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Comparison of Juvenile Feed Protocols on Growth and Spawning in Zebrafish

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Over the past 2 decades, zebrafish, *Danio rerio*, have become a mainstream laboratory animal model, yet zebrafish husbandry practices remain far from standardized. Feeding protocols play a critical role in the health, wellbeing, and productivity of zebrafish laboratories, yet they vary significantly between facilities. In this study, we compared our current feeding protocol for juvenile zebrafish (30 dpf to 75 dpf), a 3:1mixture of fish flake and freeze-dried krill fed twice per day with live artemia twice per day (FKA), to a diet of Gemma Micro 300 fed once per day with live artemia once per day (GMA). Our results showed that juvenile EK wild-type zebrafish fed GMA were longer and heavier than juveniles fed FKA. As compared with FKA-fed juveniles, fish fed GMA as juveniles showed better reproductive performance as measured by spawning success, fertilization rate, and clutch size. As adults, fish from both feeding protocols were acclimated to our standard adult feeding protocol, and the long-term effects of juvenile diet were assessed. At 2 y of age, the groups showed no difference in mortality or fecundity. Reproductive performance is a crucial aspect of zebrafish research, as much of the research focuses on the developing embryo. Here we show that switching juvenile zebrafish from a mixture of flake and krill to Gemma Micro 300 improves reproductive performance, even with fewer feedings of live artemia, thus simplifying husbandry practices.

Abbreviations: ACUC, Animal Care and Use Committee; EK, Ekkwill strain of zebrafish; FKA, Juvenile feed protocol consisting of a 3:1 fish flake: krill mixture and artemia each fed twice daily; GMA, Juvenile feed protocol consisting of Gemma Micro 300 and artemia each fed once daily; NICHD, National Institute of Child Health and Human Development

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Zebrafish are an increasingly important animal model for the study of genetics and development, cancer, and disease treatment.[28] Short generation time, embryonic optical clarity,[33] external fertilization and small embryonic size likewise make zebrafish an excellent model for drug screening[21] and for classic forward and reverse genetic screens.[5,29] Currently, over 1,300 zebrafish laboratories are registered on The Zebrafish Model Organism Database (zfin.org June 2020). Despite the rapid increase in the popularity and impact of zebrafish in research, the husbandry practices of laboratory zebrafish remain far from standardized.[3,16]

Water quality, housing, feed, and nutrition are key elements that must be monitored and modulated to provide the optimal level of care for all aquatic research species. Some research has been done to determine appropriate water quality parameters and housing requirements for zebrafish,[3,30] with many research facilities reporting similar ideal set points and ranges. However, feed and nutrition standards remain vague and unstandardized.[26,35] The need to understand the nutritional requirements of zebrafish,[15] and to use a standardized diet[25] are important to the expanding zebrafish community. Recent strides have been made to develop a chemically defined zebrafish reference diet[8] that will open the door to understanding the nutritional requirements for zebrafish and creating an optimal diet. In the meantime, performance-based studies of feeding protocols can help the research community design the best husbandry practices for their facilities. Using nonstandardized or untested diets introduce contaminants, as occurred at the University of Utah Centralized Zebrafish Animal Resource when they discovered that the nonhatching decapsulated brine shrimp cysts being fed to the fish were contaminated with chromium.[34]

In the wild, zebrafish are omnivorous and feed on zooplankton, insects, arachnids, phytoplankton, and vegetation.[7,32] In the laboratory, zebrafish are typically fed commercially available fish feed designed for commercial aquaculture species (often larval rearing diets) or diets designed for home aquarists. Many laboratories using aquatic species and aquaculture facilities also supplement processed feed with live feed; one of the most common live feeds is freshly hatched (instar II) artemia nauplii.

A commonly accepted feeding protocol among laboratories using zebrafish is to provide 5% of the fish's body weight of the selected fish feed, administered 2 to 5 times daily.[18] According to the manufacturer, Gemma Micro 300 (Skretting) can replace live artemia and provide similar or better spawning. Recommendations are to feed at 5% of the biomass up to twice a day to subadults (juveniles), and at 3% once a day to adults.[31] While some studies have cited use of this particular formula for zebrafish juveniles,[12] currently no standardized nutritional requirements or feeding protocol are available for zebrafish of any stage of development.[35]

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To select a diet for our zebrafish, we compared our previous feeding protocol for juveniles with the formulated Gemma Micro 300 diet. A previous study[19] showed that replacing artemia nauplii with rotifers, combined with Gemma Micro 300, resulted in a modest decrease in growth rate and reproductive performance in their facility, but remained within reasonable ranges and still met their research goals. Our study compared flake and krill with Gemma Micro 300, while maintaining live feeding with artemia with both diets. Although some laboratories are attempting to move away from live feeds for zebrafish, artemia nauplii are still fed in more than 85% of zebrafish facilities worldwide.[20]

The goal of our study on modifying our juvenile feeding protocol was to provide useful information for the zebrafish community when selecting the protocol that is best for growth and reproductive performance and most feasible from a husbandry and labor perspective in their facilities.

