Shining a light on rearing pigmentless zebrafish

Jacqueline Glover*, Thom Berriman*, Dimitra Mantzorou*, William Havelange*, Sam Berry* and Bruno Correia da Silva*

*King's College London, Zebrafish Facility, Biological Services Unit.

KING'S College LONDON

1. Introduction

Zebrafish (*Danio rerio*) strains with mutated pigment cells allow clear *in-vivo* imaging to be carried out. In 2016, Crystal mutants, which lack most pigment cells in both their body and eyes, were first produced at King's College London (Antinucci and Hindges, 2016).

However, evidence shows strains with mutated pigment cells exhibit reduced survival during the early rearing stages in comparison to wildtype strains (Wilson, 2012; Barwood *et al.,* 2017). The Crystal strain, in particular, has poor survival in the nursery compared to wildtypes, and anecdotal evidence suggests lower spawning success compared to other strains.

Light has been shown to influence the survival of wildtype zebrafish larvae (Villamizar *et al.,* 2014), however research of its effect on other zebrafish strains is limited (Barwood *et al.,* 2017). Light is also well known to influence spawning behaviour in zebrafish, as their reproductive cycle is photoperiod-dependant, with most spawning at dawn (Tsang *et al.,* 2017).

This experiment aimed to look at the impact of light intensity as a way to **refine** methods of rearing pigmentless zebrafish and to **reduce** the number of fish needed to maintain important genetic lines, such as the Crystal line.

2. Materials and Methods

Three strains of zebrafish, varying in their degree of pigmentation, were selected (Wildtype AB, Albino^{b4} and Crystal Mutant $^{(nacrew2/w2;albb4/b4;roya9/a9)}$).

Twelve rows, on the same recirculating system, were filled with empty tanks. A lux meter was used to calculate the average light intensity (LUX) of each row from inside the tanks. Three rows were selected to represent high (259 LUX), medium (128 LUX) and low (38 LUX) light intensity levels (Figure 1).

