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# Histological Study in the Digestive Tract on Larval Development of Rainbow Trout (*Oncorhynchus mykiss,* Walbaum, 1792)

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**Abstract:** In this study the development of the digestive tract of newly hatched rainbow trout (*Oncorhynchus mykiss*) larvae were examined. The digestive tract was attached to the anus a few hours after hatching. On the second day their mouths were open and the differentiation in the esophagus started. Mucosal folds contained a large quantity of cubic epithelium and goblet cells. Goblet cells were stained positively with Alcian blue and also stained negatively with P.A.S. The wall of the esophagus consisted of a circular muscle layer and a thin tunica serosa. It was possible to see a thin longitudinal muscle layer in the inner side, on the tenth day.

Two days after hatching, the stomach was formed as a continuing part of the esophagus mucosa. Goblet cells were not observed. The wall of the stomach consisted of a circular muscle layer and a thin tunica serosa. The first signs of the stomach glands were observed on the 22nd day.

In the intestine the basophilic granular cytoplasm of epithelial cells was stained lightly with P.A.S. on the second day after hatching. The first goblet cells were seen on the third day.

The liver sinusoids were observed in the first 3-4 days. The cells were stained positively with P.A.S. because of the glycogen concentration.

**Key Words:** Rainbow trout *(Oncorhynchus mykiss)*, development, histology.

## Alabalıkda (*Oncorhynchus mykiss,* Walbaum, 1792) Larval Gelişimde Sindirim Kanalının Histolojik Olarak İncelenmesi

**Özet:** Bu araştırmada yumurtadan yeni çıkan alabalık larvalarının gelişimleri incelendi. Yumurtadan çıktıktan birkaç saat sonra larvada sindirim sistemi, anüse bağlanır ve 2. günde ağızları açılıp, özofagus farklılaşmaya başlar.

Mukoza kıvrımı çok sayıda kübik epitelyum ve kadeh hücrelerini içerir. Kadeh hücreleri Alcian mavisi ile pozitiflik, P.A.S. ile negatiflik gösterir. Özofagusun duvarı sirküler bir kas tabakası ile ince bir tunika serozadan oluşur. 10. günde iç kısımda ikinci bir ince longitudinal kas tabakası görülür.

Yumurtadan çıktıktan 2 gün sonra larvada mide, özofagusun devamı şeklindedir. Goblet hücreleri gözlenememiştir. Mide duvarı sirküler bir kas tabakası ile ince bir tunika serozadan oluşur. Mide bezlerinin ilk belirtisi 22. günde gözlenmiştir.

Yumurtadan çıktıktan sonraki 2. günde epitel hücrelerin bazofilik granüler sitoplazması ile apikal mikrovilluslar, P.A.S. ile hafif derecede boyanmıştır. İlk goblet hücreleri 3. günde görülmüştür.

Karaciğer sinusoidleri 3. veya 4. günlerde görülür. Glikojen yoğunluğundan hücreler P.A.S. pozitiflik göstermiştir.

**Anahtar Sözcükler**: Gökkuşağı alabalığı *(Oncorhynchus mykiss),* larval gelişim, histoloji.

## Introduction

The rainbow trout, *Oncorhynchus mykiss* is an important food fish and is extensively cultured in freshwater fish farm in Turkey. There are a lot of cold freshwater sources in which to do rainbow trout culture and it is very easy to adapt to environment, breeding and also marketing. While the rainbow trout culture industry is rapidly expanding, the present supply of fry is inadequate to meet the growing demand. The mortality during the larval period, mainly due to starvation, is often regarded as one of the key factors accounting for survival in wild populations.

The histology of the digestive system and larval development of many fish specimens have been investigated (1-22).

Several authors have described the organs of rainbow trout in macroscopical studies and histological, histochemical, microscopic studies of the whole anatomy (23-29).

The aim of this study was to examine the digestive tract of rainbow trout, *Oncorhynchus mykiss* in larval development.

## Materials and Methods

In this study rainbow trout, *Oncorhynchus mykiss* larvae were obtained from the hatchery of the Cip Fisheries Institute, Faculty of Fisheries, Fırat University, Elazığ, Turkey.

