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Biological Rhythm Research

ISSN: 0929-1016 (Print) 1744-4179 (Online) Journal homepage: http://www.tandfonline.com/loi/nbrr20

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To cite this article: Omar Caballero-Hernández, Miguel Hernández-Patricio, Itzel Sigala-Regalado, Juan B. Morales-Malacara & Manuel Miranda-Anaya (2015) Circadian rhythms and photic entrainment of swimming activity in cave-dwelling fish Astyanax mexicanus (Actinopterygii: Characidae), from El Sotano La Tinaja, San Luis Potosi, Mexico, Biological Rhythm Research, 46:4, 579-586, DOI: 10.1080/09291016.2015.1034972

To link to this article: http://dx.doi.org/10.1080/09291016.2015.1034972

Biological Rhythm Research, 2015
Vol. 46, No. 4, 579–586, http://dx.doi.org/10.1080/09291016.2015.1034972

Circadian rhythms and photic entrainment of swimming activity in cave-dwelling fish *Astyanax mexicanus* (Actinopterygii: Characidae), from El Sotano La Tinaja, San Luis Potosi, Mexico

Omar Caballero-Hernández, Miguel Hernández-Patricio, Itzel Sigala-Regalado, Juan B. Morales-Malacara and Manuel Miranda-Anaya*

Unidad Multidisciplinaria de Docencia e Investigación, Facultad de Ciencias, Universidad Nacional Autónoma de México, Juriquilla, Querétaro 76230, Mexico

(*Received 10 March 2015; accepted 18 March 2015*)

Circadian regulation has a profound adaptive meaning in timing the best performance of biological functions in a cyclic niche. However, in cave-dwelling animals (troglobitic), a lack of photic cyclic environment may represent a disadvantage for persistence of circadian rhythms. There are different populations of cave-dwelling fish *Astyanax mexicanus* in caves of the Sierra El Abra, Mexico, with different evolutive history. In the present work, we report that fish collected from El Sótano la Tinaja show circadian rhythms of swimming activity in laboratory conditions. Rhythms observed in some of the organisms entrain to either continuous light–dark cycles or discrete skeleton photoperiods tested. Our results indicate that circadian rhythm of swimming activity and their ability to entrain in discrete and continuous photoperiods persist in some organisms that might represent one of the oldest populations of cave-dwelling *A. mexicanus* in the Sierra El Abra.

Keywords: *Astyanax mexicanus*; circadian rhythm; photic entrainment; cave-dwelling; fish; La Tinaja; Sierra el Abra

Introduction

Circadian rhythms of behaviour and other biological functions exist in a wide variety of living organisms, from prokaryote to mammals; it is a common trait that has arisen independently in all known Phyla as a result of a natural selection of the daily changes in our planet. Circadian regulation has a profound adaptive significance in timing the best performance of biological functions in a cyclic niche. The adaptive significance of a circadian clock depends on the possibility to entrain to environmental cyclic cues (zeitgeber) and anticipate cyclic events. Constant conditions are required to observe persistence of circadian rhythms. However, it is possible that natural constant environments represent a selective pressure where some phenotypic features suffer regressive evolution, and then natural selection may negatively affect the circadian organization. Therefore, cave-dwelling organisms are considered as an opportunity to test it (Cavallari et al. 2011; Mena-Barreto & Trajano 2014).

Circadian locomotor activity has been detected in laboratory conditions in diverse species of cave-dwelling animals such as fish (Pati 2001; Trajano et al. 2005, 2012; Cavallari et al. 2011; Beale et al. 2013), crustaceans (O-Martínez et al. 2004), crickets

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*Corresponding author. Email: miranmanuel@ciencias.unam.mx

(Hoenen 2005), millipedes (Koilraj et al. 2000), spiders (Hoenen & Gnaspini 1999; Soriano-Morales et al. 2013), beetles (Pasquali & Sbordoni 2014), salamanders (Hervant & Mathieu 2000), frogs (Espino Del Castillo et al. 2009) and bats (Joshi & Vanlalnghaka 2005; Vanlalnghaka et al. 2005) indicating that the mechanisms underlying circadian rhythms in these species are still functional in a diverse range of expression from nearly arrhythmic to a well-defined cyclic activity. Such diversity in expressions seems to be related with the animal's niche if they are troglophile, trogloxene (both may be part of their life cycle in surface conditions) or troglobitic (all their biological cycles occur in caves).

