorable microclimate in the inhospitable environment of the twilight zone during this time. As conditions improve, the population increasingly inhabits the twilight zone, waiting for favorable epigeic conditions to commence nocturnal foraging in the forest.

This migratory pattern is like those found for other plethodontids and occurs for similar reasons (i.e., breeding, foraging, and finding suitable climate). However, some Cave Salamanders inhabited the cave year round in this study, i.e., the population did not entirely leave the subterranean environment. Many individuals exited the cave for nocturnal foraging when the epigeic climate was suitable (JGB, pers. obs.), but some of these individuals were found back in the cave the following day during a regular survey. Perhaps individuals left the cave to

inhabit adjacent areas just outside the entrance for some time, but whether individuals emigrated from this cave is unknown; however, Cave Salamanders do not migrate away from Virginia caves (Hutchison, 1958). Thus, the migratory pattern of this population of Cave Salamanders is unique in that it occurs primarily within a subterranean system.

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APPENDIX 1. Seasonal summary statistics for all measured macroclimatic variables by area.

Area	Season	Mean (\pm SD)	<i>n</i>	Range		
Cave twilight zone	Temperature ($^{\circ}$ C)	Preforaging	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)	Preforaging	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 ₂ O)	Preforaging	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 ($^{\circ}$ C)	Preforaging	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)		Preforaging	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 ₂ O)		Preforaging	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	
Epigeal		Temperature ($^{\circ}$ C)	Preforaging	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)	Preforaging	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 ₂ O)	Preforaging	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 ($^{\circ}$ C)	Preforaging	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)		Preforaging	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)		Preforaging	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.)		Preforaging	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	