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Morphological and histological structure of hepatopancreas in rock goby *Gobius paganellus* on the western coast of Libya

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Abstract

Background: The rock goby, *Gobius paganellus*, is not a commercial species. This species has an essential role in the coastal ecosystem as a biological indicator. Therefore, it has been selected as the study's model species.

Aim: Due to the insufficient studies that have described the hepatopancreas of *G. paganellus*, this study aimed to provide information on the anatomical and histological structure of the hepatopancreas of the alimentary canal of this species on the western coast of Libya.

Methods: Fifty mature *G. paganellus* specimens were collected from the northwest of Libya (Tajoura, Jodaem, and Farwa Island). Total length and total weight of the samples were measured and performed by using gross anatomy and histology. Then, the histological sections (3–5 µm) were stained with hematoxylin and eosin (H and E).

Results: Morphologically, the liver has a large pyriform lobe. The dorsal surface of the liver is shiny and smooth, and the ventral surface contains shallow sulci; each sulcus has a large blood vessel. Histologically, the liver is wrapped with a thin capsule of fibro-connective tissue. The hepatic parenchyma is made of hepatocytes with blood sinusoids. The hepatocytes are polygonal-shaped cells and have no hepatic lobules or portal triads. Melano-macrophage centers are distributed next to the blood vessels and bile ducts. The bile ducts are lined by columnar epithelial cells. The exocrine pancreatic tissue was observed in the liver parenchyma, and it consists of acini that are composed of pyramidal cells and contain zymogen granules.

Conclusion: The liver of this species has both pancreatic and liver tissue, which was discovered in this investigation for the first time.

Keywords: *G. paganellus*, Hepatopancreas, Architecture, Libya.

Introduction

The liver is a large vital organ connected to a vertebrate's digestive system. The size, shape, and volume of the liver are adapted to the available space between other visceral organs in the general cavity, and it has a wide range of functions in all vertebrates (Faccioli *et al.*, 2014). Fish organ systems vary to some degree from that of mammals due to the aquatic environment (Ferguson, 1989).

The liver regulates the fish metabolism during anabolism and catabolism (Bruslé and Anadon, 1996; Lall and Kaushik, 2021). In addition, it plays a significant role in storing the metabolites, as well as producing most of the plasma proteins. It breaks down old red blood cells, and one of its products and gall secretion is eliminated through a duct into the duodenum (Bertolucci *et al.*, 2008; Faccioli *et al.*, 2014; Stori *et al.*, 2014). However, the liver is a prominent organ that is well-recognized for being sensitive to a wide range of environmental factors. Because of the liver's capacity for detoxification and storage of harmful components,

which affect its morphological characteristics, it is often used as an environmental biomarker (Al-Yousuf *et al.*, 2000; Bruslé and Anadon, 1996; Gochfeld, 2003; Petcoff *et al.*, 2006; Rocha *et al.*, 1994).

Nevertheless, there are variations in the number of liver lobes among the various fish species (Sales *et al.*, 2017). The predominant cellular type of the liver, hepatocytes are arranged as cords to form cellular plates that separate a network of biliary canaliculi and many vascular sinusoids (Eurell and Haensly, 1982; Bruslé and Anadon 1996; Bombonato *et al.*, 2007; Faccioli *et al.*, 2014). The melano-macrophages are visible in the parenchyma of the fish liver, and unique clusters of pigment-containing cells are often arranged in melano-macrophage centers (Hartley *et al.*, 1996). Melano-macrophage centers enlarged in conditions of environmental stress and have been proposed as accurate biomarkers for water quality in terms of both deoxygenation and iatrogenic chemical pollution (Agius and Roberts, 2003). The liver is a target organ for research on how the environment affects hepatic

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structure and/or physiological function Bruslé and Anadon (1996).

