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Physiological, and Biochemical Responses of Shubunkin Goldfish, *Carassius auratus* (Linn.) Exposed to Inland Saline Groundwater

Deepa Bhatt, Vaneet Inder Kaur, Meera D. Ansal, Shanthanagouda, A. Holeyappa[1] and Neelam Bansal[2]

Department of Aquaculture, [1]Department of Aquatic Environment, [2]Department of Veterinary Anatomy
College of Veterinary Science, College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141 004, India
E-mail: deepabhatt94@gmail.com

***Abstract*:** Goldfish (*Carassius auratus*) are valued in ornamental aquaculture for their vibrant coloration and adaptability. This study examined their tolerance to inland saline water (ISW) and its effects on stress, growth, and survival. Over 120 days, goldfish were exposed to ISW, with an initial salinity of 12 ppt. Fish were conditioned and gradually acclimated to salinities of 0, 2, 4, 6, 8, and 10 ppt, increasing by 1 ppt every 2 hours. that salinity levels above 4ppt led to significant deviations in stress indicators, with marked changes in haematological, biochemical, and antioxidant responses at levels up to 10 ppt. Goldfish were able to adapt and grow in salinities up to 6 ppt, though levels below 4 ppt were optimal for health and performance. This research offers insights into goldfish adaptability in ISW, suggesting that salinities up to 6 ppt are manageable, with levels below 4 ppt most favourable, thereby supporting the potential of inland saline water in aquaculture.

***Keywords*:** *Carassius auratus*, Fish physiology, Growth, Goldfish, Inland saline water, Salinity

Salinization affects approximately 1,125 million hectares of global land, with about 76 million hectares impacted by human activities (Hossain 2019). This process occurs naturally but is often exacerbated by human factors, particularly in arid and semi-arid regions where practices such as excessive irrigation, waterlogging, and indiscriminate fertilizer use drive salt accumulation in soils (Verma et al., 2013). As agricultural productivity declines on these lands, they are increasingly being repurposed for inland saline aquaculture, providing an alternative use for degraded areas. Inland saline aquaculture leverages salt-affected regions to support fish farming, however, elevated salinity imposes physiological challenges for fish, impacting growth, survival, and metabolism. High salinity induces osmotic stress and ionic imbalances that can disrupt normal cellular processes, which are critical for growth and homeostasis (Khan 2019, Zhang et al., 2023). These stressors are compounded by the unique ionic profiles of inland saline water, which differ from seawater and require species to exhibit specific adaptive responses (Kim et al., 2017, Tian et al., 2020). Therefore, understanding which species can thrive in these environments is essential to optimize inland saline aquaculture practices. Freshwater species like goldfish, crucian carp, and molly have shown tolerance to saline conditions (Schofield et al., 2006, Singh et al., 2023). In Punjab, koi carp and Shubunkin goldfish have demonstrated adaptability to saline water, supporting potential for inland saline aquaculture in India (Bhatt 2018, Bhatt et al., 2018) and expanding inland aquaculture (Dhawan et al., 2010, Li et al., 2024). Goldfish (*Carassius auratus*) are highly adaptable to varying water conditions, making them ideal candidates for saline aquaculture. Their resilience offers a model for studying salinity's effects on growth and survival, with benefits for ornamental fish production and land rehabilitation. This study examines goldfish responses to different salinity levels in inland saline water to assess their viability in saline aquaculture.

Currently, no studies address the effects of inland saline water on the survival, growth, behavior, physiological responses, coloration, and stress responses of ornamental fish. Given the waterlogging and natural salinity issues in certain areas of North-Western India, it is essential to optimize rearing techniques for freshwater goldfish (*C. auratus* Linn.). Enhancing goldfish salinity tolerance could improve the socio-economic status of local farmers. Understanding how ISW's ionic composition differs from seawater and affects various fish species is also crucial. This study thus aims to assess the adaptability of goldfish to ISW by examining survival, growth, behavior, physiological responses, and coloration.

