A. Espino del Castillo, G. Castaño-Meneses, M.J. Dávila-Montes, M. Miranda-Anaya, J.B. Morales-Malacara, and R. Paredes-León – Seasonal distribution and circadian activity in the troglophile long-footed robber frog, *Eleutherodactylus longipes* (Anura: Brachycephalidae) at Los Riscos Cave, Querétaro, Mexico: Field and laboratory studies. *Journal of Cave and Karst Studies,* v. 71, no. 1, p. 24–31.

SEASONAL DISTRIBUTION AND CIRCADIAN ACTIVITY IN THE TROGLOPHILE LONG-FOOTED ROBBER FROG, *ELEUTHERODACTYLUS LONGIPES* (ANURA: BRACHYCEPHALIDAE) AT LOS RISCOS CAVE, QUERÉTARO, MEXICO: FIELD AND LABORATORY STUDIES

Adriana Espino del Castillo[1], Gabriela Castaño-Meneses[2,4], Mayra J. Dávila-Montes[1,3], Manuel Miranda-Anaya[3], Juan B. Morales-Malacara[1,4,6], and Ricardo Paredes-León[5]

Abstract: Los Riscos Cave belongs to the El Abra limestone and its geographical location is in the Sierra Gorda in the State of Querétaro, Mexico. The cave has a high faunal diversity that includes arthropods and some vertebrates, such as vampire bats and anurans, and includes the robber frog *Eleutherodactylus longipes* (Baird, 1859). The abundance of the robber frog changes non-randomly between dry and rainy seasons and is related to the search for humid conditions inside the cave. In addition, the robber frog was located in areas where some scattered light may influence its dispersion inside the cave; and therefore, its activity. Frogs displayed spontaneous circadian rhythms of locomotor activity from the first days of the experimental observation in constant darkness. The average period of circadian rhythms was 24.85 $\pm$ 0.93 h indicating, in isolated conditions, a diurnal activity. When exposed to artificial light-dark cycles, the animals lacked daily activity rhythms, and ultradian activity was observed. The preference for high humidity and low illumination in the cave and a partial endogenous circadian rhythmicity confirm the troglophilic affinity of the robber frog to cave environments.

Introduction

Caves represent windows to the lithosphere where different habitats are characterized by partial or total darkness, nearly constant temperature, often high levels of humidity, and a low flow of nutrients. Nevertheless, a variety of organisms have long colonized these subterranean environments. There are still constant incursions to caves by trogloxenes and troglophiles. In addition, there are unique troglobitic species that have developed diverse and specialized adaptations during their evolution to the lack of light.

In Mexico, there are records of 27 species of anurans that inhabit caves (Reddell, 1981; Hoffmann et al., 1986), which corresponds to approximately 7% of the total amphibian diversity of the country (363). This high percentage of use of this habitat for anurans is more frequent than expected (López-Ortega and Casas-Andreu, 2005).

Los Riscos Cave has been investigated for its fauna over many years and has a large diversity of fauna that includes arthropods and vertebrates, such as vampire bats *Desmodus rotundus* (Geoffroy, 1810) and *Diphylla ecaudata* Spix, 1823, the frugivorous bat *Artibeus lituratus* (Olfers, 1818), an insectivorous bat *Corynorhinus mexicanus* (Allen, 1916), and the fish *Astyanax mexicanus* (De Filipi, 1853), and anurans as *Incilius valliceps* (Wiegmann, 1833) (previously known as *Bufo valliceps*, see Frost, 2008), and the robber frog *Eleutherodactylus longipes* (Baird, 1859) (Fig. 1), which is a member of the family Brachycephalidae, following Frost et al., (2006).

Eleutherodactylus longipes is endemic to Mexico, being distributed along the Sierra Madre Oriental from the states of Nuevo Leon through Hidalgo, Tamaulipas, San Luis Potosí and Querétaro in isolated localities (Lynch, 1970; IUCN, 2006). It inhabits moderate elevations from 650 to 2000 m, and it shows a strong tendency to occupy caves (Taylor, 1939; Lynch, 1970; IUCN, 2006). The biology and natural history of this frog are unknown.

[1] Acarología & Bioespeleología, Departamento de Biología Comparada, Facultad de Ciencias, Universidad Nacional Autónoma de México, Coyoacán 04510, Distrito Federal, México
[2] Ecología y Sistemática de Microartrópodos, Departamento de Ecología y Recursos Naturales, Facultad de Ciencias, Facultad de Ciencias, Universidad Nacional Autónoma de México, Coyoacán 04510, Distrito Federal, México
[3] Biología animal experimental, Departamento de Biología Celular, Facultad de Ciencias, Universidad Nacional Autónoma de México, Coyoacán 04510, Distrito Federal, México
[4] Additional Address: Unidad Multidisciplinaría de Docencia e Investigación, Facultad de Ciencias, Universidad Nacional Autónoma de México, Campus Juriquilla, Boulevard Juriquilla 3001, C.P. 76230, Querétaro, Querétaro, México
[5] Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, Coyoacán 04510, Distrito Federal, México
[6] Corresponding Author (email: jbmm@hp.fciencias.unam.mx).

