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Histological characterization of different organs in *Acipenser ruthenus* juveniles

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Abstract. This study aims to highlight new information on histology of sturgeons in general and of sterlet in particular. Some organs, such as: the esophagus, the stomach, the anterior intestine, the posterior intestine, pyloric caeca, the liver, the pancreas, gonads and the gills were observed under the microscope. Samples from 11 months old sterlets were taken from different individuals that were in full growth in a recirculated aquaculture system. Cultured in these conditions, it appears that the sterlet is developing harmonious. The proofs are the large spaces between the muscular fibers, both smooth and ribbed.

Key words: sterlet, histology study, recirculated aquaculture system, sturgeon.

Rezumat. Prezentul studiu se doreşte a aduce informaţii noi în domeniul biologiei celulare a sturionilor în general şi a cegăi în special. Mai multe organe precum esofagul, stomacul, intestinul anterior, intestinul posterior, cecumurile pilorice, ficatul, pancreasul şi branhiile au fost studiate sub lupa microscopului. S-au utilizat probe recoltate de la indivizi cu vârsta de 11 luni, crescuţi în condiţii de fermă în sistem recirculat. Crescută în aceste condiţii în urma studiilor noastre, se pare că cega se dezvoltă armonios aflându-se la momentul studiului în plin proces de creştere, dovadă fiind spaţiile largi dintre fibrele musculare striate şi netede.

Cuvinte cheie: cegă, studiu histologic, sistem recirculat de acvacultură, sturioni.

Introduction. The sterlet, (*Acipenser ruthenus*), was not long time ago a sought after trophy by many anglers. Also, it was, and still is very appreciated by the gourmands for its special flavor, being considered to be one of the most delicious sturgeon in Europe (Muscalu & Muscalu 2009; Moyle & Cech 2004).

Because the demand for sterlet meat is still high in Eastern Europe, solutions in order to stop the excessive fishing from the natural waters were developed. The solution is relatively simple: aquaculture (Bura & Szelei 2009). Not very long time ago, the sterlet was reared in Romanian aquaculture, but technical difficulties and high production costs, made this species to be forgotten by Romanian aquaculturists. Still, in the present days, it seems that the sturgeon aquaculture in Romania is returning in force (www.anpa.ro), especially in intensive and super-intensive systems. But, for the aquaculturists to successfully rear sturgeons, and especially sterlet, advanced knowledge on the sterlet biology is essential. This paper shows some histological aspects on different organs from this species and also tries to elucidate if the culture system has any negative effect on the sterlet's development. The scientific literature in the field of sturgeon histology is scarce.

Material and Method. In order to approach this issue, 5 sterlets (*Acipenser ruthenus*) were used at the age of 11 months. They were picked in such way, that to have a high variability in size. Until the time of the assay, they were fed with commercially available extruded sturgeon feed (Muscalu & Muscalu 2009). The fish were measured and then samples from different organs were collected. These pieces were fixed in 80º alcohol for a period of three days, processed according to histological techniques (Botarel et al

1982) and stained by Malory trichromic method. The morpho-structure of these segments were studied and photographed at the microscope.

The fish weighted 79.1 grams and had an average standard length of 29.2 cm.

Results and Discussion. Next we will describe each studied organ from the five sterlets. The digestive system has been studied histologicaly at other species of sturgeons, having a high importance in understanding the formulation of sturgeon diets (Ostos-Garrido et al 2009). There were absolutely no difference between the individuals, and unfortunately, the male gonad could not be studied in good conditions, therefore we will resume only in description of the ovaries.

Esophagus structure

The esophagus is a digestive channel that has a wall structured in 4 tunics: mucosis membrane, sub-mucosis membrane, muscular membrane and tunica adventitia. The lumen of the channel is wrinkled due to numerous circular folds that the mucosis and sub-mucosis form (Figure 1). These folds allow the dilatation of the esophagus during the swallowing process and the leading of food into the stomach. The esophagus mucosis also has a large number of folds that resemble the intestinal villosites, planked with single- layered columnar epithelium that has at the apical pole highly developed microvillus (Figure 1). Among the columnar cells, there are a large number of calceiform cells that are involved in the mucosis secretion. Their accumulation at the surface of the esophagus mucosis facilitates the sliding of the food to the gastric compartment.

At the base of the villosity folds come off the esophagus glands, simple and tubular, that enter the mucosis of the chorion. They cross it until they reach its border with the sub-mucosis. The glandular epithelium resembles the gastric one and has replacement cells and numerous basophilic cytoplasm cells, is granular and produces mucosis (Figure 2).

The mucosis chorion is formed by the lax vascularization conjunctive tissue that has an infiltrated developed lymphoid (Figure 3).

The sub-mucosis if mainly formed by collagen fibers that are organized into a support network for the esophagus glands.

The musculature is strong, organized on three layers of smooth muscular fibers: internal- circular, middle- plexiform and external- longitudinal. In the conjunctive tissue of the perymisium, between the collagen fibers, there are numerous elastic fibers concentrated (Figure 4).

