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EFFECTS OF STOCKING DENSITY ON GROWTH OF ZEBRAFISH (*DANIO RERIO*, HAMILTON, 1822)

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Abstract: The effect of stocking density on growth of zebra fish was examined. Total five different stocking densities (5, 15, 25, 35 and 45 individuals per 2 liters of water) were maintained in triplicate for a period of 60 days. One month old zebrafish were randomly stocked into 15 tanks and fish were fed with commercial diet. The mean weight gain, specific growth rate and length gain for treatment 1 (5 fish/2liter) and treatment 2 (15 fish/2 liter) were significantly ($p < 0.05$) higher than treatment 3 (25 fish/2 liter), treatment 4 (35 fish/2 liter) and treatment 5 (45 fish/2 liter). The gender weight gain, survival rate and condition factor did not show any significant ($p > 0.05$) difference among treatments. The result of this study suggests that the stocking density of zebrafish could be 15 fish per 2 litre of water in a laboratory system with aeration.

Key words: Effect, stocking, zebrafish, growth

INTRODUCTION

The zebrafish (*Danio rerio*, Hamilton, 1822) is a small freshwater fish species broadly distributed in India, Bangladesh and Nepal (Engeszer *et al.* 2007). They are most commonly found in freshwater ponds, marshes, canal, flood plain and paddy fields (Spence and Smith 2006). Now it has become a laboratory model in the area of developmental biology, medicine, genetics, aquaculture etc. for its favorable characteristics like short life span, transparent eggs, high tolerance in wide variable environment, etc.

In fish culture, rearing density is a very important factor that affects fish development, feeding behaviour (Wocher *et al.* 2011), feed utilization and water quality (Ellis *et al.* 2002). Fish production cost can be minimized by the maximum utilization of tank or rearing space. On the other hand, relatively high rearing density hampers fish health and welfare as well as their production due to stress, predation and competition for food, so the determination of optimal rearing density is a precondition for maximum growth for highest production that leads to maximum profit. Therefore, the impact of rearing density on growth and reproduction of some fish species has been recorded like Atlantic salmon *Salmo salar* (Hosfeld *et al.* 2009), rainbow trout *Oncorhynchus mykiss* (McKenzie *et al.* 2012), Nile tilapia *Oreochromis niloticus* (Azaza *et al.* 2013) and Japanese

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Medaka *Oryzias latipes* (Rosemore and Welsh 2012). An interesting work was carried out by Castranova *et al.* (2011) who worked on zebrafish and suggested that using densities as high as 12 fish/liter does not have a negative effect on reproductive performance. They also suggested that further study is needed to determine the maximum stocking density, effect of water quality parameter, feed type and genetic strain on growth and reproduction of zebrafish in laboratory condition, so the study was designed to investigate the effects of stocking density on growth and survival of zebrafish (*Danio rerio*).

MATERIAL AND METHODS

The zebrafish *Danio rerio*, Glo fish (genetically modified strain) type was used for this experiment. In August 2014, one month old zebrafish were collected from an aquarium shop of Katabon aquarium fish market at Dhaka city. The experiment was conducted in glass made 15 (3375 cm^3) tanks situated in a laboratory, Department of Fisheries, University of Dhaka. The experiment was designed with 5 stoking densities and 3 replications for each. Fish were stocked randomly in15 tanks at five treatments stocking densities: treatments 1, 2, 3, 4 and 5 corresponding to 5, 15, 25, 35, 45 fish/2 liters of water. Fish were fed to satiation point twice in a day at 7 a.m. and 6 p.m. with commercial dry pellet (TetraBits® Complete, Tetra GmbH, Germany). Satiation point is a point within a 5-min period, where fish were no longer actively searching for food. (Gonzales 2012). The nutrients composition of the commercial dry pellet was protein 47.5%, oil 6.5%, ash 10.5%, moisture 6%; additives: vitamin A 29770 IU/kg, vitamin D31860 IU/kg, vitamin E 200 mg/kg, L-ascorbyl-2-polyphosphate 137 mg/kg. Aeration was provided to each tank by using air pump. Water was exchanged by siphoning method in every two days. Water quality parameters such as temperature (°C), pH, dissolved oxygen (mg/l), total dissolved solids (ppm), ORP (oxidation reduction potential), conductivity (ms/cm), and resistivity (Ω cm) were measured using multiparameter machine (HI 9828 multiparameter, HANNA Instruments, Woonsocket RI-USA).

