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Care, breeding and management of laboratory zebrafish (*Danio rerio*)

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Abstract

During the past few decades, zebrafish has gained respectable popularity as a vertebrate animal model in modern biology research. Several biological features inherited in zebrafish have invited attention of scientific community to use them in genetics and developmental biology research to substitute laboratory mouse. The origin of the laboratory zebrafish is traced to Ganges in East India and Burma, a fresh water fish found in slow stream and rice paddy. The zebrafish can easily adopt laboratory conditions and breed very well even in fluctuating environmental conditions. In research laboratory, zebrafish can be reared in less expensive glass aquarium or using more advance recirculating water system. The recirculating water system has many inbuilt features which help to provide optimum physical environment necessary for rearing fish in the healthy conditions. In the present paper, attempts have been made to compile brief information about zebrafish breeding, embryo & larval culture, feeding, water quality control, physical environment and fish health monitoring necessary for rearing zebrafish in the research laboratory.

Key words : zebrafish, management, breeding

Introduction

The laboratory zebrafish (*Danio rerio*) as a research model of human biology is more and more evident each year. In the recent years, zebrafish has emerged as a model organism for studying genetic mechanisms of vertebrate development and diseases. Its rapid embryonic development, transparency of its embryos and large number of offspring together with several other advantages make it ideal for discovering and understanding the genes that regulate embryonic development as well as the physiology of the adult organism.

Zebrafish is freshwater fish that were originally found in slow streams, paddy fields and in the Ganges River in East India and Burma (Fig. 1). Zebrafish is available now a days at pet store throughout the world. Considering their potential use as a model organism in biomedical research, they were brought to the laboratory and started rearing in the aquaria by providing optimum conditions necessary for their survival and breeding. Proper care and management of zebrafish in the laboratory conditions is therefore necessary for the survival of the fish, keeping them free from diseases and maintaining their breeding potential.

Aquaria

Zebrafish can be easily maintained in 40-50 litres glass aquaria in the laboratory. Mouse and rat cages can also be used to rear zebrafish. The aquaria or cages can be placed on table top or stacked on shelves in the racks (Fig.2). The aquaria water gets turbid due to uneaten feed and fish faeces that may lead to elevation of ammonia level in the water. The aquaria should be cleaned manually by siphoning up debris from the bottom of the tank. If one third of aquaria water is replaced every day, a separate tank filtering system will not be necessary. Otherwise, water filtration requires every day with the replacement of 50 % water once a week. The aeration of the aquaria water is necessary for providing dissolved oxygen to the fish.

In case of recirculating system, water is recycled continuously in the aquaria after filtering through mechanical, chemical and biological filters. There will be provision for sterilization and heating of water in the system. The debris accumulated at the bottom of the aquaria is removed with the water current and separated on filter pads. The machine has inbuilt provision to carry out operations such as water filtration, sterilization, aeration and heating automatically. However, the viable functioning of the system is dependent on the regular maintenance of various accessories in the system. The machine takes care of many of the cleaning operations, which obviously reduces manpower requirement in day to day care of the fish (Fig.3).

Temperature

Aquaria water temperature ranges between 26-29^0 C, most suitable for survival and breeding of zebrafish. The fish do not breed and develop normally when the aquarium temperature goes below 25^0 C or above 31^0 C. In cold season, temperature of aquaria water should be maintained between 26-29^0 C by providing room heaters. During summer when outside temperature is high, water temperature should be maintained by providing room air conditioners. In recirculation system, temperature of aquaria water is maintained by inbuilt water heating and cooling system.

Photoperiod

Zebrafish are photoperiodic in breeding and lay eggs shortly after sunrise in natural conditions. Therefore, maintenance of constant light and dark period in the room especially for the breeding fish is essential. The artificial light should be provided by fluorescent lights with the control of day-night cycle (14 hours light/10 hours dark) by automatic timer.

Aquarium water

Different types of water are used for maintaining adult fish, developing embryos and young larvae. In general, adults can be maintained in tap water, by conditioning it properly, however, this depends on the quality of the local water source and the demands for embryo production. The poor quality water will affect the health of the fish, increases the susceptibility to diseases and decrease breeding efficiency. If the quality of local water is not good, deionised or distilled water mixed with small amount of salts and minerals can be used. Embryos and young larvae have special requirement and should be raised in egg water.

Feeding of zebrafish

They are fed with variety of diets. The types of feed available in the market are larval diet, brine shrimp cyst, baby food and adult diet. Enough food should be added to each tank to get all the fish some food. Adult fish are fed twice a day, however multiple light feeding allow the fish better opportunity to utilize the food. Live adult brine shrimp is best possible food for breeder fish and growing babies.

Zebrafish breeding

Sexual differentiation in zebrafish occurs at the age of 10-12 weeks; males are longer, slimmer and more yellow on the belly whereas females look like plumber and more silvery. Adult zebrafish aged between 7 to 18 months are good for breeding purpose, though they become sexually mature at the age of 10-12 weeks. In laboratory conditions, maintenance of constant light and dark cycle is essential for breeding females. The females usually lay eggs next morning soon after the start of light cycle, if the constant light and dark photo period is provided. The adult fish (4:8) are paired 1-2 hours before the end of light period and allow them to remain overnight in 30-40 litres tank. The bottom of tank is covered with layer of marbles to prevent fish from eating their eggs. Specialized tanks with false bottom can be fabricated for obtaining eggs from individual fish by pair wise breeding.

