EFFECTS OF SALINITY AND HARDNESS ON THE GROWTH OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*) IN NORTHERN PUNJAB REGION OF PAKISTAN

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ABSTRACT: *The tolerance of salinity and hardness was checked on the growth and survival of Nile tilapia (Oreochromis niloticus) in National Agriculture Research Council (NARC) Islamabad. Fry of the Tilapia measuring between 2.5cm to 2.9cm in total length and weighing between 0.435g to 0.603g (Body Weight BW) were used for the salinity and hardness treatment. Different levels of salinity from 0-15ppt were developed for the three months. 100 % survival rate was observed in 0-7.5ppt while in 12.5ppt and 15ppt all the fish died. Salinities between 0-10ppt were endured by the fish however the most appropriate salinity level was 5ppt on which maximum growth was recorded. For hardness treatment different concentrations of 350mg/lit, 450mg/lit, 550mg/lit and 650mg/lit of CaCl2 were used. No mortality was observed in this case and the most preferred concentration on which the fish showed maximum growth was 550 mg/lit.*

KEYWORDS: Growth performance, survival, water quality, tilapia growth, saline resistance,

INTRODUCTION

Pakistan is located in subtropical belt and a major part of the country faces shortage of rainfall throughout the year especially in summer season due to dry and extremely hot weather. This results in excessive water evaporation which consequently leads to accumulation of salts in soil and water reservoirs (Ahmed, 1993). This water is considered unfit for both agricultural products and aquatic life. It is the dire need of the hour to look for feasible alternatives of salt tolerant species in order to utilize these abandoned water resources. Although saline soil is not productive for agriculture, but it may be used as a substitute for culturing a salt resistant fish (Mateen, 2007). The demand to create aquaculture in saline water has been increased due to the shortage of it in fresh water when compared with agriculture and other urban activities (El-Sayed, 2002).

Nile tilapia (*Oreochromis niloticus*), is a standout amongst the most refined species on the planet. Generation of Nile tilapia is done in raceways and tanks through different culturing system (Little, 2004; Hernandez et al, 2014). It has been effectively cultivated under an extensive variety of natural conditions and is a critical aquaculture fish species

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in numerous parts of the world, especially in tropical and sub-tropical countries (Githukia et al, 2014; El-Sayed, 2002). It is thought to be the most beneficial and globally accepted fish as food (Ogello et al, 2014; Siddik et al, 2014).

In approximately 75 countries Tilapia have been cultured (Josupeit, 2005). By the end of 2010 tilapia production reached 3.7 MT globally (FAO, 2004). However, it appears that aquaculture industry is now confronted with large challenge of climate change. Over the past century the global sea level has increased from 10 to 20 cm (Parry et al, 2007). Saltwater intrusion is expected to have higher rate than that of the early time (Jermy et al, 1996; Abdel-Tawwab, 2011). The blow of sea level rise in the area and its consequences to agriculture production had long been investigated (Wassmann et al, 2004). Under the effect of change in climatic condition and rising of sea water, saline water-intruded inland in the area is expanding (Parry et al, 2007; Lawson and Anetekhai 2011). It appears that efficient use of brackish water and abundant sea water is an important alternative. Moreover a decreased dependence on freshwater and increased use of brackish and sea water for fish aquaculture, allows the utilization of unsuitable area.

Hardness of water is a serious issue in the fish production, as it fluctuates noticeably from area to area. Hardness of calcium (CH) and magnesium are the major contributor of total water hardness. More than 20 mgL^{-1} of $CaCO_3$ is the proposed value for water hardness in ponds of aquaculture (Buttner et al, 1993; Schofield et al, 2011). To access some vital processes such as bone formation, blood clotting, and numerous other processes of cell, Ca^{+2} is crucial for fish (Flik et al, 1994).Fish fulfils its demand for calcium by ingesting it with food and branchial absorption (Flik and Verbost, 1995).
Keeping in view, the increasing interest in tilapia farming this project was planned to evaluate the consequence of varied levels of salinity on the growth and survival of Nile tilapia. The expected outcomes of the study will be helpful in designing strategies not only to grow this commercially important fish but also help to strengthen the country’s economy.

