Title page

Title: MORPHOLOGY OF THE ORO-PHARYNGEAL CAVITY AND OESOPHAGUS OF THE FARMED ADULT AFRICAN CATFISH (Clarias gariepinus BURCHELL, 1822).

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Running title: African catfish oro-pharynx and oesophagus histology

Summary

The morphology revealed cornified horny plates lining the buccal surfaces of the lips. The tongue was not free moving, and the oesophagus was a narrow tube connecting the oro-pharyngeal cavity to the stomach with no valve or constriction separating it from the later. Histologically, the cavity wall was lined by stratified squamous epithelium containing eosinophilic club cells, mucous cells and taste buds. The laminar propria contained dense collagen fibres. No taste bud was seen on the tongue suggesting absence of role in food selection through gestation like the cavity wall which contained taste buds. The oesophagus was lined by stratified mucous epithelium containing club cells. Histochemistry revealed the presence of neutral, acid and combination of the two. The large extent of oesophageal mucification indicates need for mucin lubrication of food since teleost is known to lack salivary glands.

KEY WORDS: ORO-PHARYNGEAL CAVITY, OESOPHAGUS, HISTOLOGY, HISTOCHEMISTRY, MUCIN ,AFRICAN CATFISH
Introduction

The form and positions of the mouth, dentition on the jaws and oro-pharynx; and the gill rakers show close relation with the mode of feeding and kinds of food (Podoskina, 1993; Platelli et al., 1998; Salvador-Jr et al., 2009). In the *Chondrostoma nasus* L., an herbivorous fish, the ventral mouth position predisposes the fish to help up food overgrowing submerged stones (Sysa et al., 2006).

The lips, the primary food procuring organ assumes different forms and may be adhesive in some teleosts (Delariva and Agustinho, 2001). Girigis (1952 a,b) observed a stratum corneum and even horny protuberances on the lips of the herbivorous bottom feeder *Labeo horie*. Two sharp hornycutting edges in the upper and lower borders of the mouth immediately inside the lips enable the fish to take up food. Horny plates on the inner face of the lips in *Noemacheilus barbatulus* L. are used in trituration (Mester, 1971). The granular processes on the very board queer lips of bottom feeding *Pseudogobio esocinus* are important in food finders (Suzuki, 1956). Bransons and Hake (1972) observed the rich vascularization on the lips (and oro-pharyngeal) tissues of *Piaractus nigripinnis*, indicating a respiratory function in this fish which inhabits water deficient in oxygen. The oro-pharynx bears a variety of specialized organs for specific functions. The organs include- lamellar organ, buccal valves, tongue, pharyngeal pads and epibranchial organ (Al-Husain, 1949; Saxena, 1958; Kapoor, 1957a).

The oesophagus is usually a short and narrow tube connecting the bucco-pharynx to the stomach (Sinha, 1977; Hirji, 1983; Diaz et al., 2008; Cao and Wang, 2009). It is lined by stratified cuboidal to simple columnar epithelium with mucous cells and communicates with the swim bladder through the pneumatic duct in the rainbow trout *Salmo gairdneri* (Ezeasor, 1984), but psuedostratified epithelium with PAS and AB positive mucous cells in the *Leporinus taeniofasciatus* (Albrecht et al ,2001). The oesophagus of walking catfish *Clarias batrachus* according to Raji and Norouzi (2010), presents numerous deep longitudinal folds, lined by few layers of stratified squamous epithelium with numerous superficial mucous cells that changed to columnar epithelium at the end of oesophagus.
Despite the increasing interest in the commercial production of the African catfish, there is
dearth of information on the morphology of its basic digestive tract, unlike most teleosts in
available literature. In this paper we present our findings on the anatomy of the oro-pharyngeal
cavity and oesophagus of the farmed African catfish. The knowledge will enhance our
understanding of its adaptive digestive physiology.