In deepest regions of caves, poor fluctuant environmental conditions may exist; however, signals able to entrain endogenously generated circadian rhythms may be caused by behaviour such as migration of troglophile and or trogloxene species (Poulson & White 1969; Lamprecht & Weber 1992; Mena-Barreto & Trajano 2014).

Cave-dwelling animals represent unique models for studying the evolutionary change of structures such as eyes and pigmentation and also physiological functions such as circadian photoreception. The Neotropical blind fish *Astyanax mexicanus* [De Filippi 1853; junior synonym *Anoptichthys antrobius* = *Astyanax jordani*] (Reiss et al. 2003) is of particular interest, because it is widely distributed in Mexican karst area in Sierra El Abra, North Eastern Mexico (Reddel 1964; Gross 2012). Diverse blind pigmentedless populations of fish have been reported (Cabej 2012). Evidence from molecular data suggests that the 29 currently characterized cave populations were originated from two or three major waves of colonization during the past 2–8 million years (Gross 2012; Strecker et al. 2013). Each of these populations seems to be isolated in a particular cave, which also gives the opportunity of reviewing bottleneck selection of traits in a particular population. Circadian locomotor activity has been the subject of study in other populations of some caves in the El Abra System (Erkens & Martin 1982a, 1982b; Thines & Weyers 1978). Recent studies confirm that circadian activity at molecular clockwork still can be detected in specimens collected at the Chica and Pachon caves (Beale et al. 2013), but some fish collected in these locations also show attenuated and arrhythmic metabolism (Moran et al. 2014). When cyclic motor activity persists, it is consistent with the expression of clock genes monitored in the fins of fish from La Chica cave (Beale et al. 2013). Nonetheless, it is perhaps the fish population phylogenetically most recent in comparison with Sabinos, Tinaja and Pachon caves (Gross 2012; Strecker et al. 2013), and then it is possible that differences in circadian locomotor activity and photic entraining between organisms that belong to different caves may show different strengths. In this study, we explore whether the circadian locomotor activity rhythm and its ability to entrain to light cycles persist in *A. mexicanus* collected from the population of fish located in El Sótano de La Tinaja cave in San Luis Potosí, México. We also explored whether skeleton photoperiod (SP) can entrain a possible rhythmicity detected in swimming behaviour in order to reduce the effects of masking produced by light. The results indicate that noisy circadian rhythms in free running are detected, but also photic entraining in continuous and skeleton photoperiods, indicating fully functional circadian photic entrainment in some organisms studied.

Methods

Animals

Eight fish were collected from the El Sotano de la Tinaja Cave using regular hand nets. Fish were kept for transportation in independent bags, half-filled with water of their original niche. Organisms were stored in a thermic isolated box inside a second recipient with iced water in order to reduce drastic temperature changes. Once in the laboratory, fish were kept in aquaria (10 × 10 × 30 cm) with dechlorinated water inside an environmentally controlled chamber at the Facultad de Ciencias, UNAM, as indicated elsewhere (Soriano-Morales et al. 2013). Fish were fed with regular leaflets once a week. Aquaria were filled with water previously bubbled with an air pump. Cleaning was performed once a week in dim red light, replacing half of the water at the bottom with a regular siphon.

At the end of the experiments (some fish died before finishing), samples were deposited in the collection of the bio-speleology group at the Facultad de Ciencias, UNAM.

Activity recordings

Locomotor activity was recorded using an infrared light beam detector, coupled to an acquisition data board in a PC. Data were collected by means of the software ACTIBIO (Facultad de Psicología, URIDES, UNAM) as indicated elsewhere (Soriano-Morales et al. 2013).

