Analysis and Importance of Hard Water Compositions for Potential Housing of Zebrafish as Research Model

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Abstract:

Background and Aim: Nowadays the zebrafish has become an important research model for the study of many diseases and drug discovery due to convenience and less expense. Analysis of composition of hard water is of paramount importance because it plays a vital role in cellular and molecular level activities and may give negative impacts on the results of research studies. So it is necessary to check and maintain the concentration of basic water parameters for good research purpose and it maybe varying between the places. So we aim to identify and draw attention to factors likely to affect the welfare of maintaining laboratory zebrafish.

Methods: All the bioassays were conducted with zebrafish maintained water sample. The parameters were assayed as per the Indian Standard methods of sampling and test for waste water by (IS 3025 (part60)-2008 (Reaffirmed 2013), mean ± S.E. error bar in percentage compared with acceptable range.

Results: In the result, there were observed that significant fall in the concentrations of chloride, sulphate, iron calcium and significant rise in alkalinity, total hardness in the collected tap water when compared with the acceptable range.

Conclusions: Our results gave additional information, importance of concentration of basic hard water parameters to provide the best potential housing of zebrafish for research purpose.

Keywords: Zebrafish, Hard water, water quality parameters.

Introduction

Currently the zebrafish is a key experimental model worldwide and millions of there are used for studies in human diseases and drug discovery, because of similarities in genes that are found in zebrafish. Many researchers who are interested in an embryological and genetically tractable disease model have now turned to zebrafish model. This also acts as emerging model for proving several acquired human disease studies at the molecular and cellular levels [1,2,3]. One of the recent article showed that importance of assessing the concentration of well water parameters and it significantly associated with waterborne disease [4]. Hence, it is important to monitor the water quality parameters for proper maintenance, care, survival rate of zebrafish to use as potential research model. Some studies suggested lack of standard protocols and guidelines for housing and maintenance of zebrafish

which needs to be immediately addressed [5,6]. So, the maintenances of poor water quality can have direct effects on growth, metabolism, development of cells and molecular markers. Likely, recent studies proved that these factors can affected in brain development, organ functions and disease [7]. At least minimum basic parameter concentration should be maintained which is most important for the welfare of zebrafish. Untill now importance of water quality is largely unknown and no study was done on that [8,9]. So, therefore minimal numbers of controlled studies should be needed to evaluate the water quality, which is best for captive zebrafish. Taking into account of these previous study effects, the objective of the present study is to analyse the effects of concentration of water quality parameters and its importance to potential housing and survival rate of zebrafish for research purpose. So our aim and the objective of present study was a). To evaluate the concentration and importance of water quality parameters of tap water, b). To evaluate tolerance of zebrafish to survive in this water with above parameters.

Material and Methods

Adult Zebrafish (Danio rerio) were obtained from a local aquarium store at Palakkad Kerala, India. After getting approval from the institute committee, we done preliminary analysis in hard water parameters according standard operating procedures provided by the CPCSEA for experimentation on fishes, government of India, ministry of fisheries, animal husbandry and dairying, 2021[10]. All animals were treated humanely and with the aim of alleviating any suffering. Water samples were collected on bore well tap water (referred as hard water) at Government Medical College (GMC), Palakkad, Kerala, India. First, the fish were divided into three set (10 fish/tank) and housed the adult zebrafish in 10 L glass aquariums with bio filter sponge filtration systems, with a 12h:12 h light/dark

photoperiod at room temperature. Fish tanks were cleaned regularly and renewed with fresh water two to three times a week, due to maintain the water nutrition which required for maintaining healthy aquatic environment. Fish were fed commercial dry food (food size 300/400 microns for adult fish) five times a week based on requirements. The water samples were collected and maintained on ice while transported to Quality Control District lab, Kerala Water Authority (KWA), Kalmandapam, Palakkad, Kerala on the same day, where execution of bioassays were done. The parameters were assayed as per the Indian Standard methods[11] of sampling and test (physical and chemical) for water and waste water is Fluoride by (IS 3025 (part60)-2008 (Reaffirmed 2013), Iron by (IS 3025 (part53)-2003 (Reaffirmed 2014), Colour by (IS 3025 (part4)-1983 (Reaffirmed 2017), Turbidity by (IS 3025 (part10)-1984 (Reaffirmed 2017),PH by (IS 3025 (part11)-1983 (Reaffirmed 2017), Sulphate by (IS 3025 (part24)-1984 (Reaffirmed 2017),Total Dissolved solids by (IS 3025 (part 16)-1984 (Reaffirmed 2017), Alkalinity by IS 3025 (part 23)-1986 (Reaffirmed 2019), Total Hardness by IS 3025 (part 21)-2009 (Reaffirmed 2019), Calcium by IS 3025 (part 40)-1991 (Reaffirmed 2019), Magnesium by IS 3025 (part 46)-1994 (Reaffirmed 2019), Chloride by IS 3025 (part 32)-1988 (Reaffirmed 2019), Acidity by IS 3025 (part 22)-1986 (Reaffirmed 2019), Electrical conductivity at 25°C by IS 3025 (part 14)-2013 (Reaffirmed 2019) and nitrate is assayed by APHA method [11]. Water quality reports usually express hardness levels in terms of parts per million (ppm) or milligrams per litre (mg/l). Data are presented as means ± S.E. Statistical comparisons were made with a two-tailed t test for independent observations error bar in percentage.

