

# Comparison of zebrafish embryo collection using various methods.

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**Intro:** The increased scientific need for large numbers of good quality embryos has seen a rise of various different breeding strategies. From a scientific and **welfare** perspective, a good breeding strategy is key; where stress is reduced and fish allowed to have a choice in mating preference as well as a choice on whether to spawn or not. Using data from a previous comparisons of breeding strategies it was determined that there was a difference between the mass embryo methods in relation to the quality of embryos produced. (Figures 1 and 2). It was hypothesized that stress during breeding maybe a factor in the use of these units.



Fig 1: image of non viable embryos with viable ones



Fig 2: Images of viable embryos

**Methods:** 3 mass embryos units were used in this experiment one with no choice preference (MEM1) (fig3), one modified MEM1 to allow some choice preference (MEM2) (fig4) and a third that allow for full choice preference (MEM3) (fig5). These units were set up using 6 month old hybrid wildtype fish from the same generation. The fish were set up according to the unit recommendations in a mixed group of 40 females and 20 males for each unit the night before. The next morning the fish were allowed to breed and the eggs collect. The embryos were then placed into petri dishes until 24 hours where they were assessed and separating out into fertilised, unfertilised and non viable. Embryos were measured using a 15ml falcon tube (fig6).

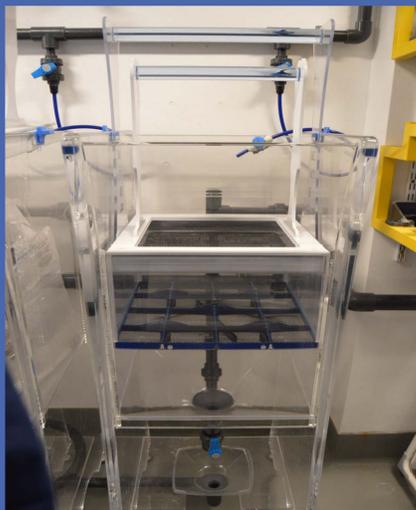


Fig 3: MEM 1

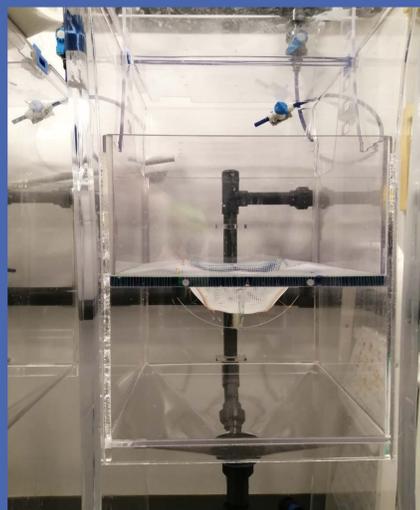


Fig 4 MEM 2



Fig 5: MEM 3



Fig 6: Embryo counting

**Results:** A comparison of the 3 MEM units (fig 7) shows that there is no difference in the total number of viable embryos produced by the MEM units. However there does seem that there is a difference between MEM1 and MEM3 in the number of non viable embryos (fig 8 ) which was also found in the initial data from 2013 (Fig 9 ).

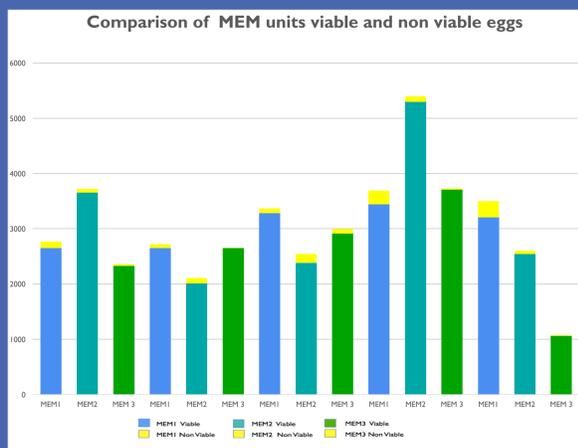


Fig 7: Comparison of the 3 MEM units between the Viable and non viable embryos.

