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Study of Impact of Diet Conditions on Egg Laying Behavior of Zebra Fish

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ABSTRACT

Zebra fish is one of the most used model organisms in biomedical sciences and other research fields. Despite the huge importance of having an efficient and high throughput zebra fish aquaculture, little is known on its specific feeding requirements and very few studies have focused on the impact of feeds on the overall fish performance throughout their lifecycle. At the end of the diet, the body mass index of the 60 mg arm was 1.5 fold greater than the 5 mg arm. The intervention also had a marked impact on fertility; breeding success and egg production in the 60 mg arm were increased 2.1- and 6.2-fold compared to the 5 mg arm, respectively. Mean spawning success was significantly higher in zebra fish fed diet 5 than in those fed diets 1 - 4. Mean fertilization and hatching rates were higher in the fish fed diet 3 - 5 than in diets 1 and 2. Zebra fish consumed commercial pellet feed only resulted in more viable offspring's and grown better. Results suggested that commercial pellet is suitable in zebra fish culture for maximum growth and production of viable offspring in laboratory condition.

1. Introduction

Zebra fish, Danio rerio, is a small tropical freshwater cyprinid. Its small size, external development, high fecundity, embryo transparency, short generation time, easy management, physiological similarity to mammals and the amount of research tools available, have turned this species into one of the main model organisms for developmental studies. In the last two decades the use of this research model has dramatically widespread to various fields of biological sciences, being currently used in ecotoxicology, neurosciences, behavior, cancer, genetics, nutrition, aquaculture, as a model for human diseases, drug discovery, among others.

This boom in research has led its aquaculture to grow exponentially worldwide without sufficient accompanying studies on novel methods for husbandry and larval rearing which would optimize an intensive production of fish for research with adequate standardization and fish welfare. With new transgenic tools being rapidly developed, the zebra fish mutation project as well as its current role as a powerful tool in drug screens, it became essential to find better protocols and methods to breed and raise zebra fish faster, more reliably and with better welfare. However, little is known on the nutritional requirements of zebra fish, being mostly reared with information available for Cyprinaformes. This is becoming a major concern within the research community as it imposes a difficulty to standardize a husbandry protocol in different facilities. Proper nutrition is not only important for individual growth and survival but also to the reproductive success which directly affects offspring fitness. Traditionally, zebra fish have been raised with a combination of live feeds and processed dry feeds. In the first 5-7 days post-fertilization (dpf), larvae extract nutrients from the yolk sac not requiring exogenous feeding. After that period they are commonly fed with paramecium or rotifers until 9-15 dpf. Thereafter their diet is based on artemia (Artemia nauplii) complemented with processed dry feed.

Growth and reproduction are the main parameters that are directly linked with profitability and productivity of fish production. Zebra fish are omnivorous and growth rates mainly depend on diet as well as age and season, with rapid growth in early life stage in monsoon. Although pertinent literatures are available on zebra fish as model animal in research but a few documents are available in nutritional requirement specially protein requirement of zebra fish. Thus there is a need to study protein requirement of zebra fish for proper growth and reproduction. Therefore, the study was conducted to determine the effect of 3 natural and 1 commercially available diet on growth, reproduction and embryogenesis of zebra fish.

2. Literature Review

MD GOLAM RABBANE (2017) This study evaluated the effects of five diets (diet 1: Dried tubifex, diet 2: Artemia, diet 3: Artemia and commercial pellet feed, diet 4: Spirulina and commercial pellet feed; diet 5: commercial pellet feed) on growth, reproductive performances and embryogenesis of zebra fish Danio rerio for a period of 62 days. Significantly higher specific growth rate was found in diet 5 when compared with diet 1 but no significant difference was observed between diet 2, 3, 4 and 5. Mean weight and length gain were significantly greater in zebrafish fed diet 5 than diets 1 - 4. While 100% survival was found in diet 4, the lowest level was 90.26 ± 1.06% in diet 1.

Marc Tye (2019) Dietary contaminants are often an over-looked factor in the health of zebra fish. Typically, water is considered to be the source for most contaminants, especially within an aquatic environment. For this reason, source water for zebra fish recirculating systems is highly regulated and monitored daily. Most facilities use reverse osmosis or de-ionized water filtration systems to purify incoming water to ensure that contaminants, as well as pathogens, do not enter their zebra fish housing units. However, diets are rarely tested for contaminants and, in the case of manufactured zebra fish feeds, since the product is marketed for aquaculture or aquarium use it is assumed that the feed is acceptable for animals used for research. The following provides examples as to how contaminants could lead to negative effects on development and behavior of developing zebra fish.

