Chapter - 2

REVIEW OF LITERATURE

Ascertaining the sequence of changes in maturity stages in fishes during the year are of considerable importance in building a thorough knowledge of the reproductive cycle and breeding mechanism of the exploited fish stocks and culturable fishes. This is the basic requirement for their successful propagation, management and optimum exploitation.

Fishes are major aquatic food sources and are indicators of the health of aquatic ecosystem but their number has reduced drastically due to extensive exploitation (Gjerde et al., 1983), environmental fluctuations (Tegner and Butler, 1985), predation (Tegner et al., 1992) and natural mortality (Guzman-del proo, 1992). The decline in fish production has lead to the development of aquaculture stocks and genetic management programs. These programs are carried out by capturing wild breeding animals, inducing them to spawn in hatchery, and releasing larvae into natural habitat only when they are competent to survive. The genetic diversity of hatchery reared organisms is low as compared to natural populations (Smith and Carnoy, 1992). This loss in genetic diversity is due to small effective number of parents for producing subsequent generations (Vega et al., 1997). Further, this decrease in genetic variability limits the potential for genetic gain from artificial selection. (Allendorf and Phelps, 1998). Hence, fishes can be managed optimally only when stocks are properly defined.
One of the reasons in the decline of culturable and natural fishes in the nature is the indiscriminate use of pesticide, which made its modest beginning in India in 1948-49 (McKim, 1985). Pesticides are now recognized as environmental pollutants of potential technological concern to fishes as diagnosed by their acute toxicities (Saxena and Mani, 1987). The use of pesticides has increased with growing awareness about their utility in agriculture, animal husbandry and welfare of mankind. The pesticides even when applied in restricted areas are washed by rains to larger water bodies influencing the reproductive physiology of fish adversely. Heavy contamination of pesticides in water lead to oxygen depletion and cases of poisoning and mass mortality of fishes are not uncommon.

The present work i.e. “Toxic Effects of Organophosphorus Pesticide Monocrotophos on Developmental Stages of Oocyte in Ovary and Ultrastructure of Egg of Cyprinus carpio communis L. (1758)” has been undertaken keeping in view the above mentioned facts. The findings of the present investigations shall help in giving the alarm so that the use of this pesticide is optimized and the aquatic biodiversity is saved from such poisonous agrochemicals.

2.1 MONOCROTOPHOS TOXICITY

The pesticide is defined under the Food and Environmental Protection Act (USEPA, 1985) as “any substance, preparation or organism prepared or used for destroying any pest”. The use of natural chemicals was started in during the middle nineteenth century (Penny and Adams, 1863), however, the usage of synthetic pesticide commenced only about seventy years ago.

Extremely important classes of inorganic insecticide, the organophosphates are extremely used worldwide to combat pests. Malathion, an organophosphate pesticide was introduced is 1950 as a broad spectrum pesticide. Monocrotophos was registered in 1965 in the sixty countries of the world, developed by Ciba Geigy (Novarties). Monocrotophos is a nonspecific insecticide and acaricide belonging to vinyl phosphate group (Skripsky and Loosli, 1994).

The evaluation of the toxicity of monocrotophos has been reviewed in the ‘Hand Book of Pesticide Toxicity’ (Gallo and Lawryk, 1991a), International Program on Chemical Safety (IPCS, 1993) and the American Conference of Government Industrial Hygienists (ACGIH, 2003). Monocrotophos is classified by WHO as highly
hazardous and has been responsible for mortality resulting from accidental or unintentional exposures. It is highly toxic and cause disorders related to respiration, reproduction etc. Inhalation or skin contact may increase the susceptibility to the pesticide without showing immediate symptoms (Senanayake and Karalliede, 1987).

Cyclohexanone is a solvent that adds viability to monocrotophos to kill the target and has major health hazard when it comes in direct contact or is ingested causing depressing effect on the nervous system of the organisms and cause allergies (Material Safety Data Sheet). However, no ophalamological, pathological and histopathological effects on rats have been observed when monocrotophos was intravenously administered with cyclohexanone (Greener et al., 1982).

The health hazard assessment of monocrotophos is based mainly on toxicology reviews (FAO/WHO, 1992, 1994, 1996; ACGIH, 2003). Skripsky and Loosli (1994) showed that monocrotophos is a water soluble organophosphate insecticide with high oral and moderate dermal toxicity. Lifetime chronic feeding studies indicated reduced acetyl cholinesterase in blood plasma, brain and other tissues in dogs, rats and mice (Rao et al., 1992).