Larval samples were taken on days 3, 5, 6, 10, 12, 17, 22, 27 and 31 during metamorphosis at hatching for the histological study. At least three individuals from each group were examined. A total of twenty-seven larvae were fixed in 10 % formalin solution and embedded in paraffin (30,31). Sections 5-6 µm thick were prepared and stained according to standard histological techniques: hematoxylin-eosine (H&E). Masson trichrome stain, Alcian blue and Periodic acid schiff reagent (P.A.S.) supplemented by amyloglucosidase digestion control for glycogen (30-32). The sections of rainbow trout larvae were examined under a Nikon inverted microscope and photographs taken with a Nikon photomicrographic attachment.

## Results

The pharynx has epithelium folds microscopically (Fig. 1). The esophagus can be distinguished from other regions 3 days after hatching. The liver sinusoids were seen on the third and fourth day. They appeared clearly after those days. The cells were stained positively with P.A.S. due to glycogen concentration. Light colored secretory cells were seen in stained mucosal folds. The longitudinal muscle layer were seen in the 10-day old larvae. The lamina propria made up of fibrous connective tissue were clearly formed in 6-day old larvae. There was a thin tunica serosa layer in the outer side. The secretory cells were stained negatively with P.A.S. and stained positively with Alcian blue. However, some epithelial cells near the lumen were light pink when stained with P.A.S. in larvae after 17 days. Mucosal folds were determined in 13-day-old larvae. The primer folds were fully formed in 17-day-old larvae (Fig. 2).

The stomach is like an extension esophagus. 3-day-old larvae have mucosal folds. In the epithelial layer there are cubic epithelium cells. Subepithelial connective tissue begins to be clear three days after hatching. In 10-day old larvae lamina epithelialis turns into one layer cubical cells. These cells contain small granules. Serous cardiac glands were observed in larvae 20 days old (Fig. 3-5). They were like simple alveolar glands in larvae 23 days old. During the whole development brush border was not encountered on the luminal side of the lamina epithelialis. In the 29-day-old larvae stomach glands were numerous. The inner muscle layer was about twice as much as the outer layer. Apical layer was stained positively with Alcian blue (Fig. 5).

The intestine can be distinguished from the stomach three days after hatching. Brush border was present on the luminal layer of epithelial layer with one. The goblet

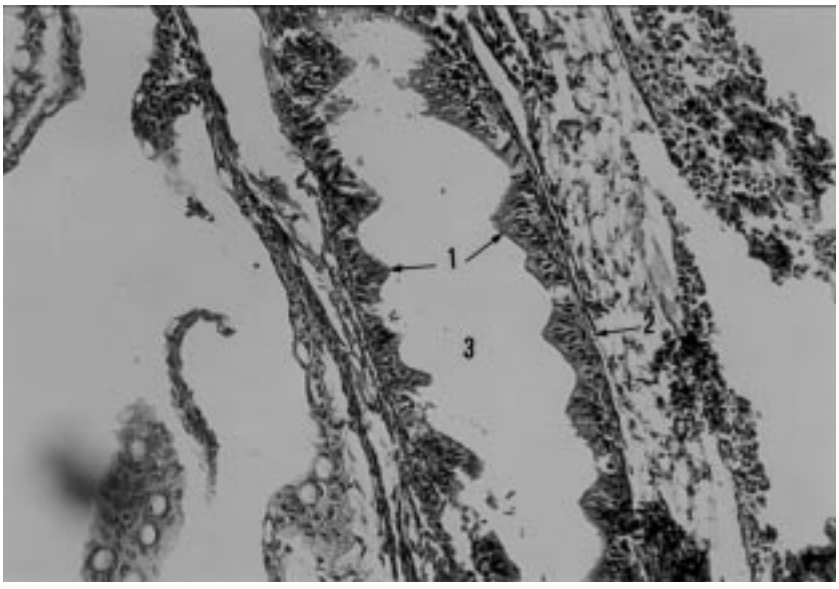


Figure 1. Pharynx of a 6-day-old *O. mykiss* larva. 1. Mucosal epithelium processes, 2. Muscle layer, 3. Lumen of the pharynx. (H&E). X100.

