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Preliminary Evaluation on the Effects of Feeds on the Growth and Early Reproductive Performance of Zebrafish (*Danio rerio*)

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This study evaluated the effects of several commercially available feeds and different feeding regimes on the growth and early reproductive performance of zebrafish (*Danio rerio*). Juvenile zebrafish (n = 20; 5.06 ± 0.69 mg) were stocked into each of 24 tanks (volume, 2 L); 3 tanks were assigned to each of 8 feeding combinations for a period of 60 d. At the end of 60 d, 2 male and 2 female fish from each tank were pooled by dietary treatment (n = 6) and used to evaluate the effects of feeding combinations on early reproductive performance. Zebrafish fed dietary treatments 3 and 7 had significantly greater weight gain than zebrafish fed diet 5. Mean spawning success was significantly greater in zebrafish fed the control diet (*Artemia* only) than in those fed diet 1. Mean hatch rates were greater in zebrafish fed the control feed and diets 1, 2, 3, 5, and 6 than zebrafish fed diet 4. Additional results suggest that female zebrafish are sexually mature after 90 d post fertilization and that fertilization rates are the limiting factor in early reproduction.

The use of zebrafish (*Danio rerio*) in genetic and developmental biology research has been increasing globally.[8,23,26,38,42] The continuation and expansion of such research relies on a consistent supply of fertilized embryos for injection experiments, RNA extraction, in situ and other research procedures.[10] Researchers are therefore dependent on the timely development of sexually mature zebrafish and availability of fertilized embryos.

Nutrition has been identified as a key factor affecting oogenesis, reproductive performance, and larval development in aquatic vertebrates[4-6,18,20,22,40] and invertebrates.[2,46] To date, most zebrafish feed-related research has been conducted on larvae,[3,33,41] with little attention given to juvenile and sexually mature zebrafish.[27] Knowing more about the nutritional requirements of zebrafish at various developmental stages will help to determine which feed or feed combination is best for raising these animals.

In the absence of understanding the nutritional needs of zebrafish, several commercially available products are implemented in the feeding practices of zebrafish culture.[45] These include, but are not limited to, the use of *Artemia*, fish flake, various formulated fry diets, and rotifers.[25] In some instances, different feeds with similar nutritional values are used in feeding programs, despite the lack of evidence to warrant their inclusion. The National Institute of Child Health and Human Development (Bethesda, MD) uses 4 different diets for juvenile zebrafish, including *Artemia*, Hatchfry Encapsulon (AP Hatch 300 Ziegler Brothers, Gardners, PA), and a krill–fish flake mixture. Determining whether a feed or feeding regime is beneficial in culturing zebrafish or unduly encumbers husbandry staff is difficult in the absence of intentional evaluations.

An important part of analyzing any diet for the rearing of fish is the evaluation of any effects on reproductive performance.[22] Therefore, the goal of the current study was to determine the effects of various feeds and their combinations on the growth and early reproductive performance of zebrafish in a research setting.

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Materials and Methods

Diet preparation. The study evaluated a control diet of decapsulated *Artemia* (San Francisco strain, Brine Shrimp Direct, Ogden, UT) and 7 additional diets: 1) *Artemia* + krill:flake mix, 2) *Artemia* + AP Hatch 300 (Ziegler Brothers, Gardners, PA), 3) *Artemia* + Hatchfry Encapsulon (Argent Chemical Laboratories, Redmond, WA), 4) *Artemia* + krill:flake mix + AP Hatch 300, 5) *Artemia* + krill:flake mix + Hatchfry Encapsulon, 6) *Artemia* + AP Hatch 300 + Hatchfry Encapsulon, and 7) *Artemia* + AP Hatch 300 + Hatchfry Encapsulon (Figure 1). Diet 2 was made by using a 1:3 ratio (dry weight/dry weight) of krill (Argent Chemical Laboratories) to flake feed (Ocean Star International, Hayward, CA). In the cases of diets 4 through 7, AP Hatch 300, Hatchfry Encapsulon, and krill–flake mix were added in equal ratios (dry weight/dry weight) with the respective dry feeds of that particular dietary treatment. Once all components of a dietary treatment were combined, 100-g aliquots of each diet were mixed by shaking the container for 1 min to ensure homogeneity and then stored in labeled food-grade plastic cell-culture flasks at 4 °C until needed. Each flask was refilled once empty, and flasks were not aerated while storing dietary treatments. Before each feeding, flasks were shaken by hand for 1 min to ensure homogeneity of the diets. The feed expiration date used in this study was 6 mo after the stock cans were first opened. Fresh cans were used to prepare the diets for this study, and all feeds originated from the same sources throughout the entire study.

