

## Original Article

# Comparative histological and histochemical studies on the pancreas of *Labeo rohita* (Hamilton, 1822), *Mystus vittatus* (Bloch, 1790) and *Notopterus notopterus* (Pallas, 1769)

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**Abstract:** The histological analysis, disposition and histochemical localization of tryptophan were investigated in the pancreas to compare the cellular organization and histochemical characterization in the pancreas of *Labeo rohita* (Hamilton, 1822), *Mystus vittatus* (Bloch, 1790) and *Notopterus notopterus* (Pallas, 1769) having different feeding habits. Histological analysis demonstrated that the exocrine pancreatic tissues were dispersed within the hepatic parenchyma and spleen in *L. rohita*. Thin septa of connective tissue separated parenchyma of liver and also the spleen from exocrine pancreatic cells. However, in *M. vittatus*, the discrete pancreatic tissue formed distinct oval or elongated acini interspersed with small area of islet of Langerhans and blood vessels. In *N. notopterus*, the rhomboidal acinar cells of discrete pancreatic tissue intercalated with comparatively clear and large islet of Langerhans. The exocrine acinar cells in all the three species were provided with prominent nuclei and densely packed zymogen granules. Histochemical localization revealed that the zymogen granules of exocrine acinar cells of all species exhibited varied intensities of tryptophan reaction, the precursor of various pancreatic enzymes which may be related to the food and feeding habits of the fishes under study.

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## Introduction

One of the most important digestive glands in teleosts is pancreas consisting of exocrine and endocrine tissue with various degrees of morphoanatomical and cellular variations (Eurell and Haensly, 1982; Youson et al., 2006; El-Said et al., 2010; Chakrabarti and Ghosh, 2012). Pancreas in teleosts shows numerous species variations with respect to their cytological structures as well as functional aspects (Sinha and Moitra, 1975; Dwivedi and Pandey, 1982; Vicentini et al., 2005; Petcoff et al., 2006). A hepatopancreas has been reported in the iridescent shark catfish *Pangasius hypophthalmus* (Seyrafi et al., 2009) and a spleenopancreas in *Barbus pectoralis* (Khaksar Mahabdy et al., 2012). The formation of glandular acinuses is a regular part of the exocrine pancreas; their cytological details and chemical nature have been investigated in fishes

having different feeding habits (Shafi, 1973; Bremer, 1980). Studies relating to precise cellular details, including the precise histochemical nature and functional aspects of the pancreas in Indian freshwater teleosts are limited.

The aim of our research was to explore the presence of pancreas in discrete or diffused pattern and to examine the structural and functional status of pancreatic tissues in *Labeo rohita*, *Mystus vittatus* and *Notopterus notopterus* having different feeding habits by histological analysis and histochemical localization. These species are of great interest to fish culture, including their considerable growth and excellent quality.

## Materials and methods

Mature specimens of *L. rohita* (28-30 cm in total length), *M. vittatus* (10-12 cm in total length) and

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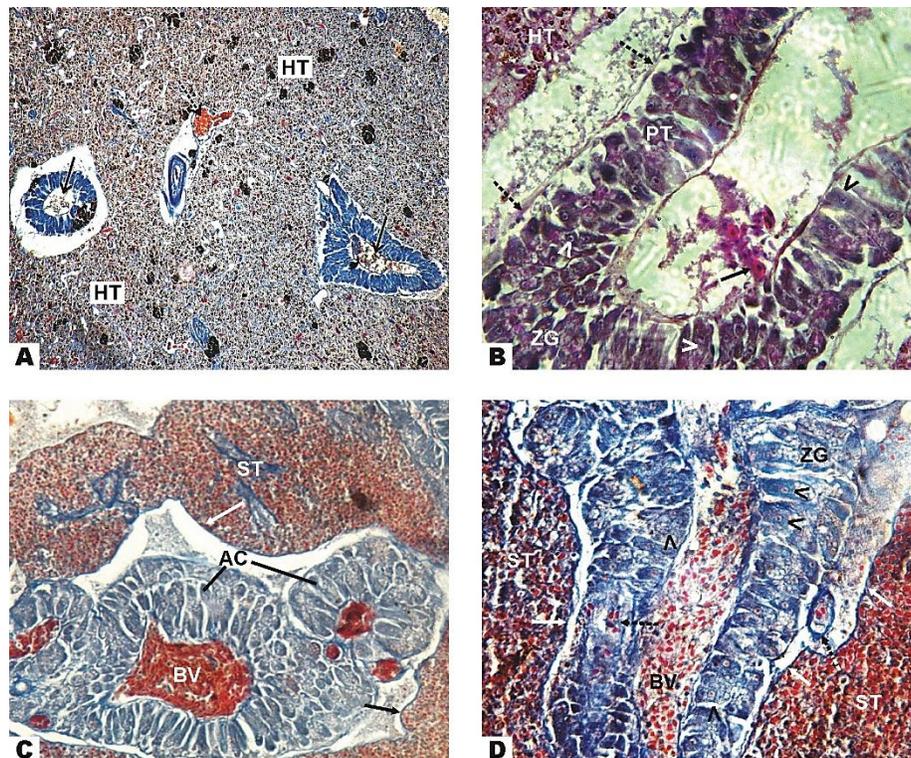