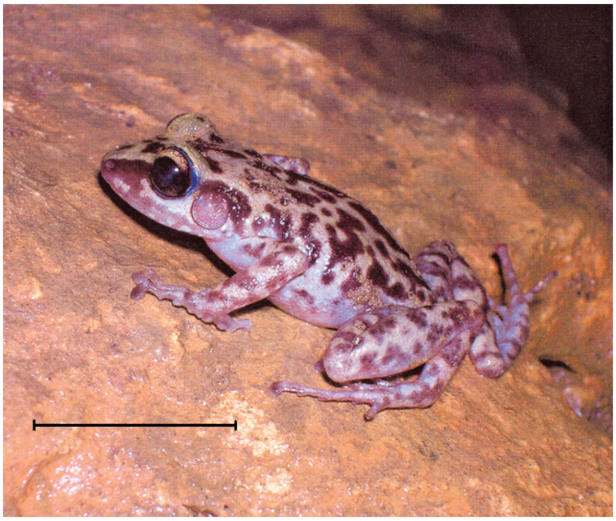

Figure 1. Long-footed robber frog *Eleutherodactylus longipes* at Los Riscos Cave, Querétaro, Mexico. Scale bar is 2 cm.

Caves provide a diversity of habitats where environmental signals may have different influences upon the circadian properties of the organisms that inhabit them. Near the main entry of a cave, natural day-night cycles, as well as changes in temperature and humidity, may play a significant role in the daily organization of animal activity. In deep regions of caves, constant or poorly fluctuating conditions lack the temporal periodicity to entrain endogenously generated circadian rhythms, and poor light conditions can also affect other physiological traits such as vision or skin color (Poulson and White, 1969; Lamprecht and Weber, 1992).

Diverse studies of circadian activity rhythms have been performed under laboratory conditions by monitoring the locomotor activity of fish (Trajano et al., 2005; Pati, 2001), crustaceans (De la O-Martínez et al., 2004), crickets (Hoenen 2005), millipedes (Koilraj et al., 2000), spiders (Gnaspini et al., 2003) and salamanders (Hervant et al., 2000), among others. Results have varied: some species are arrhythmic and others remain capable of displaying low-amplitude circadian rhythms, able to synchronize to artificial light-dark cycles.

Studying different morphological, ontogenetic or ethological adaptations to underground environments may help us understand the adaptive meaning of having a functional circadian clock in organisms that live in such unusual habitats.

Our aim is to present the habitat and seasonal distribution of *E. longipes* inside Los Riscos Cave, as well as its locomotor activity pattern in laboratory conditions, for understanding long-term circadian locomotor activity in natural settings and their response to artificial light-dark cycles.

Materials and Methods

Locality

Los Riscos Cave (21° 11′ 38″ N, 99° 30′ 50″ W: Lazcano Sahagún, 1986b) belongs to the El Abra limestone and its geographical location is in the Sierra Gorda in the State of Querétaro, Mexico (Alencaster et al., 1999). It is located 3 km northeast from Puente de Dios up the river Jalpan at 1122 m asl. Water persistence inside the cave varies between dry and rainy periods. Maximum precipitation occurs from June to November, and the driest period is from December to May. Average annual precipitation is about 48.9 mm d^{-1} (CETENAL, 1986; CNA, 2004), and the average annual temperature is 24 °C (INEGI, 1996; CGSNEGI, 2004). The climate of the area is warm subhumid type (A)C_1(wo)(w) sensu Köppen and modified by Garcia (1981) and the category of vegetation is tropical dry forest (Carabias Lillo et al., 1999).

This cave represents a mixed underground system (horizontal and vertical) with eight zones, denoted with the letters A to H, including tunnels and galleries (Fig. 2). The cave is mainly horizontal, with a depth of 35 m and a length of 550 m (Lazcano Sahagún, 1986a, 1986b). Criteria for defining each zone are based on different topographical events and features inside the cave, like tunnels, areas of collapse or changing climatic conditions. The main entry, labeled as zone A, is surrounded by local vegetation and sunlight scatters about 35 m inside. Other zones have diminished amount of light or are totally dark. Zones B, C, D and E represent the largest transects, therefore average values of humidity, temperature, and light indicate the proximal (about 40 m deep, designated as 1) and distal (about 80 m deep, designated as 2) locations. Zone B2 represents the upper chamber at the end of this zone, which is 30 m high.

Zones G and H include narrow corridors and vertical pits and thus presented access challenges. These areas were not considered for this study because no frogs were found there.

Field Techniques

All sampling activities were performed seasonally every three months from November 2005 to March 2008. Physical parameters such as temperature, humidity, and luminosity were recorded in different locations inside the cave using a thermohydrometer (IAQ- Calc 8760), and a luxometer (EXTECH instruments 0–2000 luxes). Readings were obtained every 20 m along search transects in both dry and rainy seasons.