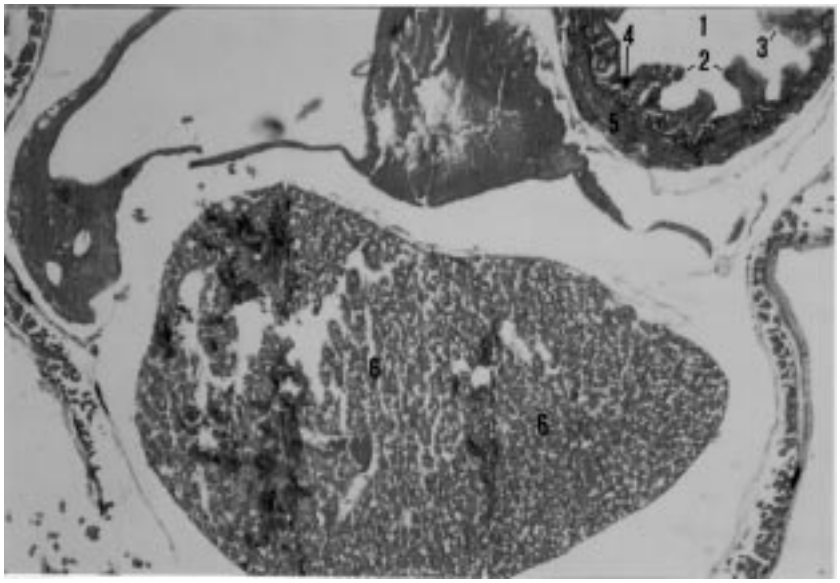


Figure 2. Transversal section of a 17-day-old *O. mykiss* larva. 1. Lumen of the esophagus, 2. Primer folds, 3. Lamina epithelialis, 4. Lamina propria, 5. Circular muscle layer, 6. Liver (Masson trichrome). X100.

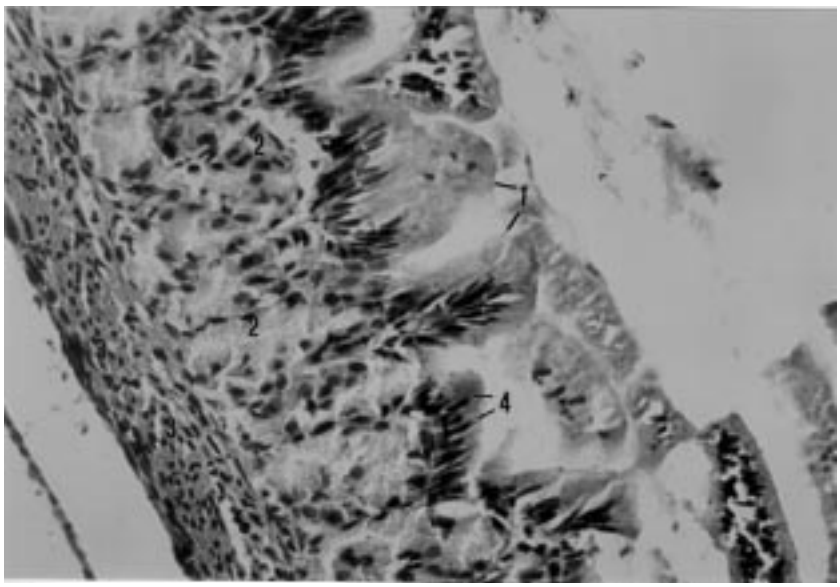


Figure 3. Cardiac stomach of a 22-day-old *O. mykiss* larva. 1. Primer folds, 2. Cardiac glands, 3. Muscle layer, 4. Prismatic cells. (P.A.S.). X100.

cells occurred during the metamorphosis. They appeared in 10-day-old larvae. The apical of epithelial cells was stained slightly pink with P.A.S. (Fig. 4-6). The goblet cells were stained positively with Alcian blue (Fig. 7-9).

