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Effect of Different Salinity Level on Breeding, Fertilization, Hatching and Survival of Nile Tilapia, *Oreochromis niloticus* (Linnaeus, 1758) in Captivity

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Authors' Contribution
GA conceived and designed the study and wrote the manuscript. AM did experimental work. AG analysed the data. SF and LD edited the manuscript. SSAA assisted in brooder collection.

Key words
Nile tilapia, *Oreochromis niloticus*, Breeding, Salinity, Captivity, Survival.

ABSTRACT

This study was conducted to assess optimal salinity level among 0‰, 5‰, 10‰, 15‰, 20‰ and 25‰ for successful breeding of Nile tilapia, *Oreochromis niloticus*. The duration of study was 56 days. Brooders (48) having mean weight (male 162±0.2 g and female 160±2.5 g) were selected and stocked into hapa nets in 12 fiberglass tanks (2000-liter). Ratio among male and female was 1:3. They were fed with commercial floating pelleted feed constituting 35% crude protein with 5% body weight twice a day. Eggs were collected on weekly basis by cultch removal method. Results showed that the highest fecundity, fertility, hatchability and survival of fry were obtained in salinity of 0‰ and 5‰ and significantly decreased on 20‰ and 25‰. The eggs per gram body weight were also recorded in all treatments and highest eggs were obtained *i.e.* 4.0-4.3 per female on 0‰-15‰. Water temperature (28.02±0.12°C), dissolved oxygen (6.4±0.02 mg/L), pH (7.47±0.04) and ammonia (less than 0.022±0.001 mg/L) were monitored throughout the study period. Water quality parameters remained within the recommended range. Our results suggest that Nile tilapia, *O. niloticus* may have maximum eggs up to 15‰ salinity with 92% survival of fry.

INTRODUCTION

Aqua farming is the fastest growing food creating sector, now accounts for 50 percent of the total world's food fish (FAO, 2014; Kevin *et al*., 2015; Malik *et al*., 2017). Demand for fin fish is expected to exceed all available supplies soon owing to the revolutionary changes taking place in the dietary habits of people all over the world and medical community has promoted fishery products as healthy food (Dawczynski *et al*., 2010; Siriwardhana *et al*., 2012; FAO, 2014). Fish and fishery products have recorded the highest increase in price, both in national and international market during recent years, compared to any other food item (Kevin *et al*., 2015; De Silva, 2016).

In order to control high prices, aquaculture development has become an urgent need to fulfil shortage of animal protein for human being. In Pakistan, marine or brackish water aquaculture does not exist still now. The climate of Pakistan is arid and semi-arid with scarce and irregular rainfall (Iqbal *et al*., 2012). Much of its land is affected with salinity and water-logging and the underlain water is brackish (Jarwar, 2006). Such areas can be used for fish culture which will act as a tool for desalinization of the soil through brackish water fish farming (Jarwar, 2014), for which fish seed will be required in plenty. Traditionally, tilapia is cultivated extensively in Sindh. These fishes breed fall under stress in brackish water as salinity affected ecological factors and natural food production (Mateen *et al*., 2004; Chaughtai *et al*., 2015; Malik *et al*., 2017). The breeding of tilapia on optimum salinity level may become useful for its seed production in bulk.