Stomach's structure

The mucosis and the sub-mucosis of the stomach wall form numerous circular wrinkles. The mucosis epithelium, single- layered prismatic, invaginates in the chorion, generating gastric crypts that continue with the gastric glands. Morphologically, the gastric glands are simple tubular, rarely branched (Figure 5).

The epithelium of the gastric mucosis is formed by prismatic cells with a ribbed plateau developed at the apical pole, overlapped by a mucosispolysaccharide „film" and the basic subephitelium membrane is fine (Figure 6).

The glandular epithelium is formed by cubical small cells located at the level of the gastric crypts, with the role of replacement for the used cells; the cells that secrete the mucosis located at the level of the neck of the glands; the large parietal cells with red cytoplasm, located along the glands; main cells with undefined shape and slightly basophile cytoplasm, loaded with secreted granulations, prevalent at the basis of the glands (Figure 7).

The interglandular chorion is reduced to fine conjunctive septum, capillarised, and the basic one is lax, with fine collagen fibers, fibroblasts and capillary vessels.

The sub- mucosis is a thick layer of conjunctive tissue in which the collagen fibers prevail, fibroblasts and a rich vascular network.

The muscular tunic (Figure 8) is organized of three layers of smooth myocytes, internal- oriented longitudinal, middle oriented circular and more developed, external – longitudinal and reduced. The myocytes are large, spindle- shaped and a stick-shape nucleus. The interfibers spaces are large and contain collagen and elastic fibers and include large vascular plexuses.

The external tunic of the stomach wall is whey- like, formed by lax conjunctive tissue, vascularized and innervated.

In the esophagus part, the gastric mucosis has smaller wrinkles and the sub-mucosis contains many vascular and nervous plexuses.

Anterior intestine structure

The anterior intestine structure has the wall structured on four overlapped tunics: the mucosis, the sub- mucosis, muscular and serous.

The mucosis presents a large number of intestinal villosities, very tall and very tight (Figure 9), planked with a single-layered prismatic epithelium that has at the apical pole highly developed microvillus. Among the absorbent cells from the structure of the villosity epithelium, there are, randomly, calceiform cells.

The villosity chorion is formed by lax conjunctive tissue that contains a small number of smooth myocytes, forming the villosity muscle, a very rich capillary network and numerous infiltration cells (Figure 10).

The basic chorion contains a rich infiltrated lymphoid (Figure 10).

Intestinal gland are not many, they have a wide lumen and are mostly planked with enterocytes with a visible ribbed plateau, among which there are located rare calceiform cells (Figure 11).

The sub-mucus tunic is reduced to a fine pellicle of conjunctive tissue with a collagen structure, vascularized and innervated.

The musculature is structured on two layers of smooth myocytes: intern- circular and external-longitudinal. The two layers are formed by reduced fascicle of smooth myocytes, with a large perimysium, in which there are collagen and elastic fibers, but also sanguine capillaries with a large lumen (Figure 11).

Posterior intestine structure

The wall of the posterior intestine is structured by the same organization plan as in the digestive segments described above, presenting from the interior to the exterior the mucosis, the sub-mucosis, muscular and serous.

The mucosis and the sub-mucosis are elongated in the channel lumen shaping into a spiral. In its top, in the center, it presents a strong formation with a lymphoid aspect (Figure 12). The mucosis does not present villosities, it has a single-layered epithelium, which, by penetrating into the chorion, generates numerous simple tubular glands. It their structure prevail the calceiform cells that secrete mucosis (Figure 13).

The chorion is reduced to a fine layer of conjunctive lax tissue, located peri and interglandular.

Sub-mucosis is also reduced and it is populated with rich infiltrated lymphoid (Figure 13).

The spiral generated by the mucosis and sub-mucosis is superficially planked by mucosis, that the same as the mucosis that defines the channel lumen, does not present villosities, but presents tubular glands larger in comparison to the ones around the lumen. The epithelium of these glands is mainly formed by calceiform cells. The interglandular spaces are reduced (Figure 14).

The conjunctive tissue of the sub-mucosis penetrates in the depth of the helix and, by its set out, divides the lymphoid structure in two areas that are similar to the areas of secondary lymphoid follicles: with the cortical and medullary areas.

The cortical area, of a darker color, is a dense structure, rich in lymphocytes and poor in reticular- epithelium cells (Figs 14-15).

The medullary area is rich in reticular-epithelium cells, with extended ramifications that generate through anastomosis a network. In the network's meshes are located the lymphocytes (Figure 16).

The reticular-epithelium cells have role to secrete a series of humoral factors, that control the function of this lymphoid organ. The muscular tunic is made from two overlapping smooth muscular layers; an internal layer – circular, more developed and an external layer-longitudinal, less developed. The endomisium and the perimisium are wide.

Pyloric caeca structure

The wall of the Pyloric caeca (Figure 17) has the mucosis planked with a prismatic single- layered epithelium. This penetrates in the mucosis' chorion and forms simple or ramified tubular glands.

The epithelium of the mucosis is single- layered prismatic (Figure 18) and it has a highly developed ribbed plateau. Among the epithelium cells there are numerous calceiform cells and the chorion is highly vascularized.

The muscular tunic is organized on two overlapped ample smooth myocytes layers: the internal layer with a circular disposition and the external layer with a longitudinal disposition.

