DISCUSSION

The absolute essential thing for life is water. It is the most precious resource that exists on our planet (Abowei and George, 2009). Industrialization and urbanization together with ending up of wastes and pollutants alter the water quality. Condition of water bodies depends on changes in concentration of physico-chemical parameters like temperature, pH, dissolved oxygen (Gulson et al., 1997). Bioavailability and toxicity of metals to aquatic organisms is dependent on physico-chemical features of water like hardness, pH, temperature. The toxicity of any substance depends upon its concentration and physico-chemical conditions under which it is tested (Eisler, 1970).

Temperature is the limiting factor in the aquatic environment (Odum 1971; Boyd 1979). It is the most important environmental variable that affects metabolic activities, growth, feeding, reproduction, distribution and migratory behaviours of aquatic organisms (Largler et al., 1977; Suski et al., 2006). Permeability of tissue for poisons increases as the water temperature increases. The metabolic rate, water and oxygen consumption also increase with increase in temperature (Chauhan and Saxena, 1992). Hence, the toxins which are not active at low temperature will prove fatal in increased temperature. Temperature plays a key role in physiology of ectotherms, as their body temperature changes with the temperature of environment (Hochachka and Somero, 2002).

Studies indicate that an increase in environmental temperature results in elevated mortality rates in metal exposed fishes. Hazel (1995), Hochachka and Somero (2002) reported alterations in membrane integrity, permeability to metals as well as the mobility and the functions of membrane associated receptors due to elevated temperature. McLeod and Pessah (1973) observed increased mercury toxicity with increase in temperature in rainbow trout. Sarkar (1991) reported the effects of temperature on eggs, fry and fingerlings of *Labeo rohita* exposed to urea. At high temperatures the development of egg was ceased and fingerlings exhibited abnormal behaviour. Reviews suggest that elevated temperatures tend to enhance toxic effects of metals on organism (Cairns et al., 1975; Mc Lusky et al.,1986; Heugens et al., 2002, Gordon, 2005; Sokolova and Lannig, 2008). Wilmer et al., (2000) and Portner...
(2001, 2002) reported that rise in temperature results in a profound increase in metal uptake rates. It further increases the metabolic rates which contribute to metal accumulation. This may result in energy deficits due to elevated maintenance cost. Acute toxicity of mercury in relation to temperature in fingerlings of Indian major carps catla, rohu and mrigal was studied by Kumar and Gupta (2006). The safe concentrations of mercury for the fingerlings of the three carps were observed. It was found that all the three fishes were more sensitive at high temperature. Rathore and Khangarot (2002) reported that acute toxicity of mercuric chloride increased with increase in temperature. Similar trends were reported in other metals by Cairns et al., (1981).

In the present study, temperature of reservoir water and laboratory water showed little variation during June 2012-May 2013. During the acute and chronic toxicity experiment temperature did not fluctuate widely.

Hydrogen ion concentration or pH is one of the vital physico-chemical characteristics, which is related with the composition and life processes of the organisms within. pH is a measure of strength of hydrogen and determines alkalinity and acidity of water which influences the survival, metabolism, physiology and growth of aquatic organisms. All biological and chemical processes in nature are completed in a normal range of pH.

Toxicity of metals is immensely influenced by the physico-chemical characteristics of the environment. Water pH influences the metal uptake and their toxicity index by fresh water fish (Alabaster and Lioyd, 1980; Spry and Weiner, 1991; Kock et al, 1996). Loon (1982) concluded that temperature and pH govern the methylation of elements like lead and mercury. Bradley and Sprague (1985) reported that hardness and pH of water have been the important modifying physico-chemical factors of zinc toxicity. Further lethality of dissolved zinc increased with concomitant increase in pH. Soft waters of low pH exhibit greatest toxicity of waterborne metals to aquatic organisms (Penttinen et al., 1995). Rouleau et al., (1996) studied manganese uptake in brown trout Salmo trutta and concluded that at acidic pH manganese uptake was more. The detrimental effects of zinc and cadmium were seen at lowered pH.
(DWAF, 1996). Hansen and Welsh (1996) investigated a positive correlation between zinc toxicity and pH of water in rainbow trout. Datta and Das (2003) studied the lead toxicity in *Cyprinus carpio* and *Catla catla*. The result showed increase in pH during acute exposure from 7.6 to 7.9, increased lead accumulation in *Cyprinus carpio* and *Catla catla*. Further increase in pH reduced the accumulation thus showed inverse relation. Ramnathan et al., (2005) recommended optimum range of pH for maximum growth in shrimp and carp. Adhikari et al., (2006) demonstrated a linear relation between increasing pH and decreasing accumulation of lead and chromium at variable exposure periods in *Labeo rohita* a fresh water major carp. Cadmium, however was showed to have no linear relationship between its accumulation and pH.

In the present investigation, the pH showed a narrow variation from 7.2 to 8.6.

Dissolved oxygen is one of the most important parameters in water quality assessment and reflects the physical and biological processes happening in water. Reduction in DO increases the toxicity of zinc, lead and copper salts in rainbow trout (Llyod, 1961). The median lethal concentration of aqueous ammonia at reduced dissolved oxygen was tested with rainbow trout, *Salmo gairdneri* fingerlings (Thruston et al., 1981). Dissolved oxygen decreases due to increase in temperature and also due to the increased microbial activity (Sangu, 1987; Kataria, 1996). Low oxygen in water is generally associated with heavy contamination of organic matter. Ammonia toxicity increased as dissolved oxygen decreased.

In the present study, the DO of reservoir water and laboratory water showed a narrow range of variation during different seasons.

Hardness is a measure of the quantity of divalent ions such as calcium, magnesium and/or iron in water. Since long it has been known that most heavy metals become less toxic in harder water (Chapman and Mc Cardy, 1977; Howarth and Sprague, 1978). Toxicity of copper to various fish species decreased with an increase in water hardness (Erickson et al., 1997; Lauren et al., 1986; Taylor et al., 2000). A several fold difference in the acute toxicity of lead was observed between hard and soft water by Davies et al., (1976). Heavy metals are more soluble in soft water hence are more toxic in soft water than in hard water. Aquatic toxicologists believe that
water hardness substantially affects metal toxicity but has little effect on the toxicity of organic contaminants (Sprague, 1985; Mayer and Ellersieck, 1988). Miller and Mackay (1980) studied copper toxicity in juvenile rainbow trout, *Oncorhynchus mykiss* and believed that calcium hardness protects the fish from copper toxicity. Pascoe et al., (1986) investigated the toxicity of cadmium to rainbow trout and confirmed the protective effect of hard water at different levels of cadmium. Short term toxicity of heavy metals decreases when the pH and/or hardness increases (Lucan-Bouche et al., 1999). Rathore and Khagarot (2002) concluded that metals are less toxic in hard water, provided pH is kept constant.

In the present study, the total hardness of reservoir water and laboratory water showed little fluctuation during different seasons.