MATERIALS AND METHODS:

Study site

The experiments were conducted in the Aquaculture and Fisheries Research Institute (AFRI), National Agricultural Research Center (NARC) Islamabad (33.6720° N, 73.1280° E) from July, 2016 to October, 2016.

Acclimatization

Fry of mono sex Nile tilapia, *Orechromis niloticus* were imported from the Thailand weighing about 0.2g. Fish (0.4354-0.603g) BW and (2.5- 2.9cm) TL were acclimatized in cemented raceways having volume of 7380.5 liters, for about one week in July. The raceways were supplied with water from tube well having a temperature between 24-28^{o}C.After which, fish were shifted to the aquaria filled with forty liter of water. These aquaria were acclimatized, twenty four hours before transferring fish into them. Fish aquaria (30x35x30 cm length x width x height) were used. All the aquaria fed on 0.5gms of 40% CP diet twice in a day. Temperature of aquaria was between 28-29^{o}C.

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Weighing of fish

Before shifting the fish into aquaria it was weighed on electrical balance of model BL-150S (Sortorius), length of the fish was also measured with the help of scale.

Aeration

All the aquaria were supplied with aquarium air pump of model SB-348A (SOBO).

Stocking density

Ten fish per aquaria was placed. Ten aquaria were for experimental group and one was for control group. Fish was acclimatized for a week in the room temperature condition before starting the treatment.

Salinity levels

In the first six aquaria salinity treatment was started while in the remaining four aquaria treatment for hardness was practiced. One aquarium was chosen as control group. Stock solution of sodium chloride maintained with salinity refractometer (0-100ppt) range model 300011 made in China. In the first aquaria 37.5 liter of pure water was taken along with2.5 liter stock solution. In the second aquarium 35 liter of pure water was taken along with 5 liter of stock solution. In the third aquarium 7.5 liter of stock solution was taken with 32.5liter of pure water. In the fourth aquarium 30 liter of pure water was taken along with 10 liter of stock solution. In the fifth aquarium 35 liter of pure water was taken with 15 liter of stock solution. Survival rate of *Oreochromis niloticus* was also checked at 12.5ppt. In other four aquaria hardness was maintained by adding $CaCl_2$ 350, 450, 550,650 mg/L. Concentrated Solution of $CaCl_2$ was prepared and added in each aquarium. Electrochemical analyzer model S/N 104158 was used to maintain desired value of hardness.

Water quality analysis

Water samples for all aquaria were collected for chemical analysis between 9:30am at 30cm depth. Dissolved oxygen, water temperature, salinity, PH and hardness were analyzed at 30cm depth with an electrochemical analyzer model S/N 104158 before and after treatments on daily bases. In all treatment, dissolved oxygen amount ranged from 3.3mg/L to 3.61mg/L. The range of water temperature range was 28-31.4^oC. The pH was ranged between 6.95-8.15 and before treatment salinity and hardness were 0.2mg/L and 230-250mg/L respectively. Water pH, calcium hardness, temperature and salinity were monitored daily. From each aquarium for water quality analysis a 500 ml water sample was withdrawn daily.

Growth performance

Growth performance was calculated on weekly basis as:

Weight gain = W2-W1

Where W1-W2 are the initial and final fish weight respectively

Length gain =L2- L1

Where L1-L2 are the initial and final fish length and survival were reported for all experimental aquaria.

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Feed of fish

All stocked fish were provided with the same commercial feed which was used during the period of acclimatization. Fish were fed with diet twice a day. The ratio of diet for each aquarium was adjusted weekly with an increase in the body weight of fish.