Materials and Methods

This study was performed in an AAALAC accredited facility under an active research project (ASP 15-010), overseen by the NICHD ACUC. The study took place in a large 26,000-L recirculating aquaculture system designed for zebrafish. Water quality parameters were stable and continuously monitored and maintained as follows: water temperature of 26.7 °C, pH of 7.0, conductance of 1000 µS/cm, and a dissolved oxygen level of 7.30 mg/L. Ammonia levels were between 0.00 and 0.04 mg/L, nitrite ranged between 0.040 and 0.120 mg/L and nitrate values ranged between 30.0 and 37.0 mg/L. Alkalinity ranged between 8 and 20 mg/L $CaC0_3$ and total hardness stayed between 56 to 72 mg/L. The air temperature averaged 26.4°C for the initial study period. The standard light cycle was 14 h of light with 10 h of darkness, set to ramp up and down in intensity to mimic sunrise and sunset. Health evaluations were made twice daily by a dedicated staff member, who removed moribund fish and fish with a Body Condition Score (BCS)[4] of either 1 (emaciated) or 5 (distended). Sentinel fish exposed to pre- or post-filtration water are evaluated twice annually for the presence of common pathogens.

We used juvenile fish that were collected as embryos from a mass breeding of several hundred EK wildtype zebrafish in a Mass Embryo Production System (MEPS Pentair/Aquatic Habitats). Prestudy husbandry was standardized for all larval fish. From 5 d post-fertilization (dpf) to 9 dpf, larvae were housed in 6-L tanks and were fed a mixture of type-L saltwater rotifers in a static environment, as previously described.[2] From 10 dpf to 28 dpf, the zebrafish were fed to perceived satiation with artemia nauplii (*Artemia fransiscana*) in the morning and afternoon, with additional feedings of powdered diets: Larval AP100 (Zeigler Bros) in the morning and Hatchfry Encapsulon 3 (Argent Aquaculture) in the afternoon. Artemia nauplii feeding was done with a standard squeeze bottle; the amounts dispensed were based on previously observed satiation amounts for known numbers of fish. In our facility, fish are considered juveniles when they have definitive black stripe formation, around the onset of posterior squamation (scale formation).[24]

At the start of the study (29 dpf), 2 dietary groups were formed by netting and counting fish from 6-L tanks into subgroups in new, clean tanks. Five 6-L tanks and nine 1.8-L tanks were used, per diet, with starting stocking densities of 64 fish per 6-L tank (10.6 fish/L) and 28 fish per 1.8-L tank (15.6 fish/L). After the tanks were filled, the diets for each tank were arbitrarily chosen, and each tank was placed on one of 2 rows on the same rack.

One diet (FKA) received the current feeding protocol of commercially available Freshwater Aquarium Flake Food (Ocean Star International), supplemented with freeze dried krill (Argent Aquaculture) that was ground in a food processor at a 3:1 ratio. This mixture was then fed to satiation twice daily, with 2 additional feedings of live artemia nauplii to perceived satiation, for a total of 4 feedings. Each liquid artemia feeding averages to be approximately 0.2 grams (dry weight) of hatched artemia per 10 fish.

The other diet (GMA) received a morning feeding of Gemma Micro 300 and an afternoon feeding of artemia nauplii to perceived satiation. The Gemma was fed at a rate of 10 mg per 10 fish, with the liquid artemia feedings being equivalent to approximately 0.2 grams (dry weight) of hatched artemia per 10 fish.

The rear portion of the tank lids used in our facility (and for this study) were modified to improve efficiency of husbandry practices. Gemma waste tended to accumulate on the back horizontal space and spouts of the tanks. Cutting the rear portion of the lids effectively fixed this problem, with any debris being cleared out with a quick squirt of system water.

At 32 dpf and 72 dpf, tanks were taken off the system and photographed at a fixed distance from the camera. All 6-L tanks were split into two 1.8-L tanks for the photographs. Each photograph included the base of the tank (177.8 millimeters) and at the given resolution and distance, Adobe Photoshop CS2[1] was calibrated to scale the pixels to the known length of the base. The software was then used to measure the fork lengths of all fish in each tank facing perpendicular to the camera. Fish lengths were not differentiated by sex. Initial and final length measurements were used to determine average growth rates for each tank and diet.

