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Morphological and anatomical characterization of the digestive tract of *Centropomus parallelus* and *C. undecimalis*

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ABSTRACT. *Centropomus parallelus* and *C. undecimalis* are the most common species of Centropomidae in Brazil. Some authors attribute their failure to grow under in artificial conditions due to limited knowledge on the morphological and functional development of their digestive system and on nutrition requirements of both species. Current research characterizes histologically and histochemically the digestive tract of *C. parallelus* and *C. undecimalis* to provide a histophysiological assessment for studies related to their nutritional needs. In the esophagus, the mucous cells in the epithelium stained positively for PAS and AB. In the stomach, positive reactions to PAS and negative to AB respectively occurred on surface and mucous cells in the epithelium and in the lamina propria. In the intestine, goblet cells showed ratio similarity, with mucus being positive in AB and PAS. A greater concentration of these cells existed in the middle and distal intestine, with a greater increase of secretions when compared to those in the proximal intestine. Results show that *C. parallelus* and *C. undecimalis* display the main features of the digestive tract of carnivorous fish, a well-developed stomach and a relatively short intestine. In fact, they give insights for future studies on the mechanisms related to the species's digestive and absorptive physiology.

Keywords: Digestive tract, feeding habits, histology, Centropomus.

Caracterização Morfológica e Anatômica do Tubo Digestório de *Centropomus parallelus* e *C. undecimalis*

RESUMO. *Centropomus parallelus* e *C. undecimalis* são as espécies mais comuns de Centropomidae no Brasil. Alguns autores atribuem o insucesso no seu cultivo devido ao escasso conhecimento sobre o desenvolvimento morfológico e funcional do sistema digestório, assim como o desconhecimento das exigências nutricionais das espécies. Dessa forma realizou-se o presente trabalho, com o objetivo de caracterizar histológica e histoquimicamente o tubo digestório de *C. parallelus* e *C. undecimalis*, para estudos histofisiológicos relacionados às necessidades nutricionais deste peixe. No esôfago, as células mucosas, presentes no epitélio coraram-se positivamente tanto em PAS como em AB. No estômago, as células superficiais e mucosas presentes no epitélio e na lâmina própria respectivamente, apresentaram reações positivas em PAS e negativa em AB. No intestino as células caliciformes apresentaram similaridade na proporção entre os mucos sendo positivos tanto em PAS como em AB; nos intestinos médio e distal observou-se uma maior concentração destas células, resultando em uma quantidade maior de secreções em comparação ao intestino proximal. Através dos resultados observados, concluise que o *C. parallelus* e *C. undecimalis* exibem as principais características do trato digestório de peixes carnívoros, como um estômago bem desenvolvido e um intestino relativamente curto. Estes resultados darão subsídios para futuros estudos sobre mecanismos da fisiologia digestiva e absortiva desta espécie.

Palavras chaves: trato digestivo, hábito alimentar, histologia, Centropomus.

Introduction

Centropomus parallelus and *C. undecimalis* are euryhaline fish species and are defined as carnivores (they feed on fish, shrimp and small crustaceans during the juvenile stage). Centropomidae, a fish family widely distributed from southern Florida (Gulf of Mexico) to southern Brazil (Rio Grande do Sul State) (CERQUEIRA, 2005), have opportunistic habits since they feed on what is available in the environment.

Adult fish reproduce preferably near the mouth of rivers and estuaries and young individuals benefit from the rich waters of mangroves and estuarine regions for their development (TAYLOR et al., 2000). When juvenile and adults fish of these species are bred in confinement, they become highly resistant to handling procedures and changes in physical-chemical parameters of water (CERQUEIRA et al., 2005) and their feed consumption improves.

The snook is a genus with acknowledged ecological importance and high economic potential for fishing, aquaculture and sports. Their meat presents high organoleptic quality and thus a high market value (CERQUEIRA, 2005). Due to their high commercial value, the species have been captured intensely and a decrease of stocks occurred in natural environments (ALIAUME et al., 2000).