The rock goby, *Gobius paganellus*, is one of the most abundant among all fish species. This species lives in Mediterranean Sea, Black Sea, Gulf of Eilat, and the Red Sea, as well as the northwest of Atlantic Ocean (Amores *et al.*, 1990; Engin and Seyhan, 2009; Louiz *et al.*, 2016; Ragheb *et al.*, 2019), Portuguese rocky shores (Henriques *et al.*, 1999), Western Scotland to Senegal, and the Atlantic islands of the Azores, Madeira, and Canary Islands (Miller, 1986). *G. paganellus* exists on the subtidal rocky areas at about five meters on Ireland's coast and fifteen meters depth in the Azores (Dunne, 1978; Miller, 1984; Patzner *et al.*, 1992; Miller, 1986).

Family Gobiidae includes the benthic species *Tripterygion delaisi*, *Parablennius incognitus*, and *P. ruber*, it is considered one of the biggest marine families (Azevedo and Simas, 2000). In British Island; goby individuals reach maturity in their second or third year (Manuel *et al.*, 2000). However, Gobiidae plays a significant role in the ecosystems of temperate and tropical coral reefs (Louiz, *et al.*, 2016). Although many Gobiidae species are poorly understood in their environments and have limited descriptions of their eggs and larvae, the beginning of reproduction is also influenced by the water temperature throughout the winter (Miller, 1984; Ruple, 1984; Compaire *et al.*, 2018). However, *G. paganellus* started the reproductive period in October, and the spawning season was from January to March. (Hajji *et al.*, 2012).

Despite *G. paganellus* species being adapted to tidal environments, they can be stressed at high temperatures of 30°C and above, which may damage the normal biological functions of organisms (Madeira *et al.*, 2014). In addition, they may be particularly sensitive to high thermal waves that happen in low tide, which happens to be the warmest time of the day in the summer. However, the biological component of rock pond fish like *G. paganellus*, is of considerable importance in evaluations of environmental stress since it will help to create more plausible scenarios when dealing with the consequences of stress on tidal pond animals (Barton, 2002; Portner and Farrell, 2008; Sokolova, 2013; Pörtner *et al.*, 2017; Ruple, 1984; Louiz *et al.*, 2016; Paul *et al.*, 2018). Therefore, this study investigates the liver of *G. paganellus* to test the hypotheses that the liver has a distinct structure.

Materials and Methods

Animals

Fifty mature *G. paganellus* specimens were collected from the northwest of Libya (Tajoura, Jodaem, and Farwa Island). The total length and total weight of the samples were measured in grams and millimeters, respectively. A longitudinal incision was made a longitudinal incision along the ventral region of the fish.

Gross anatomy

The liver's features were examined and photographed alongside digestive tract organs. Samples were dissected, and the liver was then preserved in 4% formaldehyde for examination and documentation using a Leica M50 stereomicroscope (Germany).

Histological procedures

After fish dissection, liver pieces from ten random samples were fixed in a 4% formaldehyde solution, and then the samples were washed with 70% ethanol, dehydrated in graded ethanol solutions, and then embedded in paraffin. Histological sections (3–5 µm) were stained with hematoxylin and eosin stain (H&E) (Suvarna *et al.*, 2012). The sections were analyzed and photodocumented using an Olympus BX50 microscope (Japan).

Ethical approval

Not needed for this study.

Results

Gross anatomy

The results of this study showed the range of total length and total weight of the samples are 81–196 mm and 6.13–101.6 gm, respectively. The liver of *G. paganellus* lies ventral to the stomach and is located cranially to the stomach and posterior of the esophagus on the left side in the general cavity. The liver is pale reddish-brown (Fig. 1A and B). It is pyriform-shaped, with one lobe that is wide in the anterior portion and narrows in the posterior portion; a thin layer of serous membrane covers it and is completely suspended by mesenteries. The dorsal surface of the liver is smooth, whereas the ventral surface has a shallow fissure surrounding the large blood vessels (Fig. 1C). There is a main shallow fissure that appears along the ventral surface of the liver and branches to smaller on both sides of the liver, but it does not penetrate deep into the liver (Fig. 1D). Therefore, the lobes are less evident on both surfaces (Fig. 1C and D) and there is no appearance of a separate pancreas organ in the body cavity.