Monocrotophos has low environmental persistence but it is extremely toxic to birds and is commonly used as bird poison (Smith, 1993; Tomlin, 1994), whereas it is moderately toxic to aquatic organisms like fishes e.g. rainbow trout and bluegill sunfish (Tomlin, 1994; Meister, 1997), aquatic invertebrates like Daphnia (Woodbridge, 1996). Monocrotophos is mutagenic (Bhunya and Behera, 1988; Kumar and Janardhan, 1988), genotoxic (Bhunya and Jena, 1993; Peitl et al., 1996) and carcinogenic but not embryotoxic or teratogenic even though it may cause reproductive toxicity (Fuchs, 1992).

Various workers have tried to assess the monocrotophos toxicity on different parameters of fish in the past. Some of their published work includes the effect of monocrotophos on behaviour of Anabas testudineus (Santhakumar and Balaji, 2000); leucocyte count of Channa punctatus (Seth and Saxena, 2003); erythropoietic activity of Channa punctatus (Agrahari et al., 2006); haematological indices of Clarias gariepinus (Yaji and Auta, 2007); oxidase stress and locomotor behavior response of Gambusia affinis (Kavitha and Venkateswara, 2007); genotoxic effect in Channa
punctatus (Ali and Kumar, 2008) and inhibition of Na\textsuperscript{+} K\textsuperscript{+} ATPase in Channa punctatus (Agrahari and Gopal, 2008).

2.2 EFFECTS OF TOXICANTS ON REPRODUCTIVE POTENTIAL OF FISH

The first paper on toxicological studies of fish was published by Penny and Adams in 1863. Among the aquatic organisms, fish (Stephen and Mount, 1973) and their eggs have attracted considerable interest for toxicity testing, particularly because early life stage test represent the life cycle test (McKim, 1985).

The morphology of fish’s eggs or ovaries changes during reproductive cycle of fish helps to recognize seven phases based on gross morphological changes in ovary, percentage of oocytes, ova diameter and GSI (Guraya et al., 1975) and into five phases based on appearance of nucleus and nucleoli and distribution of cytoplasmic organelles in Garra ruffa (Bardakei, 1987). The maturity stages of fresh water fishes have been described by Lagler (1978) and of Cyprinus carpio communis by Alikunhi (1966).

The effect of chemicals on gonad development has been the subject of various workers in past like effect of urea on ovarian cycle of Mystus vittatus (Srivastava, 1994; Jyothi and Narayan, 1996; Bawah and Das, 2002; Sharma, 2002; Srivastava et al., 2002; Sohal et al., 2003). The process of oocytes development in teleost fish was studied by Tyler and Sumpter (1996) and in Fundulus heteroclitus by Archie (1938). The ultrastructure of oocytes growth (Chaudhary, 1956; Nicholls and Maple, 1972; Hirose, 1975; Cruz and Cruz, 1992), the cellular envelopes of developing oocyte (Iwaskai, 1973; Anderson, 1974; Wourms and Shelden, 1976; Shockley and King, 1977; Brusle, 1980) and accellular covers during growth and after eggs maturation (Dumont and Brummett, 1980; Lapes et al., 1982; Anderson, 1966) has been discussed earlier.

The teleost egg has membranous envelope which is made up of two layers (Ivankov and Kurdyayeva, 1973; Balon, 1977). Structure and thickness of eggs envelope vary depending on developmental stages and environment (Riehl, 1978). Ultrastructure of micropyle on egg surface is a criterion for egg identification in teleosts (Riehl and Schulte, 1978). Rottmann et al. (1991) discovered an attachment apparatus at animal pole of three cling fishes, however some fishes like Cyprinus
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carpio and Apoyon inberbis have completely smooth eggs (Riehl and Patzner, 1994; Breining and Britz, 2000; Lahnsteiner, 2003). The diameter of eggs of C. carpio has been found to be 0.9–1.2 mm (Breining and Britz, 2000). Ultrastructure of eggs membrane and micropyle of Hypothalmicthys molitrix was worked out by Esmaeili and Johal (2005). The diameter, appearance of eggs and position of nucleus in eggs are the indicators of type of egg development (Riehl, 1978).

The effects of various toxicants on the egg have also been studied. Zinc affects the enzymatic process that softens the egg capsule (Grande, 1967). Fertilization rate is effected by PCB in Atlantic salmon (Jensen et al., 1971), chlorinated hydrocarbons on the eggs of winter flounder (Smith and Cole, 1973) and heavy metals in herring egg (Kihlstrom et al., 1971). The low pH impaired egg production in Flagfish (Craig and Bakshi, 1977). Phenyl mercuric acetate affects the number of eggs spawned in Zebra fish (Ojaveer et al., 1980). The eggs of common carp were found to be more sensitive to Zn effluent at higher developmental stages (Sharma and Sharma, 1994). Synthetic pyrethroids in common carp eggs resulted in residue accumulation (Dhawan and Kaur, 1996).