Figure 1. Photomicrographs of section of pancreas in *L. rohita* showing histological architecture stained with Delafield's Haematoxylin-Eosin (HE) and Mallory's triple (MT) stain. (A). Scattered round or triangle shaped pancreatic tissue (PT) distributed in between the hepatic tissue (HT). Note the presence of blood vessels (BV) (solid arrows) encircled by AC. Broken arrow indicates central sinusoid with erythrocytes (MT X100), (B). Higher magnification of hepatopancreas showing pyramidal or triangular acinar cells (AC) of PT arranged in several rows. AC are provided with basally located nucleus (N) with conspicuous nucleolus (arrow heads) and numerous zymogen granules (ZG). Note the presence of thin septa of connective tissue (broken arrows) which separates PT from HT. Solid arrow indicates blood vessels (BV) encircled by AC (HE X400), (C). Spleenic pancreas showing orientation of pancreatic AC surrounding BV. AC are distinguished from splenic tissue (ST) by thin septa of connective tissue (Solid arrows) (MT X200) and (D). Higher magnification of spleenopancreas showing AC around BV contains prominent nucleolus in nucleus (N) (arrow heads) and acidophilic ZG. PT is distinguished from HT by thin septa of connective tissue (solid arrows). Broken arrows indicate BV adjacent to AC (MT X400).

*N. notopterus* (23-25 cm in total length) were collected from local freshwater fish farm of Burdwan (23.2333° N, 87.8667° E), West Bengal, India during April-October, 2013. Fishes were anesthetized with an overdose of Chloral hydrate following the guidelines given by Institutional Ethical Committee.

**Histological preparation:** The body cavity was cut open through abdominal incision and small pieces of the discrete pancreas, pancreatic tissues associated with liver and spleen were removed and fixed in aqueous Bouin's fluid for 18 hrs. The tissues were then placed in 70% ethanol and subsequently dehydrated through ascending series of ethanol, followed by acetone and cleared with xylene, impregnated and embedded in paraffin wax (56-58°C). Serial sections of 4 µm thick were obtained with Leitz microtome. Deparaffinised sections were

brought to distilled water through descending series of ethanol and were stained with Delafield's Haematoxylin-Eosin (HE) and Mallory's Triple (MT) stain (Mallory, 1936).

**Histochemical preparation:** For histochemical study, the pieces of pancreas, hepatopancreas and spleenopancreas were removed and fixed in 10% neutral formalin for 16 hrs. The tissues were passed through graded series of ethanol, cleared in xylene and embedded in paraffin wax at 52-54°C in vacuum embedding bath. Serial sections of the tissues were cut at 8-10 µm in thickness. Deparaffinised sections were brought to distilled water and then subjected to histochemical demonstration of tryptophan by Dimethylaminobenzaldehyde (DMAB) Nitrate method (Adams, 1957).

