CHAPTER - III

STRUCTURAL CHANGES IN RESPONSE TO INCREASED ENVIRONMENTAL CALCIUM AND MAGNESIUM CONCENTRATION
(A) PITUITARY GLAND

INTRODUCTION
OBSERVATIONS
DISCUSSION
BIBLIOGRAPHY
INTRODUCTION

The present study has been planned to observe the effect of different calcium concentrations in the pituitary gland of the fish, *Heteropneustes fossilis* (Bloch) during its reproductive cycle. The structure of pituitary gland has been elaborately described by various workers. Aida (1988) has studied a review of plasma hormone change during ovulation in cyprinid fishes. Assem (1984) has studied a comparison between the effects of cortisol and prolactin on the euryhaline tilapia (*Sarotherodon mossambicus*). Ayala (1998) has shown immunocytochemical and ultrastructural characterization of somatolactin cells from the mediterranean yellowtail (*Seriola dumerili*, Risso, 1810). Bage et al. (1975) have studied the pituitary gland of the roach, *Leuciscus rutilus*. Benjamin (1979) has reported the cell types in the adenohypophysis of the marine teleost *Crenilabrus melops*. Bern (1967) has reported the hormones and endocrine glands of fishes. Bern et al. (1992) have selectively surveyed the endocrine system of the rainbow trout (*Oncorhynchus mykiss*) with emphasis on the hormonal regulation of ion balance. Bonga and Greves (1978) have shown the relationship between prolactin cell activity, environmental calcium and plasma calcium in teleost *Gasterosteus aculeatus* in stannietomized fish. Bonga and Meij (1980) have shown the effect of ambient calcium on prolactin cell activity and plasma electrolytes in *Sarotherodon mossambicus*, Whereas Bonga et al. (1983) have studied effects of external Mg²⁺ and Ca²⁺ on branchial osmotic water permeability and prolactin secretion in teleost fish, *Sarotherodon mossambicus*. Evans (1940) has shown the seasonal
changes in the pituitary gland of the eel. Fargher and McKeown (1989) have studied the effect of prolactin on calcium homeostasis in coho salmon, *Oncorhynchus kisutch*. Figueroa et al. (1994) have studied prolactin gene expression and changes of prolactin pituitary level during the seasonal acclimatization of the carp, *Cyprinus carpio*. Flik et al. (1986) have studied effects of ovine prolactin on calcium uptake and distribution in *Oreochromis mossambicus*. Grau et al. (1986) have shown the role of calcium in prolactin release from the pituitary of the teleost (*Oreochromis mossambicus*) in vitro. Hendee and Mueller (1985) have studied the histology of the adenohypophysis of the kelp greenling, *Hexagrammus decagrammus*. Joshi (1980) studied the cytology of the pituitary gland of the fresh water carp, *Labeo ciuris* (Ham.). Kakizawa et al. (1993) have shown the activation of somatolactin cells in the pituitary of the rainbow trout, *Oncorhynchus mykiss* by low environmental calcium. Kakizawa et al. (1996) have reported effects of hypothalamic factors on somatolactin secretion from the organ-cultured pituitary of *rainbow trout*. Kaneko et al. (1989) have shown the localization of calcium regulatory hormones in fish. Kaneko et al. (1993a) have studied gene expression and intracellular localization of somatolactin in the pituitary of rainbow trout. Kaneko et al. (1993b) have also reported that pituitary of 'cobalt' variant of the rainbow trout separated from the hypothalamus, lacks most pars intermedial and neurohypophysial tissue. Kaneko (1996) has studied cell biology of somatolactin. Kausel et al. (1998) have shown the effect of seasonal acclimatization of the expression of the carp transcription factor pit-1. Kausal et al. (1999) have reported that transcription factor pit-1
expression is modulated upon seasonal acclimatization of eurythermal ectotherms: Identification of two pit-1 genes in the carp (*Cyprinus carpio*). Kawauchi et al. (1989) have studied the isolation and characterization of sub units of two distinct salmon gonadotropins. Lopez et al. (2001) have recently studied in situ hybridization of somatolactin transcripts in the pituitary glands from acclimatized carp (*Cyprinus carpio*). Olivereau (1968) has studied the functional cytology of prolactin secreting cells in *Salmo gairdneri*. Olivereau and Olivereau (1982) have shown kinetics of the response of prolactin cells to environmental changes in the eel. Olivereau et al. (1980) have reported the specific effect of calcium ions on the calcium sensitive cells of the pars intermedia in the gold fish. Olivereau et al. (1982) have reported the response of the gold fish adapted to diluted sea water with different calcium and magnesium contents. Pang et al. (1971) have reported the hypocalcemic and telanic seizures in hypophysectomized killifish. Pang et al. (1973) have studied the pituitary regulation of serum calcium levels in killifish. Prasada Rao (1969) has made a comparative study of the pituitary gland of certain fresh water teleosts. Prasada Rao (1970) has also reported the hypophysis of two fresh water teleosts *Labeo calbasu* (Ham.) and *Puntius sarana* (Ham.). Prasada Rao (1999) has recently shown the regulation of hypophysial gonadotropin by neuropeptides of the brain and gonadal secretions in teleosts. Pang (1981) has studied the hypercalcemic effects of ovine prolactin on intact killifish, *Fundulus heteroclitus* subjected to different environmental calcium challenges. Pandolfi et al. (2000) have also studied immunocytochemical localization of different cell types in the
adenohypophysis of the cichlid fish, *Cichlasoma dimerus*. Pandolfi et al. (2001) have recently reported the ontogeny of immunoreactive somatolactin, prolactin and growth hormone secretory cells in the developing pituitary gland of *Cichlasoma dimerus*. Quesada et al. (1988) have reported the immunocytochemical and ultrastructural characterization of the cell types in the adenohypophysis of *Sparus aurata*. Rai (1966) has reported the histophysiology of pituitary gland in correlation with the ovarian cycle in Tor tor (Ham.). Raizada (1973) has studied the structure of the pituitary gland of *Rasbora daniconius* and its cyclical changes in correlation with reproductive cycle. Raizada and Bhargava (1974) have reported the pituitary gland of a teleost, *Nandus nandus* (Ham.). Saksena (1979) has studied the pituitary gland and reproductive cycle of Indian fresh water goby, *Glossogobius giuris* (Ham.). Singh (1980) has shown the experimental identification of the cell types in the pituitary gland of air breathing fish *Trichogaster fasciatus*. Srivastava and Swarup (1985) have shown the structure and behaviour of the corpuscles of stannius, the ultimobranchial gland and the prolactin cells in response to prolactin induced hypercalcemia in male cat fish, *Clarias batrachus*. Swarup (1985) has reported the endocrine regulation of calcium in vertebrates. Sower (2001) has also reported Update: brain and pituitary hormones of lampreys. Suzuki et al. (1988) have studied the isolation and characterization of two distinct gonadotropins from chum salmon pituitary glands. Swanson et al. (1991) have studied the isolation and biochemical characterization of two distinct pituitary gonadotropins from coho salmon. Toubeau et al. (1990) have shown the
immunocytochemical study of cell type distribution in the pituitary of *Barbus barbus*. Vissio et al. (1997) have studied the structure and cell type distribution of the pituitary gland of *Odontesthes bonariensis*. Volckaert et al. (1999) have reported the immunohisto-chemically detected ontogeny of prolactin and growth hormone cells in the African cat fish *Clarias gariepinus*. Wurts and Stickney (1989) have studied responses of red drum, *Sciaenops ocellatus* to calcium and magnesium concentrations in fresh and salt water.
OBSERVATIONS

The present study on the pituitary gland of *Heteropneustes fossilis* (Bloch) deals with:

MORPHOLOGY OF THE PITUITARY GLAND

The pituitary gland of the *Heteropneustes fossilis* is a small oval shaped structure and creamy white in colour. It is situated at the base of the brain anterior to the lobi inferiores. It is seen connected to the hypothalamus by a solid stalk of nerve fibres forming the central core of pituitary gland. Thus it is of leptobasic type.

HISTOLOGY AND CYTOLOGY OF THE PITUITARY GLAND

In the following description for the cytological details of the pituitary gland in *Heteropneustes fossilis*, the terminology by Green (1951), which is widely accepted by the majority of endocrinologists like Gorbman (1965), has been used. The pituitary gland of *Heteropneustes fossilis* has two separate components which are recognised as the glandular part (adenohypophysis) and neural part (neurohypophysis). The adenohypophysis is further differentiated into three regions, viz.; the rostral pars distalis, the proximal pars distalis and pars intermedia. These regions are in close association with each other and are only distinguished by the tinctorial behaviour of their cell types. (Fig.1)

The orientation of the adenohypophysis is of simple type. Here the glandular regions are arranged one behind the other on antero-posterior axis with rostral pars distalis being the anterior part
and pars intermedia being the posterior part whereas the middle region is occupied by proximal pars distalis, which is largest of all the three glandular regions.

**Rostral pars distalis**

It is the smallest part of the glandular region situated in the anterior region of the pituitary gland. It consists of two types of acidophils and one type of basophil cells. The two types of acidophils present in the rostral pars distalis have been designated in the present study as Acidophil I and Acidophil II.

The Acidophil I cells are found as groups of closely packed cells which are oval in shape and contain granulated cytoplasm and a distinct centrally placed round nucleus. These cells are known as PAS positive prolactin secreting cells which play important role in hypercalcimia (Pang, 1981).

The Acidophil II are smaller in size than the Acidophil I. These are oval in shape with homogenous cytoplasm and nucleus is round in shape. Few basophils are found scattered in the rostral pars distalis in between the groups of Acidophil I. Basophils are small in size and polygonal in shape and found in groups of two to three in between the acidophils. The cytoplasm of these basophils is homogeneous and the nucleus is centrally placed.

**Proximal pars distalis**

Cell types of this region consists of two types of acidophils (Acidophil I and Acidophil II), two types of basophils (Basophil I and
Basophil II) and chromophobes. The Basophil I are oval or elliptical in shape and found in various groups. The cytoplasm of Basophil I cells is granulated and more deeply stained with the basic dyes like haematoxylin. The number, size and shape of the Basophil I show seasonal variations which is seen to be associated with the maturation of gonads or calcium secretion during spawning period or other environmental factors (Bern, 1967). The Basophil I are similar to basophils of rostral pars distalis.

The Basophil II are also polygonal in shape and are smaller in size and lesser in number in comparison to Basophil I and are found singly or in small groups of two or three cells. They do not show marked seasonal variations in shape, size and number.

The Acidophil I are deeply stained with acidic dyes (PAS and eosin) and similar to Acidophil I of the rostral pars distalis. Perhaps these Acidophil I cells are also responsible for calcium sensitive cells. These are round in shape containing densely granulated cytoplasm and are mostly found in groups.

The Acidophil II are smaller in size and oval in shape. Tinctorially, these cells are similar to the Acidophil II of rostral pars distalis.

Apart from acidophils and basophils, another type of cells, chromophobes are also found scattered in between the groups of acidophils and basophils. The cytoplasm of these cells does not respond to the stains used. These cells are only identified by their nuclei which are acidophilic in nature.
Pars Intermedia

It is the posterior region of the pituitary gland which is composed mainly of acidophils with a few basophils. The acidophils are present in groups of large masses of cells around the ramification of highly branched neurohypophysis in this region. These acidophils are similar in their cellular structure and tinctorial properties to the Acidophil I of rostral pars distalis and proximal pars distalis. These cells are known as calcium sensitive (PAS positive) cells (Olivereau et al. 1982). The basophils are scattered singly or in groups of two or three in between the groups of acidophil cells. The basophils are similar to the basophils present in the rostral pars distalis. The pars intermedia varies in size during spawning period. In Heteropneustes fossilis, ten cell types are distinguished in different glandular regions. However, only five different cell types have been identified on the basis of their tinctorial properties.

Neurohypophysis

The pituitary stalk enters the pituitary gland as neurohypophysis. Its main trunk runs posteriorly giving off few neurohypophysial recesses to rostral and proximal pars distalis. Posteriorly it arborises extensively with the pars intermedia.

The neurohypophysis is composed of neurosecretory fibres laden with neurosecretory material, non-neurosecretory fibres without neurosecretory material, pituicytes and Herring bodies. Pituicyte are the acidophilic cells which are identified by their nuclei only. Herring bodies are the large masses of neurosecretory material having a central core of oval exoplasm.
SEASONAL CHANGES IN THE PITUITARY GLAND

The present study towards the seasonal changes of the pituitary gland in *Heteropneustes fossilis* is based on the cytological study of this gland (specially proximal pars distalis) from the seasonal samples of the reproductive cycle of this fish.

There are sufficient evidences to show that the teleost pituitary undergoes certain changes in different seasons and such changes are associated, in some way or the other, with the reproductive cycle and calcium regulation.

The cells of rostral pars distalis as well as of pars intermedia do not seem to exhibit any marked changes which may be correlated with the reproductive cycle. However, certain significant changes are observed in the Basophil I of proximal pars distalis which show a close correlation with the reproductive cycle of the fish.

Post-spawning Period (September to December)

During this period the Basophil I of proximal pars distalis are small and round in shape with a large and round nucleus. The cytoplasm of most of these basophils is lightly stained and smooth in appearance due to the scanty production of secretory granules. This degranulation persists even in September in a few basophils. In October and November all the Basophil I respond to basic dyes but remain smooth in appearance (Fig.2). In the month of December the Basophil I become smaller in size and rounded in shape with a smooth cytoplasm. The cell and nuclear outlines are quite distinct. (Fig.7)
Pre-spawning Period (January to April)

In January, no remarkable change takes place in the cytology of Basophil I in comparison to those of December, while in February and March the secretory granules begin to appear in the cytoplasm giving it a deep stained appearance. (Fig.3). In April, the Basophil I become highly granulated. They become much coarser in appearance due to the accumulation of secretory granules all over the cytoplasm. It may perhaps be that during this period the rate of production of secretory granules is much more than the rate of their release. The cell and nuclear outlines are distinct. (Fig.13).

Spawning Period (May to August)

During this period the deeply stained basophils (Basophil I) gradually show degranulation in their cytoplasm which becomes maximum by the end of August. In May and June degranulation of cytoplasm increases. (Fig.4). In July and August, the degranulation process is at its maximum due to which the degranulated basophils appear blurred although the cell outlines are recognisable. The nuclear membrane is also distinct. (Fig.19 and 5) It can be inferred that the rate of release of secretory granules is more than the rate of their production which accelerates gradually from May and becomes maximum in August.
Seasonal changes in the physiology of calcium regulation in correlation with the cytology of pituitary gland

It is a well established fact that seasonal changes of the pituitary gland can be correlated with the seasonal changes of the gonads and the reproductive cycle. Such changes can also be correlated with the calcium regulation of the fish.

