Successful Replacement of *Artemia salina* nauplii with Marine Rotifers (*Brachionus plicatilis*) in the Diet of Preadult Zebrafish (*Danio rerio*)

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Abstract

Previously established rearing protocols for zebrafish begin feeding with marine rotifers (*Brachionus plicatilis*), followed by *Artemia* nauplii until the fish reach subadult stage, the developmental time point at which they can be most easily transitioned onto a processed diet. However, the inclusion of *Artemia* is less than ideal, given its fluctuating availability and high costs. We tested whether or not we could replace *Artemia* with rotifers during our normal rearing sequence and still meet published performance standards for (i) weaning fish onto a processed diet by 25 days postfertilization (dpf) and (ii) successful breeding by 60 dpf. Here, we present the results of trials where wild-type and *casper* zebrafish were fed exclusively with rotifers (R) or rotifers followed by *Artemia* (RA) for the first 25 dpf after which point all fish were transitioned to a processed diet (Gemma Micro 300). We measured growth and survival at days 25 and 60, and tested for reproductive capability at 60 dpf. While growth performance was significantly better in the RA groups, we were still able to meet goals for both weaning and generation time in the R groups without compromising survival or sex ratios.

Introduction

ZEBRAFISH LARVAE AND JUVENILES are well adapted to feed on zooplankton within the water column,^{1,2} and feed most efficiently when they are given the opportunity to capture small, slow moving prey that move within their field of vision.² As the fish develop, they become more adept at consuming larger and more diverse items, not only within the water column, but also at the surface and, later along the benthos.² By the time the fish have reached the subadult stage, they feed at all three levels with equal efficiency. Given these observations, it is not surprising that the feeding protocols that promote the highest rates and growth and survival in this species typically progress from a diet of small live prey to larger, inanimate processed feeds, with the transition between diet types being coincident with the fish attaining the subadult stage.^{3–5}

We previously reported a method for rearing zebrafish that begins with a diet of saltwater rotifers (*Brachionus plicatilis*) enriched with algal paste (Rotigro; Reed Mariculture, Inc.),³ and then progresses to live *Artemia* sp. nauplii that is then fed to apparent satiation until the subadult stage. At this developmental time point (~30 days postfertilization [dpf]), the fish can be easily weaned onto a processed pelleted feed (Gemma Micro 300 [GM300]; Skretting) that serves as the sole dietary item for the rest of the life cycle.⁵

While this approach supports high rates of growth, survival, and reproduction, it has drawbacks. In general, the inclusion of live feeds in the diet of zebrafish is not ideal; cultured zooplankton can be expensive and labor intensive to produce, may vary considerably in nutritional profile,⁶ and can potentially be a source of toxic contaminants and/or disease.^{7,8} However, because it is far more labor intensive—and less effective^{9,10}—to rear zebrafish on processed diets alone, we have adopted a general strategy of employing first rotifers, followed by *Artemia*, until the fish grow to a size where they can be easily weaned onto a processed diet that allows for greater control in quantity, nutritional profile, and purity.⁵

Still, the method could be refined, particularly if *Artemia* could be eliminated. Of the two live items in the diet, *Artemia* is far more labor intensive, expensive, and challenging to control nutritionally.¹¹ It is also considerably more difficult to successfully present to first feeding stage zebrafish larvae than are rotifers, due to their larger size and swimming speed.³ The exclusive use of rotifers represents a more economical, less labor intensive, and simpler solution, especially given the fact that our rotifer cultures are continuous and naturally produce an excess of 20 to 30 million rotifers per culture on a daily basis.¹²

Given these realities, we devised a simple set of trials to determine the feasibility of replacing *Artemia* with rotifers in

our zebrafish rearing protocols. Here, we present the results of trials where wild-type (WT) and hypopigmented zebrafish were grown up on diets consisting of rotifers followed by *Artemia* or rotifers exclusively, until they were weaned onto a processed diet. Our intention was not to directly compare performance of fish fed on rotifers versus those fed on *Artemia*. Rather, we simply wanted to determine whether we could use rotifers alone to grow the fish at a rate that would allow us to (i) effectively transition them to a processed diet by 30 dpf or less, and (ii) meet our standard generation time of 60 dpf.⁴