Proximate analysis

After growth trial, four fish were collected from each aquarium and according to the standard methods of AOAC (1990) the proximate chemical analysis of whole-fish body was done for moisture, protein, total lipids, and ash. In drying oven (GCA, model 18EM, Precision Scientific group, Chicago, USA) moisture content was determined by drying the samples to at 85°C for about 24 hours. Nitrogen content was estimated using a micro kjeldahl apparatus (Labconco, Labconco Corporation, Kansas, USA) and for the crude protein estimation nitrogen content was multiplied by 6.25. Determination of lipid content was made by ether extraction in multi-unit extraction Soxhlet apparatus (Lab-Line Instruments, Inc., Melrose Park, Illinois, USA) for 16 hours and ash content was determined by combusting dry samples in a muffle furnace (Thermolyne Corporation, Dubuque, Iowa, USA) at 550°C for 6 hours.

Statistical analysis

Data was analyzed by analysis of variance (ANOVA) and means were compared by LSD. Data was also presented in the form of graph and tables.

RESULTS

Water quality parameters

During study average temperature of different aquaria ranged from 24-30 ^{0}C, range of average pH was 6.52-7.34 while average dissolved oxygen ranged from3.33-3.61(Table 1).

Table 1. Average water quality parameters during experimental period

Time period	Average temperature(^{0}C)	Average pH	Average dissolved oxygen(mg/L)
Week1	30.16±0.6	6.92±1	3.55±0.3
Week2	28.42±3.5	6.91±1	3.61±0.3
Week3	25.64±0.6	6.89±1	3.33±0.4
Week4	27.53±0.6	7.33±4	3.55±0.3
Week5	28.54± 0.7	7.22±3	3.5± 0.3
Week6	29.21±0.2	6.62±2	3.44±0.3
Week7	27.56±0.3	7.34±2	3.5±0.4
Week8	24.28±0.5	6.52±3	3.55±0.3

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Week9	25.72±0.3	7.31±2	3.55±0.3

Impacts of saltiness on the survival of *O. niloticus*

Rate of survival of *O. niloticus* on different salinities are shown in Fig. 1. In this study, 100% survival rate was monitored in 0 to 7.5ppt while 100% death rate in 12.5 and 15ppt. At 10ppt within 24 hours after salinity treatment 60% and in second week 70% survival rate were observed. After this no mortality was seen. So it is concluded that 0-7.5ppt is the optimum salinity level for the growth of *O. niloticus* at fingerling stage. Survival percentage at various levels of salinity is also given in table 2.

Table 2. Survival percentage of *Oreochromis niloticus* at different salinity levels, Salinity levels (Survival %)

Time period	2.5ppt	5.0ppt	7.5ppt	10.0ppt	12.5ppt	15.0ppt
Week1	100	100	100	60	0	0
Week2	100	100	100	80	0	0
Week3	100	100	100	100	0	0
Week4	100	100	100	100	0	0
Week5	100	100	100	100	0	0
Week6	100	100	100	100	0	0
Week7	100	100	100	100	0	0
Week8	100	100	100	100	0	0
Week 9	100	100	100	100	0	0

Effects of salinity on the growth of *O. niloticus* body weight (bw)

Growth of *Oreochromis niloticus* was observed at different salinity levels. At all salinity levels significant raise in body weight was observed (p< 0.05). At 0ppt, it was 2.63±1.94, at 2.5ppt, 3.44±2.76, at 5ppt, 3.773±3.25, at 7.5ppt3.72±2.90 at 10ppt3.41±2.59 (Table 3). A significant enhance in average body weight gain (P≤ 0.05) was observed. At 0ppt weight gain in grams was 5.61, at 2.5ppt 7.37, at 5ppt8.80,at 7.5ppt7.97 at 10ppt7.07(Table 4). Figure (1) depicts the maximum and minimum weight gain at different salinity levels.