Unlike temperature and dissolved oxygen, the nitrates do not have direct effect on aquatic life. Excess levels of nitrates in water however, cause obstacle to survive and a number of adverse health and ecological effects. Nitrogen is in the form of nitrate, nitrite or ammonium. Excess nitrogen can cause overstimulation of plant growth, which brings about eutrophication, which ultimately results in fish kills (Akan et al., 2012).

In the present work, the nitrate content in both reservoir water and laboratory tap water showed little variation.

Phosphorus is the nutrient of short supply and one of the key elements in the water. Natural water contains very small quantity of phosphorus. The abundance of phosphorus depends on geochemical cycles. Phosphorus load to lakes and rivers come from nonpoint sources like runoff from pasture and croplands. Unless phosphates are present in very high levels are not toxic to organisms (Akan et al., 2012).

In the present investigation, the phosphate in reservoir water and tap water was in normal range.

This study offered information about present status of physico-chemical parameters of reservoir water and laboratory tap water. It was observed from the available information that, temperature and water hardness are the key factors
affecting the response of organisms to toxins. Among all the environmental factors, the effects of water hardness and temperature on the toxicity of chemicals are studied most often (Welsh et al., 1993, 2000).

Toxicity tests are experiments or trials designed to assess the concentration of xenobiotics and the duration of exposure required to produce a criterion effect. The aquatic toxicity tests are usually referred as bioassays. Bioassay is merely a dose response evaluation (Cairns, 1980). Acute toxicity studies are designed to determine the median lethal dose/concentration of the toxicant. LC$_{50}$ is a statistically derived expression of a single dose of a toxicant that is expected to kill 50% of the test animals in 24-96 hours. The evaluation of acute toxicity test is worthwhile to know the levels below which it is considered safe. The safe concentration, which permits successful reproduction, growth and all other normal life processes in the animal’s natural habitat, is usually much lower than LC$_{50}$.

Several workers have studied various invertebrate and vertebrate animal species for acute toxicity. Several workers have revealed the toxicity of heavy metals including lead and mercury in various aquatic organisms and indicated different limits. Shrivastav et al., (1988) determined the LC$_{50}$ values of mercury in developing stages of *Cyprinus carpio* and *Cirrhinus mrigala* and reported delayed development in mercury exposed eggs in both the fish species and swimming abnormalities in the fingerlings. The LC$_{50}$ for *C. carpio* hatchlings, fry and fingerlings was 0.05, 0.50 and 1.50 ppm respectively and that for *C. mrigala* was 0.10, 0.10 and 1.00 ppm. Hatchlings and fry were more susceptible as compared to fingerlings. Further behavioural changes like increased opercular movement and decreased swimming were reported. Agarwal (1991) estimated the LC$_{50}$ value for mercuric chloride in *Channa punctatus* and reported behavioural changes during the acute exposure. The LC$_{50}$ estimated was 2.11 ppm. LC$_{50}$ value for cadmium in *Channa punctatus* was estimated by Shastry and Shukla (1993) as 11.2 ppm. Gupta and Rajbanshi (1995) reported LC$_{50}$ for mercury in *Rasbora daniconius* as 0.80 ppm. The estimated LC$_{50}$ for mercury in fish *Boleophthalamus dussumieri* was 2.00 ppm (Manoj and Ragothaman, 1999). Leblond and Hontela (1999) observed the acute toxicity of mercury, zinc and cadmium in rainbow trout and reported that mercury was more
toxic to rainbow trout as compared to zinc and cadmium. Pandey et al., (2005) estimated LC$_{50}$ of mercury for *Channa punctatus* as 1.21 ppm. Gupta and Kumar (2006) quoted the LC$_{50}$ of mercury for *Cirrhinus mrigala* as 1.11 ppm. Selvanathan (2011) chose mercuric chloride and cadmium chloride as xenobiotics for lethality estimation in *Clarias batractus*. The determined LC$_{50}$ value for mercuric chloride was 1.43 ppm and for cadmium chloride it was 8.13 ppm. Hedayati et al., (2012), investigated sensitivities of different heavy metals to fish *Rutilus rutilus*. The lowest LC$_{50}$ after acute exposure of 96 hours was reported for mercury among the three, mercury, lead and zinc. The LC$_{50}$ values reported for mercury, lead and zinc for *Rutilus rutilus* were 0.35 +0.16, 39.9+0.76 and 48.3+0.48 ppm respectively. Hedayati et al., (2012) reported that mercury was more toxic than lead and zinc for *Rutilus rutilus*. The acute toxicity of cadmium and mercury in the juveniles *Lates calcarifer* (Bloch) for 96 hours was estimated by Mohan Raj (2013). The LC$_{50}$ values were 6.08 ppm for cadmium and 1.03 ppm for mercury. The dose dependent hyperactivity and behavioural changes were reported during the exposure. Mercury was reported to be more toxic than cadmium.

Notable differences in sensitivity of lead among fish species have been reported by Salmeron-Flores et al., (1990). It was concluded by Pickering and Henderson (1966) after studies on fat head minnows *Pimephales promelas* and bluegill *Lepomis macrochirus* that LC$_{50}$ for lead is low in soft water and high in hard water. Srivastava and Mishra (1979) reported 19 ppm as 96hour LC$_{50}$ for lead for a fresh water teleost, *Colisa fasciatus*. Demayo et al., (1981) reported that lead toxicity is a function of water hardness, species tested and age of fish. High water hardness reduces lead toxicity to fish due to a significant inorganic process that controls lead availability to fish (Hodson et al., 1984). Bhilave (2001) obtained 21.849 ppm as LC$_{50}$ for lead acetate for fingerlings of *Cirrhinus mrigala*. Martinez et al., (2004) reported 96 hour LC$_{50}$ value for lead in juveniles of *Prochilodus lineatus* as 95 mg Pb.L$^{-1}$. The estimated LC$_{50}$ for lead acetate in *Clarias gariepinus* was 122 mg/l (Al-Balwi et al., 2011). Rout et al., (2013) reported that lead toxicity to aquatic organisms varies with the stages of life of organism, test water criteria and duration of exposure.
Chronic toxicity is also known as long term but definite toxicity. The toxicity involves repeated administrations over a considerable period of life span of the test animals. Chronic toxicity tests are arranged with the objective to study adverse effects of chemicals on structure and function of organs, tissues and cells. A long term or chronic exposure for various heavy metals in different animals was studied extensively by various workers.