Materials and Methods

Fish

Two strains of zebrafish were used in the feeding trials: AB and *casper* (*mitfa*^{w2}; *roy*^{ag}). The AB strain was chosen because it is the most commonly used wild-type strain in experimental applications, whereas *casper* was selected because it is also widely employed (due to its transparent skin) and is generally more challenging to rear than a typical wild-type strain (author's personal observation). A breeding population of AB fish maintained at Boston Children's Hospital (BCH) for >25 generations¹³ was used to generate the embryos used in the trials. The *casper* embryos used in the trials were produced from a breeding stock of animals that was created by crossing fish from the original lineage of *casper*¹⁴ to a group of fish originating from a wholesale tropical fish supplier (Ekkwill Waterlife Resources) and subsequently maintaining them in the same fashion as we do the AB and other wild-types.¹³ This stock has been maintained for greater than five generations at BCH, using the same crossing strategy that we employ to maintain AB and other wild-type stocks.¹³ The AB and *casper* embryos for the trials were obtained from group spawning events involving ~100 fish per strain. The use of these animals in these trials was approved by the Institutional Animal Care and Use Committee at Boston Children's Hospital (IACUC Protocol No. 14-05-2763R). Water quality conditions during the trials are outlined in Table 1.

Rearing trials

Zebrafish embryos collected from the group spawning events detailed above were arrayed into 24 (12 AB, 12 *casper*) replicate groups of 15 individuals each on 1 dpf and then

incubated at 28°C in 50 mm Petri dishes until gas bladder inflation at 5 dpf, the developmental milestone that coincides with the onset of exogenous feeding.² At this point the larvae were transferred to 0.8 L holding tanks, yielding holding densities of ~18 fish per liter. The fish in each tank were photographed with a digital camera (Panasonic Lumix LX4) as a group. From 5 to 8 dpf, all 24 replicate groups were reared in static polyculture with saltwater rotifers (*B. plicatilis*, type L) and *Nannochloropsis* algae paste (RotiGro Plus; Reed Mariculture, Inc.).³ Mean rotifer density in rearing tanks during this period was ~800/mL.

At 9 dpf, six of the AB replicate groups and six of the *casper* groups were placed on recirculating flow at a slow drip (~14 mL/min rate) and were assigned to be fed rotifers exclusively (R-WT, and R-*casper*, respectively) (Table 2). The remaining six AB and six *casper* groups were also placed on flow at the same drip rate, and were assigned to be fed *Artemia* (RA-WT, and RA-*casper*, respectively). R groups were fed collected, concentrated rotifers 3× daily to apparent satiation from 9 to 25 dpf. RA groups were fed collected, concentrated first instar *Artemia* nauplii 3× daily to apparent satiation from 9 to 25 dpf. At 25 dpf, the flow rate into all tanks was increased (~40 mL/min rate) and the fish were fasted for 24 h. From the following day onward, all tanks were fed a pelleted diet (Gemma Micro 300; Skretting) once per day, using a modified spice dispenser (Pentair Aquatic Ecosystems) set at the "pinch" setting, which results in a dose of feed that averages ~0.05 g per plunge of the dispenser. At 32 dpf, we changed the setting on the feed dispenser to the one-eighth setting, which averages ~0.25 g per plunge of the dispenser. At the same time, the flow rate into all of the tanks was increased to ~150 mL/min. Finally, on 54 dpf, we changed the setting on the dispenser to one-fourth, which dispenses ~0.54 g per plunge. The flow rates remained at ~150 mL/min rate. The feeding and flow remained at this level for the duration of the trials (Table 2).

