

ARTICLE

The Effect of Inland Saline Groundwater on Growth, Maturation, and Osmoregulation of Common Carp

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Abstract

A 90-day experiment was conducted on Common Carp *Cyprinus carpio* in eight rectangular earthen ponds (21 × 10 × 1.50 m) for testing the effect of four groundwater salinities (0, 5, 10, and 15‰). At the end of the experiment, 100% survival was observed in all of the treatment groups. Somatic growth and reproductive performance were significantly ($P < 0.05$) influenced by salinity. Both males and females showed a significantly higher gonadosomatic index ($24.46 \pm 4.11\%$ [mean \pm SE]) at 5‰ ($P < 0.05$) compared with the fish that were kept in freshwater ($15.92 \pm 2.43\%$). The gonadal osmolality and osmolytes of the seminal and ovarian fluids increased in proportion to salinity. Ova diameter was inversely related to salinity. The incidence of spawning at 5‰ was significantly higher than at 0, 10, and 15‰. The gonadosomatic index was also significantly higher ($30.15 \pm 3.44\%$) at 5‰ followed by 0, 10, and 15‰, respectively. We concluded that the optimum salinity for the maturation of Common Carp is 5‰.

The salinization of arable land and groundwater is a major threat to agriculture across the world, as it has adverse effects on ecology and production (Williams 2001). The main causes of soil salinization in both coastal and inland states are waterlogging, indiscriminate use of inorganic fertilizers, and overirrigation (Beresford et al. 2004). Globally, around one-third of the cultivable land is reported to be under the influence of salinity (Singh et al. 2017). In India alone, an estimated 8.62 million hectares are affected by soil salinization (Lakra et al. 2014). The chemical

composition of inland saline groundwater (ISGW) differs from that of natural seawater, typically being low in potassium (K^+) and high in calcium (Ca^{2+}), whereas magnesium (Mg^{2+}) is highly variable (Saoud et al. 2003). Fortifying inland saline water with these ions could rectify the ionic deficiency problem and allow its use for aquaculture. Inland saline aquaculture has been developed in several countries, and a number of practices have been developed to culture a range of euryhaline and stenohaline species (Saoud et al. 2003; Rahman et al. 2005).

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In India, freshwater fish such as goldfish *Carassius* spp., tilapia *Oreochromis* spp., and riverine catfish *Pangasianodon hypophthalmus* were identified as potential candidate species for inland saline aquaculture (Wang et al. 1997; Saoud et al. 2003; Kumar et al. 2017). Common Carp *Cyprinus carpio* is generally considered to be a freshwater stenohaline fish (occupying water with salinity <0.05‰) that grows rapidly at a temperature range of 23–30°C and has a broad range of thermo tolerance (Wang et al. 1997). Common Carp has been reported to have moderate tolerance to salinity changes (Kulijev and Agayarova 1984; Lam and Sharma 1985). Recent studies have reported relatively poor growth in Common Carp that are reared in saline waters (2–3‰) due to physiological changes (Ghelichpour et al. 2018; Ghelichpour and Mirghaed 2019). A few studies have reported that the osmolality of fish gonads is a signal of stress that interrupts gonadal growth (Li et al. 2012). Numerous previous studies have reported an adverse effect of salinity on the maturation of Mrigal Carp *Cirrhinus mrigala* and Striped Mullet *Mugil cephalus* (Rajender 2000; Baxevanis et al. 2004; Barman et al. 2005). Inland saline water has also been used for breeding a few stenohaline species such as Common Carp and Mrigal Carp (Rajender 2000; Malik et al 2018).

To use the inland saline water resources in India, Common Carp culture practices are being standardized (Malik et al. 2018). However, procuring Common Carp seed from long distances creates an economic burden for the farmers. In this regard, the development or refinement of hatchery technology for Common Carp that uses inland saline groundwater might be a feasible option. In this respect, the present study assessed the effect of different salinities of ISGW on the survival, growth, maturation, osmoregulation and eggs, and sperm quality of Common Carp.

METHODS

Experimental Fish

Common Carp fingerlings with a weight of 32 ± 2.50 g (mean \pm SE; \approx 2.5 months old) were procured from an earthen pond at Sampla, Haryana, India. The experimental fish were transported to the farm of the ICAR-Central Institute of Fisheries Education (CIFE) regional center, Rohtak, Haryana, India. The fish were acclimatized to four salinities (0, 5, 10, and 15‰) in four 1,200-L force-resin plastic tanks for 2–3 weeks, and there was no mortality during this period.

