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DEVELOPMENTAL CHANGES OF DIGESTIVE SYSTEM STRUCTURES IN PIKE-PERCH (*SANDER LUCIOPERCA* L.)

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Abstract: The present study was undertaken to observe the morphological and functional development of pike-perch digestive system during larval period. The newly hatched pike-perch larvae were used, and the experiment lasted until the 30th day post hatching. The larvae were fed from the 5 day with *Artemia* sp. nauplii. The fish were sampled and preserved for histological and histochemical analyses. At hatching, the digestive tract of pike-perch was a straight tube and consisted of undifferentiated cells. The mouth was closed, and esophagus was not connected with the intestine. Undifferentiated liver and pancreas cells were situated over the yolk sac. Between the 5 and 7 day post hatching, the digestive tract developed, and liver and pancreas became active. Digestion and absorption of exogenous food in the intestinal enterocytes was observed before the stomach development. The esophageal goblet cells of the pike-perch larvae developed in 1-2 days after the mouth opening, at the onset of endo-exogenous feeding. Lipids were found in the enterocytes of anterior intestine, while protein absorption – in the epithelium of posterior intestine section. Development of stomach, its glands, and pyloric caeca took place between the 15 and 30 day post hatching.

Key words: development, digestive system, histology, pike-perch, larvae, juvenile

Introduction

The pike-perch (*Sander lucioperca* L.) is one of the most valuable freshwater fish species. It inhabits rivers, lakes, ponds, and dam reservoirs. In the Central Europe, prices for pike-perch are high, due to its fast growth and very tasty meat.

Pike-perch rearing has a long tradition but the interest in this species considerably increased in the eighties. Pond production of pike-perch is usually extensive or semi-intensive (Hilge and Steffens 1996; Ruuhijärvi and Hyvärinen 1996), and its main purpose is production of fingerlings for stocking open waters. Two methods are the most frequently applied in pike-perch fingerling production: traditional pond rearing in polyculture with common carp, or monoculture which is more costly and laborious.

Throughout recent years, attempts of intensification of pike-perch production were undertaken in some European countries. Experimental rearing of larvae under controlled conditions was described by Ruuhijärvi et al. (1991), Schlumberger and Proteau (1991), Proteau et al. (1993). These experiments, however, did not produce satisfactory results due to high larval mortality. High biological sensitivity of pike-perch in the period from hatching to the juvenile stage makes rearing of the larvae very difficult. The origin and causes of larval mass mortality are unknown which makes efficient rearing impossible. However, rearing of pike-perch larvae is an extremely important issue from the commercial point of view, since it is the only way of successful reproduction of this species.

The aim of the present study was a detailed description of pike-perch early ontogenesis, with particular attention paid to the digestive system development. Description of morphological changes in correctly developing digestive system is essential for optimization of rearing conditions. Histological methods are very

useful for observation of yolk sac resorption process, and the changes in digestive system. They also allow for accurate determination of the moment when fish start exogenous feeding, and detailed description of this critical moment of larval life.

Materials and Methods

The study was carried out in the Laboratory of Ichthyobiology and Fisheries of the Warsaw Agriculture University. The newly hatched pike-perch embryos were obtained from the hatchery of the Experimental Fish Farm (Warsaw Agriculture University) in Łąki Jaktorowskie. The fish were placed in 3 recirculatory glass aquaria of 20 dm^3 volume, at the stocking density of 500 individuals per tank (25 ind./dm^3). The larvae were fed from the fifth day post hatching (dph) *Artemia* sp. nauplii, twice a day *ad libitum* (about 100 nauplii per fish). Continuous illumination of tanks did not exceed 100 lx.

The larvae were reared at constant water temperature of 20ºC, until the 30th day post hatching.

Every 24 hours, 10 fish were sampled for histological analyses. They were anesthetized using MS 222, and preserved in buffered formaldehyde and Bouin solution. Then, they were dehydrated in a series of ethanol solutions, and embedded in paraffin. The series of 5 µm thick paraffin sections were obtained using the microtome MicroTec CUT 4050. For morphological analyzes, the sections were stained with eosin and hematoxylin, while for glycogen and neutral mucins – using histochemical methods PAS and Alcian Blue pH 2.5, 1, 0.5 (for acidic glycoproteins – carboxyl and sulfated). The used staining techniques were described by Pearse (1985).

All measurements were done using the Nikon-Alphaphot-2 YS2 microscope connected with the Mintron camera and computer image analysis system MicroScan for Windows (v.1.5). The micrographs were done using the Nikon 4300 camera. Total body length of larvae was measured with 0.01 mm accuracy, using the light microscope.

Results

Development of larval digestive system

Total body length (TL) of newly hatched pike-perch larvae was 5.04±0.05 mm. The body was transparent and surrounded by a fin fold. The larvae showed oval yolk sacs divided into two sections: oil globule in the anterior part, and yolk in the posterior part. Both the oil globule and yolk were enclosed within the yolk syncytial layer (YSL). Two syncytial zones were observed: one surrounding the oil globule, and another surrounding the yolk. No blood vessel network was observed on the yolk sac.

The period of endogenous feeding of the pike-perch lasted until the 6th day post hatching, mixed endo-exogenous feeding occurred from the 6th to the 17th day, and from the 17th day on the fish were exclusively fed exogenous feeding.

I. Endogenous feeding period

The newly hatched pike-perch larvae showed acidophilic yolk sac. Endodermal epithelium surrounding the yolk sac was eosinophilic (H-E) and PAS-positive showing the presence of neutral glycoproteins. The mouth and esophagus were closed (Figure 1a).

