

# Food trials conducted to improve the survival and development of Zebrafish

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## Introduction

Zebrafish has joined the mouse as one of the most important model organisms (MO) to study biological processes *in vivo*, due to their high fecundity, rapid generation time and optical translucency during early embryogenesis.

The mouse, due to its genetic tractability, has been a stable research tool for over a century. In this time much has been done to understand the husbandry requirements of the species including dietary requirements. While there is a considerable amount of literature about Zebrafish research there is very little with regards to their husbandry requirements, most importantly their nutritional needs. Many research facilities suffer with high mortality rates within their Zebrafish fry as well as deformities and gender bias. Poor dietary provision such as over or under feeding or a poor quality of diet is a major contributing factor.

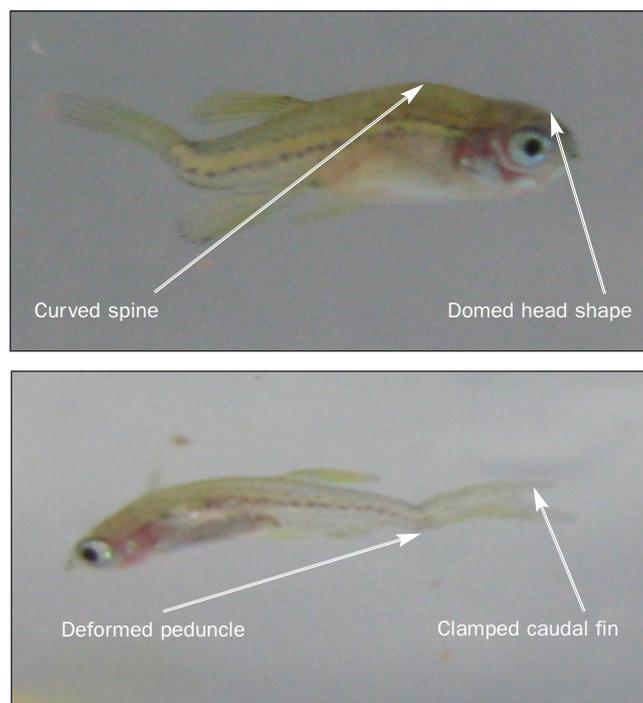
Here we have looked at a variety of feeding regimes that can potentially enhance the survival rate and quality of life for Zebrafish fry. The overall aim is to define the optimal breeding diet and regimes to support larval and fry development by improving survival rates and providing adequate nutrients to



**Figure 1.** How a normal healthy Zebrafish should appear at 3 months of age.

reduce the chance of deformities occurring as well as improving gender ratio.

## Deformed fish – lack of nutritional requirements?



**Figures 2 & 3.** The deformities found in the Zebrafish reared on ZM at 3 months of age.

## Zebrafish diets

Over 100 dry fish foods are commercially available. Only around 5% of these diets claim to be a specialist Zebrafish diet. Invariably these diets have different nutritional values and can be in multiple forms e.g. granules, pellets, flake or sticks. These are available in

various sizes from 5microns or above with variable sink and float rates. An alternative to a manufactured feed is live food. Of these the 3 most commonly used are Artemia (Brine shrimp), Paramecia (Protozoa) and Rotifers.



Figure 4. Examples of diets.

### Paramecia

Live food is commonly used within Zebrafish facilities, with Paramecia being the most popular. Paramecia is a single celled ciliate protozoa which ranges from 50-350µm in length. It propels its body by the simple cilia at speeds of 20 body lengths per second. It is cultured within our facility in Petri dishes and fed yeast and wheat grains. Survival rates for fry reared on Paramecia have been recorded as high as 100%.

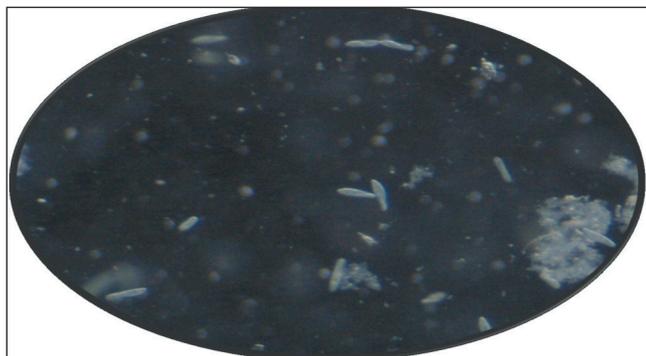


Figure 5. Close up of live food.