L. Adele Fowler (2019) The value of the zebra fish (Danio rerio) as a model organism continues to expand. In developing the model, current feeding practice in zebra fish laboratories includes the use of commercially available diets. In this study, we compared outcomes in growth, body composition, and reproduction among zebra fish fed five highly utilized commercial diets and one formulated chemically defined

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reference diet. Wild-type zebra fish larvae were raised on live feed until 21 days post-fertilization and then fed diets for 16 weeks. All fish received a daily ration of >5% of body weight (adjusted biweekly). Growth varied among diets throughout the feeding trial, and at study termination (week 16), significant differences among diets were observed for terminal weight gain, body condition index, body fat deposition, and reproductive outcomes. In addition, the proportion of viable embryos produced from females fed the formulated reference diet was high relative to the commercial diets. These data suggest that metabolic profiles, most likely reflecting nutrient/energy availability, utilization, and allocation, vary relative to diet in zebra fish. Undefined differences in metabolic profiles could result in erroneous predictions of health outcomes and make comparisons among laboratories more challenging. We recommend that dietary standards should be defined for zebra fish to support their common utility in biomedical research.

Stephen A. Watts, (2020) The value of the zebra fish model has been well established. However, culture variability within and among laboratories remains a concern, particularly as it relates to nutrition. Investigators using rodent models addressed this concern several decades ago and have developed strict nutritional regimes to which their models adhere. These investigators decreased the variability associated with nutrition in most studies by developing standardized reference and open formulation diets. Zebra fish investigators have not embraced this approach. In this article, we address the problems associated with the lack of nutritional information and standardization in the zebra fish research community. Based on the knowledge gained from studies of other animals, including traditional research models, other fish species, domesticated and companion animals, and humans, we have proposed an approach that seeks to standardize nutrition research in zebra fish. We have identified a number of factors for consideration in zebrafish nutrition studies and have suggested a number of proposed outcomes. The long term-goal of nutrition research will be to identify the daily nutritional requirements of the zebrafish and to develop appropriate standardized reference and open formulation diets.

Avdesh Avdesh (2012) this protocol describes regular care and maintenance of a zebra fish laboratory. Zebra fish are now gaining popularity in genetics, pharmacological and behavioural research. As a vertebrate, zebra fish share considerable genetic sequence similarity with humans and are being used as an animal model for various human disease conditions. The advantages of zebra fish in comparison to other common vertebrate models include high fecundity, low maintenance cost, transparent embryos, and rapid development. Due to the spur of interest in zebra fish research, the need to establish and maintain a productive zebra fish housing facility is also increasing. Although literature is available for the maintenance of a zebra fish laboratory, a concise video protocol is lacking. This video illustrates the protocol for regular housing, feeding, breeding and raising of zebra fish larvae. This process will help researchers to understand the natural behavior and optimal conditions of zebra fish husbandry and hence troubleshoot experimental issues that originate from the fish husbandry conditions. This protocol will be of immense help to researchers planning to establish a zebra fish laboratory, and also to graduate students who are intending to use zebra fish as an animal model.

3. Material and Methods

Animals

Zebra fish research was approved by the University of Otago Animal Ethics Committee. Mature zebrafish were maintained in 3.5 L tanks on a Palletized Centralized Life Support System (Tecniplast). The water in this recirculating system was pumped through mechanical filtration, charcoal filtration, and UV-treatment; and 10% of the water was replaced every hour. The water was kept at 26–30˚C, with pH 7.6–8.0 and a conductivity of 300–600 µS. The facility environment maintained a 14-hour light and 10-hour dark circadian cycle. Water quality parameters were automatically measured and adjusted, and remained within acceptable limits for the duration of the study.

