The influence of ontogenetic dietary fluctuations on zebrafish size and swimming performance

Chris Marks *, Steven M. Lombardo, Kristie L. Formanik, Francisco B.-G. Moore † and Brian Bagatto †

Department of Biology, The University of Akron, Akron, OH, USA

INTRODUCTION

Phenotypic plasticity is critical in determining fitness. As conditions change during ontogeny, continued responsiveness is necessary to meet the demands of the environment. Studies have shown that subsequent ontogenetic periods of development can interact with one another and shape developmental outcomes. The role genetic variation within populations plays in shaping these outcomes remains unclear. Four full-sib families of zebrafish Danio rerio were raised under for dietary regimes: high food rations for 60 days (HH), low food rations for 60 days (LL), high food rations for 30 days followed by low food rations for 30 (HL), and low food rations for 30 days followed by high food rations for 30 (LH). While the low ration diet significantly reduced body length at 30 days, diet was no longer a significant factor at day 60. Only family level variation influenced body length. Furthermore, there was significant family level variation in the manner in which swimming performance responded to fluctuating dietary conditions. Some families increased swimming performance in response to dietary change, while others did not. These results suggest that plastic responsiveness to subsequent environmental changes can be trait specific and vary significantly within populations.

Keywords: zebrafish, Danio rerio, ontogeny, swim, quantitative genetics
strains were maintained according to standard procedures (Westerfield, 1994). Adults were maintained and bred at 26 ± 0.5°C with a 14L:10D light cycle.

**BREEDING DESIGN**

Males and females were randomly paired resulting in four full-sib families. Mating pairs were placed in 2-L containers lined with a marble substrate and supplied with a common water source (Z-Mod housing system, Marine Biotech, Beverly, MA).

**TREATMENTS**

Siblings were raised together in 2-L containers with a common water source (Z-Mod housing system, Marine Biotech, Beverly, MA) and were maintained at 26 ± 0.5°C with a 14L:10D light cycle for the duration of the experiment. Food consisted of pulverized Zeigler™ adult zebrafish diet supplemented with equal parts of <100 and 100–150 micron Zeigler™ larval diet (1:1:1). After 30 days, the <100 and 100–150 micron supplements were replaced with 150–250 and 250–450 micron supplements. For all feedings, 500 mg of food was mixed with 250 ml of system water. From this solution, fish were fed at 0.1 mg/fish (low-ration treatment) and 0.2 mg/fish (high-ration treatment). We chose these rations based on a standard dry food recipe from a protocol available at the Zebrafish International Resource Center (http://zebrafish.org/zirc/documents/protocols/pdf/Fish_Feeding/Flake_Food/Dry_Food_Recipes.pdf). We assigned 0.1 mg/fish to the low-ration treatment since it was the amount designated by this protocol. Therefore, the terms “high” and “low” are relative and apply only to the confines of this particular study. Feedings were conducted once daily and excess food was removed before each feeding. After two weeks, we noted all food was being consumed within 24 h. After 30 days, half of the individuals from each treatment were switched to a separate 2-L tank and subjected to the opposite feeding treatment for the remainder of the experiment. In this resulting four feeding treatments: high food rations for 60 days (HH), low food rations for 60 days (LL), high food rations for 30 days followed by low food rations for 30 (HL), and low food rations for 30 days followed by high food rations for 30 (LH). These four treatments were applied to all four families. Individuals from each family were housed together according to feeding treatments.

**MEASUREMENTS**

**Body size**

To ensure feeding treatments had an initial effect, we measured the total length (TL) of each subject at 30 days. Fish were placed individually in a small petri dish filled with system water. A ruler was included in each picture. Photography was conducted with a Nikon D300 camera under standard lighting conditions. We measured TL from the most anterior point to the posterior point of the caudal fin. We observed no damage to caudal fins at any point in the study. At 60 days, each subject was euthanized with MS-222 (300 mg/l tricaine methane sulfonate buffered to a neutral pH with sodium bicarbonate) and photographed on the subject’s right side with a length standard in each picture. We measured standard length (SL) from the most anterior point to the base of the hypural plate at caudal flexion. Maximum depth (MD) was measured as the maximum dorsal–ventral distance measured along the flank. All measurements were made using ImageJ (Version 1.42, NIH). Measurements were made five times on each subject and the mean was recorded.

