2000

The life history of the cave salamander, Eurycea lucifuga Rafinesque, in West Virginia

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The life history of the cave salamander,
Eurycea lucifuga Rafinesque, in West Virginia

Thesis submitted to
the Graduate College of
Marshall University

In partial fulfillment of the
requirements for the Degree of
Master of Science in
Biological Sciences

by

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Huntington, West Virginia

05 May 2000

This thesis was accepted on May 05, 2000
Month Day Year

as meeting the research requirements for the master's degree.

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Acknowledgements

I wish to thank my advisor, Dr. Thomas K. Pauley, for the opportunity to attend graduate school at Marshall University. The amount of knowledge and understanding I gained from him could not be found anywhere else in the world. He is truly a blessed and remarkable man and I feel honored to be one of his students.

A special thanks to all the people that have supported me, mentally and financially, through graduate school. My family and Erica Moyer have showed undying support to me throughout the entire graduate experience. Without their constant support I would have never finished my education. They believed in me when even I did not.

Thanks to my committee members, Dr. Tarter and Dr. Strait. The guidance they gave during my research was very appreciated. Thanks to all my lab mates in 310. The crazy times we had in that room helped make every day go by just a little bit faster. Forty years from now when I look back at my Marshall experience, I will always think of those times as some of the best ever.

Thanks to all the people who have helped me on my thesis. These include Zach Felix, Jayme Waldron, Sandra Raimondo, Brian Lindley, Jeff Humphries, Jennifer Wykle, Jessica Wooten, Andrew Johnson, and Laura Gould. A special thanks goes to Zach Felix who, not only is the best wildlife photographer I know, but also a great friend. Without his help in my field work I would of never of finished my project. Thanks also to Sandra Raimondo who helped identify stomach contents.

Finally, I would like to thank the West Virginia Division of Natural Resources – Nongame Wildlife and Natural Heritage Program for their funding. All animals were collected and handled under scientific permit number 78-1999 issued by the West Virginia Division of Natural Resources.

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Abstract

In West Virginia, as throughout its range, there is limited information about the life history of the cave salamander (*Eurycea lucifuga*). The purpose of this study was to describe the natural history of this species in West Virginia. The objectives of this study were to determine habitat selection of the cave salamander, to ascertain its environmental characteristics, and to study its reproductive and non-reproductive biology. Three study caves were located in Greenbrier County, West Virginia. All sites were examined at least once each month from May 1999 through April 2000. Caves were divided into three zones: entrance, twilight, and dark. The only environmental parameter that determined the cave salamanders habitat choice was the relative humidity of the crevices. Eggs were observed between 12 September and 9 November 1999. All eggs hatched within 52 days (+/- 6 days). Larvae were found all months of the study. Larval period was determined to be over 22 months. Adult prey items consisted of twelve prey taxa, with the order Diptera comprising the largest percentage of food. Salamander movements, habitat use, and community ecology are also discussed.

Chapter 1: Species Description

Rafinesque first described the cave salamander (*Eurycea lucifuga*) in 1822. The type locality is "near Lexington" in Fayette County, Kentucky (Hutchison 1979). *Eurycea lucifuga* is classified in the Kingdom Animalia, Phylum Vertebrata, Class Amphibia, Subclass Lissamphibia, Order Caudata, Family Plethodontidae, Genus *Eurycea*, Species *lucifuga*.

The adult cave salamander is a large, slender salamander primarily found in caves or regions where caves are located. Adults have bright orange or reddish-orange coloration on their dorsal surface and are heavily marked with irregularly spaced dark spots (Petranka 1998). Cave salamanders have large, prehensile tails and can reach a total length of 178 mm (Fig. 1). Males can be distinguished from females by the more prominent cirri, presence of mental glands, distinctly margined vents, and slightly longer legs (Smith 1961). Adults are primarily terrestrial and lay their eggs in water.

Larval *Eurycea lucifuga* have a bulbous head typical of most *Eurycea* larvae with a black line extending from the eyes to the nose (Fig. 2). The aquatic larvae have gills inserted anterior to the legs and contain approximately three gill rakers. The body is uniformly pigmented a tannish-brown on the dorsal surface and the ventral surface is a creamy white. Three longitudinal rows of small light spots occur on each side with the more dorsal spots being more obvious. The tail is keeled from the tip to just posterior to the hind legs. Larval period lasts 6-18 months before metamorphosis occurs at a size of 56-60 mm total length (Petranka 1998).

Cave salamanders are considered troglophiles (facultative cavernicoles) and are commonly found both in and out of caves and many complete their life cycle in either environment (Green and Brant 1966). Inside caves, adults are found under rocks, boards, and other debris but they most commonly inhabit crevices formed in the walls of the caves. Although *E. lucifuga* is found mostly in caves, it can also be found above the ground in the epigean environment. Here, adults can be found under logs, rocks, and in forest litter. During my searches I found adults outside caves at night actively searching for food.

The cave salamander occurs in limestone regions from Indiana southward to northern Alabama and Mississippi, and from western Virginia to eastern Oklahoma (Petranka 1998)(Fig. 3). There are over 400 caves of notable size found in West Virginia. Although caves are found in all the physiographic provinces of the state, it was traditionally thought that the cave salamander occurs only in caves of the Greenbrier Limestone Series which forms extensive outcrops within the Allegheny Plateau Province (Green et al. 1967). These caves are located in the southeastern portion of the state. Hutchison (1958) and Green et al. (1967) found that the correlation of the distribution of the cave salamander is not due to the limestone, but probably the habitat of the caves afforded by the nature of the rock. Recently the cave salamander has been observed in areas other than the region listed above. Surveys in the New River Gorge National River in 1991 and 1992 revealed that *E. lucifuga* occupies abandoned coal mines in sandstone formations (Pauley et al. 1993). Distribution of the cave salamander in West Virginia extends from Pocahontas County south along the southeastern border of the state through Greenbrier, Monroe, Fayette, Summers, and Mercer counties (Green and Pauley 1987).

Chapter 2: Introduction and Overview

Caves are very sensitive ecosystems that consist of a relatively small number of species. The cave environment is so fragile because it is remains very constant throughout the year. In addition to the absence of light, the physical environment is characterized by silence, relatively constant temperature which approximates the mean annual temperature of the region in which the cave is located, and in the great majority of caves, an unusually high relative humidity which, except near entrances, is accompanied by an exceptionally low rate of evaporation (Barr 1967). Any environmental or human perturbations can cause catastrophic changes to the fauna contained in the caves.

The cave salamander is listed as a species of special concern in West Virginia. The reason for this listing is the salamander's limited habitat within its range. Population levels of this salamander are thought to be declining in West Virginia (WVDNR 1987).

A better understanding of the life history and requirements of *E. lucifuga* are needed to assess the current status and future conservation of this species in West Virginia. Salamanders are excellent indicators of environmental health (Dunson et al. 1992) and the cave salamander is no exception. Long-term monitoring of a habitat specialist like the cave salamander can signal local perturbations (Heyer et al. 1994).

Very few life history studies have been conducted on *E. lucifuga*. Banta and McAtee (1906) were the first to do a life history of the species. Their paper, while very informative, dealt only with certain aspects of the ecology of the cave salamander. Hutchison (1958) did the most complete study on the distribution and ecology of the cave salamander. The study was conducted in caves in Giles County, Virginia and his data is very useful in comparison to a West Virginia study. No other studies could be found that dealt primarily with this species.

Other studies that dealt with the cave salamander include Williams (1980) who noted the seasonal variation in cave salamander populations in Illinois. Extensive food analysis studies of *E. lucifuga* were conducted by Peck (1974), Peck and Richardson (1976) and Smith (1948). Notes on different aspects of courtship, breeding, and egg development were presented by Myers (1958a), Organ (1968), McDowell (1988), as well as Barden and Kezer (1944). Additional aspects of *E. lucifuga* have been discussed with reference to other species. Such topics included larval ecology of five plethodontid species (Rudolph 1978) and salamander antipredator postures (Brodie 1977).

In West Virginia, as throughout its range, there is very little information about the life history of the cave salamander (Green and Pauley 1987). Although there has been some research in West Virginia, it is mostly incomplete. Reese (1933) first reported on the fauna and occurrence of *E. lucifuga* in West Virginia caves. Almost all data about the species in West Virginia is from studies performed in the 1960s. Green and Brant (1966) noted the distribution of the cave salamander throughout the state. Green et al. (1967) gave a brief report on the overall distribution, ecology, and life history of the cave salamander in West Virginia. While aspects of the natural history are available, additional research is needed to provide a complete understanding of this secluded species of salamander.

The overall objective of this study was to obtain a complete understanding of the cave salamander in West Virginia. Specific objectives of this study were to establish the habitat selection of the cave salamander, to ascertain its environmental characteristics, and to determine its reproductive and non-reproductive biology. A measurable increase in knowledge of the life history of the cave salamander in West Virginia was gained since little or no information is known about major aspects of the life cycle of the species. The information provided will help with the future conservation and monitoring of this species and its habitat.

Chapter 3: Study Site Description

This study was conducted in three caves in Greenbrier County, West Virginia (Fig. 5). This area was chosen because of the high number of limestone caves. One-fourth of all known caves in the state are found in this region. All caves are developed in the limestones of the Greenbrier Series which outcrops in a broad upland, two to four miles wide, in the central part of the county (Davies 1958). Each cave is described below:

Buckeye Creek Cave 37° 58" 33" N.; 80° 24'03" W.

This site, owned by Gene Turner, is eight kilometers east of Williamsburg (elevation, 600.5 meters) (Davies 1958). The cave follows a stream passage but does have several side rooms and passages (Fig. 6). The cave can become flooded in the winter and spring with heavy rain and snowmelt. Between June 1999 and January 2000 the stream entering the cave dried up and became non-existent. Approximately ten meters into the cave another stream starts to flow out the east wall. This joins with another stream that flows out of west wall approximately 50 meters farther into the cave. The stream is characterized by rock rubble at the entrance and slowly changes to a stream bottom of medium-sized gravel with occasional sandbars farther into the cave passage. This cave was chosen because of its easy navigability and presence of larvae in my initial search of the cave in May 1999. The cave floor consists of rock rubble at the entrance and then is covered with heavy deposits of alluvial soil and sand farther into the cave.

Higginbothams No.2 Cave 37° 55' 59" N.; 80° 24' 30" W.

This site is located in a low ridge (elevation, 685.8 meters), 2.1 kilometers west of Frankford (Davies 1958). The Higginbothams Caves are a series of four caves owned by John Mooney. Higginbothams Number 2 cave was chosen because it contains several rimstone pools that have historically contained eggs of the cave salamander (Green et al. 1967). The floor of the cave is soft clay with some rock rubble near the entrance. The entrance to the cave is one meter high and opens into a room that is approximately eight meters wide and 15.25 meters long (Fig. 7). The room gently slopes down after this room for approximately 15 more meters into a second room that contains a series of nine rimstone pools (Fig. 8). A low passage beneath flowstone in the second room leads to a third, larger room with a high ceiling. This room was not included in the study because of its limited and difficult access.

Norman Cave 38° 01' 06" N.; 80° 19' 12" W.

This site is part of the Bone-Norman cave system located 1.6 kilometers southwest of Julia. The entrance is at the top of a steep rise (elevation, 655 meters), 91.4 meters west of a sharp bend in county road 7 (Davies 1958). The cave is 120 meters long with a floor that is covered with large rock slabs and slopes downward (Fig. 9). Approximately 80 meters from the entrance there is a narrow crawl-way that drops into a shallow underground stream. The stream passage only runs about 25 meters long before it drops off of a 4.6-meter waterfall.

Chapter 4: Adult Population Demography

Introduction

Adult population dynamics provide data on population densities, population size and structure, movements, seasonal population fluctuations, and other aspects of the adult life history of the cave salamander. This data can be used as a baseline to determine the health and status of the population of the cave salamander. Petranka (1998) noted that data are currently unavailable on the status of populations of the cave salamander throughout their range. This holds true for populations of *E. lucifuga* in West Virginia.

The purpose of this chapter is to gain an understanding of cave salamander adult population dynamics. Due to the declining nature of this salamander population, it should be carefully monitored (WVDNR 1987). The estimates obtained can provide a baseline of information upon which the future status of these populations can be determined.

Methods and Materials

During each visit, several environmental parameters were measured at each of the three study caves. Environmental data were collected at three stations at every study site. Station 1 was located at the entrance of the cave, station 2 was within the twilight zone, and station 3 was in the dark zone, where no light could be detected with a light meter. The range of station 2, which was located in the twilight zone, could be highly variable according to the amount of foliage outside of the cave, season, and time of the day. The approximate midpoint of where sunlight penetrated into the cave is where station 2 was located. Environmental data collected at each station included air temperature (°C), light (Lux), and relative humidity (% RH). Air temperature and relative humidity were measured using a Pocket Hygro-Thermometer by Extech Instruments and light was calculated by using an Extech Instruments light meter.

A mark-recapture survey of adult *E. lucifuga* was conducted twice a month at the three study caves during the summer and early fall (May through September) and surveyed once a month during the fall, winter, and spring (October through April). Distribution and habitat selection were determined by performing terrestrial searches for adult and juvenile cave salamanders inside and outside of the caves. Approximately a 10-meter radius in front of the cave entrance was searched for salamanders. This area included the outside rock face of the cave since the rock displayed the same characteristics as the inside cave. Inside the cave the entire expanse of the cave was searched for salamanders, including the entrance, twilight zone, and dark zone of the cave. Walls, floor, and ceiling were searched since this is the primary habitat of adults. When a salamander was observed in a crevice, a wire hanger fashioned into a hook was utilized to extract the animal from the crack.

Each *E. lucifuga* captured was marked using the Visible Implant Fluorescent Elastomer (VIE) tagging system manufactured by Northwest Marine Technology. This mark-recapture technique was used in place of the more common toe-clipping technique for several reasons. Two reasons for using this alternative mark-recapture technique are the evidence that toe-clipping has harmful effects on amphibians (Clark 1972) and salamander toes can regenerate which can be detrimental to a long-term study. The primary reason for using the VIE tagging system is that cave salamanders are excellent climbers which are often found at great distances from the ground and on perfectly vertical rock faces. I felt that toe-clipping would hinder salamander mobility and climbing skill.

The VIE tagging system utilizes a specially developed, bio-compatible, two part fluorescent elastomer system (color and curing agent). After mixing the color and the curing agent, the elastomer is a liquid and can be placed in a 0.3 cc syringe. Three colors were used for this study; red, orange, and yellow. Four tag locations were used to obtain unique marks for each salamander tagged (Fig. 10). The combination of three elastomer colors and four tag locations gave each individual *E. lucifuga* a unique mark. A hand-held UV light was employed to determine if the marks were visible. Each cave salamander collected was checked for VIE marks and was tagged if never captured before.

Each cave salamander captured was measured for snout-vent length (SVL), total length (TL), and cranial width to the nearest 0.1 mm with a Scienceware dial Vernier caliper. Each salamander was also weighed using a 10 gram Pesola scale. For all other salamander species SVL and weight were measured.

Location of each capture was recorded as a distance from the cave entrance and height from the cave floor. Flagging was placed at five-meter intervals on each side of the cave from the entrance. Location of each capture could then be accurately estimated using the flags.

Mark-recapture data were used to assess population size and seasonal movements. There is at present no evidence to indicate that dispersal of cave salamanders takes place overland between caves. At most, there may be occasional dispersal across small surface streams when floods wash the larval salamanders from some caves. It can therefore be assumed that each cave contains an individually closed population with little to no emigration or immigration.

Despite the assumption that the study site represented a closed system, both open and closed population estimates were used. Assuming a closed model, the Schnabel method (Schnabel 1938) was used to determine population size. The computer program JOLLEY 3.6 (Center for Conservation Biology, Stanford University) was also used assuming an open population. Both methods are useful because they can provide a series of population estimates, which can be repeated until the investigator is satisfied with the results (Smith 1996).

Results

Seventeen mark and recapture survey days were performed between 21 May 1999 and 8 April 2000. All surveys were used in the population estimates and escape data were used in monthly movement observations. Sites were surveyed 194.5 total hours during the study period.

Average air temperature (°C), light (Lux), and relative humidity (%) are given for all three caves in Tables 1-3. Average temperature ranged from 16 °C in the entrance of Norman Cave to 11.2 °C in the dark zone of Buckeye Creek Cave.

Average light varied from 227 Lux in the entrance of Buckeye Creek Cave to perpetual blackness in the dark zones of all three caves. Relative humidity ranged from 90 percent in the dark zone of Higginbothams Cave No. 2 to 63 percent in the twilight zone of Buckeye Creek Cave.

Temperature fluctuations for the three stations in all study caves are shown in Figures 11-13. Temperature fluctuations in the entrance and twilight zone varied greatly from month to month in both Higginbothams Cave No. 2 and Norman Cave. The following maximum and minimum temperatures were recorded: Higginbothams Cave No. 2 – Station 1, 24 °C (September) and 4 °C (January); Station 2, 23 °C (September) and 7 °C (January). Norman Cave – Station 1, 24 °C (August) and 9 °C (January); Station 2, 24 °C (August) and 11 °C (January). Air temperature of the dark zones of the Higginbothams No. 2 and Norman Caves remained fairly constant throughout the year with an average of 11.6 °C and 15.9 °C, respectively. Air temperatures in Buckeye Creek Cave varied greatly throughout the study period. Minimum and maximum for the three stations were recorded: Station 1 – 24 °C (October) and 3 °C (January); Station 2 – 19 °C (June) and 4°C (January); Station 3 – 16°C (July) and 5 °C (January).

Relative humidity fluctuations for all three stations in the study caves are shown in figures 14-16. In Higginbothams Cave No. 2, relative humidity fluctuated greatly at the entrance and twilight zones, being higher in the summer months. Maximum and minimum relative humidity was recorded: Station 1, 96 percent (July) and 46 percent (January); Station 2, 99 percent (July) and 64 percent (September). Relative humidity of the dark zone of Higginbothams Cave

No. 2 stayed fairly constant at an average of 90 percent. Relative humidity of Norman and Buckeye Creek caves varied greatly throughout the study at all three stations. Maximum and minimum were recorded: Norman Cave – Station 1, 98 percent (November and April) and 56 percent (February); Station 2, 98 percent (November and April) and 63 percent (February); Station 3, 98 percent (April) and 51 percent (November). Buckeye Creek Cave – Station 1, 90 percent (July) and 35 percent (November); 89 percent (July) and 36 percent (November); Station 3, 89 percent (April) and 43 percent (May).

Light fluctuations for station 1 and 2 for all caves in the study are shown in Figures 17-19. Light readings in the entrance varied from the highest in the winter months to the lowest in the summer months. A reading of zero denotes that the caves were visited at night. Maximum and minimum light readings were recorded for station 1. Higginbothams Cave No. 2, 678 lux (October) and 24 lux (July); Norman Cave, 234 lux (November) and 34 lux (July); Buckeye Creek Cave, 372 lux (April) and 18 lux (July). Light readings for the twilight zone were fairly constant with an average reading of 3.2 lux for Higginbothams Cave No. 2, 67.2 lux for Norman Cave, and 50.4 lux for Buckeye Creek Cave.

Statistical analyses were performed to determine significant differences among the different zones of the caves and their environmental variables. A one-way ANOVA was used to test temperature and relative humidity readings. There was no significant difference in any of the study caves for relative humidity when the different zones were compared. There was no significant difference between the three zones when temperature was compared except in

Higginbothams Cave No. 2. A Dunn's multiple comparison test was used to isolate the differences between the zones. It was found that there was a significant difference between the dark zone and the entrance as well as between the dark zone and the twilight zone ($p=0.031$). Light readings were compared between the entrance and the twilight zones using a Mann-Whitney rank sum test. It was found that there was a significant difference in all three caves ($p=<0.001$).

A one-way ANOVA was used to test for significant differences between the individual zones when compared to the other caves in the study. It was found that there was a significant difference between temperatures of the dark zones of the caves. A Dunn's multiple comparison test was used to isolate where differences occurred. It was found that significant differences were between Buckeye Creek and Norman caves ($p=<0.001$), as well as between Higginbothams Cave No. 2 and Norman Cave ($p=<0.001$). In addition, one-way ANOVA, using the Dunn's multiple comparison test, showed that there was a significant difference between the relative humidity of the dark zone of Buckeye Creek Cave and Higginbothams Cave No. 2 ($p=0.014$).

A one-way ANOVA was used to determine any significant differences between the twilight zones of the three caves and their environmental parameters. It was found that there was a significant difference when relative humidity ($p=0.010$) and light readings ($p=0.035$) were compared. A Tukey multiple comparison test determined that relative humidity differences occurred between Buckeye Creek and Norman caves as well as between Buckeye Creek and Higginbothams No. 2 caves. A Dunn's multiple comparison test determined that differences between the light readings occurred between Norman and Higginbothams No. 2 caves and also between Norman and Buckeye Creek caves. No significant differences were found between environmental variables in cave entrances.

