



**MOSQUITO LARVAE (*Culex Spp.*)  
STARTLE RESPONSES TO  
VIBRATION STIMULI**

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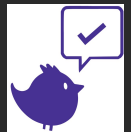
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# WHY STARTLE?

- Rapid, defensive response
- Facilitate escape from a negative experience or hazardous exposure = *ESCAPE RESPONSE*
- Contributes to a species' survival
- Reliable behaviour



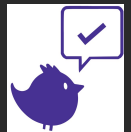
# STARTLING IN MOSQUITO LARVAE (1/3)

- **Perform stereotypical escape responses**

1. *Resting at surface*

2. *Dive with onset of danger*

3. *Return to surface*



# STARTLING IN MOSQUITO LARVAE (2/3)

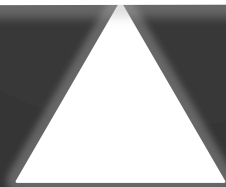
## TRADE OFF

### ESCAPING POTENTIAL DANGER

- e.g. predation,
- e.g. hazardous exposure

### ENERGETIC COSTS

- Increased mortality (*Lucas & Romoser, 2001*)
- Impacts development (*Tuno et al., 2004*)



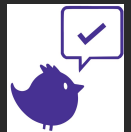
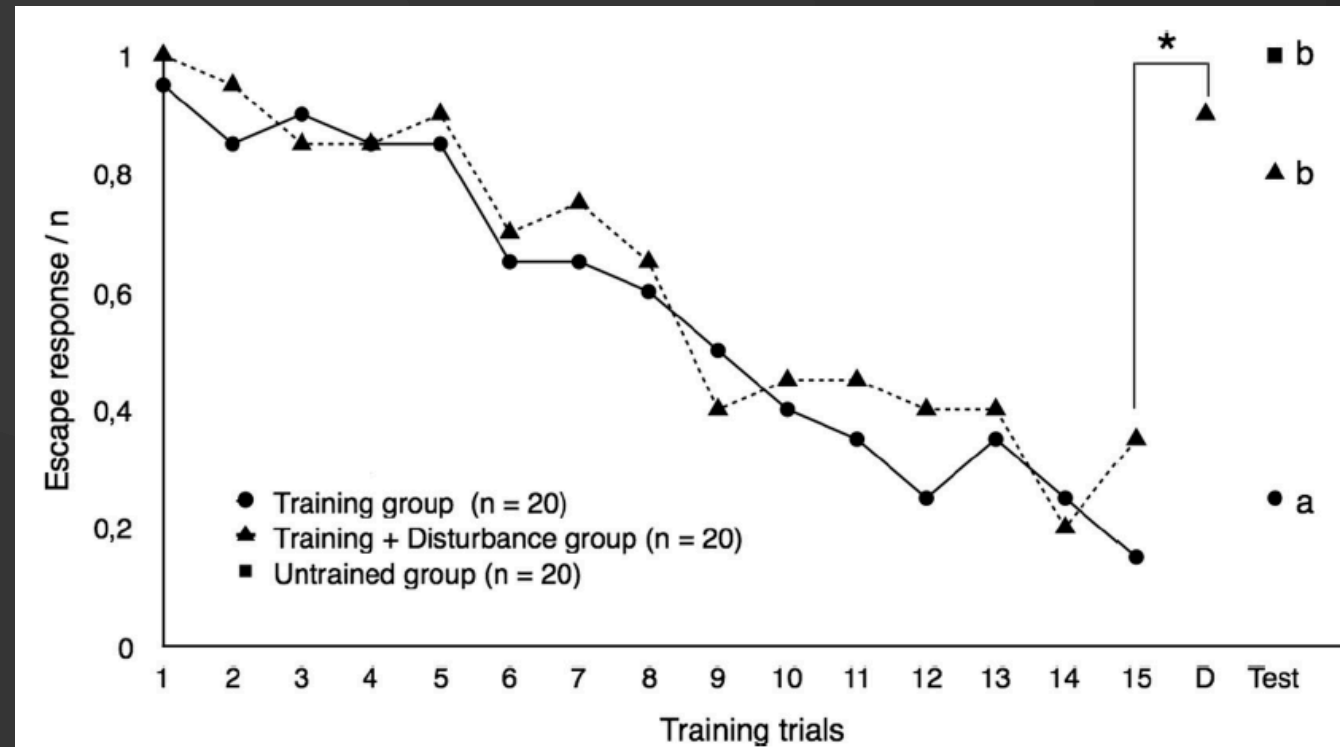
# STARTLING IN MOSQUITO LARVAE (3/3)

