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The effects of feeding frequency on growth and reproduction in zebrafish (*Danio rerio*)

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ABSTRACT

In the present study, we examined the effects of feeding regime on growth and reproductive performance in wild-type zebrafish (*Danio rerio*) maintained on a recirculating aquaculture system. Starting at 30 days post-fertilization (dpf), we fed 8 replicate groups of age and strain matched fish a pelleted formulated diet (Gemma Micro 300, Skretting) either once every other day (EOD), one time (1×), three times (3×) or five times (5×) a day to achieve a total daily feed input of 5% of body weight per day compared against a "standard" control (C) of *Artemia salina* nauplii/metanauplii three times daily to apparent satiation. Fish in each treatment group were weighed and measured for fork length once every other week until 150 dpf. We evaluated the effects of these feeding regimes on reproductive performance (breeding success, fecundity, and embryo viability) by setting up randomly sampled fish from each replicate groups in small group crosses (2 males, 3 females) once every other week starting at 76 dpf until the experiment was terminated at 191 dpf. Growth performance was significantly affected by feeding regime, especially in female fish, where fish in the 1×, 3×, and 5× groups were significantly ($p<0.05$, one way ANOVA) longer and heavier than fish in control and EOD groups at the end of experiment. Feeding regime had a less clear effect on reproductive performance. Mean fecundity and embryo viability varied little between groups, but the fish fed EOD, 1× and 3× showed significantly higher rates ($p<0.05$) of breeding success than the control and 5× groups. These results suggest that feeding regimes most conducive to growth do not necessarily maximize reproductive success in this species.

1. Introduction

The zebrafish (*Danio rerio*) is an important experimental model organism used in many fields of science, including human disease (Lieschke and Currie, 2007), developmental biology and genetics (Grunwald and Eisen, 2002), environmental toxicology (Scholz et al., 2008), drug screening (Barros et al., 2008), evolution (Cañestro et al., 2007), and, increasingly, aquaculture (Ulloa et al., 2011). Despite this fact, the science underlying strategies for the culture and management of this species in research settings has been poorly developed (Lawrence, 2007, 2011). During the past few years, attempts have been made to improve current understanding of key biological characteristics of zebrafish in the areas of nutrition (Jaya-Ram et al., 2008; Siccardi et al., 2009); reproductive biology (Adatto et al., 2011; Castranova et al., 2011; Sessa et al., 2008); larviculture (Best et al., 2010); behavior (Filby et al., 2010; Paull et al., 2010); and natural history (Spence et al., 2006; Spence et al., 2007). These and other emerging data sets hold the promise of being used as a framework for developing more sophisticated, performance driven management practices more befitting of the animal's status as a mainstream research model.

Still, some of the most basic questions remain unanswered. Among the most notable of these involves matching feeding practices to the digestive biology and normal feeding behaviors of the fish. Indeed, the simple act of feeding a single zebrafish facility with hundreds or even thousands of individual tanks has important implications for growth and reproductive performance of stocks, and also comprises a major percentage of the labor devoted to maintaining such an operation. The latter factor is especially critical, because the "standard" practice in the field is to feed post-larval fish anywhere from 2 to 5 times a day, 365 days a year, usually with some combination of *Artemia salina* nauplii and a formulated diet (Brand et al., 2002; Varga, 2011; Westerfield, 2007). While many laboratories have employed this extremely labor-intensive feeding strategy for decades, its effects on performance have never been evaluated.

Zebrafish are euryphagous ominivores possessing a long intestine with a large absorption area and no true stomach (Ulloa et al., 2011). The fact that the species is agastric has led to speculation that under controlled conditions, the fish will perform best when fed frequent, small meals throughout the course of a day (Lawrence, 2007). This hypothesis is generally supported by data from several feeding studies that show superior growth and survival rates when formulated diets (Carvalho et al., 2006) or live prey items (Best et al., 2010) are presented to fish on a continuous basis during the larval stage of development. This is not surprising; larvae of many cultured fish species must be fed

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frequently and to excess because of their small body size, high energy demands, and simple digestive systems (Craig and Helfrich, 2002; Dabrowski, 1986). It is also generally true that fish need to be fed less frequently and at a lower amount of feed per feeding as they mature (NRC, 2011). The standard practices for post-larval zebrafish referenced above are reflective of this pattern, but have never been formally tested for their effects on growth and reproductive function.

In an attempt to begin to address this information gap, we tested five different feeding regimes for their effects on growth and reproductive performance in a group of wild-type, age-matched zebrafish, from the sub-adult stage onwards. These trials are the first formal evaluation of feeding practices in zebrafish beyond the juvenile stage.

2. Material and methods

2.1. Strain selection

The AB strain was selected for use in these feeding trials because it is among the most common and widely used strains in the zebrafish research (Spence et al., 2008). The fish used in this experiment originated from a breeding population of approximately 100 six-month old animals that were housed in an 80-liter polycarbonate cylindrical tank connected to a 4500-liter recirculating aquaculture system. This population of the AB strain has been maintained at the research aquaculture facilities at Children's Hospital Boston (CHB) for >20 generations in accordance with a breeding program that maximizes genetic diversity and minimizes inbreeding (Lawrence, 2011). The use of the animals in this experiment was approved by the Institutional Animal Care and Use Committee at CHB (IACUC protocol # 11-05-1964R).