Gradual fast transfer in different Calcium concentration during experimental period

To understand the physiology of calcium regulation which can be associated with the seasonal changes of the pituitary gland, gradual fast transfer of different calcium concentrations was done in the live fishes during different periods of the reproductive cycle (as described in material and methods). For that purpose, experimental fishes were gradually adapted from 2.5 m mol l\(^{-1}\) with an increase of 2.5 m mol l\(^{-1}\) at every step. Each step lasted for a day. In 65.0 m mol l\(^{-1}\) calcium chloride (CaCl\(_2\cdot2\)H\(_2\)O) solution in post-spawning period and pre-spawning period, whereas during spawning period in 62.5 m mol l\(^{-1}\) solution of Calcium chloride the fish could not survive for more than 5 to 6 hours and is found lethal. Cytological changes in the pituitary gland of experimental fishes were compared with those of normal (control) fishes during different periods of the reproductive cycle.

It has been observed that in experimental fishes basophils of rostral pars distalis and pars intermedia and Basophil II of proximal pars distalis and Acidophil I of rostral - and proximal pars distalis and acidophils of pars intermedia do exhibit certain changes
after administering calcium during different periods of the reproductive cycle which can be compared with the control fishes.

Post-spawning period (Experimental group)

During this period the acidophils become hypertrophied and have large nucleus in comparison to the control group. Whereas basophils do not exhibit any change (Fig. (control) 6, 7 and 8 (exp.) 9, 10 and 11).

Pre-spawning period (Experimental group)

The acidophils contain highly granular cytoplasm with distinct large nucleus and exhibit hypertrophy in comparison to control group. Basophils of all the three region are granular and smooth (Fig. (control) 12, 13 and 14 (exp.) 15, 16 and 17).

Spawning period (Experimental group)

During this period the acidophils are feebly degranulated and exhibit hypertrophy in comparison to control group while basophils cell contain highly degranulated cytoplasm with eccentrically placed nucleus. These cells also become hypertrophied due to the effect of calcium. (Fig. (control) 18, 19 and 20 (exp.) 21, 22 and 23).

Gradual fast transfer in different magnesium concentrations of experimental group

The fish *Heteropneustes fossilis* belonging to experimental group were gradually adapted from 10 m mol l⁻¹, 20 m mol l⁻¹, 35 m mol l⁻¹, 55 m mol l⁻¹ and 80 m mol l⁻¹ (each step lasted for a day). In 80 m mol l⁻¹ magnesium chloride (MgCl₂.6H₂O) solution, the animal could not survive for more than 7 to 8 hours during post-spawning and pre-spawning periods.
whereas during spawning period, in 55.0 m mol l⁻¹ solution of magnesium chloride, the fish could not survive for more than 7-8 hours. Cytological changes in the pituitary gland of magnesium treated fishes were compared with those of normal (control) fishes during different periods of the reproductive cycle. Important cytological changes were observed in the acidophils (Acidophil I of rostral-and proximal pars distalis and acidophils of pars intermedia) and basophils (basophils of rostral pars distalis proximal pars distalis and pars intermedia) during different periods of reproductive cycle.

**Post-spawning period (Experimental group)**

During this period the acidophils become hypertrophied having indistinct large nucleus in comparison to control group. However, basophils show lesser affinity towards stain and the cell size and number become reduced. (Fig (control) 6,7 and 8 (exp.) 24,25 and 26)

**Pre-spawning period (Experimental Group)**

During this period also acidophils exhibit hypertrophied condition and at places these cells become clumped together. Again, during this period basophils are feebly stained. The cell size and number is also reduced. (Fig (control) 12,13 and 14 (exp.) 27,28 and 29)

**Spawning period (Experimental group)**

During this period also the acidophils are clumped together showing hypertrophied condition. In comparison to control group, basophils show lesser affinity towards stains. As during the post-spawning and pre-spawning periods, the cell size and number of basophils is reduced in this period also. (Fig.(control) 18,18 and 20 (exp.) 30,31 and 32)
DISCUSSION

The pituitary gland of fish, *Heteropneustes fossilis* (Bloch) has been described in detail. Seasonal changes in the Basophil I (gonadotrops) of the proximal pars distalis have been correlated with the reproductive cycle of the fish. Besides this effect of calcium and magnesium on various cells types of the pituitary gland has been observed during different periods of the reproductive cycle.

The cells of rostral pars distalis and pars intermedia of the pituitary gland in fish, *Heteropneustes fossilis* (Bloch) do not exhibit marked cytological seasonal changes which can be correlated with the reproductive cycle. In *Channa punctatus* (Belsare, 1967) seasonal changes in the basophils of rostral pars distalis are correlated with the annual reproductive cycle suggesting a possible role in gonadotropic secretion. No such basophils are observed in the rostral pars distalis in the present fish. The basophils of the proximal pars distalis of several fishes like *Clarias batrachus* (Anant Prakash, 1976), *Channa punctatus* (Belsare, 1967), *Labeo giuris* (Joshi, 1980), Chum salmon (Kawauchi et al. 1989 and Suzuki et al., 1988), *Labeo calbasu*, *Puntius sarana*, *Labeo rohita*, *Catla catla*, and *Cirrhinus mrigala* (Prasada Rao, 1969, 1972; Prasada Rao et al. 1972, Prasada Rao, 1999), *Rasbora daniconius* and *Nandus nandus* (Raizada, 1973 and Raizada and Bhargava, 1974), *Glossogobius giuris* (Saksena, 1980) and coho salmon (Swanson et al. 1991) were
found to exhibit seasonal changes which were correlated with the changes in the gonads during reproductive cycle.

In *Clarias batrachus*, Anant Prakash (1976) has noticed that the degranulation process in basophils appears in late pre-spawning period which indicates that the release of hormones is accelerated during this period which helps in progressive maturation of the gonads. The degranulation is maximum in July and August (spawning period) in this fish. In *Channa punctatus* (Belsare, 1967), granulation appears during November and December, storage and progressive discharge take place during January to August and vacuolisation and degranulation in basophils is observed during September to November. Maximum vacuolisation takes place in the basophils after the spawning thus giving a sieve-like appearance to them. In chum salmon (Kawauchi et al. 1989; Suzuki et al., 1988) two distinct gonadotropins, designated as GTH-I and GTH-II were identified. In *Labeo calbasu*, *Puntius sarana*, *Labeo rohita*, *Catla catla*, and *Cirrhinus mrigala* (Prasada Rao, 1969, 1972; Prasada Rao et al. 1972, Prasada Rao, 1999), seasonal changes in the basophils or cyanophils (gonadotropin secreting cells) are correlated with gonadal cyclic changes. These cells underwent correlative seasonal cyclic changes such as degranulation and vacuolisation. In *Rosbora daniconius* and *Nandus nandus* (Raizada, 1973 and 1974), the degranulation (release of hormones) of the basophils has been found to be associated with
the maturation of the gonads and spawning behaviour of the fish. During the process of degranulation the vacuole like structures appear in the basophils and these structures increase enormously in size as well as in number when the spawning activity of the fish is greatest. In Glossogobius giuris (Saksena, 1980) the process of granulation and degranulation in these basophils closely follows the process of maturation of the gonads and spawning of the fish. The release of hormones, as evinced by the process of degranulation of the basophils, during the spawning period (June to September) and the highest degranulation in July and August results in the evacuation of ripe eggs and sperms from gonads. In coho salmon, Swanson et al. (1991) have identified that two types of gonadotropins (GTH I and GTH II) distinctly different from each other in chemical characteristics and structurally homologous to tetrapod FSH and LH respectively. Each gonadotropin consists of α and β sub units. GTH I was shown to be predominant in the plasma and pituitary of vitellogenic females, whereas GTH II was predominant at the time of final oocyte maturation.

In the present study on fish, Heteropneustes fossilis, it has been observed that the degranulation is associated with the release of hormones. During the early post-spawning period (September to October) the basophils are at their lowest ebb as far as their secretory activity is concerned whereas in the late post-spawning period (November to December) the resumption of the secretory process in the cytoplasm of these basophils takes
place. The gonads show spent condition in early post-spawning period while their rebuilding process takes place during later post-spawning period. During the pre-spawning period (January to April), the accumulation of secretory granules in the cytoplasm of basophils progresses and the cells become deeply stained during the spawning period (May to August). The degranulation process beings from the month of June which implies that the discharge of hormone is greater than its elaboration. The gonads during this period undergo process of final growth and maturation. The process of degranulation is at its highest degree in July and August, In August fish the basophils are in dull condition as far as their secretory activity is concerned. Thus the process of granulation and degranulation in the basophils are in intimate relationship with the process of gametogenesis and the spawning periodicity of the fish.

Fishes do not possess a parathyroid gland even then they are able to maintain plasma calcium levels in calcium depleting environment. It is therefore essential that they possess a hypercalcimic factor. It was the pioneer work of Olivereau (Olivereau and Chartier-Baraduc, 1965; Olivereau and Olivereau, 1970, 1978; Olivereau and Lemoive, 1973) which has established that the pituitary is an important hypercalcemic organ and prolactin is a hypercalcemic hormone in teleost. Pang et al. (1971, 1973 and 1978) and Pang (1981) have confirmed these observations in the killifish, *Fundulus heterochitus*. When killifish
were adapted to artificial calcium deficient sea water, hypophysectomy elicited a significant decrease in plasma calcium only but in no other electrolyte (Pang et al., 1971). When calcium was present in the environment, hypophysectomy did not cause hypercalcemia. In a later study Pang et al. (1973) further observed that replacement therapy or injection of pituitary homogenates were both effective in correcting the hypercalcemia. Srivastava and Swarup (1985) have administered calcium intraperitonially in *Clarias batrachus* and found that prolactin cells become inactive and show signs of atrophy.

Bonga and Meij (1980) have shown that low prolactin cell activity in sea water fish is related to high calcium and magnesium concentration in sea water. After transfer of fresh water fish to sea water, prolactin cell activity was markedly reduced. A similar reduction occurred in fresh water fish after increasing the ionic calcium concentration to that of sea water. Magnesium ions, although considerably less effective than calcium in the same concentration, had a similar effect.

Olivereau and Olivereau (1981) have demonstrated through various experimental procedures that PAS +ve cells of pars intermedia of the gold fish are sensitive towards the calcium which results in the inhibition of release of secretory granules. Through another experiment on the same fish, Olivereau et al. (1982) have observed that the stimulation of calcium sensitive cells appears specific in calcium free water. However, they state
that role of calcium sensitive cells in the hypertonic calcium free environment remains enigmatic.

The acidophils of pars intermedia of most of the teleosts are PbH -ve and PAS +ve and often these cells are referred to as PIPAS-cells (Pars intermedia PAS cells). Kakizawa et al. (1993) have shown hypercalcemic action of PIPAS cells or calcium sensitive cells. The chronic effects of changes in environmental calcium on SL cell activity were examined in rainbow trout. The results suggested that exposure to high calcium environment reduced the SL cell activity of these cells.

In the present fish, Heteropeustes fossilis the prolactin cells (acidophils) show hypertrophy after the administration of calcium during the process of gradual fast transfer throughout the reproductive cycle of the fish. It suggests strongly the role of prolactin in calcium regulation.

Effect of magnesium on the cell types of pituitary gland has been even less observed. Olivereau et al. (1982) have also observed that in magnesium deficient water, the prolactin cells become stimulated. With the supplementation of 0.2 m M of magnesium in water, decrease of plasma osmolarity and sodium level in the gold fish is reduced. Although no clear inhibitory effect could be detected on the calcium sensitive cells. In the present fish, hypertrophy is quite evident in the calcium sensitive acidophil cells with the effect of magnesium. Basophils show unusual behaviour. They become lesser and lesser positive towards stains which indicates their inactivity resulting into inhibition of general metabolism and reproduction of the fish.
It may be concluded that prolactin cells (Ca sensitive cells) are very active in fresh water fish which reduce the permeability of the fish integument for ions and water. These cells show hypertrophy with the addition of calcium or magnesium in water resulting in the reduction of high prolactin cell activity.
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Fig. 1 Photomicrograph of the vertical longitudinal section of the pituitary gland of *Heteropneustes fossilis* (Bloch) showing the orientation of the different regions

A. F. 10 x 10

Abbreviations

N. : Neurohypophysis
P.I. : Pars intermedia
P.PD. : Proximal pars distalis
P.S. : Pituitary stalk
R.PD. : Rostral pars distalis

Photomicrograph of the vertical longitudinal section of the pituitary gland of *Heteropneustes fossilis* (Bloch) showing the seasonal variations during post-spawning period (October)

Fig. 2 Showing of acidophils and smooth basophils in proximal pars distalis.

A.F. 10 x 60

Abbreviations

A : Acidophils
B : Basophils
Fig. 3  Photomicrograph of the vertical longitudinal section of the pituitary gland of *Heteropneustes fossilis* (Bloch) showing the seasonal variation of pre-spawning period (February) showing Acidophil I and Basophil I in proximal pars distalis.

H & E 10 x 60

Abbreviations
A.I. : Acidophil I
B.I. : Basophil I

Fig. 4  Photomicrograph of the vertical longitudinal section of the pituitary gland of *Heteropneustes fossilis* (Bloch) showing the seasonal variation during spawning period (June) showing acidophils and degranulated Basophil I in proximal pars distalis.

H & E 10 x 60

Abbreviations
A : Acidophils
B.I. : Basophil I

Fig. 5  Photomicrograph of the vertical longitudinal section of the pituitary gland of *Heteropneustes fossilis* (Bloch) showing the seasonal variation during spawning period (August) showing acidophils and degranulated Basophil I in proximal pars distalis.

H & E 10 x 60

Abbreviations
A : Acidophils
B.I. : Basophil I
Photomicrograph of the vertical longitudinal section of the pituitary gland of *Heteropneustes fossilis* (Bloch) during post-spawning period Control group (December).

H & E  

10 x 60

**Fig. 6**  Showing Acidophil I and Acidophil II and basophils in rostral pars distalis

**Fig. 7**  Showing acidophils and basophils in proximal pars distalis

**Fig. 8**  Showing acidophils in pars intermedia

**Abbreviations**

A. : Acidophils

A.I. : Acidophil I

A.II. : Acidophil II

B : Basophils
Photomicrograph of the vertical longitudinal section of the pituitary gland of *Heteropneustes fossilis* (Bloch) showing the effect of 65 m mol l⁻¹ of calcium chloride (CaCl₂⋅2H₂O) solution in experimental group during post-spawning period (December).