Experimental Design

The experiment was conducted in eight uniform nondrainable rectangular earthen ponds ($21 \times 10 \times 1.5$ m = 315 m³), following Mishra and Singh (2002), with a stocking density of 5,000/ha for a total of 105 fish per pond

stocked. The experimental set up consisted of three treatments—5, 10, and 15‰—and a fresh water as the control, in duplicates. The culture duration was 90 days. In preparation for the experiment, the ponds were manually cleared of weeds and pumped dry for 2 d, followed by the application of limestone at a rate of 250 kg/ha. Then, all of the ponds were filled with the required salinities by mixing the ISGW at 15‰ salinity with freshwater. The values for salinity (refractometer; S/Mill-E, ATAGO, Tokyo), pH (digital electronic pH meter), dissolved oxygen (Winkler's method), and temperature (mercury thermometer) were measured twice daily in all of the experimental groups.

The concentration of un-ionized ammonia was measured spectrophotometrically at 635 nm (phenate method), total alkalinity (titrimetric method by titrating against standard H₂SO₄) and total hardness (titrimetric method by using Eriochrome Black T) were observed twice per week by following the standard methods (APHA 2012). The concentrations of Na⁺ and K⁺ were analyzed by means of a flame photometer (Model 1382, ESICO, Parwanoo India). The fish were fed with commercial fish feed (CP AQUA, Chennai, India) with a crude protein content of 32% at an estimated rate of 2% of the body weight twice daily. The proximate analysis (Table 1) of the diet was measured with standard methods (AOAC 2012).

Growth Performance

The growth parameters (initial and final body weight, specific growth rate, weight gain percentage, feed efficiency ratio, feed conversion ratio, and protein efficiency ratio) were recorded every 15 d during the 90-d culture period. For sampling, the fish were captured by using a drag net and they were weighed ($n = 10$) in the field by using an electronic weighing balance to within 0.001 g. In each sampling, a total of six fish (3 female fish and 3 male fish) were collected for growth parameter estimation. Based on weight, the following growth parameters were analyzed as follows by using the formulae given by Tan et al. (2017):

$$\text{Percentage weight gain (WG\%)} \\ = \frac{\text{final weight (g)} - \text{initial weight (g)}}{\text{initial weight (g)}} \times 100$$

$$\text{Specific growth rate (SGR\%)} \\ = \frac{\ln(\text{final weight}) - \ln(\text{initial weight})}{\text{number of days}} \times 100$$

$$\text{Feed conversion ratio (FCR)} \\ = \frac{\text{feed given (dry weight)}}{\text{body weight gain (wet weight)}}$$

$$\text{Feed efficiency ratio (FER)} = \frac{\text{body weight gain (wet weight)}}{\text{feed given (dry weight)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{body weight gain (wet weight)}}{\text{crude protein fed}}$$

$$\text{Survival rate (\%)} = \frac{\text{total number of animal harvested}}{\text{total number stocked}} \times 100$$

Gonadosomatic Index

The fish were sampled at days 15, 30, 45, 60, 75, and 90 during the experimental period. The specimens were sexed by observing an elongated body, concave shape of the genital opening, roughness of pectoral fins later, and out white milt oozing from the genital opening in males. Females were identified by observing a genital opening that bulges out, a distinct convex surface, and a swollen belly. To collect the gonadal tissues from each pond, both male ($n = 3$) and female ($n = 3$) fish were euthanized before dissection by using clove oil at 50 $\mu\text{L/L}$ (Misra et al. 2006). The maturity of both the male and female fish was measured by calculating a gonadosomatic index (GSI). The GSI values were determined from the ratio of total body weight and gonadal weight.

Egg and Sperm Quality

Gonadal osmolality of Common Carp.—Osmolality (mOsmol/kg) of oocytes and testis was estimated (at 90 d) with a cryoscopic osmometer (Osmomat 030, Gonotec, Berlin). The oocytes and testis were blotted dry on lint-free filter paper to remove the external fluid. The gonads were macerated and centrifuged at $14,000 \times g$ for 5 min at 4°C (Mandal et al. 2017). Later, 10 μL of the supernatant was used for the analysis. The osmotic pressure was given in mOsmol/kg.

Blood serum, seminal, and ovarian plasma characteristics.—Samples of blood serum, seminal, and ovarian plasma ($n = 3$) were extracted (for 24 h at 4°C) in the Eppendorf tubes by using ice-cold 6% trichloroacetic acid. The extracts were then centrifuged (at $10,351 \times g$ for 10 min at 4°C) and the supernatants that were used for the analysis of the ionic concentrations (of Na^+ , K^+ , Mg^{2+} , Ca^{2+} , and Fe^{2+}) were further subjected to acidic digestion using a microwave digestion system (Anton Parr, Ashland, Virginia). Around 5 mL of concentrated HNO_3 , 1 mL of HCL, and 500 μL of supernatant were added to each microwave digestion vessel. The digested samples were diluted with 25 mL of triple-distilled water and additionally exposed to minerals study through inductively coupled

plasma-atomic emission spectroscopy (SPECTRO Analytical Instruments GmbH, Kleve, Germany; Thompson and Walsh 1989) at the Indian Institute of Technology, Mumbai, India.

