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Seasonal changes in the gills of Heteropneustes fossilis (Bloch) during various phases of reproductive cycle.

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Abstract :
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, chloride cells and Epithelial cell 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. During spawning period from May to July due to environmental stress of high temperature cells under consideration become highly active and enlarged.
Keywords : Fish, Gill, Pilaster cell, Mucus cell, Chloride cell, Epithelial cell

INTRODUCTION

The present study has been made to observe the seasonal 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 (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 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; Sakuragui 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.
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. 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. 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 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.

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.* Maina and Moloiy (1980) have shwon the organisation of gas exchange organs in *air breathing* catfish *Clarias* by light, electron and scanning microscope study. 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.

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 (1968, 1972) 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.K.Singh et.al., (2017) have reported morphological effect of highest calcium concentration in *Heteropneustes fossilis* (Bloch) during different periods of reproductive cyclePlayle 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 epitheliam, 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.

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.

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 gills Histology to show the clear-cut differentiations of various cell types.

OBSERVATIONS

Seasonal Changes in 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. Chloride 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. Chloride 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. Chloride cells are also seen with normal blood supply (Fig.3 and 4).

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

In the month of January and February straight primary and secondary lamellae were observed. 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 Chloride cells were clearly visible. Prominent blood supply was also observed (Fig.5). 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. Chloride cells are also clearly observed. Very prominent blood supply was also observed (Fig.6)

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 Chloride cells are observed and a very prominent blood supply is clearly seen (Fig.7, 8 and 9). 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. Chloride cells are prominent. Normal blood supply were observed (Fig.10). 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. Chloride cells are also clearly seen. Normal blood supply was seen (Fig.11).

DISCUSSION

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.

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.

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 mucus 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). (Munshi & Singh 1992; Fernandes at al 1994; Evans et al., 2005; Moraes et al., 2005; Banerjee, 2007; Fernandes et al. 2007) The higher mucus cell density in the gills of the air-breathing erythrinid, *H. unitaeniatus* than those in the water-breathing, *H. malabaricus*, may be related to mode of respiration and a possible protection against desiccation when fish is out of water Parashar & Banerjee, 1999b, 1999c). *H. unitaeniatus* is known for its moving between ponds (Saul, 1975) during dry season. This hypothesis is supported by the presence of mucus cell even in its lamellar epithelium which rarely occurs in water-breathing species but is reported in other air-breathing fish such as *Clarias batrachus* (L.) (Olson,1996) and *Channa striata*s (Bloch) (Chandra & Banerjee, 2003,2004) and the knowledge that mucus has low permeability to water (Shepherd,1989).

Similar results are observing in this fish during pre-spawning and spawning period.

Through the observation we can conclude that in the month from may to july, the spawning period fish is under environmental stress of high temperature. The study revealed that in the same period, mucus cell, Epithelial cells, Chloride cells and pilaster cells functions more rapidly and thus become highly active and enlarged.

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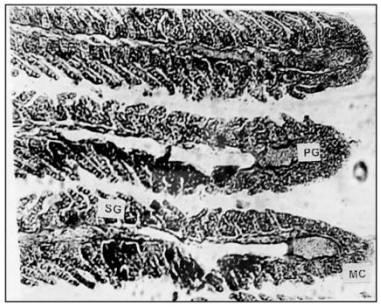


Fig. 7 Section of the gill filament of Heteropneustes fossilis (Bloch)during spawning period (May) showing straight primary and secondary gill filament.

H & E 150x

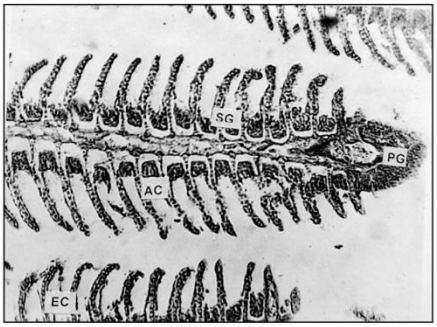


Fig. 10 Section of the dill filament of Heteropneustes fossilis (Bloch)during spawning period (June) showing well developed epithelial,chloride and pillaster cells.

H&E 150x

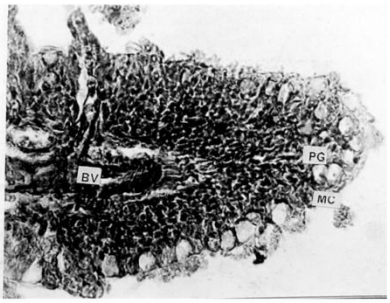


Fig. 8 Section of the dill filament of Heteropneustes fossilis (Bloch)during spawning period (May) showing well developed mucus cells.

H&E 450x

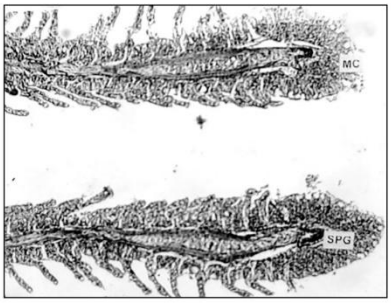


Fig. 11 Section of the dill filament of Heteropneustes fossilis (Bloch)during spawning period (July) showing highly developed mucus cells, epitheal cell and chloride cell.