Growth measurements

At 25 dpf (weaning onto processed diet age) and 60 dpf (age of first reproduction), each replicate tank was taken off the system and photographed from above with a digital camera (Panasonic Lumix LX4). Photographs were subsequently analyzed with Adobe Photoshop Cs4 software. In this fashion, at least 10 randomly selected fish in each replicate were

TABLE 1. WATER QUALITY CONDITIONS DURING THE STUDY PERIOD

Water quality parameter	Value	Testing method	Recording frequency
pH	7.50 ± 0.13	SCADA 3000	Continuous
Conductivity (μS)	1821.59 ± 74.11	SCADA 3000	Continuous
Alkalinity (mg/L CaCO ₃)	63.60 ± 11.60	LaMotte Test Kit	Monthly
Hardness (mg/L CaCO ₃)	118.01 ± 12.75	LaMotte Test Kit	Monthly
Dissolved oxygen (mg/L)	7.32 ± 0.67	LaMotte Test Kit	Monthly
Carbon dioxide (mg/L)	2.25 ± 1.64	LaMotte Test Kit	Monthly
Phosphate (mg/L)	0.25 ± 0.35	LaMotte Test Kit	Monthly
Temperature (°C)	27.77 ± 0.32	SCADA 3000	Continuous
Total ammonia nitrogen (mg/L)	0.04 ± 0.02	LaMotte Test Kit	Weekly
Nitrite (mg/L)	0.02 ± 0.01	LaMotte Test Kit	Weekly
Nitrate (mg/L)	3.84 ± 0.62	LaMotte Test Kit	Weekly

Data are mean ± SEM.

SEM, standard error of the mean.

TABLE 2. EXPERIMENTAL FEEDING REGIME

<i>Rotifers exclusively (R)</i>			<i>Rotifers + Artemia (RA)</i>		
<i>Feed type</i>	<i>Amount</i>	<i>Flow rate</i>	<i>Feed type</i>	<i>Amount</i>	<i>Flow rate</i>
<i>Brachionus plicatilis</i> (L)	Continuous Apparent satiation 3×daily	Static ~ 14 mL/min	<i>B. plicatilis</i> (L) <i>Artemia salina</i> nauplii	Continuous Apparent satiation 3×daily	Static ~ 14 mL/min
Gemma Micro 300	0.05 g 1×daily 0.25 g 1×daily 0.54 g 1×daily	~ 40 mL/min ~ 150 mL/min ~ 150 mL/min	Gemma Micro 300	0.05 g 1×daily 0.25 g 1×daily 0.54 g 1×daily	~ 40 mL/min ~ 150 mL/min ~ 150 mL/min

measured for total length (from the tip of the snout to the longer lobe of the caudal fin) 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. Fish were also weighed (at the same time that they were photographed) by transferring all of the fish from each replicate tank to a previously tared balance (Scout Pro; Ohaus, Inc.).

Determination of sex ratios

At 60 dpf, the sex ratio of each treatment group was determined by counting the number of males and females in each tank. Adult zebrafish may be reliably sexed on the basis of differences in morphology and pigmentation between males and females.^{2,15}

Breeding trials

To test whether fish in all treatments had developed at a rate that allowed them to meet our standard generation time of 60 dpf,⁴ 2 males and 3 females were randomly selected from each replicate tank and set up in a standard mating cage, yielding 6 crosses per treatment, and a total of 24 crosses. When the animals were set up in crosses, they were placed in the mating cages in the afternoon and remained there until noon on the following day, at which point they were removed and returned to their holding tanks. If crosses were successful, the embryos were collected, rinsed with system water, transferred to 50 mm Petri dishes, and incubated overnight at 28°C.

At 1 dpf, each clutch was inspected under a dissecting microscope (Leica Microsystems, Inc.). The clutches were photographed, and the numbers of viable and nonviable

embryos in each clutch were subsequently counted using Photoshop's count feature.

