The effect of different biosecurity, feed, and frequency of breeding on the fecundity and fertility of zebrafish *(Danio rerio)*

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Aim: To assess and refine the factors involved in breeding, and reduce breeding population size

Introduction

Zebrafish in research are frequently bred for many purposes but the success rate of breeding varies greatly[1]. There can be adverse effects on the welfare if breeding is unsuccessful as this can lead to further attempts. This can and does cause stress and exhaustion to the fish, through fish to fish interactions involved in breeding and the human involvment in setting up to breed, such as netting fish and placing them in a new environment. These are all causes of stress, which must be reduced wherever possible, not only for the general wellbeing of the fish but also to decrease the risk of health issues, such as mycobacterial infection[2]. Understanding the variables involved with breeding leads to refinements in process and subsequent reductions in numbers of fish in a breeding population.

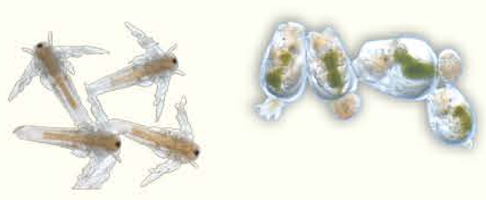
Methods

We aimed to compare breeding success between different variables: strains AB, which is more genetically isolated, and TL, which is more recently outbred; habitat, using two different systems; diet using two diffierent live foods; frequency of breeding, occuring weekly, fortnighly, monthly, and quarterly.

Strain

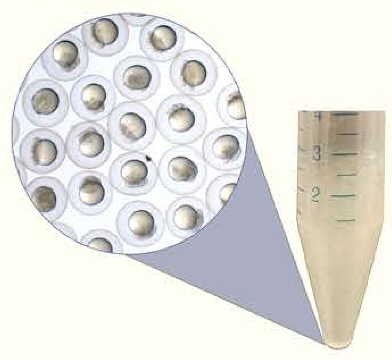
Habitat

Diet



Frequency Weekly Fortnightly Monthly Quarterly

Fig. 1 (below): the collected embryos were counted by an estimate based on the total mLs of embryos by using a falcon tube.



When bred, they were netted out and placed into paired breeding boxes (fig. 2); this was usually done between 3 pm and 5 pm and then embryos were collected the next day between 12 am and 2 pm. The number of pairs from each tank that successfully laid embryos was recorded. All embryos from the tank were then combined and measured using a falcon tube to get an estimate of the number (fig. 1). A sample was taken from this and used to determine a fertilisation rate.

Fig. 2: fish paired in a breeding box.