Staining slides were dehydrated with graded series

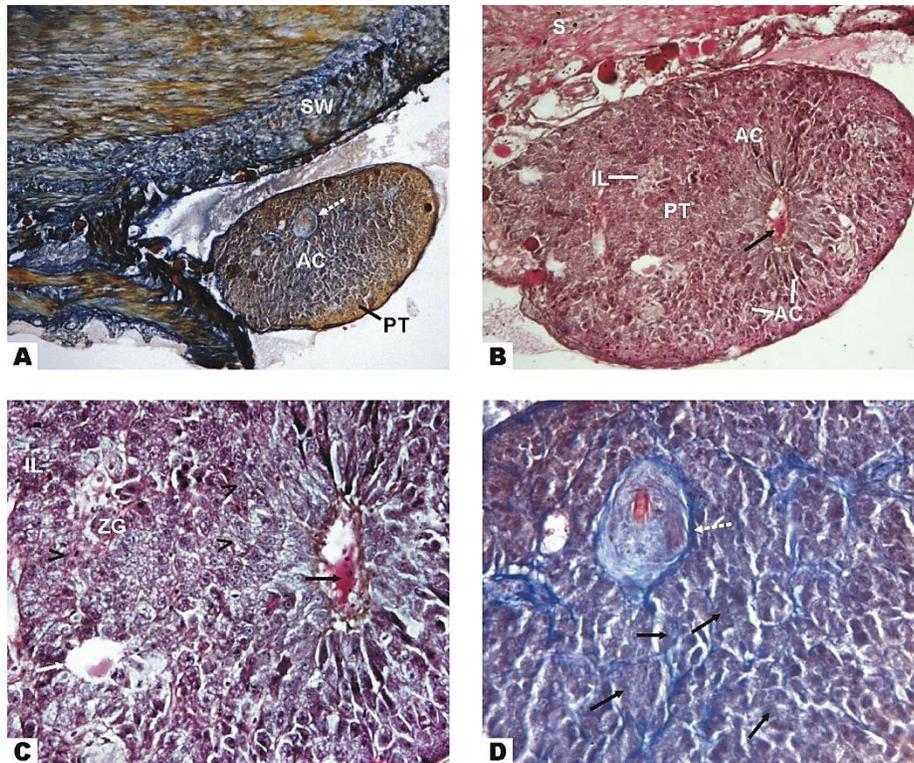


Figure 2. Photomicrographs of section of pancreas in *M. vittatus* showing histological architecture stained with Delafield's Haematoxylin-Eosin (HE) and Mallory's triple (MT) stain. (A). Showing pancreatic tissue (PT) having clusters of acinar cells (AC) adjacent to the stomach wall (SW). Broken arrow indicates small islet of Langerhans (IL) (MT X100), (B). PT attached with the wall of stomach (S) having dense organization of AC around blood vessels (BV) (arrow) and scattered IL (HE X200), (C). Higher magnification of AC with prominent zymogen granules (ZG) and conspicuous nucleolus in the nucleus (arrow heads). Note the presence of BV (solid arrows) and IL encircled by AC (HE X400) and (D). Presence of IL (broken arrow) in between AC (solid arrows) (MT X400).

of ethanol, cleared in xylene and mounted with DPX. The slides were examined and photographed under an Olympus-Tokyo PM-6 compound microscope.

## Results

***Labeo rohita*:** The pancreas in *L. rohita* is distributed within the liver parenchyma forming hepatopancreas and within the splenic tissues. The triangle oval or elongated patches of pancreatic tissues are randomly distributed within the hepatic parenchyma. The hepatocytes are arranged in cords around central sinusoids, which contained mainly erythrocytes (Fig. 1A). The hepatic cells contain granular cytoplasm with prominent nuclei (Figs. 1A, 1B). The hepatopancreas in this fish is separated from the hepatic parenchyma by a thin layer of connective tissue (Fig. 1B). The pancreatic patches are made up of exocrine acini, which are bounded by connective tissue, existed around hepatocytes (Figs. 1A, 1B) and the blood vessels and spleen (Figs. 1C, 1D). The

pyramidal or triangular acinar cells are large and arranged in two or more rows. A typical exocrine cell contains a broad basal region and a narrow apical part. The basal region contains a large spherical nucleus with a distinct nucleolus. The apical parts of the exocrine pancreatic cells contain acidophilic zymogen granules, which are relatively larger in size (Figs. 1B, 1D). In the present study, the patches of the islet of Langerhans cannot be detected in the hepatopancreas and spleenopancreas.