Liver's structure

The liver is wrapped in a thin conjunctive capsule, manly formed by collagen fibers and fibroblasts (Figure 19). From the capsule fine conjunctive septums unwrap and they penetrate through the interior of the organ and represent the stroma or the support structure for the hepatic parenchyma.

The parenchyma of the organ is structured in hepatic lobules, morphological formations made from hepatocytes organized in rows radially oriented towards a central vessel- the centrolobular vein (Figure 20).

The hepatic rows are separated by small conjunctive spaces and the hepatocytes have a polygonal aspect, with a diameter of approximately. 20 microns. The morphologic aspect of the hepatocytes is slightly heterogeneous. Hence, there are hepatocytes with a spherical nucleus, central or slightly exocentric, with an obvious nucleolus and heterochromatin granules, and the cytoplasm is fine granular.

The second morphological type is represented by the hepatocytes with a spherical nucleus, but located totally exocentric, on the internal forepart of the plasmatic membrane. Around the nucleus there is set a fine pellicle of granular cytoplasm, while the largest part of the cytoplasm is filled with lipoid vacuoles, this aspect being a general one. A great number of this kind of cells become hypertrofic due to the overload of lipids, the exocentric nucleus gets fuddled and this process is followed by pycnosis and caryorrhexis. These aspects are correlated with a process of hepatic degenerative by overload of lipids.

The third morphological type is represented by the binucleate hepatocytes, with spherical nuclei and fine granular cytoplasm, lightly loaded with lipid drops.

From the three morphological types, the dominant ones are the hepatocytes that have the cytoplasm loaded with lipidic vacuoles.

The interlobular spaces (Kiernan) (Figure 21) are tight and contain a small quantity of conjunctive tissue, support for the elements of the portal triad, respectively the hepatic arteriola, the hepatic venule and the biliary canaliculi.

Pancreas structure

The pancreas is located adjacent to the liver as described by Weisel (1979). At the exterior, it is wrapped in its own conjunctive capsule from which unwrap septums that enter in the organ, separating it into lobules.

The pancreatic lobules are formed by secreting units- the pancreatic acini- of a spherical or oval are separated by the large secreting cells, with a spherical nucleus and cytoplasm loaded with secreting granulation (Figure 22).

The interlobular conjunctive spaces are wide and include groups of small endocrine, with a random spread, forming together the endocrine pancreatic parenchyma.

Gonad's structure

The microscopic sections through the gonads show their different aspect in female gonads, respectively ovaries. These are wrapped in a conjunctive capsule, under which there is located a thick layer of conjunctive tissue made from numerous collage fibers, fibroblasts.

Under the albuginea there is located the ovarian parenchyma formed by primordial ovarian follicles organized in nests or rows (Figure 23).

The period of primordial follicle is characterized by the presence of the elementary ovum, a cell of spherical shape, with the size of approximately 12 microns. In the nucleus, the nucleolus is obvious, with the aspect of a spherical corpuscle, strongly basophile.

The elementary ovum is wrapped in a layer of flat mesoderm cells.

Gill's structure

The microscopic sections through the gills (Figure 24) show the abundance of the gristly tissue that grants resistance and stiffness.

Through the longitudinal section, one can notice that in the respiratory lamellae there is a rich lymphoid infiltrate, with a role in the defense of the organism, as gills are the first gate to infections.

On the length of the respiratory lamellae, through transparency, one may observe a bronchial arteriole.

Conclusions. The esophagus mucosis presents a large number of wrinkles, similar to the intestinal villosites, planked with planked with single-layered columnar epithelium that has at the apical pole highly developed microvillus.

The muscular tunic of the esophagus wall is organized of three layers of smooth myocytes overlapped and in the perimisium and endomisium there are numerous elastic fibers.

The epithelium of the gastric glands is formed by replacement cells located at the level of the gastric crypts, cells that secrete the mucosis, parietal and main cells.

The mucosis of the anterior intestine presents a large number of intestinal villosities, very tall and very tight, planked with a single-layered prismatic epithelium that has at the apical pole highly developed microvillus. The development of the villosities and of the ribbed plateau is correlated with a highly active absorption process at the level of this segment.

The glands of the anterior intestine are not many, they have a wide lumen and are mostly planked with enterocytes with a visible ribbed plateau, among which there are located rare calceiform cells.

The mucosis and the sub-mucosis of the anterior intestine are elongated in the channel lumen shaping into a „helix". In its top, in the center, it presents a strong formation with a lymphoid aspect.

The conjunctive tissue of the sub-mucosis of the posterior intestine penetrates in the depth of the helix and, by its set out, divides the lymphoid structure in two areas that are similar to the areas of secondary lymphoid follicles: the cortical and medullar areas.

