

Ontogeny of Digestive System of Egyptian Sole (*Solea vulgaris*): Morphological and Histochemical studies of Larvae and Juvenile

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ABSTRACT

The aim of the present work is clarifying the ontogeny of the Egyptian Sole digestive system during its larval stage (0DAH, day after hatching) until it become a juvenile (40DAH). The main changes in the morphological digestive tract was detected during its early life at 2DAH (both the mouth and anus are opened). Another day after, the digestive tract of *S. vulgaris* became longer. After metamorphosis (19-22DAH), the stomach is sac-shaped and limited in its concave side by the liver, occupying the wider part of the cavity. The larvae and juveniles of *S. vulgaris* have a small stomach, a relatively long intestine. Histochemically, a few acidophilic goblet cells appeared scattered between the epithelial cells of the both buccopharynx and esophagus regions. The incipient stomach is distinguished at 3DAH and the mucosa is composed of sulphated mucousubstances. After metamorphosis, an increase in the number of the goblet cells in the esophagus epithelium mucosa is detected. The mucus probably plays an important role in lubrication. Incipient stomach is differentiated into two regions the cardiac and pyloric stomach. The brush border of the intestinal epithelium exhibited acidic mucousubstances activities. Sulphated mucousubstances is mainly localized in the intestinal epithelium. Finally, the digestive system of *S. vulgaris* is differentiated into 5 portions, buccopharyngeal cavity, and extends through the body as esophagus, cardiac and pyloric stomach and intestine. After 30DAH, the digestive tract shows the same histological organization as in the adult one.

Keywords: *Solea vulgaris*; Histochemistry; Digestive system.

INTRODUCTION

Members of the genus *Solea* sp. are recorded among the most important and valuable commercial flatfishes in Egypt and greatly appreciated by consumers of sea products (Gabr *et al.* 2003). In Egypt, common sole, *S. solea*, was successfully hatched for the first time in 1986 and juveniles were obtained and maintained in healthy conditions (Zaki and Hamza 1986 and Assem *et al.*, 2012). One of the

main features which determine the end of the transformation from larvae to juvenile stage in teleosts is the development of a complete functional adult-style digestive system (Yúfera and Darías, 2007). Generally, the digestive system of the marine fish larvae that hatch from small pelagic eggs, doesn't reach fully development at first-feeding but already functional (Segner *et al.*, 1994; Moyano *et al.*, 1996; Martínez *et al.*, 1999, Falk-Petersen, 2005). In teleost fish, digestion during the first

stages takes place in the intestine. The first gastric glands can be detected through a few days or weeks after hatching and their number increases progressively partially or completely covering the stomach epithelium depending on the species (Ribeiro *et al.*, 1999; Ortiz-Delgado *et al.*, 2003; Elbal *et al.*, 2004).

As a Pleuronectiformes, the transformation to juvenile implies profound changes in morphology and feeding behaviour. Eye migration and acquisition of benthic habits occurs early in the development (Yúfera *et al.*, 1999; Fernández-Díaz *et al.*, 2001), several days before the stomach starts to develop (Ribeiro *et al.*, 1999). The juveniles and adults fed mainly on benthic invertebrates such as polychaetes, crustacean and mollusks (Cabral, 2000), and consequently, a carnivorous-mode acid digestion could be expected in this species.

The main aim of the present study is to clearly show the morphological and histochemical changes in digestive tract of Egyptian sole (*Solea vulgaris*) during the transition from larvae to juveniles.

MATERIALS AND METHODS

Larvae rearing: Larvae used in the present study were obtained from the marine hatchery of the National Institute of Oceanography and Fisheries, Alex., Egypt. After hatching the larvae were transferred to the rearing tanks at density 60 ± 12 larvae per liter. The water temperature was about $16 \pm 1^\circ\text{C}$ and sea water salinity was 37 ± 2 ppt throughout the experiment.

Feeding regime: *Solea vulgaris* larvae feeding regime from first feeding until 28DAH were based on live food only. Larvae were fed Rotifer 10 ind./ml from 3DAH until 12 ± 2 DAH. From 14 ± 1 DAH, larvae fed on *Artemia*, newly hatched nauplii 3-5 ind./ml. Instars I, II and III were gradually introduced to the larvae. At the age 25DAH, it fed on *Artemia* metanauplii 8-12 ind./ml. Weaning trial performed on juveniles at 122

age 28 ± 2 DAH up to the ending of the study period 40DAH, using weaning diet, in addition to the continuous supplemental of live food.

Morphological studies: The morphological characteristics of larval digestive tract at ages 0, 2-5DAH was observed under the light microscope. The larvae clarified by treated with glacial acetic acid: glycerin, 1:1 (v:v), for 1-2 days. The post-larvae (15DAH) and juvenile (50DAH) needed to dissection the abdominal cavity.

Histochemical studies: Five larvae were fixed in 10% formal saline at ages 2, 3 and 10DAH and each 10 days until it transformed to a juvenile (40DAH). After fixation, the samples were dehydrated and embedded in paraffin wax and sectioned at 6-7 μm . After removing the wax with xylene and hydration in ethanol series sections were stained for general histochemical techniques for the identification and differentiation of carboxylated and/or sulphated mucosubstances were applied using Periodic Acid Schiff and Alcian Blue (PAS/AB) for carboxylated glycoconjugates.

Morphometric measurements: Histological sections of intestine were submitted to measurements of: thickness of mucosa, thickness of muscularis, height of the mucosal fold and diameter of goblet cells. Five observations per larvae/juvenile were used for morphometric evaluation under X100 magnification. Measurements were performed with light microscope using an eye piece micrometer previously calibrated for the magnification used, with a stage micrometer. All data were expressed as mean \pm SE.

RESULTS

Digestive system

Morphological ontogeny of the digestive tract in S. vulgaris

S. vulgaris larval development is dividing into two main stages endogenous and exogenous larval stages. The feeding behavior throughout

MORPHOLOGICAL AND HISTOCHEMICAL ONTOGENY FOR LARVAL *SOLEA VULGARIS* DIGESTIVE SYSTEM

its early life; the endogenous feeding behavior (pre-larval stage) followed by the exogenous larval feeding behavior. It's notable that, when the larvae completely take the asymmetric shape, which occurs with the end of the metamorphosis stage, at that time, it is known as a juvenile, which is similarly looks like its parents.

After hatching at 0DAH, the digestive tract of *S. vulgaris* pre-larvae is merely a tubular segment laying dorsally to the yolk sac with closed mouth and anus (Fig. 1, a).

First feeding started at age 2DAH, both the mouth and the anus opened. Also, the incipient stomach is distinguished from this age forward. The posterior portion of digestive tube bent slightly and the yolk sac volume decreased. It gets completely absorbed by the end of 5DAH (Fig. 1, b). For the meantime, the abdominal cavity had a prominent round shape with a ventral position. Until the start of a benthic life style a similar disposition in all the structures was maintained apart from increases in size.

At the age 3DAH, the digestive tract becomes longer (Fig. 1, c). Another day past (4DAH), the liver was noticed in the anterior portion of the digestive tract, at dorsal portion of the digestive loop. The remind yolk sac also surrounds the abdominal portion of the digestive tract (Fig. 1, d).

The exogenous feeding larvae at 5DAH, all larvae absorb their yolk sac depending completely on the external feeding. The intestinal loop is observed in the third portion of the digestive tract. The loop of the digestive tract occupied the right side of the larvae, the residues of the yolk sac remained in the frontal part of the abdominal cavity (Fig. 2, a).

By 15DAH, the portions of the digestive tract located in the abdominal cavity are the stomach, still poorly developed, the anterior portion, which defined the lower part of the cavity bending over itself to reach the posterior portion of the cavity. The liver is enlarged and

occupies more than the half of the digestive cavity (Fig. 2, b).

The benthic way of life was acquired around 19 to 22DAH. At this point an increase in the length and fold of the digestive tract is observed. The abdominal cavity in a metamorphosed larva is positioned on the right side of the dorsal side, beginning just after the gill arch. It exhibited a triangular shape, wider near the head region and becoming thinner in its posterior part. The stomach is sac-shaped and is limited in its concave side by the liver, occupying the wider part of the cavity. At the pyloric valve the intestine bent slightly over the stomach and delineates the cavity periphery. When it reached the frontal right side it bent over again following in a diagonal direction until it reached the end of the cavity where it turned to end near the pelvic fin (Fig. 2, c&d).

The larvae and juveniles of *S. vulgaris* have a small stomach, a relatively long intestine and lack a pyloric caeca.

Finally, the digestive system of *S. vulgaris* is similar to other vertebrates, which consists primarily of the digestive tract, a long muscular-walled tube beginning at the mouth and terminating at the anus. The digestive tract in *S. vulgaris* is differentiated into 5 portions, buccopharyngeal cavity, and extends through the body as esophagus, cardiac and pyloric stomach and intestine.

General view of the digestive tract of S.vulgaris

The teleost gastrointestinal tract is histologically simple and some fundamental points in the architecture of this tube are remarkably constant. The typical appearance of the gut wall cut transversely. Although each portion of the alimentary tract has its own characteristic feature, the digestive tract as a whole is composed of four layers:

1. **The mucosa**, lining the lumen of the tube and consisting of an inner epithelium, a middle lamina propria (a thin cellular connective

tissue), and an outer smooth muscularis mucosae;

2. **The submucosa**, a less cellular connective tissue layer with blood vessels, lymphatic tissue and nerve plexi, binding the mucosa to the muscular wall;
3. **The tunica muscularis (a muscular coat)**, which is often divided into two perpendicular layers, inner circular and outer longitudinal of smooth muscle;
4. **The serosa or adventitia**, surrounded by a simple squamous epithelium being supported by a thin connective layer.

Histochemistry aspects of digestive tract in *S.vulgaris*

Histochemical staining is specialized staining methods are used to illustrate particular features. The Periodic Acid–Schiff (PAS) is acidophilic stain, effects on the mucopolysaccharides. The mucopolysaccharides, considered as the main material construct the wall of the digestive tract and differentiation to neutral and acidic mucosubstance. The sulphated mucosubstance is neutrophilic, stains blue. The carboxylated mucosubstance is acidophilic, stained purple.

