



Original article

Study on survival, growth, haematology and body composition of *Cyprinus carpio* under different acute and chronic salinity regimes

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ABSTRACT

Pakistan's most of the land is less productive or no productivity at all due to erosion and salinity of the soil, which can be utilized to develop fisheries. The project, "Survival, growth and body composition of *Cyprinus carpio* under different salinity regimes" was undertaken in two phases. In the first phase susceptibility of *Cyprinus carpio* at four salinity levels in triplicate within 0–10 g L⁻¹ NaCl for 96 h in each aquarium was checked after one week acclimation at 0 g L⁻¹, 2 g L⁻¹ and 4 g L⁻¹ NaCl. LC₅₀ values varied from 7.67 to 10.65 g L⁻¹ after 96 h for *C. carpio*. Percentage mortality of the fish and important water quality parameters after every 12 h were observed for a period of 96-h. Probit analysis showed that 96-h LC₅₀ values ranged from 7.67 to 10.65 g L⁻¹. During experimental period aquaria water temperature ranged from 29.6 to 33.7 °C, pH values fluctuated between 7.8 and 9.7, Electrical conductivity values ranged from 2.40 to 20.13 dSm⁻¹ and Dissolved oxygen ranged between 2.23 and 10 mg L⁻¹. Sub-lethal salt concentration i.e. 0 g L⁻¹ to 3 g L⁻¹ NaCl upto 40 days showed that growth of *C. carpio* decreased with the increase of water salinity levels and ceased at 4 g L⁻¹ salinity and increase in salinity have negatively affected hematological parameters.

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1. Introduction

Fishes are poikilothermic so any change in their ambient medium and other factors like food, space, season, salinity, temperature and physical activity affect the fish growth (Weatherly and Gill, 1987; Houlihan, 1993; Burel et al., 1996; Britz et al., 1997; Azevedo et al., 1998). Thus changes in the water temperature affect the growth of fish (Smith, 1989; Mohnen and Wang, 1992). In Punjab huge belts of saline soil which are not utilized for agriculture can be used for aquaculture by stocking suitable fish species. Salinity is main factor that affect the metabolism of fish, which in return effect the growth, feed intake and survival of fish (Brocksen and Cole, 1972; Raghuraman, 1973; Desilva and Perera, 1976). The rise in the natural salinity is often caused when evaporation is com-

bined with intrusions of ground water (Close, 1990; Williams, 1999; Kay et al., 2001; Sampaio and Bianchini, 2002). If the environmental salinity exceeds the optimal level fish may die, become stressed and lose the ability to osmoregulate. Fish growth will be maximum when osmoregulatory demands are minimum (Marshall et al., 1999). Fish reared at optimum salinity help to reduce its metabolic activity which ultimately reserves energy for its growth (Neill and Bryan, 1991). In brackish or estuarine environments salinity is an important environmental factor for disease dynamics or parasitism (Haskin and Ford, 1982; Reisser and Forward, 1991; Kesting et al., 1996; Zander, 1998; Koie, 1999; Messick et al., 1999). There is deficiency of fresh water with elevated salinity levels in the southern Punjab (McEnroe and Cech, 1985; Davidson, 2000). Salinity tolerance of fish can be determined by body size and age (maturation stage) of fish (Garcia-Gallego et al., 1998). Effect on the electrocardiogram and some of blood serum minerals in grass carp exposed to concentrations of 4, 8 and 12 g L⁻¹ salinities indicated that mineral level in the studied groups was significantly different (Enayati et al., 2013). Effect of water salinity changes on thyroid hormones level and thyroid-stimulating hormone (TSH) of grass carp held in three different salinities at concentrations of 4, 8 and 12 g L⁻¹. Thyroxine level was significantly higher only in 12 g L⁻¹ group in comparison with

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the control and other experimental groups (Peyghan et al., 2013). In this study *C. carpio* as one of the most important fishes of aquaculture industry in Asia and also as a model to determine salinity effects have been used. Objective was to determine the tolerance limits of salinity in *C. carpio* under variable conditions of arid zone subject to the gradually increase in salinity.