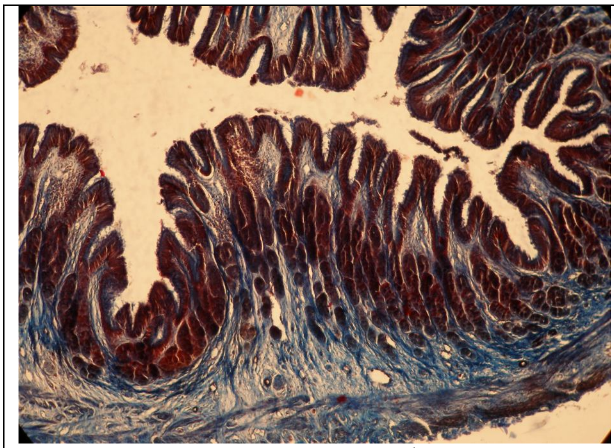
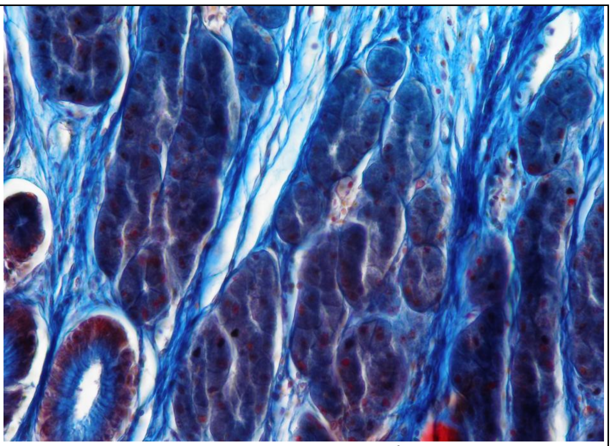
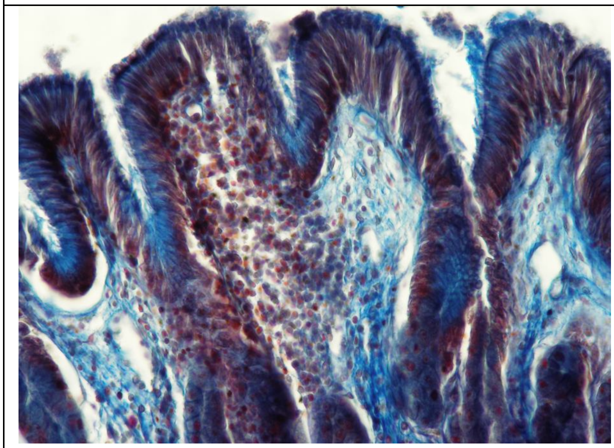
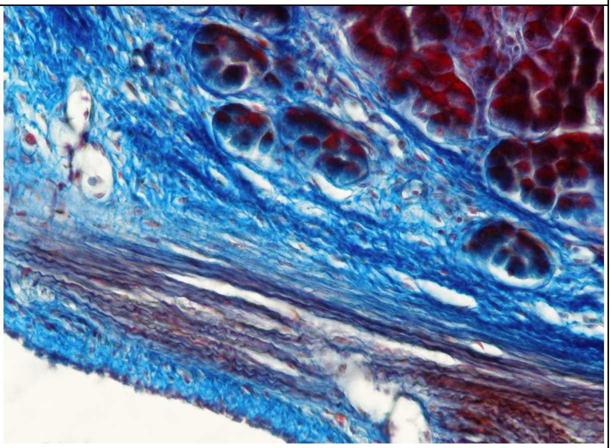
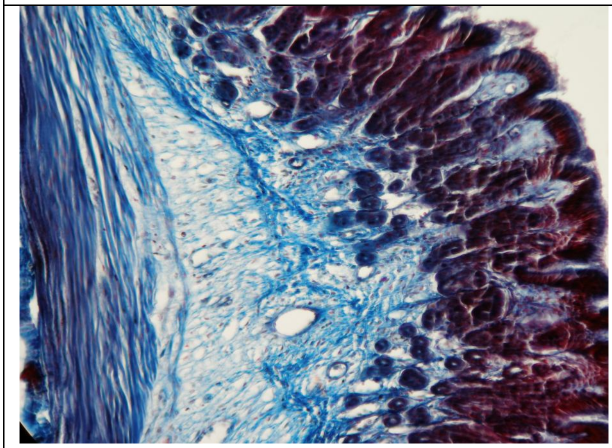
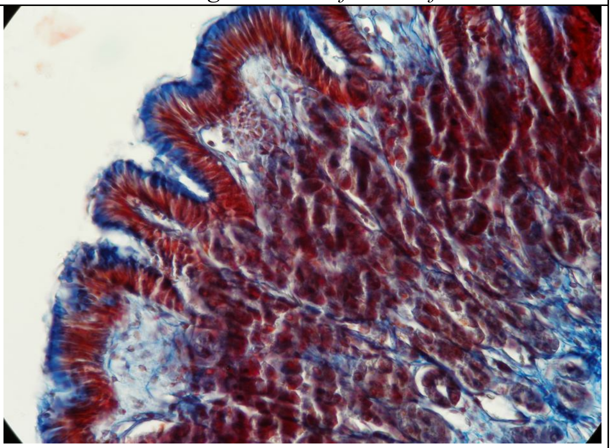
The cortical area of the lymphoid formation, of a darker color, is a dense structure, rich in lymphocytes and poor in reticular- epithelium cells. The medullar area is rich in reticular-epithelium cells, with extended ramifications that generate through anastomosis a network. In the network's meshes are located the lymphocytes. The reticular- epithelium cells have role to secrete a series of humoral factors, that control the function of this lymphoid organ.