Histological studies

The liver parenchyma is encapsulated by a thin, delicate layer of loose connective tissues and squamous epithelial cells. Branches of the connective tissues were dispatched to the liver parenchyma, and no lobulation was present. However, the loose connective tissue was primarily composed of collagen fibers. The liver parenchyma was not divided into distinct hexagonal lobules. The hepatic parenchyma consists of polygonal to rounded hepatocytes with fat droplets (Fig. 2A). The parenchyma hepatocytes are arranged in acinus-like aggregation or cords that are organized in two cells thick with small canaliculi between a double face of the hepatocyte cords and no portal triads as in higher vertebrates (Fig. 2B and D). Hepatocytes were usually polygonal in shape and had spherical nuclei, eosinophilic cytoplasm, fat droplets, transparent cytoplasm, and large clear vacuolization. These vacuoles are glycogen



Fig. 1. (A, B) liver of *G. paganellus* lies cranially ventral to the stomach (st), on the left side in the general cavity, in a pale reddish-brown color. (C, D) Pyriform-shaped, one lobe with a smooth concave dorsal surface (ds) and a convex ventral surface (vs) with shallow fissure (arrowed) surrounding the large blood vessels. The liver is wide from the cranial region and, narrow from the tail and covered with a thin layer of serous membrane.

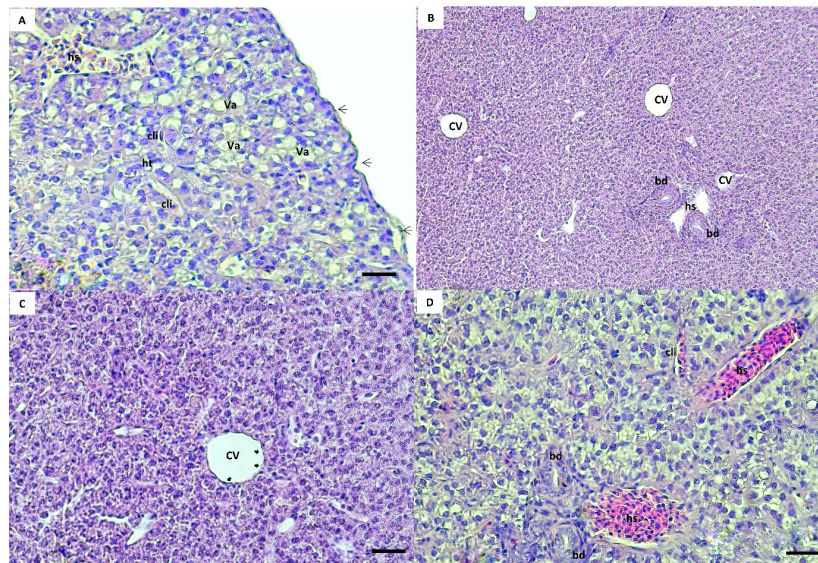


Fig. 2. (A, B, C, D) Histological sections of the liver show the hepatic tissue (ht) with discrete hepatocyte, central vein (cv), hepatic sinusoids (hs). A and C a thin layer of simple squamous epithelium with lamina propria encapsulated the liver parenchyma (black arrow). The presence of vacuolated hepatocyte (va), hs and cv lined with endothelial cells (black and white arrow), respectively. B and D bile ducts (bd) lined with simple cuboidal. The canaliculi (cli) between the hepatocytes. (Bar: B: 0.001 mm; A, C, D: 0.0005 mm).

and lipid droplets during the preparation of histologic specimens (Fig. 2A and D).