The search for frogs was carried out along each one of the cave zones during day-twilight periods because we observed in preliminary explorations that apparently the frog's activity inside the cave is the same all times of the day. The frogs are not very visible. For example, in zone B they were more commonly observed at rest on top of walls or crevices on walls, whereas in other zones (E, F), the

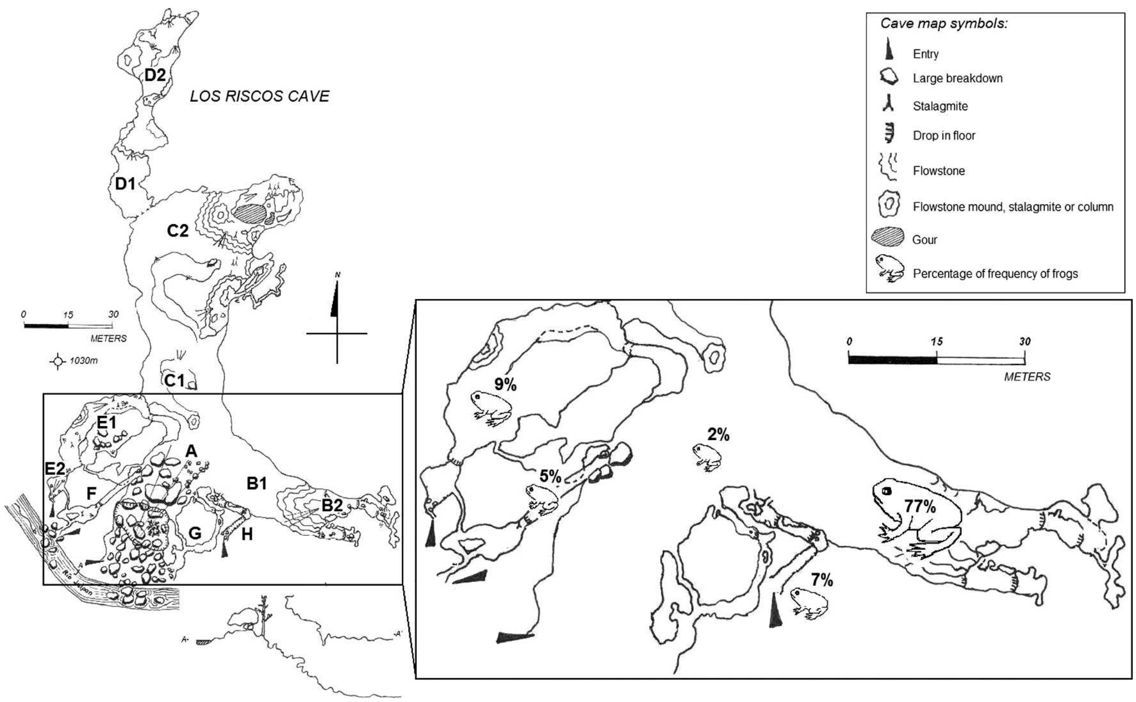

Figure 2. Distribution and relative abundance of *E. longipes* in Los Riscos Cave, Querétaro, Mexico. Arrows represent cave entry points. The map, modified from Lazcano Sahagún (1986), shows in detail the zones of the cave, where frogs were sampled. Frog-like symbols indicate percentage of frequency of observation in each zone.

frogs were found under rocks. Zones where the frogs were observed did not include puddles or pools of water. Some frogs were marked and released; others were collected and transported to the laboratory for circadian studies or to serve as voucher specimens at the Facultad de Ciencias, Universidad Nacional Autónoma de México (FC). In order to minimize possible dampening of endogenous activity rhythms, all animals used in the laboratory were transported in a dark styrofoam box slightly cooled with crushed ice and were recorded in the laboratory as soon as possible. A few individuals were examined for parasites and to confirm proper identification. These specimens will be deposited at the Universidad Nacional Autónoma de México (UNAM), in the Museo de Zoología de la Facultad de Ciencias (MZFC-UNAM) and Colección Nacional de Anfibios y Reptiles (CNAR-IBUNAM).

LABORATORY TECHNIQUES

Nine frogs collected during different seasons (five in dry season and four in rainy season) were used for this portion of the study. Frogs were individually kept in glass aquaria (2.5 L) and were each equipped with infrared light crossings to detect locomotor activity as described elsewhere (Miranda-Anaya et al., 2003). These were kept in continuously ventilated, light-proof wooden boxes, and maintained at 23 ± 2 °C in environmentally-controlled rooms at the FC facilities. In order to maintain a sufficiently high humidity inside the recording chamber, each aquarium had a wet paper towel spread on its bottom which was moistened with clean water once a week. Locomotor activity data were summarized every 10 min for further analysis by means of a data acquisition board (NAFRI disp. México D. F.).

Every frog was initially monitored for at least nine days under continuous darkness (DD). As needed, a dim red light bulb (1–2 lx) was used for visual inspection and maintenance of aquaria. Then, light-dark cycles (LD 12:12) were used during at least ten days in order to observe any possible entrainment of locomotor activity rhythms. Light cycles were provided by means of a fluorescent lamp (Tecnolite F15T8D) controlled by a timer. The lamp was partially dimmed by using black tape and located at 30 cm above aquaria in order to provide half of the light intensity (150 lx). Frogs were fed once a week with juvenile crickets that were rendered legless to avoid their interfering with frog locomotor activities.

DATA ANALYSIS

Seasonal means in abundance were compared by a student *t*-test for independent samples performed by Statistica software (StatSoft, 1999). A Fisher's exact test (Tocher, 1950) was used to evaluate the association between seasons and between different cave zones. We performed the Freeman and Halton (1951) analysis of the Fisher exact probability for a two-by-five contingency table. A two way Analysis of Variance (ANOVA) was performed in order to evaluate the effect of the season and cave zone on the environmental parameters with a *post hoc* Tukey's test used to identify significant differences. A multiple correlation was performed between temperature, humidity, luminosity, and frog abundance in sites where frogs were recorded.

