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Histological Aspects of the Early Development of the Digestive System of Burbot *Lota lota* L. (Lotidae, Gadiformes)*

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The ontogeny of the digestive tract was studied histologically in burbot, *Lota lota* L., from hatching to 42 days post-hatch (dph). At hatching, the digestive tract consisted of a straight tube with discernible digestive accessory glands (the liver and the pancreas) dorsally attached to the yolk sac. Most of the yolk sac reserves were consumed during the first 12 days and were completely depleted by 17 dph. The first PAS-positive goblet cells appeared at 6 dph, dispersed within the epithelium of the oesophagus and increasing substantially in number and distribution as development progressed. At 12 dph, the first vacuoles (neutral lipids) appeared in the intestine, indicating the functional absorption of nutrients from food. Differentiation of gastric glands was first noticed at 17 dph and was extensive by 27 dph. *L. lota* larvae have a morphologically complete digestive tract by 32 dph. These findings on the development of the digestive system in *L. lota* may contribute to a better understanding of its ontogeny and can be useful for improvement of the larval rearing techniques of this promising species for freshwater aquaculture diversification.

Key words: Fish larvae, ontogeny, liver, pancreas, stomach.

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Burbot *Lota lota* L. 1758 is the only freshwater representative of the hake and burbot family (Lotidae) found in the nearly entire Holarctic. This benthic and nocturnal fish prefers low water temperatures. Populations of this species have been steadily diminishing across its range (STAPANIAN *et al*. 2010). Burbot is both a highly valuable fish species and a promising candidate for intensive coldwater aquaculture (TRABELSI *et al*. 2011). Several attempts have been undertaken since the 1990s to restore natural *L. lota* populations, e.g. efforts to produce fry using live food (HARZEVILI *et al*. 2004).

Information on larval organogenesis of particular fish species is crucial for a better understanding of early growth and priorities during this stage. Research in this field also offers insights into fish biology and taxonomy (ZAMBONINO-INFANTE *et al*. 2008). It is commonly acknowledged that successful rearing of larval fish requires appropriate adjustment of culture conditions and feeding strategies to the ontogenetic status of larvae (SANTAMARÍA *et al*. 2004). Fish species which attract the attention of commercial producers have very small larvae that must be fed with live food for several weeks before they can be weaned onto dry feed. However, the age of weaning varies considerably between species and depends heavily on digestive tract development (ZAMBONINO-INFANTE *et al*. 2008). The digestive tract of fish larvae is morpho-

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logically, histologically and physiologically less elaborate than that of the adult fish. Development of the digestive tract from a simple, undifferentiated and straight incipient gut of the yolk-sac larva to a complex, segmented digestive tract of the adult proceeds through periodic and rapid changes rather than by continuous gradation (GOVONI *et al*. 1986).

Few reports have been published on larval culture of burbot (e.g. ŻARSKI *et al*. 2009; FURGAŁA-SELEZNIOW *et al*. 2014). Moreover, there are virtually no studies focusing on the biological aspects of the early development of burbot larvae and histological analyses of digestive tract development are rather limited and incomplete (VANHEULE 2012; PALIŃSKA-ŻARSKA *et al*. 2014a). Hence, the objectives of the present study were to describe the larval development of the digestive tract and accessory glands of *L. lota* as well as to determine the timing of organ differentiation. These findings would be useful for improvement of the larval rearing techniques of this promising species for freshwater aquaculture diversification.

Material and Methods

Larvae were obtained by artificial spawning of fish originating from the Odra River (north-western Poland). Eggs obtained from three females of an average weight of 720 ± 108 g (mean ± SD) were fertilized with semen from three males of an average weight of 332 ± 79 g. For the first month, the eggs were incubated in Weiss jars, at 3-4°C. Afterwards, the water temperature was kept at 6°C until hatching. As soon as the first hatched larvae were observed (day 32 of incubation), the eggs were transferred to water at 10°C in order to synchronize the hatching (ŻARSKI *et al*. 2009; KUPREN *et al*. 2014).

After hatching, the larvae were moved to a semi-closed laboratory recirculating system, where they were stocked in two 25 dm^3 aquaria. The initial stocking density of larvae was 60 ind. dm^{-3} as suggested for experimental rearing by HARZEVILI *et al*. (2004) and KUPREN *et al*. (2014). The system was equipped with biological filtration, aeration and automatic temperature regulation (± 0.1°C). For the first week of the study, the water temperature in the tanks was 10°C. Following swim bladder inflation (from 8 days post-hatch; dph), the water temperature was gradually increased from 10 to 16°C over 3 days, and maintained until the end of the experiment. Such a thermal regime has been recommended as optimal for burbot larvae (WOLNICKI *et al*. 2002; HARZEVILI *et al*. 2004). Specimens were exposed to a 12 L:12 D photoperiod. The ammonia content during the whole rearing period was below 0.01 mg dm^{-3} and dissolved oxygen saturation was always above 80%. The larvae were fed three times a day *ad libitum* with freshly hatched *Artemia* sp. nauplii (San Francisco origin – SFO, Argent USA) from 8 dph, which coincided with swim bladder inflation, until the end of the experiment. The rearing tanks were cleaned every morning. A random sample of 20 larvae from each tank was collected every other day from hatching to 12 dph and every five days from 12 to 42 dph. They were submitted to growth analysis and 10 specimens were taken for further histological studies.

The sampled larvae were anesthetized with tricaine methanesulfonate (MS-222, Sigma, dose of 150 mg dm^{-3}), while those for histological studies were sacrificed with an overdose of the anaesthetic. Digital photographs of each specimen were taken using Jenoptic ProgRes® 3 and processed using the ProgRes® CapturePro 2.5 software (Jenoptic, Germany). In each digital photograph, the total body length (TL) was measured to the nearest 0.01 mm. After taking photographs, the weight of larvae was determined to the nearest 0.1 mg (±0.0001 g) with an analytical microbalance KERN ALJ 220-4M. By day 17, the larvae were gently drained on filter paper and weighed three times (10 fish at a time) and the mean wet weight of a single specimen was then calculated. From 22 dph, fish were individually weighed. Afterwards, they were fixed in Bouin solution, dehydrated in graded ethanol, embedded in paraffin and cut into serial cross- and sagittal sections (5-8 μm thick) with a Leica RM2265 microtome. Sections were stained according to the Mayers' Haematoxylin and Eosin (HE) procedure for general histomorphological observations, while periodic acid-Schiff (PAS) was used to detect neutral glycoconjugates in mucous cells and glycogen deposits in the liver. Histological sections were photographed with a Leica DFC420 digital camera (Leica Microsystems Switzerland Ltd.) coupled to a Leica DM 2500 microscope and processed using software designated by the manufacturer (Leica Application Suite v 3.5.0).

Results

Larval growth

The larvae showed exponential growth for wet weight and total length from the beginning of exogenous feeding at 8 dph (TL: 4.38 ± 0.14 mm) until the end of the study (42 dph; TL: 23.96 ± 2.41 mm) (Fig. 1). The shape of both curves is defined most precisely by an exponential function with a natural logarithm base. The curve for body length growth was defined by the equation $TL = 2.4059e^{0,1787*dph}$ (R^2=0.91), while the individual body gain relies on the equation $TW = 0.082e^{0,5651*dph}$ (R^2=0.85).

