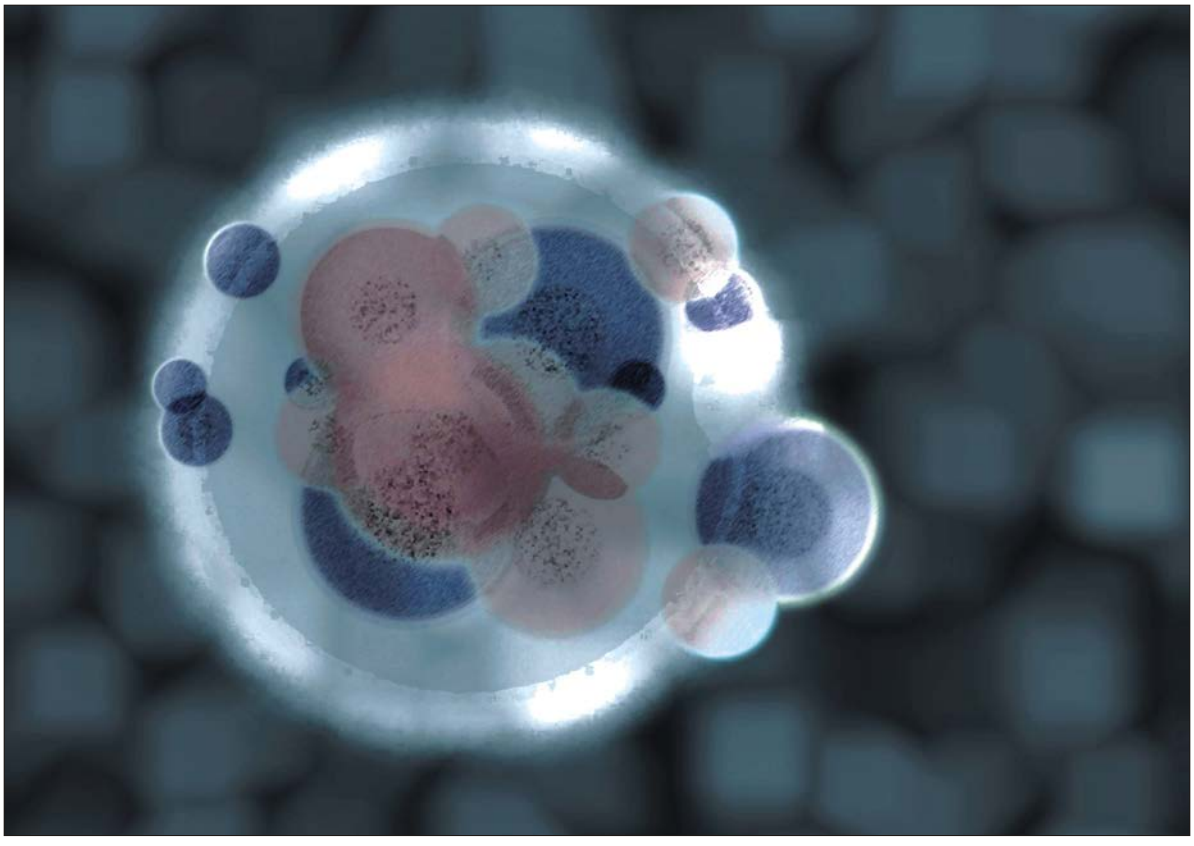

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Histochemical Distribution of Lipase and Acid Phosphatase in the Intestinal Tract of the Snow Trout, *Schizothorax curvifrons* Heckel.

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Abstract: Histochemical localization of lipase and acid phosphatase has been investigated from the entire intestinal tract of the snow trout, *Schizothorax curvifrons* by utilizing histoenzymological techniques with the objectives to investigate occurrence and cellular localization of the selected enzymes in the entire intestinal tract of *Schizothorax curvifrons* and their contribution to intracellular digestive, absorptive and transport processes that will be of vital utility while going for the cultural practices of *Schizothorax curvifrons*. Lipase was localized in the brush border and cytoplasm of enterocytes of both intestinal bulb and intestine. The submucosal lymph spaces, blood vessels and blood capillaries of the intestinal bulb also proved to be lipase positive, whereas the entire intestinal submucosa and the rectal region did not reveal any sign for the presence of lipase. Activity of acid phosphatase was detected in brush borders, supranuclear regions of enterocytes, lamina propria and the submucosal region of both the intestinal bulb and intestine. The activity of acid phosphatase gradually decreases in posterior direction and in the rectal region its activity was very weak and confined only to the enterocytes forming the brush border of microvilli. The possible roles of these enzymes in intracellular digestion, absorption and transport have been discussed in this study.