The wall of the pyloric caeca has the mucosis planked with a prismatic single-layered epithelium. This penetrates in the mucosis' chorion and forms simple or ramified tubular glands.

The liver is wrapped on the exterior in a thin conjunctive capsule, manly formed by collagen fibers and fibroblasts.

The morphologic aspect of the hepatocytes from the structure of the hepatic lobules is slightly heterogeneous depending of the loading degree of their cytoplasm with lipidic vacuoles, the formation of lipidic sediment being a characteristic of this species. Hence, there are hepatocytes with a spherical nucleus, central or slightly exocentric, with an obvious nucleolus and heterochromatin granules, and the cytoplasm is fine granular.

The pancreas is located near to the liver, has a lobules structure, the exocrine component of the parenchyma being represented by the pancreatic acini (continued on page 207).

 **Figure 1.** *Acipenser ruthenus.* *Esophagus – general view.*	 **Figure 2.** *Acipenser ruthenus.* *Esophagus – esophagian glands.*
 **Figure 3.** *Acipenser ruthenus.* *Esophagus limphoid infiltrate in mucosas chorion.*	 **Figure 4.** *Acipenser ruthenus.* *Esophagus – conjunctive tissue of the muscular tunic with a large number of elastic fibers*
 **Figure 5.** *Acipenser ruthenus.* *Stomach – general view*	 **Figure 6.** *Acipenser ruthenus.* *Stomach – gastric mucosis with prismatic single layered epithelium with striated plateau and lymphoid infiltrate in the chorion*

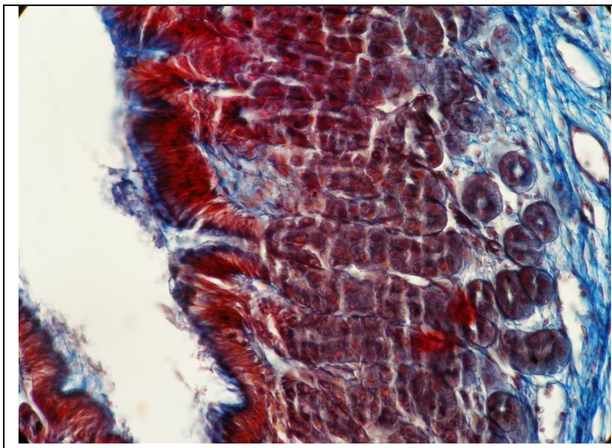


Figure 7. *Acipenser ruthenus.*
Gastric glands

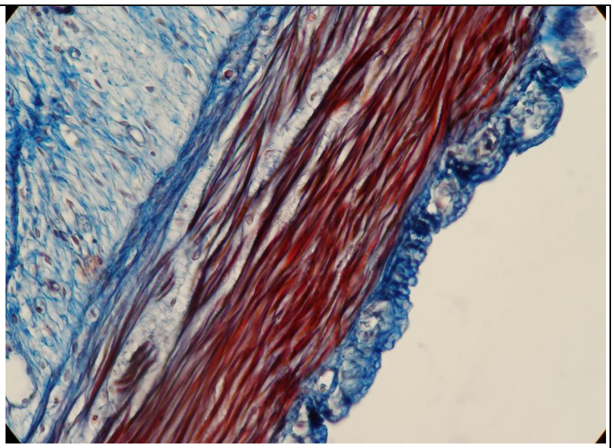


Figure 8. *Acipenser ruthenus.*
Stomach – muscular tunic

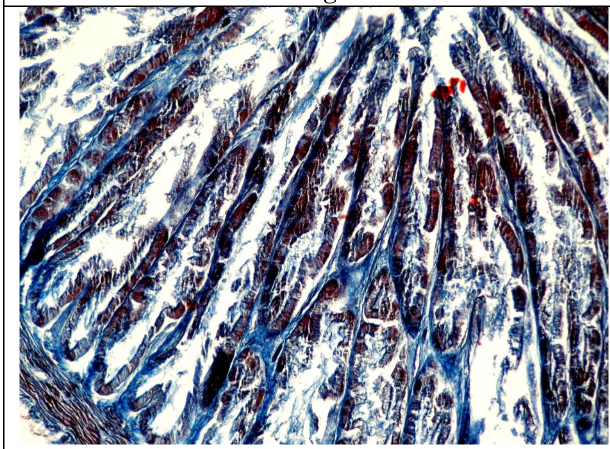


Figure 9. *Acipenser ruthenus.*
Anterior intestine – intestine vilosities

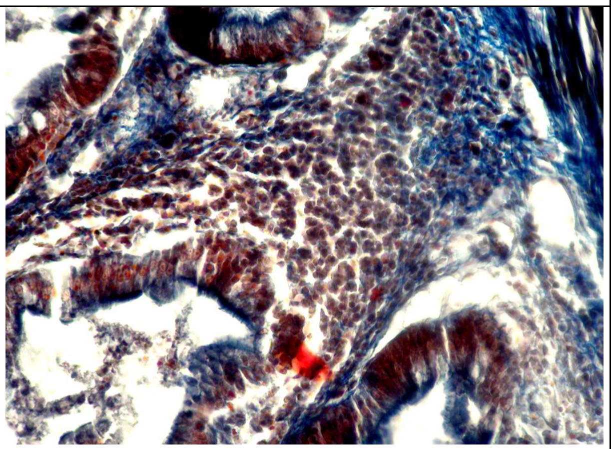


Figure 10. *Acipenser ruthenus.*
Anterior intestine – basal chorion with lymphocyte infiltrate