At the end of the study, 30 percent fish of each tank were randomly collected and euthanized using 2-phenoxy ethanol and weighed to determine growth parameters. To evaluate the growth of fishes following parameters were measured:

Weight gained = Mean final fish weight – mean initial fish weight

$$\text{Gender weight gain (g)} = \frac{Wf}{Wm}$$

Wf = Female weight gain, Wm = Male weight gain.

$$\text{Specific growth rate (g)} = \frac{\text{In } W_2 - \text{In } W_1}{T_2 - T_1}$$

where,

W_1 = Initial live body weight (g) at time T1 (day)

W_2 = Final live body weight (g) at time T2 (day)

T_2-T_1 = Number of days of the experiment

$$\text{Food conversion ratio, FCR} = \frac{\text{Feed (g) consumed by the fish}}{\text{Weight (g) gain of the fish } (W_2 - W_1)}$$

$$\text{Survival rate (\%)} = \frac{\text{Number of fish harvested}}{\text{Number of fish stocked}} \times 100$$

$$\text{Condition factor, K (\%)} = \frac{\text{Fish weight}}{(\text{Fish length})^3} \times 100$$

Length enhancement (cm) = Mean final length (cm) - mean initial length (cm).

The data obtained in the experiment were analyzed statistically by one-way ANOVA followed by Tukey test using statistical software (SPSS, version 16.0, SPSS Inc., Chicago, USA). Values were given with mean ± standard error (SE).

RESULTS AND DISCUSSION

The mean values of water quality parameters of different treatments have been presented in Table 1. The water quality parameter like pH, ORP and resistivity did not show any statistical significant difference among different stocking densities. Whereas other parameters like temperature, TDS, DO and conductivity were significantly affected by stocking densitities (ANOVA, $p > 0.05$; Table 1). Highest temperature recorded 27.65°C in treatment 3 in and lowest one was 26.81°C treatment 5.

There was significant difference for TDS (ppm) among the treatment densities. The highest value was 208 ppm found in treatment 5 and the lowest was 184.33 ppm in treatment 1. No significant difference for ORP was found among the treatment densities. Significant difference was recorded for DO (ppm) and the lowest value was 2.39 ppm in treatment 5. Significant difference was recorded for conductivity (ms/cm) among the treatment densities. In treatment 4, the value was 0.4 (ms/cm) which was highest and in treatment 5, the value was 0.33 (ms/cm) which was lowest. No significant difference for resistivity (MΩcm) was found among the treatment densities.

Matthews *et al.* (2002) recommended that the appropriate wider range of temperature for zebrafish as 24 - 30°C. But the maintenance temperature of zebrafish at laboratory condition is 28.5°C recommended by Westerfield (1995). Most aquatic animal involving fish fauna can tolerate TDS levels of 1000 mg/l (Boyd 1999), so TDS was in acceptable range for all tanks. DO is very important water quality parameter for fish culture because low levels of DO are responsible for higher fish mortalities in culture than any other water quality parameters (Timmons *et al.* 2002 and Boyd 1979). Popma and Masser (1999) reported that a number of tropical fish species, such as tilapia is tolerant of lower levels of dissolved oxygen, and it may likely that zebrafish fall into this group and they are likely to encounter poor-oxygen habit in nature. Timmons *et al.* (2002) stated that most teleost fish can tolerate a wider pH range from 6 to 9.5. It is normally practical to maintain most freshwater fish at a pH in the 7 – 8 range to promote better condition of filters and stable water quality.

Table 1. Water quality parameters (Mean± SEM).

Parameter	Treatment					ANOVA
	1	2	3	4	5	
Temperature (°C)	27.02± 0.05^{ab}	27.08± 0.195^{ab}	27.65± 0.08^{a}	27.09± 0.20^{ab}	26.81± 0.10^{b}	*
pH	8.53± 0.21	8.31± 0.06	8.37± 0.03	8.35± 0.05	8.27± 0.08	NS
TDS (ppm)	184.33± 1.85^{a}	187.67± 1.20^{a}	193.67± 0.8^{a}	204.33± 2.3^{b}	208± 3.05^{b}	*
ORP	56.16± 14.48	53.00± 1.04	54.76± 0.61	59.33± 0.48	62.00± 0.88	NS
DO (ppm)	3.42± 0.09^{a}	2.54± 0.04^{b}	2.40± 0.05^{b}	2.40± 0.14^{b}	2.39± 0.09^{b}	*
Conductivity (ms/cm)	0.37± 0.008^{ab}	0.37± 0.01^{ab}	0.38± 0.003^{ab}	0.4± 0.008^{a}	0.33± 0.017^{b}	*
Resistivity (MΩcm)	.0027± 0.00003	.0026± 0.00006	.0026± 0.00003	.0027± 0.00003	.0027± 0.00003	NS

