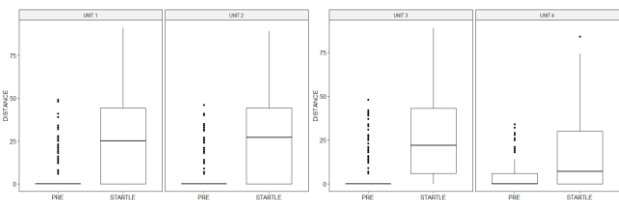


## *Drosophila adults*

- The plots (below) compare the 'PRE'-startle distance to the 'STARTLE' distance for each unit, both recorded over one second.
- Units 1 & 2 were situated in Location 1, whereas units 3 & 4 were placed in Location 2.
- Operator error led to inconsistent temperatures of Unit 4. As a result, only Units 1-3 were included in the ANOVA analyses.



- The plots (above) indicate the distance travelled in the second after the light-off stimulus was greater than during the 'PRE' second beforehand. This is reiterated by a very significant effect of type in the ANOVA (below), confirming that the startle response is effectively recorded by the video-tracking system
- Most importantly, the effect of unit was not significant, suggesting that distance travelled was recorded reliably across units.
- Ideally, we would not observe a type:unit interaction. However, we believe differences in experimenter handling may have contributed to the significance of this effect. We note that the type:unit effect is of a far lower order of magnitude than the type (startle) effect.

Drosophila melanogaster ANOVA				
	Sum Sq.	Df	F value	p
Intercept	425	1	1.61	.205
TYPE	24362	1	92.1	< 2.2e-16 ***
UNIT	857	2	1.62	.198
TIMESLOT	4814	11	1.65	.0774
TYPE:UNIT	2371	2	4.48	.0114 *
TYPE:TIMESLOT	12868	11	4.42	1.18e-6 ***
UNIT:TIMESLOT	5674	22	0.975	.493
TYPE:UNIT:TIMESLOT	22949	22	3.94	1.50e-9***
Residuals	1351952	5112		

## A Tale of Two Locations

We demonstrate replication of startling behaviour with *Drosophila* & mosquito larvae at 2 different locations, in 6 different units, on 2 consecutive days.

The Zantiks MWP unit is an automated and controlled environment, permitting standardisation of animal behaviour experiments. Using the integrated startle stimulus generation, we recorded distance travelled with the built-in real-time video-tracking.

### Location 1 Garden Office

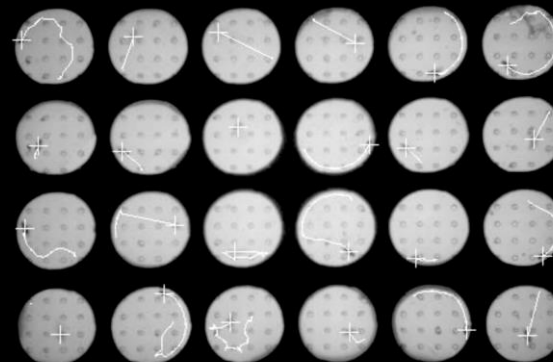
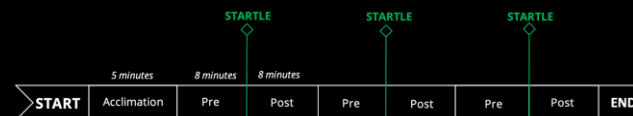


### Location 2 Farm Barn



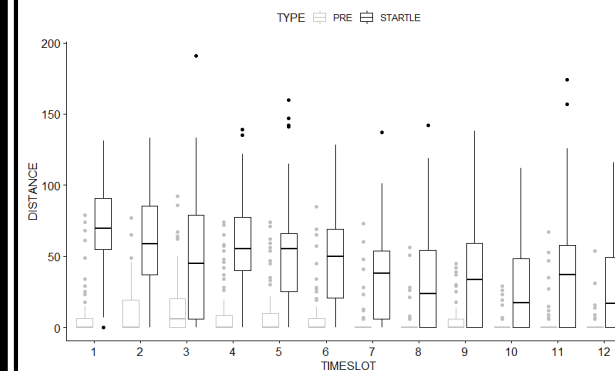
## Methods

- Startle responses of *Drosophila melanogaster* adults were induced by 15ms light-off stimuli using 530nm green LEDs, whereas *Culex spp.* larvae were startled with 464Hz, 500ms vibrations.
- 24-well plates of animals were run according to the procedure, depicted right. 2 plates of *Drosophila* & 2 plates of mosquitoes were at each location.
- Plates were run in 12 time slots: 6 on Day 1 (2x morning, 2x afternoon, 2x evening), 6 on Day 2.
- Plates of animals were swapped between locations from Day 1 to Day 2.
- Temperature was kept constant using the Zantiks temperature control add-on. Flies were run at 22°C, mosquitoes at 20°C.
- The integrated tracking recorded second-by-second distance travelled by each animal.
- Flies startle to the light-off stimulus by taking flight and mosquito larvae move downwards. The straight lines in the figure to the right depict the movement of flies via flight.



## Mosquito Larvae

- We ran 4 mosquito plates on 2 units, 1 at each location. Upon initial data screening, it became apparent that the well-plate holder accessory at Location 1 was not successfully transmitting the startle vibration due to manufacturing tolerance errors.
- Therefore, we focused our analysis on demonstrating replicability within one unit over time.
- The graph (below) shows a decrease in startle distance with time.



Mosquito larvae ANOVA				
	Sum Sq.	Df	F value	p
TYPE	592630	1	827	< 2.2e-16 ***
TIMESLOT	111042	11	14.1	< 2.2e-16 ***
TYPE:TIMESLOT	50353	11	6.39	1.84e-10 ***
Residuals	1220680	1704		

- As predicted, mosquito larvae exhibited greater movement in the second after the vibration stimulus than in the 'PRE'-vibration second, again confirmed by the significant main effect of type (above).
- We note the significant effects of timeslot and type:timeslot. Graphical analysis of these effects suggests that larvae startled less over time. This may be due to a decline in the general health and vitality of subjects.