MATERIAL AND METHODS

Experimental environments: The study was conducted at the Instructional cum Research Farm, College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India (30°90'45.1"N, 75°80'16.4"E) over a four-month period. Inland saline water, stock water: 15 ppt was obtained from the salt-affected and waterlogged region

of Shajrana, District Fazilka, Punjab (30°33'53.7"N, 74°11'92.2"E). To ensure water quality, the stock water was continuously aerated for 15 days and subsequently filtered through muslin cloth (20 µm). The filtered water was then diluted with freshwater (borewell water) to achieve salinity levels of 2, 4, 6, 8, and 10 ppt, with freshwater (0 ppt salinity) serving as the control. A total of one hundred eighty goldfish (*C. auratus var. shubhunkin*) were procured from a local market and kept in indoor cemented tanks for a one-month conditioning period. During this time, the fish were gradually acclimatized to the experimental salinities by increasing the salinity by 1 ppt every 2 hours until the desired salinity levels were reached. After acclimatization, the fish were stocked in glass aquaria (50 liters capacity) at a density of 10 fingerlings per aquarium. The experiment involved six treatments (three replicates for each treatment), with salinity levels ranging from 2, 4, 6, 8, and 10 ppt. All aquaria were continuously aerated with a diaphragm air blower (RS-15000, 220-240 V/50Hz, 20 Watt, 0.024 MPa). The experiment was conducted following a completely randomized design (CRD) to ensure proper randomization of treatments. The goldfish were provided commercial feed (OPTIMUM: 28% crude protein, 4% crude fiber, 3% crude fat, and 10% moisture) ad libitum twice a day, at 9–10 AM and 4-5 PM. Before each feeding, any remaining feed and faecal matter were siphoned out to maintain water quality. Water samples for physico-chemical analysis were collected daily between 09:00 and 10:00 AM. The parameters measured included temperature, pH, electrical conductivity (EC), dissolved oxygen (DO), total hardness (TH), total alkalinity (TA), ammonia nitrogen (NH_3-N), and ionic composition (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, and SO_4^{2-}), using standard methods outlined in APHA (2012).

Behavioral and survival observations: Fish survival and behavior were monitored daily across all treatments. The survival rate was determined using the following formula (Sultana et al., 2018):

Survival rate (%) = (Number of fish alive / Number of fish stocked) × 100.

Behavioral responses were categorized based on swimming activity (very active, less active, erratic swimming), feeding behavior (high, moderate, low appetite), and threat response (normal, modest, weak). The swimming behavior was assessed by observing the fish's movement within the water column, while feeding response was determined by the amount of uneaten feed remaining at the bottom of the tank (Lawson and Alake 2011). Stress-related behavioral reactions were noted periodically throughout the 120-day salinity exposure.

Sampling and analysis: At the end of the 120-day experimental period, the fish were fasted for 24 hours. Following this, they were anesthetized using 0.4 mL L^{-1} of 2-phenoxyethanol (1 mL L^{-1}) administered via 26G x 1/2" (0.45 × 13 mm) syringes (Hindustan Syringes & Medical Devices Ltd.). After the fish were anesthetized, they were weighed and measured before being returned to their respective tanks. Blood samples were obtained from the caudal veins of three fish per tank using heparinized syringes. The collected blood was centrifuged at 4000 g for 3 minutes at 4°C to separate the serum. The resulting serum was stored at -80°C for subsequent analysis (Bhatt et al., 2024).

Growth performance

Total body length and weight: These were measured monthly following standard procedures (Halver 1957).

Specific growth rate (SGR, % weight gain day^{-1})

SGR= log(final body weight (g)- log(initial body weight (g)/ t (time interval in days) x 100

Feed conversion ratio (FCR) = feed intake (g)/ weight gain (g)

Weight gain (%) = Final body weight (g) − Initial body weight (g)/Initial body weight (g) x 100

Blood metabolic profile

Reagents and materials: Total erythrocyte count (TEC), total leukocyte count (TLC), hemoglobin (Hb), hematocrit (HCT), total protein, albumin, and glucose (Cat. #120235 ERBA Mannheim Kit) were measured from fish serum according to the reference manual provided by ERBA Lachema s.r.o. A 50 µL serum sample was analyzed using a biochemical analyzer (CHEM-7, ERBA, Mannheim), following the standard protocol. Additionally, antioxidant parameters, including superoxide dismutase (SOD), lipid peroxidation (LPO), and glutathione reductase (GR), were assessed based on standard methodologies (Nicholls 1962, Nishikimi et al., 1972, Placer et al., 1966).

