ORIGINAL RESEARCH

Ultrastructure and cellular organization of longsnout seahorse *Hippocampus reidi* liver

Luis A. Romano[1], Luana B. Giesta[1, *], Virginia F. Pedrosa[1], Michael H. Schwarz[3], Luís A. Sampaio[2] and Ricardo V. Rodrigues[2]

[1]Laboratório de Imunologia e Patologia de Organismos Aquáticos, Instituto de Oceanografia, Universidade Federal do Rio Grande (FURG), Rio Grande, Brazil. [2]Laboratório de Piscicultura Estuarina e Marinha, Instituto de Oceanografia, Universidade Federal do Rio Grande (FURG), Rio Grande, Brazil. [3]Virginia Tech, Virginia Seafood Agricultural Research and Extension Center, 102 S. King Street, 23669 - Hampton, USA.
ORCID *Luis A. Romano* https://orcid.org/0000-0003-3144-3579, *Luana B. Giesta* https://orcid.org/0009-0007-2189-8357, *Virginia F. Pedrosa* https://orcid.org/0000-0003-3353-1994, *Michael H. Schwarz* https://orcid.org/0000-0002-9618-6262, *Luís A. Sampaio* https://orcid.org/0000-0001-6533-1837, *Ricardo V. Rodrigues* https://orcid.org/0000-0002-8809-4739

ABSTRACT. *Hippocampus reidi* is an endangered tropical species, as well as being one of the most widely traded marine fish globally. The cellular organization of the normal liver in the longsnout seahorse (*H. reidi*) was studied using electron microscopy to provide an overview of the liver's fine structure. Two hundred and fifty seahorses were collected and prepared for transmission electron microscopy. Sections were cut using an LKB ultramicrotome and then washed with PBS, stained with uranyl citrate for 2-3 min, and then with lead acetate for 20 s. The grids were then observed using an electron microscope Zeiss EM 109. The cytoplasm of the hepatocyte contains a nucleus surrounded by a double membrane, diffusely scattered mitochondria and a network of the endoplasmic reticulum of rough and smooth types. Most of the rough endoplasmic reticulum appear to be in parallel to the nuclear envelope or the cell membrane and was situated relatively in the center part of the cytoplasm. The cellular organization of the longsnout seahorse liver is quite similar to that of other mammalian species.

Key words: Hepatocyte, electron microscopy, histological morphology.

∗Correspondence:
luanabortolinigiesta@gmail.com

Received: 3 October 2025
Accepted: 17 November 2025

ISSN 2683-7595 (print)
ISSN 2683-7951 (online)

https://ojs.inidep.edu.ar

Journal of the Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP)

Ultraestructura y organización celular del hígado del caballito de mar de hocico largo *Hippocampus reidi*

RESUMEN. *Hippocampus reidi* es una especie tropical en peligro de extinción y uno de los peces marinos más comercializados a nivel mundial. Se estudió la organización celular del hígado normal del caballito de mar de hocico largo *H. reidi* mediante microscopía electrónica, lo que proporcionó una visión general de la estructura fina del hígado. Se recogieron 250 *H. reidi* y fueron preparados para microscopía electrónica de transmisión. Se cortaron secciones con un ultramicrótomo LKB y se lavaron con PBS, se tiñeron con citrato de uranilo durante 2-3 min y después con acetato de plomo durante 20 s. Por último, las rejillas se observaron con un microscopio electrónico Zeiss EM 109. A lo largo del canalículo biliar se proyectan numerosas microvellosidades. El citoplasma del hepatocito contiene un núcleo rodeado por una doble membrana, mitocondrias dispersas y una red de retículos endoplásmicos de tipo rugoso y liso. La mayoría de los retículos de tipo rugoso parecen estar en paralelo con la envoltura nuclear o la membrana celular y se sitúan relativamente en la parte central del citoplasma.

Palabras clave: Hepatocito, microscopía electrónica, morfología histológica.

INTRODUCTION

Seahorses are considered endangered species and have been listed in the Convention for the International Trade in Endangered Species (CITES 2009) and as Near Threatened (NT) on the IUCN Red List of Threatened Species (Oliveira and Pollom 2017). Seahorse populations have decreased worldwide owing to their use in traditional Chinese medicine, souvenir commerce, habitat degradation and sales in the ornamental trade (Foster et al. 2016; Koning and Hoeksema 2021). In order to reduce fishing pressure and supply the ornamental trade, many seahorses have been considered for commercial aquaculture production (Koning and Hoeksema 2021). There is information available on *Hippocampus reidi* Ginsburg, 1933 wild populations, indoor-reared reproduction, and larviculture (Freret-Meurer and Andreata 2008; Hora and Joyeux 2009; Koning and Hoeksema 2021). However, no information was found in the literature related to liver ultrastructure of the longsnout seahorse.