Tilapia culture has been growing fast during the past

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two decades and is becoming the world's second most important finfish group after the carps, presently called as 'aquatic chicken' because of fast growth, easy adaptation into a widespread ecological condition, cultivated within a wide range of densities and reproduce in captivity (SEAFISH, 2011; FAO, 2014; Al-Feky *et al*., 2015). Tilapia are native to Africa, but have been introduced in 140 countries of the world including Pakistan. Tilapia belonging to genera, *Oreochromis* are identified as economically valuable fish species for aqua farming and represent a major source of protein in many regions of the world like China, Thailand, Indonesia, Philippian, Vietnam, Africa, Europe, USA, Japan, UAE and Latin America (Chowdhury, 2011; Jaspe *et al*., 2011; Daudpota *et al*., 2014, 2016; Malik *et al*., 2017). Global production of tilapia has reached about 4 million tons in 2013 and is growing at the annual rate of 3%-5%. China is the largest producer followed by Egypt, Indonesia, Thailand, Philippines, Brazil, Vietnam and Bangladesh (Fitzsimmons *et al*., 2011; FAO, 2011). Tilapia are more resistant against diseases. They can breed easily in captivity and there is no need of induced spawning. They are well known for eating variety of foods and can grow best in wide range of ecological situations such as water-temperature, pH, salinity, dissolved oxygen (Daudpota *et al*., 2014, 2016). Several studies have been done on the culture of tilapia in saline areas (Cnaani and Hulata, 2011; Jaspe *et al*., 2011; Ahmadi *et al*., 2015). Due to shortage of fresh water and increased load to provide food for growing population, tilapia species are now being cultivated in brackish water ponds and in sea cages as well (Cnaani and Hulata, 2011).

The Nile tilapia can only tolerate brackish water with salinity up to 25‰, while the black tilapia (*Oreochromis mossambicus*) can tolerate salinity up to 40‰ and red tilapia can survive in pure seawater up to 32‰ (Jaspe *et al*., 2011). Due to this, tilapia species are the best option because they are omnivorous and can be easily adapted on artificial feed, survive at low oxygen levels, tolerate a wide range of salinity and can be cultured on low volume with high densities. Tilapia species are productive breeders, they complete their life cycle in confined environment, and have high tolerability against atmospheric stress than carps (Iqbal *et al*., 2012; Ronald *et al*., 2014). For sustainable aquaculture of tilapia, availability of good quality seed in mass quantities is the basic requirement. It can be possible through introducing its artificial breeding methods to meet the demands of the fish culturists (Kevin *et al*., 2015; Iqbal *et al*., 2016). Therefore, the present study was planned to determine optimal salinity level for breeding of Nile tilapia, *Oreochromis niloticus* in captivity.

MATERIALS AND METHODS

Experimental setup

Experiment was conducted at Seed Production Unit, Hawks Bay, Karachi, Pakistan, for a period of 56 days. Twelve fiberglass tanks (2000-liter water holding capacity) and 12 nylon made hapa were used for this trial. Salinity was maintained by adding some freshwater in the tanks to get desired concentration of salts. Water depth was kept at 3 feet in all experimental tanks.

Brood-stock selection and stocking

Brood-stock of experimental fish *i.e.* 36 females and 12 males ranging from 18.6 ± 0.15 cm in length and 160.2±0.2 g in weight, respectively, were selected on the basis of morphological characters. Subsequently, they were released into the breeding nylon made hapa in breeding tanks for spawning in different treatments like T_1 (0‰), T_2 (5‰), T_3 (10‰), T_4 (15‰), T_5 (20‰) and T_6 (25‰), where T represents treatment.

Experimental diet

Brood-stock were fed artificial floating pelleted feed (35% crude protein, 5.8% crude fat, 6.7% crude fiber, 9.8% moisture and 8.4% ash) at 2% body weight with feeding frequency of 2 times in a day (at 9:00 and 16:00).

Egg collection and incubation

After 12 days of stocking, all brooders were gathered at the corner of the hapa by means of bamboo and mouth of female's tilapia were checked one by one to get fertilized eggs. These eggs were collected from the mouth of incubating females weekly. After that, these eggs were cleaned and then stocked in incubatory jars separately for further development and hatching process (Ahmed *et al*., 2007; Valeta *et al*., 2013). The quantity, length and weight of these eggs were noted. Each incubator was stocked with different egg densities like 2180, 2120, 2100, 2090, 1008 and 560 eggs per incubator. Hatched yolk-sac fry was transferred into rectangular plastic nursing tubs for further development till egg yolk absorption.

Water quality parameters

Temperature of the tanks water was monitored daily with mercury thermometer. Dissolved oxygen (DO) was noted by using a portable test kit (Merck KGaA, 64271, Germany). The pH was determined by using pH meter (EzDO 6011, Taiwan) and ammonia was estimated by portable test kits (Merck KGaA, 64271, Germany) on weekly basis.