***Mystus vittatus*:** The pancreatic tissue consists of the clusters of acinar cells observed adjacent to stomach or attached to the wall of the stomach (Fig. 2A). In *M. vittatus*, the pancreas is relatively more organized and composed of a large number of acinar cells (Figs. 2B, 2C, 2D). Each acinus cell contains zymogen granules, relatively smaller in size than those of *L. rohita* and a spherical nucleus with a distinct nucleolus (Fig. 2C). Limited patches of islet of Langerhans and blood vessels are present in

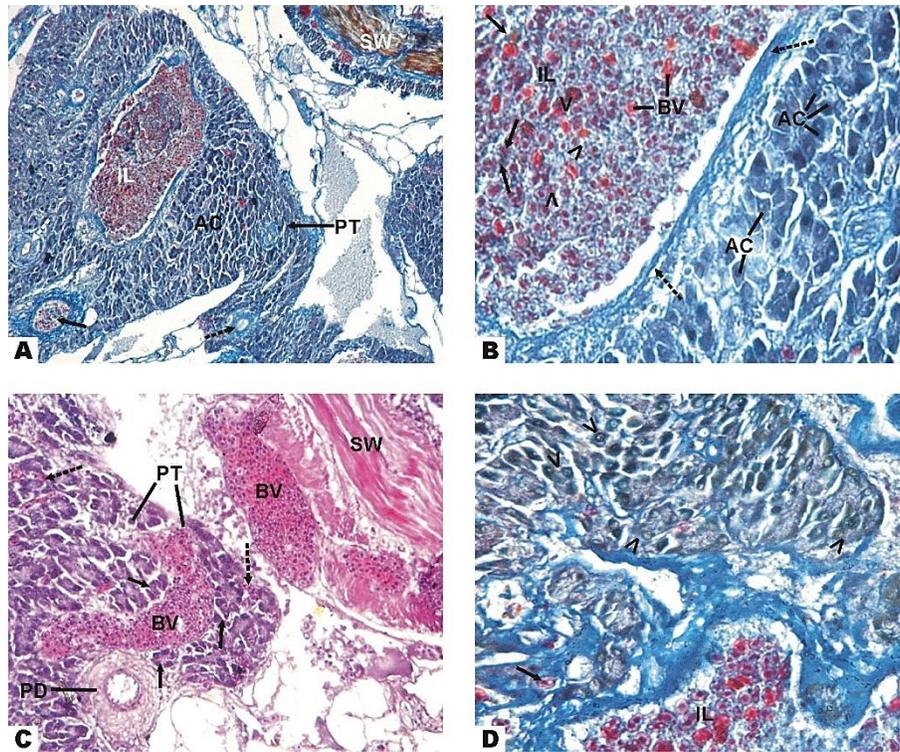


Figure 3. Photomicrographs of section of pancreas in *N. notopterus* showing histological architecture stained with Delafield's Haematoxylin-Eosin (HE) and Mallory's triple (MT) stain. (A). Showing pancreatic tissue (PT) adjacent to stomach wall (SW) having bunch of acinar cells (AC) encircling islet of Langerhans (IL). Note the presence of blood vessel (BV) (solid arrow) and pancreatic ducts (PD) (broken arrows) in the PT (MT X100), (B). Higher magnification showing IL separated from AC by connective tissue septa (broken arrows). IL showing  $\alpha$  cells (solid arrows) and  $\beta$  cells (broken arrows) in between BV (MT X400), (C). Showing exocrine PT consisted of cluster of AC (solid arrows) attached to SW. Note the presence of BV among AC and attached to the SW. Broken arrows indicate scattered BV in between AC. PD indicates pancreatic duct (HE X200) and (D). Rhomboidal shaped AC (arrow heads) provided with diffused zymogen granules. Note the presence of IL and scattered blood cells (solid arrow) among AC (MT  $\times$  400).

between the exocrine cells (Figs. 2B, 2C). Pancreatic duct is also observed, encircled by acinar cells (Figs. 2A, 2D).

***Notopterus notopterus*:** In *N. notopterus*, the pancreatic tissues are sandwiched in between pyloric caeca and the wall of the stomach in distal region. The exocrine pancreatic tissue consists of clusters of rhomboidal type of cells mostly organized in acini. The acinar cells are provided with basophilic cytoplasm, distinct nuclei and many eosinophilic or aniline blue positive zymogen granules (Figs. 3B, 3C, 3D). Blood vessels and pancreatic ducts are present here and there with in the ground pancreatic tissue (Figs. 3C, 3D). The zymogen granules are diffused and/or relatively smaller in size than those of *L. rohita* in the pancreatic acinar cells (Figs. 3B, 3D). Moreover, the pancreas of *N. notopterus* also presents the endocrine tissues as islet organ in between the exocrine cells

(Figs. 3A, 3B, 3D). Langerhan's islets are surrounded by a delicate connective tissue. In the islet, cells are arranged in scattered way, between which were capillaries. The  $\alpha$  and  $\beta$  cells are clearly differentiated in the islets. The  $\alpha$  cells are bigger with the prominent nucleus while  $\beta$  cells are smaller, spherical with rounded nuclei (Figs. 3B and 3D).

