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Effect of highest Calcium concentrations in the gills of Heteropneustes fossilis (Bloch) during different phases of reproductive cycle.

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Abstract :

Effect of highest Calcium concentrations in the gills of fish *Heteropneustes fossilis* have been studied. The gill surface area was observed during highest Calcium concentrations. Presence of prominent chloride cells during the exposure to highest Calcium concentrations i.e., 65.0 and 62.5 m mol l^{-1} was naturally expected and noted. In this experiment, in calcium enriched exposure, the Hyperplasia of secondary lamellae, hyperplasia of chloride cells and active mucous cell during various phases of reproductive cycle. pointing optimum ionic transport during such treatment.
Keywords : Fish, Gill, Calcium , chloride cells, mucous cell.

INTRODUCTION

The present study has been made to observe the effect of highest Calcium concentration changes in the structure of gill filament in fish *Heteropneustes fossilis* (Bloch) during different phases of reproductive cycle. The structure of gill filament in fish has been elaborately described by various authors. (Munshi & Singh 1992; Fernandes at al 1994; Evans et al., 2005; Moraes et al., 2005; Banerjee, 2007; Fernandes et al. 2007) The main cells that constitute the filament epithelium from the inner to the outer cess layer are non-differentiated, neuroepithelial, chloride, mucous and pavement cells. Several studies on the teleost gill epithelium have emphasized the pavement cells (PVCs) of the lamellar epithelium which are directly related to gas exchange and the chloride cells(CCs) which are related to the ion regulation as well as the changes of these cells in response to the internal and/or external ionic or acid-base environment(Munshi,1964; Moron et al., 2003). Mucous cells present in the gill filament epithelium and their secretion may be a mechanism for adaptation to different conditions of the aquatic environment. Changes in the density of the mucous cells of gills and skin(Paul & Banerjee, 1997). Banerjee, 2007; reported that Mucous cells are active cells present in the gills and they respond to environmental changes.

Ahmet R. Oguz(2015) has reported Histological changes in the gill epithelium of endemic Lake Van Fish *(Chalcalburnus tarichi)* during migration from alkaline water to freshwater.

Adinarayana P, et al.,(2017) have reported the Histopathological changes in the gills of fresh water fish *Channa striatus* (Bloch) infected with Epizootic Ulcerative Syndrome. Sandro Estevan Moron et al.,(2009) have observed response of Mucous cells of the gills of traira *(Hoplias malabaricus)* and jeju *(Hoplerythrinus unitaeniatus)* (Teleostei: Erythrinidae) to hypo-and hyper-osmotic ion stress.

Conklin et al.(1992) have studied the effect of chronic exposure to soft acidic water on gill developmental morphology, number, location, size of chloride cells and mucous cells in embryo of larval brook trout, *Salvelinus fontinalis.*

Dunel-Erb and Laurent (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

Evans (1974) 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 mucous cell distribution in the gill epithelium and also noted the rate of mucous production under normal environment. Handy and Eddy (1989) have pointed that mucous layer is evident on the primary lamellae and may have indirect effect on the branchial microenvironment because mucous is an ion exchange material which rapidly absorb H^+. They have further reported (1991) different mucous cell distribution on the gill epithelium and their function in different fish and also pointed the absence of mucous on secondary lamellae of unstressed rainbow trout, *Oncorhynchus mykiss* (Walbaum). It was also shown that mucous function in the branchial microenvironment of rainbow trout is limited to stress situations where mucocytes discharge is stimulated to form distinct mucous layer on the gill surface. This may not be the case in other fish species which have different mucous cell distribution on the gill epithelium and probably different mucous production rate under normal environmental condition.

Laurent and Dunel-Erb (1977) 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*. 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 Moloiy (1980) have shwon the organisation of gas exchange organs in *air breathing* catfish *Clarias* 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".

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. (1970) 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.

Olson et al. (1989) have pointed the location of angiotensin covering enzyme in gill tissue and determined whether pillar cells might also be the sites of angiotensin covering 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 Marlusa (1988) 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.

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

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

Zaugg (1981) has studied the photoperiod and temperature effects on gill Na^+/K^+ ATPase activity and migration in juvenile steel head *Salmo garidneri*.