PAS 10 x 60

Fig. 9  Showing hypertrophied Acidophil I in rostral pars distalis

Fig. 10  Showing hypertrophied Acidophil I in proximal pars distalis

Fig. 11  Showing hypertrophied acidophils in pars intermedia

**Abbreviations**

A : Acidophils
A.I. : Acidophil I
A.II. : Acidophil II
B : Basophils
B.I. : Basophil I
Photomicrograph of the vertical longitudinal section of the pituitary gland of *Heteropneustes fossilis* (Bloch) of control group during pre-spawning period (April).

H & E 10 x 60

Fig.12  Showing two types of acidophils in rostral pars distalis

Fig.13  Showing Acidophil I and Bbasophil I in proximal pars distalis

Fig.14  Showing acidophils & basophils in pars intermedia

**Abbreviations**

A. : Acidophils

A.I. : Acidophil I

A.II. : Acidophil II

B.I. : Basophil I
Photomicrograph of the vertical longitudinal section of the pituitary gland of *Heteropneustes fossilis* (Bloch) showing the effect of 65 m mol l⁻¹ of calcium chloride (CaCl₂·2H₂O) solution in experimental group during pre-spawning period (April).

H & E  
10 x 60

Fig. 15 Showing hypertrophied Acidophil I in rostral pars distalis

Fig. 16 Showing hypertrophied Acidophil I in proximal pars distalis

Fig. 17 Showing hypertrophied acidophils in pars intermedia

**Abbreviations**

A : Acidophils
A.I. : Acidophil I
Photomicrograph of the vertical longitudinal section of the pituitary gland of *Heteropneustes fossilis* (Bloch) of control group during spawning period (July).

**H & E**  
10 x 60

**Fig. 18**  
Showing normal Acidophil I and normal basophils in rostral pars distalis

**Fig. 19**  
Showing normal Acidophil I and degranulated Basophil I and Basophil II in proximal pars distalis

**Fig. 20**  
Showing acidophils and basophils in pars intermedia

**Abbreviations**

A : Acidophils  
A.I. : Acidophil I  
B : Basophils  
B.I. : Basophil I  
B.II. : Basophil II
Photomicrograph of the vertical longitudinal section of the pituitary gland of *Heteropneustes fossilis* (Bloch) showing the effect of 62.5 m mol l⁻¹ of calcium chloride (CaCl₂·2H₂O) solution in experimental group during spawning period (July).

H & E 10 x 60

**Fig.21** Showing hypertrophied Acidophil I and degranulated hypertrophied basophils in rostral pars distalis

**Fig.22** Showing hypertrophied Acidophil I and degranulated Basophil I and hypertrophied Basophil II in proximal pars distalis

**Fig.23** Showing hypertrophied Acidophil I and degranulated hypertrophied basophils in pars intermedia

**Abbreviations**

<table>
<thead>
<tr>
<th>A.I.</th>
<th>Acidophil I</th>
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<td>B</td>
<td>Basophils</td>
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<td>B.I.</td>
<td>Basophil I</td>
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<td>B.II.</td>
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Photomicrograph of the vertical longitudinal section of the pituitary gland of *Heteropneustes fossilis* (Bloch) showing the effect of 80 m mol l⁻¹ of magnesium chloride (MgCl₂·6H₂O) solution in experimental group during post-spawning period (December).

H & E 10 x 60

Fig. 24  Showing hypertrophied Acidophils I in rostral pars distalis

Fig. 25  Showing hypertrophied Acidophils I in proximal pars distalis

Fig. 26  Showing hypertrophied acidophils in pars intermedia

**Abbreviations**

A. : Acidophils

A.I. : Acidophil I

A.II. : Acidophil II
Photomicrograph of the vertical longitudinal section of the pituitary gland of *Heteropneustes fossilis* (Bloch) showing the effect of 80 m mol l\(^{-1}\) of magnesium chloride (MgCl\(_2\).6H\(_2\)O) solution in experimental group during pre-spawning period (April).

H & E 10 x 60

Fig. 27  Showing hypertrophied Acidophil I in rostral pars distalis

Fig. 28  Showing hypertrophied Acidophil I basophils in proximal pars distalis

Fig. 29  Showing hypertrophied acidophils in pars intermedia

**Abbreviations**

A : Acidophils
A.I. : Acidophil I
B : Basophils
Photomicrograph of the vertical longitudinal section of the pituitary gland of *Heteropneustes fossilis* (Bloch) showing the effect of 55 m mol l⁻¹ of magnesium chloride (MgCl₂.6H₂O) solution in experimental group during spawning period (July).

H & E 10 x 60

Fig. 30  Showing hypertrophied Acidophil I in rostral pars distalis

Fig. 31  Showing hypertrophied Acidophil I and feebly stained basophils in proximal pars distalis

Fig. 32  Showing hypertrophied acidophils in pars intermedia

**Abbreviations**

A : Acidophils
A.I. : Acidophil I
B : Basophils
(B) ULTIMOBRANCHIAL GLAND

INTRODUCTION
OBSERVATIONS
DISCUSSION
BIBLIOGRAPHY
INTRODUCTION

The present study has been planned to observe the effect of different Calcium concentrations in a teleost fish *Heteropneustes fossilis* (Bloch) during its reproductive cycle. The ultimobranchial gland has a vesicular structure in fishes. It contains follicles or cords of cells which show feature of endocrine secretion. This gland was first described in elasmobranch as the suprapericardial bodies because of its position in relation to pericardium. In teleosts this gland has a granular structure mixed with degenerated nuclei.

In *Heteropneustes fossilis* (Bloch), the gland is located in between the heart and oesophagus. It is situated in the connective tissue mass dorsal to oesophagus and posterior to sinus-venosus. The ultimobranchial gland of fish is a rich source of calcitonin (CT) which exert a powerful effect of hypocalcemia in mammals (Pang 1971).

Many workers have shown that fish or mammalian calcitonin can reduce plasma Calcium in fish (Peignoux-Deville et al., 1975). Several attempts using either ultimobranchiallectomy or calcitonin injection, failed to produce a consistent effect on hypocalcemic regulation in teleosts.

Ahmad and Swarup (1988) recognised seasonal changes in the functional morphology of ultimobranchial gland in fish in relation
to the reproductive cycle and changes in serum Calcium level of a fresh water female cat fish, *Mystus vittatus* (Bloch).

Bonga (1980) has worked on synthetic salmon calcitonin and low ambient Calcium on plasma Calcium, ultimobranchial cells, stannius bodies and prolactin cells in the teleost *Gasterosteus aculeatus*. He recognised that the ultimobranchial body in sticklebacks contain granular secretory cells and nongranular supporting cells. The supporting cells separate the secretory cells over large areas from surrounding connective tissue.

Chakroborti and Mukherjee (1993) have observed "Hypocalcemic action of Salmon Calcitonin and ultimobranchial gland extracts in fresh water teleost *Cyprinus carpio*".

Das (1990) has also studied hypocalcemic action on the ultimobranchial gland of *Anabas testudinus*.

Hongwen and Hinging (1986) have reported that ultimobranchial gland localized in the granular cells of the follicular epithelium. They have further stressed that ultimobranchial gland have important role in the sexual cycle of fish.

Lopez (1973) has recognised that calcitonin probably acts on the Calcium exchange at the site of the "bone lining cellular layer" which controls Calcium ion transport (Lopez et al. (1976) have worked on effect of calcitonin and ultimobranchia-lectomy (UBX) on Calcium and bone metabolism in the Eel *Anguilla anguilla* L.

Pang (1971) has described in his paper "Calcium and ultimobranchial gland in fishes contain" follicles of cords of cells
which show features of hypocalcemic activities and such cells are present in ultimobranchial gland of shark, bony fish and lung fish. In most teleosts, the ultimobranchial gland exists as a sheet of tissue in the interseptum between the paricardial and the peritoneal cavities. It is usually present beneath the oesophagus and may be single or paired. The ultimobranchial gland can be seen microscopically as a white band of tissue on interseptum, in small fishes. It can not be seen by naked eyes, serial sections of the interseptum usually reveal the glandular tissue. In many species such as sharks, trout, gold fish and eel, the ultimobranchial gland has a follicular structure. It is also described by Robertson (1969). However in the Killifish, *Fundulus heteroclitus*, this gland appears as a cord of cells. Similar features were also described in rainbow trout by Copp (1969).

Patel and Das (1994) have reported the hypocalcemic activity in sharks, teleost and lungfishes. They extracted the hormone calcitonin from ultimobranchial gland of a number of fish species; Salmon, eels, sharks, rays and carps and stressed the ultimobranchial gland has seasonal changes in the circulating and glandular levels of calcitonin.

Peignoux-Deville et al. (1975) have studied "Responses of the ultimobranchial body in Eel (*Anguilla anguilla* L.)" maintained in sea water and experimentally matured to injections of synthetic salmon calcitonin.
Rasquin and Rosenbloom (1954) were the first to recognised that the ultimobranchial gland takes important part in Calcium metabolism. Later Copp et al. (1967), extracted calcitonin (CT) from ultimobranchial gland of dog fish *Squalus suckeyi* and hypocalcemic responses were subsequently observed with ultimobranchial gland extract from sharks (Urist 1967) and teleosts (Copp et al. 1968).

Roberson (1969) has observed that the ultimobranchial gland in rainbow trout, *Salmo gairdneri* is associated with the hypocalcemic hormone calcitonin while is ductless central cavity contain cellular debris as a result of a holocrine of apocrine secretion.

Sasayama and Shimura (1986) have shown the morphometrical study of the development of corpuscles of stannius and ultimobranchial gland in the gold fish.

The ultimobranchial gland could not be located in the embryo before hatching while in fry just after the hatching unpaired ultimobranchial gland was found to be attached to the ventral portion of oesophagus. The volume of ultimobranchial gland increased linearly three months after hatching, thereafter, however only very small increase in the volume of ultimobranchial gland could be noted, even in the fully grown adult. Corpuscles of Stannius are always found larger than ultimobranchial gland throughout the development and also in adult (Sasayama and Shimura 1986).

Sasayama et al. (1989) have studied ultimobranchial gland in fry of the chum salmon, *Onchorhynchus keta*, immunohistochemically by peroxidase-antiperoxidase method and noted the parenchymal cells
of the gland showing weak reaction to anti-salmon calcitonin in antiserum. During larval development the calcitonin immunoreactive cells increased in number and stainability.

Sehe (1960) has worked on "Radioautographic studies on the Ultimobranchial gland in vertebrates, fishes and Amphibians". He observed that the ultimobranchial body is embedded in the loose connective tissues at the lateral margin of the transverse septum. The ultimobranchial gland consists few follicles, irregular in shape, with occasional appearance of several solid cell strands. In all fishes numerous capillaries and sometimes pigment cells are penetrating the ultimobranchial body region. He has also confirmed that no storage of iodine is indicated by the radioautographs.

The effect of Calcium metabolism in fishes has also been studied by Lopez et al. (1976), Milhaud et al. (1977), Peignoux-Deville et al. (1978), who have suggested some important function for calcitonin of Calcium regulation in teleosts.

Shinohara, et al. (1998) have reported the unpaired ultimobranchial glands of African lungfish, Protopterus dolloi. They have reported that ultimobranchial gland exist only on the left side of the phyanx.

Srivastava et al. (1987) have studied a effect of glucagon treatment in ultimobranchial gland of male Clarias batrachus for fifteen days and observed the decrease in cytoplasmic granulation.

Swarup and Ahmad (1983) have recognised the ultimobranchial gland of Mystus vittatus (Bloch) in response to experimental hypercalcemia.
Previous workers have shown some definite function to the ultimobranchial gland in fish (Fenwick, 1978). The ultimobranchial gland present in all jawed fishes, is known to be homologus with the calcitonin cells of mammals and is rich source of calcitonin (Copp et al. 1987), Swarup et al. (1980).

Swarup and Alim (1990) have studied sexual difference through histology of the ultimobranchial gland of mature teleosts, *Puntius sophore* and *Crossocheilus lutius*. Their studies suggested that activity of the gland increased during the period of sexual maturation in females. The unpaired ultimobranchial gland in *Puntius sophore* is located in the ventral profile of the oesophagus. In females the gland is very large, lobulated the thinly encapsulated. The gland has oval follicular structure with basal nuclei. In some of the follicles the lamina is occupied by colloidal like material. In males the ultimobranchial gland is small and spindle shaped structure, less vascular and only few follicles with luminal colloid is evident. According to Swarup and Alim (1990) it is observed that ultimobranchial glands of matured females have higher activity than males ie in females the glands are large and display hypertrophy and highly vascularised. The ultimobranchial gland of female animals are in follicular fashion while that of males are cord like.

Suzuki (1995) has reported that the calcitonin like substance in plasma of the hagfish *Eptatretus burgeri* (Cyclostomata). They have observed that the CT like substance present in hag fish plasma appears to be very similar to salmon CT.
OBSERVATIONS

SEASONAL CHANGES

Post-spawning period (September - December)

In the month of September and October cytological details were observed in the ultimobranchial tissue as follows:

Gland possesses highly compact follicular cells with indistinct boundaries. Granular cytoplasm was observed. Nuclei were also observed (Fig. 1). During the month of November and December, it possesses follicular cells with indistinct boundaries. Granular cytoplasm with large nuclei were evident (Fig. 2).

Gradual fast transfer in different Calcium concentrations during post-spawning period

The fish *Heteropneustes fossilis* (Bloch) belonging to experimental group were gradually adapted from 2.5 m mol l\(^{-1}\) upto 65 m mol l\(^{-1}\) with an increase of 2.5 m mol l\(^{-1}\) at every step. (each step lasted for a day). In 65 m mol l\(^{-1}\), Calcium chloride (CaCl\(_2\).2H\(_2\)O) solution the animal could not survive for more than 5 to 6 hours and is found lethal. Important cytological changes observed in the ultimobranchial tissue. The gland possesses follicular structure with distinct boundaries. Granular cytoplasm with prominent nuclei were observed (Fig. 3).

In control group structural details are same as described earlier. (Fig. 2)
Pre-spawning period (January - April)

In the month of January and February important cytological details were observed in the ultimobranchial tissue. The gland possesses follicular structure with distinct boundaries. Slightly granular cytoplasm with nuclei of normal size (Fig.4) were seen. In March and April it is observed that gland possesses large follicular structure with distinct boundaries. Slightly granular cytoplasm with large nuclei were also evident (Fig.5).