In both Osteichthyes and Mammalia, the liver is a very large, discrete, encapsulated, sinusoidally perfused gland that is relatively homogeneous at the subgross level. The fish liver features the same general circulatory components as the mammalian liver; that is, blood is supplied by hepatic arterioles and portal veins and is drained by hepatic veins. The biliary apparatus of fish is also comparable to that of mammals (Gingerich 1982). Although subtle differences in sinusoidal structure exist, the most variable aspects of the fish liver involve the biliary system, in which there are considerable interspecies differences in the length and position of ducts (Gingerich 1982). For example, in fish such as salmon, the common bile duct and cystic duct are intra- rather than extrahepatic, and intracellular canaliculi have been observed in some cyprinids (Gingerich 1982). Consequently, the aim of the present study was to describe the ultrastructure and the cellular organization of normal longsnout seahorse *Hippocampus reidi* liver using electron microscopy.

MATERIALS AND METHODS

Immediately after hatching, 250 seahorses were collected using a 1 l beaker and transferred to a 50 l tank, connected to a semi-static system. During the larviculture, temperature was maintained at 27 ± 0.5 °C, salinity 23, pH 7.85, Total Ammonium Nitrogen (TAN) 0.22 ± 0.03 mg l^{-1}, NO_2 0.05 ± 0.01 mg l^{-1}, NO_3 4.7 ± 0,4 mg l^{-1}, alkalinity 143 ± 9 mg l^{-1} as $CaCO_3$, and constant photoperiod was provided. Seahorses were fed enriched rotifers *Brachionus plicatilis* (Protein Selco Plus, Inve, Belgium) (5-10 ind. ml^{-1}) from day 1 to 6 post-hatching (ph); *Artemia* nauplii (Inve, Belgium) (2-5 ind. ml^{-1}) introduced from day 5 to 8 followed by enriched *Artemia* (Easy Selco, Inve, Belgium) (3-10 ind. ml^{-1}) from day 8 to the end of the experiment. During the rotifer feeding stage, larviculture was carried out using the 'green water' system, adding microalgae *Nannochloropsis* sp. paste (Instant algae, USA) (100-200.000 cel ml^{-1}). At day 50 ph, five seahorses were euthanized with 100 mg ml^{-1} MS 222.

For transmission electron microscopy, 1-2 mm sections of liver tissue were fixed in 0.2% glutaraldehyde for 2 h, then washed with PBS and processed in increasing concentrations of ethanol. Tissue fragments were then embedded in Lowicryl K4M (Chemische Werke Lowi, Waldkraiburg, West Germany) for 48 h at 4 °C with ultraviolet light exposure. Sections were cut using an LKB ultramicrotome, washed with PBS, stained with uranyl citrate for 2-3 min and lead acetate for 20 s. Grids were then observed using a Zeiss EM 109 electron microscope.

RESULTS

The polygonal hepatocyte was surrounded by a cell membrane, part of which faced the Disse space, and numerous microvilli projected along the bile

canaliculus. The cytoplasm of the hepatocyte contained a nucleus surrounded by a double membrane, diffusely scattered mitochondria, and a network of endoplasmic reticulum of rough and smooth types. Most of the rough reticulum appeared to be in parallel to the nuclear envelope or the cell membrane and were situated relatively in the center part of the cytoplasm. The smooth endoplasmic reticulum ribosomes were situated mostly in the peripheral region and frequently associated with deposits of stored glycogen. Free ribosomes and glycogen particles were also numerously encountered. A few lysosomes and Golgi complexes were observed around the bile canaliculus, located between two or three hepatocytes (Figure 1 A).