One hundred forty-eight different *E. lucifuga* were found on the study sites during the mark-recapture study. Of these, 52 were found in Higginbothams Cave No. 2, 89 were observed in Norman Cave, and seven were found in Buckeye Creek Cave. Of the 148 cave salamanders found 45 were male, 49 female, and 51 juveniles. Gender could not be determined for three specimens because of a lack of sexual characteristics. The sex ratio for the study was 1:1.21 (Table 4).

Males had an average cranial width of 8.7 $\pm$ 1.23 mm, SVL of 60.1 $\pm$ 4.85 mm, total length of 140.5 $\pm$ 18.53 mm, and weight of 3.3 $\pm$ 0.85 grams (Table 5). Females had an average cranial width of 8.6 $\pm$ 1.03 mm, SVL of 60.9 $\pm$ 4.56 mm, total length of 142.8 $\pm$ 22.31 mm, and weight of 3.4 $\pm$ 0.73 grams. Juveniles had an average cranial width of 6.6 $\pm$ 1.27 mm, SVL of 45.2 $\pm$ 6.69 mm, total length of 104.6 $\pm$ 23.84 mm, and weight of 1.4 $\pm$ 0.57 grams. A regression analysis was used to compare snout-vent length to cranial width (Fig. 20) and snout-vent length to total length (Fig. 21). A positive relationship was found with both comparisons with R^2 values of 0.6651 and 0.6032, respectively.

Seasonal movement of *E. lucifuga* was observed by recording the distance each salamander was found from the cave entrance (Fig. 22). Mark-recapture data and escape data were both used. Only 17 salamanders were found outside the caves during monthly searches. Location of salamanders ranged from 3.0 meters outside the cave (June) to 60 meters into the cave (March). The majority of the cave salamanders were 10.9 meters from the cave entrance. This distance lies within the twilight zone of all study caves.

The Schnabel method was used to calculate the population sizes of *E. lucifuga* in each study cave. The population was assumed to be closed and estimates for Higginbothams Cave No. 2 was 116 individuals, Norman Cave was 182, and Buckeye Creek Cave was 14 individuals.

Populations were also estimated assuming an open population with the program JOLLEY. Estimated population sizes for Higginbothams Cave No. 2 was 215, for Norman Cave was 123, and for Buckeye Creek Cave was 20 individuals.

Peak abundance of the visual population of *E. lucifuga* occurred in July and slowly declined to zero in December (Fig. 23). When sexes were separated it was found that peak abundance for males, females, and juveniles also occurred in July 1999 (Fig. 24). Adult abundance experienced a sharp drop in September 1999 but juvenile numbers stayed rather high. The lowest abundance for both sexes and age classes occurred in December 1999 and January 2000 when no salamanders were observed in the study caves.

Discussion

The physical environment of caves becomes more stable with increased distance into a cave. The entrance zone is characterized by wide fluctuations in temperature and relative humidity, which normally reflects the outside weather.

The environment of the twilight zone is characterized by variable light intensity from the epigean sunlight at the cave mouth to zero candle-meters at incipience of the total darkness zone, as well a tendency to less variable climatological conditions than the entrance zones (Barr 1949). The dark zones of caves are more or less characterized by constant temperature and relative humidity, as well as total and complete darkness.

Caves in this study reflected these environmental trends in the different zones. Environmental readings of the dark zone remained fairly constant while the environmental readings from the twilight zone seemed to reflect weather fluctuations of the outside environment, but with less degree of variation.

Monthly movement patterns of the *E. lucifuga* populations within the cave can be attributed to the environmental fluctuations. Salamanders were found nearest the entrance in April and May and showed a definite movement farther into the cave with the onset of warmer temperatures. During this time there was an observed dryness towards the entrance of the cave with higher temperatures and relative humidity. High temperatures were determined to be the significant factor influencing this movement because the relative humidity readings during this time were comparable to that of the dark zones of the cave. Deeper recesses of the twilight zone and the dark zone were where most salamanders were found, so it can be assumed that relative humidity was not a factor for their movement deeper into the caves.

Populations moved somewhat closer to the entrance in September and October but then moved farther into the cave with the onset of cold temperatures.

Temperature and a drier environment can again be attributed to this movement into the deeper recesses of the caverns. Many caves "breathe", exhaling cave air from their mouths in the summer and inhaling colder, drier air in the winter (Barr 1967). Cave salamanders exchange gases and lose water through their skin, like all amphibians; therefore, they are very vulnerable to drier conditions. Drier conditions and colder temperatures explain the movement farther into the cave in winter months.

The wide range of distances from the entrance that salamanders were caught in March and April shows the general progression and movement of salamanders toward the cave opening with the onset of warmer temperatures. Eventual progression towards the entrance, with the onset of warmer spring temperatures, could be due to the increased availability of the food supply. Entrance and twilight areas are characterized by a marked increase in food supply introduced from the outside environment (Barr 1949). Warmer temperatures would bring more food into the cave in this area.

Seasonal movements of cave salamanders have been noted in other studies. Hutchison (1958) observed a progression of the salamanders deeper into the twilight zone with the onset of warmer temperatures. He concluded that the governing factor influencing the distribution of *E. lucifuga* was moisture. Williams (1980) detected the movement of an Illinois *E. lucifuga* population deeper into the caves during the summer.

Even with seasonal movement, it was found that the majority of the population inhabited the twilight zone of the caves. Hutchison (1958), Peck and Richardson

(1976), Banta and MacAtee (1906), and Williams (1980) observed the same trend. One of the most obvious reasons *E. lucifuga* is found in this area is that it has a relatively stable environment, compared to the outside and entrance area. A preference for environmental stability cannot be the only factor or else the animal would choose the dark zone with its constant environment. Another factor that determines the high abundance of the species in this location may be the food supply. The entrance and twilight zones, as mentioned previously, are much more abundant with food items because of the introduction of prey from the outside environment. It would be expected that cave faunas, relying on food input from the external environment, would have a greater species diversity and larger population sizes in the part of the cave with the greatest food availability (Peck 1976). Therefore, a combination of food availability and a stable environment contribute to the high abundance of this species in the twilight zone.

Green et al. (1967) postulated a random distribution of *E. lucifuga* throughout the cave system. My data supports this hypothesis because I found salamanders as far into caves as 60 meters. Conversations with spelunkers have revealed the presence of this salamander deep into the recesses of the cave. Banta and McAtee (1906) found a cave salamander 1.5 miles within Wyandotte Cave. I believe that *E. lucifuga* occur throughout the caves but the highest abundance is near entrances to the outside environment.

I observed a steady increase in monthly abundance of the populations inside the study caves until July when a sharp decrease in the visual population was observed. Hutchison (1958) noted a sharp decrease in the visible population of cave salamanders in Virginia during July and August, and remained low during the fall and winter months. Williams (1980) and Ives (1951) also noticed a fluctuation in the visible population of cave salamanders in an Illinois cavern.

Seasonal fluctuations of cave populations have been observed in other amphibian species as well. Fowler (1951) observed seasonal fluctuations with cave populations of *Plethodon dixi*. Mohr (1944) noticed emigrations and migrations of *E. longicauda* from caves affecting the visual population size. Mohr (1952) also noted a seasonal fluctuation in the cave population of *P. cinereus dorsalis*.

Fluctuations in population size must be the cause of some factor independent of the environmental parameters. It becomes apparent what is happening when Figure 24 is viewed. Adult salamanders have a steep drop in numbers beginning in July and at the same time juvenile numbers increase greatly. It can be postulated that the adult salamanders are moving farther into the caves for mating and courtship.

Petranka (1998) stated that the mating season probably occurs during the summer and early autumn prior to the initiation of egg laying. Banta and McAtee (1906) noted that adults move farther away from the cave mouths and into the deeper recesses of caves to oviposit. The time period coincides with the sudden disappearance of the visual population of adults.

Further evidence that shows that the decline in adult numbers is due to the onset of mating and courtship is evidenced in the size of the follicles in gravid females. In July and August, the majority of the females captured were gravid and contained large follicles that were observed through the skin of the venter. Hutchison (1958) captured most gravid females during the same time. Increased follicle diameter indicates that eggs are nearing oviposition size. Females need water to deposit their eggs, which was not found in the immediate areas that were searched, except for the few small aquatic areas previously described. Therefore, females must travel further into caves to unknown water sources to deposit their eggs.

Hutchison (1958) and Williams (1980) noticed a marked reduction in the size of the visible population of *E. lucifuga* after the first visit to caves. Both authors concluded that the reason for this was because of researcher handling. My study did not have that problem, which is evidenced by the increase in the visible population during the first few months.

Population estimates were estimated using an open and closed model. There is probably no completely accurate way to determine the population size of this species. Censusing is not effective because capture results indicate that most of the population is accessible for most of the year (Juterbock 1998). Regardless, a population count is ideal to serve as baseline data to compare to future population counts.

In all likelihood these populations are closed. Very little migration in and out of the cave is thought to occur. There is at present no evidence to indicate that dispersal of cave salamanders takes place overland between caves. Barr and Peck (1965) studied a cave beetle that is comparable in isolation and dispersal potential to the cave salamander. They found that there was limited dispersal of this beetle and at most there may be occasional dispersal across small surface streams when floods wash the beetles from caves. Cave salamanders are very comparable to the cave beetle in that dispersal probably does not happen unless the larvae are washed away in a flood to a different cave system.

A population estimate is still needed to give a count of at least a proportion of the population to measure its health and status. Population estimates vary widely and should be looked at cautiously. The Schnabel method was used to estimate the size of a closed population. Numbers obtained seem to be more accurate with a higher population in Norman, which is the larger cave. Numbers estimated for Buckeye Creek Cave should be ignored because they are grossly inaccurate with only two recaptures. The visible population in Buckeye Creek Cave was nonexistent for most of the year, which is evidenced by only seven cave salamanders being caught during the study.

Buckeye Creek Cave had very low numbers of cave salamanders compared to the other two study sites. The population size was very uncharacteristic of a cave of such size. It can be hypothesized that low visible population size in Buckeye Creek Cave could be due to the stream flowing through the cave. The cave periodically floods in the spring filling almost the entire cavern with water. Any salamanders would be swept away farther into the cave. Myers (1958b) noted similar situations where cave salamanders were not present in tunnel-like caves that had entrances drained by fairly large streams. One recapture was caught at the entrance to the cave during April 2000. This is evidence that the species can survive in the cave, but only in limited numbers and in protected crevices.

Rudolph (1978) observed that severe floods caused a great reduction on the visible populations of salamanders. Actual population decreases resulting from floods were commonly on the order of 50-1000 percent, based on estimates of the visual populations after salamander behavior had returned to normal. Rudolph found that only 54 percent of larval *E. lucifuga* survived after flooding experiments. Larval cave salamanders in Buckeye Creek Cave would undoubtedly suffer the same fate, providing more evidence for the low numbers on this site.

There are no data to compare population estimates against other studies. Hutchison (1958) determined that the population sizes in his caves ranged from 36 to 63. Population estimates from my study cannot be compared because the size of Hutchison's caves were not given. Juterbock (1998) gave a population estimate of one individual per meter in epigean ravine habitat. His data cannot be compared either because this study dealt with salamanders found on the surface instead of in caves.

Estimates would indicate a strong population of salamanders on the study sites, with the exception of Buckeye Creek Cave. The high number of juveniles found would also indicate an increasing population. Norman Cave has the healthiest population of *E. lucifuga* because of its high percentage of juveniles. Juveniles were not found in Buckeye Creek Cave. The future of *E. lucifuga* Buckeye Creek Cave is uncertain.

Females were slightly larger than males. Morphometrics in this research were similar to other studies. Minton (1972) found that males (60.1 mm) were slightly larger than females (59.6 mm) in Indiana. Guttman (1989) reported that males in Ohio average 60 mm SVL and females measured 62 mm SVL.

Smith (1961) noticed variation of several morphological features in Illinois cave salamanders. He observed that southeastern species were more heavily spotted above, are shorter-legged, have fewer vomerine teeth, and have less prominent secondary sexual characteristics than salamanders from the Lower Mississippi Border counties. Grobman (1943) reported on finding significantly larger specimens in the Nashville, Tennessee basin, with most averaging 168.2 mm total length. Few of the cave salamanders in my study exceeded 160 mm total length.

Various notes have been published describing variations in the cave salamander. Reese and Smith (1951), Eigenmann and Kennedy (1903), and Minckley (1959) reported on atypically pigmented *E. lucifuga*. Banta and McAtee (1906) suggest that these aberrant patterns represent different stages in arrested transformation of pattern from the typical, wholly diffuse larval type to that of the adult.

No salamanders were found with such extreme abnormalities in this study but a gravid female was found on 19 August 1999 that had traits both of *E. lucifuga* and *E. longicauda*. The color of the body was yellowish like that of *E. longicauda* and the tail markings were similar to herring bone patterns but with spots interspersed in between. Smith (1964) reported that interbreeding of the cave salamander and the long-tailed salamander subspecies (*E. l. melanopleura*) does rarely occur. This specimen might have been a hybrid of these closely related species. Both species have different breeding seasons but they could overlap. Cave salamanders have a fall breeding season and long-tailed salamanders have a winter breeding season. Further genetic work would have to be performed to determine if this is a hybrid salamander.

Females outnumbered males by a ratio of 1:1.21. Most other studies observed numerical superiority of males. Hutchison (1958) observed a 1.51:1 ratio in Virginia populations. Williams (1980) observed a 1.13:1 ratio in an Illinois population. Juterbock (1998) reported higher female numbers with a 1:1.5 ratio in an Ohio population. There is no apparent reason for the larger number of females in my study. The ratio is very close to the theoretical 1:1 ratio in most animal populations.

Chapter 5: Mating, Egg-Laying, and Larval Development

Introduction

Certain aspects of the cave salamander life history are unknown or not well documented. Perhaps the biggest gap in cave salamander data occurs in reproductive and early development information. The secretive nature of this species makes it difficult to observe egg-laying and larval development. This information is critical to gain a full understanding of the species. Conservation and protection of a species is dependent upon a complete understanding of the study organism.

The primary purpose of this chapter is to complete the gaps of information so that a comprehensive life history can be obtained of the cave salamander in West Virginia. An additional purpose of the chapter is to determine larval population size and determine requirements for larvae of this species. Study sites were chosen because historical records show the presence of eggs in past observations.

Study Site Description

Each study cave contained an aquatic area that could be used for larvae and egg searching. Buckeye Creek Cave had a small stream entering it at the entrance and two other streams entering the cave from underground. Also found in the cave are temporary pools and one permanent rimstone pool. Norman cave contained an underground stream that was difficult to access. This stream was located approximately 80 meters into the cave through a deep crevasse.

The stream was not discovered until half way through the study period. Higginbothams Cave No. 2 contained many rimstone pools that contained various amounts of water during the study.

The floor of the second room in Higginbothams Cave Number 2 is covered with nine rimstone pools (Fig. 8). These rimstone pools would go through various stages of inundation of water and drying depending on the amount of moisture in the cave. Pools one and two were always filled with water and the others were filled or dry at various times. The other pools would fill in a pattern (3 to 9) as the water level would rise in the pool that proceeded it.

Rimstone pools are formed from calcite growing around the edges of the pools. It builds upward and inward, squeezing the pool into an even smaller space and pushing the water to a higher level. The versatile stone actually constructs dams that keep growing higher and higher (Green et al. 1967). Excellent egg deposition sites are provided by the nature of the rimstone to slant toward the center of the pools and the rough texture of the pool walls. The bottoms of the pools were covered with fine, silt-like mud. Limited debris was found in the pools ranging from old boards to pieces of rock that had fallen from the ceiling. Animal scat was also found in some of the pools indicating the occasional visitation of a large mammal. The landowners' cat (*Felis domesticus*) and an opossum (*Didelphis virginiana*) were observed in the cave. The owner also observed a bobcat (*Lynx rufus*) hibernating in the cave the previous winter.

Methods and Materials

Environmental Data

During each visit several environmental and physical parameters were measured at the pools in Higginbothams Cave Number 2. Water temperature (°C) was measured with a Lamotte armored thermometer, air temperature (°C) with Reotemp thermometers at the edge of the pools, pH with a pHTestr 2 with ATC, and dissolved oxygen (mg/L) was measured using a YSI 55 oxygen probe. Water depth (cm) and which rimstone pools were filled were also recorded.

The reason for noting which pool was filled was that it showed a direct correlation to how much water was in the cave. The rimstone pools in Higginbothams Cave Number 2 were all connected but did not have a constant flow of water through them. Water entered an empty pool once the level of water exceeded the capacity of the pool next to it. Water filled the pools in order from one to nine. The more pools that filled with water indicated the more water in the cave. This is comparable to stream bank width in other salamander studies (Lindley 1999) in epigean environments.

Egg-Laying and Development

Three aquatic areas contained on the study sites were searched monthly for eggs. Locations of eggs and substrate that eggs were attached to were recorded. When egg deposition occurred, the cave was visited once a week until larvae emerged. During each visit the number of eggs observed was noted and eggs were measured to the nearest 0.1 mm with a Scienceware dial Vernier caliper. Weekly egg development was compared and described using the Harrison stages of development (Harrison 1969).

In addition, clutch size of gravid females was determined. This was observed by dissections of preserved specimens found in the West Virginia Biological Survey, Marshall University, West Virginia. Eggs were counted and recorded along with SVL and total length of gravid females.

Larval Data

Larval *E. lucifuga* searches were conducted twice a month during the summer and early fall (May through September) and surveyed once a month during the fall, winter, and spring (October through April). Larval salamander searches were conducted using dip nets and turkey bastes. The turkey baste was useful for this purpose in that it could capture the larvae without disturbing the surrounding substrate. When larvae were captured, their total length, snout-vent length (if possible), and location of capture were recorded.

Results

Environmental Data

All aquatic environmental data were obtained from pool two in Higginbothams Cave No. 2 because it was the only pool in all three study caves that contained water and was easily accessible throughout the study. Pool one in this cave also contained water throughout the study but access to it was too difficult to obtain proper environmental readings. Temporary pools containing larvae were found in Buckeye Creek Cave in May 1999 but dried up by 6 June 1999. An underground stream was discovered in Norman cave but not until late in the study, preventing any useful environmental data from being obtained.

Table 6 shows the occurrence of water in the different pools in Higginbothams Cave No. 2. Only pool one and two contained water throughout the study. January 2000, February 2000, and May 1999 were the wettest months in this cave which is indicated by all pools having some water in them. July, September, and November were the driest months, which is evidenced by only pools one and two containing water.

Pool two environmental data were as follows (Table 7): average water pH ranged from 7.5 in February 2000 to 8.5 in May 1999; average water temperature ranged from 9.8 °C in January 2000 to 10.8 °C in December 1999; average air temperature of the area around the pool ranged from 8.6 °C in January 2000 to 14.7 °C in August 1999; average dissolved oxygen ranged from 3.7 mg/L in June 1999 to 6.8 mg/L in January 2000; and average water depth ranged from 12.7 cm in June 1999 to 35 cm in January 2000.

<u>Egg-Laying and Development</u>

Nine gravid females were found during dissections of West Virginia Biological Survey specimens. Out of those nine, only four had egg follicles large enough to count. Average SVL of gravid females was 64.7 mm, ranging from 59.9 to 70.3 mm SVL. Average clutch size was 55 eggs for the four gravid females with large enough follicles to be counted (Table 8). Gravid females were first noticed in field observations in April with tiny follicles formed. In July 1999, follicles were big enough to measure through the skin averaging 1.7 mm in diameter. This suggests that eggs develop from April to deposition in the fall.

Streams within Buckeye Creek and Norman caves were searched on a monthly basis as well as the pools in Higginbothams Cave No. 2. No eggs were found in the two streams but they were found in the pools of Higginbothams Cave No. 2.

Eurycea lucifuga eggs were found between 12 September and 9 November 1999 in pools one and two of Higginbothams Cave No. 2 (Fig. 8). All eggs were attached singly by a pedicel to the edges and undersides of rocks or to the sides of the rimstone pools. Several eggs were observed laying on the bottom of the rimstone pools towards the later stages of development. Egg diameter ranged from 2.9 to 5.2 mm.

During egg development, average water pH in pool two ranged from 8.0 to 8.2 with an average of 8.1 (Table 9). Water depth ranged from 19 to 34 cm with an average depth of 27.7 cm. Average water temperature ranged from 10.5 to 11.1 °C with and average of 10.8 °C. Average air temperature above pool two ranged from 11.7 to 12 °C with an average of 11.8 °C. Average dissolved oxygen content of water in pool two ranged from 5.5 to 6.6 mg/L with and average of 6.0 mg/L.

Eggs described and illustrated (Figs. 25-29) were initially found on 18 September 1999. These eggs were not observed on 12 September 1999, so I assumed that they were less than one week old. Eggs were monitored until they hatched between 30 October 1999 and 9 November 1999. Eggs developed and hatched within 52 days (+/- 6 days).

