Unique localization of disseminated pancreas in the esophagus of catfish (*Clarias gariepinus*) with reference to sexual dimorphism

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Running title

Disseminated (esophageal) pancreas in African catfish

Abstract

Background: The fish pancreas composed of two portions: compact and disseminated. However, little has been elucidated in catfish. The present study describes a unique localization of the disseminated pancreas in African catfish. Methods: Paraffin sections were processed for either routine histological examination following staining with hematoxylin and eosin (H & E), periodic acid Schiff's, or were subjected to immunohistochemical staining for detection of both insulin producing β-cells and glucagon producing alpha cells. Results: Our investigation showed that the pancreas of catfish consisted of both compact and disseminated portions. The compact pancreas was embedded in the mesenteric adipose tissue between the spleen, stomach, and liver. However, the disseminated one showed unique localization in the tunica adventitia of the middle portion of the esophagus. The pancreas consisted of two portions, exocrine and endocrine. Furthermore, in both types of pancreas, the female showed a significantly higher ratio for the endocrine islet area/pancreatic tissue area than that of the male. Also, a significantly higher ratio for both insulin and glucagon positive area/islet area in the female pancreas (Compact and disseminated) than that of the male. In conclusion: The present study provides evidence on a unique localization of the disseminated pancreas in the esophagus of catfish. Furthermore, we revealed sex-related difference in the endocrine portion in both pancreatic tissues with more development in the female. The study suggests that sex hormones could be contributed to such sexual dimorphism. However, further investigation is required to compare the degree of development during the spawning and resting seasons.

Keywords: Esophagus, Glucagon-secreting alpha cells, Insulin-secreting beta cells, Islets of Langerhans, Pancreas.

1. INTRODUCTION

The Catfish is considered as one of the major species of the freshwater teleost fish and it represents 12% of all teleost (Wilson and Reeder, 2005). Additionally, the African Catfish (*Clarias gariepinus)*, despite their name, is widely distributed in Africa as well as in some areas of Asia and Europe (FAO; Turan, 2016). Generally, the catfish in Africa, especially in Egypt showed high economic value as it has been considered as an important source of food, and their significant contribution to income due to their export to other countries all over the world (Zaghloul et al., 2017) . In the last three decades, the catfish farming and production have been increased dramatically in Egypt due to their relatively high growth rate and its ability to withstand hard environmental conditions (Shaalan et al., 2017) as well as their high resistance to diseases (Shourbela et al., 2020) .

The fish species had more numbers of endocrine glands than that in mammals including Corpuscles of Stannius, Ultimobranchial, Pituitary, pineal, chromaffin and interrenal tissue and islets of Langerhans (Prasad et al., 2017). The pancreas is one of the most important glands associated with the gastrointestinal tract and consists of both the exocrine (acini and pancreatic duct) and the endocrine portion (islets of Langerhans) (Kaptaner, 2019). The exocrine part responsible for the digestion of carbohydrates, lipids, and protein via secretion of enzymes (Senoo, 2000). The endocrine portion releases hormones directly into the bloodstream that regulate the blood glucose level (Geyer et al., 1996). On contrary to mammals, the pancreas in the majority of teleosts and carp fish is composed of a compacted (main) portion (observed as a white mass associated with mesenteries between spleen, gall bladder, and stomach), hepatopancreas (exocrine tissue scattered between hepatocytes), and disseminated portion (Brinn, 1973; Naguib et al., 2009; Youson and Al-Mahrouki, 1999). These areas or islets are also known as Brockmann bodies consisting of both endocrine and exocrine tissue, which can be found in variable percentage among the species often in conjunction with smaller accessory islets (Agulleiro et al., 1993; Brinn, 1973; Epple, 1969; Mokhtar, 2015).

Even though investigations of the pancreas localization and structure in most teleost have already been reported, little attention has been paid to the distribution and percentage of both endocrine and exocrine part of the pancreas among both compact and disseminated types of the pancreas in African catfish. Furthermore, there is a dearth of information about the sex-related differences in the percentage of endocrine to exocrine portion as well as the ratio of both glucagon and insulin positive cells in the islets among both types of the pancreas. Therefore, the aim of our research is to investigate the distribution of pancreatic islets in different regions in catfish, with a comparison of the area ratio of the islet and both α and β-cells among both sexes by both histological and immunohistochemical means.

2. MATERIAL AND METHODS

2.1 Experimental animals and ethics statement

A total of twenty apparently healthy African catfish (Clarias gariepinus) of both sexes (10 males and 10 females) with average one-year age were purchased from Behira commercial fish farms, and They were transported in water tanks equipped with an air pump. The age was determined from the recorded data by the commercial fish farms that was dealt with. The average length and weight of male fishes were 46.0 ± 2.8 cm, and 600.0 ± 5.8 g, respectively, but that of females were 39.0 ± 3.5 cm, and 490.0 ± 5.8 g, respectively. The catfish sex was externally determined by examination of the urogenital region (supplementary figure 1A and 1D). All experimental procedures were performed in accordance with the ethics and regulations guide of the Animal Care and Use for laboratory animals, including fish approved by the Institutional committee at Zagazig University, Faculty of Veterinary Medicine with the approval number: ZU-IACUC/2/F/116/2018).