The nucleus, round or oval in shape and measuring around 10 μm in diameter, was usually located at the center of the hepatocyte. It was surrounded by the nuclear membrane. The nucleoplasm contained a dense round nucleolus that was not limited by a lamella. The nuclear membrane consisted of two lamellae, the external and the internal, separated by a perinuclear space of around 40 to 50 μm in width. The external lamellae were occasionally observed to be continuous with the lamellae of the endoplasmic reticulum. Thus, this nuclear membrane seemed to be a part of the endoplasmic reticulum and developed from it. The nuclear membrane was perforated by a large number of pores of 400 to 1,000 Å or more in diameter. A tangential section of the nuclear membrane showed pores as small annuli of the same size as seen in a cross section. Pores area occasionally appears to be denser, suggesting the possibility of a diaphragm. The external and internal lamellae were joined at their edges. A large number of pores in the nuclear envelope, and the continuation of the nuclear membrane with the endoplasmic reticulum, provided the exchanges of

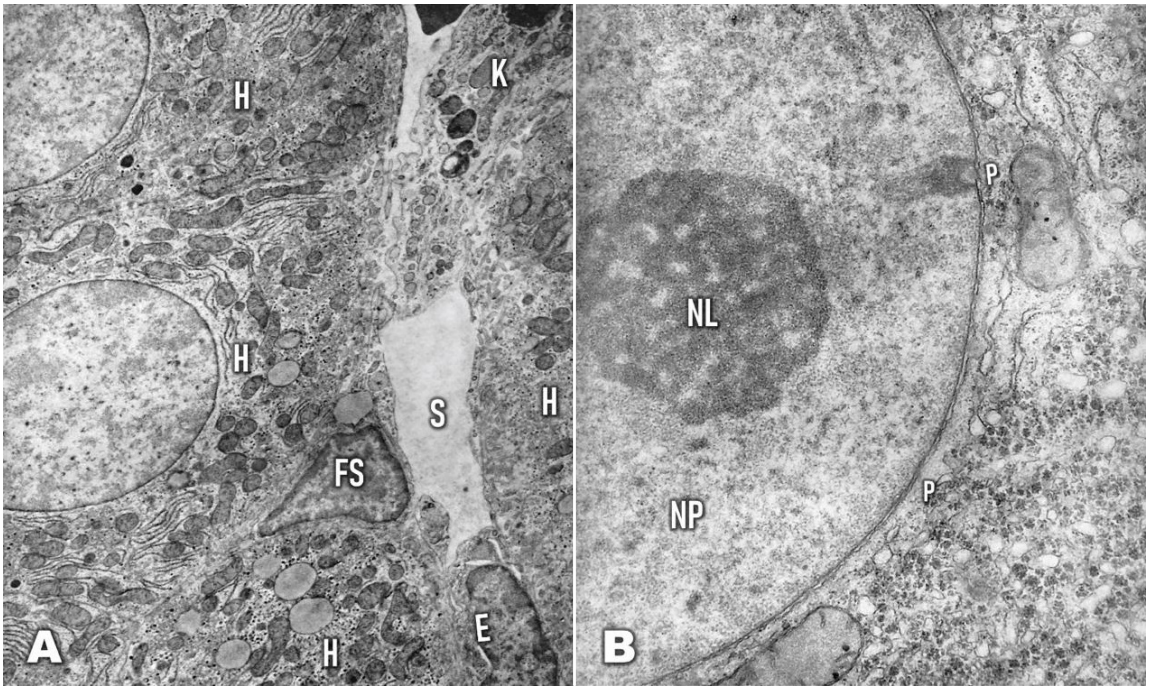

Figure 1. A) Photomicrograph showing general structural components of *Hippocampus reidi* liver. H: hepatocytes, K: Kupffer cell. E: endothelial cell. FS: spindle cell ×5,000. B) The nuclear membrane consists of two lamellae, in part, perforated by pores (P). The nucleoplasm (NP) in the vicinity to a nuclear pore appears to be dense. The nucleolus (NL) consists of the nucleolemma and the pars amorpha. The former was made of dense filaments, and latter was a diffuse matrix. ×25,000.

substances between the nucleoplasm and the cytoplasm. Therefore, substances synthesized in the nucleus or in the cytoplasm could pass either through the canal of the nuclear envelope, connected with the endoplasmic reticulum or through numerous nuclear pores (Figure 1 B and Figure 2 A).

The nucleoplasm contained numerous fine granules ranging from 150 to 200 Å in diameter, primarily composed of DNA and arranged in the nucleoplasm (Figure 2 A). The nucleolus, not surrounded by a membrane, consisted of two major components, a nucleolemma and a pars amorpha. The nucleolemma was an aggregate of anastomosing filaments of 100 to 200 μm in diameter, composed of dense 100 to 150 Å in diameter particles. The pars amorpha was a diffuse matrix.

