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Morphological changes in digestive tract of Atlantic sturgeon *Acipenser oxyrinchus* during organogenesis

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Abstract

The histological development of the digestive tract of Atlantic sturgeon *Acipenser oxyrinchus* larvae were studied from 6th to 49th day post hatching (dph). The majority of developmental changes in the Atlantic sturgeon larvae took place during the first days after hatching. In Atlantic sturgeon larvae, digestive tract development started from the spiral intestine and proceeded frontward, similarly as in other sturgeons. During the endogenous feeding phase, the wall of the yolk sac differentiated into the stomach (glandular and non-glandular regions) and the anterior and intermediate intestine, while the hind-gut primordium differentiated into the spiral valve and rectum. At on first exogenous feeding (10 dph), the digestive tract consisted of a well developed buccal cavity, oesophagus, glandular and non-glandular stomach, anterior intestine, spiral intestine and anus. The absorptive surface area of the sturgeon intestine increased during development due to the elongation of the intestine and formation of pyloric caeca between 6th -20th dph. At the onset of exogenous feeding, the organization and cytoarchitecture of the digestive system in Atlantic sturgeon larvae was generally similar to those of juveniles and adults.

Keywords: Digestive tract, Organogenesis, Atlantic sturgeon

Introduction

Extensive research has recently been conducted on postembrional of larvae of different sturgeons species development (Gisbert et al. 1998; Boglione et al. 1999; Domeneghini et al. 2002). However, there is lack of research concerning Atlantic sturgeon (*Acipenser oxyrinchus*) larvae development. Creating appropriate rearing strategy requires knowledge on changes in gastrointestinal tract and its associated processes of digestion and absorption of food during development of larvae. The most important information for breeders is recognition of the moment when feeding should begin as larvae require exogenous nutrition.

Lack of information on these aspects for larvae became an inspiration for conducting research which takes into consideration of developmental changes taking place in the gastrointestinal tract of fish in early life stages.

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Materials and methods

The research took place in rearing facilities of Inland Fisheries Institute in Olsztyn (Experimental Fish Farm in Dgal). The rearing period began at hatching and continued until the 49th day of development. Larvae were reared in tanks (1× 0.6× 0.3 m), under recirculation system. A total of 350 animals stocked in each tank and the average water temperature was 19 °C ± 0.5 °C. The larvae were fed from 10th day after hatching with Artemia salina ad libitum. Every 14 days (from 6th to 49th day post hatching) 10 larvae were collected for anesthetization, preserving in Bouin's solution and embedding in Paraplast. They were thereafter cut into 5 µm sections and subjected to histological and histochemical procedures and morphometric analyses.

Results

The experiment started on 6th day post hatching (dph) and the gastrointestinal tract of larvae consisted of a large glandular (cardiac), non-glandular (pyloric) stomach, anterior intestine, intermediate intestine, spiral valve and rectum (with ciliate cylindrical epithelium) (Fig. 1).

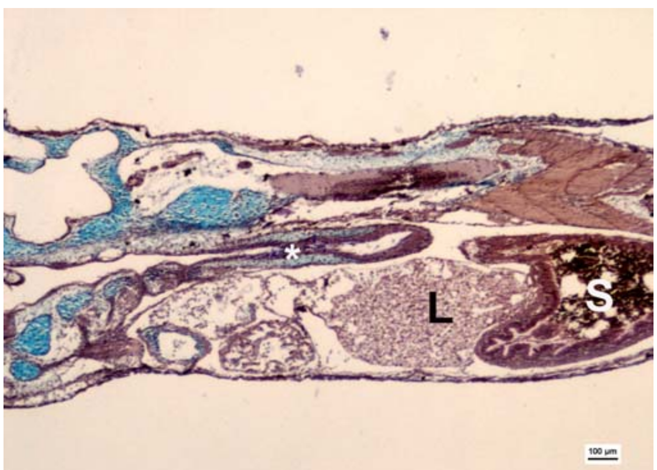

Fig. 1. Sixth day post hatching. Longitudinal section of buccal cavity, liver (L), non glandular and glandular stomach. No connection between the buccal cavity and stomach (artistic). The yolk present in the stomach lumen (S).