Stouthart et al. (1998) studied PCB induced stress response in larvae of common carps mediated by hyderomineral balance. Ramesha et al. (1997) studied the combined toxicity of Hg and Cd to embryos of common carp. Some work on the effects of fertilizers on early developmental stages of Cyprinus carpio has been undertaken by Gupta, (2000). The toxicity of two broadly used pesticides, valour and match on fry of C. carpio was studied by Bhatnagar et al. (2003).

Along with the reproductive potential of fish, the toxicants are also found to affect the various aspects of behavior of the fish. Some of these studies include the decrease in oxygen consumption and feeding energetic upon exposure to urea in Oreochromis mossambicus (Palanivelu and Vijayavel, 2004); decreased swimming speed of Gambusia affinis by mercury (Jakka et al., 2007) and Artemia salina by four organophosphorus insecticides (Rao et al., 2007); genotoxicity in the form of DNA breaks by endosulfan in Mystus vittatus (Sharma et al., 2007). The altered behavioural responses in variety of fishes has been reported by many workers like in Heteropneustes fossilis by temperature fluctuations (Pandey et al., 2008); altered behavioural response in Labeo rohita by cypermethrin (Marigoudar et al., 2009), in Rasbora daniconius by paper mill effluent (Pathan et al., 2009), in Cyprinus carpio
by profenofos (Ismail et al., 2009), dimethoate (Singh et al., 2009) and quinalphos (Chebbi and David, 2009).

2.3 HISTOLOGICAL STUDIES

The process of oocyte development in teleost fish was studied by Tyler and Sumpter (1996). On the basis of the histological study of oocyte development, a number of distinct developmental stages are delineated. Microscopic examination of ovaries revealed five developmental stages in sword fish (Yamamoto, 1956), seven phases in Mystus tengara (Guraya et al., 1975), seven stages in Dicentrarchus labrax (Mayor et al., 1988), nine stages in Hemiodus species (Brandao et al., 2003), five stages in bullet tuna (Macias et al., 2005), five stages in Symphysodon and megrim (Chellapa et al., 2005; Robson and King, 2006).

Some workers tried to evaluate the effect of pesticides on ovary of fishes. Some of them observed the effects of carbofuran in fish (Carten and Groves, 1973; Kabir and Ahmed, 1979; Karpagaganopathy and Sukumar, 1988; Ram and Singh, 1988; Sukumar and Karpagaganopathy, 1988). Others reported the prevention of reproduction by carbaryl (Carlson, 1973), toxic effect of Zn and Cd on Phoxinus phoxinus (Bengtsson, 1974, 1975), effect of submithion on common carp eggs (Kapur et al., 1978), effect of Zn on Poecilia reticulata (Pierson, 1981), effect of submithion on ovary of Garra mulya (Pawar and Katdare, 1983) and toxicity of HgCl, CdCl, phenol and NH3 to Channa punctatus (Bhattacharya, 1985).

The deleterious effects of pesticides have been reported in few studies such as delayed maturity (Crandall and Goodnight, 1962), abortion in Gambusia (Boyd, 1964), reduced cholesterol and lipid metabolism in ovary (Singh and Singh, 1980), total atresia and presence of haematoma in ovary (Kling, 1981), expansion of renal tubules (Gupta and Rajbanshi, 1982), deformity in size and reduction in GSI (Kulshrestha and Arora, 1984), decline in percentage of different stages of oocyte (Pandey and Shukla, 1985), desquamation in epithelial cells of renal tubules (Mani and Saxena, 1985), increase in interfollicular space (Singh and Sahai, 1985), degeneration of ovagerous lamellae (Harilal and Sahai, 1986), benign cystic transformation of ovarian cells (Kumar and Janardhan, 1988), necrosis and fibrosis in connective tissue of ovary (Rastogi and Kulshrestha, 1990), wrinkled oocytes in retrogressive type of ovaries (Sukumar and Karpagaganopathy, 1992), reduced...
growth of oocytes (Srivastava and Srivastava, 1994), altered biochemical composition of fish (Vincent et al., 1995), deposition of granular material in hepatocytes (Sharma and Sharma, 1994), vacuolation of cytoplasm (Das and Hazarika, 1998) and apoptosis in ovarian follicle (Virk and Dhawan, 1997; Virk and Kaur, 1999; Nanda et al., 2000; Tanz et al., 2001).

Bieniarz and Epler (1976) studied the condition of ovary in sexually mature common carp which failed to spawn showed resorption of mature oocytes and absence of early maturation stages. *Cyprinus carpio* eggs exposed to pyrethroids yields distorted larvae and reduced hatching rate (Dhawan and Kaur, 1996).

Liney et al. (2006) have reported severe health effects in fish on biochemical, hormonal and morphological parameters upon long term exposure to effluents of waste treatment water. Pesticides pose threat even at the fingerling stages by inducing histopathological changes in *Cyprinus carpio* (Dincel et al., 2009). Cypermethrin is reported to induce histological changes in gonads of *Heteropneustes fossilis* (Singh and Singh, 2008) and *Oreochromis niloticus* (Korkmaz et al., 2009). The similar effects have been noticed in ovary of *Lepomis macrochirus* on exposure to endosulfan (Dutta and Dalai, 2008).

