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Effect of salinity on lipid profile of *Cyprinus carpio* reared in inland saline water

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Abstract

Aim : The present study was undertaken to investigate the influence of different salinity levels on gonadal tissue and serum biochemical parameters of *Cyprinus carpio reared* in inland saline groundwater to standardize the optimal salinity level for better maturation and seed production.

Methodology : A 90 days experiment was conducted in non-drainable rectangular earthen ponds (n= 8) to study the effects of different salinity (0, 5, 10 and 15 ppt) levels on distribution of lipid class profile in *Cyprinus carpio* reared in inland saline groundwater. Gonads and serum were collected on 15 days interval and lipid classes, cholesterol, phospholipid and triacylglycerol were analyzed by using extracted total lipid.

Fish culture pond

Cholesterol, phospholipid and triacylglycerol varies to salinity and maturity stages of *C. carpio* in inland saline groundwater

High content of lipid class at 0 and 5 ppt as compared to 10 and 15 ppt of inland saline groundwater

Optimum salinity for *C. carpio* culture in inland saline groundwater at 5 ppt

Results : The concentration of different forms of lipids were significantly influenced by salinity and their concentration increased with development of maturity stages but lowest level was recorded at ovulation or spermiation stages. It was observed that significantly ($p < 0.05$) highest level of all lipids was found at 0 and 5 ppt compared to 10 and 15 ppt salinity.

Interpretation : The present study revealed that various lipid class fatty acids varied according to salinity and maturity stages of *C. carpio* in inland saline groundwater. Further, it also indicated that *C. carpio has* a tendency to adapt, survive, tolerate and reproduce in inland saline environment salinity ranging from 0-15 ppt and was found optimal at 5 ppt based on the reproductive ability.

Key words: *Cyprinus carpio*, Environmental stress, Lipid profile, Salinity

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Introduction

Common carp (*Cyprinus carpio*) is a stenohaline freshwater fish (Wang *et al.,* 1997). It gains 0.6 to 1.0 kg body weight within one season in the polycultural fish ponds of subtropical and tropical areas. It may spawn throughout the year in tropical areas of India, with a peak in January-March and July-August (FAO, 2013). Earlier studies have revealed that silver carp and common carp had a lower oxygen consumption rate and standard metabolic rate under 3 ppt salinity level as they spent less energy on maintaining internal equilibrium (Von Oertzen, 1985). Additional interest in *C. carpio* culture has been created by the problem of salination of agricultural land. Salinization of land and ground water is a common problem in India. In India, 8.62 million ha has been reported to be affected by soil salinization (Lakra *et al.,* 2014; Jahan *et al.,* 2018). It is well documented that the environmental factors such as temperature, rainfall, salinity play a key role in regulating the reproductive behavior of aquatic species (Haddy and Pankhurst, 1998).

Salinity imbalance is one of the environmental stress which directly effects several biological and physiological actions. It is a detrimental factor for ionic balance, homeostasis, and subsequently immune imbalance, growth, maturation and reproduction. Stress due to salinity disturbs homeostasis and has further determinable effects on growth, reproduction etc. (Morales *et al.,* 2005). Therefore, understanding the influence of variable salinities on levels of reproductive lipid profile of *C. carpio* will be important for potentially use of salt affected areas and it might be used as indicators of physiological stress response in fish subsequently helpful for expansion of aquaculture. Numerous fish species exhibit poor reproductive performance under unfavorable environmental conditions. that generally causes male or female brood stock fails to endure oocyte or testicular maturation, spermiation or ovulation following spawning (Zohar and Mylonas, 2001). Common carp has the capability to develop biochemical and physiological adaptation to cope up with the adverse environmental impacts. Several biochemical compounds such as lipid (phospholipid, cholesterol and triacylglycerol) act as osmolytes and energy source during the gonadal development which is mainly contributed by change or fluctuation in salinity (Hosoi *et al.,* 2003); however, different inland saline groundwater response studies of fresh water fish *C. carpio* are scanty, hence, the present study was undertaken to investigate the influence of different salinity range (0-15 ppt) of *C. carpio* reared in inland saline groundwater and its subsequent impact on gonadal tissue and serum biochemical parameters to standardize the optimal salinity level for better maturation and seed production of *C. carpio* in inland saline groundwater.