Hepatocytes were limited by a single membrane that appeared in different directions. Under high power magnification, the cell membrane had a unit membrane structure around 75 Å in width, composed of two dense outer layers and a light intermediate one. The cell membrane facing the Disse space and along the bile canaliculus had numerous microvilli, which effectively increase surface for uptake or secretion, and probably regulate plasma flow in the Disse space (Figure 3 A). The cell membrane bordering the adjacent hepatocyte membrane was straight and smooth. Pinocytic invaginations of the cell membrane, about 0.1 μm in width, were frequently observed at the cell membrane facing the Disse space with increased density at the concaved part (Figure 2 B).

The width of the intercellular space between two hepatocytes was usually constant and about 100 to 200 Å. The ‘studlike’ projections and junctional complexes, which were commonly observed surrounding the bile canaliculus, attached the hepatocyte to the next cell.

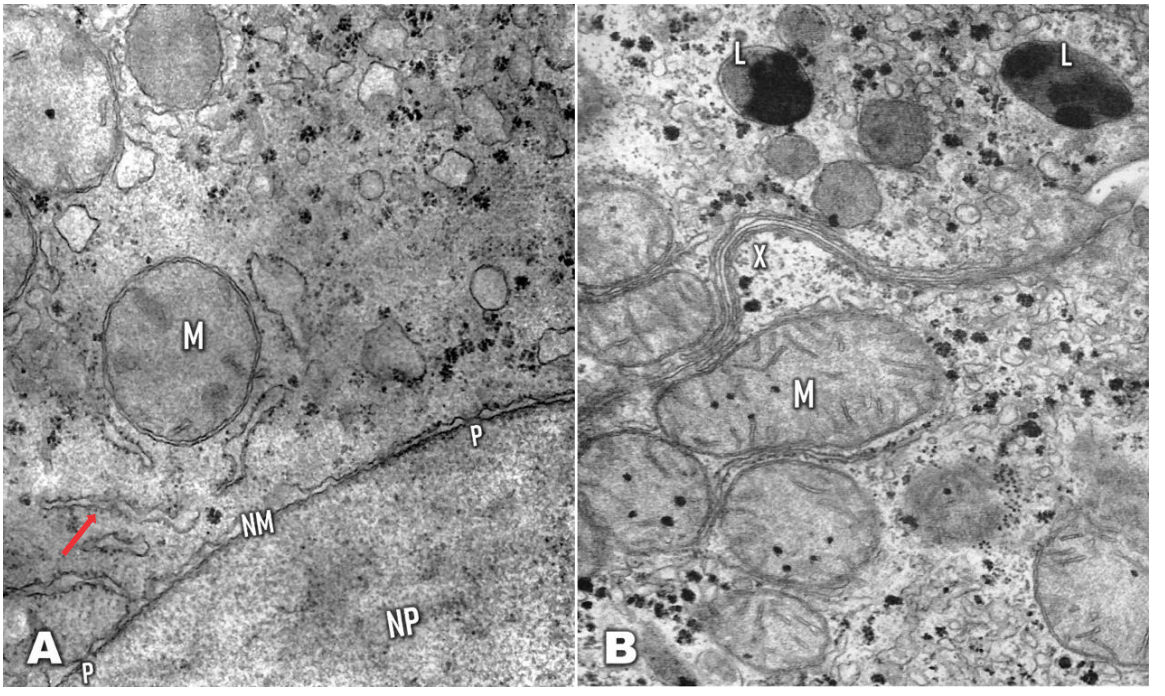

Figure 2. A) Nuclear membrane (NM) is perforated by pores (P), appearing to be somewhat denser than the surroundings. It was observed with the smooth surface of endoplasmic reticulum close and in parallel to the membrane (arrow). NP: nucleoplasm. M: mitochondria. ×48,000. B) Projection of the cellular membrane (X) of the hepatocyte. M: mitochondria. L: lysosome. ×40,000.

