Investigation of Zebrafish Diets Leads to Nutritional Refinements and Reduction of Fish Numbers

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Aim: To assess the impacts of different feeding regimens on zebrafish development refine husbandry and reduce animal numbers.

Introduction

When utilising animal models in research it is of paramount importance that reliable and reproducible science is produced. In order to achieve this model organisms are ideally maintained in standardised conditions, thus minimizing environmentally induced variability and the potential for human error.

As a poikilothermic ectotherm, standardisation of zebrafish husbandry is difficult to achieve. Their internal temperature is determined by environmental conditions. Thus, they have a great capacity for adaption. Often associated with this adaptability is a high level of environmentally induced plasticity. Zebrafish express this plasticity at all levels – genetic, individual, and population. It has also been shown that genetic variation affects responses to nutrients and diet influences gene expression.

Zebrafish husbandry, particularly diets and feeding practices vary greatly, presenting a range of refinement opportunities. Factors that need to be considered relating to feeding are - environmental conditions such as water quality, lighting, system design, food availability, food attraction, and feeding behaviour and how the food is digested, assimilated and metabolised.

Variation occurs because of different husbandry practices. There is potential for reduction in animal numbers through the refinement and standardisation of feeding practices.

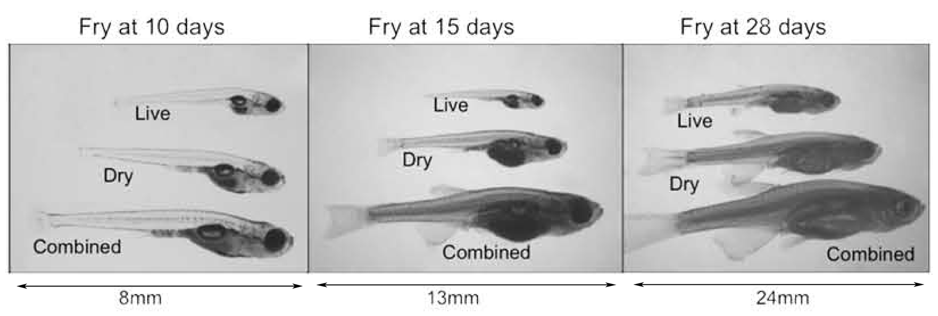


Fig. 1: A trial carried out by Maley et al. 2008 highlighted the impact of diet on early growth in zebrafish. This trial compared their growth when reared on diets consisting of exclusively dry food, exclusively live food and a combination of both.

Methods

Zebrafish larvae were divided into five groups, with each group consisting of triplicates of 40 fish. The fish were AB wildtypes and randomly grouped together from various clutches. Each group was fed five different commercially available dry diets, all supplemented with live rotifer. The diets were administered four times a day until 56 days post fertilisation (dpf). Standard length (SL) and width measurements (fig 2 and 3) were taken approximately every six days; weights were recorded at 112 dpf. Survival was recorded at 28 (data not shown) and 56 dpf.

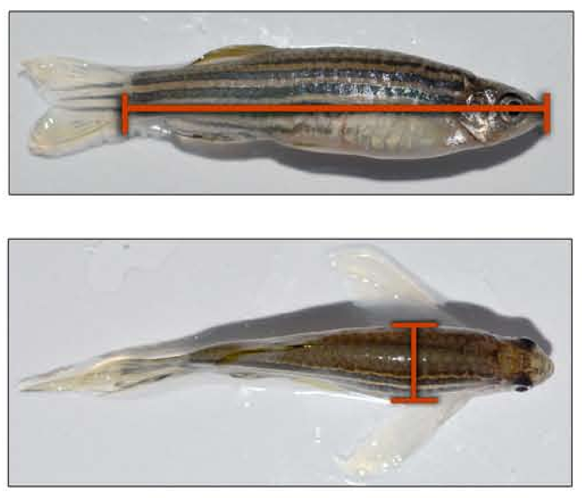


Fig. 2 (above): Standard length (SL) is measured from the tip of the snout to the base of the caudal fin. (Below) Width was measured across the widest part of the body when viewed dorsally.