Materials and Methods

Experimental site: The experiment was conducted at the Wet Laboratory of ICAR- Central Institute of Fisheries Education (CIFE), Rohtak, India, from April to August. The juvenile fish of *C. carpio* weighing 30±5.0g were collected from earthen ponds of Sampla village of Rohtak (Haryana) in early April. After collection, fish were transported to the experimental site and were acclimatized in different salinities, viz. 0 ppt (C), 5 ppt (T1), 10 ppt (T2), and 15 ppt (T3) in 1200 l capacity Fibre-reinforced plastic (FRP) tanks for a period of two weeks.

Experimental set up: The experiment was conducted in non-drainable rectangular earthen ponds (n= 8) of dimension 21x10x1.50 m. which consist of four treatment groups, *viz.* 0 ppt (C), 5 ppt (T1), 10 ppt (T2), and 15 ppt (T3) in duplicate. The salinity of pond water was adjusted as 5 and 10 ppt by adding freshwater. Salinities were held constant thereafter, and were checked daily with a hand- held refractometer. Throughout the experiment, most of the water quality parameters remained at optimal levels among the different experimental treatments. Fish were stocked in the last week of April with a stocking density of 5000 numbers per ha in each pond and fed to satiation twice daily.

Sampling of experimental fish: *C. carpio* were captured by drag net at 15 days interval in the month of May to August. Fishes were sexed by male oozes out white milt from its genital opening and female with its swollen belly. Both male and female (n =3) fishes were anesthetize by using clove oil (50 $\mu l l^{-1}$) (Misra *et al.,* 2006). The Ethical Guidelines of the Animal Care for ICAR-Central Institute of Fisheries Education, Mumbai India were strictly followed and sampled on 15 days at regular interval for serum and gonads collection. Gonad tissues were collected and total lipids were extracted and determined gravimetrically by the method described by Folch *et al.* (1957) and lipid classes, cholesterol, phospholipid and triacylglycerol were analyzed by using extracted total lipid (sample). From the same fishes, blood was collected from caudal peduncle region by using syringe (No. 23) and was kept for clotting in tilted position after centrifuged at 3000g for 10 min at 4°C, the supernatant was collected and stored at -20 °C in Eppendorf tube till further analysis of serum cholesterol, phospholipid and triacylglycerides was conducted.

Lipid profile analysis of fish serum: The cholesterol, phospholipid and triacylglycerol level in serum of both sexes were determined by Innoline assay kit (Merck India Ltd) by following enzymatic colorimetric method. The analysis was carried out by following the kit manufacture's instruction. Briefly, test tubes labelled as Blank (B), Standard (S) and Test (T) were taken and in all tubes, 1 ml working reagent was added. A 0.01 ml of cholesterol standard was taken in the standard test tube and 0.01 ml of plasma was added in the test tube and 5 µl of distilled water in test tube marked as blank. It was mixed well and incubated at 37°C for 325 sec. The absorbance of Standard (S) and Test (T) was measured against blank (B) in a spectrophotometer at 505 nm. Similar steps were followed for estimating phospholipid and triacylglycerol by adding their specific standard and working reagent.

Lipid profile analysis of fish gonads

Cholesterol analysis: Gonadal cholesterol was estimated according to Zlatkis *et al.* (1953). Briefly, sample (0.3 ml), acetic

acid (5.7 ml) and colouring agent (4 ml) were taken and mixed well at room temperature. The concentration of cholesterol in gonads were read against the blank at 575 nm.

Phospholipid analysis: Estimation of gonads phospholipid was done according to Wargner *et al.* (1961). Briefly, test tubes containing sample (0.1 ml) and 60 % perchloric acid (0.4ml) were placed in aluminum (150 0C) block for 2 hrs. A 5ml colouring agent was added and kept in water bath (37 °C) for 2 hrs. The concentration of phospholipid in gonads were read against the blank (0.1 ml chloroform) at 650 nm.