**Swim velocity**

Prior to terminal measurements (above), maximum swimming velocity (Umax) was measured according to Widmer et al. (2006). Briefly, individual fish were placed in a clear acrylic flume (44.7 mm inner diameter by 30 cm long) which drew system water. System water was maintained at 26.5°C with a Lifegard heater module (Pentair Aquatics). Water was oxygenated to 6.8 mg/l with airstone bubblers. Baffles placed at the anterior portion of the swim chamber maintained consistent laminar flow throughout the length of the flume. Individual subjects were allowed to acclimate to the tunnel for 5 min prior to measurement. With an initial flow velocity of 4 cm/s, flow (Blue-White Industries, Huntington Beach, CA, USA; flow rate meter F-1000-RB) was increased every 5 s by 2 cm/s until the fish spent >50% of the time increment touching the back mesh of the chamber (Brett, 1964). Maximum swim velocity was calculated based on the inner diameter of the tube and the final flow measurement and was determined based on the SL of the fish tested. Subjects were selected randomly from families/treatments.

**STATISTICS**

To test for the effects of feeding for days 0–30 on TL, we used a Two-Way ANOVA. Family, treatment, and their interaction were included as sources of variation. To test for the effects of feeding throughout the experiment on SL and swim velocity, we used a Three-Way ANOVA. Family, food treatment for days 0–30 (Diet0–30), food treatment for days 30–60 (Diet30–60), and all possible interactions were included as potential sources of variation. For swimming velocity, comparisons across all families and treatments we made using Tukey’s HSD. TL and SL were log transformed to meet normality assumptions. Statistics were performed in JMP version 9.0.2 (SAS institute).

**RESULTS**

**SURVIVAL**

Ninety-three subjects survived the experiment. Chi-squared tests revealed that survival shared no contingencies with feeding treatments ($X^2 = 0.56, P = 0.91$) or families ($X^2 = 2.91, P = 0.41$). Sample sizes for treatments and families are shown in Tables 2 and 3.

**BODY SIZE**

At 30 days, family (F) and diet were both significant factors in influencing TL (Table 1). Individuals from the high food treatment were significantly longer (4.68 ± 0.20 mm vs 3.87 ± 0.17 mm; Figure 1). At 60 days, however, the only significant source of variation on SL and MD was family (Table 1). Mean standard lengths (untransformed) are presented in Table 2.

**SWIMMING VELOCITY**

Many factors contributed to variation in swim velocity. Besides variation among families (F), diet for days 30–60 also contributed...
Table 1 | ANOVA results for total length (TL; n = 136), standard length (SL; n = 93), maximum depth (MD; n = 93), and swim velocity (n = 93).

| Variable          | Source                   | DF | MS    | F     | P     |
|-------------------|--------------------------|----|-------|-------|-------|
| Total length      | Family                   | 3  | 16.4850       | 7.8045 | <0.0001 |
|                   | Diet0–30                 | 1  | 19.8648       | 9.4046 | 0.0026 |
|                   | Family × Diet0–30        | 3  | 0.7649        | 0.3621 | 0.7805 |
|                   | Error                    | 128| 2.1122        |        |       |
| Standard length   | Family                   | 3  | 3.4353        | 245.04 | <0.0001 |
|                   | Diet0–30                 | 1  | 0.0123        | 0.87   | 0.3526 |
|                   | Family × Diet0–30        | 3  | 0.0017        | 0.12   | 0.9477 |
|                   | Diet30–60                | 1  | 0.0424        | 3.02   | 0.0860 |
|                   | Family × Diet30–60       | 3  | 0.0013        | 0.09   | 0.9645 |
|                   | Diet0–30 × Diet30–60    | 1  | 0.0003        | 0.02   | 0.8861 |
|                   | Family × Diet0–30 × Diet30–60 | 3 | 0.0078       | 0.56   | 0.6438 |
|                   | Error                    | 77 | 0.0140        |        |       |
| Maximum depth     | Family                   | 3  | 2.6945        | 242.74 | <0.0001 |
|                   | Diet0–30                 | 1  | 0.0066        | 0.60   | 0.4426 |
|                   | Family × Diet0–30        | 3  | 0.0055        | 0.50   | 0.6858 |
|                   | Diet30–60                | 1  | 0.0370        | 3.33   | 0.0718 |
|                   | Family × Diet30–60       | 3  | 0.0043        | 0.39   | 0.7612 |
|                   | Diet0–30 × Diet30–60    | 1  | 0.0009        | 0.08   | 0.7724 |
|                   | Family × Diet0–30 × Diet30–60 | 3 | 0.0074     | 0.67   | 0.5728 |
|                   | Error                    | 77 | 0.0111        |        |       |
| Swim velocity     | Family                   | 3  | 491.9777      | 54.31  | <0.0001 |
|                   | Diet0–30                 | 1  | 7.1416        | 0.79   | 0.3774 |
|                   | Family × Diet0–30        | 3  | 5.0020        | 0.55   | 0.6482 |
|                   | Diet30–60                | 1  | 51.2372       | 5.66   | 0.0199 |
|                   | Family × Diet30–60       | 3  | 28.1016       | 3.10   | 0.0315 |
|                   | Diet0–30 × Diet30–60    | 1  | 56.8658       | 6.28   | 0.0143 |
|                   | Family × Diet0–30 × Diet30–60 | 3 | 28.3586     | 3.13   | 0.0304 |
|                   | Error                    | 77 | 9.0594        |        |       |