Results 1

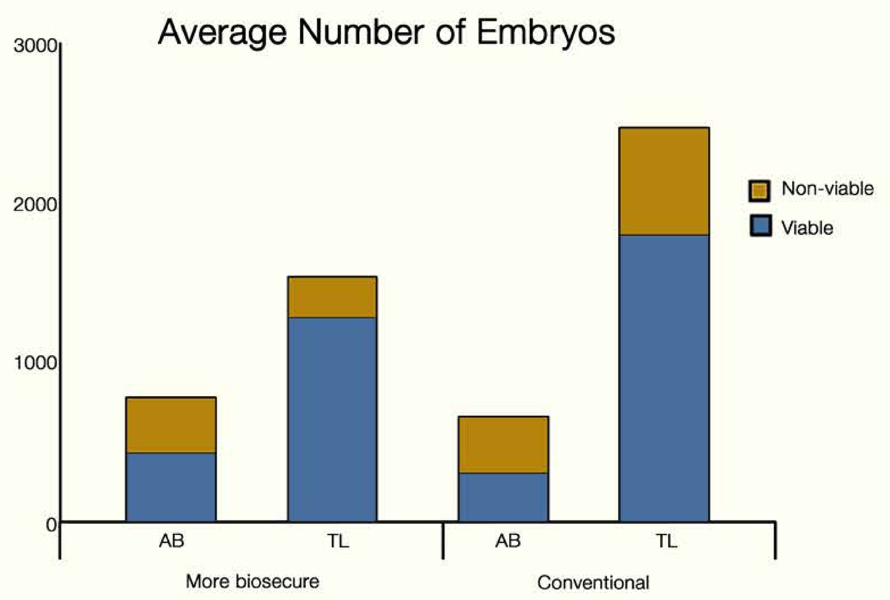


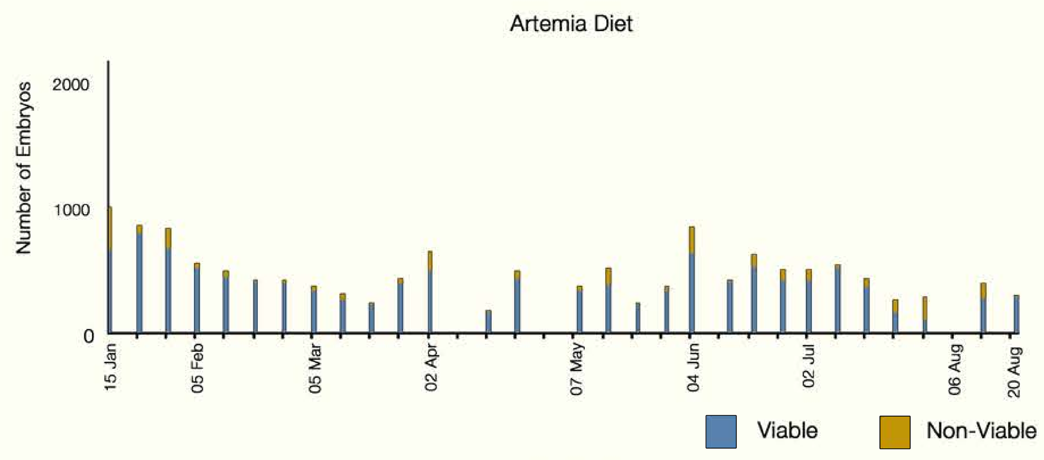
Fig. 3: Bar chart showing the differences in the average number of embryos collected between the two rooms, the lines within the rooms and showing fertilisation rate

Strain: The data shows that the more recently outbred TL strain was more likely to lay and produce embryos at a higher fertilisation rate (fig. 3). This may be due to the AB strain being significantly more inbred and therefore showing lower fertility and fecundity. It also reflects phenotypical differences - TLs have long fins, which requires more energy to swim.

Habitat: There was a higher number of both AB and TL embryos in the conventional room; however, there was a higher fertilisation rate in the more biosecure room (fig. 3). This suggests a reduced pathogen load allows for healthier fish. Other variables, such as colouration and water flow within the tanks, could impact the results.

Results 2

Diet: Artemia, which was fed throughout adulthood, unlike the rotifer which was only nursery fed, appeared to have a higher fertilisation rate, yet with a lower fecundity (figs. 4a and 4b). Since the comparison in adults is between live and dry food, further work comparing the nutritional differences are needed.



