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Histological and histochemical study on the alimentary canal in Walking catfish (*Claris batrachus*) and piranha (*Serrasalmus nattereri*)

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Summary

The purpose of this study is to describe the histological and histochemical features of digestive tract in two aquarium fishes with different types of feeding habits which are omnivorous (Walking catfish) and carnivorous (piranha), respectively. Five adult Walking catfish (L: 26.4 ± 3.4 cm, W: 265 ± 55 g) and five adult piranha (L: 23.6 ± 4.5 cm, W: 247 ± 50 g) were used for this study. The fishes were killed by decapitation method and small pieces (0.5 cm × 0.5 cm) from the esophagus, cardiac, fundus and pyloric portions of the stomach, proximal, distal parts of intestine and pyloric caeca were fixed by immersion in Bouin's solution. The specimens were processed through routine paraffin embedding technique and cut at 5-7 µm and stained with hematoxylin and eosin, Van Gieson, Verhoof, Alcian blue, PAS, and AB+PAS technique for different microstructural and microfibrilar structures. The morphological and histochemical structures of all specimens were studied with a light microscope and micrographs were prepared. The esophagus of Walking catfish and piranha are found to have numerous deep longitudinal folds, stratified squamous epithelium with numerous mucous cells which react positively to PAS and AB stains. Taste buds and muscularis mucosa were not seen in the esophagus of both species. The muscularis mucosa was organized in longitudinal and circular layers of striated muscular fibers. The mucosa of the stomach was formed by simple columnar epithelium with folds and gastric pits formed by the invagination of the mucosal layer into the lamina propria. The surface epithelium of the stomach reacted positively to PAS but negatively to AB staining. The mucosal surface of the intestine in Walking catfish and piranha has numerous folds lined by simple tall columnar cells, along with goblet cells which reacted positive to PAS and AB staining, so that goblet cells in the intestine of Walking catfish were significantly more than in piranha. The Caeca pyloric was found only in piranha and it showed an elongated fold lined with columnar cells and numerous mucous cells which reacted positively to PAS.

Key words: Histology, Digestive tract, Walking catfish, Piranha

Introduction

Fish digestive tracts show remarkable differences in morphology and function. Differences observed at specific levels are related to food, feeding habits, body weight, shape and sex (Grosh and Das, 1987; Boglione *et al*., 1992; Murray *et al*., 1996). Furthermore, the presence of a relation between feeding behavior and the basic histology view or the improbability of this (Martin and Blaber, 1984) has been argued. The histology of the digestive tract has been studied with light and electron microscopes in fish species (Hale, 1965; Caceci, 1984; Caceci and Hrubrec, 1990; Mojazi *et al*., 2005; Khojasteh *et al*., 2009). The feeding activities of fish are classified most commonly according to the type of food consumed. In simplest form, fish are either herbivore if they eat plant material, carnivores if they consume animal material or omnivores if they eat a combination of plant and animal materials (Evans, 1998).

The Walking catfish (*Clarias batrachus*) is a species of freshwater air breathing catfish found primarily in Southeast Asia, although known in English as "Walking

catfish", its "Walk" is often just a sort of wriggling motion with a snakelike movement, using its pectoral fins to keep it upright. In the wild, the natural diet habit of this creature is omnivorous (Mills, 1993). Piranha (*Serrasalmus nattereri*), which include not only the carnivorous but also large, peaceful herbivores, live in South American rivers (Bailey and Sandford, 1998).

Walking catfish has an elongated body shape. This catfish has long-based dorsal and anal fins as well as several pairs of sensory barbells, and the pectoral fins contain poisonous spines which are especially stout on the male. Piranha is snub-nosed and the lower jaw is longer than the top jaw, and both jaws contain very sharp teeth.

The main purpose of this study is to describe the histological and histochemical features of the digestive tract in two aquarium fish with different types of feeding habits which are omnivorous (Walking catfish) and carnivorous (piranha), respectively.

Materials and Methods

Five adult Walking catfish (L: 32 cm, W: 240 g) and five adult piranha (L: 22 cm, W: 190 g) were used for this study. The fishes were killed by decapitation and the entire digestive tract was exposed through a mid-ventral incision and removed from the body. Small pieces from the esophagus, cardiac, fundus and pyloric portion of the stomach, proximal and distal parts of the intestine and pyloric caeca after washing in phosphate buffer were fixed by immersion in Bouin's solution. After fixation, the specimens were rinsed in water, dehydrated in graded ethanol solutions (50% to absolute), cleared in xylene and embedded in paraffin. Transverse paraffin sections (5-7 µm) of each portion of the digestive tract were prepared and processed for staining through hematoxylin and eosin (H&E), Van Gieson (VG), Verhof (V), Alcian blue (AB), Periodic acid Schiff (PAS), AB+PAS (Lee and Luna, 1968), and technique micrographs were taken with a video camera connected to a microscope (Olympus, Tokyo, Japan). All chemicals were purchased from sigma chemical company.

