The African catfish, *Clarias gariepinus* is an exotic fish, introduced in India inadvertently in the year 1993. It is known to be a hardy carnivore that can withstand adverse environmental conditions. *Clarias gariepinus* is a threat to the native fish species, because it predates on their young ones. Although the Government of India has banned the culture and sale of *Clarias gariepinus*, it continues to be freely available in most fish markets of the country. Therefore, a more effective way of managing this fish is to conduct basic research on its reproductive biology so that suitable strategies can be devised to keep its numbers in check. The present investigation is a step in this direction and deals with an important aspect of reproduction, viz. oocyte maturation and hydration.

In oviparous fishes, the centrally located egg nucleus or germinal vesicle is temporarily arrested in diplotene stage of meiotic prophase1 during vitellogenesis. At an appropriate time meiosis resumes, germinal vesicle shifts to the peripheral position and breaks down, followed by unequal cytokinesis leading to formation of small polar bodies. Besides these changes some morphological changes like decrease in the opacity of oocyte, coalescence of oil droplets to form large globules, and depending on the species, oocyte hydration also occurs. Oocyte maturation is a well-coordinated hormonal process involving gonadotropin (LH), maturation-inducing hormone (MIH), and maturation-promoting factor (MPF) subsequently followed by ovulation. One of the important changes that occur in the teleost during oocyte maturation is the process of oocyte hydration. Oocyte hydration prepares the oocytes according to the habitat in which they will be ovulated and also directly or indirectly affects the yolk physiology that determines the nature of the embryonic development.

The study incorporated in this thesis deals with the physiological mechanism of oocyte hydration associated with the maturation in the African catfish, *Clarias gariepinus*. Cytoplasmic reorganization during meiotic resumption has been investigated. An attempt has been made to explain molecular mechanism of oocyte
hydration. Expression of aquaporin and cathepsin genes has been investigated to speculate their role during maturation.

The first part of the study deals with genes involved in water uptake and proteolysis of yolk proteins. To analyse expression of *aquaporin* (1 and 3) and *cathepsin* (B, D and L) genes, specific primers were designed for amplification of a portion of these genes. These amplified products were cloned and sequenced. From the obtained sequences, subjected to BLAST on NCBI, phylogenetic trees were constructed using the matching nucleotide sequence from different species. With the help of a translation tool, these nucleotide sequences were translated to amino acids to confirm conservancies at amino acid level.

The second part of the thesis demonstrates that C-21 steroids, dihydroxypregnenlone (DHP) and cortisol, alone or together, can initiate maturation in the oocytes of the catfish under *in vitro* conditions. A steady increase in the water content is also observed in oocytes undergoing maturation. Further, maturational response induced by DHP and cortisol, as well as water content in the respective oocytes is reduced in the presence of ion or water channel blockers. The result indicates that ion channel blocker is more effective than water channel blocker to inhibit the movement of water into the oocytes.

In gravid catfish influx of water is not restricted to oocytes only following initiation of maturation by gonadotropic releasing hormone (GnRH), but simultaneously water content of liver, kidney, gills and muscles also varies. Within four hrs of GnRH administration, water content of oocytes, liver and kidney increases substantially, which enhanced further at eight and twelve hrs; in gills significant increase in water content is evident only after ovulation; whereas water content dropped in muscles. These experiments indicate that in African catfish spawning demersal eggs in freshwater, water content of oocytes approximately increases 8-10% during maturation and ovulation.

The maintenance of body fluid homeostasis is one of the osmotic challenges faced by fishes. Aquaporin(s), water channel proteins, are expressed in osmoregulatory organs (gills, kidneys and intestinal epithelium) in response to changes in environment.
Recent works have suggested role of aquaporin-1b for facilitating water permeation and resultant swelling of oocytes during maturation. The present study correlates the expression of *aquaporin* (*aqp*) transcripts with change in water content of various tissues. Qualitative analysis of both *aqp* genes in different tissues (ovary, liver, kidney, gill and muscles) confirms expression of *aqp1* and *aqp3* in all the tissues, except muscles where *aqp3* fails to express. Although both the genes are expressed in yolky and ovulated oocytes, but level of *aqp1*transcripts is more than *aqp3*, substantiating the role of *aqp1* in oocyte hydration, whereas *aqp3* plays an important role in osmoregulation, therefore maximum expression of *aqp3* mRNA has been noticed in gills of both gravid and ovulated catfish. In addition, kidney and liver tissues from ovulated fish express comparatively more *aqp3* mRNA than samples from gravid catfish.

It is universally accepted that lysosomes play a major role in specific proteolytic cleavage of vitellogenin and process it to yolk globules/granules, which are stored under mild acidic conditions. On meiotic resumption at appropriate time, cytoplasmic reorganization due to proteolysis of egg-yolk proteins and hydration are apparent events in the oocytes. Free amino acids (FAAs) derived from yolk proteolysis contribute to the osmotic gradient, which drives the influx of water. Hence, enzymatic processing of vitellogenin and further proteolysis of egg-yolk proteins are regulated processes that are essential to provide nutrients at the right time to the developing embryo to ensure survival of the embryo until it can feed at its own.

In this investigation, lysosomal enzymes, aspartic proteases (Cathepsin D) and cysteine proteases (Cathepsin B and L) have been found to be associated with proteolysis of yolk proteins. Cathepsin enzymes have been analysed qualitatively and quantitatively with respective primers at gene expression level in the oocytes at different time points after initiation of maturation. In addition, enzyme activity of Cathepsin D and B in the ovarian tissues has been measured at corresponding time intervals. Level of *catD* and *catB* mRNA gradually increases during maturation and ovulation, however *catL* transcript reduces. On the contrary, both the enzymes Cathepsin D and B are maximally active in post-vitellogenic oocytes, thereafter enzyme activity reduces and decreases to a minimum in ovulated oocytes. Nevertheless,
Cathepsin B plays a pivotal role during maturation of oocytes in the catfish suggesting cleavage of egg-yolk proteins at specific sites.

Although electrophoretic pattern of yolk proteins is rather identical in yolky and ovulated oocytes but one peptide is definitely of smaller mass in ovulated oocytes that of yolky oocytes. Major peptides resolved on SDS PAGE are further analyzed by MALDI-TOF, MALDI LC-MS/MS. Spectrum of tryptic-digested peptides substantiates cleavage of yolk protein. MS/MS sequences of these peptides obtained from yolky and ovulated oocytes show similarity with vitellogenin of *Clarias macrocephalus*.

The last part of thesis discusses isolation and characterization of egg-yolk proteins from the gravid catfish. Egg-yolk proteins have been isolated by gel filtration chromatography on Sepharose 6B. Purified protein represents lipovitellin, which resolves into four major peptides on PAGE under denaturing conditions. These peptides were further analysed by LC-MS/MS. The peptide fragments thus obtained showed a significant similarity with vitellogenin protein of *Clarias macrocephalus*. Trypsin digested fragments of each peptide showed similarity to lipovitellin region of vitellogenin of many fishes and also to lipovitellin of *Ctenolabrus rupestris*. Among these sequences, one of the sequences matched with the N-terminal conserved domain of vitellogenin of *Clarias macrocephalus*. 