The lumen diameter of primary intestine was uniform (Figure 1b). The mouth and pharynx were lined with cubic epithelial cells of irregular shape. Over the development time, these cells gradually flattened, and on the third day they transformed into the multilayered squamous epithelium. Esophagus was undifferentiated, and not connected with the intestine. At hatching, the intestinal lumen was lined with irregular cubic cells that transformed on the second day into the unilayered cylindrical epithelium (Figure 1c). The intestinal epithelium showed basophilic cytoplasm and cell membrane (H-E). The undifferentiated liver and pancreas were separated from the yolk sac by syncytial layer. The cells of both organs adjoined the syncytium (Figure 1d).

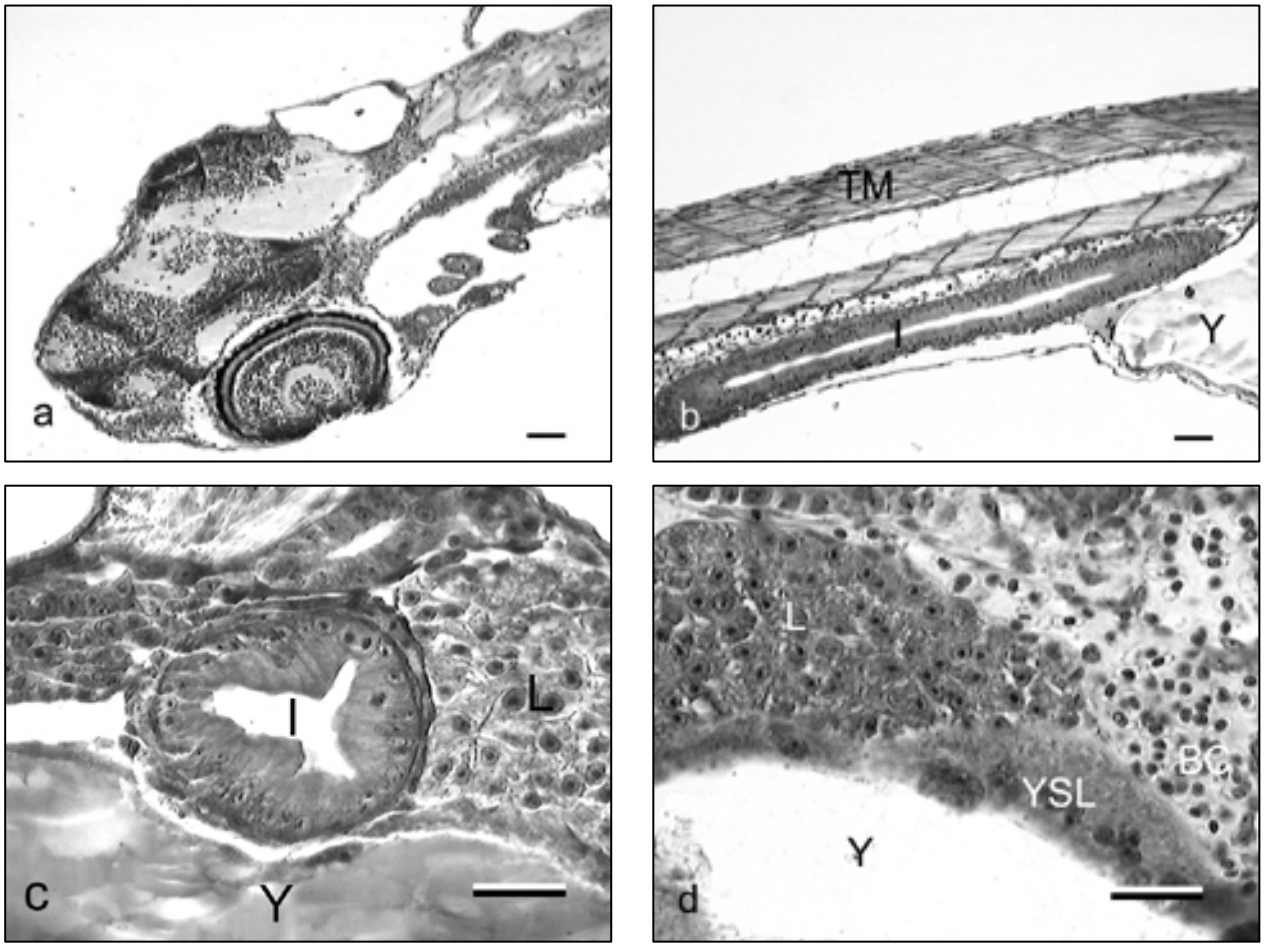

Figure 1. a). Horizontal section of larval head at hatching. The closed mouth. Bar 100µm. b). Longitudinal section of the posterior body part. Primary digestive tract (I) at hatching. Trunk muscles (TM), yolk sac (Y). Bar 100µm. c). Cross-section of the primary intestine (I) lined with single layer of columnar epithelium of variable cell height at 2 dph. Liver (L), yolk sac (Y). Bar 25µm. d). Cross-section of non-differentiated liver cells (L) adjacent to the blood vessels (BC) and syncytium (YSL) at 2 dph. Bar 100µm.

On the third day post hatching (TL = 5.39±0.03 mm) the length and lumen of larval intestine increased, particularly in the anterior section. The intestine was lined with slightly folded unilayered mucosa of cylindrical epithelial cells, the nuclei of which were situated in the basal region. The hepatocytes with centrally located nucleus, and distinct nucleolus increased which resulted in liver growth. At this stage, no glycogen storage was observed.

II. Mixed feeding period

Considerable changes occurred between the 5th and the 7th day post hatching (TL = 6.00-6.10±0.02 mm). The mouth opened, and so did the esophagus which connected the anterior intestine (Figure 2a).