Newly deposited eggs observed on 18 September 1999 were round in shape and were between Harrison stages 1 and 12 (Fig. 25). On 27 September 1999, within nine days of their original observation, eggs were still between Harrison stages 1 to 12. On 3 October 1999, 15 days from their original observation, eggs were between Harrison stages 13 and 20 (Fig. 26). On 10 October 1999, 22 days from the original observation, eggs were between Harrison stages 21-29 (Fig. 27). On 17 October 1999, 29 days from original observation, eggs were between Harrison stages 30 to 35 with an elongation occurring, but without straitening of the embryo (Fig. 28). On 24 October 1999, 36 days after original observation, embryos were still elongating and two eye "bumps" could be identified in the head region. At this point eggs were still between Harrison stages 30-35 (Fig. 29). On 31 October 1999, 43 days after original observation, eggs were showing more rapid development and were between Harrison stages 30-35. Finally, on 9 November 1999, 52 days after original observation, all eggs had hatched, except two that were between Harrison stages 41 and 46 with definite gills present.

Newly hatched larvae had a gray dorsal side with a single white row of spots (Fig. 30). Average larval size at hatching on 9 November 1999 was 13.2 mm total length (n=4), with the largest being 13.8 mm and the smallest measuring 12.2 mm total length. A large, white yolk sac was attached to the ventral surface of the larvae. Larvae had no swimming power and rested on the bottom of the pool. When disturbed the larvae would wriggle but accomplished little movement. Newly hatched larvae had no back legs, front legs were evident but without fingers, and the dorsal and ventral of the tail was clear. There was a set of three single gills on each side to aid in respiration.

On a few occasions, several adult *E. lucifuga* were observed under water in pool one. On 18 September 1999, one adult cave salamander was observed in the water walking on the bottom. I watched the animal for approximately two hours and did not observe any egg deposition. The salamander would often take about three steps and search around. Occasionally the salamander would bury its head in the silt at the bottom of the pool. Other times it would arch its back upwards and press its cloacal region into the floor while wobbling back and forth. On 27 September 1999, five adult cave salamanders were observed walking on the bottom of pool one. Again the adults were observed for approximately two hours but no egg deposition occurred. On both occasions salamanders could not be collected to determine sex because of the difficult access and deep water of pool one.

Larval Data

Larvae were only observed in Buckeye Creek Cave and Higginbothams Cave No. 2. Larvae found in Buckeye Creek cave were found in temporary pools in the "first stoopway" in May 1999. Five larvae were observed ranging in total length from 19.7 to 22.9 mm with an average total length of 21.0 mm. All larvae were found dead or dying in the dried up pools on 6 June 1999.

Also, on 27 September 1999, one fairly large larva was found in the stream just inside the entrance to Buckeye Creek Cave. This individual had a snout-vent length of 28.6 mm and a total length of 53 mm.

Larvae were observed all months of the study in rimstone pools of Higginbothams Cave No. 2. In addition, larvae were found in pools three and seven in March 2000 and pools three, seven, and nine in April 2000. Larvae were found sitting on the bottom of pools or under submerged debris. One hundred and six larvae were observed in the rimstone pools. Larvae caught in pool seven were congregated around piles of animal feces. Sizes ranged from 12.1 to 45.9 mm. Figure 31 shows the total length of the larvae during each month. Two size classes are evident from November 1999 to April 2000. Also, two larger than average *E. lucifuga* larvae were found during the summer. One larvae measured 45.9 mm total length and was observed on 21 July 1999 in Higginbothams Cave No. 2 and the other was 53.0 mm (28.6 mm SVL) found on 27 September 1999 in the stream of Buckeye Creek Cave.

Discussion

Mating of the cave salamander has never been observed in the wild. Unfortunately, I was unable to document the phenomena. However, Organ (1968) observed the courtship behavior and spermatophore of the cave salamander under laboratory conditions. In this study, he observed males and females in the tail-straddling courtship typical of most salamanders. An intact spermatophore has never been discovered, but Organ observed a decapped spermatophore. It was white in color and amorphous and measured 3.7 mm high, 6.1 mm long, and 3.6 mm wide.

No information is available on the time it takes a female to deposit her eggs after she picks up the sperm cap with her cloaca. Research has been done on the number of eggs that each gravid female contains. Barden and Kezer (1944) found 51 eggs were deposited by a female *E. lucifuga* after pituitary gland implantation. Trauth et al. (1990) found the average clutch size of Arkansas cave salamanders to be 78 eggs. Hutchison (1956) discovered that the clutch size of Virginia cave salamanders was between 49 and 87 eggs.

In this study, the average clutch size (55) was lower than in similar studies. Hutchison (1956) stated that clutch size was independent of the size of the female in his study of Virginia *E. lucifuga*. My data supports this conclusion because the second largest gravid female in this study had the second smallest number of eggs. Therefore, it can be assumed that clutch size is independent of female length and that the differences in the clutch sizes between all studies is due to random chance of each sample.

In this study all eggs were deposited singly to the edges of rimstone pools or attached to the undersides and edges of rocks within rimstone pools. Green et al. (1967) observed the same pattern in a previous study of West Virginia cave salamander populations. Myers (1958a), in a Missouri cave, found eggs deposited in the same fashion, but only in streams. Therefore, it can be concluded that cave salamanders breed in either flowing or standing water. This is probably the case in West Virginia as well but no eggs were observed in streams or springs. Further studies are needed to test this prediction.

Eggs were observed between 12 September 1999 and 9 November 1999. Deposition of eggs occurred two weeks earlier than what Green et al. (1967) observed. Myers (1958a) observed eggs as late as 20 January in Missouri caves. Mount (1975) reported that eggs are known to be laid in Alabama from September to January. Rudolph (1978) observed hatchlings in Oklahoma springs in the winter and early spring. Hatchlings are evidence that eggs just hatched, indicating a later egg deposition date than in West Virginia. Time of oviposition must be determined by some environmental factor creating a geographic difference between egg deposition times.

Egg development was typical of most salamanders. Development followed stages described in Harrison's (1969) paper that is used by most to standardize embryo development. Incubation periods are much different than similar species. Brophy (1995) found that *E. cirrigera* had an incubation period of 27 days in West Virginia. Mohr (1943) determined the incubation period of *E. longicauda* to be 85 to 90 days.

The difference between similar species is probably due to two factors; predation and temperature. *Eurycea cirrigera* is found in streams and ponds that contain a high number of predators. Their fast incubation period and high numbers of eggs ensure the success of the species. *Eurycea longicauda* has a long incubation period that can be attributed to the low temperatures and low predation associated with the egg deposition location of this species. *Eurycea* *longicauda* and *E. lucifuga* are similar in this aspect in that they both lay eggs in underground aquatic environments, which have cool temperatures. In addition, these environments contain little or no predation because of their secluded and secretive locations. The only predation on the cave salamander eggs observed in this study was by an *E. lucifuga* larvae that ate two eggs that were knocked off their pedicels and placed in a petri dish at the bottom of the pool.

Larvae were very under-developed and vulnerable at hatching. Hatchlings measured 12.1 to 15.4 mm. Their front limbs were under-developed and the back limbs were nonexistent. Only movements observed were by wriggling which produced little movement. Rudolph (1978) found these characteristics are harmful to cave salamander hatchlings that are found in streams and springs outside of caves. He found that only 54 percent of larval cave salamanders survived experimental floods. Also, Rudolph discovered that only 12 to 13 percent of *E. lucifuga* larvae could survive experimental fish predation tests.

Myers (1958a), Sinclair (1950), and Green et al. (1967) observed similar hatching sizes for *E. lucifuga* but hatching dates differed by up to three months. Sinclair (1950) found hatchlings measuring 14 mm total length on 4 February in seeps and springs of Tennessee. Myers (1958a) found 11 mm hatchlings on 2 January in Mushroom Cave, Missouri. Green et al. (1967) found similar results to my data in observations of hatchlings in West Virginia caves. Egg deposition and hatching times must therefore be regional, depending upon location and environmental factors. Streams and pools inside caves are generally warmer than aquatic areas outside of caverns during the winter months. Sinclair's data was reported from seeps and springs outside caves and the colder water and temperatures could have contributed to the later egg deposition and larval emergence times. On 17 January 2000, three larvae were observed in pool one with their yolk sack still attached and appeared to be fairly underdeveloped. Sizes could not be determined for these specimens because of the difficult access to this pool. This could indicate a later egg deposition time for species in the same cave.

Larvae retained their yolk sack for over a month and during this time they experienced fast growth. Larvae grew an average of 8 mm from 9 November 1999 to 17 January 2000. After this period, larvae experienced a slow growth rate indicated by an average size increase of less than one millimeter in three months. Green et al. (1967) observed the same trend of rapid initial growth followed by a drop in growth rate once the yolk had been absorbed. Green and his colleagues believed this to be a direct result from the lack of food in rimstone pools. Slow growth could be affected by environmental factors also. Water temperature in the pools of Higginbothams Cave No. 2 remained constantly cold and had a very low dissolved oxygen. A lack of food as well as low temperatures and dissolved oxygen are determined to cause the slow growth rates.

A slow growth rate because of lack of nutrient uptake is also evidenced in the time it takes to metamorphose. Larval *E. lucifuga* found in streams and seeps outside caves reach metamorphose in a shorter time period (Rudolph 1978). He showed that larval *E. lucifuga* obtain metamorphose size between July and October of the same year of hatching. Trauth et al. (1990) found similar results to Rudolph's data with cave salamander larvae metamorphosing in the same time period. Both Rudolph and Trauth noted that it took some larvae populations more than a year to reach metamorphose size.

Larvae found in this study took more than one year to metamorphose, which is evidenced by the two size classes illustrated in Figure 31. During March and April 2000 there were definite differences in size between two groups of larvae. Most larvae averaged 20.8 mm total length during this time period, but two other larvae measured 37.1 mm and 37.8 mm total length. Two different size classes supports the concept that the larval period for cave salamanders is longer than one year.

Petranka (1998) reported that the larval period of the cave salamander lasts six to 18 months. I think that the larval period in West Virginia is slightly longer than what Petranka proposed. One larva was found in the Higginbothams Cave No. 2 pools in July 1999 that measured 45.9 mm total length and another was found in a pool in Buckeye Creek Cave that measured 53.0 mm total length (28.6 mm SVL) in September 1999. If these larvae hatched in November 1997 they would be 20 and 22 months old, respectively.

Green et al. (1967) reported a migration of larval *E. lucifuga* from the rimstone pools where they were originally found into streams within the cave during the winter and early spring when the pools began to overflow. Larvae in my study exhibited similar movements in Higginbothams Cave No. 2. Eggs and larvae were originally found in pools one and two in November 1999. At this time only these two pools contained water. During February, March, and April 2000 the larvae were found in pools one, two, seven, and nine which all contained water. Water from pool nine followed a two-foot high passage for approximately 100 meters before it joined an underground stream.

To label this movement as dispersal or migration is difficult. If it is a migration to a stream it could be to find a better food supply. It could also be dispersal to increase spatial segregation. The cause of this movement is uncertain and warrants further investigation.

For most of the year larvae were found to be fairly sedimentary and did not move unless disturbed. *Eurycea lucifuga* larvae were mostly found on the bottom of the pools and sometimes under debris. Sinclair (1950) counted hundreds crawling over the bottom of a shallow spring in the open at all hours of the day and night. Sinclair noted that older individuals were more sensitive to light and were found more commonly under shelter during the day. I did not notice such a trend but this could be do to the fact that all the pools in Higginbothams were found in the dark zone.

Larvae found in pool seven during February, March, and April 2000 were aggregated around piles of animal feces located in the bottom of the water. It could not be determined if the animal scat provided any nutrients. A food analysis for larvae was not performed. Rudolph (1978) did research on the prey composition of *E. lucifuga* and determined that the majority of food items belonged to the order Ostracoda and order Diptera (larvae). I believe that the larvae found in Higinbothams Cave No. 2 probably consumed zooplankton more than benthic insects because aquatic macroinvertebrates were never observed in the pools.

Chapter 6: Adult Feeding Habits

Introduction

One of the central theses of cave ecology is food resources for predators or scavengers are generally more scarce in caves than epigean environments (Peck and Richardson 1976). Limited information is available on the feeding habits of the cave salamander to support this point. Data does show that cave salamanders consume many different prey items depending on the organisms contained in their microhabitat. This means that in West Virginia alone, each individual cave contains separate prey items dependent on the invertebrate fauna contained there. The purpose of this portion of the study was to determine the prey items of *E. lucifuga* found in West Virginia.

Methods and Materials

Two different methods were implored to determine feeding habits of adult cave salamanders. The first was stomach pumping of live specimens and the second was gut extraction of preserved specimens. Two methods were used because stomach pumping did not produce sufficient data alone. Also, specimens could not be obtained in the winter months to stomach pump.

Stomach pumping consisted of finding cave salamanders throughout the study sites by searching the walls and overturning objects. Stomachs were pumped in the field with a 10cc syringe fitted with 18-gauge rubber tubing. The syringe was filled with water and the tubing was inserted into the mouth of the salamander until it reached the salamander's stomach. The stomach was then flushed with water until the salamander vomited up the contents of its stomach. Stomach contents were collected in a petri dish and immediately placed into 70 percent ethanol solution. The location, snout-vent length, total length, sex, and cranial width of each salamander were recorded.

Specimens one through six that were used for stomach extraction were obtained from the West Virginia Biological Survey, Marshall University, West Virginia. Stomachs were dissected from preserved specimens, cut open, and then flushed of all contents. Locality, sex, snout-vent length, cranial width, total length, and date of capture were recorded with each dissection.

All prey items were examined with a dissecting microscope and identified to order. Invertebrate taxonomy follows Borror et al. (1992). Lengths and widths of the stomach contents were measured to the nearest 0.1 mm with a Scienceware dial Vernier caliper. Empty stomachs were included in the analyses to determine feeding efficiencies.

Results

Twelve prey taxa were observed in the gut analysis of 53 juveniles and adult cave salamanders (Table 10). Of the 32 stomachs that contained prey items, Diptera comprised the largest percentage of gut contents (34.4%). Forty percent of the stomachs analyzed were empty. Differences in seasonal feeding could not be determined because of lack of sufficient species from every month.

Table 11 shows the number of prey items found in individual *E. lucifuga* stomachs. Twenty-six stomachs had identifiable prey items. Forty-six percent of the stomachs that contained food had multiple prey items within them. Specimen

10 contained the most quantity of prey items with 12 different organisms contained within its stomach.

Discussion

Prey items observed in this study showed similarities with related research. In this study, the most common prey items of adults were dipterans and araneae. These findings are comparable to studies performed by Hutchison (1958) and Peck (1974). Both studies found dipterans to be the most common prey item in cave salamander stomachs. Conversely, a study by Peck and Richardson (1976) determined that trichopteran made up the majority of prey items found in stomachs, followed by dipteran. This data supports the concept that the cave salamander does not select for one particular food item in its habitat. Rather, *E. lucifuga* is an opportunistic feeder and consumes prey items that are most readily available.

A high percentage of stomachs with multiple prey items further supports the theory that cave salamanders are opportunistic feeders. This data suggests that cave salamanders will take advantage of any prey item that it happens to find. The high number of taxa present in this small number of specimens further supports this suggestion.

Only two prey items, dipteran and araneae, are found more than ten percent of the time in this study. Peck and Richardson (1976) consider food items that are eaten more than ten percent of the time to be the primary food item of an animal. This is not to say that the cave salamander selects for these species, but rather that dipteran and araneae are the most abundant food source in the caves of West Virginia. Hutchison (1958) found similar findings in the caves of Virginia where the most numerous insect in the cave was a Dipteran helomyzid fly, *Amoebaleria defessa.*

Another interesting observation in this study was the high occurrence of empty stomachs (40%). Laboratory studies by Peck and Richardson (1976) found that the rate of food passage through the digestive tract of *E. lucifuga* averaged six days at 12.5° C. This temperature is comparable to caves in this study. This indicates that *E. lucifuga* has a low feeding rate or that there is not a high amount of food available to this species.

Chapter 7: Individual Movements, Habitat Use, and Community Ecology

Introduction

Little is known about the movement patterns of individual cave salamanders or the interactions that *E. lucifuga* has within its habitat and the other organisms found within its environment. The cave salamander is considered a top predator in its ecosystem and encounters little to no competition from other salamanders. This goal of this chapter was to observe individual movements of this species and describe the interactions *E. lucifuga* had with other animal species in West Virginia caves.

Methods and Materials

Movements

Individual salamander movements were observed using a mark-recapture study. Cave salamanders were captured and marked using the Visible Implant Fluorescent Elastomer (VIE) tagging system described in chapter four. Location of capture was recorded as a distance (meters) from the cave entrance. Height (meters) of each capture was also recorded if the salamander was located on the wall. It was not possible to determine home range of this species because of the low numbers of recaptures and the ability of the cave salamander to live deep inside crevices found in caves. Linear movements were calculated by using multiple recaptures to determine distances an individual moved. A t-test was used to determine differences between linear movements among the different sexes.

Habitat Use

Physical and chemical characteristics were recorded with each capture to determine habitat use. Recorded with each capture were crevice temperature and relative humidity, location, and distance from the entrance. If a salamander was not found in a crevice, the ambient temperature and relative humidity were used. Air temperature and relative humidity were measured using a Pocket Hygro-Thermometer by Extech Instruments.

Community Ecology

Other vertebrate species found on the study sites were recorded. Location of salamander species was recorded in the same manner as locations of the cave salamander. Any unusual findings and observations were noted.

Results

Movements

Tables 12 and 13 show individual movements of cave salamanders in Higginbothams Cave No. 2 and Norman Cave. The mean horizontal movement of 34 cave salamanders for the two caves was 8.85 ± 9.77 meters. The mean distance of movement by males was 12.83 ± 14.28 meters, females 3.82 ± 2.50 meters, and juveniles 9.83 ± 8.23 meters. A high standard deviation is because negative numbers indicate the salamander was found outside the cave. Movements were compared between males, females, and juveniles using a one-way ANOVA. There was no significant difference found but the findings should be interpreted cautiously with a p-value equal to 0.057.

Habitat Use

Eighty-nine percent of all salamanders were found in caves. Seventy-eight percent of all *E. lucifuga* were found in crevices. Average relative humidity was 90 percent and the mean temperature was 16.2 °C of all the crevices where cave salamanders were found. A Mann-Whitney rank sum test was performed to determine any significant difference between the means of the relative humidity and temperatures of the crevices and the twilight zone, where the majority of salamanders were found. There was a significant difference found when the relative humidity was compared ($p = < 0.001$).

Community Ecology

Sixteen different animal species were found in the three study caves (Table 14). Bats and the long-tailed salamander were found in the highest amounts along with the cave salamander. Bats were mainly found only in the fall and winter months. Interesting species that were found in the cave included an opossum (*Didelphis virginiana*) and a house cat (*Felis domesticus*) because they are not normally found in this habitat.

Discussion

Movements

I could not determine the home range of cave salamanders because of the low amount of multiple recaptures and problems associated with the nature of caves. Home range is measured by using either a mean activity radius (MAR) or minimum convex polygon (MCP). Both of these techniques require that you measure the mean distances from the center of the activity of an individual to all capture points (Humphries 1999). I could not calculate the home range because this would require knowing how far cave salamanders move into the crevices.

Movement data is not available in the literature for this species. Movements of *E. lucifuga* in this study were similar to movement of red-backed salamanders (*Plethodon cinereus*). Kleeburger and Werner (1982) found that red-backed salamanders had a mean activity radius of 12.97 meters for males, 24.34 meters for females, and 12.87 meters for juveniles. A comparison was made between my data and the movements of another species that inhabits rock crevices, the green salamander (*Aneides aeneus*). Gordon (1952) found that the green salamander traveled a maximum distance of 91 meters although they are generally sedentary. This great movement was only observed in the fall when the salamanders moved to outcrops with deeper crevices.

It can be concluded from my data that the cave salamanders are very active and are capable of moving far distances. Gut analysis of cave salamanders caught in the entrance zone contained prey items that were only found in the area of perpetual darkness (Peck and Richardson 1976). This suggests that cave salamanders can move great distances in a short period of time.

<u>Habitat Use</u>

Eurycea lucifuga in this study was found mostly in caves. It is not a troglobite, a cave-obligate species, but rather a troglophile. Troglophiles are facultative species that live and reproduce not only in caves but also in cool, dark, moist microhabitats outside of caves (Barr 1967). Other researchers have found *E. lucifuga* in caves (Hutchison 1956,1958; Williams 1980; Minton 1972; Banta and McAtee 1906; Green et al. 1967), on the forest floor (Minton 1972; Green et al. 1967), on open hillsides (Minton 1972), in springs (Banta and McAtee 1906; Rudolph 1978), and in cypress swamps (Smith 1961). Cave salamanders in West Virginia are undoubtedly found in caves and on the forest floor which is evidenced by the finding of the species in the Bluestone National Scenic River (Pauley pers. comm.) and at Barger's Spring near Hinton, Summers County (Green et al. 1967). No caves are reported in this county.

