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

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Abstract :
Effect of highest magnesium concentrations in the gills of fish *Heteropneustes fossilis* have been studied. Interestingly in Magnesium exposure (55.0 m mol l^{-1} and 80.0 m mol l^{-1}), it was noted that prominent shrinked mucous cell, highly curved primary, secondary gill filament, highly dilated blood vessel. Epithelial cells and chloride cells were absent, showing acute stress during highest tolerance limit of this ion. during various phases of reproductive cycle.
Keywords : Fish, Gill, Magnesium, chloride cells, epithelial cells mucous cell.

INTRODUCTION

The present study has been made to observe the effect of highest Magnesium concentration, 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 authors 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. (Munshi & Singh 1992; Fernandes at al 1994; Evans et al., 2005; Banerjee, 2007; Fernandes et al. 2007) The main cells that constitute the filament epithelium from the inner to the outer cell 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,1960, 1964; Moron et al., 2003) have reported 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. Banerjee, 2007; reported that Mucous cells are active cells present in the gills and they respond to environmental changes.

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.*
Evans (1974) reported ionic exchange mechanism in fish gill. Franklin and 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 three 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 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".

McDonald at al, (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.

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.

K.Singh and O.P. Gupta (2009), have reported pH experimental, pH control temperature of experimental group, temperature of control group in *Heteropneustes fossilis (Bloch)*

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 one complete reproductive cycle.

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.

With the treatment in Magnesium concentration, the things are exactly same. The previous workers (Bonga et al., 1983) have mentioned that the tolerance limit of Magnesium dose not exceed beyond 55.0 m mol l^{-1} which was also noted during spawning period in *Heteropneustes fossilis* (Bloch). Interestingly this limit exceeded to large extent i.e., 80 m mol l^{-1} during pre and post-spawning phase.

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 Megnasium concentrations during post-spawning period (December). 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 80 m mol l^{-1} (each step lasted for a day). In 80 m mol l^{-1} magnesium chloride ($MgCl_2.6H_2O$, E. Merck) solution the animal could not survive for more than 7 to 8 hours and is found lethal.
2. Gradually fast transfer in different Megnasium concentrations during pre-spawning period (April). The whole set up was as described above.
3. Gradually fast transfer in different Megnasium concentrations during spawning period (July). 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 ($MgCl_2.6H_2O$, E. Merck) solution the animal could not survive for more than 7 to 8 hours and is interestingly found lethal at this phase of reproductive cycle.

At the time of sacrifice the fish were killed by a single blow on the head and important cytological details of gill 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 gills showing differentiations of various cell types.

OBSERVATIONS

Gradual fast transfer in highest magnesium concentrations of experimental group during post-spawning period (December)

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 ($MgCl_2.6H_2O$) 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. Mucous 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.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. Acidophilic cells are also seen with normal blood supply (Fig.1).

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

The experimental set up is same as described above. Highly shrinked primary and curved secondary gill lamellae were noted. Prominently shrinked mucous cells were observed on the tip of the primary gill lamella. Tip of the secondary gill were in swollen condition. Highly shrinked chain of a pilaster cells and ruptured epithelial cells were also observed. Highly shrinked blood supply was also seen (Fig.5).

Pre-spawning period (Control group – April)

Straight primary and curved secondary gill lamellae were observed in control group (April). Well developed mucous 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.2)

Gradual fast transfer in highest Magnesium concentrations of experimental group during spawning period(July)

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 ($MgCl_2.6H_2O$) 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 shrinked primary gill lamella and prominently curved secondary gill lamella were observed. But a smaller number mucous cells than the control were observed in the tip of the primary gill lamella. Ruptured pilaster cells were observed and the blood vessels were in highly 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. Acidophilic cells are also clearly seen. Normal blood supply was seen (Fig.3).

DISCUSSION

In highest Magnesium concentration the surface area of gill was decreased during variouse phases of reproductive cycle, in fish *Heteropneustes fossilis*. Both primary and secondary gill lamella are not only highly curved but 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. Adinarayana et. al (2017) have reported the structural alterations such as changes in shape, epithelial proliferation; lamellar fusion and necrosis. 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, 1985). Ahmet R. OGUZ (2015) has reported acclimatd to freshwater, a small number of mucous cell was identified in the secondary lamella. Epithelial lifting was observed in both types of aquatic environments. Gill anomalies were mostly observed in freshwater sample. Hyperplasia, lamellar fusion, vasodilation, and necrosis were observed in the gills of most fish that migrated to fishwater the primary lamella, increase in mucous cell number in the gills may be functional in fish osmoregulation. There was no hyperplasia during the maximum Magnesium concentration the mucous cells are very much shrinked condition during pre-spawning. However smaller mucous cells were observed during spawning and post-spawning period. (Banerjee, 2007) has reported the mucous cell are present in the gills and there are evidences that they respond to environmental changes. 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. In maximum Magnesium concentration chloride cells are not 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). Pillaster cells is in enhanced condition at highest magnesium concentration during all the three phases of reproductive cycle.

