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Full Length Research Paper

Histological study of the gill (gill filaments and gill rakers) in post flexion to finger ling stage of *schizothorax plagiostomus* (Heckel)

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Study of fish gills are complex and are suited for gaseous and ionic exchange in extreme conditions of their habitat. The present paper deals with the histological study of gill filaments and gill rakers from post flexion to fingerling stages of snow trout *schizothorax plagiostomus*. For collecting the post flexon to finger stage larvae induced breeding were conducted on the bank of Alaknanda during the month of October-November, 2006 by stripping method. The number of gill filaments and gill rakers increases as the size of the *S. plagiostomus* larvae increases.The gill filaments are arranged in two rows on a gill arch, while the gill rakers are arranged in one or two rows on the gill arch. Morphologically the rakers are soft and long in each gill arch. The gill filaments are also soft and long. They are directly related to their feeding nature and food quality. In the present study of post-flexion to fingerling stage of *Schizothorax plagiostomus* larvae the gill apparatus, i.e. Gill arch, filaments, and rakers were easily distinguishable around the buccopharyngeal region. Some blood channels were also recognizable in this stage and pseudobranch arch or area was more developed or elongated and having close resemblance to the gill filaments. Blood channels with R.B.Cs. like substances as in gills were noticed in pseudobranch. Formation of secondary lamellae with marginal channels was apparent in the section of 1.0-2.0cm. larvae of *S. plagiostomus.* Also in this stage, the branchial arteries were discernible in each gill arch. Gill cover was elongated. A group of pillar cells were recognizable and within these groups of cells the blood channels were apparent among the 8.0 cm. larvae. Thus, the blood channels of secondary lamellae were lined by pillar cells (mesenchymal cells).

Keywords: *Schizothorax plagiostomus*, Histological, Post flexion stage, Fingerling Stage,

INTRODUCTION

In the aquatic animals including fishes, the gills are respiratory organs, which are very efficient in removing oxygen from water. There is only 1/20 amount of oxygen present in water as in the same volume of air. Gills greatly increase the surface area for gas exchange and they occur in a variety of animal groups including arthropods, terrestrial crustaceans, annelids, fish, and amphibians (Graham, 1997 and Evans, 1998). It is now highly accepted that the fish gill is multifunctional organ and fulfills several functions in fishes mainly dealing with respiration and osmoregulation (Smith, 1929).

Gills are typically comprised of gill rakers, gill arch, and gill filament .The gill rakers serve as one of the most important food processing devices in fishes (Kapoor, 1965 and Khanna and Mehrotra, 1970). According to Iwai (1963, 1964) and Kapoor (1965), the gill rakers in fishes taste, filter or prevent the escape of food material. Gill arch contains veins, and arteries that supply blood to the attached gill filaments. The arches are a rigid structure, which provide support and protection for the attached lamellae. There are usually two types of filaments, which are attached to it i.e. primary and secondary. The primary lamellae (or gill filaments) extend perpendicular from the gill arch. The filaments are located close together arranged in rows extending from both sides of the gill arches. With usually four gill

arches side by side per side of the fish (Graham, 1997) the filaments form a "Sieve through which the ventilator water must pass" (Evans, 1998). Each primary lamella has an efferent and afferent blood vessel, which supplies blood to the secondary lamellae. Secondary lamellae extend vertically from the primary lamellae (or filaments) and are placed closely together forming small channels for water flow. Each secondary lamella is made up of two sheets of epithelial cells with pillar cells that hold them apart. The total number of lamellae constitutes the total surface area of the gills available for gas transfer. The number of lamellae per animal is correlated with their size and activity, the larger and more active the animal, the more lamellae (Evans, 1998). Gills provide a one-way for oxygen to perfuse over them. This one way flow increases their efficiency since there is not much mixing of oxygenated and deoxygenated water directly over the gills and there is no "dead air space" such as the trachea in which oxygenated and deoxygenated water can be mixed.

The present study has been under taken to know the Morpho-Histological development of gills or respiratory system among the *Schizothorax plagiostomus* larvae from post flexion to fingerling stage. The result would be a useful reference material for further investigations related to respiratory system among the fishes inhabiting the snowfed rivers including many other closely related varieties of fresh water as well as snow fed fishes. *Schizothorax, species* are the only fishes, which may be, taken for commercial production in the cold-water area of many hill places where the water bodies are going to be used for hydroelectric power projects viz. Himanchal Pradesh, Uttarakhand, Nepal, Sikkim, and Arunachal Pradesh etc. In these places, the snow trout fishes are only indigenous fishes, which can be produced for commercial purpose if the reservoirs are going to be developed.