Statistical analysis: The data was analysed using SPSS 20.0 software (IBM SPSS Inc., USA) with Duncan's multiple-range test.

RESULTS AND DISCUSSION

Water quality parameters: Na^+ and Cl^- were the dominant ions, with higher concentrations than other cations and anions ($Na^+ > Mg^{2+} > Ca^{2+} > K^+$ and $Cl^- > SO_4^{2-}$). Significant differences were observed in water quality and ionic composition (Table 1). Except for temperature, all parameters and ion concentrations increased with salinity, with the highest values at 10 ppt and the lowest at 0 ppt (control). There were no significant differences in the physico-chemical parameters across the experimental tanks. All parameters remained within acceptable ranges for fish culture, consistent with Akinrotimi et al. (2007b). Parameters such as electrical conductivity (EC), total

alkalinity (TA), total hardness (TH), and the ionic composition ($Na^+ > Mg^{2+} > Ca^{2+} > K^+$ and $Cl^- > SO_4^{2-}$) exhibited trends parallel to the stock inland saline water (12 ppt). However, elevated ammonia nitrogen (NH_3-N) levels at higher salinities could represent a major stressor for freshwater fish (Bhatnagar and Singh 2010). There was significant increase in fish mortality with rising salinity, peaking at the highest salinity exposure.

Growth parameters: The survival rates were 100% at 0ppt and 2ppt, they began to decrease from day 45 in treatments with higher salinity (Table 2). At the completion of the experiment, survival rates varied between 80 and 100%. Growth metrics—such as mean final total body length (cm), body weight (g), total length gain (TLG), net weight gain (NWG), and specific growth rate (SGR) also showed significant variations), underscoring the detrimental effect of elevated salinity on both survival and growth (Table 2). Maintaining the salinity at or below 2 ppt is ideal for the extended rearing of goldfish, as growth performance significantly declined at salinity levels above 8ppt over the 120-day trial period. Küçük (2013) also demonstrated similar tolerances and growth trends under increased salinity. The combined influence of temperature (14.2 to 30.5°C range) and salinity notably impacted survival rates during prolonged exposure. The goldfish are typically hardy, their optimal performance at lower salinities (2 ppt) reinforces their

Table 1. Mean physico- chemical parameters of stock ISW and different salinity treatments

Parameters	ISW (Stock) 12 ppt	0 ppt	2 ppt	4 ppt	6 ppt	8 ppt	10 ppt
Temperature (^OC)	29.33	24.06^a	24.14^a	24.01^a	24.0^a	24.10^a	24.07^a
pH	8.87	7.41^d	7.99^c	8.28^b	8.30^b	8.46^a	8.48^a
DO (mg/L)	5.50	9.13^a	8.47^b	8.19^c	7.99^c	7.19^d	6.49^e
EC (mS/cm)	17.92	0.84^f	5.94^e	8.82^d	10.78^c	13.45^b	17.22^a
TA ($CaCO_3$ mg/L)	254.7	260^e	279^d	295^c	300^{bc}	305^b	317^a
TH ($CaCO_3$ mg/L)	2316.7	364.60^f	1045.60^e	1550.50^d	2168.80^c	2250.80^b	2699.90^a
NH_3-N (mg/L)	0.21	0.06^c	0.15^b	0.14^b	0.14^b	0.14^b	0.18^a
Ca^{2+} ($CaCO_3$ mg/L)	360.2	91.23^f	115.00^e	141.30^d	195.70^c	233.70^b	281.10^a
Mg^{2+} ($CaCO_3$ mgl^{-1})	587.3	83.29^f	231.50^e	333.20^d	466.20^c	558.10^b	586.60^a
Na^{2+} (mg/L)	765.9	19.80^f	192.90^e	264.20^d	364.30^c	460.40^b	592.60^a
K^+ (mg/L)	91.1	0.84^f	16.21^e	24.91^d	39.73^c	58.35^b	79.56^a
Cl^- (mg/L)	3539.7	72.50^f	621.20^e	950.70^d	1571.70^c	2194.50^b	2854.90^a
SO_4^{2-} (mg/L)	87.1	5.80^d	38.56^c	43.58^c	60.21^b	68.50^b	81.79^a

Values with same superscripts in a row do not differ significantly ($p \leq 0.05$).