2. Experimental methods-general consideration

2.1. Experimental fish

Cyprinus carpio 500 fingerling were collected from the Govt. Fish Seed Hatchery Faisalabad than brought to Govt. College University, Faisalabad, Pakistan and acclimatized in the water available here for one week. The water (fish collection site) was analyzed and recorded for various physico-chemical characteristics for information. The ground water used during experimentation was also analyzed for various important physico-chemical characteristics. Fish was fed on the 30% CP formulated diet @ 4% wet body weight daily during acclimatization period.

2.1.1. Phase-I

In triplicate four salt concentrations (Control, 6, 8 and 10 g L⁻¹) at four different temperatures (Control, 30, 32.5 and 35 °C) at three salt acclimation levels (Control, 2 and 4 g L⁻¹) were prepared in the glass aquaria of each having dimensions 30L × 30 W × 45H cm (29.1 L water capacity). In each aquarium five acclimatized fingerlings were stocked for one week. The remaining fish of storage aquaria was treated with 2 g L⁻¹ of salt and was acclimatized for 96-h. Observations in behavior and mortality were recorded after every 12 h interval for 96 h. Tested fingerlings were not fed in aquaria. Aquaria were equipped with air pumps to keep the dissolved oxygen of the water near to optimum. Same procedure was repeated with the stocked fingerlings acclimatized at the 2 g L⁻¹ salt solution. The left over stocked fingerlings was shifted to further higher concentration (4 g L⁻¹) of rock salt for another week for acclimation at further higher level. After one week of acclimation the said fish was exposed to the same four concentrations along with control of rock salt as mentioned earlier for 96 h. Water was filtered to remove the excreta frequently.

2.1.2. Phase-II

a set of another four concentrations (Control, 1, 2 and 3 g L⁻¹) at four different temperatures (Control, 30, 32.5 and 35 °C) of rock salt with fish acclimatized on control water in triplicate were prepared in the above mentioned glass aquaria (29.1 L water capacity) to determine the salinity effect on growth parameters. Important physico-chemical parameters of aquaria water, such as temperature, dissolved oxygen, pH, electric conductivity, total dissolved solids and chlorides were observed using Professional meter (HANNA HI 9828).

2.1.3. Phase-III

Impact of different salinity regimes was evaluated in three steps on various fish quality parameters viz. fish blood quality and fish meat quality in following steps.

2.1.4. Step-I

Before dissecting fish, blood sample was collected by directly puncturing its heart to study the impact of salinity on various blood parameters of treated fish viz. Hemoglobin concentration (Hb Conc.), Red Blood Cell Count (RBC Count), White Blood Cell Count (WBC) within acclimations and within the different treatments as compare to control.

2.1.5. Step-II

Salinity impact on fish physiology was analyzed by using light microscopy technique which includes the histology of fish tissues viz. gill, liver and body muscles.

2.1.6. Step-III

Meat quality of fish was analyzed for evaluating the effect of salinity stresses on fish body composition viz. moisture, crude protein, crude fat, and ash contents with respect to control fish.

Statistical analysis was performed on mortality data by using regression models and LC₅₀ was calculated against various treatments and acclimations. Analysis of variance test was performed on various physico-chemical and blood parameters.

