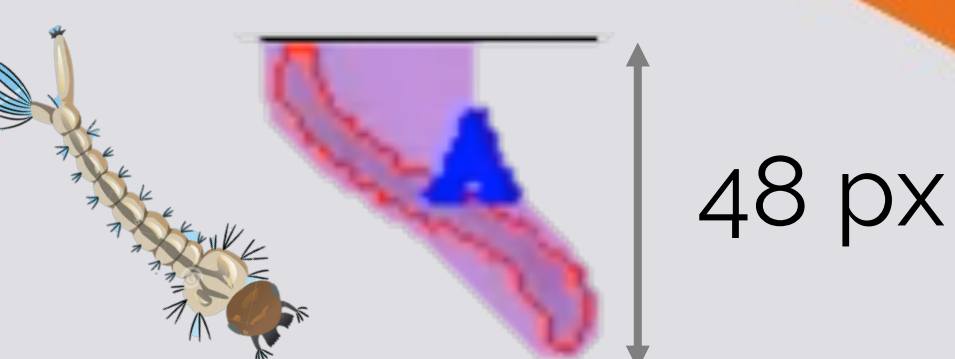


# Aedes aegypti

## A tracking system to assess activity and learning in mosquito larvae

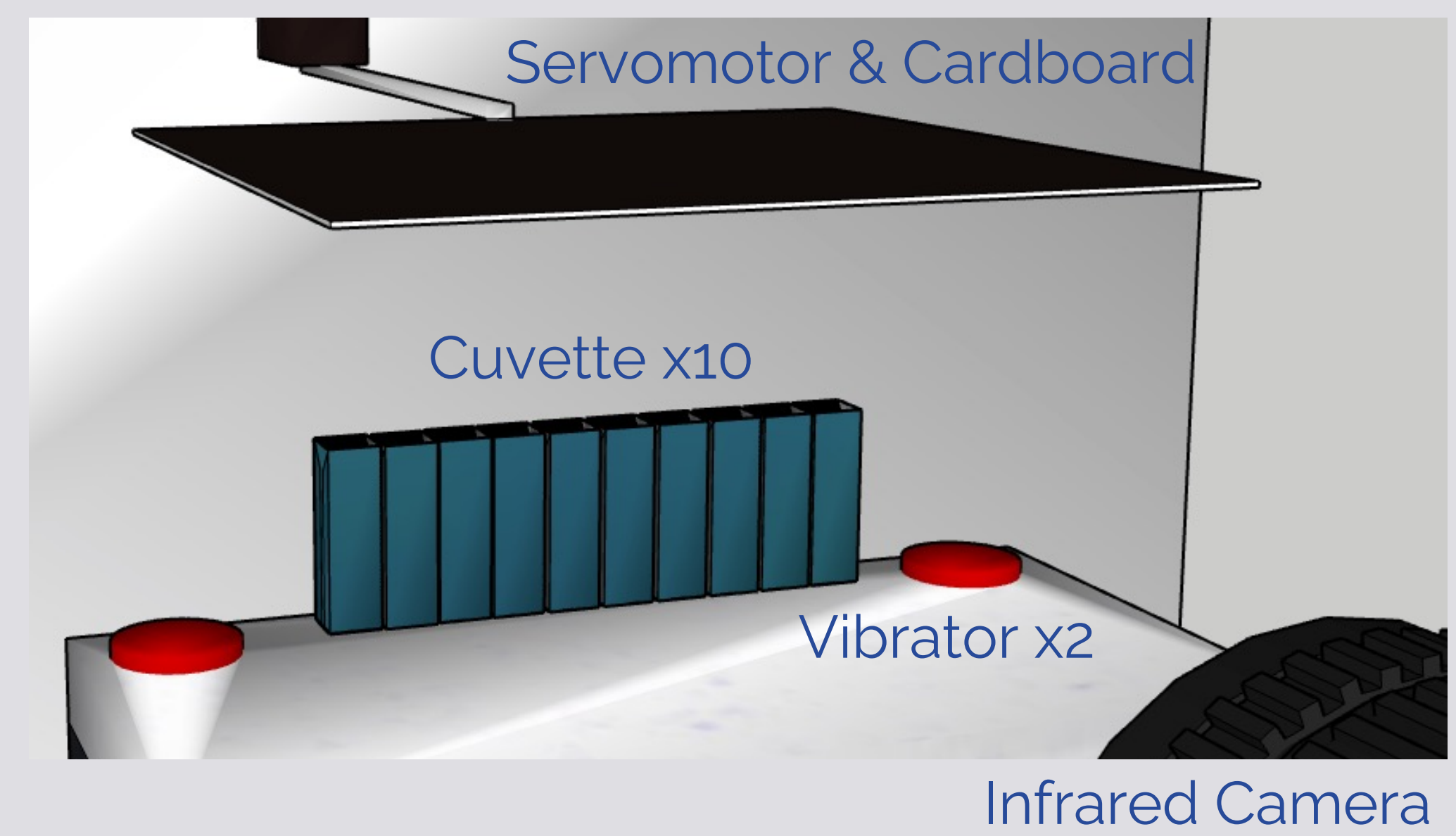


### Introduction

When **mosquito larvae** face a **predator**, represented as an **aversive stimulus**, they **dive** away from the water surface. Repeated stimuli lead larvae to **habituate**, *i.e.* they no longer dive away.

