EFFECTS OF FEEDING TIME ON THE DAILY RHYTHMS OF TEMPERATURE SELECTION IN THE BLIND MEXICAN CAVEFISH (*Astyanax mexicanus*)

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Introduction

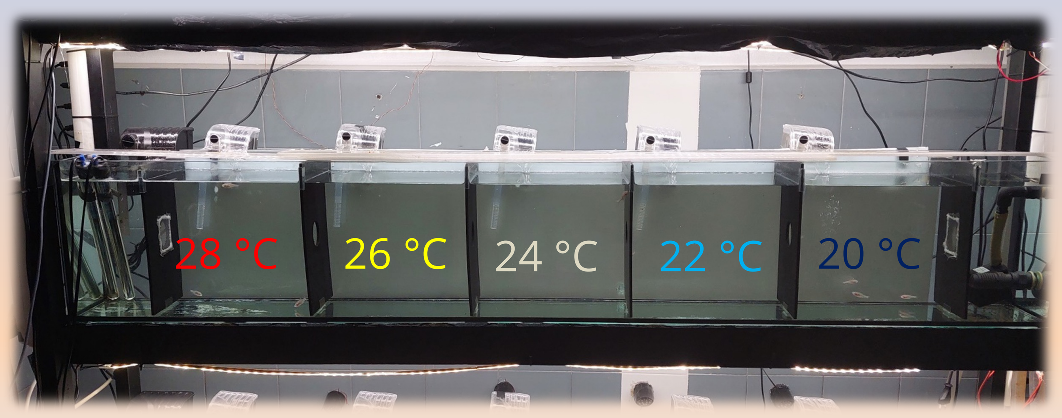
- Animals and plants live on a rhythmic planet, with regular and predictable periods of light-darkness, temperature and food. As a result, the evolution has selected the existence of endogenous circadian clocks that synchronise their physiology and behaviour with the environmental cycles.
- Besides the existence of thermocycles, fish display a thermal preference. Being ectothermic organisms, temperature selection in fish is connected to their biological processes, choosing those in which the development of these processes will be most effective.
- Over the past few millions of years, the blind cavefish *Astyanax mexicanus* has been isolated from neighbouring rivers in underground caves in Mexico. These fish have evolved and adapted to a life in complete darkness and these adaptations include also the loss of circadian rhythms to varying degrees (Steindal et al., 2018).

Objectives

The aim of this study was to investigate the existence of daily rhythms of temperature selection and the effect of feeding time on these rhythms in a fish that has evolved in an arrhythmic environment, such as *A. mexicanus*.

Methodology

- The thermal preference was evaluated using 3 multi-chamber systems in which a continuous thermal gradient (from 20 ºC to 28 ºC) was established, created by using water heaters and coolers.
- A constant temperature of 24 ºC in the whole system was used as control.



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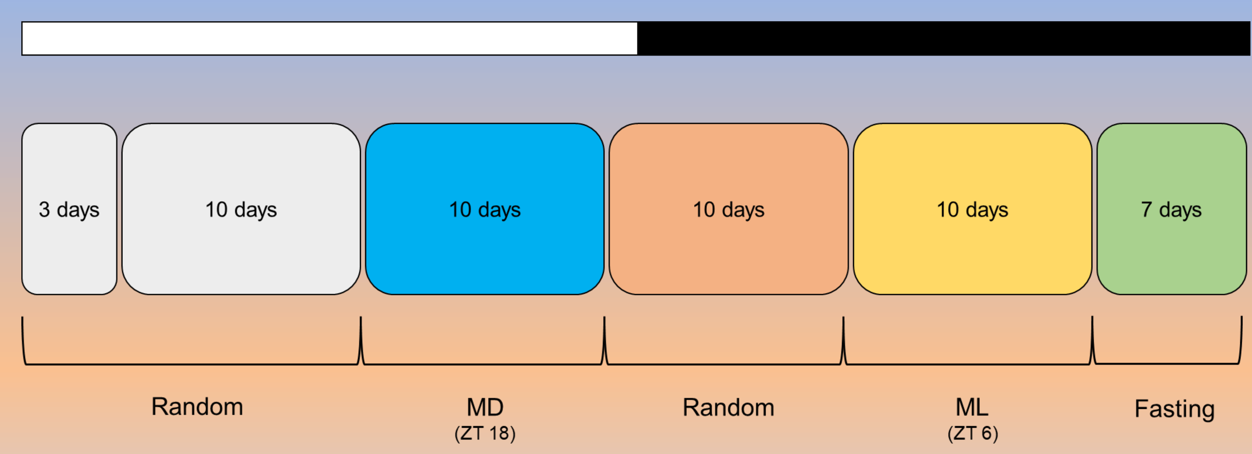