Protocols

After arrival, when in constant darkness, dim red light (1 lx) was used for visually help in maintenance. First, fish were maintained in constant darkness at least 10 days, then light photoperiod (LD) 12:12 (250 lx) or SP 11D:1L was provided by a fluorescent lamp during 10 more days. Then, constant conditions were held in constant darkness.

Data analysis

Summatory of data once every 10 min was stored and then analysed in Chronos-Fit software (Zuther et al. 2009) in double plotted actograms and power spectrum analysis. All calculated periods with a level of $p < 0.05$ were considered significant. Free running period in constant dim red light was contrasted with the complete or discrete entrainment.

Results

All of the animals tested in initial DD ($n = 8$) displayed significant free running circadian rhythms of swimming activity during at least seven days of continuous recording. Individual values of significant periods detected with its power value are plotted in Figure 1; each individual is indicated with a particular symbol, first in DD and then in either conditions LD or SP. Among the circadian periodicities, multiple random ultradian periods of activity were also detected. When exposed to LD ($n = 4$), a clear increase in the power value of circadian periods was observed in two organisms (indicated with signs − and +); but no increasing in power was observed when fish were exposed to SP ($n = 4$). In Figure 2(B), the changes in circadian period for each organism tested are shown. Individuals are labelled as A1–A8. Fish studied in SP showed a shift in the circadian period (A1–A4) to a period close to 24 h, no post entrainment was possible to test in these animals. In the group exposed to LD, a change in the period close to 24 h was noted in fish A5 and A8; the other two fish changed the period but

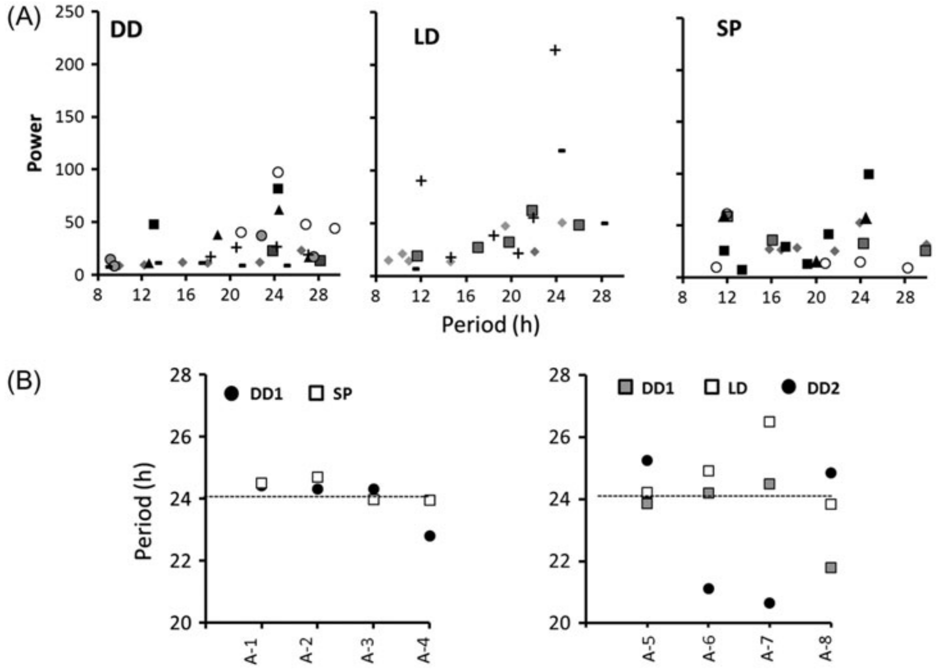

Figure 1. (A) significant periods detected by means of power spectrum analysis. Each symbol corresponds to an independent organism exposed first to DD and then complete (LD) or SP. (B) Changes in the circadian period value for each organism before, during and after LD (left panel) or SP (right panel).

did not reach 24 h. Fish in LD were healthy enough in order to explore few days in DD in a postentrainment free running. A particular example is shown in actogram of Figure 2 (fish A5) plotted in binary format as shown in Chronos-Fit program.