Key words: Lipase, acid phosphatase, *Schizothorax curvifrons*, histoenzymological, enzymes, enterocytes, brush border, intracellular digestion

INTRODUCTION

A deeper understanding of physiology of fish nutrition demands better knowledge of the digestive enzyme activities. As in other vertebrates the tendency of fish to utilize ingested nutrients chiefly depends on the presence of appropriate enzymes in appropriate locations in the wall and along the lumen of intestinal tract. Diverse intestinal enzymes engaged in digestive and absorptive processes have been reported in a variety of adult fish (Goel and Sastry, 1973; Sastry, 1974; Sinha, 1979; Kuperman and Kuzmina, 1994; Kuzmina and Gelman, 1997; Cahu *et al*., 2000; Tengjaroenkul *et al*., 2000; Kozaric *et al*., 2004). Distribution and intensity of intestinal enzyme activity along and within the intestinal tract varies with feeding habits and intestinal morphology (Hofer and Schiemer, 1981; Kuzmina, 1984; Kuzmina and Smirnova, 1992; Sabapathy and Teo, 1993; Kolkovski, 2001; Gawlicka *et al*., 2002).

The snow trout, *Schizothorax curvifrons* is a prized indigenous herbivorous cold freshwater teleost of the Kashmir valley whose population is at decline, the reason being the multiple factors. The fish belonging to the family cyprinidae proves to be morphometrically, meristically and economically most variable and valuable promising food species of the paradise vale and has an ability to utilize variety of foods. The intestinal tract is of utmost physiological importance as it is the site of temporary storage of food and most of the digestive, absorptive and transport processes. As is the case with other cyprinids, the snow trout, *Schizothorax curvifrons* lacks true stomach, the proximal end of the intestine is of greater diameter than the rest of the intestine and serves the functions of temporary storage of ingested food and this dilated region of the intestinal tract is the intestinal bulb. There are no reports available in the literature regarding the enzymehistochemistry of *Schizothorax curvifrons*, therefore the present study began the histochemical localization of lipase and acid phosphatase in different portions of the intestinal tract of adult snow trout, *Schizothorax curvifrons*. The aim of this study was to investigate occurrence and cellular localization of the selected enzymes in the entire intestinal tract of *Schizothorax curvifrons* and their contribution to intracellular digestive, absorptive and transport processes that will be of vital utility while going for the cultural practices of *Schizothorax curvifrons* at a commercial scale.

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MATERIALS AND METHODS

Adult living specimens of wild, normal and healthy snow trout, *Schizothorax curvifrons* were caught from their natural habitat and fish with a body weight of 350-400 g and body length 20-25 cm were dissected, the abdominal cavity was operated and the different portions of the intestinal tract viz intestinal bulb, intestine proper and rectum were fixed in cold (4°C) neutral formalin for a brief period of time. Frozen sections (10-15 μ thick) of the fixed tissues were sectioned at-25°C from a cryostat (Leica CM 3050S) and utilized for histochemical detection of enzymatic activities. Histoenzymological techniques for detecting enzymatic activities were taken according to (Pearse, 1972a, b), for lipase-substrate, Tween 80 (pH 7.4) and acid phosphatase-substrate, sodium β-glycerophosphate (pH 5.0). The tissue sections were incubated at 37°C for 6 h for lipase and for 1 h for acid phosphatase in their respective incubating mediums prepared from their respective substrates, while control sections were incubated in the same medium without substrate, following incubation the tissue sections were rinsed in distilled water, developed in dilute yellow ammonium sulphide and coverslipped with mounting medium, glycerin jelly. Finally the prepared slides were studied under light microscope and the enzymatic activities were visually analyzed and further evaluated as strong or intense enzymatic reaction, moderate, weak or absent depending on the staining intensity in the tissues. This study has been conducted from October 2006 to April 2007 in the ichthyology laboratory of the department of Zoology university of Kashmir, Srinagar, India.

RESULTS

Intestinal bulb is observed to be rich source of lipase bearing strong enzyme activity in brush border, cytoplasm of columnar epithelial cells, the intervillar portions and the submucosal lymph spaces, blood vessels and blood capillaries (Fig. 1). The intestine exhibits intense lipolytic activity particularly along the apical portions of the villi and throughout the cytoplasm of the mucosal epithelial cells (Fig. 2), however, the brush border possesses moderate enzyme activity whereas, the entire submucosa is devoid of any lipolytic activity. The goblet shaped mucous cells, muscularis and the serosa of the intestinal bulb and intestine and the entire rectal region are found to be lipase negative.