2.2 Sample collection and fixation

Following stunning of the catfish, a ventral midline incision was extended from the urogenital opening toward the cranial region, and the esophagus (cranial, middle, and caudal portions), liver, and adipose tissue within the mesentery in the triangular area between intestine, stomach, liver, and gall bladder (supplementary figure 1C and 1F). Then, the specimens were immediately fixed in Bouin's solution overnight at 4ºC.

2.3 Histological procedures and immunohistochemical staining

After overnight fixation, the specimens were washed and were subsequently dehydrated in ascending concentrations of ethyl alcohol, cleared in xylene (three changes/ thirty minutes each), then embedded in melted paraffin (three changes/one hour each), then paraffin blocks were prepared. Finally, 4 µm paraffin sections were obtained and used for either routine histological examination following staining with hematoxylin and eosin (H & E), periodic acid Schiff's, or were subjected to immunohistochemical staining for detection of both insulin producing β-cells and glucagon producing alpha cells.

The immunohistochemical staining was performed according to our previous protocol (Elewa et al., 2010). Briefly, the deparaffinized and rehydrated sections were subjected to antigen retrieval via heating at 105 °C in 10 mM citrate buffer, pH 6.0 for 20 min. Then, the sections were kept at room temperature for 30 min cooling. Then, peroxidase blocking was performed by incubating the sections in a solution of 3% H_2O_2 in absolute methanol for 20 min at room temperature followed by washing with distilled water and incubation with blocking serum (goat serum for insulin staining and mouse kit for glucagon staining) for 1 hour at room temperature. For negative control, we incubated sections

with PBS instead of the primary antibody (the data not show). Then, the sections were overnight incubated with the specific primary antibody at 4 ºC (polyclonal rabbit anti-insulin "Cat. No. Ab210560, Abcam", and monoclonal mouse anti-glucagon "Cat. No. 14-9743-8, Invitrogen") diluted at 1:50. Such primary antibodies raised in mammalian species have been successfully used in several previous studies to investigate the endocrine pancreas of various teleost species (Kaptaner, 2019). Then, the sections were washed in Tris-Buffered Saline (TBS); followed by incubation with biotin-conjugated secondary antibodies (goat anti-rabbit IgG antiserum for insulin stained sections and anti-mouse IgG antiserum for glucagon stained sections) for 60 min, at room temperature. Then, the sections were washed three times (5min/each) in TBS and followed by incubation with horseradish-peroxidase conjugate for 30 min. Horseradish peroxidase activity was visualized by incubating the sections for with a substrate-chromogen solution containing 3,3'-diaminobenzidine tetrahydrochloride (DAB)- H_2O_2 solution for 3 min. Sections then were next washed in double-distilled water and shortly stained with Mayer's hematoxylin for counterstaining. For negative control sections, TBS was added instead of the primary antibody.

Photographs from both immunohistochemical and H&E stained sections were captured using a Sony super steady cyber shot digital camera (Dsc-W800, Sony, Japan) connected to an Olympus BX 21 light microscope at Histology and Cytology Department, Faculty of Veterinary Medicine, Zagazig University, Egypt.

2.4 Morphometrical examination

Morphometrical measurements were performed using an Image J analysis free software (Fiji image j; 1.51 n, NIH, USA) to measure the ratio of islet area/pancreatic area, the number of the islets/ total pancreatic area in both compact (in mesenteric adipose tissue) and disseminated (that was observed in the wall of the middle portion of the esophagus) pancreas and comparing it among both sexes using photomicrographs captured from H&E stained sections at 100x magnification. Furthermore, we measured the glucagon and insulin positive areas in the compact and disseminated pancreas in both sexes using photographs that have been captured from immune-stained sections of both male and female fish at 400x magnification.

2.5 Statistical analysis

For comparison of the sex differences in the ratio of the islet area/pancreatic area, as well as the positive area ratio of either insulin or glucagon/islet area. In the current investigation, we performed the Mann-Whitney *U* test as a non-parametric test used to compare the non-parametrical Morphometrical values of samples from both male and female. The data have been presented as mean ± standard error (SE) and statistical significance was determined when *P* values ≤ 0.05.

3. RESULTS

3.1 Anatomical features

Morphologically, the adult African catfish has a long cylindrical shaped body, smooth skin bearing no scales with blackish color on the dorsal and lateral surface as well as the grayish color ventral surface. The male can be easily recognized externally from the female via the existence of the sexual papilla in the urogenital region. Such sexual papilla was observed as a nipple-like projection just behind the anal opening in males (supplementary Figure 1A) but was absent in females (supplementary Figure 1D). Furthermore, internally the male testes appear creamy white color (supplementary Figure 1B), and the female ovary had a brownish-red color (supplementary Figure 1E). The compact pancreas was observed to be concealed in mesenteric adipose tissue located in the triangular area between the spleen, stomach, and liver (supplementary Figures 1E and 1F). The esophagus of catfish is short, extending anteriorly from the pharynx and continued caudally in the cardiac region of the stomach. The esophagus of catfish was divided into 3 portions (anterior, middle, and posterior) according to shape and location. The anterior portion appeared as funnel-shaped, while the other portions were tubular in shape. The posterior portion is covered by one of the liver lobes, however the middle portion was not covered and began after the end of the funnel-shaped anterior portion (supplementary Figures 1B and 1F).