The LD cycles indicate nocturnal activity that has phase control in a secondary DD protocol (day 22 in actogram). Correspondent periodograms are shown in the right side of the figure. It is clear a change in period and increase on the power values when LD conditions are present, and a well-defined cycle in the average profile (±SEM) were noted. In Figure 3, an example of the effect on locomotor activity rhythm of the SP 11:1. In this particular example, the main activity seems to be adjusted to the second pulse after few transients. The average waveform shows also for this condition a negative masking of the light pulse.

Discussion

Diverse species of cave-dwelling (troglobitic) fish worldwide have been isolated from natural daylight for millions of years, showing a set of striking convergent "troglomorphic" phenotypes such as eye loss, depigmentation, enhancement of environmental sensory system and low metabolic rates. Despite several millions of years in a non-photic environment (Gross 2012), do cavefish still retain clocks? If they do so, why they still retain their ability to light entrain in a non-photic environment? The Sierra de El Abra has diverse caves where *Astyanax* populations can be found in diverse grades of polymorphism regarding eye reduction, metabolic rate and circadian locomotor

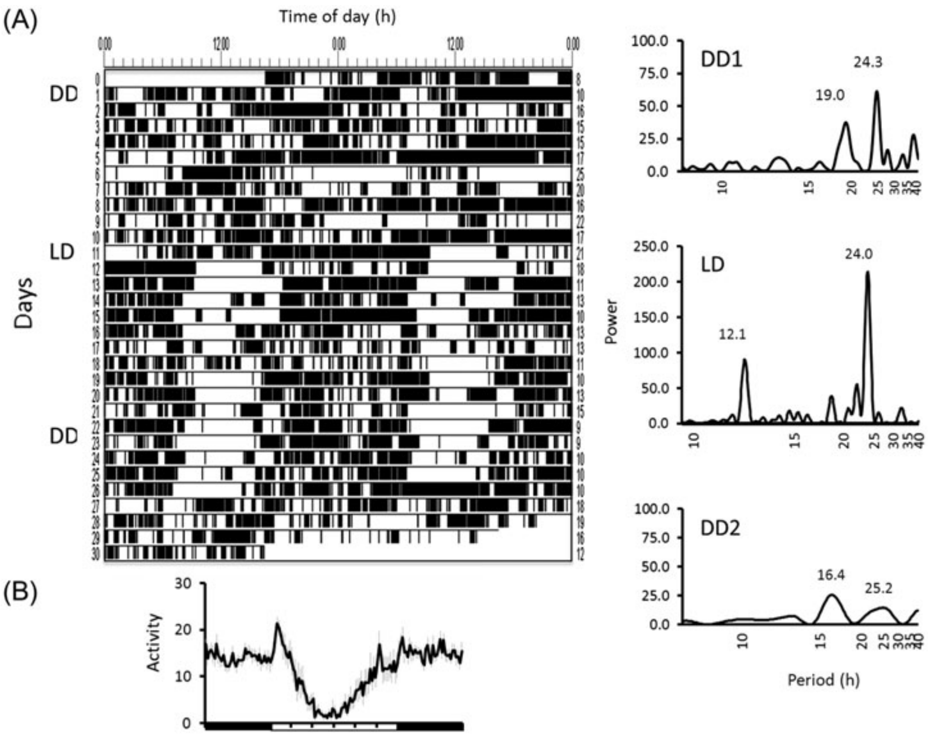

Figure 2. Circadian rhythm of locomotor activity in *A. mexicanus.* Constant darkness (DD) and complete photoperiod (LD) are indicated in the actogram. An average waveform during LD is shown below with the correspondent LD cycle. Periodogram analysis is shown for each condition at the right panels.