- LD 12:12 h
- 4 different feeding schedules: Random, MD (mid-dark), ML (mid-light) and fasting.

The 3 days prior to the start of the first phase were considered as a period of acclimation to the system.

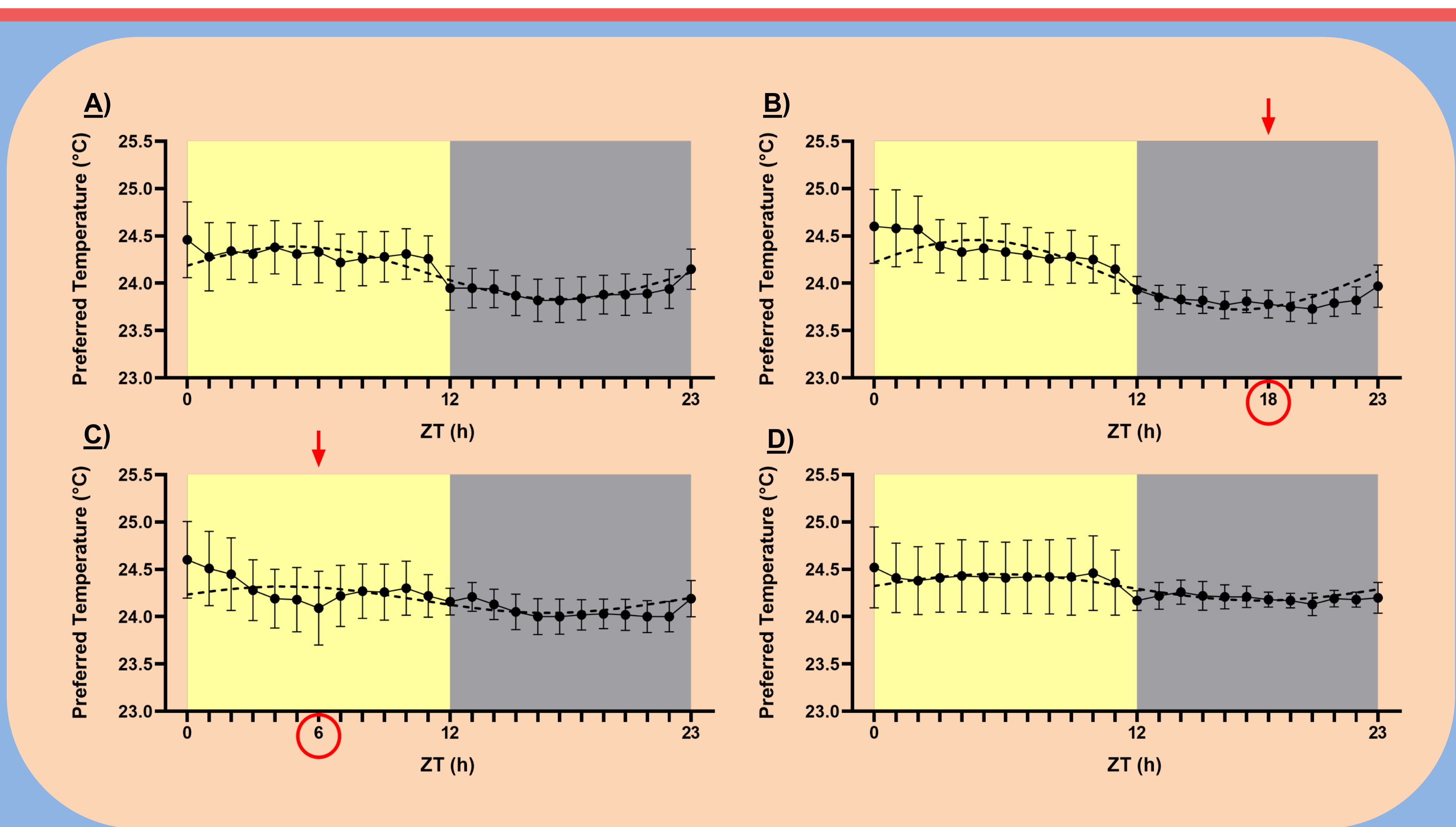
- Video analysis of animal behaviour: FishCounter (Dr. Ginés García Mateos, University of Murcia, Spain, Version 3.0).
- The thermal preference was calculated by applying the following formula:

$$Pref.Temp. = \frac{(n_1 \cdot T_1 + n_2 \cdot T_2 + n_3 \cdot T_3 + n_4 \cdot T_4 + n_5 \cdot T_5)}{N}$$

n is equal to the number of fish in each chamber (1 to 5), T is equal to the temperature of the corresponding chamber and N is equal to the total number of fish.

Results

1



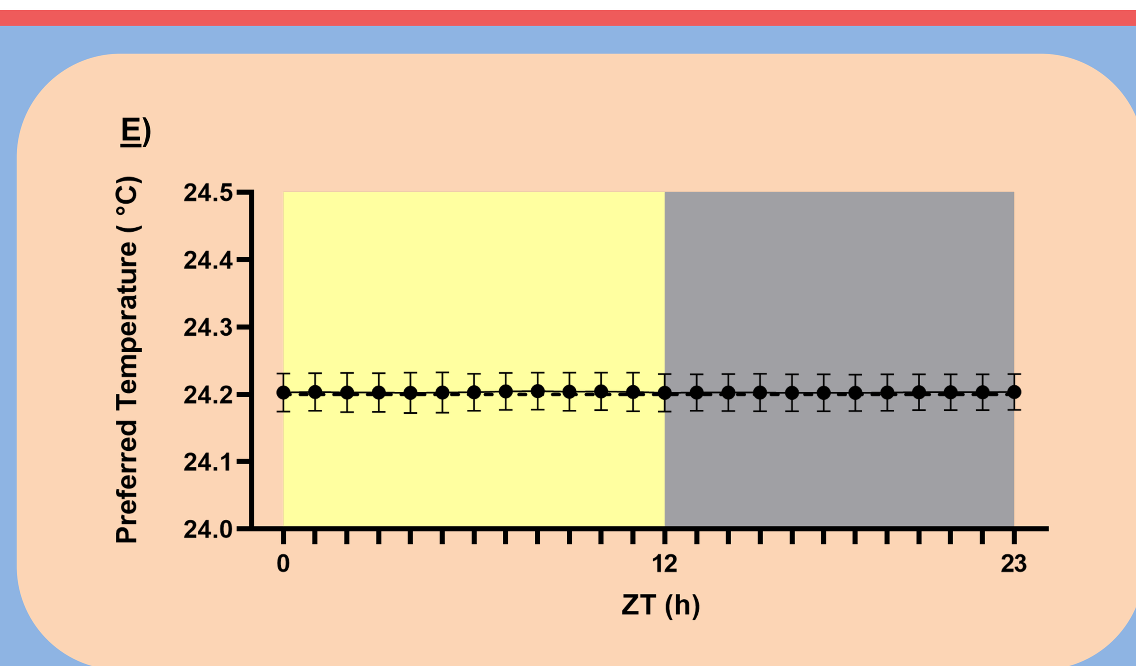


Figure 1. Daily rhythm of thermal preference of *A. mexicanus* under an LD cycle 12:12 and random feeding (A), MD feeding (B), ML feeding (C) and fasting (D). The control is represented in graph E. The yellow and gray areas indicate the periods of light and dark, respectively. The red arrows indicate the feeding time in MD (ZT 18h, B) and ML (ZT 6h, C), respectively. The dotted line represents the setting of the Cosinor function (Cosinor, p<0.05). The data (n=3 replicas) are represented as mean ±SEM.