Gradual fast transfer in different Calcium concentrations during pre-spawning period

The experimental protocol was exactly the same as described above (exposure to 65 m mol l⁻¹, CaCl₂.2H₂O). Gland possesses indistinct follicular cells. Poorly granular cytoplasm with enlarged nuclei were observed (Fig.6).

In control group structural details are same as described earlier. (Fig.5)

Spawning period (May-August)

Important cytological details were observed in the ultimobranchial tissue in May and June. The gland possesses highly compact but indistinct follicular structure with indistinct boundaries. Poorly granular cytoplasm with nuclei of normal size (Fig.7). At someplaces the nuclei become enlarged. In the month of July and August it is observed that gland possesses large follicles with distinct boundaries. Poorly granular cytoplasm with prominent nuclei were observed (Fig.8).
Gradual fast transfer in different Calcium concentrations of experimental group during spawning period

The fish *Heteropneustes fossilis* (Bloch) belonging to experimental group were gradually adapted from 2.5 m mol l\(^{-1}\), 5.0 m mol l\(^{-1}\) upto 62.5 m mol l\(^{-1}\) solution of Calcium chloride (CaCl\(_2\cdot2\)H\(_2\)O) in fresh water (each step lasted for a day). In 62.5 m mol l\(^{-1}\) solution animal could not survive for more than 5 to 6 hrs and the concentration is found lethal. Important cytological changes were observed in ultibranchial tissue. During this exposure it is observed that gland possesses prominent follicular cells with distinct boundaries. Poorly granular cytoplasm with, normal nuclei with poor staining response were also observed (Fig.9).

In control group structural details are same as described earlier. (Fig.8)

Gradual fast transfer in different Magnesium concentrations of experimental group during post-spawning period

The fish *Heteropneustes fossilis* (Bloch) belonging to experimental group were gradually adapted from 10 m mol l\(^{-1}\), 20 m mol l\(^{-1}\), 35 m mol l\(^{-1}\), 55 m mol l\(^{-1}\) and 80 m mol l\(^{-1}\) (each step lasted for a day). In 80 m mol l\(^{-1}\) Magnesium chloride (MgCl\(_2\cdot6\)H\(_2\)O) solution, the animal could not survive for more than 7 to 8 hours and therefore is found lethal. Important cytological changes were observed in ultimobranchial tissue. During this exposure the gland possesses small follicular structure with distinct boundaries at few places, while in other part indistinct follicular boundaries were noted.
Poorly granular cytoplasm with comparatively smaller nuclei were also observed (Fig.10).

In control group structural details are same as described earlier. (Fig.2)

Gradual fast transfer in different Magnesium concentrations of experimental group during pre-spawning period

The experimental protocol was exactly the same as described above (exposure to 80 m mol l⁻¹ MgCl₂.6H₂O). In this concentration, the gland possesses small follicles with indistinct boundaries. Granular cytoplasm with large nuclei were clearly visible (Fig.11).

In control group structural details are same as described earlier. (Fig.5)

Gradual fast transfer in different Magnesium concentrations of experimental group during spawning period

The fish Heteropneustes fossilis(Bloch) belonging to experimental group were gradually adapted from 10 m mol l⁻¹, 20 m mol l⁻¹, 35 m mol l⁻¹ and 55 m mol l⁻¹ (each step lasted for a day). In 55 m mol l⁻¹ Magnesium chloride (MgCl₂.6H₂O) solution, the animal could not survive for more than 7 to 8 hours and therefore is found lethal. Important cytological changes were observed in ultimo branchial tissue. Gland possesses large follicular structure with indistinct boundaries. Slightly granular cytoplasm and small nuclei were observed (Fig.12).

In control group structural details are same as described earlier. (Fig.8)
DISCUSSION

The ultimobranchial gland is a rich source of calcitonin which plays an important role in Calcium regulation in teleost. In fish *Heteropneustes fossilis* (Bloch) the ultimobranchial gland is a compact mass which has a follicular structure. Structure is highly effected during gradual fast transfer. At maximum Calcium concentration, the cytoplasm is found poorly granular during pre-spawning and spawning period. But during post-spawning period cytoplasm is granular. Cytoplasm showed decreased staining response and vacuolated during pre-spawning, spawning and post-spawning in experimental group. Regarding seasonal variations during post-spawning and pre-spawning period slightly granular cytoplasm is observed, whereas during spawning poorly hyaline cytoplasm was observed.

At maximum magnesium concentration, the poorly granular cytoplasm was observed during all the three phases i.e., post-spawning, pre-spawning and spawning period.

Ahmad and Swarup (1988) have observed the seasonal changes in the morphology of the ultimobranchial gland. During maturation period of *Mystus vittatus* serum Calcium level increased. During spawning (July and August) this level is highest. During this phase epithelial cells of the ultimobranchial body show hypertrophy and there is also an increase in the nuclear size. The follicular cells of the gland during post-spawning phase get reduced in size with smaller and rounded nuclei. The lamina of cells is completely destroyed, which is related to the ultimobranchial activity resulting in the atrophy of the
follicles, clumping of cells and reduced nuclear size. They have also recognized that serum Calcium level increases throughout preparatory, pre-spawning and spawning phases which is coupled with enhancement of the activity of the ultimobranchial body and the ovary. Epithelial cells during this phase of the ultimobranchial body show hypertrophy and an increase of their nuclear size.

Similar result was observed in our experiments during seasonal changes i.e.; pre-spawning, spawning and post-spawning period. During pre-spawning period (January to April), the ultimobranchial gland displays sign of high activity as the follicles show hypertrophied condition. The size of the nuclei is large during this period. During spawning period (May to August) the gland is at its peak of activity which is evident by hypertrophy of follicular cells. The nuclei are comparatively large and elongated. During post-spawning period (September to December) the follicular cells of the gland get markedly reduced in size with smaller and rounded nuclei granular cytoplasm was observed.

In present study of fish *Heteropneustes fossilis* (Bloch) at maximum Calcium concentration the nuclei is small during spawning period when compared to the control group. During post-spawning and pre-spawning period, the size of the nucleus become larger as compared to the control group.

Pang (1970) has also reported that fish ultimobranchial is a rich source of calcitonin. Salmon calcitonin has been studied extensively and also synthesized in vitro. It has also an important
role in osmoregulation and Calcium metabolism. Histological study of ultrastructural details indicates the secretory activities in the glandular tissue.

Pang (1981) has observed that the prolactin administration is effective when fish *Fundulus heteroclitus* L. are maintained in fresh water and Calcium rich fresh water but when fish are maintained in sea water and Calcium deficient sea water, the prolactin treatment induced hypercalcemia. In sea water the ultimobranchial gland has enhancement of nuclei and cytoplasm become poorly granular.

Srivastava and Swarup (1985) have shown that prolactin administration induced hypercalcemia in *Clarias batrachus* (Bloch). The gland remained unchanged on day 1st. On day 3rd the activity increased in the nuclear size and the poor staining response of the ultimobranchial cell. On day 5th and since 10th there is also less staining response compared with nuclear enlargement. Cytoplasmic vacuolization and shrinkage of nucleus were observed after 15th day administration.

Patel and Das (1994) have also observed that fish ultimobranchial gland is a rich source hypocalcemic factor, calcitonin and produced hypercalcemia when injected in rats. The difference in hypercalcemic and hypophosphatemic activity of ultimobranchial gland extract from male and female carps further confirmed that in fishes, the circulating level of calcitonin is higher in females during gonadal maturation and spawning phases.
In present study at maximum Calcium concentration follicular size increased and shrinkage in nuclei were observed during spawning period.

Swarup and Ahmad (1983) have observed that the ultimobranchial gland of *Mystus vittatus* become active after induced hypercalcemia. It has hypertrophied nuclei and the glandular volume increased after 22 days treatment.

Swarup and Srivastava (1984) have studied the ultimobranchial gland of *Clarias batrachus* (Bloch) treated with Vitamin D₃ and shown an increase in the nuclear size and less staining response of cytoplasm after fifth day where this gland of 0.05% CaCl₂ treated group shows degenerative changes.

Sasayama and Shimura (1986) have recognised that the volume of *Corpuscles of Stannius* and ultimobranchial gland increased linearly upto three months after hatching. *Corpuscles of Stannious* was always bigger than ultimobranchial gland throughout the development and also in adults. The ultimobranchial gland could not be found in the embryo before hatching. In 0.5 cm fry just after hatching, unpaired ultimobranchial body primordium was found to be attached to the ventral portion of the oesophagus. They have also noted very small increase in the volume of ultimobranchial gland even in fully grown adult.

Srivastava and Swarup (1986) have studied the effect of glucagon treatment on ultimobranchial gland in male *Clarias batrachus*. The gland shows decreased staining response and
cytoplasmic vacuolization was also occurred while the nuclear size displays no change throughout the experiment.

At maximum Calcium concentration i.e.; 62.5 m mol l\(^{-1}\) the follicle wall become enlarged and degenerated nuclei were prominently observed in pre-spawning and spawning period. But during post-spawning and pre-spawning period the size of nucleus become larger compared to the control group. In Magnesium concentration i.e., 80.0 m mol l\(^{-1}\) and 55.0 m mol l\(^{-1}\) during all the three stages of reproductive cycle, there is an increase in follicular size and nuclei are in degenerative condition.

These changes indicates that during spawning period ultimobranchial gland is highly active as compared to that of pre-spawning and post-spawning period in experimental group as well as in control group in Calcium exposure. Where as in Magnesium exposure the activity is high during all the three phases of reproductive cycle.

Pathak (2002) has observed that in euryhaline fish Tilapia, (*Oreochromis mossambicus*) at highest Calcium concentration i.e., 10 m mol l\(^{-1}\) the follicle wall become enlarge and degenerated nuclei are prominently observed during all the three phases of reproductive cycle i.e.; pre-spawning, spawning and post-spawning period.


Pathak, R. (2002): Structural changes in response to increased environmental salinity and calcium on some organs associated with the adjustment to environment in a teleost with special reference to its reproductive cycle and calcium regulatory organs, Unpublish Ph. D. Thesis, Dr. H.S. Gour University, Sagar.


Fig. 1: Section of ultimobranchial gland of *Heteropneustes fossilis* (Bloch) for seasonal variations during post-spawning period (October) showing highly compact follicular cells. H & E 10 x 40

Fig. 2: Section of ultimobranchial gland of *Heteropneustes fossilis* (Bloch) for control group during post-spawning period (December) showing granular cytoplasm with large nuclei. H & E 10 x 40

Fig. 3: Section of ultimobranchial gland of *Heteropneustes fossilis* (Bloch) for experimental group exposed to 65.0 m mol l⁻¹ Calcium chloride (CaCl₂·2H₂O) solution during post-spawning period (December) showing slightly granular cytoplasm with prominent nuclei. H & E 10 x 40

Abbreviations:

CT - Compact tissue
GC - Granular cytoplasm
SGC - Slightly granular cytoplasm
PN - Prominent nuclei
UBG - Ultimobranchial gland
Fig. 4 : Section of ultimobranchial gland of *Heteropneustes fossilis* (Bloch) for seasonal variations during pre-spawning period (February) showing slightly granular cytoplasm with normal nuclei. 
H & E
10 x 40

Fig. 5 : Section of ultimobranchial gland of *Heteropneustes fossilis* (Bloch) for control group during pre-spawning period (April) showing slightly granular cytoplasm with large nuclei. 
H & E
10 x 40

Fig. 6 : Section of ultimobranchial gland of *Heteropneustes fossilis* (Bloch) for experimental group exposed to 65.0 mmol l⁻¹ Calcium chloride (CaCl₂·2H₂O) solution during pre-spawning period (April) showing poorly granular cytoplasm and with enlarged nuclei. 
H & E
10 x 40

Abbreviations:

- **EN**  - Enlarged nuclei
- **LN**  - Large nuclei
- **SGC** - Slightly granular cytoplasm
- **PGC** - Poorly granular cytoplasm
- **UBG** - Ultimobranchial gland
Fig. 7 : Section of ultimobranchial gland of *Heteropneustes fossilis* (Bloch) for seasonal variations during spawning period (May) showing poorly granular cytoplasm with normal nuclei.

H & E 10 x 40

Fig. 8 : Section of ultimobranchial gland of *Heteropneustes fossilis* (Bloch) for control group during spawning period (July) showing poorly granular cytoplasm with prominent and nuclei.

H & E 10 x 40

Fig. 9 : Section of ultimobranchial gland of *Heteropneustes fossilis* (Bloch) for experimental group exposed to 62.5 m mol l\(^{-1}\) Calcium chloride (CaCl\(_2\).2H\(_2\)O) solution during spawning period (July) showing poorly granular cytoplasm and with normal nuclei.

H & E 10 x 40

Abbreviations:

- PGC: Poorly granular cytoplasm
- PN: Prominent nucleus
- UBG: Ultimobranchial gland
Section of ultimobranchial gland of *Heteropneustes fossilis* (Bloch) for experimental group exposed to 80.0 \( \text{m mol l}^{-1} \) Magnesium chloride \((\text{MgCl}_2\cdot6\text{H}_2\text{O})\) solution during post-spawning period (December) showing poorly granular cytoplasm with small nuclei.

H & E 10 x 40

Section of ultimobranchial gland of *Heteropneustes fossilis* (Bloch) for experimental group exposed to 80.0 \( \text{m mol l}^{-1} \) Magnesium chloride \((\text{MgCl}_2\cdot6\text{H}_2\text{O})\) solution during pre-spawning period (April) showing poorly granular cytoplasm with enlarged nuclei.

H & E 10 x 40

Section of ultimobranchial gland of *Heteropneustes fossilis* (Bloch) for experimental group exposed to 55.0 \( \text{m mol l}^{-1} \) Magnesium chloride \((\text{MgCl}_2\cdot6\text{H}_2\text{O})\) solution during spawning period (July) showing slightly granular cytoplasm with small nuclei.

H & E 10 x 40

Abbreviations:

- GC: Granular cytoplasm
- PGC: Poorly granular cytoplasm
- SGC: Slightly granular cytoplasm
- SN: Small nuclei
- UBG: Ultimo branchial gland
INTRODUCTION

The present study has been made to observe the effect of different Calcium and Magnesium changes in the structure of gill filament in fish *Heteropneustes fossilis* (Bloch) during its reproductive cycle. The structure of gill filament in fish has been elaborately described by various workers.