Locomotor activity data were analyzed in double-plotted actograms based on a day-by-day histogram in which each bar indicates the amount of activity observed in a time lapse of 10 min across a 24-hour series. In this presentation, the daily traces stacked vertically in chronological order give a lucid view of the activity envelope (DeCoursey, 2004).

The respective circadian periods for at least seven consecutive days (τ) were calculated using χ^2 periodograms (Sokolove and Bushell, 1978) at 0–30 h intervals. Ultradian activity was analyzed by means of Fast Fourier Transform Analysis using the software DISPAC (Instituto de Fisiología Célular (IFC), UNAM, Mexico; Aguilar-Roblero et al., 1997). Periodograms with spikes above the confidence interval ($p < 0.05$) were considered rhythmic. Results on period lengths of free-running activity bouts under different protocols were analyzed using a non-paired student *t*-test with the software program Statistica; differences were considered significant when $p < 0.05$. All results are presented as mean values ± standard deviations (SD) unless otherwise noted.

Results

Physical Parameters in Los Riscos Cave

The two-way ANOVA test showed no significant effect of season on temperature ($F_{1,\ 60} = 0.80$; $p > 0.05$), humidity ($F_{1,\ 60} = 3.18$ $p > 0.05$), or luminosity ($F_{1,\ 60} = 0.47$ $p > 0.05$). However, there were significant effects of cave zone on these parameters (temperature: $F_{9,\ 60} = 2.35$ $p < 0.05$; humidity: $F_{9,\ 60} = 5.81$ $p < 0.005$; luminosity: $F_{9,\ 60} = 37.72$ $p < 0.0005$). Temperature differences were found between zones B and D2 and between zones D1 and D2. The main differences for the humidity were found between zone A and zone D2. For the luminosity, zones B and C1 together were different from the other zones and zone A was different from all others (Fig. 3).

The zone with the highest temperature and humidity in both seasons was D2. This zone has permanent colonies of vampire bats (*Desmodus rotundus* and *Diphylla ecaudata*) present in the deepest part of the cave. Two other bat species, *Artibeus lituratus* and *Corynorhinus mexicanus*, were found in this zone, but only during a single sampling event.

Distribution of *Eleutherodactylus longipes* in the Cave During Different Seasons

A total of 43 frogs was found in the cave during the three-year study. Seasonal average abundance for the rainy season was 5 ± 1.41 SD, and for the dry season was 11 ± 4.24 SD; the difference between the two seasons was significant ($t_4 = 2.82$; $p = 0.04$).

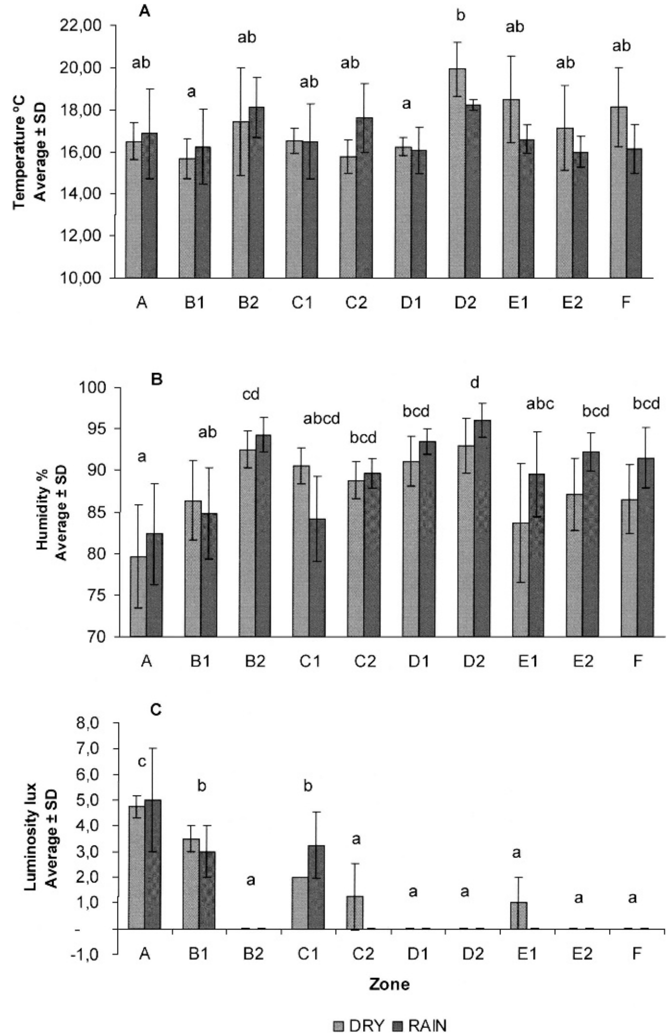

Figure 3. Average temperature (A), humidity (B), and luminosity (C) along Los Riscos Cave. Letters denote significant differences between zones according to *post hoc* comparisons using Tukey's test ($p < 0.05$). Light gray bars mean dry season and dark gray bars mean rainy season.

