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Effect of different salinity exposures on the embryonic development of zebrafish (*danio rerio*)

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Abstract

The aim of this study was to evaluate the effects of salinity stress on the embryogenesis of Zebrafish (*Danio rerio*). Zebrafish embryos were developed until hatching up to 8 ppt but the survival rates until hatching were very low at 4, 6 and 8 ppt. Hatching rates were decreased with increased salinity concentration. With the increased of developmental stages, salinity tolerance was also increased. Blastula stage embryos were more tolerant than 2 to 4 cell stages. The present study revealed that salinity stress adversely affect on the embryonic development of Zebrafish.

INTRODUCTION

Fish are subject to stress every day. Potential stressors for fish include fluctuations of water quality parameters such as temperature, salinity, pH, dissolved oxygen, insufficient food supply, predation, and exposure to toxins. Stress disturbs the internal balance, homeostasis, and has further detrimental effects on behavior, growth, reproduction, immune function and disease tolerance (1-4). Salinity, an important stressor that affects primarily on gills of fish, as the major organ involved both in osmoregulation and waste nitrogen excretion and led to decrease the immune system level. Coastal districts of Bangladesh are the victims of salinity intrusion. There are 260 species of freshwater fishes are available in Bangladesh, are sensitive to the salinity intrusion in the coastal area. The changes of tidal patterns, as well as increasing salinity intrusion into the freshwater environment, will impact on fish populations. Salinity changes may severely impair fertilization, normal development and survival of embryos and larvae of freshwater fishes.

MATERIALS AND METHODS

A. Experimental area and species

The experiment was conducted in the Wet Laboratory of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. Wild type Zebrafish (*D. rerio*), were collected from the field laboratory complex of the Faculty of Fisheries and kept in aquariun and fed with zoolpankton and commercial feed.

B. Collection of eggs

The naturally-spawned fertilized eggs were collected every morning around at 7 AM from the aquarium and transferred into petri dishes containing egg water, 0.03% brine water mix. Then the eggs were checked under a microscope attached with camera (OPTICA B350, Italy).

C. Salinity adjustment

The saline water was obtained from reserved brine water. Different salinity levels were prepared by adding tap water to brine water and adjusted using a digital refractrometer (DRSO-28, Italy). Salinities were adjusted once in a day.

D. Experimental design

The present research work have been designed with two experiments –

Experiment -1: Fifty 2 to 4 cell embryos were transferred to petri dishes containing 50 ml test solution with 2, 4, 6, 8 and 10 ppt salinity and incubated.

Experiment -2: Fifty same stage embryos were transferred to petri dishes containing 50 ml test solution with 6, 8, 10, and 12 ppt, incubated for 1–2 h and then returned to egg water.

RESULTS

When 2 to 4 cell embryos were incubated at different salinities until hatching, the eggs were developed normally at 2, 4, 6 and 8 ppt up to gastrulation stage but their next developmental stages were lower compared to control embryos (Table 1). At 10 ppt about 85% embryos were dead before gastrulation period. None of the embryos were hatched at 10 ppt.

Table 1: Development of 2 to 4-cell embryos of zebrafish incubated until hatching at different salinities

Salinity (ppt)	Exposed Embryos (no.)	Developmental stages (%)					
		Cleavage	Blastulation	Gastrulation	Segmentation	Pharyngula	Hatching
0.0	100	100	99	99	99	99	99
2.0	100	100	81	73	65	65	63
4.0	100	100	90	77	52	33	29
6.0	100	100	90	75	54	24	21
8.0	100	94	94	65	43	28	20
10.0	100	81	48	15	5	0	0

Blastulae stage embryos were exposed to 6, 8, 10 and 12 ppt for 1-2 h. Blastulae stage embryos at 6 ppt for 1 h developed synchronously and their hatching rate was 71% which was lower than that of control (Table 2). But when the embryos were exposed for 2 h at 6ppt, they developed up to blastula stage asynchronously but the hatching rate was very low (40%). All the embryos were dead at exposure of 10 and 12 ppt for 2h after gastrulation stage. With increased time of different salinity exposure, egg development and hatching were lowered.

Table 2: Development of blastulae exposed to 6, 8, 10 and 12 ppt for 1-2 h

Salinity (‰)	Exposed Embryos (no.)	Treatment duration (h)	Developmental stage (%)					
			Cleavage	Blastulation	Gastrulation	Segmentation	Pharyngula	Hatching
0	100	-	100	100	100	100	100	100
6	100	1	100	99	94	75	72	71
6	100	2	100	98	49	44	42	40
8	100	1	100	98	71	65	61	58
8	100	2	100	95	34	30	12	0
10	100	1	100	99	55	48	40	36
10	100	2	100	97	25	0	0	0
12	100	1	100	98	0	0	0	0
12	100	2	100	96	0	0	0	0

DISCUSSION

Present work was conducted to determine the effect of salinity stress on the various egg developmental stages of Zebrafish. Major findings of the present research work were a) Zebra fish embryos can develop properly less than 4 ppt salinity, b) hatching rate decreased with increased of salinity, c) cleavage rate pattern was synchronous and lower but other developmental stages were severely impaired by increased salinity, d) blastula stage was more tolerant to salinity changes than 2-4 cell stages embryos. It is supposed to the loss of osmotic imbalance and suggests that high osmotic concentration impaired the nuclear division of the embryonic cells. It is most likely that the osmotic concentration at these salinities is beyond the osmoregulatory capacity of the developing embryos, as noted by Potts and Eddy (5). The present finding is more or less similar to that.

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