Considerable work on the morphology and histology of gills in different adult fishes including snow trout fishes in India as well as abroad has been conducted by many workers i.e. Bejtel (1949); Steen and Kruysse (1964); Hughes and Grimstone (1965); Hughes (1966,1973,1984); Munshi and Singh (1968); Kempton (1969); Ahuja (1970); Bettex-Galland and Hughes (1973); Hughes and Weibel (1973); Fromm (1974); Morgan and Twell (1973); Munshi(1976); Laurent and Dunel (1976); Hughes (1979); Kendall and Dale (1979); Kimura and Kudo(1979); Boyd et.al. (1980); Munshi et.al.(1980); Hughes and Mittal (1980); Chilmonczyk and Monge (1980); Cooke (1980); Cooke and Campbell (1980); Laurent (1984); Rooj (1993); Parihar and Dubey (1995); Ferrandes and Perna (1995); etc. excepting the work of Hughes et.al. (1986); Fiky-el-Nabil et.al.(1987) and Rombough (2004) etc. But no information is available related to the development of the gills from post flexion to fingerling larvae stage in hill stream snow trout fishes. Here the trout fishes are highly available and have good value in respect to commercial production as in uttrakhand more than 42 reservoirs are going to be developed for hydroelectric power projects (Singh et.al. 1993).

Hence, an effort has been made for the first time to work out on this aspect on Himalayan Snow trout *S.plagiostomus* (Heckel) fish larvae.

MATERIAL AND METHODS

Live brooders of *Schizothorax plagiostomus* (Heckel) were collected from the Glacierfed River Alaknanda located at an altitude of 540 m, longitude 78^0 47' 26'' and latitude of 30^0 13'16'', 38.8 Km. near the Chauras-Jhulapul, Srikot, Srinagar (Garhwal) during September-October 2006 by using "cast gill net". The mature brooders were stripped for taken out the egg and melt by applying the slight pressure on the abdomen. The eggs and milt were mixed with the help of birds feathers in a cleaned enameled tray for 5-10 minutes (Bahuguna, 2000, 2006). After fertilization, eggs were plac in the hatching trays and kept in the laboratory with proper airation and controled airation. From post flexion to finger ling stages with 4-8 hrs intervals 5-10 larvae were fix in different fixatives viz. 4% formalin, 70% alcohol, calcium formal, aqueous bouins, alcoholic bouins etc. as given by Taylor (1967), Pearse (1975), Dingerkus and Uhler (1977) and Kaji, et.al. (1996) with some modifications were applied according to the local condition. After completion of fixation (18-24 hrs), the larvae were washed 2-3 times in distilled water than alcohol, till the excess of fixative comes out from the tissue. The tissue were saved in 70% alcohol till wax blocks of the different larvae of different stages were prepared by dehydration in graded alcohol (30% - 100%) and clearing by xylene than imbeded in paraffin wax (E Merck Histo Paraffin wax, 54-56^0C melting point). The wax blocks were cuts in transverselly and longitudinally serially section of 5-6µ thickness by using Erma rotary microtome (Japan).

Mounting of sections were carried by using thin layer of tissue and Mayer's egg albumin as adhesive. The serially arranged tissue sections were put on the slides and kept on the hot plate than few drops of distilled water were used for spreading the sections on the slides. After drying (40-45^0C), the spread slides were used for staining. Before staining, the waxed slides were run through xylene for dewaxing of the slides (5-10 minutes). For the staining of the slides methods followed as given by the Gray (1964), Taylor (1967), Pearse (1975), and Kaji, et.al. (1996) etc. using Ehrlich's Acid Alum haematoxylin and eosin stain (Double staining), Mallory triple staining (Triple staining), Heidenhain's Iron haematoxylin, Mercuric bromophenol blue and PAS reagent.

