DAILY RHYTHMS OF LOCOMOTOR ACTIVITY AND EXPRESSION OF NON-VISUAL OPSINS IN THE BLIND MEXICAN CAVEFISH *Astyanax mexicanus*

Conti F.[1], A. Vergès-Castillo [2], F.J. Sánchez-Vázquez [1] and J.A. Muñoz-Cueto [2]

[1]* Department of Physiology, Faculty of Biology, University of Murcia, Murcia, Spain

[2] Department of Biology, Faculty of Marine and Environmental Sciences and INMAR, University of Cádiz, Cádiz, Spain

*Email: javisan@um.es

INTRODUCTION:

✓ Most animals and plants have developed an endogenous circadian clock that synchronizes their physiology and behaviour with the environmental light-dark cycle.

✓ The opsin family is composed of visual opsins, expressed in retinal photoreceptors, and non-visual opsins expressed in both ocular and extraocular tissues.

✓ The blind Mexican cavefish *Astyanax mexicanus*, is an important model system for the study of adaptive and regressive evolutionary changes (e.g., loss of functional eyes and pigmentation) that have occurred as animals have evolved to life underground (Jeffery, 2001).

OBJECTIVES:

▼ To investigate photic entrainment and a possible circadian endogenous rhythmicity of this eyeless hypogean species.

▼ To investigate the daily expression patterns and rhythms of non-visual extraocular opsins in the *A. mexicanus* brain.

RESULTS:

◉ Daily recording of locomotor activity:

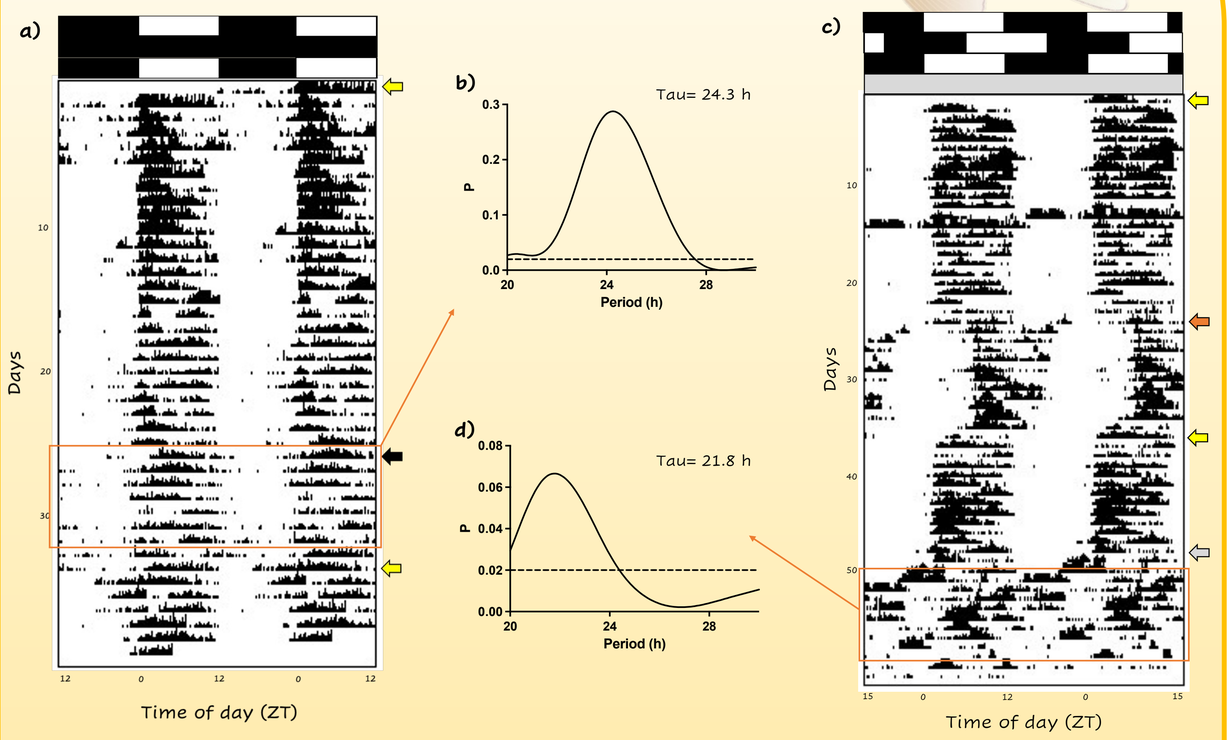


Figure 1. *Representative locomotor actograms of 2 aquaria subjected to different lighting regimes (**a**, **c**). The white and black bars above graphs represent the light and dark phases, respectively. Yellow arrows represent LD 12:12 (**a**, **c**), the orange arrow the 6 hours shift of LD 12:12 (**c**), the black arrow DD (**a**) and the grey arrow LLdim (**c**). Data are double-plotted on a time scale of 48 h, the height of each point representing the number of