Statistical analyses

Results of survival, total length, weight, sex ratios, clutch size, viability, and spawning success were subjected to one-way analysis of variance (ANOVA). All statistical analyses were done using GraphPad Prism Software version 5.0. Survival, sex ratios, and spawning success results were arcsine square-root transformed before submitting to ANOVA. When *p*-values showed significance (*p* < 0.05), individual means were compared using the Tukey's multiple comparison test.

Results

Growth and survival performance

There were no statistical differences in mean survival between R and RA treatments in the WT or *casper* groups (Table 3). The lowest mean survival rate of 0.78 ± 0.11 (mean $\pm$ standard error) observed in the R-*casper* groups was due to a nonprotocol-induced mortality event in one tank (blocked flow over a weekend) that occurred before 25 dpf. Mortality in the remaining 23 treatment tanks was extremely low (a maximum of one or two individuals per tank across treatments), and was in all likelihood also due to incidental mortality not related to study conditions.

There were significant differences in growth performance between fish in the R and RA treatments. At 25 dpf, RA-WT fish were on average significantly longer and heavier (1.89 ± 0.04 cm; 0.07 ± 0.002 g) than the R-WT groups (1.45 ± 0.01 cm; 0.02 ± 0.003 g) (Table 3). The RA-*casper* groups

TABLE 3. GROWTH AND SURVIVAL PERFORMANCE

<i>Treatment</i>	<i>R-casper</i>	<i>RA-casper</i>	<i>R-WT</i>	<i>RA-WT</i>
Age (dpf) 25				
Mean total length (cm)	1.30 ± 0.02	1.51 ± 0.06^a	1.45 ± 0.01	1.89 ± 0.04^a
Mean weight (g/animal)	0.01 ± 0.002	0.03 ± 0.007	0.02 ± 0.003	0.07 ± 0.002^a
Mean survival (prop)	0.78 ± 0.11	1.00 ± 0.00	0.98 ± 0.01	0.97 ± 0.02
Age (dpf) 60				
Mean total length (cm)	2.92 ± 0.06	2.90 ± 0.08	2.99 ± 0.05	3.23 ± 0.05^a
Mean weight (g/animal)	0.21 ± 0.02	$0.18 \pm .01$	0.22 ± 0.004	0.29 ± 0.01^a
Sex ratio (prop male)	0.36 ± 0.06	0.20 ± 0.02	0.36 ± 0.06	0.33 ± 0.03

Data are mean $\pm$ SEM. Mean weight per animal values were derived by dividing the total weight of each tank by the number of animals in tanks, across treatment groups.

^aMeans within each row are significantly different (*p* < 0.05) from each other (within strains).

dpf, days postfertilization; WT, wild-type.

TABLE 4. REPRODUCTIVE PERFORMANCE AT 60 DAYS POST-FERTILIZATION

Treatment	R-casper	RA-casper	R-WT	RA-WT
Spawning success				
No. of successful/total	4/6	0/6	6/6	5/6
Proportion	0.67	0	1	0.83
Total embryos spawned	265 ± 21.72	—	517 ± 41.04	1117 ± 164.62
MCS	66.25 ± 10.86	—	86.16 ± 20.52	223.4 ± 82.31
Mean viability (prop.)	0.96 ± 0.02	—	0.63 ± 0.13	0.70 ± 0.08

Data are mean ± SEM.

MCS, mean clutch size.

also showed superior growth; 1.51 ± 0.06 cm; 0.02 ± 0.003 g) compared with the R-casper (1.30 ± 0.02 cm; 0.01 ± 0.002 g), although the differences in weight between the treatments were not significant (Table 3). At 60 dpf, the RA-WT fish were still significantly larger than the R-WT groups, both in terms of length and weight (3.23 ± 0.05 cm; 0.29 ± 0.01 g vs. 2.99 ± 0.05 cm; 0.22 ± 0.004 g, respectively) although the differences were not as pronounced as they were at 25 dpf (Table 3). There were no significant differences in mean length or weight between the R-casper and RA-casper groups at day 60 (Table 3).