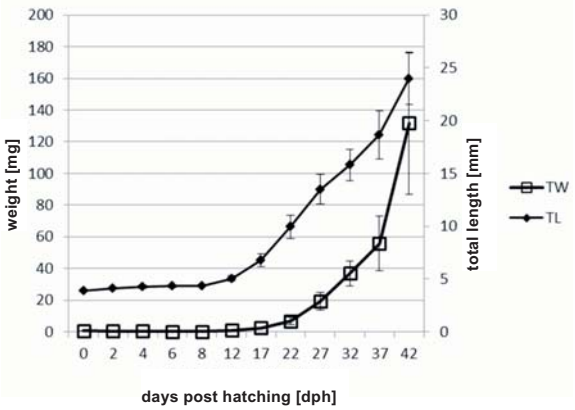

Fig. 1. Growth in weight (left scale) and total length (right scale) of *Lota lota* larvae from hatching until 42 days post-hatch (dph). Each point represents the mean of forty measures ± SD.

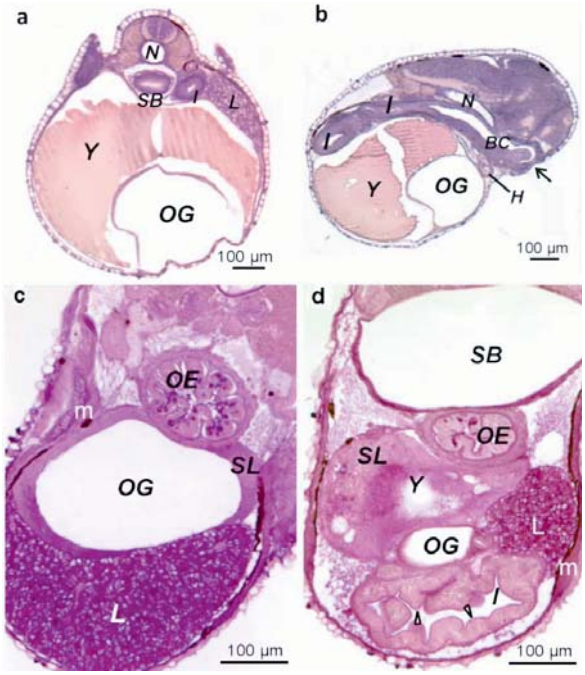

Fig. 2. Histological section of *Lota lota* larvae in different stages of depletion of the yolk sac. Yolk-sac larvae (2 dph) (a) cross- and (b) longitudinal section, buccopharyngeal cavity still closed (arrow) (HE staining). (c) cross- section by anterior and (d) posterior part of the body of 12-day old larva with remnants of the yolk and oil globule; PAS-positive brush border marked by arrowheads (PAS staining). BC – buccopharyngeal cavity; H – heart; I – intestine; L – liver; m – melanin pigment granules; N – notochord; OE – oesophagus; OG – oil globule; SB – swim bladder; SL – syncytial layer; Y – yolk.

Yolk sac

During the endotrophic stage, after hatching (0-2 dph; TL: 3.92 ± 0.09 mm – 4.12 ± 0.11 mm), yolk-sac larvae had a large, ovoid, homogenous yolk sac exhibiting an acidophilic matrix (eosin/HE affinity) (Fig. 2a, b). Immersed in the yolk sac, a cephalically located empty space (in the anterior lower part of the yolk sac, close to the heart) could be observed. This structure was the oil globule containing lipids, thus it was dissolved during the paraffin embedding process (Fig. 2). The yolk and oil globule were surrounded by a syncytial layer of squamous cells called the periblast. Depletion of the yolk was visible as soon as day 2. At 2 dph, the digestive tract appeared as a tube dorsal to the yolk sac, with a smooth lumen (primordial intestine) and closed mouth (Fig. 2b) and closed anus. Between 3-4 dph, the mouth and the anus opened and the endo-exotrophic period started. Most of the yolk sac reserves were absorbed between 6 and 12 dph (TL: 4.32 ± 0.12 and 5.06 ± 0.26 mm respectively). At 6 dph, the yolk and oil globule were still visible. At 12 dph, the yolk matrix and oil globule were restricted to the anterior region of the abdominal cavity, surrounded by the liver. Remnants of the yolk sac were surrounded by the thick yolk syncytial layer (Fig. 2c, d). Complete resorption of the endogenous reserves (the end of the endo-exotrophic stage) occurred between 12 and 17 dph, concurrently with oil globule exhaustion. Therefore, both structures were absent in larvae from 17 dph onwards (TL: 6.81 ± 0.61 mm).

Oesophagus

The oesophagus is located directly posterior to the pharynx. It connects the pharyngeal cavity with the anterior intestine. In the yolk-sac larvae, there was a short and narrow oesophageal lumen, around which the epithelial cells divided to form a stratified epithelium. Unlike the intestinal epithelial cells, the oesophageal epithelial cells of yolk-sac larvae created an irregular border. At 2 dph, the oesophagus was a straight tube with a smooth ellipsoidal lumen, pushed to the left side by the developing swim bladder (Fig. 2a). By day 4 (TL: 4.27 ± 0.15 mm), longitudinal folds could be seen along the length of the oesophagus, which gradually became more and more prominent (Fig. 3). As the fish grew, the oesophagus elongated and the circular and longitudinal layers of striated muscle surrounding the oesophageal wall thickened (Fig. 3). No goblet cells were present until day 6. At 6 dph, these PAS-positive cells were noticed sporadically. From day 12 onwards, they proliferated rapidly (Fig. 3b, c) and were numerous by 22 dph (TL: 9.95 ± 1.08 mm). From 32 dph (TL: 15.08 ± 1.47 mm), the epithelium lining this region of the digestive tract became completely covered by goblet cells. Their number was the highest just posterior to the pharynx, but they were absent at the transition of the oesophagus in the stomach. From day 22 on-

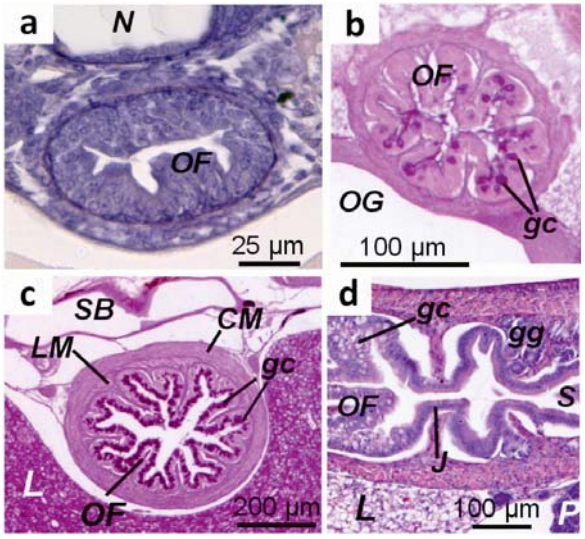

Fig. 3. Ontogeny of the oesophagus in *Lota lota* larvae. (a) 4-day-old larva, note emerging longitudinal oesophagus folds (HE staining). (b) 12-day-old larva, PAS-positive goblet cells appeared (PAS staining). (c) 37 day-old-larva, thick layers of longitudinal and circular muscle are clearly discernible, the epithelium lining the oesophagus is completely covered by goblet cells (PAS staining). (d) 32-day old larva, the junction between the oesophagus and the stomach (HE staining). CM – circular muscle layer; gc – goblet cells; gg – gastric glands; J – junction; L – liver; LM – longitudinal muscle layer; N – notochord; OF – oesophagus folds; OG – oil globule; P – pancreas; S – stomach; SB – swim bladder.

wards, a constriction between oesophagus and stomach was clearly visible in the longitudinal sections (Fig. 3d).

