Comparison of Feeding Regimes for Zebrafish (*Danio rerio*)

Paul Barwood, Carole Wilson UCL Zebrafish Facility

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

Intro

The reliability and reproducibility of research utilising animal models is paramount. Ideally, a model organism is maintained in standardised conditions, thus minimising variability. Being a poikilothermic ectotherm, zebrafish are highly adaptable, they express environmentally induced plasticity at all levels: genetic, individual, and population.

Zebrafish husbandry, particularly the diets and feeding practices in use vary greatly, presenting various refinement opportunities. Nutritional requirements differ at various life stages, but zebrafish are typically fed one type of dry diet that only varies in size, not composition. Live foods are commonly used to supplement dry diets, increasing survival rates and reproductive performance (Goolish et al., 1999) and may constitute environmental enrichment. However, live food can act as a vector for pathogens and is another source of variability. Removing this aspect of their husbandry has greater implications than the effects on growth and survival.

This poster highlights some of the variation that occurs not only because of different husbandry practices, but also from the technicians caring for the animals. The lack of standardisation between facilities means that there are many possible husbandry refinements, with these refinements resulting in a reduction of animals being used and improving welfare.

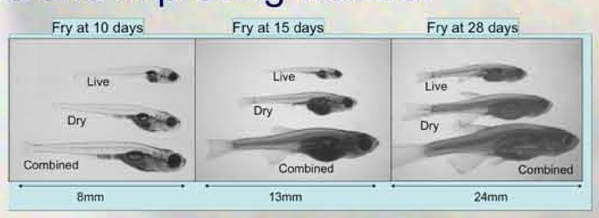

Fig. 1: A trial by Maley et al 2008 highlighted how zebrafish growth is affected by diet.

Methods

In Trial 1 zebrafish were fed four different diets containing dry food, live brine shrimp (Artemia spp.), frozen copepods, and live rotifer (B. plicatilis). Standard length (SL) was measured approximately every 8 days, with survival recorded at 28 days post fertilisation (dpf) and 56 dpf.

In Trial 2 zebrafish were fed 5 different commercially available dry diets, all supplemented with live rotifer. SL and width measurements were taken approximately every 6 days, weights were recorded at 112 dpf. Survival was recorded at 28 dpf and 56 dpf.

Fig. 2: Live food. From top left: artemia, copepods, and rotifer.

Results 1

Human error affected this trial. The dry diet feeding was found to be inconsistent, negatively impacting the reliability of the results. The diets supplemented with live artemia or live rotifer had the best growth, smallest size variation, and survival, indicating that their use can help to mitigate against suboptimal use of dry food.

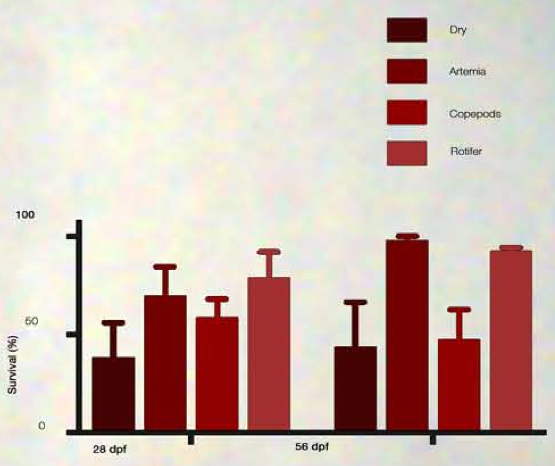

Fig. 3: Chart showing the survival rates of zebrafish at 28 dpf and 56 dpf that were reared using four different diets.

Results 2

At 56 dpf, the fish fed diet D were significantly shorter than those fed diets A, B, C, and E. There was no significant difference between diets A, B, C, and E. The survival was similar for all the diets used.

The similar survival rates of all five dry diets, and similar average SL's at 56 dpf of fish fed dry diets A, B, C, E suggest similar performance between the diets. 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. Dry diet D was found to be nutritionally deficient, resulting in poor growth rates and caused skeletal deformities, its use was stopped at 56 dpf.