### 2.4 STUDY OF VITELLOGENESIS

Vitelligenin (Vtg) is an estrogen induced yolk precursor lipophosphoprotein which is present in blood of oviparous vertebrates and invertebrates during vitellogenesis (Bergink and Wallace, 1974). The primary structure of the Vtg molecule, however, generally differs between fish species, even between closely allied species (Lee et al., 1992). The classification of Vtg as phosphoglycoprotein indicates the crucial functional groups that are carried on backbone of molecules, namely lipids, carbohydrates, phosphate group (Sire et al., 1944; Mommsen and Walsh, 1988).

In oviparous animals, accumulation of yolk material into oocytes during oogenesis and the mobilization during embryogenesis are key processes for successful reproduction. Oocyte yolk proteins and lipids are derived from enzymatic cleavage of precursors, predominantly Vtg and very low density lipoproteins (Weigand, 1996; Tyler and Sumpter, 1996; Schneider, 1996). Yolk is then secreted in late stages of oogenesis and is mobilized in the embryo to provide nutrients for embryogenesis.
(Wallace, 1985). Thus, vitellogenesis is defined as induced hepatic synthesis of egg yolk protein precursor, Vtg, its secretion and transport in ovary and its uptake into maturing oocyte (Pawleoski et al., 2000; Kwon et al., 2001).

The extra ovarian synthesis of egg yolk as a highly conserved reproductive strategy has been adopted by all egg laying vertebrates (Wallace and Selman, 2005). In fishes, vitellogenin is synthesized in liver parenchymal cells (Phartyal et al., 2006). Blood transport vitellogenin from liver to ovaries where it is selectively sequestered by receptor mediated endocytosis into developing oocytes (Tseng et al., 2001). It has been reported that ovarian estrogens stimulate the liver to synthesize vitellogenin (Wheeler et al., 2005). In fact, vitellogenesis can be induced at any time of year by administration of estrogen (Verslycke et al., 2002; Phartyal et al., 2006). Estradiol diffuses freely across membrane of liver cell and binds to estrogen receptors so initiating transcription and translation of Vtg (Sehgal and Goswami, 2004).

Sehgal and Goswami (2001) worked to determine the biochemical nature of vitellogenin in fishes. Sehgal and Goswami (2004) later reported that vitellogenin exist as charged isomers in Channa punctatus. The egg yolk is found to be composed of lipovitellin and phosvitin (Sehgal and Goswami, 2005). However considerable heterogeneity has been reported in the physico-chemical characteristics of piscine vitellogenin (Sehgal and Goswami, 2002). During vitellogenesis three phosvitins appear with intermediate contents which are eventually phosphorylated to final products (Arukwe et al., 2002). Vitellogenin and its simplified forms that is lipovitellin, phosvitin are stotred in ooplasm as yolk platelets or granules (Sehgal and Goswami, 2003).

Vtg is weighted after a lengthy purification procedure has used different protein quantification methods such as Lowry (1951), Bradford (1976) or simple absorbance measure. The vitellogenin can exist in forms with different degree of phosphorylation (Von Bohman et al., 1981). So, Craik and Harvey (1984) suggested that it is not possible to convert concentration of plasma phosphorus protein to concentration of Vtg unless protein phosphorus contents Vtg is known. The radioimmunoassays (Sumpter, 1985) and enzyme immunoassays (Harries et al., 1996) for Vtg detection in rainbow trout are available which is considered best for in situ monitoring for presence of environmental estrogens (Jobling et al., 1996; Bon et al., 1997; Thorpe et al., 2000). The sensitive and quantitative methodology for Vtg in
zebra fish, fathead minnow and medaka species have been developed by Fenske et al. (2002). Vtg was isolated by anion exchange chromatography from plasma of Danio rerio induced with 17 α ethylestradiol and zVtg. ELISA for study of environmental estrogens in zebrafish (Arukwe et al., 2002). Verslycke (2002) discovered that indirect technique like ALP, HSI, GSI have similar sensitivity as that of EIA, at least in EE₂ exposure studies and is thus a rapid, simple and cost effective alternative to immunoassays. The range obtained for Vtg in maturing female carps were similar to those observed in other cyprinid fish that could reach up to 1mg/ml (Van Aerle et al., 2001; Petrovic et al., 2002) in rivers with high concentration of estrogenic compounds.

Xenobiotically induced hepatic Zrp and Vtg synthesis may cause an imbalance in reproductive strategy of fish population (Ware, 1980). The high Vtg might cause kidney failure and increased mortality rates as a result of metabolic stress (Herman and Kincaid, 1988).