Table 2. Survival and growth of shubunkin goldfish, *C. auratus* (L.) in different salinity treatments

Parameters	Days	0 ppt	2 ppt	4 ppt	6 ppt	8 ppt	10 ppt
Av. ITBL (cm)	0	8.04^a	8.10^a	8.15^a	8.19^a	8.26^a	8.25^a
Av. FTBL (cm)	120	8.11^{ab}	8.19^{ab}	8.17^{ab}	8.23^a	8.01^{ab}	7.76^b
Av. IBW (g)	0	6.40^a	6.40^a	6.80^a	6.60^a	6.50^a	6.30^a
Av. FBW (g)	120	7.98^a	7.28^b	7.38^b	7.01^b	5.37^c	5.16^d
Survival (%)	0-30	100.0^a	100.0^a	100.0^a	100.0^a	100.0^a	100.0^a
	30-60	100.0^a	100.0^a	86.66^a	86.66^a	86.66^a	86.66^a
	60-90	100.0^a	100.0^a	86.66^a	86.66^a	80.00^b	60.00^b
	90-120	100.0^a	93.33.0^a	80.00^{ab}	80.00^{ab}	80.00^{ab}	60.00^b
TLG		0.07^a	0.09^a	0.02^{ab}	0.04^{ab}	-0.25^c	-0.48^c
NWG		1.58^a	0.89^{ab}	0.58^b	0.41^b	-1.14^c	-1.12^c
SGR		1.32^a	0.73^{ab}	0.48^b	0.34^b	-0.95^c	-0.93^c

Values with same superscripts in a row do not differ significantly ($p \leq 0.05$).
Av.= Average; ITBL= Initial total body length; FTBL= Final total body length,
IBW= Initial body weight, FBW= Final body weight, TLG = Total length gain; NWG= Net weight gain,
SGR = Specific growth rate

categorization as stenohaline freshwater fish (Luz et al., 2008). The reduction in growth at elevated salinities can likely be attributed to physiological and metabolic disruptions triggered by salinity stress (Mangat and Hundal 2014). Although goldfish could survive at a salinity of 8 ppt, their growth was compromised, likely due to a reduction in appetite and decreased feed conversion efficiency at these higher salinity levels (Luz et al., 2008).

Fish behavior was monitored through assessments of swimming activity, feeding responses, and morphological characteristics, including skin coloration, body fragility, and mucus secretion. Deviations from typical behavior, such as reduced activity, lethargy, diminished feeding responses, skin discoloration, increased fragility, and excessive mucus production, were observed at a salinity level of 6 ppt after 45 days (Table 3).

Blood Metabolic Profile

Haematological parameters: TEC, TLC and Hct showed a progressive increase with rising salinity levels from 0 ppt to 10ppt. Conversely, hemoglobin (Hb) concentrations were highest at 0 ppt and significantly decreased to their lowest at 4 ppt (Table 4). TEC, TLC, and Hct levels were observed to increase progressively with rising salinity from 0 to 10 ppt. Extended exposure to lower temperatures combined with elevated salinity brought about significant shifts in these physiological parameters, suggesting that stress induced by these conditions required compensatory adjustments to maintain homeostasis (Hosseini et al., 2011). Under salinity stress, heightened Hct levels often indicate water loss resulting from ionic imbalances between the fish's internal environment and the external conditions. Initially, during saltwater acclimation, Hct levels may increase but typically stabilize to pre-exposure values over time. Stress conditions can also elevate catecholamine release, which stimulates TEC production as a response to reduced oxygen levels, potentially resulting in increased TEC and Hct measurements. Typically, higher TEC correlate with increased Hb levels, essential for respiration. However, TEC rose with salinity, Hb levels declined, suggesting poor cell quality or reduced hemoglobin content per cell. This discrepancy likely indicates an adaptive response, with abnormal TEC formation possibly leading to anemia under salinity stress. TLC also increased at higher salinities, likely reflecting an immune response mediated by elevated cortisol (Gomes et al., 2003). These findings reveal adaptive shifts in respiratory and immune functions, underscoring the need for further study into physiological responses to salinity in fish.

