Regular Care and Aquarium Maintenance of Zebra Fish

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Abstract: ***This protocol describes regular care and maintenance of a zebrafish laboratory. Zebrafish are now gaining popularity in genetics, pharmacological and behavioural research. As a vertebrate, zebrafish share considerable genetic sequence similarity with humans and are being used as an animal model for various human disease conditions. The advantages of zebrafish in comparison to other common vertebrate models include high fecundity, low maintenance cost, transparent embryos, and rapid development. Due to the spur of interest in zebrafish research, the need to establish and maintain a productive zebrafish housing facility is also increasing. Although literature is available for the maintenance of a zebrafish laboratory, a concise video protocol is lacking. This video illustrates the protocol for regular housing, feeding, breeding and raising of zebrafish larvae. This process will help researchers to understand the natural behaviour and optimal conditions of zebrafish husbandry and hence troubleshoot experimental issues that originate from the fish husbandry conditions. This protocol will be of immense help to researchers planning to establish a zebrafish laboratory, and also to graduate students who are intending to use zebrafish as an animal model.***

Keywords: Zebra fish, Aquarium maintenance, Aquarium, Model organism

1. Introduction

The zebrafish (Danio rerio) is a tropical freshwater fish found in the rivers (mainly the Ganges) of the Himalayan region of South Asia, especially in northern India, as well as northern Pakistan, Bhutan and Nepal. It belongs to the family of cyprinids (Cyprinidae) in the class of ray-finned fishes (Actinopterygii) and to the bony fishes (Teleosts or Teleostei) in this class. Zebrafish (Danio rerio) are small, about 3 -5 cm long. They are native to rivers in India and are commonly kept as pets. The males are slender and torpedo-shaped, with black longitudinal stripes and usually a golden tinge on the belly and fins. Egg-laden females are thicker and have little, if any, gold on their underside. The zebrafish was first used as a biological model by George Streisinger (University of Oregon) in the 1970s because it was simpler than mice and easier to manipulate genetically. Streisinger's colleagues, especially Chuck Kimmel at his university, were very impressed with the idea of using the more attractive zebrafish embryo to study the development of the nervous system.

Since it was first described by Francis Hamilton in 1822 from the Ganges Delta, the small striped fish commonly known as the 'zebrafish' or 'zebra danio' has become a popular aquarium fish.

Zebrafish are easy to rear, have a generation time of 3 months, and females can lay hundreds of eggs at weekly intervals. Fertilization is external, providing easy access to the embryos for observation and manipulation. The developing embryos can be studied easily under a dissecting microscope because they are transparent.

Zebrafish embryos develop rapidly (in 2 ± 4 days), the heart beats in 24 hours and erythrocytes appear. Another advantage of studying zebrafish is that unlike other fish, which may be triploid or tetraploid (which makes genetic analysis difficult), it maintains the diploid state.

Zebrafish have many anatomical and genetic similarities with humans, including the brain, digestive system, muscles, vessels, and innate immune system. Also, 70% of human disease genes have functional similarities with zebrafish.

Scientific names	*Danio rerio*
Common names	Zebra fish
Distribution	India, Bangladesh, Bhutan, etc.
Size	1.5 – 2.5 inches
Life expectancy	3 – 5 years
Colour	Blue, green, red, purple with black & white strips
Diet	Omnivorous
Temperament	Peaceful & playful
Tank size (min.)	4 gallons
Temperature	17 – 23 C
pH	6.8 – 7.8
Hardness	5 – 7 dGH
Care level	Easy
Breeding	Egg layer

Figure 1: Adult zebrafish

Salient features of zebrafish as a model organism

D. rerio is preferred by scientists because of its variety of features that make it useful as a model organism. The embryo develops rapidly outside mother and optically clear and thus, easily accessible for experimentation and observation. The embryo develops very fast, and the blastula stage lasts only for 3 h, while gastrulation gets completed in 5 h; in an embryo that is about 18 h old, very well-developed ears, eyes, segmenting muscles, and brain can be viewed as the embryo is transparent. By 24 h, segmentation gets completed, and most primary organ systems are formed. By 72 h, the embryo hatches out from the egg shell and within the next 2 days starts hunting for food. In a period of just 4 days, the embryo converts rapidly into a small version of adult. The rapid development simplifies development and genetic studies.