Ova diameter.—The ova diameter was measured using a stage micrometer in conjunction with a microscope that was equipped with a digital camera. The striped eggs that were collected from different females were mixed, and 10 ova were taken to measure the diameter.

Absolute fecundity.—The fecundity of the fish was calculated by the gravimetric method. Absolute fecundity was calculated (after 90 d) as per the following formula:

$$\text{Absolute fecundity} = \frac{\text{number of eggs counted in the sample} \times \text{weight of both ovaries}}{\text{weight of the sample (g)}}$$

Statistical analysis.—One-way analysis of variance (ANOVA) using statistical package SPSS version 22.0 was used for the data analysis at each time interval. The significant differences between the treatments were found by using Duncan's multiple range test.

RESULTS

Water Quality

The water quality parameters were all suitable for culture of Common Carp among the different treatment groups (Table 2). No significant variation was observed in dissolved oxygen (6.6–6.7 mg/L), pH (7.5–7.8), temperature (29.2°C), alkalinity (207–319 mg/L), un-ionized ammonia (0.07–0.10 mg/L), and nitrite nitrogen (0.001–0.006 mg/L) during the experimental period.

Growth Performance

No mortality of Common Carp was observed in any of the treatment groups (Table 3). The percentage of WG and SGR was significantly different ($P < 0.05$) among the treatments, and the highest values were observed in freshwater, followed by 5‰ water (Table 3). The lowest values of WG and SGR were observed at 15‰, compared with the other treatments. No significant difference was found in FCR, FER, and PER (Table 3).

Gonadosomatic Index

A significant difference ($P < 0.05$) was observed in the GSI of Common Carp among different treatment groups. At day 90, the mean GSI of male fish was significantly higher at 5‰ than at 10‰ and 15‰. The treatment group at the salinity of 15‰ showed the lowest values for GSI (Figure 1). At the end of the experiment, the GSI for female fish at 5‰ was not different from that for female

TABLE 1. Proximate composition of experimental diet (% dry matter basis). The values are expressed as mean \pm SE ($n = 3$). The abbreviations are as follows: CP = crude protein, EE = ether extract, NFE = nitrogen free extract, CF = crude fiber, and DE = digestible energy (kcal/100 g) = (% CP \times 4) + (% EE \times 9) + (% NFE \times 4) (Halver 1976).

CP (%)	EE (%)	NFE (%)	CF (%)	Moisture (%)	DE (kcal/kg)
31.91 \pm 0.24	4.03 \pm 0.26	49.41 \pm 0.36	3.86 \pm 0.24	10.76 \pm 0.14	361.64 \pm 2.68

TABLE 2. Physico-chemical parameters for maturation of Common Carp at different salinities. Values with different lowercase letters in the same row differ significantly ($P < 0.05$), and values are expressed as mean \pm SE ($n = 180$).

Water quality parameters	Freshwater	5‰	10‰	15‰
Dissolved oxygen (mg/L)	6.6 \pm 0.30 z	6.7 \pm 0.40 z	6.6 \pm 0.30 z	6.6 \pm 0.40 z
Temperature ($^{\circ}$ C)	29 \pm 1.60 z	29 \pm 1.60 z	29 \pm 1.60 z	29 \pm 1.20 z
Free CO ₂ (mg/L)				
Ph	7.8 \pm 0.12 z	7.8 \pm 0.10 z	7.5 \pm 0.13 z	7.8 \pm 0.16 z
Hardness (mg/L)	505 \pm 58.40 x	1,362 \pm 249.40 y	1,905 \pm 281.80 zy	2,452 \pm 112 z
Alkalinity (mg/L)	207 \pm 15.5 z	279 \pm 12.5 z	302 \pm 14.7 z	319 \pm 20.10 z
Ammonia-N (mg/L)	0.07 \pm 0.02 z	0.08 \pm 0.01 z	0.07 \pm 0.02 z	0.10 \pm 0.01 z
Nitrite-N (mg/L)	0.002 \pm 0.01 z	0.001 \pm 0.02 z	0.004 \pm 0.02 z	0.006 \pm 0.02 z
Calcium (Ca ²⁺) (mg/L)	56 \pm 5.30 y	125 \pm 28.20 z	133 \pm 20.40 z	146 \pm 8.80 z
Magnesium (Mg ²⁺) (mg/L)	86 \pm 13.40 x	218 \pm 49.71 y	445 \pm 27.21 z	491 \pm 45.81 z
Potassium (K ⁺) (mg/L)	3.45 \pm 0.30 y	4.73 \pm 0.50 y	8.40 \pm 0.50 z	9.08 \pm 0.21 z
Sodium (Na ⁺) (mg/L)	298 \pm 12.50 w	902 \pm 127.70 x	1,798 \pm 50.50 y	2,595 \pm 55.30 z

fish in freshwater but was significantly greater than that of fish that were in the 10‰ and 15‰ treatments (Figure 2).