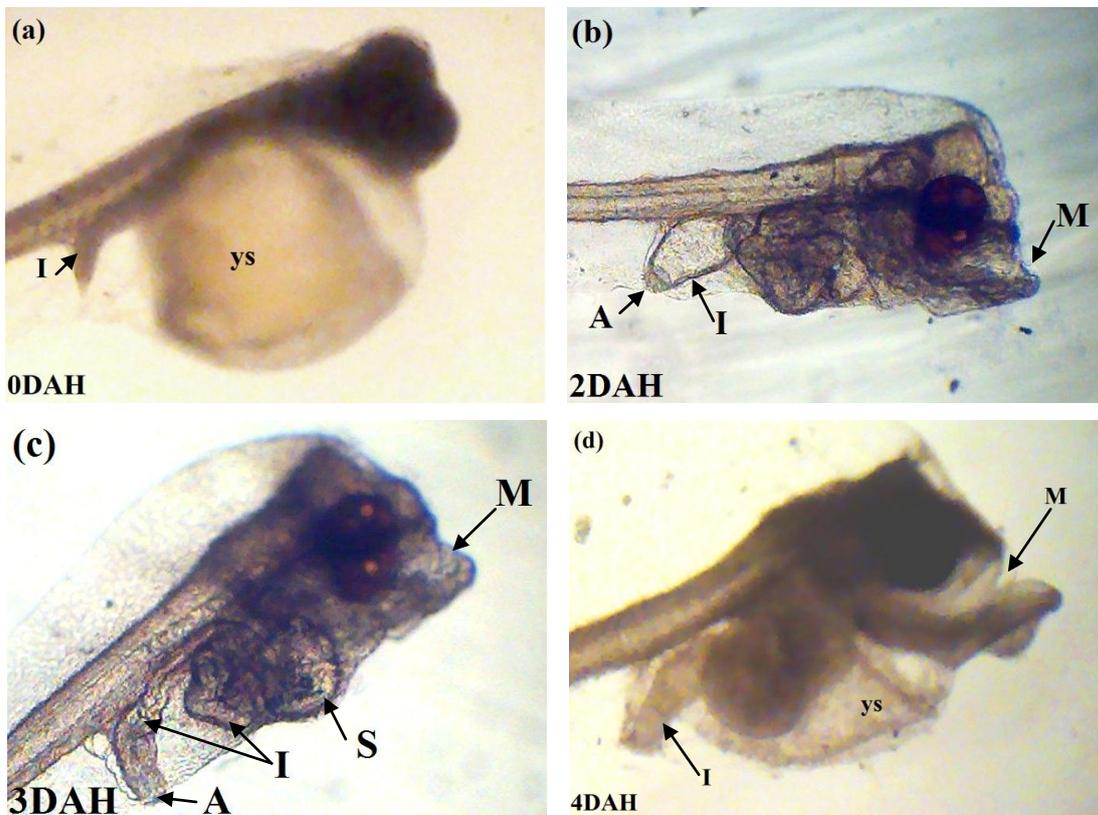


Fig.1: Photomicrograph of *S. vulgaris* larvae at different ages showing; morphological ontogeny of the digestive system. A: anus; I: intestine; M: mouth; S: stomach; ys: yolk sac.

- a) Pre-larvae after hatching, 0DAH (X400).
- b) two days larvae after hatching, 2DAH (X400).
- c) three days larvae after hatching, 3DAH (X400).
- d) four days larvae after hatching, 4DAH (X400).

MORPHOLOGICAL AND HISTOCHEMICAL ONTOGENY FOR LARVAL *SOLEA VULGARIS*
DIGESTIVE SYSTEM

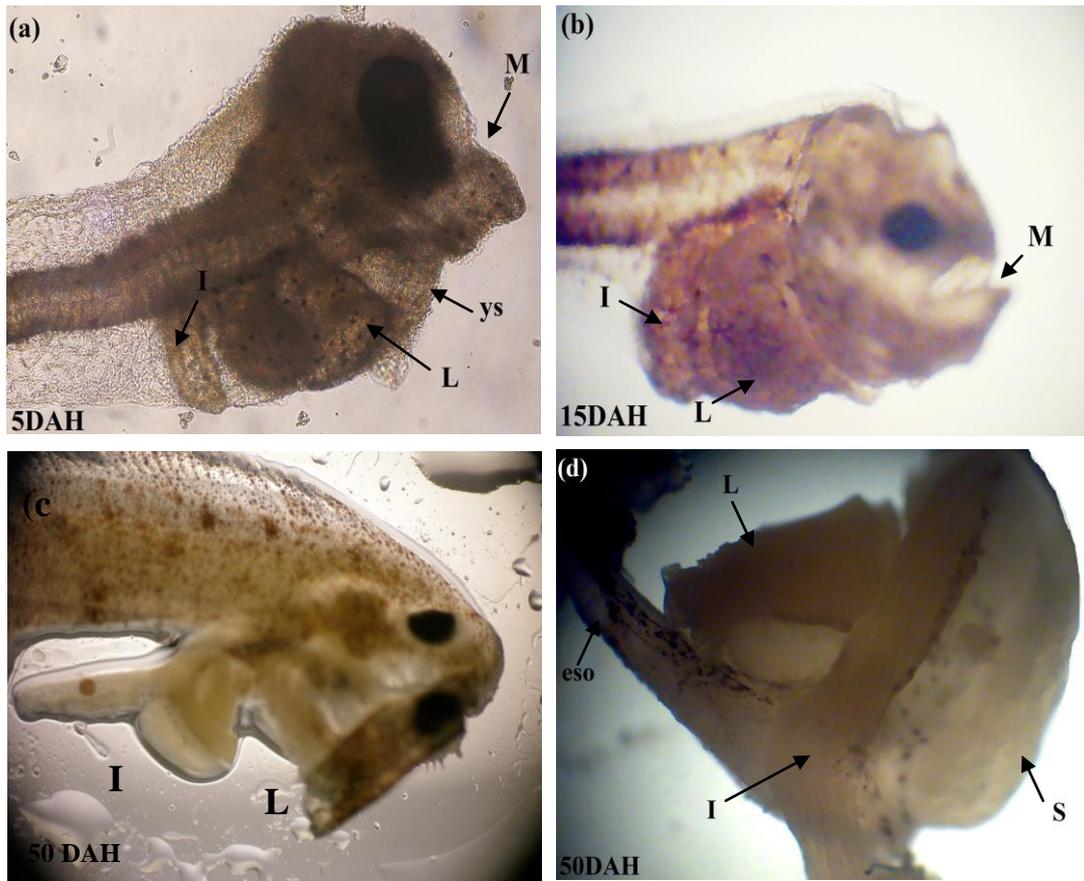


Fig.2: Photomicrograph of *S. vulgaris* at different ages showing; morphological ontogeny of the digestive system. I: intestine; eso: esophagus; L: liver; M: mouth; ys: yolk sac; S: stomach.

- a) Five days larvae after hatching, 5DAH (X400). b). 15 days larvae after hatching, 15DAH (X400). c). 50 days juvenile after hatching, 50DAH (X40). d). 50 days juvenile after hatching, 50DAH (X40).

Histochemistry studies were carried out to investigate the ontogeny of the digestive tract of *S. vulgaris* during the larval development, until it becoming completely developed. The most notable changes in the digestive tract that could be associated with larval growth were noticed during the early larval life, especially throughout the period of the metamorphosis stage.

Histochemical Characteristics of Buccopharyngeal cavity/esophagus of S. vulgaris

At hatching 0DAH, the epithelium consisted of a mono-stratified layer of cubic columnar cells. The lumen of the digestive tract was narrow with a tendency to widen at both extremities. The yolk sac contained several peripheral oil globules and exhibited homogenous acidophilic yolk.

At 2DAH, few acidophilic goblet cells were appeared scattered between the epithelial cells of the buccopharynx. Mucous cells are visible throughout the buccopharyngeal

epithelium being more abundant in the anterior region of the buccal cavity, while their size and number increased as larval grew (Fig. 3, a). The oral cavity and pharynx are lined by a stratified squamous epithelium containing abundant mucus-secreting cells.

The tunica mucosa of the buccopharyngeal cavity and the esophagus featured a great number of different sized longitudinal folds, separated by well-pronounced furrows in the esophagus. Also folds were present, albeit smaller, in the pharynx. In both organs, surface epithelium of the mucosa was stratified, with small cuboidal cells in the basal layer, columnar cells in intermediate layers and more flattened cells in the superficial layer. The cytoplasm of mucous cells was granular and contained a heavily PAS-reactive material in the buccopharyngeal cavity as well as in the esophagus. The mucous cells were acidophilic (AB positive) and stained blue.

The esophagus mucosa of the *S. vulgaris* pre-larvae at age 2DAH is appeared as a villous lined by stratified epithelium connecting to the buccopharyngeal cavity and containing about 15 ± 2 of goblet cells. The esophageal mucosa was organized in a mucosa arranged in short folds $37 \pm 3 \mu\text{m}$ and scattered acidophilic goblet cells. The number of villous was about 7 ± 1 in transversal section (Fig. 3, b). The submucosa didn't detect at this age. Primary longitudinal cells are detected to form the tunica muscularis layer (stained pink). The lumen of this digestive portion is narrower when compared with the rest of the digestive tract (Fig. 3, c).

At age 3DAH, the cross section of esophagus were arranged continuously as showing in Fig (4, a) as; a primary fold, long villi contain 11 ± 1 of goblet cells, about 2 in each villi followed by secondary fold, short villi formed by mucous cell, which unfilled with goblet cell (Fig. 4, b). The number of villous was about 9 in transversal section and reach to about $38 \pm 4 \mu\text{m}$ in high. The tunica muscularis

also is detected with no noticeable development (Fig. 4, c).

The buccopharyngeal cavity is situated in the posterior portion of the head; together with the gill arch it constituted the gill chamber that filters and retains food. The esophagus is short and possessed the thickest wall of the digestive tract (Fig 5, a). The exogenous feeding larvae at age 10DAH, the number of villi were about 10 in transversal section and reach to about $40 \pm 6 \mu\text{m}$ in high (Fig 5, b). The epithelium mucosa increased in the number of the goblet cells to reach 30 ± 2 , about 5 ± 1 in primary villi and 2 ± 1 in the secondary villi.

