Comparing Zebrafish Embryo Production Methods

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Aim: To compare embryo collection methods using two mass embryo methods and two traditional methods

Intro

The increased scientific need for large numbers of good quality embryos (fig. 1) has seen the rise of various breeding strategies. In this poster, a comparison was made between four different spawning methods. From both a scientific and welfare perspective, a good breeding strategy is key; it should reduce stress in fish, allow the fish to express mate choice and preference, as well as to choose whether or not to spawn.

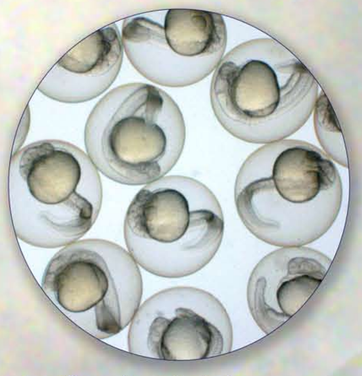


Fig. 1: Good quality embryos typically produced from healthy adults. No visible deformities nor debis in or around the embryos.

Methods

Three rounds of testing were done to compare different breeding methods: mass embryo method 1 (MEM1) and 2 (MEM2) and traditional method 1 (TM1) and 2 (TM2) (fig. 2).

In the first round a comparison of all four collection methods were done. Overall, 80 adult fish consisting of 60 females and 20 males were used for MEM1, MEM2, and TM1; 20 pairs of adult fish were used for TM2. Embryos were collected and counted for each method using a sieve and a 15 ml falcon tube, which was filled to the 2 ml marker with embryos; this was done several times to obtain an average number per ml.

In the second round a comparison was made between MEM1, MEM2 and TM1. The embryos were collected and counted in the same method. The third round was done comparing MEM1 and MEM2, specifically looking at the quality of embryos produced.

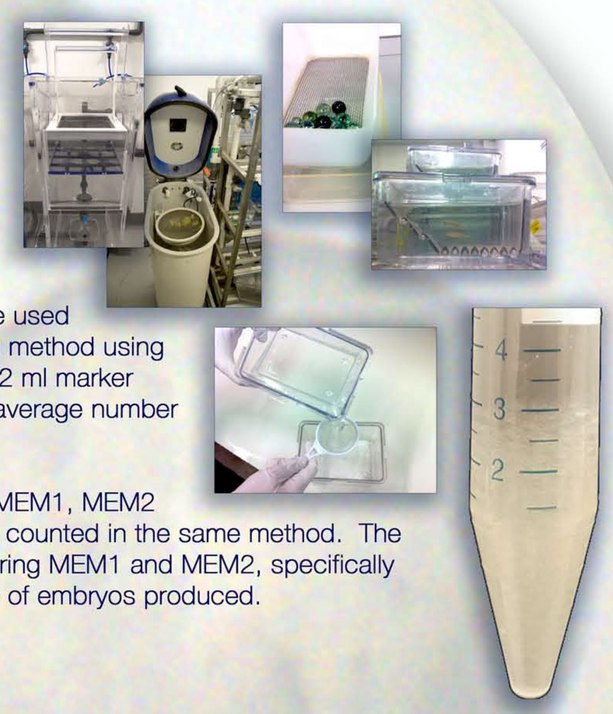


Fig. 2: From top left to bottom right: MEM1, MEM2, and TM1 and TM2. The embryos collected were measured in 2 ml units in a falcon tube.

Results

In the first round, we found no significant difference between the number of embryos produced from each method (fig. 3). In the second round, however, we found a significant difference between the number of embryos produced from MEM1 and those produced from TM1(fig.4).

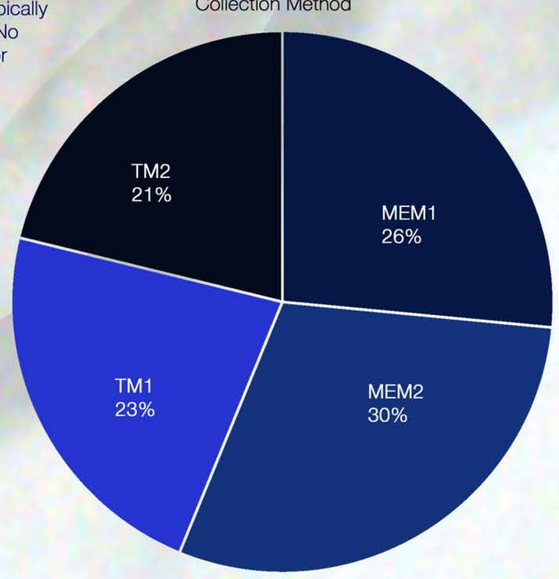


Fig 3: the total number of embryos collected across all methods, with no significant difference between any method.