The blood sinusoidal is lined with simple squamous epithelial tissue (elongated endothelial cells with flattened nuclei) and discontinuous basement

membrane forming a very thin epithelial sheet (Fig. 2D). No portal triads as in higher vertebrates or lobule division; instead, it has a structure of sinusoids that converge at the central vein (Fig. 2B). The biliary system consists of ducts and bile canaliculi (Fig. 2B

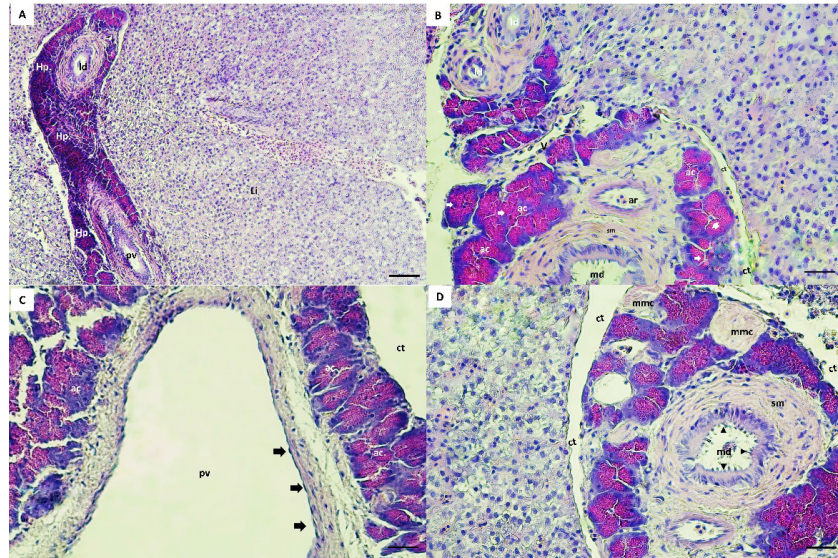


Fig 3. Hepaticpancreas tissue (hp) architectures are diffused in the liver parenchyma (Li), Hematoxylin–eosin (H&E) staining, exocrine acini (ac) readily recognized around the portal vein (pv), vein (v), artery (ar) and ducts (A, B, C, D). The lobes are surrounded by a thin layer of connective tissue (ct) (B, C, D). The main duct (md) is lined by a simple columnar with a brush border (black arrow heads), surrounded with smooth muscle (sm) and collagen fibers (B, D). The lateral ducts (ld) are lined by simple cuboidal epithelial cells (B). The acini cells are pyramidal in shape with eosinophilic zymogen granules, dark basophilic cytoplasm, and distinct basal nuclei (B). The pv is lined with endothelial cells (black arrow) (C). The melanomacrophage centers (mmc) in Hepaticpancreas are located near the blood vessels and ducts (D). (Bar: A: 0.001 mm; B, C, D: 0.0005 mm).

and D). The bile ducts and portal vein are located surrounded mainly by the parenchyma of the liver tissue. The bile duct is lined by simple columnar epithelium and structurally consists of three distinct layers: tunica mucosa of epithelial cells that are simple cuboidal with large spherical nucleus and brush border, tunica muscularis of some smooth muscles, and very thin tunica serosa of dense connective tissues. The apical border of the adjacent hepatocytes faces toward the bile canaliculi direction, where the bile ducts exist (Fig. 2D).

Microscopic structure results showed that hepatopancreatic tissue of *G. paganellus* was located in the internal surface of the liver and diffused in the liver parenchyma, arranged around the branches of the portal vein, arteries, and ducts in the portal area. Hepatopancreatic tissues were readily discernible (Fig. 3A). The hepatopancreatic tissues represented about 6% of the liver tissue. It has a cluster of an exocrine acinar arrangement, separated from hepatic parenchyma by a thin layer of connective tissues; each acinus consists of mostly organized tall pyramidal cells. The acinus cells have apical eosinophilic zymogen granules, which vary in density, as well as a dark basophilic cytoplasm and distinct basal nuclei that contact the basal membrane (Fig. 3B). Moreover, the pancreatic acinar cells are distinguished from hepatic tissue by their basophilic basal pole and eosinophilic apical cytoplasm and there was no pancreatic endocrine tissue observed among exocrine hepatopancreatic tissue. Thus, the

hepatopancreatic portion was clearly shown that it was the exocrine pancreas (Fig. 3B and C).