Inside caves, the salamander was mostly found in crevices at different heights along the walls. Banta and McAtee (1906) and Green et al. (1967) found similar findings. Hutchison (1958) and Williams (1980) reported that rarely were salamanders found without a thin film of water on them or at least on the surface of the space they occupied. I did not notice such a trend, but the high relative humidity of the crevices compared to the ambient relative humidity could explain the salamander's preference for inhabiting these spaces.

Another possible reason for the high numbers found in crevices could be the protection they afford. Salamanders were difficult to extract from these areas, especially if the crevices extended into the wall for some distance. Salamanders avoided capture by retreating farther into the crevice.

Williams (1980) collected 16 percent of adults in streams. I did not find many salamanders in the pools of Higginbothams Cave No. 2. During the egg deposition season I observed five adults on the bottom of pool one. Because of the difficult access to this pool it could not be determined what sex they were. They could have been females preparing to deposit eggs. Only on two other occasions did I find cave salamanders in the water. Green et al. (1967) only found one instance when an individual was found under water. The study by Williams (1980) was conducted in a cave with a large stream, which could explain the high numbers of salamanders found in aquatic areas.

It was originally thought that the range of the cave salamander was associated with the occurrence of limestone substrates. From observations on local distribution and from the locality records it appears that the species is not limited to limestone areas (Hutchison 1958). Two specimens of the cave salamander have been collected in northeast Georgia, in the center of a large crystalline rock, far removed from any limestone area (Green et al. 1967). In West Virginia, the cave salamander occupies abandoned coal mines in sandstone formations (Pauley et al. 1993). Heath et al. (1986) found similar findings in abandoned mines of the Ouachita Mountains, Arkansas. It can therefore be concluded that *E. lucifuga* is a secretive species that occupies caves because the caverns optimize their habitat requirements.

Community Ecology

In caves studied, only five species of salamanders were found. It is postulated that caves, especially twilight zones, seem suited for occupancy by many salamander species of the southeastern United States (Hairston 1949). The small number of salamanders associated with caves must then be because of the competitive dominance of *E. lucifuga*.

Cave salamanders appear to function as top predators in many cave communities (Petranka 1998). Peck (1974) determined that in caves where other species exist with *E. lucifuga*, the other species have been found to be comparatively undernourished, less general predators, and displaced to the low end of the spectrum of food particle size. Cave salamanders are so well adapted to the cave environment with its low food availability and limited hiding spaces that it out-competes any other species of terrestrial salamander.

Little is known about the kinds of predation that occur on the cave salamander. The black rat snake (*Elaphe o. obsoleta*), various field mice (*Peromyscus*), and a short-tailed shrew (*Blarina brevicauda*) were all found in the study caves and could be possible predators of the cave salamander. Banta (1907) indicated that the mouse *Peromyscus* was probably the chief predator on the cave salamander in Mayfield's Cave. Further investigation has to take place to determine what predators consume cave salamanders.

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Tables and Figures

Table 1. Average environmental parameters for the three stations in Norman Cave.

Station	Air Temp. (C)	Light (Lux)	Relative Humidity (%)
Entrance	16	84	78
Twilight	15.9	67	83
Dark	15.9	X	83

Table 2. Average environmental parameters for the three stations in Higginbothams Cave.

Station	Air Temp. (C)	Light (Lux)	Relative Humidity (%)
Entrance	15.4	202	72
Twilight	14.3	3	80
Dark	11.6	X	90

Table 3. Average environmental parameters for the three stations in Buckeye Creek Cave.

Station	Air Temp. (C)	Light (Lux)	Relative Humidity (%)
Entrance	14	227	65
Twilight	12.3	50	63
Dark	11.2	X	69

Table 4. Monthly sex ratios for all study caves.

Month	Total No. Males	Total No. Females	Ratio Males/ Females
May	7	8	0.88
June	14	17	0.82
July	20	22	0.91
August	4	6	0.67
September	3	7	0.43
October	2	2	1
November	0	2	0
December	0	0	0
January	0	0	0
February	0	0	0
March	2	0	2
April	5	5	1
Total	57	69	0.83

Table 5. Average metramorphic measurements of cave salamanders found on the study sites. Numbers in the parantheses represent standard deviation.

Cave	Sex	n	Cranial Width (mm)	SVL (mm)	Total Length (mm)	Weight (grams)
Higginbothams	Male	19	8.5	61.3	143.6	3.6
	Female	21	8.9	62.3	146.6	3.5
	Juvenile	11	7.0	46.3	108.8	1.6
Norman	Male	20	8.7	57.9	130.6	2.7
	Female	26	8.7	59.6	139.0	2.9
	Juvenile	40	6.2	44.1	100.3	1.2
Buckeye Creek	Male	6	8.9	61.1	147.2	3.5
	Female	2	8.1	60.9	152.9	3.7
	Juvenile	0	x	x	x	x
Total	Male	45	8.7 (1.23)	60.1 (4.85)	140.5 (18.53)	3.3 (0.85)
	Female	49	8.6 (1.03)	60.9 (4.56)	142.8 (22.31)	3.4 (0.73)
	Juvenile	51	6.6 (1.27)	45.2 (6.69)	104.6 (23.84)	1.4 (0.57)

Table 6. Occurrence of water in the rimstone pools in Higginbothams Cave No. 2. An asterick (*) represents that the pool contained water.

Month	Pool 1	Pool 2	Pool 3	Pool 4	Pool 5	Pool 6	Pool 7	Pool 8	Pool 9
21-May-99	*	*	*	*	*	*	*	*	*
6-Jun-99	*	*	*	*	*	*	*	*	*
16-Jun-99	*	*							
12-Jul-99	*	*							
21-Jul-99	*	*							
7-Aug-99	*	*	*						
19-Aug-99	*	*	*						
12-Sep-99	*	*	*	*	*				
27-Sep-99	*	*							
24-Oct-99	*	*	*	*	*		*	*	
17-Nov-99	*	*							
5-Dec-99	*	*	*		*	*	*	*	*
17-Jan-00	*	*	*	*	*	*	*	*	*
27-Feb-00	*	*	*	*	*	*	*	*	*
30-Mar-00	*	*	*	*			*	*	*
8-Apr-00	*	*	*	*			*	*	*

Table 7. Average environmental parameters of pool 2 in Higginbothams Cave No. 2.

Month	Water pH	Water Temp	Air Temp (C	DO (mg/L)	Depth (cm)
May-99	8.5	10.5	12.0	4.2	NA
Jun-99	8.4	10.2	12.7	3.7	12.7
Jul-99	8.2	10.3	11.4	4.7	24.0
Aug-99	8.4	10.8	14.7	4.7	29.5
Sep-99	8.0	11.1	11.8	5.8	19.0
Oct-99	8.1	10.5	12.0	6.5	34.0
Nov-99	8.2	10.5	11.7	5.5	30.0
Dec-99	8.3	10.8	13.1	5.9	34.0
Jan-00	8.4	9.8	8.6	6.8	35.0
Feb-00	7.5	10.0	11.2	6.6	28.0
Mar-00	8.4	10.3	11.3	6.3	33.0
Apr-00	8.4	10.5	10.7	6.2	33.0

Table 8. Snout-vent length and clutch size of gravid female *Eurycea lucifuga*.

Date Collected	SVL	Amount of Eggs
3 August 1965	67	69
6 August 1966	70.3	58
29 June 1949	66.2	53
8 August 1994	62	38

Table 9. Average environmental values in pool 2 of Higginbothams Cave No.2 for months associated with egg development.

Month	pH	Water Temp. (C)	Air Temp. (C)	Dissolved Oxygen (mg/L)	Water Depth (cm)
September	8.0	11.1	11.8	5.8	19
October	8.1	10.7	12.0	6.6	34
November	8.2	10.5	11.7	5.5	30

Table 10. Prey items observed in stomach analysis.

Prey Item	No. of stomachs in which food occurred (n=32)	Percentage of occurrence
Araneae (Spiders)	7	21.9
Acari (Mites)	2	6.3
Annelida (Earthworms)	2	6.3
Chilopoda, Scolopendromorpha (Centipede)	1	3.1
Coleoptera (Larvae)	1	3.1
Coleoptera (Adults)	2	6.3
Collembola (Springtails)	1	3.1
Diptera (Flies)	11	34.4
Gastropoda (Snails)	2	6.3
Hymenoptera - Family: Formicidae (Ants)	1	3.1
Isopoda (Isopods)	3	9.4
Lepidoptera - Larvae (Caterpillars)	1	3.1
Lepidoptera (Moths and Butterflies)	2	6.3
Orthoptera (Crickets)	2	6.3

Table 11. Quantity of prey items per individual *Eurycea lucifuga* stomach.

Specimen Number	Prey Item	Quantity of Prey Item
1	Araneae	1
2	Collembola	1
3	Acari	1
3	Lepidptera	1
4	Araneae	1
5	Hymenoptera (Formicidae)	1
6	Orthoptera (Cricket)	1
6	Coleoptera (Adult)	1
7	Isopoda	6
8	Diptera	4
9	Coleoptera	1
9	Isopoda	1
9	Gastropoda	3
9	Coleoptera (Adult)	2
9	Araneae	1
9	Diptera	2
10	Diptera	1
11	Acari	1
11	Isopoda	2
12	Diptera	9
12	Lepidoptera (Moth)	1
13	Annelida	1
14	Chilopoda, Scolopendromorpha (Centipede)	1
14	Diptera	4
15	Diptera	1
16	Araneae	3
16	Coleoptera (Larvae)	2
16	Lepidoptera (caterpillar)	1
16	Coleoptera (Larvae)	1
16	Gastropoda	5
17	Annelida	1
18	Diptera	1
19	Diptera	1
20	Diptera	1
21	Diptera	8
22	Orthoptera	1
23	Diptera	1

Table 11. (continued)

23	Araneae	1
24	Isopoda	1
25	Araneae	1
26	Araneae	4
27	Unidentifiable	1
28	Unidentifiable - Legs	1
29	Unidentifiable - Random Sclerites	
30	Unidentifiable - Random Sclerites	
31	Unidentifiable - Random Sclerites	
32	Unidentifiable - Random Sclerites	

Table 12. Horizontal movements of cave salamanders found in Higginbothams Cave No. 2.

ID #	Sex	Horizontal Distance Moved (meters)	Direction	Days Between Capture
2r,3y	female	2.5	Into Cave	36
		1.6	Into Cave	62
		3.4	Into Cave	15
1r,2o	juvenile	12.5	Into Cave	75
		14.4	Toward Entrance	24
2r,3o	male	1	Into Cave	26
2r,4o	male	1.2	Into Cave	26
2o,4o	female	2.5	Into Cave	26
3r,4o	female	1.4	Into Cave	26
1r,4r	juvenile	6.3	Into Cave	90
2o,4y	female	2	Toward Entrance	53
1r,3y	juvenile	11.4	Into Cave	15
2y,4o	juvenile	12.4	Into Cave	221
1o,2y	male	0	0	9
1o,4o	juvenile	0	0	26

Table 13. Horizontal movement of cave salamanders in Norman Cave.

ID #	Sex	Horizontal Distance Moved (meters)	Direction	Days Between Capture
3r,4r	female	6.6	Towards Entrance	36
		2.9	Towards Entrance	9
		8.4	Into Cave	98
2y,3y	juvenile	12	Into Cave	61
		6.7	Towards Entrance	17
2y,4y	juvenile	1	Into Cave	26
		1.5	Into Cave	26
1r,2o	juvenile	3	Into Cave	26
		11	Into Cave	26
2r,3y	male	0	0	26
		6.2	Into Cave	9
1r,2y	juvenile	7.5	Into Cave	26
3o,4o	male	29	Into Cave	26
1y,4r	female	2.5	Towards Entrance	26
3o,4r	male	20.4	Into Cave	26
1r,3r	male	40	Into Cave	62
3r,4y	female	2.2	Into Cave	64
1o,3o	female	8.4	Into Cave	85
1r,4o	male	1.8	Into Cave	77
2y,3o,4o	male	20	Towards Entrance	35
1y	juvenile	30.6	Into Cave	129
1o,3r	juvenile	6.9	Towards Entrance	77
2y,3o,4y	male	30	Into Cave	165
1o,2o,3y	juvenile	25	Towards Entrance	209
3r,4o	male	4.4	Towards Entrance	261
1y,2o	juvenile	14.7	Towards Entrance	245
4y	female	5.3	Into Cave	323
1o,2y,3y	juvenile	0	0	35

Table 14. Additional species observed in the three study caves.

Species		
Comman Name	**Scientific Name**	**Number Found**
Amphibians		
Eastern American Toad	*Bufo a. americanus*	1
Southern Green Frog- Adult	*Rana clamitans melanota*	1
Southern Green Frog- Tadpole	*Rana clamitans melanota*	1
Long-tailed Salamander - Adult	*Eurycea longicauda*	32
Long-tailed Salamander - Larvae	*Eurycea longicauda*	7
Mountain Dusky Salamander	*Desmognathus ochrophaeus*	1
Northern Dusky Salamander	*Desmognathus fuscus*	6
Pickerel Frog	*Rana palustris*	1
Red-backed Salamander	*Plethodon cinereus*	1
Slimy Salamander	*Plethodon glutinosus*	20
Birds		
Eastern Phoebe	*Sayornis phoebe*	1
Reptiles		
Black Rat Snake	*Elaphe o. obsoleta*	2
Mammals		
Big Brown Bat	*Eptesicus fuscus*	Many
Cat	*Felis domesticus*	1
Eastern Pipestrel	*Pipistrellus subflavus*	Many
Little Brown Bat	*Myotis lucifugus*	Many
Opossum	*Didelphis virginiana*	1
Peromyscus	*Peromyscus*	4
Short-tailed Shrew	*Blarina brevicauda*	1

Figure 1. Typical adult *Eurycea lucifuga* found on the study sites.

Figure 1. Typical adult *Eurycea lucifuga* found on the study sites.

Figure 2. Typical larval *Eurycea lucifuga* found on the study sites.

Figure 3. Range of *Eurycea lucifuga* (Petranka 1998). An "X" designates the occurrence of a disjunct population consisting of a single published record.

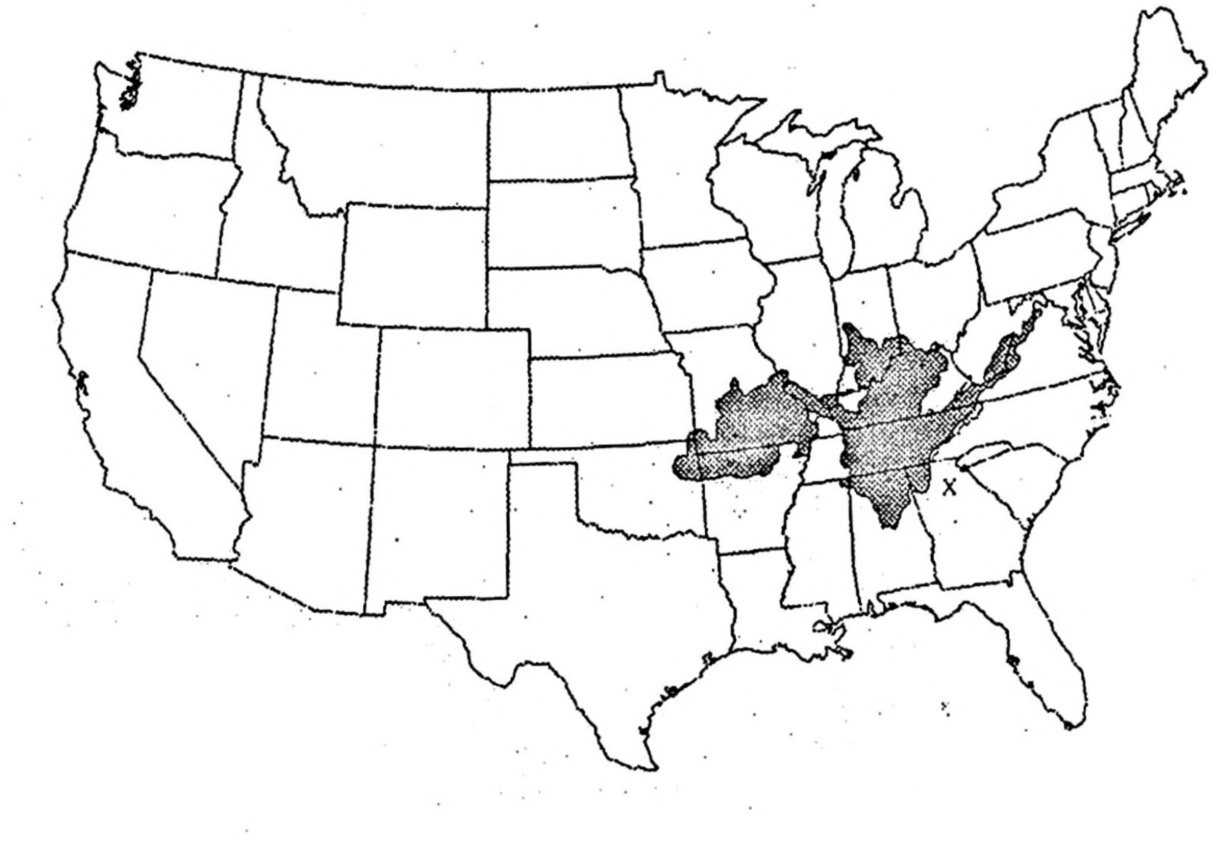

X

Figure 4. Range of *Eurycea lucifuga* in West Virginia.


0 5 10 15 20 miles
SCALE

Figure 5. Location of study caves in Greenbrier County, West Virginia. Fig. 5a shows location of Buckeye Creek Cave and Higginbothams Cave No.2 (Williamsburg Quadrangle). Fig. 5b shows location of Norman Cave (Droop Quadrangle).


Buckeye Creek Cave
Higginbothams Cave

a.


FALLING SPRING
Norman Cave

b.

Figure 5. Location of study caves in Greenbrier County, West Virginia. Fig. 5a shows location of Buckeye Creek Cave and Higginbothams Cave No.2 (Williamsburg Quadrangle). Fig. 5b shows location of Norman Cave (Droop Quadrangle).


Buckeye Creek Cave
Higginbothams Cave
a.
FALLING SPRING
Norman Cave
b.

Figure 6. Entrance and map of searched areas in Buckeye Creek Cave. Map courtesy of West Virginia Association for Cave Studies.

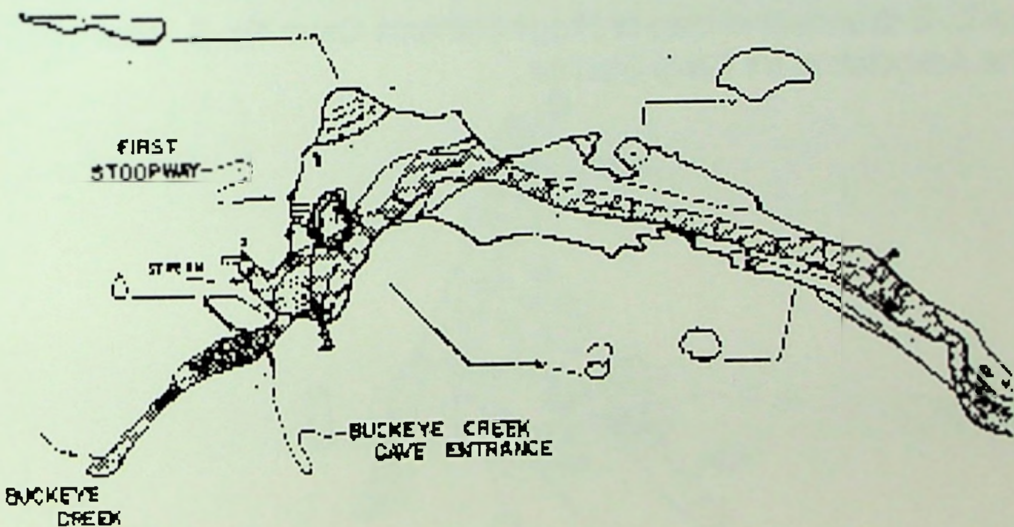

FIRST
STOOPWAY
BUCKEYE CREEK
CAVE ENTRANCE
BUCKEYE
CREEK

Figure 7. Entrance and map of Higginbothams Cave No. 2. Map courtesy of West Virginia Association for Cave Studies.

