

# Light microscopy and ultrastructure of the liver of *Astyanax altiparanae* Garutti and Britski, 2000 (Teleostei, Characidae)

Bruno Bertolucci<sup>1</sup>, Carlos Alberto Vicentini<sup>1,2\*</sup>, Irene Bastos Franceschini Vicentini<sup>1,2</sup> and Maria Terezinha Siqueira Bombonato<sup>1</sup>

<sup>1</sup>Departamento de Ciências Biológicas, Faculdade de Ciências, Universidade Estadual Paulista, Cx. Postal 473, 17033-360, Bauru, São Paulo, Brazil. <sup>2</sup>Centro de Aquicultura, Universidade Estadual Paulista, Jaboticabal, São Paulo, Brazil. \*Author for Correspondence. E-mail: carlosav@fc.unesp.br

**ABSTRACT.** Livers of thirty specimens of *Astyanax altiparanae* obtained from a commercial fish farm were subjected to light and transmission electron microscopy, in order to describe the hepatic parenchyma and the intrahepatic exocrine pancreatic tissue. Anatomically, the liver showed only three hepatic lobes. Histological analysis demonstrated that the hepatocytes were spread out as anastomotic cords, arranged in two cellular layers and surrounded by sinusoids. The intrahepatic exocrine pancreatic tissue exhibited an acinar arrangement and was diffused in the hepatic parenchyma. Ultrastructural analysis showed that the hepatocytes had a rounded nucleus and a rough endoplasmatic reticulum, with a parallel disposition to the nuclear membrane. The exocrine pancreatic cells showed secretion granules at the apical portion, and the rough endoplasmatic reticulum was concentrically distributed.

**Key words:** liver histology, hepatocyte ultrastructure, exocrine pancreas, teleost.

**RESUMO.** Histologia e ultraestrutura do fígado de *Astyanax altiparanae* Garutti e Britski, 2000 (Teleostei, Characidae). Fígados de 30 exemplares de *Astyanax altiparanae*, obtidos de pesqueiros, foram submetidos à microscopia de luz e à microscopia eletrônica de transmissão para descrição do parênquima hepático e do tecido pancreático exócrino intra-hepático. Anatômica, o fígado apresenta somente três lobos hepáticos. A análise histológica demonstra que os hepatócitos se encontram arranjados na forma de cordões anastomosados, dispostos em duas camadas celulares, sendo que estes estão cercados por sinusóides. O tecido pancreático exócrino intra-hepático apresenta arranjo acinar difuso pelo parênquima hepático. A análise ultraestrutural demonstra que os hepatócitos possuem um núcleo arredondado e retículo endoplasmático rugoso, com disposição paralela à membrana nuclear. As células pancreáticas exócrinas apresentam grânulos de secreção na porção apical e retículo endoplasmático rugoso com distribuição concêntrica.

**Palavras-chave:** histologia hepática, ultraestrutura hepática, pâncreas exócrino, teleosteo.

## Introduction

Fish are especially susceptible to environmental variations and respond more sensitively to pollutants than numerous mammals. Their liver is then a very interesting model for the study of interactions between environmental factors and hepatic structures and functions (Bruslé and Anadon, 1996). Thus research on fish liver is important especially in the field of problems induced by aquaculture conditions and aquatic pollution (Gochfeld, 2003; Mela *et al.*, 2007).

The liver in fish is a dense organ ventrally located in the cranial region of the general cavity. Its size, shape, and volume are adapted to the available space between other visceral organs. In

many Teleostei species, the liver is divided into three lobes. However, lobulation was not found in some Teleostei (Bruslé and Anadon, 1996). The hepatic parenchyma in fish is made up of two cellular plates surrounded by sinusoids. Between two neighboring sinusoids, the hepatocytes are arranged as cords, generally two cells thick. The cords extend between central and portal zones (Hinton *et al.*, 1972; Kendall and Hawkins, 1975; Hinton and Pool, 1976; Robertson and Bradley, 1992; Bruslé and Anadon, 1996).