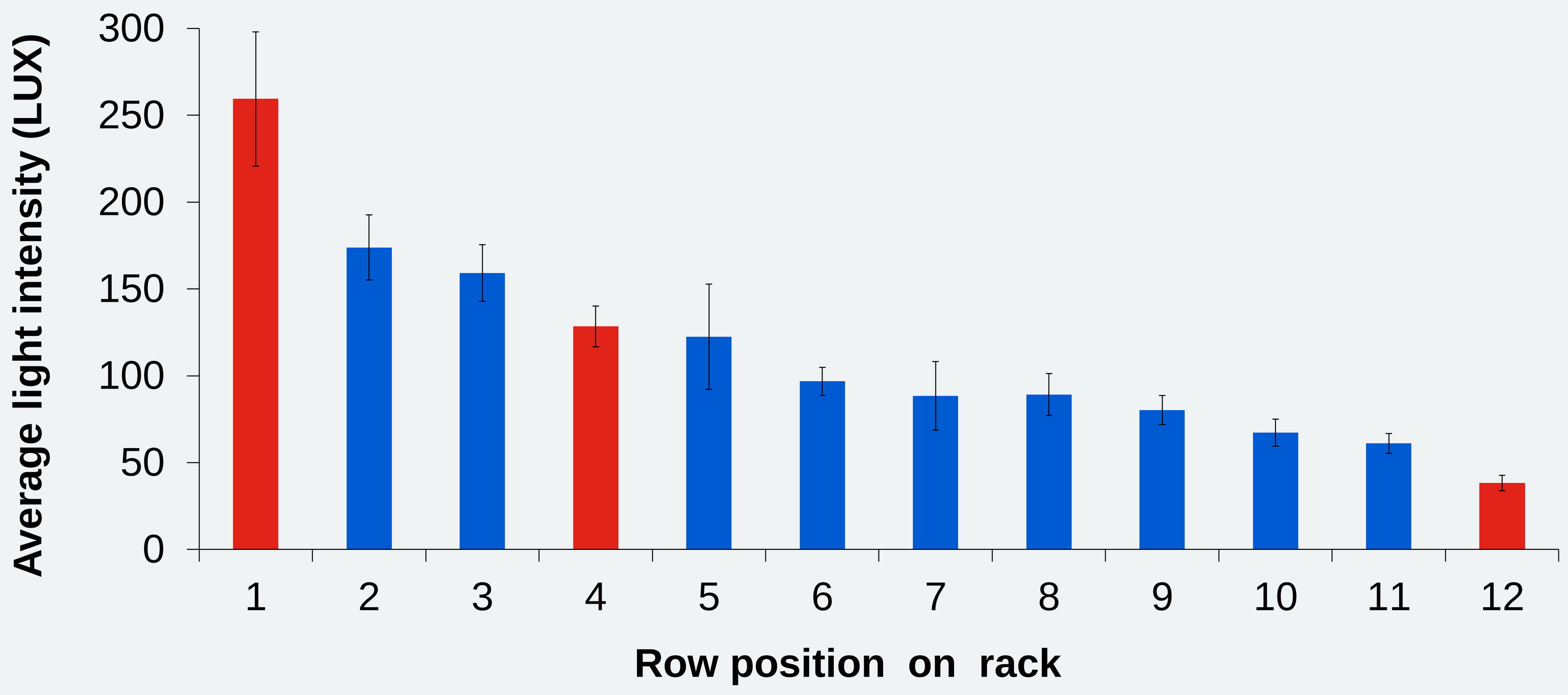

Figure 1 Average light intensity (LUX) of tanks across two racks. High, medium and low light intensity used for the purpose of this study are shown in red.

Figure 2 Wildtype AB (left), Albino (centre) and Crystal Mutant (right) zebrafish strains.

Three tanks of each zebrafish strain were then assigned to each of the three rows. At 5dpf, fry were transferred into 3.5 litre tanks (n=25 per tank), containing 1 litre of water. All fish were fed according to methods previously described by Mantzorou *et al.,* (2017).

Upon reaching sexual maturity (between 58-84dpf) the survival rate of each tank was calculated. All tanks were then reduced to 17 fish per tank. Remaining fish were then paired to spawn, a total of three times, to measure spawning success. Eggs from each pair were collected in separate petri-dishes and the fertile eggs produced by each pair were counted.

3. Results

Of the groups tested, zebrafish strain was found to significantly affect the survival rate of zebrafish during the nursery period (p<0.01) and the survival rate of each strain was found to be significantly different to each other (p<0.01). In contrast, light level had no significant effect on survival rates in any of the zebrafish strains studied (Figure 3).

Spawning success was also significantly affected by the strain of zebrafish (p<0.05), with a significant difference found between wildtype and crystal spawning success (p<0.01). In comparison, light level had no significant effect on spawning success in any of the zebrafish strains studied (Figure 4).

There was no significant effect of either zebrafish strain or light level on the fertility of any of the zebrafish studied (Figure 5).

