CIRCADIAN RHYTHMS OF LOCOMOTOR ACTIVITY AND SPAWNING IN ZEBRAFISH (*DANIO RERIO* L.)

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In this study, we investigated the existence of a circadian spawning rhythm in zebrafish exposed to different lighting conditions, using an automatic and programmable egg collector. We monitorized locomotor activity and the number of eggs released, throughout 24 hours. The results showed a diurnal activity pattern, when fish were under 14L: 10D. When we applied a 1h pulse of darkness four hours after lights on (coinciding with the peak of spawning), fish changed the time of spawning following the pulse of darkness. Feeding at night, however, they did not change diurnal spawning rhythm. Under continuous light photoperiod, we observed endogenous rhythm with $\tau = 22.3$h. These results revealed the existence of spawning rhythms and their synchronisation to light and feeding.

INTRODUCTION

Zebrafish (*Danio rerio*) has been recently used in biomedical research in vertebrates (Fishman, 2001). Not many information about its ecology and behaviour has been taken into account to develop protocols for farming. The purpose of this paper is to investigate daily spawning and locomotor rhythms in zebrafish exposed to different light conditions, and to observe whether mealtime affects the locomotor and spawning patterns.

MATERIALS AND METHODS

Different light photoperiods:

14:10 light-dark (LD)

Continuous light (LL)

Pulse of darkness

→ In experiment 1 fish were exposed to LD cycle and fed either at ZT4 or at ZT19, to know if feeding time influences spawning.

→ In experiment 2 a 1h pulse of darkness was given either at ZT3 (the peak of spawning previously observed) or at ZT7, to test the light effect on spawning.

→ Finally, fish were exposed to LL to test whether spawning rhythms persisted.

The animals were separated according to sex before crosses were made, so locomotor activity in males and females were monitorized.

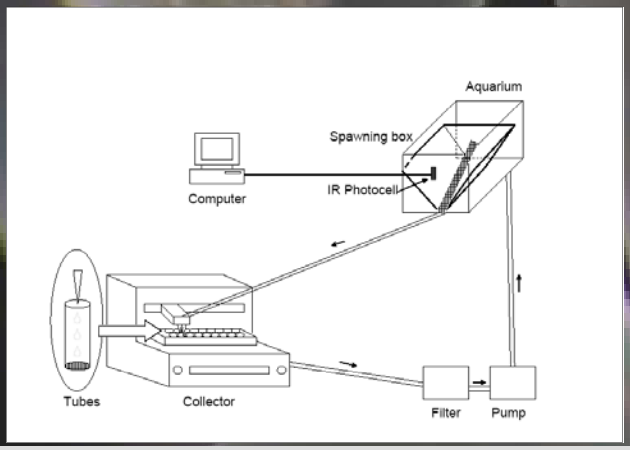

Fig 1. Detailed scheme of the system used to carry out the experiments.

RESULTS AND DISCUSSION

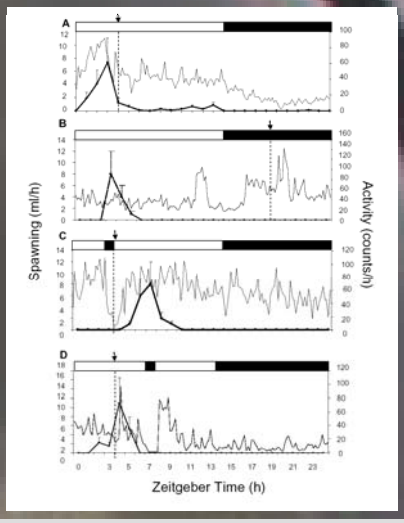

Fig 2. Average values of daily locomotor activity and spawning rhythms in zebrafish under artificial 14L:10D photoperiod, on the days on which spawning was obtained.

A pulse of darkness at ZT3 induced a shift in the daily spawning rhythm, which started after the pulse of darkness, the peak of highest volume of spawning occurring at ZT7. When the pulse of darkness was provided at ZT7, spawning returned to ZT3.

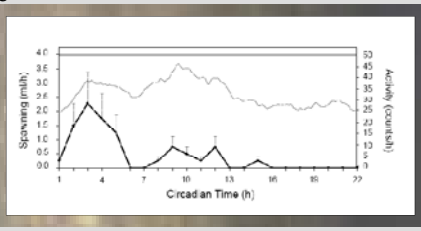

Fig 3. Average values of daily locomotor activity and spawning rhythms in zebrafish with continuous light photoperiod, on the days on which spawning was obtained.

Under LL locomotor activity rhythms persisted with a periodicity of 22.3 h, the spawning rhythm showing a circadian pattern with its acrophase at CT3

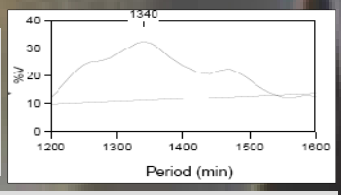

Fig 4. The periodgram, calculated using the chi-square periodogram (software, El Temps) with a confidence level of 95%, the phase (mins) is indicated at the top of the periodogram.

CONCLUSIONS

Light, but not feeding, seems to be the key environmental factor in timing the daily spawning in zebrafish, since this study revealed that spawning rhythms can be shifted by dark pulses. Besides, spawning rhythms are circadian, because they persisted under LL.

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