infrared light-beam interruptions per 10 min. Respective Lomb- Scargle periodogram analysis (**b**, **d**) of the constant conditions (DD and LLdim). The period of the free-running rhythm (τ) is indicated above the periodograms. The actograms and periodogram analyses were carried out with the chronobiology software El Temps (v. 313, Prof. Díez Noguera, University of Barcelona, Spain).*

◉ Daily expression of non-visual opsin genes:

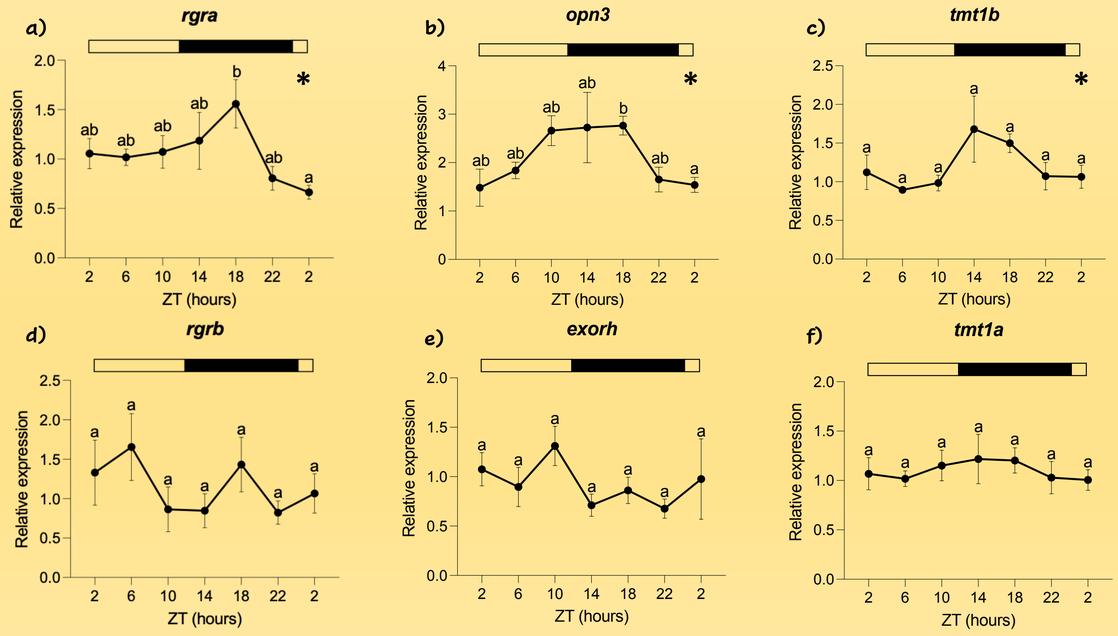
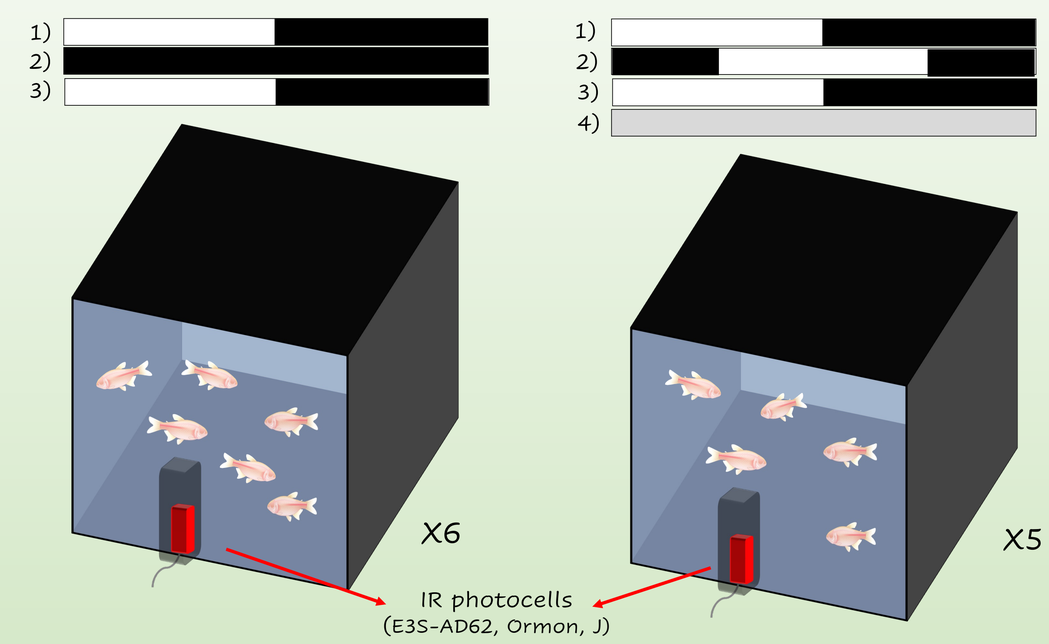