Vitellogenin has been proved to be a core endpoint to assess exposure of fish to environmental estrogens (Sumpter and Jobling, 1995; Korsgard and Pedersen, 1998) as increased vitellogenin synthesis may be related to activation of hepatopancreas estrogen receptor (Heppell et al., 1995; Kime et al., 1999). Plasma vitellogenin measurements therefore provide valuable information about exposure to endocrine disruptors and not only to environmental estrogens (Tseng et al., 2001).

A large number of in vivo studies have also been reported the vitellogenin induction by xenobiotic estrogens in fish and amphibians (Jobling and Sumpter, 1993) using rainbow trout, Pelissero et al. (1993) using trout and common carp. All these studies showed significant elevations of vitellogenin at tested dose. In other studies Sumpter and Jobling (1995), Jobling et al. (1996), Schwaiger et al. (2000) have reported in vitro induction of yolk protein synthesis (in a dose dependent manner) of several environmental chemicals, including alkyl phenol etoxylate. Heiden et al. (2006) and Lal (2007) reported that the liver produced decreased vitellogenin under the influence of pesticides due to lack of appropriate stimulus from the central nervous system.

Sehgal and Goswami (2001) reported that liver glycogen content is a net result of activities of enzymes glycogen synthetase and glycogen phosphorylase. Pereira et
al. (2005) reported that the reduced HIS, ALP on serum vitellogenin due to Cd toxicity result in lower fecundities for adults and smaller yolk sacs for larvae reducing overall reproductive success of winter founder population.

The GSI, Vtg level and histological observations were made on common carp from three rivers receiving sewage treatment plant effluents by Carballo et al. (2005).

Ruby et al., (2005) reported that elevated plasma vitellogenin levels along with decreased vitellogenin levels in the gonads suggest that exposure of female Salmon to HCN inhibits the uptake of vitellogenin at ovarian level. Zha et al. (2006) also reported increased plasma vitellogenin levels in male *Oryzias latipes* upon exposure to pentachlorophenol.

### 2.5 HORMONAL STUDIES

Improved histochemical techniques and electron microscopy have shown promise of localizing the cellular sources of steroid hormones in fish ovary (Lofts and Bern, 1972; Guraya, 1976a). In some fishes such as rainbow trout, after ovulation, granulosa, thecal and interstitial cells can synthesize steroids that may have a function in the maintenance of ovulated eggs and estradiol production (Lambert, 1978; Van der Hurk and Peute, 1979; Guraya, 1979).

The ovarian hormones are mostly synthesized by the special thecal cells which are homologous to leydig cells in males. (Nandi, 1967; Bern and Chieffi, 1968; Hoar, 1969; Ozon, 1972; Reinboth, 1972; Colombo and Clemenze, 1972). However, ovarian steroidogenesis has been attributed to the granulosa cells in *Cyprinus carpio* and *Sarotherodon aurea* (Jalabert, 1976; Sundararaj and Goswami, 1977). The teleost ovary is capable of providing C18 estrogenic steroids has been determined by experiments involving extraction and identification of estrogenic steroids from ovary (Hoar and Nagahama, 1978).

The teleost ovary is also known to produce several nonestrogenic steroids (Sandor, 1979). Multiple sites for prostaglandin synthesis are discovered within fish ovary (Geotz, 1991).

Progesterone has been identified in ovaries but its presence in the blood has not so far been reported except in winter flounder, *Pseudopleuronectes americanus* (Campbell et al., 1976).
In *Heteropneustes fossilis*, plasma levels of testosterone (Truscott, 1978) are much lower than those of estrodiol-17β during vitellogenic period, whereas higher in maintenance phase (Sundararaj et al., 1980).

An environmental endocrine disruptor is defined as an external compound that interferes with or mimics natural hormones in the body that are responsible for the maintenance, reproduction, development or behaviour of an organism (USEPA, 1997). Endocrine disruption is, therefore, likely to affect breeding dynamics and reproductive success in group spawning fish (Jon et al., 2004).

The US Geological Survey (USGS) National Water Quality Assessment (NAWQA) program recently found evidence of endocrine disruption in common carp and large mouth bass (*Micropterus salmoides*) collected from waterways that contain synthetic organic compounds. Evidence indicates concentration of sex steroid hormones and vitellogenin were different in fish from contaminated and reference sites (Folmar, 1993).

The close neural relationship of hypothalamus and pituitary gland with the brain makes them particularly vulnerable to neurotoxins such as organophosphate pesticides and heavy metals. They damage the neurons of hypothalamus which are responsible for GnRH release, leading to failure of ovaries and testes to produce yolky eggs and viable sperm (Kime, 1998).

Analysis of estrogen and testosterone in blood of carp showed a relation between concentration of waterborne pesticides and levels of sex steroid hormones (Balch et al., 2004).