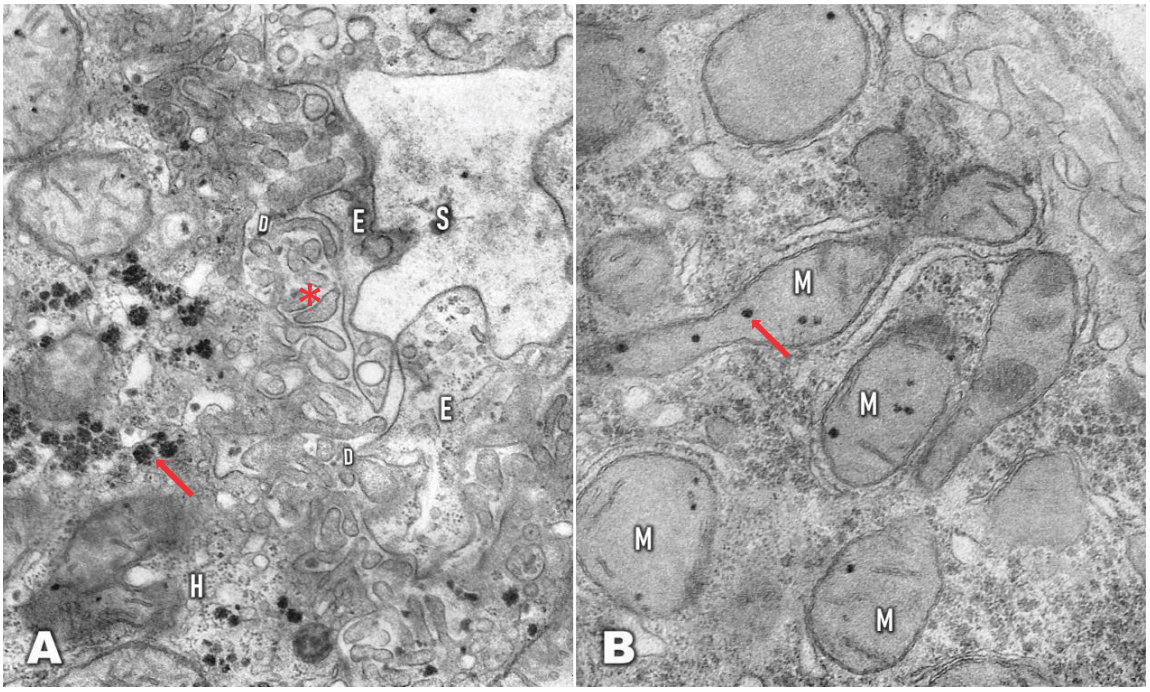

Figure 3. A) The cell membrane projects numerous microvilli (∗) into the Disse space (D). Numerous chylomicrons, appeared as small dense particles, showed to be taken up into the hepatocyte (H) by the invagination of the cell membrane (pinocytosis - arrows). E: endothelial cell. S: sinusoid. ×41,000. B) Mitochondria (M) were surrounded by double lamellae. The cristae, protruded from the inner lamella, and mitochondrial granules (arrows) were observed in the matrix. ×45,000.

The mitochondria, usually round, oval or oblong in shape, appeared to be composed of a homogeneous matrix of moderate electron density and surrounded by a double membrane, outer and inner lamellae. The width of the single lamella was about 50 to 70 Å, and the intervening low-density layer between two lamellas was around 70 to 100 Å in width. Electron-dense mitochondrial granules, roughly 300 Å in size, were visible in the matrix, and mitochondrial crests, which protruded from the inner lamella into the matrix, typically had a tubular shape. The mitochondrial matrix contained fine granules of about 65 to 100 Å in size (Figure 3 B).

The cytoplasm of the hepatocyte contains a network of endoplasmic reticulum (ER), membrane-limited channels, disposed as tubules, vesicles or sacs. The ER was subdivided into a smooth surfaced (agranular) and a rough surfaced (granular or ergaslloplasm) forms, and its cisternae appeared to be slightly more opaque than the surrounding cytoplasm, probably because of its contents. The rough surfaced ER was characterized by the presence of electron dense particles of about 150 to 200 Å in size on its surface. It was usually in regular arrangement of flattened cisternae. Electron-dense particles were referred to as ribonucleoprotein particles or ribosomes, which contained the largest part of the cytoplasmic nuclei acid. The smooth surfaced ER was less than the rough surfaced ER in normal liver and mostly observed in the peripheral region of the cytoplasm.

The Golgi complex consisted of 3-5 flat cisternae in close parallel arrangement and vesicles of varied sizes. The ends of the cisternae were often dilated and contained dense granules, 300 to 800 Å in diameter. The Golgi complex was frequently seen around the bile canaliculus. However, it was also occasionally observed near the nucleus.