Investigations on the development of the digestive morphophysiological system of the fish should therefore be conducted to understand better the diet and conduct procedures of the species under aquaculture conditions. In fact, histological studies on fish have been a tool to provide extensive information on fish gastrointestinal tract (SANTOS et al., 2007). Similarly, knowledge on their morphology, coupled with an understanding of their feeding habits, is essential for assessing the nutritional physiology of the species, inferring the functions of the process and determining the nutrient absorption (BORGES et al., 2010; SANTOS et al., 2007). The above would be a great help to plan appropriate diets for the breeding and management of animals in potential condition for fish farming.

Although Borges et al. (2010) performed histological and immuno-histochemical analyses and evaluated the absorption of lipids and proteins in the digestive system of *C. parallelus*, research on the morphology, histology, histochemistry and digestive system of histophysiology of *Centropomus* is scarce.

Despite the rustic bass management furnishes a great potential for aquaculture, lack of knowledge on the species's nutritional and physiological aspects may complicate the fattening process.

Current study characterizes the histological and histochemical aspects of the digestive system of *C. parallelus* and *C. undecimalis* and describes the anatomy of their digestive tract and possible changes in the secretion of the mucous cells to provide relevant information on the nutritional physiology of the species. A standard feeding basis with artificial diets may thus be established.

Material and methods

Twenty-five wild juvenile specimens of both species were used, with an average weight 157.5 ± 110.0 g and total length 23.6 ± 5.6 cm for *C. parallelus* and 150.9 ± 44.8 g and total length $24.8 \pm$

2.5 cm for *C. undecimalis*, respectively, collected quarterly from March to December 2010, in the river São João, district of Barra de São João, in the municipality of Casimiro de Abreu, Rio de Janeiro State, Brazil. Fish were collected with the help of local fishermen, by fishing rod and wait net, with the permission of Ibama, and packed in 1000 L boxes of fiber cement, provided with constant aeration and continuous water flow.

The farmed specimens were captured at the Polo Regional Vale do Ribeira of the Agencia Paulista Tecnologia Agronegocios (APTA), Pariquera-Açu, Brazil (24°43'S; 47°53"W) and at the Laboratory of Marine Fish Culture (Lapmar), Universidade Federal de Santa Catarina (UFSC), Florianópolis, Santa Catarina State, Brazil (27°36'S; 48°37"W). Fifteen specimens of *C. parallelus* and *C. undecimalis*, respectively, were captured at each site. *C. parallelus* specimens featured average weight 268.1 ± 183.9 g and total length 26.9 ± 8.8 cm, while *C. undecimalis* specimens featured average weight 92.0 ± 24.1 cm and standard length 23.0 ± 2.0 cm at the nursery on the same stations. Catching was undertaken with fishing nets of different mesh sizes.

Fish food in captivity consisted of commercial food for carnivorous fish, containing 45% crude protein (min.), ether extract 14% (min.) 6% crude fiber (max.) Calcium 2.5% (max.), 1% phosphorus (min.) 14% ash (max), vitamin C 300 mg.

The specimens were killed by freezing treatment in ice water, followed by disruption of the cervical spine. After obtaining body weight (g) and standard length (cm), the immediate opening of the celomic cavity was performed to remove their digestive organs.

Histological and histochemistry of the gastrointestinal tract

Fragments of the esophagus, stomach (cardic, fundic and pyloric parts) and intestine (proximal, medial and distal sections) of farmed and wild specimens were washed in 0.9% saline and then fixed in Bouin solution for 24 hours. Tissues were dehydrated by increasing concentrations of ethanol (70-100%), cleaned in xylene and embedded in paraffin paraplast (BEHMER et al., 1976). The semi-serial 5 μ m cuts of different portions of the esophagus, stomach and intestine were stained with hematoxylin-eosin (HE) for general viewing of tissues and organs.