## Potential solution = HABITUATION

➤ Progressive and reversible decrease in a behavioural response resulting to a repeated stimulus (*Rankin et al., 2009*)

- Stimulus-specific
- Distinguished from sensory adaptation & effector fatigue

- Already demonstrated to a visual stimulus in *Aedes aegypti* larvae (*Baglan et al., 2017*)



# OBJECTIVES



- Investigate habituation of *Culex spp.* larvae to a vibration stimulus
  1. Characterise optimum vibration frequency
  2. Characterise optimum vibration duration
  3. Determine if habituation occurs



# MATERIALS

## ANIMALS

- 4<sup>th</sup> instar *Culex spp. larvae*

- Wild reservoir

- Env day-night cycles & temperatures

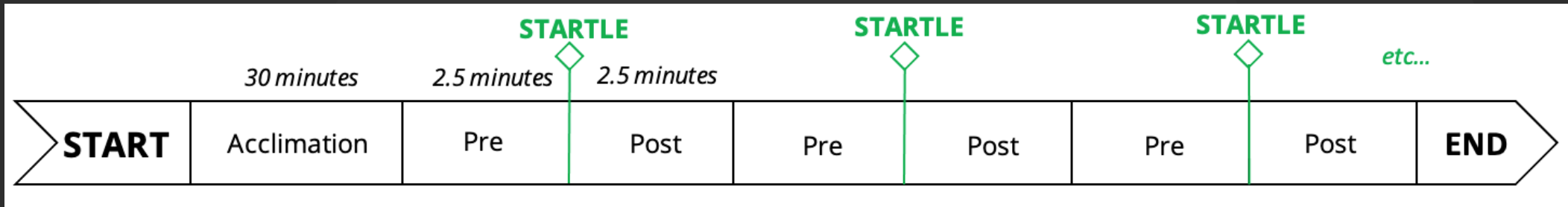
# ZANTIKS MWP UNITS



- Automated & controlled environmental set-up
  - White overhead light, 20°C temperature
- Larvae in 24-well plates
- Unit records distance travelled second-by-second with integrated video tracking