The esophageal goblet cells situated among the squamous epithelial cells started secretion of neutral glycoproteins (PAS-positive), as well as synthesizing acidic carboxyl mucins staining with Alcian Blue (pH 2,5/ PAS) and sulfate mucins staining with AB/PAS (pH 0.5 and 1.0) (Figure 2a). Secretion of neutral glycoproteins took place

mainly in the anterior part of esophagus, while the pharyngeal mucous cells, and those of posterior esophagus section produced mainly acidic carboxyl and sulfate glycoproteins. Further increase in liver volume took place, and hepatic blood vessels filled with blood cells became visible. Glycogen storage was observed (PAS-positive regions) (Figure 2b).

The pancreas was situated above the liver, and showed exocrine activity. On the fifth day post hatching, first proenzyme granules (H-E and PAS-positive) appeared in the basophilic pancreatic cells. Two days later, the number of granules considerably increased (Figure 2c), and a large Langerhans islet appeared (Figure 2c).

From the very beginning of mixed, endo-exogenous feeding (6-7 dph), a bile duct was observed, connecting the liver with intestine. It opened into the anterior intestine section (Figure 2d). Both, the gall bladder and the bile duct mucosa consisted of unilayered cubic epithelium. The gall bladder (121.5 µm long and 50.4 µm wide, on the 7 dph) was situated between the liver and pancreas.

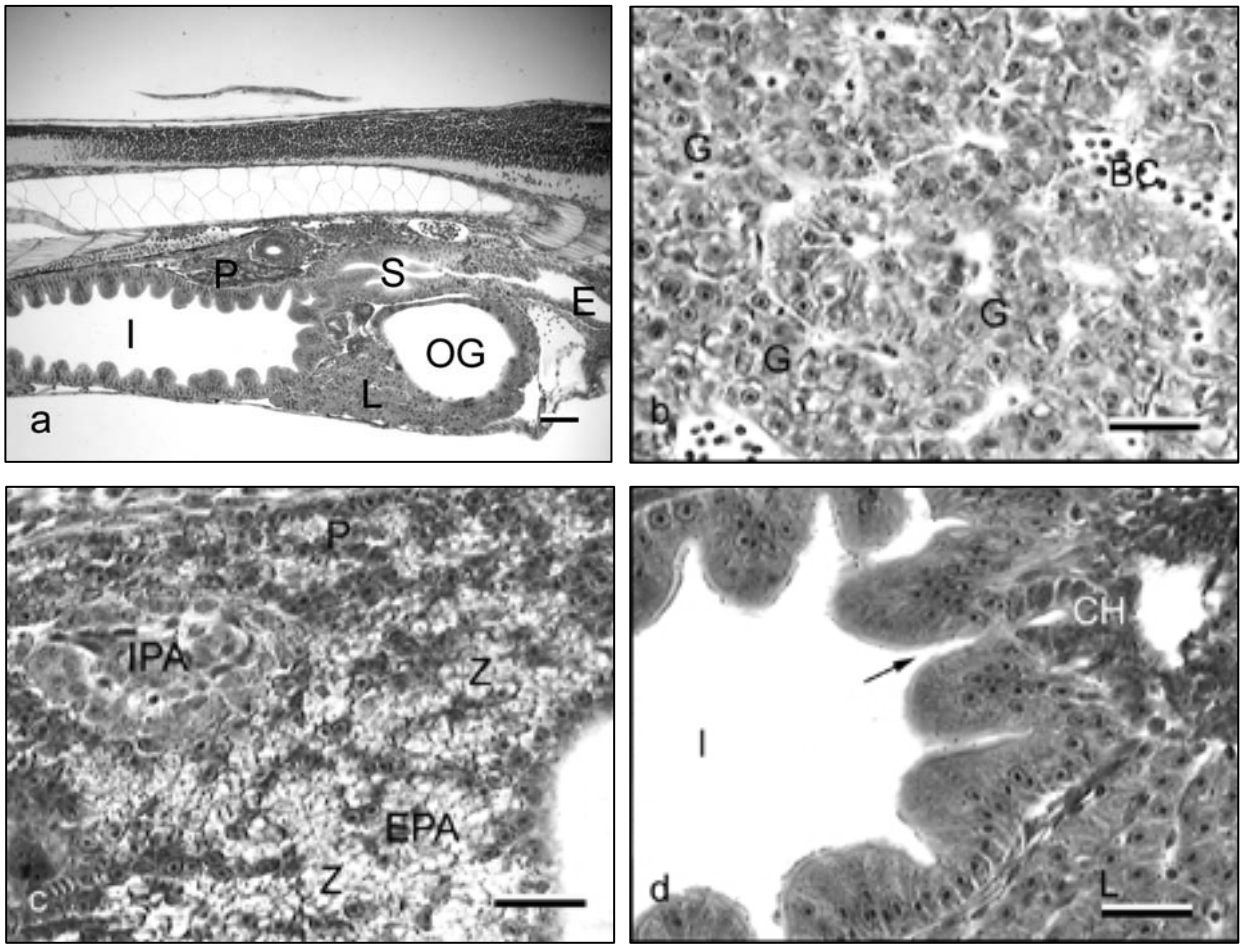

Figure. 2. a). The connection between the esophagus (E) and anterior intestine (I) at 5 dph. Liver (L), pancreas (P), stomach (S) oil globule (OG). Bar 100µm b). Blood vessels (BC) among the hepatocytes at 7 dph. Glycogen (G) PAS-positive areas. Bar 25µm. c). Longitudinal section of pancreas at 7 dph. Exocrine part (EPA) and Langerhans islet (IPA). Proenzyme (Z). Bar 25µm. d). The connection (arrow) between the bile duct (CH) with intestine (I) at 7 dph. Bar 25µm.

