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Histological Study of the Organogenesis of the Digestive System and Swim Bladder of the *Chalcalburnus tarichi* Pallas, 1811 (Cyprinidae)

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Abstract: The histological development of the digestive system and swim bladder of *Chalcalburnus tarichi* larvae and their histology in adults were studied under light microscopy. After hatching, the digestive tract is a simple undifferentiated tube. Exogenous feeding started on the 6th day. The yolk sac was absorbed completely on the 9th day. The goblet cells appeared first in the bucco-pharyngeal cavity and oesophagus on the 4th day, in the anterior on the 9th day and in the posterior intestine on the 5th day. The digestive tract was differentiated as the buccal cavity, pharynx, oesophagus post-eosophageal swelling and intestine on the 5th day. At the same time, taste buds and pharyngeal teeth also started to form. In the oesophagus, the circular muscle was observed on the 9th day and the longitudinal muscle in one-year-old fish. In the intestine, the circular muscle was observed on the 35th day and the longitudinal muscle in two-year-old individuals. The wall of the digestive tract is composed of epithelial, submucosa, muscle and serosa layers. The liver lobules formed on the 3rd day and the cells began to reserve glycogen on the 10th day. The pancreatic acina formed on the 5th day and the tubular in one-year-old fish. The liver and pancreas ducts opened into the anterior intestine before the exogenous feeding started. The swim bladder was observed as one lobuled on the 4.5th day and two lobuled on the 35th day.

Key Words: *Chalcalburnus tarichi*, digestive system, histology

Chalcalburnus tarichi Pallas, 1811 (Cyprinidae)'de Sindirim Sistemi ve Yüzme Kesesi Gelişiminin Histolojik Olarak İncelenmesi

Özet: İnci kefali larvalarında, sindirim kanalı, karaciğer, pankreas ve yüzme kesesinin histolojik gelişimi ve erginlerdeki yapısı ışık mikroskobunda incelendi. Embriyo yumurtadan çıktığında sindirim kanalı basit tüp şeklindedir. Dış beslenme 6. günde başladı. Besin kesesi 9. günde tamamıyla absorbe edildi. Goblet hücreleri ilk olarak ağız, farinks ve özofagusta 4., ön bağırsakta 9. ve arka bagırsakta 5. günde görüldü. Sindirim kanalı 5. günde ağız boşluğu, farinks, özofagus, özofagusu takip eden geniş kısım ve bağırsak şeklinde ayrılır. Aynı gün, tad tomurcukları ve farinks dişleri de şekillenmeye başlar. Özofagusta dairesel kas 9. günde, boyuna kas bir yaşında görüldü. Bagırsakta dairesel kas 35. günde; boyuna kas iki yaş grubunda şekillendi. Sindirim kanalının duvarı epitel, submukoza, muskularis ve seroza tabakalarından meydana gelir. Karaciğer lobülleri 3. günde şekillendi ve hücreler 10. günde glikojen depo etmeye başladı. Ekzokrin pankreasta asinar yapılar 5. günde, tübüler yapılar bir yaş grubunda görüldü. Karaciğer ve pankreas kanalları dış beslenme başlamadan önce ön bağırsağa açıldı. Hava kesesi 4.5. günde tek loplu, 35. günde iki lopludur.

Anahtar Sözcükler: *Chalcalburnus tarichi*, sindirim sistemi, histoloji

Introduction

The alimentary tract of teleostean fish has been studied widely and described morphologically, to determine the function of many specialized anatomical structures in relation to the different feeding adaptations (1-14).

The *Chalcalburnus tarichi* is an endemic cyprinid species of the Lake Van basin. There are a few studies about the gross morphology of the digestive system of this species (15). In the present study, the development of the digestive system of the *Chalcalburnus tarichi* larvae and the anatomy and histology of the digestive tract in adults were investigated. These results will provide a basis for future studies on the nutrition and ecology of the species.

Materials and Methods

The mature female and male *Chalcalburnus tarichi* were taken from Karasu river (Lake Van) in May. The eggs and milt were stripped from the spawner artifically and fertilized by the dry method. The fertilized eggs were incubated in an 18±1°C aquarium for approximately 4 days. Hatched larvae were transferred to another aquarium at the same temperature. On the 5th day after hatching, live foods (mainly rotifers) were added to the aquarium for the first feeding of the larvae.

Samples were taken daily up to the end of the larval period (35th day). Then, 4 month-old, 1+, 2+ aged and mature individuals were sampled for histological investigation. Larvae samples were fixed in 7% formalin, formol-saline and Bouin's fluid and embedded in paraffin. Sections 5 µm thick were stained by the Mayer's haemotoxylin-eosin (H-E), Mallory's triple stain, alcian blue-periodic acid-Schift (AB/PAS) and periodic acid-Schift (PAS) (16).

From the juveniles and adults, the digestive tract and pancreas were removed by cutting across the oesophagus and anus. The tract was divided into three parts by folding, and were fixed separately. The liver was dissected freely and fixed.

Microphotographs showing the different regions of the alimentary tract were taken using an Olympus photomicrographic system.

Results

At hatching, in free embryos the digestive tract is a straight, undifferentiated tube composed of columnar epithelium. Its mouth and anus were not opened. A large yolk reserve ventral to the digestive tube extended posteriorly two-thirds of the length of the larvae and terminated just before the posterior end of the digestive tube.

Morphologically, after 5 days of hatching, six distinct regions in the digestive tract could be defined: the buccal cavity, pharynx, oesophagus, post-oesophageal swelling (PES) and anterior and posterior intestine.

