ISSN: 2583-9365

An Episode of Acute Mortality in Laboratory Zebrafish- A Case Report

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Abstract

In the recent years, zebrafish (Danio rerio) species has been popularized as an animal model of human biology in understanding the genetic mechanism of vertebrate development and disease. Aquarium water is an immediate surrounding medium for the fish species in laboratory setup that determine their survival and normal physiological activities. Raising healthy zebrafish in the laboratory environment is prerequisite to ensure disease free stock for better research outcome. Important water parameters such as temperature, pH, electric conductivity, salinity, dissolved oxygen and CO_2 are required to be monitored regularly as a part of quality control. An episode of acute mortality was witnessed in several lines of laboratory zebrafish maintained at the facility of TIFR, Mumbai. The clinical symptoms include lethargy, swimming inability, settling down at the bottom and acute death. The acute mortality was evidenced irrespective of age, sex and strains of the zebrafish. The investigation was carried out to find the etiological agents implicated in the acute mortality. After ruling out various causative agents associated with the mortality, an investigation was zeroed on the pH of the fish aquarium water, the culprit for the acute death episode. The highly acidic pH (3.5 to 4.0) of the fish aquarium water was brought to normal level by adding buffer solution to avoid further mortality. Present paper describes an episode of acute death, timely diagnosis and successful measures taken to rescue the Danio rerio loss due to highly acidic aquarium water.

Key words: *Danio rerio*, Aquaria, CWRS, water quality

Introduction

The laboratory zebrafish (Danio rerio) as a research model of human biology is more and more evident each year. The sturdiness to survive in sub-optimum conditions, high fecundity rate in females, transparent embryos, external fertilization & development, popularized laboratory zebrafish as a promising animal model in modern biology research. The species has become a major research model used in biomedical studies to investigate vertebrate development, genetics, physiology, and behaviour (1). With the ever increasing usage of laboratory zebrafish in several areas of biomedical research, many life science laboratories, pharmacy industries and educational institutions have established zebrafish facilities across the globe. With the larger scope in research, training and education, a comprehensive understanding of the husbandry of zebrafish is essential to ensure efficient propagation and maintenance of healthy and genetically diverse colonies.

Materials & Methods

Aquaria:

Department of Biological Sciences at TIFR Mumbai, established state-of-the-art zebrafish facility with continuous water recirculating system (CWRS, Fig.1). The CWRS holds more than 1500 aquaria of 1.5 L, 3 L and 10 L capacity with total stocking density of ~45-50K adult zebrafish. The system has equipped with an inbuilt provision of water heaters, chiller, oxygen aeration, degassing, UV sterilization, biological, mechanical and chemical filters. The sterilized water is supplied in the fish aquaria continuously after filtration through biological, mechanical and chemical filters. The CWR system also provides high water exchange (5-30%) over a period of 24 hours, keeping the levels of fish metabolic products within normal range. The zebrafish lines were raised and maintained as per the standard operating protocols being exercised at the Max Plank Institute, Tubingen, Germany (2).

Water quality:

Reverse Osmosis water was obtained using Millipore (REOS-100) water purification system and used in CWR system to raise several wild type (WT), transgenic (TGN) and knockout (KO) lines of the laboratory zebrafish. Red sea salts (25gm/L) and sodium bicarbonate (16gm/L) solutions were prepared and stored in the respective dosing tanks. The sensor based dosing pumps operate automatically to mix salts and buffer solutions in the RO water to adjust conductivity and pH, respectively. The water parameters were regularly monitored for temperature, pH, conductivity, dissolved oxygen using pH/conductivity meter (Table 1). The toxic elements such as chlorine, ammonia, nitrite and nitrate were measured manually using kits (Merck India).

Photoperiod:

Zebrafish being photoperiodic in breeding and spawn eggs soon after sunrise in natural conditions, the fish aquaria were maintained on constant light-dark cycle. The facility was illuminated using fluorescent white lights with light intensity between 100 to 150 lux, regulated by timer to provide constant light and dark cycle (Table 1).