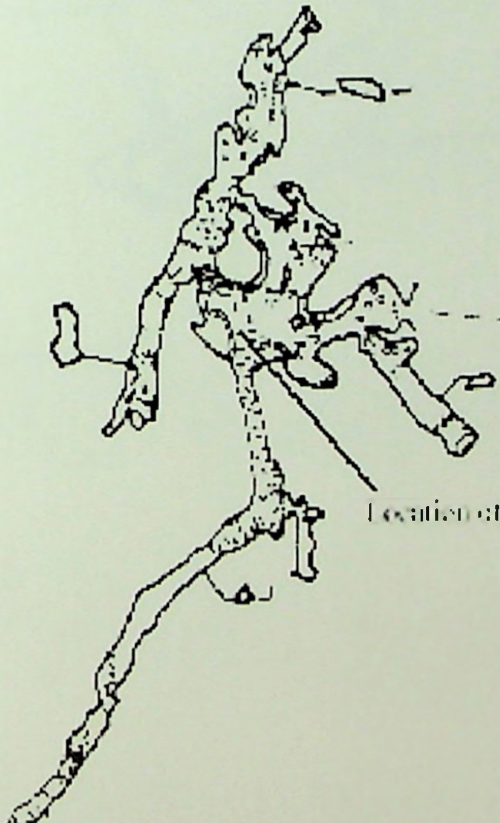
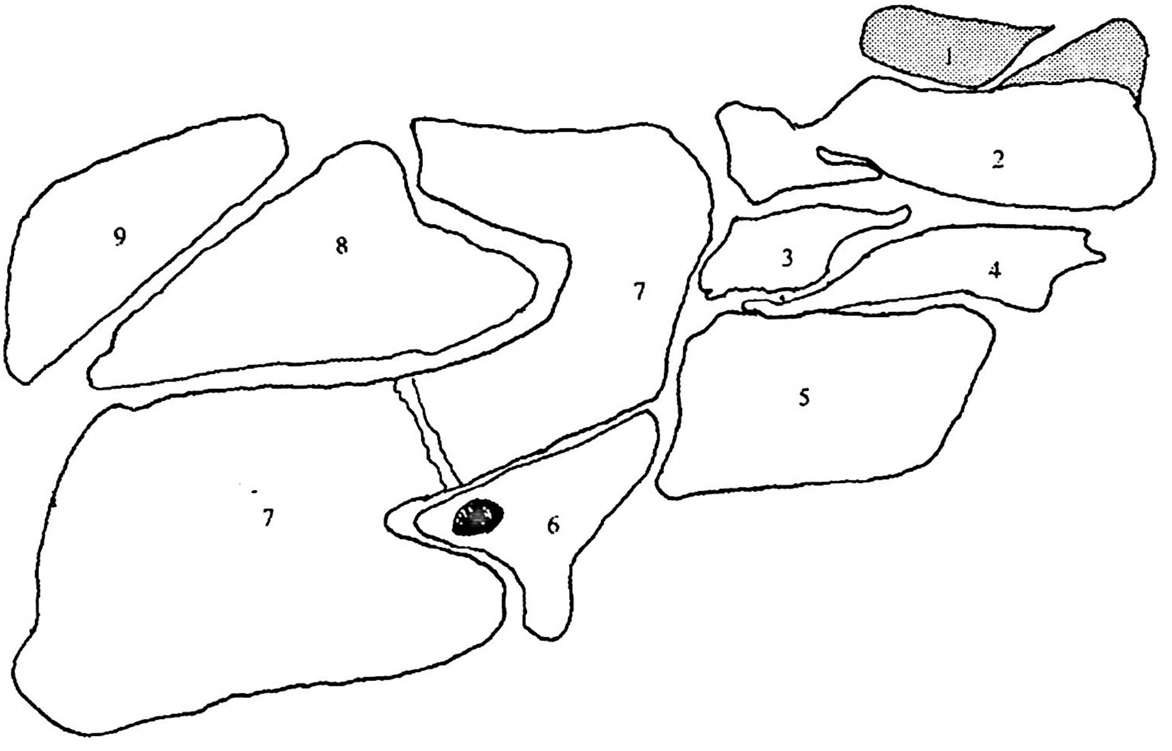

ENTRANCE

Figure 8. Map of rimstone pools found in Higginbothams Cave No. 2.



1
2
9
8
3
4
7
5
7
6

Figure 9. Entrance and map of Norman Cave. Map courtesy of West Virginia Association for Cave Studies.

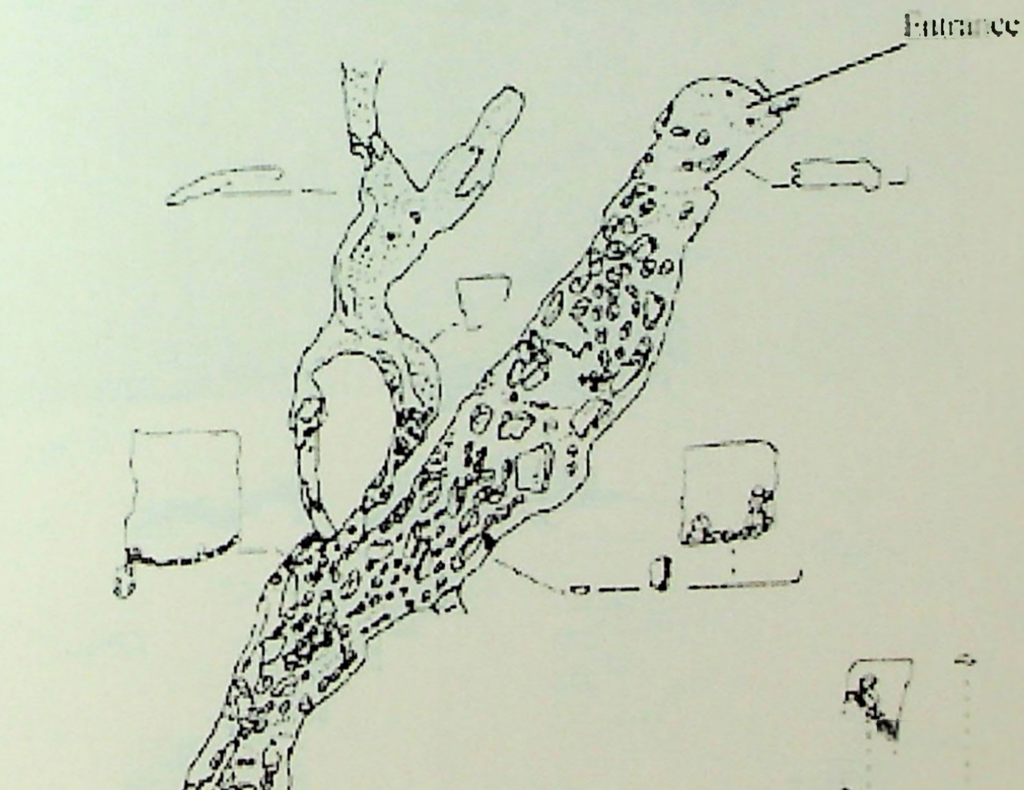

Entrance

Figure 10. Possible tagging locations on *Eurycea lucifuga* (Fig. 10a). An example of an *E. lucifuga* tagged 1 yellow, 4 red (Fig. 10b).

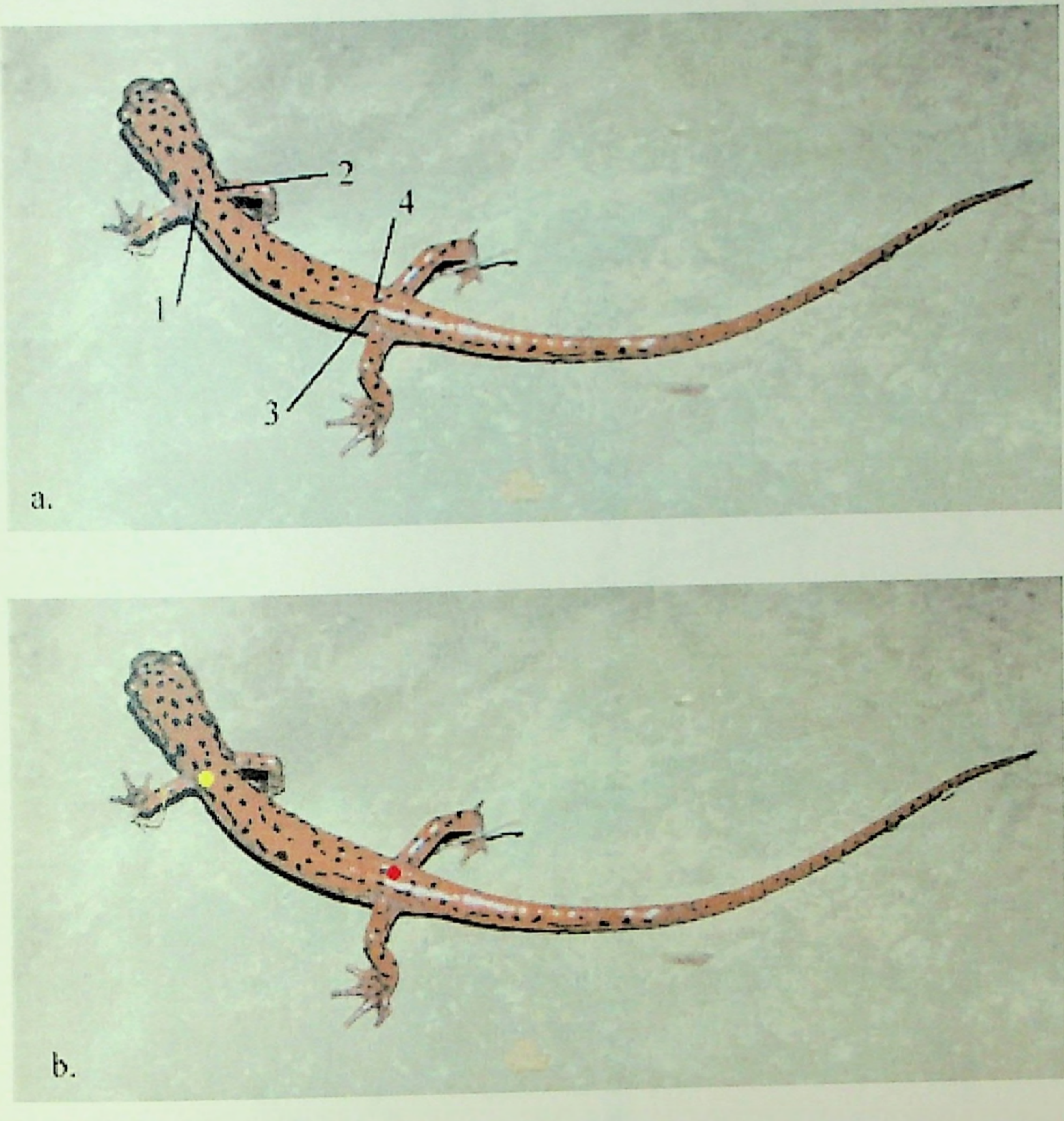

2
4
1
3
a.
b.

Figure 11. Air temperature fluctuations for three zones in Higginbothams Cave No. 2.

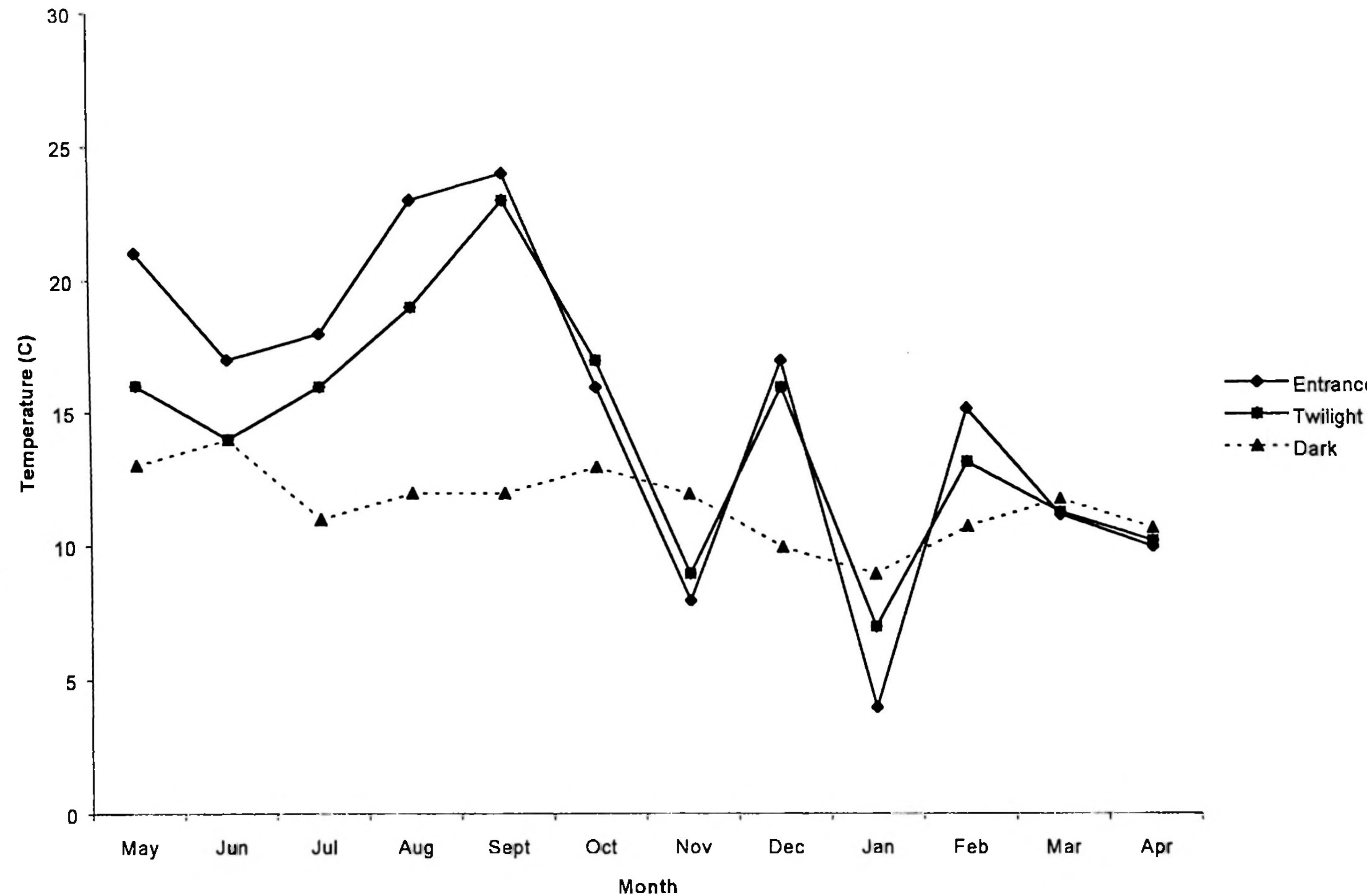
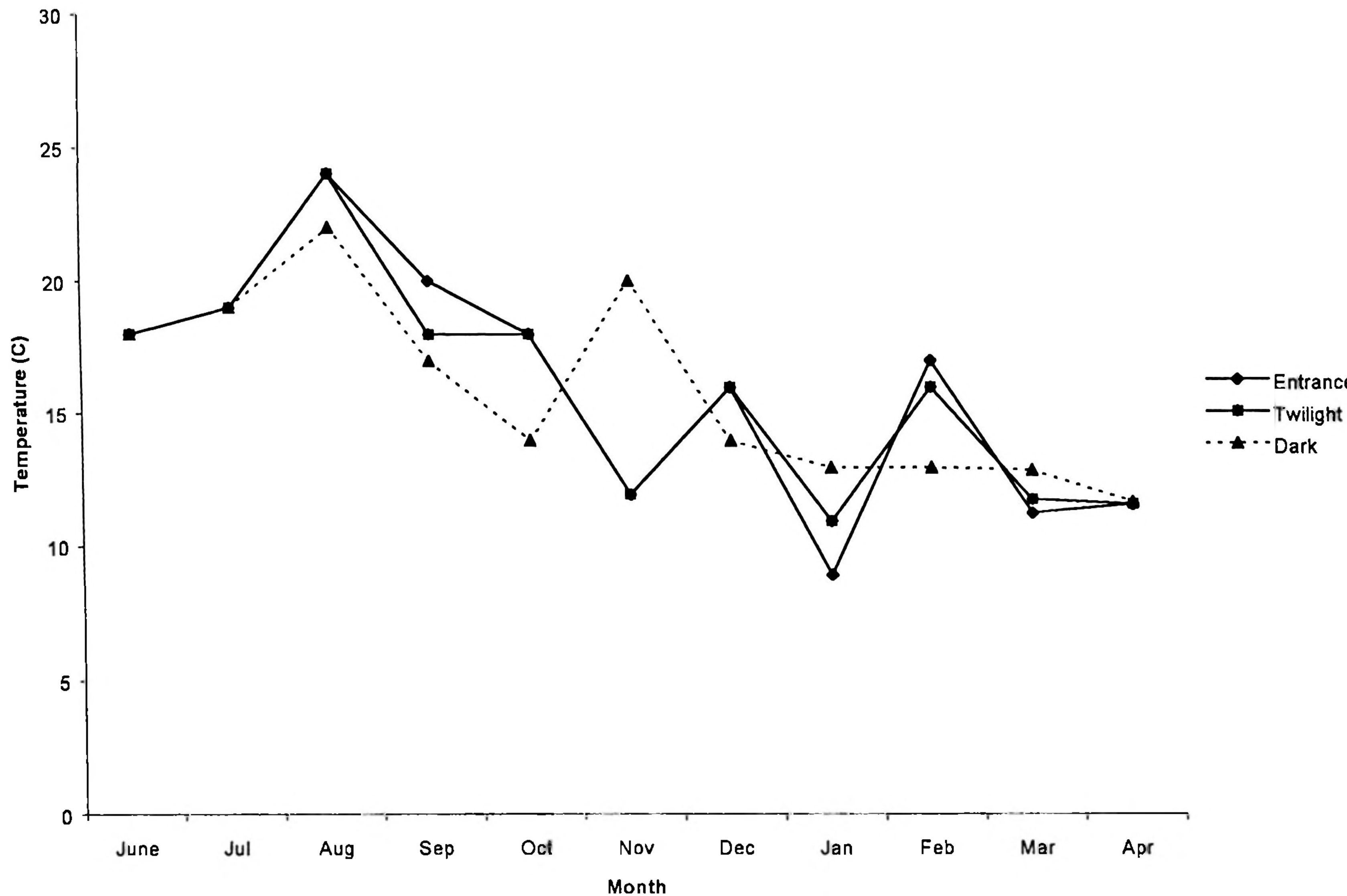

Temperature (C)
30
25
20
15
10
5
0
May
Jun
Jul
Aug
Sept
Oct
Nov
Dec
Jan
Feb
Mar
Apr
Month
Entrance
Twilight
Dark

Figure 12. Air temperature fluctuations for three zones in Norman Cave.



30
25
20
15
10
5
0
Temperature (C)
June
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
Month
Entrance
Twilight
Dark

Figure 13. Air temperature fluctuations for three zones in Buckeye Creek Cave.

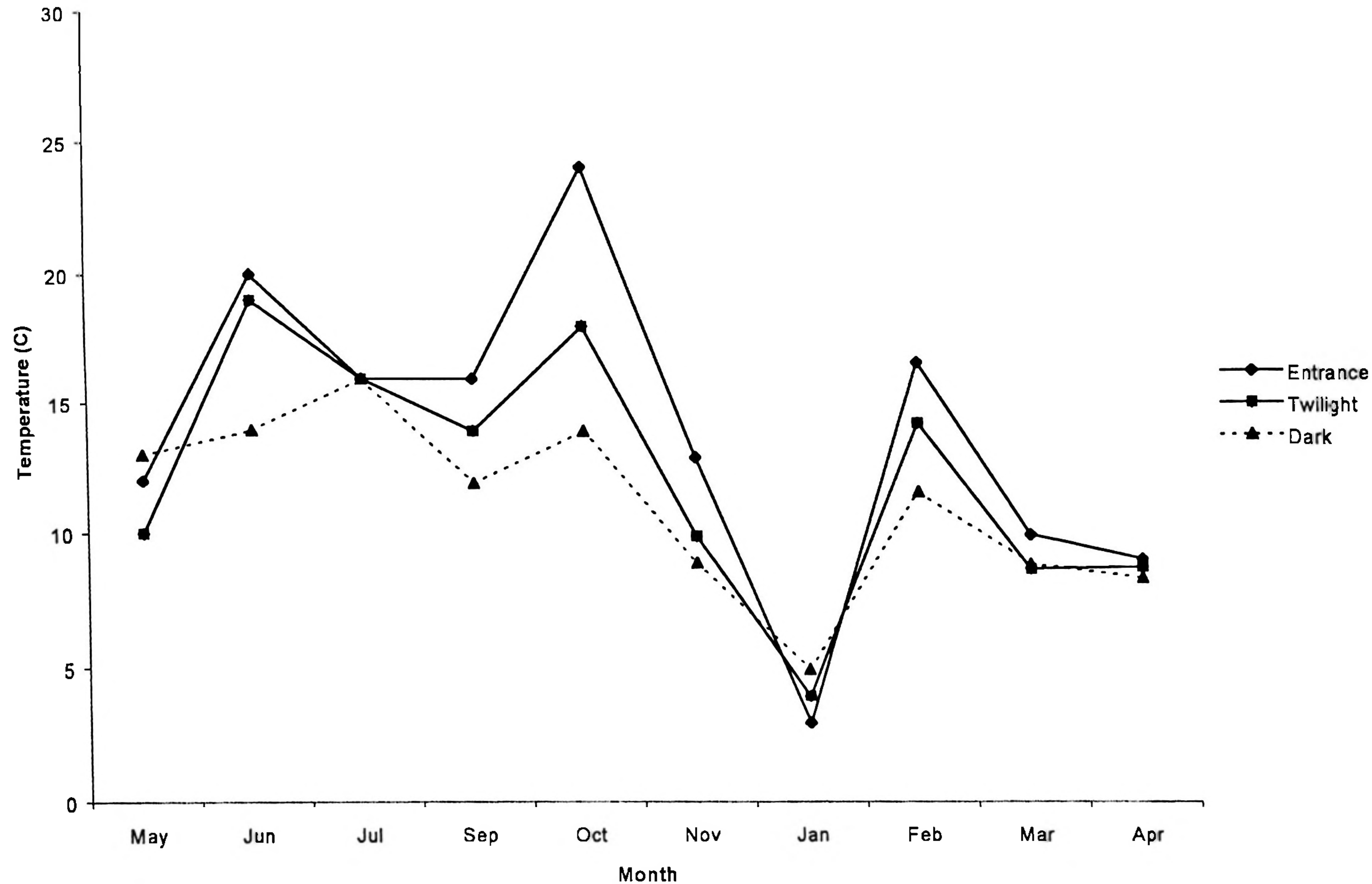

30
25
20
15
10
5
0
Temperature (C)
May
Jun
Jul
Sep
Oct
Nov
Jan
Feb
Mar
Apr
Month
Entrance
Twilight
Dark

Figure 14. Relative humidity fluctuations for three zones in Higginbothams Cave No. 2.