Bucco-pharyngeal Cavity

On the first day of hatching, the small bucco-pharingeal cavity appeared (Figure 1). The pharingeal region was easily distinguished from the buccal cavity by the development of the gill structure in the epithelium on the 2nd day. The mouth opened on day 3. At this time, the gill arch developed well (Figure 2). While the buccal cavity was composed of simple squamous epithelium, another region of the digestive tract consisted of a cuboidal epithelial layer. On the 4th day, a few goblet cells, with no secretion, interdispersed within the epithelium appeared in buccal cavity and pharynx. On the 5th day, the goblet cells were stained by AB/PAS, which indicates the presence of acid mucopolysaccharides (Figure 3). At the same time, the goblet cells in the skin epithelial layer were also stained. The stratification of the ephitelial layer in the pharynx region began and at the same time pharyngeal teeth began to grow on the ventral surface of the post-pharynx region. The first fold was observed in the post-pharyngeal region on the 6th day. The first taste bud appeared in the epithelial layer on the 5th day and the number of these increased with age (Figure 4). They are situated on the crests of the mucosal

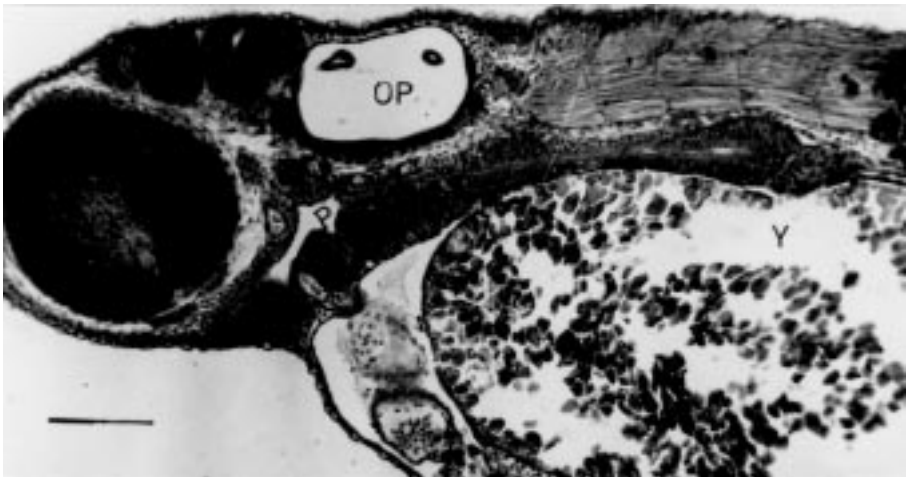

Figure1. Sagittal section of the first day after hatching. E. eye; P. pharnyx; arrow. digestive tube; OP. operculum; Y. yolk sac. Mallory's triple stain. Scale bar=120 µm

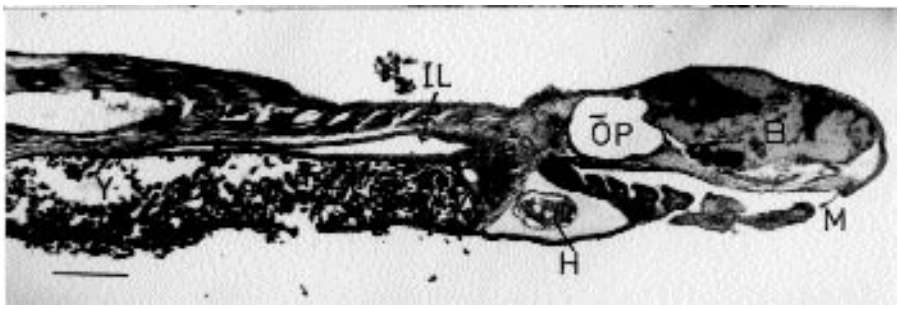

Figure 2. Sagittal section of the 3rd day after hatching. M. mouth; IL. intestine lumen; H.heart; B.brain; Y. yolk sac. H-E. Scale bar=200 µm

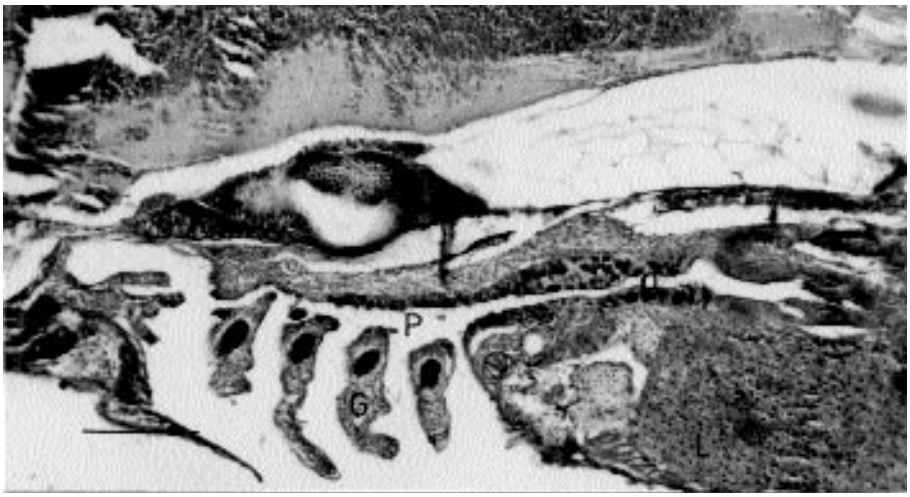

Figure 3. Pharnyx (P) and oesophagus (O) of a 5-day-old larva. G. gill. AB/PAS. Scale bar=120 µm