The exocrine hepatopancreas ductular system of *G. paganellus* is composed of a large main duct and small lateral ducts (Fig. 3A, B, and D). The main duct opens in the middle portion of intestinal lobules (Fig. 3B and C), surrounded by smooth muscle cells, collagenous fibers present between the pancreatic lobules, and lined by the mucosa consisting of simple columnar epithelium with an apical brush border. At the same time, the mucosa of the lateral ducts consists of simple cuboidal cells (Fig. 3B). The melanomacrophage centers (MMCs) of *G. paganellus*, which are often nodular in appearance, are located near the blood vessels and bile ducts and are lined by a thin connective tissue that enters the organ as septa; some dark pigments have been found in the cytoplasm of these cells, primarily along the edges of the melano-macrophage centers (Fig. 3D).

Discussion

This study describes for the first time the normal anatomy and histology of the liver of *G. paganellus*. The anatomical description of the liver organ of *G. paganellus* revealed a pyriform shape, with one lobe that is wide in the anterior portion and narrow in the posterior portion. The liver is a pale yellowish-brown, similar to *G. brasiliensis* (Bruslé and Anadon, 1996), while the color's liver and the number of lobes of *Hoplias aff. malabaricus* and *Hypostomus francisci* were different (Sales *et al.*, 2017). Maybe the liver

color is related to different habits. Whereas the adult individuals of *G. paganellus* and *G. brasiliensis* prefer areas with rocks or coral and live on the reef inhabitant of seawater, while *H. aff. malabaricus* and *H. francisci* are freshwater fish and pelagic fish (Sales *et al.*, 2017). The liver of *G. paganellus* has a smooth dorsal surface and a shallow fissure ventral surface surrounded by large blood vessels. While the digestive glands of *Neurergus microspilotus* and *N. kaiseri* consist of a big liver and a small pancreas, it is divided into two separated lobes, right and left of both species (Vaissi *et al.*, 2017). However, these results agree with the findings of Vaissi *et al.* (2017) and Akou (2019) that the gall bladder is situated just dorsal to the right side of the liver lobe of *N. microspilotus*, *N. kaiseri*, *C. carpiolinnaeus*, and *Mesopotamichthys sharpeyi*. Histologically, this result showed that a thin layer of serous membrane of mesothelium and fibro-connective tissue-rich collagen fibers surround the liver organ of *G. paganellus*; the covering epithelium may play a role in the protection of the liver fraction during movement by secreting peritoneum fluid in the abdominal cavity. The same appearance was described by El-Shammaa *et al.* (2008) of gilthead sea bream and Vaissi *et al.* (2017) of *N. microspilotus*, and *N. kaiseri*. In addition, glycogen and fat storage dissolve regularly throughout the histopathologic process, leading to substantial histological variety.

The hepatic parenchyma of *G. paganellus*' liver consists of hepatocytes spread out as irregular cords arranged radially around a central vein. These cords are not divided into distinct hexagonal lobules, nor do they have portal triads, as in higher vertebrates, agreed with the results shown by Akou and AL-Jowari (2019) and Akiyoshi and Inoue (2004). Each plate has polygonal-shaped cells with spherical nuclei usually centrally located, eosinophilic cytoplasm with presents a large amount of vacuolated; this arrangement was detected in trout (Anderson and Mitchum, 1974), in tilapia (Abd El-Fatah, 1999), and gilthead sea bream (El-Shammaa *et al.*, 2008). Hepatocytes face the biliary apically and the sinusoidal at the base. Hepatocyte cords can be organized in two cells thick or branch and/or consolidated between two neighboring sinusoids. The hepatocyte arrangement in *G. paganellus* resembles that recognized in other studies as grey mullets (Biagianti-Risbourg, 1991) and in the Atlantic croaker (Eurell and Hanesly, 1982); the former authors showed that hepatocytes surrounded a sinusoid that was arranged as tubules of hepatocytes.