Statistical analysis

Data on fecundity, fertilization of eggs, fry hatchability and its survival were evaluated by analysis of variance (ANOVA) using Minitab 17.0 version statistical software. These factors were calculated by using the following formulae (Brian, 2015; Malik *et al*., 2017).

$$\text{Fertilization (\%)} = \frac{\text{No of fertile eggs}}{\text{Total No of collected eggs}} \times 100$$

$$\text{Hatchability (\%)} = \frac{\text{No of eggs hatched (fry)}}{\text{No of eggs incubated (fertile)}}$$

$$\text{Unfertile eggs (\%)} = \frac{\text{No of whitish broken eggs}}{\text{No of eggs fertile}} \times 100$$

$$\text{Survival(\%)} = \frac{\text{Final No of fry (after yolk disappears)}}{\text{Initial No of fry (with yolk after hatching)}} \times 100$$

$$\text{Egg body weight}^{-1} = \frac{\text{Total No of collected eggs}}{\text{Weight of female (g)}}$$

RESULTS

Among six treatments, highest fecundity (number of eggs) was found in T_1, T_2, T_3, and T_4 (2180±4.2, 2120±4.2, 2100±4.5 and 2090±3.8, respectively (Table I) as compared to those of T_5 and T_6 (1008±21.0 and 560±21.0, respectively). Significant egg fertility was shown in T_1 (2050±3.2), T_2 (980±3.2), T_3 (1955±5.8) and T_4 (1944±11.0). Same results were found for hatchlings in this study. Higher survival of fry was achieved on lower salinity groups (Table I).

Regression analysis showed that the relationship between salinity and breeding component (fecundity, egg fertility, hatchability and survival of fry) was significantly higher up to 15 ‰ salinity level, after which no significant growth was observed (Fig. 1). The quantity of fertile eggs incubated in her mouth was not significantly different up to 15% salinity. Above this level of salinity, fertile eggs were found to be inversely proportional (Table II). Number of eggs in one gram of fish body weight were recorded in all treatments; higher eggs were found 4.3 to 4.0 in T_1 to T_4 (Table II).

Table I.- Morphometric and breeding performance of Nile tilapia (*Oreochromis niloticus*) on different Salinity during 56 days.

	Salinity Level					
	T_1-0‰	T_2-5‰	T_3-10‰	T_4-15‰	T_5-20‰	T_6-25‰
Morphometric parameters						
Weight (g)/ Female	160.5 ± 2.16a	160.5 ± 2.12a	160.0 ± 2.65b	160.2 ± 2.20 a	160.0 ± 2.60 b	160.0 ± 2.60 b
Total length (cm)/ Female	18.8 ± 0.75 a	18.6 ± 0.74 a	18.5 ± 0.60 a	18.7 ± 0.43 a	18.5 ± 0.29 b	18.6 ± 0.28 a
Body depth (cm)	8.2 ± 0.15 b	8.5 ± 0.13 a	8.0 ± 0.14 b	8.1 ± 0.16 b	8.0 ± 0.14 b	8.5 ± 0.14 a
Breeding parameters						
Total number of eggs	2180 ± 4.2 a	2120 ± 4.2 a	2100 ± 4.5 a	2090 ± 3.8 a	1008 ± 21.0 b	560 ± 21.0 c
Total number of fertile eggs	2050 ± 3.2 a	1980 ± 3.2 a	1955 ± 5.8 a	1944 ± 11.0 a	761 ± 4.7 b	320 ± 4.7 c
Total number of unfertile eggs	130 ± 4.4 b	140 ± 4.4 b	145 ± 9.6 b	146 ± 4.1 b	247 ± 7.3 a	240 ± 7.3 a
Total number of hatchlings	1920 ± 13.1 a	1850 ± 13.1 a	1780 ± 8.5 a	1756 ± 5.0 a	512 ± 5.0 b	152 ± 5.0 c
Total number of fry	1780 ± 5.1 a	1710 ± 5.1 a	1620 ± 10.6 a	1582 ± 7.7 a	340 ± 3.2 b	70 ± 3.2 c
Fertilization %	94.0 ± 0.7 a	93.4 ± 0.3 a	93.1 ± 0.2 a	93.0 ± 0.4 b	75.5 ± 0.3 b	57.1 ± 0.4 b
Hatchability %	93.6 ± 0.7 a	93.4 ± 0.2 a	91.04 ± 0.1 a	90.3 ± 0.2 a	67.3 ± 0.5 b	47.5 ± 0.3 b
Survival %	92.7 ± 0.0 a	92.4 ± 0.1 a	91.0 ± 0.4 a	90.1 ± 0.4 a	66.4 ± 0.4 b	46.1± 0.1 b