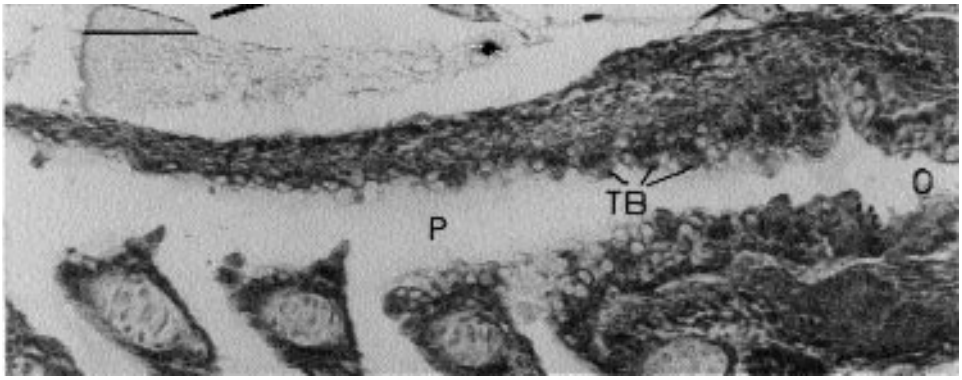

Figure 4. Sagittal section of the pharnyx (P) of the 9-day-old larva. O. oesophagus; TB. taste buds. Mallory's triple stain. Scale bar=50 µm

folds. The longitudinal muscle appeared under the mucosa on the 10th day. Its thickness increased posteriorly toward the oesophagus and increased with the age of the fish. In adult fish, the epithelial layer includes the taste buds, goblet cells. It also includes undifferentiated cells which are located in the base of the epithelial layer and are stained darkly with H-E. The longitudinal section of pharynx in adult fish is shown in Figure 5.

Oesophagus

The oesophagus was differentiated on the 5th day. The small mucosal folds which consist of a simple cubic epithelium appeared. There were a few goblet cells, but no secretion on the 4th day. On the 5th day, an increased number of goblet cells were stained by AB/PAS as in the bucco-pharyngeal region (Figure 6). At the same time, the stratification began. The collagen fibrils stained blue

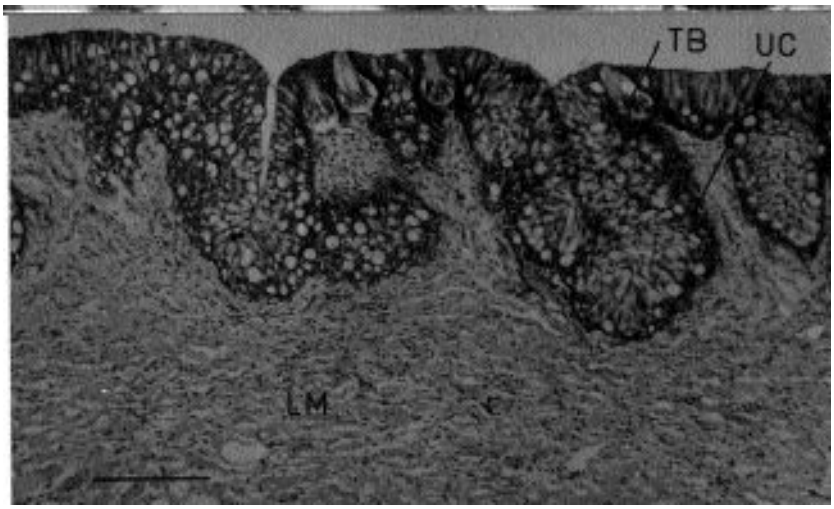

Figure 5. Longitudinal section of pharnyx in adult fish. TB. taste buds; UC. undifferentiated cells; LM. longitudinal muscle. H-E. Scale bar=120 µm

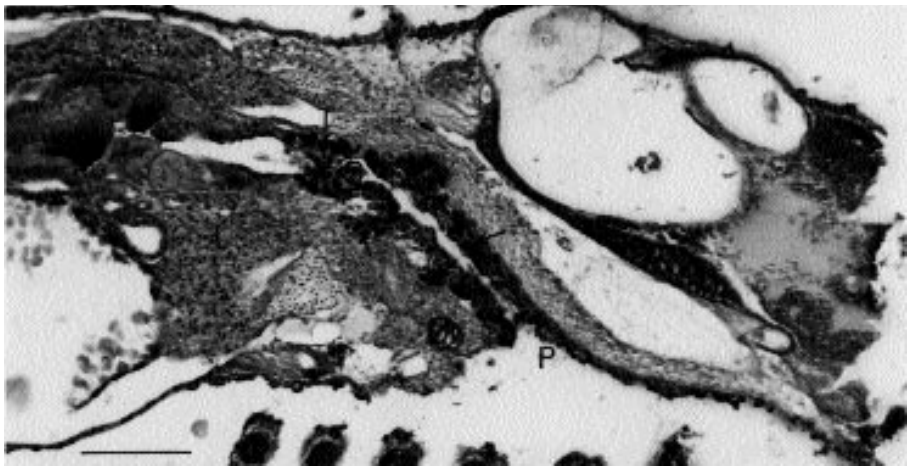

Figure 6. Mucosal folds in the pharynx (P) and oesophagus (O) of 5 day,old larva. Arrow. goblet cells; L. liver. AB/PAS. Scale bar=120 µm

with Mallory's triple stain appeared under the epithelial layer on the 6th day. The circular striated muscle was observed on the 9th day (Figure 7). By one year of age, a thin longitudinal muscle was differentiated under the circular muscle (Figure 8). The basic layers making up the oesophagus were epithelium, lamina propria, submucosa,