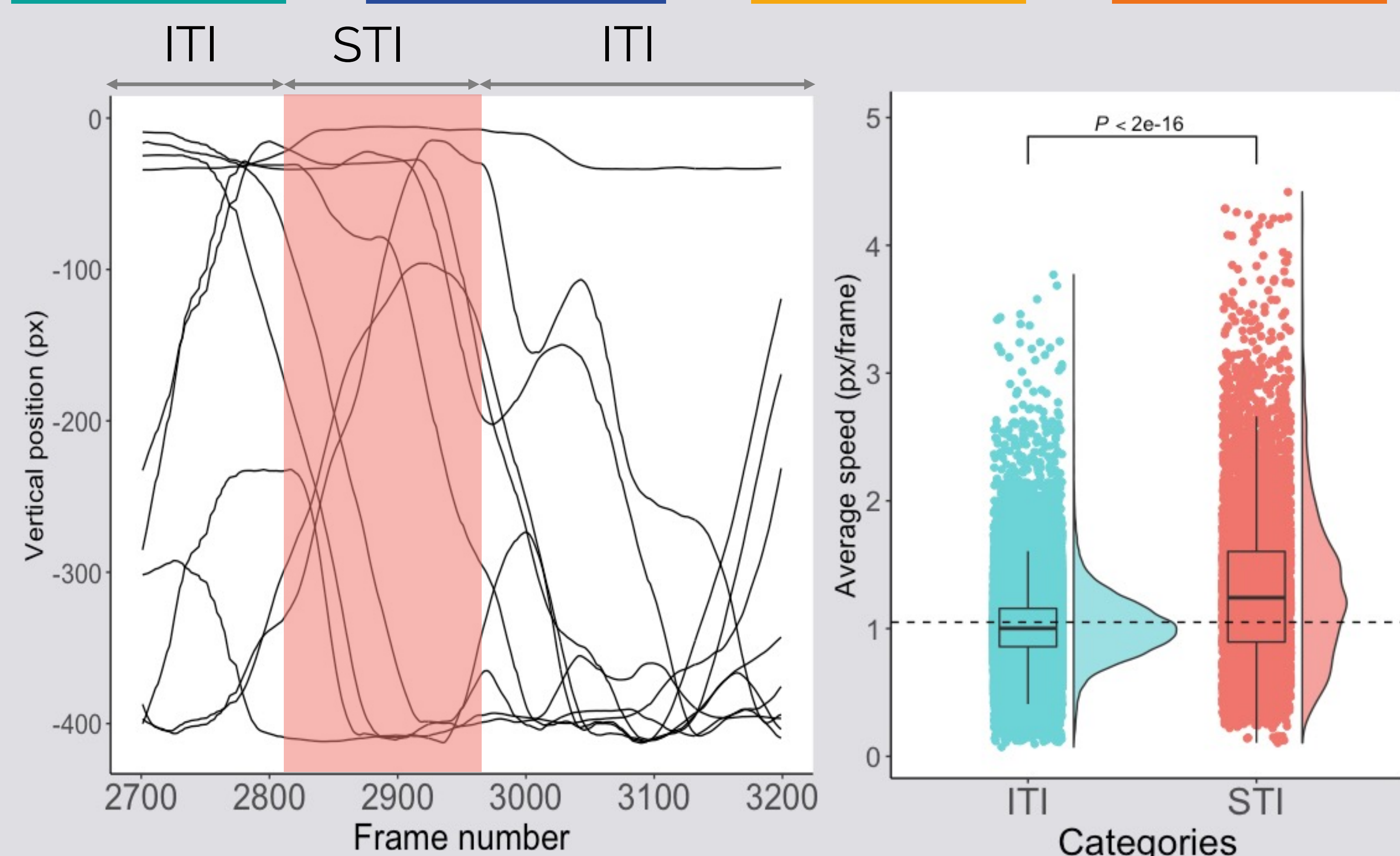
**Habituation**, a non-associative type of **learning**, has so far been studied with a bioassay using a **binary variable** (yes/no), which may hide variation in larvae's responses.

Here we present a multi-object **tracking system** to record behaviour allowing **quantitative measurements** of individual response.



### Activity tracker

An **infrared camera** recorded larvae's movements. Trajectory was extracted using **adaptive threshold** from **each frame**, and subsequently analysed. Larvae's individual **distance** corresponded to the sum of **displacements** (in px) during **stimulus presentation** (6 sec) and **average speed** to the displacement **per frame**.

