# **Histology of Arctic char**

Presentation of histological pictures of arctic char organs with emphasis on tissues and cells of the digestive system

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#### Working document

#### Histology of Arctic char (Salvelinus alpinus)

## Introduction

Arctic char is distributed in both marine and freshwater environments, and spawns in lakes, streams and rivers. Arctic char grows to over 50 cm and the dwarf type grows to around 20 cm. The female produces 2,500 to 8,500 eggs of 4 to 4.5 mm size. Arctic char is an important species for the aquaculture industry particularly in the Arctic region with a large potential for further development.

Anadromous Arctic chars spend their juvenile years in fresh water, and once mature, migrate annually to the marine environment. The first migration of Arctic char has been found to occur between 4 and 13 years of age. When in the marine environment, Arctic chars inhabit coastal and intertidal areas.

Arctic char is related to both salmon and lake trout and has many of the same characteristics. The fish is highly variable in color, depending on the time of year and the environmental conditions where it lives. The appearance of Arctic char differs between populations.

Arctic char is among the northern-most fish in the world. and is an important species for aquaculture in Nordic countries. However, like other species in aquaculture diseases occur and my cause problems and increased mortality, followed by economic problems for the fish farmers.

As there are very little information on histology of artic char, the Arctaqua project planned to develop a histology atlas on this species. The atlas was planned to be offered students and others to learn more about Arctic char histology and anatomy. An atlas could also be a valuable tool for farmers and fish health personnel in aquaculture. This is especially the case during diseases, histology may help in diagnosis of affected fish and fish organs and be an important tool to determine etiology of infectious and non-infectious diseases in fish.

The aim of this part of Arctaqua was to provide a comprehensive description of main anatomical and histological structures of organs of arctic char during early development. This was planned to be conducted by Arctaqua partner in Russia with the help of Russian students and employees, and in collaboration with Nord university/Akvatik. After the increased sanctions against Russia in March 2022, it become clear that this work could not continue with the Russians as partners.

Nord university and Akvatik had already started their work on preparations, and sampling of char organs were already conducted by Akvatik. It was agreed that the work on the atlas should proceed, and the material should be offered students at Nord university. The aim was to do the histological work at Nord by students under supervision by Nord/Akvatik, but was not possible. Thus, the work is presented as a working document. As much of the material including histological sections and pictures are available this material could be offered to a student to finalize as a bachelor/master thesis.

This report is a part of the project Arctaqua on development of arctic aquaculture of Interreg Kolarctic CBC program. This project is co-funded by Kolarctic CBC Program and Nordland County.

## Material and methods

### Sampling

Tissue samples were collected at Sigerfjord Fisk AS in Sortland. The tissues were preserved in a 10% buffered formalin solution (35% Formaldehyde) for transport and storage.

# Bone decalcification

Samples containing bones were decalcified by leaving the samples in a 10% EDTA solution to preserve the collagen structure of the samples. The samples were cut into smaller pieces before being inserted into cassettes. The cassettes containing the samples were then left inside a container with a decalcification solution for three days. The solutions were refreshed every day and they were constantly stirred using an orbital shaker plate for the entire duration of decalcification. However, the results were not optimal as some parts of the bones failed to decalcify at the end of the procedure.

## **Histological analysis**

Tissues were cut and processed using a tissue processor and paraffin embedding station (Shandon Citadel 2000 Tissue processor; Leica EG 1150 H). The samples were stored in a refrigerator designated for histology samples. The samples were taken out of the refrigerator one at a time to maintain the sturdiness of the paraffin for improved sectioning. A rotary microtome (Thermo Scientific<sup>™</sup> HM 355S Automatic Microtime Package, Brand: Thermo Scientific<sup>™</sup> 905200STS) was used to cut the paraffin blocks at 3µm thickness. A warm water bath was used to melt away the excess wax, leaving only the tissue samples to be collected on a microscope slide. The slides were stained H&E (Hematoxylin and Eosin) using a multi-stainer (Leica ST5020 Multistainer). For the final analysis, images were taken using Olympus BX 61. The images were then examined using software Cell Sense. No specific directions were followed other than obtaining quality photos of the entire slide. The photos with the most optimal representation of the sample were chosen at the end to be published. No specific measurements were taken.