Stomach

At 12 dph, the stomach anlage in burbot existed as an extension of the oesophagus. This sack-like structure had a mucosa consisting of columnar epithelium, a thick, muscular wall and neither goblet cells nor gastric glands were present (Fig. 4a). Between 17 and 22 dph (Fig. 4b), the primordial stomach started to bend, assuming the shape of a tilted letter "v" at around 27 dph (TL: 13.49 ± 1.4 mm). The first developing gastric glands in the mucosa appeared by day 17, at the beginning of the exotrophic stage (Fig. 5a). During the next days of rearing, the gastric glands proliferated actively as clusters of cuboidal cells surrounded by a thin layer of connective tissue (Figs 4b and 5b). From 17 dph, PAS-positive granules appeared in the apical part of the gastric epithelium. The production of the first PAS-positive granules started in larvae at the same time as the first gastric glands appeared. Two weeks later, the stomach's lumen was completely coated by the mucus (Fig. 5b). Meanwhile, no PAS-positive secretor granules were present in the gastric glands. By day 22, the stomach grew in size and two different regions were clearly distinguishable: glandular and non-glandular regions (Fig. 4b). Between 22 and 27 dph, the glandular region of the stomach developed substantially and abundant gastric glands were arranged along numerous longitudinal folds. No goblet cells could be observed in the stomach epithelium, although they occurred in the epithelia of the oesophagus and intestine. The muscularis propria composed of two smooth-muscle layers (outer longitudinal and inner circular) was thin in the glandular portion of the stomach. The inner layer of circular musculature became very thick in the non-glandular portion. At 12 dph, numerous non-staining epithelial vacuoles appeared in the transitional region between the primordial stomach and the intestine. They persisted until the end of this study (Fig. 5c, d). Parallel to the differentiation of the stomach, the pyloric sphincter, which separates the non-glandular stomach from the anterior intestine, evolved and was well-distinguishable on cross-sections starting at 27 dph. Its inner side was lined with highly vacuolated epithelium. The apical cytoplasm of the stomach and the pyloric

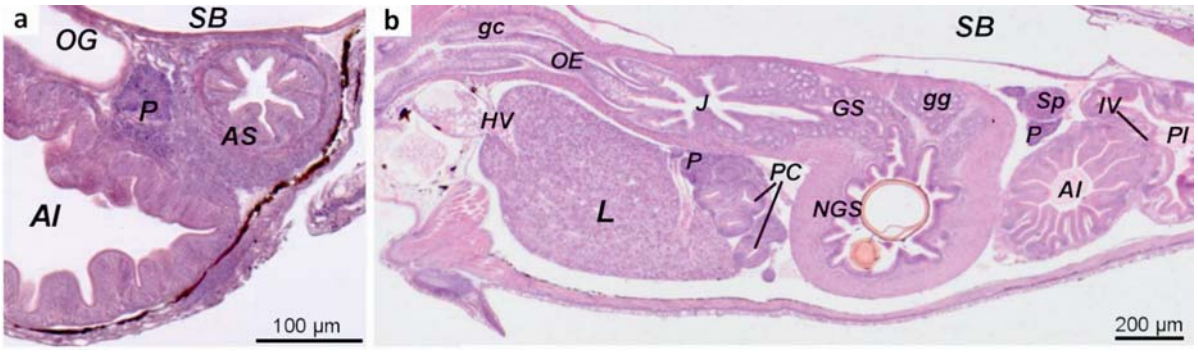

Fig. 4. Morphology of the stomach of *Lota lota* larvae (HE staining). (a) Cross-section of 12 dph larva, note thick-walled anlage of stomach. (b) Longitudinal section of the digestive tract at 22 dph, note two stomach regions – glandular and non-glandular. AI – anterior intestine; AS – anlage of stomach; gg – gastric glands; gc – goblet cells; GS – glandular stomach; HV – hepatic vein; IV – ileorectal valve; NGS – non-glandular stomach; J – the junction between the oesophagus (OE) and the glandular stomach (GS); L – liver; OG – remnants of the oil globule; P – pancreas; PC – pyloric caeca; PI – posterior intestine; SB – swim bladder; Sp – spleen.

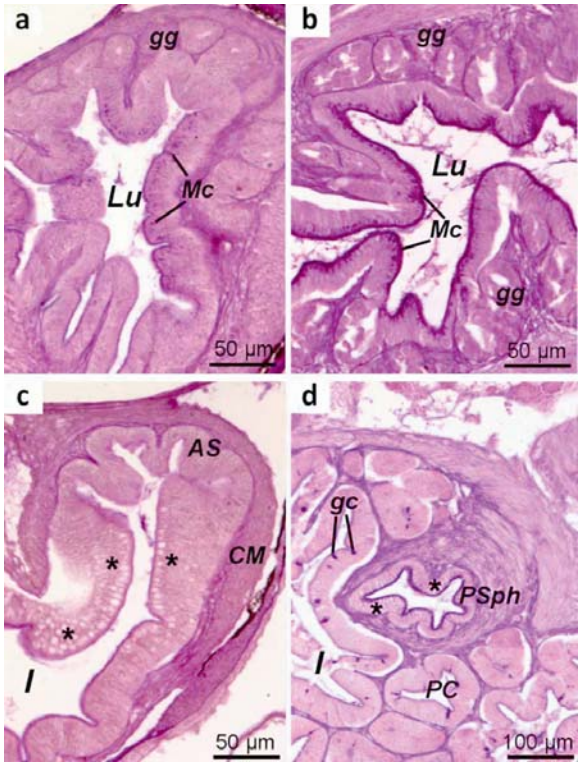

Fig. 5. Histological details of stomach development in *Lota lota* larvae (PAS staining). (a) Day 17 post-hatch, appearance of gastric glands and PAS-positive mucus in the apical part of the stomach mucosa. (b) Day 32 post-hatch, well-developed gastric glands, lumen of stomach coated by PAS-positive mucus. (c) 12 day post-hatch, note transitional region between stomach anlage and intestine coated by numerous non-staining epithelial vacuoles (*). (d) Pyloric sphincter of 42 day-old larva. (a,b,d) – cross-sections, (c) – longitudinal section. AS – stomach anlage; I – intestine; CM – circular muscle layer; gc – goblet cells; gg – gastric glands; Mc – mucus; Lu – stomach lumen; PC – pyloric caeca; PSph – pyloric sphincter.

sphincter epithelial cells were strongly PAS-positive from 32 dph, indicating the presence of neutral mucosubstances (Fig. 5b, d).

From 32 dph to the end of the study, the stomach increased in size and complexity by means of an increase in the size and number of mucosal folds in both glandular and non-glandular regions, as well as by an increase in gastric gland number. However, no relevant histological changes were observed.

Intestine and pyloric caeca

The intestine was the longest portion of the digestive tract and one of the first organs of the digestive system to differentiate in burbot larvae. By day 2, it appeared as a tube located over the yolk sac, lined by a simple columnar epithelium bordered by a layer of microvilli at the apical surface (Fig. 2b). The brush border was slightly PAS-positive just after hatching, whereas in older fish it was clearly PAS-positive (Fig. 2d). Some intensive proliferation of intestinal mucosa cells was observed (Fig. 6a). The enterocytes were arranged in a single layer and contained median to basally located nuclei. By day 6, both a contraction separating the middle intestine from the posterior intestine and the beginning of the first intestinal loop were noticeable.