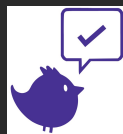
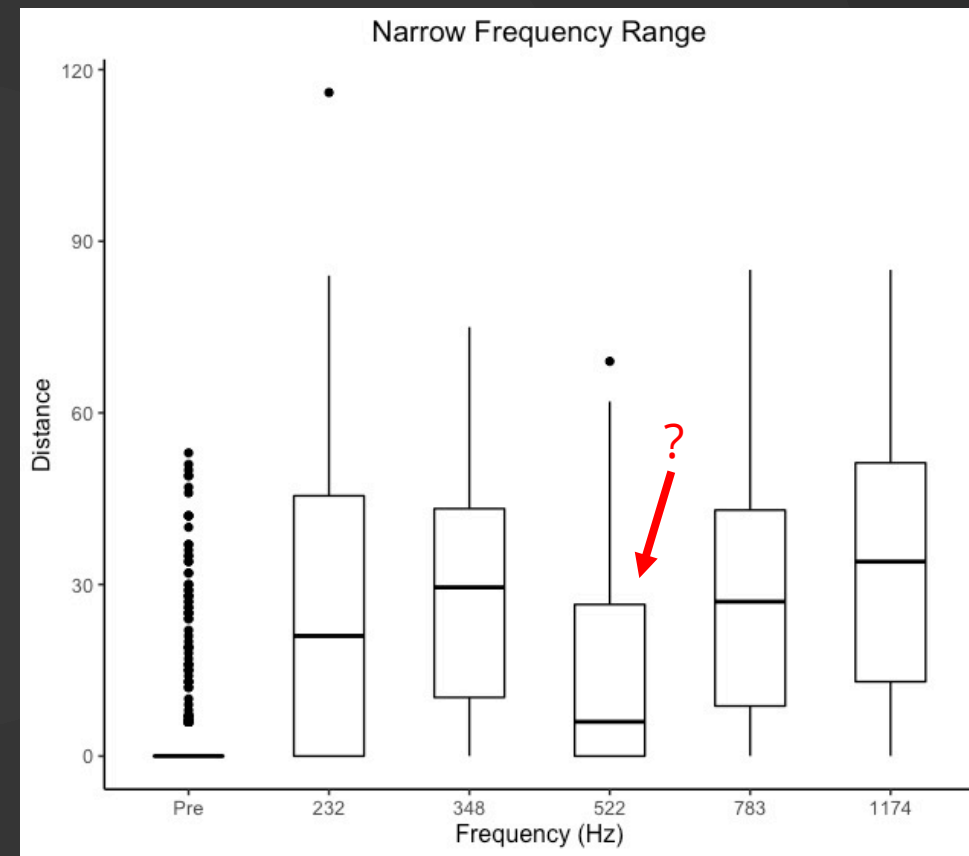
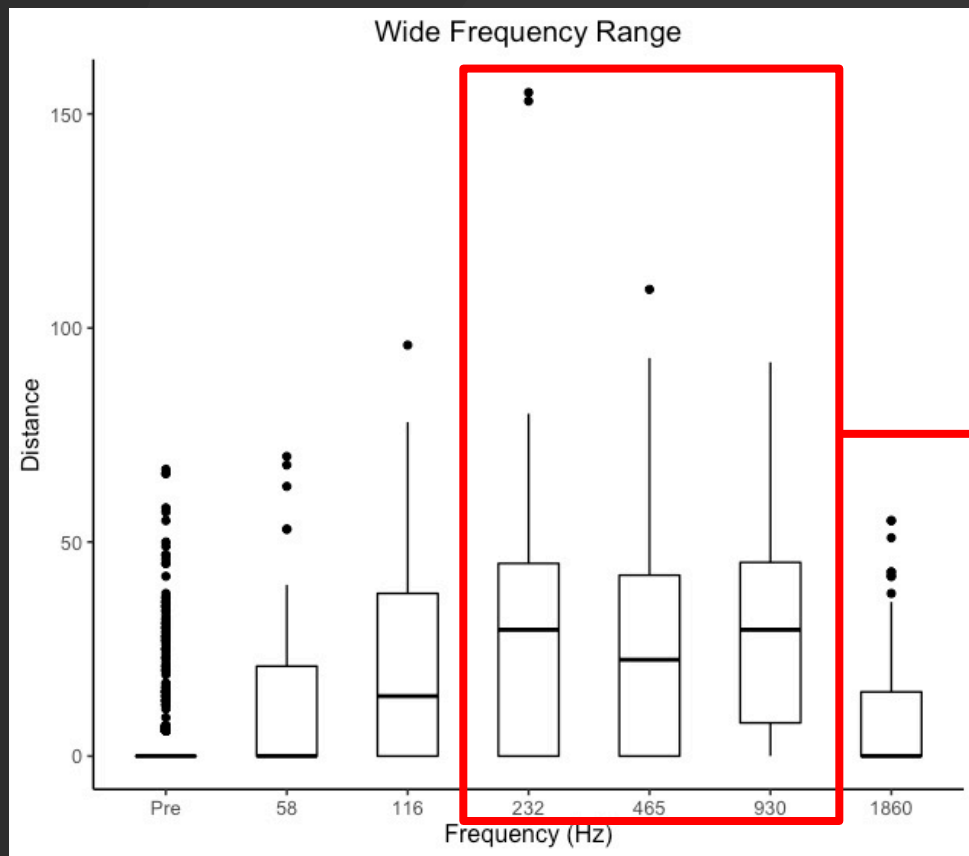
# METHODS



