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Hepatopancreas in some sea fish from different species and the structure of the liver in teleost fish, common pandora, *Pagellus erythinus* (Linnaeus, 1758) and whiting, *Merlangius merlangus euxinus* (Nordmann, 1840)

Srebrenka Nejedli[1]*, and Ivana Tlak Gajger[2]

[1]*Department of Anatomy, Histology and Embriology, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

[2]*Department of Biology and Pathology of Fish and Bees, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

NEJEDLI, S., I. TLAK GAJGER: Hepatopancreas in some sea fish from different species and the structure of the liver in teleost fish, common pandora, *Pagellus erythinus* (Linnaeus, 1758) and whiting, *Merlangius merlangus euxinus* (Nordmann, 1840). Vet. arhiv 83, 441-452, 2013.

ABSTRACT

The livers of twenty-nine species of fish from twenty different fish families obtained from the Adriatic Sea (Croatia) were investigated. In the livers of twenty species of fish hepatopancreas was found, but not always in the same species. Histology and stereological analysis was undertaken of the liver in ten teleost fish, one each of: common pandora, *Pagellus erythinus* (Linnaeus, 1758) and in whiting, *Merlangius merlangus euxinus* (Nordmann, 1840). In the liver of the common pandora there was a smallest proportion of liver tissue 85.81% and liver blood vessels 2.50% and the largest proportion of hepatopancreas tissue 14.19% and hepatopancreas blood vessels 1.70%. The liver of whiting contained the largest proportion of liver tissue 95.14% and liver blood vessels 4.60% and smaller proportion of hepatopancreas tissue 4.86% and hepatopancreas blood vessels 1.43%. A statistically significant correlation was only obtained between the percentages of the mean values of the examined tissues and blood vessels of the livers between whiting and common pandora ($P < 0.01$), but not between the groups of the same species. In whiting and common pandora the division to the liver lobuli, the Glison trias is not visible and the perisinusoidal space (space of Dissae) is large and well developed. The sinusoidal capillaries were narrow and irregularly shaped and the hepatocytes were organized in tubular form.

Key words: histology, stereology, liver, hepatopancreas, sea fish

*Corresponding author:

Srebrenka Nejedli, DVM, Department of Anatomy, Histology and Embriology, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia, Phone: + 385 1 2390 242; E-mail: snejedli@vef.hr

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Introduction

Liver cells form more than 80% of the liver parenchyma, and in fish livers they are set around the capillary space sinusoids. Between the sinusoid endothelium and the membranes of the hepatocytes there is perisinusoidal space or space of Disse of varying width. There is no basal membrane under the epithelium of the liver sinusoidals in fish. The only exception is the liver of the lamprey (*Petromyzon marinus*), in which the existence of the basal membrane has been noticed. Inside the perisinusoidal space in the liver of brown bullhead, goldfish and trout cells have been described which accumulate a large amount fatty substances - lypocites, and are also called Ito-cells (FUJITA et al., 1986; WAKE et al., 1987; ROBERTSON and BRADLEY, 1992; MUNSHI and DUTTA, 1996). The presence of Kupffer cells in fish liver still remains an open question, since they have only been found for certain in the liver of the brown bullhead (MUNSHI and DUTTA, 1996; EL-BAKARY and EL-GAMMAL, 2010).

The pancreas is an organ, which in some fishes is compact, but can be composed of lobules, in which case it mostly consists of two lobules. Histologically, the pancreas in fish is formed from an exocrine and an endocrine part. The exocrine part secretes digestive juices, while the endocrine part secretes the hormones insulin and glucagon (YOUSON et al., 2006). The formation of glandular cells or glandular acinuses is a regular part of the exocrine part of the pancreas, their cytoplasm is basophilic, except for the apical part, where it is acidophilic because of the presence of secretion granules. In some fish the pancreas is present in the form of islets of exocrine pancreatic tissue dispersed around the bowels or in the liver. If the exocrine pancreatic tissue is located in the liver, this organ is then called the hepatopancreas (EURELL and HAENSLY, 1982; GEYER et al., 1996; KOZARIĆ, 2001; PETCOFF et al., 2006; EL-BAKARY and EL-GAMMAL, 2010).