Material and Method

The fish *Heteropneustes fossilis* (Bloch) were obtained from local Sagar lake, Sagar, M.P. Twenty four adult fishes were collected during the first week of every month for one complete reproductive cycle i.e.; for continuous 12 months.

The eyes as well as the surface bones of skull were removed and an incision was given in the abdomen so as to ensure efficient fixative.

During the experimental period the mature fishes ranging between 12 to 17 cm in length and 25-30 gm in weight were placed in tap water aquarium in laboratory conditions and treated with tetracycline to control bacteria and other out breaks in Post-spawning (December), Pre-spawning (April) and Spawning period (July). Healthy fishes were selected for the experimental work. Eight fishes were kept in each aquarium which contains 24 litre tap water i.e. 3 litre/fish. They were acclimatized for about a week before starting the experiment. During this period fishes were fed with dried shrimps. However, they were not fed throughout the experimental period and the water of each aquarium was renewed twice a week.

Experiment with different Calcium concentrations

The Calcium chloride ($CaCl_2.2H_2O$, E. Merck) was used throughout the experimental period. For the preparation of Calcium chloride solutions of different concentrations the dry powder was properly weighted and dissolved in tap water.

It was very surprising and interesting to note the highest calcium tolerance i.e. 65.0 m mol l^{-1} by this fish from the preliminary experiments with gradual fast adaptation during pre and post spawning and 62.5 m mol l^{-1} during spawning period.

Even in the euryhaline fish, Oreochromic mossambicus (Pathak, 2002) and also in other teleosts the maximum tolerance limit do not cress beyond 10 m mol l^{-1} exceeding which the animals show sign of stress thereby also start onset of mortality.

Gradually fast transfer in different Calcium concentrations during post-spawning, pre-spawning and spawning period

Sixteen fishes in total were used for this experiment i.e., eight fishes are used for experimental work and eights fishes are used for control group. Each aquarium contains equal amount of water i.e., 3 litres/fish. After setting experiments the observations were taken at regular interval noting the pH experimental, pH control, temperature of experimental group, temperature of control group. The environmental temperature as well as the mortality at each animal in all the aquariums were noted.

The experiments were set in following way as per the protocol of S.E. Wendelaar Bonga et al.(1983):

1.Gradually fast transfer in different Calcium concentrations during post-spawning period (December). 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} in calcium chloride $CaCl_2.2H_2O$, E. Merck) solution in fresh water (each step lasted for a day).In 65 m mol l^{-1} solution the animals could not survive for more than 5 to 6 hrs and the concentration is found lethal.

2.Gradually fast transfer in different Calcium concentrations during pre-spawning period (April). The whole set up was as described above.

3.Gradually fast transfer in different Calcium concentrations during spawning period (July). 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 62.5 m mol l^{-1} in calcium chloride $CaCl_2.2H_2O$, E. Merck) solution in fresh water (each step lasted for a day). In 62.5 m mol l^{-1} solution the animals could not survive for more than 5 to 6 hrs and the concentration is found lethal.

At the time of sacrifice the fish were killed by a single blow on the head and important cytological details of gills was dissected carefully and fixed immediately in proper fixative Hollande's modified Bouin and 70% alcohol. It was thoroughly washed, dehydrated and then embedded in paraffin wax (melting point 60-62^{0}C) suitable sectioning at 5-6 were made to prior to specific and suitable staining.

Stains used

Following stains in addition to normal stains i.e., Hematoxylin and Eosin, Mallory's triple and PAS were used for study gill cytology show the clear-cut differentiations of various cell types.

OBSERVATIONS

Gradual fast transfer in highest 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 $CaCl_2.2H_2O$ 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 ($CaCl_2.2H_2O$) 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 mucous 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. Chloride cell were absent while highly damaged blood vessels was also seen (Fig.4).

Post-spawning period (Control group-December)

Important cytological changes were observed in the gills. In control group (December) straight primary and slightly curved secondary gill lamellae were observed. A large number of mucous cells are seen on the tip of primary gill lamella. A well developed chain of pilaster cells were observed. Chloride cells are also seen with normal blood supply (Fig.1).

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

The experimental set up is same as described earlier. Straight primary and curved secondary gill lamella were observed. Mucous cells were scanty. A chain of pilaster cells which is reduced size was clearly observed. Well developed epithelial cells are seen. Well developed chloride cells can also be clearly observed. Highly enlarged blood vessels were observed but at few places they are in damaged condition (Fig.5).