3. Results

In the acute toxicity studies mortality of *Cyprinus carpio* was observed to check the effect of different salt concentrations (Control, 6, 8 and 10 mg L⁻¹) under different acclimation levels (Control, 2 and 4 mg L⁻¹). Results showed that mortality of *Cyprinus carpio* increases with the increase of salt concentration. Probit equation of mortality for 96-h was (-6.577 + 0.689X) and LC₅₀ was 5.26345 ± 0.522 (4.32–7.11 at 95% CI) for *Cyprinus carpio* subjected to different salt concentrations after acclimation at 0 g L⁻¹. When the fish was subjected to different salt concentrations after acclimation at 2 g L⁻¹ the probit equation of mortality for 96-h was (-1.86 + 0.14X) and LC₅₀ was 13.3847 ± 3.40 (7.67–10.04 at 95% CI). In the third acclimation (4 g L⁻¹) the probit equation was (-3.53 + 0.22X) and LC₅₀ 16.3770 ± 7.985 (8.76–10.65). Various physico-chemical parameters like Temperature, pH, Electrical conductivity and dissolved oxygen does not vary too much among different treatments but statistically it caused significant differences among treatments, days and experiments. Grand mean value of temperature during acclimation at 0 g L⁻¹ was 31.1 ± 0.08 which increases to 32.0 ± 0.09 and 32.5 ± 0.22 when the fish was acclimatized at 2 g L⁻¹ and 4 g L⁻¹ respectively. In case of pH grand mean values were 8.82 ± 0.05, 9.0 ± 0.07 and 9.30 ± 0.11 when the fish was acclimatized at 0 g L⁻¹, 2 g L⁻¹ and 4 g L⁻¹ respectively. Grand mean values of electrical conductivity were 12.41 ± 0.61, 12.69 ± 0.21 and 12.32 ± 0.19 at 0 g L⁻¹, 2 g L⁻¹ and 4 g L⁻¹ acclimation level respectively. 5.9 ± 0.09, 8.02 ± 0.36 and 3.73 ± 0.36 were the grand mean values of dissolved oxygen when the fish was acclimatized at 0 g L⁻¹, 2 g L⁻¹ and 4 g L⁻¹. Analysis of variance was highly significant among acclimation levels and treatments.

In chronic toxicity average body weight and average total length decreases with the increase in the salt concentration. Grand mean values of average total length, average total weight and various physico-chemical parameters with standard error are shown in the (Table 1). Analysis of variance was highly significant, significant and non-significant among acclimation levels and treatments.

There were differences in the hematological observations of *C. carpio* as shown in the (Table 2). Analysis of variance for hematological values of *C. carpio* was highly significant. Probability for moisture content, dry matter, fat and protein was non-significant among treatments and replications.

Light microscopy showed in T₀ (0 g L⁻¹) tissues were not damaged and showed continuous strip, in T₁ (1 g L⁻¹) light microscopy of tissues shows atrophy of muscles in T₂ (2 g L⁻¹) tissues shows vascular degeneration of muscle bundles in T₃ (3 g L⁻¹) tissues were damaged and this treatment shows edema of muscle bundles. Meat Quality parameters under study showed a gradual decrease in crude protein % with the increase in salinity concentration.

Table 1

Mean values of body weight (g), total length (cm) and different physico-chemical parameters at different salt concentrations.

Parameters/Acclimation	T ₀ (0 g L ⁻¹)	T ₁ (1 g L ⁻¹)	T ₂ (2 g L ⁻¹)	T ₃ (3 g L ⁻¹)
Average body weight (g)	2.40c ± 0.20	2.35d ± 0.01	3.10a ± 0.01	2.64b ± 0.03
Average body length (cm)	6.20b ± 0.01	6.23b ± 0.00	6.58a ± 0.00	6.10c ± 0.02
Temperature(°C)	32b ± 0.09	32.1c ± 0.09	32.5a ± 0.08	32.3b ± 0.08
pH	9.5b ± 0.03	9.6a ± 0.03	9.2d ± 0.03	9.3c ± 0.04
Electrical conductivity (dSm ⁻¹)	2.04d ± 0.01	4.23c ± 0.17	6.44b ± 0.02	7.85a ± 0.01
Dissolved oxygen (mg L ⁻¹)	4.9d ± 0.09	5.1c ± 0.08	5.7b ± 0.09	6.5a ± 0.10

Table 2

Mean values of hematological and meat quality parameters at different salt concentrations.