Alteration in blood concentration of sex steroids is associated with reproductive impairment and other critical reproductive factors (Calbom and Clement, 1992) and feminized behaviour in male western gulls of southern California (Fry and Toone, 1996).

The aromatase enzyme converts testosterone to estradiol in female fish. Some compound inhibits this aromatase activity and cause decreased estrogen synthesis. Such aromatase inhibiting activity has been demonstrated for imidazole fungicides (Monod et al., 1993), pulp mill effluents (McMaster et al., 1995), PAHs (Afonso et al., 1995) and tributyltin. These compounds also affect steroid feedback to pituitary in both sexes since this is dependent on aromatic activity (Piferrer et al., 1994). Galas et
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al. (1999) studied the seasonal variation of steroid secretion by dispersed carp ovarian follicular cells as influenced by carp pituitary homogenate, hCG.

hCG stimulated germinal vesicle breakdown in oocyte in several fish species (Patino and Thomas, 1990; Degani and Boker, 1992; York et al., 1993) and steroid production in vitellogenetic and full grown ovarian follicle (Srivastava and Van der-Kroak, 1994) has been reported earlier.

Galas and Bieniarz (1989) showed characteristic seasonal fluctuations of progesterone, estradiol as measured by their level in carp plasma and ovarian tissue homogenates. Jalabert and Fostier (1984a) showed that in Onchorhynchus mykiss, a high level of estradiol could also prevent oocyte maturation before vitellogenesis has completed.

Khan and Law (2005) studied the adverse effects of various pesticides and related chemicals on enzyme and hormone systems of fishes, amphibians, reptiles and suspect them to be major cause for their decline. Xie et al. (2005) evaluated estrogenic potencies of four herbicides and surfactants using rainbow trout vitellogenin assay and reported 93-fold increase in plasma vitellogenin. Heiden et al. (2006) reported that TCDD is potent endocrine disruptor effecting reproduction specially estradiol concentration and vitellogenin level in Danio rerio. Several other workers reported the organophosphates as efficient endocrine disruptors like Laura and Dutta (2005) studied endocrine disruption in Lepomis macrochirus by diazinon. Lai (2007) reported endocrine mediated reproductive dysfunction in Indian fishes; Singh and Singh (2008) reported lowered levels of estradiol-17β in Heteropneustes fossilis by cypermethrin and lowered sex steroids in fishes during breeding phase from north india in another studies. Recently, Scholz and Kluver (2009) also revealed the effects of endocrine disrupters on sexual and gonadal development in many fishes.

Vitellogenesis provides a valuable biomarker for endocrine dysfunction in both sexes (Kime et al., 1999). The presence of Vtg in the plasma of male fish is an indication of exposure to an estrogenic stimulus (Fry and Toone, 1996). It may be pointed out here that till now the effects of pesticides on hCG have not been reported.
2.6 ELECTRON MICROSCOPIC STUDIES

A variety of aspects related to oogenesis, developmental stages, organelles in the oocyte, membrane bounding oocytes etc. have been studied by various scientists using electron microscopy.

Guraya (1965) revealed that the various organelles in the oocyte including undeveloped mitochondria and endoplasmic reticulum were found to be ultrastructurally associated to oil droplets formed during oocyte development. Nicholls and Maple (1972) proved that during vitellogenesis many proteins and lipids are actively synthesized (Te Hessen, 1977). Micropolysaccharides are now found to be the main constituent of cortical alveoli (Khoo, 1979; De Vlaming, 1983).

It has been found that some or all of the oocyte yolk has an exogenous origin, probably being synthesized in liver and transported to the ovary through circulation as revealed by electron microscopy (Droller and Roth, 1966; Narranvang, 1968; Brusle, 1980). Thiaw and Mattei (1991) found that follicular epithelium of oocyte in Aphyasemion splendopleure is made of prismatic cells in which density of yolk is variable.

Cruz and Cruz (2000) reported the presence of great quantities of electron dense intercellular material in the follicular epithelium of P. microps which is uptaken from circulation and enter the follicle through intercellular spaces and in perioocytic space. Lian-ju (2000) described a method for observation of embedded sections of fish gonad by SEM technique.

Sphenoid nucleoli and yolk vesicles and several bundles of filaments adhered on the nucleoli could be viewed by SEM technique for first time. The SEM technique was also used to examine the morphological changes in the oocyte of Japanese eel, Anguilla japonica, induced to undergo ovarian development by repeated injection of Salmon pituitary homogenate (Kayaba et al., 2001) whereas Trehan and Garg (2001) studied the seasonal reproduction activity in ovaries of Cirrhinus mrigala using SEM technique.