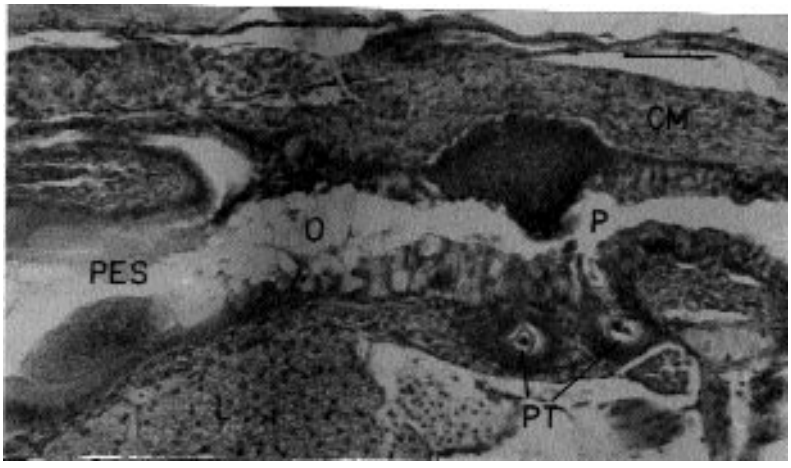

Figure 7. Sagittal section through pharynx (P), oesophagus (O) and post-oesophageal swelling (PES) of 10-day-old larva, showing circular striated muscle (CM). PT. pharyngeal teeth. H-E. Scale bar=50 µm

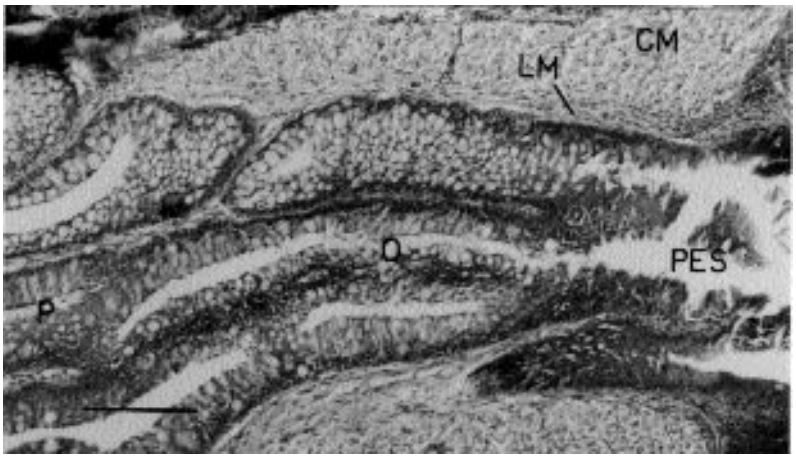

Figure 8. Sagittal section showing longitudinal (LM) and circular muscle (CM) at the oesophagus (O) and post-oesophageal swelleng (PES) of 1-year-old juvenile. P. pharynx. H-E. Scale bar=120 µm

muscularis and serosa. No distinct junction between the lamina propria and submucosa was observed. The lamina propria and submucosa contained no mucous cells, but expanded into the mucosal folds. Glands and muscularis mucosa were not observed in the wall of the oesophagus.

There were no histological differences in adult fish but the muscle layer was thicker. Goblet cells were interdispersed within the epithelial layer and some taste buds were situated on the top of the mucosal folds (Figure 9). The surface cells and undifferented cells could be distinguished easily (Figure 10).

Post-Oesophageal Swelling

On the 5th day, a construction at the posterior end of the oesophagus was followed by a swelling called the post-oesophageal swelling (PES) (Figure 11). The distinction between the oesophagus and the PES was defined by an abrupt transition from a stratified epithelium with numerous goblet cells to a simple columnar epithelium devoid of goblet cells. A duct which was differentiated from the dorsal wall, where the PES started, opened to the swim bladder on day 4.5 (Figure 12). The epithelial layer of the PES has a straight border. The longitudinal folds appeared in 8-day-old larvae. The

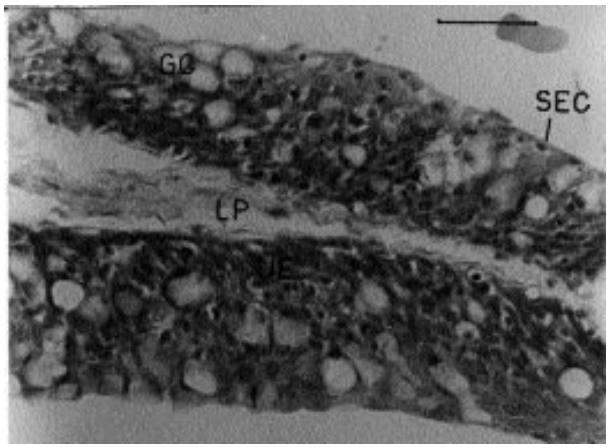

Figure 10. Oesophageal fold showing multi-layered epithelium. GC. goblet cells; SEC. surface epithelial cells; UC. undifferentead cells; LP. lamina propria. H-E. Scale bar=30 µm

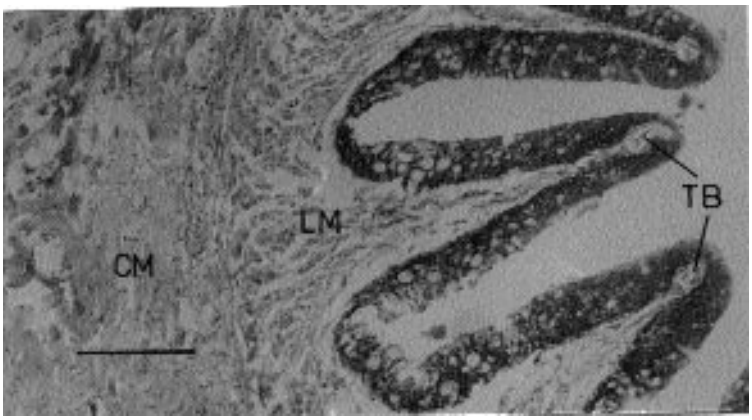

Figure 9. Transversal section of oesophagus in adult fish. TB. taste buds; CM. circular muscle; LM. longitudinal muscle; H-E. Scale bar=120 µm