The detrimental effects caused by lead include haematological, biochemical and physiological alterations in several aquatic species (Chandravaty and Reddy, 1996). Chen and Sonstegard (1984) reported reduced vitellogenin in rainbow trout *Oncorhyncus mykiss* after exposure to cadmium. Hodson et al., (1984) reported haematological and neurological effects after sub lethal exposure of lead. Tiwari et al., (1987) observed the lead poisoning on haematological and biochemical profile of fish, *Barbus conchonicus*, similar parameters were studied on eel *Anguilla anguilla* by Santos and Hall (1990). Khan and Weis (1987) reported significant reduction in sperm motility but no alterations in sperm morphology after exposure to methyl mercury in *Fundulus heteroelitus*. A delay in gonial multiplication and spermatogenesis in *Tilapia nilotica* after exposure to zinc was reported by Caring (1992). Allen (1995) reported deposition of lead in testes and ovaries of *Oreochromis aureus* after exposure to lead and cadmium. *Mystus gulio* was observed after sublethal exposure to lead and a decreasing growth rate in youngfish was reported by Kashthuri and Chandran (1997). In *Cyprinus carpio* scoliosis was reported after exposure to lead by Stominska and Jezierska (2000). Parashar and Banerjee (2002) reported toxic impact of lethal concentration of lead nitrate on gills of catfish *Heteropneustes fossilis*. Palaniappan et al., (2008) reported the morphological changes like alteration in lamellar surfaces, epithelial hyperplasia and fusion of adjacent lamellae due to lead exposure in a fresh water fish *Catla catla*. Mastan et al., (2008), reported haematological alterations like decrease in RBC counts, haemoglobin percentage and serum protein levels after a chronic exposure of 45 days to lead nitrate in *Clarias batrachus*.

Jezierska et al., (2009) reported that heavy metals induce a delay in the embryonic development of fish and bring about reduced viability. Haematological,
biochemical and immunological damage leading to altered growth due to lead in *Tilapia zilli* was assessed by Zaki et al., (2010). Lead nitrate induced histopathological changes in gills of African catfish *Clarias batrachus* were reported by Khan et al., (2011). A long term exposure of *Heteropneustus fossilis* to lead nitrate was caused reduced plasma calcium and inorganic phosphate levels (Srivastava et al., 2013). Mokhtar and Abd-Elhafeez (2013) observed histopathological alterations in liver, ovary and hepatopancreas of *Oreochromis niloticus* after chronic exposure to lead acetate and rapid alterations like detachment and lifting of epithelial lining in gills were reported. Reduced growth, altered haematological parameters and reproduction was noted by Balawi et al., (2011) in *Clarias gariepinus* after chronic exposure to lead acetate.

Mobarak (2008) documented developmental toxicity and teratogenicity in a review study of cadmium, lead and mercury. It was observed that lead accumulation occurred in various tissues after a chronic exposure of 30 days (Ahmed and Bibi, 2010). The metal ions like lead and mercury not only influence growth but also accumulate in various tissues of exposed fishes (Ahmed and Bibi, 2010; Ahmed et al., 2011). Arya et al., (2013), studied chronic exposure of 40 days to lead in *Cirrhinus mrigala* and reported significant increase in enzymes and higher metabolic rate.

Toxicity of mercury was found to be greater at elevated temperature, low oxygen content and reduced salinities in marine environments and in the presence of metals such as zinc and lead (Oliviera and Torres, 1995, Beckvar et al., 1996). Bakre (1985) reported alterations in hepatocytes after exposure to mercuric chloride in *Gambusia affinis*. Adverse effects of mercury on different fish tissues were reported by Kirubagaran (1988). Effects of low and moderate doses of inorganic mercury on gill physiology were studied in *Micropterus salmoides* and mosquito fish *Gambusia hbrooki* by Jagoe et al., (1996).

Liao et al., (2006), observed mercury accumulation, histopathological effects and choline esterase activity alterations in medaka (*Oryzias latipes*) after sublethal exposure to mercuric chloride. Mercury not only affects the motility of sperms but also inhibit the enzyme activities of spermatozoa (Sarosiek et al., 2009). Khoshnood
et al., (2011) reported cellular disorders in gill epithelium as a histopathological and pathomorphological effects of 15 day exposure to mercuric chloride on Acipenser persicus. Similar observations were reported by Ribeiro et al., (2000) in Trichomyctrus zonatus. In the present study, acute toxicity of lead acetate and mercuric chloride for 96 hour to Cirrhinus mrigala was determined.

In the present study, no mortality at the end of 96h was reported at 250 ppm of lead acetate. The value hence represents LC0. The onset of mortality was at 255 ppm and mortality increased gradually with increase in concentration. At the end of 96h, 50% mortality was observed at 282 ppm concentration of lead acetate.

Acute toxicity of mercuric chloride to fish C. mrigala showed no mortality at 400 ppb (0.400 ppm) and hence the value represents LC0. The mortality was first observed at 402 ppb (0.402 ppm) and gradual increase in mortality with increase in concentration of mercuric chloride was noted. 50% mortality was observed at 412 ppb.

The acute toxicity limits in our study for lead acetate seem to be higher than those observed earlier in other species, it is because of the fact that the acute toxicity limits mostly depend on physico-chemical parameters of water and physiological conditions of fish. Several studies have shown that the effect of heavy metals on aquatic animals are influenced by water quality. In the present study, it was observed that mercuric chloride was more toxic than lead acetate to fish C. mrigala, as the LC50 value for mercuric chloride was lower than that of lead acetate.

For the chronic toxicity study, the 1/20th and 1/10th of LC50 concentration of both the toxicants, lead acetate and mercuric chloride were selected. The 1/20th (14.1 ppm) and 1/10th (28.2 ppm) of LC50 concentration of lead acetate and 1/20th (0.0206 ppm) and 1/10th (0.0402 ppm) concentration of mercuric chloride were chosen. The chronic toxicity study was done for 30 days. The acute and chronically exposed fishes were subjected to study their behavioural changes, histopathological changes on pituitary gland and gonads and effect on gonadotropin hormones with reference to the control fishes. The results indicated that both, lead acetate and mercuric chloride
prove lethal to *C. mrigala* after a limit. The limitation standard for both, the toxicants is different.

Behavioural responses to the environment is a crucial part of survival of the organisms. Behaviour is one kind of response at the level of individual organism. Behaviour is a complex phenomenon through which animal adjusts its various functions to a constant or changing environment. Contaminants affect the fish behaviour including locomotion, swimming performance, respiratory behaviour, feeding, social interactions and predator avoidance. Behaviour responses to toxicant help in predicting the intensity of toxicant and predicting the water quality. Behavioural changes in fish are important indicators of contamination of aquatic media (Richmands and Datta, 1992). Fishes are more vulnerable to metal contaminantion and show quick responses as compared to other organisms. It is because metals are highly soluble in water and fishes are intimately associated with water.