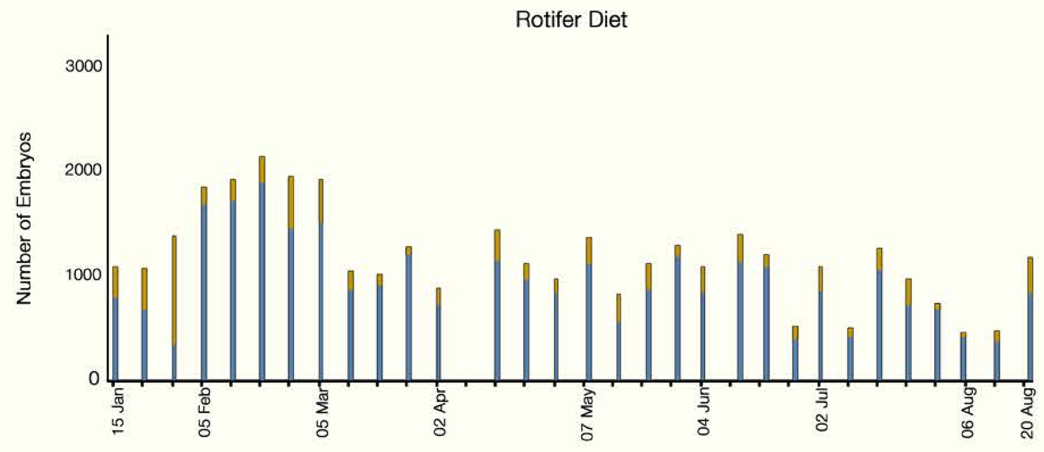
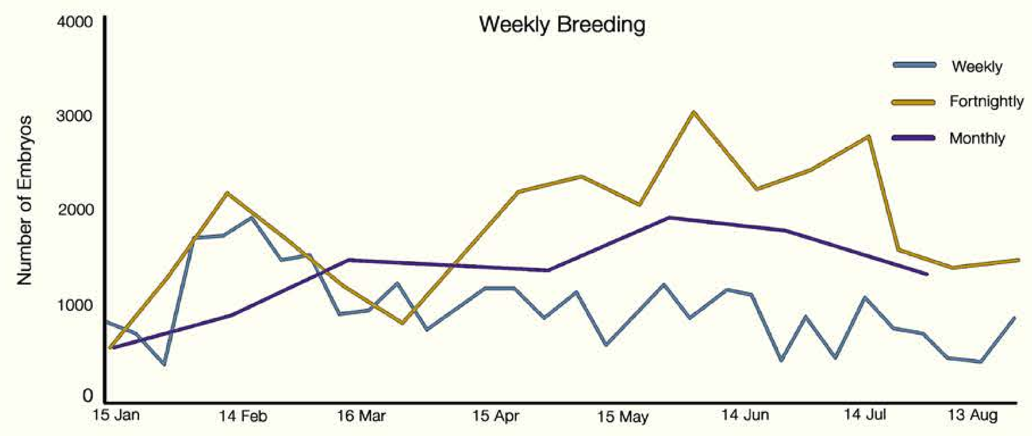


Fig. 4a (above) and 4b (below): Number of embryos collected over nine months from the TL strain on the artemia diet (above) and the rotifer diet (below).

Fig 5 (below): overall embryo production over eight months comparing weekly, fortnightly, and monthly breeding frequencies



Frequency: Those bred weekly gave a lower yield compared to fish bred less frequently (fig. 5). Weekly breeding had a constant fluctuation in number of embryos, which suggests stress and exhaustion from high frequency breeding and handling, and more time may be needed to recover from a session.

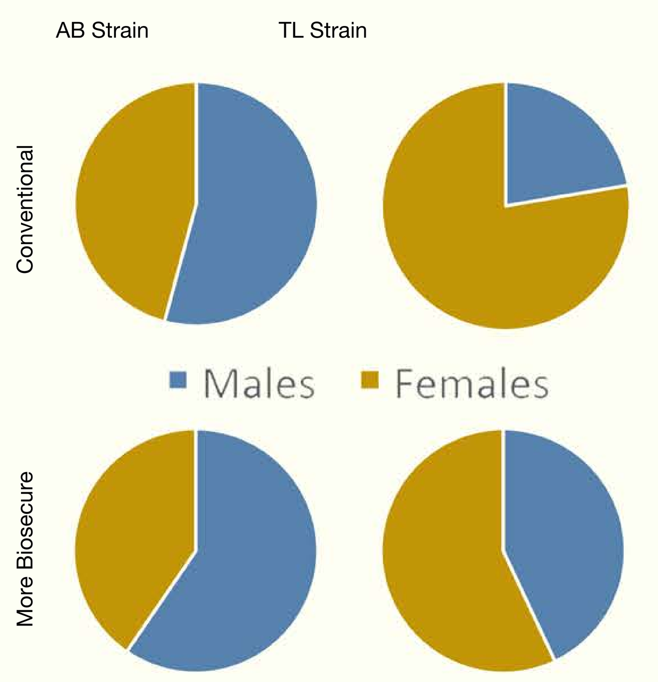


Fig. 6: Live and dead fish after 11 months as a percentage of total population. Comparing the two strains and two rooms.