*Significant ($p < 0.05$), NS- Not significant ($p > 0.05$). Mean values with different superscript letters in each row indicate significant difference.

The mean weight gain for treatments 1 and 2 was significantly ($p < 0.05$) higher than treatments 3, 4 and 5 (Fig. 1). But there was no significant difference between treatments 1 and 2. The mean weight gain was highest (0.188 ± 0.004 g) in treatment 1 (5 fish/2 liter water) and lowest (0.048 ± 0.001 g) in treatment 5 (45 fish/2 liter water). Again the mean weight gain was not significantly different among treatments 3, 4 and 5.

Sirakov and Ivancheva (2008) found higher weight gain in lower stocking density for trout culture. Kithsiri *et al.* (2010) reported 0.39 gm weight gain in

stocked 60 guppy fish per 160 liters of water. This was comparatively greater value when compared with zebrafish because the stocking density of guppy was too low.

The specific growth rate was highest in treatment 1 (5 fish/2 liter water) and it was significantly higher than other treatments (Fig. 2). There was no significant difference between treatments 4 and 5. Significant lowest specific growth rate was found in treatment 4 (35 fish/2 liter water) and treatment 5 (45 fish/2 liter water). This parameter (SGR) decreased with increasing density which was 1.71 ± 0.02 to 0.56 ± 0.02. Azaza *et al.* (2013) also reported that SGR is affected significantly by stocking density and they observed highest SGR for lowest stocking density for Nile tilapia (*Oreochromis niloticus*).

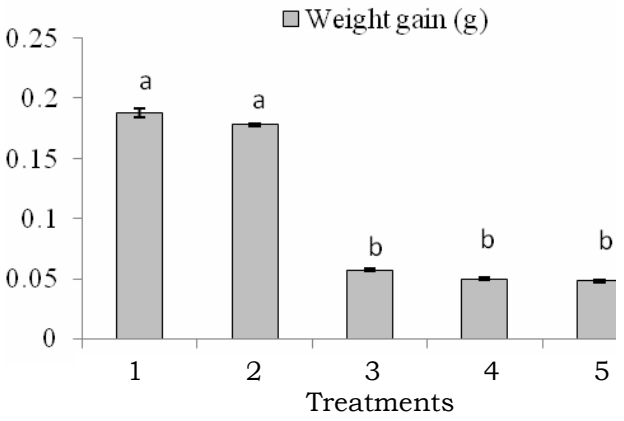

Fig. 1. Weight gain among the treatment densities. Bar with different letters indicate significantly ($p < 0.05$) different.

FCR increased with increasing stocking density and the range of FCR was 1.85 ± 0.02 to 2.56 ± 0.11 (Fig. 3). Among the treatments, FCR was significantly different ($p < 0.05$) when analyzed by ANOVA. There was no significant difference between treatment 4 and 5. The lowest value was found in treatment 1 (5 fish/2 liter water). The range of mean value of food conversion ratio was 1.85 ± 0.02 to 2.56 ± 0.11 (Table 5). Food conversion ratio and feed efficiency were negatively correlated with stocking density in *Chrysichthys nigrodigitatus* larvae (Pangni *et al.* 2008). They found 1.16, 1.17 and 1.43 for 5, 6 and 7 fish per litre of water. This result indicates that high stocking density reduced feed efficiency. Similar results had been reported in both *Cyprinus carpio* larvae (Jha and Barat 2005).

Survival rate did not show any significant difference among different stoking densities (Fig. 4). The mean survival rate was highest in treatment 1 (5 fish/2

litre water) and lowest in treatment 5 (45 fish/2 liter water). The range of mean value of survival rate was 78.67 ± 1.66 to 100 ± 0 (Table 6). Survival rate was almost better for all treatments. As zebrafish is very hardy fish by nature so they are very capable of surviving in a very crowded condition. As a result stocking density did not affect survival rate significantly. The survival rate was found 94 - 100% in zebrafish (Gonzales 2012). They stocked 10 matured zebra fish per liter of water. Szkudlarek and Zakes (2007) found survival rate which ranged from 79.2 to 72.3% in pikeperch, *Sander lucioperca* larvae under controlled conditions when stocked at 6, 10 and 15 individuals per liter.