Triacylglycerol analysis: Gonadal triacylglycerol was estimated according to Foster and Dunn (1973). Gonad sample (1 ml), isopropanol (4 ml) and washed alumina (400 mg) were placed in a mechanical rotator for 15 min and centrifuged. The supernatant (2 ml) was collected in screw-capped tubes and were mixed with potassium hydroxide, incubated at 70°C for 15 min. Later after cooling, metaperiodate solution (1 ml) and acetone reagent (0.5 ml) was added and incubated at 50°C for 30 min. The concentration of triacylglycerol in gonads was read against the blank (1 ml distilled water) at 405 nm.

Statistical analyses: The data were analyzed with a statistical package SPSS version 22.0 in which data were subjected to One-way ANOVA followed by Duncan's Multiple Range Test in order to determine significant differences between the means.

Results and Discussion

Several research has been reported on variation in environmental factors such as salinity on cultured species. Previous studies have demonstrated that several biochemical parameters such as lipids profile has a direct impact on several physiological processes such as osmoregulation and transport of nutrients. Therefore, the nature and quantity of these parameters vary according to species, environmental conditions (temperature, pH, salinity), maturation cycle etc. (Kheriji *et al.,* 2004). Stenohaline fishes (*C. carpio*) can live in a narrow range of environmental salinities due to lack of their ability to synthesize new salt transporting proteins (Kidder *et al.,* 2006). Though, these fishes are able to sustain ionic and osmotic homeostasis of their body fluids across a range of environmental salinities by using osmoregulatory mechanisms which are energy demanding processes (Sampaio *et al.,* 2002). As reported by Sampaio *et al.* (2002) the maturation process is an energy demanding process which requires a significant amount of energy diverted in this process. During this period lipids act as energy (osmotic modulator) source for salinity acclimation.

In order to understand the mechanisms involved in the acclimation and subsequently optimization of salinity of *C. carpio* to different salinities, the concentration of lipid profile in the serum and gonads of both male and female acclimated at different salinities for various time periods was determined (May to August). Fresh water fish, *C. carpio* was exposed to 0-15 ppt saline medium and on exposure for 90 days no mortality occurred and it was found that *C. carpio* could successfully sustain in saline water condition. During the experiment, water quality parameters were found optimal among different experimental treatments. Hardness (505-2451 mg l^{-1}), potassium (3.45-9.08 mg l^{-1}) , magnesium (86.66-490.88 mg l^{-1}), calcium (56-820 mg l^{-1}), sodium (396.53-2595 mg l^{-1}) alkalinity (206.66-318.66 mg l^{-1}), dissolved oxygen (6.58- 6.75 mg l^{-1}), temperature (29.16°C), pH (7.46-7.85), total ammonia nitrogen (0.068 - 0.1mg l^{-1}) and nitrite nitrogen (0.001- 0.006 mg l^{-1}) remained within the acceptable limits for rearing of *C. carpio.*

The present study reveals that the concentrations of cholesterol, phospholipid and triacylglycerol changed (Fig. 1,2) following alteration of acclimating salinity in inland saline groundwater, and subsequently the concentration of lipid content (phospholipid, cholesterol and triacylglycerides) increased with development of maturity stages from May to July, however, the lowest level was recorded at ovulation or spermiation stages in August. It was observed that significantly highest level of lipid profile was found at 0 and 5 ppt compared to 10 and 15 ppt saline water. It has been reported that concentration of these forms of lipid were highest at 0 and 5 ppt, further as salinity increased, their levels reduced. Similar results were observed by Arjona *et al.,* 2009; Kavya *et al.,* 2015; suggested that these biochemical energy sources decrease as salinity level increases. The highest mean cholesterol content were recorded at oocyte spent stage (587 mg dl^{-1}) at 0 ppt salinity (Fig.1A); however, in contrast with male serum highest concentration (179 100 g^{-1}) was noted at 5 ppt (Fig. 2A). Significantly, the lowest cholesterol content was recorded at 15 ppt both in males and females. Gonadal cholesterol level was highest at spent stage in both females (500 100 g^{-1}) represented in Fig.1 (B) and males (415 100 g^{-1}) at 5 ppt (Fig. 2B) followed by 0, 10, however, lowest at 15 ppt saline water.