3.2 Histological observations

3.2.1 Histomorphometrical characterization of the compact pancreas

The compact pancreas was observed to be embedded in the mesenteric adipose tissue. It consisted of a poorly demarcated lobulated gland, with very delicate connective tissue (CT) septa extending from a thin CT capsule. The parenchyma of the pancreas comprised of exocrine and endocrine parts. The exocrine unit consists of tightly packed pancreatic acini and duct system. The endocrine portion appeared as pale staining islets distributed among the exocrine tissue (Figures 1A to 1D). The pancreatic islets in female were oval to ovoid-shape, but that of a male was of irregular shape and of an apparently smaller size than that of females (Figures 1C and 1D). Interestingly, the average area ratio of islet/pancreatic area was significantly higher in the female than that of males (Figure 1E). On the contrary, the male showed higher numbers of islets/pancreatic areas but of non-significant differences (Figure 1F).

3.2.2 Histological characterization of various esophageal regions

As shown in (supplementary Figure 2), the wall of the esophagus consisted of 4 tunics (mucosa, submucosa, muscular, and adventitia). Histologically, the anterior, middle and posterior portions of

the esophagus could be distinguished by their mucosal folds, lining epithelium, luminal area. The anterior portion of the catfish esophagus is characterized by the presence of highly folded mucosa (elongated leaf-like folds that were consisted of primary, secondary, and tertiary folds) with a narrow lumen (251.67± 14.84 µm). The lining epithelia of such folds was characterized by the presence of large polyhedral shaped club cells that showed a negative PAS reaction, as well as numerous PAS positive goblet cells (supplementary Figure 2A and 2B). However, a moderate size lumen (573.82±43.76 µm) and less folded mucosa was observed in the middle portion of the esophagus than that of the anterior portion. Furthermore, the lining epithelia characterized by the absence of club cells as well as less PAS positive goblet cells. Interestingly, a disseminated pancreatic tissue was observed in their adventitial layer, in close contact with their muscular layer. Such pancreatic tissue consisted of both endocrine and exocrine tissue similar to that of the compact pancreas (supplementary Figure 2C and 2D). The posterior part of esophagus has the widest lumen (1235.61±74.76 µm) and characterized by the absence of such disseminated pancreatic tissue (supplementary Figure 2E and 2F).

3.2.3 Histomorphometrical characterization of the disseminated (esophageal) pancreas

The disseminated pancreas in catfish was noted to be in close contact with the tunica muscularis of the middle portion of the esophagus in both sexes, where it was observed to be dispersed in their adventitia (Figure 2A to 2D). Similar to the compact pancreas, the disseminated pancreas consisted of exocrine and endocrine portions. The parenchyma of the former portion consisted of serous secretory acini and excretory duct. The endocrine portion consisted of islets of Langerhans. The shape of such islets was irregular to ovoid in males and oval to ovoid in females. Similar to the compact pancreas, the morphometric measurements revealed a significantly higher ratio of the islet`s of Langerhans area/ pancreatic area in the disseminated pancreas of the female than that of the male. However, contrary to the compact pancreas, the female showed a higher percentage of islet`s number/ disseminated pancreatic area ratio than that of male but of non-significant difference (Figure 2E and 2F).

3.2.4 Immunohistochemical examination of glucagon positive alpha cells and insulin positive β-cells

In the compact pancreas, the glucagon immune-positive alpha cells were spherical to spindle-shaped and showed cytoplasmic processes in both sexes. In a male, such cells were located in the peripheral area of the pancreatic islet and few cells were observed in the mantle region (Figure 3A and 3C). However, in the female such cells were observed in both periphery, mantle, and in the center and central region of the islet (Figure 3B and 3D). Statistical analysis showed a significant higher

glucagon immune-positive area ratio/total islet's area in the female than that of the male (Figure 3E). In the disseminated (esophageal) pancreas, the glucagon immunoreactive cells were arranged in the peripheral and the mantle region of the islet but were absent from the islet core (Figure 4A to 4D). Similar to the compact pancreas, the female showed significantly higher glucagon positive area ratio/ total pancreatic islet's area in the esophageal adventitia than that of males (Figure 4E).

Figure 5A to 5D showed spindle-shaped insulin immunoreactive β cells occupying most of the islet center. Furthermore, in males, a small cluster of beta cells was occasionally observed between the exocrine acini (Figure 5C). Morphometrical analysis of the insulin positive area ratio/ islet's area within the compact pancreas, revealed a significantly higher ratio of the females than that of males (Figure 5E).

Examination of the islets within the esophageal pancreas denoted that the insulin immunoreactive cells were located mainly in the islet's center as well as, occasional localized in the mantle region (Figure 6A to 6D). Statistical analysis clarified that the insulin positive area ratio/ islet's area within the esophageal pancreas was significantly higher in females than that of males (Figure 6E).

4. DISCUSSION

Catfish is one of the freshwater teleost fish that live near to the bottom. It is an omnivorous type that has a convoluted tubular gastrointestinal tract (short esophagus, stomach, and followed by intestine) and is known as stomach teleost (Lee et al., 2001; Mello et al., 2019; Moawad et al., 2017). In fish, it has been reported that the alimentary pattern could affect hormonal secretion (Navarro et al., 2002). Furthermore, two forms of the pancreas (compact and disseminated) were reported in various fish species (Chen et al., 2006; Luchini et al., 2015; Mokhtar, 2015; Tocher et al., 2008; Youson et al., 2006), however, reports explaining the localization and morphology of disseminated portion in catfish remain scarce.

