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Effect of water pH on the early developmental responses in zebrafish (*danio rerio*)

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Abstract

This experiment was conducted to evaluate the egg production performance in zebrafish (*Danio rerio*) in different pH treatments. Zebrafish were reared continuously 15 days in 3 different pH treatments like control (pH 6.8-7.4), acidic (pH 5.0-6.0) and basic (pH 9.0-10.0) media and then eggs were collected from them. Numbers of egg collection from the acidic and basic media were significantly varied from the control. Collected eggs from acidic, basic and control media were then incubated at different pH treatments ranging from pH 2.0-12.0. Significant differences were observed in hatching rate and time in control medium from two other different treatments.

Key words: Water, pH, zebrafish

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Introduction

In most fishes, fertilization and embryogenesis occur outside the body of female. Their development is readily affected by the environmental factors (Sawant *et al*., 2001) such as pH, temperature, salinity, etc. The changes in pH may severely impair egg production, fertilization, hatching, normal development and survival of embryos and larvae. The effect of temperature and salinity has been studied in broad areas but the effects of pH on the early developmental responses remain largely untested.

The introduction of chemicals into the environment by human activities can represent a serious risk to environmental and human health (Scholz *et al.*, 2008). Now a day's rapidly growing industrial activities have lead to continuous release of chemical substances which may contains acidic and basic pollutants that ultimately increase or decreases the water pH. pH is the main factor affecting water quality. Extreme pH negatively affects fish growth and reproduction (Zweig *et al*., 1999) and even causes massive mortalities in fish culture. pH may also affect general body physiology and physiology of reproduction. So it is necessary to clarify the biological response of fish to the pH especially in their early developmental studies.

Over the past twenty years, the zebrafish (*Danio rerio*) has emerged as a pre-eminent vertebrate model for studying genetics and development (Anandhan, 2009). A number of favorable attributes, including its small size, rapid development and generation time, optical transparency during early development, tractability in forward genetic screens, and genetic similarity to humans (Lamason *et al*., 2005) have fueled its rise in popularity for biomedical studies. Yet reports detailing the effects of water pH on its embryogenesis are lacking. The present study addresses the effects of different pH on egg production and embryonic development of the zebrafish.

Materials and methods

Wild-type matured zebrafish (*Danio rerio*) were collected from the Field Laboratory Complex of the

Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh and maintained at 27±l °C, at a density of one fish per litre of water in a 60 litre water tank in the backyard laboratory under this faculty. Zebrsfish were fed with zooplankton and commercial pelleted feed (Krishibid Fish Feed Ltd.) twice a day. Water exchange and tank-cleaning were made as required. The same mature fish were used repeatedly for experiments. To ensure fertility, each fish was initially permitted to spawn naturally.

A total of 10 pair of fertile zebrafish was used in a ratio of 1:1 in each aquarium. Matured male and female fish were reared in each aquarium for a period of 15 days to adjust with this environment. After that they were provided with plastic tray with two layers of marble and ornamental plastic trees to prevent the fish eating their eggs once laid (Matthews *et al.*, 2002). Thus 'breeding traps' were provided to prevent them from eating the eggs laid. Female zebrafish release eggs directly onto a bare substrate, but when provided with an artificial spawning site, such as a plastic box filled with gravel or marbles, they will preferentially use this (Spence *et al*., 2006). The fish usually laid eggs in the early morning of the following day. The naturally-spawned and fertilized eggs were transferred into petridish containing water. The eggs were counted from each aquarium and checked under a digital camera equipped microscope (Optika, Italy). Zebrafish embryos were then incubated at pH 2.0-12.0 from each treatment for the determination of hatching performances.

The desired pH was prepared by adding acetic acid CH_3COOH) and sodium hydroxide (NaOH) with the help of a portable pH meter (HANNA, HI98107 ROMANIA) and was adjusted twice in a day. All the experiments were repeated thrice, using different batches of embryos. The effect of pH exposure of embryos was assessed in terms of cleavage rate and pattern, gastrulation and hatching. The results represent the mean of three replicates per pH treatments. The standard error of mean (SEM) was also presented.

Results and discussion

The effect of different pH level on egg production of zebrafish was determined through the collection of eggs from the aquaria (Table 1). Egg production was found higher at control pH medium and lower in basic pH conditions. There was significant difference among different water pH for egg production. The highest mean number of eggs (55.5±11.39) was observed at controlled pH and the lowest number (6.6±6.5) was found at basic pH medium. The mean number of eggs at controlled pH was significantly ($P<0.01$) differ than other treatments.

Table 1. Effects of egg production at different pH treatments. Values are presented as mean±SE and are significantly different at $P<0.01$

Sl. No.	pH treatments	pH range	Mean number of eggs per day (±SE)
1.	Control treatments	pH 6.8-7.4	55.5±11.39
2.	Acidic treatments	pH 5.0-6.0	30.5±9.86*
3.	Basic treatments	pH 9.0-10.0	6.6±6.5*

The frequency of egg collection was also varied in different pH treatments (Fig. 1). Eggs collected from the control group varied significantly ($P<0.01$) from the acidic and basic treatments. Eggs were collected from the control and acidic medium on daily basis whereas interruptions in the collection of eggs were observed in the basic medium. Significant reduction ($P<0.01$) of the egg production was also observed in the alternate day in basic medium. In that day, the number of eggs reduced to zeros. This might be due to the toxic effects of basic medium. According to Lawrence (2007) zebrafish should facilitate within the pH range of 7.0-8.0 for better physiological functions though they are reported from pH 5.9-8.1 in the natural habitats (Engeszer *et al.*, 2007). Zervoudaki (2014) found similar production performance in copepod species (*Acartia clausi*) at low pH conditions. Faruk *et al.* (2012) also found similar results in case of Monosex Nile tilapia (*Oreochromis niloticus*) fry due to the effects of

temperature. Comparative scenarios of 10 days of egg production by zebrafish in three different treatments were presented in the Fig. 1.