During larval development, the intestinal folds, first seen at 4 dph, increased in complexity and length along the entire intestinal mucosa (Fig. 6b). However, the development of these folds was asymmetric with a higher abundance and depth in the middle intestine. Primordial pyloric caeca were observed at 17 dph (Fig. 6c) as small extensions of the anterior part of the intestine. By day 22, they were clearly discernible as long extensions of the anterior part of the intestine and were fully developed at the end of the experiment (Figs 4b and 5d). Pancreatic tissue was frequently present between the caecal projections.

At 12 dph, the ileorectal valve was clearly visible (Fig. 6b) and the posterior intestine terminated in a short rectal zone. The rectal epithelium of the larval *L. lota* was similar to the intestinal epithelium and became very thin toward the rectal area. The rectum was devoid of mucosal folds and microvilli were absent from its most caudal end (Fig. 6d).

No goblet cells were present in any of the intestine regions at the earliest stages (Fig. 2d). By day 22, the first PAS-positive goblet cells were detected in intestinal mucosa of the middle and posterior intestine. By day 27, they were more numerous and their number tended to increase slightly along with larval development. In general, goblet cells were less abundant in the intestine than in the oesophagus and their number was higher in the posterior part of the intestine. Few goblet cells were clearly visible in the pyloric caeca from day 32 and they became more frequent at the end of the experiment (Fig. 5d).

By day 12, empty supranuclear vesicles were detected in the intestine. Between 17 and 22 dph, a large increase in the number of these vacuoles was observed (Fig. 7). Eosinophilic supranuclear inclusion bodies in enterocytes of the posterior intestine were detected between 12 and 22 dph. With the exception of its length, size and number of mucosal folds, no significant histological changes were observed in the intestine from 27 dph until the end of the study.

Swim bladder

At 2 dph, the swim bladder was present as an evagination of the anterior intestine (Fig. 8a). The

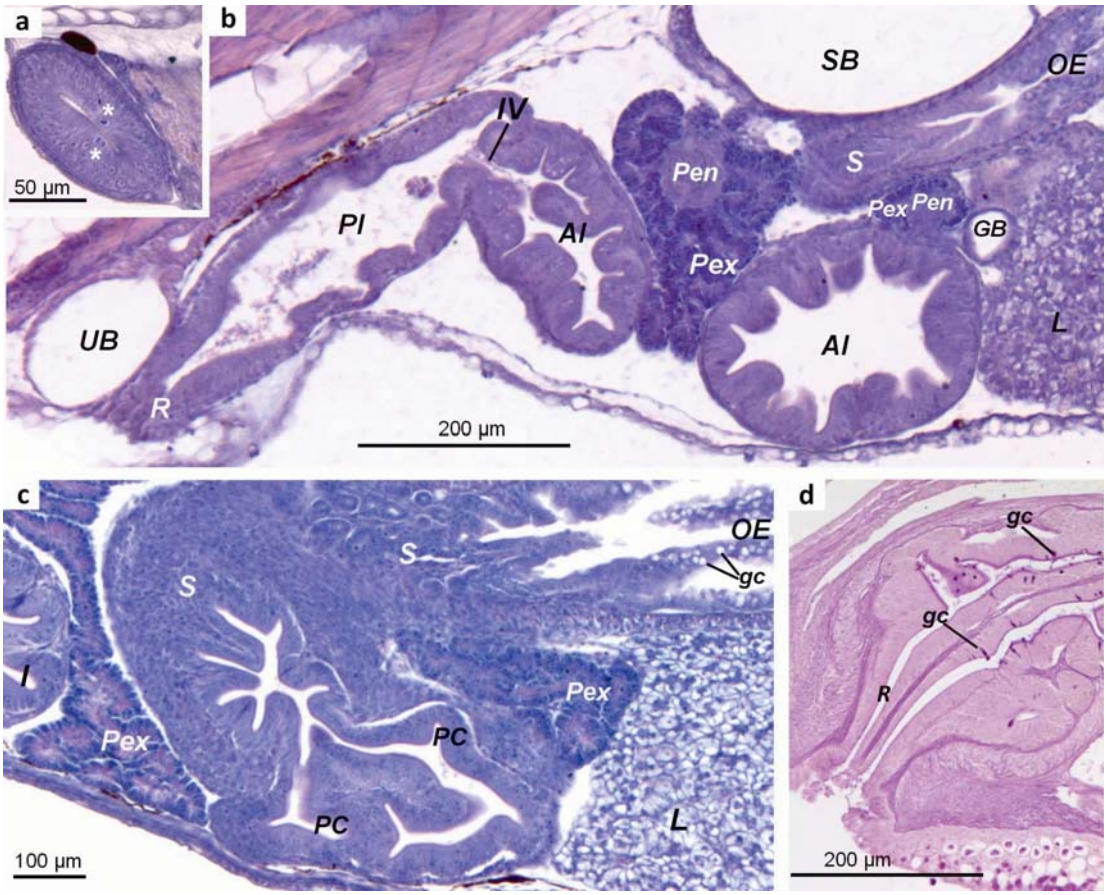

Fig. 6. Longitudinal sections of the digestive system in *Lota lota* larvae during ontogeny (HE staining). (a) Day 2 post-hatch, intensive proliferation of epithelial cells in primordial intestine (*). (b) Day 12 post-hatch, ileorectal valve and intestinal folds clearly distinguishable. (c) Day 17 post-hatch, appearance of primordial pyloric caeca. (d) Day 37 post-hatch, details of the rectal zone (PAS staining). AI – anterior intestine; GB – gall bladder; gc – goblet cells; IV – ileorectal valve; L – liver; OE – oesophagus; Pen – endocrine pancreas; Pex – exocrine pancreas; PC – pyloric caeca; PI – posterior intestine; R – rectum; S – stomach; SB – swim bladder; UB – urinary bladder.

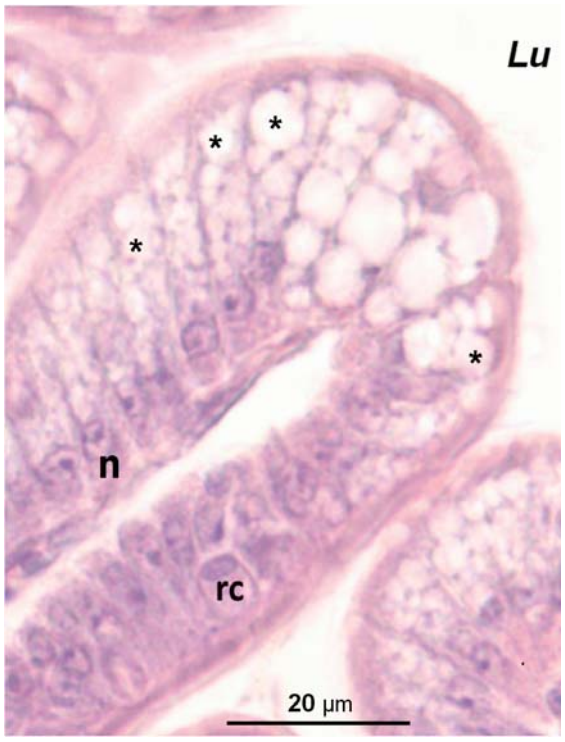

Fig. 7. Cross-section of the anterior intestinal fold at 22 dph (HE staining). Asterisks mark supranuclear vacuoles in enterocytes. Lu – intestinal lumen; n – enterocyte nuclei; rc – rodlet cells.

swim bladder chamber was lined by a cubic simple epithelial layer similar to the one in the gut. This structure possessed an irregular lumen connected to the digestive tube through a pneumatic duct entering the posterior end of the bladder cavity. The rete mirabile was present as a sparse network of capillaries among the connective tissue at the posterior ventral side of the swim bladder (Fig. 8b). About 12 dph, soon after inflation, the swim bladder started to become more oval in shape and its walls were flattened (Fig. 2d and 8c). The whole organ was completely developed by about 17 dph, being similar to that of an adult specimen. The pneumatic duct was not observed after 27 dph.