**Materials and Methods**

Twenty adult African catfish sourced from a commercial aquaculture in Eastern Nigeria were
used for the study. They weighed an average of 900g and measured a standard body length of
45cm in length. The fish were euthanized with chloroform. The oro-pharyngeal cavity was cut
open bilaterally at the junction between the mandible and maxilla. The body cavity was cut open
through the ventral surface and the alimentary tract dissected out. The specimen under study –
the oro-pharyngeal wall and oesophagus were excised and immediately fixed in 10% neutral
buffered formalin.

The tissue was passed through graded ethanol, cleared in xylene, impregnated and embedded in
paraffin wax. Sections 5 – 6µm thick were obtained with Leitz microtome model 1512. They
were stained with haematoxylin and eosin for light microscopy examination (Bancroft and
Stevens, 1977). Mucins were demonstrated using Alcian blue (AB) at pH 2.5 (Steedman, 1950;
Lev and Spicer, 1964) and Periodic acid Schiff (PAS) with and without prior digestion with
diastase (Lillie and Greco, 1947, Ikpegbu et al., 2011). In addition, the PAS technique was
employed in combination with AB for neutral and acid mucin (Bancroft and Stevens, 1977).
Photomicrographs were taken with – Motican 2001 camera (Motican UK) attached to Olympus
microscope.

**RESULTS**

Grossly, the oro-pharyngeal cavity was bounded dorsally by the palate and ventrally by the
mandibular bone, cranially by upper and lower lips. The palate that formed the roof had three
horny plates, one cranial plate and two caudal plates. The cranial plate was semilunar while the
two caudal plates were separated by a thickened mucous membrane (Fig.1). The horny plates on
the mandible which formed the floor of the mouth were separated by thickened mucous membrane (Fig.2). The floor presented a tongue that was fixed (Fig.2). At caudal end of the dorsal wall of the oro-pharyngeal cavity were located two elevated round structures referred to as the pharyngeal pads. They were located about 2cm to the aditus oesophagus. The oesophagus was a short narrow tube connecting the oro-pharyngeal cavity to the stomach. On entering the thoraco-abdominal cavity it coursed caudo-dorsally to the liver and entered the stomach. There was no marked constriction separating the oesophagus from the stomach except the enlarged nature of the later.

**HISTOLOGY**

The tongue: the tunica mucosa presented stratified squamous epithelium containing eosinophilic club cells (Fig.3). No taste bud was observed in the epithelium. Dense collagen fibres were observed in the lamina propria/submucosa. The skeletal muscles of the tunica muscularis were oriented mostly longitudinal direction. Hyaline cartilage was present at the base of the tongue.

Oro-pharyngeal wall: The adult mucosa contained stratified mucous epithelium with large eosinophilic club cells, and occasional taste buds. The lamina propria contained dense collagen fibres in irregular orientation (Fig.4).

Oesophagus: the longitudinal fold mucosa was lined by stratified mucous epithelium containing eosinophilic club cells (Fig.5, 6). The core of longitudinal folds was of densely packed collagen fibres. The lamina propria- submucosa contained collagen fibres, and bundles of striated muscle in mostly longitudinal orientation (Fig.7). The tunica muscularis was of striated muscles mostly in circular orientation interspersed with longitudinal muscle bundles. Tunica adventitia was entirely of loose connective tissue with blood vessels.

**DISCUSSION**

The oro-pharyngeal cavity in conjunction with the branchial arches may filter and keep the food for proper trituration by the pharyngeal pad in the adult. This has been documented also in *Odontesthes bonariensis* (Diaz et al., 2006).