Lysosomes, which ranged in size from 0.3 to 1.09 μm, were limited by a single membrane and contain numerous electron dense ferritin-like particles and varying polymorphous contents. They were frequently observed in the pericanalicular zone of the hepatocytc. By means of electron microscopy, glycogen particles, free ribosomes, fat droplets, various pigments and ferritin molecules, were identified in the cell matrix.

The fine structural features of seahorse showed prominent large round nuclei, and occasionally two nuclei. The major part of the cytoplasm was occupied by mitochondria, both smooth and rough endoplasmic reticulum, and scattered glycogen particles. The basolateral portion of the hepatocyte, which faces the sinusoidal capillaries, displayed a rich microvillous elaboration of the plasmalemma. This microvillous border occupied the major part of the Disse space canaliculi, formed by a widening of the intercellular space. However, the canalicular lumens were set off from the intercellular space by occluding junctions.

DISCUSSION

Data of the present study provide an overview of the fine structural organization of the *Hippocampus reidi* liver. The fundamental features of the seahorse liver resulted in remarkably similar to the features of liver from other fish species. The prominent cell type is the hepatocyte, characterized by the presence of intracellular albumin (Yokota and Fahimi 1981). Parenchymal cells in the liver are the hepatocytes, while non-parenchymal cells include the Kupffer- like cells, and endothelial cells (Naito et al. 1997).

Small profiles of the Golgi apparatus were seen occasionally in the apical cytoplasm close to canaliculi, but we did not detect secretory vesicles associated with canaliculi. The canaliculi are believed to form an anastomosing system as these small bile ducts are a consistent feature in the portal areas.

Monocyte derived macrophages are found in virtually every organ and tissue of the body and comprise the diffuse reticulo-endothelial system (Aschoff 1924; Furth et al. 1972). In the liver, these macrophages are termed Kupffer cells (Widmann et al. 1972), although originally these phagocytic cells were likely confused with the stellate cells that would later be identified by Ito (1973). Hinton and Pool (1976) noted that Kupffer cells are not distributed homogenously in the liver and appear to show some variation regarding their phagocytic activity. These authors also reported that Kupffer cells are more frequently encountered and are larger in regions around the portal areas than around the central venules. Present data corroborate this finding in the seahorse, although regional differences in the seahorse liver appear to be less pronounced than those reported for other fish liver.

Endothelial cells are an important cell type in any organ, and certainly so in the liver. Liver endothelial cells are specialized, with the presence of fenestrations that appear aggregated into groups that form ‘sieve plates’ (Gingerich 1982). The very sparse nature of a basal lamina beneath the endothelial cells, along with the absence of diaphragmatic coverings of the fenestrations, allows for apparent relatively free movement of small molecules (less than 125 nm diameter) between the Disse space and the capillary lumen.

The present analysis indicates that a considerable portion of hepatocytes is mononucleated. Gupta (2000) however, reported that the population of hepatocytes includes many double nucleated cells, which functional significance is not clear. There was no evidence to indicate that any other cell type within the liver included double nucleated cells (Smedsrod et al. 1985, 1994). The thin squamous endothelial cells display extensive spread of cytoplasm and membranes, making the detection of two nuclei within one cell a very difficult endeavor. We are not aware of any examples of double nucleated endothelial cells and believe they are not likely to exist.

The general histological organization of the longsnout seahorse liver comparable to livers of

other fish species. The hepatocytes contained one or two nuclei; none of the Kupffer or Ito cells were double nucleated. The presence of canaliculi and a bile duct system appear similar to that reported for other mammalian species. The cellular organization of the longsnout seahorse liver is quite similar to that of other mammalian species, confirming that the longsnout seahorse presents a useful animal model for studying liver structure and function.

ACKNOWLEDGEMENTS

The authors acknowledge the support of this project from the Virginia Tech - VSAREC and the International Initiative for Sustainable and Biosecure Aquafarming (IISBA). L. A. Sampaio and L. A. Romano are supported by Brazilian CNPq. Partial fundings for this study were provided by MCT/CNPq/CT-Agronegócio/MPA and EMBRAPA.