The present study provided a unique localization of the dispersed pancreas in the esophageal wall of catfish (middle esophageal portion). Moreover, we revealed the sex-related difference in the endocrine portion. Histologically, we could identify three portions of the esophagus in catfish (anterior, middle, and posterior) according to lumen size, mucosal folds, and lining epithelium. Conversely, only two esophageal portions (anterior and posterior) were reported in both catfish and grass carp (Abd El Hafez et al., 2013). The anterior esophageal portion is characterized by highly folded mucosa with a narrow lumen. The lining epithelia of the folds has large polyhedral club cells and goblet cells. This was inconsistent with previous report by (Abd El Hafez et al., 2013). However, in the anterior portion of sea bream esophagus, the club cells were absent and only two types of goblet cells (mature and immature) was reported in (Abidi and Parwez, 2015) and (Abumandour and El-

Bakary, 2018). The middle and posterior portions showed moderate, and large size lumen, less folded mucosa than anterior part, with the absence of club cells and a smaller number of PAS-positive goblet cells. Interestingly, our results clarified a unique localization of the disseminated pancreas in the adventitia of the middle portion of the esophagus in both sexes. However, in *Labeo calbasu,* Ghosh and Chakrabarti, 2016 reported the presence of the exocrine pancreatic acini and ducts within the hepatic parenchyma tissue as hepatopancreas and in spleen as spleenopancreas. In *Mystus gulio*, the same authors stated that the disseminated pancreas was attached to the outer wall of the stomach.

Histologically, both compact and disseminated pancreas consisted of poorly lobulated glands with very delicate connective tissue septa. Each lobule is composed of exocrine and endocrine portion. The exocrine portion consisted of secretory pancreatic acini and pancreatic duct. Similar findings were reported in the compact pancreas (Mokhtar, 2015; Naguib et al., 2009; Sheibani and Pahlavan Yali, 2006). The endocrine portion appeared as pale staining islets distributed among the exocrine tissue, similar to that recorded in other previous reports (Chakrabarti and Ghosh, 2015; Luchini et al., 2015). Endocrine tissue consisted of Large principal islet and numerous smaller islets, as in most previous studies (Groff and Youson, 1997; Stefan and Falkmer, 1980). The islets of Langerhans were responsible for the secretion of insulin hormone by beta cells to maintain blood glucose level normal. Alpha cells were responsible for the secretion of glucagon hormone that works to raise the concentration of glucose and fatty acids in the bloodstream (Geser, 1976). On the other hand, it has been reported that the islet organ in the hagfish consisted only of insulin and somatostatin cells with absence of glucagon cells (Heller, 2015).

The present study revealed an oval to ovoid shaped islets in the female pancreas and of irregular shaped in males, similar to that reported in grass carp (Mokhtar, 2015) and in lake van fish (Kaptaner, 2019), but showed large circular shaped in both sexes of the toadfish pancreas (Palazón-Fernández et al., 2011). Islet cells were spherical and spindle in shape, as in red-eadred slider (Ku et al., 2001). A recent report from humans and animal models revealed sexual difference in the ratio of different hormone-secreting cells in the pancreas of the same species (Parchami and Kusha, 2015). Furthermore, it has been reported that prolactin secreted from the pituitary gland and placental lactogen during pregnancy contributes to the expansion of β-cell mass (Huang et al., 2009). Additionally, rapid enlargement of β-cell mass was observed during pregnancy (Gannon et al., 2018). Similarly, in the current investigation, significant higher ratio of the area of pancreatic islet was observed in female catfish than that of males. Additionally, the current investigation revealed significant higher glucagon and insulin positive area in the islets of female catfish pancreas than that of males. These results were inconsistent with other reports of an increase in glucagon content in

pancreases of female mice (Vagn Bonnevie-Nielsen, 1980, 1982). Therefore, we suggested that more hormonal secretion from the female islets could be associated with the ovulation and spawning period.

In the male, glucagon positive cells were detected in peripheral and mantle regions of the islets (Lee et al., 2003). On the contrary, in the female, the alpha cells were observed to be arranged mainly in the peripheral, mantle. However, few cords were detected in the center of the islet. This was consistent with other reports in *Lepisosteus osseus,* (Youson et al., 2001); in *Amia calva,* (Kong et al., 2002) in *Cyprinus Carpio,* and in splenic pancreatic lobe of ddN mouse (Lee et al., 2010). Moreover, similar to that reported in Bowfin (Scheuermann et al., 1991) and in *Silurus asotus and Siniperca scherzeri* (Lee et al., 2001), the insulin-immunoreactive cells in both compact and disseminated pancreas, were localized mainly in the center of the islet in both sexes. On the contrary, the beta cells were reported to be localized in the periphery of pancreatic islets in angler fish (Johnson et al., 1976) and *in Mugil auratus and Mugil saliens* (Lozano and Agulleiro, 1986).