Fish Nutrition:

The laboratory zebrafish of all the age group were fed with highly nutritious and balanced diet. Four types of diets (larval diet, brine shrimp cyst, micropellete and adult diet) were provided at four different timings (9.0, 12.0, 16.0 and 19.0h) to achieve proper growth of the fish.

Case History:

As per the SOP, all the fish aquaria were being examined daily for any abnormal, sick, moribund or dead fish in order to monitor health of the zebrafish. During routine examination, one day several fish tanks were witnessed with moribund and dead fish. The dead fish were settled down at the bottom of the tanks. The death toll was very high and fish in several tanks were affected with 5-10 death in each tank. The clinical manifestation includes lethargy, swimming inability and settling down at the bottom leading to the acute death. The episode of acute death was continued till the appropriate measures were taken.

Investigations:

The literature was searched to find out the various pathogenic organisms causing acute mortality in the laboratory zebrafish. Literature search found that one of the fish pathogens (Mycobacteriosis species) can cause acute mortality, however, the associated clinical symptoms were absent (3). The involvement of any pathogenic agent in the existing episode of acute mortality was ruled out and the focus of investigation was diverted to the non specific agents. The levels of nitrogenous compounds such as ammonia, nitrite and nitrate in the aquarium water were measured and found within the normal range. The aquaria water was found free of chlorine content. The recording of temperature, pH, conductivity, dissolved oxygen in aquarium water displayed on the monitor was within accepted levels. However, pH of the system water was in the sub normal level when measured manually using hand operated portable pH meter (Eutech, Singapore). There was significant reduction in the pH (3.5 to 4) level making the system water highly acidic. Further investigation revealed that the pH sensor was defective and was providing wrong message to the pH dosing pump.

Treatment:

The ailing and dead fish were removed immediately from the tanks to avoid further deterioration of the system water quality. The sick fish were euthanized using ice cold water and the carcasses were disposed of through incineration. The buffering of the fish tank water was carried out by adding solution of sodium bicarbonate (16gm/L) at the rate of 50 ml after every half an hour. The pH sensor was replaced immediately and pH level of the system water was monitored manually after every hour till it was restored to the normal level.

Conclusion

Despite the widespread use of zebrafish as an animal model for understanding the genetics of human development and disease for the past several years, relatively little information is available about common health issues, disease susceptibility, diagnosis, prophylactic and therapeutic measures in wild type as well as mutant lines used in research colonies. Laboratory zebrafish are susceptible to both infectious and non-infectious agents which causes subclinical or clinical illness. Zebrafish develop stress due to bad quality aquarium water, overcrowding and transport. Good husbandry practices, nutrition and water quality are therefore necessary for raising the healthy zebrafish in aquarium environment. Regular monitoring of aquaria water for important parameters such as temperature, pH, conductivity, hardness, dissolved oxygen, chlorine and nitrogenous compounds such as ammonia, nitrite and nitrate is prerequisite to raise the healthy laboratory fish. Regular calibration of the temperature, pH, conductivity and dissolved oxygen sensors is also necessary for accurate recording of the above parameters and keeping the CWR system in good working condition.

Acknowledgement

The author deeply acknowledge the support of Dr. Mahendra Sonawane, Associate Professor, his research group and helpers at the Fish Facility, DBS, TIFR, Mumbai.

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Fig-1: Continuous Water Recirculating System (CWRS), Zebrafish Facility, TIFR, Mumbai.

Table 1: Fish aquarium water parameters and normal levels.

Sr. No.	Water parameters	Normal range
1	Temperature (°C)	28.5 ± 1
2	pH	7.5 ± 1
3	Conductivity (uS)	350 ± 50
4	Dissolved oxygen (mg/L)	6.0 ± 1
5	Hardness (mg/L)	100-150
6	Chlorine (mg/L)	Nil
7	Nitrite (mg/L)	< 0.1
8	Nitrate (mg/L)	25 to 50
9	NH_3 (mg/L)	<0.03
10	Photoperiod (Light: Dark)	13h :11h