Biochemical and antioxidant parameters: There was results reveal a significant decrease in serum blood glucose and total protein levels up to 8 ppt salinity, followed by a

Table 3. Behavioural and morphological responses in shubunkin goldfish, *C. auratus* (L.) in different salinity treatments during the experimental period

Behaviour	Days	0 ppt	2 ppt	4 ppt	6 ppt	8 ppt	10 ppt
Swimming activity	0-15	A	A	A	A	A	VA
	30	A	A	A	A	LA	LA
	45-75	A	A	LA	LA	LA	S
	90-120	A	A	LA	LA	S	S
Feeding response	0-45	HAp	HAp	Hap	HAp	Hap	Hap
	60-75	HAp	HAp	Hap	HAp	Hap	Lap
	90-105	HAp	HAp	Hap	HAp	Lap	Lap
	120	HAp	HAp	Lap	LAp	Lap	Lap
Colouration	0-30	NC	NC	NC	NC	NC	NC
	45	NC	NC	NC	NC	DC	DC
	60-120	NC	NC	NC	DC	DC	DC
Body fragility	0-30	NF	NF	NF	NF	NF	NF
	45-60	NF	NF	NF	HF	HF	HF
	75-120	NF	NF	HF	HF	HF	HF
Mucus	0-30	NM	NM	NM	NM	NM	NM
	45-120	NM	NM	NM	EM	EM	EM

Swimming Activity - A = Active, LA = Less Active, S = Sluggish
Feeding Response – HAp – High Appetite, LAp = Low Appetite
Colouration – NC = Normal colouration, DC = Dull colouration
Body fragility- NF = Normal fragility, HF = High fragility
Mucus- NM = Normal Mucus, EM = Enhanced Mucus

marked increase at 10 ppt (Table 4). Albumin and globulin levels displayed significant modulation across salinity levels, with maximum values at 10 ppt (Table 4). Correspondingly, the Alb/Glb ratio decreased up to 4 ppt, increased at 6 ppt, and then significantly declined at 8 ppt and 10 ppt (Table 4). Salinity stress may trigger glucogenolysis to meet energy demands, leading to elevated blood glucose and reduced liver glycogen. Huang et al. (2006) reported that increased salinity raises energy consumption, initially met through glucose and lipid metabolism, with proteins utilized when glucose is insufficient. Total serum protein decreased up to 8 ppt salinity, possibly due to altered amino acid metabolism and protein breakdown, as observed in common carp and rohu under transport stress. Albumin and globulin levels followed a similar trend, decreasing up to 4 ppt and increasing thereafter, indicating immune system activation. Elevated protein levels and reduced Alb/Glb ratios at higher salinity suggest enhanced defense mechanisms. The results highlight the adaptive capacity of goldfish at salinities of 2–4 ppt, where homeostasis is maintained, also reflected in optimal growth.

Among antioxidant parameters, superoxide dismutase (SOD) reached its highest levels at 6 ppt, while lipid peroxidation (LPO) and glutathione reductase (GR) peaked at 6 and 10 ppt, respectively. LPO and GR levels showed a significant increase (across all salinity treatments (Table 4). The activities of antioxidant enzymes (SOD, LPO, and GR) increased noticeably at higher salinity levels, indicating oxidative stress. This suggests that an imbalance between reactive oxygen species production and the antioxidant defenses of the fish occurs under these stressful conditions. These findings are consistent with prior studies (Lushchak and Bagnyukova 2006, Birnie-Gauvin 2017) which also reported elevated antioxidant enzyme levels as a response to environmental stressors, underscoring the importance of antioxidant mechanisms in protecting cells from oxidative damage.

Carotenoid analysis of fish skin and muscle: Carotenoid content demonstrated a significant reduction at salinity levels above 4 ppt, accompanied by a visible bleaching of yellow pigmentation in the skin. The increase in carotenoid levels was observed at 8 ppt, although no significant differences were detected between the 0 and 2 ppt treatments. At the higher salinity range (8–10 ppt), both skin brightness and carotenoid content declined further, intensifying the bleaching effect (Table 4).