Different letters in the same row represent significant difference ($P< 0.05$) values are mean ± standard error.

Table II.- Average data record of collected fertilized eggs of Nile tilapia (*Oreochromis niloticus*) with respect to the female parent tilapia size (cm) weight (g) on different salinity levels.

Salinity (‰)	Total length (cm)	Total weight (g)	Fertilized eggs/Female	Fertilized eggs/(g)*
0	18.8 ± 0.75	160.5 ± 2.16	683 ± 6.0	4.3 ± 0.14
5	18.6 ± 0.74	160.5 ± 2.12	660 ± 4.1	4.1 ± 0.13
10	18.5 ± 0.60	160.0 ± 2.65	652 ± 4.0	4.1 ± 0.13
15	18.7 ± 0.43	160.2 ± 2.20	648 ± 2.4	4.0 ± 0.12
20	18.4 ± 0.29	160.0 ± 2.60	254 ± 6.1	1.6 ± 0.12
25	18.6 ± 0.28	160.0 ± 2.60	107 ± 7.0	0.7 ± 0.1

For statistical data, see Table I. *Number of eggs per gram= total number of eggs/weight of female (g).

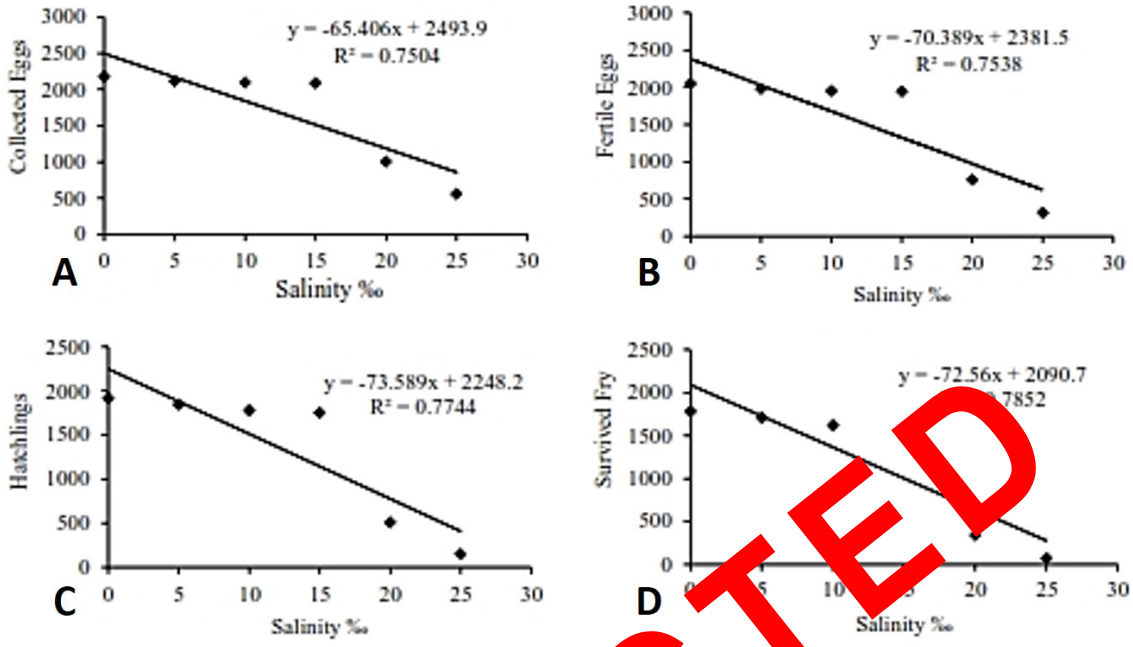

Fig. 1. Regression of salinity on fecundity (A), fertility (B), hatchability (C) and fry (D) of Nile tilapia (*Oreochromis niloticus*) among all treatments.