5. CONCLUSION

In the catfish (Clarias gariepinus), two types of pancreas were observed; compact and disseminated one. The former was detected pancreas in the triangular area between liver, stomach, spleen and gallbladder. The later type was embedded in the adventitia of the middle portion of the esophagus. Interestingly, we clarified some regional distribution of both insulin and glucagon positive cells in the pancreatic islets of both types. Additionally, we reported sex-related differences in the size of the islet's as well as the positive area ratio of both insulin and glucagon cells with more significant values in the females than that of males.

Data availability statement

on what request the data that is presented in this manuscript will be available to the readers. It can be on "requesting the corresponding author request".

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476

Figure1

Male

Female

A

EX

250 μm

B

EX

EX

250 μm

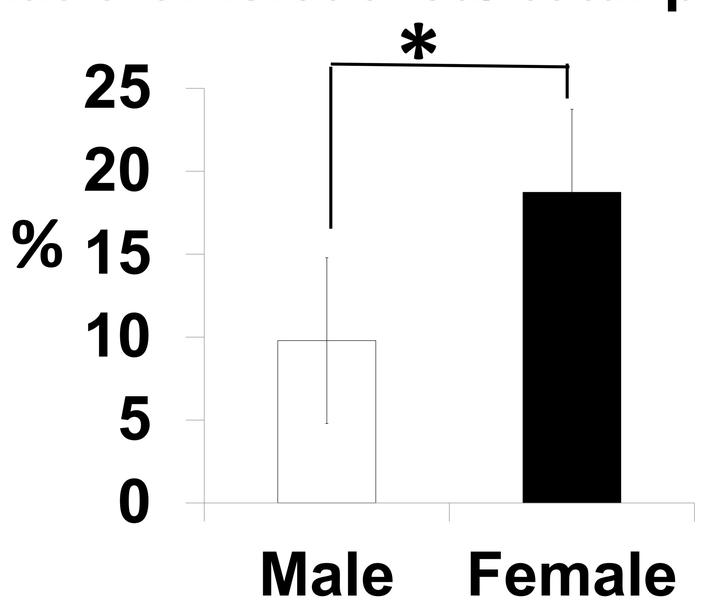
C

25 μm

D

25 μm

E Ratio of islet area/ total pancreatic area



F Islet number/ total pancreatic area

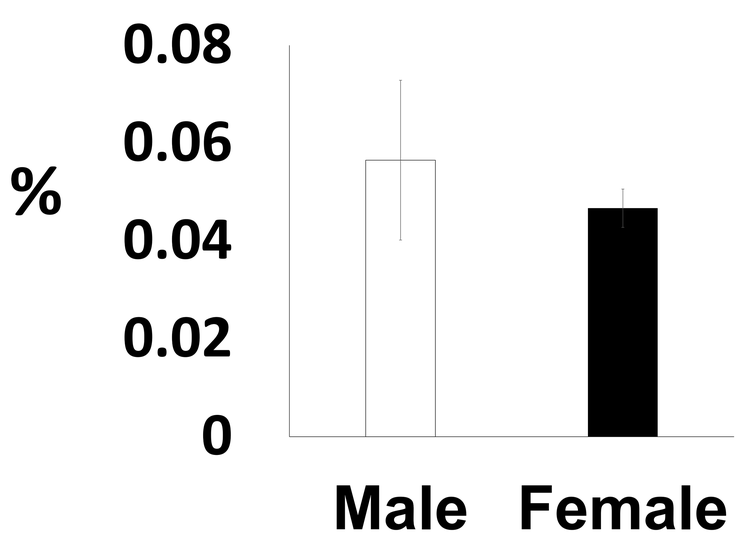


Figure 2

Male

Female

A

EX

M

250 μm

B

EX

M

250 μm

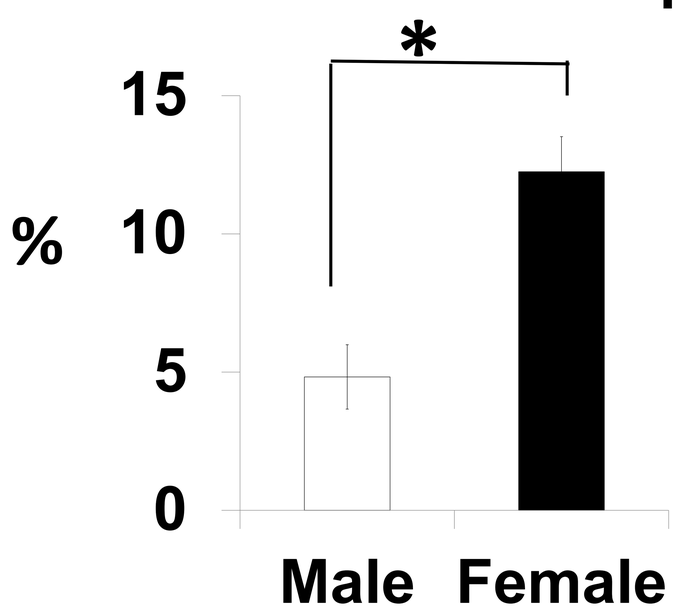
C

25 μm

D

25 μm

E Ratio of Islet area/ total pancreatic area



F Islet number/ total pancreatic area

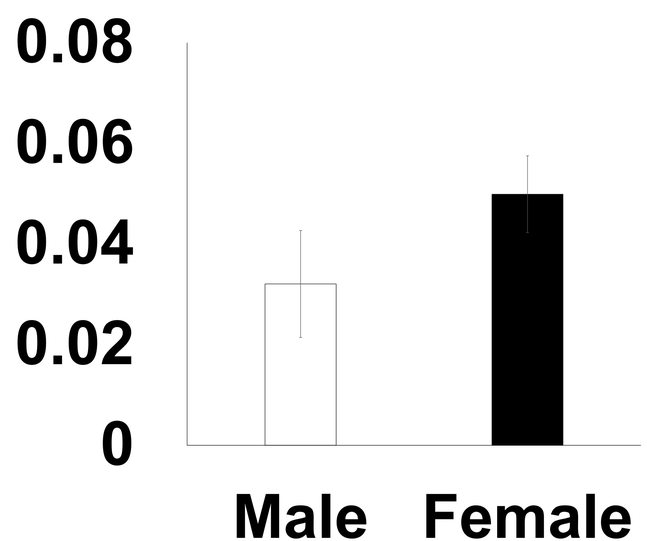


Figure 3

Male

Female

A

B

100 µm

100 µm

C

D

25 µm

25 µm

E Ratio of glucagon positive area/ islet area in compact pancreas

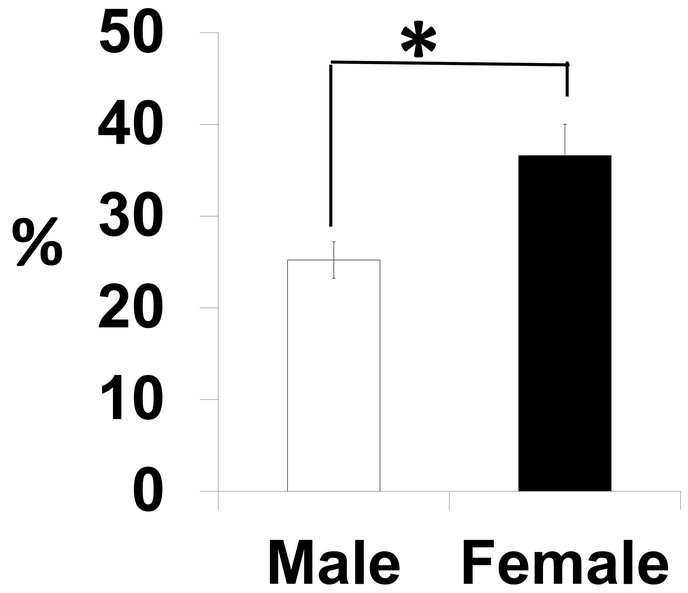


Figure 4

Male

Female

A

250 μm

B

250 μm

C

25 μm

D

25 μm

E Ratio of glucagon positive area/ islet area in esophageal disseminated pancreas

