Chapter 6

Summary and Conclusion
6.1. SUMMARY

The present investigations cover the histochemical and ultrastructural aspects of the gills, intestinal tract and liver of a naturally feeding freshwater teleost of Kashmir, *Schizothorax curvifrons* Heckel.

6.1.1. Histochemical Studies

Histochemical observations on the localization and distribution of inorganic constituents, organic components and enzymes in the gills, intestinal tract and liver of *Schizothorax curvifrons* have been made. Among the inorganic elements localized include iron and calcium, whereas the organic components studied are proteins, tryptophan, elastic fibres, DNA, glycogen, neutral and acid mucin and neutral lipids. In addition enzymes viz. alkaline phosphatase, acid phosphatase, adenosine triphosphatase (ATPase) and lipase have been demonstrated histochemically.

Intense reaction for iron is observed in the cytoplasm of the hepatic cells. The gills, intestinal bulb, intestine and the rectum however, react negatively to prussian blue stain.

With respect to the histochemical detection of calcium, only the intestinal bulb and the intestine of the fish studied reveal positive results. The submucosal lymph spaces, blood vessels and blood capillaries are intensely localized sites. The columnar epithelial cells of the mucosa on the other hand exhibit weak reaction, whereas the muscularis and serosa are devoid of any reaction.

Proteins are demonstrated in the gills, entire intestinal tract and liver. The chloride and pavement cells of the gills, the columnar epithelial cells of mucosa, the submucosal core, muscularis, serosal layer and the hepatocytes exhibit well pronounced reaction for proteins. No reaction is observed in the mucous cells.
Except rectum, rest of the tissues studied show intense presence of tryptophan. The sites of localization include the afferent epithelia, chloride and pavement cells of the gills, brush border, epithelial cells, submucosal blood vessels and blood capillaries and the hepatic cells. On the contrary, the efferent epithelia of the gills, mucous cells, muscularis and serosa are noted to be tryptophan negative.

Elastic fibres are localized around the walls of the blood vessels and the submucosal connective tissue of the entire intestinal tract, whereas the gills and the liver do not exhibit any reaction.

The nuclei of the chloride cells, pavement cells and mucous cells of the gills, the basal nuclei of the columnar epithelial cells of the entire intestinal tract and the centrally placed nuclei of the hepatic cells reveal well intense reaction for DNA. The scattered oval nuclei of the submucosal connective tissue react moderately whereas, the muscularis is weakly positive. The serosa exhibit negative reaction.

Glycogen is found to be intensely distributed throughout the cytoplasm of the hepatic cells. The gills and the entire intestinal tract react negatively to Best's carmine stain.

The mucous cells of the gills stain intensely for both neutral and acid mucin exhibiting uniform stain intensity. The mucous cells interspersed between epithelial cells of the entire intestinal tract are positive for both neutral and acid mucin. However, the stain intensity for acid mucin is noted to be intense than the neutral mucin confirming the presence of greater amount of acid mucin. The liver of the fish investigated reacts negatively for both neutral and acid mucin.

Except for the gills, which stain negatively to Sudan black B. neutral lipids are localized in the entire intestinal tract and liver. The sites of
localization include the brush border, epithelial cells, submucosal lymph spaces, blood vessels, blood capillaries, muscularis and the hepatic cells.

Histoenzymological studies reveal the presence of alkaline phosphatase in the gills, entire intestinal tract and liver of *Schizothorax curvifrons*. In the gills, intense enzyme activity is present in the lamellae and core compartments of the secondary lamellae whereas, the pavement cells are devoid of any activity. In the intestinal bulb and intestine, the brush borders, columnar epithelial cells, lamina propria and the crypts or the bases of the villi exhibit intense alkaline phosphatase activity. Moderate activity is discernible in the submucosal blood vessels and blood capillaries. In the rectum, alkaline phosphatase is localized only in the brush borders. In the liver, enzyme activity is observed to be pronounced in the cytoplasm of hepatic cells. However, it has been observed that the muscularis and the serosa of the entire intestinal tract do not show activity for the enzyme.