The adult zebrafish attains sexual maturity very quickly, having generation time of about 10 weeks, and also this tiny fish has good fecundity rate. When kept under optimal conditions, the zebrafish can lay about 200 eggs per week. Under laboratory conditions the zebrafish can spawn throughout the year that ensures the constant supply of offspring from designated pairs that makes this transparent fish a quintessential choice for large-scale genetic approaches to identify novel genes and to discover their specific functions in vertebrates. The zebrafish is a very hard fish and is very easy to raise.

In addition to the features of zebrafish mentioned above, it requires very low space and maintenance cost. These features make this fish an attractive model organism for developmental, toxicological, and transgenic studies.

Being a transparent vertebrate, the zebrafish has emerged as a convenient alternative to study the early development of the cardiovascular system and observe the flow of blood. In zebrafish larvae the vessels and blood flow can easily be visualized by using simple dissecting microscope and also by using fluorescent proteins; the development of the blood vascular system could be examined in great details

The zebrafish exhibits remarkable capacity of regeneration even in adult stages. The caudal fin especially provides an ideal tissue for vascular regeneration studies due to its simple and fine architecture and relative transparency.

Being a vertebrate, the zebrafish is an ideal model to study cancer, though humans and fishes are separated from their common ancestry but biology of the cancer in both groups of organisms is the same.

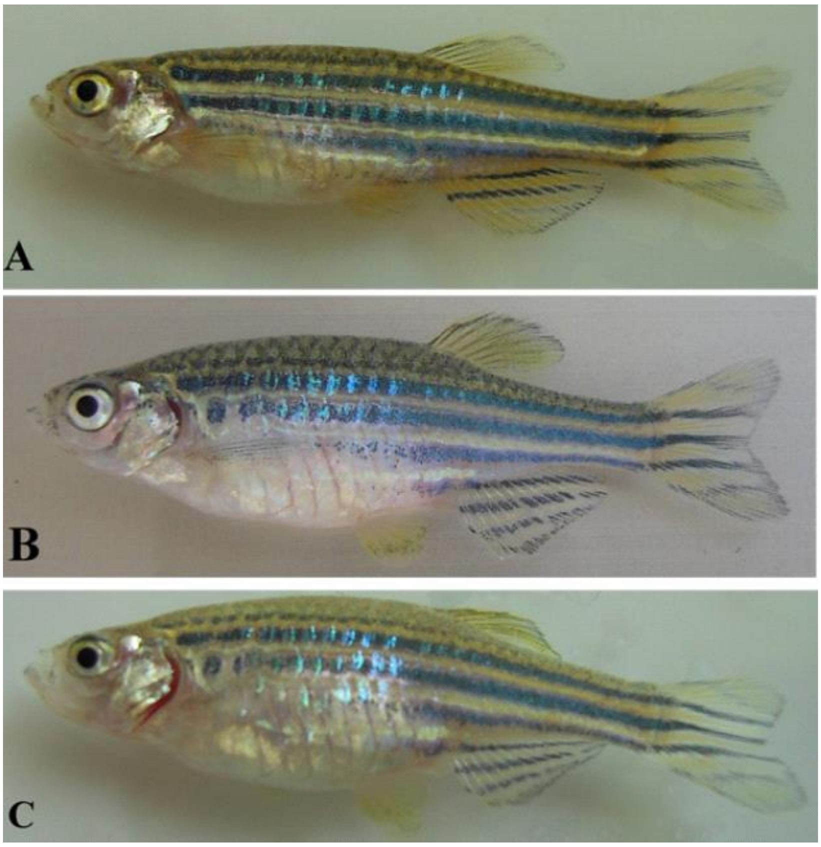

Figure: An illustration of a male zebrafish (A) and female zebrafish (B, C).