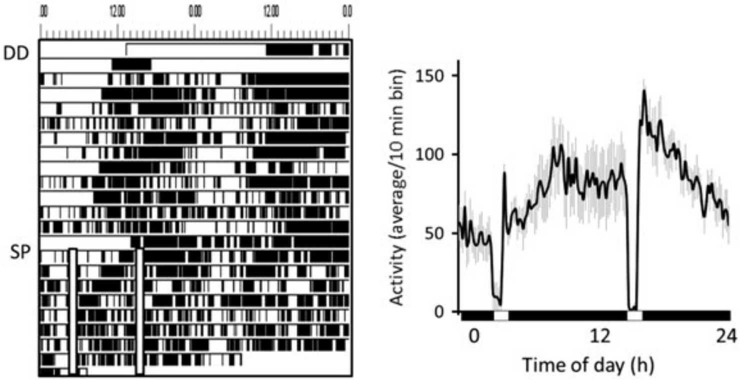

Figure 3. An example of the circadian rhythm of swimming activity in *Astyanax* is shown in DD and its response to skeleton photoperiod. The average waveforms in the right panel (SE is shown as grey lines along the curve) of the last days during SP show also a negative masking of the light pulse on the swimming activity.

activity, indicating that organisms in each cave have been genetically isolated by allopatric or parapatric speciation mechanisms. This is a quite interesting opportunity regarding the persistence of circadian rhythms in a non-photo cyclic environment. In the present work, we confirm that the fish found in El Sotano Tinaja cave, a group considered among the oldest populations of *Astyanax* in the karst of the Sierra El Abra (Erkens & Martin 1982a, 1982b; Beale et al. 2013; Strecker et al. 2013), also retain its ability to show light entrainable circadian rhythms of locomotor activity. In the present study, the circadian rhythmicity was detected through the use of the software Chronos-Fit (Zuther et al. 2009); the actograms plotted consider a binary representation regarding data above the average. This visual representation makes easier to see the rhythm free running along several days and its response to photoperiod, either complete or discrete. Previous reports that indicate the absence of circadian rhythms in cave organisms may be related with the way in which activity was studied or as a failure to detect them (Hoenen & Marques 1998). It has been recently indicated that aerobic metabolism is reduced and arrhythmic in continuous darkness in *Astyanax* collected at Pachon (Moran et al. 2014), the oldest population of the cave system in Sierra El Abra (Gross 2012). In the present report, we show that circadian activity persists during 10 days before being exposed to light cycles in fish collected in La Tinaja, and some of them were able to show phase control with the previous LD when released in DD (Figure 1) indicating a true entrainment. Compared with other reports, we noted nocturnal activity rather than diurnal, and when SP was tested, main activity was organized near the second pulse that could be considered as the "evening." It has been noted that diurnal or nocturnal patterns of activity in fish may depend on a diversity of factors (Reebs 2002) even in *Astyanax* (Erkens & Martin 1982b). We also noted that activity is reduced in constant darkness in comparison with the behaviour observed in LD. In DD, activity is low amplitude but persistent compared with the spontaneous activity observed on a surface specimen (data non shown) also in agreement with the observed in other studies regarding sleep regulation in *Astyanax* as an adaptation to increase the effective time searching for food (Duboué et al. 2012). This last study indicates that behaviour in organisms collected in La Tinaja could be explained as an endogenous behavioural adaptation to an environment with extreme low food resources, in order to stay in *quasi*-permanent alert at any time but with potential induction of cycles when changing environmental conditions.

Cave-dwelling adult *Astyanax* in La Tinaja is characterized by anophthalmy, complete loss of the lens as well as partial loss and reduction of the retina due to programmed cell death (for review, see Protas & Jeffery 2012). Movement in the retinal pigment epithelium in the reminiscent retina of *Astyanax* from Chica cave has been reported (Espinasa & Jeffery 2006), indicating that reminiscent retinae still presents clock functions. Photoentrainment in *A. mexicanus* may not require of functional retinae as an input pathway but a functional photosensitivity in pineal gland may also still exist: so far, no studies on pineal photosensitivity have been reported in blind fish, therefore such question remains unanswered. The present work is complement to previous works on circadian studies and expression of circadian rhythms in other populations of *A. mexicanus* in the Sierra El Abra caves. The entrainment response of SP is tested for first time as well as a free running after entrainment in LD. Also, we show that such responses vary between individuals indicating that the possible fading of the circadian clock may occur differentially among organisms of the same population.