When setting up zebrafish breeding stock, sex ratios are rarely even (fig. 5). The desired number of repeats were not set up because of this. The variables that contribute to sex determination in zebrafish is still not fully understood[3]; the habitat appears to have an impact as well as influencing survival rates (fig. 7) due to the lower pathogen load.

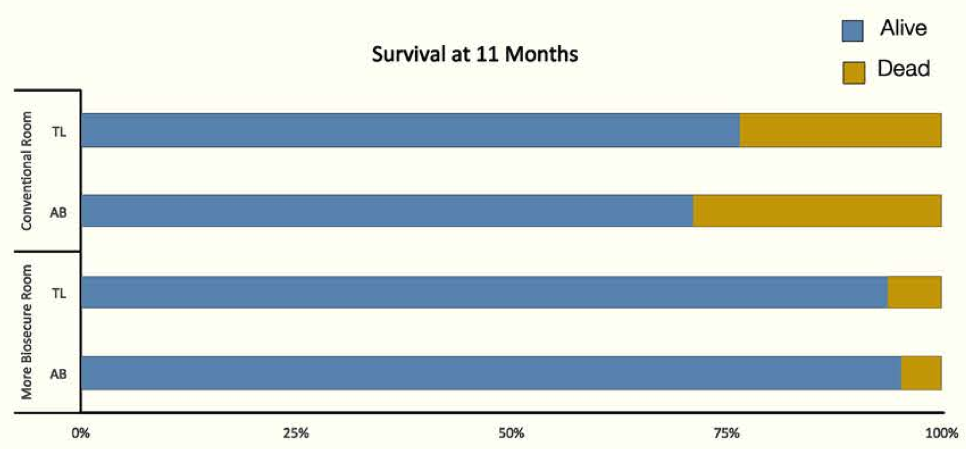


Fig. 7: the mortality rate across the rooms. The more bioseure room had a lower mortality rate overall due to the lower pathogen load.

Discussion

Understanding breeding success between different variables can help refine the process. Our results suggest a live food diet fed throughout both nursery and adult stages may result in higher fertility rates, in terms of fertilised to unfertilised embryos produced. However, those fish fed rotifer at larval and juvenile stages and then a more complete diet at adult stages produced more embryos in general.

These results also suggest a more biosecure habitat with a lower pathogen load also increases fertility and improves overall health.

We also found a reduction in the yield of embryos from breeding fish frequently, due to stress from increased fish to fish contact time, netting and other factors involved when placing them in a new environment. This is confirmed through higher mortality rates (figXX).

Breeding techniques can best be **refined** by: consideration of diet; increased attention to the health of the fish through lower pathogen levels; minimised stress to fish through handling and breeding. It would then dramatically improve the welfare of the fish whilst at the same time achieve better and more reproducible scientific results through higher numbers of viable embryos in **reduced** sized breeding populations.

Further Work

We will look closer at the nutritional differences of different dry food diets and different gut loads of rotifer. We will now feed fish on a rotifer diet throughout their life span in order to look specifically at the nutritional differences between rotifer and artemia. We will also compare the success rates of different breeding methods, such as larger breeding boxes and breeding trays. As this study continues, we will also assess the impact of ageing, hence we will continue the current study until the fish are 18 months old.

References:

1 Nasiadka, A. Clark, M.D. (2012). Zebrafish Breeding in the Laboratory Environment. ILAR Journal, Vol 53, No. 2, 161-168
2 Ramsay JM, et al. (2009). Husbandry stress exacerbates mycobacterial infections in adult zebrafish, Danio rerio (Hamilton). Journal of Fish Diseases, Vol 32, No. 11, 931-941
3 Liew, W.C. Orbán, L. (2013). Zebrafish sex: a complicated affair. Briefings in Functional Genomics, Vol 13, No. 2, 172-187

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