Diets

The five commercial diets utilized in the study were acquired from commercial vendors and consisted of the following: Tetramin Tropical Flakes (Spectrum Brands, Blacksburg, VA), Otohime C1 (Marubeni Nisshin Feed Co. Ltd, Tokyo, Japan), Gemma Micro 300 (Skretting Zebrafish, Westbrook, ME), Ziegler Larval AP100 (Zeigler Bros, Inc., Gardners, PA), and Artemia cysts (INVE Aquaculture, Inc., Salt Lake City, UT). Z12 represents a formulated reference diet that was developed and manufactured in our laboratory (Table 1). Z12, Tetramin, and Otohime were ground to a size that did not exceed 300 lm. Proximate analysis of all diets was performed by Eurofins Scientific Laboratories, Inc. (Table 2). Stage I Artemia nauplii were harvested at 09:00 and 17:00 hours daily. Artemia cultures were maintained in two 2 L brine shrimp hatching cones (Pentair Aquatic EcoSystems Inc., Apopka, FL) at a water temperature of 25C– 26C. Cultures were set up 24 h before harvest, with 1.5 L of purified water, 15 g of synthetic sea salt, and 3 g of nondecapsulated cysts added to each cone. Before feeding, harvested nauplii were strained, rinsed, and resuspended to 200 mL with system water.

Table 1. Dietary Composition of Z12

Ingredient	*g/100 g*
Fish protein hydrolysate (82%)[a,b]	20.00
Casein (vita-free) (96%)[a,c]	25.00
Soy protein isolate (92%)[a,d]	5.00
Wheat gluten (80%)[a,e]	7.00
Wheat starch	9.60
Dextrin	5.00
Soy lecithin	4.00
Canthaxanthin	2.31
Ascorbyl palmitate	0.04
Vitamin premix BML-2[e]	4.00
Mineral mix BTm[f]	3.00
Betaine	0.15
Potassium phosphate monobasic	1.15
Alginate	5.38
Cholesterol	0.12
Menhaden fish oil	4.67
Corn oil	2.33

Composition of the vitamin premix (%): ascorbic acid, 12.5; butylated hydroxyanisole, 0.1; biotin, 0.1; cellulose, 60.0; calcium pantothenate, 1.5; cobalamin, 0.1; folic acid, 0.5; inositol, 18.0; nicotinic acid, 2.6; para-aminobenzoic acid, 3.0; pyridoxine hydrochloride, 0.3; riboflavin, 0.8; thiamine mononitrate, 0.5. f Composition of the mineral premix (%): calcium carbonate, 2.100; calcium phosphate dibasic, 73.500; citric acid, 0.227; cupric citrate, 0.046; ferric citrate, 0.558; magnesium oxide, 2.500; magnesium citrate, 0.835; potassium iodide, 0.001; potassium phosphate dibasic, 8.100; potassium sulfate, 6.800; sodium chloride, 3.060; sodium phosphate, 2.140; zinc citrate, 0.133.

Collected embryos were transferred to Petri dishes (n = 50 per dish) and incubated at 28.5C until 5 days postfertilization (dpf). From 5 to 11 dpf, hatched larvae were poly-cultured in five 6 L static tanks (n = 240 larvae per tank) with the rotifer Brachionus plicatilis at a salinity of 5 ppt, and enriched with Nannochloropsis (RotiGrow Omega, Reed Mariculture). Starting at 11 dpf, each 6 L tank of zebrafish larvae was proffered 20 mL of concentrated Artemia at each feeding (equivalent to >300 nauplii per fish per day) until 21 dpf. At 21 dpf, fish from all 6 L tanks were combined and randomly distributed into 54, 2.8 L tanks at a density of 13 fish per tank.

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Each tank was then randomly assigned to one of six dietary treatments (n = 9 tanks per treatment). The feeding trial was initiated the following day, in which fish were fed the experimental diets for a 16-week period. All diet groups were provided a daily ration (split between the morning and evening feeding) consisting of no less than 5% body weight. To maintain this ration throughout the feeding trial, rations were adjusted for growth every 2 weeks. Fish fed the Artemia dietary treatment were provided >500 nauplii per fish per day (an ad libitum ration in which live Artemia were always present in the water column)

Experimental animals were maintained under a 14-h light/ 10-h dark cycle with lights turned on at 07:00 hours local time. All tanks were maintained at 28C and 1500 lS/cm conductivity in a recirculating system (Aquaneering, Inc.), with 5.4 L exchanged from each tank per hour.