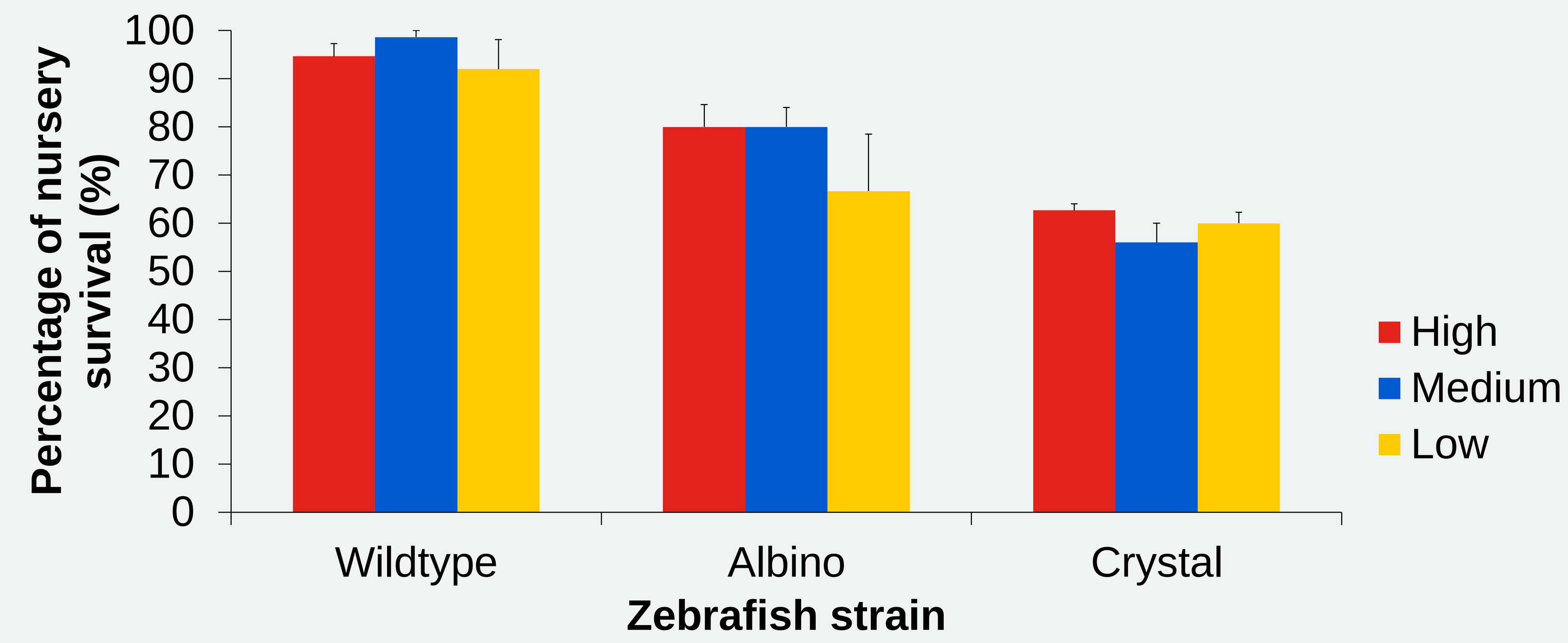

Figure 3 Average survival of three zebrafish strains during the nursery period when exposed to three LUX levels. Error bars show standard error.

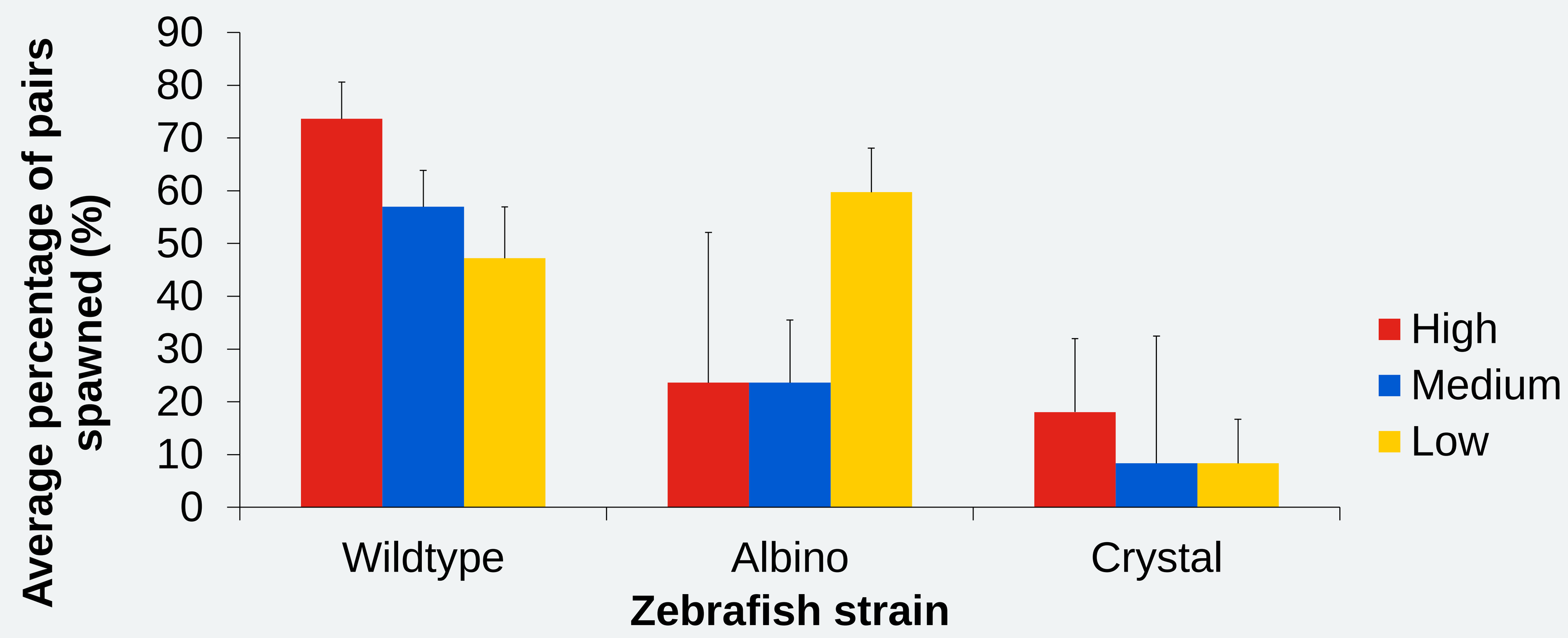

Figure 4 Average spawning success of three zebrafish strains raised at three different LUX levels. Error bars show standard error.