The experimental groups were bred on 3 separate instances (56, 70 and 91 dpf). Each tank was split into multiple static 1.5-L breeding tanks (Aquatic Habitats) filled with system water. After 24 h, the fish were returned to the original tanks on the system. Any embryos were rinsed and stored in methylene blue egg water in culture dishes. The culture dishes were stored in an incubator at 28 °C for 24 h before determining viability rates. To calculate viability, embryos were identified as viable live embryos, or as unfertilized embryos or embryos that failed to survive gastrulation. Statistical analysis was completed using Microsoft Excel[3] using 2 pair, type 3 *t* tests assuming unequal variances or ANOVA 2 factor with replication.

At 78 dpf, both of the dietary groups were sexed, weighed and started on an adult feeding protocol of Gemma Micro 300 at 20 mg per 10 fish in the morning and a single artemia feeding in the afternoon.

After the initial experimental period (91 dpf), fish from each diet were kept separated for long-term observation and periodic breeding. A random mix of fish from the 6-L and 1.8-L tanks were placed in nine 1.8-L tanks for each diet (18 tanks total). Each tank was stocked with 6 males and 6 females of normal Body Condition Score (2 to 4), with no signs of egg retention or clinical health concern. These tanks were bred with all fish from one tank placed into one 2-L static breeding tank (Techniplast USA, West Chester, PA) filled with system water. Embryos were then cleaned, stored and classified in the same manner as the initial experimental period.

Results

Fish were sexed and weighed at 78 dpf. Both male and female fish from the GMA diet weighed significantly more than fish from the FKA diet ($P < 0.001$). Average male weights for the

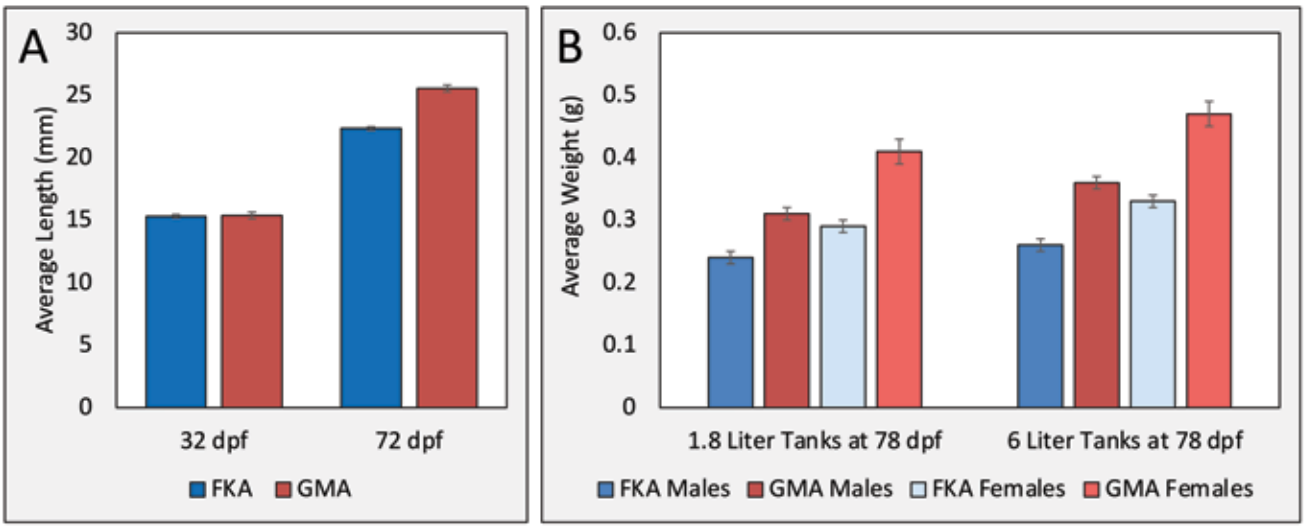

Figure 1. Length and weight measurements of zebrafish on 2 different juvenile diets (FKA & GMA). (A) Initial fork lengths were not significantly different ($P = 0.75$), however, fish fed the GMA diet grew significantly longer ($P < 0.001$) by 72 dpf than fish fed the FKA diet. (B) By 78 dpf, same sex fish weighed significantly more ($P < 0.001$) for the GMA diet than those fed the FKA diet. For both diets, same-sex fish were heavier under the 6 L housing conditions ($P < 0.001$ to $P = 0.02$), except for the FKA males ($P = 0.09$).

FKA and GMA diets were 0.20 and 0.32 grams, respectively, with female weights being 0.30 and 0.43 grams (Figure 1). To replicate multiple rearing conditions, the 2 dietary groups were split and reared in subgroups of 6-L tanks and 1.8-L tanks. Fish reared in 6-L tanks weighed more than fish reared in 1.8-L tanks regardless of the diet.

Laboratory zebrafish lack a chromosome responsible for sexual differentiation and environmental factors have been shown to cause sex ratio skewing in zebrafish.[23] Some evidence shows that differences in sex ratios can occur with drastic differences in stocking density[27] so we calculated sex ratio for both diets and did not find a significant difference in sex ratio (Figure 2), nor did we observe a difference in sex ratio between tank sizes or significant incidences of illness or mortality in either diet or subgroup.