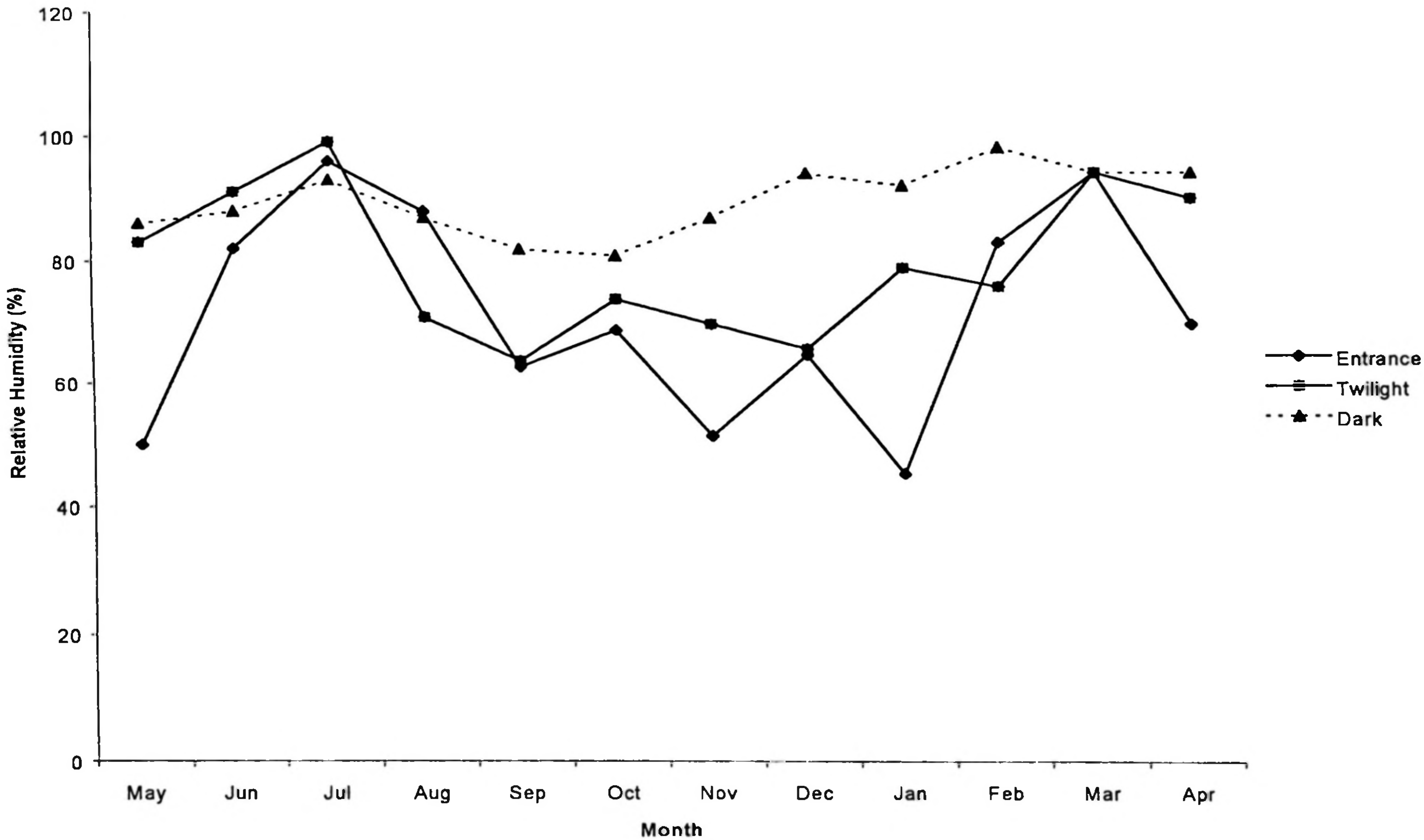

120
100
80
60
40
20
0
Relative Humidity (%)
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
Month
Entrance
Twilight
Dark

Figure 15. Relative humidity fluctuations for three zones in Norman Cave.

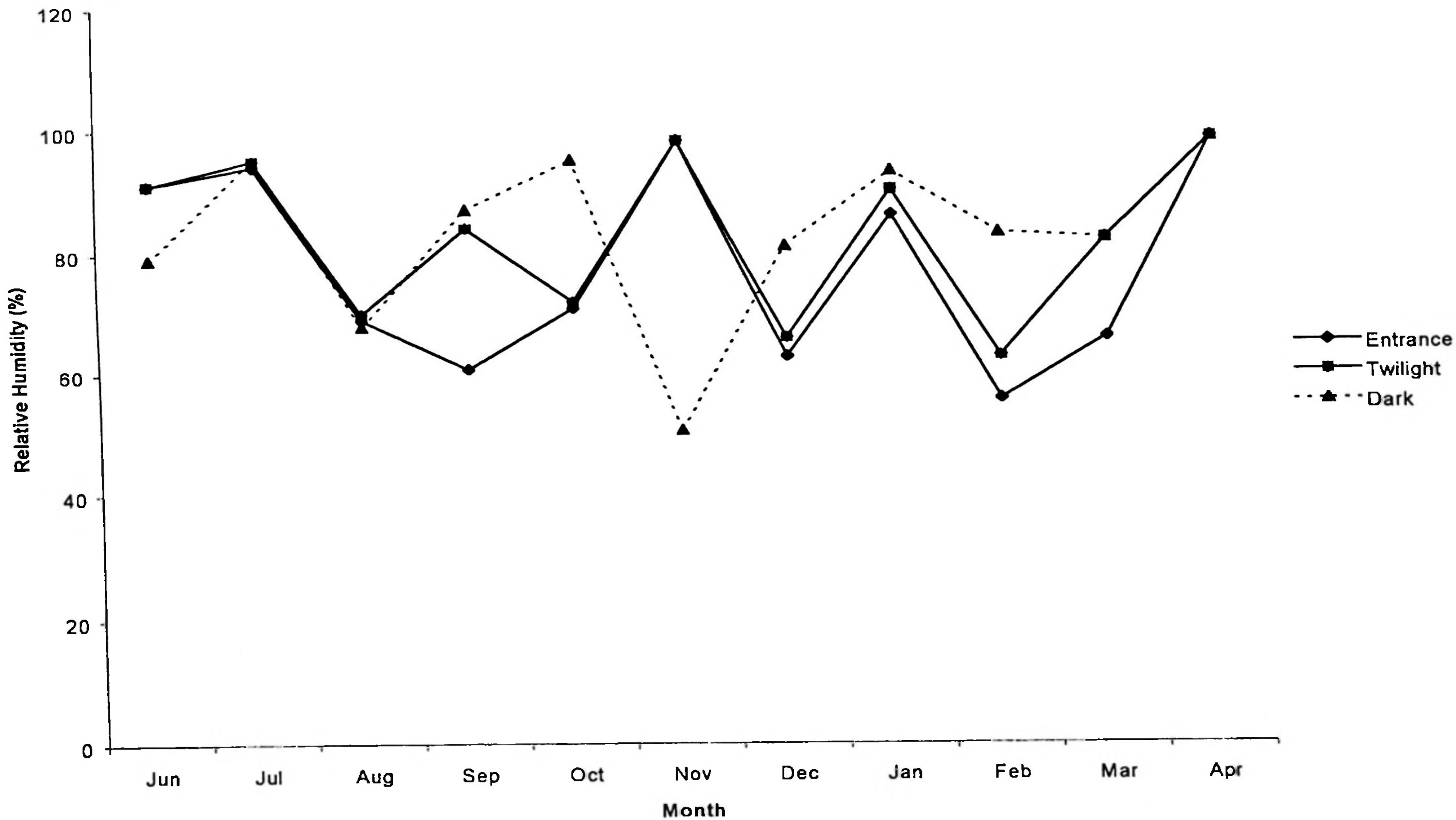
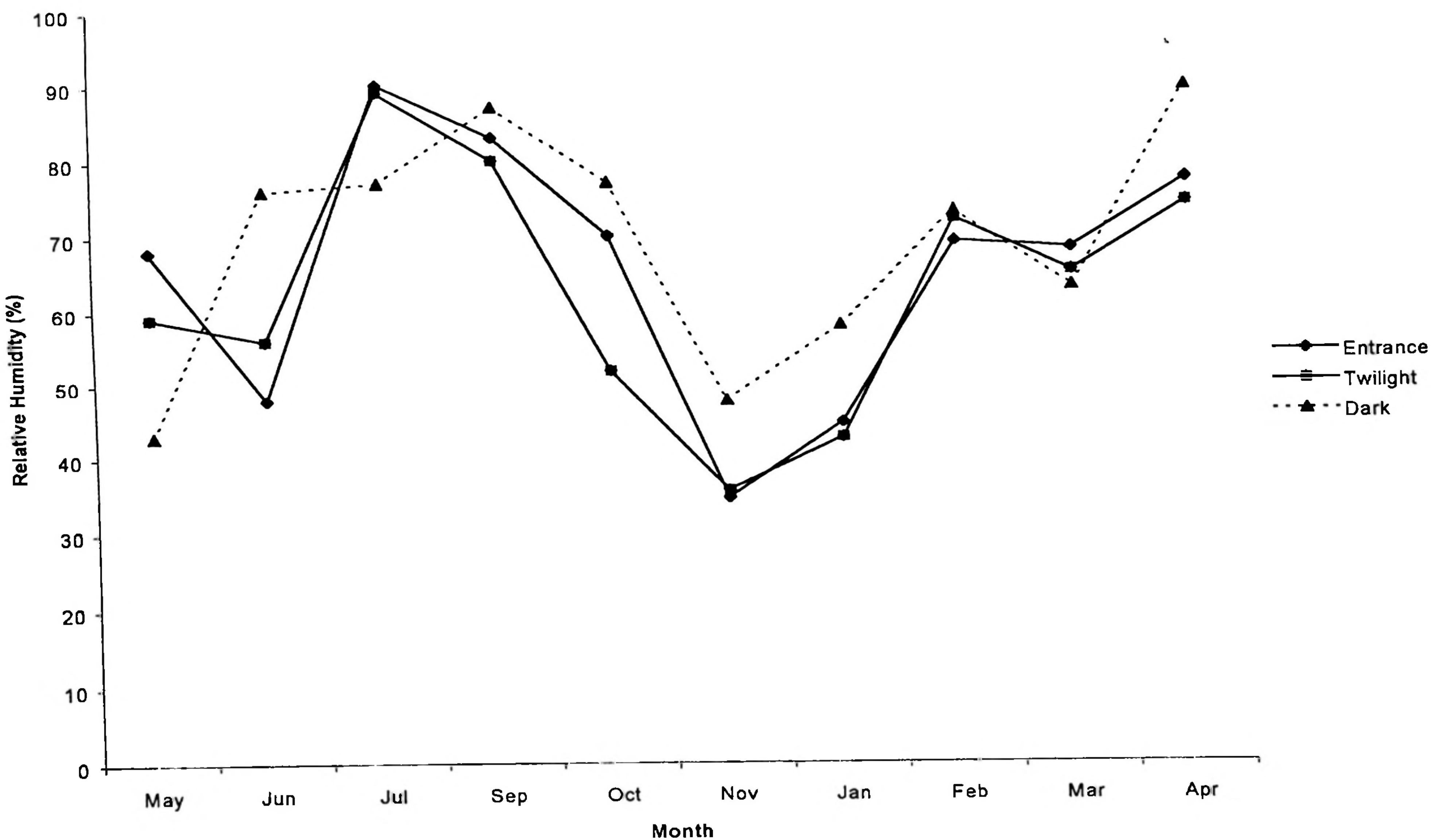
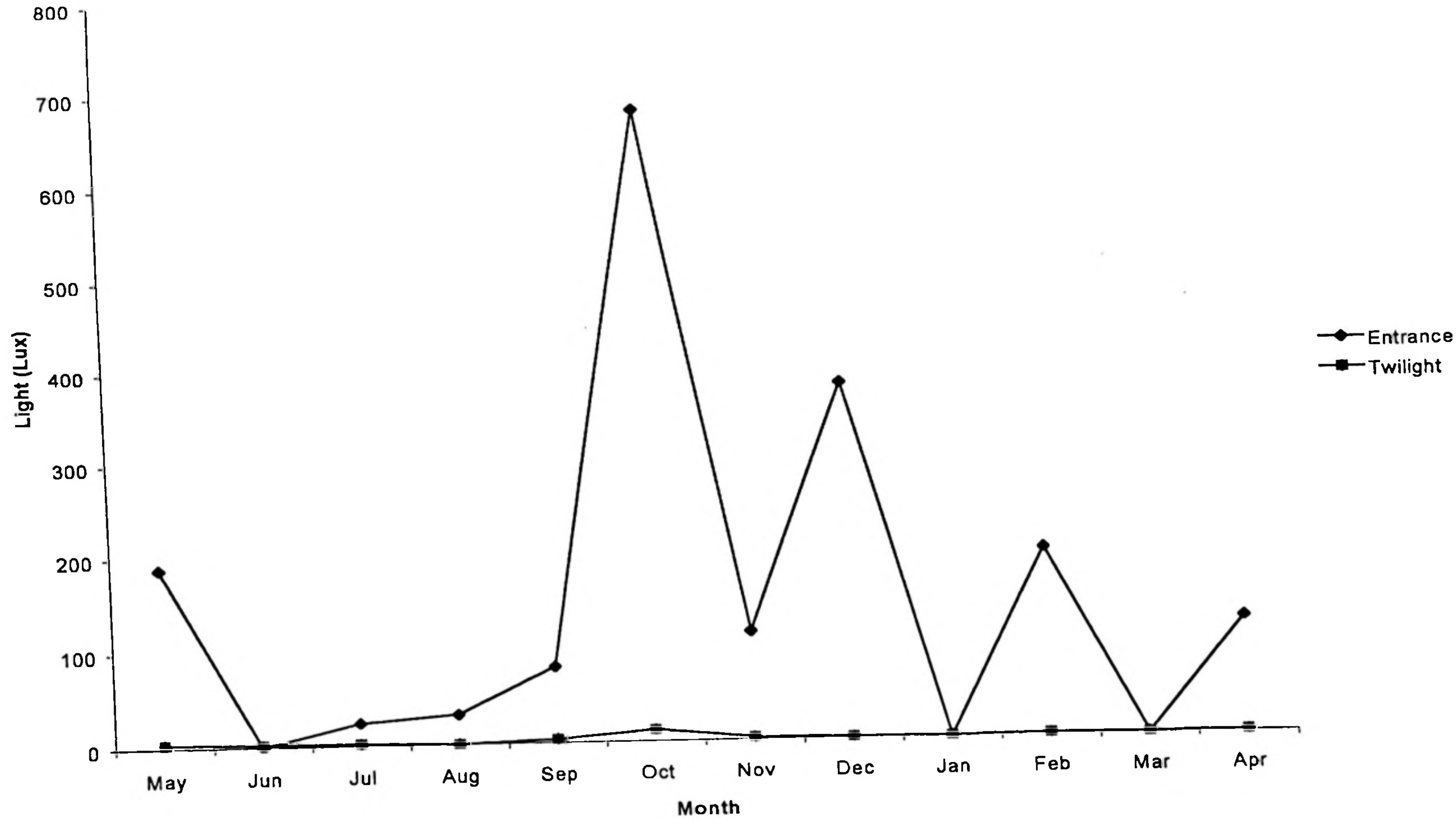

120
100
80
60
40
20
0
Relative Humidity (%)
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
Month
Entrance
Twilight
Dark

Figure 16. Relative humidity fluctuations for three zones in Buckeye Creek Cave.



100
90
80
70
60
50
40
30
20
10
0
Relative Humidity (%)
May
Jun
Jul
Sep
Oct
Nov
Jan
Feb
Mar
Apr
Month
Entrance
Twilight
Dark

Figure 17. Light fluctuations for two zones in Higginbothams Cave No. 2.



800
700
600
500
400
300
200
100
0
Light (Lux)
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
Month
Entrance
Twilight

Figure 17. Light fluctuations for two zones in Higginbothams Cave No. 2.