Intestinal bulb and the intestine are found to be the most active portions secreting acid phosphatase. The brush borders, supranuclear regions of enterocytes particularly along the basal portions of the villi, lamina propria and the submucosal region are coated with a black precipitate, thereby confirming a strong acid phosphatase activity (Fig. 3, 4), whereas a progressive decrease in the enzyme activity is noticeable in the mucosal epithelial cells towards the apices of the villi and the tips of the villi are found to be acid phosphatase negative. The rectum on the other hand follows a marked decrease in the enzyme activity (Fig. 5) and the acid phosphatase activity

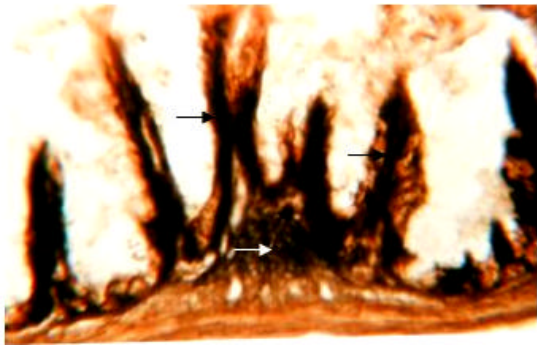

Fig. 1: Activity and location of lipase in the intestinal bulb (→). X280

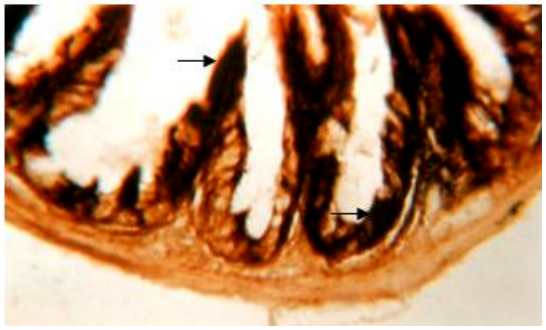

Fig. 2: Location and intensity of lipase in the intestine (→). X320

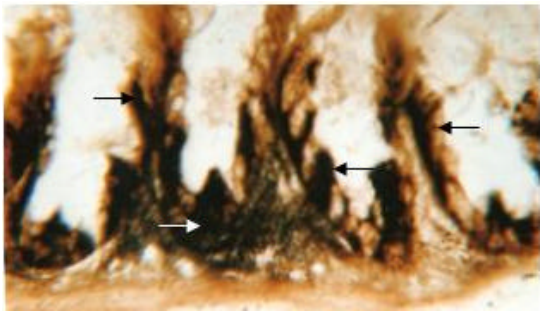

Fig. 3: Location and intensity of acid phosphatase in the intestinal bulb (→). X200

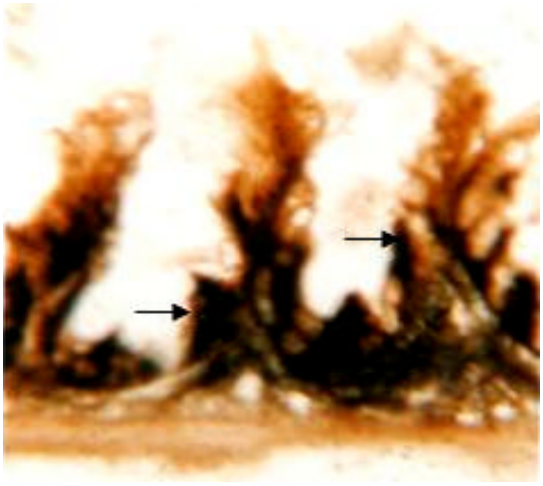

Fig. 4: Activity and location of acid phosphatase in the intestine (→). X200

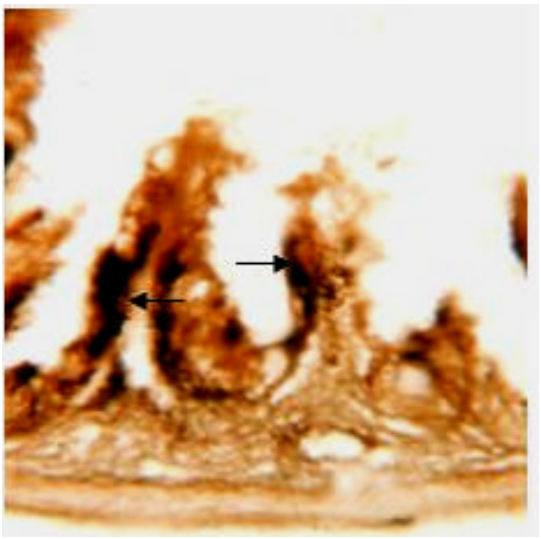

Fig. 5: Activity of acid phosphatase in the rectum (→). X200

is restricted only to the enterocytes forming the brush border of the microvilli. The mucous secreting goblet cells, muscularis and the serosa of the entire intestinal tract did not reveal any sign for the presence of acid phosphatase.