The emphasis of the present histological work is on the digestive system, and is mostly covered by figures 1-19. Figures 20 to 50 are on other organs, tissues and cells.







*Figure 1.* Sections of mid intestine of juvenile Arctic Char at 4x magnification. H&E stain. Intestinal fold (IF), lumen (L) and muscularis (MS).



Figure 2. Section of mid intestine of juvenile Arctic char at 10x magnification. H&E stain. The mucosa consists of; epithelium that consists mainly of enterocytes (E), lamina propria (LP), stratum compactum (SC). Goblet cells (G) can be observed throughout the epithelium. The longitudinal muscle layer (LM), circular muscle layer (CM) and serosa (S) can be observed in the muscularis.



*Figure 3.* Section of mid intestine of juvenile Arctic char at 20x magnification. H&E stain. Goblet cells (G), lamina propria (LP), stratum compactum (SC), longitudinal muscle layer (LM), circular muscle layer and serosa (S).



*Figure 4.* Section of mid intestine of juvenile Arctic char at 20x magnification. H&E stain. Goblet cells (G), lumen (L), lamina propria (LP), nucleus of goblet cell (arrow) and enterocyte (double-headed arrow).



*Figure 5.* Section of posterior intestine of juvenile Arctic char at 4x magnification. H&E stain. Intestinal fold (IF), lumen (L) and muscularis (MS).



Figure 6. Section of posterior intestine of juvenile Arctic char at 10x magnification. H&E stain. Lumen (L), lamina propria (LP), epithelium (E), stratum compactum (SC), longitudinal muscle layer (LM), circular muscle layer (CM) and serosa (S). Goblet cells (G) can be observed throughout the epithelium.



Figure 7. Section of posterior intestine of juvenile Arctic char at 10x magnification. H&E stain. Lumen (L), lamina propria (LP), epithelium (E), stratum compactum (SC), longitudinal muscle layer (LM), circular muscle layer (CM) and serosa (S). Goblet cells (G) can be observed throughout the epithelium.



*Figure 8.* Section of posterior intestine of juvenile Arctic char at 20x magnification. H&E stain. *Serosa (S), longitudinal & circular muscle layer (LM & CM) stratum compactum (SC).* 



*Figure 9.* Section of posterior intestine of juvenile Arctic char at 20x magnification. H&E stain. *Lumen (L), lamina propria (LP), epithelium (E) and goblet cells (G)* 



*Figure 10. Horizontal section of pyloric caeca of juvenile Arctic char at 2x magnification. H&E stain. Pyloric caeca (PC) and Exocrine pancreas (P)* 



*Figure 11. Horizontal section of pyloric caeca of juvenile Arctic char at 4x magnification. H&E stain. Pyloric caeca (PC), lumen (L), villi (V) and exocrine pancreas (EP)* 



*Figure 12. Horizontal section of pyloric caeca of juvenile Arctic char at 10x magnification. H&E stain. Lumen (L), villi (V), internal muscle layer (IM) (longitudinal), external muscle layer (EM) (circular), serosa (S), submucosa (SM) and lamina propria (LP). Exocrine pancreas can be observed next to the pyloric caeca showing acinar cells (\*).* 



Figure 13. Horizontal section of pyloric caeca of juvenile Arctic char at 20x magnification. H&E stain. The photo shows various layers of intestinal wall. Lumen (L), microvilli (MC), layers of enterocytes (E), lamina propria (LP), internal muscle layer (IM) (longitudinal), external muscle layer (EM) (circular), serosa (S) and sub mucosa (SM). Exocrine pancreas (EP) and acinar cells (\*).