Pre-spawning period (Control group – April)

In control group (April). Well developed mucous cells are present on the tip of the primary gill lamella. Epithelial cells are seen. Chloride cells are also clearly observed. Very prominent blood supply was also observed (Fig.2)

Gradual fast transfer in highest 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 ($CaCl_2.2H_2O$) 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 mucous cells are present on the tip of the primary gill lamella, when compared to the pre-spawning, the size of the mucous cell was considerably large. Well developed chain of pilaster cell and prominent epithelial cells were also observed. Blood capillaries are in shrinked and damaged condition (Fig.6).

Spawning period (Control group – July)

During the control group (July) primary and secondary gill lamellae were found straight. A large number of mucous 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. Chloride cells are also clearly seen. Normal blood supply was seen (Fig.3).

DISCUSSION

The surface area of the gill was normal in highest Calcium concentration during pre-spawning and spawning and post-spawning period. During maximum Calcium exposure the primary and secondary gill lamellae are found slightly curved during pre-spawning, spawning and post-spawning period.

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 report the surface area and structure of primary and secondary lamellae.

Sala and Marlasca (1986) described the specialized epithelia of juvenile turbot *Scopthalmus 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). Same result obtain in fish *Heteropneustes fossilis* highest exposure of calcium during various phases of reproductive cycle. (Fernandes and Perna Martines, 2002) reported that Hypostomus plecostomus adapted to water with high ion concentration (NaCl and Ca2+) had a significant increase in the density of the mucous while their exposure to distilled water resulted in hypoplasia and hypotrophy of these cells.

At maximum Calcium concentration the mucous cells are in very much active condition and epithelium cell are showing hypertrophy during all the three phases of reproductive cycle. Banergee, 2007 was study the mucous cell are active cell present in the gills and there are evidences that they respond to environmental changes. Moron at.al. 2009. Study to quantify the mucous cell and identify mucousbstances (glycoproteins) present in these cell in gills in the two ecologically distinct erythrinid species, H. Malabaricus and H. Unitaeniatus as well as their responses to ion challenge consisted of exposure to absence and high concentration of Na^{+}, Cl- and Ca^{2+} in the freshwater environment.

Same results were also obtained by Shukla (1993) with gradual slow and direct transfer experiments in different salinity concentrations where the reduction of mucous 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 the gill arches.

In our experiments with at highest Calcium concentration similar results were observed during post-spawning, pre-spawning and spawning period, Hypertropheid chloride cells are observed. Copeland (1948) 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 at "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 mucous cells (Copeland, 1948). 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). Hypostomus plecostomus adapted to water with high ion concentration (NaCl and Ca2+) had a significant increase in the density of the Mucous while their exposure to distilled water resulted in hypoplasia and hypotrophy of these cells (Fernandes and Perna-martins, 2002). At maximum Calcium concentration well developed epithelial cells are observed in all the three phases of reproductive cycle.

It can be concluded that enriched calcium exposure, the Hyperplasia of secondary lamellae, hyperplasia of chloride cells and active mucous cell during various phases of reproductive cycle, pointing optimum ionic transport during such treatment.

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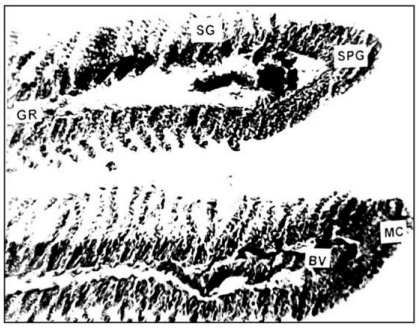


Fig. 1 Section of the gill filament of *Heteropneustes fossilis* (Bloch)during post-spawning period (Decemaber) in control group showing straight primary and slightly curved secondary gill filament.

Mallory's triple 150x

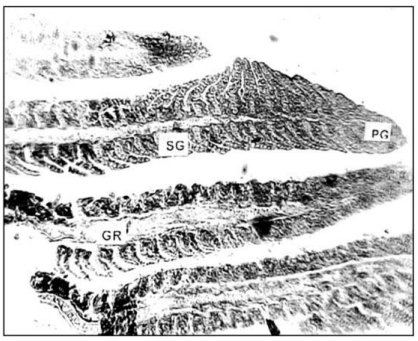


Fig. 2 Section of the gill filament of *Heteropneustes fossilis* (Bloch)during pre spawning period (April) in control group showing mucus cells on the tip of the primary gill filament .Epithelial and chloride cells are observed.