2. Review of Literature

Since it was first described by Francis Hamilton from the Ganges Delta in 1822, the small striped fish commonly known as the 'zebrafish' or 'zebra danio' (Figure 1.1) has become variously a popular aquarium fish (Talwar & Jhingran, 1992) an important bioassay (Von Hertell et al., 1990) and in the last few years a major model in vertebrate physiology and developmental genetics (Vascotto et al., 1997). Several attributes of the species explain its popularity in all these contexts: it reaches a length of only 30mm, is relatively easy to rear and breed in captivity, and produces large numbers of offspring with a generation time as short as four months.

As a result of the use of the zebrafish as a developmental genetic model, a dense microsatellite map is available, which will greatly facilitate future mapping of loci (e.g., Shimoda et al. 1999, Postlethwait et al. 1998).

The zebrafish exhibits a striking pattern of up to five blue longitudinal stripes on the sides of the body, alternating with gold interstripes and extending into the caudal fin. The anal fin is similarly striped whilst the dorsal fin has a blue upper edge, bordered with white. In D. kyathit, the stripe pattern typical of D. rerio is broken up anteriorly into spots, with only three stripes extending into the caudal fin and weak striping on the anal fin. There appears to be variation in the degree of spotting, with some individuals perhaps approaching more closely the typical D. rerio striped pattern (Fang, 2000).

Within two years, the complete zebrafish genome sequence is expected to be known (Duyk & Schmitt, 2001). Much progress has been made in elucidating the genetic basis of the zebrafish colour pattern (e.g., Parichy et al., 2000), a trait which appears to be important in speciation via reproductive isolation in other teleost fish (e.g., Seehausen & van Alphen, 1998).

The recent interest in zebrafish genetics has resulted in molecular phylogenies of the Danio genus (Meyer et al., 1993, 1995) and the taxonomy of Danio is receiving renewed attention (e.g., Fang, 1998).

Despite these advantages, there are reasons why the zebrafish might not prove to be a good model for studying the genetic basis of prezygotic isolation. Primary amongst these is the current lack of knowledge about mate choice in this species. Behavioural work on the zebrafish has primarily concentrated on its shoaling behaviour (e.g., Krause et al., 1999) and its response to aquatic pollutants (e.g., Vogl et al., 1999).

3. Methodology

Study Material

Zebrafish Husbandry

Zebrafish were housed in 15-20 litre tanks, which were arranged on shelves in a self-contained stock room within the School of Biology aquarium unit. The room was heated to a temperature of 17–23 °C using an 'Auto heat' fan heater in combination with a built-in room temperature control. Lighting was provided by fluorescent ceiling lights and was set to a 14h light: 10h dark cycle. Compressed air was supplied to the room from a central source in the aquarium. Fish in the tanks were provided with a gravel substrate and imitation plastic plants made out of green mesh. Filtration was supplied by one Algard 'Bio foam 45' air-driven biological filter unit per tank. Maximum density of fish was 35 adults per tank. Tank water was topped up where necessary, with a 30% water change done every two to three days. All tap water was aged in buckets for a minimum of two days before introduction to tanks, Fish were monitored daily for signs of distress, with extra water changes being performed when required.