Extensive secretion of mucus, erratic swimming, surfacing were some behavioural patterns when animals were exposed to zinc and cadmium separately (Spehar, 1976; Benoit et al., 1976). Gupta and Gupta (1979) observed *Colisa fasciatus* and *Notopterus notopterus* exposed to aldrin and ethyl parathion and erratic swimming, jerky movements, loss of equilibrium and excess secretion of mucus were changes reported in behaviour. Benett and Dooley, (1982) considered secretion of mucus as a defense and excretory response while studying on *Fundulus heterclitus* a non migratory atlantic killi fish. Increased opercular movements associated with decreased swimming activity was reported by Qasim (1983) in *Tilapia mossambica* exposed to mercury. Abnormal swimming behaviour and altered movements are considered the result of excessive elimination of skeletal minerals (Pragatheeswaran et al., 1987). Pandey et al., (1990) suggested that behaviour like extensive secretion of mucus and faded body colour after exposure to stressors are due to dysfunction of melanophores of pituitary gland. James (1990) individually and in combination observed behavioural changes of *Oreochromis mossambicus* after exposure to sublethal and lethal concentration of zinc, chromium and copper; further erratic swimming, jerky movements, frequent surfacing, and restlessness were some
responses reported during the exposure. *Heteropneustes fossilis* showed an increase in oxygen uptake, which may be due to extra oxygen demand due to metal stress (Sampath et al., 1990). Camargo and Tarazona (1991) observed behaviour of rainbow trout exposed to sub-lethal concentration of inorganic fluoride characterized by darkening of the skin and an increased mucous secretion, hypoexcitability and a decreased respiratory rate. Weber et al., (1997) suggested that, lead is a neurotoxic element that causes behavioural dysfunction in fish. Low concentration of heavy metals alters behaviours like food gathering, navigation, defense and reproduction and reduces their survival (Sandahl et al., 2007; Tierney et al., 2010; Mc Intyre et al., 2012). The locomotary, feeding and social responses of fish are essential for their survival. Changes in these responses result in the reduced ability to grow survive and reproduce which finally causes change in their population. Mokhtar and Hanan, (2013) reported decrease in swimming activity and increase in mucus secretion in *Oreochromis niloticus* exposed to sub lethal concentration of lead acetate. Similar behavioural changes were observed in the present study.

In the present study, changes in behaviour of *C. mrigala* were observed during acute and chronic exposure to lead acetate and mercuric chloride as compared to the control. Fish exposed to different concentrations of lead acetate and mercuric chloride showed marked behavioural changes compared to control. Respiratory upset by increased opercular movement, increased surfacing with open mouth, loss of equilibrium, irregular swimming, vertical swimming, rapid jerks to jump outside and finally settling to the bottom were the general responses in all exposures. With increasing concentration of toxicant, the abnormal activities increased initially and finally reduced, showing the sign of distress. Similar behaviour was observed when the exposure time was increased i.e. chronic studies of 30 days. Fishes exposed to lead acetate showed certain typical behavioural changes like excessive mucus secretion and yellowness on body. Body colour was faded and scales shed. The marked behavioural changes after exposure to mercuric chloride were gulping of air directly, increased vulnerability, rapid jerks and loss of equilibrium.

As the exposure to toxicants extended fishes started showing signs of distress and lethargy. The swimming movements decreased. When the toxicant concentration
became more lethal fish showed struggle for breathing and died. Severe behavioural changes were seen in response to mercuric chloride than to lead acetate during present study.

The reproductive physiology of fish is better understood by a thorough knowledge of pituitary gland, as the pituitary gland controls several other glandular secretions. Matty and Matty (1959) studied the histochemistry of pituitaries of number of teleosts and provided a basis for the further investigations of the histophysiology of the gland. Khan (1962) described the morphology and histology of pituitary gland of *Labeo rohita* and further commented on functional anatomy of gland. Lal (1964) studied histological and histochemical details of pituitary gland of *C. mrigala*. Further, a correlation between gonadal state and changes in meso-adenohypophysis (PPD) was revealed. Moitra and Sarkar (1976) studied pituitary gland of major carps *Labeo rohita* and *Cirrhinus mrigala*. Joy and Sathyanesan (1980) studied pituitary gland of *Tilapia mossambica*. Cell types in the pituitary of roach *Rutilus rutilus* were studied by Jafri and Ensor (1980). An appropriate cell localization by a new combined staining technique was offered and six glandular cell types were localized. The pituitary gland of exotic carp, *Carassius carassius* was studied by Bhatt and Negi (1986) and localization of different cell types as well as tinctorially different cells were recognized. The morpho histology of pituitary gland of estuarine teleost *Velamugil cunnesius* was studied by Narayan et al., (1984) and reported, corticotropes in rostral pars distalis (RPD), somatotropes in proximal pars distalis (PPD) and gonadotropes in ventral and lateral regions of PPD. Cells of anterior pituitary and their multifunctions were observed by Yeung (2006). Further, the differentiation of anterior pituitary cells and their committed nature was reported.

Ekici and Timur (2013) studied *Cyprinus carpio*, a seasonal breeder for the anatomical and histochemical details of pituitary gland. The acidophilic, basophilic and chromophobich cells were described further. Gonadotrophes of a stream fish *Nemacheilus mooreh* were reported to be active, changing in size and with cytoplasmic granulation by Kharat and Khilare (2013) after the study of morphology and histology of pituitary gland.
In the present study, the histology of pituitary gland of *C. mrigala* was observed. In general, the cells of pituitary gland were differentiated into chromophobes and chromophils. Chromophobes showed a faint staining, while chromophils like basophils and acidophils showed differential staining. The results resembled the earlier studies of different workers.

In agreement with the results of Lal, (1963) the pituitary gland of *C. mrigala* showed attachment with the brain and a well developed sella turcica. The cells in RPD were predominated acidophils. The PPD showed much variation with regard to size and cell components at different maturity stages. Basophils outnumbered the acidophils in this region. Change in cellular abundance of pituitary gland is in agreement with observation of earlier authors (Lal, 1963; Bhatt, 1989b; Ursani et al., 2012). The degenerative changes such as vacuolization, pycnosis in gonadotropes of *C. mrigala* were similar as reported by Joy and Sathyanesan, (1979); Prakash et al., (1984); Farbridge et al., (1985).

The physiology of reproduction in fish has been extensively reviewed by several workers (Fontaine, 1975; Harvey and Hoar; 1979; Peter and Crim 1979; Devlin and Nagahama, 2002; Yashizaki et al., 2002). It is well established that the pituitary gland is indispensible for the maturation of gonads in all vertebrate organisms. In teleost fishes, the gonadal activities depend on the function of gonadotropes of pituitary gland (adenohypophysis). As the gonadal development proceeds to maturity certain morphological and histological changes take place in the carp’s pituitary (Lal, 1963). Seasonal variations in the histology of pituitary gland of *C. mrigala* in relation to gonadal activity were studied by Moitra and Sarkar, (1976). A great seasonal variation in percentage composition of acidophils and basophils was reported. Further the average weight of the pituitary gland was related with maturation state of gonads.

Scruggs (1951), reported striking seasonal changes in basophils of pituitary gland in *Cyprinus carpio* and *Carassius carassius*. Lal (1963) noted changes in the cellular content of adenohypophysis (proximal pars distalis) after morphological, histological and histochemical studies on pituitary gland of *C. mrigala*. Further, the
cells exhibit degenerative changes such as vacuolization and degeneration in basophils like gonadotropes. Basophils of various shapes were seen in *Labeo rohita* (Khan, 1962). Large basophils during maturation of gonads were reported by Belsare (1967) in *Channa punctatus*. A correlative seasonal change in the gonadotropes of proximal pars distalis (PPD), gonadosomatic index (GSI) and the gonadotropic activity was suggested by Singh (1970) in female *Mystus vittatus*. The basophils in the pituitary of *Clarias batrachus* were studied by Joy and Sathyanesan (1979). Further, little increase in pituitary basophil during initial gonadal maturation and peak in ripe gonads was reported.