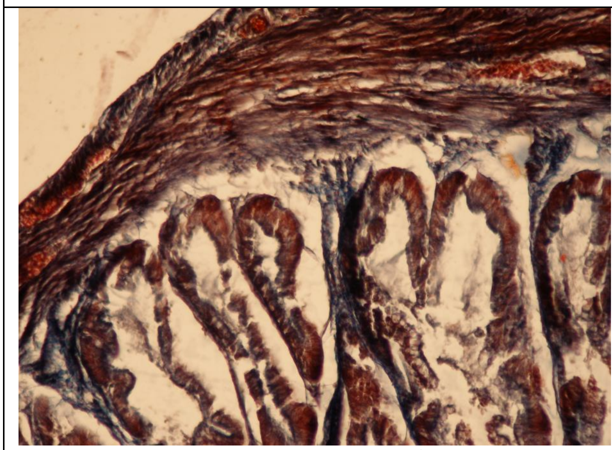


Figure 11. *Acipenser ruthenus.*
Anterior intestine – intestinal glands

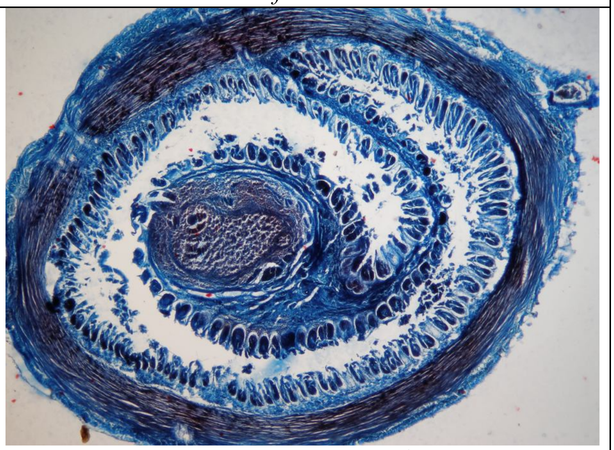


Figure 12. *Acipenser ruthenus.*
Posterior intestine –General view of spiral valve

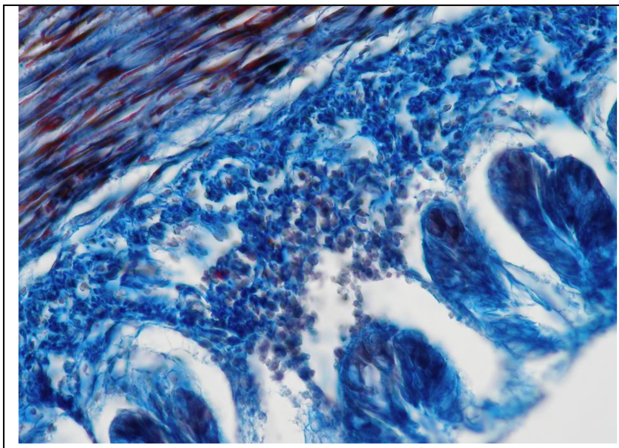


Figure 13. *Acipenser ruthenus.*
Intestinal mucosis – lymphoid infiltrate

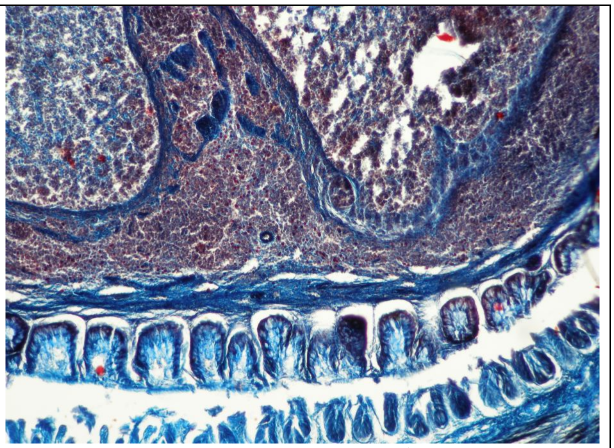


Figure 14. *Acipenser ruthenus.*
Posterior intestine – spire section: lymfoid formation

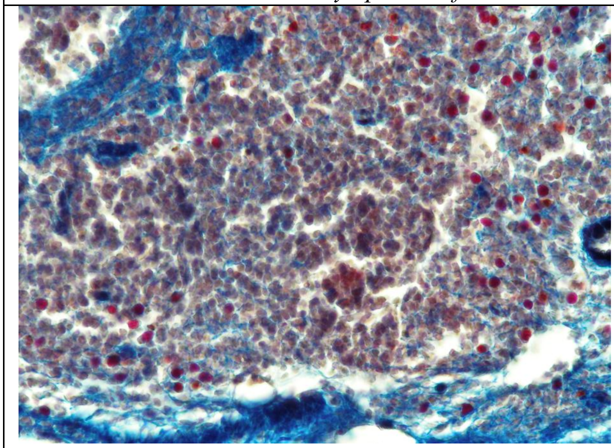


Figure 15. *Acipenser ruthenus.*
Posterior intestine –cortical zone of the lymfoid formation