Embryonic development of Nile tilapia was divided into 6 phases: zygote to cleavage (Fig. 2A), blastula (Fig. 2B), gastrula (Fig. 2C), pharyngula (Fig. 2D), hatching (Fig. 2E, F), larval (Fig. 2G, H) and juvenile (Fig. 2I, J). Zygote to cleavage phase began from 0–1 h to 4 h after post fertilization and one day after post fertilization, it characterized with cytoplasmic growth, formation of blastodisc at the end of animal pole, presence of different perivitelline gape and cleavage phase categorized with a sequence of mitotic partitions that resulted in several blastomeres. Blastula phase began from 4-20 h after post fertilization and after post fertilization, categorized with 2 different coatings of blastoderm, external outer layer and a periphery multinucleate mass of cytoplasm resulting from fusion of cells. The gastrula phase began from 20-40 h after post fertilization and 2 days after post fertilization, germ ring surrounding the margin of the blastoderm; it is the main characteristics of this phase and embryonic protection layer stretched from germ ring towards the animal pole and form the neural tube. Phase pharyngula began from 40-88 h after post fertilization and 2-4 days after post fertilization, considered by primordia of pharyngeal arches, which were present but very difficult to differentiate individually at earlier periods. Hatching phase began from 88-116 h post fertilization and 4-5 days after post fertilization, categorized by the formation and differentiation of tissues and organs of pharyngeal skeleton. The phase larval development starts after hatching phase till the end of yolk absorption started from 116-274 h post fertilization and 5-12 days after post fertilization. This phase gradually started with the movement of jaws, operculum flapper, and pectoral fins. Distinguish by inflation of the hydrostatic organ (swim bladder) became functionalized and pharyngeal skeleton prior to starting exogenous feeding. The phase juvenile development began after larva stage and developed all body parts completely and looked like parents until the first maturation of gametes. This stage starts from 306-672 h after post fertilization and 13-28 days after post fertilization (Table III).

Table III.- Developmental stages of Nile tilapia (*Oreochromis niloticus*) during the study period.

Developmental stages		Hours post-fertilization	Days post-fertilization
Embryo	Cleavage (2–32 cells)	2-4	1
	Blastula	4–20	1
	Gastrula	20-40	2
	Pharyngula	40-88	2-4
	Hatching	88–116	4-5
Larva	Early larva	116–140	5-6
	Late larva	208–274	9-12
Juvenile	Early juvenile	306-352	13-15
	Late juvenile	552- 672	23-28