It can be concluded that in highest Magnesium concentration, prominent shrinked mucous cell, highly curved primary and secondary gill filament, highly dilated blood vessel. Epithelial cells and chloride cells were absent in gills, showing acute stress during highest tolerance limit of this ion. during various phases of its reproductive cycle.

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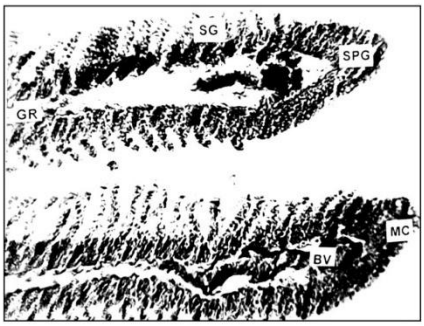


Fig. 1 Section of the gill filament of *Heteropneustes fossilis* (Bloch)during post-spawning period (Decemaber) in control group showing straight primary and slightly curvedsecondary gill filament.

Mallory's triple 150x

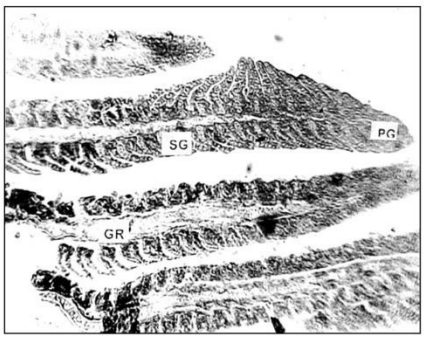


Fig. 2 Section of the gill filament of *Heteropneustes fossilis* (Bloch)during pre spawning period (April) in control group showing mucus cells on the tip of the primary gill filament .Epithelial and chloride cells are observed.

H & E 150x

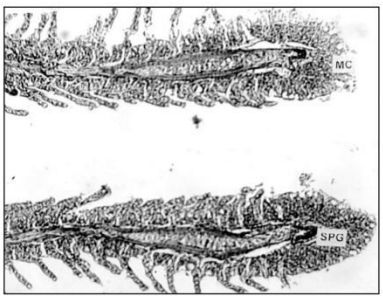


Fig. 3 Section of the dill filament of *Heteropneustes fossilis* (Bloch)during post spawning period (July) in control group showing highly developed mucus cells and pilaster cell.

H & E 150x

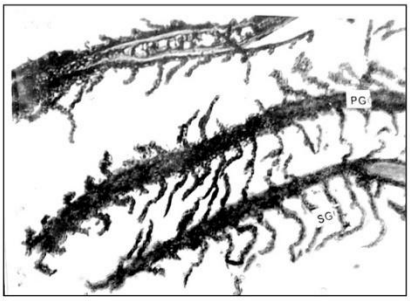


Fig. 4 Section of the gill filament of *Heteropneustes fossilis* (Bloch)during post spawning period (December) exposed in 80 m mol l-1 of Magnesium chloride (Mgcl2.6H2O) solution showing highly curved primary and secondary gill filament and highly dilated blood vessel.

H & E 150x

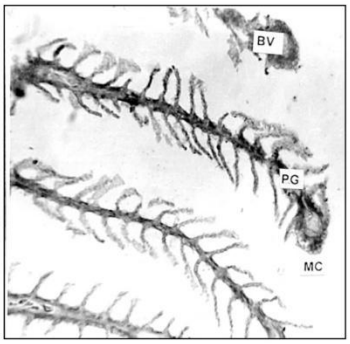


Fig. 5 Section of the gill filament of *Heteropneustes fossilis* (Bloch)during pre spawning period (April) exposed in 80 m mol l-1 of Magnesium chloride (Mgcl2.6H2O) solution showing prominent shrinked mucus cells and highly shrinked blood suplly

