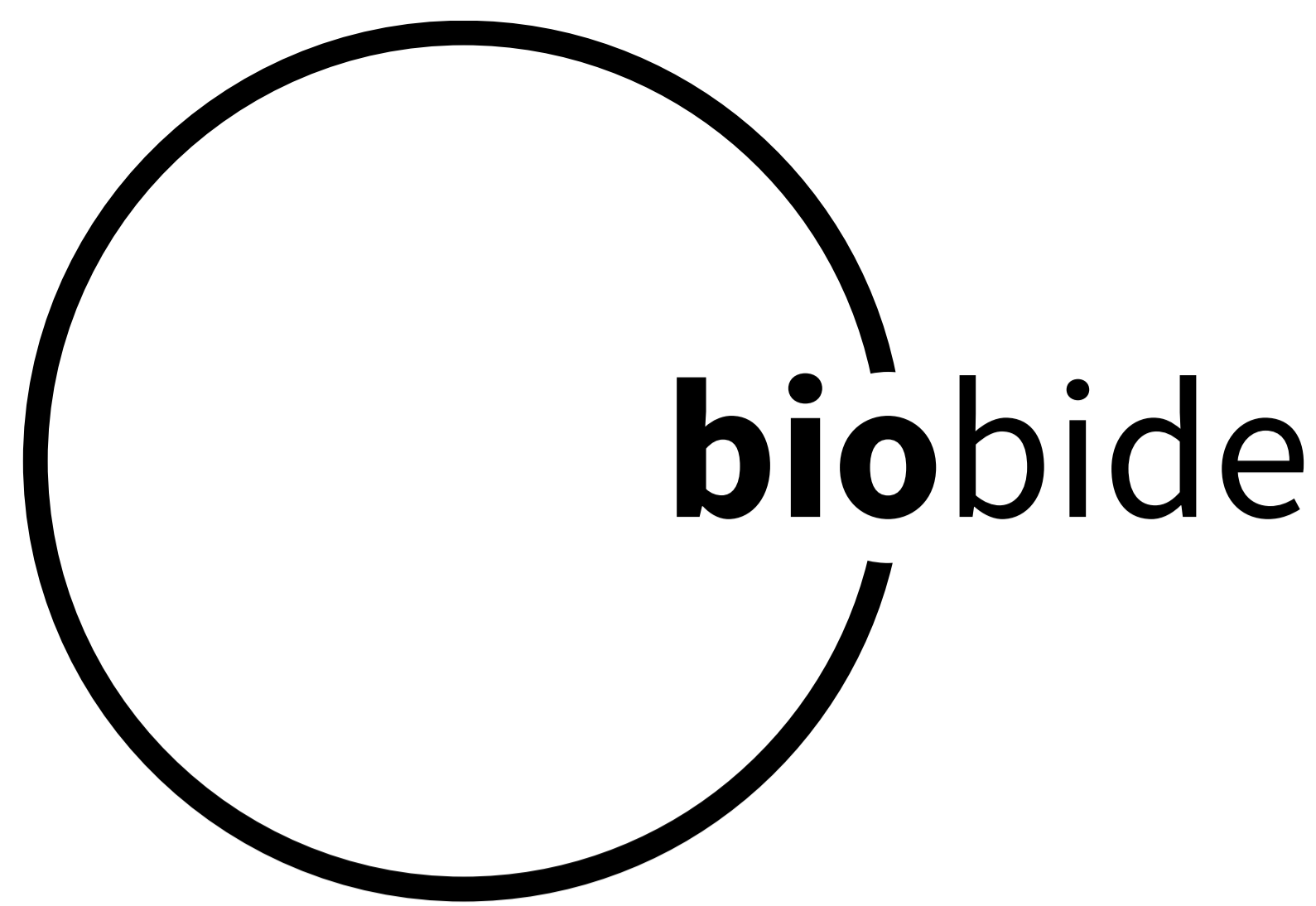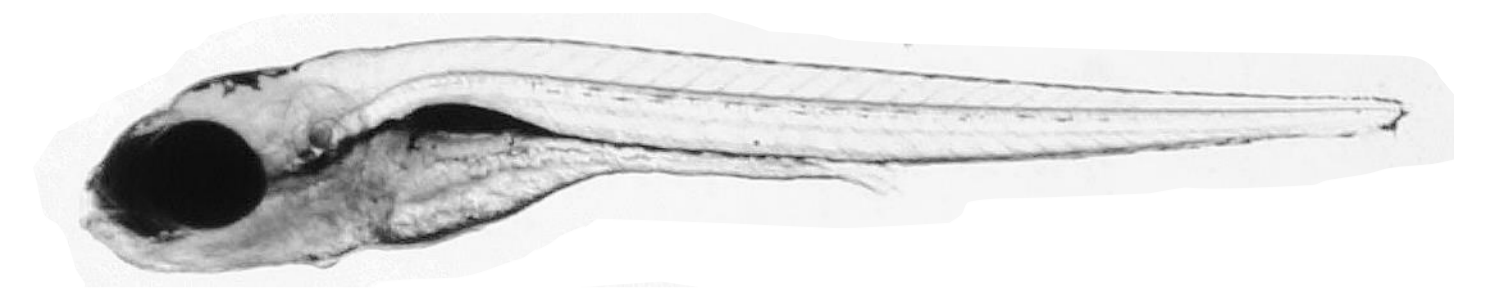# STANDARDIZED LIGTH/DARK PREFERENCE TEST FOR ANXIETY AND STRESS RESEARCH USING ZEBRAFISH LARVAE