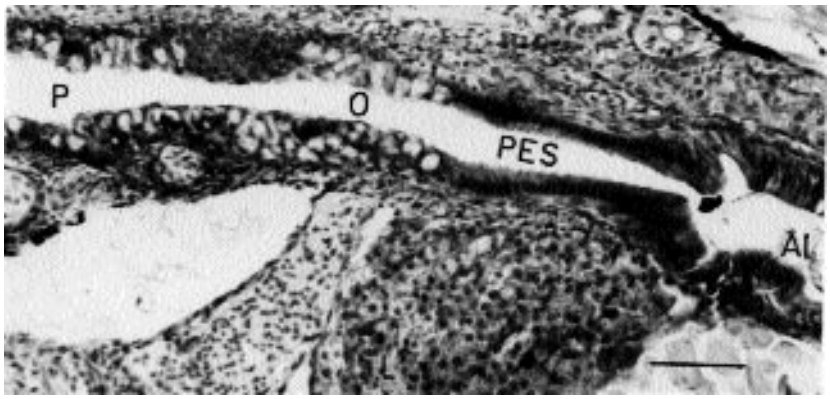

Figure 11. The post-oesophageal swelling (PES) in 6-day-old larva. O. oesophagus; Al. anterior intestine; L. liver; P. pharnyx. Mallory's triple stain. Scale bar=50 µm

Figure 12. Swim bladder duct (arrow) in 7-day-old larva. PES. post-oesophagel swelling; O. oesophagus; SB. swim bladder. AB/PAS. Scale bar=120 µm

layers of the oesophagus also extended until the end of the PES, and PES shortens depending on the development of the fish.

Intestine

Until the 4th day, the intestine was a straight tube composed of cubic epithelium. It can be separated into two morphologically distinct regions called the anterior and posterior intestine. The lumen of the intestine began to widen in the 3-day old larvae (Figure 2). The anterior intestine began at the end of the PES and it was distinguished from the posterior intestine by the small folds composed of columnar cells, on the 4th day. The mucosal folds in the posterior intestine were observed on the 8th-10th day. Although many goblet cells including secretion in the posterior intestine appeared on the 5th day (Figure 13), only a few goblet cells in the anterior intestine were observed on the 9th day (Figure 14). The circular smooth muscle cells were defined under the epithelial layer of the intestine in the 35-day-old larvae. The lamina propria and submucosa were not

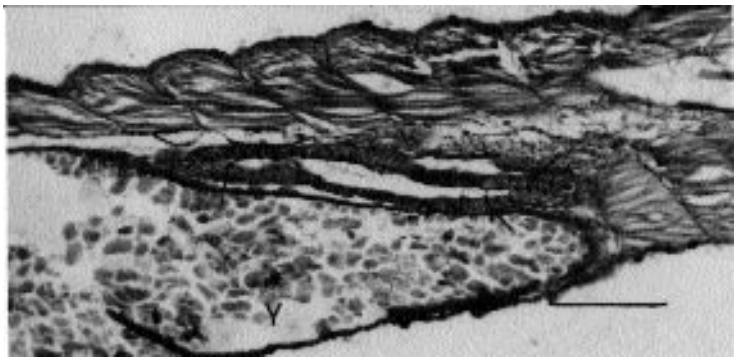

Figure 13. Posterior intestine of 5-day-old larva. Arrow. goblet cell; Y. yolk sac. AB/PAS. Scale bar=120 µm

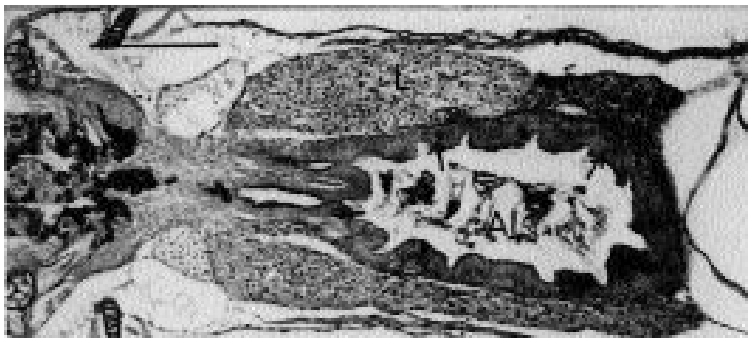

Figure 14. Anterior intestine (AI) of 9-day-old larva. Arrow. goblet cells; L. liver. AB/PAS. Scale bar=120 µm

distinguished as in the oesophagus and pharynx but they expanded in the epithelial folds.

The intestine started to coil in 40-day-old fish and it divided into three regions (1st, 2nd, 3rd). In 2+ old fish thin longitudinal muscle developed around the circular muscle. In adults, the thickness of longitudinal muscle was almost half that of the circular. The mucosal folds got smaller as the intestine diameter got narrower. It was deepest in the first region (Figure 15). They were uniform in shape in the 2nd and 3rd regions. The number of goblet cells increased toward the anus. While the goblet cells in the 1st region were only stained blue with AB/PAS, they were stained blue and red in the 2nd and 3rd regions.

The histological appearence of the intestine did not vary significantly in adults, as in the bucco-pharyngeal cavity and oesophagus. Only the thickness of the layers increased with age. The intestine wall was composed of an epithelial layer including many goblet cells, lamina propria, submucosa, muscularis and serosa (Figure 16). The epithelial layer was composed of goblet cells, columnal cells and undifferentiated cells (Figure 17).