	FREQUENCY	DURATION	HABITUATION
<i>Aim</i>	Establish vibration frequency resulting in greatest escape response	Establish cut-off of vibration duration for escape response	Determine if habituation to vibrations occurred
<i>Variables</i>	Ascending freqs from 58-1860Hz. 500ms duration	Ascending duration from 2ms to 550ms, 900Hz frequency	4 blocks of 15 startles at 10s intervals, 5 mins between blocks



# RESULTS (1/3): FREQUENCY



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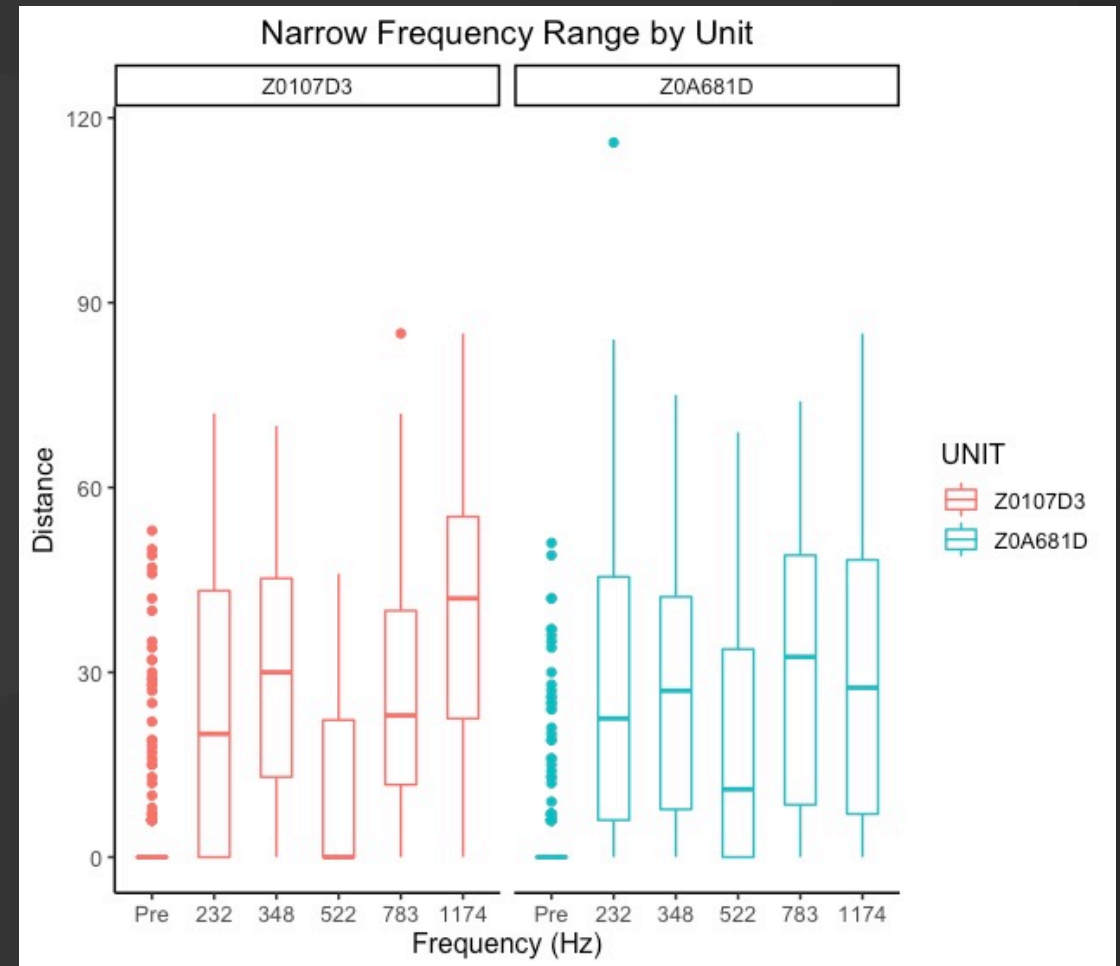
- Consistent drop at 522Hz in both units
- Harmonics within unit?
- Still strong significant effect of **TYPE** (vibrations vs Pre) in ANOVA analysis

