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A HISTOLOGICAL STUDY OF THE ZEISSL MEMBRANE FOUND IN THE DIGESTIVE TRACT OF THE DANUBE SALMON *Hucho hucho* (Linnaeus, 1758)

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Abstract

The digestive system of fish is different from one species to another, depending on the type of food ingested. Danube salmon Hucho hucho (Linnaeus, 1758) is a critically endangered species of the Salmonidae family with the threat of extinction looming large, therefore conservation efforts are urgently needed. This study presents a detailed microscopic analysis of the Zeissl membrane in Danube salmon, through histological means. The biological material analyzed consisted of six fish raised in a specialized breeding farm for restocking purposes. Samples were collected from the gastrointestinal tract and processed using histological paraffin embedding. The Zeissl membrane was present in all studied segments, but its structure and development varied. It comprised the stratum compactum (SC) and stratum granulosum (SG). The SC formed a continuous membrane with unequal thickness in most of the studied segments, except for the cardia area of the stomach, where it appeared discontinuously, and the duodenum, where it only appeared between the apertures of the pyloric caeca. Aside from its role in limiting gastrointestinal distension, it may have other implications for the morphophysiology of the Danube salmon digestive tract.

Key words: *stratum compactum, stratum granulosum, Zeissl membrane.*

INTRODUCTION

Danube salmon *Hucho hucho* (Linnaeus, 1758) is a critically endangered species of the Salmonidae family with the threat of extinction looming large (Holcík et al., 1988; Ihuț et al., 2014). Conservation efforts are being made to protect this species both in its natural habitat (in situ) (Zitek et al., 2004, 2008; Schmutz et al., 2002; Ratschan, 2014) and outside of it (ex-situ) (Holcík, 1995; Holzer, 2011; Stráňai, 2012). To adopt conservation measures for any species, it is important to understand its biological characteristics (Holcík et al., 1988; Ihuț et al., 2014; 2017; 2020), which may help to improve the species' conservation efforts.

One important biological characteristic is the particularities of the digestive apparatus in fish (Moisa et al., 2022). This is different from one species to another, being also dependent on the type of food ingested. Danube salmon can ingest up to 50% of its body weight in a single feeding session since it is known as a voracious predator (Šubjak, 2013). The digestive system of fish is divided into four segments, as stated by Harder (1975) and Teresa & Maciej (2019). The headgut, which includes the oral cavity and pharynx, is responsible for capturing food. The foregut, consisting of the esophagus and stomach, is where food digestion begins. The midgut, located between the pyloric caeca and the rectum, is responsible for continuing digestion and nutrient absorption. Finally, the hindgut is the rectum. From the foregut to the hindgut, the digestive wall consists of four layers (mucosa, submucosa, muscularis, and serosa).

According to histological literature, there is a unique membrane within the inner mucous layer (Adlersberg et al., 1955; Vitanov et al., 1995; Bacha & Bacha, 2006; Sapundzhiev et al., 2017), which is not present in all species, and its presence could distinguish the gut wall across species. It is present in carnivorous species and has an important role in limiting stomach distension and preventing perforation when consuming large amounts of food, including bones (Zahariev, 2011). Lim (1922) identified in the gastric mucosa of the cat a thick condensation in the inner part of the mucosa as Zeissl membrane and Oppel's stratum compactum. Adlersberg et al. (1955) and Gherman (1993) describe the Zeissl membrane as a dense connective tissue compressed at the base of the tubules that form the subglandular layer. The membrane is not elastic and separates the muscle fibers within the inter-glandular tissue from muscularis mucosae (Lim, 1922). Some authors referred to this structure only as lamina subglandularis (Vitanov et al., 1995; Bacha & Bacha, 2006), composed of two different layers: the stratum compactum (SC) and the stratum granulosum (SG) (Bacha & Bacha, 2006).