Results

In the present study the digestive tract of Walking catfish and piranha were divided into three parts: esophagus, stomach and intestine (Figs. 1 and 2). Although each portion of the digestive tract has a special structure, the wall of the digestive tract as a whole is composed of the mucosa, tela submucosa, tunica muscularis and tunica serosa.

The esophagus of Walking catfish was found to have numerous deep longitudinal folds; it was lined by a few layers of stratified squamous epithelium with numerous superficial mucous cells, so that it changed to simple columnar epithelium at the end of the esophagus. Taste buds were absent in the epithelium of the esophagus of Walking catfish. The lamina propria was formed by loose connective tissue without elastic fibers. The lamina muscularis mucosa was not present. The tunica muscularis with striated muscular fibers had two layers; a thick inner circular and thinner outer longitudinal layer. Outer serosa was a thin layer of connective tissue surrounded by mesothelium. The mucous cells were stained with AB and PAS in the lower part of the mucosal fold, but stained only with AB in the upper part of the mucosal folds, indicating the presence of neutral and acidic (carboxyl and sulphate groups) glycoprotein (Fig. 3).

The stomach of Walking catfish included the cardiac, fundus and pyloric region. The mucosa of the cardiac and fundus were formed by a single layer of columnar epithelium with folds (Fig. 4). There were mucosal folds in three portions that were shallow in the pyloric region. There were numerous gastric pits of the stomach formed by the invaginations of the mucosal layer into the lamina propria, which were lined to the opening of tubular branched glands formed by the columnar cells (Fig. 4). The columnar epithelial cells contain a layer of cells with pepsinogen granules. In the pyloric region the gastric glands were scarce, but did not disappear

completely. Although the lamina muscularis mucosa was inconspicuous, a few muscular fibers were present and in the pylorus, the muscularis mucosa was complete. The submucosa is formed by loose connective tissue. The surface epithelium of the stomach reacted positively to PAS but was negative to AB.

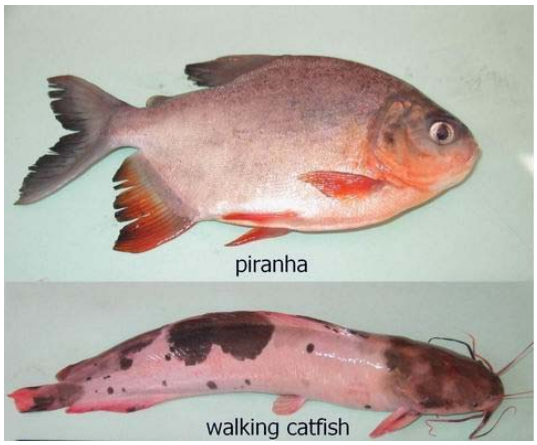

Fig. 1: Walking (a) catfish and piranha (b)

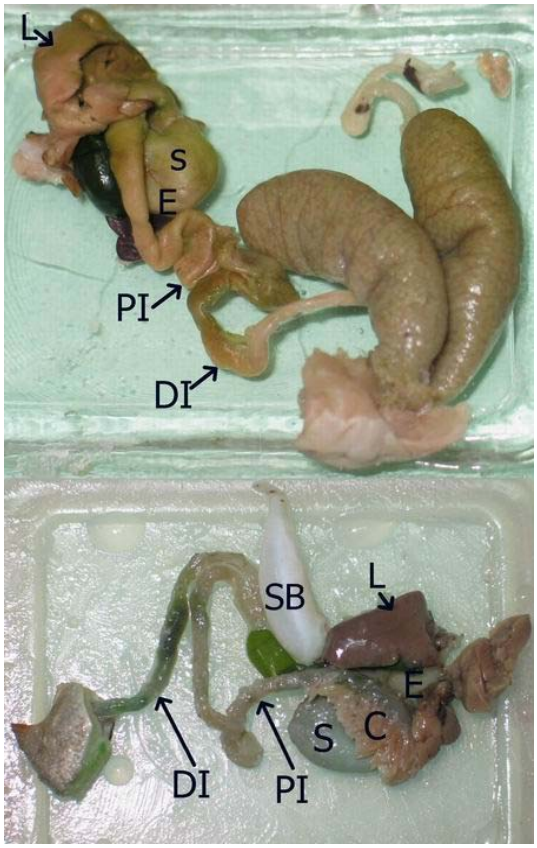

Fig. 2: Gross photograph of digestive tract in Walking catfish (a) and piranha (b). Esophagus (E), stomach (S), caeca pyloric (c), liver (L), proximal intestine (PI), distal intestine (DI), and swim bladder (SB)