The changes in phospholipid constituents both in serum and gonads of *C. carpio* is considerably analogous to the maturation stage and salinity variance in both sexes. Phospholipid content in male serum (252 mg dl^{-1}) was relatively three fold less (Fig. 2C) as compared to female serum (712.22 mg dl^{-1}) at same salinity (5 ppt) level Fig.1(C). Significantly ($P<0.05$) the highest level of phospholipid was recorded at 0 and 5 ppt at gonadal maturing stage in July, both in serum and gonadal tissues. Phospholipid content in the blood serum and gonads of females (Fig. 1D) showed a trend similar to males (Fig. 2D) in contrast to salinity except for the increase in the value was noticed in females. The highest mean triacylglycerol content was observed in the serum of females (Fig. 1E) collected in July at ripe stage just before ovulation (381.525 mg dl^{-1}) at 5 ppt followed by 0 ppt (213.015 mg dl^{-1}), 10 ppt (166.75 mg dl^{-1}) and 15 ppt (151.75 mg dl^{-1}); similar trend was found in male serum (Fig. 2E). Triacylglycerol content in the blood serum and gonads of females (Fig. 1F) showed a trend similar to males (Fig. 2F) in contrast to salinity, except for that increase in the value was more pronounced in females. However, as compared to males (396.515 mg 100 g^{-1}), female gonads treated with 5 ppt showed 2

