

Morphological Description of Blue Shark Liver, *Prionace glauca* (Linnaeus, 1758), Elasmobranchii, Carcharhiniformes

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Abstract— *The liver is the largest gland in the body and plays a central role in metabolic and immune homeostasis. This body is responsible for more than 200 functions such as detoxification, storage, energy production, nutrient conversion, hormonal balance and coagulation. A study of the morphology of the liver of the blue shark (Prionace glauca) during the development phase was carried out. To describe it was used light microscopy, scanning electron and counting of liver cells in this species. The liver occupies 20% of the size of the animal. Fat is gradually accumulated in the liver tissue with the development of the animal, reaching almost 60% of the liver in animals ready to be born.*

Keywords— *Liver. Microscopy. Morphology. Lipids.*

I. INTRODUCTION

In fish, the liver has a function very similar to terrestrial vertebrates as an important role in the metabolism of lactate in pyruvate [1]. This organ can store up to one-eighth of the total glycogen reserves that are consumed during some physical exercise, being used as a source of energy, or even in food when there is a shortage of food [2].

In the marine environment, the blue shark (*Prionace glauca*) is a species of shark that is present in tropical and subtropical waters and is easily captured [3][4][1][5][6]. This species is widely used in culinary consumption and has been decreasing marine stocks bringing ecological concern [7]. Replacement of this species becomes very difficult because it is K-strategist, that is, low level of fecundity and late sexual maturation [8].

To understand the liver of the *Prionace glauca* focus of the study, we begin by understanding the embryonic development of the liver in sharks and bone fish

that forms a ventral evagination of the floor of the digestive tract behind the stomach, the more caudal evagination forms the gallbladder and the evagination cranial, being even in most fish, it branches and expands to give rise to the liver [9]. The mesenchyme found in the coelomic cavity induces the hepatic evagination endoderm to proliferate, branch out and differentiate into hepatocytes, liver glandular cells [10][11][12].

Still in these species it presents the format of a horseshoe. The right and left lobes are the same and are connected by an isthmus. The gallbladder is elongated and usually included in the right lobe [13]. They are formed by right and left lobe presenting microscopically lobes formed by hepatic cords, vascularization anastomosed with the central lobular vein [12]. It appears as in all other vertebrates, a key organ that will control many vital functions and play a prominent role in fish physiology, both in anabolism (proteins, lipids and carbohydrates) and in catabolism (nitrogen, glycogenolysis and detoxification). important role in vitellogenesis [14]. On the other hand, it should be considered as a target organ of different physical and environmental aggressions leading to structural and metabolic modification, causing death of the animal [15]. The liver can be considered the starting point for comparative and phylogenetic studies among vertebrates [14][16].

The differences in the organization of the fish liver are due to the complexity of the liver organization that requires a three-dimensional approach, taking into account the plans of histological cuts.

II. MATERIAL AND METHODS

The 18 specimens of blue shark were 6 (six) of 13 cm, 6 (six) of 26 cm, 6 (six) of 45 cm, were found in the department of Anatomy of the Tuberculosis and wild of the Faculty of Veterinary Medicine and Animal Science at the University of São Paulo. Animals were measured according to Sadowsky [17], and divided into different sizes of embryos and fetuses.

For the collection of the liver, the tubers were opened to the alva line, cranial-cranial direction, breaking the pelvic girdle until the rupture of the scapular waist, located ventrally between the pectorals.

The histochemical analyzes were performed on the samples of the medial lobe D and E of the liver, fixed in 10% and dehydrated in the increasing series of ethanol (70 to 100%), diaphanized in xylol, including paraffin. 5 μ m cuts were performed on microtome (Leica, German) and stained with hematoxylin-eosin (HE).

Part of the samples were prepared for semifinished cuts, as they were dehydrated in series with increasing growths (50 to 100%), placed in propylene oxide, embedded in Spurr resin. The blocks were sectioned in a glass knife 300nm thick and stained with toluidine blue. All microscopes of light microscopes of microscopes of light Nikon Eclipse E- 800 of the Advanced Center of Diagnostic by Image - CADI-FMVZ-USP.

For scanning electron microscopy (SEM), the islands were dehydrated in increasing series of humidity of 50% to 100%, with low sulfuric acid content (FMVZ-USP) and carbon colonies in metallic (stub) and metallic bases (*Sputtering*) with silver on the EMITECH K550 metallizer (FMVZ-USP), analyzed and photographed using a scanning electron microscope LEO 435VP (FMVZ-USP).