After proper dehydration and clearing of stained sections, they were covered with cover slip by using DPX as a mounting medium and than photomicrographs

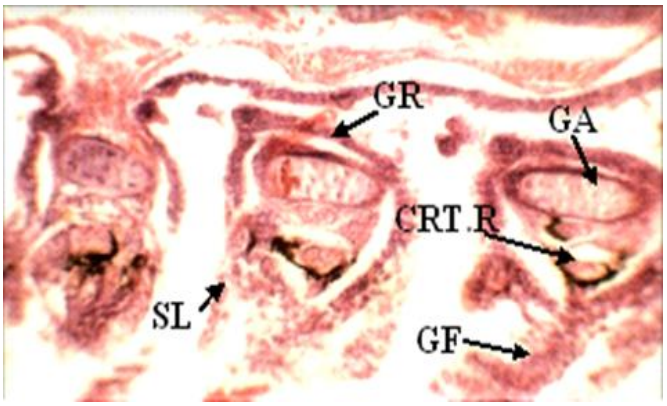

Figure 1 L.S. of 1.0 cm.larvae showing gill, gill raker with gill arch and gill filaments (400x Haematoxylin Eosin stain).

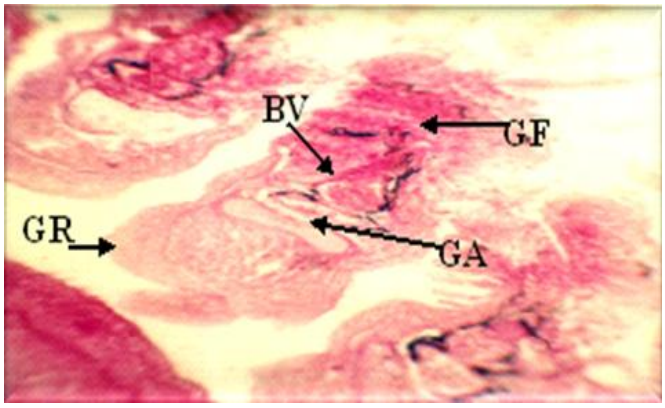

Figure 2: L.S. of 2.0 cm. larvae showing gill arch, gill filament, gill raker and blood vessels. (400x Eosin stain).

of some prepared slides were taken with the help of Olympus- photomicroscope (PM-6 and PM -10).

OBSERVATION

Fish gills are complex and are suited for gaseous and ionic exchange in extreme conditions of their habitat. The limnological characteristics of the habitat are responsible for various modifications in functional organization of fish gills (Hughes; 1984). In *Schizothorax plagiostomus* the gills consist of a longer lower limb, supported by ceratobranchial and a shorter upper limb supported by the epibranchial. Each gill arch in the larvae of *S. plagiostomus* bears gill rakers towards the inner (buccal) side and very thin long gill filaments towards the outer (opercular) side. The gills are so arranged that they separate the buccal cavity from the opercular cavity. The gill rakers are present in one or two rows towards the buccal side. They are soft, thin thread like structure. Morphoquantitative variations have been recorded in the larvae of *S.plagiostomus*.

In the present study on post-flexion to fingerling stage of *S.plagiostomus* larvae the gill apparatus i.e. Gill arch, filaments, and rakers were easily distinguishable around the buccopharyngeal region of larvae having 1.0 - 2.0cm. length (Figure 1). Later on as the size of the larvae increases this organ becomes prominent and developed. Some blood channels were also recognizable at this stage, pseudobranch was more developed or elongated and having close resemblance to the gill filaments. Blood channels with R.B.Cs. like substances as in gills were also noticed in pseudobranch (Figure 1). Formation of secondary lamellae with marginal channels was apparent in the section of 1.0-2.0cm. larvae of *S. plagiostomus.* Also in this stage, the branchial arteries were discernible in each gill arch. Gill cover was elongated. Cartilaginous rods in gill arch and cartilage in branchiostegal membrane start to form. Gill rakers are present towards the buccal sides, which were covered by an epithelial layer (Figure 2). When the larval size increases from 3.0-4.0 cm morphologically the gill filaments become elongated. The secondary lamellae and branchial arteries were more complecated as well

