Shacking up together – trialling tent-style enrichment and how it affects breeding success in Zebrafish

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Introduction

Adding environmental enrichment to animal housing is important for improving the mental and physical well-being of our animals, thereby improving the quality of the data we get from our research.[1] While enrichment is widely used and studied in rodent and other mammal models,[2] it is less well implemented in Zebrafish husbandry.

A survey of Zebrafish facilities conducted in 2017 found that only 24% of respondents actively used enrichment such as gravel or plants in their Zebrafish tanks.[3] Common reasons cited against the use of enrichment in Zebrafish tanks include:[4]

- a perceived lack of evidence regarding welfare
- the potential for increased variability
- risk of increased biofilm
- cost
- staff workload

However research does show that Zebrafish do likely benefit from the introduction of environmental enrichment, showing:

- a preference for enriched tanks[5]
- improved survivorship and body condition[6]
- reduced stress[6]
- reduced aggression[7]

As Zebrafish use increases, testing the effects of enrichment will help us to increase welfare in our facilities.

At the King's College London BSF Zebrafish Facility many of our tanks are barren, although we do provide daily live feeds to all tanks as well as pictures of gravel and plastic aquarium plants that can be used at the researcher's discretion. As part of our commitment to continually improve the welfare of our Zebrafish, we wanted to trial some new enrichment options that we could offer.

We decided to trial some new tent-style environmental enrichment (Figure 1). It appealed to us because of its simple design, the material could be easily cagewashed and would not degrade in water and external testing suggested an increase in embryo production after the enrichment was added.[8]

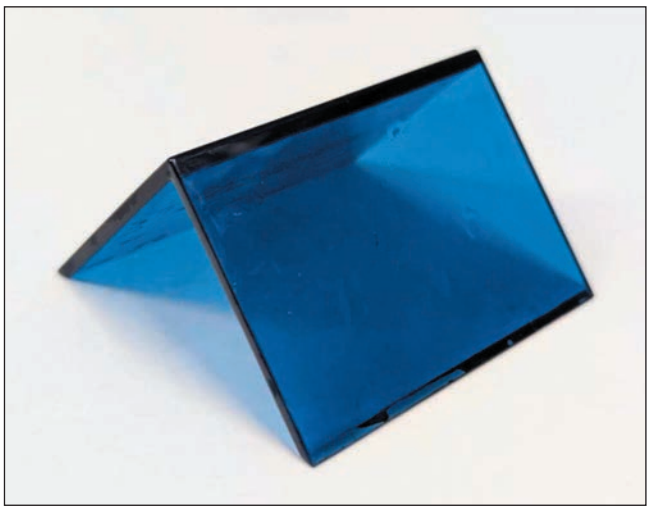

Figure 1. Tent-style enrichment.

Method

Ten of our housing tanks were set up, five control tanks and five enriched tanks (Figure 2), with twelve AB wildtype Zebrafish for each tank – six males and six females at three months post fertilisation. They were given three weeks to acclimatise to the new tanks and enrichment. The placement of the enriched and control tanks on the home rack was alternated to try and alleviate variability.

Figure 2. The set up of the enrichment tank.

Every two weeks, three pairs from each tank were set up in a one litre breeding tanks in the afternoon with a gate to separate the male and female. Breeding tanks were alternated on the shelf to alleviate variability (Figure 3). The Zebrafish were left separated overnight to allow for pheromone transmission without the possibility of spawning.

The next morning at 09:00 during the start of the dawn light-cycle, the gates were opened and the fish were able to spawn until 12:00 at which point the fish were returned to their home tanks.

