Does tank enrichment improve the mental status of zebrafish?

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Introduction

Tank enrichment is a mandatory component in the housing of all animal species under directive 2010/63/EU. Within zebrafish facilities, the question arises whether tank enrichment provides an improvement in the welfare of zebrafish and whether there are associated risks. The tank enrichment should be easily applicable in multiple systems and should not pose a risk to tank hygiene.

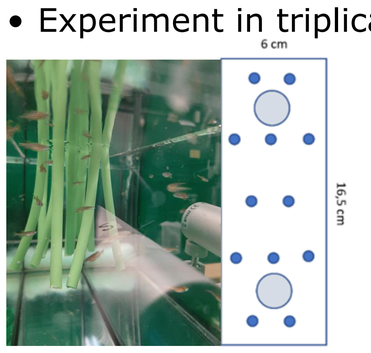
Objective

The objective of this research is to determine which tank enrichment is most suitable for zebrafish facilities, taking into account the standard housing for zebrafish breeding. Tank enrichment was assessed based on:

- Welfare improvement using the Judgment Bias Test (JBT)
- Tankhygiene through qPCR biofilm swab analysis for bacteria.
- Applicability: animal control, cleaning, replacement.

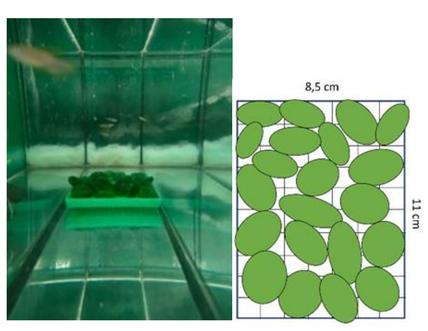
Tank enrichment

- During a period of 6 weeks (4 – 10 weeks post-hatching).
- Young, non-sexually mature fish.
- 30 fish / tank of 8,3L (49,6 x 9,3 x 18 cm).
- 3 types of enrichment compared to blank(no additional enrichment)
- Experiment in triplicate, 15 fish per tank trained for JBT.



Tubes: Solid silicone tubes attached to the lid. Hanging from the lid into the water column, free from the bottom.

Plant: Plastic aquarium plant with weight. Extending from the bottom into the water column.



Marbles: Green flat glass marbles on the bottom of the tank in a dish with a mesh bottom.

Judgement Bias Test (JBT)

The judgement bias test determines the mental status of a fish. A positive mental status is a result of better animal welfare.

The test consist of three phases:

- Training (1 week;16 rounds): Fish learn to push a disk to receive a reward (artemia).
- Color discrimination training: A red and bleu disc are presented, with only one color providing a reward.
- Test: Only a yellow disc is presented with a reward. How quickly does the fish choose?

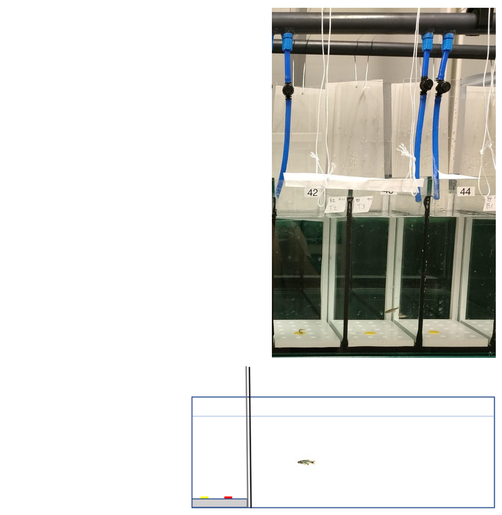


Figure 1.: JBT setup with a hole board containing the disks in the test compartment, separated from the aquarium by a double sliding door. One transparent and one non-transparent.

Results JBT and Tank hygiene

In the judgement bias test, the time to touch the unknown color (yellow) disc is measured. All three tank enrichments are below the average of the control group. After a Mann-Whitney U test, the plant group is the only one showing a significant difference from the control group.

The tubes and marbles have too large standard deviation to prove any significant effect (Fig. 4).