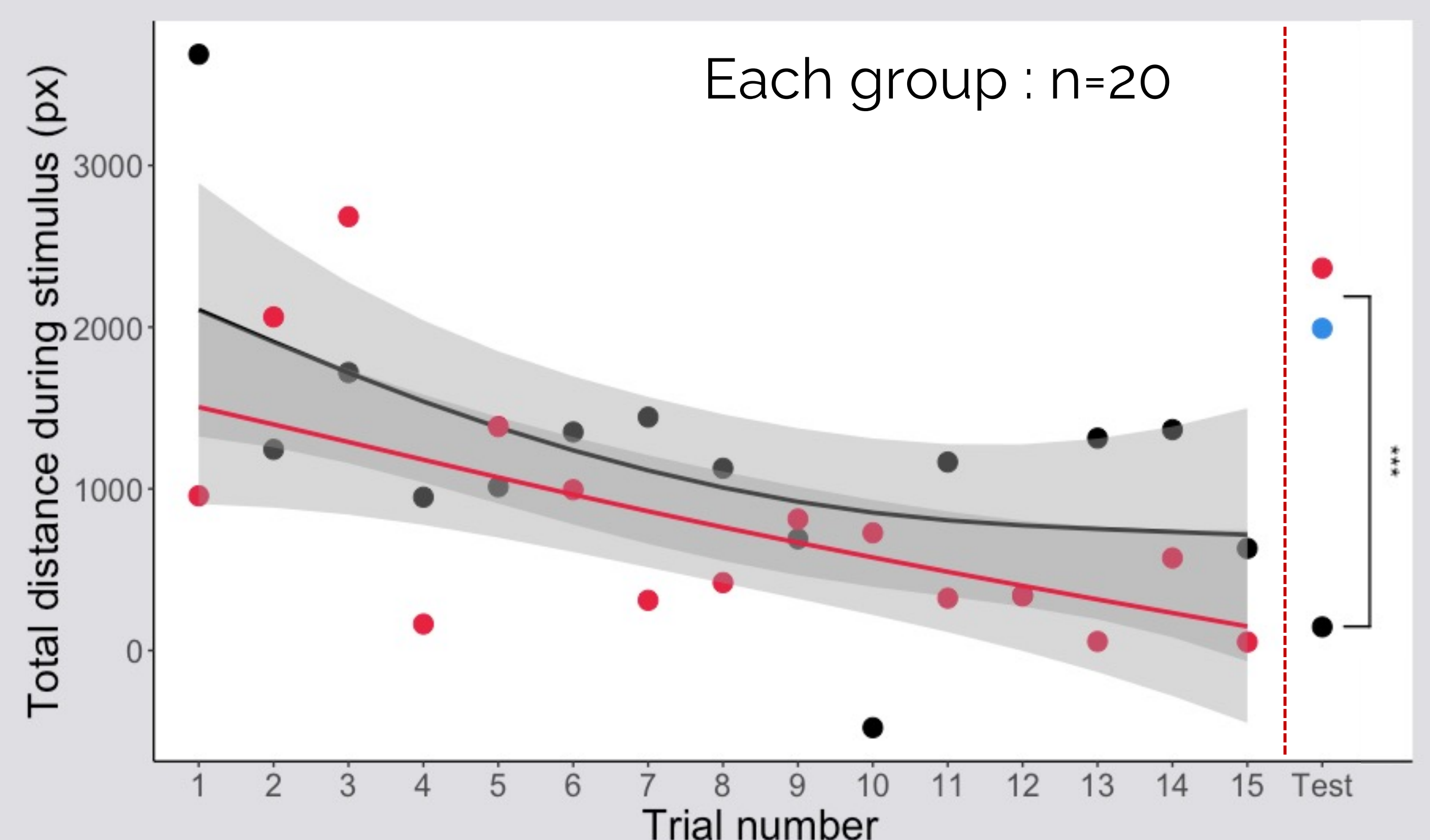


During the **first** stimulus presentation (**STI**), larvae tends to dive away and **reach the bottom** of the cuvette. Larvae's speed during **intertrial interval** (**ITI**) was lower than larvae's speed during **stimulus presentation** (**STI**).

### Learning assessment

Fourth-instar **Aedes aegypti** larvae were separated in 3 groups and tested on individual **cuvettes**. An electronic-controlled cardboard projected its **shadow** over the surface, inducing individual **diving response**.

Group   Phase	Familiarisation	Training	Disturbance	Test
● Experimental	30 min	15 trials	Rest	1 trial
● Control n°1			Shadow 6 sec	
● Control n°2		Rest	Vibration 6 sec	Shadow 6 sec



- After 15 trials, the response was **lower** than in the 1<sup>st</sup> trial.
- Control n°1 showed that **disturbance**, after 15 trials, **reversed habituation**.
- Control n°2 showed that **rested** larvae that did not face aversive stimuli presented **high response** to the test.

### Conclusion

High-throughput quantitative analysis of **behaviour kinematics** of mosquito larvae improves **replicability** by avoiding **observer's bias** and allowing **reproducible workflows**. The **absence of experimenter** during the protocol may also cut down **processing time**.

Diminution in larvae's response was due to **habituation** and neither to effector **fatigue** nor **sensory adaptation**.

This multi-tracking system can be used to study the **effect of environmental factors** (e.g. chemical pollutants) on **activity** and **cognitive abilities** in mosquito larvae.