Gall bladder

By 2 dph, the gall bladder anlage in *L. lota* yolk-sac larvae was evident and the choledochus connected to the intestine (Fig. 9). At that time, it was lying on the left side of the fish, close to the anterior part of the intestine, the yolk sac and the liver. This organ was completely developed by 17 dph, when it adopted its typical pear shape. The epithelial cells of the gallbladder were cuboidal to squa-

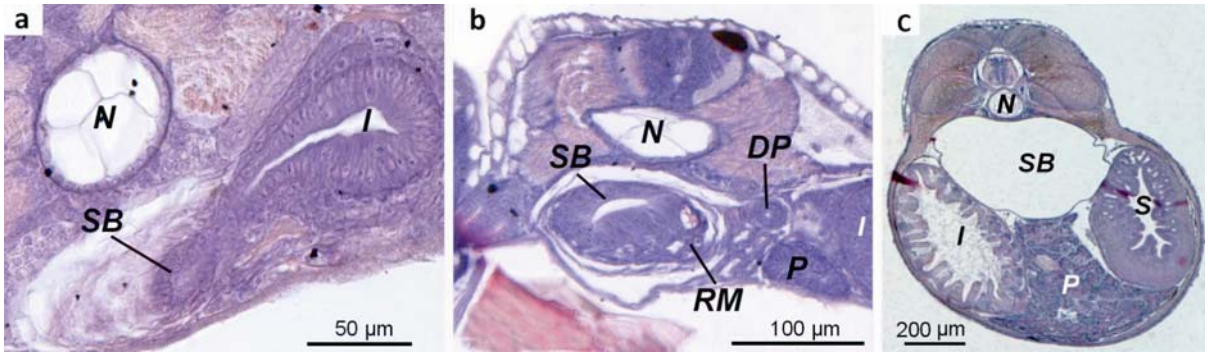

Fig. 8. Cross-sections of the swim bladder (HE staining). (a) Swim bladder as an evagination of the anterior intestine (2-dph). (b) Details of the swim bladder anlage, note rete mirabile and pneumatic duct (2 dph). (c) The inflated swim bladder of the 22-day-old larva. DP – pneumatic duct; I – intestine; N – notochord; P – pancreas; RM – rete mirabile; S – stomach; SB – swim bladder.

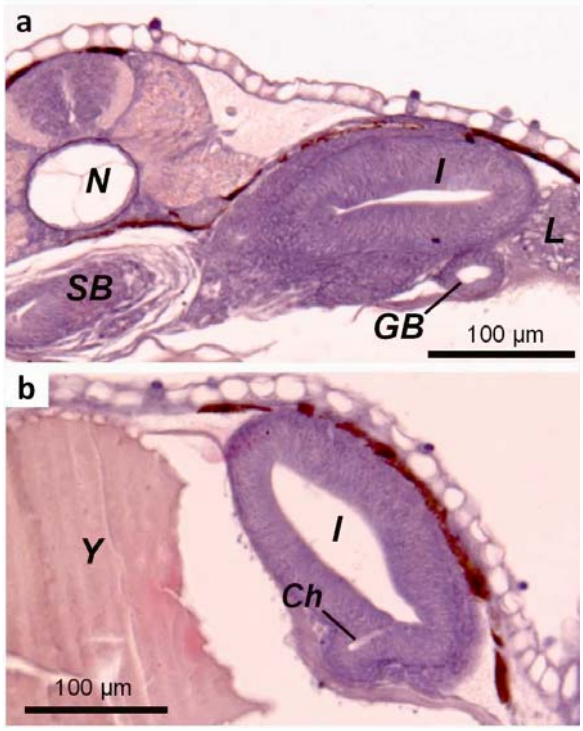

Fig. 9. Cross-histological sections of the gall bladder of *Lota lota* (2 dph) (HE staining). (a) The gall bladder anlage lying between intestine and liver and (b) choledochus connecting gall bladder to the intestine. Ch – choledochus; GB – gall bladder; I – intestine; L – liver; N – notochord; SB – swim bladder; Y – yolk.

mous, and the cells appeared stretched and thin when the bladder was distended. The simple epithelium was surrounded by a layer of connective tissue with smooth muscle cells present in between the collagen fibers. During the exotrophic stage of fish development, the gallbladder is frequently surrounded by hepatic tissue and has a wide lumen. Bile flows to the intestine via a common bile duct (Fig. 9b) which is also lined by a layer of cuboidal or columnar cells.

Liver

The liver was clearly distinguishable as early as the yolk-sac stage (2 dph). Morphologically, it was a small, triangular, well-vascularized mass lying on the yolk sac on the left side of the fish (Figs 2a and 10a). Histologically, this accessory digestive gland appeared as a mass of polyhedral hepatocytes. By day 4, hepatocytes divided into distinct lobules and arranged themselves along sinusoids with visible blood cells (Fig. 10b). The lipid vacuolisation of hepatocytes started at 2 dph and by day 6 the vacuoles were evident in all the examined *L. lota* larvae (Fig. 10b, c). As the larvae grew, the liver continued to differentiate and the lipid vacuoles became more abundant and more prominent within the cytoplasm of hepatocytes. Glycogen

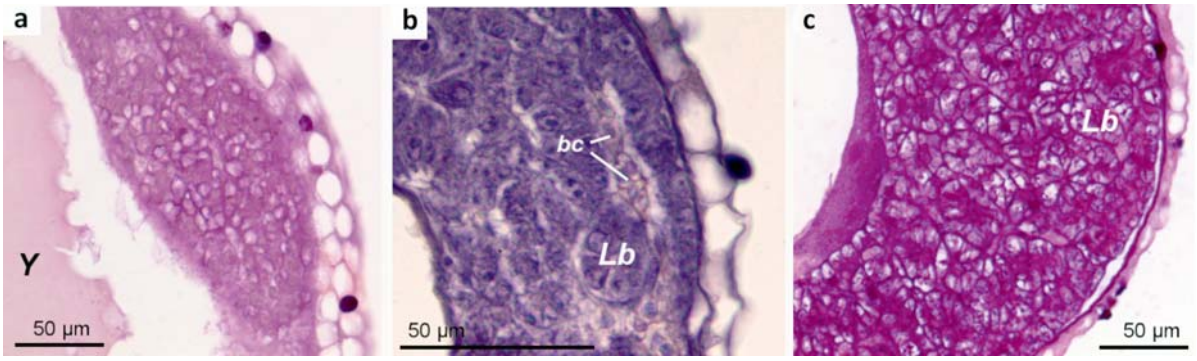

Fig. 10. Histological sections of the liver of *Lota lota* at different stages of development. (a) Hepatocytes with low amount of glycogen in 2-day-old larva (PAS staining). (b) Day 4 post-hatch, hepatocytes divided into distinct lobules, arranged along sinusoids with blood cells (HE staining). (c) Hepatocytes with intensive glycogen PAS positive staining (magenta purple) in 12-day-old larva. bc – blood cells; Lb – hepatic lobules; Y – yolk.