After metamorphosis 20DAH, the esophagus appears to take the major form of the main layers (Fig. 6, a&b). The lumen of the esophagus is lined by a protective stratified epithelium containing about 34 ± 2 of goblet cells. The mucosa is thrown into longitudinal folds. The mucus probably plays an important role in lubrication. The number of villous was about 10 in transversal section.

The lamina propria and submucosa are more or less separated by some fascicule of the muscularis mucosa. The muscularis mucosa consists of rather small bundles of longitudinally coursing smooth muscle cells. The submucosa filed the villi cone with both collagenous and elastic fibers. Also, the lymphoid tissue is present in the submucosa (Fig. 6, c&d).

The tunica muscularis comprising interweaving striated muscle fibers that extends as far as the stomach. Scattered through the various layers of the epithelium are single secretory cells which release large amounts of neutral and acidic glycolconjugates which could have a role in the lubrication of the epithelium, in the interaction between mucosa and viruses and/or bacteria.

The esophagus development of the *S. vulgaris* juvenile at ages 30DAH was still in progress (Fig. 7, a&b). At 40DAH, the mucosa increased in thickness and number of goblet

MORPHOLOGICAL AND HISTOCHEMICAL ONTOGENY FOR LARVAL *SOLEA VULGARIS*
DIGESTIVE SYSTEM

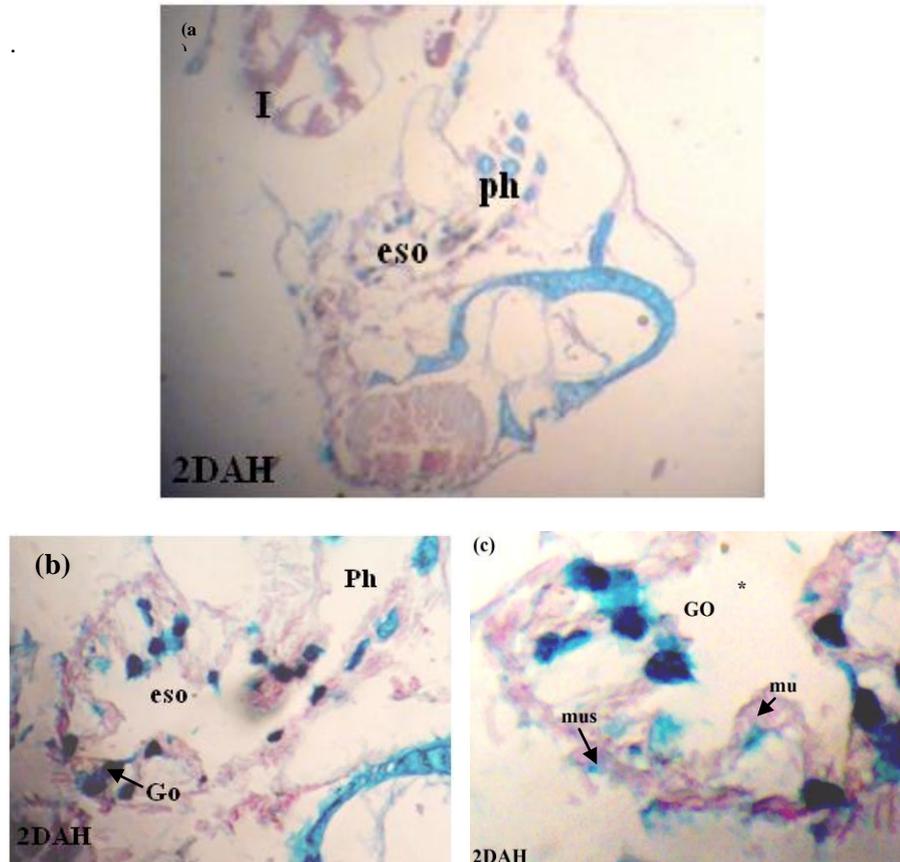


Fig. 3: Photomicrograph of transverse section (TS) in digestive tract of larvae *S. vulgaris* at 2DAH showing; the pharynx (ph) opened in the esophagus (eso) through narrow lumen (*) and esophagus was lined by goblet cells (Go), react positively to Alcian blue (dark blue). Notice that not all villus have Go in its mucosa layer (mu). Muscularis layer (mus) has a positive reaction to the PAS and gave violet color as mu, (PAS/Alcian blue). a). TS for esophagus at 2 days after hatching larvae (X100) , b). Magnification of (a) (X250) and c). Magnification of (a) (X400).

cells to reach more than 85. The tunica muscularis is divided into two layers; the inner circular (stained rose and surrounded blue) and the outer one is longitudinal (stained blue), of smooth muscle. The number of villous was about 12 in transversal (Fig 7, c&d)

Histochemical Characteristics of Stomach of *S. vulgaris*

The transition from esophageal to gastric mucosa was gradually. The stratified epithelium

of the esophagus is replaced by the secretory columnar epithelium that lines the whole surface of the stomach. Surface columnar epithelium is secretor throughout the stomach. Mucous histochemical properties are uniform all over the stomach.

Incipient stomach is distinguished at age 3DAH as around muscular structure situated at the end of the esophagus (Fig. 8, a). The mucosa is lining the lumen of the tube with the

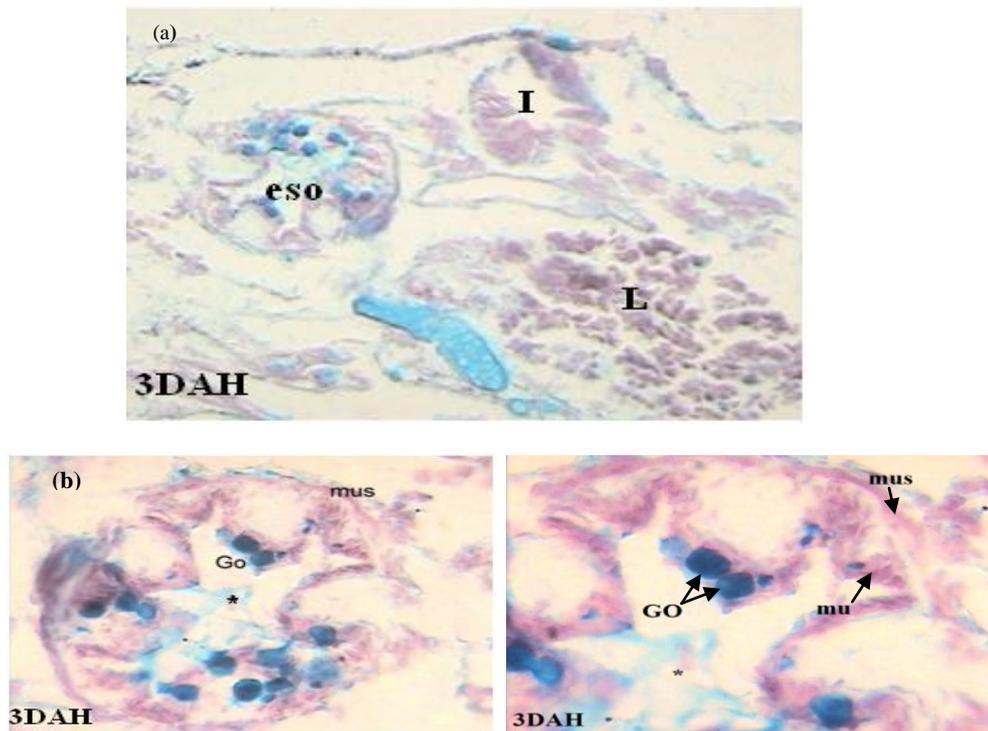


Fig. 4: Photomicrograph of transverse section (TS) in digestive tract of larvae *S. vulgaris* at 3DAH showing; the esophagus (eso) located beside the intestine (I) in the anterior portion of the abdomen, left the liver (L) in the posterior. Showing: lumen (*), goblet cells (Go), mucosa layer (mu), muscularis layer (mus), (PAS/Alcian blue). a). TS for esophagus at 3 days after hatching larvae (X100) , b). Magnification of (a) (X250) and c). Magnification of (a) (X400).

epithelium, which formed by a single layer of elongated columnar mucus-secreting cells. The stomach mucous cells are composed of sulphated mucosubstance. PAS/AB positivity was visualized by an intense purple-violet staining in surface columnar cells. The mucus protects the stomach from self-digestion and fills the apical cytoplasm of all cells (Fig. 8, b).

At the age 10DAH, the stomach lumen cavity is increased in the width (Fig. 8, c&d). At age 20DAH, the stomach mucous is organized in folds, lined by columnar epithelium and devoid of goblet cells (Fig. 9, a-d). After metamorphosis, two different regions are identified according to their histological organization: the cardiac (anterior) (Fig. 10, a&b) and pyloric (posterior) (Fig. 10, c&d). The

mucosa of the cardiac stomach was organized in folds, lined by simple columnar epithelium and give positive PAS reaction. The villi height is about $34 \pm 3 \mu\text{m}$ in the cardiac region and $35 \pm 2 \mu\text{m}$ in the pyloric region.