From the qPCR analysis, it can be concluded that the plant enrichment exhibits the highest bacterial growth. This is observed in both total bacterial count and Mycobacterium spp. Bacteria seem to adhere better to plastic and silicone than glass. However, due to a large standard deviation among triplicates, this effect is not statistically significant. (Fig. 5)

Results Training

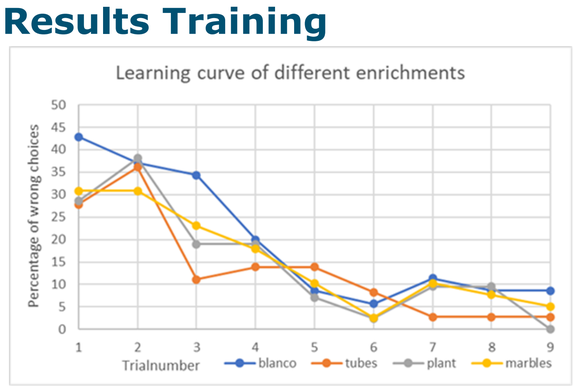


Figure 2.: The speed at which fish learn to enter the test compartment and push a disc for a reward.

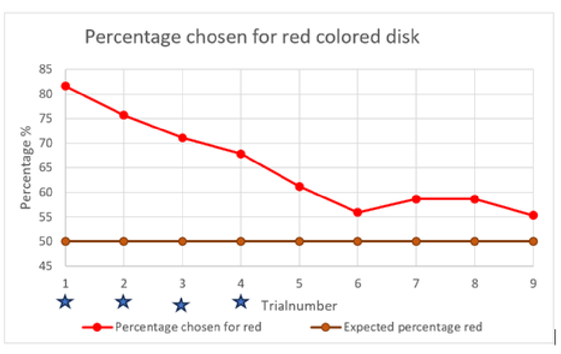


Figure 3.: An unexpected effect: Zebrafish (N=152) show a preference for a red disc over a blue one.

Growing up in an enriched environment can have a positive effect on the learning ability of zebrafish. During the training, the learning curve was examined for each enrichment. Each enrichment was individually compared to the control group (Figure 2). Each trial number was analyzed separately using a Mann-Whitney U test. It can be observed that there are some differences at certain points, but these are insufficient to conclude that there is a correlation between an enriched environment and improved learning ability. During the color discrimination training, zebrafish exhibited a significant (*) preference, up to trial 4, for the red disc as their first choice (Figure 3).

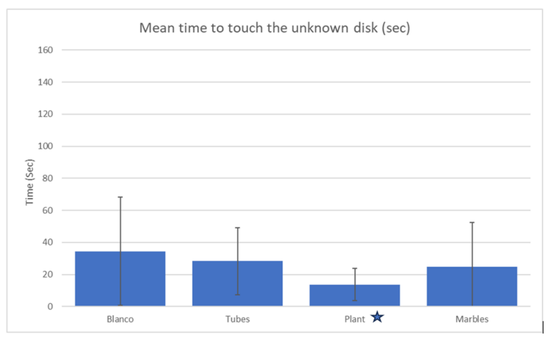


Figure 4.: The average time to touch the unknown disc. Significance (*) is determined compared to the control blanco (P < 0,05).

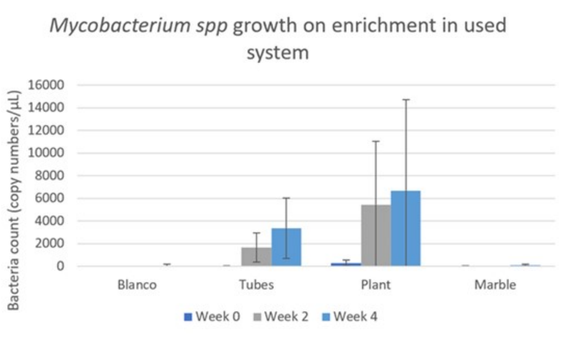


Figure 5.: qPCR results of Mycobacterium spp. growth on the tank enrichments. (Mean *± STDEV)*.

Conclusions

- No significant effect of tank enrichment on learning speed.
- Zebrafish show a preference for the color red.
- Only the tank enrichment "Plant" shows a significant effect in JBT.
- On glass tank enrichment there is less bacterial growth than others.

Acknowledgement

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