No statistically significant differences were found in the initial lengths of any diet or subgroup for the initial lengths (Figure 1). A highly significant difference in growth was identified ($P < 0.001$), with average growth from 32 and 72 dpf between the 2 diets. Average growth was 7.11 mm for the FKA diet and 10.10 mm for the GMA diet. Average final lengths were 22.33 mm for the FKA diet and 25.54 mm for the GMA diet at 72 dpf. Breaking down the results into tank size subgroups demonstrated that the FKA fish in 6 l tanks grew significantly faster than their counterparts in 1.8 l tanks ($P = 0.02$) while no significant difference in growth was found between the tank sizes in the GMA diet ($P = 0.10$). Both GMA subgroups had much greater growth than either of the FKA subgroups ($P < 0.001$).

Breeding and viability improved after the initial spawning for both diet groups. The spawning success rate (at least 1 viable embryo at 24 hpf) was significantly higher in the GMA diet group for the first breeding event, and each individual tank for both diets successfully spawned in the next 2 events (Figure 3). Tank size subgroups were closely controlled for differences in density. Fish housed in the 6-L tank subgroup tended to outperform fish in 1.8-L tanks, regardless of diet, especially in the first (56 dpf) spawning (Figure 3), although performance was not statistically different as a whole. However, viability was significantly higher ($P < 0.001$) for each of the initial 3 breeding events with the GMA diet as compared with the FKA diet (Figure 3), even though both diets were switched to a Gemma diet with no artemia feedings (standard adult diet) at 78 dpf. ANOVA showed a highly significant difference ($P < 0.001$) in the number of viable embryos produced by both diets after the initial breedings.

Assessing the cumulative total number of viable embryos produced for each diet provides a metric that combines spawning success rate, fecundity, and viability throughout the experiment. Over the 3 breeding events, fish fed the GMA diet produced 16,738 total viable embryos as compared with 5,282 viable embryos produced by fish fed the FKA diet ($P < 0.001$) (Figure 4 A). Clutch sizes per spawning event (Figure 4 B) also showed a significant difference between the GMA and FKA fish overall ($P = 0.001$); this difference was particularly pronounced at 70 dpf. The 6-L tanks showed larger clutch sizes and earlier spawning success regardless of feeding protocol (Figure 4 C).

After the initial experimental period, at around 3 mo of age, fish were maintained and bred at approximately 6 wk intervals from 7 mo until 2 y of age (Figure 5). Although differences were seen within individual spawning events, ANOVA showed no overall significant differences in breeding success ($P = 0.21$), clutch size ($P = 0.63$) or the total number of viable embryos ($P = 0.96$) were found between the 2 diets. The total number of viable embryos produced exclusively during long-term observation were 21,044 for FKA and 21,152 for GMA. No relevant differences were found between the diets in the number of long-term illnesses or deaths.

Discussion

The lack of standardization of zebrafish husbandry makes it difficult for facility managers to choose the best feeding regime. In addition, choosing the regime that produces the fish with the best reproductive health, practical feasibility, and reproductive lifespan are factors that have a direct effect on research output. The published literature on nutrition and feeding in zebrafish is difficult to use in this regard.

The Aquaculture Industry has studied the relationship between feeding and stocking densities for many years, and this work can provide important insights relevant to zebrafish raised for research purposes. One study[10] showed that rainbow trout (*Oncorhynchus mykiss*) did not grow as well at very high