Author contributions

Luis A. Romano: conceptualization; formal analysis; writing-original draft; writing-review and editing; supervision. Luana B. Giesta: writing-original draft; writing-review and editing. Virginia F. Pedrosa: methodology; formal analysis; writing-original draft. Michael H. Schwarz: resources; funding acquisition. Luís A. Sampaio: investigation; resources; funding acquisition. Ricardo V. Rodrigues: conceptualization; methodology; investigation; writing-original draft; writing-review and editing; supervision.

REFERENCES

Aschoff L. 1924. Das reticulo-endotheliale system. In: Kraus F, Meyer E, Minkowski O, Müller F, Sahli,H, Schittenhelm A, Czerny A, Heubner O, Langstein L, editors. Ergebnisse der inneren medizin und kinderheilkunde. Ergebnisse der inneren medizin und kinderheilkunde. Vol 26. Berlin: Springer. p. 1-118.

[CITES] Convention on International Trade in Endangered Species of Wild Fauna and Flora. 2009. Appendices II listing proposal for *Hippocampus*. https://www.cites.org/eng/resources/species.html.

Foster S, Wiswedel S, Vincent A. 2016. Opportunities and challenges for analysis of wildlife trade using CITES data-seahorses as a case study. Aquat Conserv Mar Freshwat Ecosyst. 26 (1): 154-172.

Freret-Meurer NV, Andreata V. 2008. Field studies of a Brazilian seahorse population, *Hippocampus reidi* Ginsburg, 1933. Braz Arch Biol Technol. 51 (4): 743-751.

Furth R, Cohn ZA, Hirsch JG, Humphrey JH, Spector WG, Langevoort HL. 1972. The mononuclear phagocyte system: a new classification of macrophages, monocytes, and their precursor cells. Bull WHO. 46 (6): 845-852.

Gingerich WH. 1982. Hepatic toxicology of fishes. In: Weber LJ, editor. Aquatic toxicology. New York: Raven Press. p. 55-105.

Gupta S. 2000. Hepatic polyploidy and liver growth control. Semin Cancer Biol. 10 (3): 161-171.

Hinton DE, Pool CR. 1976. Ultrastructure of the liver in channel catfish *Ictalurus punctatus* (Rafinesque). J Fish Biol. 8: 209-219.

Hora MSC, Joyeux J-C, 2009. Closing the reproductive cycle: growth of the seahorse *Hippocampus reidi* (Teleostei, Syngnathidae) from birth to adulthood under experimental conditions. Aquaculture. 292: 37-41. DOI: https://doi.org/10.1016/j.aquaculture.2009.03.023

Ito T. 1973. Recent advances in the study on the fine structure of the hepatic sinusoidal wall: a review. Gunma Rep Med Sci. 6: 119-163.

Koning S, Hoeksema BW. 2021. Diversity of seahorse species (*Hippocampus* spp.) in the international aquarium trade. Diversity. 13 (5): 187.

DOI: https://doi.org/10.3390/d13050187

Naito M, Hasegawa G, Takahashi K. 1997. Development, differentiation, and maturation of Kupffer cells. Microsc Res Tech. 39: 350-364. DOI: https://doi.org/10.1002/(SICI)1097-0029(19971115)39:4<350::AID-JEMT5>3.0.CO;2-L

Oliveira T, Pollom R. 2017. *Hippocampus reidi*. The IUCN Red List of Threatened Species 2017: e.T10082A17025021.

Yokota S, Fahimi HD. 1981. Immunocytochemical localization of albumin in the secretory apparatus of rat liver parenchymal cells. Proc Nat Acad Sci USA. 78 (8): 4970-4974. DOI: https://doi.org/10.1073/pnas.78.8.4970

Smedsrod B, de Bleser PJ, Braet F, Lovisetti P, Vanderkerken K, Wisse E, Geerts A. 1994. Cell biology of liver endothelial and Kupffer cells. Gut. 35: 1509-1516. DOI: https://doi.org/10.1136/gut.35.11.1509

Smedsrod B, Pertoft H, Eggertsen G, Sundstrom C. 1985. Functional and morphological characterization of cultures of Kupffer cells and liver endothelial cells prepared by means of density separation in Percol, and selective substrate adherence. Cell Tissue Res. 241: 639-649.

Widmann JJ, Cotran RS, Fahmi HD. 1972. Mononuclear phagocytes (Kuffer cells) and endothelial cells: identification of two functional cell types in rat liver sinusoids by endogenous peroxidase activity. J Cell Biol. 52: 159-170. DOI: https://doi.org/10.1083/jcb.52.1.159