Table 1. Summary of the increased in total weight (g) and length (cm) of *O. niloticus* in varied salinities

Salinity level in ppt	**Average final weight (g)**	**Average initial weight (g)**	**increase in average weight(g)**	**Average final length(cm)**	**Average initial length (cm)**	**increase in average length(cm)**
0	6.3	0.68	5.61	8.5	3.7	4.8
2.5	8.02	0.64	7.37	9.4	3.5	5.9
5.0	9.43	0.62	8.80	10.1	3.9	6.2

7.5	8.63	0.65	7.97	7.9	3.2	4.7
10.0	7.99	0.91	7.07	8.5	3.7	4.8

Table 2. Average increase in weight and length at different hardness levels

Parameters	350 mg/L	450 mg/L	550mg/L	650mg/L
Weight(g)	2.47±1.84	3.15±2.22	4.18±3.43	3.31±2.53
Length(cm)	5.65±1.84	5.91±1.57	6.61±2.17	5.86±2.0

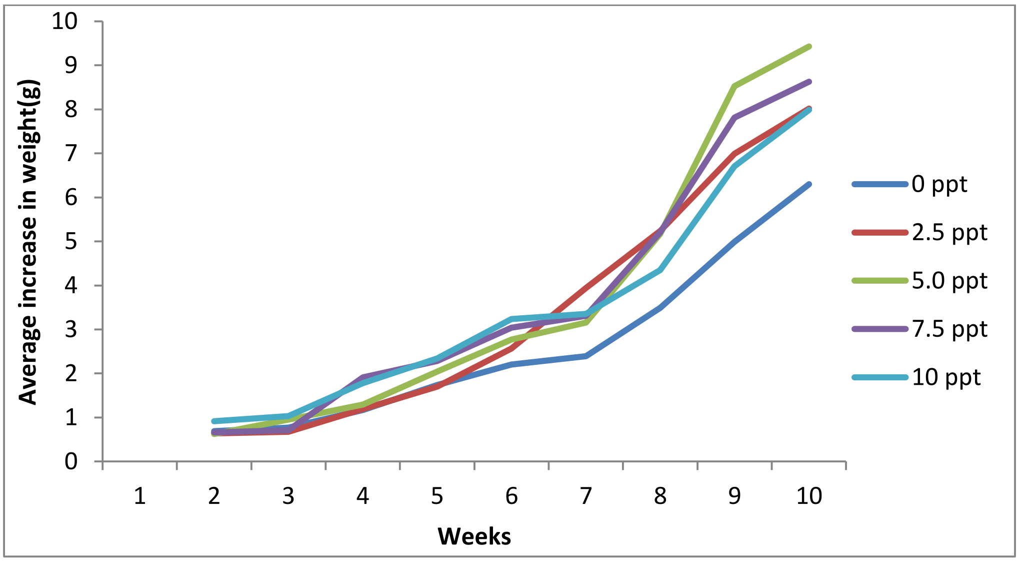

Figure 1. Effect of salinity on growth (weight) of *Oreochromis niloticus*

Total length (tl)

It was observed that there is non-significant increase in total length at salinity level 0ppt,2.5ppt,7.5ppt and 10ppt (P>0.05), while significant increase in length was observed at salinity level 5.0ppt (P< 0.05) At 0ppt it was 5.72±1.45 ,at 2.5 ppt5.41±1.95, at5 ppt6.44±2.14, at 7.5ppt5.74±1.79,at 10ppt. 5.96±1.96 (Table 3). At 0ppt length gain in centimeters was 4.8,at 2.5ppt 5.9, at 5ppt6.2,at 7.5ppt4.7 at 10ppt4.8 (Table 4). Figure (2) depicts the maximum and minimum increase in length at different salinity levels. So it is concluded that salinity levels have significant effect on growth performance of *O. niloticus* (P<0.05).