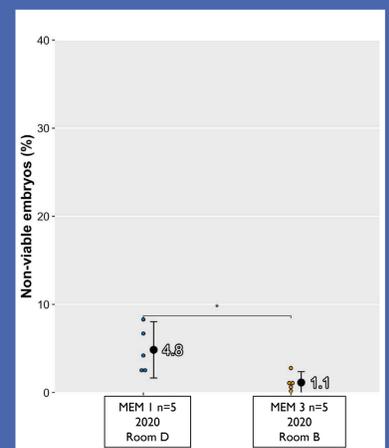


Fig 8: Comparison of non viable embryos between MEM units

A comparison between the locations of the MEM1 and MEM 3 units was performed. With the MEM3 there was no significant difference between the number of non viable embryos (fig 10). However the comparison of MEM1 (fig 11) shows that there is a difference between the non viable embryos produced in different rooms.

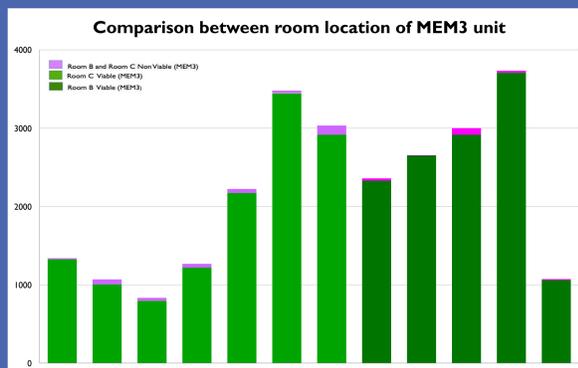


Fig 10: Comparison of non viable embryos produced by MEM3 in 2 room locations.

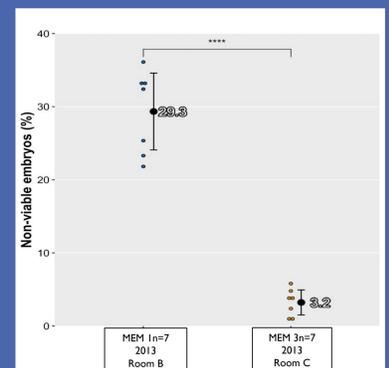


Fig 9: Comparison of non viable embryos between MEM units 2013

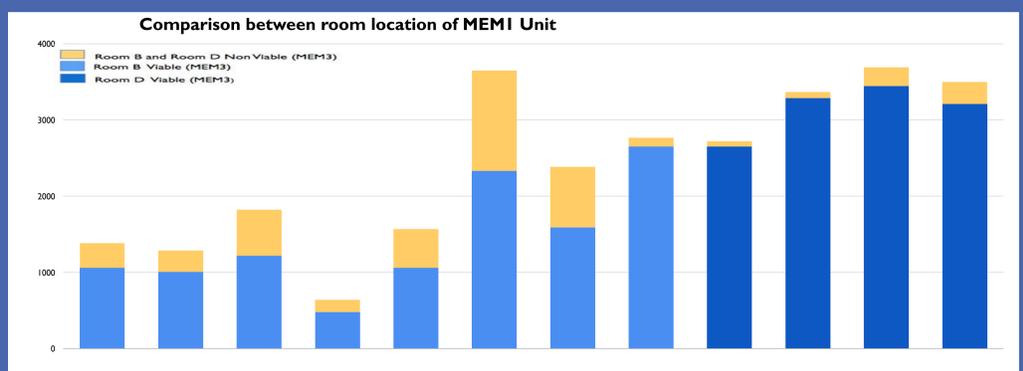


Fig 11: Comparison of non viable embryos producing by unit MEM1 in 2 different rooms.

**Discussion:** From this and previous trials performed using the MEM units we have found that there is a percentage of embryos produced that are non viable, this percentage seems to vary according to which unit is used. It appears that the more **choice preference** the fish have for breeding, the number of non viable embryos is reduced per breeding session. However in this newest trail when comparing MEM 1 and it's location placement we found that there was an additional **reduction** in the non viable embryos produced. This suggest other possible sources of **stress** could be causing the non viable embryos, one of these possible sources could be people. In the initial trial the MEM1 unit placed in the middle of the room with a large number of researchers in and out at varying times of day and night, especially after the unit was set up. For the new trail the unit had been moved into a room and located in a low level traffic area. This seems to suggest at least with the MEM1 that placement in a low traffic area could reduce stress thereby given a better quality of embryos. Although there are many more factors that could be contributing to the non viable embryos from this trial would suggest choice preference and unit places can reduce stress thereby improving the fish **welfare** while in the MEM units.

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