In the SG, there are cells with granular cytoplasm, which some authors consider immune cells (Khojasteh et al., 2009). The Zeissl membrane has been observed in several mammalian species, including cats, tigers, rats, and horses (Lim, 1922; Trautman & Fiebiger, 1957; Bacha & Bacha, 2006; Zahariev et al., 2010; Sapundzhiev et al., 2017). In these cases, it was present in the stomach and appears to be disposed of between the bottom of the gastric glands and muscularis mucosae (Singh & Kannabathula, 2018). In carnivores, it can extend into the intestine (Bacha & Bacha, 2006). Regarding fish, the Zeissl membrane has been reported in Rainbow trout, *Oncorhynchus mykiss* (Khojasteh et al., 2009; Yonkova et al., 2017), Chinook salmon, *Oncorhynchus tshawytscha* (Greene, 1912), Atlantic salmon, *Salmo salar* (Gulland, 1898), Northern pike, *Esox lucius* (Bucke, 1971; Sadeghinezhad et al., 2015), Brown trout, *Salmo trutta* (Burnstock, 1959), Common dentex, *Dentex dentex* (Carrassón et al., 2006), Mosquitofish, *Gambusia affinis* (Cengiz & Unlu, 2006) and Asian knifefish, *Notopterus notopterus* (Khadse & Gadhikar, 2017).

Since there is no information in the literature regarding the presence of the Zeissl membrane in Danube salmon, this study aimed to verify the structure and development of this layer in various segments of the digestive tract microscopically.

MATERIALS AND METHODS

All procedures involving animals were conducted following Romanian (Law 43/2014) and European legislation (EU Directive 63/2010). The Ethics Committee of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca (No. 117/2018) approved the study. The six specimens of Danube salmon used in this study were raised in captivity in a trout farm from Transylvania, Romania, to create a breeding stock for repopulating the region's mountain and submontane rivers. For microscopy assessment, several transversal fragments in the form of slices with a thickness of 4 mm for each were collected from the following regions: the foregut (the cardia and pyloric region of the stomach), the midgut (duodenum, pyloric caeca, and the posterior portion of the medium intestine), and the hindgut (anterior portion of the rectum). The samples were fixed by immersion in Stive solution for 24 hours, dehydrated in ethanol alcohol, clarified with n-Butanol, and finally embedded in paraffin. Thin sections of 5 µm were taken and stained with Goldner’s trichrome method. The histological slides were examined under an optical microscope (Olympus BX41) and images were captured using a digital camera (Olympus E330). The images were further processed using Adobe Photoshop CS 2.0.

RESULTS AND DISCUSSIONS

The microscopic analyses proved the presence of the Zeissl membrane in Danube salmon, in all studied segments with some differences between components.

In the histology structure of the cardiac stomach, the Zeissl membrane appears to be composed of both SC and SG. The SC was discontinuous and uneven in thickness over the entire circumference. The SC is present on about 30% of the stomach circumference in the cardiac region. The SG was better represented than the SC, being present on about 80% of the circumference. In certain regions, the thickness of the SC is roughly half that of the lamina propria. Meanwhile, in areas where the SC is present, SG is about double in thickness, reaching up to the muscularis mucosae. It is worth noting that in these regions, the muscle cells from the muscularis mucosae seem to be tightly compacted with a mostly longitudinal alignment (Figure 1).

The SG exhibits a more pronounced thickness in regions where the SC is not present. It is located between the lamina propria at the bottom of the glands and the muscularis mucosae. The ratio of thickness between lamina propria and SG is approximately equal. In these regions, the muscularis mucosae are composed of either smaller or larger fascicles, spaced apart one from another (Figure 2).

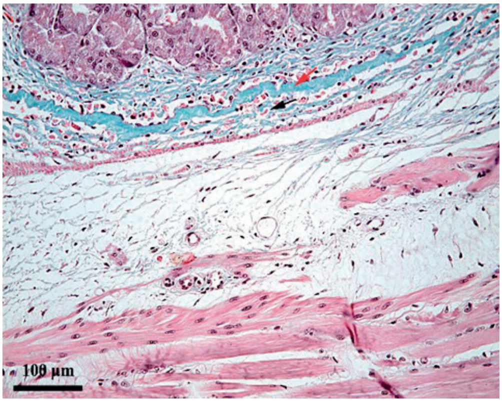

Figure 1. Stomach cardia area, Goldner's trichrome stain; red arrow – *stratum compactum*; black arrow – *stratum granulosum*