A large number of teleost species gonadotropes show hypertrophy, vacuolization, granulation, numerical increase and other signs of extensive activity in relation to maturation of gonads (Joy and Sathyanesan, 1979; Prakash et al., 1984; Farbridge et al., 1985; Kaneko et al., 1986). Ahsan (1966), in lake chub *Couesius polnbeus*, reported coincided maximal GSI and increased activity of gonadotropes. Seasonal changes in gondotropin cells activity and GSI was reported by Van Oordt et al., (1987) in African cat fish *Clarias gariepinus*. Cyclic changes in pituitary of gonadotropes were studied in relation to the ovarian cycle in *Puntius sarana* (Ham) by Bhat and Dutt (1990). Quantitative and qualitative variations in gonadotropes during ovarian cycle were reported by Zaki et al., (1996) in *Siganus rivulatu* suggesting seasonal quantitative variation in gonadotropes indicating maximum number and activity during fully mature stage and minimum number and activity in spent stage. Similar results were reported in *Oreochromis mossambicus* by Ursani et al., (2012). Gonadotropes in *Oreochromis niloticus* were reported to be larger than other cell types during different season (EI Sakhawy et al., 2011). Further, small gonadotropes were reported in winter. Similar observations were reported by Mousa and Mousa (1998) in *Mugil cephalus* and EI-Zoghby et al., (2008) in catfish *Clarias lazera*.

Two types of gonadotropes were described in Japanese eel (*Anguilla japonica*) by Yoshiura et al., (1999) and in Nile tilapia *Oreochromis niloticus* by Kasper et al., (2006).
In the present study, the pituitary gland of *C. mrigala* showed seasonal variations in gonadotropes. Gonadotropes showed maximum number during sexually mature stage and minimum number during spent stage. Gonadotropes also showed hypertrophy, granulation, vacuolization in relation to maturation of gonads. The results thus, are in agreement with the earlier workers. Gonadotropes were observed to be larger than other cell types during breeding season while smaller and numerous during rest of the seasons of year. These observations are similar with the results reported by EI Sakhawy et al., (2011).

The diagnostic significance of immunohistochemistry (IHC) has developed during last quarter of the century. Immunohistochemistry is an umbrella term, which encompasses many methods used to determine tissue constituents with the employment of specific antibodies that can be visualized through staining (Haines and West, 2005).

Immunohistochemical study of the sturgeon (*Acipenser baerii*) was done by Pellssero (1988). The results demonstrated the resemblance of structure of pituitary with the teleost pituitary in distribution of the cell types. Alterations of gonadropes in the pituitary gland during the annual reproductive cycle of the female sand goby (*Oxyeleotris marmoratus*) were studied by IHC by Vongvatcharanon et al., (2005). Further, the study showed strong anti GTH II β labeled gonadotropes in pituitary gland in all stages. The number of GTH II β positive gonadotropes showed increase towards mature ovarian stage. Similar immunocytochemical observations were demonstrated in *Salmo gairdneri irideus* by Nozaki et al., (1990 b). The anti GTH II β labeled gonadotropes showed ovarian maturity related patterns of activity. Nozaki et al., (2013) demonstrated intensely stained adenohypophysial cells with anti ovine-LH β in *Paramyxine atami*.

Immunohistochemical study of pituitary cells in wild and captive *Salminus hilarii* females during the annual reproductive cycle was studied by Honji et al., (2013) and characteristic presence of strongly immunoreactive growth hormone cells (GH/somatotropes) and weakly immunoreactive gonadotropes in PPD were reported. The pituitary gonadotropes were more immunoreactive to β LH antibodies than to β
FSH antibodies because, molecular structure and primary sequence may be more conserved in β LH subunit than in β FSH subunit (Levavi-Sivan et al., 2010). Results indicated that gonadotropin or GH cells in vitellogenic females were significantly larger and had larger nucleus than the GH cells in previtellogenic females. Studies on seasonal variations on the GH cells were done by Vargas-Chacoff et al., (2009) and Fiszbein et al., (2010).

In the present study, the gonadotropes showed immunoreactivity with anti-HCG. The immunoreactivity variations were in relation with the seasonal variations in the gonadotropin family cells, which is similar with the reports of Fiszbein et al., (2010).

Heavy metals exposure produces a wide range of adverse effects and can impair reproduction and disrupt the expression of estradiol and testosterone in vertebrates (Tan et al., 2009; Frederick and Jayasena, 2011). Lead absorption lead to high mortality rate and cause many biochemical and histological alterations in fish (Coetzee, 1988). Heavy metals such as lead, cadmium and mercury reported to have an endocrine disruptive potential. Mercury was found to be deposited in the pituitaries of laboratory animals like rat after exposure to mercury by different routes (Thorlacius et al., 1985).

Pundir and Saxena (1992) studied pituitary gland of fish *Puntius ticto* after chronic exposure to cadmium. Further, loss of structural organization and change in shape and size of pituitary gland was reported. Prominent vacuolization was displayed by PPD cells. Singh and Singh (1980) studied pituitary gland of *Heteropeustes fossilis* in response to cythion and hexedrin treatment. Observations were similar in agreement with Pundir. Karabanova (1983) studied the pituitary gland of rainbow trout *Salmo gairdneri* fingerlings in response to hypotonic environment and thiourea and reported cellular lysis and vacuolization. Remarkable changes in pituitary gland and inflammation of pituitary gland of *Puntius ticto* exposed to weedone were observed by Verma et al., (1984). Gradual accumulation of secretary granules in gonadotropes was reported in cyprinid fish *Puntius sarana* exposed to high concentration of cadmium chloride (Kumari and Gopal, 1991). Ronis et al., (1998)
showed alterations in pituitary activity due to metals. Effect of cadmium exposure on the pituitary gland of Italian wall lizard *Podarcis sicula* was studied by Favorito (2010). Cadmium showed a cytotoxic effect on the gland with an evident alteration in adenohypophyseal cells.

Murrel and walking catfish exposed to 10-50 µg/l of methyl mercury, inorganic mercury or a mercurial fungicide were studied by Ram and Joy, (1988) and smaller, inactive and fewer gonadotropes were reported, in both species. Atrophic changes of the pituitary corticotropes in cortisol impaired fish from sites contaminated with heavy metals were shown by Hontela et al., (1992). The reduced cell size, reduced cell area and presence of large intercellular spaces indicate the atrophy and structural impairment. Hontela, (1997) observed structural and functional impairement of pituitary in fish exposed to craft mill effluent with smaller corticotropes and larger intercellular spaces in the exposed fish. Favorito (2010) suggested a direct correlation between accumulation of cadmium in the brain and alteration of the normal occurance and distribution of the corticotropes, lactotropes and gonadotropes cells and their secretary activity. Sakly and Hachfi (2010) reported that cadmium could be the toxicant principally acting on hypothalamic pituitary axis.

Simultaneous exposure to cadmium and lead lower the membrane fluidity in pituitary gland. It affects the membrane function and cause alterations in receptor binding and secretory mechanisms of pituitary hormones (Pillai et al., 2002).