The intestine in Walking catfish had no macroscopically differentiable regions. However, there were microscopic differences that characterize the intestine

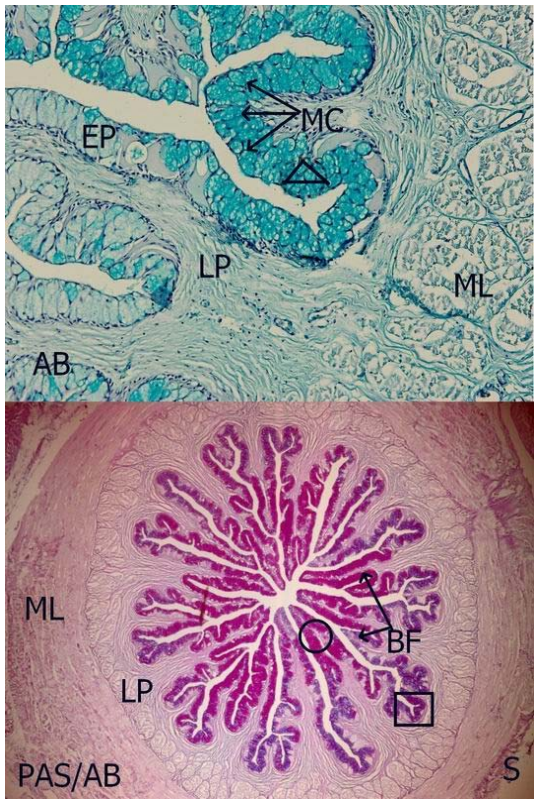

Fig. 3: Transverse section of the esophagus in Walking catfish with alcian blue (AB) and periodic acid schiff + AB (PAS+AB) staining. Epithelium (EP), lamina propria (LP), mucosal cells (MC), muscular layer (ML), serosa (S), circle (PAS positive), triangle (AB positive), and rectangle (AB and PAS positive, ×64)

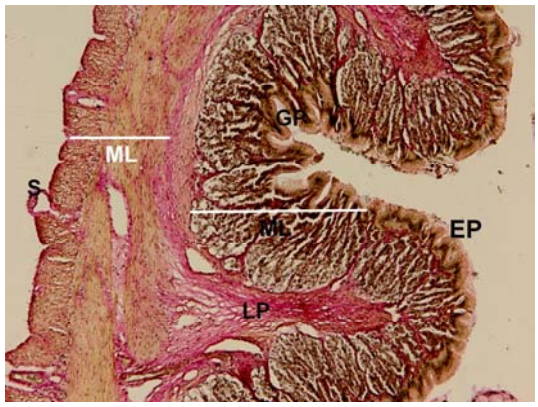

Fig. 4: Transverse section of the cardiac region in piranha showing epithelium (EP), gastric pit (GP), lamina propria (LP), mucosal layer (ML), sub mucosa (SM), muscularis layer (ML) and serosa (S), (PAS+VG, ×320)