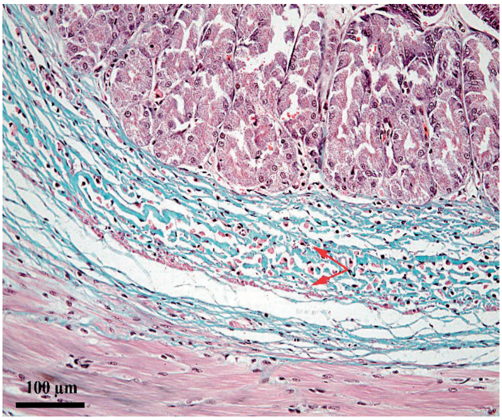

Figure 2. Stomach cardia area, Goldner's trichrome stain; red arrow – *stratum granulosum*

The presence of the Zeissl membrane in the pyloric stomach was continuous. The SC was noticeably thicker and uneven in thickness throughout its entire circumference. The SG was well developed in areas where the SC is thicker. In these areas, the fibril component of the SG appears to be quite compact to the muscularis mucosa, giving the appearance of doubling, and the cells from the SG occupy the space between the two lamellas (Figure 3). In the areas where the SC is thinner, the SG is approximately four times thicker. The granular cells are numerously represented in the areoles delimited by the fibrillar component in this layer (Figure 4).

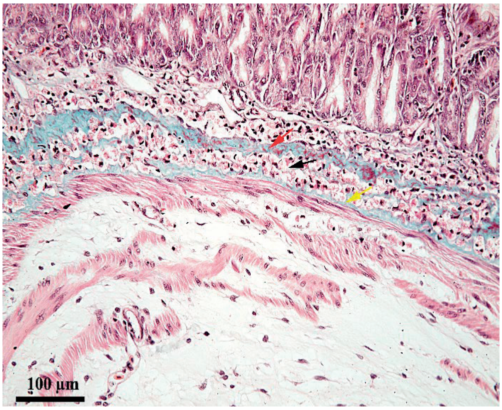

Figure 3. Stomach pyloric area – double layered aspect of the *stratum compactum*, Goldner's trichrome stain; red arrow – *stratum compactum*; black arrow – *stratum granulosum*; yellow arrow – double layered aspect of the *stratum compactum*

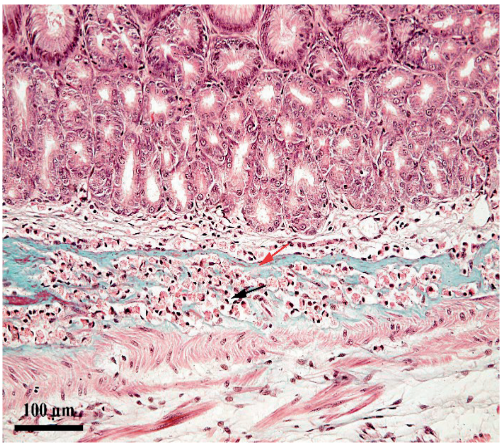

Figure 4. Stomach pyloric area, Goldner's trichrome stain; red arrow – *stratum compactum*; black arrow – *stratum granulosum*

In the duodenum, the Zeissl membrane was present and appears to consist of two layers: the SC and the SG. In the areas between the openings of the pyloric caeca, the SC appears well-developed. Its thickness represents approximately 30% of the thickness of the lamina propria, which is situated between the SC and the basal membrane of the epithelium. The SG was approximately three times thicker than the SC (Figure 5). In areas where the SC is highly developed, the fibers from the SG are quite compact, giving in some cases an aspect of doubling the SC. Between the two bands, fibrous branches with predominantly oblique layouts were observed. Toward the opening orifice of the pyloric caeca, the SC becomes thinner until it disintegrates.

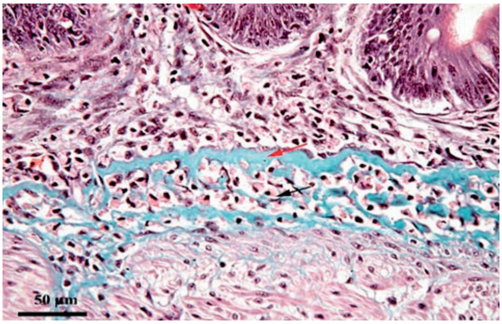

Figure 5. Duodenum, Goldner's trichrome stain; red arrow – *stratum compactum*; black arrow – *stratum granulosum*

The Zeissl membrane was formed only by the SG in the pyloric caeca. The fibrillary component of the SG was disposed quite compactly, forming a structure similar to the SC, but it appears disposed in direct contact with the muscularis externa. From it to the lamina propria, the granular cells typical of this layer were observed. Few granular cells were also observed between the fibrous membrane of the SG and the muscularis externa. The muscularis mucosae are absent and the SG comes in direct contact with the muscularis externa. Therefore, the submucosa is missing in this segment (Figure 6).