Previous studies have indicated that in teleost fish, the pancreatic exocrine tissue develops around the portal vein during ontogenesis. It remains extrahepatic or penetrates somewhat deeply into the liver parenchyma depending on

the species, such as *Ictalurus punctatus* (Kendall and Hawkins, 1975; Hinton and Pool, 1976), *Pimelodus maculatus* (Marconi Stipp et al., 1980), *Micropogon undulatus* (Eurell and Haensly, 1982), *Serranus cabrilla* (Gonzalez et al., 1993) and *Oreochromis niloticus* (Vicentini et al., 2005). Pancreatic tissue can be differentiated from hepatic tissue by its acinar arrangement. In addition, a thin septum of connective tissue separates the hepatocytes from the exocrine pancreatic cells (Bruslé and Anadon, 1996).

The species *Astyanax altiparanae* is found in abundance in the Paraná river system, and it is appreciated in sport fishing for human consumption. It also plays an important role in the local food chain, especially for carnivorous fish (Smith, 2003), and presents potential to aquaculture (Godinho, 2007). Then, the present study was to describe the morphological characteristics of the liver and the intrahepatic exocrine pancreas in *Astyanax altiparanae*.

## Material and methods

Thirty specimens of *Astyanax altiparanae* – 15 males ( $7.24 \pm 0.7$  cm) and 15 females ( $7.51 \pm 0.6$  cm), were obtained from a commercial fish farm in Bauru, São Paulo State, and transported to the Department of Biological Sciences, Faculty of Sciences, Unesp. The fishes were anaesthetized with 0.02% methanesulfonate and the peritoneal cavity was opened for liver exposure, which was then removed for light microscopy and transmission electron microscopy studies. For light microscopy, the liver samples were fixed in Karnovsky solution and embedded in historesin (Leica, Germany). The histological sections were stained with hematoxylin-eosin, toluidine blue and analyzed and documented photographically using an Olympus BX50 microscope (Japan).

For transmission electron microscopy, liver fragments were fixed in glutaraldehyde 2.5% in 0.1 M phosphate buffer, pH 7.2, for 3h, postfixed in 1% osmium tetroxide in phosphate buffer, washed in the same buffer, dehydrated in a growing acetone series and embedded in Araldite resin (Durcupan ACM, Fluka, Sigma-Aldrich, St. Louis, MO, USA). Resin polymerization was then completed in an oven at 60°C for 48 h. Ultrathin sections (60 and 80 nm) were cut and transported to copper grids, contrasted with uranyl acetate and lead citrate, analyzed and documented photographically using a Philips CEM 100 transmission electron microscope (TEM), at the

Electron Microscopy Center of the Biosciences Institute of Botucatu, Unesp.

## Results

The liver of *A. altiparanae* is a dense organ located in the cranial region of the general cavity. It is divided into three lobes: the right lobe, the left dorsal lobe and the left ventral lobe (Figure 1A). The gallbladder is well developed and has an elongated shape.