Quantitative analysis of hepatic cells was performed on 13cm, 26cm and 45cm size tubes, evaluating 18 random photos by capturing computerized images, positioned in a point system containing 1148 points equidistant to 0.5cm. Determination of hepatocyte areas, hepatic nucleus, intracytoplasmic vacuoles, lobular center vein. Statistical analysis was performed by the ANOVA, TUKEY and Pearson correlates. The level of significance was set at $p < 0.05$.

III. RESULTS AND DISCUSSION

In all samples, the liver of *P. glauca*, located ventrally in the celoma cavity, consists of two long, pointed lobes with a half-moon shape, yellowish and connected by the isthmus. in touch. The liver corresponds to 20% of the total size of the animal. The gallbladder is inserted into the more cranial portion of the right lobe with elongated shape (Figure 1A).

Sharks, like cartilaginous fish, do not have a swimming bladder and are forced to remain in constant motion to avoid sinking [18]. The mean density of the liver is related to the amount of lipids contained therein, and the high oil content allows these animals to float more easily in the water column, acting as a hydrostatic organ [19], corroborating with the where the liver presented 20% of the size of the animal, macroscopically, the size, shape and disposition of its evident liver filling the space available in the celoma cavity of the shark.

Microscopically the liver presented hepatocytes with its central nucleus and hepatocytes with different sizes of vacuoles inserted into the cells displacing their nucleus to the periphery (figure 1B).

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In the six 13cm embryos he observed in the liver a disorganized cellular arrangement without dividing the liver into lobules and some vacuolated hepatocytes of translucent staining and small denominated of microgoticulares (figure 1C, D, E).

In the 26cm animals, there was an increase of the intracytoplasmic vacuoles in all the hepatocyte cells, even with the presence of vacuoles, he observed an organized cellular arrangement evidencing the hepatic cords (Figure 1F, G, H).

In fetuses of 45 cm, it is observed a better organization, when compared to the smaller animals, in hepatic cords with large intracytoplasmic translucent vacuoles denominated of macrogoticulares occupying all hepatic cells and displacing the nucleus to the cellular periphery, causing a balloon format in the hepatocytes (figure 1I, J, K).

In mammals the fat accumulation disorder within liver cells, hepatocytes, is characteristic of a pathological condition, hepatic steatosis, and when there is excess fat and for a long time, the liver cells may suffer damage, becoming inflamed [20]. In sharks, it is a healthy and necessary condition, since sharks use this stock of fat as an adaptation to aid in their buoyancy, as well as serve as a stock of glycogen to transform into glucose for energy, since they are animals that have a great energy expenditure. Another function of the fat of your liver is the importance during vitellogenesis, where the females use this fat for the

maintenance and nutrition of the puppies. Thus, over time, this accumulation of fat pushes the hepatocyte nucleus to the periphery of the membrane, a characteristic action of hepatic steatosis [20].

In the morpho-quantitative analysis, in the counting of the points of the test system, there was a

significant increase of intracytoplasmic vacuoles micro and macrogoticulares, according to the growth of the animal. The analysis of variance (** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$), observed in figure 1.L.