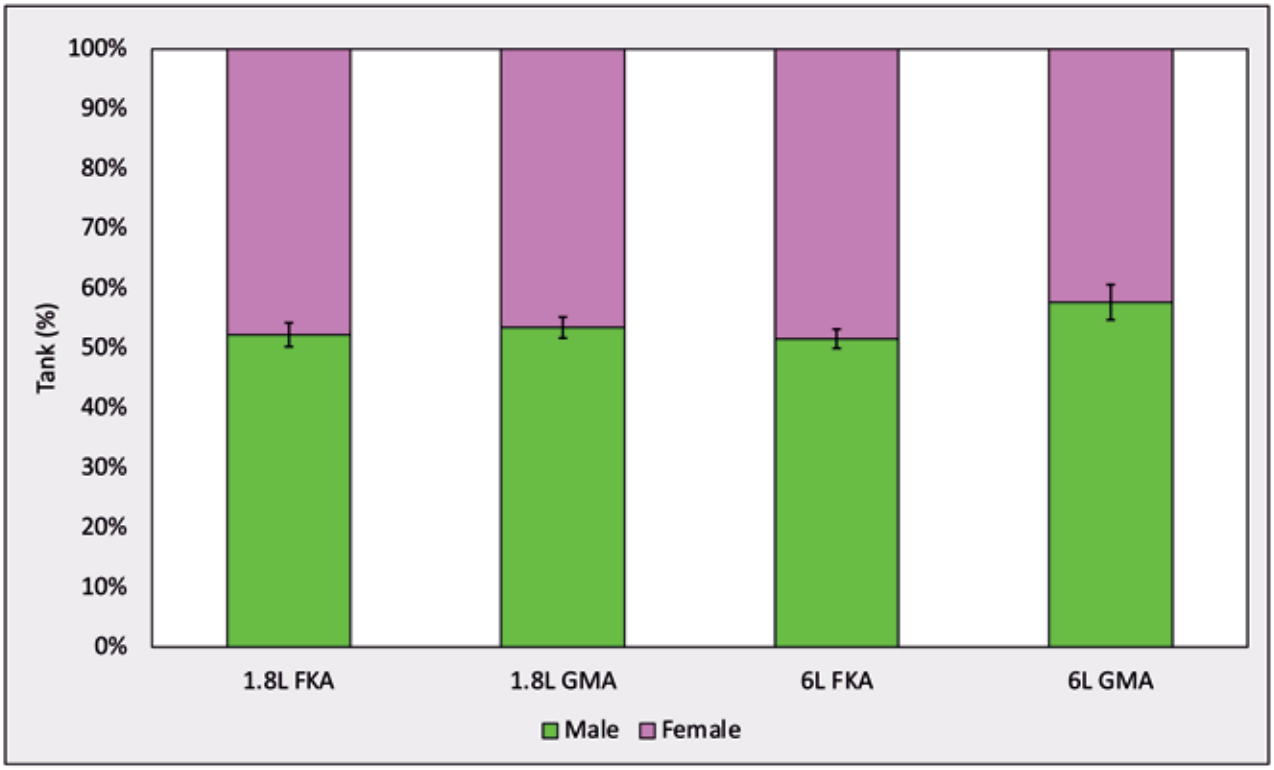

Figure 2. Sex distribution of 78 d post fertilization zebrafish on 2 different juvenile feed protocols. The diet of Gemma Micro 300 and artemia once a day (GMA) and the diet of fish fed a 3:1 mixture of Flake to Krill, and Artemia twice a day (FKA) showed no significant sex ratio skewing between one another ($P = 0.18$), even when comparing the subgroups of 1.8 L and 6-L size tanks ($P = 0.12$ to 0.87). 1.8 L FKA $n = 247$; 1.8 L GMA $n = 262$; 6 L FKA $n = 320$; 6 L GMA $n = 326$.

densities, with the change being less dramatic if the fish were fed continually, but fish in this study were stocked at a range of 107 to 450 kg/m,[22] which is 20 to 90 times higher than average zebrafish stocking densities. Alternatively, evidence from Arctic char (*Salvelinus alpinus*) showed that fish at lower stocking densities had worse growth rates, possibly due to behavioral changes including lack of schooling.[11] In that study, fish were housed between 15kg/m^3 and 120 kg/m^3, 3 to 24 times typical zebrafish stocking densities. A different study showed that rainbow trout (*Oncorhynchus mykiss*) held at 56 or 267 g/l had no differences in the stress parameters or growth parameters measured;[13] these stocking densities are 11 to 53 times higher than zebrafish stocking densities. A review on the relationship between stocking density and welfare in rainbow trout[6] provided interesting data, but the stocking densities used in commercial aquaculture are orders of magnitude higher than those used in zebrafish research. This, coupled with species' social/behavioral preferences, makes direct comparisons difficult.

The adult feeding protocol used in our facility has been changed to include Gemma Micro 300, based on published research[20] and inhouse comparisons. To assess the feasibility of including Gemma Micro 300 in our juvenile feeding protocol, we compared Gemma Micro 300 and artemia (GMA) to our current protocol of twice-daily feedings of 3:1 fish flake:krill mixture and artemia (FKA). The aim of this study was to evaluate the 2 feeding protocols holistically, including an assessment of immediate and long-term animal health, growth, reproductive performance, husbandry, and facility considerations that come with any change.

Growth, weight gain, and reproductive performance were used as the primary indicators of development and fecundity. The juvenile fish from the GMA diet grew longer, gained weight faster (Figure 1), and reached developmental milestones, such as sexual maturity and viable egg production, more quickly than did juvenile fish raised on the FKA diet.

An important variable for zebrafish research programs is the time it takes for fish to achieve sexual maturity.[17] Decreasing the time it takes to go from egg-to-egg speeds up the rate at which research can be conducted, which can directly impact the research success. We found that a juvenile feeding protocol that included Gemma Micro 300 significantly reduced the time it took for zebrafish to reach sexual maturity. This accelerated animal development rate allows researchers to perform higher throughput experiments and increases the robustness and rate of research. Possible reasons for lower egg-to-egg development time and reproductive performance could involve the nutritional profile and/or palatability of Gemma Micro 300, which may have higher levels of lipids, protein and micronutrients.