Acid phosphatase is found to be absent in the gills of the fish studied. However, the intestinal tract and liver are noted to be positive for acid phosphatase. The brush borders, mucosal epithelial cells along the bases of the villi, submucosal blood vessels and blood capillaries and the hepatic cells are the locations of intense enzyme activity. The mucous cells, muscularis and the serosa do not exhibit any activity.

Adenosine triphosphatase (ATPase) is demonstrated in the entire intestinal tract and liver of *Schizothorax curvifrons*. The gills are however, devoid of any activity. The activity is observed to be intense in the brush borders, mucosal epithelial cells, submucosal blood vessels, blood capillaries and the connective tissue of the intestinal bulb and intestine, whereas in the rectum the entire submucosa is noted to be devoid of any activity though the mucosal border and the epithelial cells exhibit pronounced activity. In the liver, intense ATPase activity is associated with both the cytoplasm and nuclei.
of polyhydral hepatic cells. No activity for ATPase is found in the muscularis and serosa.

Intestinal bulb, intestine and liver are observed to be rich sources of enzyme lipase. The localized sites include, brush borders, columnar epithelial cells of the mucosa and submucosal lymph spaces, blood vessels, blood capillaries and cytoplasm of hepatic cells. The gills, goblet shaped mucous cells, muscularis and serosa are observed to be lipase negative.

6.1.2. Ultrastructural studies

6.1.2.1. Scanning Electron Microscopic (SEM) Studies

Scanning electron microscopic studies reveal that each gill comprises of gill arch, gill rakers and gill filaments. The gill filaments are found to be arranged in a single row and equidistant from each other hence, giving it a leaf like structure. Projecting at right angles to the long axis of the filament are the secondary lamellae lying parallel to adjacent lamellae. A number of rakers are observed to radiate from both sides of the gill arch. The inner sides of the rakers are equipped with minute projections. The filament consists mainly of pavement cells with mucous cells and chloride cells spread between them.

The mucosa of the intestinal bulb and intestine when examined under SEM shows the presence of numerous major or primary mucosal folds which are associated with several minor or secondary mucosal folds. A distinct concavity is noted to be present between the mucosal folds. Mucous cells, columnar epithelial cells and the microridges are quite prominent.

The rectal mucosal surface on the other hand exhibit highly irregular pattern of major mucosal folds which are noted to be covered with a thin film of mucin. The pores through which the mucous cells discharge their contents
are quite distinct. However, the concavity and the secondary folds are not observed.

6.1.2.2. Transmission Electron Microscopic (TEM) Studies

Transmission electron microscopic studies distinctly reveal the presence of mucous cells, pavement cells and the chloride cells on the gill epithelia. The mucous cells are entirely occupied by polygonal round shaped and tightly packed mucous globules of varying electrodensities as a result of which their nuclei are displaced towards the basal portions of the cell and the cytoplasm is restricted towards the periphery. Delicate cytoplasmic extensions and islets are interposed among the mucous globules. Desmosomal complexes are not found though cellular interdigitations are quite prominent. The release of mucin by exocytosis is observed.

The pavement cells of the primary lamellae are beset with distinct microridges covered with glycocalyx. Abundant filament bundles are noticed to circumscribe a cytoplasmic region of higher electrondensity containing dark vesicles of variable electron density in the apical cytoplasm of pavement cells. The shape of the pavement cells vary from squamous to polygonal and are characterized by elongate nuclei, rough endoplasmic reticulum and a small number of mitochondria. The pavement cells of the secondary lamellae are found to be devoid of microridges.

The chloride cells of the gills are characterized by the abundance of mitochondria and tubular system ramifying through the cytoplasm. The accessory and the rodlet cells are not found during the present investigation.