Frogs were located mainly between 1200 h and 1600 h in cave zones A, B, E, and F. Density was highest in zone B and lowest in zone F for both seasons (Fig. 4). In zone A, only one individual was found during the dry season, while during the rainy season, frogs were found outside of the cave on the external wall of zone B at midnight just one time. There was no association between cave depth and season (Fisher's exact test, $p = 0.29$). For both the dry and rainy seasons, the distribution of frogs in the cave was similar. The coefficient of multiple correlation between the environmental parameters and frog abundance was highly positive and significant ($r = 0.94$; $p < 0.05$). Humidity explained most of the observed variation in abundance ($r = 0.72$; $p < 0.005$), followed by luminosity ($r = 0.35$; $p <$

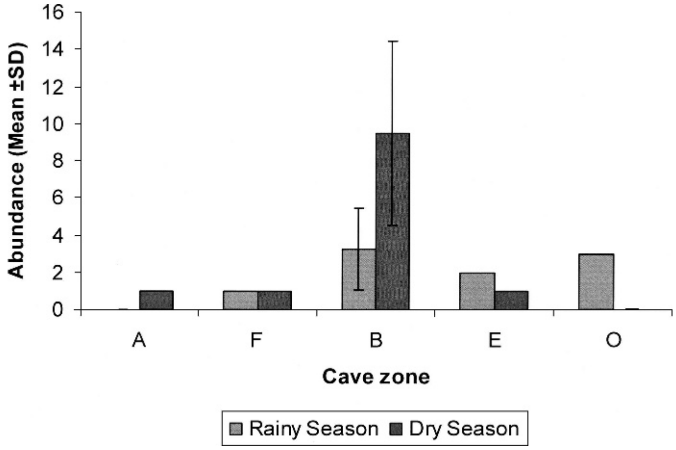

Figure 4. Seasonal distribution of *E. longipes* in different zones at Los Riscos Cave, Querétaro, Mexico. Light gray bars mean rainy season and dark gray bars mean dry season.

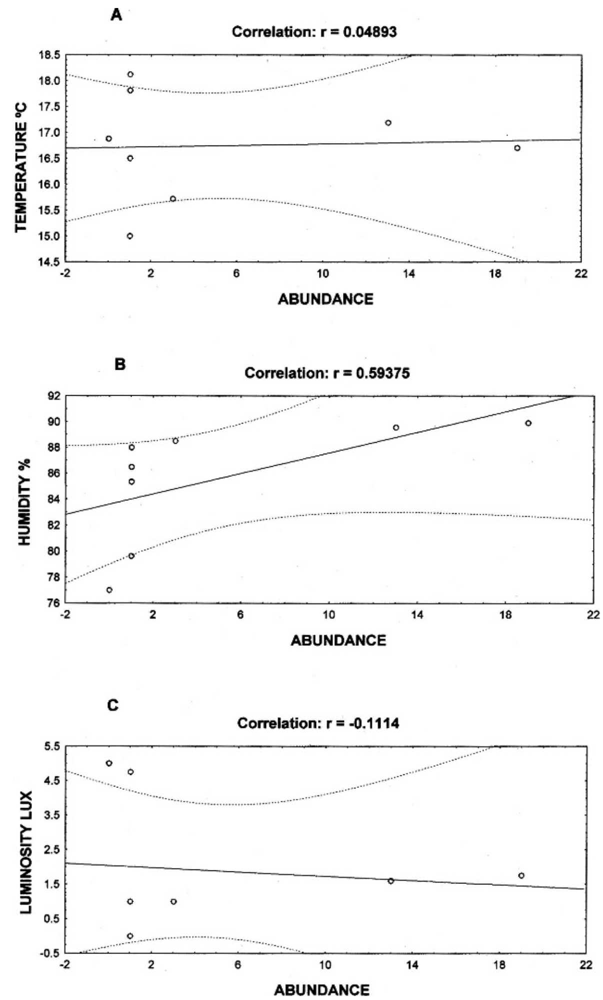

Figure 5. Simple correlation analysis between temperature (A), humidity (B), and luminosity (C) with abundance of *E. longipes* in Los Riscos Cave. Data from November 2005 to March 2008 at Los Riscos Cave, Querétaro, Mexico. Solid lines mean the correlation that is adjusted to the data, and the dashed lines mean the range of dispersion of the data with regard to the correlation line.

0.05). Temperature was not significantly correlated with abundance ($r = 0.24$; $p > 0.05$). This is unsurprising because this parameter was constant throughout the cave (Fig. 5). The simple correlation between luminosity and abundance was negative and significant ($r = -0.11$; $p < 0.05$).

Circadian Locomotor Activity Rhythms

Figure 6 shows a double-plotted actogram (A) and correspondent activity profiles (B) for a typical recording of locomotor activity under different light conditions for a single frog. A significant circadian rhythm oscillated during the first nine days of recording in DD. The main bout of activity is projected for the external-light phase. When exposed to LD cycles (day 10), activity was still maintained during the diurnal phase of the cycle and crepuscular activity was observed in this first LD condition, as shown in the correspondent activity profile (see B in Fig. 6). However, when exposed again to constant DD, the rhythm faded away and a non-significant circadian period was observed. The next exposure to LD cycles did not show circadian activity.

In a second group of animals tested for a longer period, the initial free-running circadian rhythm in DD was observed again. However, the rhythm was dampened after 10 to 20 days and LD conditions did not reorganize the activity. Frequency analysis of ultradian rhythms by means of Fast Fourier transform shows that in LD, circadian frequencies were absent while ultradian rhythms of 1 to 6 h were observed.