Table 2. Proximate Analysis for Diets

Component	Artemia	*Z12*	*Tetramin*	*Gemma*	*Otohime*	*Zeigler*
Moisture, %	9.56	9.31	7.00	6.21	5.89	3.19
Crude protein, %	58.37	47.9	48.17	60.63	58.85	54.03
Crude fat, %	14.66	12.24	11.01	19.20	14.08	14.37
Crude fiber, %	5.00	2.10	0.80	0.40	1.20	1.30
Ash, %	7.20	6.28	9.34	11.69	13.98	15.49
Carbohydrate, %[a]	5.21	22.17	23.68	1.87	6.00	11.62

Municipal tap water passed through mechanical filtration (5 lm sediment filter and charcoal), reverse osmosis, and a cation/anion exchange resin (Kent Marine). Synthetic sea salts (Instant Ocean) were then added to adjust conductivity for the system water source. Sodium bicarbonate was added as needed to maintain pH of the system water at 7.4. Total ammonia nitrogen, nitrite, and nitrate were measured colorimetrically once weekly (Mars Fish Care, Inc.). Water quality parameters during the experiment are given in Table 3. To help sustain adequate water quality, a minimal water exchange of 20% was performed on the recirculating system once per week, and tanks were siphoned every other day to remove any excess uneaten feed or debris. Tanks were maintained on the same recirculating system throughout the duration of the experiment; however, to reduce environmental confounding effects from noise, light, vibration, or other unidentified sources related to location, tanks were moved to a new position within the recirculating system every 2 weeks.

RNA sequencing

Gamete samples were collected from fish during the weight gain measurements that followed the dietary intervention. Anaesthetized females were blotted dry, placed in a dish, and pressed gently on the belly to release the eggs. The eggs were gathered with a spatula, transferred to tube containing RA1 buffer (Macherey-Nagel, cat. 740955.250) with β-mercaptoethanol, and stored at -80˚C until RNA extraction. Total RNA was prepared from gamete samples taken from group 3 (dictated by the number of samples obtained from the 5 mg arm). The RNA was filtered with the NucleoSpin RNA kit (Macherey-Nagel, cat. 740955.250) and bound and eluted with columns (17–23,000 nt size range) from the RNA clean and concentrator kit (Zymo cat. no. R1017). The resulting RNA was checked for quality and quantity by Nanodrop, Qubit, and Bioanalyzer. Library preparation and RNA sequencing was performed by New Zealand Genomics Limited. Total RNA libraries were prepared for the egg samples using the TruSeq stranded total RNA library kit with Ribozero (Illumina). The libraries were run on the HiSeq 2500 (Illumina) to generate single-ended 100 bp reads. The sequence reads were analyzed using the Tuxedo suite [27]. BiNGO, a Cytoscape plugin, was used for gene ontology analysis [28,29]. For comparing diet-induced changes in gene expression with other datasets, the HCOP: Orthology Predictions Search was used to obtain the human orthologs [30]. The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus [31] and are available through GEO series accession number GSE81007.

The next generation

The fish used in the F1 group were derived from the offspring produced by the incrosses performed in the second spawning experiment (in the first spawning experiment there were no breeding pairs in the 5 mg arm). The eggs from multiple pairs across the three F0 groups were pooled and separated out into 12 dishes of 27 embryos for each treatment arm (dictated by the number of offspring produced by the 5 mg arm). The F1 larvae from both F0 arms were then raised under the same standard facility conditions at 7.7 fish/L with offspring mortality and growth (length) measured over two months of maturation. At two mpf, the fish within each treatment arm were pooled and randomly allocated into two treatment arms, a 5 mg food per fish arm and a 60 mg food per fish arm. This treatment crossover resulted in four F1 groups: "5–5" (F0: 5 mg, F1: 5 mg), "5–60" (F0: 5 mg, F1: 60 mg), "60–5" (F0: 60 mg, F1: 5 mg), and "60–60" (F0: 60 mg, F1: 60 mg). There were three tanks within each treatment arm; these each contained 16 fish that had not been screened for fertility (Table 2). The dietary intervention and measurements were conducted in the same manner as for the F0 fish, described above.

4. Data Analysis

Statistical analysis was carried out using R statistics. The Student's unpaired t-test was used for the comparison of two means. One-way analysis of variance (ANOVA) with Tukey's post-hoc comparisons was used for comparing more than two samples. The fertility data was analysed using generalized linear models with clustering at the level of the tank. The chisquared test was used to determine whether the sex ratio was affected in the F1 generation. When determining statistical significance, a p-value of 0.05 was considered significant.