Gonadal Osmolality

The osmolality was observed to increase with an increase in salinity (Figure 3). The highest values for osmolality were observed at 15‰ for both male and female fish. In all of the treatment groups, female fish showed high osmolality values compared with male fish.

Alterations in Blood Serum and Inorganic Osmolytes in Seminal and Ovarian Fluid in Relation to Salinity

The ionic concentration of blood serum of both males and female fish increased linearly with salinity (Table 4). Among all of the serum osmolytes, the Na⁺ ion concentration was significantly ($P < 0.05$) higher than that of the other ions (Mg²⁺, Ca²⁺, and Fe²⁺). A significantly ($P < 0.05$) high level of Na⁺ was found at 15‰ (3,163 \pm 21.85 mg/L), and the lowest occurred in the control group (2,427 \pm 20.27 mg/L). A similar pattern was observed in rest of the treatment groups in both males and females.

The sodium ions (Na⁺) in seminal and ovarian plasma exhibited significant differences ($P < 0.05$) at different salinities (Figures 4 and 5). The concentration of Na⁺ was the highest found of the osmolytes in all of the treatments in both the seminal and ovarian plasma. Concentrations of the Fe²⁺ ion were found to be lowest among the

treatment groups. The results revealed a linear relationship between salinity and the concentrations of the different osmolytes in the seminal and ovarian plasma samples.

Ova Diameter

A number of distinct variations were found in the oocytes of Common Carp at various developmental stages throughout oocyte measurement. The oocytes count among the females that were reared at 5‰ was significantly greater than that in the other three groups on day 90. The frequency distribution for ova diameter decreased significantly ($P < 0.05$) with increases in salinity (Figure 6).

Absolute Fecundity

A significant difference ($P < 0.05$) was found in absolute fecundity in relation to variations in salinity (Figure 7). The highest absolute fecundity was recorded at 5‰ (54,277) followed by the control (38,356), 10‰ (21,729), and 15‰ (8,657) groups.

DISCUSSION

The present study was carried out to assess the influence of salinity on the growth, maturation, and osmoregulation of Common Carp. Many studies have been conducted to investigate the effect of salinity on freshwater fish species and reported higher production (8‰) in

TABLE 3. Comparative analysis of survival and growth performance of Common Carp at four salinities for 90 days at 27–29°C. The abbreviations are as follows: WG% = weight gain percentage, SGR = specific growth rate, FCR = feed conversion ratio, FER = feed efficiency ratio, PER = protein efficiency ratio. Values with different lowercase letters in the same column differ significantly ($P < 0.05$) and are expressed as mean \pm SE ($n = 10$).

Treatment	Survival (%)	Initial weight (g)	Final body weight (g)	WG%	SGR	FCR	FER	PER
Freshwater	100 \pm 0.00 z	182 \pm 24.30 z	3,706 \pm 248 z	1,961 \pm 139.23 z	1.46 \pm 0.03 z	0.62 \pm 0.18 z	1.61 \pm 0.04 z	5.03 \pm 0.14 z
5‰	100 \pm 0.00 z	200 \pm 33.40 z	3,385 \pm 60.0 z	1,646 \pm 321.03 zy	1.37 \pm 0.08 z	0.67 \pm 0.96 z	1.51 \pm 0.21 z	4.77 \pm 0.67 z
10‰	100 \pm 0.00 z	171 \pm 3.40 z	2,425 \pm 110 y	1,320 \pm 63.41 zy	1.18 \pm 0.03 zy	0.66 \pm 0.21 z	1.51 \pm 0.04 z	4.73 \pm 0.15 z
15‰	100 \pm 0.00 z	173 \pm 2.59 z	1,835 \pm 140 y	965 \pm 126.06 y	1.13 \pm 0.04 y	0.75 \pm 0.04 z	1.33 \pm 0.72 z	4.17 \pm 0.22 z

TABLE 4. Serum ion concentrations for male and female Common Carp at different salinities (%). Values with different lowercase letters differ significantly ($P < 0.05$), and values are expressed as mean \pm SE ($n = 3$).