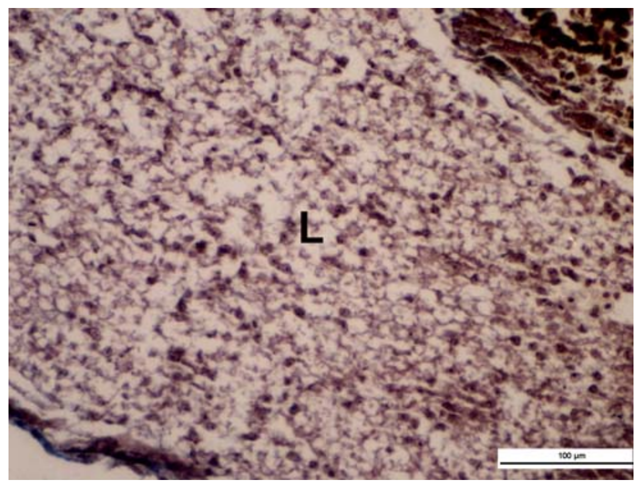

Fig. 2. Transverse section of liver (L). Numerous lipid vacuoles in hepatocyte cytoplasm. Sixth day post hatching.

In stomach region, liver and pancreas were visible. Hepatocyte nuclei were located peripherally, and cytoplasm was almost completely filled with lipids (Fig. 2). As larval development proceeded, further pancreas differentiation took place. On the 6th dph it distinctly increased and first zymogen granules appeared (Fig. 3). At that moment the evolving glandular stomach of larvae was filled with yolk and buccal cavity was closed (Fig. 1). Development of glandular stomach started on the 6th dph and at that time the yolksac epithelium transformed from squamous into cylindrical. The first gastric glands became visible on the 6th dph and their number increased during further larval development. After 6 day from hatching, the differentiation of intermediate intestine began and the spiral valve was fully developed and a melanine plug formed from pigment granules (Fig. 4).

The endmost part of spiral intestine developed into a short anal channel lined with ciliated cylindrical epithelium without mucous cells. The mucosal cells produced acidic mucins. First mucosal cells and teeth appeared in the oral cavity and pharynx at 6th dph. First taste buds also formed in the oral cavity at the same day and the differentiation of anterior intestine began. The glandular stomach began to form between 6th and 20th dph (Fig. 5). The non-glandular stomach was fully developed and separated from anterior intestine by pyloric sphincter during this period of time.

The esophagus and rectum became open at 10th dph; at the same time the melanine plug was excreted and larvae began feeding on exogenous food. The absorptive surface area of the sturgeon intestine increased during development due to the elongation of the intestine and formation of pyloric caeca between 6th -20th dph (Fig. 6).

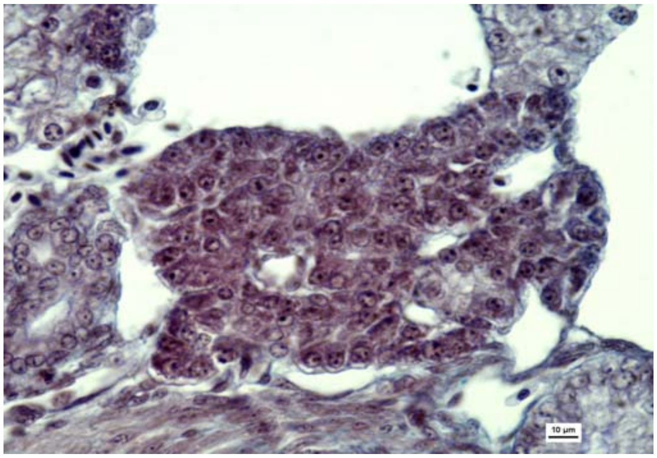

Fig. 3. Longitudinal section of pancreas. Proenzyme granules in the pancreatic cells. Sixth day post hatching.

Fig. 4. Longitudinal section of the posterior part of spiral intestine. Sixth day post hatching, developing melanin plug.