5. Results

No apparent differences in diet consumption were observed throughout the duration of the experiment. No behavioral or morphological features showed clinical nutritional deficits, as all fish appeared healthy. Survival was calculated at >95% in all diet treatments. At the termination of the experiment, the sex distribution in all diets was heavily skewed toward females (Table 4). Increases in weight gain and length over the course of the experiment were observed in all dietary treatments (Fig. 1A, B). Initially, Artemia-fed fish showed the highest rate of growth in terms of weight gain and length; however, this rate decreased after week 6. SGR varied with diet and week, ranging from 13% to 22% body weight gain per day within the first 2 weeks of feeding, and decreasing with size to <1% by week 14 (Fig. 2).

After controlling for the effects of sex and tank, significant differences among diet treatments were observed for body weight ($F_{5,44} = 24.14$, $p < 0.0001$), total body length ($F_{5,44}$ =

33.41, p < 0.0001), and BCI (F5,44 = 8.46, p < 0.0001). Mean body weight was largest in the Otohime group (927.27 – 38.14 mg) than all other diets, whereas the Artemia group had the smallest mean body weight (641.07 – 18.89 mg)

Table 3. F1 group The number of male and female fish used in each F1 treatment arm. In total there were 16 fish (4.6 fish/L) in each tank.

Arm	Sex	Tank 1	Tank 2	Tank 3
5–5	M	6	4	4
	F	9	11	10
5–60	M	6	7	8
	F	10	8	7
60–5	M	7	3	2
	F	9	13	14
60–60	M	7	4	6
	F	9	12	10

The treatment arms had a very similar BMI before the diet but after the intervention the BMI of the fish in the 60 mg arm was 1.5 fold greater than the fish in the 5 mg arm (Fig 1B). While the weight of the fish increased relative to the length, it should also be noted that the fish in the 60 mg arm were longer in length after the diet (S1 Table). For example, in group 3, the average standard fish length before the diet was 22.5 mm, but following the diet the lengths of fish in the 60 mg arm had increased 3.2 mm (95% CI: 1.6– 4.7, p = 0.0007) while the lengths of fish in the 5 mg arm had not changed significantly. Fish in the 60 mg arm also appeared more colorful, with increased sexual divergence in their coloration (S1 Table). This difference was most apparent in group 1, where the sum of the RGB (red, green, blue) values differed by 7% (95% CI: 4–10, p = 0.0078) between the males and females in the 60 mg arm but only non-significantly by 2% in the 5 mg arm. Weight loss in the 5 mg arm indicated that these fish could not represent a normal-fed control, as described previously by Oka et al., and instead represented conditions of dietary restriction. In group 3, the BMI in the 60 mg arm increased 0.07 kg/m2 (95% CI: 0.04–0.11, p < 0.0001) for males and 0.08 kg/m2 (95% CI: 0.05–0.12, p < 0.0001) for females (Fig 1B). In contrast, in the 5 mg arm the BMI decreased by 0.10 kg/m2 (95% CI: 0.07–0.14, p < 0.0001) for males and 0.11 kg/m2 (95% CI: 0.08–0.15, p < 0.0001) for females (Fig 1B). This decrease in the BMI reflected a drop in the actual weight of the fish in the 5 mg arm (S1 Table). In the 60 mg arm, the males gained 123 mg (95% CI: 81–0.165, p < 0.0001) and the females gained 120 mg (95% CI: 74–165, p < 0.0001). In the 5 mg arm, the males lost 61 mg (95% CI: 19–103, p = 0.007) and the females lost 63 mg (95% CI: 18–109, p = 0.008). For reference, the males in group 3 weighed 220 mg and the females weighed 245 mg before the diet. The diets thus reflect two ends of a spectrum of nutrient availability, from abundance to scarcity.

The dietary intervention had a similar effect on the size of the clutches of eggs produced by the fish (Fig 2B). First, it should be noted that, compared to group 1, the younger fish in group 3 produced smaller clutches; for in crosses in the 5 mg arm this rate was 0.8 fold (95% CI: 0.6– 1.0, p = 0.03) lower. As with breeding success (above), the clutch sizes were unchanged when males from the 60 mg arm were crossed to females from the 5 mg arm (F: 5 mg x M: 60 mg), relative to in crosses within the 5 mg arm. However, when females from the 60 mg arm were crossed to males within the 5 mg arm (F: 60 mg x M: 5 mg), in group 3 the eggs were produced at a rate 1.6 fold (95% CI: 1.0–2.7, p = 0.049) higher than in crosses within the 5 mg arm (Fig 2B). This result is consistent with the observation that the females in the 60 mg arm of group 3 showed a non-significant trend towards having larger ovaries; these were 47 ± 14 mg compared to 21 ± 3 mg in the 5 mg arm (S1 Table). In summary, following the dietary intervention, females in the 60 mg arm were more likely to breed and produce larger clutches of eggs than the females in the 5 mg arm.