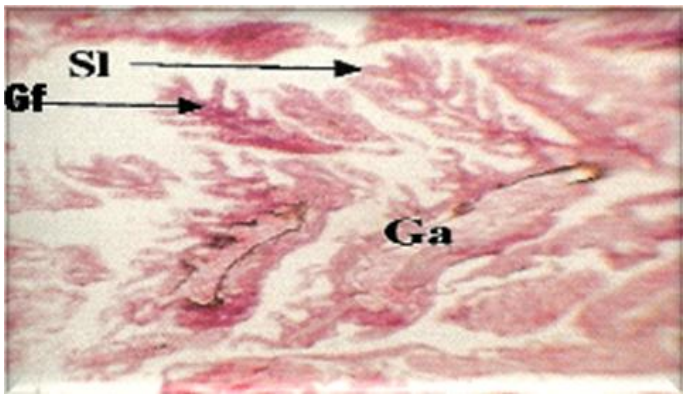

Figure 3: L.S. of 3.0 cm. larvae through branchial region showing gill filament with well developed secondary lamellae (100x Haematoxylinstain).

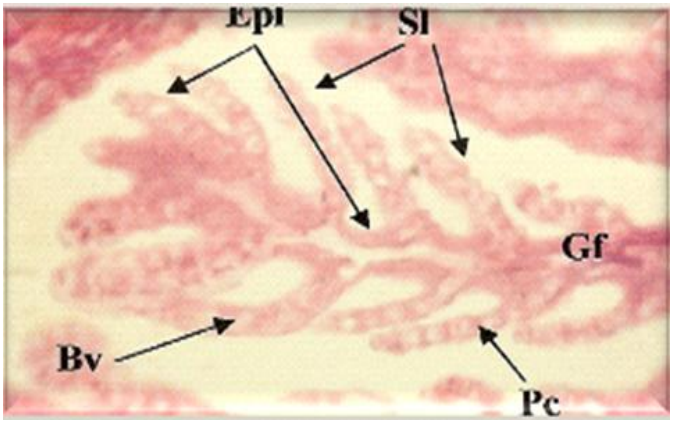

Figure 4: L.S. of 4.0 cm. larvae through branchial region showing well organized gill structures with lamellar blood vessels surrounded by pillar cells (280x Haematoxylin stain).

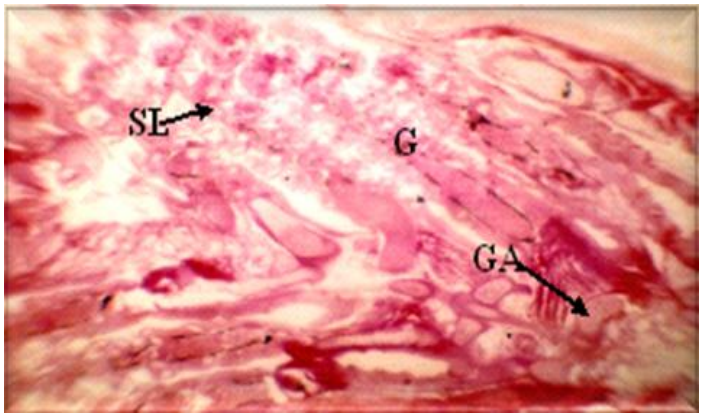

Figure 5: L.S. of 7.0 cm. larvae showing gill with secondary lamellae. (400x Eosin stain).

as developed (Figure 3 and 4). The blood cells (R.B.Cs.) were clearly visible along with lymphocytes (granulocyte and agranulocyte). Branchiostegal membrane becomes cartilagenous and pseudobranch was more developed. When the larvae of *S. plagiostomus* becomes 5.0-8.0 cm long, the gill histology was well developed (Figure 5). In this stage, the gill arch is differentiated in to base, middle and upper zones. The middle zone has well-developed taste buds. These taste buds are pear shaped and its opening is at its apical part. (Figure 6). A group of pillar cells was recognizable and within these groups of cells the blood channels were apparent on 8.0 cm. larvae.

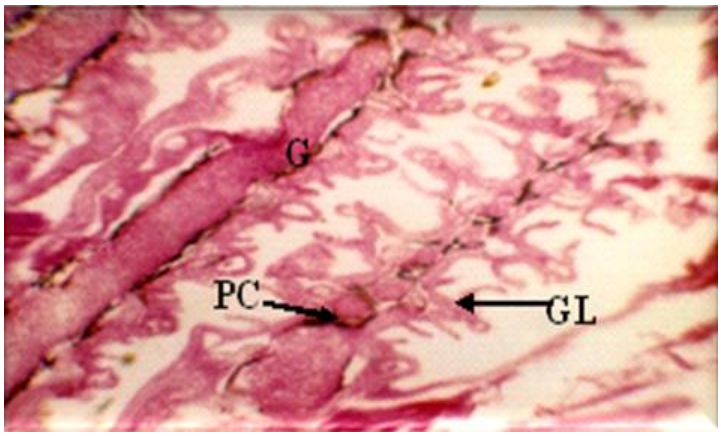

Figure 6: L.S. of 7.0 cm. larvae gill with well developed gill filaments and gill lamellae.