Sections were submitted to histochemical reactions for the identification of mucopolysaccharides (glycoconjugates) (BANCROFT; GAMBLE, 2002). The technique of periodic acid-Schiff (PAS) was applied to identify cells secreting glycoproteins and to detect simultaneously the neutral mucopolysaccharides

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in goblet cells (blushing neutral glycoconjugates). For the detection of mucopolysaccharides rich in acidic sulfate groups the technique of Alcian Blue (AB) pH 1.0 was used, whereas the reaction used was combined with periodic acid-Schiff (PAS) and Alcian Blue (AB) pH 1.0 for the joint detection of acid and neutral mucopolysaccharides.

The histological slides were examined under a light microscope OLYMPUS BX 51 and documented digitally.

Results

The esophagus, stomach and intestine of farmed and wild specimens of the arrow snook (*C. undecimalis*) and peva snook (*C. parallelus*) were analyzed and the same histological organization for most fish with a carnivorous diet was found.

The intestine was almost 70% of the total length of the digestive tract of *C. parallelus* and *C. undecimalis*. The species presented a short esophagus connected to the stomach, in the trophy. In the cranial region of the intestine, pyloric cecum, there were four finger-like appendages. The intestine was short and rolled into the abdominal cavity, forming three segments, namely: the proximal intestine, which was connected to the pyloric caecum, and the medial and distal intestine which formed the final part of the last segment, ending in a sphincter (Figure 1).



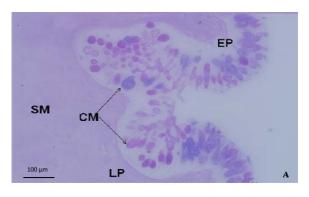
Figure 1. The digestive system of *Centropomus undecimalis*, showing: a) esophagus; b) stomach c) cecum; d) proximal intestine, and) medial; f) distal intestine.

Histologically, the esophageal mucosa presented a stratified squamous epithelium surrounded by a lamina propria with numerous taste tissues, in which two cell types may be identified: the mucosa and claviform epithelium. The epithelium comprised large quantities of mucous glands which were prepared in a homogeneous way throughout its whole surface, with positive response to the combination periodic acid-Schiff (PAS) and Alcian Blue (B), indicating neutral and acidic mucopolysaccharides, respectively (Figure 2A).

The presence of club-shaped cells in the epithelium of the esophagus in the wild and farmed animals was reported, as in some areas extending from the mucous

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glands and reaching the surface epithelium, with a strong reaction in HE (Figure 2B).



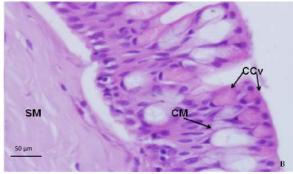


Figure 2. (A) Photomicrographs of transverse sections of the esophagus of *Centropomus parallelus*, showing the stratified squamous epithelium (EP), lamina propria (LP), mucous cell (CM) and submucosa (SM), stained with periodic acid - Schiff (PAS) + Alcian Blue (AB). (B) Club-shaped cells (CCv) and mucous cell (CM), stained with hematoxylin-eosin (HE).

The transition from the esophagus to the stomach is clearly perceived by changes from the stratified squamous epithelium into simple cylindrical epithelium (Figure 3).



Figure 3. Photomicrograph of the transition from the esophagus to the cardic stomach of *Centropomus parallelus*, showing mucosal cells of the esophagus (E) and apical of the cardic stomach epithelium (EC), stained with periodic acid- Schiff (PAS) + alcian blue (AB).

The stomach may be histologically divided into three regions: the trophy, the fundic and pyloric. Variations in the number of glands were observed for each region and regions analyzed showed histologic changes which were important for their identification. The region known as the trophy lay after the transition region of the esophageal-gastric mucosa and was continuously coated by a simple epithelium of cylindrical surface mucous cells, basement membrane, lamina propria and submucosa. There were many simple and tubular gastric glands, consisting of cells called oxintopeptics, secreting H + and Cl- and pepsinogen (Figure 4A).

