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(*Eurycea lucifuga*) in Sauerkraut Cave, Kentucky

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Movement Patterns of the Cave Salamander (*Eurycea lucifuga*) in Sauerkraut Cave, Kentucky

Abstract. The Cave Salamander, *Eurycea lucifuga,* is a common but poorly understood species of salamander that inhabits limestone features and karst systems across the eastern and midwest United States. As a species that can bridge the gap between subterranean and epigean environments, *E. lucifuga* may behave as a top predator and act as a significant facilitator in the flow of energy from the outside environment into the dark zone. The goal of this study was to document the seasonal movements of a local population of cave salamanders in Louisville, Kentucky, and the relationship between those movements and abiotic factors. Secondarily, I explored how the movements of individuals among the cave zones affect the food web of the cave as a whole. Weekly or biweekly surveys were undertaken in Sauerkraut Cave from March 2015 to February 2016. Each encountered salamander was photographed, and I recorded its distance from the entrance (m), height upon the cave wall (cm), side of the passage (left or right, when viewed from the entrance), and substrate upon which it occurred. I also measured temperature (°C) and relative humidity (%) at regular intervals throughout the cave. Salamander activity generally followed variation in temperature and humidity, with proportionally more individuals sighted in the twilight zone during the warmer months, followed by a shift to the dark zone during the colder winter months. The number of salamanders recorded per survey increased as the year progressed, with the most current numbers being more than double the average number of salamanders seen per survey for the entirety of 2015. Individuals that were recaptured more than twice exhibited more variable movement patterns than the general population, perhaps due to courtship and breeding behavior. Further study of the seasonal fluctuations of cave salamander behavior in local populations such as this could be crucial in understanding the complexities of cave ecosystems across the temperate United States.

INTRODUCTION

Since the 1970s amphibians have been valued in the scientific community for their roles as indicators of environmental health and as essential members of a complex biosphere (Hopkins 2007). Burton and Likens (1975) reported that during the breeding season in some eastern deciduous forests, the biomass of salamanders can be equal to that of small mammals and even twice the biomass of all passerine birds. This large biomass suggests that salamanders significantly influence ecosystem processes (Davic and Welsh 2004). An example of this effect was described by Schmitz *et al*. (2000), wherein predatory salamanders increased plant productivity by reducing herbivore abundance.

With widespread global population decline in amphibians ongoing, it is imperative that we understand the ecological functions of amphibian clades in their respective niches (Davic and Welsh 2004). Previous studies of salamanders focused on only a small subset of Urodela species, resulting in a deficit of information regarding some species that may be keystone species in their respective habitats.

One such imperfectly understood species is the Cave Salamander, *Eurycea lucifuga,* of the family Plethodontidae. Adults of this species are large and conspicuous, with bright reddish or orange dorsum coloration marked by erratic black spots, the pattern of which is unique to each individual. This species also possesses long limbs, a prehensile tail, large eyes, and a flattened body profile, all of which assist the animal in moving and living within the limestone and karst features with which it is most habitually linked (Petranka 1998).

A meager number of life history studies have been carried out across the eastern United States that detail the distribution and behavioral habits of adult *E. lucifuga* within karst systems (Petranka 1998, Briggler and Prather 2006, Hutchison 1958). Populations tend to move seasonally within cave networks over the course of a year, and there is spatial variation in the distribution of life stages (Petranka 1998, Camp *et al.* 2014). In temperate regions, such as Kentucky, Virginia, and Georgia, cave salamander populations distribute themselves in their preferred range of temperature and humidity—with adults following warmer, more saturated air into the dark zone during the winter months, and returning to the twilight zone as the surrounding environment warms and cool, humid air shifts to the mouth of the cave (Hutchison 1958, Camp *et al.* 2014, Petranka 1998).

While these salamanders are not exclusively restricted to cave environments (Banta and McAtee 1906, Green *et al.* 1967), they can have a significant presence in limestone karst landscapes, in terms of both biomass and niche function. Petranka (1998) suggested that cave salamanders are top predators in caves and may facilitate the flow of energy from the outside environment into the dark zone—but there has not been further investigation or testing of this hypothesis. It is estimated that only about 7,500 of the 200,000 or more caves in North America have been surveyed or are accessible for study (Barr 1968). Likewise, there is relatively little known about the complex interaction of nutrient webs between caves and their surroundings throughout the United States. As

such, understanding the movements, behavioral patterns, and the specifics of microhabitat preferences of species like *E. lucifuga* could further our knowledge of community structure within karst systems.