Decapsulated *Artemia* were hatched overnight in a conical tank by using reverse-osmosis–treated water and by maintaining a salinity of 25 ppm. According to the facility's standard operating procedure, 1 mL volume of Spirulina microfine powder (Argent Chemical Laboratories) was added as enrichment media for the *Artemia*. Aeration was provided at the base of

Diet	1100 and 1530 feedings
Control	*Artemia*
1	Krill–flake mix
2	AP hatch 300
3	Hatchfry encapsulon
4	Krill–flake mix + AP hatch 300
5	Krill–flake mix + hatchfry encapsulon
6	AP hatch 300 + hatchfry encapsulon
7	Krill–flake mix + hatchfry encapsulon + AP hatch 300

Figure 1. Weekday feeding schedule for various diets. All diets also included *Artemia* at 0830 and 1330 on weekdays. During weekends and holidays zebrafish received only the 1100 and 1530 feedings. See Materials and Methods for additional details regarding nutrient sources.

the hatching tank. *Artemia* were collected, rinsed with reverse-osmosis–treated water, and collected in a squirt bottle containing culture water. Oxygen was provided to *Artemia* by using an aquarium pump and plastic tubing until *Artemia* were fed to the zebrafish in the study.

Environment and husbandry. The aquatic system was a 7.2-m^3 recirculating system equipped with submerged mechanical and biologic filtration, a fluidized bed, carbon and cartridge filters, and UV sterilization (200,000 µws/cm^2). This system exchanged 90% of the culture water and added 10% of the total volume daily. Temperature (°C), dissolved oxygen (mg/L), pH, and conductivity (µmhos/cm) were tested daily by using a multiparameter water quality-monitoring system (model 6500, YSI, Yellow Springs, OH). Ammonia-N (mg/L), nitrite-N (mg/L), nitrate-N (mg/L), and hardness (mg/L $CaCO_3$) were tested weekly by using LaMotte test kits (Chestertown, MD). Water flow rate was maintained at 100 mL/min throughout the study.

The juvenile zebrafish used in this study were acquired by conducting a group cross with adults raised inhouse. A total of 100 adult wildtype zebrafish, AB* strain, were established in group crosses in 1-L tanks (Aquatic Habitat, Apopka, FL) overnight. Each spawning tank consisted of a false-bottom tank containing a mesh screen bottom that was seated within a larger tank to allow separation of released eggs and sperm and the spawning fish. A male:female ratio of 1:1 and a total tank density of 20 fish per spawning tank was maintained. The next day, embryos were collected and rinsed with a 60-ppm saline solution containing 2 ppm methylene blue. Fertilized embryos were placed in culture dishes (100 × 20 mm) at a density of 40 embryos per dish and incubated at 28 °C. At 5 d after fertilization, the larvae were placed in 2-L tanks and raised until the start of the study, according to facility larva-rearing protocols.

Fish in this study were used in accordance with animal study protocol 09-039, which was approved by the IACUC of the National Institute of Child Health and Human Development.