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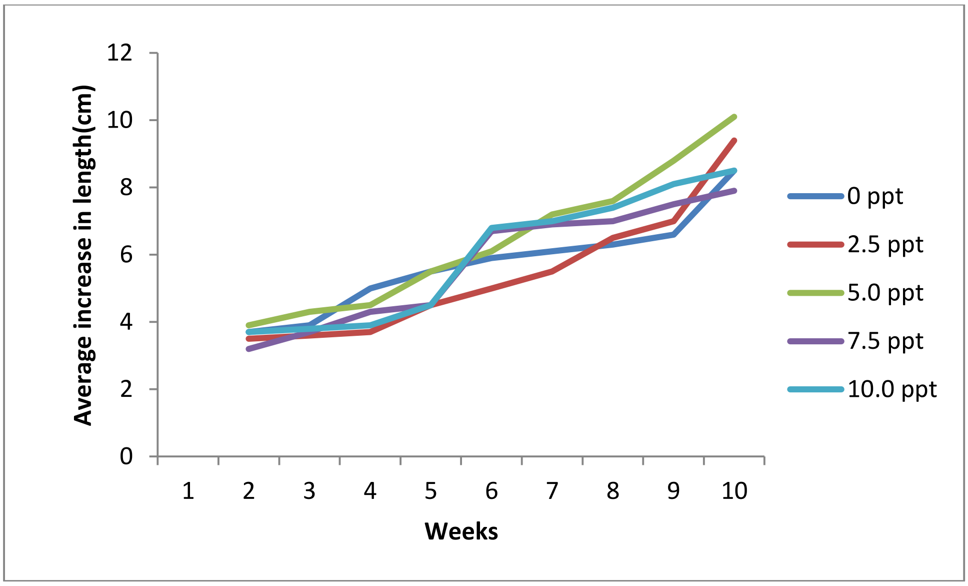

Figure 2. Effect of salinity on the growth (length) of *Oreochromis niloticus*

Effects of hardness on the growth of *O. niloticus* body weight (bw)

Growth performance of *Oreochromis niloticus* was also observed at different concentrations of hardness. At the concentration of350 mg/L it was, 2.47±1.84 at 450 mg/L 3.15±2.22at 550mg/L4.18±3.43 at650mg/L3.31±2.53 (Table 5). At 350mg/L weight gain in grams was 5.478, at 450mg/L 6.568, at 550mg/L8.688, at 650mg/L6.492 was observed (Table 6). Figure (3) shows maximum and minimum average increase in weight (g) at different concentration of hardness. It is concluded that hardness has significant effect ($P<0.05$) on growth performance of *O. niloticus.*

Table 3. Summary of the increased in total weight (g) and length (cm) of *O. niloticus* at varied concentration of hardness

Hardness concentrations (mg/L)	Average final weight (g)	Averag e initial weight (g)	Increase in average weight(g)	Average final length(cm)	Averag e initial length (cm)	Increase in average length (cm)
350	6.12	0.642	5.478	8.4	3.5	4.9
450	7.27	0.702	6.568	8.0	3.8	4.2
550	9.5	0.812	8.688	10.1	3.9	6.2
650	7.07	0.578	6.492	9.0	3.4	5.6

Table 4. Proximate composition (% dry matter) for salinity treatment

Salinity Levels	2.5 ppt	5.0 ppt	7.5 ppt	10 ppt
Moisture	77.3	79.10	78.60	79.50
Dry matter	22.70	20.90	21.40	20.50
Crude protein	52.22	57.90	56.20	54.60
Crude lipids	8.20	9.11	8.33	8.41
Ash	16.24	14.25	18.2	15.14