The principal objective of this study was to investigate how abiotic microhabitat factors and their seasonal variation affected the distribution of the adults of a local population of *E. lucifuga*. These data may provide a starting point for understanding how salamanders affect energy flow into caves, and the behavior of other populations across the range of this species.

METHODS

Field Study

Fieldwork was carried out within the main passage (99 meters in length) and an adjoining side passage (82.3 meters in length) of Sauerkraut Cave in E. P. "Tom" Sawyer State Park in Louisville, Kentucky. I conducted weekly or biweekly surveys of the salamanders within this cave from 6 March 2015 to 26 February 2016. Both quantitative and qualitative data were collected to evaluate their seasonal behavior. All visible salamanders were considered to be active. Adult *E. lucifuga* of this population inhabited most portions of the cave passages that were accessible for human observation, and their spatial positions were measured along various axes. I measured the locations of individuals along the length of the cave passages by a transect marked every two meters, with the exact distance from the drip line at the cave entrance to the middle of the salamanders' body gauged to two decimal places by a handheld measuring tape. The cave zones of both passageways were defined and measured from the drip line at the cave entrance: the twilight zone (TZ, 0.00 meters to 43.00 meters) and the dark zone (DZ, 43.00 meters to 99.00 meters). These zones were subdivided into three sections each, and at each of these points air, water, and earth substrate temperatures were recorded (°C), as well as relative humidity (%) and stream depth (cm), to further define the cave zones and monitor the changes in abiotic conditions as one moved along the length of the cave.

E. lucifuga was found on the passage floor—upon substrate, or within standing or flowing water—as well as at varying heights upon both sides of the cave wall (left or right, when viewed from the entrance). I measured height above the floor in centimeters

via the handheld measuring tape. This precise measuring system, documenting for each individual the distance from the entrance and height upon the cave wall, was to ensure that the location could be easily found again to assess surface type and other abiotic specifics. Substrates within Sauerkraut Cave ranged from naturally occurring to man-made: sloping mud banks, creviced rock walls, smooth flowstone, brick walls, and piles of broken building tile were all present, with artificial surfaces phasing out around 33 m in the main passage and 24 m in the side passage.

Individual salamanders were photographed with as little disturbance as possible, and their unique spot patterns were catalogued via the Interactive Individual Identification System (I^3S-S) in order to identify them and track their movements throughout the course of the study. The complexity of their spot patterns, coupled with unique snout-vent lengths and gender assignments, made it highly unlikely that two adults would be identical or even mistaken for another individual. With the beginning of the breeding season in late August and the emergence of secondary sexual traits (first recorded on 21 August 2015), males and females were differentiated in the field by elongated cirri and prominent mental glands in males, and swollen, visible ovaries in females. Salamanders that were observed multiple times throughout the survey period had their gender assignment included with their recorded spatial data.

Data Analysis

The photographs taken of each cave salamander were entered into I^3S-S, which—by means of the animals' distinct spot patterns and snout-vent lengths—assisted in the manual identification and cataloguing of unique salamanders for recognition in subsequent field surveys. Each recaptured adult was assigned a number (alas, specific names had to be abandoned with the progression of the study) for further tracking.

All of the data taken as field notes were systematically entered into Microsoft Excel® 2010 and calibrated to reflect the total counted numbers and spatial positioning of each recognized salamander, along with the average temperature (°C) and relative humidity (%) of the dark zone, the twilight zone, and the outside environment on the date each individual was identified. Additionally, I calculated the mean and median distances from the entrance (m) of all recorded salamanders for each survey date and entered into the spreadsheet. With this raw data, various comparisons were made to determine the

presence of patterns or correlations between environmental factors and the number of active salamanders observed in those conditions. R^2 and P-values were calculated via RStudio®.

