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Salinity induced changes in growth and gill structure of freshwater carp, Cyprinus carpio Linn.

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Abstract

Aim: The present study was carried out to evaluate salinity induced changes in the growth and gill structure of cosmopolitan freshwater carp, *Cyprinus carpio* (Linn.).

Methodology: Inland saline water (15 ‰ or g Γ¹) was collected from the salt affected waterlogged areas of village Shajrana (30.3346 0N, 74.1196 0E) in District Fazilka, Punjab (India) and diluted with underground freshwater for preparing different salinity levels (2 to 10 ‰). Fingerlings (10 ±2 cm) of *C. carpio* were exposed to 0 (control), 2, 4, 6, 8 and 10 ‰ salinity after gradual acclimatization with salinity increase @ 1 ‰ hr¹.

Results: At the end of 120 days of rearing under saline conditions, $\frac{100\%}{100\%}$ fish survival was observed up to 6 % salinity, while 86.66 and 70.00% survival was recorded in 8 and 10 %, respectively. However, fish growth declined significantly at all salinity levels (p≤0.05). Gill structure was also affected at all salinity level, but pronounced changes were observed at salinity levels $\geq 6\%$, including lamellar oedema, epithelial lifting, lamellar hyperplasia, hypertrophy, lamellar fusion, hyalinisation, aneurysm, blood congestion, etc.

Interpretation: The results offer referral database to explore optimised economic production of common carp in inland saline areas of the region at salinity levels below 6 %.

Key words: Carp, Gills, Growth, Production, Salinity, Survival

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Introduction

Agricultural output in inland non-coastal states of India has been affected significantly by underground water salinity and problem of water logging. About 12 lakh hectare land in Punjab, Haryana, Uttar Pradesh, Bihar, Madhya Pradesh, Rajasthan and Jammu and Kashmir is salt-affected. Aquaculture is the most suitable option for utilizing these underproductive or unproductive waste lands for socio-economic development with a triple mission of food/nutrition security, livelihood/employment opportunities and revenue generation. Further, aquaculture productivity depends on many biotic and abiotic factors. Salinity is one of the most significant abiotic factors and its favourable range for survival and the optimum growth of aquatic organisms is speciesspecific (Mubarik et al., 2015). Previously, many brackish water finfish species have been reared successfully in inland saline water in Harvana, Punjab and Uttar Pradesh (Ansal et al., 2013; Pathak et al., 2013; Chandra and Joshi 2015). However, culture of these species could not be commercialized in these areas due to multiple constraints, including procurement of seed from coastal states, restricted culture period (April - October) owing to mortality during the winter season and high production cost. However, during last five years, brackish water shrimp (Litopaeneus vannamei) farming has developed rapidly in inland saline areas of Punjab and Haryana, which has converted zero earning wasteland into a remunerative resource, with an earning potential of Rs. 8-10 lakh/ha/crop of up to 120 days. High cost and high risk involved in shrimp farming has restricted its adoption only by the progressive farmers and small farmers are left with no option other than looking for low-cost alternatives for livelihood.

To overcome these hurdles, some attempts were made in the past to culture freshwater carps in low saline areas of noncoastal states like Punjab and Haryana (Pathak et al., 2013; Chandra and Joshi 2015;), to provide a low-cost aquaculture technology to low-income group of farmers owning these lands. In recent years, freshwater carp culture has been initiated in low saline areas of South-west Punjab. Salinity tolerance of freshwater carps is species-specific. Among the carps, common carp (Cyprinus carpio Linn.) has been reported to be highly tolerant, while mrigal (Cirrhinus mrigala Ham.) was found least tolerant to artificial as well as in inland saline water (Ansal et al., 2013: Islam et al., 2014). Common carp. C. carpio is a well-known stenohaline freshwater fish, which has been reported to tolerate a wide range of salinities (Salati et al., 2011; Singh et al., 2018) and grow well in inland saline water (Ansal et al., 2013). Salinity induced osmoregulation stress in stenohaline freshwater species affects its physiology adversely, leading to poor growth and mortality if the tolerance limit is crossed (Gholampoor et al., 2011). Under saline conditions, oxygen and energy requirement of freshwater species increases and consequently ample amount of energy is lost to compensate osmoregulatory changes (Engstrom-Ost et al., 2005). Further, gills are the essential organs for respiration, osmoregulation, acid-base balance and nitrogenous waste excretion in fish (Heath, 1987). Gills are delicate and most vulnerable to environmental changes because of

their external location and intimate contact with the water. Structural damage in the gills impair its functioning leading to serious physiological consequences and ultimately, affecting growth and survival of fish. Histopathological studies act as biomarkers in environmental monitoring, where alterations in the vital organs serve as warning signs of damage to animal health (Gernhofer *et al.*, 2001).