The aim of our research was to explore the presence of the hepatopancreas in the livers of twenty-nine fish species from twenty different fish families obtained from the Adriatic Sea (Croatia) and the structure of the liver of two teleost fish: the common pandora, *Pagellus erythinus* (Linnaeus, 1758) and the whiting, *Merlangius merlangus euxinus* (Nordmann, 1840). The whiting and common pandora are two species of great interest in the Adriatic Sea, in addition to the fact that their meat is considered of very good quality. Since the liver has a central role in metabolism and is notable for its sensitivity to a great variety of environmental factors, it is an important organ for analysis.

Materials and methods

The research was carried out on the livers of twenty-nine species of fish from twenty different fish families (Table 2) caught from the Adriatic Sea (Croatia). The livers of all species were taken from the anterior liver lobe and removed, and fixed in 10% buffered formalin. The fixed material was embedded in paraffin and serially sectioned

at 10 μm. Sections were stained with hematoxylin and eosin (ROMEIS, 1968). Histology and stereology analysis was undertaken of the liver in ten fish samples from the Central Adriatic Sea one each of: common pandora, *Pagellus erythinus* (Linnaeus, 1758) (total body length from17 to 18 cm) and whiting, *Merlangius merlangus euxinus* (Nordmann, 1840.) (total body length from16 to 23.5 cm). Fish were used without sexual distinctions. By stereological analysis of the cuts stained with hematoxyline and eosin (ROMEIS, 1968), the proportion of the liver (liver parenchyma and connective tissue), and blood vessels of the liver, and the hepatopancreas and the blood vessels of the hepatopancreas was determined in the more visible fields. This analysis was carried out by a multipurpose test system according to WEIBEL et al. (1966). The reticule drawn on a transparent film, with a 100 measure points, was set on a projection screen of a Wild M 20 microscope, at 60 X magnification. For determination of the volume thickness of the researched elements (KALIŠNIK, 1982) in the capacity units of the researched liver, we also counted in multiple visual fields the points that were classified as liver elements (liver parenchyma and connective tissue) and liver blood vessels, as well as the hepatopancreas and hepatopancreas blood vessels. From the ratio of the total number of points assigned to the researched tissue, and the total number of points counted, we gained the volume proportion of the researched tissue within the capacity unit of the researched structure and by multiplying by 100 we gained their percentage proportion.

All calculations were processed using Statistica Release 8 software and the chi-square test.

Results

On histological preparation of the liver, stained with hematoxyline and eosin, of the investigated species of fish, diffusely dispersed exocrine pancreatic tissue was found in the livers, and therefore it may be said that these researched fish had a hepatopancreas (Table 1). Table 2 and Fig. 1 show the proportion of liver tissue and blood vessels, and the proportion of hepatopancreas tissue and hepatopancreas blood vessels in the common pandora, *Pagellus erythinus* (Linnaeus, 1758) and the whiting, *Merlangius merlangus euxinus* (Nordmann, 1840). The liver of the common pandora obtained the had a proportion of liver tissue 85.81% and liver blood vessels 2.50% and the larger proportion of hepatopacreas tissue 14.19% and hepatopancreas blood vessels 1.70%. In the liver of the whiting there was a larger proportion of liver tissue, 95.14% and liver blood vessels 4.60%, and a smaller proportion of hepatopancreas tissue 4.86% and hepatopancreas blood vessels 1.43%. A statistically significant correlation was obtained between the percentages of the mean values of all tissues and blood vessels, between the livers of whiting and common pandora ($P<0.01$) but it was not statistically significant between groups of fish from the same species.