Liver

At hatching, in the free embryo, there was a cell cluster located between the intestine and yolk sac. It developed and formed the liver. Until the 3rd day of hatching, the liver cells were distributed irregularly. The characteristic lobules of the organ started to be formed in the 3-day-old larvae and the sinusoids, including blood cells, appeared among the lobules. The liver with its large cells and light stained cytoplasm and nuclei, was easily differentiated from the pancreas. The bile duct, composed of a cubic cell layer, differentiated on day 4 and opened to the anterior intestine on the 4.5th-5th day (Figure 18). On the 10th day, the glycogen reserves, revealed by PAS, began to develop. The liver lobules were

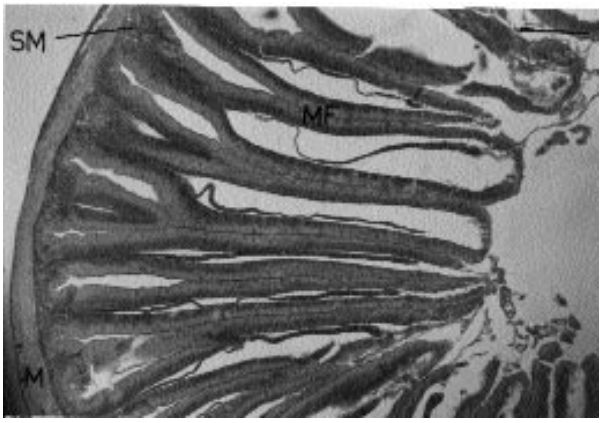

Figure 15. Transverse section from the first region of the intestine in adult. MF. mucosal folds; SM. submucosa; M. muscularis externa; Mallory's triple stain. Scale bar=200 µm

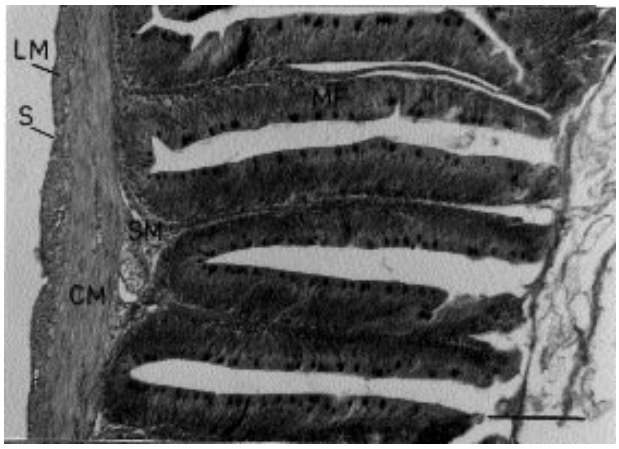

Figure 16. Transverse section from the second region of the intestine in adult fish. SM. submucosa; CM. circular muscle; LM. longitudinal muscle; S. serosa; MF. mucosal fold. PAS. Scale bar=120 µm

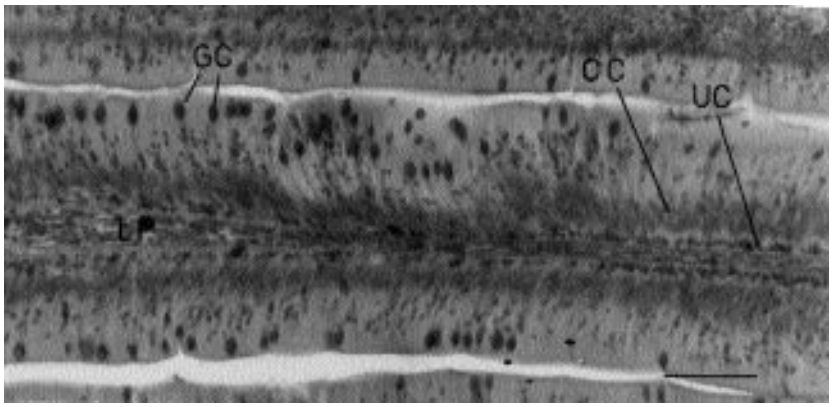

Figure 17. Intestinal fold showing epithelial layer. GC. goblet cells; CC. columnar cells; UC. undifferentiated cells; LM. lamina propria. PAS. Scale bar=50 µm

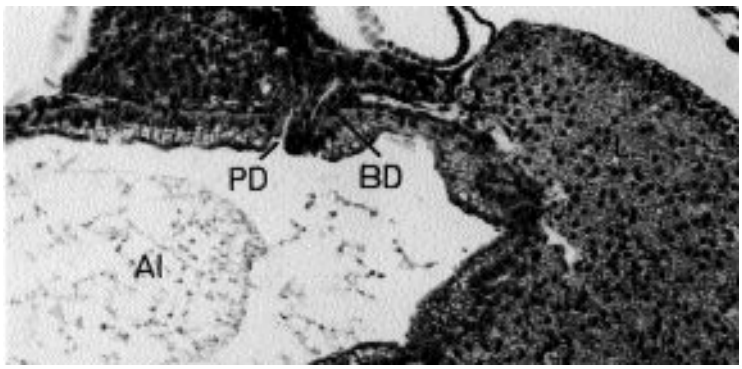

Figure 18. Pancreatic (PD) and bile duct (BD) at 18-day-old larva. P. pancreas; L. liver; AI. anterior intestine. H-E. Scale bar=120 µm

distinguished poorly, with a large central vein from the hepatocytes radially disposed and sinusoids of the fish older than 4 months.