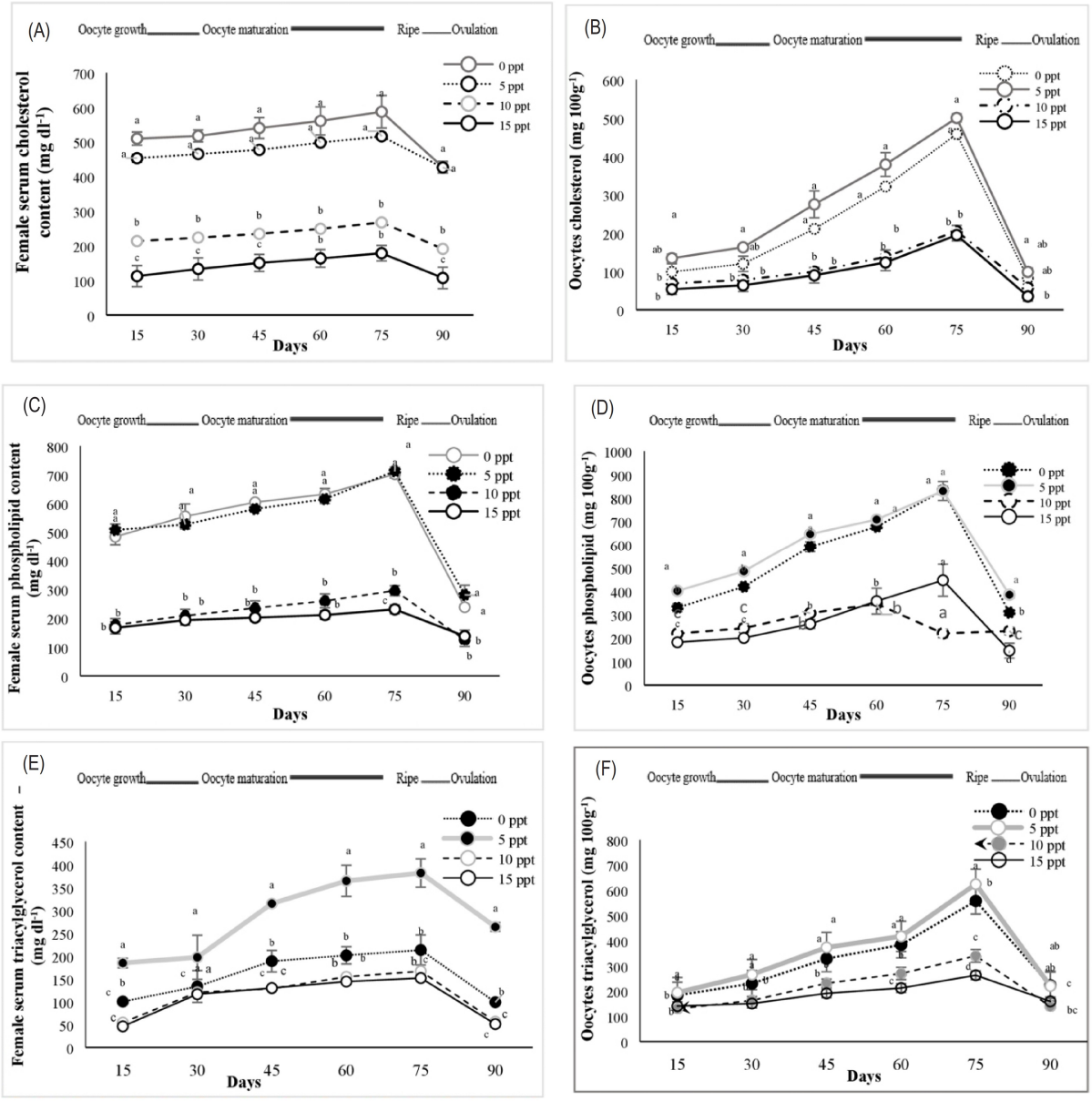

Fig. 1: Changes of lipid class content (mg dl^{-1}) in serum and oocytes (mg 100 g^{-1}) of *C. carpio* at different maturity stages subjected to different salinities in inland saline water at regular duration. Values are mean of three replicates ±S.E. Different superscripts signify statistical differences ($p<0.05$).

fold high level of triacylglycerol (624.155 mg 100 g^{-1}) at spent stage in July. Simultaneously, the concentration of these lipid increased during pre-spawning in gonadal tissues because lipid concentrations are highly affected at reproductive stage. Lipids such as cholesterol act as a precursor for the synthesis of steroids is used updуring gonad maturation; however, their concentration increases as the gonads attain maturity and reduces during ovulation or spermiation period (Sreevalli and Sudha, 2012). Some species such as *Gadus macrocephalus*, experience elevated lipid concentration during pre-spawning phase (Larsson and Fange, 1977). Serum lipid levels also peak prior to vitellogenesis in *Pleuronectes platessa* (White *et al.,* 1986), however, there is extensive variability over the season. Alternatively, some studies suggest that serum lipid concentration