Reproductive performance is the husbandry readout that has the greatest impact on research programs and is directly related to the number of fish that spawn at each spawning event, the percent of eggs that are fertilized, and the size of the clutches produced by each female. When comparing reproductive performance in GMA fed fish to reproductive performance in FKA fish, we found a significant improvement with the GMA diet (Figures 3 and 4) when evaluating the first 3 spawning events. Husbandry modifications that allow fish to become sexually mature more rapidly improve research efficiency and welfare by increasing the number of embryos produced per animal. Such changes clearly align with the "Reduction" tenet of the 3 R's for the ethical treatment of animals used in research. The ability to use fewer animals to acquire larger clutches of embryos benefits the research facility, with less space required for tanks, less time spent pairing fish for

		Percent Spawning Success			Percent Viable		
Feed Protocol	Tank Size	56 dpf	70 dpf	91 dpf	56 dpf	70 dpf	91 dpf
FKA	1.8 L	0	100	100	N/A	38	52
	6 L	40	100	100	22	50	64
GMA	1.8 L	56	100	100	64	80	74
	6 L	80	100	100	80	84	90

Figure 3. Reproductive performance of zebrafish for the first breeding events for zebrafish from 2 different juvenile feeding protocols: Gemma Micro 300 and artemia once a day (GMA) and fish fed a 3:1 mixture of Flake to Krill, and Artemia twice a day (FKA). Both diets were switched to an adult feed protocol (Gemma once a day) at 78 dpf. Percent spawning success for each tank of fish at 56 dpf was much higher in fish fed GMA than FKA. When looking at the clutches of embryos, percent viability at 24 h postfertilization was significantly higher in the GMA treatment for all three spawning events. Fish in 6 L housing conditions had a higher initial spawning success, and higher viability, than fish housed in 1.8-L tanks within each diet.