The blood sinusoidal is lined with simple squamous epithelial tissue. Endothelial cells are elongated with dark nuclei and a discontinuous basement membrane, forming a very thin epithelial sheet. The sinusoidal structure of *G. paganellus* corresponded with that of other fishes. While endothelial cells rest on a basal lamina, there are no portal triads as in higher vertebrates or lobule division; instead, they have a structure of

sinusoids that converge at the central vein. The same appearance was described in flatfish sea bream (EL-Shammaa, 2008), teleosts (Ferri and Sesso, 1981), and catfish (Hinton and Pool, 1976).

The bile duct and portal vein are surrounded by *G. paganellus* liver tissue parenchyma. The biliary system consists of ducts and bile tubules. Structurally, the bile duct consists of three distinct layers: tunica mucosa, tunica muscularis, and tunica serosa. The apical border of adjacent hepatocytes points towards the biliary tubules, where the bile ducts are present. The bile duct mucosa was lined by a simple cuboid to the columnar epithelium, consistent with Vicentini *et al.* (2005) and Faccioli *et al.* (2014), who observed that the bile ducts in the three species had a simple cuboid epithelium, which becomes columnar in large passages similar to other bony fish.

In this study, the MMCs located near the blood arteries and bile ducts also existed in the hepatic and pancreatic tissue. Many dark pigments were found in the cytoplasm of these cells, mainly along the edges of the MMCs. Similar to Mela *et al.* (2013) and Sales *et al.* (2017) in bony fish. The number of MMCs, their size, and distribution vary with species, organ, age, nutritional level, and stress conditions, as shown by Fishelson (2006) and Viana *et al.* (2021). Moreover, Van der Oost *et al.* (2003) and Steinel and Bolnick (2017) recognized the function and potential of MMCs as a histological biomarker of the immune response and to destroy, detoxify, or recycle foreign materials.

G. paganellus has intrahepatic exocrine pancreatic tissue consisting of exocrine acini. The acinus cells were arranged in clusters of columnar epithelial cells with a distinct basal nucleus and basophilic cytoplasm in the base, but the apical cytoplasm is acidophilic because of the presence of many eosinophilic zymogen secretory granules; it produces digestive enzymes. Nejedli and Gajger (2013) and Rocha *et al.* (1997) noticed that in some teleost fishes, the largest portion of the liver is liver parenchyma tissue, about 95%, and a smaller proportion of tissue about 5% is the hepatopancreas. It was not in conflict with *G. paganellus*' liver, where 6% of the hepatopancreatic tissue was present. In addition, Akou and AL-Jowari (2019) indicated that exocrine pancreatic tissue was diffused intrahepatic in *C. carpiolinnaeus*, and *M. sharpeyi* mainly consists of acini. Furthermore, the hepatopancreas has been reported in other teleosts (Vicentini *et al.*, 2005), and its percentage varies by species (Nejedli and Gajger, 2013; Faccioli *et al.*, 2014).

Conclusion

There is no unique model described for Teleostei liver, despite the fact that morphological and histological features of fish liver vary across species. For this reason, more research and in-depth analysis of each Teleostei species is needed. Overall, research on *G. paganellus* can help expand the local database and enhance Libyan

markets, making it a crucial component of the country's coastal ecology, food chain, and ecological biomarker.

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Authors contributions

SAA and HHS planned the experimental design and executed the in-vitro and in-vivo study. SAA and HHS conducted the literature review, drafted and revised the literature, and SAA wrote the final manuscript. SAA provided a hand in the histological examination and comments. HHS provided and dissected the fish, measured the length and organ weights of the fish, and calculated tissue presence ratios of the liver with ImageJ. All authors read and approved the article.

Data availability

All data supporting the findings of this study are available within the manuscript, and no additional data sources are required.

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Conflict of interest

The authors declare that there is no conflict of interest.

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