# Demonstrating the ability of a Zantiks unit to perform temperature controlled circadian rhythm experiments in *Drosophila melanogaster*

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## Introduction

Circadian rhythms can be entrained by several environmental cues including light and temperature. Temperature cycles have been shown to induce circadian rhythms in *Drosophila melanogaster*, even in the absence of light [1]. Results show the capacity of the Zantiks unit to be used for circadian rhythm studies with both light and temperature as the zeitgeber without the use of external equipment such as temperature controlled chambers.

## Method

Flies were reared at 22C in 12/12 LD lighting and collected at 1-5 days old to be transferred into the Zantiks units (pictured bottom left). Transfer was done according to the current lighting phase to avoid interference with the light cycle. Flies were loaded into standard 24 well plates with an agar/sugar/nipagin medium for sustenance and the plates were placed inside the units. 3 units were used, each with 1 plate of 24 flies.

The units were scripted to execute a light and temperature cycling program, using the LED lights equipped in the unit and an add-on temperature control module. Once loaded, the units were not interacted with until the end of the experiment.

***3 conditions were used:***

**A** - 12/12 LD at 22 C for 2 days, DD at 26 C for 8 days
**B** - 12/12 LD at 22 C for 2 days, DD at 18 C for 6 hours, DD with 12/12 temperature cycling 18C/26C for 7 days, DD at 22 C for 3 days (Figure 1)
**C** - 12/12 LD at 22 C for 2 days, DD at 18 C for 8 days (Figure 2)

The flies movement was recorded using the live internal video-tracking software. Two measure were taken - one of distance travelled by each of the flies, and one of the pixel change per frame within each well (Thresholded Mean Square Difference, MSD). Sampling was done at 1 second intervals, with autoreference images taken every hour to account for changes in the plates (condensation, shrinkage, etc.).

The high sampling frequency allows for very detailed analysis of the movement of flies, and the 2 methods can potentially distinguish between gross movement, and 'on the spot' movements such as grooming and turns.


ZANTIKS
behaviour, simply

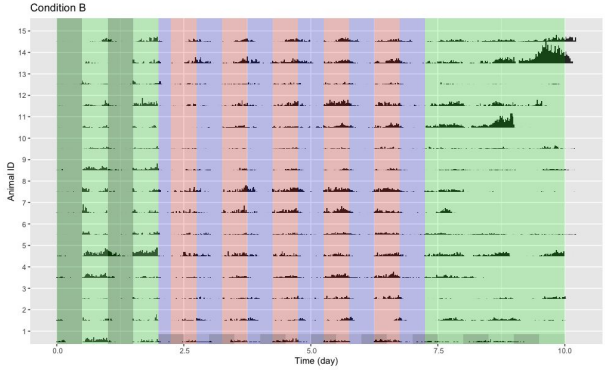


*Figure 1.* Days 0-2 (12/12 LD), Days 2-10 (DD). Colour legend: Green (22C), Purple (18C), Red (26C).

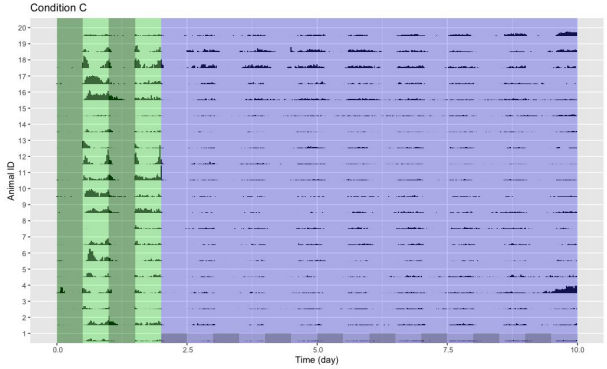


*Figure 2.* Days 0-2 (12/12 LD), Days 2-10 (DD). Colour legend: Green (22C), Purple (18C),

[1] Glaser, Franz T., and Ralf Stanewsky. "Temperature Synchronization Of The Drosophila Circadian Clock". doi:10.1016/j.cub.2005.06.056.
2] Geissmann, Quentin et al. "Rethomics: An R Framework To Analyse High-Throughput Behavioural Data". doi:10.1371/journal.pone.0209331

## Results

Data was analysed in R using the Rethomics [2] package and a custom script to import Zantiks data. All data was visualised using ggetho and examined to identify flies that died prior to the end of thermal cycling (day 7). For condition A (26C), none of the flies survived due to the medium drying out.

Curated data was visualised in bar tile plots (Figures 1, 2) and a periodogram was produced for both conditions (Figure 3). The data shows a consistent 24 hours period for both conditions (Figure 3). Condition B (Figure 1) shows a shift in the phase consistent with the 6 hour delay introduced between LD regime and temperature cycling, demonstrating that, in absence of a light cycle, the flies have adopted temperature as the zeitgeber. It can be seen that some flies have died prior to the end of the experiment in Condition B, accompanied by a spike in activity. Condition C shows consistent phases throughout the experiment, demonstrating light entrainment to be stable to temperature changes.

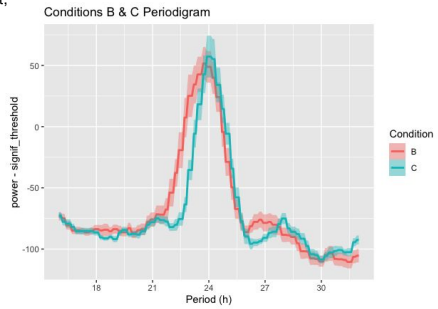


*Figure 3.* Periodogram showing average scores for each condition.

## Conclusion

Results show the capacity of the Zantiks unit to be used for circadian rhythm studies with both light and temperature as the zeitgeber without the use of external equipment such as temperature controlled chambers. The video tracking system provides clear observations of locomotive activity and is done on-board without the use of any external software or the need to store large video files, allowing the units to be used with little computational power.

Rethomics, an R package designed for circadian and sleep analysis, can be used with Zantiks data, but the customisable output format of the file can be fit to resemble the Trikinetics DAM format to be used with other analysis software built for DAM.