Bailly et al. (1992) have reported neuroepithelial cells in the gill filaments. They have also shared similar morphological functions with neuroepithelial bodies in the lungs of air-breathing vertebrates.

Beckman and Zaugg (1988) observed the copper intoxication in chinook salmon, *Oncorhyncus tshawytscha*, induced by natural spring water which also effects on gill Na⁺/K⁺ ATPase, hematocrit and plasma glucose concentration in both parr and smolt of chinook salmon.

Conklin et al. (1991) have studied the effect of chronic exposure to soft acidic water on gill developmental morphology, number, location, size of chloride cells and mucus cells in embryo of larval brook trout, *Salvelinus fontinalis*.

Copeland (1946) has studied the cytological basis of chloride transfer in the gill of *Fundulus heteroclitus*.

Couetti and Olson (1988) have shown the metabolism of non epinephrine and epinephrine in the prefused trout gill by using high pressure liquid chromatography.

Das and Srivastava (1978) have studied the responses of gill to various changes in salinity in fresh water teleost *Colisa fasciatus*.
Evans (1975) reported ionic exchange mechanism in fish gill. Franklin and Davison (1989) have shown the morphologically different chloride cells in fresh water adapted Sockey salmon, Oncorhynchus nerka. Bonga (1979) reported different mucus cell distribution in the gill epithelium and also noted the rate of mucus production under normal environment. Handy and Eddy (1989) have pointed that mucus layer is evident on the primary lamellae and may have indirect effect on the branchial microenvironment because mucus is an ion exchange material which rapidly absorb H⁺. They have further reported (1991) different mucus cell distribution on the gill epithelium and their function in different fish and also pointed the absence of mucus on secondary lamellae of unstressed rainbow trout, Oncorhynchus mykiss (Walbaum). It was also shown that mucus function in the branchial microenvironment of rainbow trout is limited to stress situations where mucocytes discharge is stimulated to form distinct mucus layer on the gill surface. This may not be the case in other fish species which have different mucus cell distribution on the gill epithelium and probably different mucus production rate under normal environmental condition. Karnakey (1986) has shown the distribution and role of smooth and invaginated chloride cells.

Laurent and Dunel-Erb (1976) have studied the functional organization of the teleost gill and have also shown the blood pathway in the primary lamellae and in the gill arch of 3 representative species of fish in trout, Salmo gairdneri, eel, Anguilla anguilla and Perch, Perca fluviatilis.
Laurent et al (1985) have studied the role of environmental sodium chloride relative to calcium in gill morphology of fresh water solmonid fish. Laurent and Hebibi (1989) have shown the gill morphometry and fish osmoregulation.

Madsen and Korsgaard (1989) have shown the time course effect of repetitive oestradiol 17 β and thyroxine injection on the natural spring smolting of Atlantic salmon, Salmo salar.

Madsen (1990) has reported the effects of repetitive cortisol and thyroxine injection on chloride cell number and Na⁺/K⁺ ATPase activity in gills of fresh water acclimated rainbow trout Salmo gairdneri. He has shown that the increased circulation of thyroxine level can modify the cortisol effect on gill chloride cell and Na⁺/K⁺ ATPase activity in the trout.

Maina and Molloy (1986) have shown the organisation of gas exchange organs in air breathing catfish Clarias mossambicus by light, electron and scanning microscope study.

Mallatt et al. (1987) observed the specific activity of Na⁺/K⁺ ATPase in hagfish gill homogenates and they have discussed “why do hagfish have gill chloride cell, when they need not to regulate plasma sodium chloride concentration”.

Maule and Schreck (1990) reported the glucocorticoid receptors in leukocytes and gill of juvenile coho salmon, Oncorhynchus kisutch and have further observed (1991) the stress and cortisol treatment changed affinity and number of glucocorticoid receptors in leukocytes and gill of coho salmon, Oncorhynchus kisutch.
McDonald and Boutilier (1989) reported that ion and acid transfer across the gill of fish rainbow trout, *Salmo gairdneri*. The mechanism and regulation were also observed by these workers.

Milet et al. (1979) have shown by the perfusion method of isolated gill arches that measurement of bidirectional gill Calcium fluxes is possible. They have also reported the effect of Corpuscles of Stannius or ultimobranchial body removal and Corpuscles of Stannius extract or calcitonin perfusion on gill Calcium fluxes in eel, *Anguilla anguilla*.

Morgan and Wright (1989) examined the morphology of the central compartment and vasculature of the gill of *Lepidosiren paradoxain* (Fitzinger) to know more about the gill ion exchange function. They have also shown the ultrastructure of the gill filament, different types of the cells, its blood vessel and function.

Munshi (1960) has reported anatomical and physiological variation both in gill and accessory respiratory organs. Structure and function was also observed in different species of air breathing fishes.

Olson et al. (1989) have pointed the location of angiotensin converting enzyme in gill tissue and determined whether pillar cells might also be the sites of angiotensin converting enzyme in trout, *Salmon gairdneri*.

Playle and Wood (1989) have made the experimental observations and proposed a theory that any gill contaminant with toxicity varying according to pH, may be more or less toxic at gills.
Sala and Marlasca (1987) reported the different type of cells in gill epithelium of juvenile turbot, *Scopthalmus maximus*. They have observed the gill filament by electron microscopic and light microscopic study and described two specialized epithelia, the thick filament or primary epithelium in contact with the arterio-venous circulation, responsible for ion extrusion in marine fish and the thin lamellar epithelium, in contact with the arterio-arterial circulation responsible for gas transfer.

Shukla (1993) has reported the effects of salinity changes and artificial stressors on kidney and gills of a cat fish *Heteropneustes fossilis* (Bloch).

Speare and Ferguson (1989) have suggested the effects of delays between death and initial exposure of gill tissue to fixation in rainbow trout *Salmo gairdneri*.

Watson et al. (1989) have compared the selected blood chemistry and haematological parameters obtained by the caudal transaction and dorsal gill incisor techniques.

Yadava and Singh (1989) reported the gross structure and dimensions of the gill in an airbreathing Estuarine Goby, *Pseudopocryptes lanceolatus*.

Zaugg (1981) has studied the photoperiod and temperature effects on gill $\text{Na}^+/\text{K}^+$ ATPase activity and migration in juvenile steel head *Salmo gairdneri*.
OBSERVATIONS

SEASONAL VARIATIONS

Post-spawning period (September – December)

Important cytological changes were observed in the gills. In September straight primary and secondary gill lamellae were observed. Mucus cells are present on the tip of the primary gill lamella. Pilaster cells are present in the form of a thin chain on the secondary gill lamella. Well developed epithelial cells are seen. Acidophilic cells are also highly developed. Prominent blood supply was observed on the tip of the primary gill lamella (Fig. 1). During the month of October straight primary and curved secondary gill lamellae were observed. Mucus cells are present on the tip of the primary gill lamella. Well developed chain of pilaster cells was observed on the secondary gill lamella. Well developed epithelial cells are also seen. Acidophilic cells are also clearly observed. Normal blood supply was clearly seen (Fig. 2). In the month of November and December straight primary and slightly curved secondary gill lamellae were observed. A large number of mucus cells are seen on the tip of primary gill lamella. A well developed chain of pilaster cells were observed. Acidophilic cells are also seen while normal blood supply (Fig. 4 and 5) was also evident.
Gradual fast transfer in different Calcium concentrations during post-spawning period

The fish *Heteropneustes fossilis* belonging to experimental group were gradually adapted from 2.5 m mol l\(^{-1}\) upto 65 m mol l\(^{-1}\) of CaCl\(_2\).2H\(_2\)O with an increase of 2.5 m mol l\(^{-1}\) at every step. (each step lasted for a day). In 65 m mol l\(^{-1}\), Calcium chloride (CaCl\(_2\).2H\(_2\)O) solution the animal could not survive for more than 5 to 6 hours and is found lethal. Important cytological changes were seen in the gills. Straight primary and slightly curved secondary gill lamellae were observed. Well developed mucus cells are present on the tip of the primary gill lamella. Shrinked pilaster cells were observed. Well developed epithelial cells are seen and there is no space between the two secondary gill filament. Acidophilic cell were absent while highly damaged blood vessel was also noted (Fig.3).

Post-spawning period (Control group-December)

In control group the structural details are same as described earlier. (Fig.4 and 5).

Seasonal changes in pre-spawning period (January - April)

In the month of January and February straight primary and secondary lamellae were observed. A large number of mucus cells are present on the tip of the primary gill lamella. Well developed pilaster cells in the form of a chain were observed in the secondary gill lamella. Epithelial and Acidophilic cells were clearly visible. Prominent blood supply was also observed (Fig.8). Straight
primary and curved secondary gill lamellae were observed in March and April. Well developed mucus cells are present on the tip of the primary gill lamella. Epithelial cells are seen. Acidophilic cells are also clearly observed. Very prominent blood supply was also observed (Fig.7)

Gradual fast transfer in different Calcium concentrations during pre-spawning period

The experimental set up is same as described earlier. Straight primary and curved secondary gill lamella were observed. Mucus cells were scanty. A chain of pilaster cells which is reduced size was clearly observed. Well developed epithelial cells are seen. Well developed acidophilic cells can also be clearly observed. Highly enlarged blood vessels were observed but at few places they are in damaged condition (Fig.6).

Pre-spawning period (Control group - April)

In control group the structural details are same as described earlier. (Fig.7).

Seasonal changes in spawning period (May –August)

Straight primary and curved secondary gill lamellae were observed in May. Highly developed mucus cell are present. A well developed chain of pilaster cells was also seen. Epithelial and acidophilic cells are observed and a very prominent blood supply is clearly seen (Fig.9, 10 and 11). In the month of June straight primary and a straight secondary gill lamellae which were curved only at the
tip observed. Mucus cells are present on the tip of the primary gill lamella. A very narrow chain of pilaster cells were seen on the secondary gill lamella. Well developed epithelial cells are seen. Acidophilic cells are prominent. Normal blood supply were observed (Fig.12). During the month of July and August primary and secondary gill lamellae were found straight. A large number of mucus cells are present on the tip of the primary gill lamella. Well developed pilaster cells in the form of a chain were also observed on the secondary gill lamella. Epithelial cells are prominent. Acidophilic cells are also clearly seen. Normal blood supply was noted (Fig.15).

Gradual fast transfer in different Calcium concentrations of experimental group during spawning period

The fish *Heteropneustes fossilis* (Bloch) belonging to experimental group were gradually adapted from 2.5 m mol l⁻¹, 5.0 m mol l⁻¹ upto 62.5 m mol l⁻¹ solution of Calcium chloride (CaCl₂.2H₂O) in fresh water (each step lasted for a day). In 62.5 m mol l⁻¹ solution animal could not survive for more than 5 to 6 hrs and the concentration is found lethal. Important cytological changes were observed during this concentration in the gills. Straight primary and highly curved secondary gill lamellae were observed. Prominently well developed mucus cells are present on the tip of the primary gill lamella, when compared to the pre-spawning, the size of the mucus cell was considerably large. Well developed chain of pilaster cell and prominent epithelial cells were also observed. Blood capillaries are in shrunked and damaged condition (Fig.13 and 14).
Spawning period (Control group – July)

In control group the structural details are same as described earlier. (Fig.15).

Gradual fast transfer in different magnesium concentrations of experimental group during post-spawning period

The fish *Heteropneustes fossilis* belonging to experimental group were gradually adapted from 10 m mol l⁻¹, 20 m mol l⁻¹, 35 m mol l⁻¹, 55 m mol l⁻¹ and then in 80 m mol l⁻¹ (each step lasted for a day). In 80 m mol l⁻¹ Magnesium chloride (MgCl₂·6H₂O) solution, the animal could not survive for more than 7 to 8 hours and therefore is found lethal. Important cytological changes were observed during this concentration in gills. Highly curved primary as well as secondary gill lamellae were observed. Mucus cells are not observed on the tip of the primary gill lamella. Highly ruptured chain of pilaster cells and damaged epithelial cells were observed. Dilated blood vessels in the primary gill lamella were also visible (Fig.16).

Post-spawning period (Control group-December)

In control group the structural details are same as described earlier. (Fig. 4 and 5).

Gradual fast transfer in different Magnesium concentrations of experimental group during pre-spawning period

The experimental set up is same as described above. Highly shrunk primary and curved secondary gill lamellae were noted. Prominently shrunk mucus cells were observed on the tip of
the primary gill lamella. Tip of the secondary gill were in swollen condition. Highly shrunken chain of a pilaster cells and ruptured epithelial cells were also observed. Highly shrunken blood supply was also seen (Fig.17).

Pre-spawning period (Control group – April)

In control group the structural details are same as described earlier. (Fig. 7).

Gradual fast transfer in different Magnesium concentrations of experimental group during spawning period

The fish *Heteropneustes fossilis* belonging to experimental group were gradually adapted from 10 m mol l⁻¹, 20 m mol l⁻¹, 35 m mol l⁻¹ and then in 55 m mol l⁻¹ (each step lasted for a day). In 55 m mol l⁻¹ magnesium chloride (MgCl₂.6H₂O) solution, the animal could not survive for more than 7 to 8 hours and therefore is found interestingly lethal at this phase of reproductive cycle. Important cytological changes were observed during this concentration in gills. Compact and highly shrunken primary gill lamella and prominently curved secondary gill lamella were observed. But a smaller number of mucus cells than the control was observed in the tip of the primary gill lamella. Ruptured pilaster cells were observed and the blood vessels were in highly damaged condition (Fig.18).

Spawning period (Control group - July)

In control group the structural details are same as described earlier. (Fig. 15).
DISCUSSION

The surface area of the gill was normal in maximum Calcium concentration during pre-spawning and spawning and post-spawning period. In higher Magnesium concentration the surface area were decreased during different phases of reproductive cycle. During highest Calcium exposure the primary and secondary gill lamellae are found slightly curved during pre-spawning, spawning and post-spawning period.

In highest magnesium concentration both primary and secondary gill lamella are not only highly curved but in a very damaged condition.

According to Laurent and Hebibi (1989) the gill lamella displayed large change in size during different ionic environment in rainbow trout. The thickness of the gill lamella epithelium is also significantly affected by external ionic concentration. Our results also agree with these workers regarding the surface area and structure of primary and secondary lamellae.

Sala and Marlasca (1987) described the specialized epithelia of juvenile turbot Scophthalmus maximus. The thick filament epithelium in contact with anterio-venuous circulation responsible for ion extrusion in marine fish and the thin lamellar epithelium in contact with anterio-arterial circulation responsible for gas transfer. A large hyperplasia of the filament epithelium is reported in trout transferred to ion poor water (Laurent and Hebibi, 1989). There was no
hyperplasia during Calcium or Magnesium exposure. However, structural degeneration or atrophy was evident in certain experiments.