For TL, factors included family, diet for days 0–30 (Diet0–30), and their interaction. For SL, MD, and swim velocity, factors included family, diet for days 0–30 (Diet0–30), diet for days 30–60 (Diet30–60), and all possible interactions. TL, SL, and MD were log transformed for analyses.

to variation in swim velocity (Diet30–60) and this effect varied significantly across families (F × Diet30–60; Table 1). Fish fed low rations for days 30–60 attained higher velocities on average than those fed high rations (Figure 2). Early diet (Diet0–30) also contributed to variation in swimming performance through its interaction with later diet (Diet0–30 × Diet30–60; Table 1). Fish raised on low rations for the duration of the experiment (LL) maintained similar velocities to those switched from low to high rations (LH; Figure 2A). Interestingly, fish switched from high to low rations (HL) attained higher swimming velocities than those maintained on high rations (HH; Figure 2A). The interaction between family and both dietary periods indicates that the quality of interactions (i.e., direction and magnitude) between dietary treatments varied across families (F × Diet0–30 × Diet30–60; Table 1). Variation in swim performance for families A and B remained consistent across diet treatments. Families C and D, however, showed variation due to both early (Diet0–30) and later (Diet30–60) food treatments. Fish from these families raised on low rations for the duration of the experiment (LL) maintained similar velocities to those switched from low to high rations (LH). Fish switched from high to low rations (HL), however, attained higher swimming velocities than those maintained on
Table 2 | Maximum depth and standard length (mm) for zebrafish (*n* = 93) at 60 days under all combinations of high and low food rations.

|               | HH (23)     | HL (25)     | LH (23)     | LL (22)     |
|---------------|-------------|-------------|-------------|-------------|
| Maximum depth | 5.86 ± 0.87 | 6.13 ± 0.90 | 5.84 ± 0.79 | 5.64 ± 0.82 |
| Standard length| 6.20 ± 0.96 | 5.32 ± 0.79 | 7.17 ± 0.97 | 6.10 ± 0.94 |

The first letter represents the food rations for days 0–30 (high vs. low). The second letter represents the food rations for days 30–60 (high vs. low). The number represents the sample size for each treatment. Data are presented as untransformed arithmetic mean ± SEM.

The performance trait addressed in this study was maximum swim velocity. Although they exhibited compensatory growth, fish raised on early low food rations maintained similar swimming velocity regardless of later dietary rations. This indicates negligible cost of compensatory growth on swimming performance in our study. Previous studies have demonstrated a tradeoff between accelerated growth and physical performance in salmon (Farrell et al., 1997) and sticklebacks (Álvarez and Metcalfe, 2007). In the case of sticklebacks, however, the associated tradeoff was present in stream rather than pond populations. This result indicates that local selective pressures can alter tradeoff trajectories among populations. Zebrafish inhabit a wide variety of habitats ranging from active streams to stagnant rice fields.
Table 3 | Tukey HSD comparisons of swimming velocities across all families and treatments.

|       | A:HH | A:HL | A:LL | B:HH | B:HL | B:LL | C:HH | C:HL | C:LL | D:HH | D:HL | D:LH | D:LL |
|-------|------|------|------|------|------|------|------|------|------|------|------|------|------|
|       | 6    | 7    | 5    | 7    | 6    | 5    | 6    | 6    | 7    | 6    | 5    | 5    | 5    |
| A:HH  | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   |
| A:HL  | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   |
| A:LL  | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   |
| B:HH  | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   |
| B:HL  | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   |
| B:LL  | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   |
| C:HH  | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   |
| C:HL  | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   |
| C:LL  | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   |
| D:HH  | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   |
| D:HL  | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   |
| D:LL  | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   | NS   |

The first row and column represents each family and treatment. The first letter represents the family. The second letter represents the food rations for days 0–30 (high vs. low). The third letter represents the food rations for days 30–60 (high vs. low). The number represents the sample size for each family/treatment group. Significant comparisons are noted with an asterisks.