RESULTS

Total Salamanders Observed

704 distinct individual cave salamanders in Sauerkraut Cave were photographed in this study. The number of salamander sightings increased over time (y = 0.1437x - 6018.8), from 19 adults seen in the initial survey (6 March 2015) to 127 adults on the last outing (26 February 2016) [Fig. 1].

Presence in the Twilight Zone

The percentage of salamanders found within the twilight zone (0 m to 33 m) ranged from 5.26% on the initial survey to 14.17% on the final excursion, with a peak of 100% on 8 May 2015. As the study progressed, the proportion of salamanders recorded in the TZ generally decreased over time and was best described by a quadratic model (y = -$0.0011x^2 + 88.755x - 2e^6$) [Fig. 2].

Regression analysis showed that the percent of salamanders present in the twilight zone depended significantly on temperature ($R^2 = 0.9351$; $P = 1.872e^{-6}$), increasing as the temperature increased from around 8°C to 18°C. In contrast, there was no significant relationship between percentages of salamanders and relative humidity ($R^2 = 0.0003$; $P = 0.935$) [Fig. 3 and 4].

Presence in the Dark Zone

Through the course of the year of study, the percent of each survey's total salamander count that was discovered within the dark (or dark) zone (43.00 m to 99.00 m) began as 94.74% on 6 March 2015 and as 85.83% on the final survey, with a maximum of 98.07% seen on 4 December 2015. This relationship followed a positive quadratic model (y = $0.0011x^2$ - 88.755x + $2e^6$), generally increasing from March of 2015 to February of 2016 [Fig. 2].

The percent of salamanders recorded in the dark zone showed a significant positive

relationship with relative humidity (%), with proportionally more individuals found to be associated with higher levels of relative humidity ($R^2 = 0.4821$; $P = 2.501$ e^{-5}) [Fig. 6]. Contrariwise, it was revealed through regression analysis that the percent of adults found in the dark zone had a negative relationship with average temperature, with proportionally more salamanders found in that region when the average temperature measurement was around 14°C [Fig. 5]. This relationship was also shown to be significant ($R^2 = 0.576$; $P = 7.057$e^{-7}).

Mean Distance from the Drip Line into the Cave

Overall, the calculated mean distances of salamanders found per survey increased over time—the mean distance (m) from the cave drip line ranged from a low of 6.41 ± 20.78 m in early May 2015 to a high of 77.97 ± 20.78 m in January 2016. The mean distance of salamanders from the mouth of the cave increased linearly (y = 0.1591x - 6684.4) [Fig. 7].

Movements of Recaptured Salamanders

Photographs of 704 distinct individual cave salamanders in Sauerkraut Cave were collected in this study, with a total of 255 recaptures over the course of 31 weekly or biweekly surveys. Through the use of the I^{3}S-S identification system, I was able to recognize individual adults and track their movements as they were encountered. Of the 28 individuals that were seen more than twice, the highest number of sightings was 9 (for one individual, salamander 407) and the lowest was 5. One of the salamanders that was sighted numerous times, individual 003, was first recorded in March 2015 a short distance into the twilight zone (22.07 m). It was witnessed a few more survey days that month, within 4 m of its initial location, before disappearing from observation until the beginning of June, wherein it was found at 73.54 m. Four subsequent recordings of 003 were within a meter of the 73 m mark. Fig. 8 highlights the variation in movement patterns seen among the recaptured individuals.

DISCUSSION

The data collected in this study illuminated the relationships between karst environmental variations and cave salamander population movement, and could easily

contribute to the evaluation of multiple aspects of the life history, behavior, and key ecological functions exhibited or performed by this species.

It is first important to note the increased number of adults seen per survey as the study progressed, from a comparatively paltry 19 individuals on the first outing to 127 in February of this year. This could perhaps be due to an effort to lessen the effects of disturbance and an increased efficiency on the part of the researchers, both in identifying and recording the required data during each excursion; but it nonetheless may suggest an increasing level of tolerance of human activity. Undoubtedly the cave, situated in a public state park, experienced regular disruption from park visitors; recollections from personal experience include the relocation of various man-made materials (bricks, building tiles, a large metal pipe, etc.) from week to week, fairly regular installations of graffiti on brick and natural cave wall surfaces, and instances of smoking within the twilight zone of the main passage. There is additional evidence of human activity, mainly in the form of discarded trash, as far back as about 80 m into the dark zone. These relatively high levels of disturbance, coupled with our consistent, comparatively low-impact surveys, do not seem to have affected the activity of this population—on the contrary, Fig. 1 illustrates how the numbers by February of this year (127 salamanders on 26 February 2016) are more than double the average number of salamanders seen per survey for the entirety of 2015 (45.16 salamanders per survey).