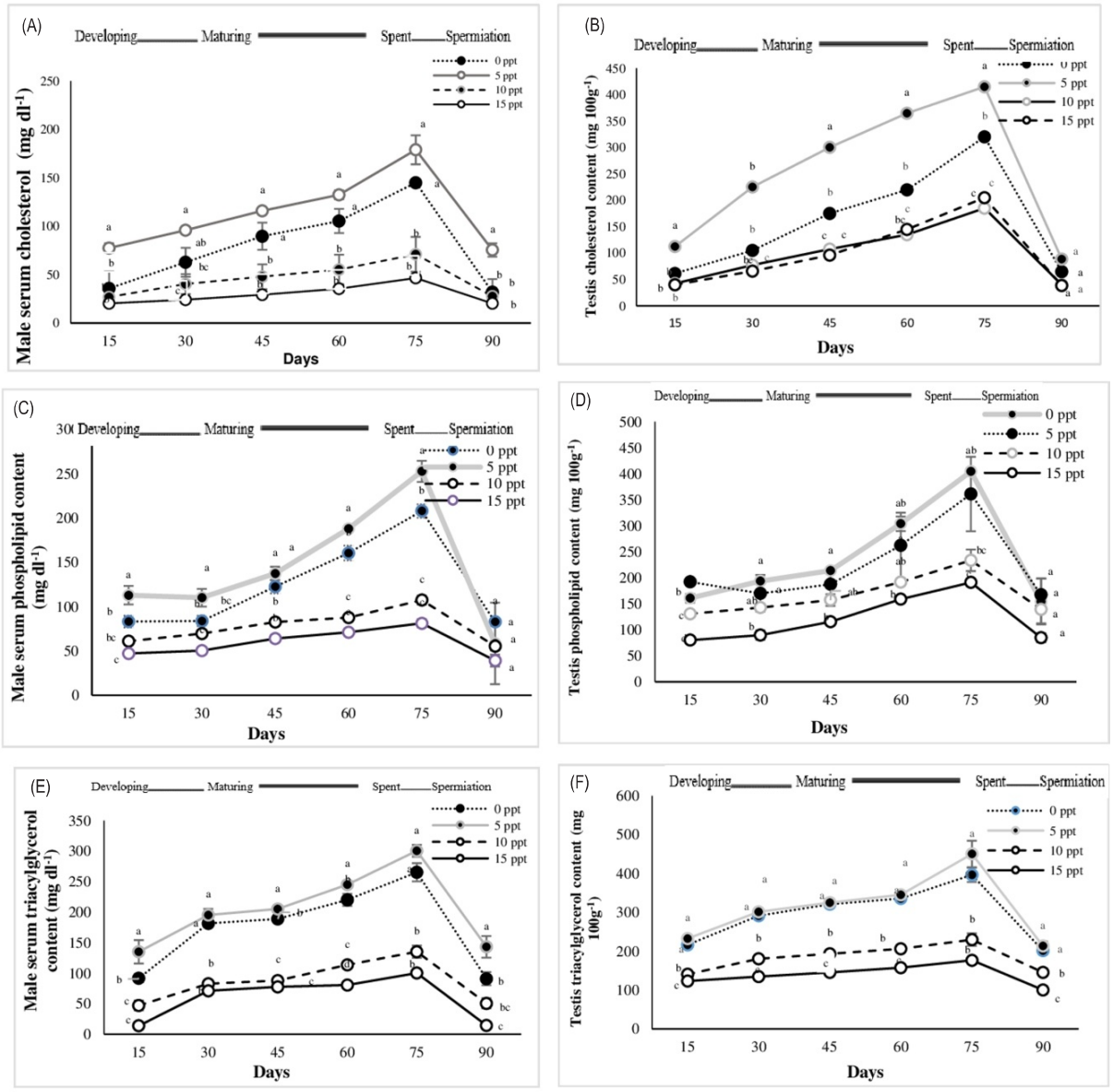

Fig. 2: Changes of lipid class content (mg dl^{-1}) in serum and testis (mg 100 g^{-1}) of *C. carpio* at different maturity stages subjected to different salinities in inland saline water at regular duration. Different superscripts signify statistical difference ($p<0.05$) (mean ± S.E.) (n = 3).

in fish are lowest during sexual maturation (Babin and Vernier, 1989), presumably due to uptake by the developing gonads. In gold fish, cholesterol concentrations are elevated throughout gonadal development and triacylglycerol concentrations peak during early recrudescence, subsequently dropping during ovulation or spermiation (Sharpe and Mac Latchy, 2007). Our results are in conformity with the findings of Assem *et al.* (2005) in *Trachinotus ovatus;* Sutharshiny *et al.* (2013) in *Scomberoides lysan,* where total lipid content in gonads attained highest value at spawning stage and lowest value at spent stage. However, they suggested that these alterations are cyclical changes in the levels of gonad lipid may be due to gonadal activity prior to and during breeding season. The present study also detected high levels of lipid class in gonads than plasma during gonadal maturation and

as compared to males, female gonads showed 2 fold high level of lipid content. (Babin and Vernier, 1989). However, it might be suggested that different constituents of lipid class in *C. carpio* as main osmolytes play an important role in osmoregulation during salinity acclimation and also contribute a significant role in the maturation of male and female in saline water rearing conditions.

The results of this study clearly reflects that *C. carpio,* has tendency to adapt, survive tolerate and reproduce in inland saline environment ranging from 0-15 ppt and found optimal at 5 ppt based on the reproductive ability. The present study shows that various class of fatty acids (cholesterol, phospholipid and triacylglycerol) vary according to salinity and maturity stages of *C. carpio* in inland saline groundwater. In addition, data presented here in on *C. carpio* reared in 0 and 5 ppt salinity had high content of different lipid class compared to 10 and 15 ppt saline water. However, future study is required to delineate its reproductive potential and stress physiology at higher salinity.

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