Between the 7 and 15 dph (TL = 6.10±0.02 mm -7.99±0.35 mm) further changes in digestive tract took place. The yolk sac content considerably decreased. Esophagus became longer and developed longitudinal folds similar to those present in the anterior intestine, and the secretory activity and number of goblet cells increased.

At this stage, anterior intestine was separated from the posterior section by the intestinal valve consisting of mucosal and submucosal layers. Both layers were extensions of the same layers of the intestine (Figure 3a). In the cytoplasm of enterocytes of anterior and central intestine section, a small number of light vacuoles were observed on the 9 dph. In the anterior part, they disappeared after 1-2 days, while in the posterior part, the supranuclear regions contained vacuoles containing acidophilic granules (Figure 3b). The supranuclear vacuoles in the posterior intestine did not disappear before the stomach development. In the fixed histological preparations, lipids are visible as empty vacuoles, while the proteins - as acidophilic granules.

The intestine grew as the fish aged: the number and size of mucosal folds, as well as the length of brush border increased. The height of mucosal folds in the anterior intestine section ranged from 24 µm to 36 µm, and in the central section - from 17 µm to 20 µm. In the posterior section, where the mucosal cells were the most diverse, the height of folds ranged from 23 µm to 29 µm. The height of the brush border was uniform in entire intestine: ~ 2 µm. The brush border covered mucosa of both, anterior and posterior intestine sections.

In the epithelium of anterior and posterior intestine mucous cells appeared on the 11 dph, and their number increased with fish age. They stained with Alcian Blue (AB pH 2.5), and showed the presence of carboxyl and sulfate carbohydrate compounds (AB pH 0.5 and 1.0).

In the hindmost section of the intestine, a short anal channel developed, lined with cubic epithelium without goblet cells.

Over the entire endo-exogenous feeding period of pike-perch larvae, the liver and pancreas gradually increased. About the 7 dph, lipid storage began in the hepatocytes. Exocrine activity of pancreas significantly decreased beginning from the 9-10 dph, and lower number of proenzyme granules was observed. On the 13 dph, first tooth germs and taste buds appeared in the mouth.

Between the 15 and 20 dph (TL = 7.99±0.35 mm-15±0.25 mm), the beginning of stomach development took place, and pyloric sphincter was observed. The primary stomach was visible from the 5 dph, as an esophagus extension located between the esophagus and anterior intestine (Figure 2a). "The stomach section" was discernible because contrary to entire esophagus and intestine, it lacked goblet cells producing acidic mucins. They were absent from the future stomach region, and pyloric sphincter (Figure 3c). At that time, the stomach size increased, and the first gastric glands developed (Figure 3d). Also the intestine elongated and developed first loop (Figure 3c). About the 20 dph the stomach was morphologically developed (Figure 3e). The number of pancreatic proenzyme granules decreased, and was less than on the 9-10 dph (Figure 3f).

III. Digestive system in juvenile period

The mouth cavity and pharynx of the juveniles between the 20 and 30 dph (TL=15±0.25 mm-31±0.15 mm), was lined with the multilayered squamous epithelium, with numerous taste cells. Teeth appeared in the upper and lower pharyngeal region.

In the endmost section of esophagus, near the stomach, the multilayered squamous epithelium was replaced by the multilayered cubic cells which were present also in the stomach wall adjacent to the esophagus. Among the cubic cells, numerous goblet cells occurred secreting acidic carboxyl and sulfate mucins staining with AB/PAS pH 2.5 and AB/PAS pH 0.5 and 1.0.

The stomach of juvenile pike-perch consisted of three parts: cardia, blind sac, and pyloric stomach.