## Discussion

In a study about larval development it was reported that the esophagus began to differentiate on the second day after hatching, the secretory cells stain negatively with P.A.S. and stain positively with Alcian blue in cubic epitelium mucosal folds. In another study (9), the authors reported that the esophagus differentiated from the other regions by mucus secretion in epithelial cells on the third day after hatching. This structure was stained blue with Alcian blue-P.A.S. In the present study it was determined that the esophagus began to appear and light colored secretory cells in mucosal folds were seen 3 days after hatching. These cells were stained negatively by P.A.S. and positively by Alcian blue which indicates the presence of glucoseaminoglycan. However, epithelial cells on the luminal surface of esophagus were slightly stained

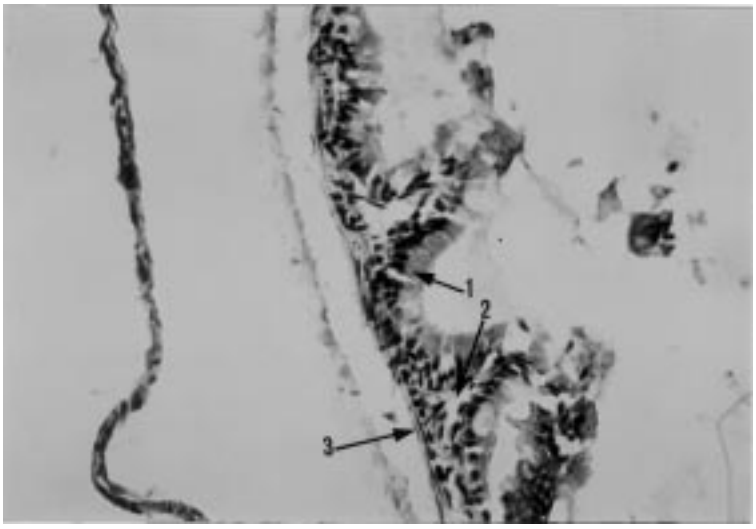


Figure 4. Intestine of a 22-day-old *O. mykiss* larva. 1. Prismatic epithelial cells, 2. Lamina propria, 3. Muscle layer (P.A.S.) X100.

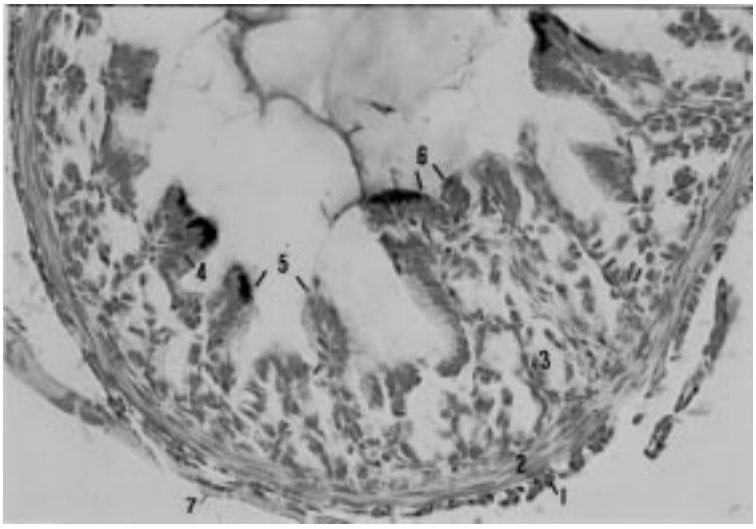


Figure 5. Stomach of a 27-day-old *O. mykiss* larva. 1. Longitudinal muscle layer, 2. Circular muscle layer, 3. Cardiac glands, 4. Prismatic epithelial cells, 5. Primer folds, 6. Mucus cells stained blue with Alcian blue, 7. Serosa. (Alcian blue-Kernechrot). X100.