Treatments	Na ⁺		K ⁺		Ca ²⁺		Mg ²⁺		Fe ²⁺	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Freshwater	2,427 \pm 20.3 w	2,721 \pm 17.4 w	22.33 \pm 1.5 w	70.66 \pm 1.8 x	25.66 \pm 1.2 w	91.00 \pm 1.0 x	4.66 \pm 0.4 x	29.36 \pm 0.7 w	4.00 \pm 0.1 w	8.13 \pm 0.2 w
5‰	2,950 \pm 20.8 x	3,107 \pm 8.8 x	167.66 \pm 4.6 x	172.0 \pm 1.2 y	56.00 \pm 1.2 x	95.00 \pm 0.5 x	28.6 \pm 0.8 y	40.0 \pm 0.6 x	6.03 \pm 0.2 x	11.0 \pm 0.1 x
10‰	3,053 \pm 27.3 y	3,172 \pm 6.0 y	265.0 \pm 2.9 y	180.33 \pm 2.6 y	73.00 \pm 1.2 y	116.6 \pm 5.7 y	38.0 \pm 0.5 z	46.26 \pm 0.5 y	8.46 \pm 0.3 y	11.9 \pm 0.1 y
15‰	3,163 \pm 21.9 z	3,357 \pm 28.5 z	332.66 \pm 6.4 z	343.33 \pm 6.6 z	109.3 \pm 2.3 z	190.33 \pm 0.8 z	38.3 \pm 0.9 z	52.30 \pm 0.7 z	10.7 \pm 0.4 z	13.7 \pm 0.4 z

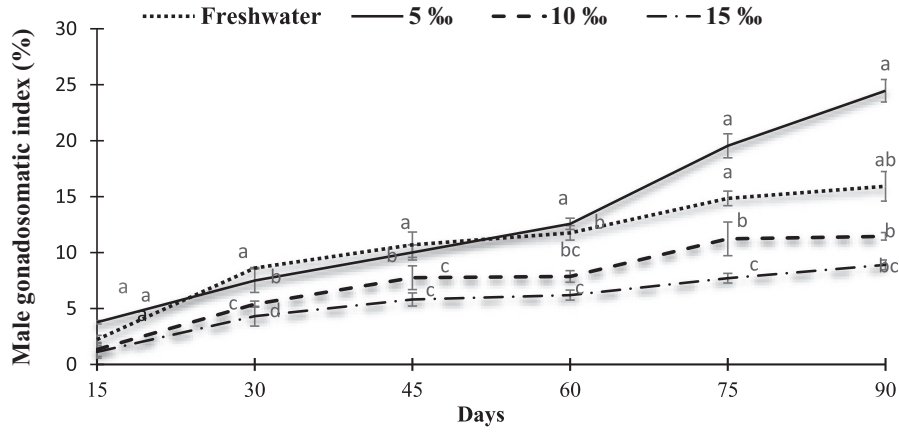


FIGURE 1. Effect of four different salinities on male gonadosomatic index of Common Carp. The data are expressed as mean \pm SE ($n = 3$). At each time interval, different superscripts signify statistical differences ($P < 0.05$).

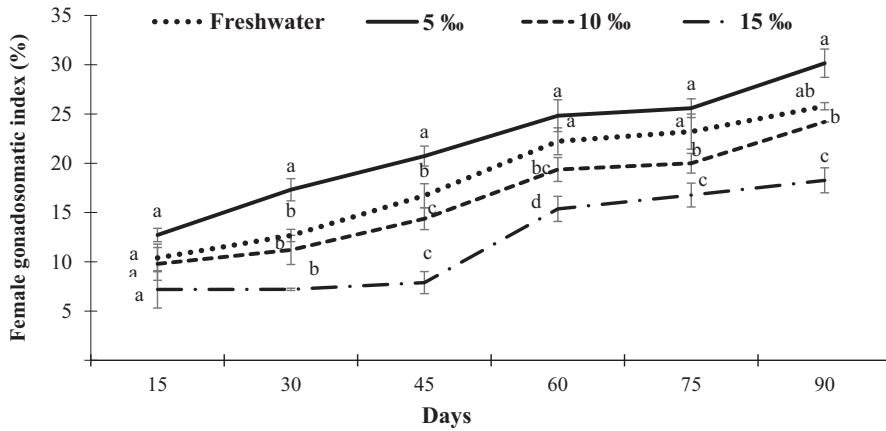


FIGURE 2. Effect of four different salinities on female gonadosomatic index of Common Carp. The data are expressed as mean \pm SE ($n = 3$). At each time interval, different superscripts signify statistical differences ($P < 0.05$).

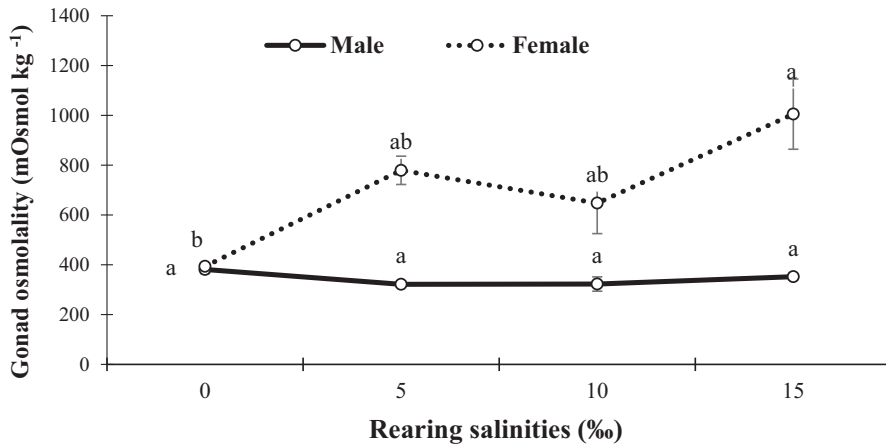


FIGURE 3. Effect of four different salinities on gonadal osmolality of Common Carp at day 90 of experiment. The data are expressed as mean \pm SE ($n = 3$). Different superscripts signify statistical differences ($P < 0.05$).