Discussion

This study suggests that nutrition can have a big impact not only on growth, as might be expected, but also sex ratios – which may be more unexpected. The impacts of varying zebrafish nutrition are poorly understood (Watts et al. 2012) but it is known that dietary nutrients can interact with molecular mechanisms and modulate physiological functions. The effect food has on gene expression (nutrigenomics) and how genetic variation affects responses to nutrients (nutrigenetics) needs more study; currently, the only factors considered are survival, growth rate, and fecundity but there are many other parameters to be considered.

The adaptability of zebrafish likely means that dietary and feeding practices cannot be considered in isolation. Their environment, along with availability and attractiveness of food, the feeding behaviour of the fish and their ability to digest different nutrient sources all play important roles. When taken cumulatively, these variables may not be so subtle and will surely impact the outcomes and reproducibility of the science.

A greater understanding of the impacts of various nutritional factors will lead to greater refinements in zebrafish feeding regimens and thereby a reduction in animal numbers. We see this trial as a stepping stone along the path to greater welfare.

Results

The SL of zebrafish reared on different diets was measured and the averages plotted over time. This creates a growth curve showing growth rates (fig. 4). At 56 dpf, the fish fed diet D were significantly shorter than those fed diets A, B, C, and E (Fig. 3); this, combined with obvious skeletal deformities, is why its use was stopped at 56 dpf. There was no significant difference between diets A, B, C, and E. The survival was similar for all the diets used (Fig. 5).

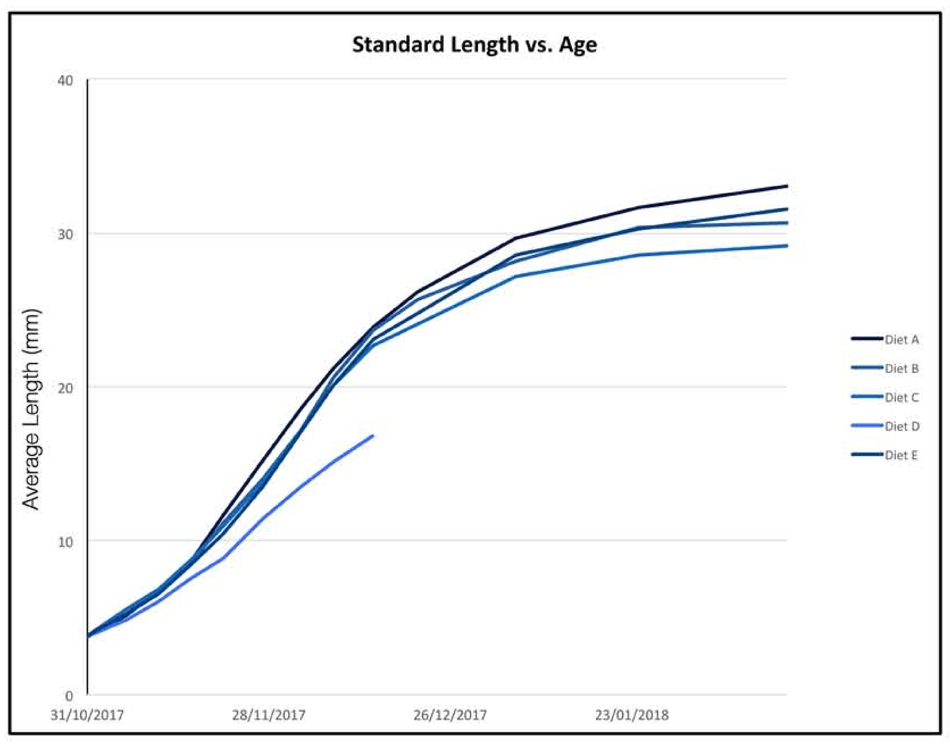
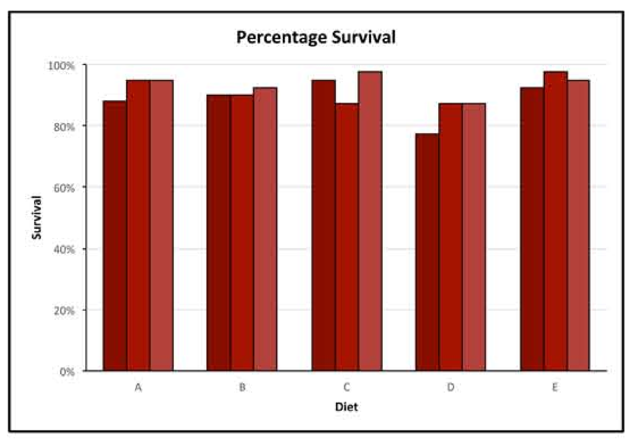