Narrow Frequency ANOVA

	Sum Sq	DF	F value	Pr(>F)
Intercept	3534	1	12.6650	0.000391 ***
<b>TYPE</b>	<b>75577</b>	<b>5</b>	<b>54.1648</b>	<b>&lt; 2.2e-16 ***</b>
UNIT	2	1	0.0059	0.939034
TYPE:UNIT	4381	5	3.1397	0.008102 **
Residuals	264552	948		

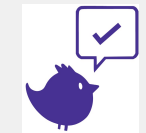
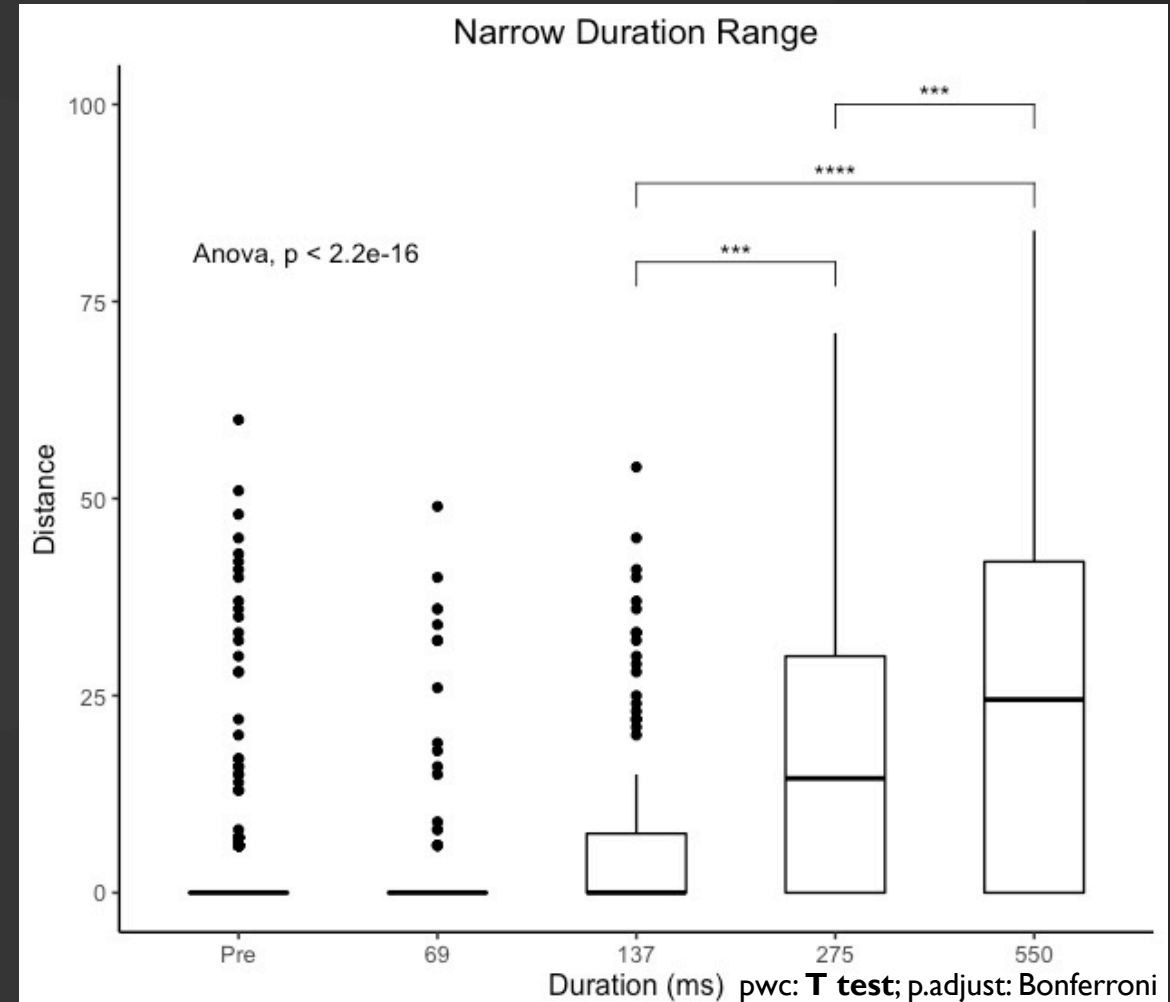
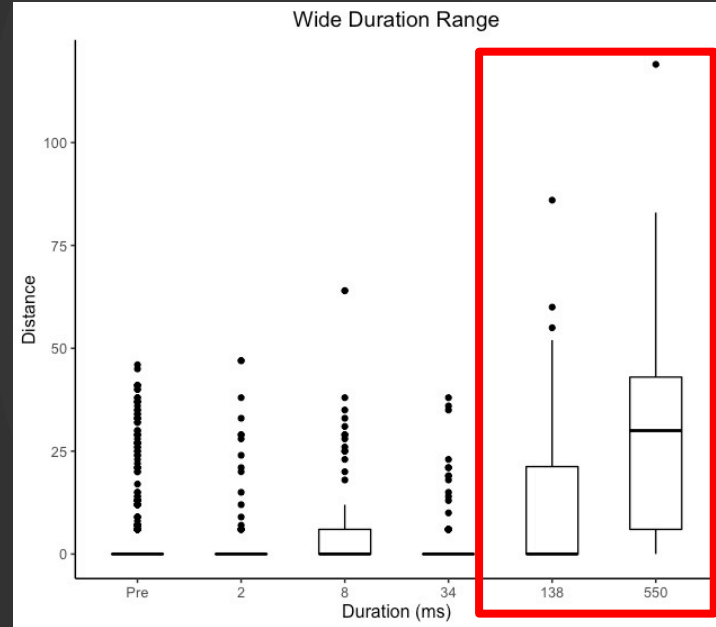
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- Decided on 900Hz for optimum frequency