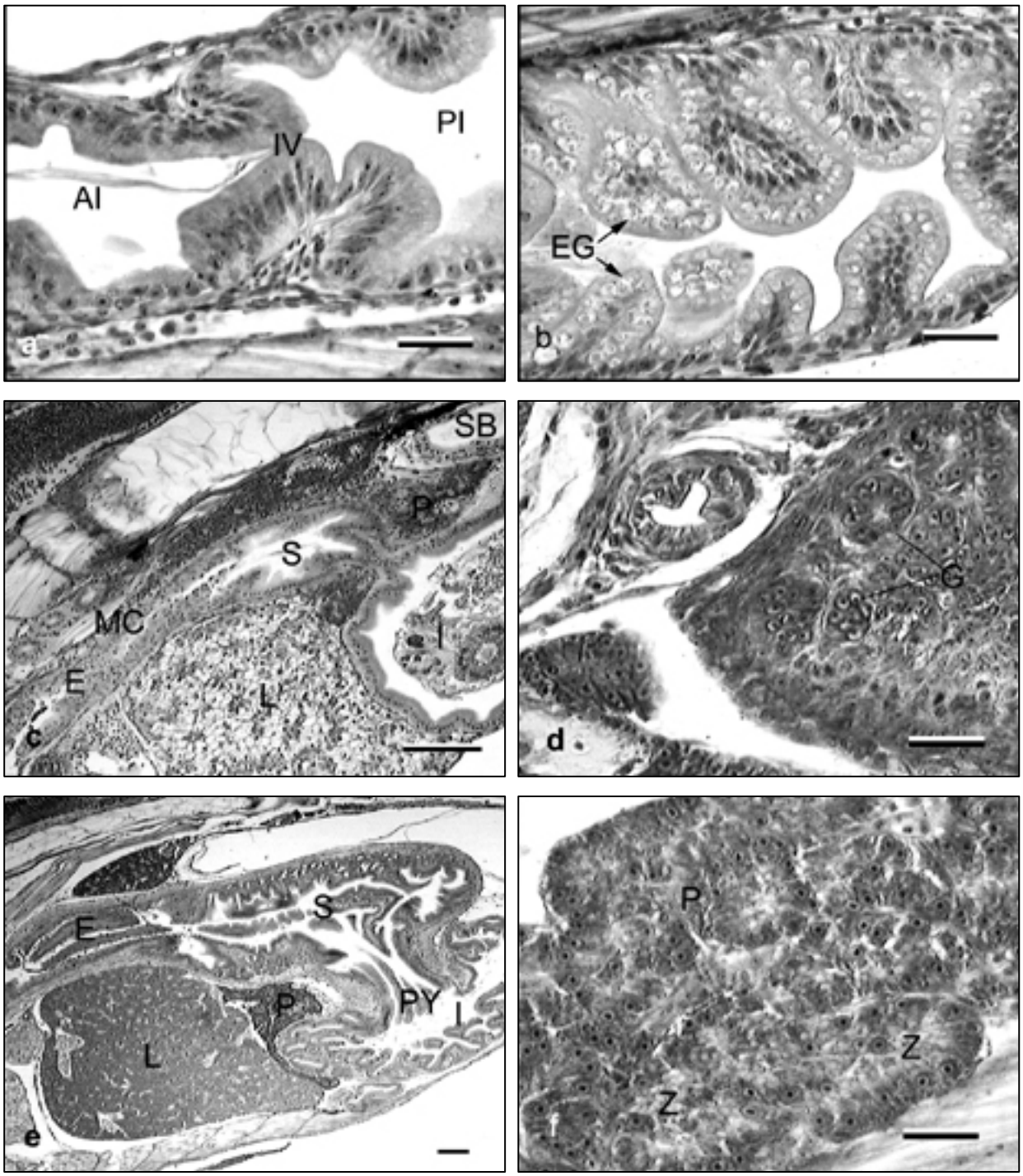

Figure 3. a). Intestinal valve (IV) between the middle (MI) and posterior intestine (PI) at 7 dph. Bar 25µm. b). Longitudinal section of posterior intestine. Small vacuoles with acidophilic granules (EG) in the supranuclear regions of the enterocyte at 9 dph. Bar 25µm. c). Longitudinal section of pike-perch larva at 16 dph. Mucous cells of esophagus (MC). "Stomach" (S) developed from esophagus extension, lipid storage in the liver (L). Swim bladder (SB). Bar 100 µm. d). The beginning of gastric gland (G) development. Bar 25µm.

e). Fully morphologically developed stomach (S) at 20 dph. Liver (L), esophagus (E), pancreas (P), intestine (I), pyloric sphincter (PY). Bar 100µm.f). Pancreas (P) – reduced number of proenzyme granules (Z). Bar 25µm.

The pyloric stomach was situated between the cardia and the blind sac (Figure 4a).

The mucosa of both, cardia and blind sac consisted of unilayered cylindrical epithelium, mucosal *lamina propria*, and muscle layer. The mucosa of cardia and blind sac contained long, tubular and divided gastric glands situated in the *lamina propria* and surrounded by a loose connective tissue. The gland bottoms consisted of polygonal secretory cells of round nuclei and cytoplasm containing acidophilic granules visible after staining with hematoxylin and eosin. The glands opened to the mucosa fold crypts. The glands of the cardiac section were considerably longer, comparing to those in the blind sac (170-280 µm, and 74-135 µm, respectively) (Figure 4b).

The pyloric stomach mucosa consisted of unilayered cylindrical epithelium, *lamina propria* and muscle layer. The long mucosal folds (105-170 µm) were developed as fanlike divisions (Figure 4c). The apical cytoplasm of cylindrical cells of cardia, pyloric stomach and blind sac was PAS-positive which indicates the presence of carbohydrate compounds.