4. Protocol

System Maintenance

- Zebrafish are kept in a circulating system that continuously filters and aerates the system water to maintain the water quality required for a healthy aquatic environment. The circulating system also helps to filter excess food and fish excreta. Different companies provide zebrafish systems. The room temperature or the tank temperature is generally maintained between 17-23 °C and the lighting conditions are 14:10 hr (light: dark). Multiple lines of fish (e.g., transgenic, mutant, wild type) can also be housed on the same system.
- A set of different kinds of filters are used in the system. In our system, water from all the tanks passes through a 120-micron filter pad, 50- micron canister filter, biological filter, active carbon absorption filter and UV disinfection filter before being circulated back into the tank. Dechlorinated/aged water is used in the zebrafish system. Water can be de-chlorinated by ageing for at least 48 hr, under ideal condition.
- The pH of the system water should be checked daily and maintained between 6.8 and 7.5. When necessary, sodium bicarbonate should be used to increase the pH.
- Fish tanks should be cleaned regularly. To clean a fish tank, close the water flow to this tank, drain excess water by tilting the tank backwards and remove the tank carefully from the system. Dirt and algae growth will be apparent on the bottom and sides of the tank.
- Carefully transfer the fish into this tank with a fish net. Close the lid and transfer the name tag of the tank. Carefully place the clean tank into the system and switch on the water supply. To decontaminate the fish net, spray with 70% ethanol, rinse in water, and let it dry before re-using. Remove the baffle from the dirty tank and spray both parts with 70% ethanol. Rinse thoroughly with tap water and allow the tank and baffle to dry fully before re-using.
- The circulating system filters have to be checked and changed regularly to ensure their proper function. These filters should be changed regularly to ensure proper and clean water supply to all the fish tanks.
- The 120-micron filter pad is usually repositioned or replaced daily; The Canister filter should be changed weekly. To change the canister filter, remove the filter unit by twisting anticlockwise with a wrench or hands. The carbon filter should be changed fortnightly (every two weeks). To change the carbon filter, remove the carbon filter unit carefully with a wrench. Discard the used activated carbon and replace it with new activated carbon. Re-fit the carbon holder and place it back into the filter unit.

Feeding

- Zebrafish can be fed with dry food (food size from 100 microns for larvae to 300/400 microns for adult fish).
- Zebrafish should never be overfed as this may increase the nitrate level in the water, possibly affecting their breeding, or viability, as some fish may die due to overeating. We recommend providing no more food during any one feeding than a tank of fish can finish within 30 min.
- When feeding on the Aquatic Habitats systems we usually turn off the water pump and air pump to allow the fish to eat the food for 30 min. This decreases the amount of food that is washed into the filters. However, users must be careful to remember to turn on these pumps again afterwards.

Breeding

Zebrafish initiate breeding at the onset of light. Fertilized eggs can be obtained either through in-tank breeding or pairwise breeding. While in-tank breeding is more labour-efficient and is implemented for regular embryo collection in our laboratory, pairwise breeding is preferred when genes or mutations are to be screened from individual fish.

For in-tank breeding, assemble the in-tank breeder and drop slowly into the fish tank after the onset of light. Alternatively, in-tank breeding set-up can be left overnight in the fish tank.

Leave the in-tank breeder for around 15 min to allow the fish to mate before removing the breeder from the tank and collecting the eggs.

Pairwise breeding is usually set up late in the afternoon after feeding.

Assemble the breeding tank and fill it with aged system water.

Transfer one female and one male to opposite sides of the breeding tank. Females can be distinguished from males because of their bigger underbelly. Males can also be distinguished from females because they are more slender and darker in colour than females. Moreover, males have more yellow colouration in the anal fin compared to females. When in doubt look for the ovipositor in female zebrafish.

Remove the divider the next morning shortly after the onset of light. Allow mating to occur undisturbed for 20 min or until sufficient numbers of embryos are laid at the bottom of the tank.

After breeding, return the fish to their tanks. Collect the eggs using a strainer.

Wash the embryos thoroughly with system water.

Transfer the embryos to a Petri dish by rinsing the strainer with embryo medium; a.k.a. EM3 (NaCl, 13.7 mM; KCl, 0.54 mM; $MgSO_4$, 1.0 mM; $CaCl_2$, 1.3 mM; Na_2HPO_4, 0.025 mM; KH_2PO_4, 0.044 mM; $NaHCO_3$, 4.2 mM).

Embryos can be observed under a microscope. Fertilized eggs are then separated from the unfertilized eggs using a needle and a pipette.