Fig. 4 (left): growth curve showing the average lengths of zebrafish reared on five different diets. Diet D notably has a smaller average length.

Fig. 5 (below): the survival rates of each triplicate over the five diets.



The similar survival rates of all five dry diets, and similar average SLs at 56 dpf of fish fed dry diets A, B, C, E suggest similar performance between the diets except diet D, which was found to be nutritionally deficient. However, differing sex ratios, SLs at 112 dpf, SL at sexual maturation, and the highly varied female weights at 112 dpf indicate greater phenotypic variation due to nutrition. .

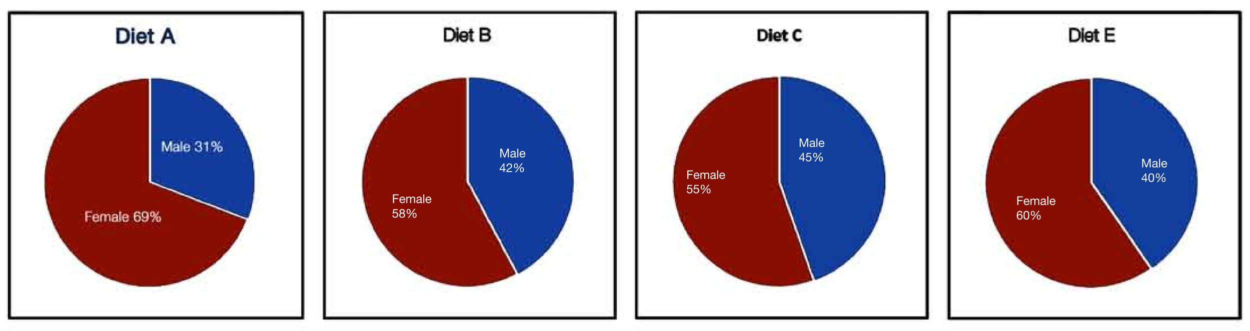


Fig. 6:The fish fed dry diets A, B and E had significant differences in the ratio of females and males

At 56 dpf the zebrafish fed dry diets A, B and E had significant differences in the proportion of female and male fish (fig. 6).

The scatter plots of width against SL suggest that zebrafish reared on different diets reach sexual maturity at different sizes. The region where the data splits indicates when the body proportions of female fish change due to ovary development. (Fig. 7). Fig 8 shows that differences in width of the males and females.