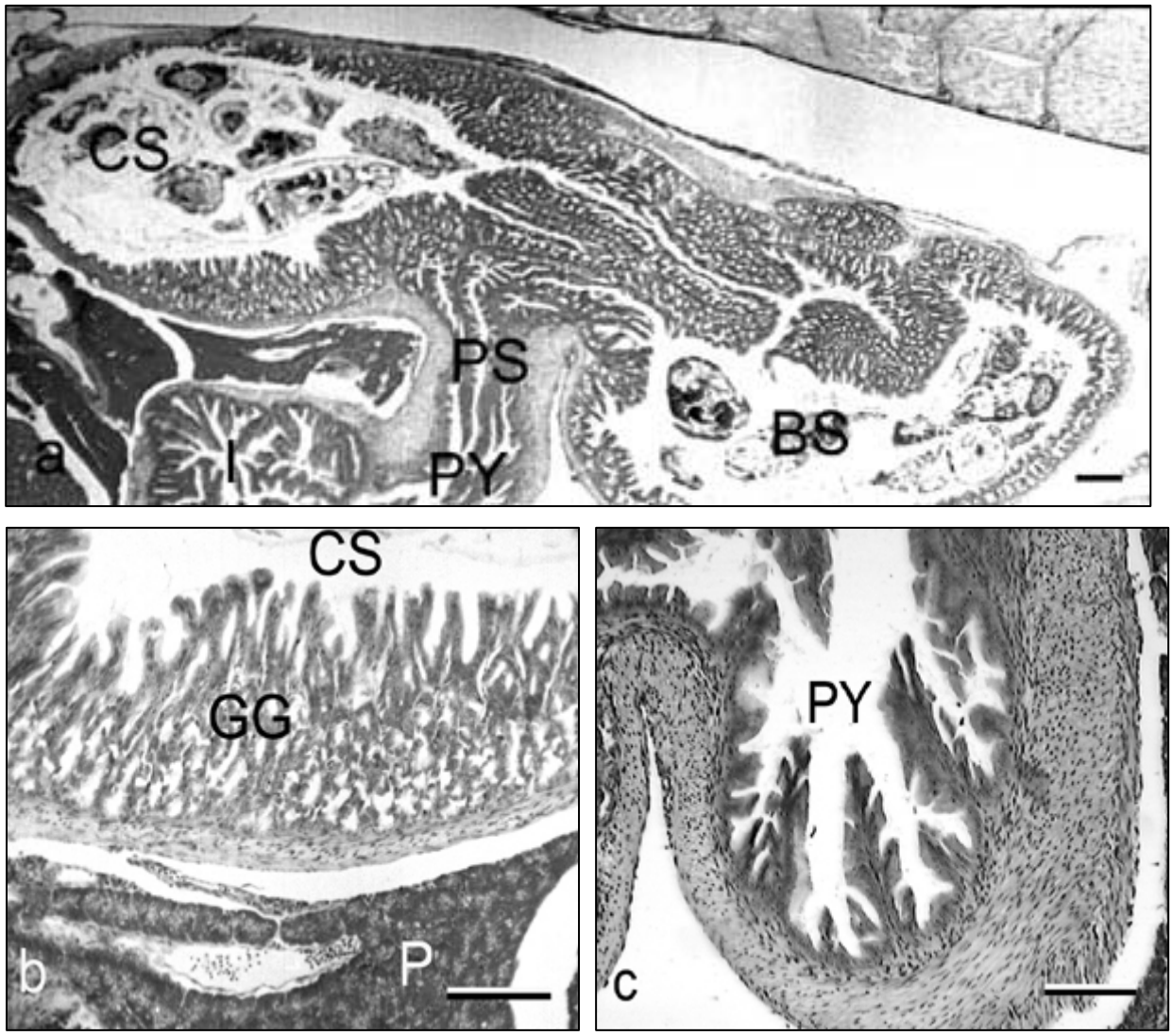

Figure 4. a). Longitudinal section of anterior stomach part (CS), pylorus sphincter (PY) and blind sac (BS) at 25 dph. Pancreas (P), intestine (I). Bar 100µm. b). Longitudinal section of cardia stomach (CS) at 25 dph. Glands (GG), pancreas (P). Bar 100µm. c). Cross-section of pyloric stomach (PY). Long, fan-shaped mucosa folds at 30 dph. Bar 100µm.

The gastric glands contained eosinophilic particles indicating pepsinogen secretion between the 25 and 30 dph. The intestine, with the pyloric caeca began with the pyloric sphincter, and its anterior section was curved downwards.

The pyloric caeca were different from the intestinal ones by higher amount of submucosa, and shorter folds (110 μm). The pyloric caeca lumen cross-section was starlike.

The intestine was histologically uniform, except for a hindmost section, separated from the central part with the intestinal valve. At that stage, supranuclear regions of posterior intestine enterocytes no more contained vacuoles with acidophilic granules.

Intestinal mucosa developed longitudinal folds, the longest in the anterior section (231-304 μm), shorter in the anterior-central part (136-159 μm), and the shortest in the posterior intestine (102 -105 μm).

The mucosal epithelium of the entire intestine contained abundant goblet cells staining with Alcian blue (AB), which indicates the presence of acidic carboxyl (AB pH 2.5), and sulfate (AB pH 0.5 and 1.0) carbohydrate compounds. The brush border of enterocytes was also AB-positive. The apical cytoplasm, just under the microvilli, was slightly PAS-positive. In all intestinal sections, AB-positive mucus was present between the mucosal folds. The AB-positive goblet cells were the most abundant in the posterior intestine.

The U-shaped liver was centrally located in the peritoneal cavity, ventrally from the esophagus, and anterio-ventrally from the stomach. The hepatocytes showed numerous light vacuoles indicating lipid storage, and dark PAS-positive glycogen granules.

The gall bladder was situated between the liver and anterior intestine with which it was connected with the bile duct.

The pancreas was dispersed within the mesentery of the anterior stomach section, among the pyloric caeca, and bile duct. On the 30 dph, the exocrine pancreatic tissue was present around the hepatic portal veins. The Langerhans islets were dispersed within entire gland.

Discussion

At hatching, the pike-perch digestive tract was a straight tube of undifferentiated cells. The mouth was closed, and esophagus unconnected with the intestine. The liver and pancreas were located over the yolk sac, as undifferentiated cells. During the endogenous feeding period, very fast development of digestive tract was observed. The results of the studies of larval development of bony fishes show that yolk sac material absorption is accompanied by an intense development of digestive system (Buddington 1985). The end of endogenous feeding of the pike-perch larvae reared at 20ºC took place at the same time as macroscopic yolk sac resorption, on the 6 day post hatching. The remaining traces of yolk nutrients visible under the microscope were absorbed until the 17 day of life.

The goblet cells developed in the esophagus of the larvae in 1-2 days after the mouth opening, simultaneously with the onset of mixed endo-exogenous feeding. At the same time goblet cells appeared also in *Solea senegalensis* (Kaup) (Sarasquete et al. 1996), and in *Solea solea* (L.) (Boulhic and Gabaudan 1992), while in the other species such as *Sparus aurata* L. developed later (Sarasquete et al. 1995). According to Gisbert et al. (1999), esophageal goblet cells in *Acipenser baeri* developed two days before the beginning of active feeding, similarly as in *Aspius aspius* L. (Ostaszewska and Wegiel 2002). The mucous cells of pike-perch posterior esophagus secreted mainly acidic carbohydrate compounds, while those in the pharynx and anterior esophagus – neutral glycoproteins. The number and size of mucous cells increased with fish age. Similar pattern of mixed secretion (of acidic and neutral mucins) was observed in the larvae of *Sparus aurata* (Domeneghini et al. 1998), *Acipenser baeri* (Gisbert et al. 1999), and *Melanogrammus aeglefinus* (Hamlin et al. 2000). According to Boulhic and

Gabaudan (1992), the esophagus of *Solea solea* larvae secreted exclusively acidic glycoproteins. The mucus produced by the fish goblet cells plays the same role as in mammals (Scocco et al. 1998), protecting the mucosa of digestive tract. According to Zimmer et al. (1992), sialic acid present in fish mucus disturbs receptor detection by the viruses, and protects the mucosa against the bacterial sialidase.