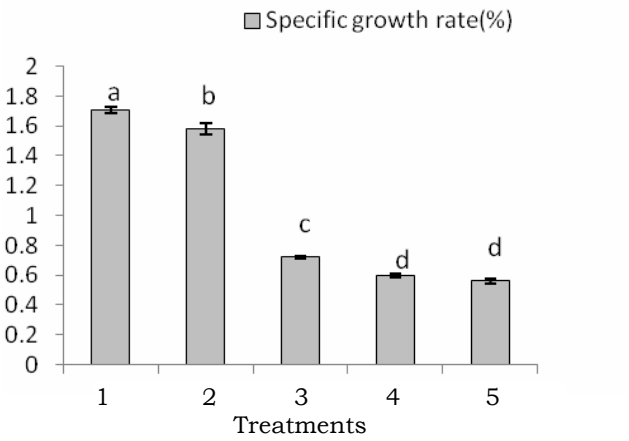

Fig. 2. Specific growth rate (Mean ± SEM) among the treatment densities. Bar with different letters indicate significantly ($p < 0.05$) different.

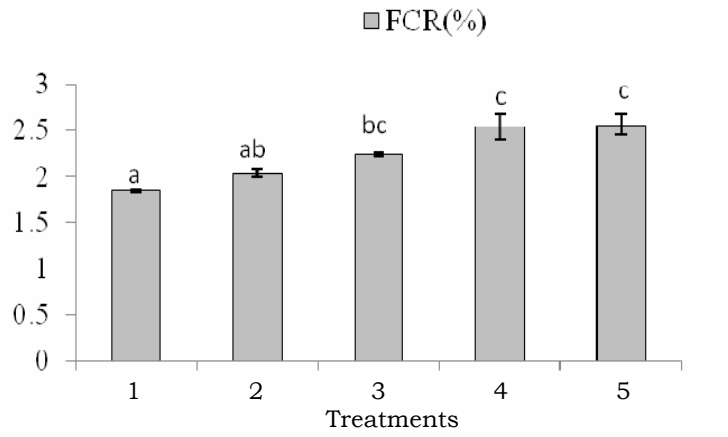

Fig. 3. Food conversion ratio (Mean ± SEM) among the treatment densities. Line with different letters indicate significantly ($p < 0.05$) different.

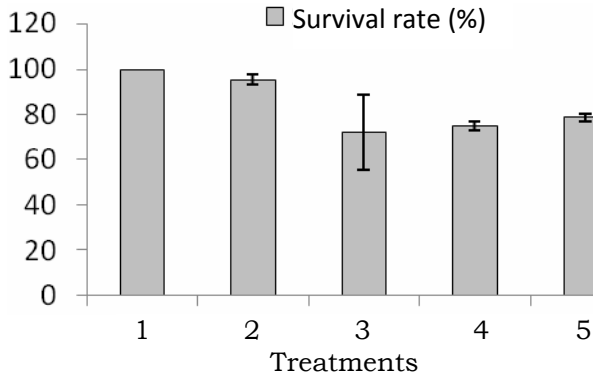

Fig. 4. Bar diagram showing the survival rate among the treatment densities.

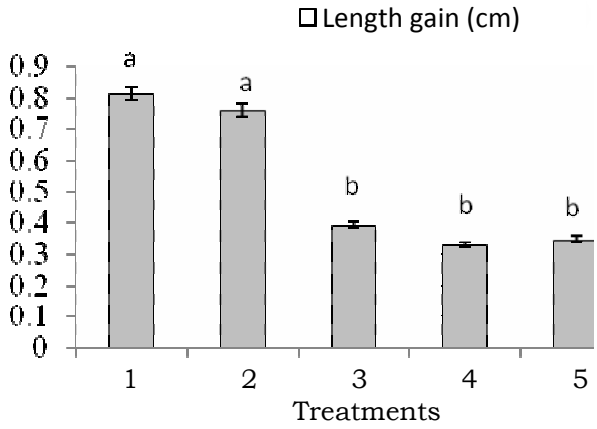

Fig. 5. Length gain (Mean ± SEM) among the treatment densities. Bar with different letters indicate significantly ($p < 0.05$) different.