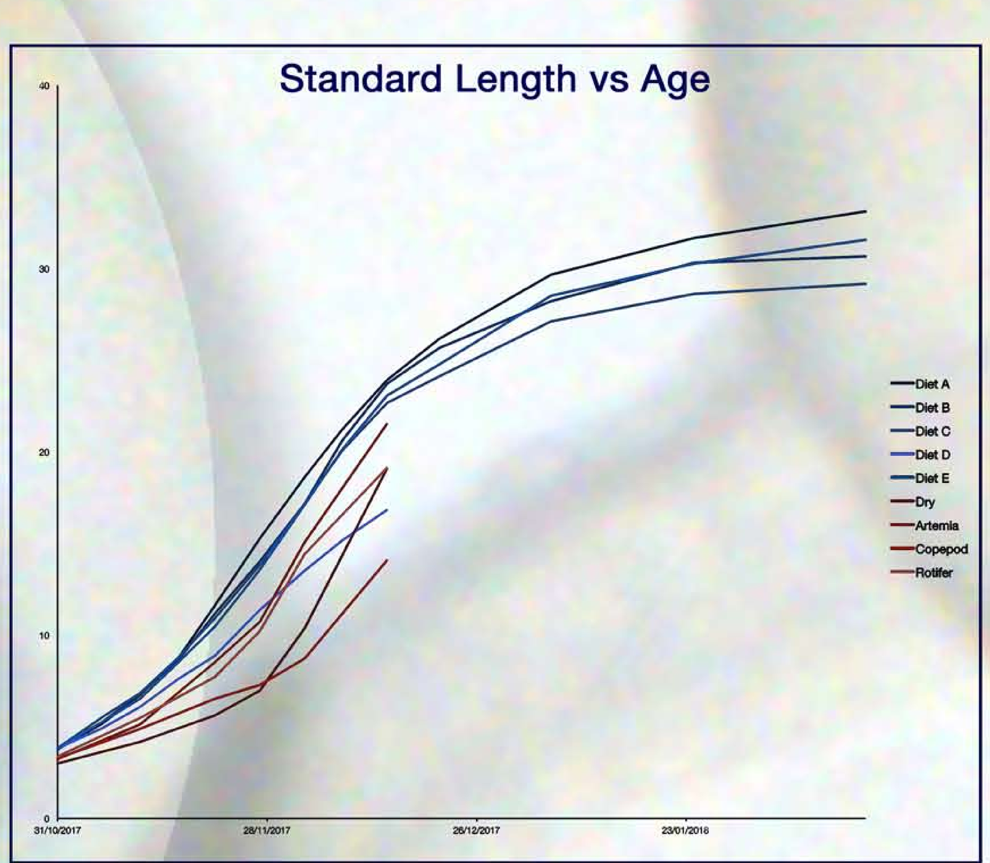

Fig. 4: growth curve showing the average lengths of zebrafish reared on eight different diets from both trials one and two.

The SL of zebrafish reared on different diets was measured and the averages plotted over time. This creates a growth curve showing growth rates. Trials one and two are shown together allowing for comparison. Trial one and diet D of trial two were stopped at 56 dpf. (Fig. 4).

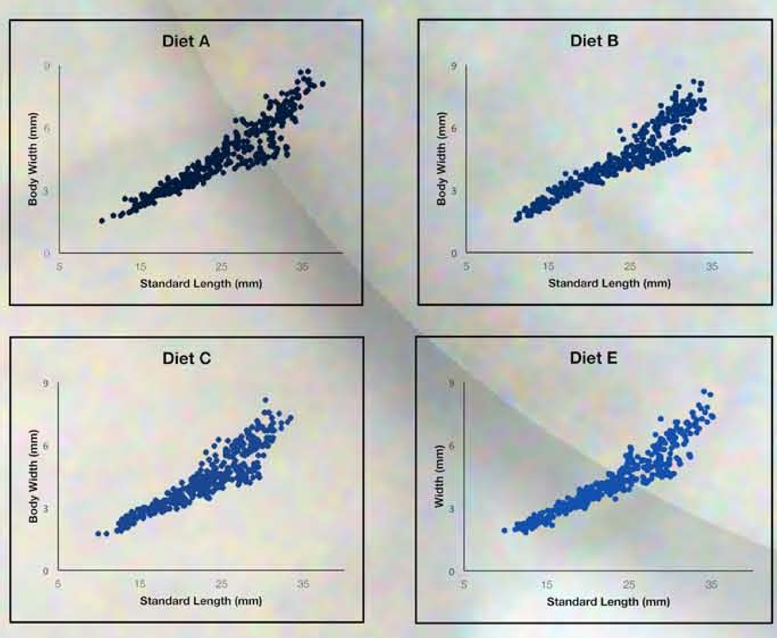

Fig. 5: scatter plots of length against width suggesting that zebrafish reared on different diets reach sexual maturity at different lengths.