Acknowledgements

Teresa Bosques-Tistler for English review; Omar Caballero was supported with a fellowship DGAPA, UNAM. Rodrigo Monjaraz Ruedas, Daniela Candia Ramírez Adrián Reyna Domínguez and The Sotano la Tinaja guides, for their assistance in the field expeditions. PAPIIT IN 226010, and IN 219113 to JBMM.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Beale A, Guibal C, Tamai TK, Klotz L, Cowen S, Peyric E, Reynoso V, Yamamoto Y, Whitmore D. 2013. Circadian rhythms in Mexican blind cavefish *Astyanax mexicanus* in the lab and in the field. Nat Commun. 4:1–10.

Cabej N. 2012. Epigenetic principles of evolution: evolution by loss. London: Elsevier. Chapter 14, p. 579–622.

Cavallari N, Frigato E, Vallone D, Fröhlich N, Lopez-Olmeda JF, Foà A, Berti R, Sánchez-Vázquez FJ, Bertolucci C, Foulkes NS, Foulkes N. 2011. A blind circadian clock in cavefish reveals that opsins mediate peripheral clock photoreception. PLoS Biol. 9:e1001142.

De La O-Martínez A, Verde MA, Valadez RL, Viccon-Pale JA, Fuentes-Pardo B. 2004. About the existence of circadian activity in cave crayfish. Biol Rhythm Res. 35:195–204.

Duboué E, Borowsky RL, Keene AC. 2012. β-adrenergic signaling regulates evolutionarily derived sleep loss in the Mexican cavefish. Brain Behav Evol. 80:233–243.

Erkens W, Martin W. 1982a. Exogenous and endogenous control of swimming activity in *Astyanax mexicanus* (Characidae, Pisces) by direct light response and by a circadian oscillator I. Analyses of the time-control systems of an Epigean river population. Z Naturforsch. 37 c: 1253–1265.

Erkens W, Martin W. 1982b. Exogenous and endogenous control of swimming activity in *Astyanax mexicanus* (Characidae, Pisces) by direct light response and by a circadian oscillator D. Features of time-controlled behaviour of a cave population and their comparison to a Epigean ancestral form. Z Naturforsch. 37 c: 1266–1273.

Espinasa L, Jeffery R. 2006. Conservation of retinal circadian rhythms during cavefish eye degeneration. Evol Dev. 8:16–22.

Espino Del Castillo AE, Castaňo-Meneses G, Dávila-Montes MJ, Miranda-Anaya M, Morales-Malacara JB, Paredes-León R. 2009. Seasonal distribution and circadian activity in the troglophile long-footed robber frog, *Eleutherodactylus longipe*s (Anura: Brachycephalidae) at Los Riscos cave, Querétaro, Mexico: field and laboratory studies. J Cave Karst Stud. 71:24–31.

Gross JB. 2012. The complex origin of *Astyanax* cavefish. BMC Evol Biol. 12:1–12.

Hervant F, Mathieu J. 2000. Metabolism and circadian rhythms of the European blind cave salamander *Proteus anguinus* and a facultative cave dweller, the Pyrenean newt (*Euproctus asper*). Can J Zool. 78:1427–1432.

Hoenen S. 2005. Circadian patterns in the activity of the Brazilian cave cricket *Strinatia brevipennis* (Ensifera: Phalangopsidae). Eur J Entomol. 102:663–668.

Hoenen S, Gnaspini P. 1999. Activity rhythms and behavioral characterization of two epigean and one cavernicolous harvestmen (Arachnida, Opiliones, Gonyleptidae). J Arachnol. 27:159–164.

Hoenen S, Marques MD. 1998. Circadian patterns of migration of *Strinatia brevipennis* (Orthoptera: Phalangopsidae) inside a cave. Biol Rhythm Res. 29:480–487.

Joshi DS, Vanlalnghaka C. 2005. Non-parametric entrainment by natural twilight in the microchiropteran Bat, *Hipposideros speoris* inside a cave. Chronobiol Int. 22:631–640.