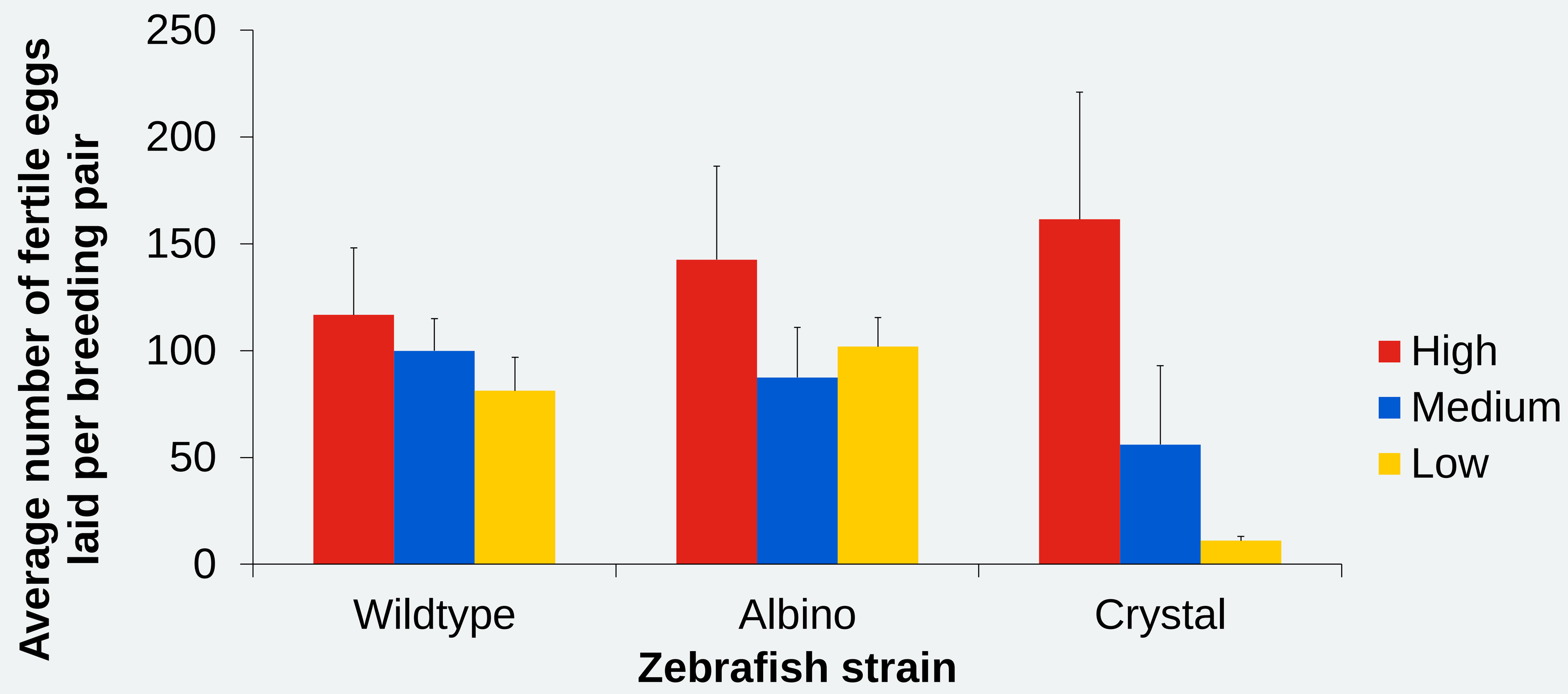

Figure 5 Average number of fertile eggs laid by three zebrafish strains raised at three different LUX levels. Error bars show standard error.

4. Discussion

The results demonstrate that **crystal mutants exhibit significantly lower survival rates** (Figure 3) in comparison to albino and normally-pigmented wildtype strains. The current study suggests lower levels of pigmentation to be associated with lower survival in zebrafish, which is in line with previous studies (Wilson, 2012; Barwood *et al.,* 2017).

It can also be seen that the **average number of spawning crystal pairs was lower than for either wildtype or albino strains** (Figure 4). Wildtype and crystal strains show a trend for increased spawning success at the highest LUX level, whilst albino zebrafish spawned best when housed at the lowest LUX level.

The study found **no significant difference in the average number of fertile eggs laid per strain**, which is in agreement with findings of Antinucci and Hindges (2016). All strains showed a tendency for increased fertility when housed at the highest LUX level (Figure 5). To conclude:

- Survival was significantly affected by zebrafish strain, but not light intensity.
- Fertility was not significantly affected by strain or light intensity.
- Spawning success was not significantly affected by light intensity.
- Crystal mutants have significantly lower spawning success compared to wildtypes.

6. References

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