Discussion

The factors determining the environmental characteristics of Los Riscos Cave are associated with the external agents imposed by local climate and season, and we found that differences of frog abundance between dry and rainy seasons can be related to humidity levels inside the cave, which is consistent with the necessity of frogs to avoid desiccation and light exposure. Also, amphibians of temperate areas display different strategies, such as hibernation, in order to avoid starvation and low environmental temperatures (Pinder et al., 1992). Among these strategies, there is use of refugia such as cracks in rocks, fallen trunks, stumps, caves and others (López-Ortega and Casas-Andreu, 2005). The study results confirmed that environmental variables such as temperature, solar radiation, and relative humidity determine the distribution of these organisms.

It is important to point out that all the individuals were found during the daytime inside the cave because Lynch and Duellman (1997) affirmed that all the *Eleutherodacty-*

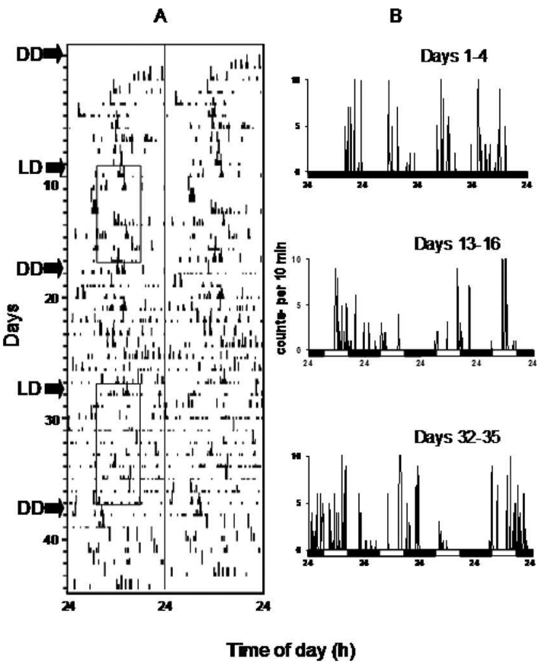

Figure 6. Double-plotted actogram (left) and corresponding activity profiles of a single frog. Light conditions are indicated by arrows (DD = constant darkness, LD = light/dark cycles), light phase is also indicated by rectangles on left side of the actogram. Activity profiles on B show also the LD cycles. Days considered for the profiles are indicated.

lus (with the exception of *E. hectus*) are nocturnal. The biotic (potential prey) and abiotic (temperature, humidity, and darkness) parameters inside the cave probably increase the range of crepuscular activity of the robber frog as shown in Figure 6B.

Inside the cave, zone B shows the main population of frogs and other records can be considered as accidental. The population living here apparently does not have contact with the exterior of the cave. However, we cannot discard the presence of little cracks that allow some movements to outside, which means the frogs could not remain in the cave full time. Additionally, the frogs were observed outside the cave at night and far from the main population of zone B.

Preliminary observations indicate that there is more diversity of invertebrates in zones A and F than in zones B and E. However, crickets and spiders in zone B can also serve as potential prey for the frogs. No frogs were observed feeding on cave animals while in the cave and, unfortunately, the contents of the few stomachs analyzed were in a state of advanced digestion, so it was not possible to identify the taxa in stomach contents. Zones C and D had appropriate temperature, humidity, and luminosity conditions, but their isolation from the external environment made these conditions difficult for the frogs to access.

Endogenous circadian rhythmicity was detected during the initial ten days of recording under laboratory conditions without acclimation, which indicates a functional circadian clock in this species. The average free-running period observed during the first 8–10 days of locomotor activity indicates a wide circadian variation, which according to Aschoff's rule, corresponds to a diurnal species (Aschoff, 1960).

This frog is visually reactive to the presence of living insects as prey. However, because the light intensity of artificial LD cycles used in the laboratory was higher than that available inside the cave, it is possible that bright light induced arhythmic patterns of ultradian expression in this species as is known to happen in vertebrates exposed to continuous bright light (Ohta, et al., 2005).

Conclusions

Robber frog distribution among cave regions, being largely concentrated in zone B, suggests that the presence of some scattered light is necessary for its activity and may influence its dispersion inside the cave. The functional circadian clocks in cave animals may have different sensitivities to light according to the species, because fish or arachnids collected in deeper zones were able to entrain their circadian rhythms on similar artificial light-dark cycles (unpublished observations). Laboratory experiments did not include interaction with other species that coexist in the same cave areas. Therefore, it is possible that timing relationships among species inside the cave might be important to sustaining circadian rhythmicity under natural conditions as observed for different cave insects (Oda et al., 2000). Non-circadian data observed in different cave species in these conditions may require a different statistical analysis that can filter the noise pattern of small peaks as seen for the rhythm of the cave cricket *Strinatia brevipennis* (Hoenen et al, 2001). The arhythmic pattern observed may require different criteria of analysis than used in the present study, and the ecophysiological significance of these findings is yet to be fully understood.

Nonetheless, this is the first study of some aspects of the biology of *E. longipes*, and we can note that the environmental preference for caves and a partial endogenous circadian rhythmicity confirms the troglophilic nature of the robber frog. The paucity of information concerning the ecology and physiology of troglophilic anurans highlights the importance of generating detailed studies of these topics.