Intestinal bulb of *Schizothorax curvifrons* is dominated by enterocytes and goblet cells. The enterocytes are observed to be long with oval nuclei and microvilli forming the brush border at the apical surface. The microvilli are covered with glycocalyx. The microfibrils of the microvilli project into the apical part of the cytoplasm where a terminal net is seen, below which
numerous invaginations of the apical membrane are evident. The enterocytes are linked to one another by desmosomes. The rough endoplasmic reticulum is noted to be present near the nucleus of the enterocyte whereas, the smooth endoplasmic reticulum is located along the basal portions. The assemblage of mitochondria and lipid droplets of different sizes are frequently observed in the cytoplasm. The mucous cells are found to be mostly filled with secretory granules of high density.

In comparison to the intestinal bulb, the microvilli of the brush border of the intestine are short and wide bearing spherical blebs on the tips. The lysosomes and rodlet cells are observed in the intestinal enterocytes. The mitochondria, lipid droplets and the goblet cells containing globules of high density are noticeable. However, the desmosomes between the enterocytes are not observed.

The enterocytes of the rectal mucosa bear short microvilli without glycocalyx covering. The nucleus with prominent nucleolus is seen to be located at the basal portion of the enterocytes. The lysosomes, mitochondria and the lipid droplets are frequently distributed in the cytoplasm though the desmosomes, the terminal web and the rodlet cells are not noted.

Under TEM, the hepatic cells show a centrally placed nucleus containing two prominent nucleoli with more condensed heterochromatin towards the periphery. The nuclear pores are quite distinct in the double nuclear membrane. Stacks of rough endoplasmic reticulum are found around the nucleus and along the plasma-membrane. The mitochondria varying in shape from circular to elongate are observed to be in close association with the rough endoplasmic reticulum. Several peroxisomes and good content of glycogen are found scattered in the cytoplasm. However, smooth endoplasmic reticulum, golgi and lipid inclusions are not observed. The exocrine pancreatic cells of the fish studied are easily differentiated from other cell
types by the presence of large number of secretory granules usually located at the apical portion of the cell. The secretory granules are uniform in density and are limited by a single membrane. The spherical nucleus of the pancreatic cells is located at the basal portion of the cell.

6.2. CONCLUSION

It may be concluded from the present histochemical findings that after detecting and localizing the various inorganic constituent, organic components and enzymes in their natural locations in the tissue sections of the gills, intestinal tract and liver of naturally feeding freshwater fish of Kashmir, *Schizothorax curvifrons*, not only their cellular architecture in respect of structure and function is established but also helps us in understanding the functional characterization of cellular and extracellular components. Further, these investigations provide an insight into the nature of various physiological and pathological processes. Moreover, histochemical investigations provide almost the only approach to certain biological problems because to a large extent the structural integrity of cells and tissues are preserved as the cells or their components can be taken as the units of investigation. The results of the present study reveal distinctly that the different constituents and enzymes are distributed differently in the tissues of the fish studied. Nevertheless, variations in the intensity of localization and distribution of the said components and enzymes noted are probably governed by multiple factors, like the nature of the mucous membrane, the time period during which the food is in contact with the digestive tract, the feeding habit of the fish, effect of temperature and the optimum efficiency of a particular organ to depict the maximum concentration of a specific substance.

The surface architecture of the gut mucosa as revealed by scanning electron microscopy (SEM) under different magnifications throws light on the modifications of mucosal epithelium and morphological characteristics of the
various cells lining the gut. These observations are useful in correlating morphological features of the region concerned with functions e.g. the presence of high primary folds along with numerous secondary folds is an adaption towards herbi-omnivore diet of the adult *Schizothorax curvifrons*. On the other hand, deep concavities formed by the anastomosis of the mucosal folds serve in the retention of ingested food for larger periods. Similarly, variations in the pattern of microridges in columnar epithelial cells in the different regions of the intestinal tract and gills can be related to their functional significance. Transmission electron microscopic studies on the other hand revealed the fine subcellular details of the tissues of the fish studied in its natural habitat and facilitated information important in interpreting the structural organization. The ultrastructural and functional characteristics of the gills, intestinal tract and liver of *Schizothorax curvifrons* studied provides a baseline for comparison with pathological and stress conditions in natural environments for further studies.