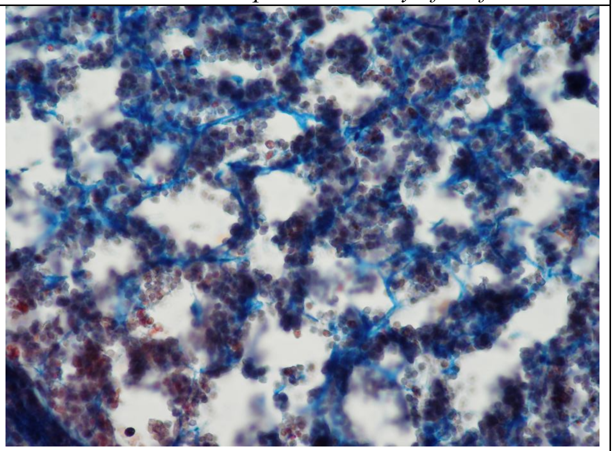


Figure 16. *Acipenser ruthenus.*
Posterior intestine –medular zone of the lymfoid formation

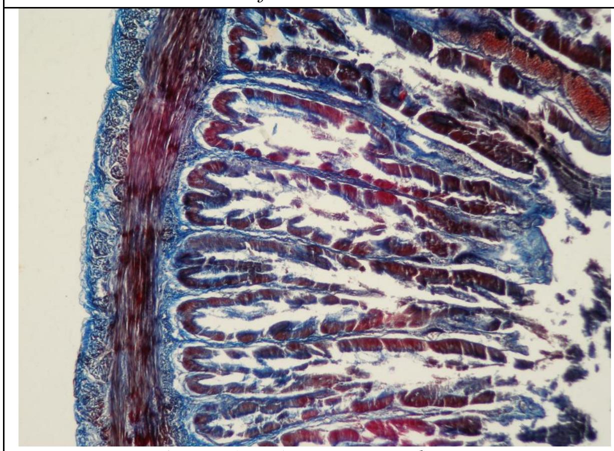


Figure 17. *Acipenser ruthenus.*
Pyloric caeca – general view

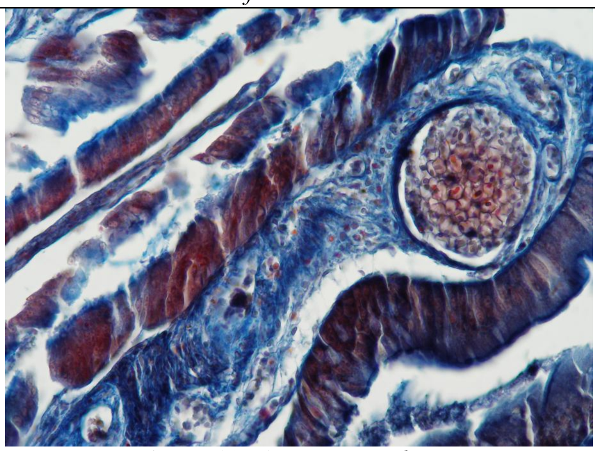


Figure 18. *Acipenser ruthenus.*
Pyloric caeca – vascularised chorion

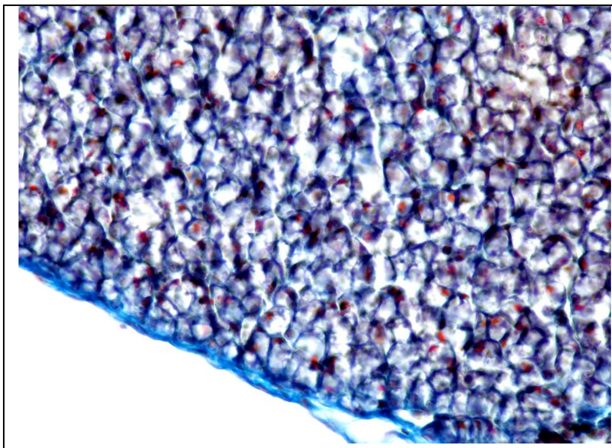


Figure 19. *Acipenser ruthenus.*
Liver – capsule and hepatic parenchime

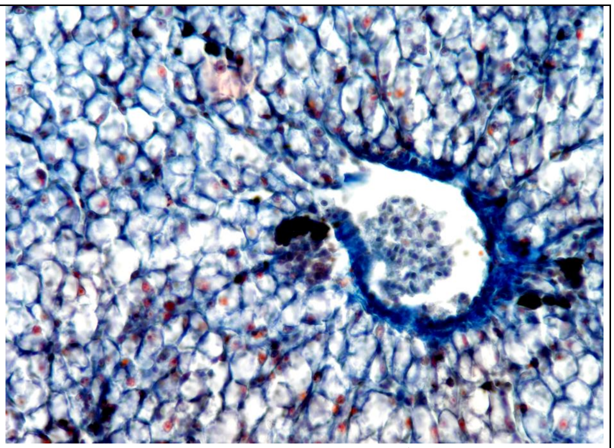


Figure 20. *Acipenser ruthenus.*
Liver – central zone of the hepatic lobe

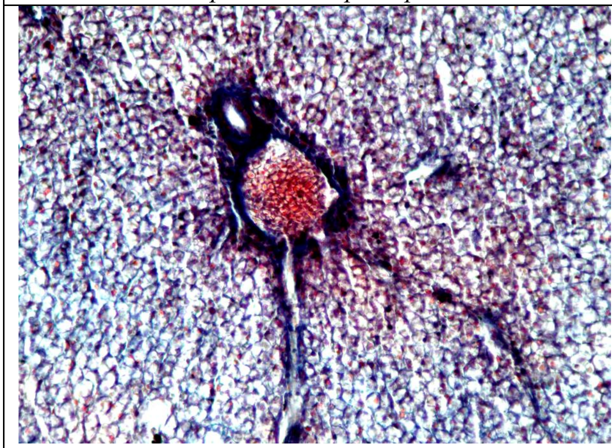


Figure 21. *Acipenser ruthenus.*
Liver – Kiernan interlobular space

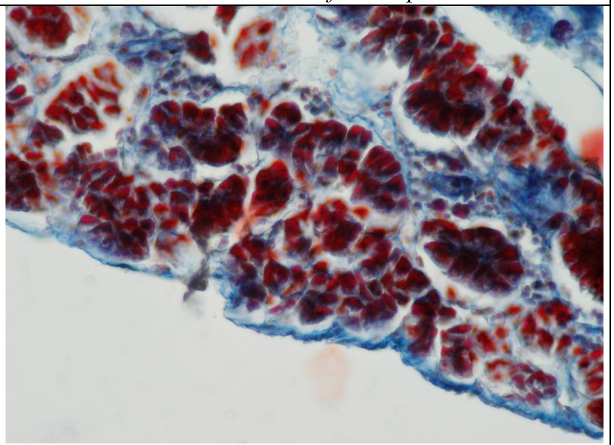


Figure 22. *Acipenser ruthenus.*
Pancreas – pancreatic acinus

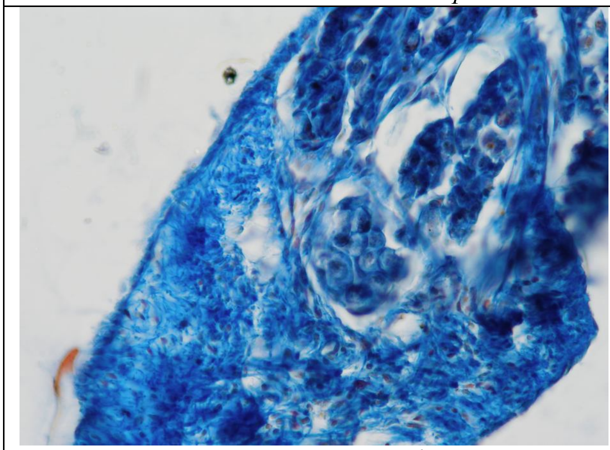


Figure 23. *Acipenser ruthenus.*
Ovary – primordial ovarian follicles organized in nests

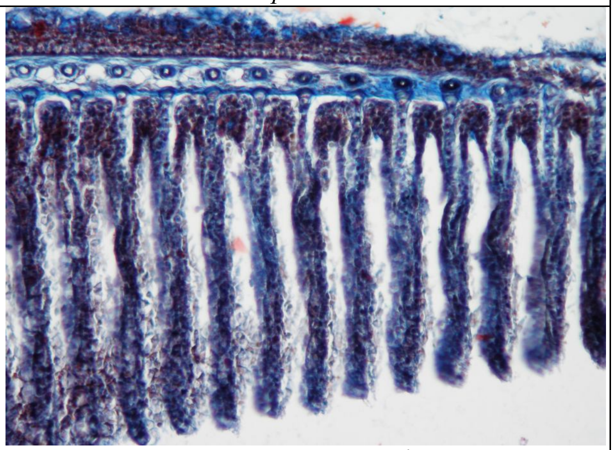


Figure 24. *Acipenser ruthenus.*
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The microscopic sections through the gonads show their different aspect in female gonads, respectively ovaries.

These are wrapped in a conjunctive capsule, under which there is located a thick layer of conjunctive tissue made from numerous collagen fibers, fibroblasts and contains a numerous population of ovogonium.

Under the albuginea there is located the ovarian parenchyma formed by primordial ovarian follicles organized in nests or rows.

The study of the microscopic sections through the studied organs do not show any negative effect of the breeding in the super- intensive system of the sturgeon in general and of the sterlet in particular. The development of sterlets in this type of system seems to be harmonious, the sterlets that have been studied being in full growth process. The proof are the large spaces between the muscular fibers, both smooth and ribbed. The same conclusions are drawn also by other authors that studied farmed sturgeons (Charmi et al 2009).

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