biobide

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OBJECTIVE
–
To set up a standardized light/dark preference test and characterize embryo behavior

Zebrafish has emerged as an exceptional animal model for many research and applications in several fields. The advantages of the zebrafish are mainly their low cost and ease of maintaining and breeding. Moreover, many of the research conducted in zebrafish has been carried out in larvae to take advantage of zebrafish fecundity, larvae small size, ease of handling and transparency. In addition, the use of zebrafish embryo/larvae is in accordance with the 3R principle. There are more and more behavioral assays traditionally used in rodents that are being adapted to zebrafish, firstly in zebrafish adults and afterwards in embryos/larvae.

Light/Dark preference Test is one of the assays that has being adapted for behavior measurement in zebrafish [1][2] for anxiety and stress research. In case of adult zebrafish there are controversial results that are supposed to be conflicting because of the differences in the testing equipment [3]. In case of zebrafish larvae there is only one paper which aimed to adapt the light/dark preference test for young juvenile (6dpf - days post fertilization) [4].

Material and Methods

Animals: 5 days post fertilization (dpf) wild type zebrafish embryos from AB strain.

Testing Plates: 8-square well plates (NuncTM, USA) combined with a mask designed by Noldus to shadow the half of each well that allows infrared lights to pass through (Figure 1)

Tracking system: Daniovision system powered by Ethovision XT9.

