Algoma University Animal Care Committee	
#AU 0038 BEHAVIOURAL TESTING WITH FISH	
Issue date: March 18, 2025	Last revised: March 2025

1) Purpose

This Standard Operating Procedure (SOP) describes acceptable procedures for the handling of fish to measure their response to cues in their environment. There are many types of mazes, but fall into two categories; (1) choice mazes, where the animal is presented numerous arms or areas from which to move into; and (2) open tank mazes, where the cue is (usually) present in the entirety of the tank. There are two categories of cues that can be used; (1) physical cues, which can include light, temperature differences, visual cues, among others; and (2) chemical cues, which can include odours and differences in pH, etc.

The size of a maze as well as the timing of the trial is specific to each experiment, and is based on size of the fish, how skittish the fish species is, and the nature of the cue. When deciding the size of the maze, it is essential that the maze is wide enough that the fish can easily turn and move in the maze, and high enough so the fish cannot jump out. All of these play a factor in the statistical power of the behavioural experiments. Each combination of fish, odour, and behavioural maze needs to be tested with preliminary trials before being employed in any research project to ensure the trials are safe and effective.

This SOP outlines how to handle the animals before and after the trial, and what to look for in terms of animal health while the trial is being conducted.

2) Policy

CCAC guidelines and regulations

3) Responsibility

Principal investigator, their research staff, and their student investigators

4) Training Required

Animal Research Ethics

Handling/training in the relevant species

Training specific to the type of maze used

Bio-methodology training in the relevant technique and species

5) Materials Required

Behavioural mazes (see pictures below for examples of mazes)

Net

Transfer container

6) Procedures

1) Fish are captured from their source tank using a net and placed into a transfer container with appropriate water
2) Fish are placed into the maze
    a) For choice mazes, there will be a defined acclimation zone where the fish will acclimate. This zone will confine the animal behind a gate (or multiple gates) to ensure the animal does not have access to the various arms of the maze
    b) For open tank mazes, the animal is either placed into a chamber to be released or directly into the tank
3) The animal is allowed to acclimate for an appropriate amount of time, this is usually between 5-15 minutes. This acclimation allows the animal to calm post transfer, the length of which is dependent on species
4) Cue administration
    a) For a choice maze, a chemical or other cue (light, warm or cold water, etc.) is administered to one arm, and a control cue is administered to the other. Some mazes will use multiple cues across multiple arms.
5) Cue dispersal
    a) For chemical cues, the cue will be allowed to disperse through the arm of the maze or through the tank depending on the maze type. The length of time this takes varies based on the volume and temperature of water in question. The length of time to diffuse through the maze arm or tank is determined using a dye trial experiment prior to working with fish, but normally takes 2-5 minutes. A dye trial involves adding food dye to the maze (without a fish present) to see how long it takes the cue to disperse.
    b) For physical cues, some are immediate, like light and the presence of a predator, some take a while to disperse, such as the addition of warm or cold water. Dispersal time can be estimated using dye trials as described above if something is physically added to the maze.

6) Behavioural monitoring. If the animal is held by gates in an acclimation zone, monitoring can be done as soon as released. Data that can be collected include time spent in each arm, time spent moving, time spent motionless, etc. It is recommended that videos are recorded to allow for the measurement of many behavioural endpoints. Monitoring can be from 2 minutes to hours, depending on the cue used and the endpoints measured. Software like ToxTrac can also be used to maximize the data collected.
7) Once the trial is over, the animal is removed via a net, transferred to the transport container, and returned to holding. The animal should be inspected for any injury prior to being placed back in their holding tank.

Skin extract production

Some cues are produced from fish themselves, one of these is the skin-damaged released alarm cue. The method to produce this cue is as follows:

1) Euthanize a fish with buffered MS-222 (250 mg/L MS-222). (NOTE: if you are using fathead minnows do not use a fish in breeding condition)
2) Thoroughly rinse the fish with water to remove any residual MS-222.
3) Remove skin from both sides of the fish and scrape away muscle or tissue from the skin.
4) Measure skin surface area and place in a petri dish with 5 mL of river water.
5) Chop the skin using scissors for 10 minutes to create homogenate.
6) Filter the mixture (can use a paper filter or filter fibre).
7) Calculate how much water must be added to bring the final concentration to bring the final concentration to 1 cm^2/100 mL of water (10 cm^2/L).
8) Rinse the petri dish and pass the water through the filter.
9) Filter water through a clean (i.e., new) filter to use as the blank cue.

7) References (if applicable)

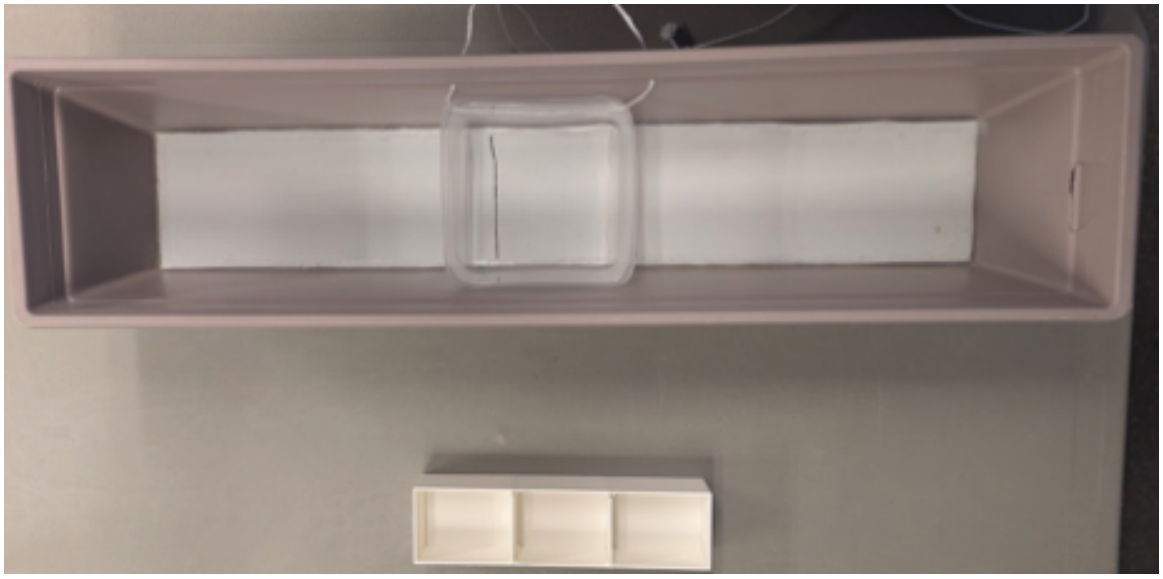

Figure 1: Picture of two sizes of trough or I-mazes. The central area is the acclimation zone where the fish can acclimate to the maze conditions (note the different ways to contain the fish in this portion of the maze), and two distal arms where cues are administered. The appropriate maze to use depends on the fish, for example the top maze is used for young of the year sturgeon, while the bottom maze is used for minnows.

Figure 2: A box maze. The fish would be held in the acclimation chamber, which is on the left hand side of the picture. The rest of the maze is bifurcated, resulting in two arms to choose from.

REVISION HISTORY		
Revision #	Revision Date	Summary of Changes
1	March 18, 2025	ACC accepted with no revisions
2		
3		
4		