From the beginning of mixed feeding (5-7 dph), the larval intestine was divided into two sections: anterior and posterior, separated by a valve. According to Pedersen and Hjelmeland (1988), the intestinal valve plays an important role at early larval stage, preventing enzyme escape from the intestine.

Histological analysis of liver and pancreas showed the level of activity of these glands. First symptoms of intestinal absorption of exogenous food were observed from the 9 day of pike-perch life.

The supranuclear vacuoles containing acidophilic granules visible in the cytoplasm of pike-perch posterior intestine were found also in the larvae of other fish species: *Gadus morhua; Dicentrarchus labrax; Sebastes melanops; Cottus nozawae; Pleuronectes ferrunginea*; (Govoni et al. 1986; Dabrowski and Culver 1991; Kjørsvik et al. 1991; Deplano et al. 1991; Baglole et al. 1998). These results suggest that the vacuoles were the result of pinocytotic absorption of protein macromolecules from the intestine. This process is typical for fish larvae showing very low secretion of digestive enzymes, and lacking the gastric protease-producing glands (Govoni et al. 1986). Watanabe (1984) suggests that acidophilic granules found in this part of epithelium indicate active intracellular digestion necessary for protein assimilation, before the stomach development. The analysis of histological preparations indicates that the posterior intestine was the most active in absorption of macroparticles at that developmental stage, before the stomach development. The mucosal folds of posterior intestine were the longest, and the most differentiated, and enterocyte cytoplasm contained the most numerous supranuclear vacuoles with acidophilic granules, that disappeared after full stomach development. The presence of supranuclear vacuoles indicates lipid storage (De Silva and Anderson 1995; Fontagné et al. 1998; Crespo et al. 2001). Lipids are hydrolyzed in the intestine to fatty acids and monoglycerides, and then absorbed. Then they are resynthesised in the smooth endoplasmic reticulum, and stored as fat droplets in enterocytes (De Silva and Anderson 1995). According to Zambonino Infante and Cahu (2001), the anterior intestine is a main site of extracellular proteolytic digestion during the larval period, due to its alkaline pH, and pancreatic trypsin presence.

The presence of brush border on the pike-perch mucosal surface indicates active epithelial transport (Bisbal and Bengston 1995). The larvae of some fish species show protein and lipid absorption immediately after first food uptake. That was observed in *Solea solea* (Boulhic and Gabaudan 1992), *Acipenser beari* (Gisbert et al. 1999), and in *Clarias gariepinus, Coregonus lavaretus* and *Scophthalmus maximus* (Segner et al. 1993). According to Cousin and Baudin-Laurencin (1986) protein absorption in *Scophthalmus maximus* larvae started on the first day of active feeding, while, absorption of lipids after the 5-6 days.

The period from the onset of exogenous feeding to first symptoms of nutrient absorption was much longer in *Melanogrammus aeglefinnus* (Hamlin et al. 2000). In this species, the mouth opened on the third day post hatching, and the fish started active feeding but absorption was observed 16 days later. The pike-perch larvae were able to pinocytose of exogenous food before the full yolk sac resorption. According to Domeneghini et al. (1998), the ability of pinocytotic protein absorption and digestion is a primitive way of food digestion. This process results in optimum utilization of dietary protein in both, agastric and gastric fishes (Sire and Vernier 1992). It

is particularly important for the vertebrates that need essential amino-acids, e.g. for muscle development (Sire and Vernier 1992; Rowlerson et al. 1995).

In the older pike-perch larvae, on the 11 dph, goblet cells appeared among the enterocytes of the anterior and posterior intestine. Similar cells were found by Domeneghini et al. (1998) in *Sparus aurata* L., and identified as basic secretory cells. They synthesize neutral and sulfate mucins, and sialomucins containing sialic acid substituted in C8. According to the same authors, carbohydrate compounds are the main component of intestinal mucus in vertebrates. Grau et al. (1992) reported that neutral mucous compounds of the intestine participate in enzymatic food digestion, formation of food mass, and absorption. In fish and in mammals, intestinal mucus plays an important protective role (Domeneghini et al., 1998). In pike-perch juveniles, after metamorphosis, mucous cells were very abundant in entire intestine, but their number was different in various sections. They were the most numerous in the posterior intestine. According to Domeneghini et al. (1998), high density of goblet cells in the posterior intestine is essential for easy defecation.

Similarly as in the other vertebrates, liver and pancreas of pike-perch are of endodermal origin. The germs of these glands appear at hatching as undifferentiated embryonic cells, between the anterior intestine and the yolk sac. First symptoms of hepatic and pancreatic activity were observed between the 6 and 7 day post hatching. Similar observations concerning the activity of these glands were reported by Boulhic and Gabaudan (1992) in *Solea solea* L. organogenesis. According to Diaz and Connes (1991), glycogen appears at the same time as hepatocyte differentiation, and its storage starts when the animal still relies on maternal nutrition (mammals) or yolk nutrients (birds, fish). Increased pancreatic activity in the pike-perch at the beginning of exogenous feeding indicates an important role of pancreatic secretory products before the stomach development (Ribeiro et al. 1999; Zambonino Infante and Cahu 2001).