As juvenile grow 30DAH, the layers of the stomach are more defined. In cross section, the wall of the stomach consists of a mucosa, submucosa and tunica muscularis (Fig. 11, a&b). The muscular coat is composed of inner circular and outer longitudinal coats of smooth muscle cells. The submucosa contains nerves, arteries, veins, and lymphatics and coarse acidophilic granulocytes. Muscularis mucosae are found and consist almost entirely of smooth muscle cells disposed longitudinally. At 40DAH, the stomach is surrounded by muscularis layer, this layer is thin in the cardiac

MORPHOLOGICAL AND HISTOCHEMICAL ONTOGENY FOR LARVAL *SOLEA VULGARIS*
DIGESTIVE SYSTEM

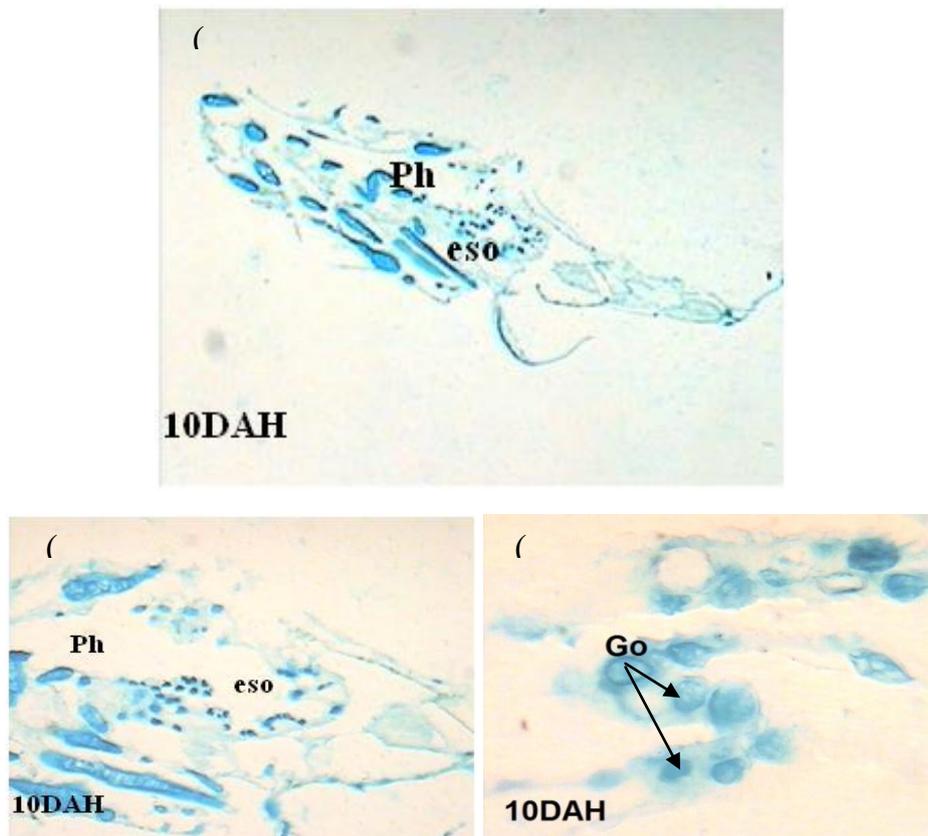


Fig. 5: Photomicrograph of longitudinal section (LS) in digestive tract of larvae *S. vulgaris* showing; buccopharyngeal cavity; pharynx (ph), esophagus (eso), goblet cells (Go), (PAS/Alcian blue). a). LS for esophagus at 10 days after hatching larvae (X140) , b). Magnification of (a) (X100) and c). Magnification of (a) (X400).

region but become thicker in the pyloric portion (pyloric sphincter). The villi height was about $127 \pm 29 \mu\text{m}$ in the cardiac region and $90 \pm 16 \mu\text{m}$ in the pyloric region (Fig. 11, c&d).

The transport of chyme from the stomach into the intestine is controlled by a muscular sphincter, the pylorus. The pyloric sphincter and the intestine create a resistance to the flow of chyme from the stomach to the intestine. At the junction of stomach with the intestine there are a large number of mucous cells, also the tunica muscularis appears as scattered longitudinal bundles in between the collagen fibers.

At age 10DAH, a pyloric ridge appeared at the junction of the stomach (pyloric sphincter)

and the intestine (Fig. 12, a&b). At age of 20DAH, the pyloric folds are increased in the number and width and gastric glands are clearly demonstrated, stained positively for PAS. The gastric glands are present in such abundance that they almost occupy the entire mucosal layer beneath the superficial epithelium (Fig. 12, c&d).

The pyloric glands (gastric glands) are separated more widely from one another than are the cardiac ones. They consist of shorter, less frequently branched tubules. Their epithelium closely resembles that of the surface of the stomach.

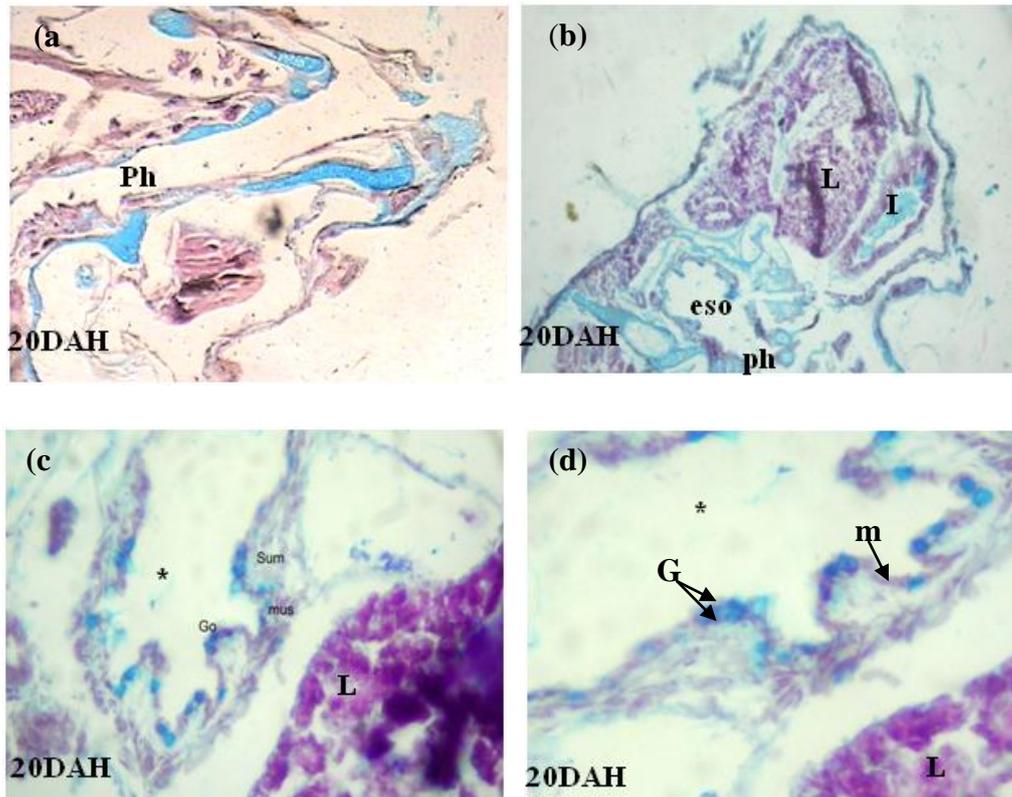


Fig. 6: Photomicrograph of longitudinal section (LS) in head and transverse section (TS) digestive tract of larvae *S. vulgaris* showing: pharynx (ph), esophagus (eso), intestine (I), liver (L), goblet cells (Go), submucosa (Sum), muscularis (mus) and lumen (*), (PAS/Alcian blue. a). LS for buccopharyngeal cavity at 20 days after hatching larvae (X40) , b). LS for buccopharyngeal cavity at 20 days after hatching larvae (X100) c).). TS for esophagus at 20 days after hatching larvae (X250). d). Magnification of (c) (X400).

Histochemical Characteristics of Intestine of *S. vulgaris*

The segment of the digestive canal following the stomach is called the intestine. At 2DAH, the brush border of the intestinal epithelium exhibited acidic mucosubstance activities. Sulphated mucosubstance is mainly localized in the intestinal epithelium (Fig. 13, a&b).

After a day (3DAH), intestinal regions show the same histological organization: a simple columnar epithelium bordered by a thin layer of acidophilic microvilli at the apical surface (Fig. 13, c&d).

The villous is finger-like is process of mucosa, varied in length between $41 \pm 4 \mu\text{m}$ (2DAH) and $45 \pm 1 \mu\text{m}$ (3DAH), which consist of an epithelium covering (stained blue) and core filled with smooth muscle cells; longitudinal in direction.

The wall of the alimentary canal is built up of several layers of simple tissues. Each layer has a definite function. These are mucosa, submucosa, muscularis and serosa. The mucosal layer is formed of glandular structure and mucous membrane. The glandular structures secrete mucous secretion that may contain digestive enzymes. The mucous membrane is usually not a simple membrane, but thrown up