Results and Discussion

In the results, there was a significant decrease in the concentrations of nitrate, chloride, sulphate, iron, calcium and

significant increases in alkalinity, total hardness in the tap water when compared with the acceptable range. Statistical comparisons were made by independent observations and error bar in percentage which is given in table and figures. Here we discuss some important basic parameters only, due to restrictions to number of pages. Currently increasing interest on zebrafish as an experimental model in biomedical research around the world and potential biological model to study the several human diseases [12]. But some studies documented that change in results of haematological parameters is probably connected with the poor maintenance of water quality, which may affect the metabolic activities, gonadal cycle and feeding intensity [13]. So, monitoring and maintaining the water quality is one of the most important factors to keep the potential housing of zebrafish for research purpose. At the very least very basic parameters that should be monitored to maintain the good water quality for housing of zebrafish research model like nitrate, pH and temperature (Table1).

The PH and Temperature level

Water PH and temperature play a major role physiological processes of fish and if any changes in these level will have a profound effect on physiological mechanisms [14]. Matthews et al documented that ideally zebrafish can survive temperatures of 18 - 32°C and PH limits 3.0 to 12.0 respectively and mention that doubles the metabolic rate for every rise of 10°C [13]. Likely some other studies showed that levels below this may reflect on metabolic activity, damages to skin and gills, leading to loss of balance and death [13,15]. Therefore, monitoring the PH and temperature level is important factors for growth, oxygen demand, survivals and intake of food etc. In our experiment we obtained that the pH is 6.6 (Table1) and average temperature is 33°C.

## Level of Metals, Hardness and Alkalinity in hard water

The ionic composition of water is important for all fish. In generally speaking the zebrafish is considered to be a hard water species. Commonly the water contains important ions source such as Cl, Ca^{2+} and Mg^{2+} which is required for fish growth and metabolism [16,17], but these are only present in very small quantities within normal freshwater. Hardness is essential for the successful survival of fish and mainly depending upon the concentration of metal ions such as calcium and magnesium ions. One study showed that the concentrations of the divalent cations Ca^{2+} and Mg^{2+} play a vital role in the ionic regulation of freshwater fish because these ions modulate the branchial permeability [18]. So any imbalance between Ca^{2+} and Mg^{2+} in water is toxic to this species and very difficult to survive. Several studies reported that low calcium levels are toxic, causes low survival rates and susceptibility to induced disease [19,20]. In our results we found that an imbalance of Ca^{2+} to Mg^{2+} that is concentration of calcium decreased when compared with magnesium in the hard water (Figure1). But severe effects were not detected in the present study, however further studies should be conducted to confirm this finding. Alkalinity and hardness values are normally similar to magnitude because calcium, magnesium, bicarbonate and carbonate ions present in water. So Alkalinity and hardness are not greatly affected by biological activity of aqua cultural operations. In the present study, we observed that similar result was obtained with lesser pH and higher alkalinity compared with the acceptable range (Table.1). Our results also proved a general rule; the most productive waters for fish culture have a hardness and alkalinity of approximately the same magnitude. Apart from that the chlorine is toxic to most of the fish at acute levels stimulate to necrosis of the gills leading to respiratory problems [17]. In freshwater zebrafish for active branchial activity uptake of ions especially

Na^+ and $Cl^-$to maintain the hypo-osmotic environment [17]. So monitoring the chlorine concentration is also important for maintaining a good healthy conditions for fish to survive in research purpose. In our results we observed that lesser amount was present when compared with acceptable range (table1).

Table 1: Concentration of basic water parameters and its different units for expressing hardness