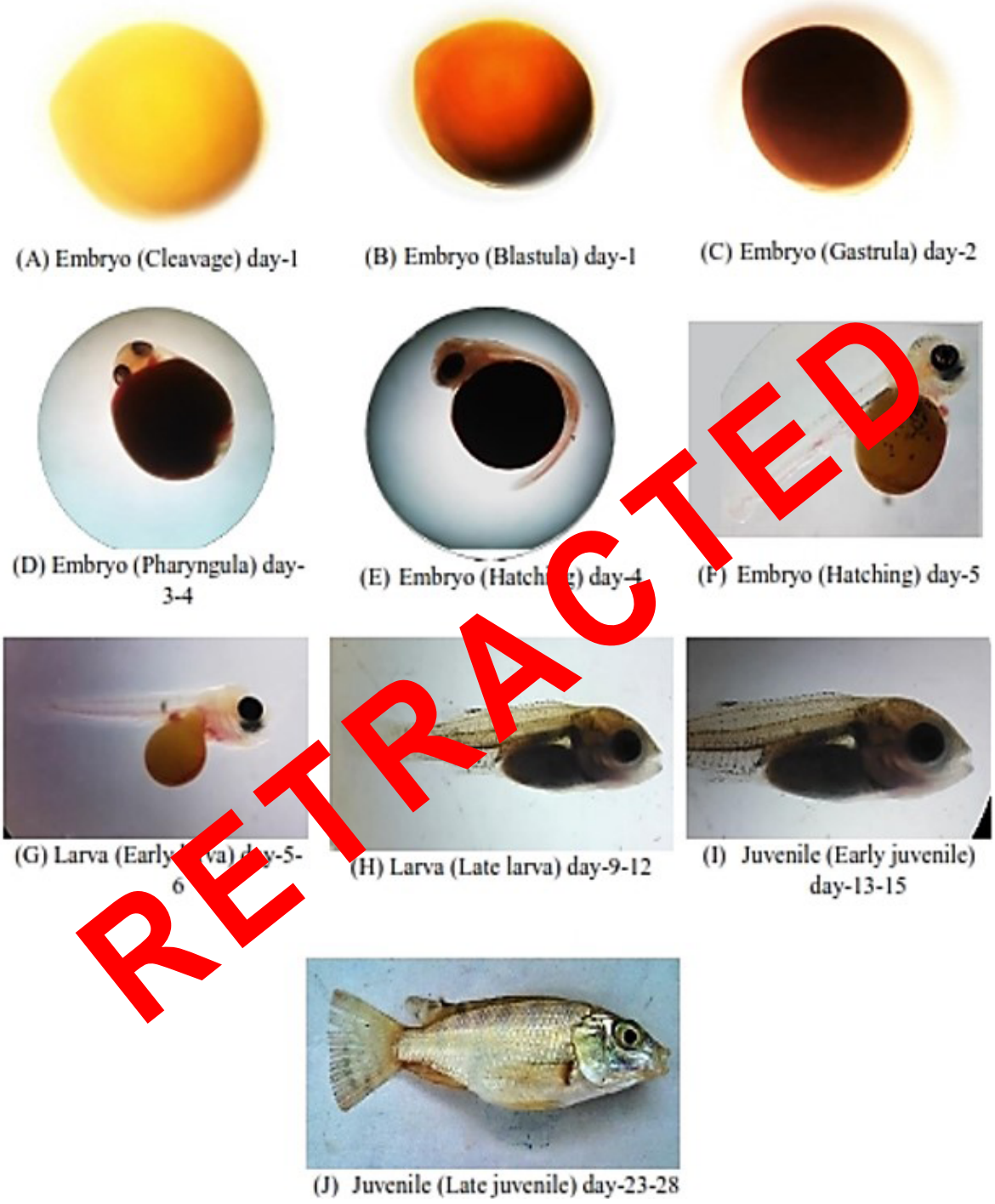

Fig. 2. Egg to juvenile development stages of Nile tilapia: fertilized egg yellow in color at the first day (A, B) than yellow brown and yolky; at second day small dark spots are appeared on the egg surface (C); from third day and fourth day eyes and hair-like tail appeared (D & E); at the fifth day head appeared (F); from fifth day to sixth day head and tail further more developed, yolk sac decreased and the embryo started slight movement (G); on ninth day to twelfth day post-yolk sac appeared at this stage yolk sac was completely absorbed and fry started to feed on artificial diet (H). On thirteenth day to fifteenth day fins and other body parts are developed completely and reached about 0.5 g in weight (I). Finally reached 1-2 g in weight and look like parent after twenty third day to twenty-eighth day (J).