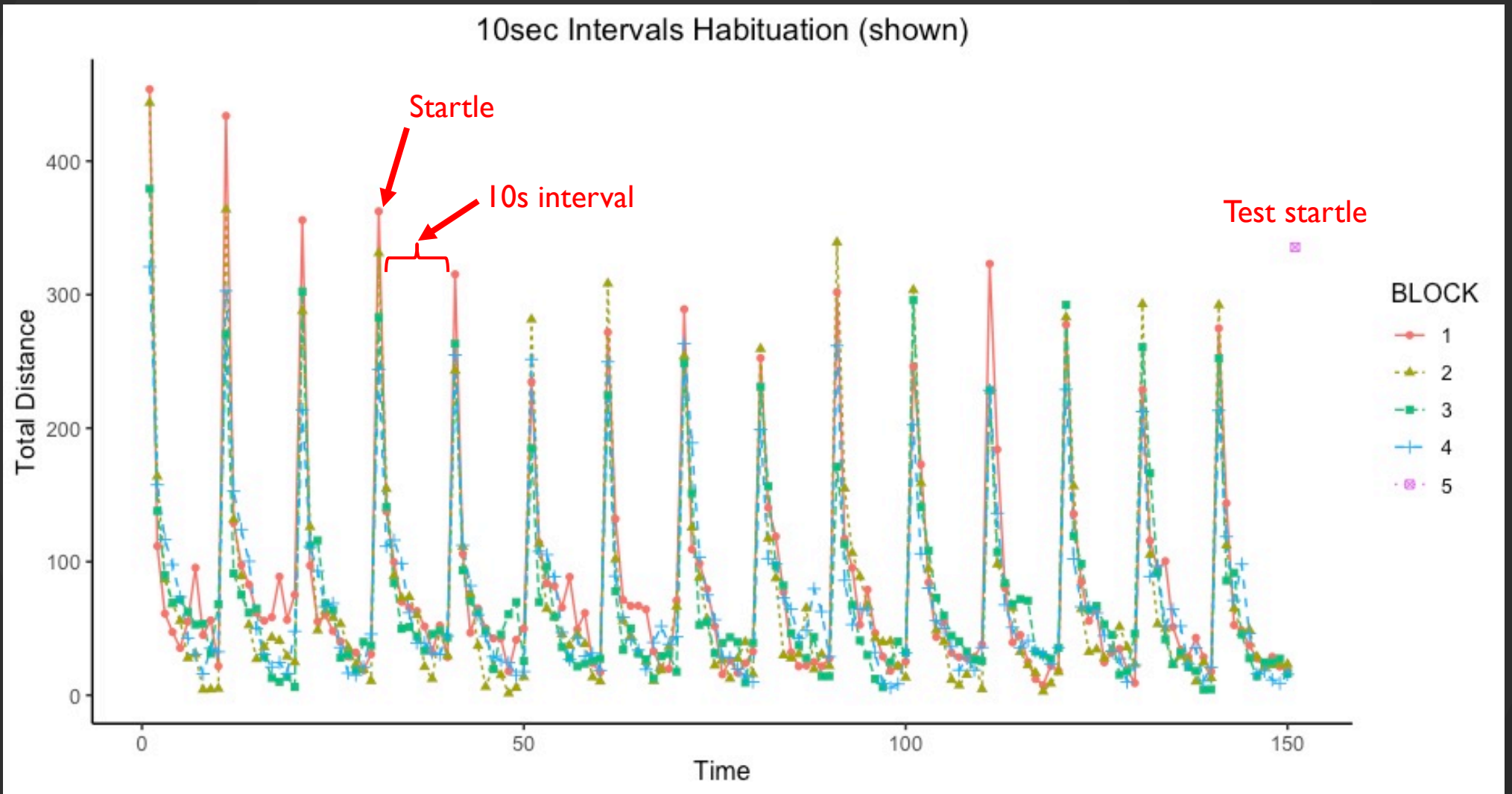


# RESULTS (2/3): DURATION

- 900Hz vibrations of varying lengths
- **1<sup>st</sup> expt** = wide range
  - 2ms to 550ms
- **2<sup>nd</sup> expt** = narrow range
  - 69ms to 550ms
- Strong significant effect of type
- Pairwise T test comparisons