Sex ratios

There were no significant differences in sex ratios between treatment groups when we assessed the sexes by morphology and skin pigmentation at 60 dpf. However, the mean sex ratios of groups across all treatments were male biased, ranging from 0.20 ± 0.02 proportion male in the RA-casper groups to 0.36 ± 0.06 proportion male in the R-casper (Table 3).

Breeding trials

The reproductive performance of the fish is detailed in Table 4. For some unknown reason, The RA-casper fish did not spawn in any of the six crosses set up at 60 dpf, even though the morphological and color differences between the sexes were clearly apparent and the females were clearly carrying eggs. In all other groups, the fish spawned successfully, ranging from 0.67 (4/6 successful) in the R-casper groups to 1.00 (6/6) in the R-WT groups (Table 4). In terms of clutch size, there was a clear association between the size of the animals in different treatment groups and the number of embryos spawned. The R-casper groups spawned the fewest number of embryos (265 ± 21.72 total embryos spawned and a mean clutch size of 66.25 ± 10.86), whereas the RA-WT showed the greatest production (1117 ± 164.62 total embryos spawned and a mean clutch size of 223.4 ± 82.31). However, the differences between the R-WT (517 ± 41.04 total embryos spawned and a mean clutch size of 86.16 ± 20.52), and RA-WT were not significant. Further, there were no significant differences in embryo viability between R-WT and RA-WT groups. The R-casper groups produced embryos with the highest mean viability across any treatment (0.96 ± 0.02).

Discussion

The “optimal” diet for laboratory zebrafish is one that efficiently promotes definition and stability in nutritional profile, biosecurity, and maximal performance (growth, sur-

vival, and reproduction). Traditional feeding paradigms^{16,17} typically do not meet all of these requirements at the same time. Recent advances made in methods for feeding larval³ and adult stage fish⁵ now support the premise that the most effective feeding strategy for this species incorporates a live prey source from the point of first feeding through the larval stage until the animals are large enough to efficiently consume a processed feed. One published example of this approach incorporates a progression from rotifers followed by *Artemia*, before finally transitioning to a processed diet when the fish reach 30 dpf and an average total length of ~ 1.8 cm⁵.

This overall approach can be further improved upon by reducing its reliance on live prey. While it is certainly possible to rear zebrafish exclusively on processed diets,¹⁸ it requires considerably more labor and does not typically promote the same levels of performance when compared against live diets.^{9,10} Given that reality, the management focus should be on reducing, rather than completely eliminating, the live prey. This may be most readily achieved by shortening the period of time during which the live prey is administered, and/or by decreasing the number of species of zooplankton that are used in the protocol. In this study, we tested whether or not it would be feasible to remove *Artemia* from the diet without sacrificing current performance standards. Most importantly, we wanted to determine whether we could use rotifers by themselves to effectively wean fish onto a processed feed by 30 dpf or less and complete a generation by 60 dpf, as we previously demonstrated with *Artemia* included in the diet.⁵

The results of these trials confirm the ability to do so, on both counts. In terms of weaning, we were actually able to successfully transition the fish onto the pelleted GM300 diet by 25 dpf, which is 5 days before our previously published standard of 30 dpf. Effective transition in this case was demonstrated by the fact that we saw no differences in survival between the groups. While the *Artemia*-fed treatment groups (both WT and casper) were significantly larger at this time point and closer in size to the total length at time of weaning reported previously,⁵ the rotifer-fed groups were still large enough (~ 1.5 cm) to consume the 300 μ M pellet with no observable effect on survival. Additionally, by 60 dpf, the rotifer-fed groups had shown compensatory growth; there were no statistically significant differences in length or weight between the casper groups, and while the differences between the RA-WT and R-WT were still significant, the gap was much smaller, and the mean total length of fish in all groups at this time point was comparable to previous reports,⁴ (Table 3). Furthermore, because the fish (in any treatment group) had yet to reach their full adult size

($\sim 3.5\text{--}4.0\text{ cm}^5$) by the time this study was concluded at 60 dpf, it is reasonable to speculate that the compensatory growth demonstrated by the rotifer-fed groups would have continued to the point where all fish across treatments would have been equivalent in size upon reaching the “plateau” of the typical growth curve for the species. This would also likely have an impact on long-term fecundity values related to female body size.¹ However, this remains to be explored.