Pancreas

The pancreas appeared between the liver and the intestine in the 4-5-day-old larvae. The pancreas, surrounded with simple squamous epithelium, was easily distinguished from the liver by the considerable amount of smaller cells and the darker stained nucleus. On day 5, exocrine cells were arranged in clusters forming characteristic acina and a Langerhans islet appeared (Figure 19). On days 6-8, the zymogen granules were seen at the apical regions of the cells. Pancreatic duct, which consisted of cubic cells, was formed on day 4 and it opened to anterior intestine, almost under the liver duct, on 4.5-5th day (Figure 18). In contrast with the liver, the pancreas was thinner at the front of the anterior intestine but it widened due to its growth toward the end of the intestine and consisted of many Langerhans islets. In the 4-month-old juvenile, large blood vessels and great number of connective tissue cells were observed in the pancreas. The fat cells appeared to be interspersed especially towards the end of the pancreas in 1+year-old

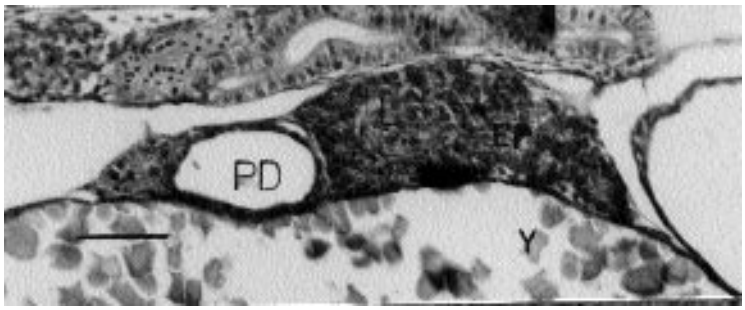

Figure 19. The pancreas of a 5-day-old larva. EP. exocrine pancreas; L. Langerhans islet; PD. pancreatic duct; Y. yolk sac. H-E. Scale bar=50 µm

fish. In the adult fish, the tubular arangement of the exocrine pancreas cells appeared also around large blood vessels (Figure 20) and the pancreas was interspersed with fat tissue (Figure 21).

Swim Bladder

The swim bladder of larvae started to be filled on day 4.5 (Figure 22). It opened into the dorsal wall of the PES with a long duct (Figure 12). The organ was made up of a simple squamous epithelial layer. 30-35 days after hatching, the swim bladder was divided into two lobules by the invagination of the epithelial layer and assumed its adult shape. The organ was lined with a cubic epithelial layer. Under the epithelial layer are fibrils and small cells.

The histological development of the digestive tract, liver, pancreas and swim bladder in *C. tarichi* is summarized in the Table.

Discussion

The development of the digestive system can be divided into three periods: the embryonic, larval and juvenile periods (9,10,17-19). The embryonic period

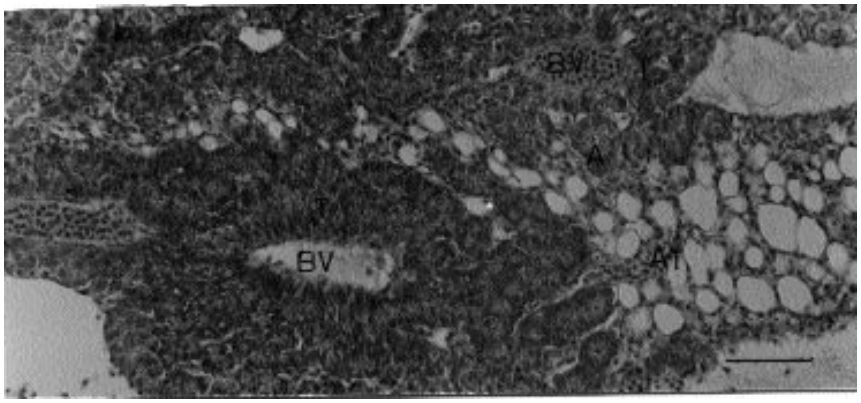

Figure 20. Tubular (T) and acinar (A) arrangements of the exocrine pancreas in adult fish. BV. blood vessels; AT. adipose tissue. H-E. Scale bar=50 µm

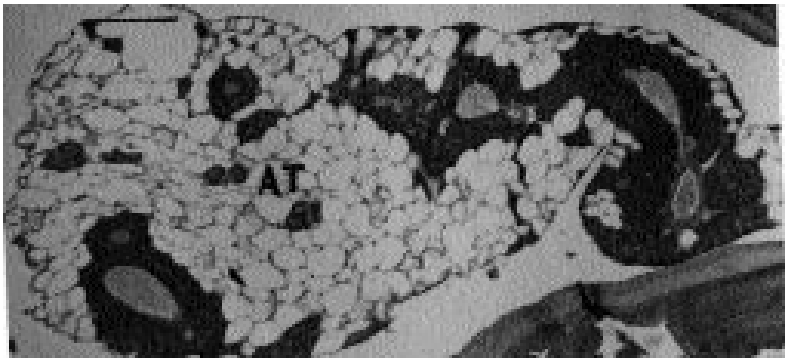

Figure 21. General view of pancreas in adult. AT. adipose tissue; PAS. Scale bar=200 µm

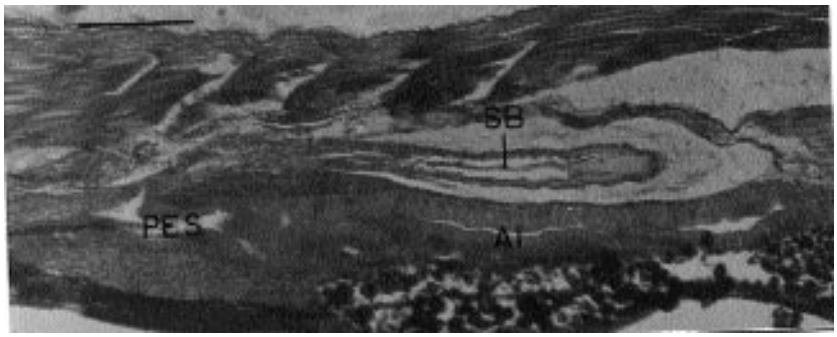

Figure 22. The swim bladder (SB) in 4.5-day-old larva. PES. post-oesophageal swelling; AI. anterior intestine. Mallory's triple stain. Scale bar=120 µm

Table. Post-hatching developmental events in the digestive tract, its associated glands and the swim bladder of *C. tarichi*.