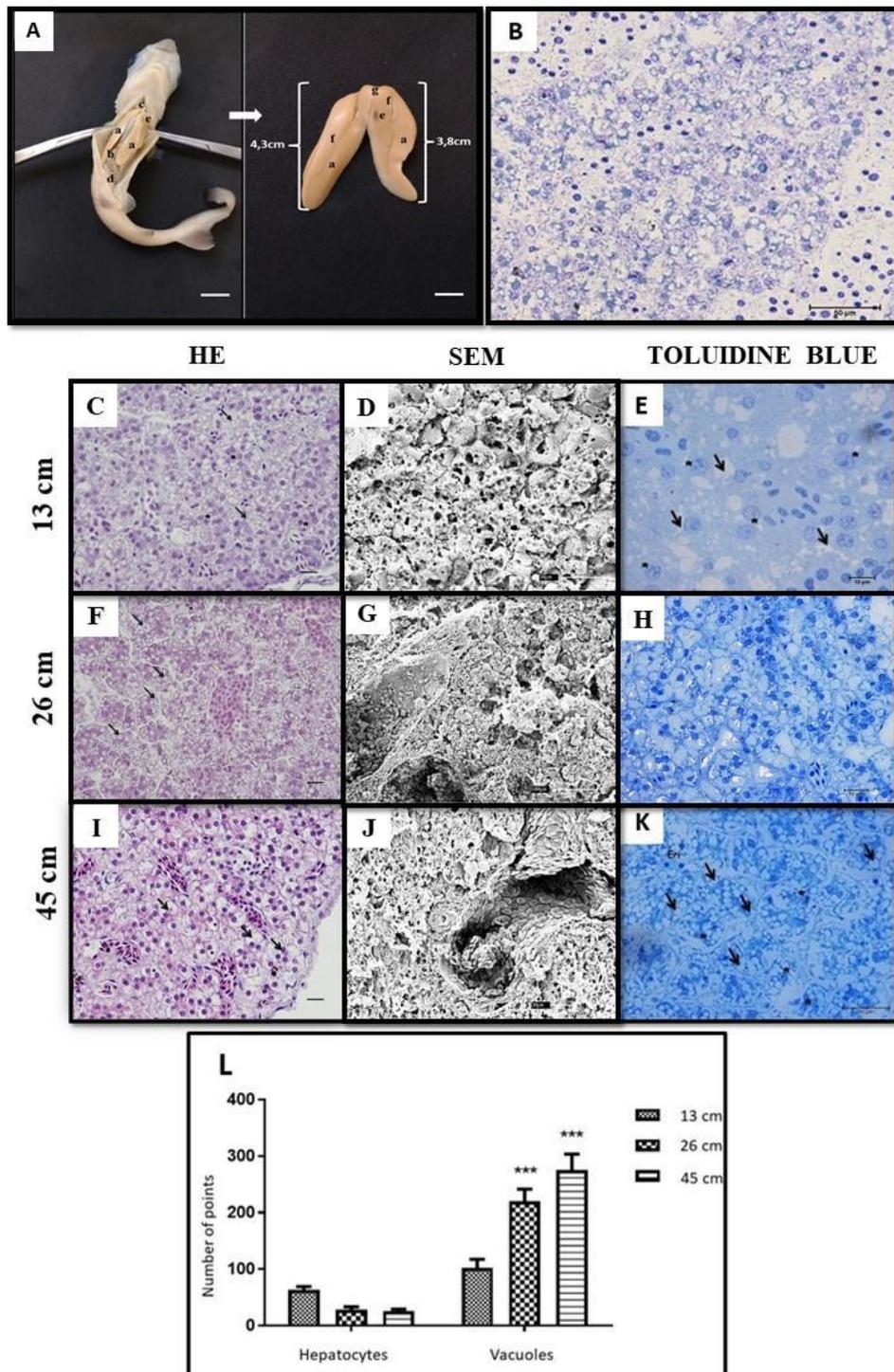


Fig.1: Morphology of *P. glauca*. In A - Opening of the cavity for access to the liver, ventrally located to the (b) stomach and intestine, caudally to (c) heart and cranially to (d) rectal gland. Connected by (g) isthmus, with (e) adhered gallbladder and (f) impressions of nearby organs. In B microscopy of the liver. Plank from C to K divided by size and staining techniques - (arrows) hepatocytes, (*) micro-articular vacuoles. Bar: 1cm. In L statistical analysis of the results.

Liver size and weight vary according to species, age and season, but almost always correspond to a fifth of their total weight and can accumulate up to 90% in oil [21]. According to BRUSLÉ and ANADON [14], there are differences in liver structures between females and males, as well as between mature and immature fish, but in our work, we could notice a nonsignificant difference between the livers of male and female embryos.

Sharks have a huge concentration of fat in their liver, in an oleic state called squalene [22]. Where in this study we could observe that this stocking of fat begins from the beginning of its embryonic stage, the hepatocytes accumulate fat in its cytoplasm according to the growth of the embryo, statistically proved by the analysis of variance where $p < 0.001$.

IV. CONCLUSION

The liver occupies 20% of the size of the animal. Fat is gradually accumulated in the liver tissue with the development of the animal, reaching almost 60% of the liver in animals ready to be born.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