***Histochemical demonstration of tryptophan:*** The zymogen granules of cytoplasm in pancreatic acinar cells of *L. rohita* exhibit maximum intensity of tryptophan reaction while hepatocytes display weak reaction for this histochemical test (Figs. 4A, 4B). The blood cells of hepatic sinusoids also show intense tryptophan reaction (Fig. 4A). The acinar cells in the splenic pancreas exhibit intense reaction for tryptophan, though comparatively less than that of hepatopancreas. The spleen tissue itself shows a weak tryptophan reaction (Fig. 4B). The zymogen granules of the acinar cells of the pancreas in

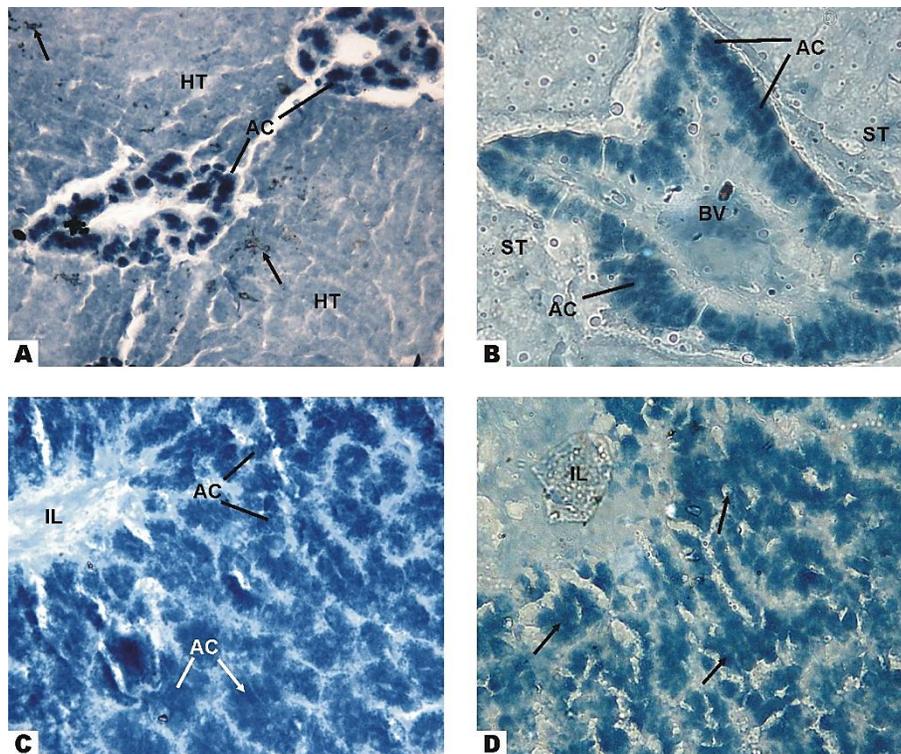


Figure 4. Photomicrographs of section of pancreas in *L. rohita*, *M. vittatus* and *N. notopterus* showing histochemical localization of tryptophan (DMAB). (A). Hepatopancreas of *L. rohita* showing intense tryptophan reaction in acinar cells (AC) of pancreatic tissue and weak reaction in hepatic tissue (HT). Solid arrows indicate positive tryptophan reaction in blood vessels (BV) (DMAB X200), (B). Spleenopancreas of *L. rohita* showing intense tryptophan reaction in acinar cells (AC) of pancreatic tissue and weak reaction in splenic tissue (ST). Note moderate reaction of tryptophan in BV (DMAB X400), (C). Strong intensity of reaction for tryptophan in zymogen granules of AC and weak reaction in islet of Langerhans (IL) of *M. vittatus* (DMAB X400) and (D). Pancreas of *N. notopterus* showing acute tryptophan reaction in the AC (solid arrows) and weak reaction in IL (DMAB X400).