Day	Mouth	Buccal Cavity	Pharynx	Oesophagus	PES	Anterior Intestine	Posterior Intestine	Liver	Pancreas	Swim Bladder
2	SqE	CuE	CuE	CuE	CuE	CuE				
3	Opened							LF		
4		GC	GC	GC		CC+MF		LDF	PDF	
4.5		Mucus	Mucus	Mucus				LDO	PDO	1 Lobule
5		St	St+TB	St+MF	CC		GC+Mucus		Acini+ LI	
6			MF+PT							
8					MF		MF			
9			CM	CM	CM	GC+M				
10								GR		
35				TB		CM	CM			2 lobule
First year				LM	LM					
Second year						LM	LM			

SqE: squamous epithelium, CuE: cuboidal epithelium, LF: lobule formation, GC: goblet cell, CC: columnar cell, MF: mucosal fold, LDF: liver duct formation, PDF: pancreatic duct formation, LDO: Liver duct opened; PDO: pancreatic duct opened; St: Stratification, TB: taste bud, LI: Langerhans ıslet, PT: pharyngeal teeth, CM: circular muscle, GR: glycogen reserve, LM: longitudinal muscle, M: Mucus.

begins with the first egg cleavage and terminates with the first exogenous feeding (17,18,20,21). The pharyngeal region of the digestive tract in *C. tarichi* embryo was distinguished with the development of the gill structure, at the 56th hour after fertilization (22). The bucco-pharyngeal cavity appeared at the end of the 2nd day after hatching and the liver and the pancreas were still in the shape of cell clusters. Feeding was exclusively endogenous.

The mouth opened on the 3rd day. The exogenous feeding began on the 6th day. The larval period ended on the 35th day. The most important developments occurred before the exogenous feeding started as in other teleost species (10,18,20). The digestive tract formed containing six morphologically distinct regions -the buccal cavity, pharynx, oesophagus, PES, anterior and posterior intestine on the 6th day after hatching. The first goblet cells appeared within the bucco-pharyngeal cavity and oesophagus on the first day after the opening of the mouth. The same developments were reported when the mouth opened in sole (18) and milkfish (9), and in some other species such as yellowtail larvae (19) in later stages. As in milkfish larvae (9), the secretion of the goblet cells in *C. tarichi* stained blue in all developmental stages with AB/PAS. The salivary glands in *C. tarichi* were not detected as in other teleosts (23).

The mucosal stratification of the pharynx began on day 5 and the thickness of stratified epithelium of the pharynx was variable in *C. tarichi*, as in some other fish species (24).

The oesophagus of the *C. tarichi* was very short as in carnivorous species, but it is an omnivorous species. The mucosal folding of the oesophagus began on the 5th day. In milkfish (9), mucosal folds develop when the larvae starts to take zooplankton or the habitat changes. In *C. tarichi*, the mucosal folds in the oesophagus, including most of the goblet cells, may be important for exogenous feeding.

The occurrence, location and distribution of the taste buds may be important for feeding type and behavior. They are absent in the giltheat sea bream *Sparus auratus* (7), and present only in the buccal cavity in yellowtail

flounder (19), in the pharynx in perch (5), in the anterior oesophagus in channel catfish (2), in the oesophagus in *Sparus aurata* (8), and in the pharynx and oesophagus in three species of Ambassidae (24). In *C. tarichi*, taste buds are numerous in the pharynx and fewer in the oesophagus. It seems that *C. tarichi* swallows food by selecting with the help of these buds.

Generally, teeth may be located on the jaws, tongue and pharynx in fish but pharyngeal teeth are the most common (23). As described by Çetinkaya and Elp (15), *C. tarichi* larvae have only pharyngeal teeth. As in sole larvae (18), they are formed almost at the same time before exogenous feeding. They are important for grinding and chewing the food.

In larvae, as in yellowtail flounder larvae (19), two distinct regions in the oesophagus are distinguished. The posterior portion of the oesophagus is composed of single layer columnar epithelium and no goblet cells. This portion is similar to the transation portion from the oesophagus to the stomach in yellowtail larvae (19).

Cyprinids do not possess a true stomach histologically, but an expansion at the anterior part of the intestine takes the place of the stomach (23). The numerous goblet cells in the posterior intestine appear before the exogenous feeding, but a few cells in the anterior appear even after the exogenous feeding.

The exocrine cells of the pancreas are arranged in a characteristic acinar manner until the age of one year. Then a tubular arrangement appears together with the acinar arrangement. In contrast to some species (12,25), the pancreas is a separate organ in *C. tarichi* larvae and adults and the adipose tissue in adult *C. tarichi* is mainly interpersed within the pancreas, but sometimes it is peripheral.

In sole larvae (18), the glycogen reserve appears in hepatocytes on the first day after the start of exogenous feeding, but on the 4th day in *C. tarichi*. This difference may be the result of insufficient feed.

The swim bladder begins to inflate on day 4.5 post-hatching. On the 5th day, it can easily be seen from outside of the larvae. In contrast, in sole (18) and coregonid larvae (10), the swim bladder starts to inflate several days after exogenous feeding starts. The inflation time of the swim bladder may be important in the catching of living prey.

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