DISCUSSION

Digestion of ingested food and absorption of digested food molecules and other metabolites generally takes place along the brush border and cytoplasm of enterocytes where numerous digestive enzymes are localized. The digestive physiology of fish larvae is well documented (Segner *et al*., 1989; Walford and Lam, 1993; Oozeki and Bailey, 1995; Baglole *et al*., 1998; Gisbert *et al*., 1999; Ribeiro *et al*., 1999) and pointed out a correlation between enzyme activities and maturation of digestive tract. In adults digestive enzyme activities are in correlation with feeding habits (Goel and Sastry, 1973; Sinha, 1979; Chakravorty and Sinha, 1982) and are also related to the diet of the fishes (Diaz *et al*., 1997; Cahu *et al*., 2000; Tengjaroenkul *et al*., 2000; Kozaric *et al*., 2006). Activity of lipase and acid phosphatase express various degrees of intensity across the intestinal tract of *Schizothorax curvifrons*.

Distribution of lipase in *Schizothorax curvifrons* varies not only along the intestinal tract but also within the cells of the various portions of the intestinal tract. According to Vonk (1937) the lipase activity in the intestine is due to the possible absorption of the pancreatic enzymes into the intestinal mucosa. The present findings however, clearly reveal that the mucosa is capable of secreting lipase as evidenced by intense enzyme activity in the brush border and cytoplasm of columnar epithelial cells. The presence of lipase in the submucosal lymph spaces, blood vessels and blood capillaries may possibly be due to the hydrolysis of fats leading to the formation of chilomicrons for easy absorption and transport in these vessels to different cells of the body. The rich lipolytic activity in the intestinal bulb of the snow trout, *Schizothorax curvifrons* lays a strong foundation for the fact that in addition to the temporary storage of ingested food, the intestinal bulb is the main site of lipid uptake from the diet. The progressive decrease in the lipase activity towards the posterior parts of the intestinal tract and its complete absence from the rectum is suggestive of the fact that the lipolytic activity in *Schizothorax curvifrons* is indeed present and occurs mainly in the anterior two third portions of the intestinal tract. The relatively restricted distribution of lipase in *Schizothorax curvifrons* concurs with previous reports that lipase activity is lowest in herbivorous fish (Opuszynski and Shireman, 1995; Tengjaroenkul *et al*., 2000) which may be correlated to the low fat content in plant material naturally consumed by the snow trout, *Schizothorax curvifrons*. The restricted distribution of lipase along the intestinal tract and within the cells support a low fat level being more appropriate for *Schizothorax curvifrons* diets.

Acid phosphatase is one of the marker enzymes of the lysosomes but its activity has also been detected outside lysosomes (Lin and Fishman, 1972). Sinha (1979) and Chakravorty and Sinha (1982) reported the presence of acid phosphatase from the brush borders, whereas Rode and Frank (1967) and Kozaric *et al*. (2006) had

revealed its presence only from the supranuclear parts of the enterocytes. Activity of acid phosphatase in investigated parts of the intestinal tract of *Schizothorax curvifrons* is however, associated with brush border, enterocytes, lamina propria and submucosal regions. Such a wide distribution of acid phosphatase may be correlated to the absorption and transport of metabolites (glucose, aminoacids) and feeding habit of the fish. Intracellular protein digestion occurs in the rectal part of the fish intestine (Kjorsvik *et al*., 1991; Segner *et al*., 1994; Sarasquete *et al*., 1995; Kozaric *et al*., 2006), however a very weak acid phosphatase activity in the rectum of the investigated fish clearly reveal that rectal part of the *Schizothorax curvifrons* plays no role in intracellular digestion of proteins. The strong acid phosphatase activity in the intestinal bulb and the intestine of the investigated fish may be explanation for the intracellular digestion and absorption of proteins of the ingested diet. Since, this enzyme is also involved in pinocytotic activity and intracellular digestion, so pinocytosis may be considered as alternative pathway of protein digestion in teleosts.

CONCLUSION

The findings of this study suggest that only the intestinal bulb and intestine participate in lipid digestion, while going for the cultural practices of *Schizothorax curvifrons* it is adequate to feed the fishes on low lipid diet. Stronger activity of acid phosphatase in the intestinal bulb and intestine than in the rectum is suggestive of the fact that the absorptive activity and intracellular protein digestion is least effective in rectum than in the rest of the intestinal tract of the snow trout, *Schizothorax curvifrons*.

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