(I) Seasonal changes in the pituitary gland of Heteropneustes fossilis (Bloch) in correlation with its reproductive cycle:

The cells of rostral pars distalis and pars intermedia do not seem to exhibit any marked change which may be correlated with the reproductive cycle.

Two types of basophils (Basophil I and Basophil II) and two types of acidophils (Acidophil I and Acidophil II) are distinguished in the proximal pars distalis. Out of these cells, only Basophil I show cytological seasonal variations.

The process of granulation and degranulation of Basophil I closely and interestingly follow the process of the maturation of gonads and spawning in this fish.

Acidophils have been identified as prolactin cells in the present study. Gradual fast transfer of Calcium during different periods of reproductive cycle results in their hypertrophy. It suggests and further confirm the role of these cells in Calcium regulation.

The calcium sensitive acidophils exhibit hypertrophy even after administering magnesium. However, the inhibitory effect of magnesium is much more pronounced on the basophils.

(II) Structural changes in response to increased environmental Calcium and Magnesium concentrations on the ultimobranchial gland of Heteropneustes fossilis (Bloch) during various phases of its reproductive cycle.

The ultimobranchial gland of fresh water fish, Heteropneustus fossilis was examined under the effect of increased environmental Calcium during various stages of reproductive cycle. In
highest Calcium concentration i.e. 65.0 m mol l\(^{-1}\) and 62.5 m mol l\(^{-1}\) (during spawning period), the large size follicles were clearly evident containing vacuolated cytoplasm and larger nuclei than control. The study suggests that in Calcium enriched environment, the ultimobranchial gland is highly active.

In Magnesium enriched environment i.e., at 80.0 m mol l\(^{-1}\) and 55.0 m mol l\(^{-1}\) (during spawning period) there is an increase in follicular size. The vacuolated cytoplasm and moderately larger nuclei compared to control were also evident.

The present study confirms an important hypocalcemic role of ultimobranchials in hypercalcemic environment and seemly hypomagnesesemic role in hypermagnesesemic concentration under lab conditions.

**(III) Seasonal variations in the ultimobranchial gland of Heteropneustes fossilis (Bloch)**

Seasonal changes in the ultimobranchial gland of *Heteropneustes fossilis* have been studied during pre-spawning (January to April), spawning (May to August) and post-spawning period (Sep-December). The activity of ultimobranchial gland is at its peak during late pre-spawning and spawning period which is evident by the hypertropy of follicular cells. Nuclei are large with granular cytoplasm. During post-spawning phase (September to December) the follicular cells of the gland get markedly reduced in size with small and rounded nuclei.
(IV) **Structural changes in response to increased environmental Calcium and Magnesium concentrations in the gills of Heteropneustes fossilis (Bloch) during various phases of reproductive cycle.**

Effect of various Calcium concentrations in the gills of fish *Heteropneustes fossilis* have been studied. The normal gill surface area was observed during highest Calcium concentrations. Presence of prominent acidophilic cells during the exposure to highest Calcium concentrations i.e., 65.0 and 62.5 m mol l\(^{-1}\) was naturally expected and noted. Interestingly in Magnesium exposure (55.0 m mol l\(^{-1}\) and 80.0 m mol l\(^{-1}\)), it was noted that epithelial cells and acidophilic cells were absent, showing acute stress during highest tolerance limit of this ion. Contrary to this experiment in calcium enriched exposure, the acidophilic cells were highly active during various phases of reproductive cycle, pointing optimum ionic transport during such treatment.

(V) **Seasonal variations in the gill of Heteropneustes fossilis (Bloch)**

Seasonal changes in the gill of fish, *Heteropneustes fossilis* (Bloch) have been studied during post-spawning, pre-spawning and spawning period. Straight primary gill and secondary lamellae were observed during the post-spawning, pre-spawning and spawning period. Well developed pilaster cells are also present and highly developed mucus cells are noted during pre-spawning and spawning period when compared to the post-spawning. Rich blood supply was also observed during all three phases of reproductive cycle.
(VI) **Structural changes in the gonads with exposure to Calcium and Magnesium concentrations in the Ovary of Heteropneustes fossilis (Bloch) during various stages of reproductive cycle.**

During the post-spawning period (September to December) the ovary shows early stages of oogeneis viz., chromatin-nucleolus stages, peri-nucleolus while yolk vesicle stage observed in large number. During pre-spawning period (January to April) further growth and development of the oocyte take place while the yolk stages are also observed in abundance. By the end of this period i.e., pre-maturation and maturation stages are seen in the oocytes alongwith a few corpora atretica. During spawning period (May to August), particularly in June and July, most of the ovas are in a mature stage amongst which are also found some early stages of oocyte as well as few corpora atretica. During July month, the post-ovulatory follicles are observed indicating the optimum condition in spawning.

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 during post-spawning period in comparison to control, 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 shrunked comparably. During spawning, the post ovulatory follicles are also found prominently shrunked.
The observations with Alizarin red S, which is specific stain for Calcium deposition, for the experimental and control group during its reproductive phase 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 of mature oocyte 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.

With Magnesium exposure in 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 from each other, are seen. The size of yolk granules has considerably increased in the oocyte. Shrinked nucleus with totally ruptured nuclear membrane were also observed during spawning than control group. Corpora atretica become also shrinked both during pre-spawning and spawning period.

(VII) Effect of Calcium concentrations in Serum Calcium, Sodium, Potassium and total Protein value.

The serum, Calcium, Sodium, Potassium and total Protein value were measured by (Shimadzu, Double beam UV-190, Japan)
Spectrophotometer. The Calcium, Protein and Potassium value are high during spawning period pointing more demand of Calcium which is necessary for the functioning of hardening enzyme thereby also help in hardening of eggs while protein meets extra energy demand during this period. An increase in sodium level was also observed during post-spawning compared to pre-spawning and spawning period.