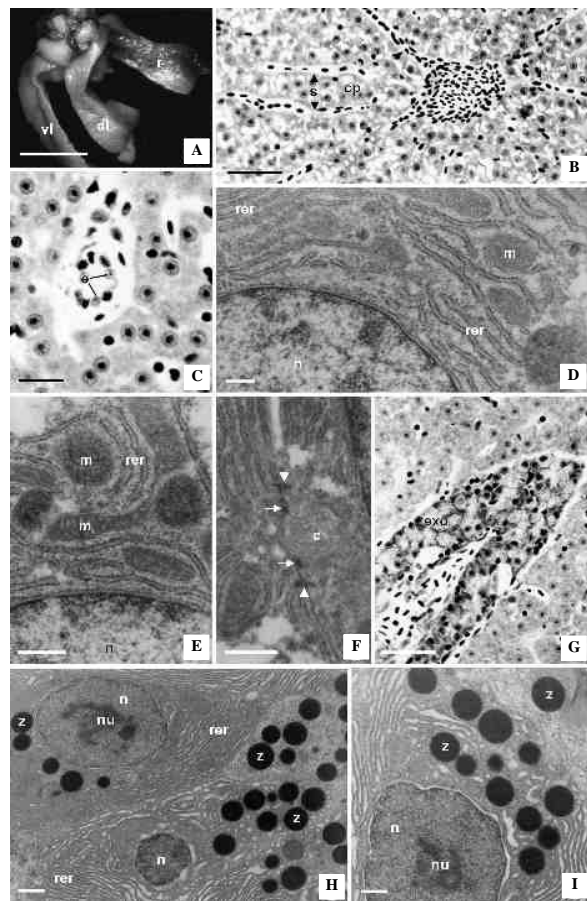
The hepatic parenchyma is made of hepatocytes spread out as anastomotic cords arranged in two cellular layers surrounding the sinusoids (Figure 1B). Light microscope observations showed that it is not possible to find hexagonal subdivisions of hepatic parenchyma or hepatic lobules. The bile ducts are usually found near the portal vein, and are lined by a simple cuboidal epithelium (Figure 1C). A concentric layer of collagen and muscular fibers were observed under the epithelium.

Ultrastructurally, the hepatocytes show a single rounded nucleus, usually centrally located. The chromatin is granular, with more condensed heterochromatin located at the periphery of the nucleus. The nucleolus is more homogenous and presents high electronic density. The rough endoplasmic reticulum is often arranged in an array parallel to the nuclear membrane. The shape of the mitochondria varies from round to elongated, and are associated to the rough endoplasmic reticulum (Figures 1D and E).

The bile canaliculi between hepatocytes typically lie in apical cell regions, toward the center of tubules or cords, although a few perisinusoidal canaliculi do occur. Microvilli from parenchymal cells often completely occlude the canalicular lumen. Desmosomal junctions commonly occur between hepatocytes near bile canaliculi, contributing to complete pericanalicular junctional complexes (Figure 1F).

Microscopic observations allowed the identification of the intrahepatic exocrine pancreatic tissue as a result of its acinar arrangement and its diffused distribution in the hepatic parenchyma (Figure 1G). The exocrine pancreatic cells were differentiated ultrastructurally from the hepatocytes by the presence of secretion granules, usually located at the apical portion of the cell. The nucleus of the pancreatic cells is generally round with a central nucleolus and it is located at the basal portion of

the cell. The rough endoplasmic reticulum is well developed, revealing dilated cisternae distributed concentrically (Figures 1H and I).



**Figure 1.** **A)** Liver of *Astyanax altiparanae* showing three hepatic lobes: right (r), dorsal left (dl) and ventral left (vl). The gallbladder is removed, Bar = 5 mm; **B)** Hepatic parenchyma consisting of two cellular plates (cp) surrounded by sinusoids (s), H/E, Bar = 0.5 µm; **C)** Bile duct showing simple cuboidal epithelium (c), H/E, Bar = 0.2 µm; **D and E)** Hepatocyte ultrastructure of *A. altiparanae*. Note: nucleus (n), rough endoplasmic reticulum (rer), mitochondria (m), TEM, Bar = 0.5 µm; **F)** A bile canaliculus (c) formed at the junction of hepatocytes. Desmosomes (arrowheads) and tight junctions (arrows) of junctional complexes occur at bile canaliculus, TEM, Bar = 0.5 µm; **G)** Organization of the intrahepatic exocrine pancreatic tissue (exo) around a blood vessel, Toluidine Blue, Bar = 0.5 µm; **H and I)** Electron micrograph of intrahepatic exocrine pancreatic cells. Note the distribution of zymogen granules (z) in the apical cytoplasm. Rough endoplasmic reticulum (rer) revealing dilated cisternae distributed concentrically. Basal cytoplasm exhibiting nucleus (n), nucleolus (nu), TEM, Bar = 0.5 µm.