Distinct histological changes took place after the onset of exogenous feeding of larvae. At that time, no more lipid vacuoles were observed in hepatocytes and their shape changed into regular (Fig. 7). Numerous blood vessels filled with blood cells appeared among hepatocytes. During the larval development, pancreas grew, and a large part of larval body cavity was filled by it. On the 20th dph it was situated between the glandular stomach, non-glandular stomach and the intestine, adjacent to the liver in front, directly under a kidney at the back (Fig. 8).

Morphometric analysis showed that the anterior intestine fold increased five times (6th dph -23 µm; 49th dph 115 µm) and posterior intestine fold increased four times (6th dph - 29 µm; 49th dph 112 µm). The height of glandular stomach mucosal layer grew three times (6th dph - 35 µm; 49th dph 109 µm). The hepatocyte area decreased between 6th dph a 49th dph (6th dph- 159 μm^2; 49th dph - 120 μm^2). At on first exogenous feeding, the digestive tract consists of a well developed buccal cavity, oesophagus, glandular and non glandular stomach, anterior intestine, spiral intestine and anus.

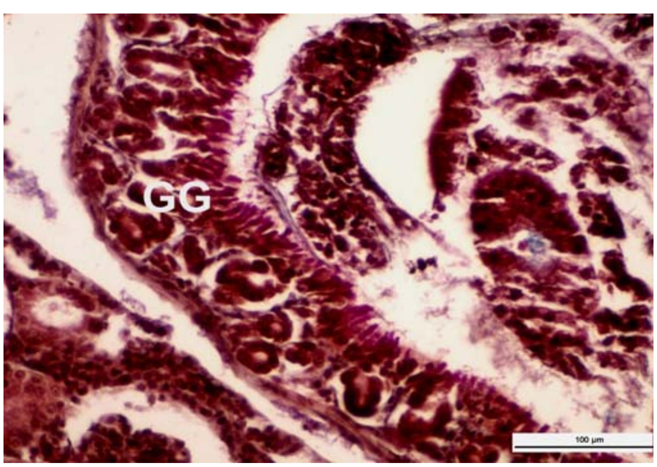

Fig. 5. Longitudinal section of glandular stomach wall. Twentieth day post hatching. The gastric glands (GG).

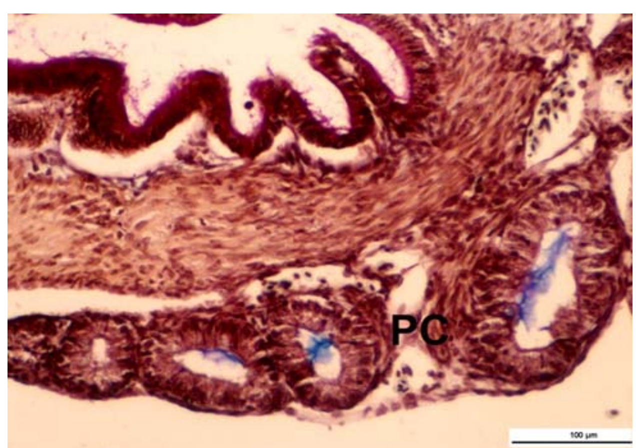

Fig. 6. Longitudinal section of non glandular stomach and pyloric caeca (PC). Twentieth day post hatching.

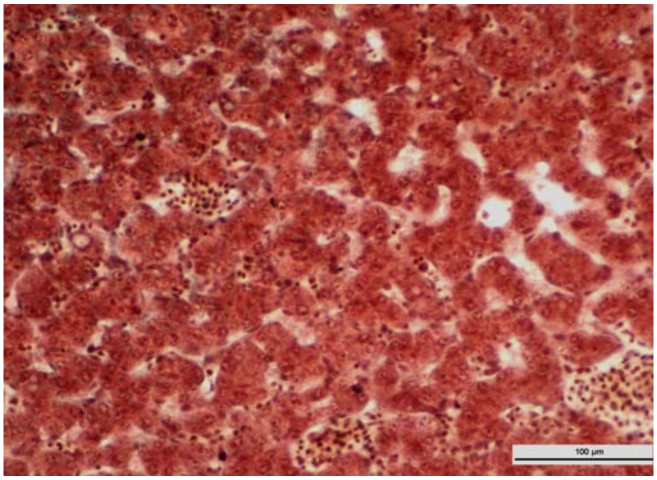

Fig. 7. Longitudinal section of liver. Thirty fifth day post hatching - no lipid vacuoles in hepatocytes.