The result of length gain is almost similar to weight gain. Significant higher length gain obtained in treatments 1 and 2 and significant lower obtained in treatments 3, 4 and 5 (Fig. 5). The range of mean value of length gain was 0.33 ± 0.008 to 0.81 ± 0.02. So length gain was maximum for lowest stocking density and minimum for higher stocking density. Similarly the length gain was higher in lower stocking density for trout culture (Sirakov and Ivancheva 2008).

Among the treatments, gender weight gain and condition factor did not show any significant difference. The mean gender weight gain was highest in treatment 5 (45 fish/2 liters water) and lowest in treatment 2 (15 fish/2 liters water) (Fig. 6). The range of mean value of gender weight gain was 1.03 ± 0.006 to 1.08 ± 0.03. The mean initial (0 day) condition factor was highest in treatment 3 (25 fish/2 liter water) and lowest in treatment 1 (5 fish/2 liter water). The

range of mean value of initial condition factor was 2.53 ± 0.38 to 2.92 ± 0.31 (Fig. 7). The mean mid (30 days) condition factor was highest in treatment 1 (5 fish/2 liters water) and lowest in treatment 4 (35 fish/2 liter water). The range of mean value of mid condition factor was 2.58 ± 0.168 to 2.26 ± 0.07 (Fig. 7). The mean final (60 days) condition factor was highest in treatment 4 (35 fish/2 liters water) and lowest in treatment 2 (15 fish/2 liters water).

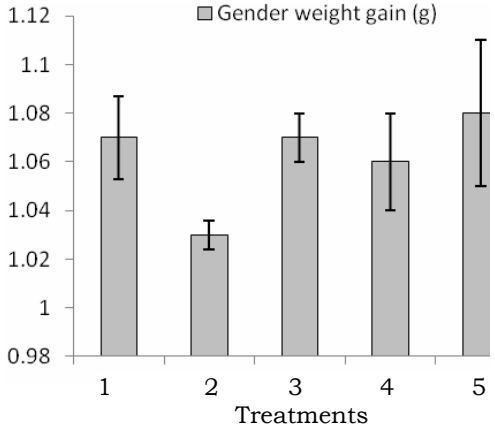

Fig. 6. Comparative gender weight gain (Mean ± SEM) among the treatment densities. The figure shows that there is no definite relation between the mean gender weight gain and stocking density.

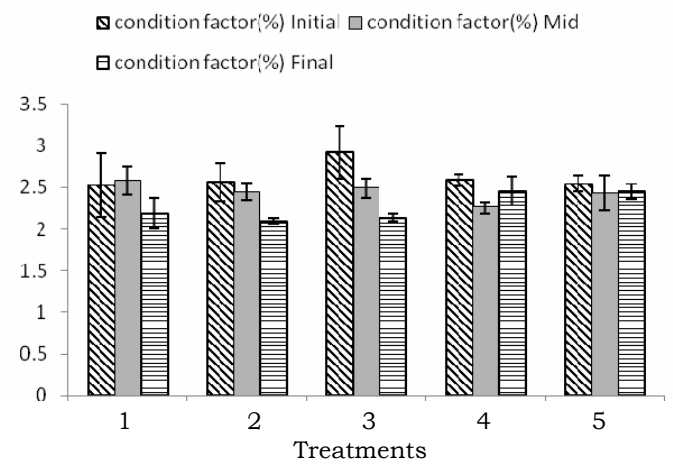

Fig. 7. Comparative condition factor (Mean ± SEM) among the treatment densities.

No significant difference of gender weight gain was also observed by Gonzales Jr (2012) for zebrafish juveniles when they cultured 20 fish per 2 liters with 8 feeding combinations for a period of 60 days. According to Heras *et al.*

(2015) the absence of statistical difference between different treatments indicates that the growth of fish in different treatments were performed equally in terms of mass and length and also fat accumulation is not produce any of the stocking densities.

Therefore, the results of the present study indicated that growth and survival rate of zebrafish decreased with the increasing stocking density. Stocking density up to 15 fish per 2 liter of water had no significant negative impact on growth and survival rate. Further work is needed to determine the effect of stocking density on reproductive performance and embryogenesis of zebrafish.

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