Histological analysis showed that the development of pike-perch digestive system: mouth opening, connecting of esophagus with the intestine, and liver and pancreas development allowed for uptake, digestion and absorption of first exogenous food on the 5-7 day of larval life. Histological and histochemical properties of the exocrine pancreas showed secretion of digestive enzymes into the intestine. Comparison of the results of the present study with the observations of liver and pancreas development in *Salmo gairdneri* (Vernier and Sire 1976), *Dicentrarchus labrax* (Diaz and Connes 1991), and *Solea solea* (Boulhic and Gabaudan 1992) revealed that development of these glands during the mixed feeding period in all species was similar. The presence of bile duct connecting the liver with anterior intestine at this developmental stage indicates hepatic activity and bile production. Lipid storage in the pike-perch hepatocytes about the 7 dph was a result of active feeding and successful food digestion which was suggested also by Diaz and Connes (1991) who obtained similar observations for *Artemia*-fed *Dicentrarchus labrax* larvae. During exogenous feeding, after a complete yolk sac resorption, histological properties of pike-perch hepatocytes changed. These changes included development of hepatic lobules arranged around the sinuses. Increased blood vessel density, and aggregation of hepatocytes around them was observed also by Hamlin et al. (2000) in *Melanogrammus aeglefinnus*. At that time, pancreas was located among the liver, intestine and stomach, and was dispersed among the pyloric caeca. At the end of the experiment, the exocrine pancreatic tissue was observed around the hepatic portal veins. According to Boulhic and Gabaudan (1992), the available commercial feeds not always meet nutritional requirements of newly hatched fish, and it is more reasonable to feed larvae natural food containing own digestive enzymes that

facilitate food assimilation by the enterocytes. Histological and biochemical analyses of digestive tracts of three fish species of different ecological position revealed that *Clarias gariepinus* and *Scophthalmus maximus* need a stomach for digestion and absorption of artificial feeds, while *Coregonus lavaretus* may take up and successfully digest feed without developed stomach (Segner et al. 1993).

Development of stomach, gastric glands, and pyloric caeca in the pike-perch larvae took place between the 15 and 30 day of life. The results of present study show that the pike-perch stomach is Y-shaped and consists of the cardia, pyloric stomach, and blind sac. These parts were identified according to the terminology developed by Harder (1975), basing on the differences in histological and histochemical properties.

Fish species showing the Y-shaped stomach with the blind sac belong, among others, to the *Engraulidae*, *Gadidae*, *Clupeidae*, and *Percidae* families (Harder 1975).

The gastric glands appeared in the pike-perch larvae about the 15-20 day post hatching, earlier comparing to the other fish species such as *Solea solea* (the 22 dph, Boulhic and Gabaudan 1992) but later than in *Scophthalmus maximus* (Cousin and Baudin-Laurencin 1986) and *Acipenser baeri* (Gisbert et al. 1999). The gastric glands in pike-perch occur in both cardia and blind sac. In *Paralabrax maculatofasciatus* larvae they were found only in the anterior part of the stomach (Perña et al. 2003). However, the development of gastric glands is not necessarily accompanied by the onset on stomach activity. In *Coregonus lavaretus* larvae, pepsin activity and acidity were observed after a long time from the development of the gastric glands (Mähr et al. 1983). Similarity, the pike-perch gastric glands contained eosinophilic particles indicating pepsinogen presence, thus digestive activity of the stomach after 10 days from the development of the gastric glands. The presence of neutral mucins in the gastric epithelium also suggested activity of gastric glands. Neutral mucins protect the epithelium against self-digestion by hydrochloric acid, and digestive enzymes produced by the gastric glands (Gisbert et al. 1999). Secretion of neutral mucins was observed in the cardia of adult *Sparus aurata* (Domeneghini et al. 1998) and larvae of *Acipenser baeri* and *Paralichthys californicus* (Gisbert et al. 1999, Gisbert et al. 2004).

Development of pyloric caeca is the last of important morphological changes in the digestive tract showing that the fish attained the juvenile stage (Bisbal and Bengston 1995). According to Balon (1975), metamorphosis is completed and fish reach the juvenile stage when they show all fins distinct and well developed. This definition is based on external morphological features, and does not take into consideration internal changes. But external morphological development is not always simultaneous with internal changes. According to Tanaka (1971) and Stroband and Dabrowski (1981), the juvenile stage begins when the gastric glands develop, stomach shows digestive activity, and pyloric caeca appear. Some fish species easily utilize artificial feed before the gastric gland development, e.g. *Coregoninae*, *Salmo gairdneri*, *Morone saxatilis*, while other ones show difficulties in feed digestion and assimilation, e.g. *Solea solea* (Boulhic and Gabaudan 1992). Therefore, the choice of appropriate food, meeting nutritional requirements of each developmental stage is one of the most important issues of intensive fish culture (Yúfera et al. 1996; Fontagné et al. 1998; Navarro and Sarasquete 1998).

The results of histological and histochemical analyses proved that in the pike-perch digestive system was developed before the complete metamorphosis, since the alimentary tract was distinctly divided into esophagus, anterior and posterior intestine, and food particles were assimilated. Also the liver and pancreas were active, and the gall bladder showed bile storage. There were no anatomical

differences between the larval and juvenile stages, except for the gastric gland and pyloric caeca presence, and intestine length.

Both personal observations and literature data (Ruuhijärvi et al. 1991; Schlumberger and Proteau 1991; Mani - Ponset et al. 1994) suggest that despite such an advanced digestive system activity, feeding of pike-perch with artificial feeds is difficult before the fish attain the juvenile stage.

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