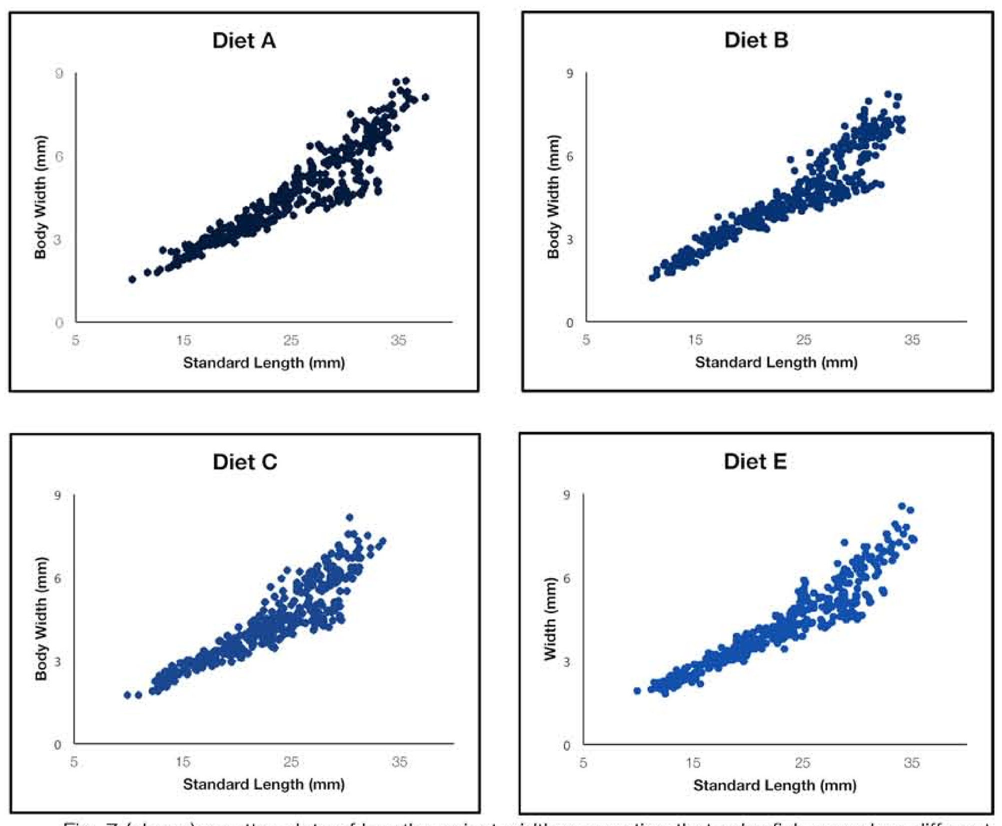
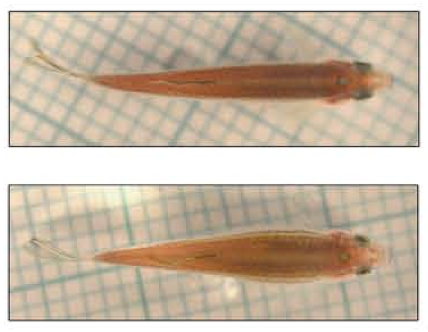


Fig. 7 (above): scatter plots of length against width suggesting that zebrafish reared on different diets reach sexual maturity at different lengths.



Figs 8 (left): The body width of a well conditioned a mature male (left) zebrafish is less than that of a female (right).

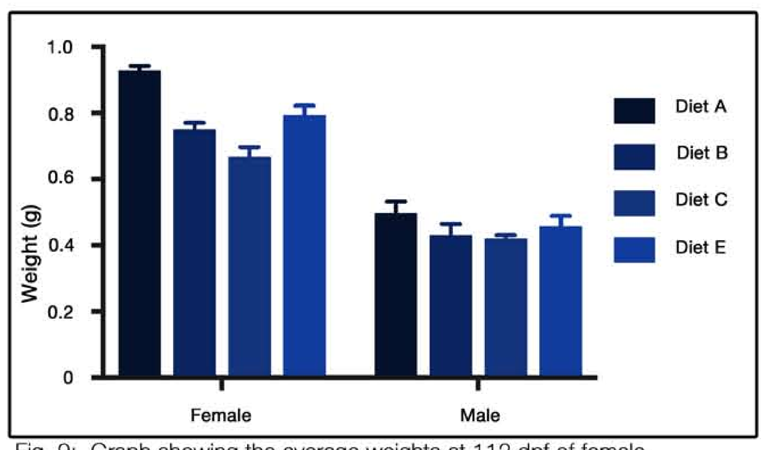


Fig. 9: Graph showing the average weights at 112 dpf of female and male zebrafish reared on four different dry diets.

The weights of female and male zebrafish were recorded at 112 dpf. The females fed dry diet A were significantly heavier than those fed dry diets B, C, and E. The females fed dry diet C also weighed significantly less than those fed the dry diets B and E. No significant differences were found between the weights of the males. (Fig. 9)

Further Work

This will run for a further 12 months. Parameters to be assessed are fecundity, long term health, body composition analysis, creating a body condition index and behavioural analysis. Following this trial, we will assess successive generations to investigate the impacts of parental nutrition and will compare the performance differences between strains to identify any potential genetic effects.

References
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