In highest Calcium concentration the mucus cells are in very much shrinked condition during all the three phases of reproductive cycle.

During the highest Magnesium concentration the mucus cells are very much shrinked during pre-spawning. However smaller mucus cells were observed during spawning and post-spawning period.

Same results were also obtained by Shukla (1993) with gradual slow and direct transfer experiments in different salinity concentrations where the reduction of mucus cells in number was evident. However, it was also noted that exposure to weak salinity even for a long duration could not transform the associated cell into the Chloride cells.

Payan et al. (1981) have discovered that in fresh water trout, the Calcium uptake is most likely to be carried out by the chloride cells.

In our experiments with at highest Calcium concentration similar results were observed during post-spawning, pre-spawning and spawning period. Acidophilic cells are prominently observed. In highest Magnesium concentration Acidophilic cells were not observed.

Copeland (1946) found the sea water adaptation of animals (previously accommodated for 1 or 2 weeks in tap water) showed cytological changes as easily as 3 hours and apparently complete changes to about 18 to 24 hours. The population and
general appearance of the chloride cells are very similar in both sea
water and fresh water adapted animals. When animals adapted to sea
water there is typically present a "Excretory vesicle" at the free
surface of the secondary filament that is almost and invariably absent
in fresh water adapted animals. The chloride cells may have dual
function, its demonstration in a number of fresh water species of
teleost does not necessarily indicate a marine origin in evolution.
There is a possibility that the chloride cells may be modified type of
mucus cells (Copeland, 1946). The chloride cell is probably concerned
only with ion transfer (Das and Srivastava, 1978). During Saline
adaptation fully developed cells (transformed cells) may be called as
chloride cells. They were found after four weeks of Saline treatment
while number of these hypertrophied cell decrease after 30 days in
sea water (Das and Srivastava, 1978).

Pillaster cells is in enhanced condition at highest Calcium
concentration during all the three phases of reproductive cycle. In
highest Magnesium concentration, these cells are in damaged condition
during post-spawning, pre-spawning and spawning phases.

In highest Calcium concentration well developed epithelial
cells are observed in all the three phases of reproductive cycle. In
highest Magnesium concentration epithelial cell were not observed
during various phases of reproductive cycle.
BIBLIOGRAPHY


Fig. 1. Section of the gill filament of *Heteropneustes fossilis* (Bloch) during post-spawning period (September) showing straight primary and secondary gill filament

H & E 15 x 10

Fig. 2 Section of the gill filament of *Heteropneustes fossilis* (Bloch) during post-spawning period (October) showing well developed epithelial and acidophilic cells, while normal blood supply can also be seen.

H & E 15 x 10

Abbreviations:

- GR = Gill ray
- SPG = Straight primary gill filament
- SSG = Straight secondary gill filament
Fig. 3  Section of the gill filament of *Heteropneustes fossilis* (Bloch) during post-spawning period (December) exposed to in 65 m mol l\(^{-1}\) of Calcium chloride (CaCl\(_2\).2H\(_2\)O) solution showing absence of acidophilic cells and highly damaged blood vessel.

H & E  15 x 10

Fig. 4  Section of the gill filament of *Heteropneustes fossilis* (Bloch) during post-spawning period (December) in control group showing straight primary and slightly curved secondary gill filament.

Mallory’s triple  15 x 10

Fig. 5  Section of the gill filament of *Heteropneustes fossilis* (Bloch) during post-spawning period (December) in control group showing well developed mucus cells and normal blood supply.

Mallory’s triple  10 x 45

Abbreviations:

- BV = Blood vessel
- GR = Gill ray
- SPG = Straight primary gill filament
- SG = Secondary gill filament
Fig. 6 Section of the gill filament of *Heteropneustes fossilis* (Bloch) during pre-spawning period (April) exposed in 65 m mol l\(^{-1}\) of Calcium chloride (CaCl\(_2\).2H\(_2\)O) solution showing highly enlarged blood vessel with well developed epithelial cells. H & E 15 x 10

Fig. 7 Section of the gill filament of *Heteropneustes fossilis* (Bloch) during pre-spawning period (April) in control group showing mucus cells on the tip of the primary gill filament. Epithelial and acidophilic cells are observed. H & E 15 x 10

Fig. 8 Section of the gill filament of *Heteropneustes fossilis* (Bloch) during pre-spawning period (January) showing well developed pilaster cells with straight secondary gill filament. H & E 15 x 10

Abbreviations:

- **BV** = Blood vessel
- **GR** = Gill ray
- **MC** = Mucus cell
- **PC** = Pilaster cell
- **SG** = Secondary gill filament
Fig. 9  Section of the gill filament of *Heteropeustes fossilis* (Bloch) during spawning period (May) showing straight primary and secondary gill filament.

H & E  15 x 10

Fig. 10  Section of the gill filament of *Heteropeustes fossilis* (Bloch) during spawning period (May) showing well developed mucus cells.

H & E  15 x 10

Abbreviations:

BV  =  Blood vessel
MC  =  Mucus cell
SG  =  Secondary gill filament
Fig. 11  Section of the gill filament of *Heteropneustes fossilis* (Bloch) during spawning period (May) showing well developed pilaster cell.

H & E  10 x 45

Fig. 12  Section of the gill filament of *Heteropneustes fossilis* (Bloch) during spawning period (June) showing well developed epithelial and acidophilic cells.

H & E  15 x 10

Abbreviations:

AC = Acidophilic cell
EC = Epithelial cell
PC = Pilaster cell
Fig. 13  Section of the gill filament of *Heteropneustes fossilis* (Bloch) during spawning period (July) exposed in 62.5 m mol l\(^{-1}\) of Calcium chloride (CaCl\(_2\).2H\(_2\)O) solution showing straight primary and highly curved secondary gill filament.

H & E  
15 x 10

Fig. 14  Section of the gill filament of *Heteropneustes fossilis* (Bloch) during spawning period (July) exposed in 62.5 m mol l\(^{-1}\) of Calcium chloride (CaCl\(_2\).2H\(_2\)O) solution showing well developed chain of pilaster cells.

H & E  
10 x 45

Fig. 15  Section of the gill filament of *Heteropneustes fossilis* (Bloch) during spawning period (July) in control group showing highly developed mucus cells.

H & E  
15 x 10

Abbreviations:

- MC = Mucus cell
- PC = Pilaster cell
- SPG = Straight primary gill filament
Fig. 16  Section of of the gill filament of *Heteropneustes fossilis* (Bloch) during post-spawning period (December) exposed in 80 m mol l⁻¹ of Magnesium chloride (MgCl₂·6H₂O) solution showing highly curved primary and secondary gill filament and highly dilated blood vessel.  
H & E  15 x 10

Fig. 17  Section of of the gill filament of *Heteropneustes fossilis* (Bloch) during pre-spawning period (April) exposed in 80 m mol l⁻¹ of Magnesium chloride (MgCl₂·6H₂O) solution showing prominent shrunken mucus cells and highly shrunken blood supply.  
H & E  15 x 10

Fig. 18  Section of of the gill filament of *Heteropneustes fossilis* (Bloch) during spawning period (July) exposed in 55 m mol l⁻¹ of Magnesium chloride (MgCl₂·6H₂O) solution showing prominence curving secondary gill filament and smaller mucus cells.  
H & E  15 x 10

Abbreviations:

BV = Blood vessel  
MC = Mucus cell  
PC = Pilaster cell  
PGL = Primary gill lamella  
SGL = Secondary gill lamella
(D) OVARY

INTRODUCTION
OBSERVATIONS
DISCUSSION
BIBLIOGRAPHY
INTRODUCTION

The present study has been planned to observe the effect of different Calcium concentrations in the ovary of *Heteropneustes fossilis* (Bloch) during its reproductive cycle. Important work on the structure of the fish ovary along with its seasonal changes in the annual reproductive cycle have been described by various workers. Abraham et al. (1984) have studied the cellular envelope of oocytes in teleosts. Anant (1976) has studies on the functional morphology of the endocrine glands in some fresh water fishes. Asahina and Hanyu (1983) have reported the role of temperature and photoperiod in annual reproductive cycle of the rose bitterling, *Rhodeus ocellatus ocellatus*. Baggerman (1980) has shown the photoperiodic and endogenous control of the annual reproduction cycle in teleost fishes. Belsare (1962) has shown the seasonal changes in the ovary of *Ophiocephalus punctatus*. Bentivegna and Benedetto (1989) have studies the Gonochorism and seasonal variations in the gonads of the Labrid Symphodus (Crenilabrus) *Ocellatus ocellatus* (Forsskal). Bieniarz et at. (1978) have reported the annual reproductive cycle of adult carp in Poland: Ovarian stage and serum gonadotropin level. Billard and Brentom (1978) have shown the Rhythms of reproduction in teleost fish. Borg and Van Veen (1982) have studied the seasonal effects of photoperiod and temperature on the ovary of the three spined stickleback *Gasterosteus aculeatus*. Bretscheider and Duyvene de wit (1947) have studied on sexual endocrinology of non mammalian vertebrates. Bromage et at. (1984) have shown the effects of constant
photoperiod on the timing of spawning in the rainbow trout. Buxton (1989) has reported the reproductive biology of Chrysoblephus laticeps, Cristiceps cristiceps Teleostei: Sparidae. Chandrasoma and De Silva (1981) have studied the reproductive biology of Puntius sarana: an indigenous species and Tilapia rendalli (Melanopleura) and an exotic fish in an ancient man made lake in Sri Lanka. Coetzee (1983) has shown seasonal, histological and macroscopic changes in the gonads of Cheimerius nufar. Davis (1977) has studied the reproductive biology of the fresh water cat fish, Tandanus tandanus in the Gwydir river Australia: II Gonadal cycle and fecundity. Duston and Bromage (1986) have studied constant photoperiod regimes and the entrainment of the annual cycle of reproduction in the female rainbow trout (Salmo gairdneri). Elliott et al. (1984) have shown the changes in reproductive function of three strains of rainbow trout exposed to constant and seasonally changing light cycles. Forberg (1982) has reported a histological study of development of oocytes in capalin, Mallotus villousus villousus (Muller). Fouche et al. (1985) have studied the female reproductive cycle of the European common carp, Cyprinus carpio at a Transval fish farm: Gonadal morphometric development. Goldberg (1981a): has studied the seasonal spawning cycle of the california tongue fish, Symphysurus atricauda (Cynoglossidae). Goldberg and Mussielt (1984) have studied the reproductive cycle of the pacific bonito, Sarda chilensis (Scombridae) from Northern Chile. Howell (1983) has also studied the seasonal changes in the ovaries of an adult yellow tail flounder, Limanda
ferruginea. Htun Han (1978) has shown the reproductive biology of
the dab, *Limanda limanda* (L) in the north sea: Seasonal changes in
the ovary. Hurk and Peute (1979) have studied the cyclic changes in
the ovary of the rainbow trout, *Salmo gairdneri* with special reference
to sites of steroidogenesis. Merwe Vander et al. (1988) have studied
the cyclic histomorphological changes in the ovary of Mud fish, *Labo
capensis*. Nakamura et al. (1989) have studied the histological and
ultrastructural evidence for the role of gonadal steroid hormones in
sex change in the protogynous wrasse *Thalassoma duperrey*. Peigiu
(1980) has shown the morphological characters and seasonal
changes in the development of the oocytes of the small croaker,
*Pseudosciaena polyactis* (Bleeker). Prabhu (1956) has reported on
Maturation of intraovarian eggs and spawning periodicity of some
fishes. Raizada (1977) has also studied the reproductive system and
the reproductive cycle in some teleosts *Rasbora daniconius* (Ham)
and Rimmer (1985) has studied the reproductive cycle of the Fork
has studies on HYpothermal-Hypophysial Neurosecretory system in
fresh water fish *Glassogobius giuris* (Ham.) in relation to reproduction.
Schreibman et al. (1982) have studied the histological and
histochemistry of the testis and ovary of the platy fish, *Xiphophorus
maculatus*, from birth to sexual maturity. Scott and Sumpter (1983)
have studied the control of trout reproduction: in basic and applied
research on hormones. Shirokova (1977) has shown peculiarities of
the sexual maturation of females of the Baltic cod, *Gadus morhua*
callarias. Subhedar and Prasada Rao (1978) have studied the seasonal changes in the corporcles of stannius and gonads of the catfish, *Heteropneustes fossilis* (Bloch). Tricas et al. (1988) have shown the sexual differentiation, gonad development and spawning seasonality of the Hawaiian butterfly fish, *Chaetodon multicinctus*. Yamamoto (1956) has studies on the formation of fish egg. 1. Annual cycle in the development of ovarian eggs in the flounder, *Liopsetta obscura*. 
OBSERVATIONS

MORPHOLOGY AND HISTOLOGY OF THE OVARY

The ovary in the *Heteropneustes fossilis* (Bloch) is an elongated and spindle shaped structure suspended in the coelom by a peritonium fold known as *mesovarian* in between the kidney and alimentary canal. The anterior portion of the ovary is conelike and tapering, the middle is wider and posterior is narrower. The peritoneal folds of the posterior side of each ovary show an extension of a hollow tube like structure forming the oviduct which ultimately joins posteriorly with the fellow of the opposite side to form a common oviduct and opens to the exterior through the genital pore.

The ovaries are enclosed in thin peritoneal covering. The ovarian wall is composed of an outer layer of fibrous connective tissue traversed by the blood capillaries and an inner layer of germinal epithelium. The ovigerous lamellae containing oocytes in various stages of development projected from the ovarian wall towards the centre of the ovary. The early stages of oocytes are found on the periphery of the lamellae. They migrate towards the ovocoel centre during their development. The ovigerous lamellae are covered by a thin layer of epithelial cells.

OOGENETIC STAGES

Different oogenetic stages in *Heteropneustes fossilis* (Bloch) can be devided into ten developmental stages on the basis of both nuclear and cytoplasmic changes with special reference to
vitellogenesis. The different developmental stages as described here are based on the work of Yamamoto (1956) which provides enough scope to study the various stages of the ovary as follows:

i. Early chromatin -nucleolus stage
ii. Late chromatin-nucleolus stage
iii. Early peri-nucleolus stage
iv. Late peri-nucleolus stage
v. Early yolk-vesicle stage
vi. Late yolk-vesicle stage
vii. Early yolk stage
viii. Late yolk stage
ix. Pre-maturation stage
x. Maturation stage

CORPORA ATRETICA & POST OVULATORY FOLLICLES

Corpora atretica

The immature oocytes which fail to attain maturity and the mature oocytes which fail to spawn ultimately undergo resorption or atresia and are called corpora atretica or atretic follicles. In the process of resorption, granulosa layer or follicular epithelium of the ovarian follicle plays an important role. Bretschneider and Duyvene de Wit (1947) were the first to make an exhaustive study in Rhodeus amarus. Bhargava (1966) has used the term corpora atretica. They produce the enzymes which
digest the yolk, the cytoplasm and the nucleus. The process of atresia of mature oocytes have been distinguished into four different stages.