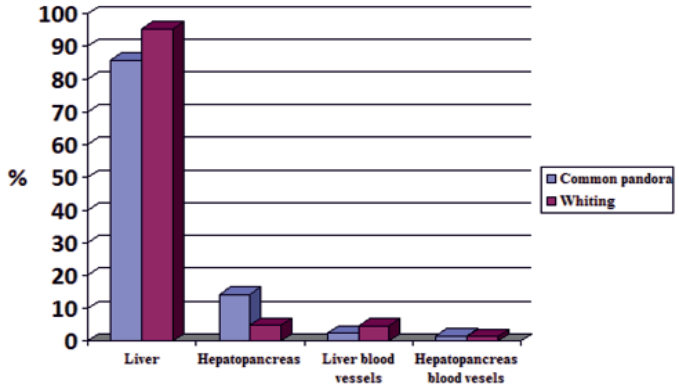

Fig. 1. Percentage of liver tissue, hepatopancreas tissue, liver blood vessels and hepatopancreas blood vessels in whiting, *Merlangius merlangus euxinus* and in common pandora, *Pagellus erythinus*.

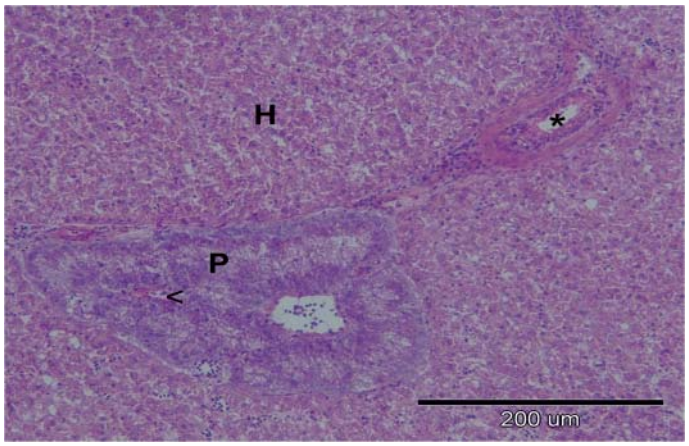

Fig. 2. Liver, common pandora, *Pagellus erythrinus*, H- liver. P- hepatopancreas; *- liver blood vessels; <- hepatopancreatic blood vessels. H&E, ×25.

Table 1. Hepatopancreas (+) or no hepatopancreas (-) in livers of twenty different fish families.

Family	Hepatopancreas
Belonidae: Garfish (*Belone belone gracilis* Lowe, 1839)	+
Congridae: Sea eel (*Conger conge*)	+
Dactylopteridae: Boat fish (*Dactilopterus volitans* Linnaeus, 1758)	+
Gobidae: Black goby (*Gobius niger* Linnaeus, 1758)	-
Gadiadae: Whiting (*Merlangius merlangus euxinus* Nordmann, 1840)	+
Labrida: Rainbow wrasse (*Coris julis* Linnaeus, 1758) Brown wrase (*Labrus merula* Linnaeus, 1758)	+ -
Merlucidae: Hake (*Merluccius merluccius* Linnaeus, 1758)	+
Moronidae: Sea-bass (*Dicentrarchus labrax* Linnaeus, 1758)	+
Mugilidae: Golden grey mullet (*Liza aurata* Risso, 1810)	-
Muraneidae: Mura (*Muraena helena* Linnaeus, 1758)	+
Mullidae: Goat fish (*Mullus barbatus barbatus* Linnaeus, 1758)	-
Serranidae: Comber (*Serranus cabrilla* Linnaeus, 1758)	+
Sparidae: Bogue (*Boops boops* Linnaeus, 1758) Dentex (*Dentex dentex* Linnaeus, 1758) White seabream (*Diplodus sargus sargus* Linnaeus, 1758) Common two-banded sea bream (*Diplodus vulgaris* Geoffroy St. Hilaire, 1817) Annular bream (*Diplodus annularis* Linnaeus, 1758) Saddled bream (*Oblada melanura* Linnaeus, 1758) Common pandora (*Pagellus erythrinus* (Linnaeus, 1758) Black sea bream (*Spondyliosoma cantharus* Linnaeus,1758) Salema (*Salpa salpa* Linnaeus,1758)	- + - + + + + + +
Sciaenidae: Brown mearge (*Sciaena umbra* Linnaeus, 1758)	+
Scombridae: Bullet tuna (*Auxis rochei* Risso, 1810)	-