Embryonic phenotype is affected by parental nutrition

To determine if the parental diet influenced the embryonic phenotype we examined several aspects of the offspring's development during their first five days of life. Of the eggs that had been fertilized, those from the fish in the 60 mg treatment arm were larger (at 6 hpf) than those produced by the fish in the 5 mg arm (S7A–S7C Fig). This effect was most apparent when comparing the cross of females from the 60 mg arm and males from the 5 mg arm (F: 60 mg x M: 5 mg) with the cross of females from the 5 mg arm and males from the 60 mg arm (F: 5 mg x M: 60 mg). In group 2, the eggs produced by females from the 60 mg arm had a yolk diameter of 675 ± 2 μm; this was significantly greater (p = 0.012) than the yolk diameter from the 5 mg arm (663 ± 2 μm; p = 0.012; S7B Fig). We also observed a nonsignificant trend towards larger chorions, which when normalized by the diameter of the yolk, were 4% larger (on average, across the three groups) for the eggs from females in the 60 mg arm compared to the eggs from the females in the 5 mg arm. The fish of both treatment arms in the three parental groups produced fertilized eggs that could successfully grow into larvae. The fertilized eggs hatched at the expected rate, typically at 2 dpf, with 99% (average across all groups) having hatched by 3 dpf. Early survival, out to 5 dpf, was high at 97% (average across all groups) and not affected by parental diet. Tail malformation in larvae, occurred sporadically at a frequency of 1% (average across all groups) and was not affected by the parental diet. However, there was acephaly (larvae that developed without a head) among 0.5% of offspring produced by incrosses within the 60 mg arm. This malformation was apparent at 1 dpf, in group 1 (n = 1 in 268 embryos), group 2 (n = 2 in 317 embryos), and group 3 (n = 1 in 227 embryos), and was never observed in crosses with the 5 mg arm. In general, however, the offspring of both treatment arms were viable. We were interested to know whether the parental diet could alter lipid deposition in the larvae. At 5 dpf, the larvae produced by the fish in the 60 mg arm showed increased staining for lipids (S7D–S7F Fig). This effect was significant in the larvae produced by group 2, where the larval staining absorbance was 1.07 ± 0.18 for incrosses within the 60 mg arm; this was larger (p = 0.002) than the staining absorbance of 0.63 ± 0.03 noted for incrosses within the 5 mg arm (S7E Fig). The females made a large contribution to amount of lipid in the larvae, with the absorbance also being greater than the incross within the 5 mg arm (p = 0.004), at 0.97 ± 0.12, when the females from the 60 mg arm were crossed to the males from the 5 mg arm (F: 60 mg x M: 5 mg). Our data show that the parental diet can alter the phenotype of the offspring even at a very early stage of development.

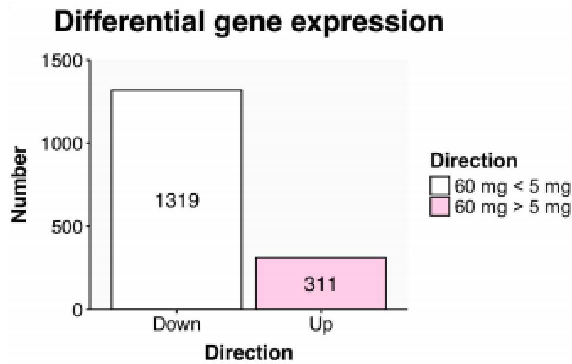

Fig 1: RNA sequencing revealed diet-induced differences in egg transcript deposition