In the present study, the pituitary gland of *C.mrigala* after exposure to lead acetate showed decrease in abundance of cellular population and distinct vacuolization in PPD cells. Smaller, inactive and fewer gonadotropes were observed in pituitary gland of treated fish. The results were similar with those reported by Pundir and Saxena (1992).

The pituitary gland of *C.mrigala* exposed to mercuric chloride showed diffused and irregular pattern of cellular arrangement in the present study. Observations such as decrease in gonadotrope population, increase in intercellular space were similar with those reported by Ram and Sathyanesan, (1983) and Favorito,
In general, it was observed that exposure to heavy metals causes impairment in pituitary gland.

Luteinizing hormone (LH) and follicle stimulating hormone (FSH) released from the pituitary gland in fish known as gonadotropic hormones control the annual cycle of gonadal growth, ovulation in females, sperm release in males and production of sex steroids in both sexes (Weltzien et al., 2004; Kamei et al., 2005). The pituitary gonadotropins synthesized by gonadotropes, enter into peripheral circulation and bring about effect on steroidogenesis and gametogenesis by binding the receptors in the gonads.

Plasma LH levels in salmonids, remained at very low levels at the start of testis development. Detectable level of LH during meiosis of germ cells and clear increase until spawning was observed. FSH on the other hand, showed a temporary increase in association with spermatogonial proliferation and spermiation and decreased before the spawning season started (Gomez et al., 1999; Campbell, 2003). Alterations in binding between gonadotropins and their respective ovary receptors in lead exposed rats were reported by Wiebe et al., (1988). Swanson, (1989) developed radioimmunoassay to quantify coho salmon FSH and LH further used to assess gonadotropin levels in Atlantic salmon. Slater et al., (1994) quantified the circulating FSH levels in Chinook salmon, Onchorhynchus tshawytscha during migration. Variable plasma LH levels in Atlantic salmon were recorded by Oppen-Bernsten et al., (1994). Mateos et al., (2002) reported significantly lowered plasma levels of FSH in rainbow trout and Mediterranean sea bass after estradiol 2 treatment.

The annual reproduction cycle in adult Poland carp was studied by Bieniarz et al.,(1978) to study the ovarian state and serum gonadotropin level. Further rise in temperature leads to rise in gonadotropin secretion and oocyte maturation. Annual cycle of gonadotropin content in common carp Cyprinus carpio was studied by Weixin et al., (1983). A definite cyclic change positively correlated with the gonadosomatic index was reported. Zohar et al., (1986) studied short term profiles of plasma gonadotropins in female rainbow trout and reported pulsatile secretion of LH. Bieniarz et al., (1992) studied gonadotropin changes during sexual maturation of
female *Cyprinus carpio*. FSH and LH roles in female reproduction are not distinct. Findings on salmonids suggest that FSH is associated with vitellogenesis while LH regulates final oocyte maturation (Swanson et al., 1991; Prat et al., 1996). Gen et al., (2000, 2003) reported that in red sea bream FSH does not have significant role in female but LH regulated complete reproduction. LH was also reported to have key role in reproduction of *Clarias gariepinus* (Koide et al., 1992).

Annual variation in plasma vitellogenin and gonadotropin II (LH) levels were studied in relation to annual ovarian cycle in female *Cirrhinus mrigala* by Maitra et al., (2007). Further, annual profile of plasma vitellogenin and gonadotropin levels showed a good correlation with GSI and oocytes during different reproductive phases.

In the present study, LH in females seemed to regulate the initiation, maturation and release of egg during female reproduction. Rise in LH was highest during June, July indicating spawning period during which maturation, and ovulation takes place. It is similar with the facts reported by Swanson et al., (1991); Vander kraak et al., (1992). Rise in LH in December indicated role of LH in initiation of next ovarian growth cycle. The results were not different from those reported by Kobayashi et al., (1986) in gold fish.

In the present study, FSH in females seemed to produce/regulate steroids as its surge was in May. FSH rise in January indicated its stimulating role in growth of ovarian follicles. The pulsating values in February, March and April indicate the continuation of growth of ovarian follicles. The fall from June suggests no further growth of oocytes and approach of ovulation and spawning. FSH and LH levels showed a correlation with GSI in female *C. mrigala* which suggest the synchronized action of pituitary gonadal axis and a interrelation between sexual maturation and somatic growth. Peak in FSH in male *C. mrigala* was observed in June during the present study indicating active spermatogenesis.

During the annual cycle, in male *C. mrigala* LH peak was displayed at April there after followed by no significant decrease and attaining low values in the beginning of December. Roughly constant LH level during February and March was
observed. A progressive non significant increase in serum LH occurred, reaching the highest levels during April-June.

The serum FSH in males initiated in January with low values. A progressive significant increase in serum FSH occurred, during February to May. Peak in FSH was observed in June there after a steady decrease in FSH was recorded until August. FSH remained below detectable level during spent stage in the month of September to December.

The present study provides evidence for the close relationship between serum gonadotropin levels, GSI values and stages of ovarian and testicular maturation during the year. The precise function of FSH and LH remains elusive in males. Studies suggest that both FSH and testosterone stimulate the different phases of spermatogenesis.

FSH may play an important role during gametogenesis in male, but not in female. LH may be involved in regulation of both early and late gametogenesis in both sexes (Gen et al., 2003). To date, the physiological significance of FSH in female reproductive process is unclear. In the present study, low initiated values of LH during December indicate the initiation of spermatogenesis. Its rise and peak in summer months indicate progression in spermatogenesis and no significant fall in monsoon indicated continuation of spawning. Below detectable level of LH during spent stage underlines its significance in regulation of early and late gametogenesis. FSH on the other hand, seems to have a role in early spermatogenesis and spawning.

Acute stressors have been found to increase gonadotropin circulating levels in brown trout, Salmo trutta (Pickering et al., 1987; Sumpter et al., 1987). However, Stacey et al., (1984), reported a decrease in gonadotropin concentration in white suckers, Catostomus commersoni. The endocrine disruptive effects of mercury were reviewed by Zhu et al., (2000). Derath et al., (2002) demonstrated changes in LH secretion in female fischer rats (F344) administered with 12mg/ml concentration of lead acetate. Findings of the study revealed that puberty was retarded in the offsprings of treated animals and a noticeable reduction in estradiol and LH. Crump and Trudeau, (2009) showed a reduced LH and sex steroid level in post spawning female
gold fish, *Carassius auratus* exposed to 0.88µg/g of methyl mercury. Tartu et al., (2013) reported altered reproductive behaviour by cadmium through modifications in the gonadotropin and sex steroid release by acting on pituitary gland.

Simultaneous exposures to cadmium and lead have been evaluated by Pillai et al., (2002) in female rats. Adult male rats treated with cadmium showed a decrease in plasma FSH level but no modification in plasma LH (Hachfi and Sukly, 2010). The exposure caused alterations in receptor binding and secretory mechanism of pituitary hormones. Ciarrocca et al., (2013) reported that cadmium exposure in urban air reduces FSH secretion in male workers. Mercury primarily acts on the ability of pituitary to secrete gonadotropin hormones (Tartu et al., 2013) and impairs reproductive behaviour.

