# Behavioral Profiling using a Modified Version of the Zebrafish Multivariate Concentric Square Field™ (zMCSF) Test

Erika Roman, Ronja Brunberg, Arshi Mustafa, Per-Ove Thörnqvist and Svante Winberg

**Uppsala University, Sweden; Erika.roman@farmbio.uu.se; svante.winberg@neuro.uu.se**

## Introduction

Increased understanding of complex psychiatric disorders may demand for more complex test arenas capturing a broader behavioral repertoire [1]. The multivariate concentric square field™ (MCSF), originally developed for rats, is unique in its design by provoking behaviors associated with exploration, risk taking and shelter seeking. Thereby a behavioral profile is generated in a single session [2, 3]. During the last decades, the zebrafish (*Danio rerio*) has become an increasingly important model organism in behavioral neuroscience. Recently, a multivariate test arena for behavioral profiling of zebrafish, i.e. the zebrafish MCSF (zMCSF) test, was described [4]. Despite demonstrating great potential for future use, the arena design was not optimal. For instance, a problem was that the arena was too large resulting in many fish spending approximately 50% or more of the time in the part of the arena that was not a designated zone, which hampered on functional description and behavioral interpretation. Herein, a modified version of the zMCSF test is described.

## Animals and method

Male and female domesticated AB fish and wild-caught fish originating from the Calcutta area, India were used. The fish were held in 2.8-liter plastic home tanks (27 ± 1.5°C) in a filtrated recirculating water system (Aquaneering, USA) where 10 % of the water was exchanged daily, and with light/dark cycles of 14/10 hours. The zebrafish were fed twice a day with tropical energy food (Aquatic Nature, Belgium) and brine shrimp eggs that had been hatched in salt water (*Artemia* cysts, Argent Aquaculture, USA). The experimental protocol and use of animals in this study was approved by the Uppsala Animal Ethical Committee, and was consistent with the Swedish Legislation on Animal Experimentation (Animal Welfare Act SFS1998:56) and the European Union Directive on the Protection of Animals Used for Scientific Purposes (2010/63/EU).

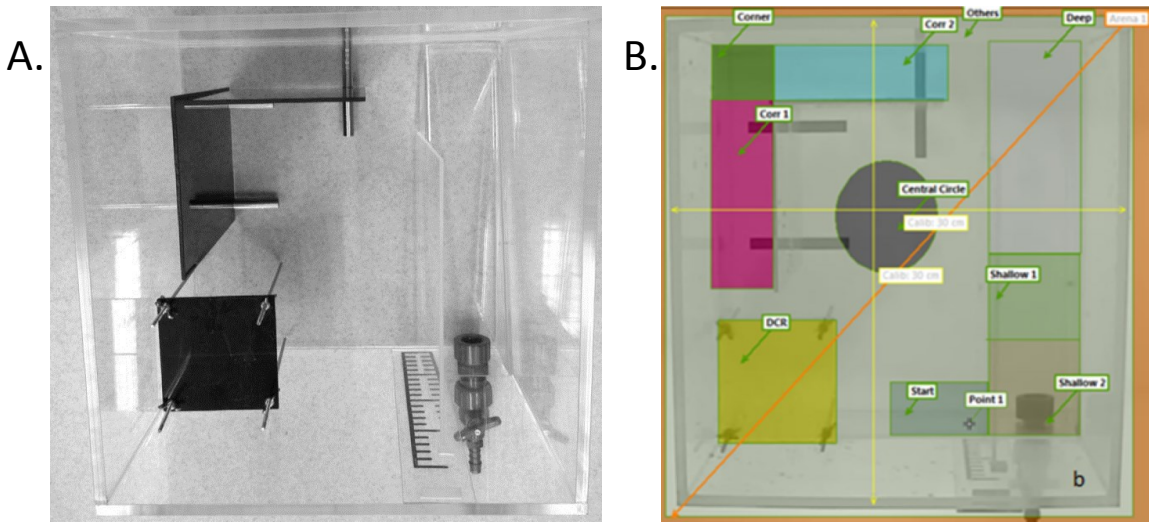


Figure 1. The modified version of the zMCSF (A) with the defined zones (B).

The modified version of the zMCSF arena consists of a tank made of Plexiglas (30 × 30 × 26 cm). The different parts in the zMCSF arena are a Plexiglas ramp with a wall covering approximately 2/3 of the side towards the open arena, a roof made of IR-transparent plastic, and two walls with weights (Figure 1A). These parts are placed in the arena to form the various zones: start, dark corner roof (DCR), central circle, corridor 1, corridor 2, corner and the ramp, which is divided into deep and shallow part 1 and part 2, respectively (Figure 1B). The walls of the arena were sandpapered after the picture in Figure 1A was taken to prevent zebrafish from reflecting in the glass. An IR-



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light table was placed underneath the arena and an IR-sensitive camera recorded the fish from above. The tank was filled with 24 ± 2°C copper-free water reaching a water depth of 9.5 cm. The fish were caught with a net, released in the arena at the mark in the start zone (Figure 1B), and allowed to freely explore the arena for 30 minutes. Between each fish, the tank was emptied, cleaned with ethanol (96%) and water, and refilled. The fish were tracked using Ethovision® XT 12.0 (Noldus Information Technology, Wageningen, The Netherlands). The number of visits, latency (s) to first visit, total time spent (s), duration per visit (s), distance travelled (cm) and mean velocity (cm/s) in each zone was registered, as well as mean velocity (cm/s) and distance travelled (cm) in the total arena. The duration (% of total trial time) in each zone and the total activity in the arena (sum of all frequencies) were calculated.

**Results**

In the modified zMCSF, the time spent in the part of the arena that was not a designated zone was decreased relative to what was seen using the previous arena set-up [4]. Moreover, the wall covering parts of the side of the ramp improved detection of the fish in that risk area since the fish had to make an active choice in order to swim up on the ramp. Finally, the inclusion of a start zone enabled detection of fish that immediately after start remained immobile before starting to explore the arena.

Individual differences in explorative strategies were evident in males and females of both strains. When observing the fish, it became evident that some fish swam back and forth on the deep part of the ramp, others moved a bit further up on the shallow part, and some swam all the way up on the shallowest part. Based on this observation the shallow half of the ramp was divided into shallow 1 and shallow 2 in order to be able to detect the most risk-taking fish, i.e. those that swam all the way up into the zone shallow 2.

In a preliminary functional interpretation of the different zones it is evident that the dark corner roof was associated with shelter seeking, while the central circle and the shallow part of the ramp are related to risk-taking behavior. Based on the observation of fish behavior on the deep part of the ramp, this zone is suggested to function as a risk assessment zone. Distance and velocity in the arena, together with the total activity are obvious measures of general activity. Activity in the part of the arena that is not a designated zone together with the corridors and the corner appear related to different explorative strategies.

**Discussion**

The results of the present study demonstrate that the smaller, modified zMCSF has great advantages compared to the previous version of the arena [4], since the detection of fish in the arena has improved, the fish spend more time in designated zones, and the smaller tank is easier to work with from a practical perspective. The functional interpretation will be further elaborated on using multivariate data analysis approaches. The results from this will set the basis for a trend analysis, which has been useful for interpretation of overall behavioral profiles in rats [3]. The zMCSF needs further validation but it clearly has a great potential in screening behavioral profiles in zebrafish, and in detecting natural variance in phenotypes observed in a population. The need for improved phenotyping strategies has recently been emphasized, and the zMCSF may constitute an important complement to conventional test used in preclinical research

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