2.2. Origin, generation, and management of experimental fish

Approximately 2000 zebrafish embryos were collected from a large group-spawning event involving the breeding population described above. At 1 day post-fertilization (dpf), 1000 viable embryos from the clutch were transferred to clean water and then incubated at 28 °C in 50 mm petri dishes at density of 100 embryos per dish until gas bladder inflation on day 5 post-fertilization, the developmental time point that coincides with exogenous feeding in this species (Harper and Lawrence, 2010). At this point, larvae from all dishes were pooled, and then distributed into twenty 1.8 liter tanks at a density of 25 fish/L. The fish were subsequently reared on a 4500 liter recirculating aquaculture system (Aquarienbau Schwarz, Gottigen, Germany) in accordance with standard feeding and water quality management protocols employed in CHB research aquaculture facilities until 30 dpf (Tables 1 and 2).

2.3. Feeding trials

At 30 dpf, the fish, which had a mean initial fork-length of 1.92 ± 0.06 cm and mean initial weight of 0.07 ± 0.01 g, were removed from their rearing tanks, pooled together in a 20 liter bucket, and then randomly allocated into 40 new 1.8 liter tanks at a density of 20 fish per tank (~11 fish/L). The fish in each of the 40 groups were photographed and weighed prior to the tanks being placed back onto the 4500 liter recirculating aquaculture system referenced above. The groups were assigned to one of 5 experimental feeding regimes, which included a control diet of *Artemia* nauplii/metanauplii fed to satiation or a formulated diet administered at 5% of body weight either once every other day (EOD), once (1×), three (3×), or five (5×) times daily (Table 3). The *Artemia* fed to control groups was hatched and collected each day according to standard methods (Sorgeloos et al., 2001) prior to feeding, and subsequently delivered to the fish in a freshwater suspension via a plastic squeeze bottle. Fish were given enough *Artemia* at each feeding such they were able to continuously consume suspended nauplii for a period of at least 5 min. This feeding approach was used as a control because it represents the closest approximation to a standard diet and feeding regime in the zebrafish research community (Lawrence, 2007). The formulated diet was weighed on a microbalance and then aliquoted into proportional meal sizes in 2 ml eppendorf tubes that were then administered to the fish accordingly. The amounts of the formulated diets fed to the animals were adjusted once every two weeks based on the mean weight of the fish in each feeding regime group so that the fish were fed at 5% of body weight throughout the course of the experiment. The fish were maintained on these diets and feeding regimes until the end of the experiment, at 191 dpf.

Table 2
Water quality conditions during the feeding trials[a].

Water quality parameter	Value range or mean	Testing method	Recording frequency
pH	7.26 ± 0.02	YSI 5200	Daily
Conductivity (μS)	1291.69 ± 10.53	YSI 5200	Daily
Alkalinity (mg/L $CaCO_3$)	36.29 ± 2.37	LaMotte Test Kit	Monthly
Hardness (mg/L $CaCO_3$)	109.57 ± 4.55	LaMotte Test Kit	Monthly
Dissolved oxygen (mg/L)	7.9 ± 0.16	LaMotte Test Kit	Monthly
Carbon dioxide (mg/L)	2.71 ± 0.26	LaMotte Test Kit	Monthly
Phosphate (mg/L)	1.79 ± 0.25	LaMotte Test Kit	Monthly
Temperature (°C)	26.69 ± 0.10	YSI 5200	Daily
Total ammonia nitrogen (mg/L)	0.001 ± 0.0006	LaMotte Test Kit	Weekly
Nitrite (mg/L)	0.02 ± 0.001	LaMotte Test Kit	Weekly
Nitrate (mg/L)	2.79 ± 0.22	LaMotte Test Kit	Weekly
H_2O exchange rate (tank change per hour)	4–6	na	na
H_2O exchange rate (daily % of total system volume)	10	na	na

[a] Values for all parameter means, when available, are mean ± standard error.

2.4. Growth measurements and determination of sex ratios

Once every other week, beginning at 30 dpf and ending at 150 dpf, each replicate tank was taken off the system and photographed from above with a digital camera (Panasonic Lumix LX4, Panasonic, Inc., Secaucus, NJ). Photographs were subsequently analyzed with Adobe Photoshop Cs4 software. In this fashion, at least 10 randomly selected fish in each replicate were measured for fork length (from the tip of the snout to the end of the middle of the caudal fin rays) using the software's ruler function. Survival was assayed at the same time by counting all fish in each replicate using the software's count function. Immediately after they were photographed, the fish in each replicate group were weighed by 1) pouring all of the fish from a given tank into a net, 2) removing the excess water from the fish and net by gently shaking the net three times, and 3) then transferring the fish to a new

Table 1
Rearing conditions for experimental zebrafish prior to start of feeding trials.

Developmental timepoint (dpf)	Diet	Feeding frequency/amount	Feeding times	H_2O exchange rate (tank changes per hour)	Salinity (g/L)	Density (fish/L)
0–4	None	na	na	0 (static)	0.5	1000
5–9	Type L saltwater rotifers (*Brachionus plicatilis*)	*Ad libitum*	Continuous[a]	0 (static)	5.0	100
10–30	*Artemia salina* nauplii/metanauplii (A)	3× daily/to apparent satiation	8 am, 12 pm, 3 pm	4–6	0.5	25

[a] Complete method described in Best et al. (2010).