Role of Live Feeds in Zebrafish Culture

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The use of the zebrafish as a model organism has grown dramatically over the last two decades. The rise in popularity of the zebrafish in research can at least be partially attributed to the relative hardiness of the species. The zebrafish, native to the flood plains of South Asia, is an adaptable and tolerant species that develops quickly and produces offspring reliably when cultured in the laboratory. Ironically, due in part to the hardiness of the species, the development of optimal husbandry protocols for zebrafish has stagnated and our understanding of their unique environmental and nutritional requirements is limited. Without this information, zebrafish culturists are often left to develop husbandry protocols based on educated guesses that can potentially result in sub-optimal culture conditions. Over the last few years though there has been a concerted effort by the zebrafish community to develop culture techniques that result in improved survival, growth and reproductive performance. Naturally, many of these studies have concentrated on determining proper feeds and feeding practices to improve growth, survival and reproductive performance of the zebrafish.

Most research facilities culturing zebrafish rely upon some type of manufactured diet to provide the majority of nutrition to their zebrafish stocks. These diets are generally assumed to be nutritionally complete and can often be used as a sole source of nutrition throughout the life cycle of the zebrafish[4,5]. It is important to note, however, that the specific dietary requirements of the zebrafish are not well defined and results with manufactured diets can be highly variable. It has been demonstrated that growth, generation time and reproductive performance of the zebrafish is improved by supplementing manufactured diets with live feeds organisms[3]. This benefit is most dramatic during the early larval stages where properly administered live feed organisms can produce significantly greater survival and growth rates, helping to set the stage for a healthy and productive adult colony. The nutritional advantage of live feeds over manufactured diets is owed to four characteristics:

1. Live feeds are a highly digestible and bioavailable source of nutrition.
2. They have a favorable amino acid profile to help support tissue growth of a growing zebrafish.
3. The swimming behavior of live feeds encourages aggressive feeding activity by the zebrafish
4. Live feed organisms can be fed specialized diets (enriched) to customize their nutritional profile for the species being fed. Thus through the use of live feeds, culturists have a tool that can greatly increase the survival, growth and spawning success of their zebrafish cultures.

The most commonly used live feeds for the culture of zebrafish are paramecia, rotifers and *Artemia sp*. Both rotifers and paramecia can be used as an initial feed item when zebrafish first begin exogenous feeding (typically around 5 days post hatch). While paramecia seem to be a reliable source of early nutrition, the use of rotifers is gaining popularity due to their ability to be raised at high densities with minimal labor requirements[1] and their ability to promote faster growth rates during the sensitive larval stages[2]. *Artemia* or brine shrimp are the most widely utilized species of live feed used for zebrafish culture. Their life history, availability and nutritional composition makes Artemia well suited as a live feed organism for zebrafish at multiple life stages.

Paramecia

Paramecia (*Paramecium sp*.) are single cell protists, 100-250 micrometers in length, that are common to freshwater environments. There small size makes them suitable as an initial food source for developing zebrafish larvae but are effective only when fed at very high levels for the first few days of feeding. While the use of paramecia is popular among some zebrafish culturists, paramecia cultures are reported to be labor intensive and subject to contamination[2]. Furthermore, their nutritional composition is not ideal, and paramecia are not ammenable to enrichment that would improve their nutritional content. Also, as paramecia are cultured in freshwater, cultures can become a source of pathogens and parasites that may adversely affect zebrafish larvae.

The Rotifer

The rotifer is a small ciliated protozoan common to freshwater and saltwater environments around the globe. Due to its size, planktonic nature and ability to be cultured in large quantities, the rotifer is a popular choice as a food item for finicky larval fishes being raised in commercial hatcheries. Today rotifers are essential as a first feed item for many commercially important species of cultured fish, crab and shrimp. While over 2200 species of rotifers have been described and many have been cultured, *Brachionus plicatilis* is used most commonly in fish hatcheries around the world and is a suitable species to be used for the initial feeding of larval zebrafish.