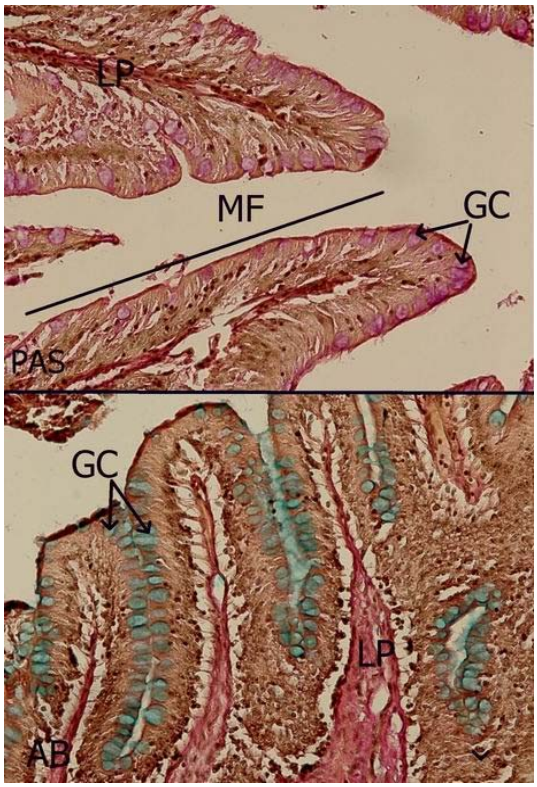

Fig. 5: Transverse section of the proximal intestine in Walking catfish with PAS and AB staining, mucosal folds (MF), goblet cell (GC), lamina propria (LP), (PAS+VG, ×320)

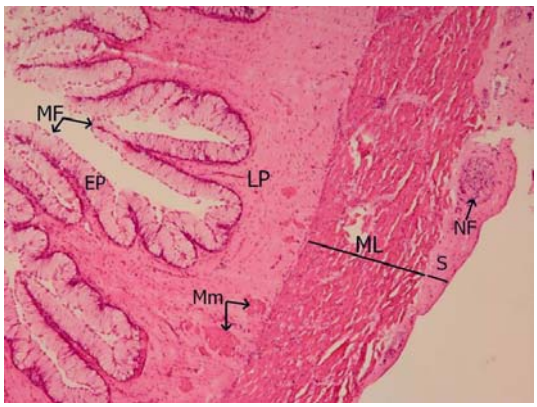

Fig. 6: Transverse section of the esophagus in piranha showing mucosal folds (MF), Epithelium (E), lamina propria (LP), muscularis mucosa (MM), tunica muscularis (ML), serosa (S) and nerve fiber (NF), (H&E, ×160)

into proximal and distal portions. The mucosal surface in the proximal intestine had numerous elongated and deep folds lined by simple tall columnar cells and goblet cells (Fig. 5). In the distal intestine, the folds were fewer and shorter containing large numbers of goblet cells. Goblet cells in

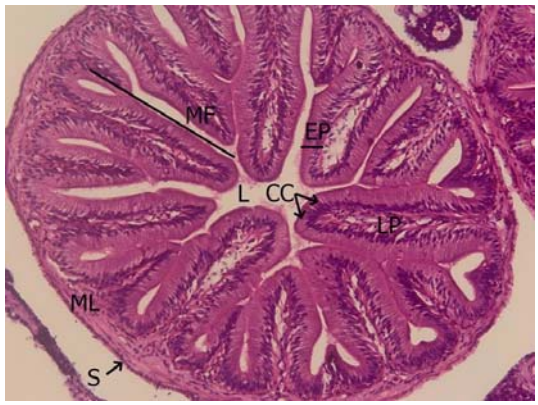

Fig. 7: Transverse sections of caeca pyloric in piranha showing lumen (L), mucosal fold (MF), epithelium (EP), and columnar cell (CC), lamina propria (LP), muscularis layer (ML), serosa (S), (H&E, ×320)

the mucosal surface were stained by AB and PAS (Fig. 5). The absence of muscularis mucosa prevented the distinct separation of lamina propria from the submucosa. The tunica muscularis was organized in two distinct layers of smooth muscle; inner circular and outer longitudinal layers.

The esophagus of piranha was characterized by numerous folds which were lined by stratified squamous epithelium filled with mucous cells. The taste buds were absent in the epithelium. The mucous cells were stained with AB and PAS. Lamina propria is formed by dense connective tissue with adipose tissue. Lamina muscularis mucosa was present so that it separated the tunica mucosa from the submucosa. Tunica muscularis had two layers of striated muscular fibers that are similar to tunica muscularis in Walking catfish (Fig. 6).

The stomach of piranha was composed of cardiac, proper and pyloric regions. The mucosa of the cardiac and gastric proper regions was formed by a simple columnar epithelium with folds and numerous gastric pits formed by the invagination of the mucosal layer into the lamina propria which is linked to the opening of simple tubular glands. The covering epithelium of the stomach (in three parts) reacted positively to PAS, but the reaction was negative to AB. In the pylorus the gastric glands were scarce but did not disappear completely. Tunica muscularis had two layers of smooth muscle fibers; a thick inner circular and a thin outer

longitudinal. Lamina muscularis mucosa was present in the stomach of piranha.