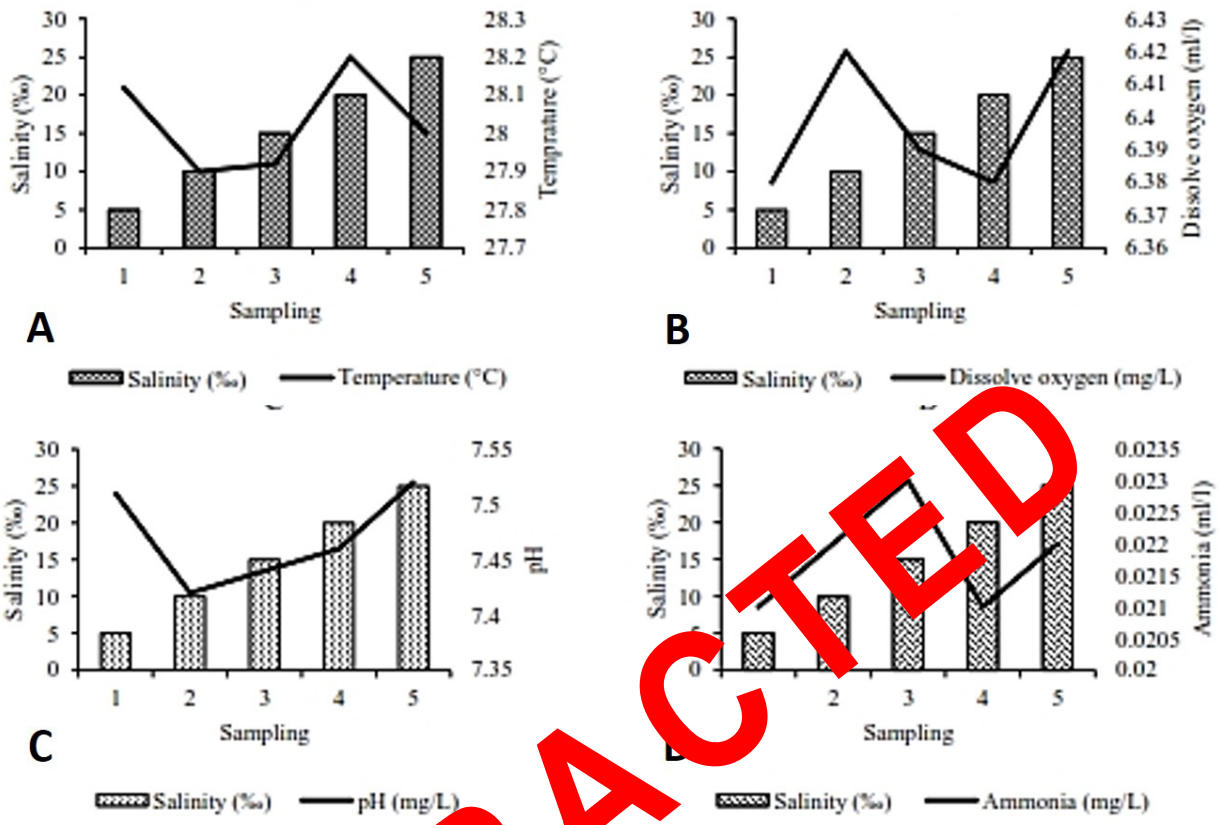

Fig. 3. Water quality parameters recorded during the experimental period. A, temperature; B, dissolve oxygen; C, pH; D, ammonia on different salinity levels.

Water quality parameters are given in Table I. Water temperature did not vary more than one degree among replicates throughout the experimental period; mean values were (27.8 °C to 28.2 °C, mean 28.03±0.2°C). Salinity of tanks water ranged from 0‰ to 25‰. No statistically significant difference (P>0.05) was found in dissolved oxygen concentration (6.38 ml/l to 6.42 ml/l, mean 6.4±0.02 ml/l). There was no significant effect of introduced feed on pH of saline water in each tank. The pH values were observed as 7.42 to 7.52 with mean of 7.47±0.04 and ammonia remained as 0.022±0.001 ml/l throughout the experiment (Fig. 3).