Embryo culture

The eggs are collected by siphoning them from the bottom of the tank and transferred into petri dish containing embryo medium. The eggs are cleaned by removing debris and washing them with embryo medium for 2-3 times. For optimal growth, eggs are kept in system water with 25-50 eggs in a 250 ml beaker during first few days. Embryos can also be raised in 90 mm petri dish containing embryo medium, keeping at 28-29^0 C temperature in laboratory incubator. Embryos should be monitored daily for the developmental stages and should remove abnormal or dead embryos from the petri dish. The embryos will hatch between 2-3 days and pec-fin stage larvae stage come out by rupturing chorionic membrane.

Larval culture

The larvae can be transferred to tank on 6th day post fertilization. Before transfer, it is necessary to ensure that swim bladder is developed in the larvae. The embryos and young larvae have special requirements and need to be raised in egg water. Deionised or distilled water is mixed with red sea salt at the concentration of 50-60 ug per ml. The larva needs artificial feeding with larval diet after 6th day because yolk is mostly depleted on 6th day post fertilization. By 11th or 12th day, the young ones are fed with freshly harvested live brine shrimp. If the larvae are maintained in recirculating system, larval tanks are supplied with system water at the rate of 25-30 drops per minute which should be increased as the age is advances.

Water quality control

The source of quality water is important for keeping fish in healthy state, maintaining their breeding potential and reducing diseases susceptibility. The water source should be free from toxic elements and microbial contamination. The fish water is polluted with uneaten feed and waste generated by the fish. This leads to rise in the levels of ammonia, nitrite and nitrate, affecting quality of water and subsequently the survival of fish. It is therefore necessary to monitor quality of water regularly using standard kits. The various parameters such as water temperature, pH, alkalinity, hardness, dissolved oxygen, chlorine, nitrite, nitrate, and ammonia levels should be monitored regularly (Table 1).

Health monitoring

Rearing zebrafish in optimal environmental conditions and practicing good quarantine procedures are essential to keep them free from most of the disease problems. Some type of environmental stress such as shipping, overcrowding or poor quality of water can lead to disease outbreaks in zebrafish. Evaluating water quality is useful in investigating aetiology of sick or dying fish. In all diagnostic procedures, parameters such as temperature, pH, ammonia, and nitrite should be measured. Additional parameters may include dissolved oxygen, gas saturation, chlorine, salinity, hardness and nitrate and metals can be measured.

For the diagnosis of fish diseases, it becomes often necessary to sacrifice fish with sampling of the individual fish. The scope for the clinical examination in live fish is limited and may be possible in anesthetized fish. Post mortem examination includes skin scrapping, fin and gill biopsies, bacteriology and histopathology. Histopathology is most useful diagnostic technique in the diagnosis of fish diseases. The small size of zebrafish allows them to be fixed and sectioned as whole mounts, thus permitting examination of all the primary organ system on a single microscope slide.

Anesthesia and Euthanasia

Mesab is widely used as an anesthetic agent for laboratory zebrafish. To achieve anesthesia zebrafish are kept in water containing 4% Mesab for some time. Zebrafish can be euthanized by overdose of Mesab or immersing in the ice water.

Summary

Laboratory zebrafish is an ideal animal model for studying complex biological mechanisms in vertebrate development and diseases. Because of potential biological characteristics, laboratory zebrafish would certainly find its place in Indian biomedical research laboratories leading to establishment of more and more zebrafish facilities in near future. Information about zebrafish husbandry practices and management in the laboratory conditions would prove highly useful in setting new zebrafish breeding facilities in this country.

Acknowledgement

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Reference

The Zebrafish Book, A guide for the laboratory use of zebrafish (*Danio rerio*), University of Oregon, USA.

Zebrafish International Resource Center, (ZIRC), University of Oregon, USA.

Table 1. Water quality parameters

Sr. No.	Parameters to be monitored	Recommended
1.	Stocking density	< 7 fish/litre
2.	Lighting	12-14 hours/day
3.	Temperature	26-28.5^{0}C (22-30^{0}C tolerated)
4.	pH	6.8 -7.5 (6.0-8.5 tolerated)
5.	Alkalinity (carbonate, KH)	~ 50-100 mg/L
6.	Hardness (general, GH)	~ 50-100 mg/L
7.	Un-ionized Ammonia (NH_3)	< 0.02 mg/L
8.	Nitrite (NO_2-)	< 0.1 mg/L
9.	Nitrate (NO_3-)	< 50 mg/L
10.	Dissolved Oxygen (DO)	6.0 mg/L- sat.
11.	Salinity/conductivity	300-1000 µS

Courtesy: The Zebrafish Book, A guide for the laboratory use of zebrafish

Fig. 1. Zebrafish (*Danio rerio*) with typical horizontal black and silver strips on body surface.

Fig. 2. The fish aquaria of tanks are stacked on the shelves in racks

Fig. 3. The recirculating water system for raising laboratory zebrafish