Major freshwater species reared under semi-intensive poly-culture system throughout in India. Hence, it is vital to understand that how well these species can perform under saline conditions, so that its productivity in inland saline water could be optimized through species selection and stocking density adjustments. The present study was, therefore, carried out to study salinity induced changes in one of the widely cultured carp species, *C. carpio* to work out suitable salinity range for its rearing in inland saline water resources of Northern India.

Materials and Methods

The present study was carried out at College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana (Punjab), India. Inland saline water of 15 % salinity (Table 1) was collected from the salt-affected- waterlogged area of village Shajrana in district Fazilka (Punjab) and was diluted with underground freshwater to prepare different salinities ranging from 2 to 10 %. Common carp fingerlings (10 ± 2 cm) were conditioned for 48 hr in a cemented pool. Preliminary acclimatization tests were conducted to evaluate suitable salinity increase rate for acclimatizing the fish to different salinity levels and based on the results, the fingerlings were acclimatized to 2, 4, 6, 8 and 10 % salinty with a gradual increase in salinity @ of 1 % hr⁻¹ interval. After acclimatization, 10 fingerlings each were transferred to aerated glass aquaria holding 20 I water of different salinities, in triplicate. Evaporation loss was compensated by adding freshwater up to marked levels to maintain volume and salinity of water in each aquarium throughout the study period of 120 days.

The fish was fed daily with pellet feed @ 1% of its body weight. Leftover feed and excreta were removed from the aquaria every alternate day through siphoning, while water siphoned out during the said process, was added back to each aquarium after filtration through a muslin cloth. Water loss during this process was compensated by adding water of same salinity. During this period, survival of fish and water quality parameters such as pH, salinity, electrical conductivity (EC), total alkalinity, total hardness, ammonical-nitrogen (NH3-N) and salt/ionic concentration viz., sodium (Na+), calcium (Ca²⁺), magnesium (Mg²⁺), potassium (K⁺), chloride (Cl) and sulphate (SO₄²), were analysed fortnightly following the standard methods APHA (2017). At the end of the study period, growth of fish was recorded in terms of total length, total length gain, body weight, net weight gain, specific growth rate and condition factor (K-value). Three specimens from each treatment were sacrificed for the histopathological examination of gills. The gills of the most external gill arches were carefully removed and preserved in 10% neutral buffered formalin solution for 24 hr. Dehydrated gills were embedded in the paraffin block and cut into 6-8µm sections with the help of microtome, because thin gill filaments and lamellae gets damaged. The gill sections were stained with haematoxylin and eosin (H&E) for visualizing salinity induced structural changes. The water quality and growth data were subjected to One-Way Analysis of Variance (ANOVA) with the help of statistical package SPSS V. 16.0. Significant ANOVA was followed by Duncan's Multiple Comparisons to determine differences among treatments (p≤0.05).

Results and Discussion

The EC, total alkalinity, total hardness and salt concentration (Ca²⁺, Mg²⁺, Cl⁻, Na⁺, K⁺, SO₄²) of water in different treatments was in accordance to salinity increase from 0-10 %. Differences in respect to mean temperature in each treatment were insignificant (p≤0.05). However, significant changes with respect to pH and NH₃-N were recorded among the treatments (Table 2), however, the values were well within the acceptable limit for carps i.e. 7-9 for pH and <0.05 for NH₃-N (Boyd and Tucker 1998; Jena et al., 2002). During 120 days of study period, no significant changes were observed in the swimming activity of C. carpio up to 4 ‰ salinity (Table 3). However, swimming activity of fish decreased in 6, 8 and 10 % salinity after 80, 60 and 40 days of exposure, respectively. Further, fish became more sluggish in 8 and 10 % salinity treatments after 100 and 80 days of exposure. Likewise, salinity levels up to 4 ‰ did not affect feed intake (appetite) of fish throughout the study period. However, reduced appetite and loss of appetite was observed in 8 and 10 ‰ treatments after 60 and 90 days of salinity exposure, respectively. The morphological changes like hypersecretion of mucus, lesions/haemorrhages on the body and deformities of the fin were observed in the fish after 100 days of exposure to 8 and 10 % salinity (Table 3). Survival of the fish declined significantly at 8 % and 10 ‰ salinity levels (p≤0.05), while significant decline in fish

growth (total length gain, net weight gain and specific growth rate) was recorded at all salinity levels (2-10 ‰) and condition factor (K-value) of fish declined significantly only at 10 ‰ (Table 4).