MORPHOLOGICAL AND HISTOCHEMICAL ONTOGENY FOR LARVAL *SOLEA VULGARIS*
DIGESTIVE SYSTEM

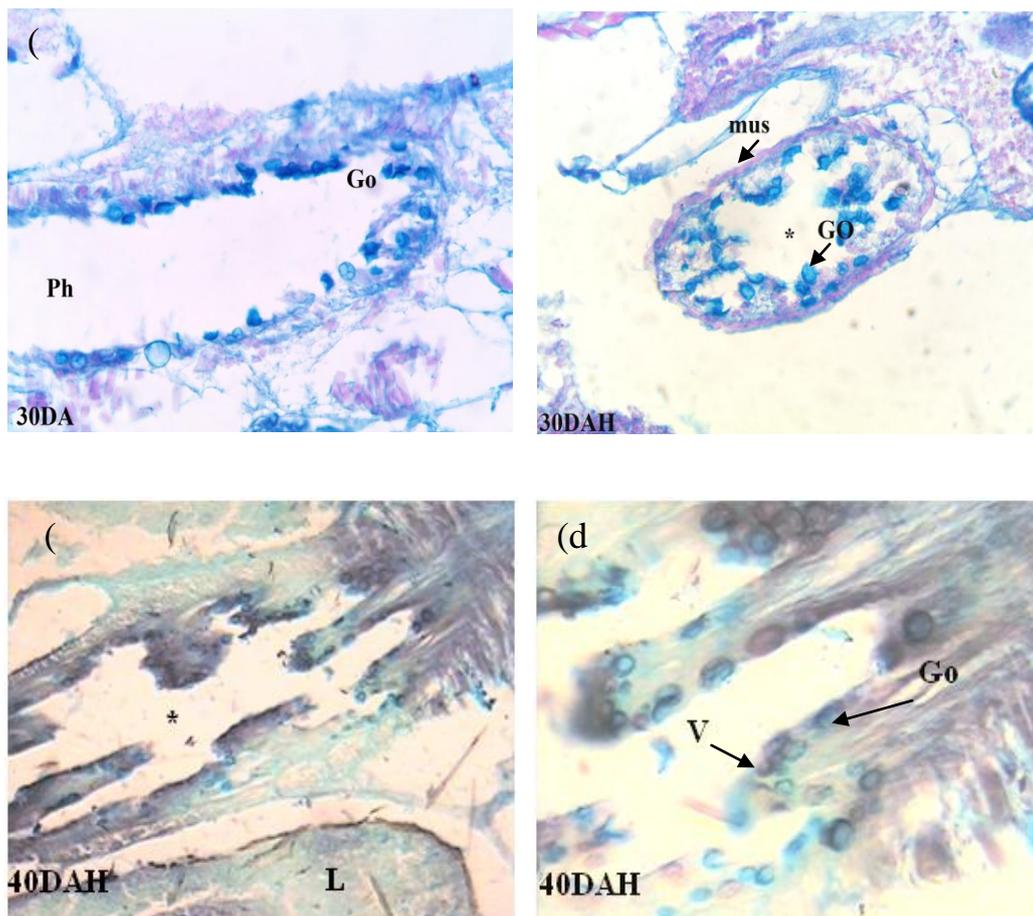


Fig. 7: Photomicrograph of transverse section (TS) in digestive tract of larvae *S. vulgaris* showing; pharynx (Ph), liver (L) and the esophagus (eso) with narrow lumen (*) lined by goblet cells (Go) placed on the villous (V), and muscularis layer (mus), (PAS/Alcian blue). a). TS for buccopharyngeal cavity at 30 days after hatching larvae (X250) , b).TS for esophagus at 30 days after hatching larvae (X100) c). TS for esophagus at 40 days after hatching larvae (X250). d). Magnification of (c) (X400).

into folds-villi, sometimes very long, to increase its surface. The mucosa functions essentially secreting a mucous substance and digestive enzymes.

At the age of 10DAH, coinciding with the increase in length of the intestine, the anterior region is convoluted in the middle part of the intestine (Fig. 14, a&b). The height of villi is about $50 \pm 3 \mu\text{m}$. No acidic mucosubstance were detected in the intestine mucous cells until age 20DAH. The goblet cells

at this age are firstly detected in the intestine, their number increased with the ontogenetic differentiation of the intestinal mucosa, being more abundant in the intestine (Fig. 14, c&d). The height of villi is about $57 \pm 2 \mu\text{m}$. The gut is elongated gradually with larval development and an increase in dense granules, vesicles and vacuoles and also the thickness of the brush border in the intestine was observed.

At age of 40DAH, the convolution of the intestine is more prominent, especially in the

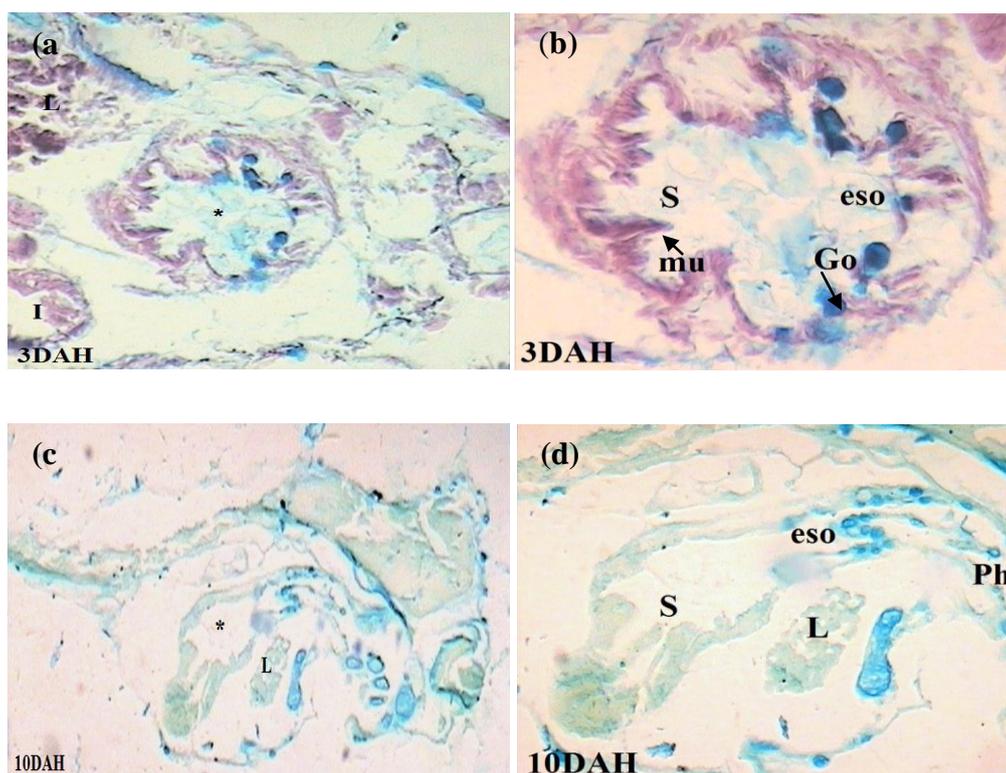


Fig. 8: Photomicrograph of longitudinal section (LS) of larvae *S. vulgaris* showing; the transition from esophagus (eso) to the stomach (S) with a replacement of goblet cells (Go) by mucosa cells (mu), (PAS/Alcian blue). a. LS at 3 days after hatching larvae (X100), b. Magnification of (a) (X250) c. TS at 10 days after hatching larvae (X40). d. Magnification of (c) (X100).

anterior region (Fig. 15, a&b). The mucosal intestine forms with the submucosa a prominent spiral fold, called the spiral valve. The height of villi is about $109 \pm 13 \mu\text{m}$ (Fig. 15, c&d).

The number of mucous cells scattered among enterocytes increased towards the posterior intestine, and histochemical properties of their secretory products varied in some aspects. Most mucous cells of the proximal intestine stained violet with PAS/AB, but others were blue only, showing variable contents of acid and neutral glycoconjugates, or acid glycoconjugates only. Very few mucous cells show PAS staining only.

DISCUSSION

The major morphological changes of the digestive system of *S. vulgaris* larvae happened during the first 2DAH, when larvae were exclusively dependent on endogenous reserves. Similarly to most marine finfish species, newly hatched *S. vulgaris* had an undifferentiated and undeveloped digestive tract. The opening of the mouth, and therefore the start of exogenous feeding determines regional gut differentiation in most teleost species (Boulhic & Gabaudan 1992; Sarasquete *et al.*, 1995; Gisbert *et al.*, 2004 and Mai *et al.*, 2005).

During the rest of the studied period the increase in size and complexity of the structures

MORPHOLOGICAL AND HISTOCHEMICAL ONTOGENY FOR LARVAL *SOLEA VULGARIS* DIGESTIVE SYSTEM

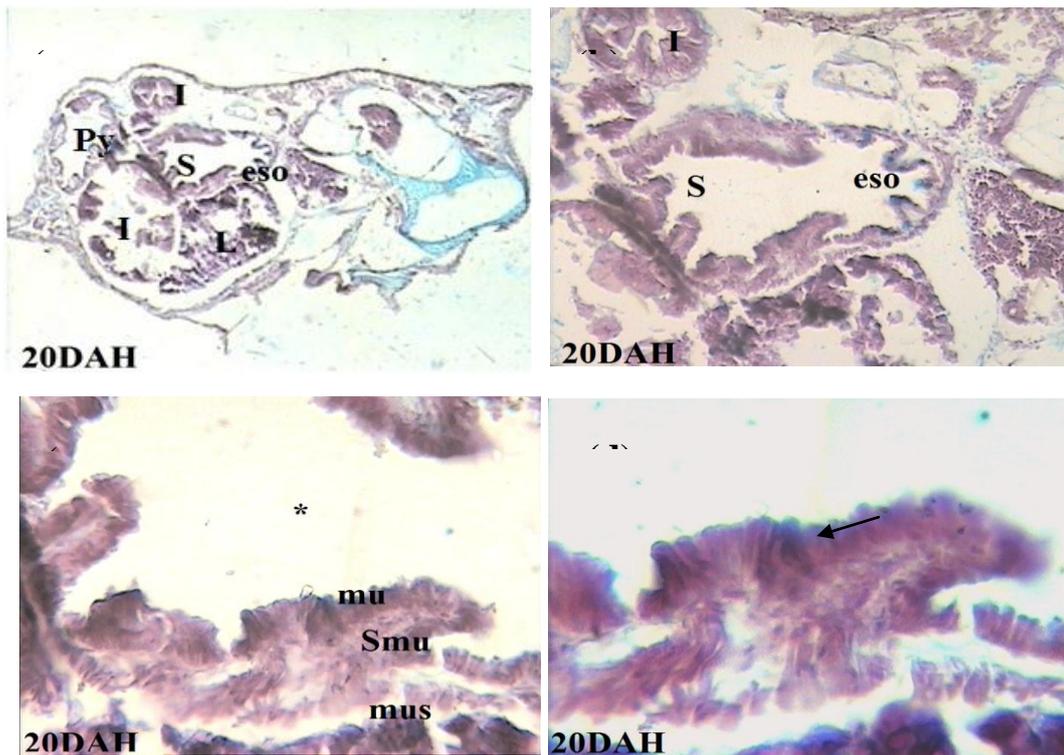


Fig. 9: Photomicrograph of transverse section (TS) in digestive tract of *S. vulgaris* larvae showing; esophagus (eso), intestine (I), liver (L), pyloric sphincter (Py) and stomach (S), which contain of mucosa (mu), submucosa (Smu), muscularis (mus), lumen (*), (PAS/Alcian blue). a. TS at 20 days after hatching larvae (X40). b. Magnification of (a) (X100). c. Magnification of (a) (X250). d. Magnification of (a) (X400).

studied were the alterations observed. The growth obtained for this species was considered normal, and was similar to the growth observed by other authors for the *S. senegalensis* (Dinis, 1992; Dinis and Reis, 1995) and *S. vulgaris* (Assem *et al.*, 2012).