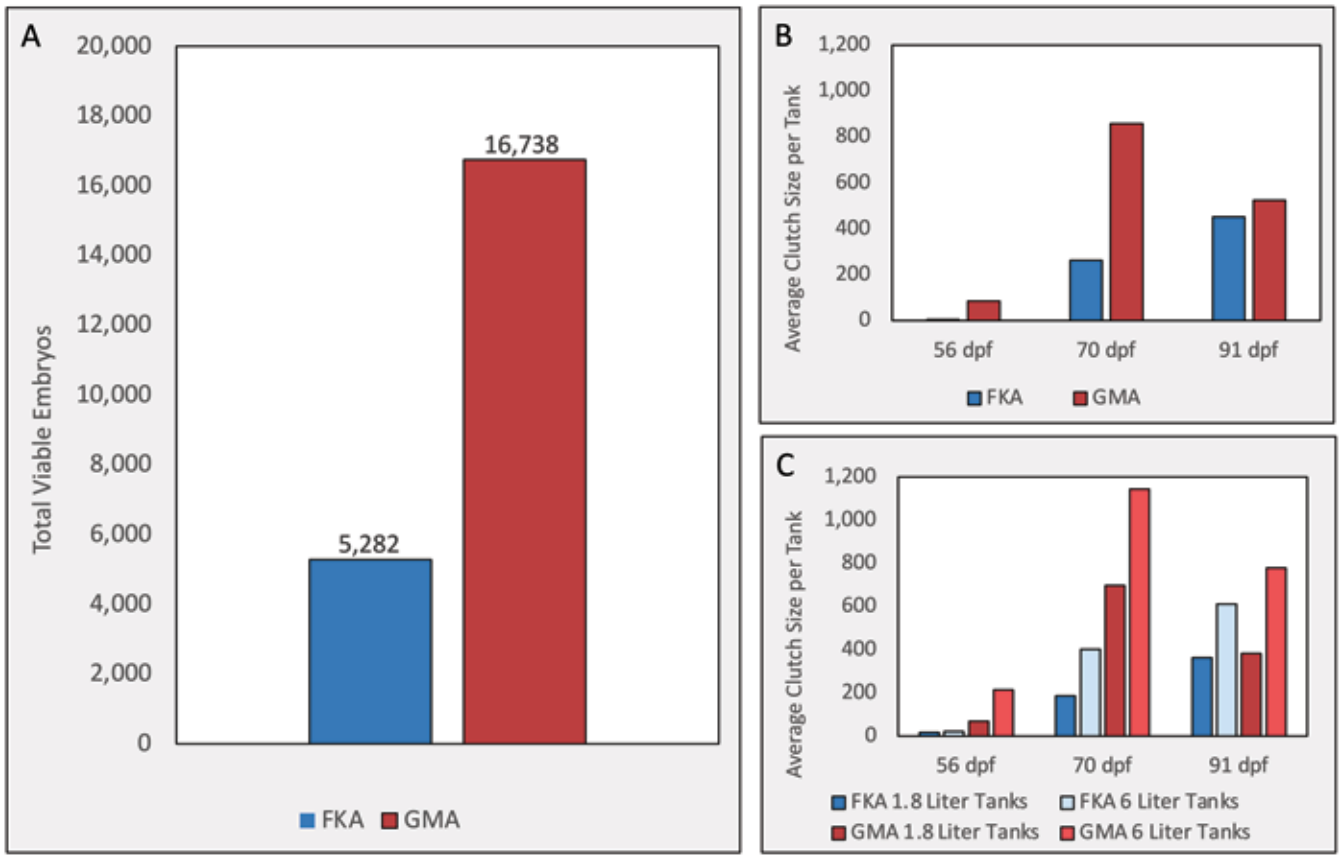

Figure 4. Reproductive performance of zebrafish from 2 different juvenile feeding protocols: Gemma Micro 300 and artemia once a day (GMA) or a 3:1 mixture of Flake to Krill, and Artemia twice a day (FKA). Both dietary groups were switched to an adult feed protocol (Gemma once a day) at 78 dpf. (A) Fish fed GMA (n = 588) produced more than 3 times more viable embryos over 3 mo compared with fish fed FKA (n = 567). (B) Tanks of fish fed GMA produced larger clutches for each spawning event. (C) Clutch size per tank trended higher in fish held in 6-L tanks (n = 10) compared with fish held in 1.8-L tanks (n = 18) regardless of diet.

breeding, fewer tanks and breeders needing to be washed, and potentially less feed needed.

At 78 dpf, fish on both diets were switched our standard adult feeding regimen of Gemma in the morning and artemia in the afternoon. A drop in viable embryo production occurred for the GMA diet group between the 70 and 91 dpf breedings, while the FKA dietary group increased production (Figure 3). While fish from both diet groups were a similar length related to adulthood,[24] the change in daily feedings could change how much energy is used for normal metabolic needs as compared with the production of gametes. The adult feed protocol is Gemma in the morning and artemia in the afternoon, which is exactly half the amount of the GMA juvenile feed diet.

The earlier reproductive success of the GMA fish introduced the concern that they would also experience an earlier decline

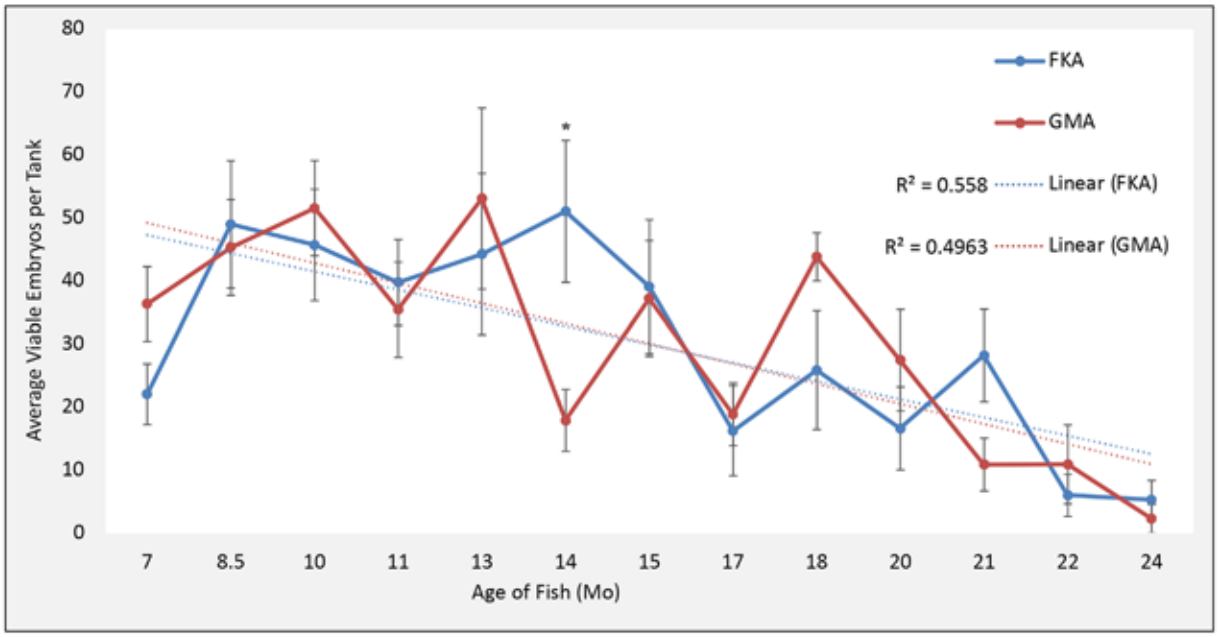

Figure 5. The average long-term reproductive performance of fish initially fed a juvenile diet of either Flake Krill and Artemia (FKA) or Gemma Micro 300 and Artemia (GMA). Each diet was reduced to nine 1.8-L tanks with 6 pairs of zebrafish in each tank. The tanks were bred approximately every 6 wk. The breeding event at 14 mo (marked with *) was the only one statistically different ($P = 0.02$) between initial dietary groups. The variance in long-term breedings did not lead to an overall difference since the total number of viable embryos produced within this timespan for FKA was 21,044 and 21,152 for GMA.