Exposure to inland saline water also induced significant histological alterations in the gills of *C. carpio* (Fig. 1). At 2 ‰ salinity, visible changes appeared in the secondary lamellae, including thickening of the tips and disorientation (bending). At 4 ‰, clubbing of secondary lamellae at some parts of the gills was seen along with blood congestion. With increase in the number of mucous cells, the onset of lamellar oedema and separation of epidermis layer from the primary lamellae was also observed. At 6 ‰, hypertrophy of cellular components was visualized. Boundaries between the individual secondary lamellae were not visible, although some secondary lamellae were found to be

Table 1: Physico-chemical parameters of inland saline water collected from Village Shajrana District Fazilka (Punjab)

Parameters	Mean ± S.E. (p≤0.05)
Salinity (‰)	15±0.05
pH	7.28 ± 0.13
EC (mScm ⁻¹)	19.78 ± 0.33
Total Alkalinity (CaCO ₃ mgl ⁻¹)	1254.70 ±6.76
Total Hardness (CaCO₃ mgl⁻¹)	2320.00 ±15.27
Ca Hardness (CaCO ₃ mgl ⁻¹)	1242.50 ±1.55
Ca ²⁺ (CaCO ₃ mgl ⁻¹)	497.40 ±62.06
Mg ²⁺ (CaCO₃ mgl ⁻¹)	482.80 ±3.60
Cl (mgl ⁻¹)	1478.70±8.89
Na [†] (mgl ⁻¹)	1176.70 ±56.46
K⁺(mgl⁻¹)	85.26 ±2.24
SO ₄ ²⁻ (mgl ⁻¹)	50.50 ±7.08
NH ₃ -N (mgl ⁻¹)	0.36 ± 0.01

Table 2: Mean values of physico-chemical parameters of inland saline water in different treatments during the rearing period of 120 days

Parameters	0‰	2‰	4‰	6‰	8‰	10‰
рН	8.57±0.03 ^b	8.51±0.06 ^b	8.54±0.05 ^b	8.56±0.03 ^b	8.63±0.01°	8.64±0.15°
EC (mS cm ⁻¹)	13.03±0.55 ^f	36.77±0.74°	61.51±0.89 ^d	93.89±1.62°	126.50±1.89 ^b	173.86±3.04°
Total alkalinity						
(CaCO₃ mg l⁻¹)	251.3±5.42°	266.73±7.88°	309.6±9.01 ^d	357.5±10.67°	460.46±22.31°	420.56±18.72 ^b
Total hardness						
(CaCO ₃ mg l ⁻¹)	284.4±6.02 ^f	503.46±10.73°	746.46±17.03 ^d	876.6±22.61°	1139.74±29.72 ^b	1358.78±37.03°
$NH_3-N (mgl^{-1})$	0.025±0.001 ^d	0.029±0.001°	0.038±0.005°	0.032±0.002 ^b	0.032±0.001b	0.029±0.01°
Ca ²⁺ (CaCO ₃ mgl ⁻¹)	61.34±1.27 ^f	90.81±3.51°	111.62±7.29 ^d	130.92±8.01°	153.49±10.47 ^b	174.09±13.03°
Mg ²⁺ (CaCO ₃ mgl ⁻¹)	35.19±0.93 ^f	78.63±1.07°	121.46±2.64 ^d	130.61±2.89°	193.4±3.42b	238.69±6.71°
Cl ⁻ (mgl ⁻¹)	55.05±7.23 ^f	285.71±11.42°	565.19±19.53 ^d	896.21±12.63°	1010.33±9.78 ^b	1252.76±12.73°
Na ⁺ (mgl ⁻¹)	60.19±10.73 ^f	128.88±10.51°	277.28±21.02 ^d	385.56±39.47°	636.60±34.66 ^b	905.14±57.23°
K ⁺ (mgl ⁻¹)	8.73±0.15 ^f	9.956±0.27°	12.61±0.40 ^d	15.93±0.69°	19.30±0.82 ^b	23.54±1.01 ^a
SO ₄ ²⁻ (mgl ⁻¹)	15.38±0.01 ^d	26.17±0.13°	33.64±0.10 ^b	34.55±0.08 ^b	35.27±0.09 ^b	46.27±0.14°