Growth evaluation. At the beginning of the study, each of 24 tanks (volume, 2 L) was stocked with 20 zebrafish (age, 30 d) in random mixed-sex groups. A random sample of 240 zebrafish that remained after all tanks were stocked were allocated into 24 replicates of 10 fish each, euthanized by using tricaine methanosulfonate (3 g/L; MS222; Argent Chemical Laboratories) buffered to neutral pH with sodium bicarbonate, patted dry, and weighed to obtain initial weights. Each tank was assigned randomly to receive 1 of 8 dietary treatments (3 tanks per diet). Each tank was fed to satiation 4 times daily (0830, 1100, 1330, and 1530) during the work week and twice daily (1100 and 1530) during weekends and holidays, according to the standard feeding protocol at the facility. Satiation was defined as the point within a 5-min period at which fish were no longer actively searching for food. Tanks were siphoned of any solid waste at least once every 2 d. The growth evaluation period lasted 60 d. The initial weight (mean ± SEM) of each fish was 5.06 ± 0.69 mg.

At the end of the growth evaluation period, 2 male and 2 female zebrafish from each tank were selected randomly and pooled by sex and dietary treatment for evaluation of initial spawning performance. Therefore, a total of 6 male and 6 female zebrafish from each dietary treatment advanced to the reproductive evaluation. The remaining fish in each group were pooled by sex, euthanized by using tricaine methanosulfonate, and weighed. Total weight gain was determined by adding the weight gains of male and female fish in each group.

Weight gain was calculated using the following equation:

$$\text{Weight gain (g)} = Wf - Wi,$$

where W_f and W_i represents final and initial weight gain, respectively. Specific growth rate was determined by using the following equation:

$$\text{Specific growth rate (mg/d)} = [(\ln Wf - \ln Wi) / T] \times 100,$$

where T represents length of the study in days. Gender weight ratio, defined as the ratio between the weight gain of female zebrafish compared to male zebrafish, was determined by using the following equation:

$$\text{Gender weight gain} = WGf / WGm,$$

where WG_f represents female weight gain and WG_m represents male weight gain. Survival rate was determined by using the following equation:

$$\text{Survival rate (\%)} = (\text{densityf} / \text{densityi}) \times 100\%,$$

where density_i represents the initial number of fish stocked in a tank and density_f represents the final number of fish stocked in a tank.

Reproduction evaluation. Fish randomly selected for the study of early reproductive performance were pooled by dietary treatment and placed in single-sex tanks before they were returned to the experimental system. Fish were fed the same dietary treatment as assigned during the growth evaluation. Fish were allowed 1 wk before spawning trials began. During each spawning trial, male and female zebrafish from each dietary treatment were randomly placed in pairwise crosses (n = 6) and allowed to spawn overnight in 0.5-L spawning tanks. Eggs were collected the next day by using the same procedure as described earlier, with each culture dish containing no more than 60 eggs. After a spawning event, fish were returned to their tanks and allowed 2 wk before initiation of the second spawning trial, which used the same procedure. Spawning success, defined as the total number of females that spawn during a spawning trial, was calculated at the end of each spawning trial as:

$$\text{Spawning success (\%)} = (\text{no. of spawning events per dietary treatment} / \text{no. of pairs established}) \times 100\%.$$

Fecundity was determined by counting the number of viable eggs released by a female zebrafish during a spawning event. A viable egg was defined as being clear and having circular shape, whereas nonviable eggs were cloudy directly after spawning

Table 1. Weight gain, specific growth rate, and survival rate of zebrafish fed various diets

Diet	Weight gain (g)	Specific growth rate (mg/d)	Survival rate (%)
Control	0.234 ± 0.018^{ab}	10.964 ± 0.134^{ab}	98.333 ± 1.666^{a}
1	0.156 ± 0.010^{ab}	10.258 ± 0.079^{ab}	96.666 ± 3.333^{a}
2	0.201 ± 0.034^{ab}	10.779 ± 0.293^{ab}	100.000 ± 0.000^{a}
3	0.262 ± 0.152^{b}	11.264 ± 0.089^{b}	100.000 ± 0.000^{a}
4	0.245 ± 0.018^{ab}	11.083 ± 0.123^{ab}	100.000 ± 0.000^{a}
5	0.149 ± 0.023^{a}	10.213 ± 0.367^{a}	96.666 ± 3.333^{a}
6	0.194 ± 0.030^{ab}	10.631 ± 0.279^{ab}	98.333 ± 1.666^{a}
7	0.275 ± 0.023^{b}	11.297 ± 0.211^{b}	98.333 ± 1.666^{a}

Values (mean ± SEM) within a column with different superscripts were significantly ($P < 0.05$, $n = 3$) different as determined by ANOVA and the Student Neuman–Keuls test.

