

## **SOP Test 5 - Measuring Novel object-Boldness in Adult Zebrafish**

### **1.0 Purpose:**

1.1 The purpose of this standard operating procedure (SOP) is to measure the boldness level of mature adult zebrafish by recording the amount of time spent close to a novel object in the tank, the “model predator”.

### **2.0 Scope:**

2.1 This protocol is suitable for individuals who have been trained in zebrafish handling and care.

2.2. Any queries, comments or suggestions, either relating to this SOP in general, or to a specific problem encountered during the procedure should be addressed to the head of the AMATrace behaviour platform, Dr. Laure Bally-Cuif.

2.3. Any deviation from this protocol should be addressed to the head of the AMATrace behaviour platform, Dr. Laure Bally-Cuif.

2.4. All zebrafish should be kept, propagated and handled in accordance with the institutional guidelines on animal safety. Please also keep in mind the principle of replacement, refinement and reduction.

### **3.0. Safety Requirements**

3.1. General laboratory safety procedures should be followed, which include: no eating, no drinking and no applying of cosmetics in the work area. Laboratory gloves must be worn at all times in the work area unless the protocol specifically notes otherwise.

### **4.0. Associated Documents:**

### **5.0 Notes:**

5.1. This protocol is designed to compare animals that have been raised under similar conditions. Fish density, feeding regimes and age will play a significant role in modifying the expression of boldness.

5.2. Adult zebrafish do not show sex-specific difference in approaches to a predator when measured using this protocol. Fish of both sexes can thus be combined in the experiment.

5.3. Environmental factors can play a significant role in changing the expression of boldness. Behaviour should be recorded in a silent behavioural room with minimal experimenter disturbance. Lighting, temperature and time of day should be kept constant during testing.

## **6.0 Quality Control:**

6.1. The boldness tank and model predator should be cleaned with both 70% ethanol and then fresh system water before starting the experiment. A similar amount of water should be placed in the tank for each recording session.

6.3. Fish should be raised in groups of a defined number (20-25 fish in a group) from larval stages onwards.

6.4. Fish need not be habituated before analysis in the behavioural setup. Fish can be brought to the testing room in their home tanks immediately before analysis begins.

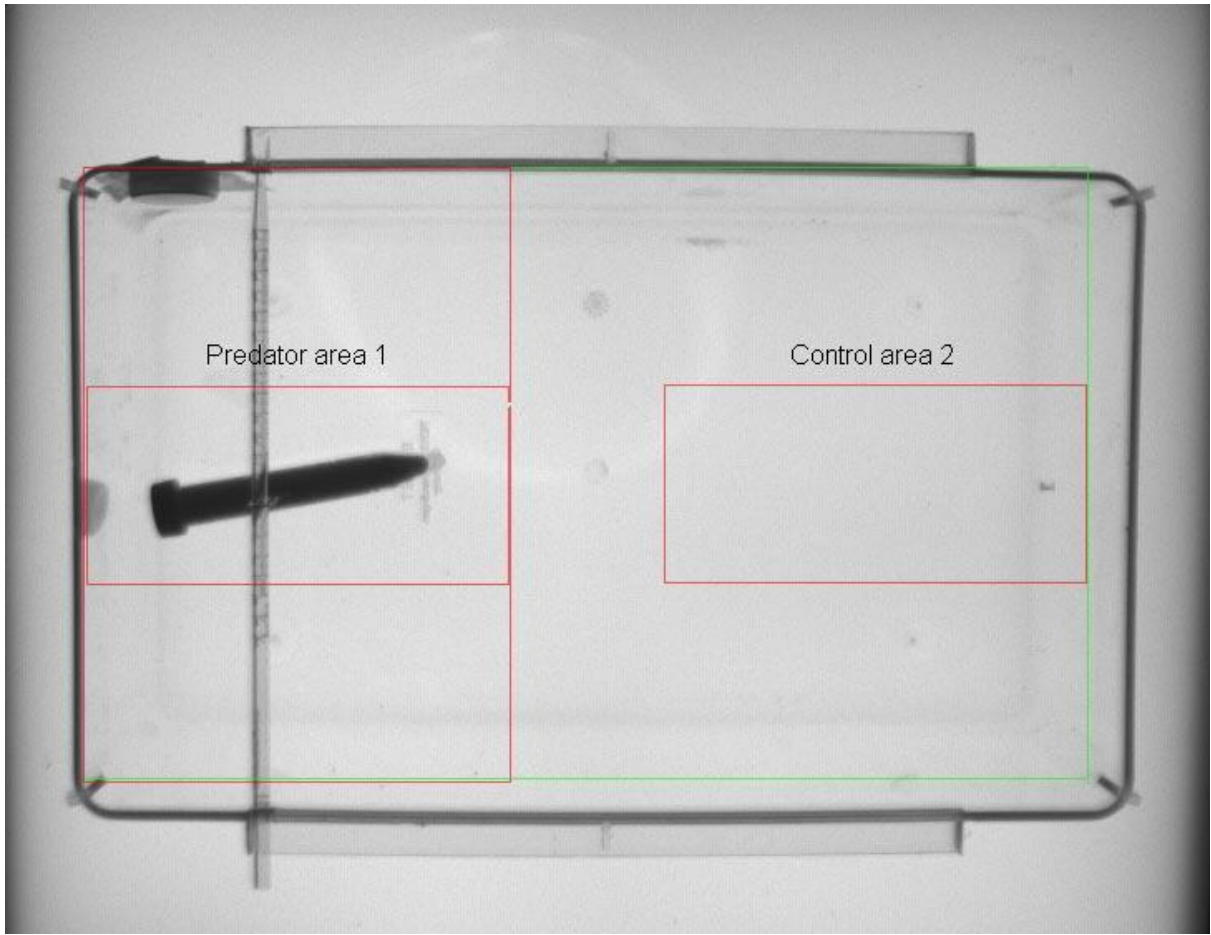
6.5. Care must be taken that the tank is illuminated evenly without shadows or reduced light at the corners. The model predator should hang in the middle of the water column and not touch the bottom of the tank.

