Investigation of preference towards bottom substrate in zebrafish

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Contact us if you want to know more or want to apply for doing the project!

Introduction

Zebrafish (*Danio rerio* Hamilton 1822) is rapidly becoming one of the most popular model organisms in biomedical research (Fig shows number of zebrafish publications per year). A search in web of science using zebrafish as the search term gave 4000 hits for the year 2017 and a 43% increase in the number of hits per year since 2012. So far, this dramatic increase in the use of zebrafish shows no signs of levelling off. In Sweden, fish is next to rodents the largest animal group used for animal experiments and among fish zebrafish is the dominating species with 24 600 fish used for experiments in 2016 (https://www.djurforsok.info/vad-ar-djurforsok/djuren/).
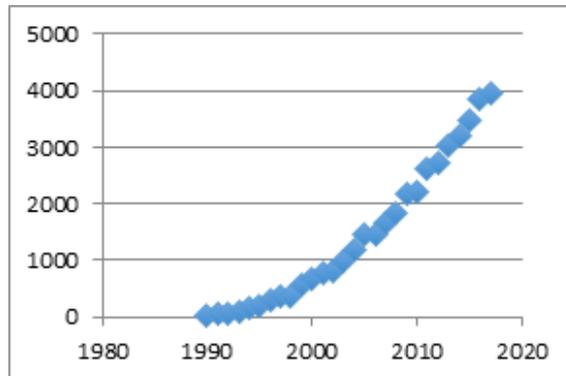
The high year-round reproductive capacity, transparent embryos and established genome rapidly made zebrafish an excellent model for studies on embryonic development. In developmental biology mainly early stages of zebrafish larvae are used. However, more recently zebrafish has also attracted considerable interest in other fields of biomedical research (e.g. neuroscience, behavioural pharmacology, toxicology etc.) and the use of adult fish as experimental animals is rapidly increasing. This puts higher demands on fish rearing (Graham et al. 2018).

Today laboratory housing of zebrafish is clearly focused on hygiene and high throughput. Fish are reared in barren, transparent tanks, usually at high densities. This is a very poor environment for the fish which is likely to have negative effects not only on fish welfare but also on the quality of the results obtained, since a barren and stressful environment will affect development and behaviour of the fish (Graham et al. 2018). The recommendations and

regulations in use today, concerning tank sizes and fish densities, are totally arbitrary and lack scientific background (SLU ID: SLU.scaw.2016.2.2-28). In most cases zebrafish rearing facilities do not use any environmental enrichment even though this is required according to the European convention no. 123, and Swedish regulations. Again, there is a total lack of knowledge on welfare effects of environmental enrichment on zebrafish.

This is the main PhD project of Oly Sen Sarma which is a first attempt to generate knowledge on zebrafish welfare, and effects of tank sizes, fish densities and environmental enrichment. The knowledge gained from this project could be used to generate scientifically validated regulations and recommendations for the rearing of zebrafish. For this project one of the important aspects is to investigate if there is any preference in zebrafish for the gravel picture (bottom substrate used for housing) under stress situation. This student project is aiming for investigating that part.

Method

3 months old zebrafish of the AB strain and offspring of wild caught fish will be used as the experimental subjects. Fish are born and reared in the Winberg lab. Fish will be reared for one month with a gravel picture positioned under the housing tanks (Sneddon et al 2014) as an artificial bottom substrate . A zebrafish skinner box setup (www.zantiks.com) will be used for the behavioural experiments. Individual zebrafish (tagged by visual implants) will be tested on top of a screen having one side with the same gravel picture used for housing and the other side with green colour, similar to the color of lids and baffles. These will flip places every 5 minutes. Finally there will be an electrical shock generated in the system for a fraction of a second under a white background after which the split screen will appear again. The camera in the setup will record the fish behavior/preference.

The hypothesis is that the fish will prefer to stay on the gravel background, i.e. to spend longer time on the gravel picture than on the green background. We also hypothesize that following the electric shock the preference for the gravel background will increase. The AB strain is a highly domesticated zebrafish strain frequently used in the lab. The hypothesis is that fish of this domesticated strain will show less preference for the gravel background than the offspring of wild caught zebrafish.

The script that we are going to run on Zantiks is called Conditioned place preference (CPP), which is a type of Pavlovian conditioning. The aim of this experiment is to measure the amount of time spent in a particular environment that has previously been familiar with the gravel picture indicating positive-reinforcing qualities of that compound.

Time planned project work

Month 1

- Tagging and acclimation
- Grouping
- Housing with/without bottom substrate (picture) for 1 month

Month 2

- Maintenance and behavioural observations
- Pilot experiments

Month 3

- Preference experiments using Zantiks boxes

Month 4-5

- Finalizing preference experiments
- Confinement stress and sampling for analysis of cortisol and brain neurotransmitter
- Data analysis and statistics
- Writing report

Reference

- Hamilton IM, Dill LM (2002) Monopolization of food by zebrafish (Danio rerio) increases in risky habitats. Can J Zool 20: 2164-2169.
- Graham C, von Keyserlingk MAG, Franks B (2018) Zebrafish welfare: Natural history, social motivation and behaviour. Appl Animal Behav Sci 200: 13-22.
- Sneddon L et al (2014) What do zebrafish want? Impact of social grouping, dominance and gender on preference for enrichment. Laboratory animals Vol. 48(4) 328-337.