Table 2. Weight gain in male and female zebrafish and female:male weight gain ratio of zebrafish fed various diets

	Weight gain (g)		
Diet	male	female	Weight ratio
Control	0.211 ± 0.027^{a}	0.271 ± 0.014^{a}	1.271 ± 0.153^{a}
1	0.142 ± 0.013^{a}	0.152 ± 0.038^{a}	1.122 ± 0.323^{a}
2	0.154 ± 0.017^{a}	0.224 ± 0.098^{a}	1.580 ± 0.707^{a}
3	0.194 ± 0.020^{a}	0.327 ± 0.011^{a}	1.729 ± 0.224^{a}
4	0.164 ± 0.017^{a}	0.324 ± 0.029^{a}	2.049 ± 0.373^{a}
5	0.094 ± 0.023^{a}	0.219 ± 0.036^{a}	2.661 ± 0.711^{a}
6	0.107 ± 0.027^{a}	0.253 ± 0.062^{a}	2.920 ± 1.154^{a}
7	0.165 ± 0.051^{a}	0.326 ± 0.082^{a}	2.031 ± 0.274^{a}

Values (mean ± SEM) within a column with different superscripts were significantly ($P < 0.05$, $n = 3$) different as determined by ANOVA and the Student Neuman–Keuls test.

and did not develop.[15] Opaque eggs and eggs with irregular shapes were discarded from the clutch.

Fertilization rate was determined 24 h post fertilization (hpf) using the following equation:

Fertilization rate (%) = (no. of fertilized embryos /total no. of embryos produced during a spawning event) × 100%,

with a fertilized embryo defined as an embryo that had undergone cellular division.[15]

Statistical analysis. All data were analyzed according to a completely randomized design. Data originating from the growth study were pooled by using each tank as an experimental unit, whereas data originating from the reproductive study used each spawning pair as the experimental unit. Survival rate, weight gain, and specific growth rate were analyzed by using one-way ANOVA.[47] Mean survival success, fecundity, and fertilization rate over the course of all spawning periods were analyzed by using ANOVA. Spawning success, fecundity, and fertilization rate for each spawning event were analyzed by using repeated-measures ANOVA. The Student Newman–Keuls test was used to separate means when significant differences were detected by ANOVA analysis. Pairwise *t* tests were used to analyze data when type I error had occurred. Differences were considered significant at a *P* value of 0.05. All statistical analyses were done by using SPSS software (version 17.0, SPSS, Chicago, IL).

Results

Water quality was maintained throughout the study at acceptable levels for aquatic animal culture. Temperature, dissolved oxygen, pH, NH_3-N, NO_2-N, NO_3-N, hardness and conductivity were maintained at 80.7 ± 0.1 °C, 7.45 ± 0.1 mg/L, 7.3 ± 0.0, 0 ± 0.0 mg/L, 0 ± 0.0 mg/L, 25.4 ± 1.5 mg/L, 97.5 ± 1.5 mg/L $CaCO_3$, and 1008.5 ± 2.7 µmho/cm, respectively.