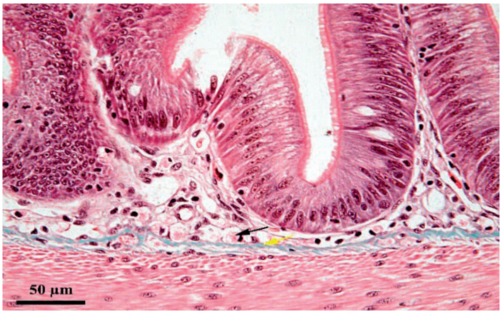

Figure 6. Pyloric caeca, Goldner's trichrome stain; black arrow – *stratum granulosum*; yellow arrow – the fibrillated component of the *stratum granulosum*

In the distal portion of the medium intestine, the Zeissl membrane was very developed and twice thicker than the lamina propria, found between it and the basal membrane of the epithelium. The SC occupies about half of the Zeissl membrane thickness (Figure 7).

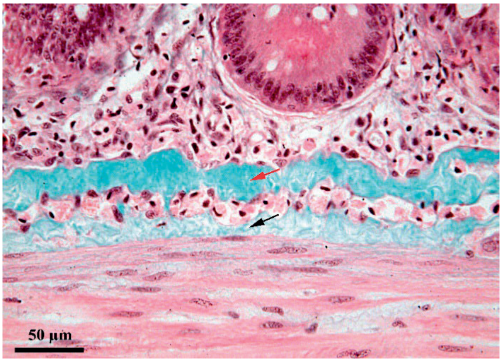

Figure 7. The distal portion of the medium intestine, Goldner's trichrome stain; red arrow – *stratum compactum*; black arrow – *stratum granulosum*

In some areas, where the SC is somewhat thinner, the fibers from the SG are arranged quite compactly to the muscularis externa, giving it the aspect of a double SC, with most of the granular cells disposed between them. Throughout the ileum, the SG comes in direct contact with the inner circular layer of the muscularis externa, so that the submucosa is absent (Figure 8).

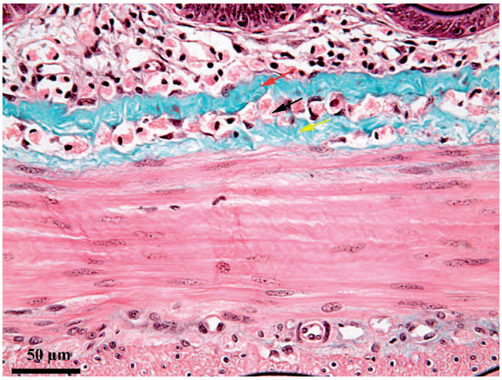

Figure 8. The distal portion of the medium intestine – doubling aspect of the *stratum compactum*, Goldner's trichrome stain; red arrow – *stratum compactum*; black arrow – *stratum granulosum*; yellow arrow – doubling aspect of the *stratum compactum*

In the anterior portion of the rectum, the Zeissl membrane was present on the entire circumference of the wall and is thinner than in the previously described segment. The SC appears more developed than the SG (Figure 9).

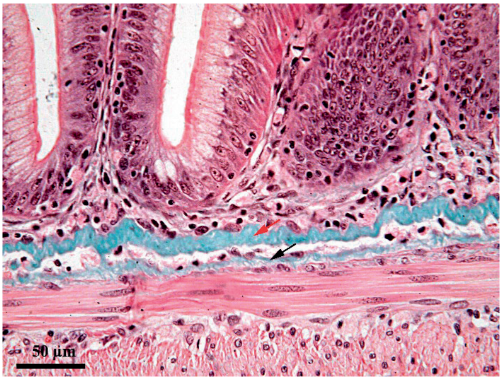

Figure 9. The anterior portion of the rectum, Goldner's trichrome stain; red arrow – *stratum compactum*; black arrow – *stratum granulosum*

In the areas where the SC is thinner, the fibers from the SG are ordered compactly resulting in an aspect of doubling the SC (Figure 10).