We also confirmed the possibility to close a generation within 60 dpf with rotifers alone, with high rates of spawning success in both the R-WT and R-*casper* groups. While overall embryo production was highest in the R-WT group, the differences between them and the R-WT were not statistically significant. While the reason for the failure of the R-*casper* groups to spawn at that time point is unclear, it is unlikely that it was related to the diets, especially since the fish were approximately the same size as the rotifer-fed *casper* groups and the females were clearly carrying eggs.

The differences we observed in growth performance between the *Artemia*-fed and rotifer-fed groups were likely related to biomass, rather than on other intrinsic (i.e., nutritional profile) differences between the two prey items. First, instar *Artemia* nauplii are on average $2\text{--}3\times$ larger than saltwater type L rotifers ($\sim 500\text{ }\mu\text{M}$ vs. $\sim 150\text{ }\mu\text{M}$),² and because we did not normalize biomass going in and instead simply fed to apparent satiation, these differences likely became manifest. Preliminary trials completed in our laboratory before this experiment showed the opposite effect when rotifer biomass was greater than *Artemia* biomass delivered to fish (unpublished, data not shown). It is important to consider that the goal of our trials was not to compare the two feed types, but rather to confirm that current performance standards could be met by using rotifers exclusively. Future studies of the comparative effect of the two feed types on larval zebrafish performance would necessitate standardizing the mass of each prey type going to the fish.

The results of these simple trials have important implications on several fronts. The elimination of *Artemia* from the diet allows for further streamlining and definition of the nutritional landscape of the experimental fish. First, the decrease from two live prey species to one reduces the complexity of the diet and dramatically cuts down on the probability of introduction of pathogens and/or toxins through the feed culture. Second, while both rotifers and *Artemia* may be variable in nutritional profile, rotifers are far simpler and less time consuming to bioencapsulate.¹¹ This makes it easier for managers to “design” a rotifer with a stable and consistent nutrient profile that can be delivered to the fish. When this approach is utilized in conjunction with a processed feed, it becomes easier to define and standardize the nutritional state of the fish for its entire lifespan. This is critical for the integrity and reproducibility of any studies where nutrition can impact the outcome of experiments.¹⁹

The replacement of *Artemia* with rotifers will likely also have a positive impact on the operating expenses of zebrafish laboratories. The current unit cost for 90% hatch rate Great Salt Lake grade *Artemia* cysts is $\sim \$50.00$ USD/pound. This may vary significantly, because as a global commodity, *Artemia* is notorious for fluctuations in price and quality depending on year to year conditions in harvest sites.²⁰ Additionally, because *Artemia* nauplii must be hatched on a

daily basis for feed-out and are not “renewable,” cysts must be purchased and brought in continuously to support production. Alternatively, rotifers are typically maintained in continuous or semi-continuous cultures,¹² so the primary material costs of production is feed, which is readily available from various sources and stable in price.

In summary, the results of these trials show that it is possible to replace *Artemia* with saltwater rotifers in the diet of larval and juvenile zebrafish without compromising performance. Further refinements to this approach (e.g., increasing the biomass of rotifers delivered to developing fish) should serve to allow for the shortening of both the period during which live prey is utilized in the diet and age of first reproduction of this important model fish species.

Acknowledgments

The authors wish to thank K. Maloney, J. Best, and S. Alvarado for technical assistance.

Disclosure Statement

No competing financial interests exist.