The transmission electron microscopy was used to study the viability of oocytes retained within the ovarian cavity and outside ovarian cavity of Prochilodus marggravii fish which was induced to spawn with carp pituitary extract by Rizzo et al. (2003). These techniques were also used to characterize the presence of nuage...
during oogenesis and early spermatogenesis of *P. mesopotamicus* in order to contribute to knowledge of behaviour of the nucleus-cytoplasm exchanges during gametogenesis by Abdulla and Cruz (2004). The evolution of nucleolus has been followed during oogenesis in teleost fish *Barbus barbus* using TEM technique (Thiry and Poncin, 2005).

Structural and functional relationships between oocytes and their envelopes were studied by means of electron microscopy in several teleost species by injecting fish with horseradish peroxidase (Abraham et al., 1984). The chorion surface has been analyzed using SEM by Johnson and Werner (1986). They described the external morphology of chorion of five freshwater fishes and concluded that SEM is a powerful tool for identifying the fish eggs.

Flegler (2004) undertook the electron microscopic studies on the development of chorion of the viviparous teleost *Dermogenys pusellus*. The ultra structural features of the micropyle and their position on the surface of egg envelope can be used as criterion for the identification of eggs in teleost. (Riehl and Schulte, 1978; Riehl, 1993; Riehl and Kokoscha, 1993). It was suggested by Riehl (1980) that the ultrastructural features of micropyle can serve as taxonomic characters which are species specific.

Chen et al. (1999) used SEM technique to study the ultrastructural features for egg identification in three genera of the order Perciformes. Esmaeili and Johal (2005) highlighted the ultrastructural features of egg envelop of silver carp, *Hypothalmichyes moltrix*. Tsukahara (2008) studied the formation of attaching filaments and villi on the surface of the oocyte of *Ozyzias latipes* using TEM technique.

The dissolved pesticides in aquatic environment may induce effects on the reproductive potential of fish is also revealed by electron microscopic studies on the ovary of fishes (Campagna et al., 2005). Many ultra structural changes are induced by these pesticides. Dutta et al. (2004) reported the dissolution of membrane along with degeneration of follicular cells between oocytes in *Heteropneustes fossilis* upon exposure to malathion. Campagna et al. (2005) not only reported increased number of mitochondria in porcine oocytes upon exposure to dimethylsulfoxide but also suggested that this change, facilitate energy formation necessary to provide
metabolizing enzymes to compensate stress induced by the pesticide. Ateeq et al. (2006) revealed vacuolization in butachlor exposed oocytes in *Clarias batrachus* by the technique of transmission electron microscope. Atteq et al. (2006) reported nuclear blebbing in *Clarias batrachus* upon exposure to butachlor. Dutta and Dalai (2008) explained that increased number of atretic oocytes on pesticide exposure is due to lack of sufficient endogenous gonadotrophin.

2.7 BIOCHEMICAL STUDIES

Organophosphorus compounds are well known for having more biochemical toxic effects in teleosts than in mammals (Scott, 1967; Jackson, 1976). They have been found to have a significant inhibitory effect on the control mechanism of reproduction in fishes (Arunachalam et al., 1980). These chemicals also affect other parameters like the swimming performance of esturine sheephead minnow, *Cyprinodon variegatus* (Cripe et al., 1984).

Enzyme inhibition has been suggested to evaluate the impact of organophosphates on carbamates in aquatic ecosystems as biological indicators in prevention of deleterious effects. (Rand and Petrocelli, 1985; Heath, 1987; Boudou and Ribeyre, 1989; Dutta et al., 1995; Luskova, 1997). The long term effects of organophosphates and carbamates on aquatic biota are difficult to assess because most of these compounds have relatively short half lives, are highly soluble, and in general have low bioaccumulation rates (Nimmo, 1985). Moreover, it is difficult to make comparisons with other species due to great range in acute toxicity levels for organophosphate insecticide (Rand and Petrocelli, 1985; Hughes et al., 1997).

The different biochemical parameters in muscle, liver and gonads were studied in *Channa punctatus* upon exposure of dimethoate pesticides (Tripathi and Singh, 2003). Similarly, Shweta et al. (2007) discovered significant alteration in various biochemical parameters and concluded that they are dose dependent.

Glucose increase is a general response in fish to acute pollutant effects, including organophosphates (Srivastava, 1981; Singh and Srivastava, 1982; Mishra and Srivastava, 1983; Natarajan, 1989; Gill et al, 1990; Balint et al., 1995; Sancho et al., 1999).

The inhibition in protein level due to increase in ALP activity was suggested by Pilo et al. (1972), Sastry and Sharma (1978), Ansari and Kumar (2007) and Das

Khattak and Hafeez (1996) studied the protein concentration in *Cyprinion watsoni* when exposed to malathion. Sancho et al. (1999) declared significant decrease in protein concentration on treatment with fenitrothion. Oruc et al. (2006) studied the effect of diazonin on lipid peroxidation and AChE activity.