Acknowledgments

For comments on an earlier draft of the manuscript, we express our appreciation to Joaquín Arroyo-Cabrales (Laboratorio de Arqueozoología, Instituto Nacional de Antropología e Historia, México), Fred Kraus (Bishop Museum, Hawaii), Gerardo López-Ortega (Universidad

Autónoma Metropolitana – Iztapalapa). We also thank Miguel Hernández, Sara Soriano, Yunuen Ávila, Marisol Vega, Itzel Sigala and Stephanie Ortega (Biospeleology group, Facultad de Ciencias, Universidad Nacional Autónoma de México) for their helpful assitance in the field expeditions. We thank Alejandro Martínez Mena and Ana Isabel Bieler Antolin, and José Antonio Hernández (Laboratorio de Microcine, Facultad de Ciencias, Universidad Nacional Autónoma de México) for their help with photographs. Financial assistance was provided by the Dirección General de Asuntos del Personal Académico, Universidad Nacional Autónoma de México, Grant IN221906 to J.B.M-M.

REFERENCES

Aguilar-Roblero, R., Salazar-Juarez, A., Rojas-Castañeda, J., Escobar, C., and Cintra, L., 1997, Organization of circadian rhythmicity and suprachiasmatic nuclei in malnourished rats: American Journal of Physiology, Regulatory Integrative Comparative Physiology, v. 273, p. R1321–R1331.

Alencaster, G., Torres-Hernández, R., Tristán-González, M., Barbosa-Gudiño, R., López-Doncel, R., Pons, J.M., and Omaña, L., 1999, El Abra formation in the western part of the Valles-San Luis Potosí Platform (Mexico): Erlanger Geologische Abhandlungen, Sonderband, v. 3, p. 7–8.

Aschoff, J., 1960, Exogenous and endogenous components in circadian rhythms, *in* Proceedings, Cold Spring Harbor Symposia on Quantitative Biology, v. 25, p. 11–28.

Baird, S.F., 1859, Reptiles of the Boundary, *in* United States and Mexican Boundary Survey under the Order of Lieut. Col. W. H. Emory, Major First Cavalry, and United States Commisioner. 2, Rept., Pt. 2. Department of the Interior, Washington, 35 p.

Carabias Lillo, J., Provencio, E., de la Maza Elvira, J., and Ruiz Corzo, M., 1999, Programa de Manejo Reserva de la Biosfera Sierra Gorda, México, Instituto Nacional de Ecología, SEMARNAT (Secretaría de Medio Ambiente y Recursos Naturales), 172 p.

CETENAL (Comisión de Estudios del Territorio Nacional), 1986, Precipitación y probabilidad de la lluvia en la República Mexicana y su evaluación, Querétaro e Hidalgo, Serie climas, Instituto de Geografía, Universidad Nacional Autónoma de México.

CGSNEGI (Coordinación General de los servicios Nacionales de Estadística, Geografía e Informática), 2004, Carta de Climas, 1:1000 000, *in* Anuario Estadístico de Querétaro de Arteaga. http://www.queretaro.gob.mx/sedesu/deseco/esteco/perfeco/anuest/PDF/Capitulos/C1.pdf

CNA (Comisión Nacional del Agua), 2004, Registro mensual de precipitación pluvial en mm. Inédito, *in* Anuario Estadístico de Querétaro de Arteaga. http://www.queretaro.gob.mx/sedesu/deseco/esteco/perfeco/anuest/PDF/Capitulos/C1.pdf

De La O-Martínez, A., Verde, M.A., Valadez, R.L., Viccon-Pale, J.A., and Fuentes-Pardo, B., 2004, About the existence of circadian activity in cave crayfish: Biological Rhythm Research, v. 35, no. 3, p. 195–204.

DeCoursey, J.P., 2004, Overview of biological timing from unicells to humans, *in* Dunlap, J.C., Loros, J.J., and De Coursey, J.P., eds., Chronobiology, 1st ed.: Sunderland, Massachusetts, Sinauer Associates Incorporated Publishers, p. 3–27.

Freeman, G.H., and Halton, J.H., 1951, Note on exact treatment of contingency, goodness of fit and other problems of significance: Biometrika, v. 38, p. 141–149.

Frost, D.R., 2008, Amphibian Species of the World: An Online Reference, Version 5.2 (19 November, 2008), New York, American Museum of Natural History, Electronic Database accessible at http://research.amnh.org/herpetology/amphibia/index.php.

Frost, D.R., Grant, T., Faivovich, J., Bain, R.H., Haas, A., Haddad, C.F.B., Channing, A., Wilkinson, M., Donnellan, S.C., Raxworthy, C.J., Campbell, J.A., Blotto, B.L., Moler, P., Drewes, R.C., Nussbaum, R.A., Lynch, J.D., Green, D.M., and Wheeler, W.C., 2006, The amphibian tree of life: New York, Bulletin of the American Museum of Natural History, no. 297, 370 p.

García, E., 1981, Modificaciones al sistema de clasificación climática de köppen: México, D.F., 252 p.

Gnaspini, P., Santos, F.H., and Hoenen, S., 2003, The occurence of different phase angles between contrasting seasons in the activity patterns of the cave harvestman *Gnosioma spelaeum* (Arachnida, Opiliones): Biological Rhythm Research, v. 34, no. 1, p. 31–49.

Hervant, F., Mathieu, J., and Durand, J.P., 2000, Metabolism and circadian rhythms of the European blind cave salamander *Proteus anguinus* and a facultative cave dweller, the Pyrenean newt (*Euproctus asper*): Canadian Journal of Zoology, v. 78, no. 8, p. 1427–1432.