## Discussion

The study of the light microscopy and ultrastructure of the liver of *A. altiparanae* showed a typical structural organization, which is similar in many teleosts. The hepatocytes are arranged in

cords, usually two cellular layers, and surrounding the sinusoids (Kendall and Hawkins, 1975; Hinton and Pool, 1976; Gonzalez *et al.*, 1993; Bruslé and Anadon, 1996; Vicentini *et al.*, 2005). The absence of division into hepatic lobules and the lack of portal triads are features of *A. altiparanae*, as evidenced in many teleosts (Hampton *et al.*, 1985; Gonzalez *et al.*, 1993; Vicentini *et al.*, 2005). However, some triads are found in *Caranx* spp. and *Lutjanus bohar* (Gonzalez, 1992).

The organization of the biliary tree in fish is similar to that observed in vertebrates. It originates in many Teleostei as an intercellular canaliculus, formed by the close apposition of two hepatocytes. The cell membrane projects numerous microvilli into the canalicular lumen and desmosomes, and tight junctions assure the cohesion (Bruslé and Anadon, 1996). According to Robertson and Bradley (1992), the canaliculi of *Salmo salar* are formed by 3 to 4 hepatocytes, and these cells do not feature filaments in the pericanalicular cytoplasm, as noted in some other teleosts (Weis, 1972; Hampton *et al.*, 1985). The structural organization of the intercellular canaliculus in *A. altiparanae* is in accordance with that reviewed in the literature (Weis, 1972; Hampton *et al.*, 1985; Robertson and Bradley, 1992; Bruslé and Anadon, 1996).

Observations by light microscopy also evidenced intrahepatic exocrine pancreatic tissue in *A. altiparanae*, associated to afferent vessels. In some species, the pancreatic tissue was identified as diffused, surrounding the digestive tract (Beccaria *et al.*, 1992; Marconi Stipp *et al.*, 1980). The pancreas in *Pimelodus maculatus* is compact, enclosed by a thin layer of conjunctive tissue and is attached to the stomach and intestine wall as small masses of glandular tissue (Marconi Stipp *et al.*, 1980). The ultrastructure of exocrine pancreatic cells of *A. altiparanae* exhibits similar characteristics to that of other teleosts (Kendall and Hawkins, 1975; Hinton and Pool, 1976; Marconi Stipp *et al.*, 1980; Beccaria *et al.*, 1992; Vicentini *et al.*, 2005).

Sea bass (Beccaria *et al.*, 1992) subjected to long fasting showed narrower lumen of excretory ducts and reduced cellular activity, demonstrated by the scarcity of zymogen granules. On the other hand, intensively fed fish were seen to have increased cellular activity and greater quantity of zymogen granules. Electron-dense zymogen granules were abundant in *A. altiparanae*. They were generally located in the apical portion of the cell. The rough endoplasmic reticulum was well developed and showed an organized pattern.

## Conclusion

The liver of *A. altiparanae* showed three hepatic lobes. Histological analysis demonstrated that the hepatocytes were spread out as anastomotic cords, arranged in two cellular layers and surrounded by sinusoids, similar to those found in other Teleostei. However, no hepatic lobules or portal triads were observed. Another characteristic observed in *A. altiparanae* was the intrahepatic exocrine pancreatic tissue associated to afferent vessels. Previous studies in teleost fish have indicated that the pancreatic tissue can remain extrahepatic or penetrate somewhat deeply into the liver parenchyma, depending on the species.

## Acknowledgments

This work was supported by a grant from the Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (300693/91-5 and 107993/2004-0), Brazil.