In *S. vulgaris*, five distinctive histologically regions: buccal-pharyngeal cavity, esophagus, cardiac and pyloric stomach and intestine are observed at the onset of exogenous feeding phase. The early differentiation and regionalization of the digestive tract in such a short period of time is a common feature of marine finfish species developing from small pelagic eggs, since yolk-sac reserves are depleted soon after hatching, and larvae need to

start to feed exogenously at early stages of development.

From hatching until early 3DAH, *S. vulgaris* larvae depended exclusively on their yolk-sac reserves, while a period of mixed nutrition based on endogenous reserves and live preys (rotifers) was noted up to 3-4DAH.

At 3DAH *S. vulgaris* larvae were sufficiently developed for successful first feeding. Similar results were described for other flatfishes; *C. fera* (Loewe and Eckmann, 1988), *S. solea* (Boulhic and Gabaudan, 1992), *P. dentatus* (Bisbal and Bengtson, 1995), *S. senegalensis* (Ribeiro *et al.*, 1999) and also *S.*

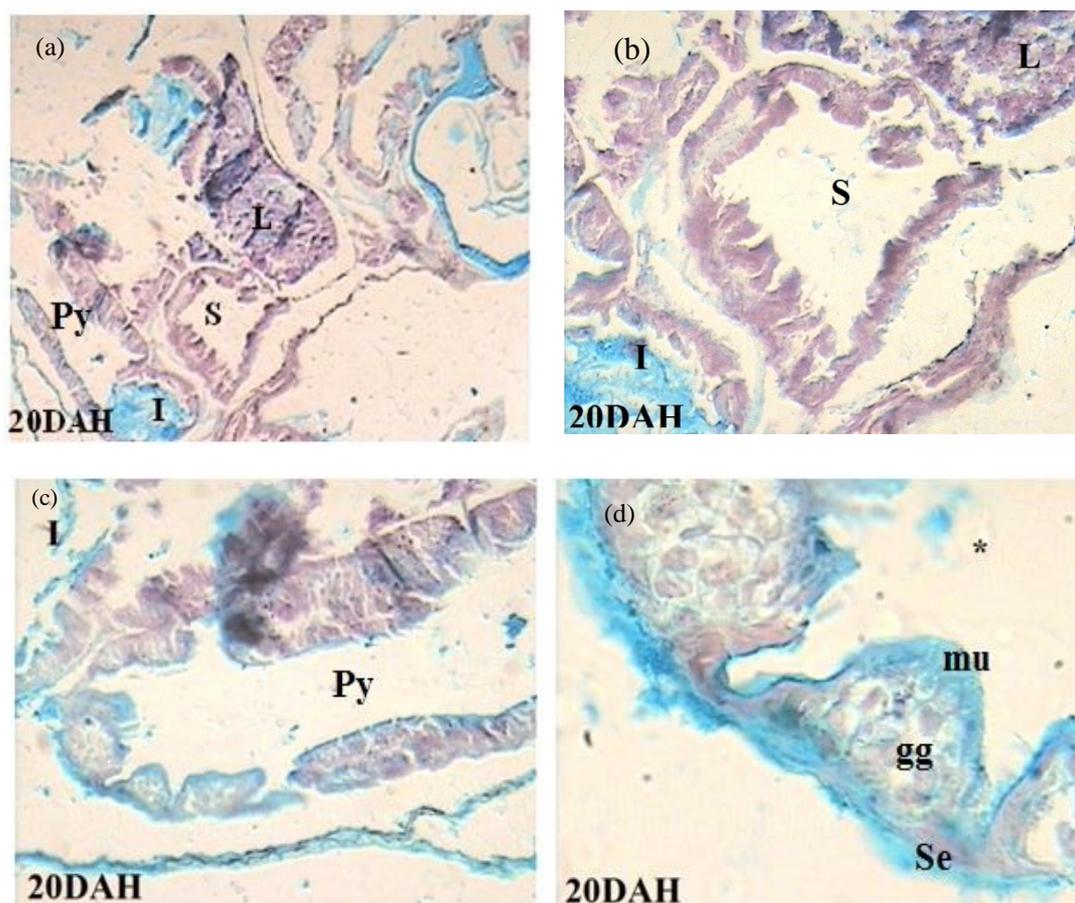


Fig. 10: Photomicrograph of transverse section (TS) of digestive tract in larvae *S. vulgaris* showing; intestine (I), liver (L), esophagus (eso), the gastro-intestinal junction. The two region of stomach; cardiac (S) and pyloric sphincter (Py), which consist of mucosa (mu) lined the lumen (*), muscularis layer contains a gastric glands (gg) and surround by the serosa (Se) . (PAS/Alcian blue). a). TS at 20 days after hatching larvae (X100) , b).Magnification of (a) (X250) c). TS at 20 days after hatching larvae (X250). d). Magnification of (c) (X400).

vulgaris (Assem *et al.*, 2012) during the yolk sac period.

This period is considered as one of the most critical events during larval early life stages, since short delays in feed availability influence posterior larval growth and survival. In the current study, the presence of functional oesophageal goblet cells secreting mucins, which are known to play an important role in

protecting the mucosa against bacterial attack and, physical and chemical damage (Mai *et al.*, 2005).

The presence of mucous cells in the esophagus on day 3DAH, previously reported for *S. solea* (Boulhic and Gabaudan, 1992), *S. senegalensis* (Sarasquete *et al.*, 1996) and *S. senegalensis* (Ribeiro *et al.*, 1999), and also the

MORPHOLOGICAL AND HISTOCHEMICAL ONTOGENY FOR LARVAL *SOLEA VULGARIS*
DIGESTIVE SYSTEM

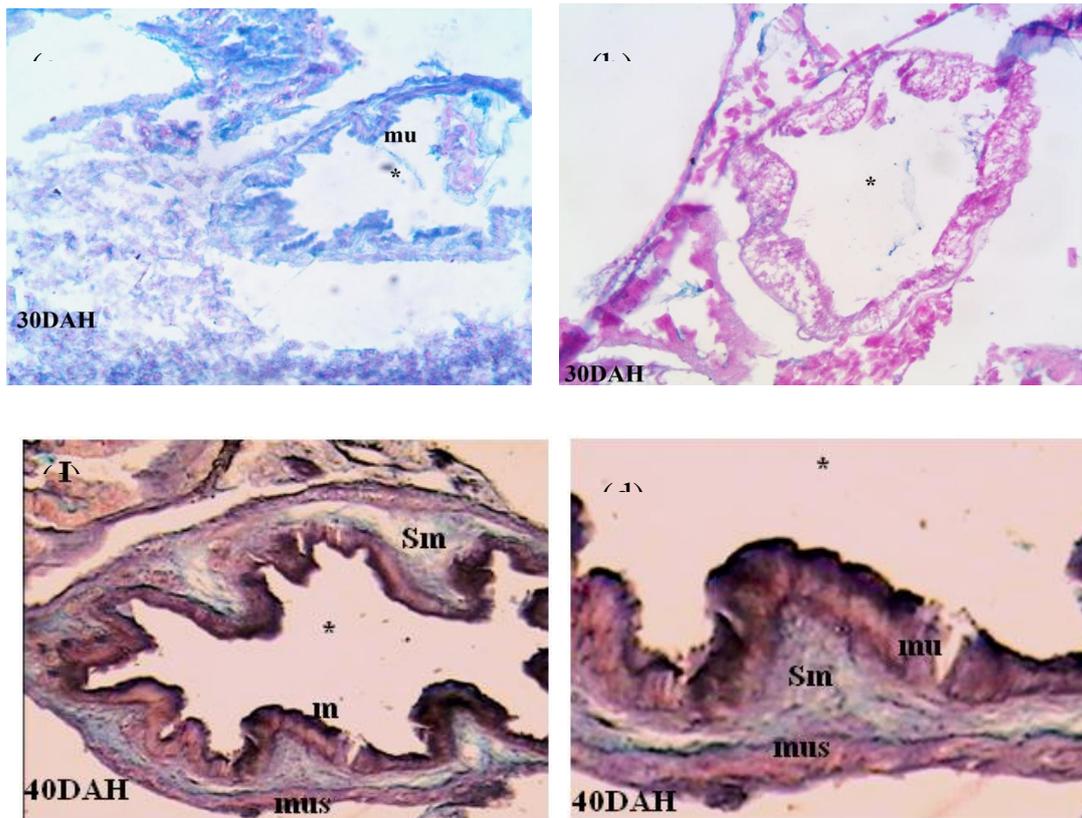


Fig. 11: Photomicrograph of transverse section (TS) of digestive tract in juvenile *S. vulgaris* showing; the two region of stomach; cardiac (S) and pyloric sphincter (Py), which contain of mucosa (mu), submucosa (Smu), muscularis (mus), lumen (*), (PAS/Alcian blue). a). TS of cardiac stomach at 30 days after hatching (X100) , b). TS of pyloric sphincter at 30 days after hatching (X100) c). TS at 40 days after hatching (X250). d). Magnification of (c) (X400).

strong muscular layer, coincides with the establishment of exogenous feeding. In other species these mucous cells appear later during ontogeny (Sarasquete *et al.*, 1995).

The number of mucous cells on the gut of *S. vulgaris* increased with larval development. The components of mucous cells were mainly sulphated mucosubstances and to a lesser extent carboxylated muco-substances. Boulhic and Gabaudan (1992) also described acidic mucosubstances for *S. solea*. Increased epithelial stratification in correspondence with the greater number of goblet cells and the acid

glycoproteins content have been related with a supportive function for the esophageal mucosa (Baglolle *et al.*, 1997). Murray *et al.* (1994), studying pleuronectid fishes, stated that apart from the lubrication role played by the mucous cells, the esophagus may have a function in pregastric digestion, through the collaboration of the elaborated mucosa folding, specialised mucous or enzyme secreting epithelial cells and a complex histochemistry of the mucous.