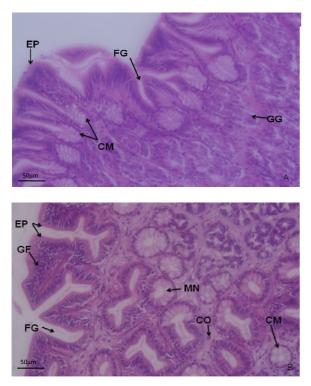


Figure 4. (A) Photomicrograph of cross-cuts of the cardic stomach of *Centropomus undecimalis*, showing the gastric glands (GG), mucous cell (CM), gastric pit (FG) and simple cylindrical epithelium (EP). (B) Photomicrograph of the fundic stomach of the *Centropomus parallelus*, showing the fundic glands (GF), gastric pit (FG), simple cylindrical epithelium (EP), cell mucous (CM), mucous cells of the cervix (MN) and oxintopeptic cells (CO), stained with hematoxylin - cosin (HE).

There were few cells in the fundic region identified as oxintopeptics and none in the pyloric region. A strong positive reaction to PAS was extant only in the apical surface of epithelial cells in three regions of the stomach, which indicated secretion of neutral mucopolysaccharides in the epithelium. The mucous layer of the fundic region of the stomach presented short gastric pits lined by a simple cylindrical epithelium consisting exclusively of mucus-secreting cells. The presence of few oxintopeptic cells may be observed (Figure 4B). The pyloric region was similar to the intestine, with a simple cylindrical and submucous epithelium composed of a thick, richly vascularized, connective tissue. The mucosa of the pyloric region of the stomach had gastric pits, which were more pronounced when compared to the cardiac and fundic regions. There was a positive reaction to PAS in the apical surface of epithelial cells and in the veins of the submucous layer, showing the regular secretion of neutral mucopolysaccharides for the protection of the mucosa. Intraepithelial macrophages were found in large quantities in the epithelium (Figure 5A). Externally, the stomach was covered by a serose.

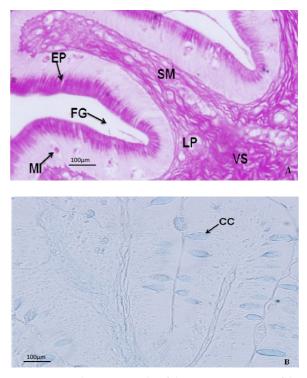


Figure 5. (A) Photomicrographs of the transverse sections of the pyloric stomach of *Centropomus undecimalis*, showing simple cylindrical epithelium (EP), lamina propria (LP), gastric pit (FG), blood vessels (VS) and intraepithelial macrophages (MI), stained with acid periodic- Schiff (PAS). (B) Photomicrograph of the proximal intestine of *Centropomus undecimalis*, showing goblet cells (CC) stained with alcian blue (AB).

The intestine was short, with the intestinal epithelium presenting a simple type with a prismatic and cylindrical aspect and a large amount of goblet cells. These cells were strongly stained by PAS and AB, with neutral and acidic mucopolysaccharides, respectively. The mucous showed villi and an intestinal wall, very thick when compared to the stomach.

Histologically, the intestine had three well-defined regions: the proximal, medial and distal sections. The proximal, medial and distal intestine showed

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histological differences. In the proximal intestine, the submucous layer consisted of richly vascularized loose connective tissue which stained intensively on AB, shown to be sulfated acid mucopolysaccharides (Figure 5B).

Numerous intraepithelial lymphocytes and macrophages and the epithelium at various times throughout the proximal intestine were reported (Figure 6A and B).

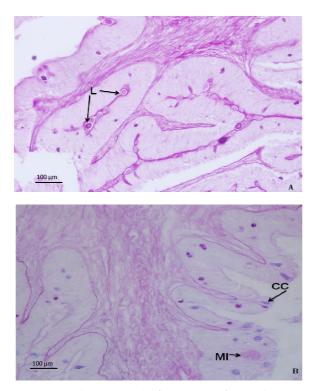


Figure 6. (A) Photomicrograph of cross section of proximal intestine of *Centropomus undecimalis*, showing the number of intraepithelial lymphocytes (L), stained with periodic acid-Schiff (PAS). (B) Photomicrograph of cross section of proximal intestine of *Centropomus parallelus*, showing goblet cells (CC) and intraepithelial macrophages (MI), stained with periodic acid-Schiff (PAS) + alcian blue (AB).