Hoenen, S., 2005, Circadian patterns in the activity of the Brazilian cave cricket *Strinatia brevipennis* (Ensifera Phalangopsidae): European Journal of Entomology, v. 102, no. 4, p. 663–668.

Hoenen, S., Schimmel, M., and Marques, M.D., 2001, Rescuing rhythms from noise: A new method of analysis: Biological Rhythm Research, v. 32, no. 2, p. 271–284.

Hoffmann, A., Palacios-Vargas, J.G., and Morales-Malacara, J.B., 1986, Manual de bioespeleología (con nuevas aportaciones de Morelos y Guerrero, México), Universidad Nacional Autónoma de México, 274 p.

INEGI (Instituto Nacional de Estadística, Geografía e Informática), 1986, Síntesis Geográfica, nomenclator y anexo cartográfico del estado de querétaro, 143 p.

IUCN (International Union for Conservation of Nature), Conservation International, and NatureServe, 2006, Global amphibian assessment. http://www.globalamphibians.org

Koilraj, A.J., Sharma, V.K., Marimuthu, G., and Chandrashekaran, M.K., 2000, Presence of circadian rhythms in the locomotor activity of a cave-dwelling millipede *Glyphiulus cavernicolus sulu* (Cambalidae, Spirostreptida): Chronobiology International, v. 17, no. 6, p. 757–765.

Lamprecht, G., and Weber, F., 1992, Spontaneous locomotion behaviour in cavernicolous animals: the regression of the endogenous circadian system, *in* Camacho, A.I., ed., The natural history of biospeleology: Madrid, Monografias del Museo Nacional de Ciencias Naturales, p. 225–262.

Lazcano Sahagún, C., 1986a, Cavernas de la Sierra Gorda. v. I, Universidad Autónoma de Querétaro, Mexico, 181 p.

Lazcano Sahagún, C., 1986b, Cavernas de la Sierra Gorda. v. II, Universidad Autónoma de Querétaro, Mexico, 206 p.

López-Ortega, G., and Casas-Andreu, G., 2005, A tunnel as hibernaculum of *Hyla plicata* (Anura: Hylidae) at Sierra Norte de Tlaxco, Tlaxcala, México: Revista de la Sociedad Mexicana de Historia Natural, 3a época, v. 2, no. 1, p. 160–167.

Lynch, J., 1970, A Taxonomic Revision of the Leptodactylid Frog Genus *Eleutherodactylus* Cope: University of Kansas, Museum of Natural History, v. 20, no. 1, p. 1–45.

Lynch, J., and Duellman, W., 1997, Frogs of the Genus *Eleutherodactylus* (Leptodactylidae) in Western Ecuador: Systematics, Ecology, and Biogeography: University of Kansas Natural History Museum, Special Publication, no. 23, p. 50–165.

Miranda-Anaya, M., Barrera-Mera, B., and Ramírez-Lomelí, E., 2003, Circadian Locomotor Activity Rhythm in the Freshwater Crab *Pseudothelphusa americana* (De Saussure, 1857): Effect of Eyestalk Ablation: Biological Rhythm Research, v. 34, no. 2, p. 167–176.

Oda, G.A., Caldas, I.L., Piqueira, J.R.C., Waterhouse, J.M., and Marques, M.D., 2000, Coupled biological oscillators in cave insects: Journal of Theoretical Biology, v. 206, no. 4, p. 515–524.

Ohta, H., Yamazaki, S., and McMahon, D., 2005, Constant light desynchronizes mammalian clock neurons: Nature Neurosciences, v. 8, no. 3, p. 267–269.

Pati, A.K., 2001, Temporal organization in locomotor activity of the hypogean loach, *Nemacheilus evezardi*, and its epigean ancestor: Environmental Biology of Fishes, v. 62, no. 1–3, p. 119–129.

Pinder, A.W., Storey, K.B., and Ultsch, G.R., 1992, Estivation and hibernation, *in* Feder, M.E., and Burggren, W.W., eds., Environmental Physiology of the Amphibians: Chicago and London, The University of Chicago Press, p. 250–276.

Poulson, T.L., and White, W.B., 1969, The cave environment: Science, v. 165, no. 3897, p. 971–981.

Reddell, J.R., 1981, A review of the cavernicole fauna of México, Guatemala and Belize: Bulletin of the Texas Memorial Museum, v. 27, p. 1–327.

Sokolove, P.G., and Bushell, W.N., 1978, The Chi-Square periodogram: Its utility for analysis of circadian rhythms: Journal of Theoretical Biology, v. 72, p. 131–60.

StatSoft Inc., 1999, Statistical user guide: Complete Statistical System Statsoft. Oklahoma.

Taylor, E., 1939, New Species of Mexican Anura: The University of Kansas, Science Bulletin, v. 26, no. 11, p. 385–401.

Tocher, K.D., 1950, Extension of the Neyman-Pearson theory of tests to discontinuous variates: Biometrika, v. 37, p. 130–144.

Trajano, E., Duarte, L., and Menna-Barreto, L., 2005, Locomotor activity rhythms in cave fishes from Chapada Diamantina, northeastern Brazil (Teleostei: Siluriformes): Biological Rhythm Research, v. 36, no. 3, p. 229–236.