Parameters/Treatments	T ₀ (0 g L ⁻¹)	T ₁ (1 g L ⁻¹)	T ₂ (2 g L ⁻¹)	T ₃ (3 g L ⁻¹)
RBCs (μL ⁻¹)	0.42b × 10 ⁶	0.38d × 10 ⁶	0.40c × 10 ⁶	0.43a × 10 ⁶
Hb (g dL ⁻¹)	3.2a	2.7b	3.1a	2.4c
HCT (%)	3.2c	2.4d	5.0b	5.3a
MCV (fL)	76.2c	73.0d	125.0a	123.3b
MCH (pg)	78.6a	77.9b	77.0b	55.8c
MCHC (dL)	80a	65b	62c	45d
PLT (μL ⁻¹)	715a × 10 ³	682b × 10 ³	439c × 10 ³	292d × 10 ³
Moisture (%)	80.97b	79.48c	80.37b	86.04a
Dry matter (%)	19.01	20.99	19.62	13.94
Fat (%)	1.15	1.56	3.59	1.55
Protein (%)	16.86	17.94	15.03	11.39

4. Discussion

In the aquatic environment, the physico-chemical conditions of water and presence of different types of ions/salts have an effective control on the regulation of gross productivity, in which sodium and chloride are the most important factors in the prevailing hydrological conditions of the area. Sensitivity of the freshwater species i.e., *C. carpio* increased with increase in salt concentrations. These fishes are the inhabitants of the hypo osmotic aquatic environment where salt content rarely exceed 500 mg L⁻¹. In hyperosmotic environment fish face the problem of osmotic loss of water and diffusional entry of salts. In this environment, the susceptibility of fish is low due to dehydration as our results are in accordance to [Peyghan et al. \(2013\)](#).

In the present project the effects of salinity on the growth and tolerance of *Cyprinus carpio* was studied. 96-h LC₅₀ values at three acclimation levels were estimated as 5.26, 13.38 and 16.37 respectively in warm water (average temperature 27 °C) through probit analysis. [Tarar \(2000\)](#) estimated that the LC₅₀ values for *L. rohita* and *C. mrigala* ranged between 5.658 and 9.174 and 5.133–8.615 g L⁻¹.

In the present study *C. carpio* showed highly significant differences in salt tolerance. For *C. carpio* it was important to determine the thresholds and to establish the ways in which other factors influence it. For this purposes *C. carpio* was subjected to various test salinities, without acclimation and with two acclimation levels. Acclimation of the fish significantly increased the tolerance of *C. carpio* likewise reported by [kefford et al. \(2004\)](#).

Significant interaction between temperature and salinity was reported by [Sardella et al. \(2004\)](#). However, it could not be ascertained. A temperature of 30 °C is clearly above the optimal temperature for carp [Elliott \(1981\)](#) and it is therefore likely that this was a stressful situation. According to [Jian-Yi et al. \(2006\)](#) pH values between 6.5 and 7.5 are appropriate for hatching and rearing of the Chinese sturgeon larvae.

The sub-lethal effects of the various grades of salinity were studied on growth parameters. Growth of *C. carpio* was observed for the period of 40 days at salinities upto 4 g L⁻¹. Comparison of different treatments was made with the help of analysis of variance. It showed significant difference in average body weight and

gain in body weight among various treatments. The growth rate decreased gradually with the increase of the salinity level. Growth in terms of change in body length was also significantly different. However, this increase was positively correlated. Salinity upto 2.25 g L⁻¹ did not affect much the growth of *C. carpio* is concerned. Growth also increased and it was difficult to mark the turning point. Growth also decreased with time as the analysis of variance showed highly significant differences when applied on average body weight.

5. Conclusion

Higher salt treatments have increased mortality rate in *Cyprinus carpio* and decreased hematology parameters and while higher salt treatment (3 g L⁻¹) caused edema in fish.

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