This conspicuous increase in population activity might also be due to climatic and weather variations that occurred early in the fieldwork. A massive flood event during the week of April 3, 2015 [Fig. 10] resulted in lower population counts in the subsequent weeks, with numbers not returning to or exceeding what had been previously recorded until the week of May 5. This year has not yet experienced a flood occurrence of the same magnitude, nor has this winter season been equivalent to 2015 in terms of low outside temperatures and quantity of snowfall [Fig. 9]. As a consequence, this local population may be exhibiting a slight change (earlier emergence or greater movement) in seasonal activity compared to what had been observed in the previous year.

The distribution of adult salamanders in the twilight and dark zones over time and through changing seasons exhibits an inverse relationship: as seen in Fig. 2, this population underwent a shift from largely twilight zone habitation in the warmer months

(April – September) to a preference for the dark zone as the temperatures approached winter conditions (October – February). There was a point in mid-autumn, around October and November, where adults were found in fairly equal proportions between the cave zones. This correlated with a shift in outside air temperature from around 19°C down to 15.8°C, and occurred when the average relative humidity and temperature measurements between the cave zones were more similar than in subsequent months. There was a 4.64 percent difference in relative humidity between zones (90.8% in the TZ and 95.44% in the DZ) when the salamander population shifted locations compared to a 7.09 percent difference from December to February (89.29% in the TZ and 96.38% in the DZ). Temperatures were also more closely equal at the transition time, with mid-autumn showing a 0.36° difference in average cave zone temperatures compared to a 4.2° difference seen from December to February.

The results of this study thus appear to agree with the findings of the few other life history studies that have been conducted, with some local variation evident: the greatest abundance observed in the Sauerkraut cave system complements the findings of Ives (1951), with the highest numbers recorded in early spring (February and March). Camp and Jensen (2007), on the other hand, found the greatest quantity of *E. lucifuga* to occur during the summer months of Georgia; again, this difference is likely a regional variation of the latitudinal locations of Georgia versus Kentucky.

Before this specific research was conducted, it was not entirely known how much this species would shift within a cave system over the course of a year. These are fairly small animals, traversing passages many dozens of meters (and more) in length. This study clarified to some degree how far an individual adult salamander could travel within the cave zones, and with what regularity. Previous researchers concluded that the movements of individuals between zones was dictated primarily by the need of those salamanders to feed and the availability of prey items, with the twilight zone offering the greatest variety and abundance (Peck and Richardson 1976). Each salamander that we recaptured was not necessarily sighted in consecutive outings, and 9 total encounters is a small maximum for a study of this duration. However, the movement patterns we were able to track highlight some unexpected results. Individuals of this population were somewhat varied in their movements, both between community members and within the

seasonal parameters seen by the population as a whole. For instance, individual 003 underwent a great shift in location between March and June 2015, from only a short distance into the twilight zone to well within the dark zone [Fig. 8]. All further sightings of this individual were confined to an area within a meter of the 73 m mark. While it cannot be assumed that this animal literally did not move for months at a time, as many surveys passed without its being sighted and identified, it is still unexpected that it continued to linger within the dark zone at a time when the general population was expected to prefer the conditions of the twilight zone. Several other salamanders exhibited the opposite phenomenon; Fig. 8 also shows how individuals 204 and 141 were repeatedly sighted in the twilight zone (distances ranging from about 11 m to 24 m) from June—which is to be expected—to late into November, a time when this species should theoretically be avoiding the colder, drier conditions near the mouth of the cave. Some animals fluctuated greatly in their locations throughout the year, sometimes within a matter of weeks, such as salamander 012; still others seemed to remain at a fairly fixed distance regardless of season or expected movement behavior (salamanders 317, 164). The explanations behind this varied movement between individuals cannot be fully explained through abiotic factors, such as temperature and humidity. Breeding or courtship behavior may have some influence over these animals' traffic patterns, a hypothesis similarly suggested by Hutchison (1958). Some small progress was made in the way of examining the role of gender in this population's seasonal movement, but with little viability; only a very small portion of the recaptures (already small in number), were identified as male or female with any level of certainty. The rest were recaptured before the emergence of secondary sexual characteristics (21 August), or did not exhibit characteristics clear enough for a positive gender assignment. As such, further examination into the effect of sex and courtship behavior on seasonal movement between zones would be a great asset to this study, perhaps accompanied with more reliable methods of sex determination in the field.