(Spence et al., 2008). It is therefore likely that ecological variation has shaped tradeoff trajectories in this species.

Interestingly, swim velocity was highest in fish switched from high to low food rations. Thus, dietary change enhanced swimming performance, but only for fish started on high rations. Fish started on low rations did not increase swimming velocity when switched to high rations. Thus, although HL and LH fish attained similar size, their physical abilities differed. Phillips (2004) performed a similar dietary switching study with mussels and found similar quantitative results. While mussels switched from high to low rations equaled those switched from low to high in terms of shell size, they differed in terms of lipid content. This result suggests that dietary order can be more critical in shaping physiological rather than morphological outcomes. Thus, the underlying physiologies of the subjects in our study may have been affected. Specifically, fish switched from high to low rations proved physiologically superior to fish from other treatments.

It should be noted that individuals from each family/treatment combination were housed together. Therefore, there remains the possibility of some influence of common rearing environment on each treatment group. This could be due to unique interactions between siblings of each family/treatment group. While we attribute the variation in this study to family and treatment effects, we do so with the understanding that these observations may be confounded. Although shared rearing space may be a component of variation in this study, we remain confident that family and treatment effects are major contributors to the observed variation. One reason for this is that mortality did not vary significantly among families or treatments. This suggests that the quality of interactions (aggressive encounters, food competition) were similar across families/treatments. However, we encourage further studies of the effects of changing dietary rations on fish physiology and physical performance to clarify these issues.

Swimming ability is a critical trait in fish. Its implications on prey capture, predator avoidance, and social interactions are evident (Videler, 1993). Thus, as swimming ability is sensitive to environmental change, the ontogenetic history of fish becomes critical in shaping their fitness. At the population level, variation in environmentally altered developmental trajectories provides the raw material for natural selection to optimize fitness in changing environments. Zebrafish inhabit a wide variety of habitats...
throughout Southeast Asia (Spence et al., 2008). Their association with a number of different habitats throughout seasonal fluctuations makes it likely that factors such as temperature, oxygen, and food availability can vary during their ontogeny. Thus, it is likely that there is some ecological component to variation in swimming ability in zebrafish. Given their small size, zebrafish are prolific swimmers that display remarkably low associated physiological costs (Plaut and Gordon, 1994). The selective factors that have shaped these abilities require further elucidation.

The role environmental complexity plays in shaping ontogenetic trajectories is receiving increasing attention. Of particular interest are the consequences multiple instances of environmental change have on developmental outcomes (Monaghan, 2008). Few studies to our knowledge have quantitatively demonstrated significant interactions between subsequent ontogenetic periods of development (Marks et al., 2005; Kotschal and Taborsky, 2010). Even less clear is the role genetic variation plays in determining the quality of phenotypic outcomes under complex conditions. Our study not only demonstrates that subsequent dietary conditions can interact in shaping zebrafish physical performance, but the quality of these effects is family specific. This result indicates at least some role of genetic variation in shaping plastic responses under complex conditions. Such variation underlies the proximate variation necessary for selection to optimize developmental outcomes in changeable environments.

In summary, we found a significant interaction between dietary environments (Diet30−60 × Diet0−30) for swimming velocity. Overall, fish switched from high to low food rations attained the highest swimming velocity. Fish started on low food rations attained similar swimming velocities regardless of later food rations. The quality of responses to dietary change varied across families, resulting in a significant Family × Diet0−30 × Diet30−60 interaction. Although early food rations influenced size at the midpoint of the experiment, fish achieved equal sizes across all food treatments at the end of the experiment. These results suggest that plastic responses to subsequent environmental changes can be trait specific and vary significantly within populations. The specific order of environmental conditions can also be critical in determining performance outcomes.

ACKNOWLEDGMENTS

This project was made possible thanks to the Choose Ohio First Tiered Mentoring Program.