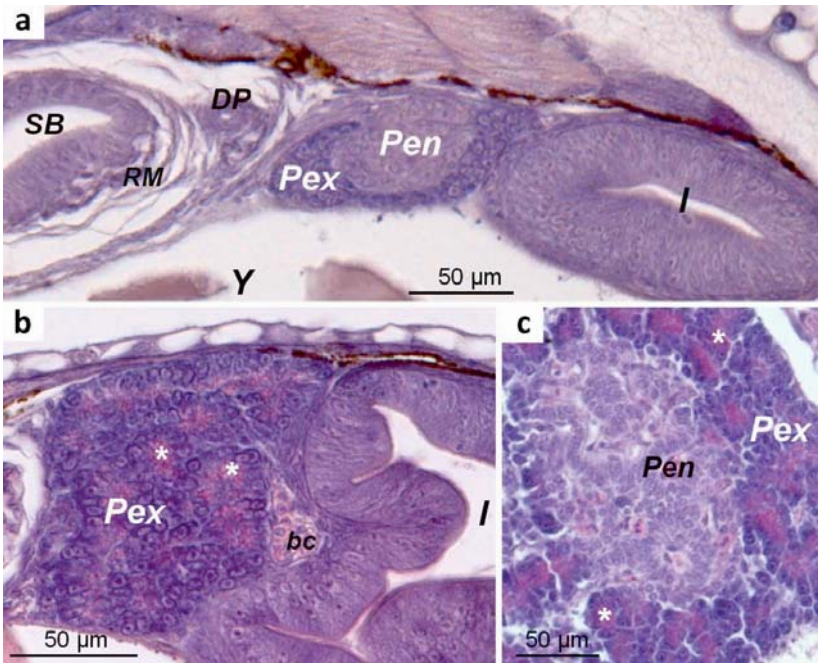

Fig. 11. Histological sections of the pancreas of *Lota lota* (HE staining). (a) Day 2 post-hatch, the pancreas lies between the swim bladder and the intestine, note the presence of two pancreatic regions. (b) Acidophilic zymogen granules (*) in the exocrine pancreas of 6-days-old larva. (c) Details of the endocrine pancreas (Langerhans' islet) at 27 dph. bc – blood cells; DP – pneumatic duct; I – intestine; Pen – endocrine pancreas; Pex – exocrine pancreas; RM – rete mirabile; SB – swim bladder; Y – yolk.

was identified after PAS staining as magenta-purple areas in the cytoplasm of hepatocytes (Fig. 10a, c). The liver progressively increased in size throughout larval development. During the exotrophic period, its most distinct features were the vacuolization of hepatocytes and the proliferation of sinusoids, which ended in the sinus venosus. The morphology of hepatic tissue was similar to that described above.

Pancreas

At 2 dph, the pancreas laid on the yolk sac, between the swim bladder and the intestine (Fig. 11a). Exocrine cells are highly basophilic and are the most abundant type of cells in the pancreas. At 2 dph, the exocrine pancreas was already differentiated into polyhedral basophilic cells forming rosette patterns, containing round-shaped eosinophilic zymogen granules. Zymogen granules became more numerous and strongly acidophilic as the larvae grew (Fig. 11b). The endocrine portion was present as a single islet in the anterior pancreas in the youngest fish (2dph) (Fig. 11a). From 12 dph until the end of experiment, two islets of Langerhans were observed. Endocrine cells had lighter cytoplasm than the surrounding exocrine cells (Fig. 11c).

As the fish grew, the pancreas extended posteriorly. By day 12, the bulk of the organ lay above the length of most of the intestine. By day 17, as the pyloric caeca appeared, pancreatic tissue could also be seen between them (Figs 4b and 6c). From 17 to 27 dph, the pancreas seemed to be very large (Fig. 8c) in comparison with the one in older *L. lota* larvae (especially those at the end of the experiment). The pancreatic duct was lined with squamous epithelium and opened into the dorsal part of the anterior intestine just after the bile duct.

Discussion

The present study provides complete information about the histological characteristics of the digestive system during burbot larval development (from hatching to 42 dph). As in other teleost fish species, three main stages of *L. lota* digestive tract organogenesis are distinguishable: endotrophic, endo-exotrophic and exotrophic (SANTAMARÍA *et al*. 2004; SÁNCHEZ-AMAYA *et al*. 2007; COMABELLA *et al*. 2013). The exotrophic stage could be divided into two phases: before the differentiation of gastric glands (exotrophic I) and with the presence of gastric glands in the stomach (exotrophic II). According to DABROWSKI (1984), larval fish fall into three groups in terms of the development and morphology of the alimentary tract and the extent of enzyme secretion in the gut. *L. lota* is a representative of the group in which larvae do not have a functional stomach or gastric glands during the larval stage, but develop digestive organs at metamorphosis. As in Atlantic cod (*Gadus morhua*) larvae, their digestive tract can be subdivided into

three parts: oesophagus and stomach (in later stages), an intestine and a short rectum (KAMISAKA & RONNESTAD 2011). Although the basic mechanisms of digestive tract development do not differ much among teleosts, the timing of developmental events and the duration of the above stages are significantly different (ZAMBONINO-INFANTE *et al*. 2008).

The development of *L. lota* yolk-sac larvae was similar to that reported for other fish species, particularly from Gadiformes such as *G. morhua* (KAMISAKA & RONNESTAD 2011) and haddock *Melanogrammus aeglefinus* (HAMLIN *et al*. 2000). At the endotrophic stage, the digestive tract was an undifferentiated, narrow tube on the dorsal part of the yolk sac, without exterior communication, as reported in other species (HAMLIN *et al*. 2000; OSTASZEWSKA *et al*. 2003; KAMISAKA & RONNESTAD 2011). Accessory glands, the liver and pancreas, were clearly distinguishable. During this phase, yolk-sac larvae lay on the bottom and occasionally made rapid movements with the entire body to swim up from the bottom, and this behaviour corresponds with observations of KUPREN *et al*. (2014). Histological analysis revealed that the mouth of *L. lota* larvae was open at 3-4 dph, indicating the onset of the endo-exotrophic stage.

In *L. lota* larvae, differentiation of the digestive tract into the oesophagus, stomach anlage, and intestine occurred within 12 dph. The first intestinal loop appeared at 6 dph and increased foldings indicated improved functionality of the gut shortly after hatching. Similar to other species, e.g. *M. aeglefinus* (HAMLIN *et al*. 2000), the exogenous feeding of *L. lota* larvae starts before the complete resorption of the yolk sac at about 8-9 dph (PALIŃSKA-ŻARSKA *et al*. 2014b; present study), whilst remnants of the oil globule were present at 12 dph.

The oesophageal cells of yolk-sac larvae are of varying heights, which gives the early oesophagus a bumpy appearance. This feature has been thought to initiate fold formation, since oesophageal folds are seen early in larval development (HAMLIN *et al*. 2000; present study). PAS-positive mucus secreting cells (producing neutral glycoconjungates) developed in the oesophagus of *L. lota* larvae starting at day 6, which is much earlier than reported for other Gadiformes, such as *M. aeglefinus* (12 dph; HAMLIN *et al*. 2000) and *G. morhua* (32 dph; MORRISON 1993), but similar to *Silurus glanis* (5 dph; KOZARIĆ *et al*. 2008) or *A. tristoechus* (4 dph; COMABELLA *et al*. 2013). Intensive proliferation of these cells in *L. lota* started after the formation of the longitudinal folds at 12 dph. HERRERA *et al*. (2010) and QU *et al*. (2012) stated that the age at which goblet cells appear varies among species, but usually it is associated with the commencement of exogenous feeding, as in ide *Leuciscus idus*, wedge sole *Dicologlossa cuneata, A. tristoechus* and *L. lota* (OSTASZEWSKA *et al*. 2003; HERRERA *et al*. 2010; COMABELLA *et al*. 2013; present study). In different vertebrates including fish, these cells are involved in transport, absorption and protection processes of the gut (ZAMBONINO-INFANTE *et al*. 2008; COMABELLA *et al*. 2013). According to SCOCCO *et al*. (1998), oesophageal mucus in fish may show the same protective functions as saliva in mammals.