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. 5). Fig 6 shows that differences in width of the males and females.

Figs 6: The body width of a well conditioned a mature male (left) zebrafish is less than that of a female (right).

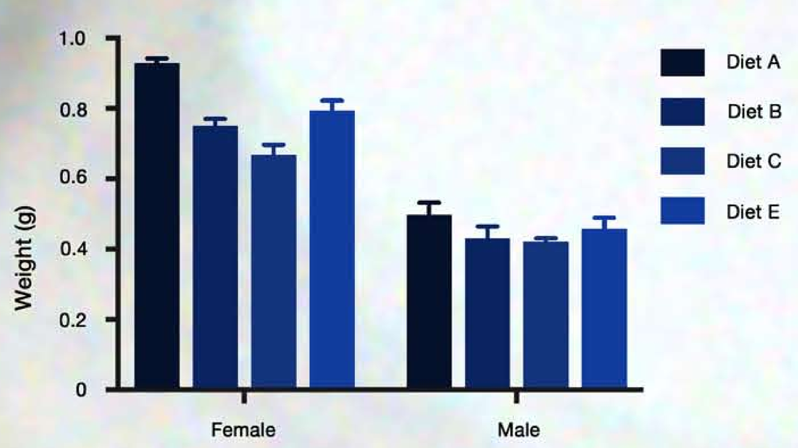

Fig. 7: Graph showing the average weights at 112 dpf of female and male zebrafish reared on four different dry diets.

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. 7)

At 56 dpf the zebrafish fed dry diets A, B and E had significant differences in the proportion of female and male fish. (Fig. 8)

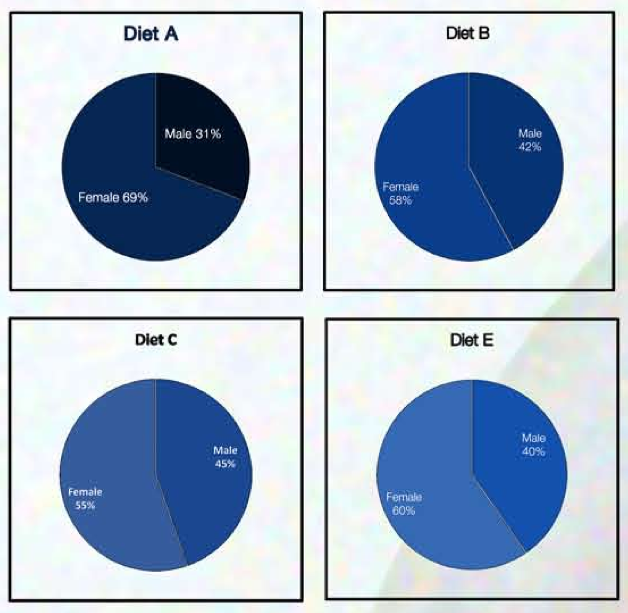

Fig. 8:The fish fed dry diets A, B and E had significant differences in the ratio of females and males

Discussion

As we know, zebrafish are very plastic and 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 – usually the only factors considered are survival, growth rate, and fecundity. The adaptability of zebrafish likely means that dietary and feeding practices cannot be considered in isolation. It is likely that small environmental changes such as tank proportions and colour, stocking density, water flow rate and flow dynamics, water temperature and quality, light intensity and spectrum, food quality and quantity, and breeding regimens will produce subtle differences. When cumulatively added they may not be so subtle and will surely impact the outcomes and reproducibility of the science.

Further Work

Further work will include repeating Trial 1 to obtain reliable results, along with testing differences between various strains to identify any potential genetic effects. Additionally, body composition testing between said strains can identify any morphological variables within the study. On top of the testing, this can also be applied to the diet variation. Body condition scoring using different parameters can also be measured, such as body depth compared to length.

Watts, S. A., M. Powell and L. R. D'Abramo (2012). "Fundamental Approaches to the Study of Zebrafish Nutrition." Ilar Journal 53(2): 144-160.
Goolish EM, Okutake K, Lesure S. 1999. Growth and survivor- ship of larval zebrafish Danio rerio on processed diets. NA J Aquaculture; 61:189–198

Acknowledgements: H. Callaway, J. Hakkesteeg, V. Moiche, K. Dunford, S. Eshmade, and E. Hitchcock.