*Figure 14. Horizontal section of pyloric caeca of juvenile Arctic char at 20x magnification. H&E stain. Layers of enterocytes (E) and their nucleus (N), microvilli (MV), lumen (L) and lamina propria (LP).* 



Figure 15. Portion of stomach in cardia region of Juvenile Arctic char at 2x magnification. H&E stained. Intestinal fold (IF) (double arrow), lamina propria (LP), sub mucosa (SM), longitudinal & circular muscle layer (LM & CM) and mucosa (M). Gastric glands can be observed throughout the epithelial folds (GG).



Figure 16. Portion of stomach in cardia region of Juvenile Arctic char at 4x magnification. H&E stained. Lamina propria (LP), sub mucosa (SM), longitudinal & circular muscle layer (LM & CM) and mucosa (M). Gastric glands can be observed throughout the intestinal folds (GG).



Figure 17. Portion of stomach in cardia region of Juvenile Arctic char at 10x magnification. H&E stained. Focused on the muscle/base of the fold. Serosa (S), sub mucosa (SM), longitudinal & circular muscle layer (LM & CM).



Figure 18. Portion of stomach in cardia region of Juvenile Arctic char at 10x magnification. H&E stained. Focused on the tip of the fold. Gastric glands (GG) (Red circle) can be observed throughout under the mucosal layer (M). Lamina propria (LP).



Figure 19. Portion of stomach in cardia region of Juvenile Arctic char at 20x magnification. H&E stained. Focused on the tip of the fold to show gastric glands (GG).



Skin of Arctic char. From the right; epidermis, scale pockets, scale (in scale pockets), dermis and muscle layer.



*Figure 101. Close up, of 1. Skin of arctic char. From the left side; Epidermis with mucous cells, epithelial cells, melanocyte, scale, basal membrane, dermis, scales and scale pockets, muscle tissue.* 



Figure 22. Skin of arctic char. From the right side of the image; Epidermis with mucous cells, epithelial cells, melanocytes, basal membrane, scales and scale pockets dermis, and muscle tissue.



Figure 23. Close up of Figure 3.



Figure 24. Swimbladder.



Figure 25. Swimbladder walls.



Figure 26. Brain structures



Figure 27. Brain structures



Figure 28. Heart tissue.



Figure 29. Heart tissue, ventricle muscle fibers, prominent nucleus in muscle cells.



Figure 11 Heart tissue



Figure 31. Heart tissue.



Figure 32. Liver tissue with hepatocytes, bile duct, blood vessels, and blood cells



*Figure 33 Close up of Figure 32. Liver tissue with hepatocytes, blood vessel, blood cells, and bile duct.* 



*Figure 12.Dorsal fin. Epidermis with epithelial and mucous cells, basalmembrane, bone matrix, and mesenchyme.* 



*Figure 13. Close up of figure 34. Dorsal fin. Epidermis with epithelial and mucous cells, basal membrane, bone matrix, and mesenchyme.* 



Figure 36. Spleen. White and red pulps.



Figure 37. Spleen. Close up of Figure 36. White and red pulps



Figure 14 Head kidney, tubules, melano-macrophages, lymphocytes, glomerulus



*Figure 39. close up of Figure. 38. Head kidney, tubules, melano-macrophages, lymphocytes, glomerulus* 



*Figure 40. close up of Figure 39. Head kidney, tubules, melano-macrophages, lymphocytes, glomerulus* 



Figure 41. Kidney tissue with tubule, blood vessel, lymphoid cells, and hematopoietic cells



Figure 42. Eye lens and retina



Figure 43. Eye, retina and rete mirabile



*Figure 44. Eye, pigment layer, photoreceptor layer, membrane, nuclear layer, inner nuclear layer, inner plexiform layer, ganglion cell layer.* 



Figure 45. Eye, optic nerve



Figure 46. Gills. Primary lamellae and secondary lamellae



*Figure 47. Close up of primary and secondary lamellae. Chloride cells, pavement cells, red blood cells, mucous cells.* 



Figure 48. Gonads of char



Figure 49. Gonads, close up of Figure 48.



Figure 50. Gonads. Close up of Figur 49.