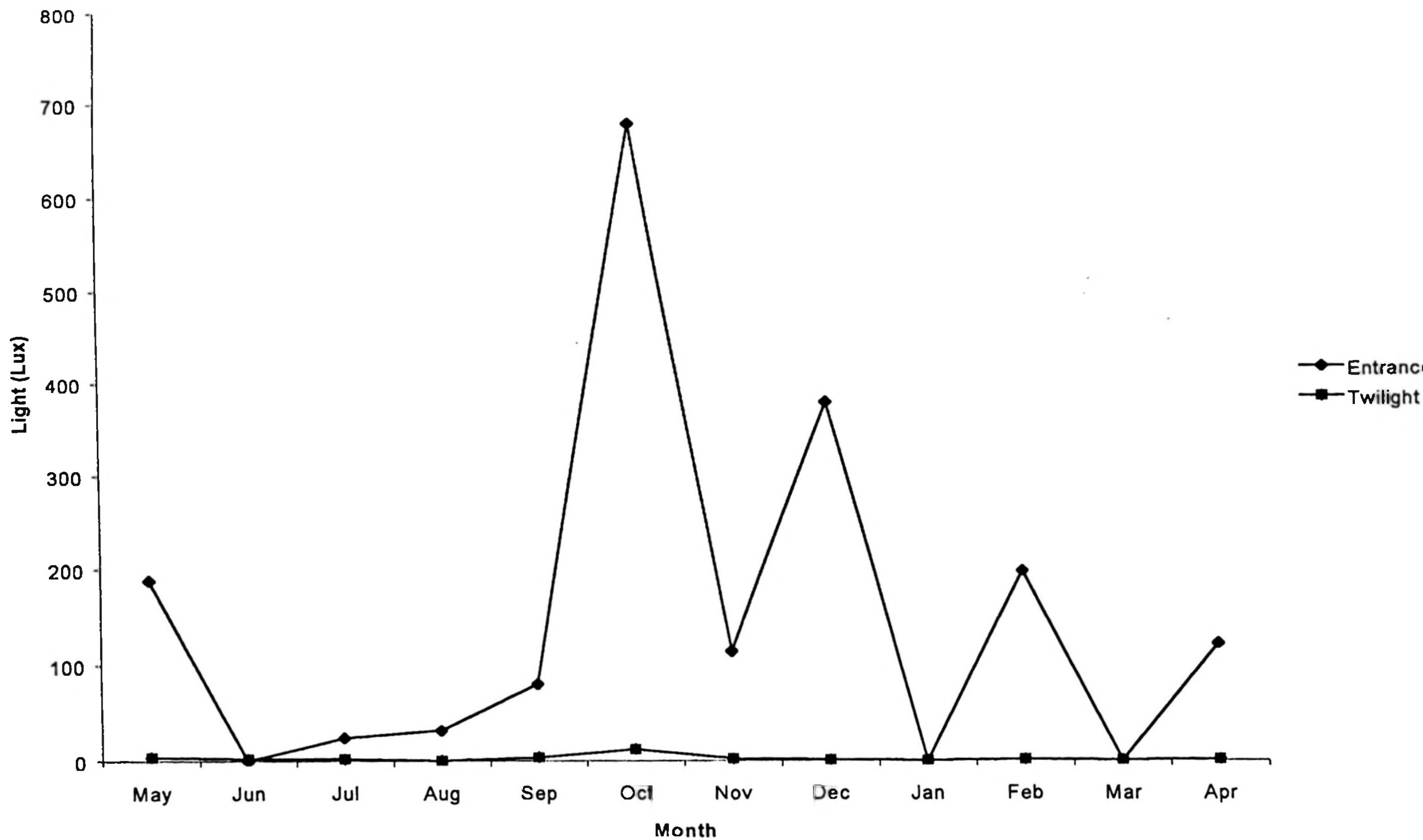
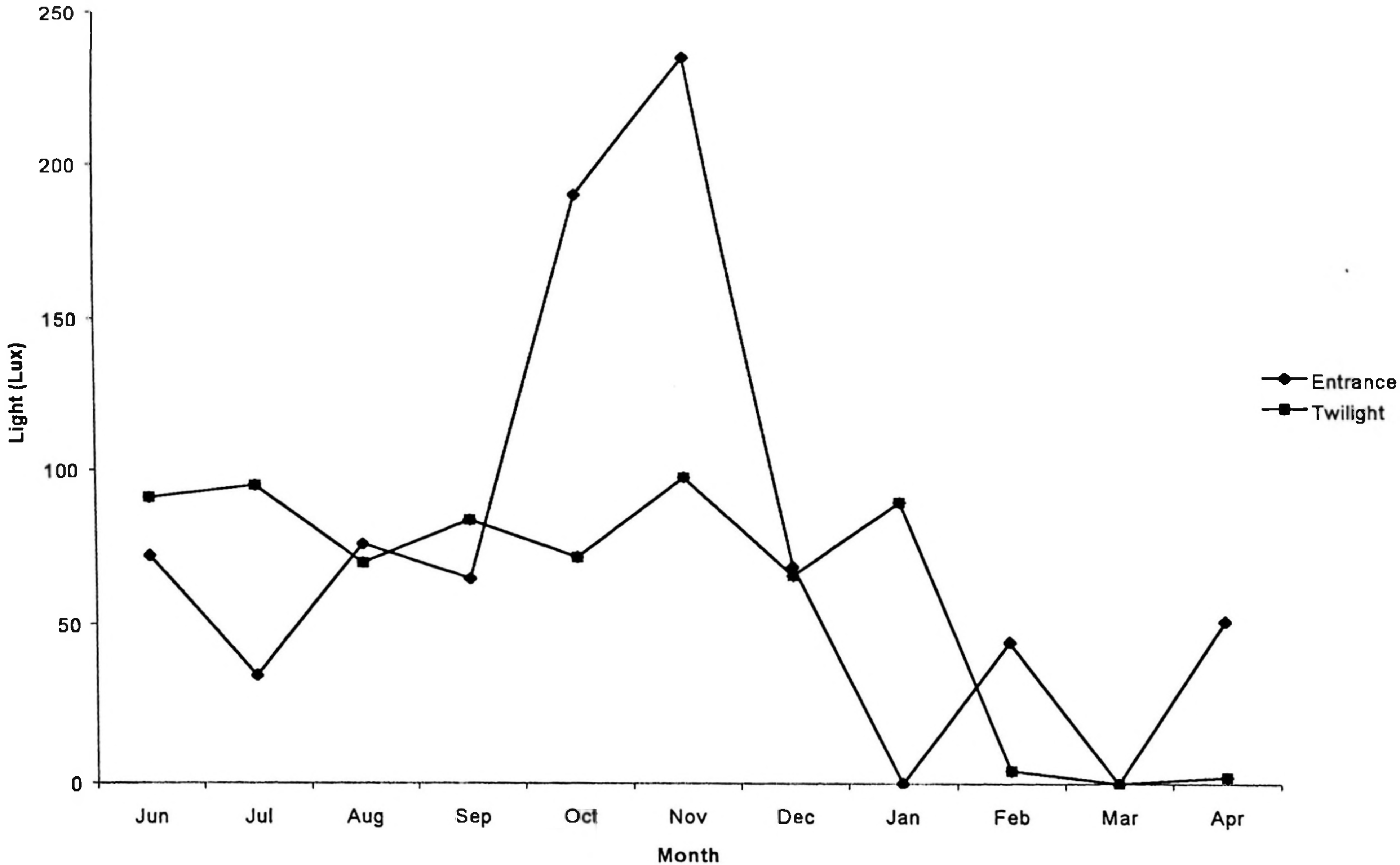
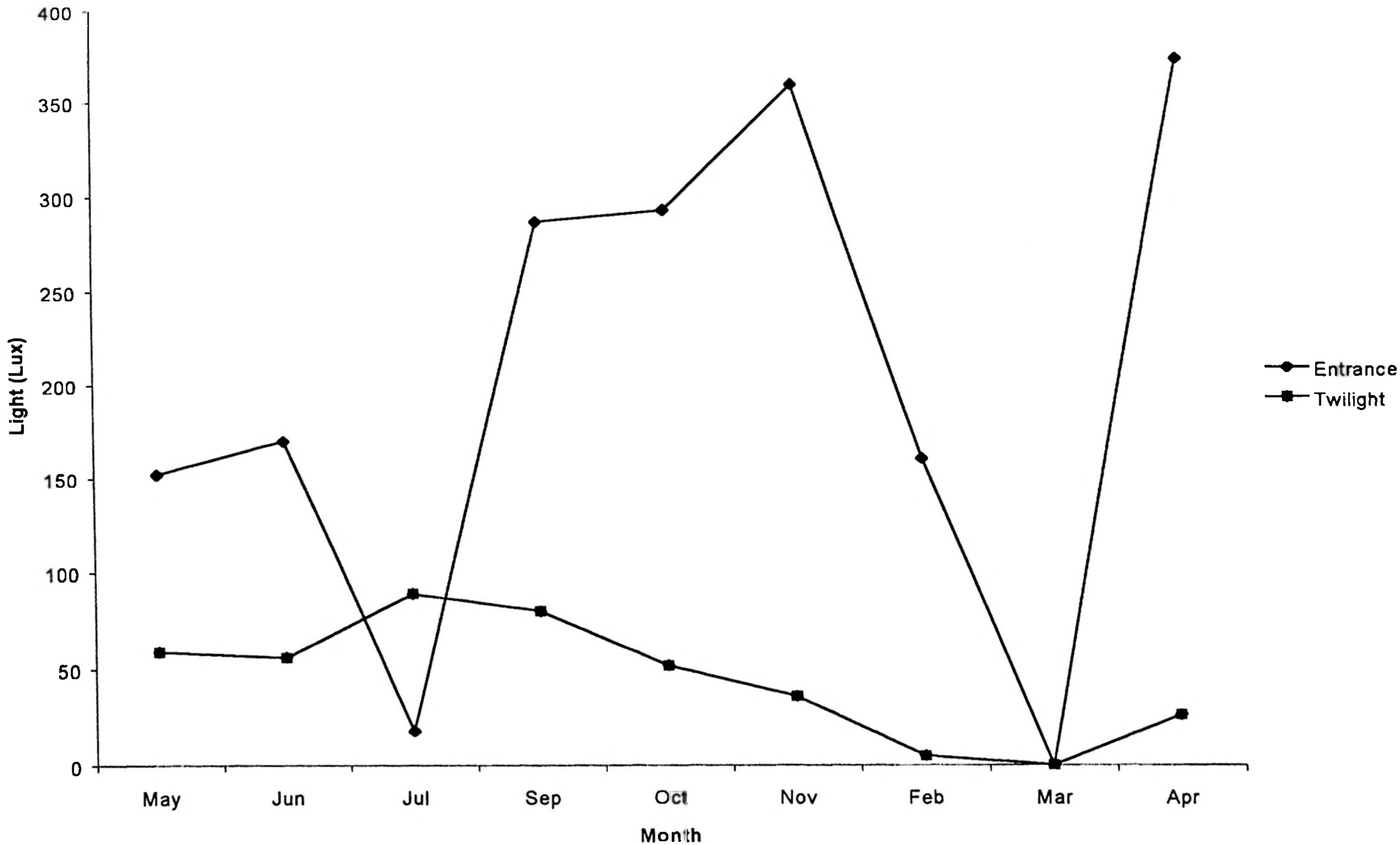

Light (Lux)
800
700
600
500
400
300
200
100
0
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
Month
Entrance
Twilight

Figure 18. Light fluctuations for two zones in Norman Cave.



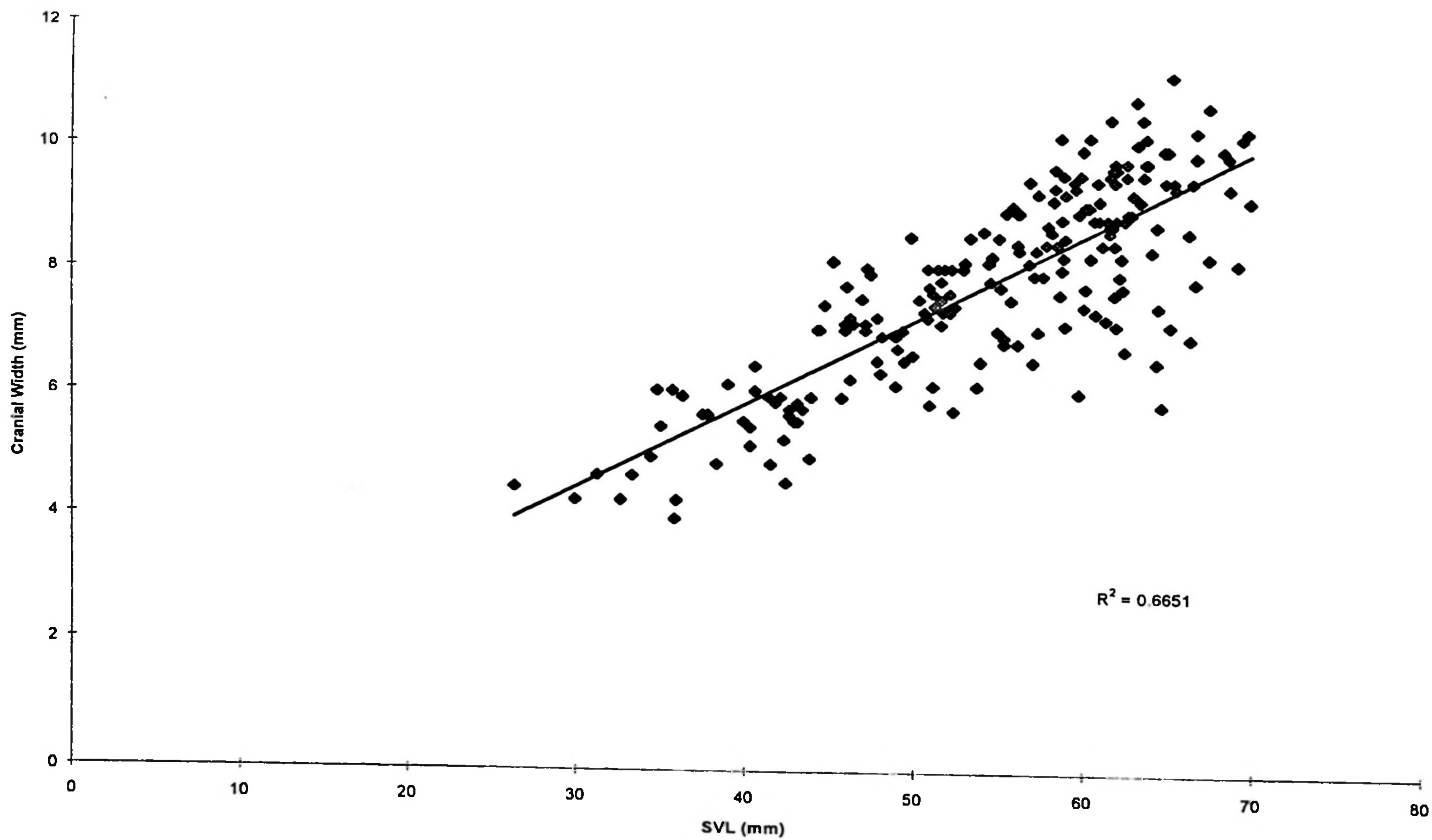
250
200
150
100
50
0
Light (Lux)
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
Month
Entrance
Twilight

Figure 19. Light fluctuations for two zones in Buckeye Creek Cave.



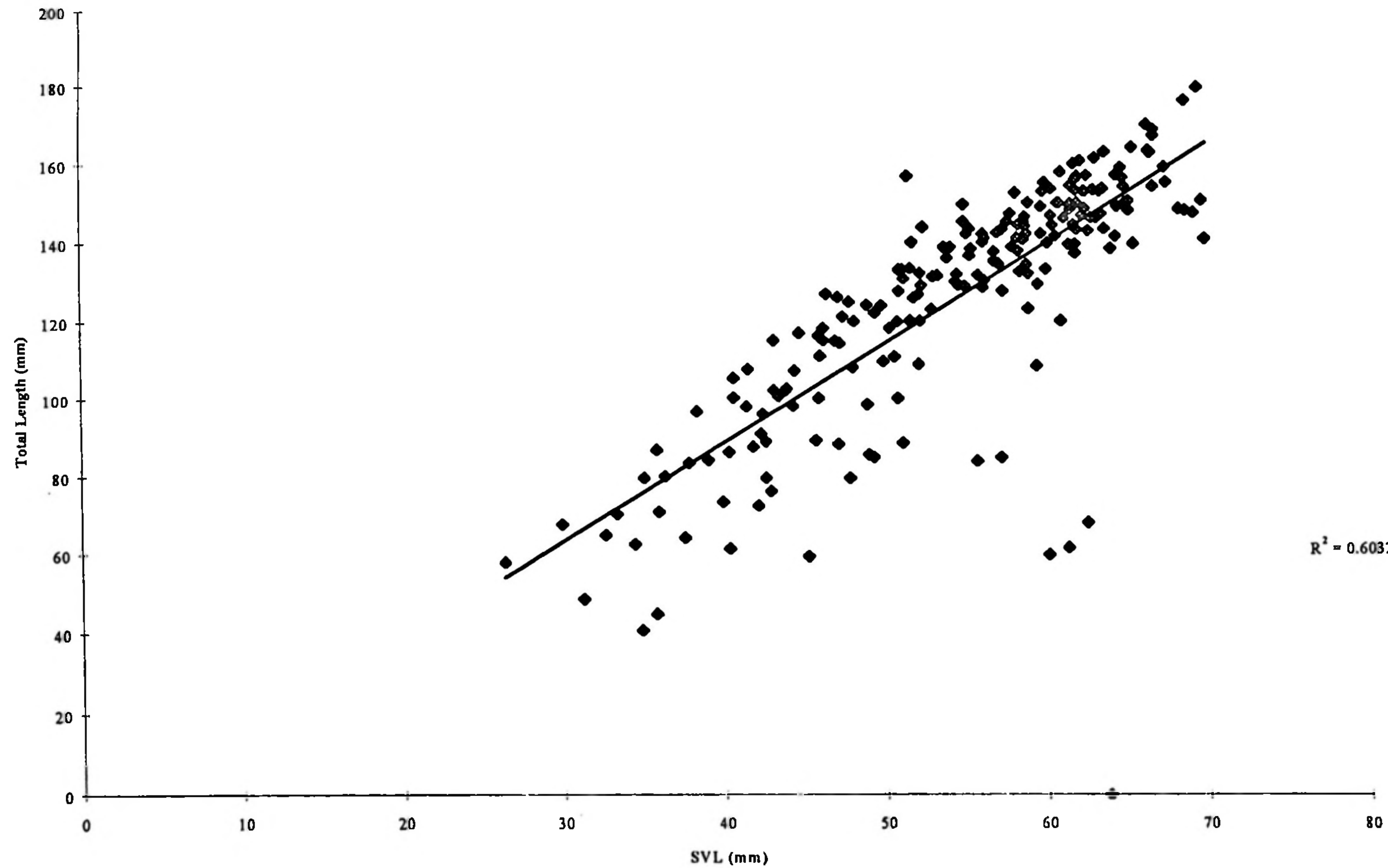
400
350
300
250
200
150
100
50
0
Light (Lux)
May
Jun
Jul
Sep
Oct
Nov
Feb
Mar
Apr
Month
Entrance
Twilight

Figure 20. Regression analysis comparing snout-vent length to cranial width of *Eurycea lucifuga*.



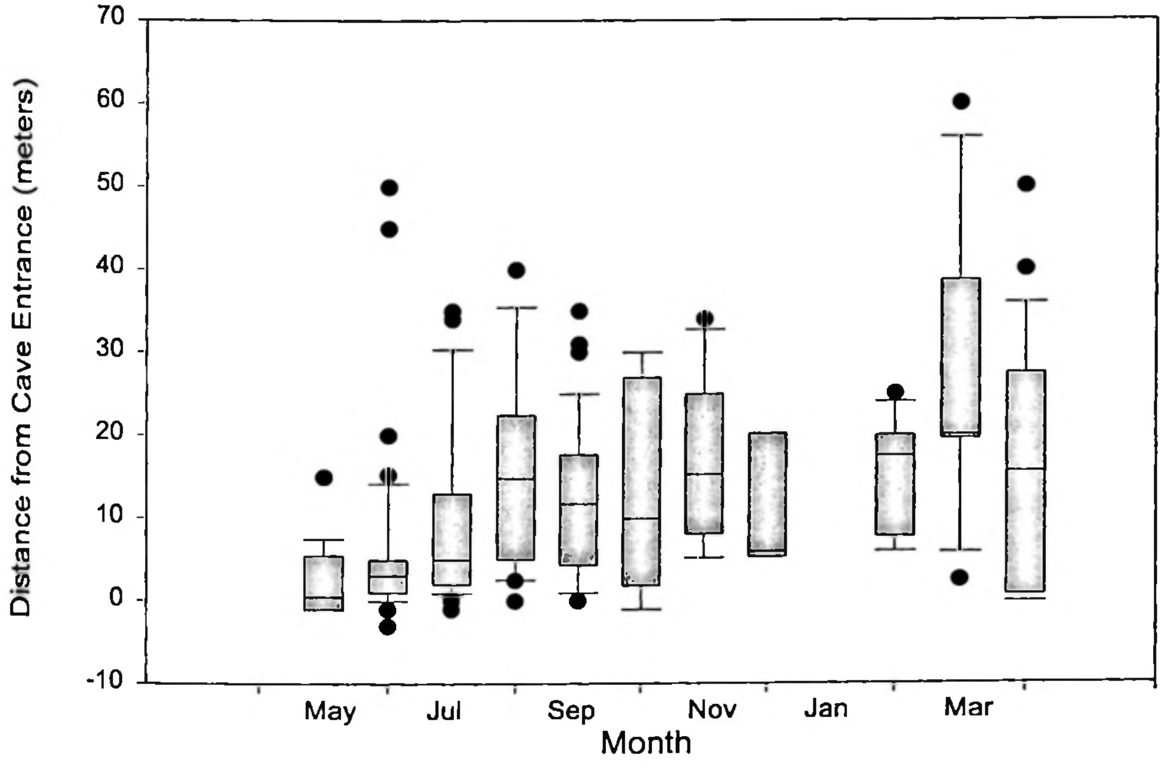
Cranial Width (mm)
12
10
8
6
4
2
0
0
10
20
30
40
50
60
70
80
SVL (mm)
R² = 0.6651

Figure 21. Regression analysis comparing snout-vent length to total length of *Eurycea lucifuga*.



Total Length (mm)
200
180
160
140
120
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80
SVL (mm)
R² = 0.6032

Figure 22. Average monthly movements of *Eurycea lucifuga* in all study caves.



Distance from Cave Entrance (meters)
70
60
50
40
30
20
10
0
-10
May
Jul
Sep
Nov
Jan
Mar
Month

Figure 23. Monthly abundance of the visible population in the three study caves.