References

1. Spence R, Gerlach G, Lawrence C, Smith C. The behaviour and ecology of the zebrafish, *Danio rerio*. Biol Rev Camb Philos Soc 2008;83:13–34.
2. Harper C, Lawrence C: The Laboratory Zebrafish (Laboratory Animal Pocket Reference). CRC Press, Eugene, OR, 2010.
3. Best J, Adatto I, Cockington J, James A, Lawrence C. A novel method for rearing first-feeding larval zebrafish: polyculture with Type L saltwater rotifers (*Brachionus plicatilis*). Zebrafish 2010;7:289–295.
4. Lawrence C, Adatto I, Best J, James A, Maloney K. Generation time of zebrafish (*Danio rerio*) and medakas (*Oryzias latipes*) housed in the same aquaculture facility. Lab Anim 2012;41:158–165.
5. Lawrence C, Best J, James A, Maloney K. The effects of feeding frequency on growth and reproduction in zebrafish (*Danio rerio*). Aquaculture 2012;368–369:103–108.
6. Siccardi AJ, Garris HW, Jones WT, Moseley DB, D'Abramo LR, Watts SA. Growth and survival of zebrafish (*Danio rerio*) fed different commercial and laboratory diets. Zebrafish 2009;6:275–280.
7. Lawrence C, Ennis DG, Harper C, Kent ML, Murray K, Sanders GE. The challenges of implementing pathogen control strategies for fishes used in biomedical research. Comp Biochem Physiol Part C Toxicol Pharmacol 2012; 155:160–166.
8. Peterson TS, Ferguson JA, Watral VG, Mutoji KN, Ennis DG, Kent ML. *Paramecium caudatum* enhances transmission and infectivity of *Mycobacterium marinum* and *M. chelonae* in zebrafish *Danio rerio*. Dis Aquat Organ 2013; 106:229–239.
9. Goolish EM, Okutake K, Lesure S. Growth and survivorship of larval zebrafish *Danio rerio* on processed diets. N Am J Aquac 1999;61:189–198.
10. Carvalho P, Arau L. Rearing zebrafish (*Danio rerio*) larvae without live food : evaluation of a commercial, a practical and a purified starter diet on larval performance. Aquacult Res 2006;37:1107–1111.

11. Lavens P, Sorgeloos P. Manual on the Production and Use of Live Food for Aquaculture. 1996:FAO Fisheries Technical Paper. No. 361. Rome, FAO. Available at: <http://medcontent.metapress.com/index/A65RM03P4874243N.pdf> \n<http://www.cabdirect.org/abstracts/19971409717.html> (accessed April 1, 2015).
12. Lawrence C, Sanders E, Henry E. Methods for culturing saltwater rotifers (*Brachionus plicatilis*) for rearing larval zebrafish. *Zebrafish* 2012;9:140–146.
13. Lawrence C. Advances in zebrafish husbandry and management. *Methods Cell Biol* 2011;104:429–451.
14. White RM, Sessa A, Burke C, *et al.* Transparent adult zebrafish as a tool for in vivo transplantation analysis. *Cell Stem Cell* 2008;2:183–189.
15. McMillan SC, Géraudie J, Akimenko M-A. Pectoral fin breeding tubercle clusters: a method to determine zebrafish sex. *Zebrafish* 2015;12:121–123.
16. Westerfield M. The Zebrafish Book. A Guide for the Laboratory Use of Zebrafish (*Danio rerio*), 4th ed. University of Oregon Press, Eugene, OR, 2007.
17. Nusslein-Volhard C, Dahm R. Zebrafish: A Practical Approach. Oxford University Press, New York, 2002.
18. Hensley MR, Leung YF. A convenient dry feed for raising zebrafish larvae. *Zebrafish* 2010;7:219–231.
19. Penglase S, Moren M, Hamre K. Lab animals: standardize the diet for zebrafish model. *Nature* 2012;491:333.
20. Lavens P, Sorgeloos P. The history, present status and prospects of the availability of Artemia cysts for aquaculture. *Aquaculture* 2000;181:397–403.

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