In the present study, in general the FSH and LH decreased significantly after the acute and chronic exposure of *C.mrigala* to lead acetate and mercuric chloride as compared to control. Decrease in serum gonadotropin (FSH and LH) level was more in fish exposed to lead acetate than those exposed to mercuric chloride. It may be due to structural and functional alterations in gonadotropes of pituitary gland. Lead acetate seemed to be more toxic.

Gonadosomatic index (GSI) is helpful in identifying gonadal maturity and spawning season of any fish species. The GSI increases with fish maturity and reaches to its maximum at the peak of maturity. The lowering of GSI indicates depletion in gonadal activity due to spawning.

Ahsan (1966) reported changes in gonadotropes in relation to gonads in lake chub. *Cirrhinus reba* was reported to have only one breeding season of short duration from June to August by Rao et al., (1972) and Gupta (1975). Lashari et al., (2007) reported peak in GSI of *Cirrhinus reba* from June to August. The air breathing *Channa gachua* from Godavari was studied for GSI and fecundity by Gaikwad et al., (2009). Further the peak in GSI was found in June indicating gonad maturation. GSI and fecundity of *Labeo calbasu* was studied by Mishra and Saxena (2012). The peak in GSI was reported in July. A small Malaysian stream fish was studied for its spawning period and fecundity by Khaironizam (2013). Two peaks for GSI in both
males and females were reported in November and April respectively and females yielded higher GSI values than males. Qazi (2001) reported the similar observations. Shaikh and Lohar (2011) reported a correlation between GSI and amount of protein and lipid during pre and post breeding seasons in *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala*. The reproductive biology of spiny eel *Macrognathus aral* was studied by Singh and Biswas (2011) and further reported one spawning peak. Similar results were reported by Nabi and Hossain (1996). *Channa punctatus* was studied for seasonal changes in GSI by Kapil et al., (2011). The peak in GSI was reported in rainy season.

Moitra and Sarkar (1976) reported a correlation between maturation of gonads in relation to average weight of pituitary gland, while studying seasonal variations in pituitary gland of *Cirrhinus mrigala*. Metal induced decline in GSI was reported by Chandra et al., (2004) in *Carassius carassius* and Srivastava et al., (2008) in *Channa punctatus*. Seasonal changes in gonadotropes activity and GSI was correlated by Van Oordt et al., (1987) in *Clarias gariepinus*. Low GSI values coincided with lowest diameter of gonadotropes during spent season, an observation reported by Zaki (1994) in *Siganus rivulatus*.

EI-Sakhaawy et al., (2011), studied Nile tilapia, *Oreochromis niloticus* and reported that during winter /non-breeding season the pituitary cells were small and less active while during mature and breeding seasons the cells became active. Ursani (2012), studied the histological changes in pituitary gonadotropes in relation to seasonal changes in gonads in *Oreochromis mossambicus* and reported heightened activity of gonadotropes when GSI was maximum.

In the present study, higher values of GSI in both sexes were reported during June to August indicating the period of maturity, spawning and its extension. Sudden decline in GSI in September indicated post-spawning period. Further decline in GSI until the recrudescence of gonads indicated spent stage. GSI showed a positive correlation with abundance in population of gonadotropes and the serum gonadotropin level during the annual reproductive cycle.
Knowledge of gonad maturation stages of fishes is required for purposes like
determination of mature stock of fish and the size and age of first maturity (Benegal,
1978). It is also required in establishing the reproduction period and length of gonadal
maturation to allow the accurate implementation of fishery legislation (Goncalves et
al., 2006). Gonad maturation has been extensively studied in different fishes by
number of researchers.

Dadzie (1974) studied *Tilapia mossambica* for cyclic changes in histology of
ovary. Six different developmental stages were distinguished. Morphological
development of the gonads in zebrafish, *Danio rerio* (Ham) was studied by Maack
(2003). Histological changes during the annual reproductive cycle and the stages of
maturity in yellow fin seabream were studied by Abou-seedo et al., (2003). Studies
indicated a prolonged spawning period extending from December to April. The
development followed the basic pattern as described in other teleosts.

Mollah, (1982) studied the cyclic changes in the ovary of catfish *Clarias
microcephalus*. The studies indicated two spawning seasons in *Clarias
microcephalus*. Colombian cat fish, *Pimelodus grosskopffí* was studied by Cala (1996)
to observe histological changes in ovary during annual cycle. The biology of gonadal
development, sex differentiation, maturation and sex reversal in fish was reviewed
with respect to cellular, molecular and endocrinological aspects by Guraya (2000).
Transformation of oocytes with peripheral cortical alveoli, lobated nucleus and
several nucleoli into large hydrated oocytes during pre spawning and spawning period
was reported by Loir et al., (2001) in *Dentex dentex*. Gonadal development in catfish
*Clarias gariepinus* was studied under laboratory conditions by Cek and Yilmaz
(2007). Ovarian development during active reproductive phase was studied in *Laboe
dyocheilus* under captive and wild conditions by Singh (2008).Correlated changes in
GSI and HIS and histological features of ovary during the study, demonstrated that
ovarian development was normal in captivity in *Laboe dyocheilus*. Spawning season
was determined by studying the GSI and histology of gonads in *Epinephelus
areolatus* and *Lethrinus vebulosus* by Mahmoud (2009). The results suggested that
one-year-old *C. gariepinus* as a brood stock for seed production. No major difference
was observed between the natural and confined spawning of the fish. Annual cyclic
changes in ovaries of featherback *Notopeterus notopterus* was studied by Chakrabarti and Chowdhary (2013). The seasonal changes in the ovary were described according to GSI variations and cytological changes in germ cells, as growth phase, maturation phase, spawning phase, post-spawning phase and resting phase. Montchowui et al., (2012), studied the gonadal maturation in *Labeo parvus*. Further, seasonal histological trend in gonad development in *Labeo parvus* was proved to be a synchronous, iteroparous spawners from the study.

The spermatogenesis showed the typical pattern of gonadal maturation as described by Takashima and Hibiya (1995). Asynchronous process of spermatogenetic cell formation was observed in *Tilapia nilotica* by Latif and Saady (1973), in *Oblada malanura* by Asem (1992) and in *Mugil seheli* by Zaki et al., (1994). Pollard (1972) found the large amount of residual sperms in the tubules during spent stage in *Galaxias maculates*. After completion of spawning season phagocytes invade the lumen of testis lobule and sperm ducts and ingest the residual sperm (Henderson, 1962). In the present study, with C. mrigala no phagocytosis was observed. This is in agreement with EL. Boray (1997, 2001) and Zaki et al., (1994).

Annual reproductive cycle of male *Labeo rohita* was studied by Kumar et al., (2003). Further, testicular development of *Labeo rohita* was associated with increasing day length and temperature. Long photoperiods and increasing temperature provide favourable condition for gonadal development in *Cirrhinus mrigala* (Singh and Singh, 1984). Singh and Singh (1984) reported a positive relationship between increasing temperature and day length and gonadal development and fall of temperature due to rainfall and surge of gonadotropin level during spawning phase.