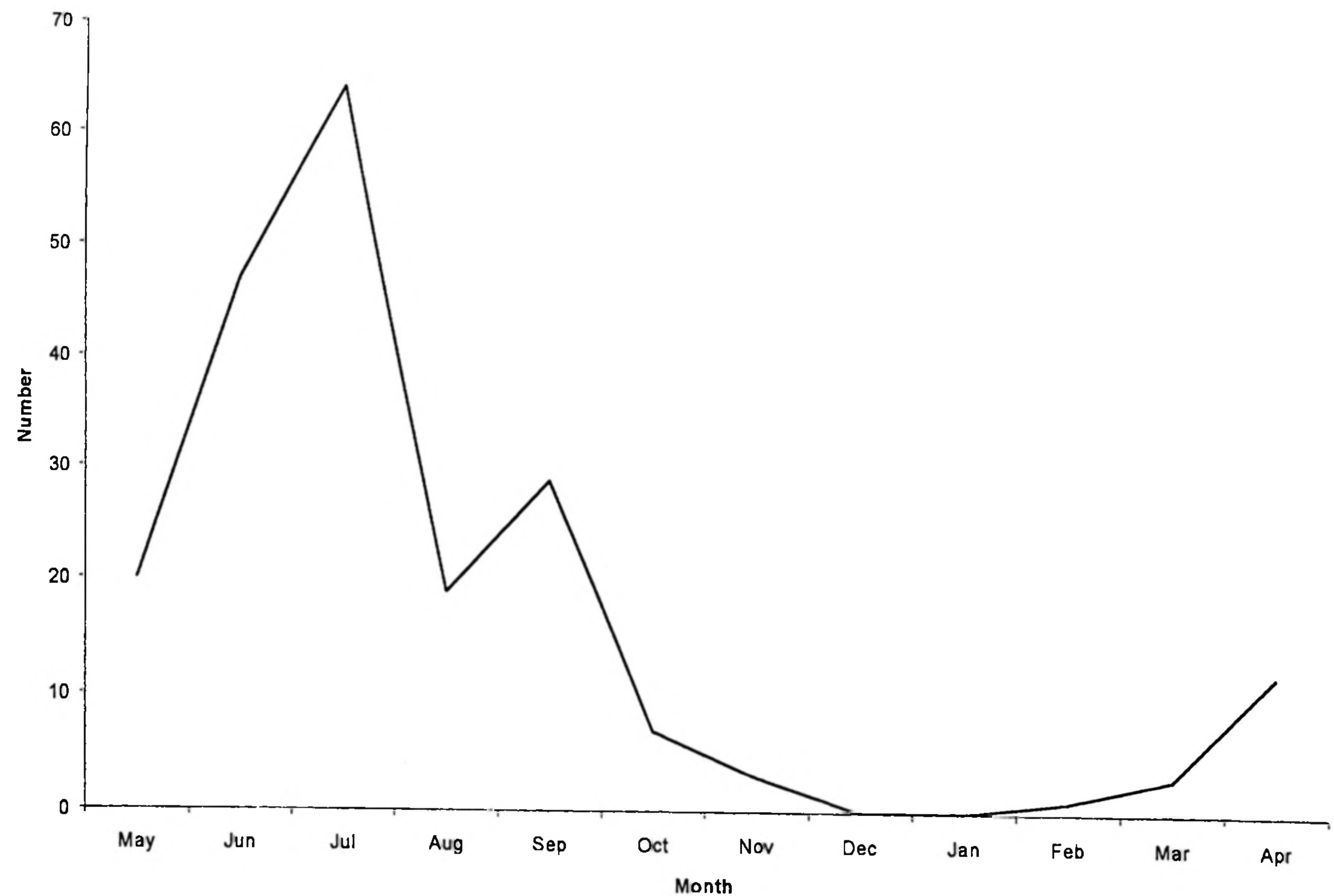

70
60
50
40
30
20
10
0
Number
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
Month

Figure 24. Monthly abundance of males, females, and juveniles in the three study caves.

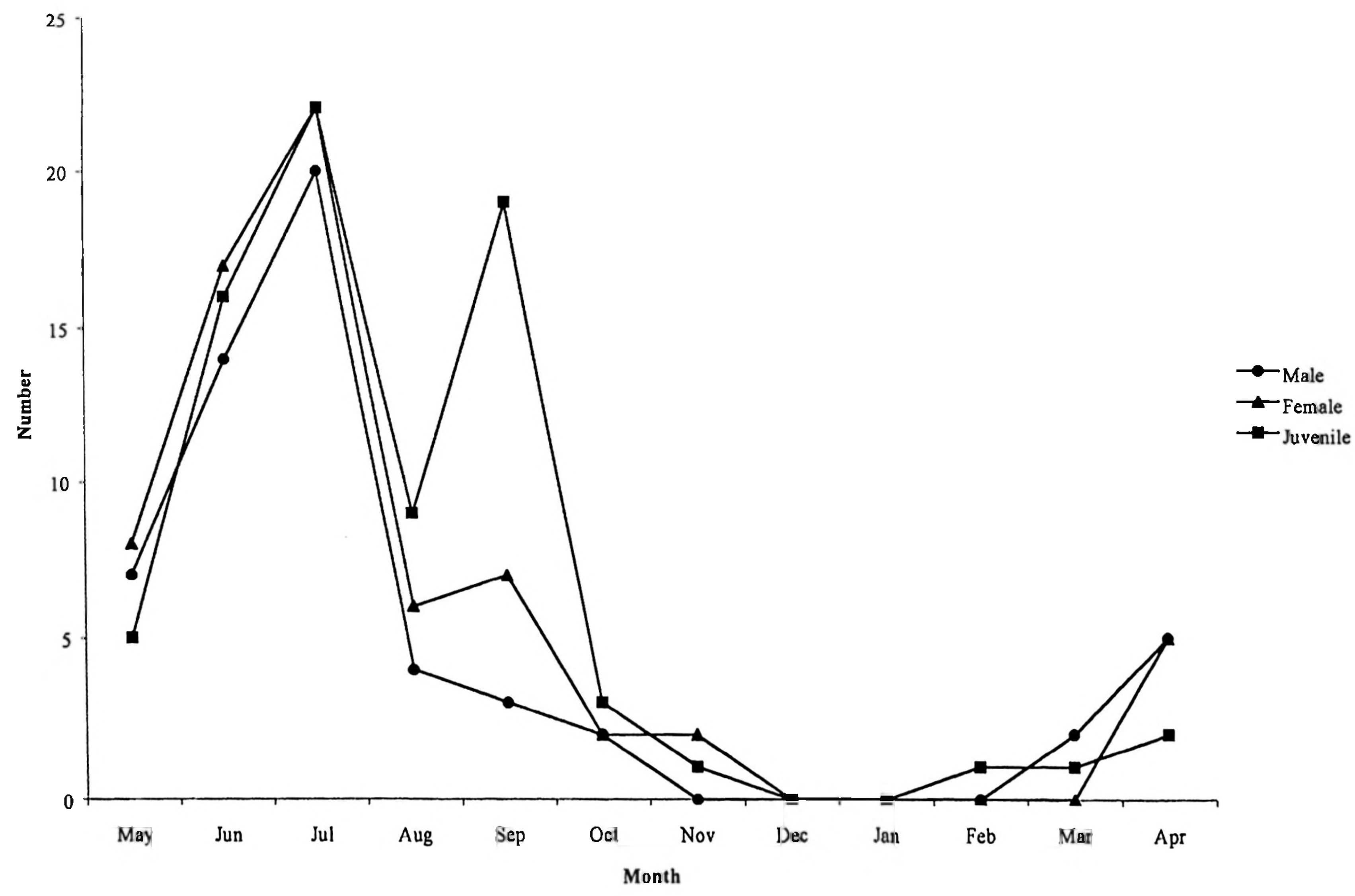
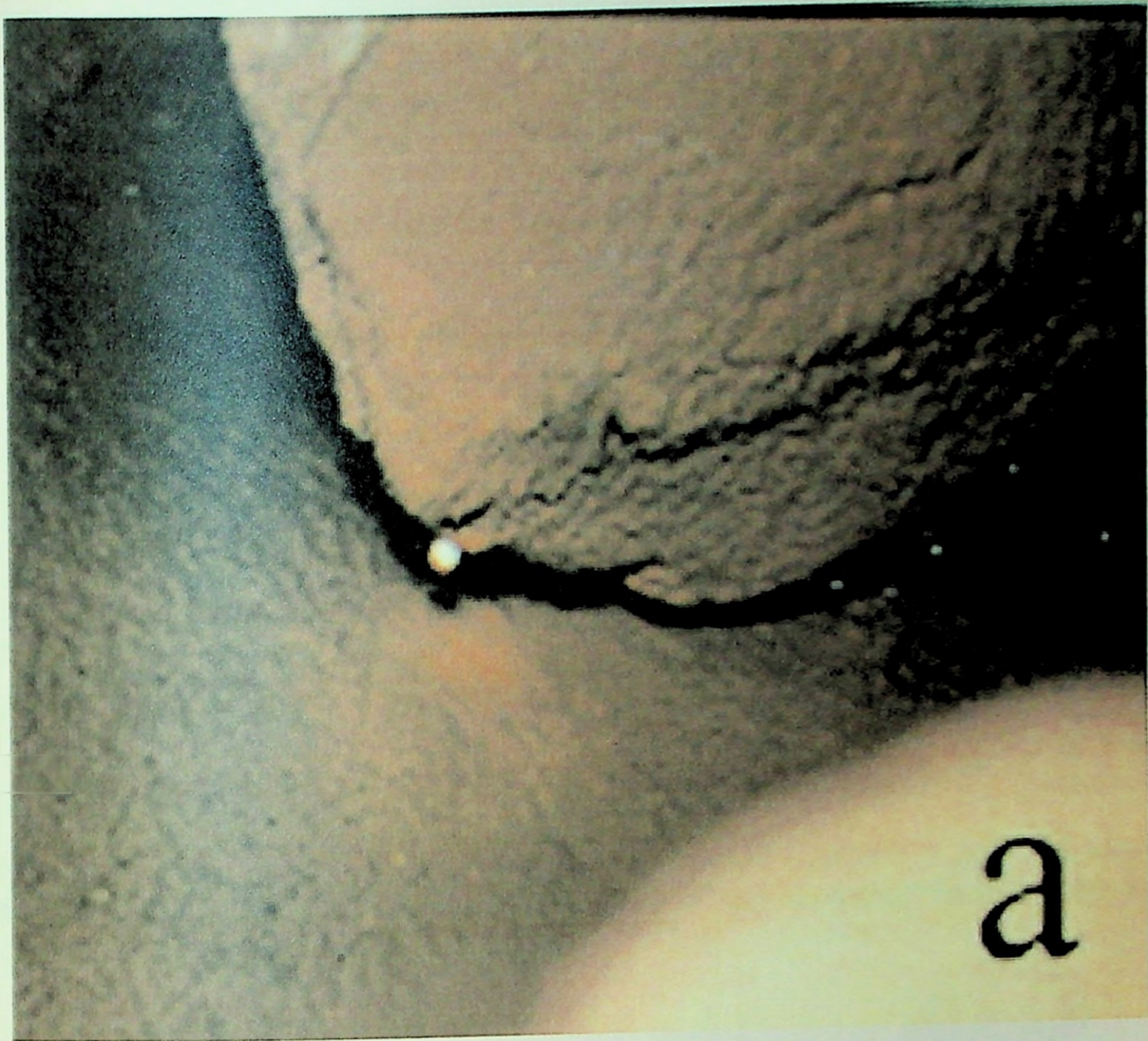

25
20
15
10
5
0
Number
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
Month
Male
Female
Juvenile

Figure 25. Egg in Harrison Stage 10 found on 18 September 1999 (Fig. 25a). Figure 25b is an example of an embryo in Harrison Stage 10 (Harrison 1969). Photo by Zach Felix.



в

Figure 26. Egg in Harrison Stage 18 found on 3 October 1999 (Fig. 26a). Figure 26b is an example of an embryo in Harrison Stage 18 (Harrison 1969). Photo by Zach Felix.

b

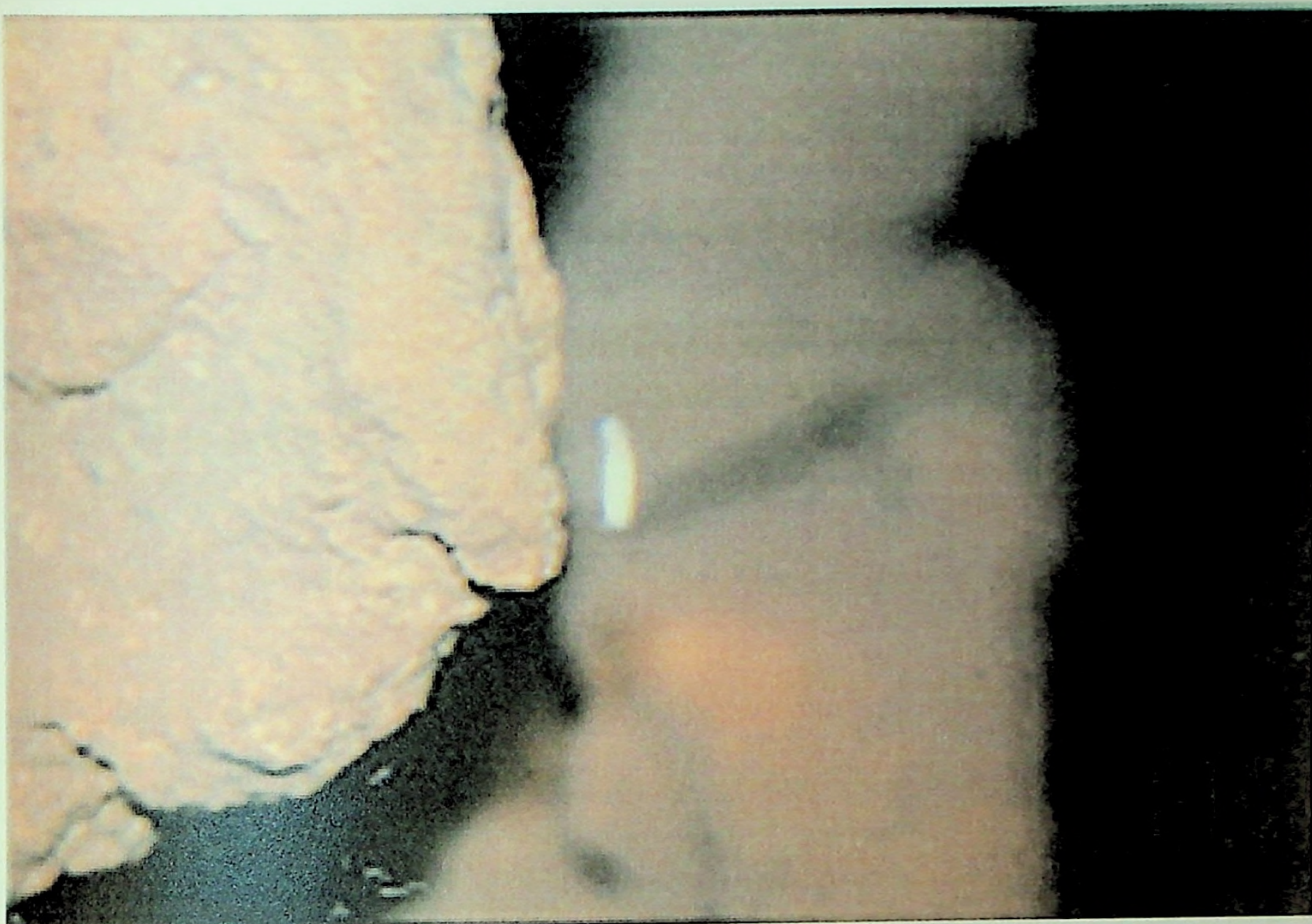
Figure 27. Egg in Harrison Stage 24 found on 10 October 1999 (Fig. 27a). Figure 27b is an example of an embryo in Harrison Stage 24 (Harrison 1969). Photo by Zach Felix.

b

Figure 28. Egg in Harrison Stage 30 found on 17 October 1999 (Fig. 28a). Figure 28b is an example of an embryo in Harrison Stage 30 (Harrison 1969). Photo by Zach Felix.

b

Figure 29. Egg in Harrison Stage 35 found on 31 October 1999 (Fig. 29a). Figure 29b is an example of an embryo in Harrison Stage 35 (Harrison 1969). Photo by Zach Felix.



b

Figure 30. Hatchling found on 9 November 1999 in pool two of Higginbothams Cave No. 2. Photo by Zach Felix.

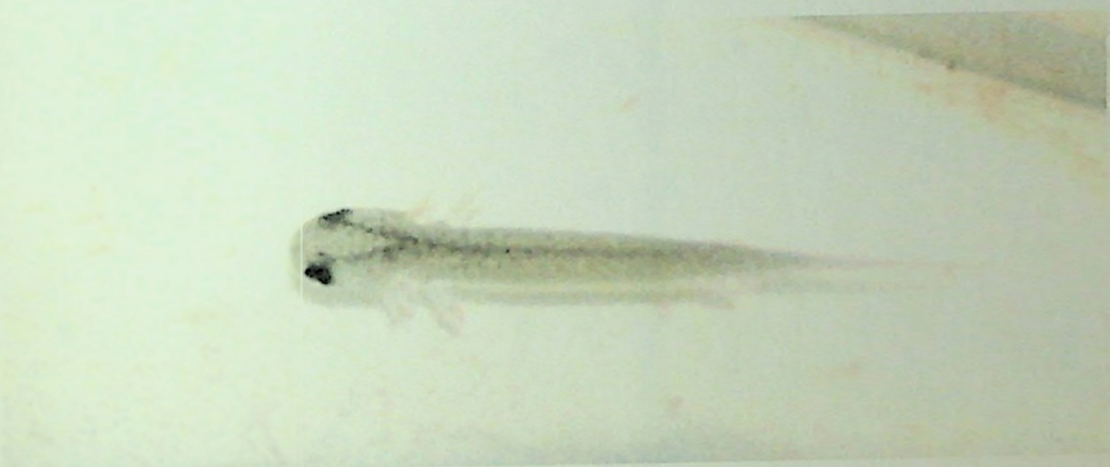
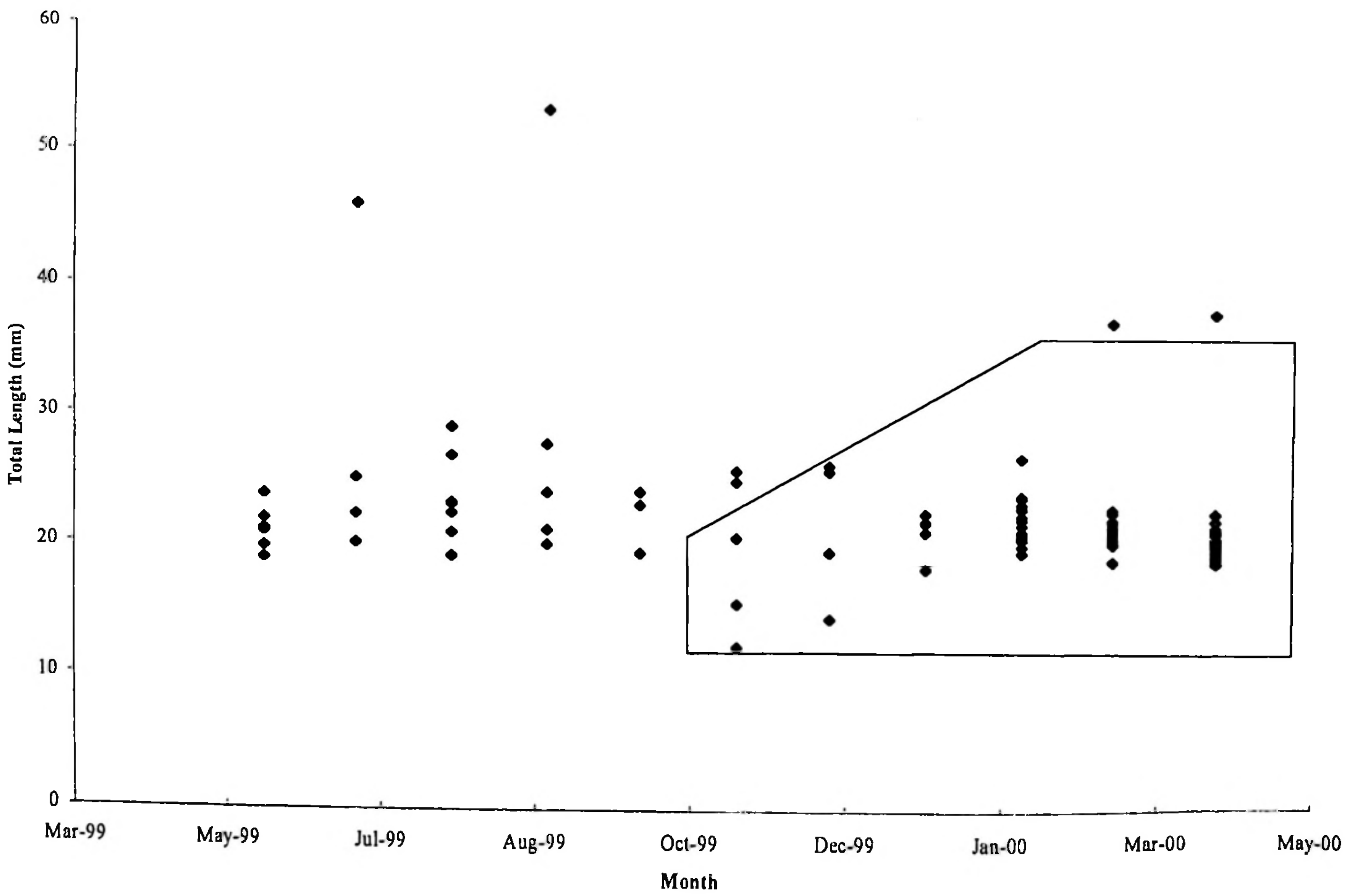


Figure 31. Monthly captures of *Eurycea lucifuga* larvae and their total length. The enclosed area represents young of the year.


60
50
40
30
20
10
0
Total Length (mm)
Mar-99
May-99
Jul-99
Aug-99
Oct-99
Dec-99
Jan-00
Mar-00
May-00
Month