(a) Stage I
(b) Stage II
(c) Stage III
(d) Stage IV (Fig.15 and 18)

Post-ovulatory follicles

The post-ovulatory follicles (empty or ruptured follicles) are formed after the extrusion of mature oocyte from the ovary. The frequency of the number of post-ovulatory follicles in the ovary is an useful measure to estimate the spawning periodicity of the fish.

Post-ovulatory follicles of *Heteropneustes fossilis* are convoluted structure containing an irregularly shaped lumen. (Fig.18)

Post-spawning period (September to December)

During this period the ovarian wall is thick and distinct. Ovigerous lamellae are present in the month of September and few ripe ovas are also present. The interfollicular space is now quite distinct, corpora atretica and post ovulatory follicle are present. Early oocyte stage though few in number, shows the chromatin nucleolus stage. In the month of October and November few blood vessels are found in the ovarian wall as well as within the ovigerous lamellae. The Chromatin-nucleolus stage and peri-nucleolus stage are abundantly seen in the stroma of the ovary. (Fig.1) During month of December, the ovigerous lamellae contain numerous oogonia. Interfollicular space is still distinct.
Late peri-nucleolus and yolk vesicle stage were also observed. Corpora atretica and post-ovulatory follicle are totally absent. (Fig.2)

Gradual fast transfer in different Calcium concentrations of experimental group during post-spawning period

The fish *Heteropneustes fossilis* (Bloch) belonging to experimental group were gradually adapted from 2.5 m mol l⁻¹, 5.0 m mol l⁻¹ upto 65 m mol l⁻¹ solution of Calcium chloride (CaCl₂.2H₂O) in fresh water (each step lasted for a day). In 65 m mol l⁻¹ solution animal could not survive for more than 5 to 6 hrs and the concentration is found lethal. Important cytological changes were observed during this concentration. In ovary thick ovarian wall was noticed while connective tissue was ruptured. In late chromatin nucleolus stage the nucleolus are in shrinked condition. Disorganised early perinucleolus stages are also seen. Yolk vesicles are noticed in shrinked condition. Corpora atretica and post ovulatory follicle are totally absent. (Fig.3 & 4).

Post-spawning period (Control Group)

In control group the structural details are same as described earlier. (Fig.2)

Alizarin red S treatment to show Calcium deposition of experimental group during post-spawning period

Experimental group were treated with Alizarin red to show deposition of Calcium during post-spawning period. Thick ovarian wall, connective tissue, ovigerous lamellae, early chromatin nucleolus and the yolk vesicle are all stained deeply with Alizarin red S. (Fig.5)
Alizarin red S treatment to show Calcium deposition in control group during post-spawning period

Control group was treated with Alizarin red S to show the deposition of Calcium during post-spawning period. The thick ovarian wall, connective tissue, ovigerous lamellae, yolk vesicle stage and late chromatin stage are moderately stained, whereas blood vessels take deep stain. Follicular epithelium and the nucleus show totally negative response towards this stain. (Fig.6)

Pre-spawning period (January to April)

During this period the ovarian wall is thick and highly vascular. In the month of January and February the vacuoles started to appear in the oocyte. A large number of early yolk vesicle stage were also observed. A few late chromatin nucleolus stage are seen. Corpora atretica and post ovulatory follicle are not seen (Fig.7.).

In the month of March and April, the ovarian wall becomes thinner as compared to that of preceding month. The late yolk vesicles stage showed vaculation while yolk formation also started in the cytoplasm. Early yolk stage lies towards the border of the ovigerous lamellae. A few corpora atretica are present. Post-ovulatory follicle are completely absent. (Fig.8 &11).

Gradual fast transfer in different Calcium concentration of experimental group during pre-spawning period

The fish *Heteropneustes fossilis* belonging to experimental group were gradually adapted from 2.5 m mol l$^{-1}$, 5.0 m mol l$^{-1}$ upto 65 m mol l$^{-1}$ (each step lasted for a day). In 65 m mol l$^{-1}$, solution of
Calcium chloride (CaCl$_2$.2H$_2$O). The animal could not survive for more than 5 to 6 hrs and the concentration is found lethal. Important cytological changes were observed during this concentration. The thinner ovarian wall was noticed while connective tissue found ruptured. Follicular epithelium and thin vitelline membrane are separated with each other. Yolk vesicle at the periphery of mature oocyte become shrunked and also found ruptured. Yolk granules are shrunked in mature oocyte. Corpora atretica become also shrunked. Disorganized nuclei are observed with in the shrunked nuclear membrane. Late Chromatin nucleolus was found in shrunken condition. (Fig. 9 &10).

Pre-spawning period (Control Group)

In control group the structural details are same as described earlier. (Fig.11)

Alizarin red S treatment in experimental group during pre-spawning period

Experimental group was treated with Alizarin red S during pre-spawning period. The thinner ovarian wall and corpora atretica were stained lightly while vitelline membrane and yolk granules were deeply stained. However follicular epithelium and late chromatin nucleolus show a negative response to the Alizarin red S stain. (Fig.12).

Alizarin red S treatment in control group during pre-spawning period

Control group was treated with Alizarin red S to pre-spawning period. Thinner ovarian wall, follicular epithelium, immature oocyte and nucleus showed a totally negative response to the Alizarin
red S whereas mature oocyte, corpora atretica, vitelline membrane and yolk granules are all lightly stained with Alizarin red S. (Fig.13)

Spawning period (May to August)

During the spawning period, the ovarian wall is very thin a large number of ripe oocytes are present alongwith few immature oocytes. Their number are fewer than in the preceding month. In month of May, interfoliccular space is further reduced. The yolk granules appear in the oocyte. Corpora atretica and some post ovulatory follicles are also evident. In June and July, majority of the ova in the ovary become fully ripened and are closely packed together. A very few immature oocyte are also present in between the ripe ones. The corpora atretica and post ovulatory follicles are also seen during this period. (Fig.14)

In the month of August corpora atretica and post ovulatory follicles are again noticed but their number has increased. (Fig.15)

Gradual fast transfer in different Calcium concentrations of experimental group during spawning period

The fish *Heteropneustes fossilis* belonging to experimental group were gradually adapted from 2.5 m mol l⁻¹, 5.0 m mol l⁻¹ upto 62.5m mol l⁻¹ in Calcium chloride (CaCl₂.2H₂O) solution in fresh water (each step lasted for a day). In 62.5m mol l⁻¹ solution the animals could not survive for more than 5 to 6 hrs and the concentration is found lethal. Important cytological changes are observed during this exposure. In ovary the ovarian wall is in ruptured condition. The follicular epithelium and vitelline membrane are distinct with each other. The inter follicular space has also increased. Vacuoles are present at the
periphery in the cytoplasm, but they are in shrunken condition specially in mature oocyte. Nuclear membrane is ruptured and nucleus has shrunked. Disorganised nuclei inside the nucleus are seen. Corpora atretica become shrunked while the post ovulatory follicles are in ruptured condition. (Fig.16 & 17)

**Spawning period (Control group)**

In control group the structural details are same as described earlier. (Fig.18)

**Alizarin Red S treatment to show Calcium deposition of experimental group during spawning period**

Experimental were treated with Alizarin red S to show the deposition of Calcium during spawning period. Thinner ovarian wall with follicular epithelium and corpora atretica are stained lightly with Alizarin red S. Vitelline membrane, mature oocyte, yolk granules are stained deeply with Alizarin red S. Post-ovulatory follicle and mature oocyte nucleus show a totally negative response to the Alizarin red S. (Fig.19 & 20).

**Alizarin red S treatment to show Calcium deposition in control group during spawning period**

Control group is treated with Alizarin red to show the deposition of Calcium during spawning period. The thinner ovarian wall, follicular epithelium, immature oocyte, post-ovulatory follicle and the nuclei showed a totally negative response to the Alizarin red S, whereas the mature oocyte, vitalline membrane and corpora atretica are deeply stained. (Fig.21)
Gradual fast transfer in different magnesium concentrations of experimental group during post-spawning period

The fish *Heteropneustes fossilis* belonging to experimental group were gradually adapted from 10 m mol l⁻¹, 20 m mol l⁻¹, 35 m mol l⁻¹, 55 m mol l⁻¹ and 80 m mol l⁻¹ (each step lasted for a day). In 80 m mol l⁻¹ magnesium chloride (MgCl₂.6H₂O) solution, the animal could not survive for more than 7 to 8 hours and therefore is found lethal. Important cytological changes were observed during this concentration in ovary. The thick ovarian wall, follicular epithelium and the ovigerous lamellae are in ruptured condition. The nuclear membrane is ruptured and nucleus is shrinked. Early chromatin nucleolus stage is also found in shrinked in condition and is basophilic in nature. Late chromatin nucleolus stage is also highly basophilic. In yolk vesicle stage, the number of vacuoles has considerably increased. (Fig.22).

Post-spawning period (Control Group)

In control group the structural details are same as described earlier. (Fig.2).

Gradual fast transfer in different magnesium concentrations of experimental group during pre-spawning period

The fish *Heteropneustes fossilis* (Bloch) belonging to experimental group were gradually adapted from 10 m mol l⁻¹, 20 m mol l⁻¹, 35 m mol l⁻¹, 55.0 m mol l⁻¹ and 80 m mol l⁻¹ (each step lasted for a day). In 80 m mol l⁻¹ magnesium chloride (MgCl₂.6H₂O) solution the animal could not survive for more than 7 to 8 hours and is found lethal. Important cytological changes were observed during this concentration in ovary. The thin ovarian wall and the corresponding follicular epithelium are
ruptured. Vitelline membrane and follicular epithelium get separated. The size of yolk granules considerably increased. Shrinked nucleus with totally ruptured nuclear membrane were also observed. Late perinucleus stage is basophilic though stained light due to magnesium exposure. The corpora atretica become shrinked in comparison to the control group. (Fig.23)

Pre-spawning period (Control Group)

In control group the structural details are same as described earlier. (Fig.11)

Gradual fast transfer in different magnesium concentrations of experimental group during spawning period

The fish Heteropneustes fossilis (Bloch) belonging to experimental group were gradually adapted from 10 m mol l\(^{-1}\), 20 m mol l\(^{-1}\), 35 m mol l\(^{-1}\) and 55 m mol l\(^{-1}\) (each step lasted for a day). In 55 m mol l\(^{-1}\) Magnesium Chloride (MgCl\(_2\).6H\(_2\)O) solution the animal could not survive for more than 7 to 8 hrs and is found lethal. Important cytological changes were observed during this concentration in ovary. The thinner ovarian wall alongwith follicular epithelium is ruptured. The follicular and vitelline membrane became isolated. The membrane of mature oocyte is highly shrinked and the size of yolk granules increased considerably. These granules became degranulated and seem to be dissolved in the cytoplasm. Corpora atretica and post-ovulatory follicle are interestingly found shrinked (not shown in figure) Nucleus is highly damaged condition. (Fig. 24)

Spawning period (Control Group)

In control group the structural details are same as described earlier. (Fig.18)
DISCUSSION

The origin of the new crop of oocytes in faced with divergent opinions and the observations in this direction are variable.

Yamamoto (1956) states that the new crop of germ cells originates from the follicles. Cells contained in the empty follicle are left behind after the extrusion of the mature oocytes. Raizada (1971) has observed that the new proliferation of germinal epithelium while Belsare (1962) has noticed the origin of new crop of the oocytes take place from the existing oogonia. Saxena (1974) has suggested that the new crop of oocytes is derived from the germinal epithelium. In Heteropneustes fossilis it has been observed that the new oogonia appear from the germinal epithelium and transformed into oocyte during a short rest period i.e. the early chromatin-nucleolus stage is always found associated with the germinal layer.

The process of the yolk formation is also described by various workers. Yamamoto (1956) has studied the formation of the fish egg with reference to vitellogenesis and histochemistry of yolk granules. Saxena (1974) has been noticed that the yolk granules start deposition in between the yolk vesicles. The yolk granules or globules are at first lightly basophilic but soon become light acidophilic. Tricas and Hiramoto (1989) have suggested that the yolk uptake rapidly accelerated oocyte enlargement. In early vitellogenesis, the vitelline membrane appeared as a thin eosinophilic band around the oocyte. Yolk spheres first appeared near ooplasm periphery and migrate towards the cell interior. As cell enlarged, the vitelline membrane thickened. In later stages of
vitellogenesis, enlarged yolk spheres filled the oocyte interior and lipid droplets aggregated near the nucleus. Coetzee (1983) and Buxton (1990) have reported the acidophilic secondary yolk globules which are next to appear first in the region of the primary yolk vesicles but later as extra vesicular yolk throughout the cytoplasm. In the tertiary yolk vesicle stage the zona radiata is clearly visible and cytoplasm become entirely filled with yolk vesicles. At the end of development the nucleus migrates towards the periphery of the cell which is followed by a degeneration of nuclear membrane, formation of oil droplet and the coalescence of yolk will follow accordingly.

In Heteropneustes fossilis (Bloch) the yolk formation starts at the primary yolk stage of oocyte as small granules of yolk inside each visible. These small granules are of first lightly acidophilic in nature. Later on they fused to form large yolk granules which are strongly acidophilic. The yolk granules or globules are of different shapes and size and therefore show differential staining property to acidic dyes.

In Heteropneustes fossilis (Bloch) the oocytes are covered with a layer of follicle cells, below which lies a vitelline membrane as also observed in Ophiocephalus punctatus, Balsare (1962), Rasbora daniconius, Raizada (1971) and Glossogobius giurus, Saxena (1974). The present study showed no other layer comparable to the theca layer.

Certain follicle cells i.e., granulosa cells take parts in the absorption of yolk. The above observation is confirmed in the present study with Heteropneustes fossilis (Bloch) where the hypertrophied cells, by their phagocytic activity, first destroy the vitelline membrane and then
invade the yolk through it. The disintegration ferment, Bretschneider and Duyvene de wit (1947) are present in the form of granules, are also seen throughout the stages of atresia.