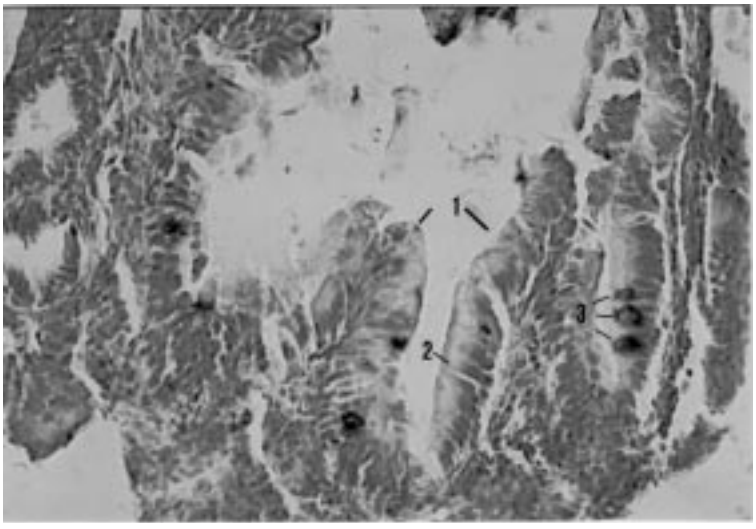


Figure 6. Intestine of a 27-day-old *O. mykiss* larva. 1. Mucosal folds, 2. Prismatic epithelial cells, 3. Goblet cells stained blue with Alcian blue. (Alcian blue-Kernechrot).X50.

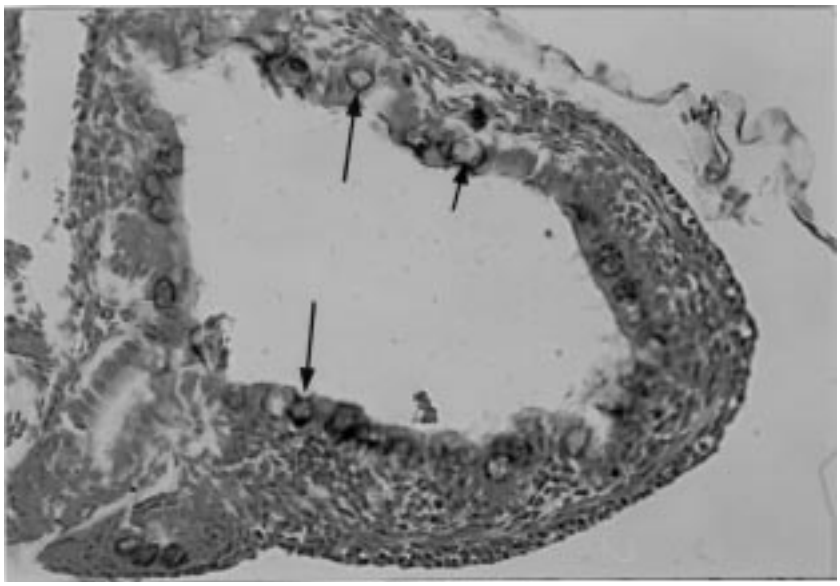

Figure 7. Goblet cells stained blue with Alcian blue from intestine of a 31-day-old *O. mykiss* larva. (Alcian blue-Kernechrot). X100.

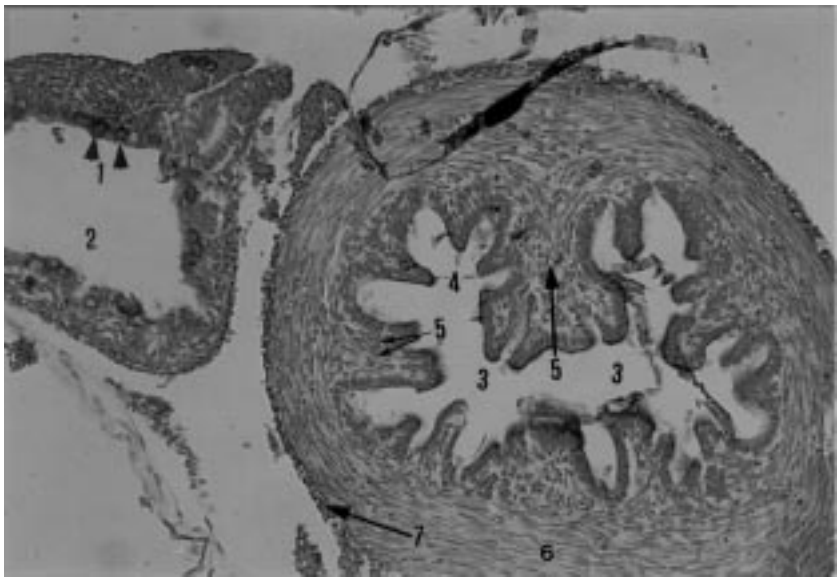


Figure 8. Transversal section of a 31-day-old *O. mykiss* larva. 1. Goblet cells of intestine, 2. Lumen of intestine, 3. Lumen of esophagus, 4. Primer folds of esophagus, 5. Lamina propria, 6. Circular muscle layer, 7. Longitudinal muscle layer. (Alcian blue-Kernechrot). X50.