Juvenile zebrafish fed diets 3 and 7 (Figure 1) had significantly ($P < 0.05$) greater weight gain and specific growth rates than did zebrafish fed diet 5 (Table 1). No significant differences were detected in survival rate (Table 1), weight gain of male or female zebrafish, or the ratio of the weight gain of female zebrafish to that of male zebrafish between any of the evaluated diets (Table 2). Mean spawning success values over the course of the study were significantly ($P < 0.01$) greater in zebrafish fed the control diet than in those fed diet 1 (Table 3). Female zebrafish the control diet or diet 1, 2, 3, 5, 6, or 7 had significantly ($P < 0.05$) higher spawning success at 95 d after fertilization than did female fish fed diet 4 (Table 4). Female zebrafish fed the control diet or diet 1, 3, 4, 5, or 6 had significantly ($P < 0.05$) greater fecundity at 109 d after fertilization than did female zebrafish fed diet 2 or 7 (Table 5). The fecundity of female zebrafish fed diet 5 improved significantly ($P < 0.05$) with age. Significant differences were not detected in fertilization rates (Table 7) of zebrafish at any spawning period. (Table 6). Zebrafish fed *Artemia* were the only group that exhibited predator–prey feeding behavior.

Discussion

The growth and early reproductive performance of zebrafish in the current study did not suggest that a particular feed or feed combination was better than the others. In general, all of the diets evaluated in this study resulted in favorable growth and survival of juvenile zebrafish. Female zebrafish grew more than 1- to 3-fold greater in size than male zebrafish. This growth difference between female and male zebrafish may have contributed to the early reproductive performance noted in this study. Female zebrafish were sexually mature at 3 mo of age, as demonstrated by spawning success and fecundity data (Table 3). This conclusion is further supported by the fact that the sexual performance did not improve significantly over the 6-wk spawning evaluation (Tables 4 and 5). However, the poor fertilization rates suggest that male zebrafish were not sexually mature at 3 mo and require more time (Table 6) and possibly a better diet to reach sexual maturity.[22]

Nutrition has been identified as a major factor in the development of breeding fish.[20,22] Which feeds and feeding combinations achieve the best growth and reproductive per-

Table 3. Mean spawning success, fecundity, and fertilization rate from 3 spawning events of zebrafish fed various diets

Diet	Spawning success (%, n = 18)	Fecundity (n)	Fertilization rate (%, [n])
Control	94.44 ± 5[a]	275.15 ± 31 (17)	58 ± 6 (17)
1	50.00 ± 12[b]	278.00 ± 46 (9)	70 ± 7 (9)
2	83.33 ± 9[ab]	178.00 ± 29 (15)	69 ± 7 (15)
3	88.89 ± 7[ab]	262.50 ± 31 (16)	50 ± 8 (16)
4	61.11 ± 12[ab]	191.20 ± 52 (10)	43 ± 11 (11)
5	72.22 ± 11[ab]	340.00 ± 48 (10)	57 ± 7 (13)
6	88.89 ± 8[ab]	265.62 ± 32 (16)	58 ± 7 (16)
7	66.67 ± 11[ab]	231.00 ± 61 (12)	50 ± 9 (12)

Values (mean ± SEM) within a column with different superscripts were significantly ($P < 0.05$, n = no. of zebrafish per event) different as determined by ANOVA and the Student Neuman–Keuls test.

Table 4. Spawning success (%) of zebrafish (age: 95, 109, or 123 d postfertilization [dpf]) fed various diets

Diet	95 dpf	109 dpf	123 dpf
Control	100 ± 0[a]	83 ± 16	100 ± 0
1	50 ± 22[a]	50 ± 22	50 ± 22
2	83 ± 16[a]	83 ± 16	83 ± 16
3	83 ± 16[a]	100 ± 0	83 ± 16
4	33 ± 21[b]	83 ± 16	66 ± 21
5	66 ± 21[a]	100 ± 0	50 ± 22
6	100 ± 0[a]	66 ± 21	100 ± 0
7	66 ± 6[a]	77 ± 6	83 ± 16

Values (mean ± SEM) within a column with different superscripts were significantly ($P < 0.05$, $n = 6$) different as determined by repeated-measures ANOVA and the Student Neuman–Keuls test. Spawning success did not differ by age for any diet.