S.No	Characteristics	Unit	Acceptable limit	Result
1	Colour	CU	5.000	5
2	Turbidity	NTU	1.00	0.58
3	PH	---	6.5to8.5	6.64
4	Acidity	mg/litre	---	1
4	Alkalinity	mg/litre	200.00	261.25
5	Sulphate (as SO_4)	mg/litre	200.00	27.09
6	Total Dissolved Solids (TDS)	mg/litre	500.00	409.00
7	Total Hardness (as $CaCO_3$)	mg/litre	200.00	276.00
8	Chloride (as Cl^-)	mg/litre	250.00	54.70
9	Nitrate (as NO_3)	mg/litre	45.00	0.37
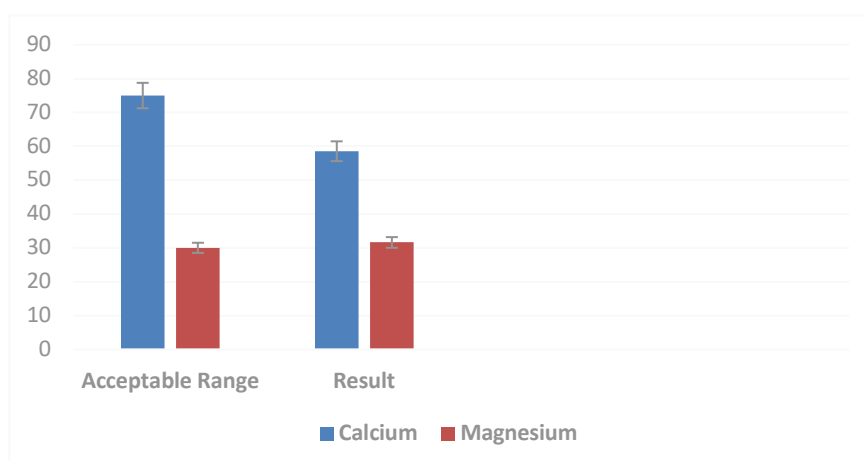
Figure 1: Concentration of Calcium (Ca) and Magnesium (Mg) (mg/L) in hard water acclimated zebrafish, mean ± S.E. error bar in percentage ($p < 0.05$) (n = 10/group), (*Acceptable limit as per IS 10500-2012 in table 1)

The Nitrite and Nitrate Levels

Water is one of the essential components required for both human and animal's life. So chemical contaminants of water can cause acute and chronic diseases in animals. So the evaluation of chemical characteristics of well water is important for routine use of research purpose [21].

One study showed that the chronic exposure of nitrate can damage gills, skin, kidneys, liver and intestines [22], these studies also indicated exchange of water frequently is more important for reducing nitrate, ammonia toxicity to the fish. Likely, one study reported that bio filters play a pivotal role in aquatic system to remove the toxic ammonia excreted by fish in the form of nitrate [23].

Similarly, we observed two adult fish died within three days due to lack of bio filter system, but after fixing the bio filter no similar result were observed, which indicates bio filter is more important for fish to survive in hard water as it maybe removes toxic materials (change water trice per week) (table 1). Which may be the

reason for our fish to survived up to three months.

The Level of Fluoride and iron

In between we have to consider the level of fluoride and iron, because of its co-existing with other ions such as Na^+, K^+, HCO_3^-, Ca^{2+} and Mg^{2+} [24]. Some studies reported that fluoride has both positive and negative

correlation with other ions[25,26,27]. In our result we observed the Fe level of the hard water samples was decreased significantly compared to the acceptable range may be due availability of magnesium ions (fig 1, 2). Hence, it is essential to monitor the concentration of chloride.
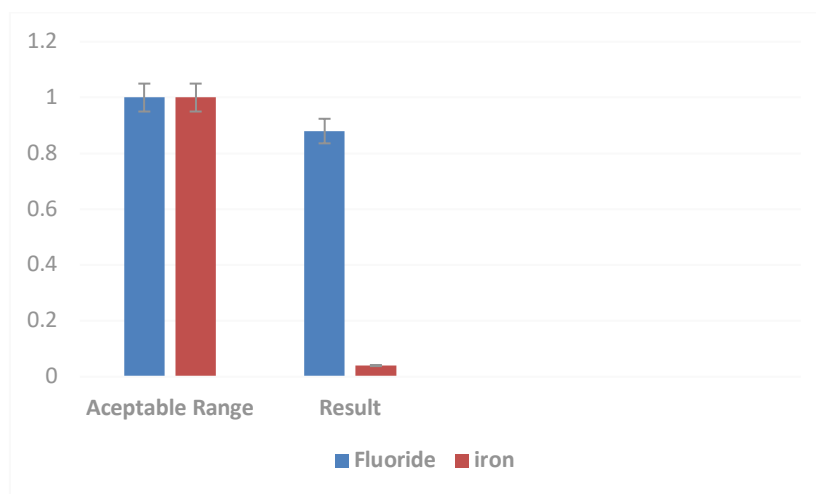
Figure 2: Concentration of Fluoride (F) and Iron (Fe) (mg/L) in hard water acclimated zebrafish, mean ± S.E. error bar in percentage (p< 0.05) (n = 10/group), (*Acceptable limit as per IS 10500-2012 in table 1)

Apart from the chemical test, care should be taken like in case of any holidays, emergency, weekend and during festival by providing at least minimal precautions include having backup equipment and an electrical generator for electrical power outages and mechanical equipment failures, feedings. Because, big sized fish can tolerate up too few days without food compare with small sized fish; similar effect were observed in our experiment four fish died at a time, due to lack of proper maintenance during regional festival holidays and feedings by others.

Conclusion

From the above obtained results we observed that many elements are essential for growth and development of aquatic organisms but above the normal levels which may had negative influence on health and also outcome of research results. So, it indicates that analysis and importance of

monitoring the concentration of metals ions present in the hard water and related effect on zebrafish to set good experimental model. Even though detailed study is needed to understand the underlying mechanism of water parameters induced adverse health effects in fishes. So, we recommend that water quality is the most important to maintaining a zebrafish as a good research model.

Acknowledgement

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