Aggression (Adult zebrafish)

The aim of the experiment is to measure the aggression levels of adult zebrafish, by measuring the interaction with their mirror image (mirror-induced stimulation, or MIS).

Fish are placed separately into the aggression setup and their behaviour is recorded using a high-speed videocamera (set to 100 frames per second). On the recording day, fish are placed singly into the aggression setup and their reaction to the mirror is recorded for 5 or 10 minutes. The data is stored as AVIs. Films are then replayed in Labwatcher (from Viewpoint Lifesciences; other software could be used for this step) at half the normal speed to make analysis more accurate. Films are manually annotated by pressing keyboard buttons to record the number of bites; tail thrashes and pushes against the mirror.

Experimental setup

The aggression tank is custom made of glass. Three walls of the tank are covered in a white opaque material (white craft rubber, Ref 6 2245 500 from Glorex). The craft rubber is inert and does not distract the fish. A mirror placed outside of 4th (clear) wall, offset at an angle of 22.5 degrees.

The tank is 15 x 10 x 30cm and filled with system water at ambient room temperature (25C). The tank is lit from both below (infrared light) and above (white light). All experiments were conducted in the afternoon (1pm onwards). The video camera (including an infrared filter) was bought from PointGrey Research Inc. and the films recorded using Videotrack from Viewpoint. Other cameras and software programmes may be used as appropriate.

Experimental procedure

1) Care is needed in order to prepare the fish before recording aggression. The fish should be handled in a fish net for one week prior to starting the experiment. Fish are gently caught, retained in the net for a few seconds and then released into a second fish tank, in order to reduce the stress associated with moving between tanks.

2) On the night before recording aggression, the fish should be placed in a home tank with white walls (in order to habituate the fish to the white walls of the recording tank).

3) Fish are placed singly into the aggression setup and their behaviour is recorded for 5 or 10 minutes. If a fish freezes for more than 30 seconds during the recording period then it should be excluded from the final analysis of results.

4) The films should be analysed blind (so that the genotype or treatment of fish is not known to the observer) and by two independent observers. The accuracy of their results can be judged using the Pearson correlation.

5) The results are manually copied into Microsoft Excel for analysis. Parameters that can be compared include number of bites, pushes and tail thrashes directed against the fish in the mirror.

6) Appropriate statistical tests should be used to analyse the data. For a comparison of two genotypes, a T-test may be sufficient. For analysis of drug treatments in different genotypes, ANOVA followed by a posthoc test may be needed.

Associated publications

For a published example of this experiment, refer to Norton et al., 2011 (J. Neurosci. 31(39):13796-13807). The setup is modified from Gerlai et al., 2000 (Pharmacol Biochem Behav. 2000 Dec;67(4):773-82.).