H & E 150x

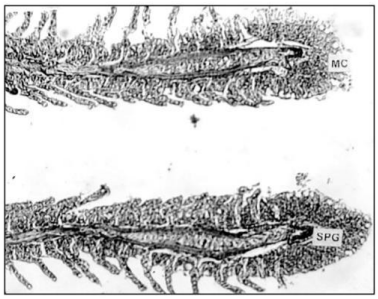


Fig. 3 Section of the gill filament of *Heteropneustes fossilis* (Bloch)during spawning period (July) in control group showing highly developed mucus cells.

H & E 150x

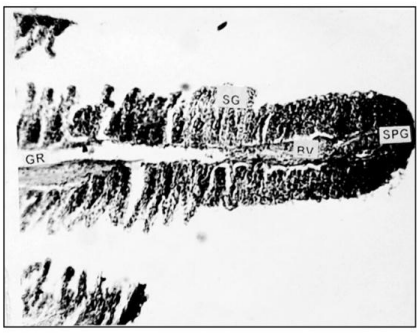


Fig. 4 Section of the gill filament of *Heteropneustes fossilis* (Bloch)during post-spawning period (Decemaber) exposed to in 65 m mol l^{-1} of Calcium chloride (CaCl2.2H2O) solution showing of and higly damage blood vessel Hypertrophid chloride cell.

H & E 150x

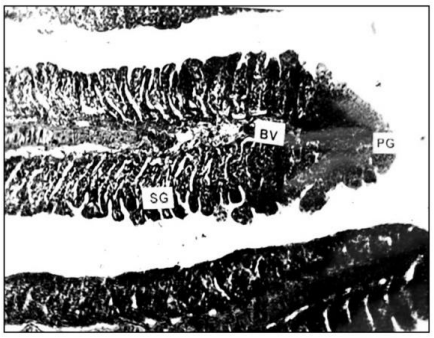


Fig. 5 Section of the gill filament of *Heteropneustes fossilis* (Bloch)during pre-spawning period (April) exposed to in 65 m mol l of Calcium chloride (CaCl2.2H2O) solution showing highly enlarged blood vessel with well developed epithelial cells and Hyperplasia of secondary lamelle

Mallory's triple 150x

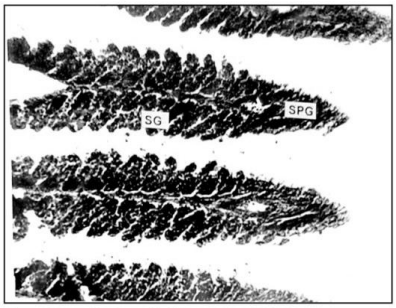
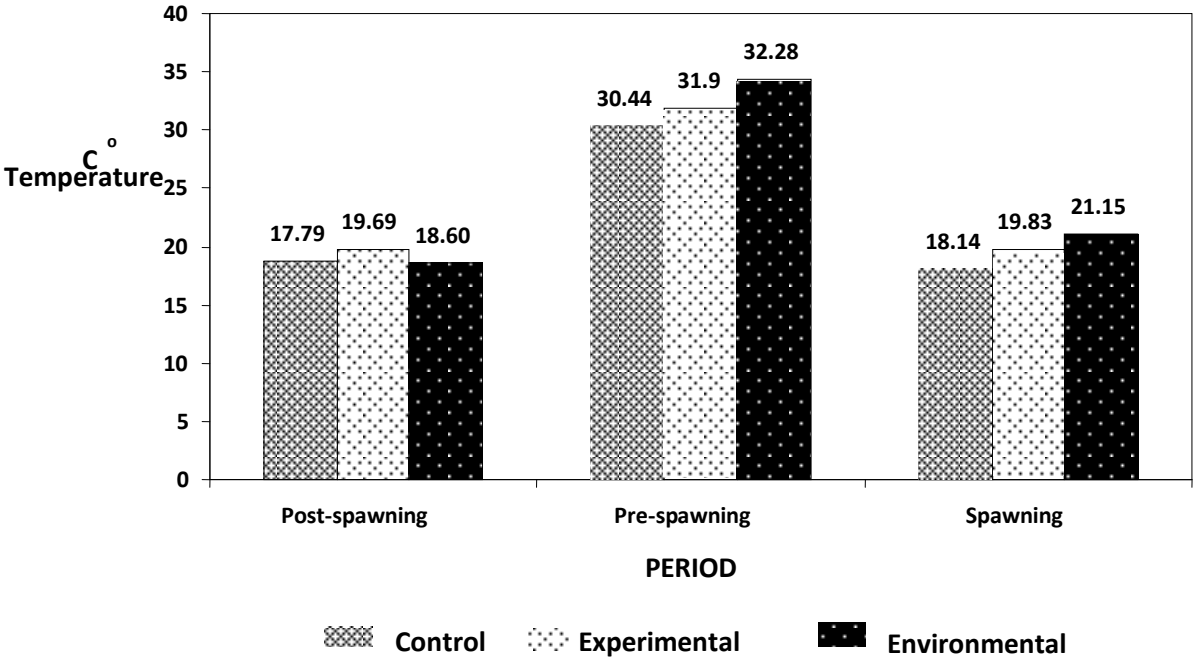