Photomicrograph detailing a rotifer with egg.
Photo credit: Reed Mariculture

Culture Methods

B. plicatilis which has a size of 150-300 micrometers (µm) are prolific animals that can be cultured at densities exceeding 1,000/mL in laboratory-scale cultures and can potentially double their population in a day. B. plicatilis is a euryhaline species and is most often cultured at zebrafish facilities at 5-20 parts per thousand (ppt). Rotifers are highly sensitive to environmental conditions and should be monitored in a similar fashion to the zebrafish colony for optimal health and production. Rotifers are most productive when cultured at temperatures of 26-30°C. Dissolved oxygen levels should be kept above 4ppm. Total ammonia nitrogen should remain below 5 parts per million (ppm). There are two basic methods that can be employed for culturing of rotifers: The batch method, where a given volume of water is inoculated with a rotifer starter culture, the rotifers are then allowed to reach a terminal density before being harvested and the culture is then restarted at regular intervals. The continuous method employs specialized filters or chemicals to remove waste particles and minimize the buildup of nitrogenous wastes from the water increase the density of rotifers cultured while minimizing the need to restart cultures. Rotifer cultures should be assessed daily. Observation of swimming speed, population counts, fecundity and culture cleanliness can be made with a microscope and are used to evaluate the health of the culture. Rotifer cultures can be started from cysts, but most zebrafish facilities simply inoculate new cultures with live rotifers from previous cultures. Under ideal conditions, a rotifer culture contains primarily females that reproduce asexually. It is not until conditions in a culture deteriorate that males will appear in abundance and sexual reproduction takes place. The result of sexual reproduction is resting cysts which are not ideal for continuous operation of culture.

Rotifers actively graze the water column feeding on particles approximately 1-10 µm in size and will need a regular supply of food for maximum productivity. There are a number of yeast- or algae-based diets suitable for culturing rotifers that are commercially available. Over the last few years, the use of concentrated algal pastes has grown in popularity within the zebrafish community. These products are favored due to their inherent nutritional quality and cost effectiveness.

Application

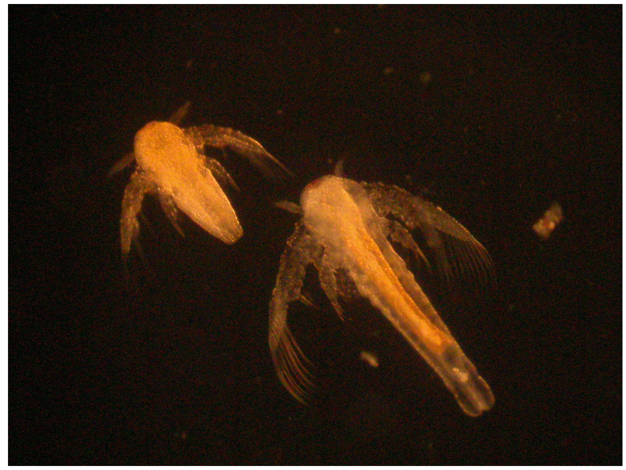
Rotifers are an effective food source for larval zebrafish. They promote rapid growth during the first few weeks of feeding allowing fish to effectively consume Artemia. It is best to enrich or gut load rotifers for a few hours prior to feeding them to zebrafish in order to improve their nutritional quality. Enriched rotifers that are not fed to fish immediately should be returned for further enrichment or stored at a temperature below 10°C in order to maintain nutritional quality for later use. Rotifers should be carefully harvested using a 55 micron mesh and should remain submerged in aerated water at all times to maintain quality. Drastic changes in pH, temperature and salinity can also adversely impact survival and health of rotifers. Rotifers will quickly settle out of the water column when exposed to salinities below 5ppt so frequent additions may be required. Feeding rates for larval zebrafish vary depending on techniques but success has been reported as low as 10-20 rotifers/ml using a rotifer-zebrafish polyculture technique 1.

Artemia

Artemia are the most popular live food organism used for the culture of zebrafish as a supplement to prepared rations in the vast majority of culture facilities. *Artemia* are small aquatic crustaceans native to inland saline environments around the globe. During a portion of their life cycle, *Artemia* produce a resting cyst that is able to withstand extreme environmental conditions. The cysts are cleaned, desiccated and graded based on hatch rate before they are canned for shipment. The vast majority of *Artemia* cysts available to zebrafish culturists are *Artemia franciscana* harvested from the Great Salt Lake in Utah. Annual harvests of *Artemia* cysts are unpredictable and can fluctuate greatly due to environmental factors. As a result, the quality, availability and price of *Artemia* cysts can fluctuate from year to year.