ISGW (Lam and Sharma 1985; Sawant et al. 2001; Dhawan et al. 2010; Imanpoor et al. 2012; Kucuk 2013). Malik et al. (2018) reported that Common Carp can survive in salinity within the range of 0–10‰. In the present study, the results revealed that Common Carp can tolerate higher salinity (15‰), but better body growth and maturation performance occurred at 5‰. Jana et al. (2006) reported a positive effect of salinity on the nutritional physiology and growth performance of brackish water fish species (Milkfish *Chanos chanos*) up to a certain salinity. The present study revealed a significant difference in growth at various salinity levels, and it was high among fish in the control (freshwater) and 5‰ treatment groups. Above this salinity level, growth declined linearly. Furthermore, no mortality was observed in the treatment groups even up to 15‰. Many studies have been conducted to investigate the effect of salinity on freshwater fish species (Goldfish *C. auratus*, Crucian Carp *C. carassius*, Common Carp, and Zebrafish *Danio rerio*) and reported higher production (at 8‰) in ISGW (Lam and Sharma 1985; Sawant et al. 2001; Dhawan et al. 2010; Imanpoor et al. 2012; Kucuk 2013).

For understanding maturation, GW and GSI have been used widely as the most reliable indicators of maturation (Lawson and Olagundoye 2011). An adverse effect of salinity on the maturation of freshwater fishes has been reported by many researchers (Rajender 2000; Baxevanis et al. 2004; Barman et al. 2005). Rajender (2000) reported better maturation among fish at higher salinity (10‰) rather than among freshwater fish Mrigal Carp. Apart from this, a few studies have also been conducted on effects of salinity in salt-affected areas on the growth and maturation of Black Bream *Acanthopagrus butcheri* (Doupe et al. 2005) and brine shrimp *Artemia* spp. (Baxevanis et al. 2004). In the present study, maturation was higher among the fish at 5‰ than among those at 0‰. However, there is lack of information on the maturity stages of freshwater fish in ISGW at salinities up to 15‰; thus, the current study is the first report on the effects of salinity on growth and maturation of Common Carp.

It is well established that parameters such as an increase in ova diameter, histological changes, and GSI are helpful in identifying the breeding season, as the ovaries of gravid females and testis of males increase in size due to hyperplasia and hypertrophy just prior to spawning. The growth of somatic and gonadal tissues represents the stages of maturation and reproduction in fish, which is in accordance with the findings of the current study. The results of the present study indicate that Common Carp (2.5 months old) attain maturity within three months of rearing at 5‰ salinity (90 d). The current study also revealed that females have more adaptability to grow early in terms of somatic growth and gonadal maturity

than males in ISGW at different salinities. Common Carp have shown effects on reproduction and maturation due to fluctuations of water temperature, photoperiod (Davies and Hanyu 1986), and different regions (Sivakumaran et al. 2003). However, research on the effect of salinity on somatic growth, enzymatic activity, survival, and other variables for the brackish water species Pacific white shrimp *L. vannamei* and Striped Mullet in ISGW were carried out by Barman et al. (2005). The size of the oocytes in the freshwater teleost changes to some extent during the process of maturation due to the water influx. Generally with the progress of maturation the size of oocytes increases as a result of hydration. However, in the current study ova diameter decreased significantly with an increase in salinity. M. A. Amer, A. M. Akar, O. Saleh, and A. E. Eissa (paper presented at the Egyptian Society for Animal Reproduction and Fertility 21st Annual Congress, 2009) detailed that fish reproduction is influenced by many environmental conditions such as salinity that enhances the breeding performance either by placing them in suitable environment or controlled by hormonal or stimulating factors. In the Sumatra Barb *Barbus tetrazona*, the diameter of the oocytes increased from 0.85 to 0.90 mm (5.9%); and in a Three-lined Rasbora *Rasbora trilineata*, from 0.74 to 0.80 mm (8.1%; Greeley et al. 1986). Finn (2007) reported an increase in the size of prehydrated oocytes resulting from hydration in Atlantic Halibut *Hippoglossus hippoglossus*. Similarly, in the current study a significant effect of different salinities on spawning behavior was detected in Common Carp. The present study revealed a significantly higher quality of eggs in terms of fecundity, stripping response, and quantification of eggs at 5‰, followed by freshwater and subsequently lowest at 15‰. Similar findings were reported by Malik et al. (2018) wherein the highest fecundity and hatching occurred at salinity 0–10‰ in marine or brackish water.