Table 5. Fecundity of zebrafish (age: 95, 109, or 123 d postfertilization [dpf]) fed various diets

Diet	95 dpf (n)	109 dpf (n)	123 dpf (n)
Control	242 ± 36 (5)	184 ± 22[a] (6)	242 ± 36 (5)
1	230 ± 59 (3)	191 ± 13[a] (3)	230 ± 59 (3)
2	124 ± 36 (4)	130 ± 33[b] (5)	124 ± 36 (4)
3	277 ± 42 (6)	180 ± 39[a] (5)	277 ± 42 (6)
4	127 ± 83 (4)	159 ± 91[a] (2)	127 ± 83 (4)
5	404 ± 86 (6)	252 ± 67[a] (4)	404 ± 86 (6)
6	161 ± 35[c] (6)	318 ± 59[a,d] (3)	334 ± 46[d] (6)
7	229 ± 26 (3)	89 ± 53[b] (4)	229 ± 26 (3)

[a,b]Values (mean ± SEM) within a column with different superscripts were significantly ($P < 0.05$) different as determined by repeated measures ANOVA and the Student Neuman–Keuls test.
[c,d]Values (mean ± SEM) within a row with different superscripts were significantly ($P < 0.05$) different as determined by repeated measures ANOVA and the Student Neuman–Keuls test. Pairwise t tests were used when type I error was encountered.

formance of zebrafish are a matter of debate. The optimal diet of zebrafish is likely to change as they transition from the juvenile stage to adulthood in the laboratory setting. A change in diet would most likely change in development and reproductive capabilities[13,22,32,36,37] due to differences in nutrient digestibility or in the nutritional composition of the different diets that would change the metabolic response and performance of the cultured fish. This study did not identify one feed or feeding combination as superior to the other diets evaluated, although a case can be made for the use of *Artemia* as the sole food for rearing juvenile zebrafish. Several studies have evaluated live and formulated feeds for the rearing of larval zebrafish. Two studies[17,29] reported that feeding *Artemia* resulted in bigger larval zebrafish, whereas another study[27] concluded that 7-mo-old zebrafish fed *Artemia* produced similar spawning results as those from zebrafish fed other commercially available diets. *Artemia* can be enriched prior to being fed to zebrafish,[9,11,28,30-35], thereby allowing modifications in the nutritional content fed to zebrafish as research identifies nutrient needs.[29] In the current study, *Artemia* was the only diet that elicited a predator–prey feeding response[9,12] and resulted in cleaner tanks. Cleaner tanks after feeding enables husbandry staff to focus on other tasks than cleaning or changing out tanks. Although the total tank weight of zebrafish fed diet 3 or 7 (Figure 1) was greater than that of zebrafish fed *Artemia* (Table 1), male weight gain was greatest in zebrafish fed *Artemia* (Table 2). The gender weight ratio of zebrafish fed *Artemia* was one of the lowest recorded in the current study, although the importance of this measure is not well understood at this time. In addition, mean spawning success over the course of the study was significantly greater in female zebrafish fed *Artemia* as compared to female zebrafish fed other diets suggesting that *Artemia* may be a better diet for rearing juvenile zebrafish.

The age of sexual maturity for zebrafish has been debated and is reported to range from 3 to 5 mo.[25,39,44-45] Results from the current study suggest that female zebrafish are sexually mature at 3 mo, as indicated by spawning success (Table 4) and fecundity (Table 5) data. The age at sexual maturity may reflect the fact that female zebrafish develop faster than do males.[43] The current results confirm a previous report[39] that female zebrafish gained more weight than did males when fed 4 of 8 dietary treatments. In that study,[39] zebrafish were fed commercially available and purified diets, whereas the current study evaluated only commercially available diets and their possible combinations. Only female zebrafish fed diet 6 improved significantly with age (Table 5), further supporting the idea that female zebrafish are sexually mature at 3 mo. The use of a large recirculating system, the spawning success and fecundity data in the current study (Tables 4 and 5, respectively), and previously published data[15] support the idea that spawning performances were not adversely affected by any pheromones that may have been released.