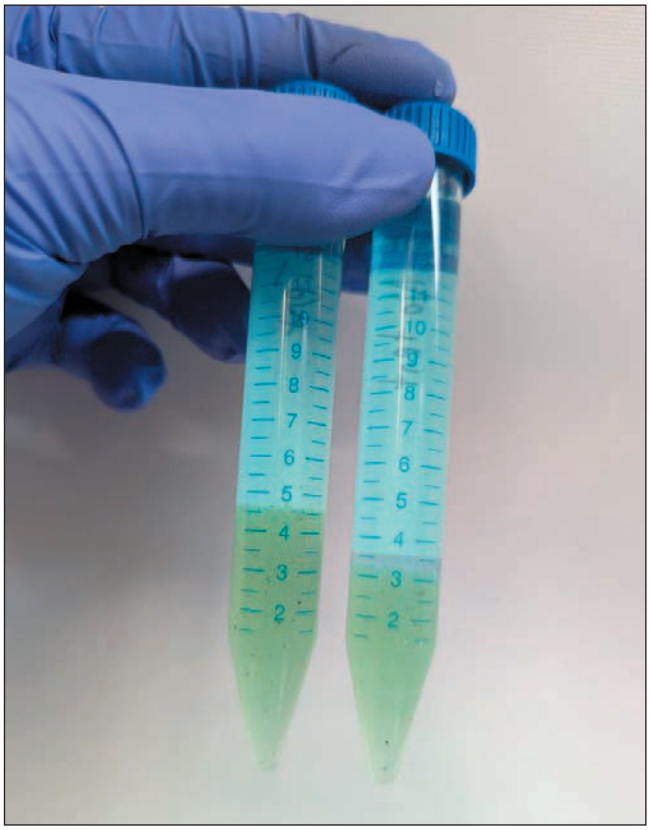

Figure 4. Volume of embryos inside the falcon tubes, the enriched tube is on the left and the control tube is on the right-hand side.

Embryos were then collected from the bottom of the breeding tank and carefully rinsed to remove detritus. The embryos from each of the tent and control tanks were pooled together and pipetted into 15ml falcon tubes and a measure of the total volume of the embryos collected from each (Figure 4).

Figure 3. Thirty breeding tanks on a shelf, alternating control and enriched.

The total volume was then multiplied by a known concentration to give us our result and we performed a t-test to check for significance.

Two sets of fifty embryos from each sample were taken and stored overnight in an incubator set at 28.5°C. The samples were then checked for fertilised embryos the next day to measure the fertilisation rate and t-test performed to check for significance.

Results

	Enriched	Control
Mean Spawning Success	82.8% ± 2.0	86.1% ± 1.4
Total No. Embryos Laid	120480	129502
Mean No. Embryos Laid	408 ± 27.5	420 ± 25.0
Mean Fertilisation rate	84% ± 2.1	86% ± 1.8

Table 1. The collected data we used to help us determine the overall breeding success of the Zebrafish within the trial. Means shown with standard error.

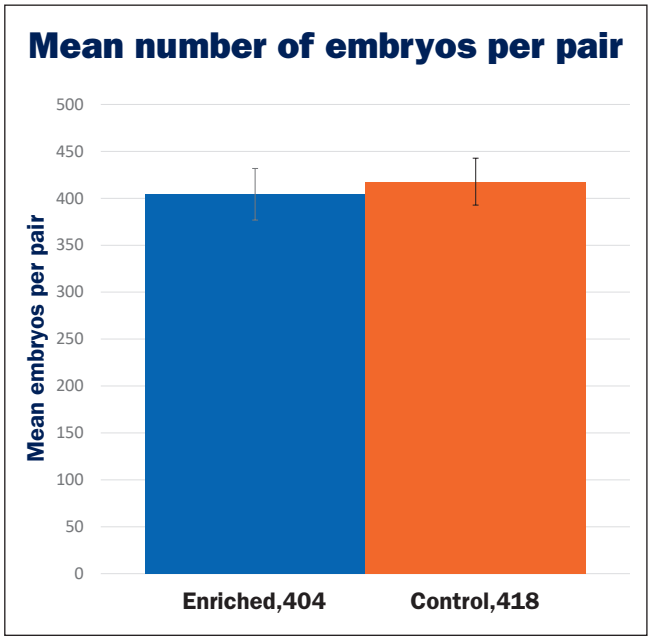

Figure 5. Chart showing the mean number of embryos laid per pair. Bars show standard error.

Discussion

Based on our results (Table 1 and Figure 5) we see that the fish from the control tanks produced slightly more embryos on average than the fish from the enriched tanks. However after conducting t-tests, we see the enrichment did not significantly impact embryo yield (P =.74). Nor did we see significant difference in the number of pairs that successfully spawned (P =.18) or in the fertilisation rate (P =.63).

Embryo production over time (Figure 6) does generally follow what we would expect given historical data from the facility. Embryo production increased over time as the Zebrafish became used to the set up and mature, before beginning to fall off again as the Zebrafish get older.