Annual gonadal cycle in catfish *Heteropneustes fossilis* was studied for spermatogenesis by Sunderraj and Vasal (1976). Spermatogenesis in rainbow trout *S. gairdneri* was reported to occur twice in year by Billard and Bretn (1978). Marcelo et al., (2009) studied zebra fish, *Danio rerio* for spermatogenesis with an emphasis on spermatogonal generation and reported short duration for the process. Verma et al., (2009) studied seasonal variation in semen characteristics and biochemical composition of seminal plasma of *Cirrhinus mrigala*. Spermatogenesis in fish under
the influence of various factors was studied by Routrav et al., (2011) in Indian major carps. Further, modulation and manipulation of spermatogenesis by various factors was possible to get viable sperms for aquaculture practice.

In the present study, spermatogenesis in *C. mrigala* was studied for the annual cycle. Histological structure of testes showed seminiferous tubules containing nests. The nests showed different spermatogenetic stages. Spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa/sperms were five different spermatogenetic cells found in the tubules at different stages of testicular maturity. The results were similar with El-Boray, (2001) and Kumar et al., (2003).

In the present study, the gonadal development showed the usual teleost pattern. Six oogenesis stages like formation of primordial germ cells (PGCs), transformation of PGCs into oogonia and transformation of oogonia into oocytes (onset of meiosis), vitellogenic growth of oocytes while under meiotic arrest, maturation and ovulation are associated with gonadal development. The process of development of the ovary in the present study did not differ from that described by Dadzie, (1974); Cala, (1996) and Ursani, (2012). In the present study, results regarding the transformation of oocytes of *C. mrigala* were similar with the results of Loir et al., (2001). Ovarian development peak in the present study, was similar with that of sharptooth cat fish *Clarias gariepinus* described by Sehriban (2007).

The physiology of the relationships between stress and reproduction for the poikilothermic vertebrates was reviewed by Greenberg and Wingfield (1987). The gross production of fishes is hampered by toxicological factor which adversely affects the anatomy of goands (Coats, 1979). Reproductive consequences of lead and mercury exposure are widely spread. Almost all aspects of reproduction are affected by lead (Zheng et al., 2003). Lead toxicity effects are more profound on nervous, digestive and circulatory systems (Pokras, 2003). The transmission of impulse needs calcium and lead is handled by the body in the same way as calcium in human, condors or fish, hence the visible effects are seen.
Marked alterations like degenerative changes in tubules, reduction in interstitial cells and varying degree of necrosis were reported in *Colisa fasciatus* exposed to arsenic by Shukla and Pandey (1984). Effect of zinc, lead and copper was studied by Kumar and Pant (1984). All the three metals induce atresia in ovary. Katti and Sathyanesan (1985) observed exposure dependent and concentration mediated changes in the testis of *Clarias batrachus* treated with lead. Saksena and Agarwal (1986) reported resorption of yolk in *Channa punctatus* exposed to mercury. Atretic changes in ovary were reported by Kirubagaran and Joy (1988) after exposure to mercury in *Clarias batrachus*. Tulsi et al., (1989) showed altered hypothalamo-hypophysial ovarian axis resulting in decreased reproductive potential of fish after lead accumulation in brain of *Anabas testudines*. Sublethal lead exposure can cause endocrine dysfunction in fish. Ruby et al., (1993) reported decreased transformation of spermatogonia to spermatocytes in sexually maturing male rainbow trout exposed to 10 µg/l lead for 12 days. Two year old female rainbow trout exposed to 10 µg/l lead for 12 days showed significantly reduced oocyte growth as compared to control (Ruby et al., 2000). Mokhtar et al., (2013) reported histological changes in ovary of *Oreochromis niloticus* exposed to lead acetate for 21 days. Further, the histopathological changes were atresis and wrinkling of follicles. The observations coincided with the results of Kumar and Pant (1984), Adeyemo (2008) and Mazrouh and Mahmoud (2009). Saksena and Agarwal (1991) studied the ovary of freshwater catfish *Clarias batrachus* exposed to mercuric chloride for 45 and 90 days. Further decreased activity of enzymes like acid phosphatase and alkaline phosphatase was reported.

Martinez, (2004) reported that acute exposure of *Prochilodus lineatus* to lead made the organism less able to survive in combination with other stressors. Ovaries of *Oreochromis mossambicus* exposed to nickel sulphate for 30 days showed ovarian impairment. Further, prominent degeneration was observed in *Oreochromis mossambicus* by nickel intoxication by Sioson and Herrera, (1996). *Clarias gariepinus* exposed to sublethal exposure of lead acetate studied by Balawi et al., (2011) showed changes in reproductive behaviour. Furthermore, reduction in abundance and size of eggs, sperm motility was reported. Generally, metal exposure
might result in damage to reproductive organs. *Oreochromis niloticus*, after exposure to sublethal concentration of cadmium showed, deterioration of structures in the gut and in the hepatic tissue (Younis et al., 2013).

Concentration dependent effects of nonylphenol on testicular structure of fish *Xiphophorus maculates* were documented by Kinnberg et al (2000). A distinct reduction was recorded in the percentage of ovarian follicles and increase in atretic follicles after hexavalent chromium exposure in *Channa punctatus* (Mishra, 2009). Vergilio et al., (2013) studied male *Gymnotus carapo* after acute exposure to mercury. Testicular dose dependent damage was reported with respect to testicular arrangement and sperm count. Patil and Dhanade (1990) studied the toxic effect of zinc and cupric chloride on *Channa punctatus* (Bloch). A long term exposure arrested oocyte maturation and several degenerative changes in both the sexes. Gonadal impairment in *Channa punctatus* after exposure to devicyprin was studied by Srivastav et al., (2008). Deshaped oocytes, disrupted follicular epithelial cells in ovary and gross inflammation in testicular region was observed. Cytotoxic damage in testis of *Heteropneustes fossilis* after exposure to sublethal concentration of an anionic surfactant was documented by Kumar et al., (2007). In the present study, acute and chronic exposure of *Cirrhinus mrigala* to lead and mercury showed marked alterations in the ovary and testis under development. The alterations were profound and the degree and changes in histoarchitechture showed variations during exposure to different concentrations. The alterations were more severe at higher concentrations of both the toxicants.

In males, after exposure to various concentrations of lead acetate alterations like the degeneration of seminiferous tubules, vacuolization in spermatogonia, loss of integrity of spermatocytes, indistinguishable arrangement of tissue were observed. These observations were similar with those reported by Katti and Sathyanesan (1985), Patil and Dhanade (1990), Kinneberg, (2000). The degenerative effects in ovary after exposure to lead acetate did not differ from Mokhtar et al., (2013), Kumar and Pant (1984), Adeymo and Mazrouh and Mahmoud (2009).
In the present study, the alterations produced by mercuric chloride in males were similar with those reported by Shukla and Pandey (1984) in *Colisa fasciatus*, and Vergilio et al., (2013) in *Gymnotus carapo*. Exposure to various concentrations of mercuric chloride showed various degenerative changes in the ovary of *C. mrigala* in the present study.