The wall of the oro-pharyngeal cavity of the adult African catfish presented epithelium of stratified mucous type. This epithelium has a protective function (Albrecht et al., 2001; Diaz et al., 2008). The dense collagen bundle seen in the lamina propria-submucosa region maybe
analogous to stratum compactum reported in some teleost, that support, strengthen and preserve the entirety of gut wall against sudden and violent extension (Burnstock, 1959, Ezeasor, 1986). The skeletal muscles may be involved in voluntary trituration. The presence of taste buds in the oro-pharyngeal wall as observed in this study has also been reported in the \textit{Odontesthes bonariesis} Diaz \textit{et al.}, (2006), and their presence suggest that the oro-pharyngeal wall may be involved in food selection or rejection by gustation (Ezeasor, 1982; Linser \textit{et al.}, 1998). The mucous cells produce mucin which is involved in lubrication and defence against pathogens (Albrecht \textit{et al.}, 2001; Micale and Mughia, 2011). The club cells present in teleost have been described as having a role in flight of fish from danger by secreting alarm substances (Singh and Kapoor, 1967). The hyaline cartilage is for support and provides point of attachment for the skeletal muscle seen (Falk – Petersen and Hansen, 2001).

The microanatomy of the tongue presented stratified mucous epithelia with club cells, collagen fibres and skeletal muscle. The tongue lacked taste buds. These features suggest an organ involved in mechanical function of voluntary feed trituration (Bishop and Odense, 1966; Maggese, 1967). The presence of rich vascularization suggest an active organ that needs nutrient and oxygen supply (Singh, 2006). The lack of taste buds as was observed in this study differs from the report on the presence of taste buds on tongue of \textit{Salmo garidneri} by Ezeasor (1982). This variation may be due to difference in species under study.

The oesophagus as documented by other researchers is a short narrow tube connecting the oro-pharyngeal cavity to the stomach (Hirji, 1983; Ezeasor, 1984; Sinha, 1986; Cao and Wang, 2009). Grossly the esophagus in this study is located directly caudal to the pharynx, and extends from the most caudal gill arch to the anterior opening of the stomach. This observation has been reported by Hamlin \textit{et al.}, (2000) in the work on haddock, \textit{Melanogrammmus aeglefinnus}. The longitudinal folds of the mucosa present in the oesophagus has been reported and provide the necessary distensibility during food intake (Ezeasor and Stokoe, 1980; Arellano \textit{et al.}, 2001; Trevino \textit{et al.}, 2011). The lining epithelium of stratified mucous has been seen in \textit{Micropogonias furnieri} but a stratified cuboidal to low columnar epithelia has been reported in \textit{Salmo gairdneri}, simple cuboidal epithelia has been reported in \textit{Perca fluviatilis L} , stratified columnar has been reported in yellow catfish \textit{Pelteobagrus fuladraco} (Hirji, 1983; Ezeasor, 1984; Diaz, \textit{et al.},
This variation may be phylogenetic or environmental. The presence of abundant mucous cells observed have also been reported and signifies the large requirement of mucin for lubrication during swallowing and increase in viscosity related to protection against abrasion and pathogens, as teleosts lack salivary gland (Scocco et al., 1998; Arellano et al., 2001; Kozaric et al., 2008). The club cells are involved in non-specific defense mechanism (Singh and Kapoor, 1967).

The presence of mostly circularly oriented striated muscle has been reported (Falk-Petersen and Hansen, 2001; Raji and Norouzi, 2010) and is associated with ability to voluntarily reject unwanted material (Jaroszewska et al., 2008). The lymphocytes seen are involved in specific defense mechanism (Ezeasor, 1984; Diaz et al., 2008). Absence of taste bud in the oesophageal epithelia as observed in this study has been reported (Diaz et al., 2006; Arellano et al., 2001; Raji and Norouzi, 2010; Albrecht et al., 2001; Hirji, 1983 and Diaz et al., 2008)). But taste buds presence have been reported in some teleosts oesophagus (Ezeasor, 1982, 1984). The varying shapes of mucous cells in the esophagus seen here have been reported in other teleosts (Hirji, 1983; Ezeasor, 1984). The presence of only circular striated muscle as seen in adult has been reported (Yang et al., 2010). This circular muscle may help produce uniform muscle contraction, thereby producing a synctium-like effect. Adventitia with loose connective tissue and adipose tissue seen in this region has been reported in literature also (Diaz et al., 2008).