Endpoints: time spent (in seconds) and distance moved (in milimeters) in light and dark zones. Both endpoints gave similar results.
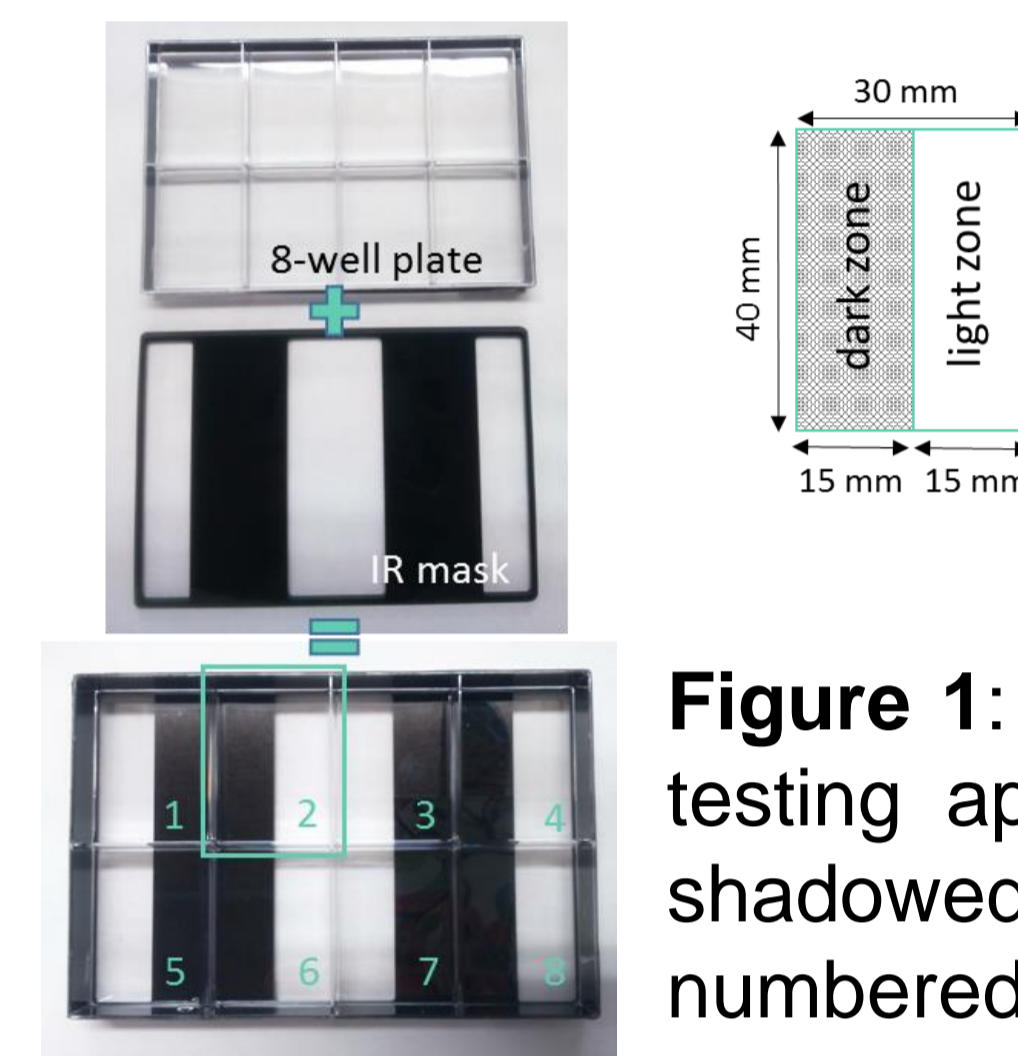

Figure 1: Images of the different parts of the testing apparatus. Half of the P8 wells are shadowed by an IR mask. Each well is numbered from 1 to 8.