Culture Methods

Artemia cysts (approximately 2 grams per Liter) are incubated in warm seawater (25-35 ppt). Aeration is required in the tank to maintain proper oxygen levels (>4 ppm) and to keep the cysts suspended in the water column. The pH of the hatching medium should be maintained between 8.0-9.0 with sodium bicarbonate additions. Bright light (approximately 2000 Lux at water surface) will help to maximize hatch rates. After approximately 16-24 hours of incubation, Artemia nauplii (Instar I phase) will emerge from the cyst and can be harvested from the culture tank. Harvesting of Artemia nauplii is conducted by draining the culture through a 100-125 micron bag or sieve and rinsing well with clean water. It is important to keep artemia submerged in well oxygenated water to maintain quality. After harvesting, Artemia can then be fed directly to zebrafish, placed in cold storage (>10C) for use later in the day or further on-grown to increase size and nutrient density for older fish. The use of Artemia cysts is not without its problems, however. The outer cyst layer or chorion is indigestible and has the potential to cause intestinal blockage when consumed by fish larvae. Artemia cysts have also been identified as a potential vector for unwanted pathogens. As a result, it is recommended to use decapsulated cysts whenever possible. Decapsulated Artemia cysts have had the outer chorion removed via a chemical process involving chlorine and sodium hydroxide and can be produced in-house or purchased through commercial suppliers. The benefits of the decapsulation process include improved hatching rates, improved energy reserves in the nauplii and prevention of cysts entering the fish culture tanks.



Newly hatched Artemia nauplii (left) with 48 hour old enriched Artemia meta nauplii

Application

While it has been shown that *Artemia* can be used as a suitable feed organism throughout the life cycle of the zebrafish, it is generally accepted that Artemia are not an ideal food source for the early feeding of zebrafish larvae. The size and swimming speed of newly hatched Artemia nauplii can make it difficult for larvae to feed effectively. As such, Artemia are better suited as a feed for later stage larvae, juveniles and adult. Artemia nauplii are generally offered to zebrafish at the rate of 1-10/ml depending on zebrafish age and stocking rate. It is important to not overfeed with Artemia since they will perish within a few hours of stocking into freshwater. Although Artemia nauplii are an excellent protein source for zebrafish they are deficient in essential fatty acids (EFA) such as omega -3 and omega-6 which have been shown to be vital nutrients for proper growth and reproductive functioning in zebrafish1. The level of these EFA’s as well the protein and vitamin content can be increased within Artemia through the process of enrichment. During enrichment, Artemia nauplii are further cultured for an additional 24-36 hours after hatching. During this time Artemia increase in size and develop working mouth parts which allows them to feed on the enrichment diet. While enriched Artemia are too large to be fed to larvae and early juveniles they can be an excellent source of nutrition for sub-adults and broodstock. Despite the benefits, the use of live feeds does pose some challenges. Live feed cultures must be maintained by trained staff on a regular basis. Cultures can be unpredictable and can experience die-offs if environmental and nutritional parameters are not maintained. Standardization of protocols is critical to maintain optimal quality of live feed cultures. Variation in how live feeds are cultured, stored and administered can have a negative impact on the overall quality of the live feeds by affecting size, nutritional composition and survival. Live feed cultures can also become contaminated with a range of organisms some of which may be pathogenic towards zebrafish. Cleanliness and biosecurity should always be a priority when working with live feeds and regular microbiological sampling can be utilized as an effective tool to monitor bacteria loading of cultures.

It's important to remember our fish are what they eat; feeding a sub optimal diet will eventually impact the quality of our fish. As the zebrafish community continues to moves forward and we gain a better understanding of zebrafish dietary requirements there will likely come a day when zebrafish-specific diets become available and can effectively eliminate the use of live feeds. Until that time comes however live feed organisms will likely play an integral role in zebrafish culture for the foreseeable future.

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