Gastric glands started to differentiate in *L. lota* at 17 dph, and the stomach was completely developed by 32 dph. In other gadiform species the appearance of gastric glands occurred slightly later. For example in *M. aeglefinus* and *G. morhua*, gastric glands developed at 33 and 37-39 days after hatching, respectively (MORRISON 1993; HAMLIN *et al*. 2000; KAMISAKA & RONNESTAD 2011). In some species of catfish this occurred earlier (KOZARIĆ *et al*. 2008, PRADHAN *et al*. 2012).

According to HAMLIN *et al*. (2000), the presence of gastric glands indicates the beginning of chemical digestion processes. On the other hand, the results obtained by VEGA-ORELLANA *et al*. (2006) suggest that stomach morphology is not necessarily indicative of its functionality. Low activity of pepsin-like enzymes was detected as early as hatching in *G. morhua* and *M. aeglefinus*, but pepsinogen transcripts were detected by RT-PCR only in the oldest larvae sampled, a few days after the gastric glands were identified morphologically (PEREZ-CASANOVA *et al*. 2006).

The status of the *L. lota* stomach function could be determined by the contents of neutral mucosubstances in the gastric epithelial cells detected by the PAS reaction. The amount of PAS-positive mucus increased gradually since the first appearance of the gastric glands (17dph) to 32 dph. The same results were obtained in *P. auriga* (SÁNCHEZ-AMAYA *et al*. 2007), and in *S. glanis* (KOZARIĆ *et al*. 2008). Several authors indicate the involvement of mucous in protecting the mucosa from autodigestion by hydrochloric acid and gastric enzymes (ELBAL *et al*. 2004; GISBERT *et al*. 2004).

The present findings showed that the first unstained vacuoles began appearing in the absorptive cells of both the anterior and the posterior intestine on 12 dph, a few days after the onset of exogenous feeding. Lipid inclusions in the enterocytes of the anterior intestine have been reported within the first hours after onset of exogenous feeding in *Clarias gariepinus* (VERRETH *et al*. 1992) and *T. fulvidraco* (YANG *et al*. 2010), whereas in most freshwater and marine larvae such as *G. morhua* (KJØRSVIK *et al*. 1991), *M. aeglefinus* (HAMLIN *et al*. 2000), *Chondrostoma nasus* (SYSA *et al*. 2006) and *O. bimaculatus* (PRADHAN *et al*. 2012), they appeared a few days later. These vacuoles are

thought to be a lipid accumulation site following luminal digestion to fatty acids and monoglycerides, transport into the enterocytes and subsequent resynthesis within the cell (STROBAND & DABROWSKI 1981; GOVONI et al. 1986). An important increase in the intestinal mucosa lipid deposition between 17 and 22 dph were observed during *L. lota* larval development, as in *O. bimaculatus* (PRADHAN *et al*. 2012). According to PRADHAN *et al*. (2012), these changes in *O. bimaculatus* seemed to be due to the nature of the dietary lipids (*Artemia nauplii* vs. natural zooplankton). In cod larvae, lipid inclusions were most frequent in the midgut and the frequency of digestive vacuoles gradually increased with time (KJØRSVIK *et al*. 1991).

The posterior part of the intestine appears to be most active in the absorption of macronutrients in this study. No vacuoles were found in the rectum of *L. lota* in contrast to most other histologically studied fish. KJØRSVIK *et al*. (1991) claim that fish larvae without a functional stomach are known to have immature digestion mechanisms. Pinocytosis has been suggested as an alternative pathway for digesting proteins in teleost larvae as their enzymatic digestive system was poorly developed (SARASQUETE *et al*. 1995). Protein absorption vacuoles in the posterior intestine have been described in both gastric and agastric fish (HAMLIN *et al*. 2000; OSTASZEWSKA *et al*. 2003; SANTAMARÍA *et al*. 2004; SYSA *et al*. 2006; HERRERA *et al*. 2010; YANG *et al*. 2010). In some teleosts, they are present only until the formation of the gastric glands, suggesting a transient mechanism for protein digestion, related to the immaturity of the digestive tract.

The acinar structure of the pancreatic tissue just after hatching and the early presence of zymogen granules in *L. lota* is similar to other Gadiformes species such as *G. morhua* and *M. aeglefinus* (MORRISON 1993; HAMLIN *et al.* 2000). In contrast, zymogen granules were not detected until the transition to exogenous feeding in *Sander lucioperca* (OSTASZEWSKA 2005), *C. nasus* (SYSA *et al.* 2006), *S. glanis* (KOZARIĆ *et al.* 2008) and *T. fulvidraco* (YANG *et al.* 2010). The relatively large size of the pancreas in *L. lota* larvae between 17-27 dph may indicate high activity of this gland while the gastric glands have only started their development and activity.

In conclusion, the histological findings on the development of the digestive system in *L. lota* could lead to a better understanding of the digestive physiology of this species. Future research should focus on the ontogeny of enzymatic secretions to provide more details about digestive tract function. Such information might be useful for improving the current larval rearing practices for this species under aquaculture conditions.

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References

COMABELLA Y., HERNÁNDEZ FRANYUTTI A., HURTADO A., CANABAL J., GARCÍA-GALANO T. 2013. Ontogenetic development of the digestive tract in Cuban gar (*Atractosteus tristoechus*) larvae. Rev. Fish Biol. Fisheries **23**: 245-260.

DABROWSKI K. 1984. The feeding of fish larvae: present state of the art and perspectives. Reprod. Nut. Dev. **24**: 807-833.

ELBAL M.T., GARCÍA-HERNÁNDEZ M.P., LOZANO M.T., AGULLEIRO B. 2004. Development of the digestive tract of gilthead sea bream (*Sparus aurata* L.). Light and electron microscopic studies. Aquaculture **234**: 215-238.

FURGAŁA-SELEZNIOW G., SKRZYPCZAK A., KUCHARCZYK D., KUJAWA R., MAMCARZ A., ŻARSKI D., TARGOŃSKA K. 2014. Food selection of burbot (*Lota lota* L.) larvae reared in illuminated net cages in mesotrophic Lake Maróz (north-eastern Poland). Aquacult. Int. **22**: 41-52.

GISBERT E., PIEDRAHITA R., CONKLIN D.E. 2004. Ontogenetic development of the digestive system in California halibut (*Paralichthys californicus*) with notes on feeding practices. Aquaculture **232**: 455-470.

GOVONI J.J., BOEHLER G.W., WATANABE Y. 1986. The physiology of digestion in fish larvae. Environ. Biol. Fish. **16**: 59-77.

HAMLIN H., HURT I., KLING L.J. 2000. Histological and morphological evaluations of the digestive tract and associated organs of haddock throughout post-hatching ontogeny. J. Fish Biol. **57**: 716-732.

HARZEVILI S.A., DOOREMONT I., VUGHT I., AUWERX J., QUATAERT P., DE CHARLEROY D. 2004. First feeding of burbot, *Lota lota* (Gadidae, Teleostei) larvae under different temperature and light conditions. Aquacult. Res. **35**: 49-55.