Table 1. Hepatopancreas (+) or no hepatopancreas (-) in livers of twenty different fish families (continued)

Family	Hepatopancreas
Squalidae: Spiny dogfish (*Squalus acanthias* Linnaeus, 1758)	+
Scorpaenidae: Sea scorpion (*Scorpaena porcus* Linnaeus, 1758)	+
Scyliorhidae: Rough dog (*Schyliorhinus canicula* Linnaeus, 1758)	-
Trachinidae: Lasser weever (*Echiichthys vipera* Cuvier, 1829)	-
Zeidae: John Dory (*Zeus faber* Linnaeus, 1758)	+

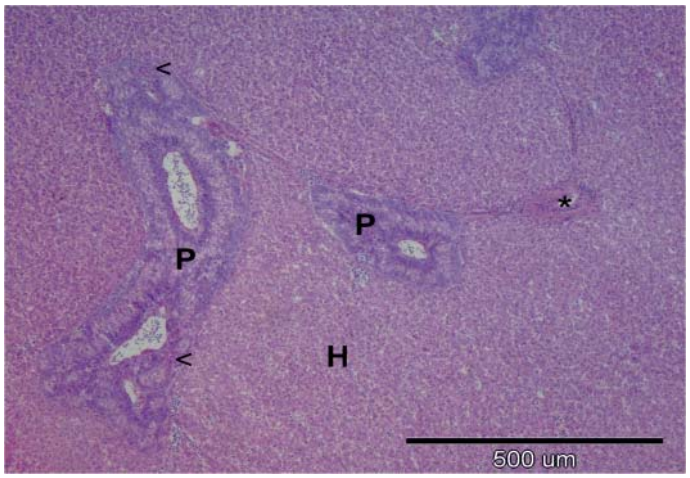

Fig. 3. Liver, common Pandora, *Pagellus erythrinus*. H- liver. P- hepatopancreas; *- liver blood vessels; <- hepatopancreas blood vessels. H&E, ×10.

The exocrine pancreatic tissue is diffusely set inside of the liver. Liver tissue, blood vessels and the pancreatic tissue with its blood vessels were seen in both the common pandora, and whiting (Figs. 2 and 3). The investigated fish differ only in the percentage of individual structural components inside the hepatopancreas. In both the investigated fish species the division into liver lobules and Glison trias is not visible and the perisinusoidal space (space of Dissae) is large and well developed. A well-developed billiary tract was detected.The hepatopancreas in this fish is separated from the hepatic parenchyma by a thin layer of connective tissue. The apical parts of the exocrine pancreatic cells

contain acidophilic zymogen granules. The hepatic parenchyma is homogenous and the hepatocytes are polygonal-shaped cells, appearing hexagonal.

Table 2. The proportion of liver tissue and its blood vessels, and the proportion of hepatopancreas hepatopancreatic blood vessels in common pandora, (*Pagellus erythinus* Linnaeus, 1758) and in whiting (*Merlangius merlangus euxinus* Nordmann, 1840).

	Common pandora				Whiting			
	Liver tissue	HP tissue	Liver blood vessels	HP blood vessels	Liver tissue	HP tissue	Liver blood vessels	HP blood vessels
Mean (%)	85.81	14.19	2.50	1.70	95.14	4.86	4.60	1.43
Median	85.94	14.06	2.53	1.70	95.20	4.82	4.60	1.34
Minimum	85.10	13.58	2.08	1.21	94.20	4.33	4.01	1.21
Maximum	86.82	14.90	2.79	2.16	96.30	5.80	5.26	1.85
Lower quartile	85.23	13.88	2.19	1.54	95.06	4.60	4.32	1.24
Upper quartile	86.12	14.77	2.78	1.86	95.54	4.94	4.97	1.55
Variance	0.31	0.24	0.08	0.11	0.32	0.18	0.19	0.06
Std. Dev.	0.55	0.49	0.28	0.34	0.56	0.42	0.43	0.25
Standard Error	0.18	0.15	0.09	0.11	0.18	0.14	0.14	0.08

In the common pandora and the whiting livers, the sinusoidal capillaries were narrow and irregularly shaped sinusoids, appearing throughout in the interstitial connective tissue between the hepatic plates. The hepatocytes were organized in tubular form and appeared as plates two or more hepatocytes thick.