Values are mean \pm S.E., n = 9 (3 from each replicate); Alphabetic subscripts (a, b, c...f) indicates significant differences within a row (p \leq 0.05)

Table 3: Behavioural changes in common carp, C. carpio reared in different salinity treatments

Behaviour	Days	Treatments						
		0 ‰	2‰	4 ‰	6 ‰	8 ‰	10 %	
Swimming activity	0	А	Α	А	Α	А	А	
	20	Α	Α	Α	Α	Α	Α	
	40	Α	Α	Α	Α	Α	LA	
	60	Α	Α	Α	Α	LA	LA	
	80	Α	Α	Α	LA	LA	S	
	100	Α	Α	Α	LA	S	S	
	120	Α	Α	Α	LA	S	S	
Feeding behavior	0	N	N	N	N	N	N	
•	20	N	N	N	N	N	N	
	40	N	N	N	N	N	RA	
	60	N	N	N	N	RA	RA	
	80	N	N	N	N	RA	RA	
	100	N	N	N	RA	RA	RA	
	120	N	N	N	RA	LA	LA	
Morphological changes	0	N	N	N	N	N	N	
	20	N	N	N	N	N	N	
	40	N	N	N	N	N	N	
	60	N	N	N	N	N	N	
	80	N	N	N	N	N	Υ	
	100	N	N	N	N	Υ	Υ	
	120	N	N	N	N	Υ	Υ	

Swimming Activity: A = Active, LA = Less Active, S = Sluggish; Feeding behaviour: N = Normal, RA = Reduced Appetite, LA = Loss of Appetite Morphological changes: N = No, Y = Yes

Table 4: Growth performance of common carp, C. carpio (L.) at different salinity levels

Growth parameter	0 ‰	2‰	4 ‰	6 ‰	8‰	10 ‰
Initial length (cm)	11.27° ±0.07	11.22° ±0.07	11.46° ±0.10	11.34°±0.10	11.25°±0.10	11.32°±0.08
Final length (cm)	13.38° ±0.24	12.45 ^b ±0.27	12.15 ^{bc} ±0.17	11.98 ^{bc} ±0.25	11.48°±0.19	11.40°±0.12
Initial weight (g)	17.33° ±0.33	16.44° ±0.29	17.33°±0.33	17.33°±0.33	17.11°±0.35	17.11°±0.35
Final weight (g)	34.44°±1.04	28.22 ^b ±0.52	23.33°±0.66	21.11 ^d ±0.75	19.33 ^{de} ±0.47	17.88°±0.42
TLG (cm)	2.11°±0.25	1.23 ^b ±0.23 (-42%)	0.68 ^{to} ±0.14 (-68%)	0.64°±0.19 (-70%)	0.23°±0.10 (-89%)	0.76°±0.02 (-64%)
NWG (g)	17.11°±0.94	11.77 ⁶ ±0.52 (-31%)	6.00°±0.66 (-65%)	3.77 ^d ±0.90 (-78%)	2.22 ^{de} ±0.40 (-87%)	0.77°±0.32 (-95%)
K-Value	1.45° ±0.07	1.49° ±0.10	1.30 ^{ab} ±0.05	1.25 ^{ab} ±0.09	1.29 ^{ab} ±0.07	1.15 ^b ±0.05
SGR(%)	0.57° ±0.02	0.45 ^b ±0.01 (-21%)	0.24°±0.02 (-58%)	0.16 ^d ±0.04 (-72%)	0.10 ^{de} ±0.01 (-82%)	0.03°±0.01 (-94%)
Survival (%)	100.00°±0.00	100.00°±0.00	100.00°±0.00	90.00° ±5.77	86.66 ⁶ ±3.33	70.0°±5.77

Values are mean \pm S.E., n = 9 (3 from each replicate); Alphabetic subscripts (a, b, c...f) indicates significant differences within a row (p \leq 0.05); Values in () indicates % change over control (+/-)

normal. Additionally, epithelial lifting, lamellar oedema and blood congestion in the vascular axis of the primary lamellae was observed. At 8 ‰, cellular hypertrophy, primary lamellae epithelial layer hyperplasia, secondary lamellae fusion, aneurysms and connective tissues hyalinization (extending towards the tips of the lamellae were observed. The lamellar size decreased with vasodilation and congestion of blood vessels. At 10 ‰, complete

fusion of the filaments was seen along with cellular hypertrophy, epithelial layer hyperplasia and blood congestion in the primary lamellae. Although, common carp belongs to the stenohaline group of freshwater species and grows well in a hypotonic environment, but has been reported to survive in the low salinity environment (Kasim 1983; Wang *et al.*, 1997; Ansal *et al.*, 2013; Islam *et al.*, 2014). In the present study, common carp was able to