A few goblet cells (mucus-secreting) in the proximal and middle regions, increasing in quantity in the distal section, were observed in the intestinal mucus (Table 1).

Table 1. Intensity mucosubstances secreted by goblet cells along the intestine of *Centropomus parallelus* and *Centropomus undecimalis* for each of the histochemical stains used.

Techniques	Regions		
	Proximal Intestine	Medial Intestine	Distal Intestine
Periodic Acid Schiff (PAS)	(+) to (++)	(++) to (+++)	(+++)
Alcian Blue (AB) pH 1.0	(++) to (+++)	(++) to (+++)	(+++)
PAS/AB pH 1.0	(+)	(+) to $(++)$	(+)

The positive reaction to the dye is represented by "+" and negative reaction by "-" weak reaction to the dye is represented by (+), intermediate reaction by (+ +) and intense reaction by (+++).

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In the medial intestine of the *C. parallelus* (Figure 7A) and in the distal intestine of the *C. undecimalis*, goblet cells were detected by positive reaction to HE and PAS, respectivelly, indicating the production of neutral mucopolysaccharides (Figure 7B).

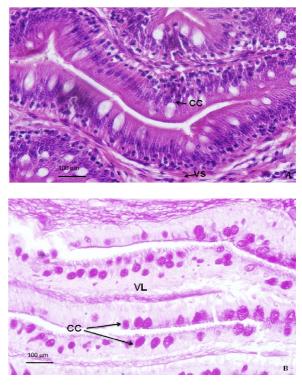


Figure 7. (A) Photomicrograph of transverse sections of the medial intestine of *Centropomus parallelus*, showing goblet cells (CC) and blood vessels (VS) stained with hematoxylin-eosin (HE). (B) Photomicrograph of distal intestine of *Centropomus undecimalis*, showing goblets cells (CC) and villi (VL) stained with periodic acid- Schiff (PAS).

Discussion

The snook's esophagus is a short body, similar to that found in *Pimelodus maculatus* (SANTOS et al., 2007). The epithelium of the esophagus is of a stratified squamous type with large amounts of mucous glands and claviform cells. They are the defense cells, or body's 'alarm' system, providing protection against abrasion and esophageal epithelial injury caused by the passage of food. Since the snook feeds on live prey, such as shrimps and fish, the passage of these foods is facilitated by the mucus produced, which lubricates the lining of the esophagus.

The stomach, the body's digestive system that stands out when it is replenished with food, taking up more than half of the abdominal cavity, is divided into trophy, fundic and pyloric sections. The cardiac and fundic regions have thick folds that store large amounts of eaten food (MENIN; MIMURA, 1992). In current study it was impossible to confirm the existence of these regions as well as a widelydeveloped muscular coat in the pyloric region, which facilitates the expulsion of gastric emptying and food to the foregut. According to Stroband and Van der Veen (1981), the main functions of the stomach are the storage of food and defense against microorganisms for the production of hydrochloric acid which initiates digestion process within the stomach (GARTNER; HIATT, 2007).

The mucus of the stomach covered with simple epithelium was composed of cylindrical tubular gastric glands in the lamina propria. The apical surface of epithelial cells in three regions of the stomach showed a strong reaction to PAS, indicating the place of secretion of neutral mucopolysaccharides in the epithelium, for the protection of the mucosal surface. According to Diaz et al. (2003), the mucopolysaccharides secreted by the epithelial cells seemed to be important for the protection of the organ wall and for the inhibition of the action of microorganisms, also involved in enzymatic digestion of food. The neutral mucopolysaccharides, found in current study, may have a protective effect against high mucosal acidity of stomach contents.