Regardless of breeding behavior, the spatial shifts seen by members of this population likely affect the flow of energy from the outside environment to the comparatively barren dark zone. Besides the random flush of plant debris that accompanies a heavy rainfall, the cave salamander may be one of the only facilitators in

the transfer of organic material to obligate troglobites—both from the outside environment and between aquatic and terrestrial habitats within caves. On occasion, flying insects (Dipterans) were observed fairly deep into the cave (personal observation of around 67 m), but never in abundance greater than one or two. Cave salamander fecal material, however, could be detected (and was often, especially in the spring) along any length of the cave passage where adult salamanders could be found. This waste product was easily collected, and could even be visually examined to reveal partially digested insect fragments. Additionally, there were occasions when recently deceased salamanders were found along the passage, and the rate of their decomposition was monitored with each consecutive survey. In most cases the available soft tissue was entirely disintegrated in the matter of a week, with the skeletal structure remaining for perhaps one week more. This demonstrates the importance of the cave salamander in a cave's energy food web, directly via mortality and the transfer of what would be inaccessible outside prey to opportunistic detritivores within the dark zone.

This study is ongoing, and much more information is needed in order to gain a better understanding of the ecology of Sauerkraut Cave. For instance, the total population size of this species within this one cave is unknown; however, continued surveys and calculated rates of recaptures could provide an estimate of population size that will presumably become more accurate over time.

In the future this highly disrupted and altered cave system may experience a restoration to a more natural state. The current outgoing stream is confined to brick barriers set in place more than fifty years ago, and restoring the natural flow of water, in stream width, depth, and direction from within the cave, could substantially influence the community structure of this karst system. The reproductive success of this species may very well be improved, as it has been suggested that cave salamanders prefer slow moving water for egg deposition (Ringia and Lips 2007). It would be extremely informative to study how a large-scale cave restoration would affect the dynamics of this population of salamanders.

The results of my study suggest the movement patterns of this top predator follow seasonal variation and are closely connected to abiotic conditions within caves. Cave communities are unique and may be dependent on species like *E. lucifuga* for nutrient

circulation (Petranka 1998). Kentucky and Tennessee contain almost 20% of all the known and accessible karst systems in North America (Barr 1968), and as such increased study and comprehension of the connections between abiotic and biotic factors and cave salamander behavior in local populations could be crucial in understanding the complexities of cave ecosystems across the temperate United States (Davic 2004, Peterman and Semlitsch 2013).

ACKNOWLEDGMENTS

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Figure Captions

Fig. 1. Number of cave salamanders observed in weekly surveys from March 6th 2015 to February 26th 2016.

Fig. 2. Percentage of salamanders located in the twilight zone (TZ) versus the dark zone (DZ).

Fig. 3. Relationship between temperatures (°C) and percentage of salamanders found in the twilight zone (TZ).

Fig. 4. Relationship between percent relative humidity and percentage of salamanders found in the twilight zone (TZ).

Fig. 5. Relationship between temperatures (°C) and percentage of salamanders found in the dark zone (DZ).

Fig. 6. Relationship between percent relative humidity and percentage of salamanders found in the dark zone (DZ).

Fig. 7. Mean distance of salamanders observed in weekly surveys from March 6th 2015 to February 26th 2016.

Fig. 8. Individual salamander recapture movements within the cave tracked over the course of the study period.

Fig. 9. The entrance of Sauerkraut Cave on the first survey, March 6th 2015.

Fig. 10. The entrance of Sauerkraut Cave after the severe flooding during the week of April 3rd 2015.