SETTING UP

Light preference/dark avoidance increases as light intensity decreases

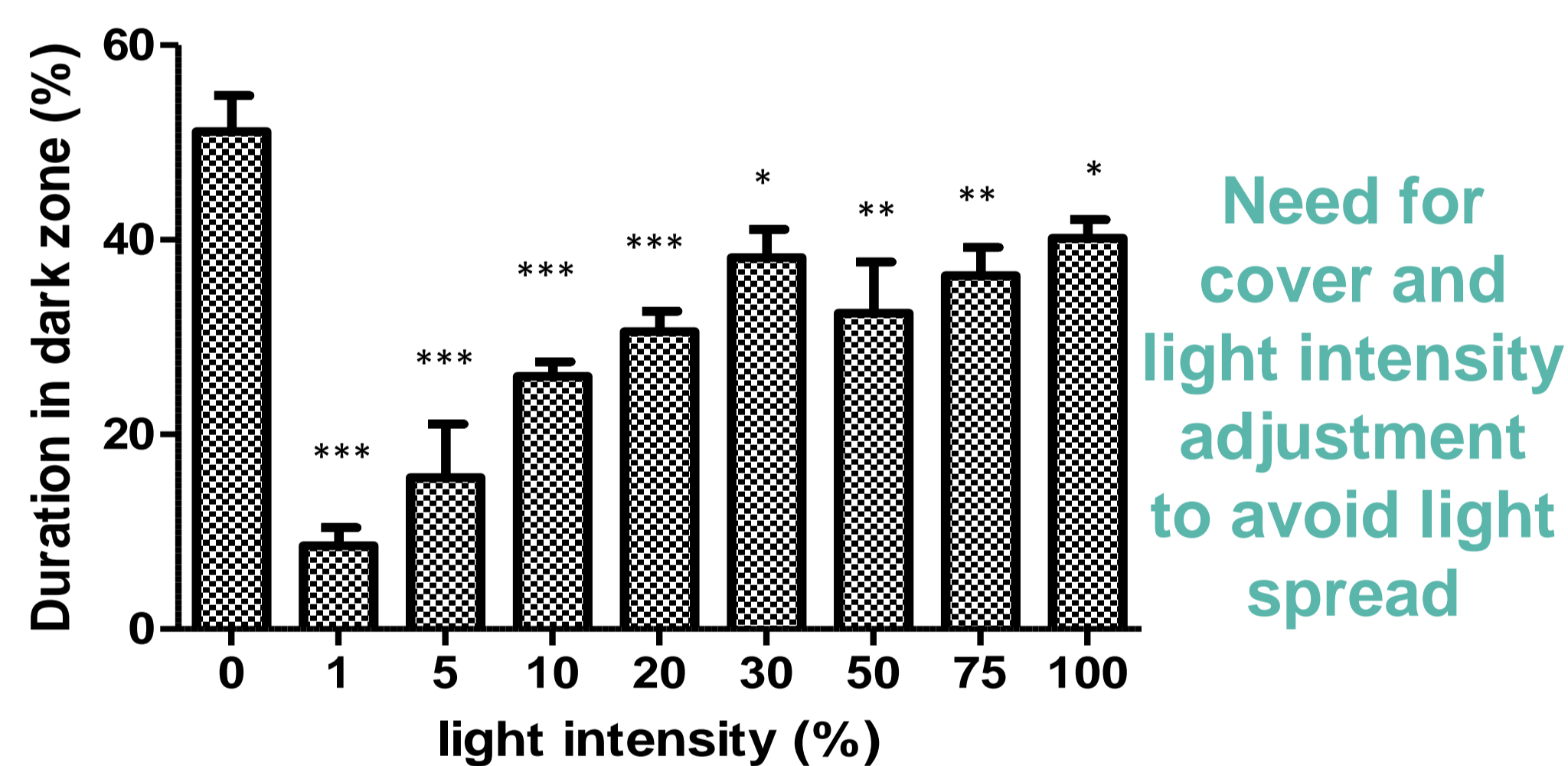*Need for cover and light intensity adjustment to avoid light spread*

Figure 2: Mean (±SEM) of time spent in dark zone in ten minutes. Embryos did not show any preference when the light is off (50% duration).

At 100% of light, time spent in dark zone is 40% and it decreased to 8.6% at 1% of light.
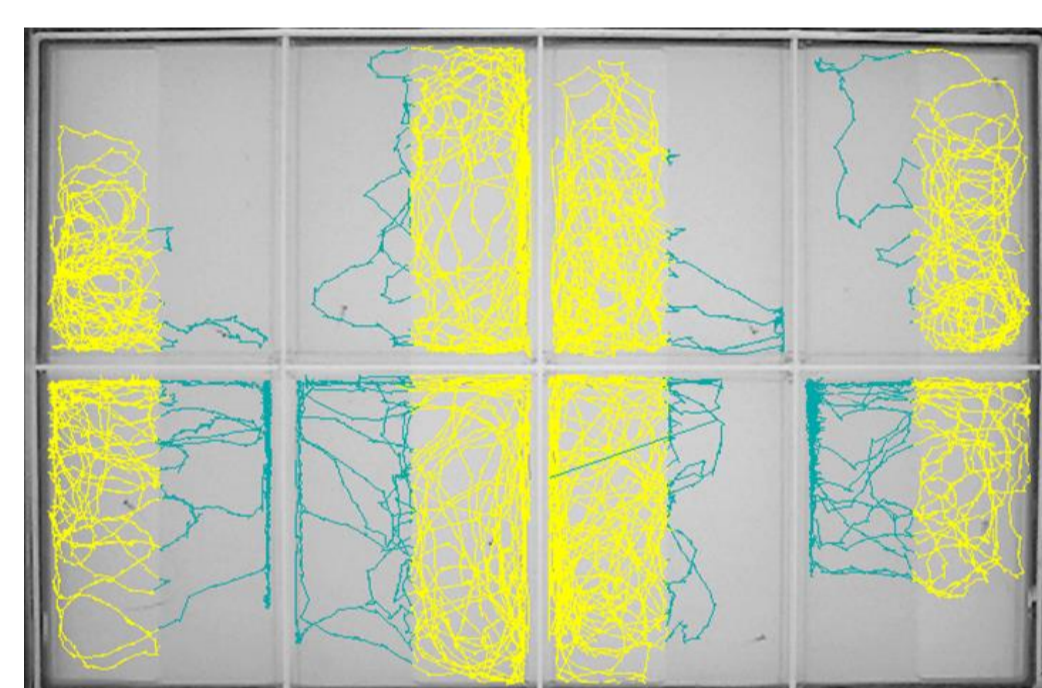
Track visualization