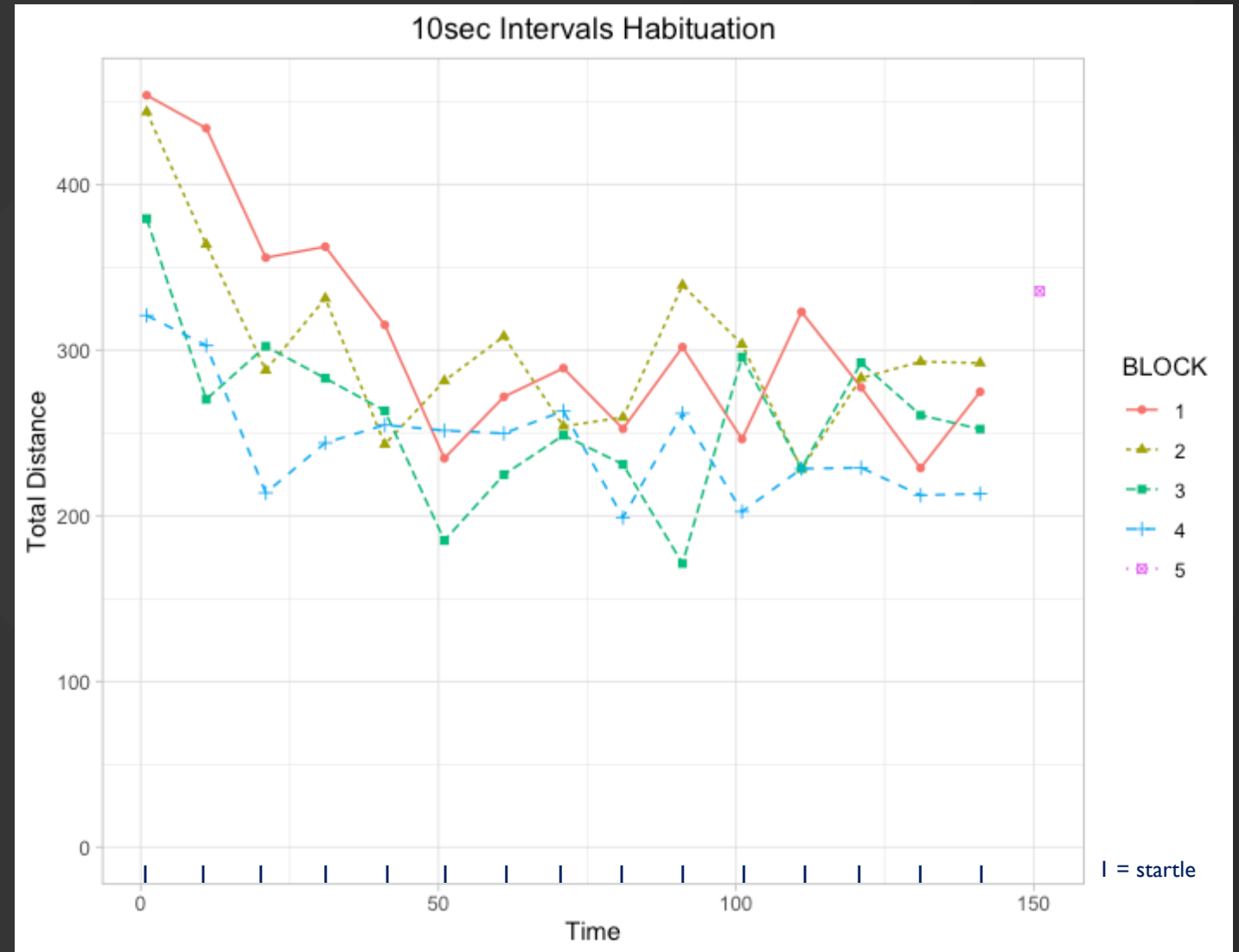


# RESULTS (3/3): HABITUATION



# RESULTS (3/3): HABITUATION

- Downward trend for all 4 blocks
  - Not complete – only ~30% decrease
- Test startle does not elicit starting distance
  - Fatigue? Memory?
- Need alternative stimulus modality
  - Accounts for effector fatigue or sensory adaptation
- Unable to replicate Baglan et al's 2017 results



# DISCUSSION

## **1. Successfully characterized vibration frequency & duration to elicit startling**

- Duration cut-off has potential use in Pre-Pulse-Inhibition (PPI) studies

## **2. Used these values to investigate habituation**

- Inconclusive results, further analysis required

## **3. Method has potential for more sophisticated environmental effect testing**

e.g. eco-toxicology

## **4. Relevant with predicted increase of vector borne diseases with climate change**

*(European Commission Joint Research Centre, 2018)*



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