## References

- BECCARIA, C. et al. Effects of dietary conditions on the exocrine pancreas of the sea bass, *Dicentrarchus labrax* L. (Teleostei). *Aquaculture*, Amsterdam, v. 101, p. 163-176, 1992.
- BRUSLÉ, J.; ANADON, G.G. The structure and function of fish liver. In: MUNSHI, J.S.D; DUTTA, H.M. (Ed.). *Fish Morphology*. North-Holland: Science Publishers, 1996. p. 77-93.
- EURELL, J.A.; HAENSLY, W.E. The histology and ultrastructure of the liver of Atlantic croaker *Micropogon undulatus* L. *J. Fish. Biol.*, London, v. 21, p. 113-125, 1982.
- GOCHFELD, M. Cases of mercury exposure, bioavailability, and absorption. *Ecotox. Environ. Safe.*, New York, v. 56, p. 174-179, 2003.
- GODINHO, H.P. Estratégias reprodutivas de peixes aplicadas à aquicultura: bases para o desenvolvimento de tecnologias de produção. *Rev. Bras. Reprod. Anim.*, Belo Horizonte, v. 31, n. 3, p. 351-360, 2007.
- GONZALEZ, G. *Contribution à la connaissance des processus ciguatérigènes*. 1992. Thèse (Doctorat Spécialité Oceanologie)–Université de Perpignan, Perpignan, 1992.
- GONZALEZ, G. et al. Histo-cytological study of the liver of the cabrilla sea bass, *Serranus cabrilla* (Teleostei, Serranidae), an available model for marine fish experimental studies. *J. Fish. Biol.*, London, v. 43, p. 363-373, 1993.
- HAMPTON, J.A. et al. Functional units in rainbow trout (*Salmo gairdneri*) liver: I. arrangement and histochemical properties of hepatocytes. *The Anat. Rec.*, New York, v. 213, p. 166-175, 1985.
- HINTON, D.E. et al. Morphology and enzyme histochemistry in the liver of largemouth bass (*Micropterus salmoides*). *J. Fish. Res. Board Can.*, Ottawa, v. 29, p. 531-34, 1972.
- HINTON, D.E.; POOL, C.R. Ultrastructure of the liver in channel catfish *Ictalurus punctatus* (Rafinesque). *J. Fish. Biol.*, London, GB, v. 8, p. 209-19, 1976.
- KENDALL, M.W.; HAWKINS, W.E. Hepatic morphology and acid phosphatase localization in the channel catfish (*Ictalurus punctatus*). *J. Fish. Res. Board Can.*, Ottawa, v. 32, p. 1459-64, 1975.
- MARCONI STIPP, A.C. et al. Fine structural analysis of a teleost exocrine pancreas cellular components – a freeze-fracture and transmission electron microscopic study. *Anat. Anzeiger, Alemanha*, v. 147, p. 60-75, 1980.
- MELA, M. et al. Effects of dietary methylmercury on liver and kidney histology in the neotropical fish *Hoplias malabaricus*. *Ecotox. Environ. Safe.*, New York, v. 68, p. 426-435, 2007.
- ROBERTSON, J.C.; BRADLEY, T.M. Liver ultrastructure of juvenile Atlantic salmon (*Salmo salar*). *J. Morphol.*, New York, v. 211, p. 41-54, 1992.
- SMITH, W.S. Os peixes do rio Sorocaba: a história de uma bacia hidrográfica. Sorocaba: TCM – Comunicação, 2003.
- VICENTINI, C.A. et al. Morphological study of the liver in the teleost *Oreochromis niloticus*. *Int. J. Morphol.*, Temuco, v. 23, n. 3, p. 211-216, 2005.
- WEIS, P. Hepatic ultrastructure in two species of normal, fasted and gravid teleost fishes. *Am. J. Anat.*, Philadelphia, v. 133, p. 317-332, 1972.

Received on May 15, 2007.

Accepted on January 15, 2008.