HERRERA M., HACHERO-CRUZADO I., NARANJO A., MANCERA J.M. 2010. Organogenesis and histological development of the wedge sole *Dicologoglossa cuneata* M. larva with special reference to the digestive system. Rev. Fish Biol. Fish. **20**: 489-497.

KAMISAKA Y., RONNESTAD I. 2011. Reconstructed 3D models of digestive organs of developing Atlantic cod (*Gadus morhua*) larvae. Mar. Biol. **158**: 233-243.

KJØRSVIK E., VAN DER MEEREN T., KRYVI H., ARNFINNSON J., KVENSETH P.G. 1991. Early development of the digestive tract of cod larvae, *Gadus morhua* L., during start-feeding and starvation. J. Fish Biol. **38**: 1-15.

KOZARIĆ Z., KUZIR S., PETRINEC Z., GJURCEVIC E., BOZIC M. 2008. The development of the digestive tract in larval European catfish (*Silurus glanis* L.). Anat. Histol. Embryol. **37**: 141-146.

KUPREN K., TRĄBSKA I., ŻARSKI D., KREJSZEFF S., PALIŃSKA-ŻARSKA K., KUCHARCZYK D. 2014. Early development and allometric growth patterns in burbot *Lota lota* L. Aquacult. Int. **22**: 29-39.

MORRISON C.M. 1993. Histology of the Atlantic cod, *Gadus morhua*: an atlas. Part four: eleutheroembryo and larva. Can. Spec. Publ. Fish. Aquat. Sci. **119**: 496-499.

OSTASZEWSKA T. 2005. Developmental changes of digestive system structures in pike-perch (*Sander lucioperca* L.). Elect. J. Ichth. **2**: 65-78.

OSTASZEWSKA T., WEGNER A., WĘGIEL M. 2003. Development of the digestive tract of ide *Leuciscus idus* (L.) during the larval stage. Arch. Pol. Fish. **11**: 181-195.

PALIŃSKA-ŻARSKA K., ŻARSKI D., KREJSZEFF S., NOWOSAD J., BIŁAS M., TREJCHEL K., BRYLEWSKI A., TARGOŃSKA K., KUCHARCZYK D. 2014a. The effect of age, size and digestive tract development on burbot, *Lota lota* (L)., larvae weaning effectiveness. Aquacult. Nutr. **20**: 281-290.

PALIŃSKA-ŻARSKA K., ŻARSKI D., KREJSZEFF S., NOWOSAD J., BIŁAS M., TREJCHEL K., KUCHARCZYK D. 2014b. Dynamics of yolk sac and oil droplet utilization and behavioral aspects of swim bladder inflation in burbot, *Lota lota* L., larvae during the first days of life, under laboratory conditions. Aquacult. Int. **22**: 13-27.

PEREZ-CASANOVA J.C., MURRAY H.M., GALLANT J.W., ROSS N.W., DOUGLAS S.E., JOHNSON S.C. 2006. Development of the digestive capacity in larvae of haddock (*Melanogrammus aeglefinus*) and Atlantic cod (*Gadus morhua*). Aquaculture **251**: 377-401.

PRADHAN P.K., JENA J.K., MITRA G., SOOD N., GISBERT E. 2012. Ontogeny of the digestive tract in butter catfish *Ompok bimaculatus* (Bloch) larvae. Fish Physiol. Biochem. **38**: 1601-1617.

QU M., DING S., XU X., SHEN M., YOU Y., SU Y. 2012. Ontogenetic development of the digestive system and growth in coral trout (*Plectropomus leopardus*). Aquaculture **334-337**: 132-141.

SÁNCHEZ-AMAYA M.I., ORTIZ-DELGADO J.B., GARCÝA-LOPEZ A., CARDENAS S., SARASQUETE C. 2007. Larval ontogeny of red banded seabream *Pagrus auriga* (Valenciennes, 1843) with special reference to the digestive system. A histological and histochemical approach. Aquaculture **263**: 259-279.

SANTAMARÍA C.A., MARIN DE MATEO M., TRAVESET R., SALA R., GRAU A., PASTOR E., SARASQUETE C., CRESPO S. 2004. Larval organogenesis in common dentex, *Dentex dentex* L. (Sparidae): histological and histochemical aspects. Aquaculture **237**: 207-228.

SARASQUETE C., POLO A., YÚFERA M. 1995. Histology and histochemistry of the development of the digestive system of larval gilthead seabream, *Sparus aurata* L. Aquaculture **130**: 79-92.

SCOCCO P., ACCILI D., MENGHI G., CECCARELLI P. 1998. Unusual glycoconjugates in the oesophagus of a tilapine polyhybrid. J. Fish Biol. **53**: 39-48.

STAPANIAN M.A., PARAGAMIAN V.L., MADENJIAN C.P., JACKSON J.R., LAPPALAINEN J., EVENSON M.J., NEUFELD M.D. 2010. World-wide status of burbot and conservation measures. Fish Fish. **11**: 34-56.

STROBAND H.W.J., DABROWSKI K. 1981. Morphological and physiological aspects of the digestive system and feeding in fresh-water fish larvae. (In: Nutrition des poissons. M. Fontaine ed. C.N.R.S., Paris): 355-376.

SYSA P., OSTASZEWSKA T., OLEJNICZAK M. 2006. Development of digestive system and swim bladder of larval nase (*Chondrostoma nasus* L.). Aquacult. Nutr. **12**: 331-339.

TRABELSI A., GARDEUR J.N., TELETCHEA F., FONTAINE P. 2011. Effects of 12 factors on burbot *Lota lota* (L., 1758) weaning performances using fractional factorial design experiment. Aquaculture **316**: 104-110.

VANHEULE D. 2012. Rearing and weaning of burbot, investigation of a specific head deformity and histological development of the digestive tract. Dissertation, Faculty of Bioscience Engineering, Ghent University, Belgium.

VEGA-ORELLANA O.M., MACHADO FRACALOSSIA D., KIYOKO SUGAI J. 2006. Dourado (*Salminus brasiliensis*) larviculture: weaning and ontogenetic development of digestive proteinases. Aquaculture **252**: 484-493.

VERRETH J., TORRECLE E., SPAZIER E., SLUISZEN A.V.D. 1992. The development of a functional digestive system in the african catfish *Clarias gariepinus* (Burchell). J. World Aquacult. Soc. **23**: 286-298.

WOLNICKI J., KAMIŃSKI R., MYSZKOWSKI L. 2002. Temperature-influenced growth and survival of burbot *Lota lota* (L.) larvae fed live food under controlled conditions. Arch. Pol. Fish. **10**: 109-113.

YANG R., XIE C., FAN Q., GAO C., FANG L. 2010. Ontogeny of the digestive tract in yellow catfish *Pelteobagrus fulvidraco* larvae. Aquaculture **302**: 112-123.

ZAMBONINO-INFANTE J., GISBER E., SARASQUETE C. 2008 Ontogeny and physiology of the digestive system of marine fish larvae. (In: Feeding and digestive functions of fish. J.E.O. Cyrino, D. Bureau, B.G. Kapoor eds. Science Publishers Inc. Enfield): 277-344.

ŻARSKI D., SASINOWSKI W., KUCHARCZYK D., KWIATKOWSKI M., KREJSZEFF S., TARGOŃSKA K. 2009. Mass initial rearing of burbot *Lota lota* (L.) larvae under controlled conditions. Pol. J. Nat. Sci. **24**: 76-84.