The caeca pyloric of piranha show elongated folds lined with columnar and numerous mucous cells which reacted positively to PAS but not to AB. Lamina propria, tela submucosa, muscularis mucosa and tunica serosa in caeca pyloric were very thin in this part of the digestive tract (Fig. 7).

The intestine of piranha was characterized by mucosal folds lined by a simple columnar and goblet cells which reacted positively to PAS AB. Goblet cells in the intestine of Walking catfish were more than that of piranha. The distributions of goblet cells increased toward the distal portions of intestine.

Discussion

Although there are great differences in the histology of intestinal tract among different fish species, the wall of the tract of Walking catfish and piranha, as also occurs in other fish, is composed of the four layers described for vertebrates (Kumar and Tembhre, 1996). In those species, as well as in other freshwater fish, the esophageal wall shows a more regular structure and longitudinal folds, which is similar to the esophagus of the *silverside odontesthes bonariensis* (Diaz *et al*., 2006) and more simple than those found in marine teleosts (Abaurrea-Equisoain and Ostos-Garrido, 1996). The esophageal epithelium is stratified squamous with mucous cells. The mucous secreting cells, arranged in a continuous sheet in the esophageal epithelium, are important for continues sheet and consistent lubrication of food particles during swallowing, and protect the epithelial surface against mechanical damages and bacteria invasion, being also related to ionic absorption (Grau *et al*., 1992; Albrecht *et al*., 2001).

The present study revealed that esophagus epithelium of Walking catfish and piranha have two types of mucous cells, the superficial mucous cells react positively to PAS, indicating that their contents are of neutral glycoproteins (GPs) and the other cells react positively to Alcian blue, indicating their contents of acidic GPs. This secretion is similar to Tilapia fish (*Tilapia spilurus*), Sea Bream (*Mylio cuvieri*) (Abdulahadi, 2005) and European catfish (*Silurus glanis*) (Kozaric *et al*., 2008).

Although the taste buds are present in the esophagus of species of the family Ambassidae and in *Gadus morhua* (Martin and Blaber, 1984; Morrison, 1987), in the esophagus of Walking catfish and piranha taste buds were not seen, which is similar to *Silverside odontesthes bonariensis* (Diaz *et al*., 2006) and *Engraulis anchoita* (Diaz *et al*., 2003). Glands and muscularis mucosa were not observed in the wall of the esophagus in the Walking catfish and piranha, which is similar to the paddle fish (*Polyodont spathula*) (Weisel, 2005). But only in the piranha, is a thick layer of collagenous materials present under the epithelium. Collagenous fibers would constitute a layer for protection, support and strengthening in many carnivorous fishes (Kapoor *et al*., 1976).

The present results revealed that mucosa of the stomach of Walking catfish and piranha reacts positively to PAS but not to AB; neutral GPs have a buffering effect on the acidity of the stomach content (Scocco *et al*., 1996). Tubular glands in the stomach of Walking catfish and piranha also reacted positively to PAS. Generally, the stomach of fish is divided into categories depending on their shape; no stomach, straight with an enlarged lumen, U-shaped and Y-shaped. The stomach is absent in many fish, including *Cryprinids* and *Labrids*, where straight stomachs are rare, and seen in Carnivorous fishes, including in pike, channel catfish and halibut, while *salmonide* are an example of fish with a U-shaped stomach (Evans and Claiborne, 2006).

The presence of caeca pyloric in piranha and the absence of it in Walking catfish is not related and affected by the nature of the habitat. The mucosa of caeca pyloric in piranha also reacts weak positively to PAS but not to AB. The caeca pyloric in piranha formed blind, fingerlike projections off the proximal intestine. They serve to increase the effective absorptive surface area of the proximal intestine without increasing intestinal length or thickness, but caeca tend to be better developed in carnivores than herbivores, especially in carnivores with short guts (Evans, 1998).

According to the results obtained from present study, the intestine of Walking catfish and piranha contains numerous goblet cells in the mucosa which react positively to PAS and AB. In both species, increased population of goblet cells in the distal intestine are in accordance with the reports in flower fish (*Pseudophoxinus antalyae*) (Cinar and Senol, 2006). The increased population of goblet cells may imply the need for increased mucosa protection and lubrication for faecal expulsion (Murray *et al*., 1994). More studies should be carried out for better understanding of the histology of digestive tract in other species of fish.

In conclusion, the taste buds were not observable in catfish and piranha. Furthermore, goblet cells in the small intestine of piranha were abundant compared to piranha. Finally, pyloric caeca could not be detected in catfish.

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