Thus, the blood channels of secondary lamellae were lined by pillar cells (mesenchymal cells) with branchiostegel membrane (Figure 5).

DISCUSSION

In the larvae of *Schizothorax plagiostomus* from post flexion to fingerling stage the gill filaments and gill rakers start to develop. As the larvae grow; the number and size of gill filament and gill rakers also increase. The number and size of gill filament and secondary lamellae, which is the actual site for gas exchange with groups of pillar cells surrounding the blood channels also increased. In 2.0 cm. length fish larvae the supply of oxygen demand gradually increases due to movement of the larvae and it increases as the size of larvae increases. Same opinion was given by Evans, 1998; Greco, et.al. 1995; Thomas et al., 1988; etc. in some other fresh water fish. In the secondary lamellae of 3.0 cm. long larvae the large number of pillar cells around the blood channels were present. These cells form small tunnels within each secondary lamella that act as channels for blood to perfuse through it. Pillar cells are used to regulate gas exchange across the secondary lamellae surface and have the ability to expand or contract by increasing or decreasing the size of the blood flow tunnels. This allows more or less blood to perfuse through the tunnels. The same functional process have been described to increase or decrease the channel size between two secondary lamella, allowing more or less water to perfuse through them by Laurent and Dunel, 1976; Evans, 1998; Hughes and Wright, 1970 etc.in some other fishes.

In water with high oxygen content the pillar cells will expand allowing more blood to rush through the lamellae to pick up oxygen while at the same time slowing the amount of high oxygenated water that flows through the channels in order to prevent the fish from getting too much oxygen, while in low oxygen content the pillar cells contract widening the water flow channels to allow more water to perfuse through it, at the same time allowing less blood to move through the lamellae, so that it can only pick up as much oxygen that is present in the water. Water flows through these lamellae channels in one direction while blood flows in the opposite direction through the epithelial cells. This creates a countercurrent flow that maximizes oxygen transfer (Newstead, 1984; Graham, 1997; Evans, 1998). This also supports our findings in hill stream snow trout larvae which inhabit high O_2 and low temperature environment with expanding pillar cells and allowing more blood to rush through the lamellae. With increasing surface of the gills the chances of ion loss may also increase. To solve this problem the number and size of goblet cells, which control the ion loss or water-influx in *S.plagiostomus* larvae began to increase after post flexion stage when the length of larvae is more than 4.0 cm., the same finding supports the opinion of Laurent, 1984; Perry, et.al. 1992; in some other fresh water fishes.

Young (1962); Walters (1966), have assumed that the gill-rakers perform exactly like a sieve in straining food items from the water and thus it is expected that a precise relationship may exist between gill-rakers; gill-rakers length and the proportion or size classes of various food types in the diet. In *S.plagiostomus* larvae the gill rakers is closely spaced. Suyehiro (1942) also indicated that fish with closely spaced gill-rakers are plankton feeders. The post flexion stage of *Schizothorax* larvae are plankton micro-algae feeder but as the size of larvae increases they move towards the deep water body and they start to feed on more algal phytoplanktonic food, so the gill rakers become longer in size and more in number. The same observation was given by Singh et.al. (1993), Bahuguna and Maithani (2005), Bahuguna (2006) in some other hill stream fishes. The relationship between food items and gill morphology has also been explained in detail by Bahuguna and Singh (1984) in some other hillstream fishes. The same morphological changes appear in the

fingerling stage of *Schizothorax plagiostomus* larvae and show a very good relationship between feeding adaptation in gill rakers.

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ABBREVIATIONS

BV = Blood Vessels, CRT.R = Cartilaginous Rod, EPL = Epithelial Layer, G = Gill, GA = Gill Arch, GF = Gill Filament, GR = Gill Raker, PC = Pillar Cells, SL = Secondary Lamellae.