As previously mentioned in *S. vulgaris*, esophageal mucous cells are developed throughout the early life stage during the

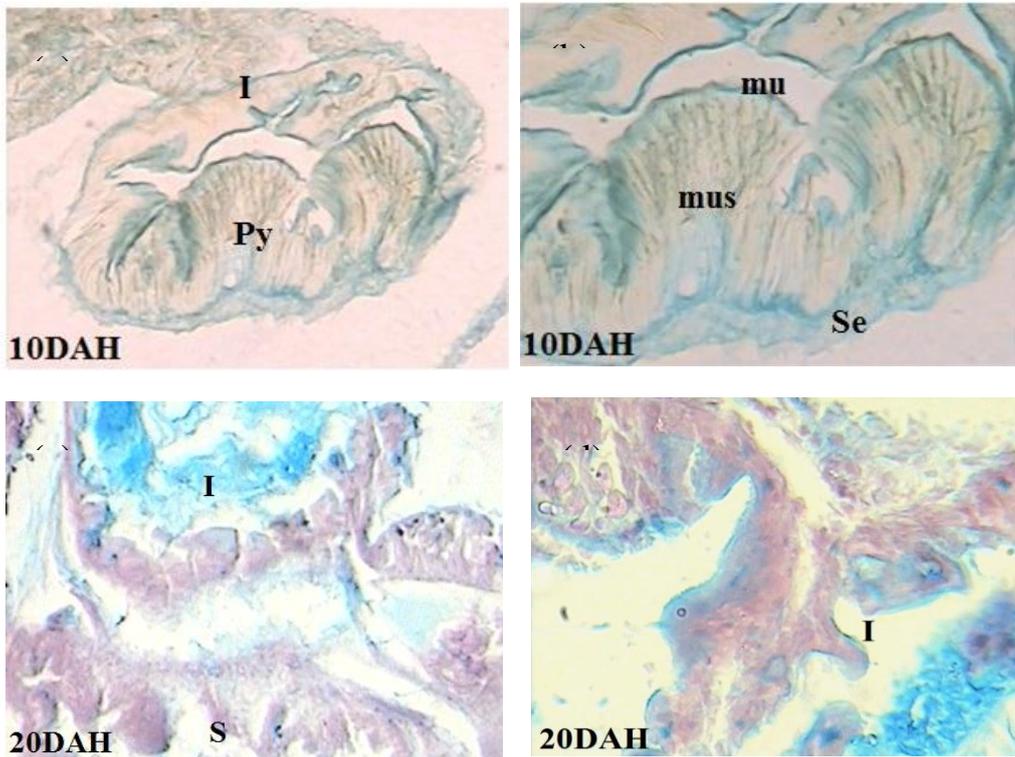


Fig. 12: Photomicrograph of transverse section (TS) in digestive tract of *S. vulgaris* showing; the transition from the stomach (pyloric sphincter, Py) to the intestine (I). mucosa cells (mu) lined the lumen (*), muscularis (mus) and serosa (Se), (PAS/Alcian blue). a). TS of larvae at 10 days after hatching (X250) , b). Magnification of (a) (X400) c). TS of larvae at 20 days after hatching (X250). d). Magnification of (c) (X400).

transition from endogenous to exogenous feeding. The differentiation of esophageal mucous cells is a species-specific process in marine teleosts, which normally takes place coinciding with the onset of exogenous feeding, as it has been noted for *S. vulgaris* (present study), *Solea solea* L. (Boulhic & Gabaudan, 1992), *Paralichthys dentatus* L. (Bisbal & Bengtson, 1995), *Pleuronectes ferruginea* (Storer) (Baglole *et al.*, 1997) and *S. senegalensis* Kaup, 1858 (Ribeiro *et al.*, 1999), or at later stages of development, as observed in *Melanogrammus aeglefinus* (L.) (Hamlin *et al.*, 2000) *Paralabrax maculatofasciatus* (Peña *et al.*, 2003) and *P. californicus* (Ayres) (Gisbert *et*

al., 2004). The reasons for such different schedules in the esophagus development remain not clear, but they might be related to the different ecology, feeding habits and/or taxonomical position of species (Mai *et al.*, 2005).

Although a cardiac stomach appears in *S. vulgaris* (3DAH) soon after first feeding, the development of gastric glands in the cardiac stomach, as well as the presence of neutral mucosubstances within stomach mucous cells, which protect the digestive mucosa from auto-digestion processes caused by hydrochloric acid and enzyme secretions produced by gastric glands, suggested that the stomach was not

MORPHOLOGICAL AND HISTOCHEMICAL ONTOGENY FOR LARVAL *SOLEA VULGARIS*
DIGESTIVE SYSTEM

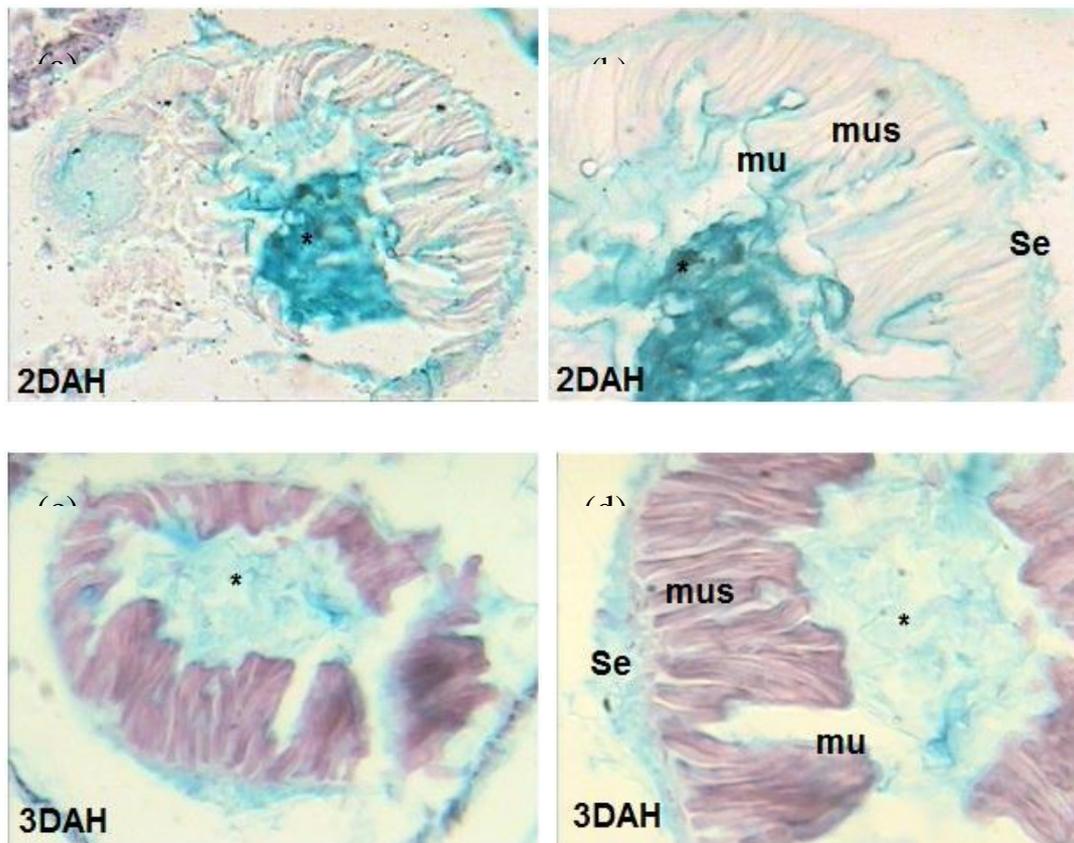


Fig. 13: Photomicrograph of transverse section (TS) in digestive tract of larvae *S. vulgaris* showing; intestine, which contain of mucosa cells (mu) lined the lumen (*), muscularis (mus) and serosa (Se), (PAS/Alcian blue). a). TS of larvae at 2 days after hatching (X250), b). Magnification of (a) (X400) c). TS of larvae at 3 days after hatching (X250). d). Magnification of (c) (X400).

functional until 21 ± 2 DAH, after the metamorphosis.

The incipient stomach is distinguished from 2DAH onwards; it was only after metamorphosis that we observed the presence of gastric glands. According to Tanaka (1973), the appearance of gastric glands indicates the beginning of the juvenile stage. Vu (1983), working with *Dicentrarchus labrax* described the differentiation of gastric glands around day 25 after hatching, but these were only functional when the fish became adults. In other study by Ribeiro *et al* (1999), the gastric glands were

detected in *S. senegalensis* around 27DAH. Fish larvae without a functional stomach are known to have immature digestive mechanisms, especially in terms of protein digestion. This has been presented by several authors as one of the problems for the development of fish larvae diets (Segner *et al.*, 1994).

Walford and Lam (1993) reported that in the absence of a functional stomach, the anterior intestine is responsible for food digestion, with a pH that remains alkaline and where trypsin-type activity is responsible for proteolytic digestion.