In the Danube salmon stomach, the Zeissl membrane presents some differences between the two studied areas. Thus, the SC inside the pyloric area is more developed than in the cardiac area and it is continuous throughout the circumference. As for the SG, it is also more developed in the pyloric segment, where in certain areas the fibril component is compactly arranged giving the aspect of doubling the SC.

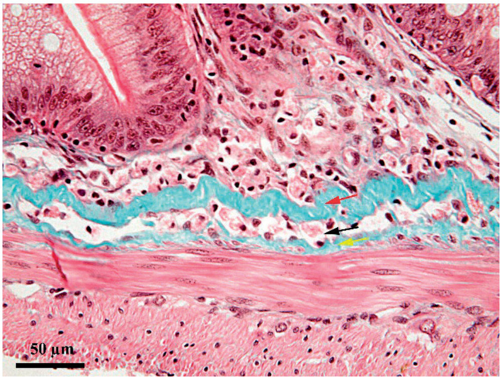

Figure 10. The anterior portion of the rectum – double layered aspect of the *stratum compactum*, Goldner's trichrome stain; red arrow – stratum compactum; black arrow – *stratum granulosum*; yellow arrow – double layered aspect of the *stratum compactum*

The double-layered aspect of the SC was observed in the duodenum, but only in areas where the SC is developed, including the ileum and the anterior portion of the rectum. Some differences between the two areas of the stomach analyzed in the study are also found in the layout of the two layers of the Zeissl membrane.

Thus, in the cardiac area, the SC was discontinuous while in the pyloric region, it was continuous. In the parts where the SC is missing, the muscularis mucosae appear discontinuous, while in areas where the SC is present, the muscularis mucosae is continuous.

Greene (1912) describes SC in the stomach of Chinook salmon as a continuous layer, but not as developed as in the intestine and pyloric caeca. The difference in thickness of the SC between the stomach and the intestine was also reported for brown trout (Burnstock, 1959). The study states that SC limits the elongation of the muscle cells in the stomach wall to 75% and in the gut to 25%. Differences in the thickness of the SC between the stomach and the intestine were also observed during the present study for Danube salmon, with the distinction that, in this case, at the level of the cardiac area of the stomach, SC is discontinuous.

In the pyloric caeca, the Zeissl membrane occurs only as SG, similar to the one described in the intestine of horses (Rajput, 2006). In Chinook salmon, at the level of the pyloric caeca, the SC is continuous and the SG is well-developed (Greene, 1912). The results obtained during this study on Danube salmon are different from those described for Chinook salmon (Greene, 1912). Thus, for the pyloric caeca, the SC is absent and the fibrillary component from the SG is quite compact. This study shows that the muscularis mucosae and the submucosa are missing in the structure of the wall of the pyloric caeca similar to the Chinook salmon (Greene, 1912). Similarly, the muscularis mucosae and the submucosa are missing in the intestine. The structure of the intestinal wall in Danube salmon is similar to the one described for Chinook salmon (Greene, 1912), Brown trout (Burnstock, 1959), and rainbow trout (Khojasteh et al., 2009). In the intestine, the SC is the thickest in the distal portion of the medium intestine, but it does not have the same size over the entire wall circumference. In the areas where the SC is thinner, it has been observed that the fibrillar component of the SG is placed next to the muscularis externa, giving the aspect of a double layer of the SC. The same features appeared in the pyloric section of the stomach, in the duodenum, and the anterior part of the rectum. As a detail, the double-layered aspect of the SC was not described in the literature. According to this, the Zeissl membrane seems to have an important role in reinforcing the digestive tract wall in some voracious predators such as Danube salmon.

CONCLUSIONS

In Danube salmon, the Zeissl membrane is present in all organs assessed in our study, with some regional peculiarities. It is the most developed in the distal portion of the medium intestine and the thinnest in the pyloric caeca, where it appears only by SG. The SG was present in all studied segments, while the SC is the thickest in the distal portion of the medium intestine, discontinued in the cardiac region, and missing in the pyloric caeca. In the areas where the SC is thinner, the fibrillar component on the outer side of the SG is dense, with both layers displaying a similar density. Finally, the structure and distribution of the Zeissl membrane are different among species.

The membrane's unique structure makes it possible for fish to consume a high amount of food at once. Aside from its role in limiting gastrointestinal distension, it may have other implications for the morphophysiology of the digestive tract. Other similar reports are required for a better understanding of the role of the Zeissl membrane in other fish species.

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