Optimizing zebrafish welfare –
Effects of social isolation on behaviour and stress responses in male and female zebrafish

Background
Zebrafish (*Danio rerio*, Hamilton 1822) is rapidly becoming one of the most popular model organisms in biomedical research. 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 through put. 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.

The project 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.

Student project information

Aim
To Investigate welfare effects of rearing zebrafish in isolation.

Brief project description
Isolation is common laboratory procedure which could be expected to affect welfare in social species like zebrafish. Adult zebrafish (4 months post hatching) will be reared in isolation with or without visual contact with a conspecific and with or without environmental enrichment for 48 days in 1.8 or 2.8 litre tanks. Environmental enrichment will be selected based on the results from our previous work and only one type of enrichment will be used. Males and females will be studied separately (16 treatments with 12 replicates per treatment, in total 192 isolated fish).

I. **Observation of feeding.** The behaviour and feed intake of individual fish will be monitored once a day during feeding.

II. **Behavioural profile.** Behavioural profiling of all isolated fish will be performed in the zebrafish multivariate concentric square field™ (zMCSF, Roman et al. 2016,2018) and/or by novel tank diving test.

III. **Cortisol secretion in response to disturbance.** Will be performed on all isolated fish.

IV. **Effects on whole body cortisol, brain monoaminergic activity and brain gene expression.** Half of the fish (n=6) from each treatment will be sampled directly from their isolation tank whereas the other half will be transferred to a novel tank and sampled after 30 min.

**Hypothesis:**
1. Isolated fish having visual contact with conspecific will show signs of improved welfare.

   Additional hypothesis will be formulated based on the results from our previous related work.

**Approximate timeline:**

- **September-October:** Breeding of the experimental fishes
- **November-December:** Transportation of juveniles to Svante Winberg Fish Facility at BMC
- **January-February:** Housing the juveniles in respective isolation tanks.
- **March-May:** Observation of feed intake.
- **May-July:** Behavior profiling and cortisol secretion response (post stressing), Brain and body sampling.

The project is best suited for a 30 hp, or longer, project. However, the design can be discussed and possibly modified according to project length.

**Reference:**


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