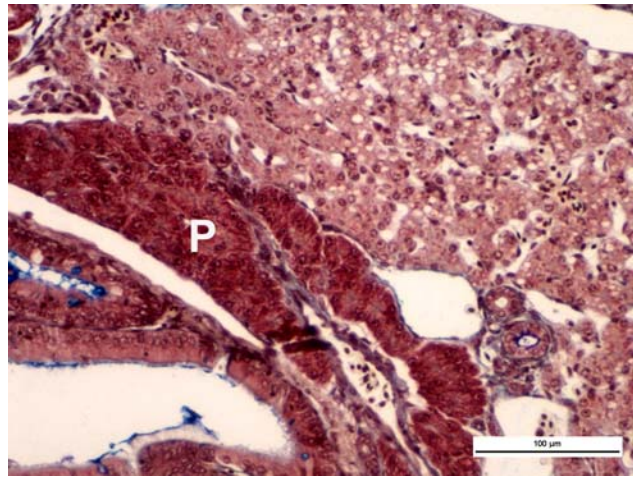

Fig. 8. Longitudinal section of liver (L) and pancreas (P). Forty ninth day post hatching.

Discussion

The Atlantic sturgeon larvae began feeding on exogenous food on 10th dph and at that time their gastrointestinal tract was fully developed and functional. The differentiation process of the elements of gastrointestinal tract in Atlantic sturgeon larvae was similar to other sturgeon species and progressed cranially, starting at the spiral valve (Buddington and Christofferson 1985). According to Gisbert and Williot (1997) excretion of melanin plug is not an appropriate sign to begin exogenous feeding of larvae. In contrast, recent research proved that excretion of melanin plug is the most appropriate moment to start feeding the Atlantic sturgeon larvae. However, some differences concerning timing of differentiation of various organs, functional ability of food uptake, digestion, and absorption were observed. The majority of developmental changes in the Atlantic sturgeon larvae took place during the first days after hatching.

In Atlantic sturgeon larvae, digestive tract development started from the spiral intestine and proceeded frontward, similarly as in other sturgeons including white, green and Siberian (Buddington and Doroshov 1986; Gisbert et al. 1998; Gisbert and Doroshov 2003). Buddington and Doroshov (1986) supposed that the presence of yolk material inside the epithelial cells of developing digestive tract indicates pinocytosis and intracellular digestion. According to Dettlaff et al. (1993), it results from holoblastic cleavage and contribution of yolk-rich endodermal cells in digestive tract development. The beginning of exogenous feeding was accompanied with evacuation of melanin plug. Some authors suggest that the onset of exogenous feeding should coincide with melanin plug excretion (Gawlicka et al. 1995). However, according to Gisbert and Williot (1997) this is not an appropriate moment for food supply; feeding should be accompanied with yolksac exhaustion and appearance of food search behavior. Feeding before the end of endogenous period was recommended by Conte et al. (1988), while the results obtained by Gisbert and Williot (1997) for Siberian sturgeon larvae indicated that food supply before the yolksac exhaustion is completely ineffective.

Non glandular stomach in Atlantic sturgeon developed first, while the first gastric glands appeared on the 6th dph, 4 days before the start of exogenous feeding. The differentiation of glandular stomach started earlier than in other sturgeon species. In Siberian, Adriatic and white sturgeons, gastric glands start to develop between the 8th and 9th dph, while in green sturgeon they appear on 12th dph (Buddington and Doroshov 1986; Gisbert et al. 1998; Gisbert and Doroshov 2003). Atlantic sturgeon larvae at the onset of exogenous feeding showed completely developed digestive glands including liver and pancreas.

Liver differentiated from the moment of hatching and when the fish started exogenous feeding it was already a large organ occupying much space in the peritoneal cavity. Pancreas became visible on the 6th dph, and from the 6th dph proenzyme granules were observed in the exocrine part. Zymogen granules in exocrine pancreas were also observed immediately before first feeding in many fish species (Chen et al. 2006; Ribeiro et al. 1999).

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