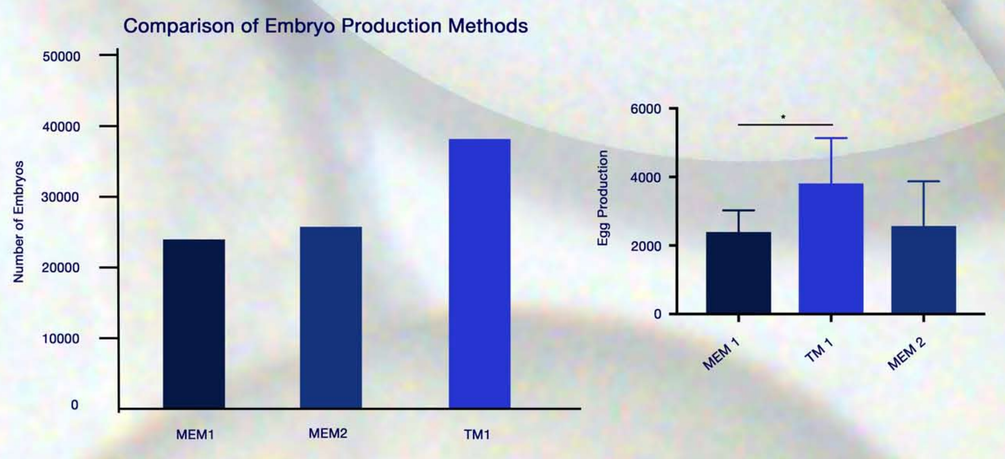


Fig. 4: Above left: TM1 yielded the highest number of embryos over the mass production methds in the second round. Right: Egg production was signficantly different between MEM1 and TM1. * indicates $p<0.05$. (One-way ANOVA with Tukey's HSD post-test).Data are shown as mean ± SD.

In the third round, we found no significant difference between the number of embryos produce by MEM1 and MEM2 (data not shown) but there was a significant difference in the quality of embryos produced by the two different methods (fig. 5). Quality was measured by eggs that were discarded due to debris or abnormalities (fig. 6).

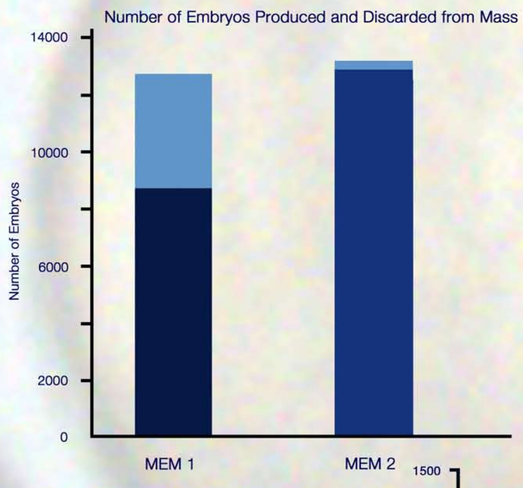
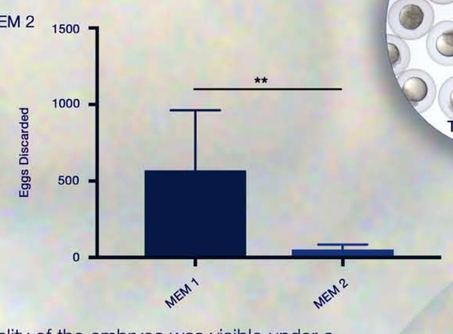


Fig 5: Above: The total amount of embryos collected from both MEMs were not entirely viable and some were discarded (light blue). Right: the total number of eggs discarded was significantly different between MEM1 and MEM2. ** indicates $p<0.01$ (Student's t-test. Data shown as mean ± SD.



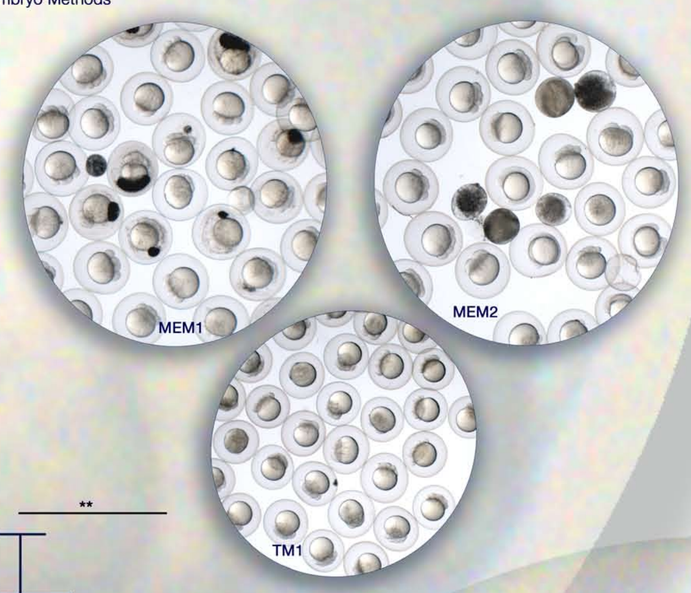


Fig. 6: Above right: the quality of the embryos was visible under a microscope. MEM1 had debris within the chorion; MEM2 had various stages of embryos; TM1 has more uniformity.

Discussion

From this investigation, there is little difference in the numbers of embryos produce in each method; however, there appears to be an embryo quality and welfare issue between the MEM1 and MEM2. Not only were more embryos discarded from MEM1, but the fish also have no choice to spawn. In fact, it could be said that they are forced to breed, which could cause stress in the group. Compared to MEM1, MEM2 allows the fish to exhibit more natural breeding behaviour, and thereby could lessen potential stress, as the structure of the unit allows the fish to choose between breeding or not by removing themselves from the breeding area.

In adhering to the 3Rs, all factors should be considered to determine the best method for breeding of zebrafish, including potential stress. Whilst all current methods appear to provide a high number of embryos required by research, the structure of equipment may have a negative impact on both parents and embryos.

Further Work

Further work to be done will include modification of MEM 1 to allow for a refuge away from the breeding area. The aim of this will be to determine if it will produce embryos of a higher quality than the current method.

References:
1. Lawrence, C., C. Harper. (2010). *The Laboratory Zebrafish*. CRC Press

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