positively with P.A.S. in the 17-day old larvae. The occurrence of longitudinal muscle layer on the 9-10 th day (1, 21) showed parallelism with this research.

In this study, it was determined that the stomach is covered with cubic epithelial cells in the 2-day old larvae (1, 9) and the first folds were seen in the stomach of 3-day-old larvae (1). Some researchers (1, 21) have explained that the stomach epithelium turned into a prismatic cell structure in 10-day-old larvae. In our study, lamina epithelialis was observed as prismatic cells with one layer.

The stomach glands were reported to be under the epithelium as simple glands on the 22nd day for the first time (1).

We observed that simple alveolar serous glands occurred in rainbow trout larvae twenty days after hatching. Moreover the muscle layer in the stomach was determined to be twice as much as two layers of the outer layer.

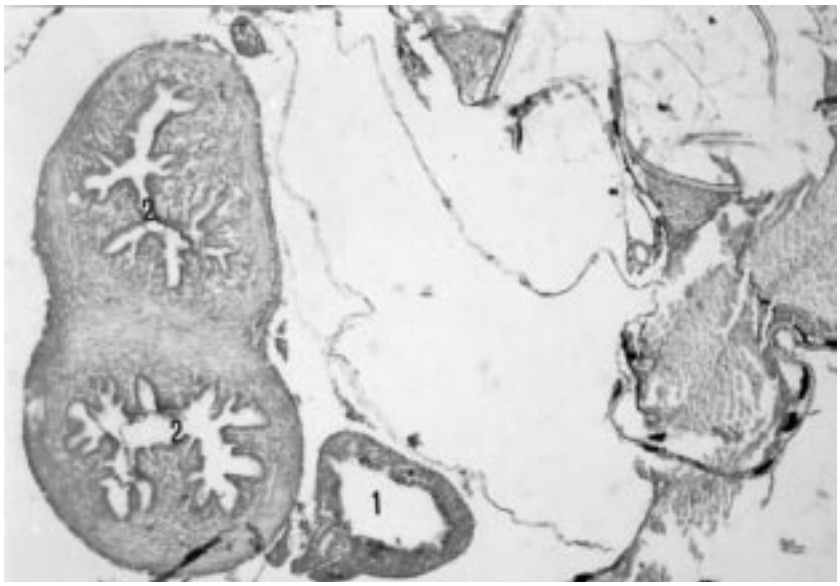


Figure 9. Transversal section of a 31-day-old *O. mykiss* larva. 1. Intestine, 2. Esophagus, (Alcian blue-Kernechrot). X20.

It was reported that the intestine has regular mucosa consisting of a prismatic layer of cells and within the posterior of the intestine epithelial cells show an apical striated border stained slightly with P.A.S. (1, 21). In the same studies, it was also reported that on the 3rd day the first goblet cells can be observed. At the same time, the alimentary canal of pike eel larvae was morphologically and functionally differentiated 3 days after hatching and before exhaustion of the yolk (20). In another study (15) on the 6th day some circles were observed within the digestive system. Some other researchers determined that goblet cells of all developmental stages stain blue with Alcian blue-P.A.S. (9, 21). On the other hand, by the end of the prelarvae stage of *Tilapia nilotica*, there are no essential changes histologically in the alimentary canal (22).

In the present study the intestine was distinguished from the stomach on the third day after hatching. It was also observed that goblet cells began to occur on the tenth day, apical regions of epithelial cells stained light pink with Alcian blue which indicated the presence of glucoseaminoglycan. In another study, the authors reported that (21), intestinal goblet cells and enterocytes of the digestive epithelium of *Sparus aurata* were rich in carboxylated and neutral mucosubstances.

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