Koilraj AJ, Sharma VK, Marimuthu G, Chandrashekaran MK. 2000. Presence of circadian rhythms in the locomotor activity of a cave-dwelling millipede *Glyphiulus cavernicolus sulu* (Cambalidae, Spirostreptida). Chronobiol Int. 17:757–765.

Lamprecht G, Weber F. 1992. Spontaneous locomotion behaviour in cavernicolous animals: the regression of the endogenous circadian system. In: Camacho AI. The natural history of biospeleology. Madrid: Monografias del Museo Nacional de Ciencias Naturales; p. 225–462.

Mena-Barreto L, Trajano E. 2014. Biological rhythmicity in subterranean animals: a function risking extinction? In: Aguilar-Roblero R, Díaz-Muñoz M, Fanjul-Moles M. Mechanisms of circadian systems in animals and their clinical relevance. Heidelberg: Springer; p. 55–68.

Moran D, Softley R, Warrant EJ. 2014. Eyeless Mexican cavefish save energy by eliminating the circadian rhythm in metabolism. PLoS ONE. 9:e107877.

Pasquali V, Sbordoni V. 2014. High variability in the expression of circadian rhythms in a cave beetle population. Biol Rhythm Res. 45:925–939.

Pati AK. 2001. Temporal organization in locomotor activity of the hypogean loach, *Nemacheilus evezardi*, and its epigean ancestor. Environ Biol Fishes. 21:119–129.

Poulson TL, White WB. 1969. The cave environment. Science 165:971–981.

Protas M, Jeffery WR. 2012. Evolution and development in cave animals: from fish to crustaceans. Wiley Interdiscip Rev Dev Biol. 1:823–845.

Reddel J. 1964. Biology of the caves of the northern El Abra Range. Assoc Mexican Cave Stud Newslett. 1:19–21.

Reebs SG. 2002. Plasticity of diel and circadian activity rhythms in fishes. Rev Fish Biol Fish. 12:349–371.

Reiss RE, Kullander SO, Ferraris CJ. 2003. Check List of the Freshwater Fishes of South and Central America. Porto Alegre: Pontificia Universidade Católica do Rio Grande do Sul; p. 742.

Soriano-Morales S, Caballero-Hernández O, Dávila-Montes M, Morales-Malacara JB, Miranda-Anaya M. 2013. Circadian locomotor activity and entrainment by light cycles in cave spiders (Dipluridae and Ctenidae) at the cave Los Riscos, Qro. México. Biol Rhythm Res. 44:949–955.

Strecker U, Bernatchez I, Wilkens H. 2013. Genetic divergence between cave and surface populations of *Astyanax* in Mexico (Characidae, Teleostei). Mol Ecol. 12:699–710.

Thines G, Weyers M. 1978. Résponses locomotrices du poisson cavernicole *Astyanax jordani* (Pices, Characidae) á des signaux périodiques et apériodiques de lumiére e de température [Locomotor responses of cave fish *Astyanax jordani* (Pices, Characidae) to periodic and aperiodic signals of light and temperature]. Int J Speleol. 10:35–55.

Trajano E, Duarte L, Menna-Barreto L. 2005. Locomotor activity rhythms in cave fishes from Chapada Diamantina, Northeastern Brazil (Teleostei: Siluriformes). Biol Rhythm Res. 35: 195–204.

Trajano E, Ueno JCH, Menna-Barreto L. 2012. Evolution of time-control mechanisms in subterranean organisms: cave fishes under light-dark cycles (Teleostei: Siluriformes, Characiformes). Biol Rhythm Res. 43:191–203.

Vanlalnghaka C, Keny VL, Satralkar MK, Khare PV, Pujari PD, Joshi DS. 2005. Natural twilight phase-response curves for the cave-dwelling Bat, *Hiposideros speoris*. Chronobiol Int. 22:793–800.

Zuther P, Gorbey S, Lemmer B. 2009. Chronos-Fit 106. Available from: http:www.ma.uni-heidelberg.de/inst/phar/lehre/chrono.html