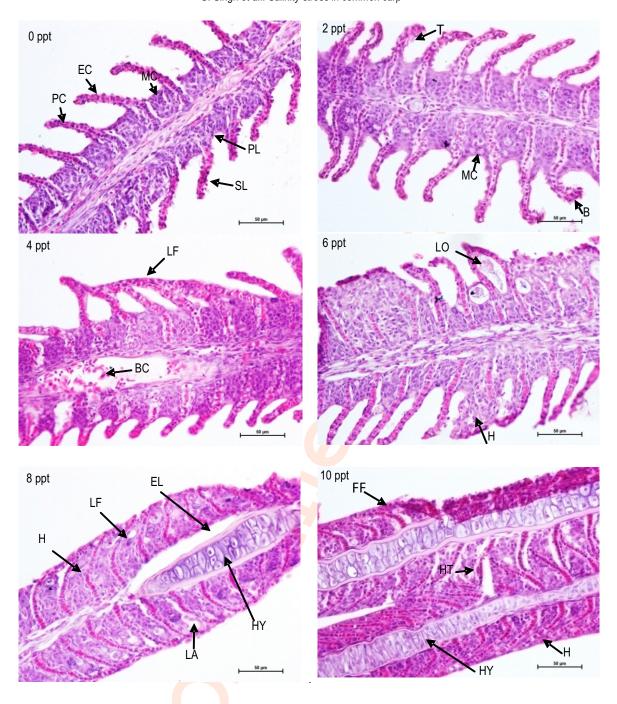


Fig. 1: Photomicrographs of gill of *C. carpio* reared in different salinity treatments (0% –10%). Showing Epithelial Cell (EC), Mucosal Cell (MC), Pillar Cells (PC), Primary Lamellae (PL), Secondary Lamellae (SL), Thickening of tips of lamellae (T), Bending of Lamellae (B), Lamellar Fusion (LF), Blood Congestion (BC), Lamellar Oedema (LO), Hyperplasia (H), Epithelial Lifting (EL), Lamellar Fusion (LF), Lamellar Aneurysm (LA), Filament Fusion (FF), Hypertrophy (HT), Hyalinisation (HY). H&E Stained, Magnification 40X.

tolerate 6 ‰ salinity for 120 days, without any significant effect on survival. The findings agree with an earlier report of 100% survival of *C. carpio* fingerlings after 60 days of rearing in 6 ‰ saline water (Mangat and Hundal, 2014) prepared from commercial-grade sodium chloride and calcium chloride salts. Apart from carps,

some other freshwater fishes have also been reported to survive well under saline conditions. Dubey and Trivedi (2016) reported 88.33% survival in spotted snakehead, *Channa punctatus* exposed to 10 % diluted seawater, while 100% mortality occurred at 20 % within 96 hr. Further, pangas catfish, *Pangasianodon*

hypophthalmus reared in inland saline water for 60 days, was able to survive up to 15 % salinity, while 100% mortality was observed at 20 % (Kumar et al., 2017).

Some stenohaline species can tolerate and grow in saline water if its internal salt concentration remains hypertonic. Once the isotonic concentration is crossed and fish becomes hypotonic to its external environment, reverse osmoregulatory changes force the fish to make unwanted physiological changes (Mustafayev and Mekhtiev, 2008). Although, common carp has the ability to adapt to salinity variations, but the degree of tolerance depends on the salinity level and exposure span. A long-term exposure to salinities above 6 ‰ appeared to have a more pronounced effect on fish movement, possibly due to exhaustive physiological compensations made to maintain its internal homeostasis. Normal appetitive behaviour indicates that fish body metabolism can still be managed or regulated, while reduced or complete loss of appetite is an indication of near or total body metabolic breakdown. Present study indicates that salinity levels \geq 6 % were near or outside the tolerance limit of the fish. Similar behavioural changes were observed by Mangat and Hundal (2014) in C. carpio during 60 days of rearing in 6% artificial saline water. Slimy appearance of fish due to excessive mucus production in saline water is attributed to adaptive changes or response to irritation caused by the enhanced concentration of salts in the water. Similar changes were reported by Thai silver barb, Barbodes gonionotus, exposed to NaCl and Diluted sea water (5-20 %) for 4 days (Akhter et al., 2009). Mucus provides primary defence in aquatic organisms by acting as a barrier between the organism and the environment (Dash et al., 2018) and salt application is a common health management practice in aquaculture to protect fish against pathogens through enhanced mucus production.