in reproductive performance or earlier mortality. To examine this, we kept the diet groups separate for long-term observation and periodic spawning. The 2 diet groups, when given identical feed and husbandry, showed very similar reproductive output, indicating that the FKA diet group was only delayed and not permanently impaired. These long-term spawning data documented the previously anecdotal rise and decline in egg production in our facility relative to the age of the zebrafish (Figure 5) but did not reveal an early decline in reproductive performance in either dietary group. The decline in the number of eggs produced with advanced age requires researchers to decide if fish this old are still valuable to their research programs.

Despite being housed under similar conditions, the fish raised on the GMA diet were heavier and had a faster growth rate than did those raised on the FKA diet. Although both diet subgroups were fed amounts relative to the tank's biomass, fish raised in 6-L tanks had a faster growth rate and bred earlier than those raised in 1.8-L tanks. However, this difference did not result in a statistically higher number of viable embryos produced per female ($P = 0.71$). This could be attributed to the lower stocking density of a 6-L tank in this study, reducing competition for food or space. A prior feed study[14] found an effect of housing density and diet on the weight of fish, also without a notable difference in the number of viable embryos. Other research has shown that major differences in growth occur in laboratory zebrafish when the amount of feed provided remains the same, but less dramatic differences occur when food is increased along with stocking density.[9] The differences we saw in our feed study could be due to a difference in overall nutrition (calories, fat, or protein) being provided to each fish or to feeding behavior or palatability. Higher weight of the GMA fish in the 6-L tanks could also indicate of excess body fat, suggesting a limit to the need for additional food during this stage, such that additional feedings would be counter-productive. Although increased weight alone is not necessarily an indicator of better health, the increased length that was observed in the GMA diet may indicate accelerated skeletal development.

From a facility and husbandry standpoint, Gemma Micro 300 produces a higher food cost over the flake feed and extra artemia. It also required the facility staff to modify the lids of the tanks from that provided by the manufacturer. However, our husbandry technicians found that less time was needed for the GMA tanks than the FKA tanks. Two of the GMA tanks needed to have their filter screens changed during the experimental period, while 8 FKA tanks needed changed screens. In the FKA diet, uneaten food would lie on the bottom of the tank, forming mats of debris. It would also accumulate between the screen and back tank wall. This uneaten food would have to be siphoned out, and occasionally, the entire tank would have to be changed for a new, clean one if biofilm was obscuring visibility. The GMA tanks required less care and maintenance. Leftover food was more likely to be eaten, and many food particles could still pass freely through the tanks' screens.

Besides cleanliness of the tanks, feeding time was also reduced with the GMA diet. With the FKA diet, fish were fed twice in the morning and twice in the afternoon; a total of 4 feedings. The GMA diet required only 2 feedings, once in the morning and once in the afternoon. Although the manufacturers of Gemma Micro 300 market this product as an artemia replacement, we continued to provide the afternoon live artemia for supplemental nutrition and environmental enrichment. This reduced the number of daily feeds from 4 to 2. With the reduced feeding

time, comes reduced labor costs or increased time for other husbandry tasks.

Our results indicate that *Danio rerio* raised on the GMA diet during the juvenile stage of fish growth, showed increased body weight, length, and improved reproductive performance than those raised on the FKA diet. In addition to direct benefits to the fish, there are indirect benefits to the researchers and husbandry technicians including, but not limited to, reduced feeding time, reduced cleaning time, reduced need for cultured live feed, and potentially a reduction in the number of animals needed to be maintained. Combined with the decreased time to go from egg to egg, and the improved reproductive performance during the first 3 spawning events in the GMA fish, we believe that switching to a protocol similar to that provided to the GMA diet group is a significant improvement over the existing protocol represented by the FKA diet.

Being able to make research-based husbandry decisions will be critical to the growth and success of the zebrafish research model. Following in the same experimental vein as this study, the diet of earlier stages of zebrafish could be compared against other feeds. Also, given that the zebrafish in the GMA diet grew longer than the FKA fish within the same timeframe, a deeper look into the dietary variance of the 2 feeds could reveal nutrients essential for musculoskeletal development for this life stage. Neither of these diets changed the long-term reproductive success of zebrafish, but greater transparency and reporting of long-term fecundity within facilities could help isolate water quality, dietary, or even husbandry practices that do provide improvements.

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