*M. vittatus* display a strong tryptophan reaction while the islet of Langerhans cells exhibit very weak reaction (Fig. 4C). In *N. notopterus*, the large number of acinar cells in the pancreatic tissue shows maximum intensity of reaction for tryptophan. The islet of Langerhan cells in between the acinar cells exhibit weak reaction for tryptophan (Fig. 4D).

### Discussion

The diffused form of the pancreas as an organ in teleosts has been studied by different authors (Chen et al., 2006; Tocher et al., 2008). Gonzalez et al. (1993) reported the presence of diffuse exocrine pancreas, which expanded through the mesentry, around some digestive organs of disseminates within the intraperitoneal adipose tissue in *Serranus cabrilla*. El-Said et al. (2010) also described the acinar arrangement of hepatopancreas in *Mugil cephalus* and *Sparus aurata*. In the present investigation, in *L. rohita* the pancreas exists as

intrahepatic as well as in the splenic tissues. On the other hand, in carnivorous *M. vittatus* and *N. notopterus*, the pancreas is a compact gland extended over the surface of the stomach and anterior part of the intestine. Khanna (1961; 1963) also observed compact nature of the pancreas in *Clarias batrachus*, *Mystus aor*, *Silonia silondia* and *Heteropneustes fossilis*.

In the present study, the exocrine acinar cells in the hepatopancreas and spleenopancreas of *L. rohita* are arranged in two or more rows surrounding a central blood vessel. The zymogen granules in the acinar cells are numerous and large in size than those in *M. vittatus* and *N. notopterus*. In herbivorous, *L. rohita* the intestine is enormously coiled, an adaptation for retention of food materials for a large period of time and the only source and copious amount of digestive enzymes are needed for effective of enzyme is the hepatopancreas digestion of food materials. The pancreatic juices along with

bile juice after being secreted from the hepatopancreas is collected in the gall bladder and ultimately emptied in the alimentary canal. Yamane (1973) gave an account of the presence of amylase in the cytoplasm of the acinar cells in diffuse pancreas and confirmed that these cells were the centre of amylase production in the carp. Ray (1988) observed that gall bladder of three species viz., *Colisa fasciata*, *Ambassis nama* and *A. ranga* with hepatopancreas, exhibited higher enzymatic activities between pH 7.5 and 8.5. Further, in the present study the concentration of the zymogen granules in the pancreatic acini of hepatopancreas in *L. rohita* appears to be higher than that of the spleenopancreas. Therefore, it may be assumed that the acinar cells of hepatopancreas are physiologically more active in respect to secretion of digestive enzymes. Alboghobeish and Khaksar Mahabdy (2005) emphasized that pancreatic tissue in *Ctenopharyngodon idella* gradually invaded the liver along the branches of the portal vein. The combined hepatic and pancreatic tissue are collectively called hepatopancreas.

Based on the results, in *L. rohita* the scattered splenic lobules along with pancreatic acinar cells enclose with the wall of the intestine may have similar digestive function like that of hepatopancreas. In *Barbus pectoralis*, Khaksar Mahabdy et al. (2012) recorded that the spleenopancreas performed many functions such as lymphatic cell production and digestion of food materials. In *M. vittatus* and *N. notopterus* the basophilic zymogen containing cells of the exocrine pancreas is for the production and storage of pancreatic enzymes that are delivered to the digestive tract through a network of ducts for effective digestion of protein-rich food materials. Field et al. (2003) reported that the exocrine pancreatic tissue produces digestive enzymes, such as trypsin, amylase and carboxypeptidase A for effective digestion of food in zebra fish (*Danio rerio*).

The results showed the prominent  $\alpha$  and  $\beta$  like cells in the endocrine component of *N. notopterus* and

*M. vittatus* may have some role in carbohydrate metabolism. Ikpegbu et al. (2012) observed islet of Langerhans interspersed in the majority of exocrine cells in *Clarias gariepinus* responsible for hormone production like insulin.

In the present study, the distribution and localization of tryptophan content was observed in the exocrine acinar cells at varied intensities in *L. rohita*, *M. vittatus* and *N. notopterus*. Tryptophan, the precursor of pancreatic enzymes mainly associated with the zymogen granules of acinar cells. The varied intensities of tryptophan reaction in the acinar cells of the aforesaid fishes may be related with the synthesis and secretion of pancreatic enzymes in different proportions according to their requirement relating to their feeding habits.

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