H & E 150x

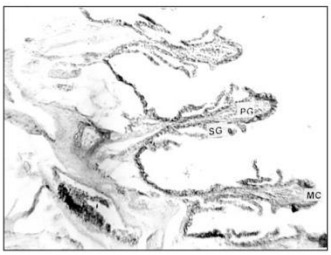
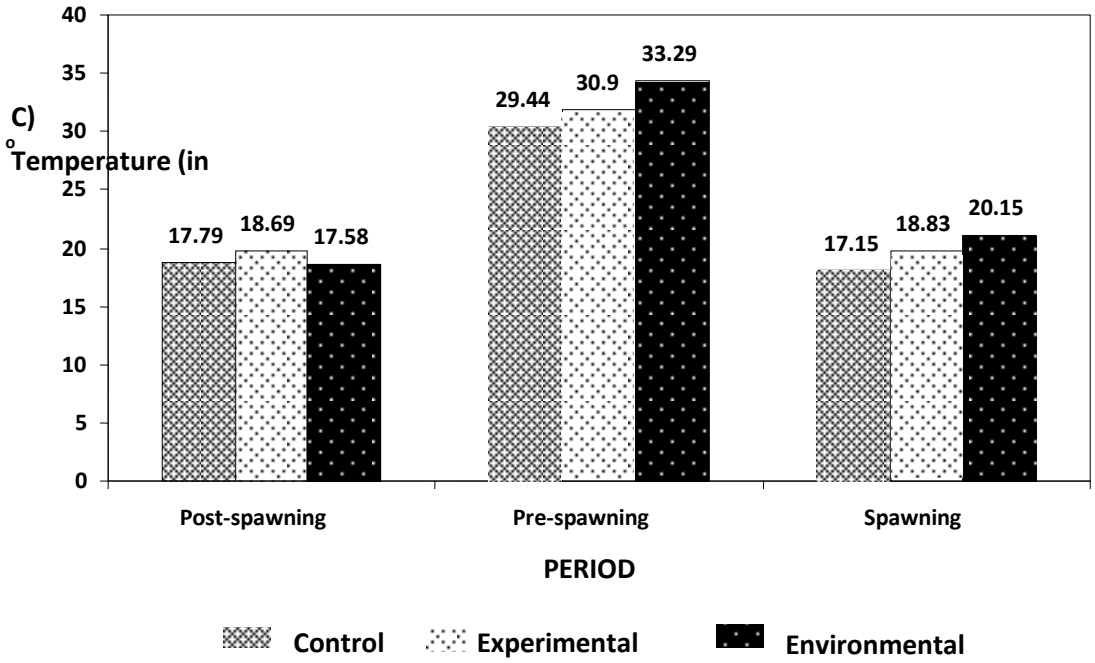


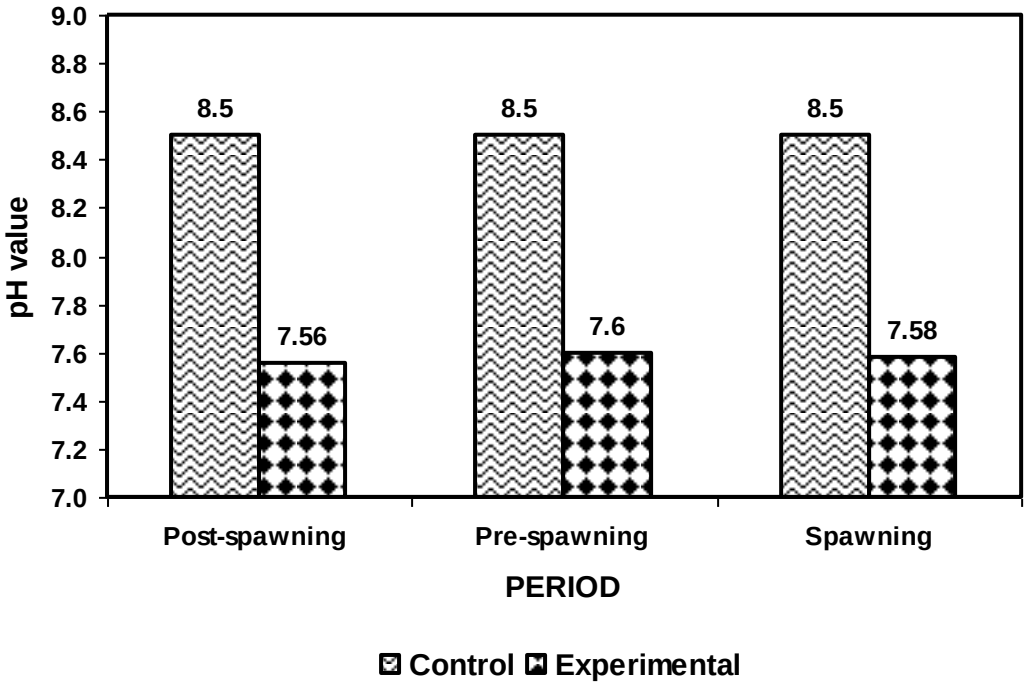
Fig. 6 Section of the gill filament of *Heteropneustes fossilis* (Bloch)during spawning period (July) exposed in 55 m mol l-1 of Magnesium chloride (Mgcl2.6H2O) solution showing prominently curved secondary gill filament and smaller mucus cells.

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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