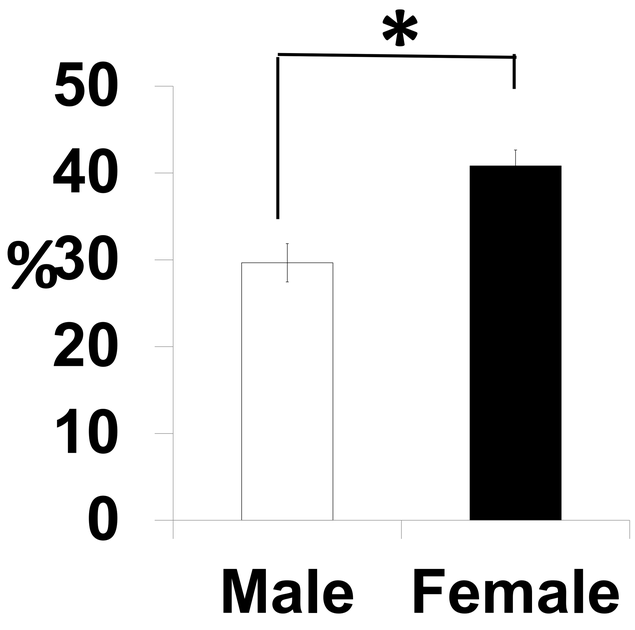


Figure 5

Male

Female

A

100 μm

B

100 μm

C

25 μm

D

25 μm

E Ratio of insulin positive area/ islet area in compact pancreas

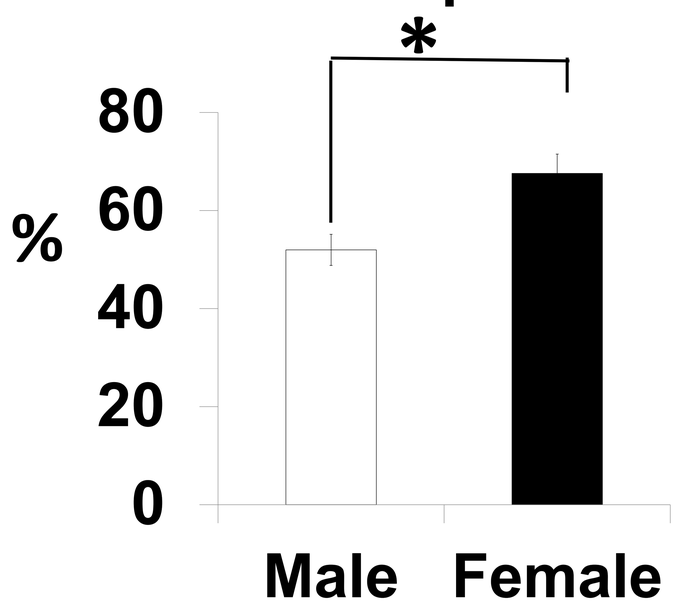


Figure 6

Male

Female

A

100 μm

B

100 μm

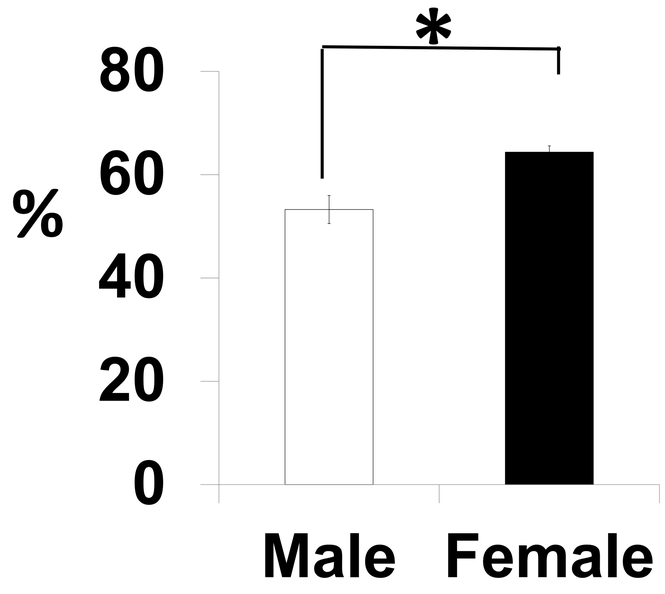
C

25 μm

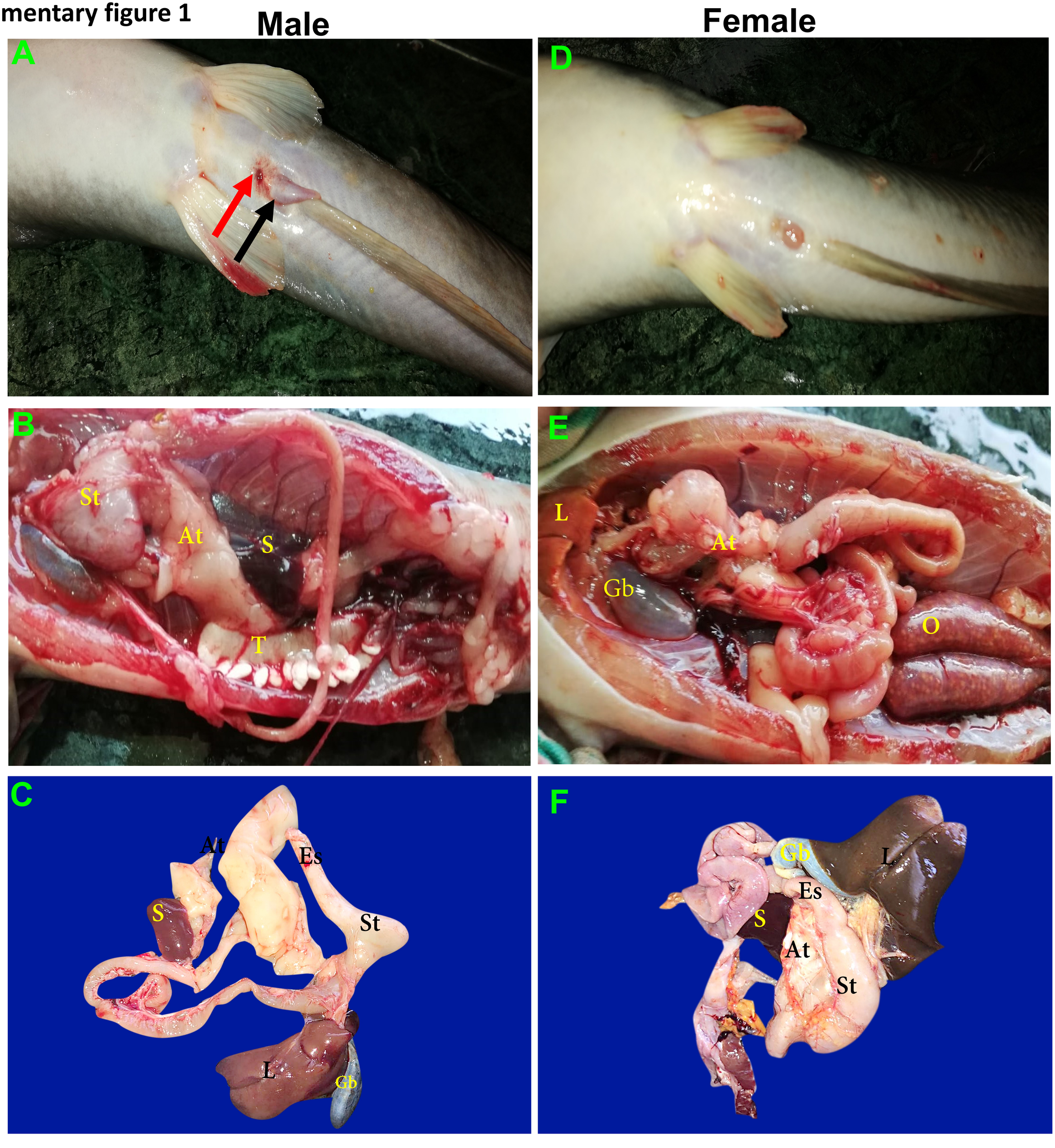
D

25 μm

E Ratio of insulin positive area/ islet area in esophageal disseminated pancreas



Supplementary figure 1



A
T. mucosa
T. submucosa
T. musculosa
T. adventitia
500 µm
B
gc
cc
50 µm
C
LI
L
EX
500 µm
D
gc
T. musculosa
EX
50 µm
E
L
500 µm
F
gg
50 µm

Figures legend

Figure 1: Photomicrographs of H&E stained sections for the catfish compact pancreas within the mesenteric adipose tissue in both sexes: (A, B) showed the pancreas lobules with endocrine pancreatic islets (arrows) between exocrine tissues (EX). (C, D) showed higher magnification for rectangle areas in (A, B). (E) Graph showing (*) significant differences in the ratio of islet area/total pancreatic area in the mesentric adipose tissue in both sexes. (F) Graph showing no significant difference in the ratio of islet number/ total pancreatic area in both sexes.

Figure 2: Photomicrographs of H&E stained sections for the dissiminated pancreatic tissue within the esophagal adventitia tissue. (A, B) showed the pancreatic exocrine tissues (EX) with the endocrine pancreatic islets (arrows) located within esophagal adventitia layer and inclose contact with the tunica musculosa of the esophagus (M). (C, D) showed higher magnification for rectangle areas in (A, B). (E) Graph showing (*) significant differences in the ratio of islet area/ total pancreatic area within the esophagal adventitia tissue in both sexes. (F) Graph showing no significant difference in the ratio of islet number according to total pancreatic area in both sexes.

Figure 3: Immunohistochemical staining of the glucagon- positive cells in the compact pancreas within the mesentric adipose tissue. (A) Glucagon-immunopositive cells in male catfish are arranged mainly in the periphery with few cells in the mantle regions. (B) Glucagon-immunopositive cells in female are detected in the periphery, the mantle and in the central region. (C, D) showed higher magnification for rectangle areas in (A, B). (E) Graph showing (*) significant differences in the ratio of glucagon positive area/ islet's area.

Figure 4: Immunohistochemical staining of the glucagon- positive cells in the disseminated pancreas. (A, B) showed the exocrine and endocrine pancreatic islets within the esophageal adventitia layer in both male (A) and female (B) catfish. (C, D) showed higher magnification for