In the current study, it was established that the gonadal osmolality of both sexes was significantly different ($P < 0.05$) among the treatment groups. The highest gonadal osmolality was verified in the 15‰ treatment groups of both male and female fish, and the lowest osmolality was observed in the freshwater group. In addition to this, significantly higher osmolality was found in ripened females compared with matured males. The results showed a gradual increase in gonadal osmolality (osmotic pressure) with increased salinities. Usually, osmolality is affected by the fluctuation of the salinity medium (Gong et al. 2004), not by dietary supplementation or the ions profile (Jahan et al. 2018). It results from the loss of body water content due to the difference in osmotic pressure. To mitigate the osmotic pressure, organisms spend considerable energy in maintaining the higher plasma ion concentrations, which may lead to a reduction in growth and maturation rates in

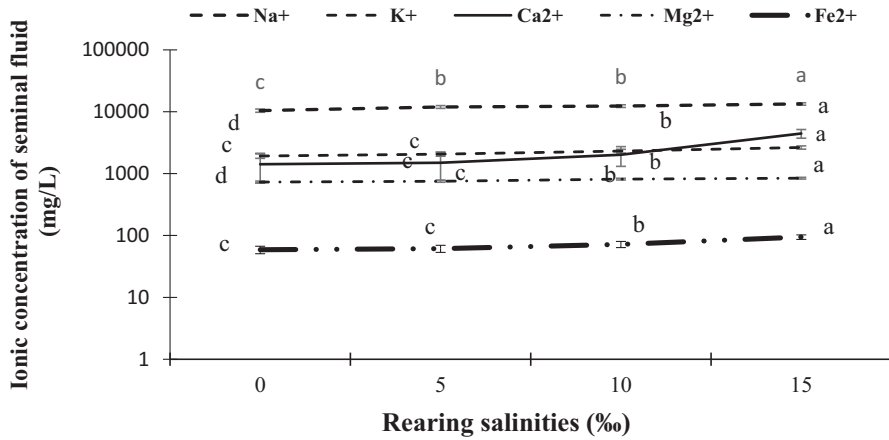


FIGURE 4. Effect of four different salinities on osmolytes of seminal fluid of Common Carp at different salinity levels (at 90 days). The data are expressed as mean ± SE ($n = 3$). For each ion, different superscripts signify statistical differences between salinities ($P < 0.05$).

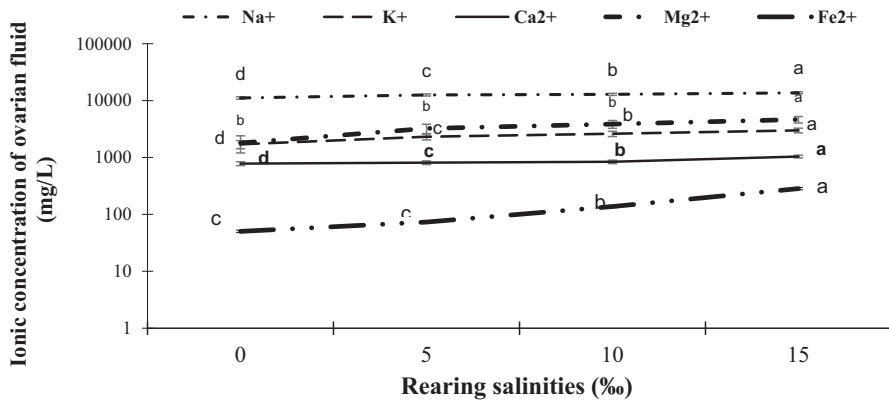


FIGURE 5. Effect of four different salinities on osmolytes of ovarian fluid of Common Carp at different salinity levels (at 90 days). The data are expressed as mean ± SE ($n = 3$). Different superscripts signify statistical differences ($P < 0.05$).

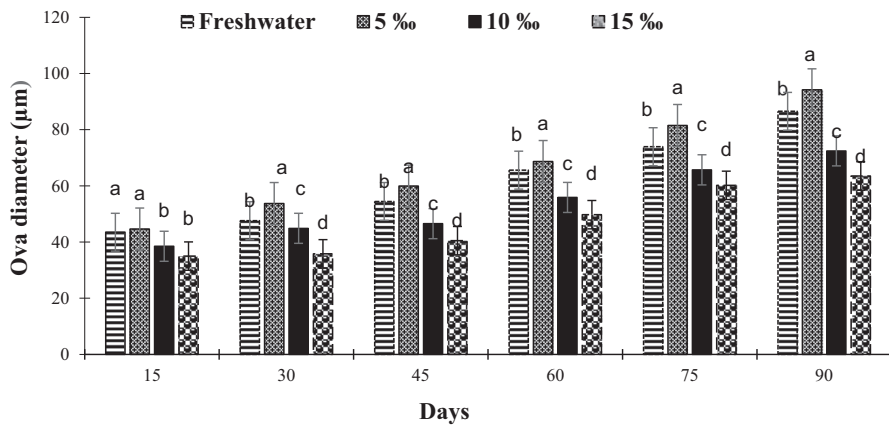


FIGURE 6. Effect of four different salinities on ova diameter (μm) of Common Carp at regular intervals of maturity. The data are expressed as mean ± SE ($n = 10$) (total number of eggs) at each time interval, and different superscripts signify statistical differences ($P < 0.05$).