Figure 6 shows a higher percentage change between subsequent set ups of the enriched fish initially with a maximum increase of 139% and a maximum decrease

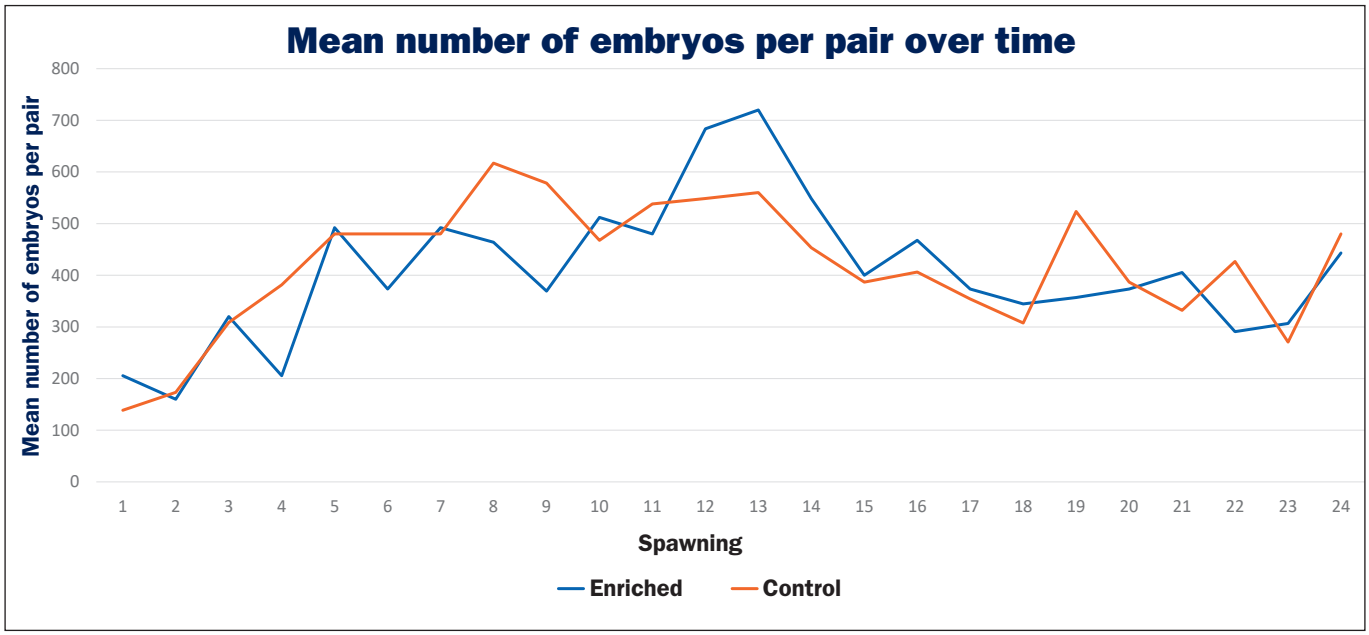

Figure 6. Chart showing the mean number of embryos laid per pair during each spawning event over the course of the trial.

of 36% compared to a maximum increase of 79% and no decrease in the control tanks. We theorise this change between set ups could be due to increased time to adapt from being placed into a novel environment (the breeding tank) from an enriched tank. Or from increased time to net the fish due to using enrichment as a hiding place. This may need to be considered in future testing with an adjustment period.

While our trial focussed on the effects the enrichment had on breeding success, we did keep observational records on the fish's behaviour. We often saw the fish were not interacting with the enrichment, preferring to swim in the top two-thirds of the water column, unless they were showing signs of distress and sheltering. This could help with identifying potential health issues early, allowing for intervention. We also had some observations of females presenting spawning behaviour on the enrichment which may have reduced their yield when set up. This may present a potential upside in facilities where embryo yield is less important or set ups are less frequent as it may help to reduce cases of eggbound females.

While the outcome of our trial was not significant, as our facility needs to maximise embryo production regularly, the slightly lower yield might negatively impact us. However the enrichment may be beneficial for facilities that do not set up their fish as often or require lower yields. There may also be alternative set ups for the tents we have not considered in this trial, such as in large breeding tanks, which might produce a different outcome and could potentially provide shelter if pairs are aggressive.

This shows the importance of trialling enrichment before widespread use as there may be unforeseen consequences. We intend to keep trialling the tents and other forms of enrichment this year to find more options to improve the welfare of our fish.

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