Enzyme cholinesterase activity related to pesticides effects has been measured in blood (Meeter and Walthius, 1968; Ahdaya et al., 1976; Chattopadhyay et al., 1982; Honkakosh et al., 1988), brain (Coppage, 1972; Nemcsok et al., 1984; Heath, 1987; Carr et al., 1995; Dutta et al., 1995; Bianchini et al., 1997), heart (Nemcsok et al., 1984), RBC (Malla Reddy et al., 1992), gills (Straws and Chambers, 1995), liver (Cunha Bastos et al., 1998) and plasma (Parma de Croux et al., 2002; Hughes et al., 1997). Weiss (1961) reported that acetylcholinesterase inhibition as low as 8% is lethal to some fish species whereas other studies reported the tolerance level of 70-90% in some fishes (Gruber and Munn, 1998).

Similar studies on exposure to anti ChE agents in fish found that chronic ChE inhibition alters swimming (Matton and Laham, 1969), feeding (Bull, 1974), social interaction (Symons, 1973) and causes flared opercula and hyper-excitability (Zinkl et al., 1987). Organophosphates and carbamates act as anticholinesterase agents by binding to esteric site of the cholinesterase enzyme (Matsumura, 1985).

Lidman et al. (1976) studied change in liver body weight ratio in rainbow trout when exposed to PCB. Singh and Singh (1980) studied total lipid in liver in *Heteropneustes fossilis* in response to Cythion and Hexadrin, Asztalos et al. (2005) confirmed liver damage by change in activities of GPT and GOT in *Cyprinus carpio*.

The protein containing yolk has been reported to develop in a number of fish oocytes (Chopra, 1958; Malone and Hisoka, 1963; Guraya, 1965; Guraya et al., 1975; Khoo, 1979). Gjessing (1963) showed the levels of several amino acids in eggs of *Clupea herangus* increased with maturity. Kapur et al. (1978) have observed reduced 3β HSD activity in gonads of common carp in response to fenitrothion treatment. The common carp was also studied for the fluctuation in chemical composition of the muscle in relation to maturity by Masurekar and Pai (1979).
The latest research work conducted by different scientists also highlights the effects of various organophosphate pesticides on various biochemical parameters of fish. Some of them are the effects of chlorpyrifos in liver of *Channa punctatus* (Jaroli and Sharma, 2005); diazinon on brain of *Cyprinus carpio* (Oruc et al., 2006); monocrotophos on biochemical parameters of *Oreochromis mossambicus* (Venkateswara and Rao, 2006; Vijayavel et al., 2006) etc. Rao (2006) studied the toxic effect of RPR-II and RPR-V on biochemical parameters of *Oreochromis mossambicus*. The monocrotophos induced effects on biochemical parameters of *Cyprinus carpio* (Neelamegam et al., 2007) and *Channa punctatus* (Agrahari et al., 2007) have also been studied.

Fatma and Nahed (2008) reported decline in ALP, protein and glycogen in *Tilapia zillii* on exposure to environmental pollution. A descending trend in ACh levels were reported in *Labeo rohita* by malathion (Patil and David, 2009) and *Cyprinus carpio* by quinalphos (Chebbi and David, 2009). The decrease in protein content, cholesterol and glycogen were reported in *Tilapia mossambica* by cypermethrin (Logaswamy and Remia, 2009), in *Oreochromis mossambicus* by heavy metals (Firat and Kargin, 2009) and in *Channa punctatus* by monocrotophos (Agrahari and Gopal, 2009) etc.

From the above review, it is inferred that the modifications in physiological controls are fully as numerous and diverse as those of anatomical nature. In a sense, the reproductive system of animal is independent of the other organ systems and can, perhaps, respond to evolutionary pressures more freely; although reproduction is indispensable to the survival of the species, it is not a matter of life and death for the individual. Hence, there is considerably need for more investigations using advance techniques and endocrine procedure of oocytes along with thorough analysis and understanding of normal behaviour of the species under investigation not only do we require more "in depth" studies but also it is essential to consider wider variety of fishes.

The stocks of most of the fishes in nature are declining due to the presence of various pesticides and effluents in the water, the abode of fishes. Sufficient information is available on the impacts of these pollutants on the various vital fish tissues, but little information is available on the effects of the pollutants on the various development stages of fish egg, which develop inside of the body of fish. These
changes may be responsible for the low fecundity, abnormal development of the eggs responsible for the either no fertilization or poor fertilization of the eggs. In both the cases, the ultimate result is the formation of either deformed embryos, which will die after some time or low auto stocking in the nature. This shall lead to the failure of aquaculture practices and decline of overall fish diversity.

The finding of the proposed study shall give clear picture how the pesticides alter the developmental process of the eggs of a culturable fish. On the basis of these observations, some suggestions can be given to the fish farmers and the State Fisheries Department for the rational use of pesticides in the vicinity of the fish farm where these fishes are being cultured.