HISTOCHEMISTRY
The adult oro-pharyngeal wall showed the presence of acid and neutral mucin, but the acid predominated showing more bluish colour in AB/PAS reaction. This may be explained by the need for acid mucin to act as protective coat to invading agents in the cavity, the tongue presented similar reaction (Diaz et al., 2006).

The Oesophageal mucins seen were both acidic and neutral, but the acidic mucin was slightly higher indicating need for more protection against pathogenic agents, prevention of damage to gut epithelium, and acting as lubricant to fibre-rich materials being an omnivorous fish (Pedini et al., 2001; Albrecht et al., 2001; Kozaric et al., 2008). The neutral mucins in the esophagus have been associated with pre-gastric digestion (Baglole et al., 1997). The purple colouration seen in some longitudinal folds signifies the presence of both acid and neutral mucin in equal quantities (Ezeasor, 1984; Diaz et al., 2006; Trevino et al., 2011). The esophagus of Sparus aurata
presented only neutral mucins (Saresquete et al., 1995). In the *Sola sola* only acid mucopolysacharides were seen in oesophageal mucous cells. In the *Salmo gairdneri* the anterior segment of the esophagus after AB/PAS procedure presented equal neutral and acid mucin, middle mostly purple while distal stained purple mostly (Ezeasor, 1984). Raji and Norouzi (2010) reported the presence of both neutral and acid mucin in the esophagus of both *Clarias batrachus* and *Serrasalmus natterieri*. Esophageal mucin has been suggested to play a role in regulating the pH of the stomach (Hirji, 1983).
REFERENCES


Fig. 1. Gross appearance of the roof of the oropharyngeal cavity showing the cranial dorsal horny plate (AHP) conforming to the outline of the upper lip (UL). Immediately caudal to this are bilateral oval shaped horny plates (CHA, CHb).

Fig. 2. Gross appearance of the lower jaw showing the lip (LP), bilateral ventral horny plates (VHP) and the tongue (T). B-Barbel.
Fig.3. Section of the adult tongue showing stratified squamous epithelium (EP) containing large eosinophilic club cells (CC) and mucuous cells (MC). Note collagen fibres (CF) in the lamina propria. H.&E. X 400.

Fig.4. Section of adult oropharyngeal mucosa showing stratified mucous epithelium containing eosinophilic club cells, and occasional taste buds (TB). Note collagen fibres (CF) in the lamina propria. MC- mucous cells. H. & E. X 400.
Fig. 5 Transverse section adult esophagus showing longitudinal fold (LF), which gave the lumen (EL) a narrow stellate appearance. Note the absence of muscularis mucosae. CM- circular skeletal muscle of tunica muscularis. H. & E. X40

Fig. 6. Section of adult oesophagus showing stratified mucous epithelium (EP) containing eosinophilic club cells (CC). Lamina propria contains collagen fibre (CF). Note MC- mucous cell. H. & E. X 400
Fig. 7. Transverse section of adult esophagus showing skeletal muscle bundles in the lamina propria-submucosa (SKM). H. & E. X100

Fig. 8. Section of oesophagus showing PAS positive mucous cells (MC). PAS X100
Fig. 9. Section of oro-pharyngeal wall showing PAS positive mucous cells (MC). PAS X400

Fig. 10. Section of oesohagus showing AB positive mucous cells (MC). PAS X400
Fig. 11. Section of adult oro-pharyngeal mucosa showing epithelial mucous cells that contain neutral (N) or acid (A) mucin. AB/PAS x400

Fig. 12. Section of adult oesophagus showing longitudinal fold epithelial that contain neutral (N) or acid (A) mucin. AB/PAS X100
Fig. 13. Section of adult oesophagus showing longitudinal fold epithelia that contain neutral (N) or acid (A) mucin or combination of both (C). AB/PAS x400