Discussion

The liver is an organ notable for its sensitivity to a great variety of environmental factors (BRUSLE and ANADON, 1996), it is a biomarker of environmental pollution, it has central role in the metabolism and the liver is an important organ for the analysis of fish diseases (OKIHIRO and HINTON, 2000; JUNG et al., 2002; AKIYOSHI and INOUE, 2004). Fish have evolved some organs that mammals have not. One of them is exocrine pancreatic tissue in liver. Pancreatic tissue inside the liver is not present in all kinds of fish, but if it is, this organ is called the hepatopancreas (EURELL and HAENSLY, 1982; GEYER et al., 1996; PETCOFF et al., 2006; EL-BAKARY and EL-GAMMAL, 2010). This exocrine pancreatic tissue is formed around the portal vein, and during ontogenesis it penetrates

deeply into the liver parenchyma (VICENTINI et al., 2005), while its proportion depends on the species of fish (MARCONI et al., 1980; EUREL and HAENSLY, 1982; GONZALEZ et al., 1993). In our investigation, a hepatopancreas was found in several different fish species (Table 1). The hepatopancreas, in all the investigated fish, was separated from the hepatic parenchyma by a thin layer of connective tissue.

Livers were investigated to compare the portion of liver tissue and its blood vessels, and the proportion of hepatopancreas tissue and hepatopancreas blood vessels in two fish from different families: the Gadiadae (whiting, *Merlangius merlangus euxinus*) and the Sparidae (common pandora, *Pagellus erythrinus*) with the same feeding habits (both are carnivores). By stereological measurements of histological preparations of the liver in the common pandora a smaller proportion of liver tissue (85.81%) and liver blood vessels (2.50%) was found and a larger proportion of hepatopacreas tissue (14.19%) and hepatopancreas blood vessels (1.70%). In the liver of whiting a larger proportionof liver tissue (95.14%) and liver blood vessels (4.60%) was found and a smaller proportion of hepatopancreas tissue (4.86%) and hepatopancreas blood vessels (1.43%).

The liver is an organ important for metabolism because one of its products, gall, is excreted through a system of ducts into the duodenum, so it is considered to be an exocrine gland, while it is also an endocrine gland because most of its products flow directly into the blood (ROSS et al., 1989). In common pandora and whiting liver a well-developed biliary tract was detected. In our research, we were trying to determine the proportion of the liver blood vessels, which was larger (4.60%) in the whiting and smaller in the common pandora (2.50%). In whiting the proportion of liver tissue was 95.14% and in the common pandora it was 85.81%. These correlations were statistically significant ($P<0.01$).

The hepatopancreas in the investigated fish has an acinar arrangement, separated from the hepatic parenchyma by a thin layer of connective tissue, as in grey mullet, *Mugil cephalus* and sea bream, *Sparus aurata* (EL-BAKARY and EL-GAMMAL, 2010). In the exocrine cells, zymogen granules are located in the apical parts of these cells. Pancreatic exocrine tissue in some species develops around the portal vein during ontogenesis. It remains extra hepatic or penetrates somewhere deep into the liver parenchyma, depending on the species (EURELL and HAENSLY, 1982; GONZALEZ et al., 1993). In the common pandora the proportion of hepatopacreas tissue is larger (14.19%) and in whiting it is smaller (4.86%), which is statistically significant ($P<0.01$).