Goldfish were able to preserve their normal coloration and carotenoid levels up to 2 ppt salinity, indicating that they could maintain their regular metabolic and physiological processes at lower salinity concentrations. However, as salinity increased beyond this threshold, metabolic

Table 4. Haematological, biochemical, antioxidant parameters and carotenoid content of shubunkin goldfish, *C. auratus* (L.) in different salinity levels at the completion of the experiment

Parameters	0 ppt	2 ppt	4 ppt	6 ppt	8 ppt	10 ppt
Haematological parameter						
Hb (g %)	5.83^{a}	4.50^{b}	3.50^{b}	3.56^{b}	3.53^{b}	3.60^{b}
Hct (%)	5.96^{d}	7.23^{c}	7.90^{b}	8.26^{b}	8.33^{b}	9.03^{a}
TEC ($x10^{6}$ mm^{3-1})	1.31^{e}	2.10^{d}	2.90^{c}	3.56^{b}	3.86^{ab}	4.46^{a}
TLC ($x10^{3}$ mm^{3-1})	3.32^{c}	3.40^{c}	4.04^{bc}	4.83^{b}	6.19^{a}	6.28^{a}
Biochemical parameters						
Glucose (g dl^{-1})	98.03^{b}	44.63^{d}	41.04^{f}	43.40^{e}	61.50^{c}	191.2^{a}
Protein (g dl^{-1})	4.24^{b}	3.75^{c}	1.97^{e}	2.85^{d}	3.74^{c}	9.10^{a}
Albumin (g dl^{-1})	1.88^{a}	1.09^{b}	0.45^{c}	1.12^{b}	1.15^{b}	1.13^{b}
Globulin (g dl^{-1})	2.35^{c}	2.65^{b}	1.52^{d}	1.73^{d}	2.59^{b}	7.97^{a}
Alb/Glb ratio (g dl^{-1})	0.79^{a}	0.41^{b}	0.29^{bc}	0.74^{a}	0.44^{b}	0.14^{c}
Antioxidant parameters						
SOD (U $mgHb^{-1}$)	0.15^{b}	0.20^{b}	0.22^{b}	0.50^{a}	0.40^{a}	0.47^{a}
LPO (nmol MDA G Hb^{-1})	0.10^{d}	0.14^{d}	0.40^{d}	2.07^{c}	3.22^{b}	4.43^{a}
GR (Mm l^{-1})	2.01^{e}	2.49^{d}	3.03^{c}	3.41^{b}	3.92^{a}	4.29^{a}
Carotenoid content						
Carotenoid (µg g^{-1}) 35. 74 (At initiation-0 day)	49.33	37.15	36.9	36.45	38.41	35.58

Values with same subscripts in a row do not differ significantly ($p \leq 0.05$)
TEC= Total erythrocyte count, TLC= Total leucocyte count, Hb= Haemoglobin, Hct= Haematocrit value, SOD= Superoxide dismutase, LPO= Lipid peroxidation and GR= Glutathione reductase

disruptions occurred, leading to a breakdown in physiological functions. These changes likely contributed to the observed decline in skin pigmentation, as well as reduced growth and survival. Lawson and Alake (2011), also observed that goldfish exposed to varying salinity levels experienced alterations in their coloration and overall health. The salinity-induced stress appears to have impaired pigmentation, which may have been a consequence of compromised physiological functions under higher salinity conditions.

CONCLUSION

This study highlights the adaptability of *Carassius auratus* to inland saline water, demonstrating that goldfish can grow and survive in salinities up to 6 ppt. However, optimal performance based on survival, growth, haematological, biochemical, antioxidant responses, and coloration was observed at salinity levels ≤4 ppt. These results suggest that maintaining salinity levels at or below 4 ppt is ideal for the long-term rearing of freshwater ornamental goldfish in inland saline water. the experiment was conducted under controlled laboratory conditions, further field-based studies are necessary to evaluate the effects of salinity in more dynamic, natural environments, accounting for additional ecological factors.

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