Male zebrafish did not perform as well as females did during the spawning evaluation and appear to require more time to develop sexually. During the spawning evaluation, mean

Table 6. Fertilization rate (%) of zebrafish (age: 95, 109, or 123 d post-fertilization [dpf]) fed various diets

Diet	95 dpf (*n*)	109 dpf (*n*)	123 dpf (*n*)
Control	56 ± 13 (6)	74 ± 5 (5)	46 ± 10 (6)
1	59 ± 10 (3)	80 ± 18 (3)	69 ± 12 (3)
2	58 ± 29 (5)	71 ± 12 (4)	74 ± 10 (4)
3	29 ± 12 (5)	52 ± 17 (6)	62 ± 9 (5)
4	23 ± 24 (2)	38 ± 17 (4)	59 ± 20 (4)
5	53 ± 13 (4)	51 ± 11 (6)	73 ± 16 (3)
6	53 ± 17 (6)	60 ± 15 (3)	60 ± 5 (6)
7	70 ± 15 (4)	54 ± 8 (3)	30 ± 16 (5)

No significant differences between values (mean ± SEM) within rows or columns as determined by repeated-measures ANOVA and the Student Neuman–Keuls test.

fertilization rats were consistently low, although they increased with age in male zebrafish fed diets 2 through 4. Although not evaluated, these poor fertilization rates may have been due to poor sperm quality, poor sperm motility, or insufficient nutrition.[20-22,27,29] Low fertilization rates are undesirable when using zebrafish in developmental biology research. If the fertilization rate is low and relatively few fertilized embryos are produced, only a small number of injected fertilized embryos may remain after an injection of a transgene or morpholino; this situation could require repeating the experiment, thus increasing labor and reagent consumption. The current data suggest that male zebrafish require more than 4.5 mo of growth before fertilization rates consistently would be 80% or greater, a desired range at the study facility.

Historically, attention has focused on developing diets that improve the reproductive performance of female aquatic animals.[6,14,38,46] The current study illustrates the importance of focusing on the nutritional needs of male zebrafish. Various studies document the role of fatty acids,[16,21] in particular arachidonic acid,[1] in improving fertilization rates in aquatic vertebrates[24] and invertebrates,[2,7,19] with a few focused on zebrafish.[21,22,29] The slower development in male zebrafish may underlie the long-held notion that zebrafish must be 4 to 5 mo old, if not older, before spawning.[39] This requirement is clearly a bottleneck in the logistical framework involved in maintaining zebrafish cultures for biomedical research and warrants more attention.

The appropriate gender weight ratio for spawning zebrafish is unclear. To my knowledge, the current study is the first to report the ratio between female and male growth rates, although a previous study reported that female zebrafish develop faster than do male zebrafish.[43] The size of the female fish is a key factor in the amount of sperm released by male zebrafish during spawning.[31] A large disparity between spawning partners can lead to aggressive behavior by the larger fish, sometimes resulting in the death of the smaller fish, during a spawning event.[9] Although modifications of the spawning environment may alleviate abusive spawning behavior, additional work is needed to tailor feeds for male and female zebrafish[22] and to elucidate the relationship, if any, between gender weight ratio and reproductive performance.

Logistical constraints to using more replicates of the numerous dietary treatments, combined with previous success in using triplicate groups, led to the use here of 6 replicates per dietary treatment, although the data suggest that additional replicates could be valuable. Nonetheless, the current findings demonstrate that male development and fertilization rates are limiting factors in early reproduction and that gender weight ratio can affect reproduction.

This study aimed to identify which among several dietary treatments maximized juvenile growth and early reproductive performance. The results of the current study do not conclusively identify any particular diet tested as the best for rearing juvenile zebrafish, although a case can be made for the use of *Artemia* as the sole food source. The study did indicate that female zebrafish mature sooner than male zebrafish. This disparity may play an important role in the early reproductive performance of zebrafish and warrants more attention.

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