After the vitelline membrane is destroyed, the ooplasm is invaded by the follicle cells. Thus making possible a rapid absorption of yolk by their phagocytic activity.

Buxton (1990) has reported the two distinct atretic events which are recognized in the ovaries of Chrysoblephus laticeps and Cristiceps cristiceps. The first is a resorption of yolk oocytes during normal ovarian cycle in the breeding season but is most common immediately after spawning. The second is a condition associated with sex reversal which includes a degeneration of all oocyte stages and the progressive absorption of the entire ovary. Merwe et al. (1988) have suggested that in the Labeo capensis the atretic oocytes are present during primary oocyte development (summer and autumn). The degeneration first occurs in the oocyte membrane where after ooplasm and nucleoplasm material extrude in to the interstitial tissue. Raizada (1971); Saxena (1974) have used the term corpora atretica for the degeneration of unspawned ripe oocytes or the immature oocytes undergoing oolysis. This term has been also followed in the present work in Heteropneustes fossilis (Bloch), the follicles undergoing resorption after the discharge of the ripe ova have been formed as the post-ovulatory follicles or ruptured follicles. Corpora atretica and post-ovulatory follicle are present in the ovaries of Heteropneustes fossilis (Bloch) during spawning phase. Their number is largest during this
period. The post-ovulatory follicle cells undergo hypertrophy and proliferation but the whole mass later on merged into the ovarian stoma. The significance of the presence of the corpora atretica and post-ovulatory follicles in the determination of the reproductive cycle of the fish has been suggested by Saxena (1974).

Seasonal changes in the ovary have been studied by many workers. Bantivegna and Benedetto, (1989); Belsare, (1962); Buxton, (1986); Coetzee, (1983); Merwe et al., (1988); Prabhu, (1956); Raizada, (1977); Subhedar and Prasada Rao, (1978); Saxena, (1974); Tricas and Hiramoto, (1989) in several fishes. Similar observations have also been found to occur in Heteropneustes fossilis (Bloch). Bantivegna and Benedetto (1989) have noticed the study of the seasonal changes in gonads of Symphodus ocellatus and revealed four phases is pre-reproductive (March to April), reproductive (May to August), post-reproduction (September to October) and quienscence (November to February). Female gonads showed maximum activity during the reproductive period. Merwe et al. (1988) have reported that the reproductive cycle of Labeo capensis can therefore be divided into four phases, namely, the primary oocyte developing phase (summer), yolk production phase (autumn), maturation phase (winter) and the final maturation and spawning phase (spring). Prabhu (1956) has studied the spawning periodicities of several species and found variations in the spawning periods in different species. He distinguished four types of spawning viz., spawning once in a year with a short period, spawning once in a year with longer durations, spawning twice a year and spawning throughout the year.
In *Heteropneustes fossilis* (Bloch) the reproductive cycle has also been divided into three phases. The post-spawning (September to December), the pre-spawning (January to April) and the spawning period (May to August). During post-spawning period, the thick ovarian wall, ovigerous lamellae, chromatin nucleolus stage, peri-nucleolus stage and yolk vesicle stage were observed, whereas corpora atretica and post-ovulatory follicles were totally absent in late past-spawning period (December).

During pre-spawning and spawning period, thinner ovarian wall containing follicular epithelium, vitelline membrane as important membrane are noted while yolk granules and corpora atretica were also seen. The post-ovulatory follicles are present in late spawning period (August month). The fish breeds only once in a year while the spawning period extend from May to August.

In experimental group and with Calcium exposure, the thick ovarian wall, ovigerous lamellae, connective tissue, early peri-nucleolus, late peri-nucleolus stage and early yolk vesicle stage are found highly ruptured in comparison to control during post-spawning period, whereas during pre-spawning and spawning period only thinner ovarian wall and follicular epithelium are found ruptured in comparison to control group. But yolk granules, corpora atretica and nucleus of mature oocyte are found shrinked comparably. During spawning, the post ovulatory follicles are found prominently shrinked.

With Alizarin red S stain, which is specific for Calcium deposition, the experimental and control group during its reproductive
phase i.e., post-spawning, pre-spawning and spawning period, the
observation are as follows: During post-spawning and in experimental
group, thick ovarian wall, ovigerous lamellae, early chromatin nucleolus
and yolk vesicles are deeply stained in comparison to control. However,
during pre-spawning and spawning period in experimental group,
vitelline membrane, yolk granules, mature oocytes are also deeply
stained with Alizarin red S, when compared to control, whereas thinner
ovarian wall and corpora atretica do not respond and could stained
lightly in comparison to control group.

With Magnesium exposure of experimental group, thick
ovarian wall and ovigerous lamellae are in ruptured condition in
comparison to control group. But early chromatin nucleolus and late
chromatin nucleolus stage are found highly basophilic than control
during post-spawning period. During pre-spawning and spawning period,
thinner ovarian wall with follicular epithelium which get separated are
seen. The size of yolk granules has considerably increased than centre.
Shrinked nucleus with totally ruptured nuclear membrane were observed
than control group. Corpora atretica become shrinked during pre-
spawning and spawning periods.
BIBLIOGRAPHY


Fig. 1 Photomicrograph of Transverse Section of the Ovary of *Heteropneustes fossilis* (Bloch) showing seasonal variations during post-spawning period (October)

H & E 10 x 6

Abbreviations

B.V. : Blood vessel
C.T. : Connective tissue
E.PN. : Early peri-nucleolus stage
L.PN. : Late peri-nucleolus stage
O.L. : Ovigerous lamellae
T.O.W. : Thick ovarian wall

Fig. 2 Photomicrograph of T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) in control group during post-spawning period (December) showing Late Peri-nucleolus and early yolk vesicle stage.

H & E 10 x 6

Abbreviations

C.T. : Connective tissue
E.PN. : Early Peri-nucleolus stage
E.YV. : Early Yolk vesicle stage
L.PN. : Late Peri-nucleolus stage
O.L. : Ovigerous Lamellae
T.O.W. : Thick Ovarian wall
Fig. 3  Photomicrograph of T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) showing the effect of 65 m mol l⁻¹ of calcium chloride (CaCl₂·2H₂O) solution during post-spawning period (December).

H & E  10 x 6

Abbreviations

C.T. : Connective tissue

O.W. : Ovarian wall

T.O.W. : Thick Ovarian wall

Fig. 4  Showing ruptured and disorganised nuclei

H & E  10 x 10

Abbreviations

N : Nuclei

P.N. : Peri-nucleolus stage
Fig. 5 Photomicrograph of T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) showing Alizarin red S treatment for calcium deposition in experimental group during post-spawning period (December). Showing positive response with Alizarin red S in few structures.

Abbreviations
C.T. : Connective tissue
E.C.N. : Early Chromatin Nucleolus stage
O.L. : Ovigerous Lamellae
T.O.W. : Thick Ovarian wall
Y.V. : Yolk Vesicle stage

Fig. 6 Photomicrograph of T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) showing Alizaring res S treatment for calcium deposition in Control group during post-spawning period (December) showing poor response with Alizarin red S treatment.

Abbreviations
B.V. : Blood vessel
C.T. : Connective tissue
O.L. : Ovigerous Lamellae
T.W. : Thin Ovarian wall
Y.V. : Yolk Vesicle stage

Showing negative stain with Alizarin red S

Abbreviations
F.E. : Follicular epithelium
N : Nucleus
Fig. 7  Photomicrograph of T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) showing seasonal variations during pre-spawning period (February).

H & E  10 x 6

Abbreviations

E.YV. : Early Yolk Vesicle stage

O.L. : Ovigerous Lamellae

T.O.W. : Thick Ovarian wall

Fig. 8  Photomicrograph of T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) showing seasonal variations during pre-spawning period (March).

H & E  10 x 6

Abbreviations

E.YV. : Early Yolk vesicle stage

L.YV. : Late Yolk vesicle stage

O.L. : Ovigerous Lamellae

T.W. : Thin Ovarian wall
Fig. 9 Photomicrograph of T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) showing the effect of 65 m mol l⁻¹ of calcium chloride (CaCl₂·2H₂O) solution during pre-spawning period (April).

H & E 10 x 6

Abbreviations

C.T. : Corpora atretica
F.E. : Follicular epithelium
O.W. : Ovarian wall
N : Nucleus
N.M. : Nuclear Membrane
V.M. : Vetelline membrane
Y.G. : Yolk granules

Fig. 10 Photomicrograph of T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) showing the effect of 65 m mol l⁻¹ of calcium chloride (CaCl₂·2H₂O) solution during pre-spawning period (April).

H & E 10 x 10

Abbreviations

F.E. : Follicular epithelium
N : Nucleus
V.M. : Vetelline membrane
Y.G. : Yolk granules

Fig. 11 Photomicrograph of T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) showing control group during pre-spawning period (April).

H & E 10 x 6

Abbreviations

F.E. : Follicular epithelium
O.W. : Ovarian wall
V.M. : Vetelline membrane
Y.G. : Yolk granules
Fig. 12  Photomicrograph of T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) showing Alizarin red S treatment for calcium deposition in experimental group during pre-spawning period (April).

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Showing poor response with Alizarin red S  
10 x 6

Abbreviations
- C.A. : Corpora atretica
- T.W. : Thin ovarian wall

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Showing positive response with Alizarin red S

Abbreviations
- V.M. : Vitelline membrane
- Y.G. : Yolk granules

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Showing negative response with Alizarin red S

Abbreviations
- F.E. : Follicular epithelium
- L. CN. : Late Chromatin Nucleolus stage

Fig. 13  Photomicrograph of T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) showing Alizarin res S treatment for calcium deposition in Control group during pre-spawning period (April).

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Showing poor response with Alizarin red S  
10 x 6

Abbreviations
- C.A. : Corpora atretica
- M.O. : Mature Oocyte
- V.M. : Vitelline membrane
- Y.G. : Yolk granules

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Showing poor response with Alizarin red S

Abbreviations
- F.E. : Follicular epithelium
- IM.O. : Immature oocyte
- N : Nucleus
- T.W. : Thin Ovarian wall
Fig. 14  Photomicrograph of T.S. of the Ovary of *Heteropeustes fossilis* (Bloch) showing seasonal variations during spawning period (June).

H & E  10 x 6

**Abbreviations**

IM.O. : Immature stage

M.O. : Mature Oocyte

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Fig. 15  Photomicrograph of T.S. of the Ovary of *Heteropeustes fossilis* (Bloch) showing seasonal variations during spawning period (August).

H & E  10 x 6

**Abbreviations**

C.A. : Corpora atretica

D.G. : Degranulated granules

F.E. : Follicular epithelium

M.O. : Mature oocyte

V.M. : Vitelline membrane
Fig. 16  Photomicrograph of T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) showing the effect of 62.5 m mol l\(^{-1}\) of calcium chloride (CaCl\(_2\).2H\(_2\)O) solution during spawning period (July).

H & E  

10 x 6

Abbreviations

F.E.  :  Follicular epithelium  
N  :  Nucleus  
T.W.  :  Thin ovarian wall  
V  :  Vacuole  
V.M.  :  Vetelline membrane  
Y.G.  :  Yolk granules

Fig. 17  Photomicrograph of T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) showing the effect of 62.5 m mol l\(^{-1}\) of calcium chloride (CaCl\(_2\).2H\(_2\)O) solution during spawning period (July).

H & E  

10 x 10

Abbreviations

F.E.  :  Follicular epithelium  
N  :  Nucleus  
V  :  Vacuole  
V.M.  :  Vetelline membrane  
Y.G.  :  Yolk granules

Fig. 18  Photomicrograph of T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) showing control group during spawning period (July).

H & E  

10 x 6

Abbreviations

C.A.  :  Corpora atretica  
F.E.  :  Follicular epithelium  
I.M.O.  :  Immature oocyte  
M.O.  :  Mature oocyte  
N  :  Nucleus  
P.F.  :  Post-ovulatry follicle  
T.W.  :  Thin ovarian wall  
V.M.  :  Vetelline membrane
Photomicrograph of T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) showing Alizarin red S treatment for calcium deposition in experimental group during spawning period (July).

Fig. 19  Showing poor response with Alizarin red S  
Abbreviations  
C.A. : Corpora atretica  
F.E. : Follicular epithelium  
T.O.W. : Thick Ovarian wall

→  Showing positive response with Alizarin red S  
Abbreviations  
M.O. : Mature Oocyte  
V.M. : Vitelline membrane  
Y.G. : Yolk granules

Fig. 20  Showing totally week response  
Abbreviations  
P.F. : Post-ovulatory follicle

Fig. 21  Photomicrograph of T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) showing Alizarin red S treatment for calcium deposition in Control group during spawning period (July).

Showing positive response with Alizarin red S  
Abbreviations  
C.A. : Corpora atretica  
M.O. : Mature oocyte  
V.M. : Vitelline membrane

Showing totally negative response  
Abbreviations  
F.E. : Follicular epithelium  
I.M.O. : Immature Oocyte  
M.O.N. : Mature Oocyte Nucleus  
P.F. : Post-ovulatory follicle
Fig. 22 Photomicrograph of the T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) showing the effect of 80 m mol l\(^{-1}\) of magnesium chloride (MgCl\(_2\)·6H\(_2\)O) solution during post-spawning period (December).

H & E 10 x 6

Abbreviations
C.T. : Connective tissue
E.CN. : Early Chromatin-nucleolus stage
L.CN. : Late Chromatin-nucleolus stage
N.M. : Nuclear membrane
P.N. : Peri nucleolus stage
O.L. : Ovigenous lamellae
T.O.W. : Thick ovrarian wall
Y.V. : Yolk vesicle

Fig. 23 Photomicrograph of T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) showing the effect of 80 m mol l\(^{-1}\) of magnesium chloride (MgCl\(_2\)·6H\(_2\)O) solution during pre-spawning period (April).

H & E 10 x 6

Abbreviations
C.A. : Corpora atretica
F.E. : Follicular epithelium
L.PN. : Late Peri-nucleolus stage
T.O.W. : Thin ovrarian wall
V.M. : Vetelline membrane
Y.G. : Yolk granules

Fig. 24 Photomicrograph of T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) showing the effect of 55 m mol l\(^{-1}\) of magnesium chloride (MgCl\(_2\)·6H\(_2\)O) solution during spawning period (July).

H & E 10 x 6

Abbreviations
F.E. : Follicular epithelium
N.M. : Nuclear membran
N : Nucleus
V.M. : Vetelline membrane
Y.G. : Yolk granules