Figure 2. Daily expression of non-visual extraocular ospin genes in the blind *A. mexicanus*: *rgra* (**a**), *opn3* (**b**), *tmt1b* (**c**), *rgrb* (**d**), *exorh* (**e**) and *tmt1a* (**f**). Data are shown as mean ± SEM (n=7/ZT). *The white and black bars above graphs represent the light and dark phases, respectively. The time scale (X-axis) is expressed as Zeitgeber Time (ZT), when ZT0 corresponds to lights on and ZT12 corresponds to lights off.* Different letters indicate significant differences (one-way ANOVA; $p<0.05$). Statistical analysis were performed with GraphPad Prism 9.2. Asterisks indicate the existence of significant daily rhythms (Cosinor, $p<0.05$).

MATERIALS AND METHODS:

- Daily recording of locomotor activity:



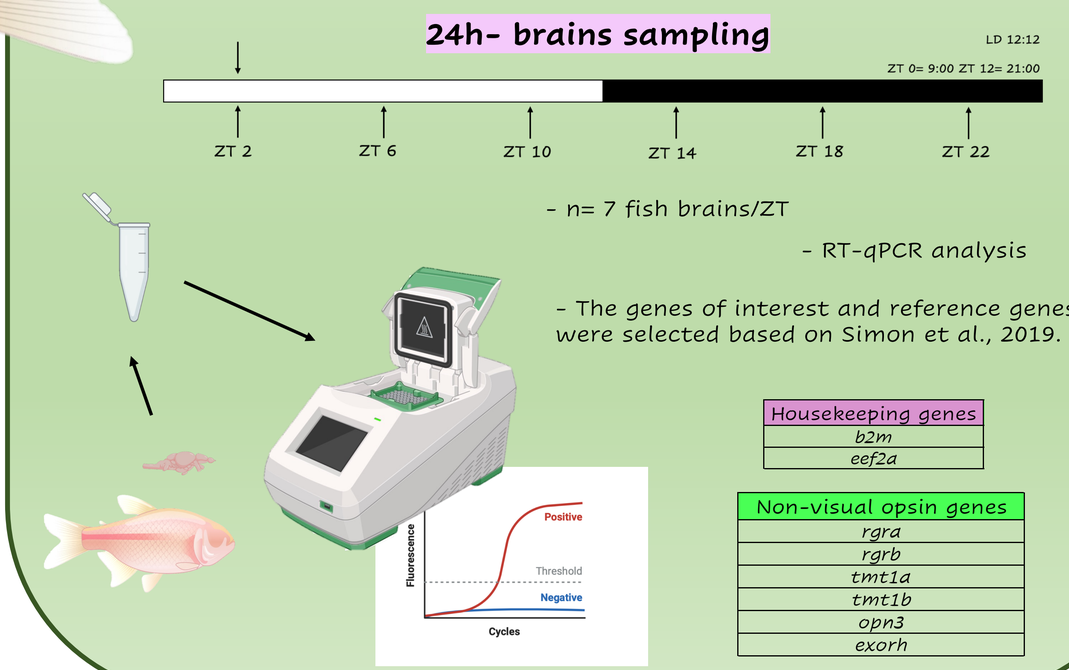
- n=6 juveniles/aquaria
- Random feeding
- Temperature 24 °C ± 0,5 °C

1) LD 12:12 (ZT 0= 9h, ZT 12= 21h)
2) DD
3) LD 12:12

- n=5 juveniles/aquaria
- Random feeding
- Temperature 24 °C ± 0,5 °C

1) LD 12:12 (ZT 0= 9h, ZT 12= 21h)
2) LD 12:12 (ZT 0= 15h, ZT 12= 3h)
3) LD 12:12
4) LLdim

- Daily expression of non-visual opsin genes:



- n= 7 fish brains/ZT
- RT-qPCR analysis
- The genes of interest and reference genes were selected based on Simon et al., 2019.

Housekeeping genes
b2m
eef2a

Non-visual opsin genes
rgra
rgrb
tmt1a
tmt1b
opn3
exorh

CONCLUSIONS:

✦ Animals showed an entrainment to the light-dark cycle with a diurnal activity pattern, i.e., increased locomotor activity during daytime and lower locomotor activity during night time (Fig. 1a, c).

✦ Under constant conditions (DD and LLdim) the fish exhibited self-sustained circadian rhythms (Fig. 1b, d).

✦ We found significant daily variations and/or rhythmic expression for 3 genes encoding non-visual opsins (Fig. 2a-c), which exhibited nocturnal acrophases:

GENE	CONDITION	MESOR (r.e)	AMPLITUDE (r.e)	ACROPHASE (ZT)	SIGNIFICANCE (p. value)
exorh	LD 12:12	-	-	-	n.s
rgra	LD 12:12	1.08	0.26	15.15	*
rgrb	LD 12:12	-	-	-	n.s
opn3	LD 12:12	2.19	0.71	13.48	**
tmt1b	LD 12:12	1.22	0.32	16.34	*
tmt1a	LD 12:12	-	-	-	n.s

Our results suggest that daily rhythms in extraretinal non-visual opsins are still able to transduce daily photic cycles and could be sustaining behavioural and other light-entrained rhythms in blind cavefish species.

REFERENCES:

Jeffery, W. R. (2001). Cavefish as a model system in evolutionary developmental biology. *Developmental biology*, *231*, 1-12.

Simon, N., Fujita, S., Porter, M., & Yoshizawa, M. (2019). Expression of extraocular opsin genes and light-dependent basal activity of blind cavefish. *PeerJ*, *7*, e8148.

All pictures were taken with Biorender.

This research was supported by the European Union's Horizon 2020 research and innovation programme under Marie Skłodowska-Curie grant No 956129 (EasyTRAIN) to FJS-V and by the Spanish Ministry of Science and Innovation under grants No PID2021-123640OB-C21 and PID2021-123640OB-C22 (BBLUEAQUA) to FJS-V and JAM-C, respectively.