DISCUSSION

It is well known fact that sustainable aquaculture requires healthy seed in mass quantity of commercially important specie which can be possible through artificial egg incubation and nursing at small to large scale hatcheries. This can also be monitored through several aspects such as brood-stock management, breeding methods, nursing techniques, farming and marketing. This research work provides information about ability to produce maximum eggs and survival of Nile tilapia (*O. niloticus*) fry on different salinity levels in captivity. Maximum percentage of fertilized eggs were obtained among treatments; 1, 2, 3 and 4 (93%-94%). These results are more or less similar with the findings of Brian (2015). He got 82%-85% fertilized eggs from *O. niloticus* stocked in seawater tanks. Rodriguez-Montes de Oca *et al*. (2015) found 66.7%, 71.8% and 65% fertilized eggs from Nile tilapia on different salinity levels *i.e.* 0‰, 5‰ and 15‰. Rehman *et al*. (2015) reported 67%–81% fertilization rate with HCG+HMG and HCG+Ovaprim artificial stimulating hormones on snakehead fish (*Channa marulius*). Furthermore, Akinwande *et al*. (2012) achieved 80% fertilization rate of *Clarias* species (intraspecific hybrids). These results are in agreement with the findingds of the present study. Evidence to support this is available in another study of Martins *et al*. (2015) who investigated the effect of salinity on artificial reproduction of silver catfish (*Rhamdia quelen*) and reported 85%–93% fertilization rate. Similar results have been reported by Abdel-Hakim *et al*. (2008).

Significant research on different strains of Nile tilapia showed 89%–92% hatching (Almeida *et al*., 2013) which are in contrast with the results of 1-4 treatments of our study. Akinwande *et al*. (2012) reported hatchability rate 79.1%-83.3% in *Clarias* spp. Martins *et al*. (2015) while studying on some other fish species reported significant results (83.3%) at 0‰ salinity for silver catfish, *Rhamdia quelen*. Young-Sulem *et al*. (2008) obtained maximum hatchability (65.3%) at various turbidity levels for *Clarias gariepinus*.

In the present study, highest survival rate of Nile tilapia, *O. niloticus* fry was found as 90.1% to 92.7% in treatment groups of 1-4. These obsevations are in coincidence with the results of Abdel-Hakim *et al*. (2008). Mubarik *et al*. (2015) have documented survival rate (39.4%-91.0 %) of common carp fry on different rock salt concentration (0-30 mg/L). On the other hand, Brian (2015) reported survival rate of fry as 71.4% in Nile tilapia with subject to red background color of the tank. Moreover, Olufeagba and Okomoda (2015) obtained survival rate of 10.47%-90.4% on parental and experimental crosses in *Heterobranchus longifilis*. However, our results are in between of these findings. The number of eggs (1.7–4.05) per gram body weight were similar to the study of Ahmed *et al*. (2007). They obtained 1-5 eggs per gram body weight in *Tilapia niloticus* and greater than Mashaii *et al*. (2016). They got 2.77±0.13 eggs/g on Nile tilapia in brackish water. In the present study, embryonic development took 552-672 h post fertilization (hpf) period and 23-28 days' post fertilization (dpf) period and it showed short time from the previous findings reported 600-720 hpf and 25-30 dpf on similar specie by Fujimura and Okada (2007). The variations in our results might have been due to climatically and geographical changes or environmental factors such as temperature, dissolved oxygen etc. Water quality parameters were suitable for Nile tilapia during the breeding period and more or less similar with the findings of previous scientists (Nandlal *et al*., 2004; Hussain, 2004; Ahmed *et al*., 2007; Khalfalla *et al*., 2008; Valeta *et al*., 2013; Ahmadi *et al*., 2015; Daudpota *et al*., 2016). They recommended that water temperature (22°C-30°C), dissolved oxygen (4.0mg/L-8.0mg/L, pH (6.5-9.0), ammonia (0.01mg/L-0.1mg/L) are suitable for the normal growth and successful breeding of tilapia.

CONCLUSION

The findings of the present study suggest that Nile tilapia, *O. niloticus* can breed successfully up to 15‰ salinity and may get maximum fertilization, hatchability and survival rate of fry. Owing to climate change and sea intrusion our agricultural land in Sindh has become saline, due to which agriculture production may be effected as well. These areas may be utilized for fish farming to overcome the protein deficiency especially animal origin and will be the source of income for the peoples of these areas. In this way, our aquaculture sector will be promoted.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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