Liver in fish is composed from two components of parenchyma, which is formed by an extracellular space (sinusoids, space of Disse), cellular space, cells (hepatocytes, bile duct epithelium) and interstitial connective tissue with blood vessels (HINTON and LAURÉN, 1990). In our research in the common pandora and in the whiting the proportion of the liver was over 85%. By analyzing histological preparations in both species of

fish, common pandora and whiting, a division into liver lobules and Glison trias is not visible, as evidenced in many telosts (HAMPTON et al., 1985; GONZALEZ et al., 1993; AKIYOSHI and INOUE, 2004; PETCOFF et al., 2006). The perisinusoidal space (space of Dissae) is large and well-developed. The hepatic parenchyma is homogenous and the hepatocytes are polygonal shaped cells, appearing hexagonal, which is also visible in tiger fish, *Hydrocynus forskahlii* (GEYER, 1996).

The sinusoids are localized in the space between the hepatic plates in which the hepatocytes are arranged (MOTTA, 1984). The hepatocyte sinusoidal structures of the fish livers were classified into three different types: a cord-like form, a tubular form and a solid form (AKIYOSHI and INOUE, 2004). In the liver of the common pandora and whiting the sinusoidal capillaries were narrow and irregularly shaped sinusoids, appearing throughout in the interstitium between the hepatic plates, similar to what is found in many teleost (HINTON and POOL, 1976; BRUSLE and ANADON, 1996; VICENTINI et al., 2005). The hepatocytes were organized in tubular form and appeared as plates, two or more hepatocytes thick.

Research into the livers of brown trout (*Salmo trutta fario*) has shown that females have a higher number of hepatocytes than males, but no significant difference was detected in the cell surface of the liver (ROCHA et al., 2001). The presence of lipid and glycogen in the cytoplasm of hepatocytes does not significantly affect the size of the cells (EL-BAKARY and EL-GAMMAL, 2010). In our research, fish were used without sexual distinctions and with the same feeding habits (both are carnivores) and the results of investigation were not statistically significant between fish from the same species.

Conclusion

In the livers of twenty species of fish a hepatopancreas was found, but not always in the same species. A statistically significant correlation was only found between the percentages of the mean values of the examined tissues and blood vessels of livers between whiting and common pandora ($P<0.01$), but not between the groups of the same species. In whiting and common pandora the division into liver lobuli and Glison trias is not visible and the perisinusoidal space (space of Dissae) is large and well-developed. The sinusoidal capillaries were narrow and irregularly shaped, and the hepatocytes were organized in tubular form.

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SAŽETAK

Istraživanja su provedena na jetri 29 vrsta iz 20 različitih porodica riba iz Jadrana (Hrvatska). U jetri 20 vrsta riba hepatopankreas je pronađen, ali ne uvijek u istih porodica. Provedene su histološke i stereološke analize jetre u deset arbuna, *Pagellus erythinus* (Linnaeus, 1758) i pišmolja, *Merlangius merlangus euxinus* (Nordmann, 1840). U jetri arbuna prisutan je manji udio jetrenog tkiva 85,81% i krvnih žila 2,50% te veći udio hepatopankreasnog tkiva 14,19% i krvnih žila u hepatopankreasu 1,70% u odnosu na jetru pišmolja koja sadrži veći udio jetrenog tkiva 95,14% i krvnih žila u jetri 4,60% te manji udio hepatopankreasnog tkiva 4,86%

i krvnih žila u hepatopankreasu 1,43%. Statistički značajne razlike ustanovljene su u udjelu istraživanog tkiva i krvnih žila jetre između arbuna i pišmolja ($P<0,01$), ali ne između riba unutar iste vrste. U arbuna i pišmolja podjela jetrenih režnjića i Glisonov trias nisu vidljivi, a perisinusoidalni prostor (Dissaeov prostor) je velik i dobro razvijen. Sinusoidalne kapilare su uske i nepravilnog oblika, a hepatociti su organizirani u cjevastom obliku.

Ključne riječi: histologija, stereologija, jetra, hepatopankreas, morske ribe