Figure 3: Track visualization of ten minutes tracking under 1% of light intensity. Movements in light and dark zones are marked in yellow and green lines respectively.

Embryos in the center wells (2,3,6,7) showed a marked light preference

Only embryos placed in the center wells show light preference.

Figure 4: Time spent by each embryo in dark and light zone during 10 minutes at 10% of light intensity. Light preference/dark avoidance is shown by embryos placed into the center wells (2,3,6 and 7) (p<0,0001) (A) but not by those in the edge well (1,4,5 and 8) (p=0,27) (B).

VALIDATION

Treatment with Fluoxetine (selective serotonin reuptake inhibitor antidepressant)
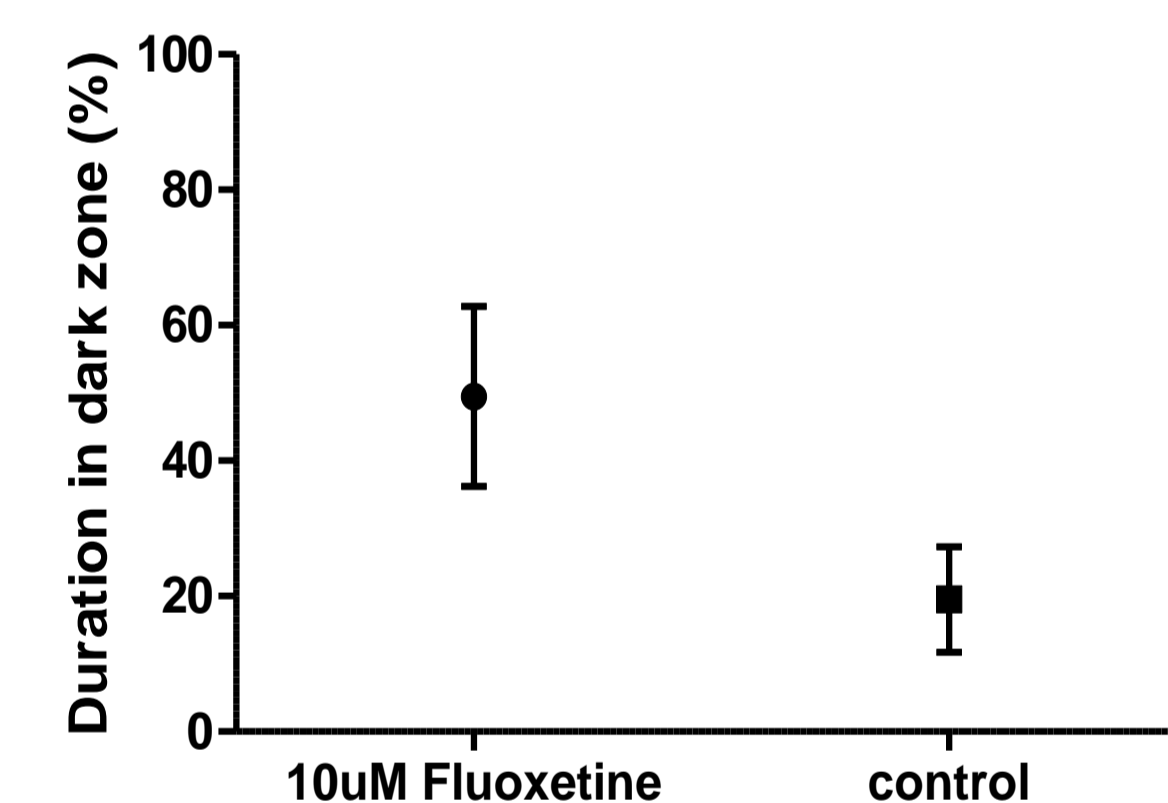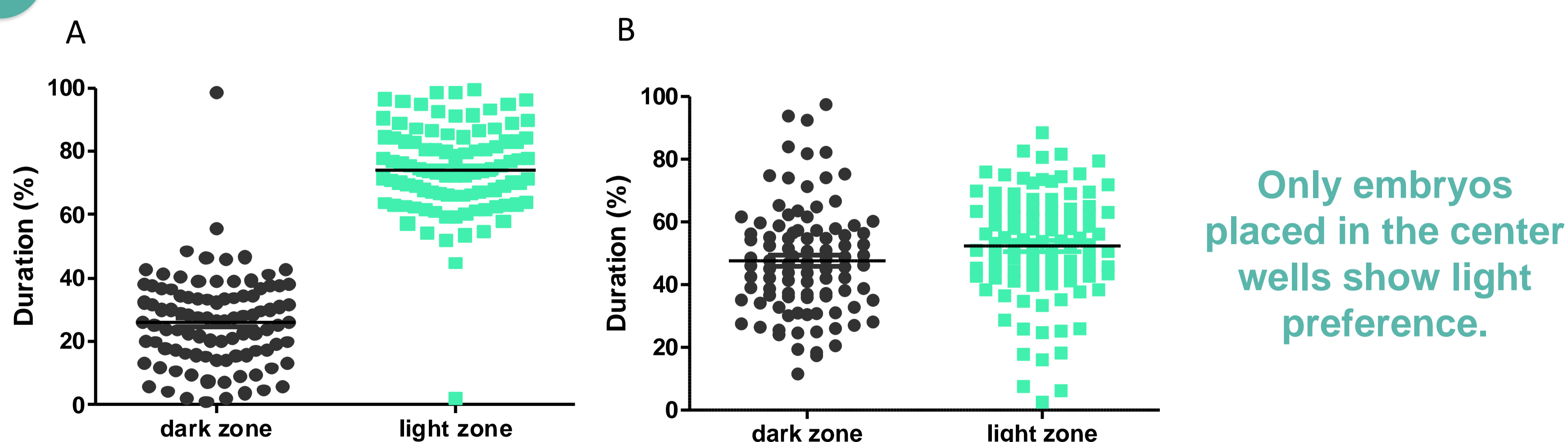

Figure 5: Percentage of time spent in dark zone by embryos treated with Fluoxetine at 10µM and untreated controls. 1% of light intensity was applied. Preliminary results.

Light preference/dark avoidance is reduced by Fluoxetine treatment (p=0,046).

CONCLUSIONS

- **Light preference / dark avoidance** is shown by 5 dpf zebrafish larvae

- Light /dark preference test for anxiety and stress research **complies with 3R's.**

- Repeatable **standardized** light/dark preference test to avoid controversial results obtained due to differently designed homemade testing apparatus

REFERENCES
[1] Serra, E.L., Medalha, C., Mattioli, R. (1999) Natural preference of zebrafish (Danio rerio) for a environment. Braz. J. Med. Biol. Res. 32(12):1555-1553.
[2] Gerlai, R. (2003) Zebra fish: an uncharted behavior genetic model. Behav. Genet. 33(5): 461-468.
[3] Blaser, R.E., Peñalosa, Y.M. (2011) Stimuli affecting zebrafish (Danio rerio) behavior in the light/dark preference test. Physiology & Behavior 104: 831-837.
[4] Steenberger, P.J., Richardso,n M.K., Champagne, D.L. (2011) Patterns of avoidance behaviours in the light/dark preference test in young juvenile zebrafish: A pharmacological study. Behavioural Brain Research 222:15-25.