Parental diet affects F1 survival and energy expenditu

To this end we raised an F1 group from a pool of offspring that was produced by incrosses within the treatment arms of the three parental groups. The survival and growth of this F1 group was tracked through juvenile development (Fig 4A and 4B). While survival out to 5 dpf was not affected by the parental diet (above), a difference in survival did became apparent at 10 dpf (Fig 4A). By 18 dpf, the survival in the progeny of the 5 mg arm was significantly lower ($p < 0.0001$) at 58 ± 2%, than the survival in the progeny of the 60 mg arm (79 ± 3%). At the end of the measurement period, at 63 dpf, the survival in the progeny of the 5 mg arm was 28% lower (95% CI: 16–41, $p < 0.0001$) than the survival in the progeny of the 60 mg arm. The parental diet did not, however, result in any significant differences in the growth of the progeny, in terms of their body length over time (Fig 4B). The resulting progeny of the 60 mg arm did show a skew towards females in the progeny, but this difference was not ($p = 0.2$) significant (Fig 5C). Therefore, the F1 generation represented a group of fish that differed according to their parental nutritional environment and that showed altered survival into adulthood. To find out how nutrient availability in the parental generation might affect the response to nutrient availability in the offspring we conducted an intergenerational crossover study.

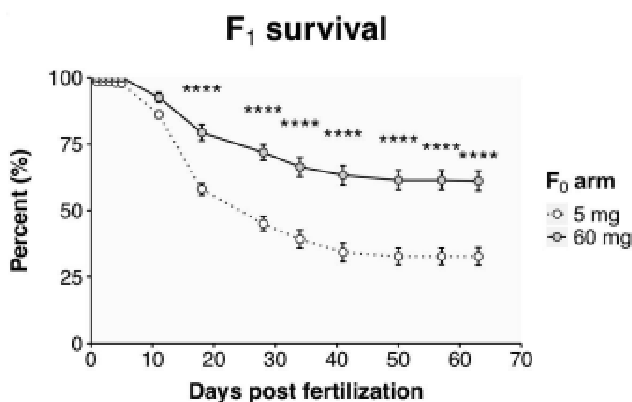

Figure 2: Parental diet affects F1 survival.

The F1 progeny, from both the 5 mg arm and the 60 mg arm, were again given either 5 or 60 mg of food each day, resulting in four F1 treatment arms. The F1 fish consumed the majority of their food with the fish given 60 mg each day (5–60 and 60–60) eating, on average, 98 ± 0.4% of the Artemia they were given (Fig 5A). In the last meal of the day, the fish given 60 mg each day consumed 2.5% (95% CI: 1.5–3.5, $p < 0.0001$) less food than their second meal. It was noticed that at the start of the diet, in week 1, the fish from the 5 mg parental arm (5–60) ate 4% less of Interestingly, an intergenerational effect was observed in swimming activity (Fig 5C). At the start of the diet, those fish in the 60 mg arm (5–60 and 60–60) were travelling a slightly larger distance than those fish in the 5 mg arm (5–5 and 60–5), but this was not significant. As in the F0 parental generation, at the end of the diet, those fish in the 60 mg arm showed increased swimming activity. However, of note is the difference between the 5–60 and 60–60 arms. At the end of the diet those fish in the 5–60 arm collectively travelled 7.0 ± 0.4 m, while those fish in the 60–60 arm travelled 9.2 ± 0.5 m ($p = 0.029$). The high dietary intake in the parental generation therefore appeared to allow for increased physical activity in the subsequent generation when exposed again to a high dietary intake.

6. Conclusion

Though diet is a principal route for contaminants to enter laboratory animals, testing and reporting of dietary contaminants is essentially nonexistent within the zebrafish community. Granting organizations and industry journals must demand detailed reporting of diet regimes including feed type, quantity fed, nutrient content, and contaminant levels. These details go beyond what is specified in the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines, which is endorsed by many peer-reviewed journals. Reporting diet regimes by simply stating “fed per standard zebrafish protocol” or “fed brine shrimp nauplii” does not provide sufficient detail to reproduce the study and should not be acceptable in peer-review publications. A feasible way to accomplish the amount of detail that is needed would be to establish open formula diets and conduct batch testing of nutrient and contaminant content. An open formula diet is a feed where the concentrations of all ingredients are publicly available. Open formula diets are needed for zebrafish to control for extrinsic dietary factors and increase reproducibility of research results, particularly for studies monitoring gene expression, embryo/larval development, oxidative stress, and behavior. Formulation of one standardized zebrafish diet is not considered feasible since research needs, and opinions as to what is best, vary greatly. However, the number of diets that are currently used by the zebrafish community is far too great to maintain consistency between facilities. Reducing the number of diets to even 10, would be a drastic improvement in standardization, though open formula diets would be the most appropriate.

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