Large amounts of gastric glands were reported in the stomach of the snook, composed of oxintopeptic cells, secreting H^+ and Cl^- and pepsinogen. According to Rotta (2003), the characteristics of the gastric glands vary and depend on the feeding habits of the fish. They are more ramified and developed in carnivorous species, as was observed in this study. According to Santos et al. (2007), the largest number of oxyntic glands may be related to higher demand in the production of secretion and ability to digest proteins since the fish eats live prey or food rich in protein.

Albrecht et al. (2001) studied the histology of the digestive tract in *Scomberomorus cavalla* and described the gastric glands as being few in number. In the anterior intestine (trophy), their number decreased considerably in the pyloric region; in the next portion of the intestine they did not occur or just looked as gland remnants. As different results found in current work, large amounts of gastric glands appeared in the trophy area, decreased in quantity in the fundic region and did not occur in the pyloric region.

The histochemical tests carried out in the gut of *Centropomus* revealed the presence of neutral and acid glycoproteins produced by epithelial cells that may protect the mucosa of the acid contained in the stomach. Santos et al. (2007) found the same result for *Pimelodus maculatus*.

The division of the gut according to the methodology of current study agrees with the division adopted by histophysiology in Stroband and Van der Veen (1981). In fact, these regions were not distinct with regard to the distribution of goblet cells.

The intestine is short, typical of carnivorous fish, with the intestinal epithelium presenting a simple type of cylindrical and prismatic goblet cells. According to Gartner and Hiatt (2007), the absorptive cells showed numerous microvilli where several enzymes were secreted. These cells acted in the process of the absorption of lipids, amino acids and carbohydrates.

Large numbers of goblet cells in the distal intestine were observed. The goblet cells produced intense lubrication and may be associated to the small bowel. According to Kierszenbaum (2008), goblet cells are cylindrical cells, mucus-producing, distributed among the enterocytes of the intestinal epithelium. These cells contain glycoproteins and are released by exocytosis, as observed in this study.The abundance of these cells and their secretory products may vary, depending on environmental conditions (BURKHARDT-HOLM et al., 1989). Different mucopolysaccharides have been correlated with the digestive functions. The presence of acid mucopolysaccharide secreted by goblet cells indicated a secretory function of the intestinal epithelium. In addition, the acid mucopolysaccharide-rich groups are sulfated peptic protease inhibitors which prevent bacterial infections and protect mucous membranes from mechanical actions (ULIBARRIE, 1982). Neutral mucopolysaccharides are involved with food digestion and emulsification of the chime and absorptive functions (CLARKE; WITCOMB, 1980). Moreover, it is possible that the quantity and the chemical composition of mucus may reflect the nature of ingested food (BURKHARDT-HOLM et al., 1989). This would possibly be an explanation for the intensity of mucus secretion in goblet cells observed along the proximal to distal intestine in this study.

Many infiltrated intraepitelial lymphocytes were found in the intercellular space of all segments of the proximal intestine. These lymphocytes are likely to have regulatory function, suppressing the immune response to intestinal antigens and inducing an immune response in the lamina propria (PABST, 1987). Thus, the large number of lymphocytes in the intestinal mucosa of fish suggests the existence of a local immune system, namely, mucosal (ROMBOUT et al., 1989).

Intraepithelial macrophages were found along the proximal and middle intestine, similar to those described by Santos et al. (2007). Since macrophages

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were found in the pyloric stomach, their presence may indicate the existence of a cellular process that acts as a cellular barrier of defense, or rather, as an immunological component in these regions. According to Santos et al. (2007), the presence of intraepithelial macrophages may suggest intraepithelial transport and degradation of proteins.

Conclusion

Centropomus undecimalis and *C. parellelus* display the main features of the digestive tract of carnivorous fish, such as a short esophagus and muscular stomach, with a relatively short intestine.

Both species show no difference in histological and histochemical features in wild and farmed specimens.

The goblet cells secrete neutral and acidic mucopolysaccharides, rich in sulfate groups, along the entire length of the intestine.

The presence of macrophages within the intraepithelial layer along the proximal intestine and transport medium may suggest intraepithelial degradation of proteins.

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