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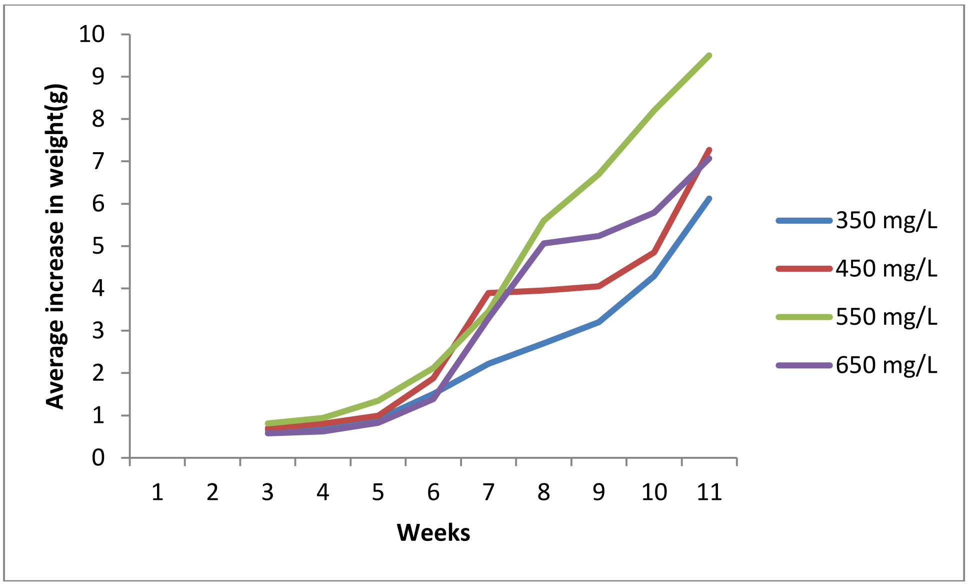

Figure 3. Effect of water hardness on the growth (weight) of *Oreochromis niloticus*

Total length (tl)

Significant Increase (P<0.05)) in average length was observed at all four levels of hardness in *O. niloticus*. At 350mg/L it was 5.65±1.84, at 450 mg/L5.91±1.57, at550mg/L6.61±2.17, at 650mg/L5.86 ±2.0 9 was recorded (Table5). At 350mg/L length gain in centimeters was 4.9, at 450mg/L 4.2, at 550mg/L6.2, at 650mg/L 5.6 was observed (Table 6). Figure (4) shows maximum and minimum average increase in length (cm) at different concentration of hardness. So it is concluded that hardness has significant effect (P<0.05) on growth performance of *O. niloticus*.

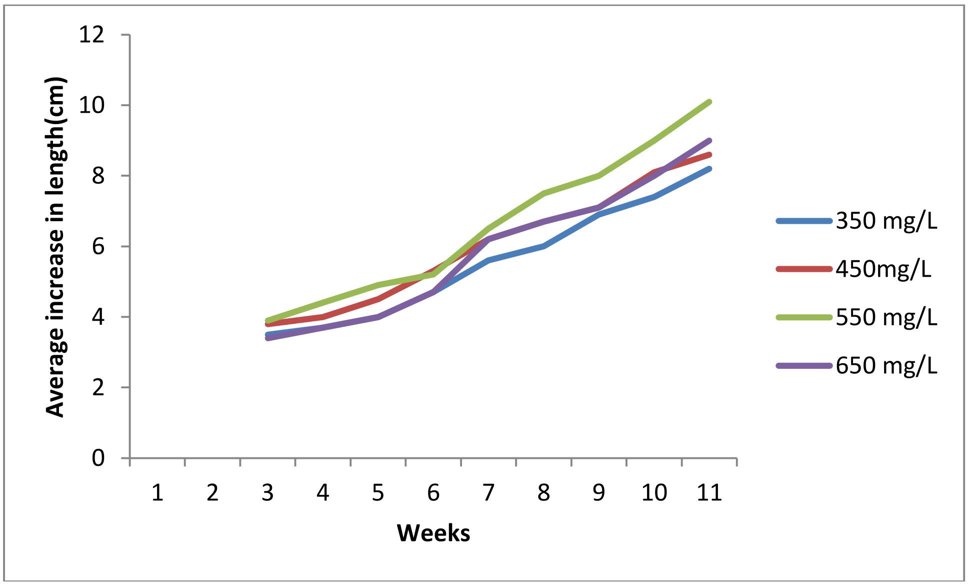

Figure 4. Effect of water hardness on the growth (length) of *Oreochromis niloticus*

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Table 5. Proximate composition (% dry matter) for hardness concentrations

Hardness concentrations	350mg/L	450mg/L	550 mg/L	650 mg/L
Moisture	75.70	77.87	75.30	73.3
Dry matter	24.30	22.12	24.70	26.20
Crude protein	51.20	53.80	58.30	54.22
Crude lipids	7.20	8.30	9.40	8.52
Ash	15.24	13.22	19.20	18.14