## **7.0 Equipment:**

7.1. The boldness setup contains three parts:

a) A computer recording system that contains VideoTrack software from ViewPoint S.A.

b) A standard large plastic fish tank (30 x 30 x 50 cm) filled with 20L of water. A plastic model predator is made by filling a 15ml Falcon tube with dark blue modelling clay. The model is suspended from a 5ml plastic pipette which is balanced across the top of the tank. A thin metal wire is attached to the middle of the model, allowing it to be suspended in the middle of the water column at one end.



c) A mounting apparatus, made of a floor that allows infra-red illumination (ViewPoint S.A.), with a video camera and a uniform white light source arranged about it.

#### **8.0. Supplies:**

Zebrafish for analysis, 12 – 15 for each genotype or treatment group.

(Optional) drugs or chemicals to modify behaviour,

System water to fill setup.

#### **9.0. Procedure:**

9.1. Adult fish are raised to adulthood in groups of 15 or more. Immediately before testing, fish are transported to the behaviour room (ideally in their home tank, or if not possible then in a 10L plastic mouse cage).

9.2. The predator boldness tank is filled with system water up to a depth of 10cm and the model is suspended from the pipette at one end. The observation tank is lit from both beneath (providing Infrared light for camera) and above by a circular white-light light bulb.

9.3. The VideoTrack programme needs to be started before behaviour is recorded. Switch on the computer and double-click on the ViewPoint space rocket icon. Launch the "Videotracking" option within the VideoTrack menu. In the detection threshold menu, set animal colour to black and detection threshold to 12 (this value may need to be calibrated for each new experimental setup). Make sure that the programme is able to track the fish smoothly, without either miss-tracking or losing the animal.

9.4. In order to measure time spent near to the predator, several areas need to be delineated within the videotrack programme. Draw one area ("area 1") that encompasses the entire tank, a second "area 2" around the model predator that is one fish body-length larger than it.

#### **Measurement Process:**

9.5. Fish are placed singly into the predator boldness setup using a standard fish net. Their position in the tank is then videotracked for the next 10 minutes. Fish are then placed in a holding tank, or back in their home tank following testing.

9.6. The VideoTrack software should be started next. Choose "Execute" from the Experiment menu and input an name for the experiment – e.g. WT 1. Press the background and then start buttons.

9.7. Record the behaviour of fish from all treatment groups or genotypes separately.

#### **Results Analysis:**

9.8. The results should be exported into Microsoft Excel and the data points analysed. For predator boldness, the amount of time spent in area 2 (near to the model predator) can be compared for each genotype or treatment group.

9.9. Plot the data as a histogram, and use appropriate statistical tests to compare the different groups (either a Student's *t*-test, or ANOVA followed by an appropriate post hoc test).

#### **10.0 Supporting Information:**

#### **11.0 History Review:**

#### **12.0 Emergency Procedures:**