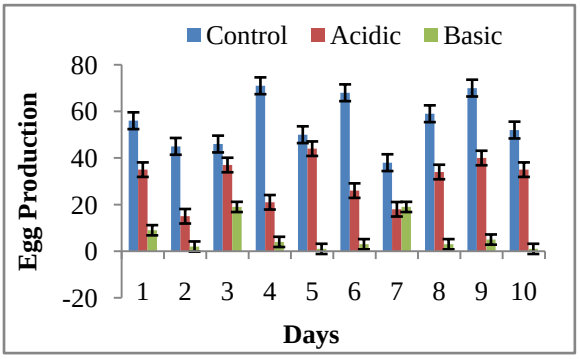

Figure 1. Frequency of egg deposition in the control (pH 6.8-7.4) and acidic (pH 5.0-6.0) and basic treatments (pH 9.0-10.0). Values are presented as mean±SE and are significantly different at P<0.01

When embryos were incubated till hatching at different pH level (2.0-12.0), the embryos cleaved and developed normally, as did the control, although the hatching was slightly lower (Table 2). In contrast, none of the embryos developed normally at pH 2.0 and 12.0. The embryos incubated at pH 5.0, 7.0, 8.0 and 10.0 cleaved synchronously. Apparently, the embryos incubated at pH higher than 10.0 and lower than 5.0 were not able to undergo gastrulation. When the control embryos reached 32 to 64-cell stage, they were at 16 to 32-cell stage. When embryos were exposed to pH 2.0 and 12.0 most of them turned to opaque and succumbed within 2hr.

Highest hatching rate (87±1.67) was observed of collected eggs at pH 7.0 from the controlled medium and lowest hatching rate (14±1.97) was at pH 10.0 of basic medium. Similarly highest hatching time (87±1.89 hr) required at pH 10.0 of basic medium and lowest required time (68±1.76) for hatching was pH 7.0 of controlled medium.

Table 2. Hatching of eggs at different pH treatments. Values are presented as mean±SD and are significantly different at P<0.01

Sl. No.	Egg collected medium	Exposed embryos	pH	Hatching rate (%)	Hatching time (hr)
1.	Control	40	2.0	0	7±1.67
		40	4.0	31±2.11	82±1.12
		40	5.0	68±1.22	75±1.36
		40	7.0	87±1.67	68±1.76
		40	8.0	83±1.32	71±1.58
		40	10.0	62±1.62	77±1.87
		40	12.0	0	9±2.23
2.	Acidic	40	2.0	0	5±2.56
		40	4.0	17±2.32	83±1.32
		40	5.0	42±1.87	79±1.84
		40	7.0	52±1.32	73±1.52
		40	8.0	48±1.52	76±1.65
		40	10.0	37±1.23	81±1.11
		40	12.0	0	8±1.70
3.	Basic	40	2.0	0	5±2.16
		40	4.0	0	11±2.34
		40	5.0	22±1.92	84±1.32
		40	7.0	32±1.37	78±1.45
		40	8.0	29±1.56	81±1.56
		40	10.0	14±1.97	87±1.89
		40	12.0	0	4±3.12

In this experiment hatching of eggs were observed at pH 4.0-10.0. There was a wide range of pH in which hatching took place, suggesting that zebrafish eggs were more tolerant to extreme pH. Hatching rate was significantly higher ($P < 0.01$) at pH 7.0 compared to those of pH 4.0, 5.0, 8.0 and 10.0 in each individual medium. The hatching rates generally declined with decreasing acid concentrations and increasing alkaline concentrations. Eggs became opaque and white within in a few minutes at pH 2.0 and 12.0. *Gao et al. (2011) conforms a significant relationship to the present study in case of pH effect on fertilization and hatching of Far Eastern Catfish (Silurus asotus) at pH ranging 2.0 to 13.0 under laboratory condition and reported the highest hatching rates was at pH 7.0 and any increase or decrease of pH from 7.0 reduced the hatching rates. Sawant (2001), Anandhan (2013) also found similar findings in zebrafish (Brachydanio rerio*) by salinity and aluminium effects, respectively. Zebrafish eggs can be hatched between 48 and 96 hr depending on the environmental temperature. In present study it was found that minimum hatching time (68 hr) was required at controlled pH. Highest hatching time was required at pH 4.0 of the basic medium. Results related to hatching time are in agreement with Gao *et al.* (2011).

pH contamination to the environment, which has yet not been recognized as a serious pollution problem, is capable of exerting considerable biological effects in nature. In the present study the data revealed that very low and very high pH effects on the early developmental responses to the test organisms. So, research on further investigations about their growth, survivability and reproductive performance of the hatched larvae should also be evaluated.

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