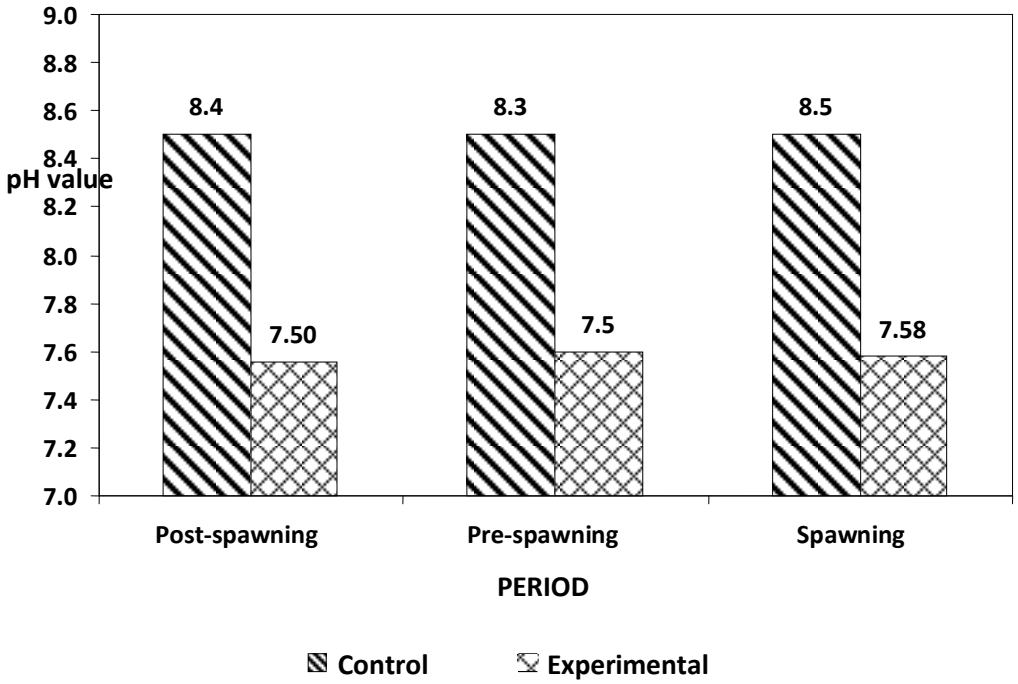
Fig. 6 Section of the gill filament of *Heteropneustes fossilis* (Bloch)during spawning period (July) exposed in 62.5 m mol l-1 of Calcium chloride (CaCl2.2H2O) Solution showing straight primary and Hypertrophy secondary gill filament

H & E 150x

PG. primary gill lamellae PC. Pilaster cell SSG. Straight secondary gill GR. gill ray SG. Scecondry gill CC. Chloride cell BV. Blood vessole MC. Mucus cell SPG. Straight primary gill



Graph: 1 showing the variation in temperature during different phases of reproductive cycle



Graph: 2 showing the variation in pH of during Different phases of reproductive cycle

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