REFERENCES

- [1] BORNATOWSKI, H.; SCHWINGEL, P. R. (2008). Feeding and breeding of the blue shark, *Prionace glauca* (Linnaeus 1758), caught on the southeast coast and south of Brazil. *Archives of Science of the Sea*. v. 41, n. 1, p. 98-103.
- [2] STOSKOPF, M. (1993). Anaesthesia. In: Brown, L. (ed.). *Aquaculture for veterinarians: fish husbandry and medicine*. Pergamon Veterinary Handbook Series. London. p.161-168.
- [3] COMPAGNO, L.J.V. & DANDO, M. & Fowler, S. (2005). *Sharks of the World*. Princeton University Press.
- [4] NAKANO, H.; SEKI, M. P. (2003). Synopsis of biological data on the blue shark *Prionace glauca* Linnaeus. *Bulletin of Fisheries Research Agency*, v. 6, p. 18-55.
- [5] QUAGGIO, A. L. C.; KOTAS, J.E.; HOSTIM, M. (2008). The catches of the blue shark, *Prionace glauca* Linnaeus (Elasmobranchii, Carcharhinidae), in the surface longline fishery (monofilament), based in Itajaí (SC), Brazil. *Pan-American Journal of Aquatic Sciences*. v. 3, n. 1, p. 61-74.
- [6] LEGAT, J.F.A.; VOOREN, C.M. (2008). Distribution and relative abundance of the Blue Shark, *Prionace glauca*, in southern Brazil. *Bulletin of the Institute of Fisheries*. v. 34, n. 3, p. 425-432.
- [7] VOOREN, C.M., CASTELLO, J.P., DE BEM JR, R.T., GOMEZ, I.C., HELLENBRANDT D., & ISOLDI, M. I. (1999). Assessment of the fishery resources of large pelagic fish. Distribution and abundance of fish. Part 1. ARGO Project Survey of the living resources of the EEZ pelagic environment - Southern Region Final Report Volume 2. Rio Grande University Foundation Rio Grande - RS.
- [8] JENNINGS, S., KAISER, M.J. & REYNOLDS, J. D. (2001). *Marine fisheries ecology*. Black well Science, Oxford. 417 p.
- [9] HILDEBRAND, M. and GOSLOW, G.E. (2006). *Analysis of vertebrate structure*. Ed. Atheneu.
- [10] ROMER, A.S.; PARSON, T.S. (1993). *Comparative anatomy of vertebrates*. Ed. Saunders, Philadelphia.
- [11] KARDONG, K.V. (2011). *Vertebrates. Comparative anatomy, function and evolution*. Ed. Roca.
- [12] LIEM, K.F.; BEMIS, W.E.; WARKER, W.F.; GRANDE, L. (2013). *Functional anatomy of vertebrates. An evolutionary perspective*. 3rd Ed. North American Publishing House.
- [13] GRASSÉ, P.P. (1958). *Traité de Zoologie. Anatomie, Systématique, Biologie. Tome XIII. Agnathes et Poissons*. Ed. Masson. Paris.
- [14] BRUSLÉ, J.; ANADON, G.G. (1996). The Structure and Function of Fish Liver. In: *Fish Morphology*. Science Publishers. pp 77-93.
- [15] ZAVALA-CAMIN, L.A. (2004). *The planet water and its fish*. Author's edition. Santos.
- [16] COMPAGNO, L.J.V. (2001). *Sharks of the World. An annotated and illustrated catalog of the shark species known to date. Volume 1 2 and 3*. FAO Species Catalog for Fisheries Purposes No. 1, Vol.2. FAO, Rome.
- [17] SADOWSKY, V. (1968) On the measurement of the total length of sharks. *Zoologischer Anzeiger*, 181: 197-199.
- [18] SUZUKI, Z., WARASHINA, Y., and KISHIDA, M. (1986). The comparison of catches by regular and deep tuna longline gears in the western and central equatorial Pacific. *Bull. Far Seas Fish. Res. Lab*, 15: 51-89.
- [19] VAN VLEET JE, FERRANS VJ. (1984). Ultrastructural alterations in skeletal muscle of pigs with acute monensin myotoxicosis. *Am J Pathol* 114: 461.

- [20] SANTOS VN, LEITE-MÓR MMB, PARISE ER *et al.* (2001). Serum concentration of basement membrane proteins and hyaluronic acid in the non-invasive diagnosis of FGNA fibrosis. *GED*, 20: S21.
- [21] KREUZER, R. AHMED, R. (1978). Food and Agriculture Organization of the United Nations. International Trade Center UNCTAD / GATT. Food and Agriculture Organization of the United Nations. vii, 180 p.
- [22] SIMOPOULOS AP. (1986). Historical perspective, conference conclusions and recommendations, and actions by federal agencies. In: Simopoulos AP, Kifer RR, Martin RE, editors. *Health Effects of Polyunsaturated Fatty Acids in Seafoods*. Orlando, FL; Academic Press, 3-29.