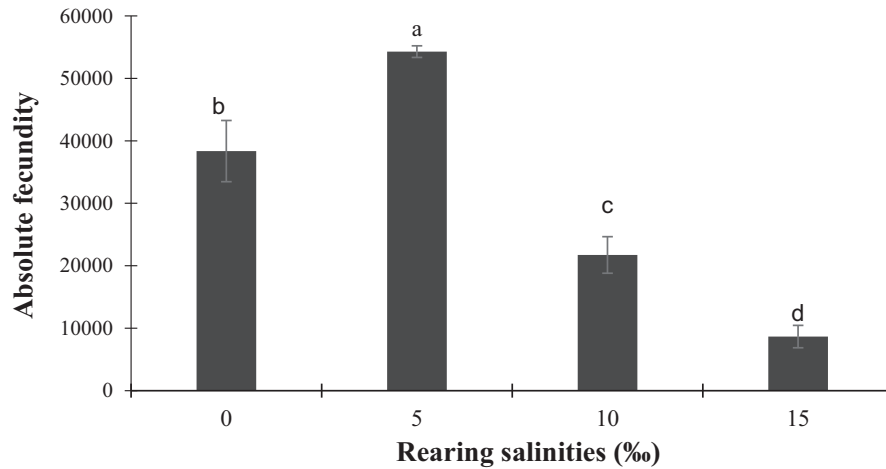


FIGURE 7. Effect of four different salinities on absolute fecundity of Common Carp at day 90. The data are expressed as mean \pm SE ($n=3$). Different superscripts signify statistical differences ($P < 0.05$).

fish—thus, the observed lower growth and maturation rate of Common Carp in the treatment group at 15‰ salinity compared with the treatment groups in freshwater and 5‰ salinity. Fish in an isotonic medium have the lowest metabolic rates; whereas, osmoregulation in seawater seems to be dynamically more costly than freshwater, as is predictable from the NaCl gradient amid the internal and external medium (Boeuf and Payan 2001).

The inorganic ionic content (osmolytes) of the seminal and ovarian plasma increased significantly with increases in salinity during the maturation period, and this may be the reason for the hyperosmolality and consequent water influx. The main reason for water uptake by oocytes both in vivo and in vitro is the influx of potassium ions, and to a lesser extent, of sodium ions (Greeley et al. 1991; Wallace et al. 1992). However, the gradual increase in the osmolytes of the seminal and ovarian fluid with increases in salinity was caused by the increasing osmoregulatory energy expenditure that is related to the regulation of ions under this salinity circumstance, causing a reduction in the growth and maturation rates in fish. Seminal fluid provides an ionic environment that helps to maintain the viability of spermatozoa, and it subsequently facilitates external fertilization when it is released into the water with eggs (Ciereszko 2008). Potassium and sodium ions may serve as the main or even the only osmolytes that provide an influx of water into the oocytes of fish that produce benthic eggs (Greeley et al. 1991). For instance, in Banded Killifish *Fundulus diaphanus*, both potassium and sodium ions participate in water uptake by oocytes maturing in vitro (Greeley et al. 1991; Wallace et al. 1992). The concentration of sodium ions increased during the maturation period, while the potassium ion concentration increased only until the germinal vesicle breakdown stage.

Therefore, a lower growth and maturation rate of Common Carp was observed at a higher salinity (15‰).

Stress because of salinity alteration can also change the concentration of serum ions and immune mechanisms (Gabriel et al. 2011). We observed that Common Carp is a stenohaline species, even though it is able to tolerate salinity fluctuations. The present study showed that Common Carp are able to maintain the mechanisms for osmotic and ionic regulation to maintain homeostasis in the body up to 15‰ salinity. Similarly, East Java strain of Nile Tilapia *Oreochromis niloticus*, a euryhaline brackish water species, showed salinity tolerance capability (Soegianto et al. 2017).

CONCLUSION

The present study indicates that rearing fingerlings at 5‰ salinity is most suitable for the growth and maturation of Common Carp. The findings of the current study could be refined further for large-scale production of Common Carp for the sustainable application of nonarable ISGW. This will offer livelihood and nutritional benefits to stakeholders that have unfertile lands that are affected with saline groundwater.

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