DISCUSSION

It has been observed that, tilapia are euryhaline fish and the most significant candidate for tropical aquaculture. It can flourish and survive in varied level of salinity as it has greater level of endurance (Guner et al, 2005; Kamal and Mair, 2005). In our study level of endurance of salinity in Nile Tilapia ranged from 0 to 7.5ppt and it did not cause any mortality. It clearly showed that the fish adapted itself very well at this salinity level.100% mortality recorded in 12.5 and 15ppt.This alarming increase in mortality rate in fish was due to anxiety pressure and less level of endurance to the salinity.

In present experiment it has been demonstrated that water salinity is a crucial factor and maximum growth execution in terms of normal weight pick up (g) and normal length increase (cm) was observed at 5.0ppt while the minimum was seen in control group. All this is testimony to the fact that saltiness is a key variable in controlling the growth in tilapia. Similar results were reported by Sparks et al, (2003).

Previously it has been reported that salt tolerance range for Nile tilapia (*Oreochromis niloticus*) is 0-15ppt (Kamal and Mair, 2005; Avella et al, 1993). Our results are slightly contradictory not due to gradual increase in salinity. Gradual shifting of fish to varied salinities let fish to adapt itself in a better way (Watanabe et al, 1984; Lemarie et al, 2004) while sudden fluctuations can cause stress, failure in osmoregulation and even mortality. Wang, (2001) reported that sudden shifting of *O. mossambicus* from 0 to above 30ppt resulted in complete mortality in a time limit of six hours. In another study, Nile tilapia reared 0ppt, 2.5ppt, 5.0 ppt, 7.5ppt have perfect chances of survival. Similar observation on tilapia reared at 0 ppm and 5000 ppm (5.0 ppt) has been reported by Ridha (2008) and Jamil et al, (2004).

Current study not only examined the result of varied salinity level on the life and resistance level of Nile tilapia (*Oreochromis niloticus*) but also focused on the result of hardness of water by developing different concentrations of $CaCl_2$. Varied level of hardness concentration can result in a noteworthy increase with reference to body

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weight and length of fish. Maximum rate of growth was noticed at 550 mg/L while this dropped to the least at 350 mg/L.

Mateen et al, (2007) reported that the growth rate of rohu varied under different hardness concentration. They reported that hybrid performed best at 450 mg/L. Cavalcante et al, (2009) also detected that the fish growth increased to a great extent when they were maintained in $CaCO_3$-limedaquaria. All these findings clearly reflect that an increase in water hardness combined with rise in alkalinity by the application of $CaCO_3$ plays a significant role in better fish growth while just water alkalinity rise doesn't affect it so well. El- Sherif and El-Feky (2009) observed that pH of water between 7-8 can be more appropriate for tilapia culturing and is considered as optimum range both for growth and survival of the fish. The current study also revealed that pH for Nile tilapia (*Oreochromis niloticus*) is 6-7 (Table 1).

In our study the water temperature was ranged between 24-30°C. Watanabe et al, (1997) reported that survival and growth in fish are not influenced at different levels of saltiness when rise in temperature was above 27°C but it has a marked effect if temperature is lower than 25°C. Similar observation on tilapia reared at 0 ppm and 5000ppm(5ppt) has been reported where salinity tolerance is greatly influenced by temperature since both these parameters alter together in environment and these changes can have positive or negative effect on growth and reproduction of cichlids (Jamil et al, 2004; Iqbal et al, 2012).

CONCLUSION

It has been concluded that if the condition of temperature is ideal, a good growth response in fish could be achieved at 5 ppt salinity level and 550 mg/L of hardness concentration. Nile tilapia was able to survive at 10 ppt saltiness concentration. Keeping in view, the increasing interest in aquaculture this study was performed not only to meet the demand of protein but also to utilize the increasing salt water resources.

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