### Trial 1

ZM Larval (Current) vs. Kyowa Larval (Specialist). These are both a dry powdered diet of the same micron. From 5 days of age; half of the fry were reared on ZM Laval and half reared on Kyowa. All fry were held in 2 litre tanks at a density of 50 fry per tank on a non-

Diet	Av. Fry into nursery	Av. Transferred	Survival %	No. of Male	No. of Female	% of deformed fry
Kyowa	90	73	58-100%	37	36	0-4%
ZM	90	57	49-91%	25	31	5-44%

Av, survival for fry reared on Kyowa	82%
Av, survival for fry reared on ZM	66%

Table 1. Fry survival rate when reared on ZM Laval diet vs. Kyowa Laval diet.

drip flow system until 15 days of age. Artemia was also fed from 15 days onwards. The results were collected on the day of transfer to adult modules – 84 days of age. 6 study groups were assessed.

### Trial 2

Comparison between ZM Laval diet and Zeigler Laval diet. Zeigler Laval is defined as a highly digestible diet which is available in sizes <100 to 500 microns. Due to the particle size and digestibility it is said to be suitable for very young fry.

Diet	Av. Fry per study	Av. Alive day 30	Av. Transferred	Spread of % survival	No. of male	No. of female	% of deformed fry
Zeigler	150	104	97	61-77%	46	51	5-20%
ZM	150	55	45	12-55%	30	15	32-61%

Av. survival for fry reared on Zeigler	65%
Av. survival for fry reared on ZM	30%

Table 2. Fry Survival rate when reared on ZM Laval diet vs. Zeigler Laval diet.

### Trial 3

100 fry were reared on Paramecia and Zeigler Laval diet. The controls were fed Zeigler from 5 days of age whereas the trial fry were fed Paramecia from 5-15 days of age, before they were then also reared on Zeigler. All fry were given Artemia from 15 days of age. As the paramecia are fed within 40mls of system water twice each day the controls were also dosed with the same amount of water. Fry were reared in 2 litre tanks under the same conditions.

Diet	Av. Fry placed into Nursery	Av. Alive day 30	Survival rate range	% of deformed fry
Paramecia	83	76	87-94%	0
Zeigler	83	52	20-78%	2%

Average survival rate for Zeigler reared Fry	60%
Average survival rate for Paramecia reared Fry	91%

Table 3. Survival rates of fry reared on Paramecia vs. Zeigler.

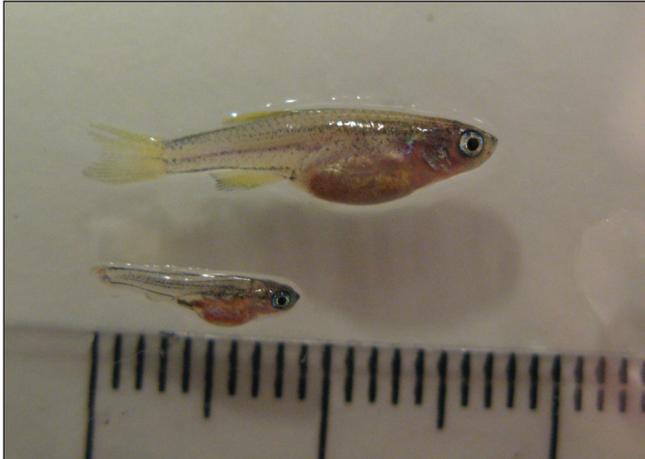
## Results and discussion

Trials 1 and 2 clearly show that the diet fed to fry can have a profound effect on their development, their potential for growth deformities and their likely survival rate and sex ratio.

Of the powdered diet, Kyowa Laval showed good survival rate, tightest sex ratio and lowest deformity

levels. ZM Laval diet has the lowest survival rate and a heavy sex bias towards males. Zeigler Laval showed a consistent survival rate which is important when predicting breeding programs and experimental cohorts.

From the data we gathered it was clear that feeding the Zebrafish fry the live paramecia up to 15 days of age gave us the greatest survival rate by as much as a 31% increase when compared to the 3 dried foods – Zeigler, ZM and Kyowa.



**Figure 6.** Growth difference of Fish raised on ZM Laval diet at 3 months of age.

Growth rate was seen to be highly variable for all fry raised on the dry diet while those raised on Paramecia grew at a more controlled and comparable rate. It should also be noted that a lower incidence of deformities was also observed, suggesting that Paramecia is an accessible and nutritious food source for the fry.

This improvement in growth and consistency along with survival allows us to plan and execute better breeding and experimental programs which, within a large scale aquatics production facility, is essential. It should also be noted that the lower levels of deformities is highly desirable from a welfare perspective.