5. Discussion

System maintenance

To maintain zebrafish in a healthy condition, it is important to provide them with a clean environment in a properly functioning aquarium system. An important part of this is changing system filters regularly so that all the tanks receive proper water flow and clean water. It is vital to avoid failure of the cycling water supply to each tank due to blocked system pipes. The pipes can be cleaned using a higher-than-normal water pressure and flow if blockage does occur. Ideally, around 10% of the system's water should be replaced daily to maintain good water quality. Alternatively, water can be replaced while changing the Canister or Carbon filter. This ensures that dirt deposited in the pipes connecting these filters is removed. The quality of water should be checked on a regular basis. Parameters such as alkalinity, pH, temperature, hardness, ammonia, dissolved oxygen, salinity, and conductivity should be considered as important factors in representing the quality of the system water. At the very least nitrate, pH, and temperature should be monitored on a regular basis to ensure good water quality for housing zebrafish. Ideal nitrate (NO_3^-) levels are <50 mg/L11; if high these levels can be reduced by replacing the water in the circulating system with the fresh system water. Occasionally, filters do not fit well and leak so it is recommended to check for any leaks after a filter change. If the water flow from the main reservoir is blocked either after changing the water pump or changing a filter, the water flow can be restored by either loosening or removing the filter for a few seconds to release any vacuum being generated in the pipes. The time required to change filters can vary depending on various factors such as total biological load on the system, cleanliness of other filters, and dirt deposited in the pipes. Hence, filters should immediately be changed if they appear dirty or if all the tanks are not receiving the correct water supply. It is also recommended that the fish net be cleaned with 70% ethanol, and rinsed in water to decontaminate it, and allowed to dry before being re-used. Drying ensures evaporation of ethanol which otherwise is toxic to fish.Most of the zebrafish systems use de-chlorinated tap water.

Feeding

Zebrafish should never be overfed as this may increase the nitrate level in the water, possibly affecting their breeding11, or viability, as some fish may die due to overeating. We recommend providing no more food during any one feeding than a tank of fish can finish within 30 min. It is very important to remove salt from the brine shrimps before feeding them to the zebrafish as excess salt concentration causes death. If more zebrafish eggs are required, fish can be fed three times a day. Cleaning the breeder fish tanks daily also improves levels of egg production.

When feeding on the Aquatic Habitats systems we usually turn off the water pump and air pump to allow the fish to eat the food for 30 min. This decreases the amount of food that is washed into the filters. However, users must be careful to remember to turn on these pumps again afterwards.

Breeding

Zebrafish are usually at optimal breeding condition between ~3 and 18 months of age. Pairwise breeding should not be performed for two consecutive days11; however, in-tank breeding can be performed daily as a tank can hold many fish which reduces the chance of the same pair of fish being bred for two days in a row. Breeding should be undertaken at regular intervals even if eggs are not required. This process will ensure the breeding cycle of the fish is maintained. It is recommended that there are more females than males in a breeding set-up. Male zebrafish change their female partners on a daily basis12 which further supports this recommendation. Furthermore, within our laboratory we initially experienced problems with breeding, however, using more females than males in a breeding set-up helped solve the problem. Moreover, feeding with a high protein content diet and brine shrimp two-three times a day, mixing fish from different tanks (from different parents), maintaining the temperature of the breeding set-up between 27 and 28 °C, and squeezing the bellies of females with blocked ovary tubes using gentle massage further improved egg production. We recommend keeping a record of fish lines/ origins to avoid in-breeding between siblings. This further improves embryo production. Keeping a record of the number of embryos laid by fish from each tank is also recommended. This assists with keeping a track of the best breeding fish tanks and taking measures to improve breeding in the fish not laying eggs.

6. Conclusion

The zebrafish Danio rerio has proven to be an invaluable model organism in scientific research due to its unique features such as rapid development, genetic similarities with humans, and ease of maintenance. Its transparent embryos allow for detailed observation of developmental processes, making it a key species for studies in developmental biology, toxicology, and genetics. Moreover, its ability to breed yearround under controlled conditions ensures a steady supply of specimens, further solidifying its role in various research fields. The zebrafish continues to be a critical tool for advancing our understanding of vertebrate biology and disease mechanisms.

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