square areas in (A, B), glucagon positive cells are detected in the periphery and few in the mantle regions. (E) Graph showing (*) significant differences in the positive area ratio of glucagon/ pancreatic islets within the esophageal adventitia tissue.

Figure 5: Immunohistochemical staining of the insulin- positive cells in the compact pancreas within the mesentric adipose tissue. (A, B) showed the pancreatic islets in male and female catfish with insulin positive cells in the center of islet. The female islet is apparently larger than in the male. (C, D) showed higher magnification of square and rectangle areas. (E) Graph showing (*) significant differences in the positive area ratio of insulin/islets area.

Figure 6: Immunohistochemical staining of the insulin- positive cells in the disseminated pancreas. (A, B) showed the exocrine and endocrine pancreatic islets within the esophageal adventitia layer in both male (A) and female (B) catfish. The pancreatic islet is apparently larger in female than that in male. (C, D) showed higher magnification of square areas. (E) Graph showing (*) significant differences in the positive area ratio of insulin/ total islets area within the esophageal adventitia tissue.

Supplementary figure 1: Gross morphology of catfish (Clarias gariepinus) and pattern of gastrointestinal tract and distribution of pancreatic tissues. (A) adult male catfish with genital papillae (black arrow), caudal to the anal opening (red arrow). (D) adult female catfish. (B, E) Internally, the gastrointestinal tract in male (B) & female (E) include stomach (St), liver (L), spleen (S), gall bladder (Gb), pancreatic tissues within the mesenteric adipose tissue (At), testes (T) and ovary (O) in male and female, respectively. (C, F) showed esophagus (Es) and pancreatic tissues localized in the triangular area between stomach (St), liver (L), spleen (S) and gall bladder (Gb).

Supplementary Figure 2. Photomicrographs of periodic acid Schiff's stained sections for the catfish esophagus: (A, B) Anterior portion of the esophagus. Notice the layers of esophagus (tunica

mucosa, submucosa, musculosa, adventitia), highly folded mucosa, numerous goblet cells (gc), clear cells (cc). (C, D) Middle portion of the esophagus. Notice moderate sized lumen (L), moderately folded mucosa with less goblet cells (gc), with the presence of disseminated pancreas including exocrine (EX) and endocrine portions represented by islets of Langerhans (IL) in the tunica adventitia and in close contact to the tunica musculosa. (E, F) Posterior portion of the esophagus. Notice large sized lumen (L), less folded mucosa, with the presence of gastric glands (gg) at the junction with the cardiac region of the stomach.