In the present study, growth of C. carpio fingerlings declined significantly at all salinity levels (2-10 %). Freshwater fish has evolved in a hypotonic environment with an ability to compensate for the passive loss of salts through active absorption and passive gain of water by excreting large volumes of dilute urine. Some of the species adapt and grow well in the saline environments; however, when the isotonic point is crossed, and the internal salt concentration of fish becomes hypotonic to the external environment, it compels the freshwater fish to make extensive physiological changes for osmoregulatory adjustments (Mustafayev and Mekhtiev 2008), leaving little energy for growth. Although, no similar supportive study with inland saline water is available for discussion, however some studies with seawater and artificial saline water are available for reference purpose. Wang et al. (1997) recorded significant decline in growth of C. carpio fingerlings during 92 days rearing in diluted sea water (0.5 to 14.5 %), while Mangat and Hundal (2016) reported reduced growth in C. carpio fingerlings during 60 days rearing in artificial saline water (1.5, 3, 6 and 12 % Nacl and CaCl₂). Further, Islam et al. (2014) recorded significantly reduced growth in another stenohaline freshwater carp species, Labeo rohita (rohu), when reared in artificial saline water (2-12 % NaCl) for 90 days. Growth of some other stenohaline freshwater species (other than carps) has also

been reported to decline under saline conditions. In spotted snakehead (C. punctatus), salinity up to 10 % did not affect growth performance of fish significantly (Dubey and Trivedi, 2016), while 90 days salinity exposure (4 and 8 %) reduced weight gain in fingerlings of an air breathing catfish, Clarias batrachus by 13% (Sarma et al., 2013). Earlier, Sahoo et al. (2003) also found C. batrachus to withstand a narrow range of salinity (2-4 %) with a significant decline in growth at 4 ‰ and weight loss at salinity levels above 6 ‰. Further, Kumar et al. (2017) found pangas catfish, P. hypophthalmus to tolerate and survive in 15 % inland saline water, but it grew well only up to 10 % salinity. Salinity tolerance and growth response of fresh water fish under salinity stress also varies with species and size of fish (Ansal et al., 2013; Gills and Payan, 2001). The present study reveals that although, C. carpio was able to tolerate and survive well in inland saline water up to 6 ‰, but fish growth was affected significantly at all salinity levels.

In the present study, salinity induced changes in the gills included lamellar fusion due to the proliferation of cells and thickening of gill filament epithelial layer, and epithelial lifting due to oedema. Oedema induced lifting of lamellar epithelium is a defence mechanism to create a barrier between the pollutant or toxicant (salinity in this case) and the bloodstream (Arellano et al. 1990). Other significant changes observed in the present study were aneurysms, clubbing of lamellae, hyalinization etc. All these changes resulted from rupturing of pillar cells and capillaries. In a euryhaline tilapia species Oreochromis niloticus, significant changes in the gills, such as chloride cell hypertrophy, epithelial lifting, structure alteration, telangiectasia and aggregation of cells of primary lamellae and aneurysms in secondary lamellae were observed at higher salinity levels (20 %), being more adaptive to saline water as compared to stenohaline species (Azevedo et al., 2015; Kucuk et al., 2013). Hassan et al. (2013) also reported lamellar lesions (necrosis) in tilapia exposed to higher salinity of 35 %. The lamellar degeneration impairs gas exchange across the gills, consequently leading to anoxic and acidic blood conditions (Ajani et al., 2007), which ultimately kills the fish. The present study suggests detrimental histological changes in the gills of C. carpio at salinity levels ≥6 % (Table 3,4). Although, longterm rearing (120 days) of common carp (C. carpio) fingerlings in inland saline water revealed 100% survival of fish up to 6 ‰ salinity, but significant decline fish growth was observed at all salinity levels 2-10 ‰. Salinity induced changes in the gills indicate that salinity levels ≥ 6 ‰ are not suitable for growth and health of fish on a long-term basis. The present study, hence, serves as a reference to develop suitable grow-out technologies for rearing C. carpio in inland saline water.

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experiment, evaluation of data and writing of manuscript; **A.H. Shanthanagouda:** Gave assistance in preparation of histological slide; **V. I. Kaur:** Provided the technical input **and N. Bansal:** Contributed in scientific evaluation of histological slides.

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