2

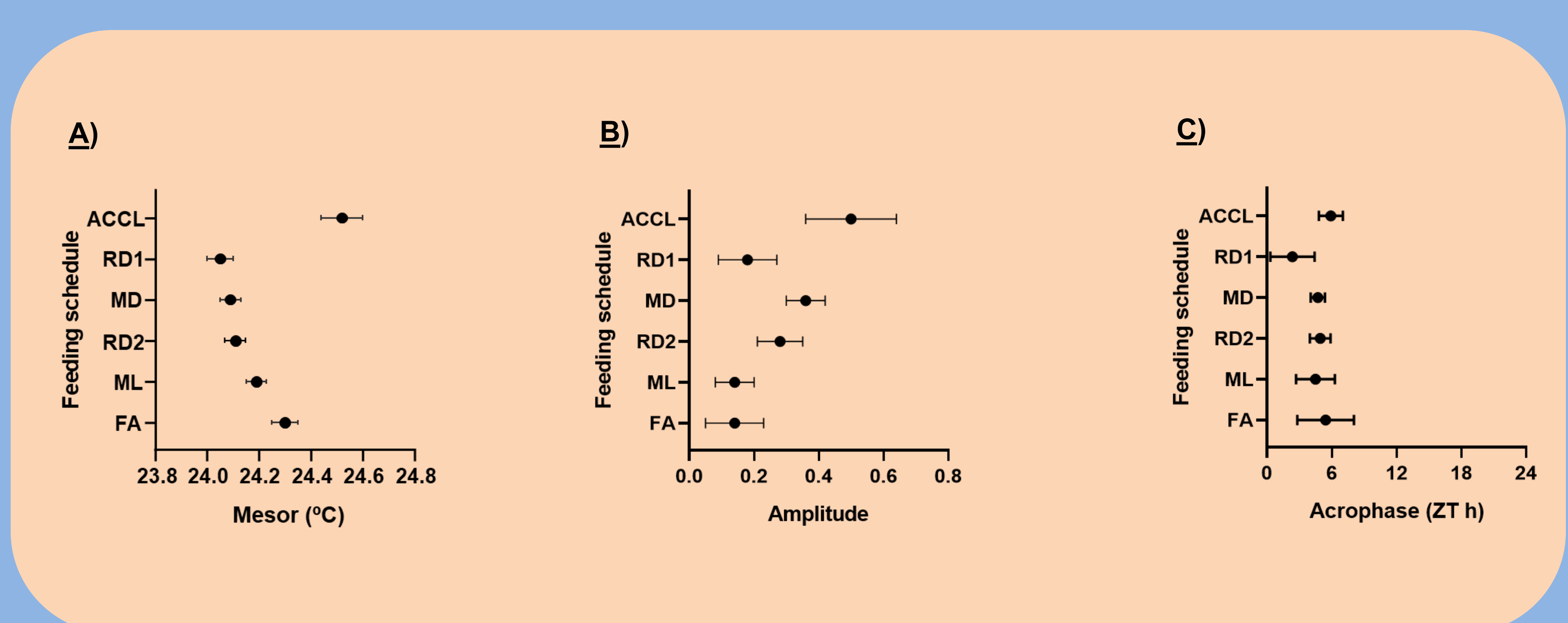


Figure 2. Representation of the analysis of the mesor (A), amplitude (B) and acrophase (C) of the daily rhythm of thermal preference of *A. mexicanus* for each feeding schedule/experimental phase. The black circles represent the mesor (A), amplitude (B) and acrophase (C) values with their respective fiducial limits (95% confidence intervals) (n=3 replicas).

Conclusion

- The fish showed a daily rhythm of thermal preference, regardless of the feeding protocol. they selected warmer temperatures during the light phase and cooler temperatures during the dark phase.
- As can be seen from the calculation of the mesor, during acclimation the fish selected a higher mean temperature than that observed in the other experimental phases, showing a possible emotional fever in response to the new environment (Rey et al., 2015), as already observed in other species of fish. The amplitude of the rhythm is also greater.
- Fish fed at ML showed a compensatory thermal preference, selecting lower temperatures in the hours before feeding and warmer temperatures afterwards.
- During the fasting phase, the thermal preference rhythm has a lower amplitude but still significant (Cosinor, p= 0.00061).

References and Acknowledgements

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