H & E 150x

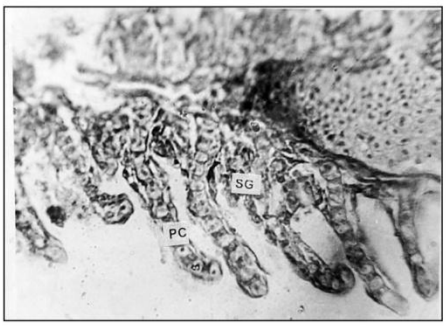
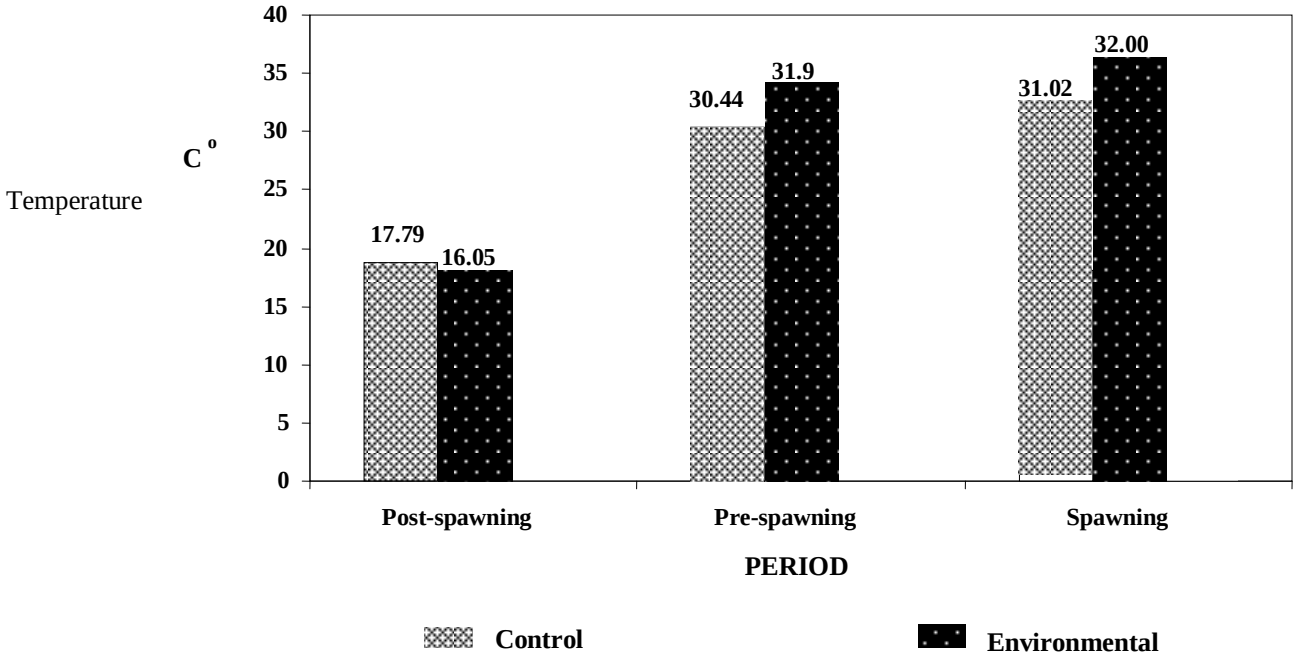
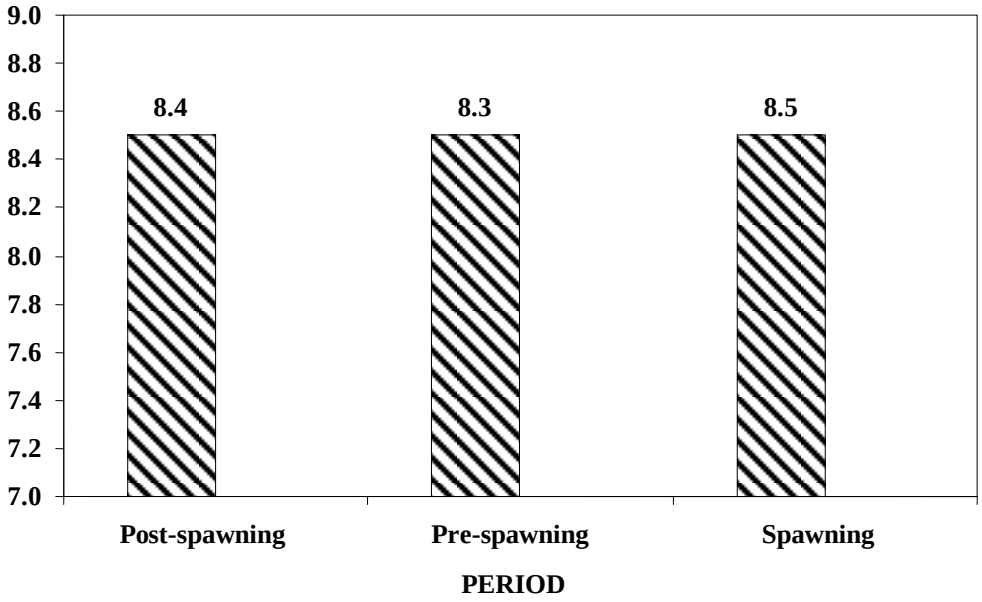


Fig. 9 Section of the gill filament of Heteropneustes fossilis (Bloch)during spawning period (May) showing well developed pilaster cell.

H&E 450x



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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