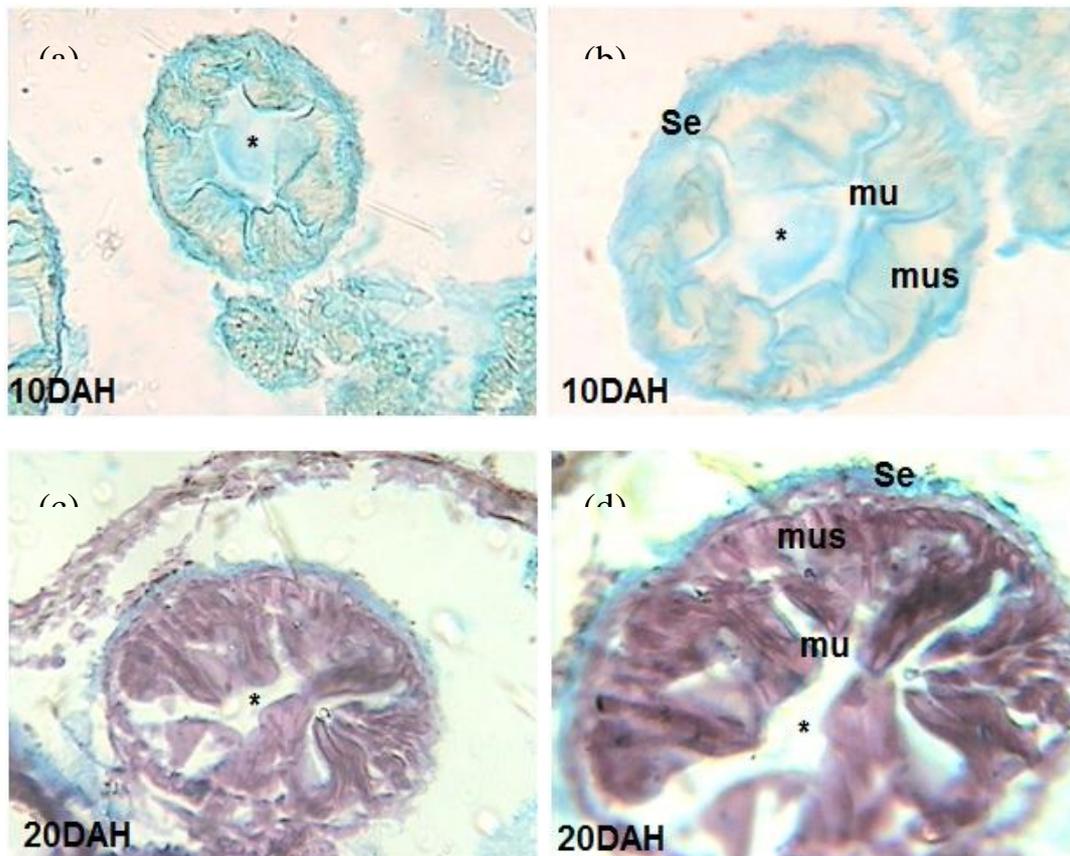


Fig. 14: Photomicrograph of transverse section (TS) in digestive tract of larvae *S. vulgaris* showing; intestine (I), which contain of mucosa cells (mu) lined the lumen (*), muscularis (mus) and serosa (Se). pharynx (Ph), upper (UJ) and lower (LJ) jaw, (PAS/Alcian blue). a). TS of larvae at 10 days after hatching (X250) , b). Magnification of (a) (X400) c). TS of larvae at 20 days after hatching (X250). d). Magnification of (c) (X400).

In the present study, no lipid inclusions are observed in the anterior segment of the intestinal mucosa of *S. vulgaris*, although lipid vacuoles are increased in the liver throughout development. These results are similar to those already reported in *Hipoglossus hipoglossus* (Linnaeus, 1758) (Luizi *et al.*, 1999), *P. californicus* (Gisbert *et al.*, 2004) and large yellow croaker (Mai *et al.*, 2005). The above-mentioned authors suggested that the lack of such lipid vacuoles in the intestine of *Artemia*-fed larvae could be interpreted as a sign of

reduced intestinal lipid digestion due to the rapid passage of *Artemia* through the alimentary canal of larvae (Luizi *et al.*, 1999); whereas, Gisbert *et al.* (2004) hypothesized that the lipid content of feed did not exceed the fatty acid absorption and exporting capacities of enterocytes, resulting in no accumulation of lipids in the intestinal mucosa.

The complete differentiation of the digestive system in *S. vulgaris* is achieved with the occurrence of metamorphosis between 19 and 22DAH, indicating the end of the larval

MORPHOLOGICAL AND HISTOCHEMICAL ONTOGENY FOR LARVAL *SOLEA VULGARIS*
DIGESTIVE SYSTEM

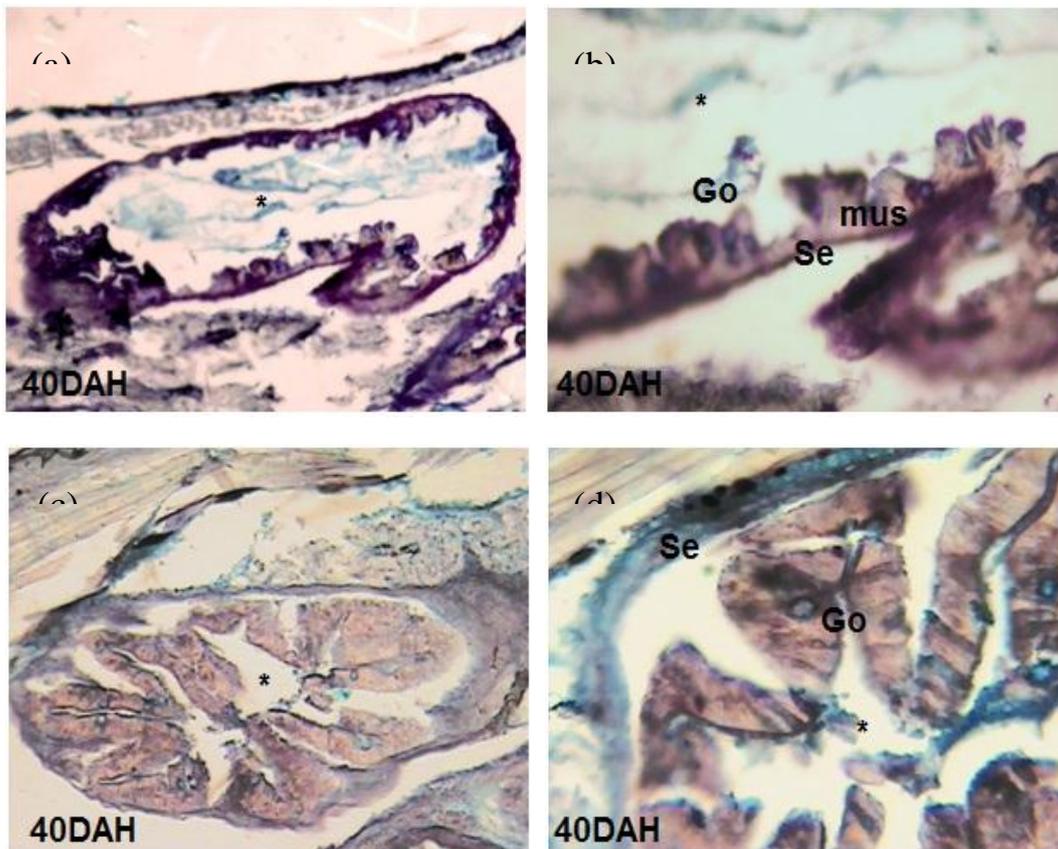


Fig. 15: Photomicrograph of transverse section (TS) in digestive tract of juvenile *S. vulgaris* showing; intestine (I), which contain of mucosal layer lined the lumen (*) by goblet cells (Go), muscularis (mus) and serosa (Se), (PAS/Alcian blue). a). TS of juvenile at 40 days after hatching (X250) , b). Magnification of (a) (X400) c). TS of juvenile at 40 days after hatching (X250). d). Magnification of (c) (X400).

period and the transition to the juvenile stage. The lack of a glandular stomach has generally been considered as a limiting factor in feeding larvae with compound diets (Segner *et al.*, 1993; Bagogle *et al.*, 1997). However, the late differentiation of a functional stomach in *S. vulgaris* could be prevent weaning larvae onto a dry diet until metamorphosis, and further studies must be conducted to determine the best moment to feed larvae with compound diets.

Until the end of the study period an increasing of the folding of the digestive tract

mucosa, mainly of the stomach and the intestine and the thickness of brush border was observed, indicating an increase in digestive and absorption area that come in agreement with study by Ribeiro *et al.*, (1999) on *S. senegalensis* larvae. However, according to Segner *et al.* (1989), this increase could be related to the increase of intestinal surface volume.

The analysis of data obtained in this study suggests that after 30DAH *S. vulgaris* larvae are capable of ingesting, digesting and

absorbing nutrients, having a morphologically complete digestive tract and are equipped with digestive enzymes that come in agreement with study by Ribeiro *et al.*, (1999) on *S. senegalensis* larvae. Nevertheless, Segner *et al.* (1989) reported that *Coregonus laietanus* larvae, with a complete digestive tract and possessing digestive enzymes, were unable to survive on dry diets. So, as this author pointed out, perhaps the larval digestive enzymes have difficulty in adapting to nutrients in dry diets.

CONCLUSION

In conclusion, the ontogenetic development of the digestive system in *S. vulgaris* followed the pattern in marine species described thus far, although there existed some differences in the differentiation time between different species, which reflect variations in the factors affecting larval development, such as egg size, incubation temperature, breeding conditions, larval nutrition or genetic origin. Further studies comparing the development and functionality of the digestive system of larvae fed different diets (i.e. compound vs. live feed) will enable a better understanding of the required feeding procedures and rearing protocols.

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MORPHOLOGICAL AND HISTOCHEMICAL ONTOGENY FOR LARVAL SOLEA VULGARIS
DIGESTIVE SYSTEM

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تطور الجهاز الهضمي في أسماك موسى المصري (سوليا فولجارس): دراسات مورفولوجية وهستوكيميائية في اليرقات والأسماك اليافعة

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الملخص العربي

ان الهدف من هذه الدراسة هو توضيح مراحل تطور الجهاز الهضمي ليرقات أسماك موسى من بعد الفقس وحتى تصبح أسماك يافعه (40 يوم بعد الفقس). من اهم التغيرات المورفولوجية التي تم ملاحظتها خلال مراحل تطورها المبكرة، أن بعد مرور يومين من الفقس فتح كلا من الفم وفتحة الشرج. بمرور يوم آخر على الفقس نلاحظ ان الجهاز الهضمي زاد في الطول. بعد التحول (19- 22 يوم بعد الفقس) ، نجد أن المعدة أخذت الشكل الكيسي ومحدده من الجانبين المقعريين بالكبد مشكلين معا أوسع جزء في التجويف البطنى. كذلك تتميز اليرقات والاسماك اليافعة لاسماك موسى بانها تحتوى على معدة صغيرة وأمعاء طويلة. خلال دراسة الانسجه كيميائيا، ظهر عدد قليل من خلايا جوبلت الحامضية مبعثرة ما بين الخلايا الطلائيه فى قطاعى التجويف البلعومى والمرئ على حدا سواء. تم تمييز بدايات المعدة بعد مرور 3 ايام على الفقس، نجد أن طبقة الميكوزا تتكون من مواد مخاطيه كبريتيه. بعد التحول، نجد أن الخلايا الطلائيه المكونه لطبقة الميكوزا فى المرئ زادت فى إحتوائها على خلايا جوبلت. فى أغلب الإعتقاد أن المادة المخاطيه المفرزه تلعب دور كبير فى عملية التشحيم. تنقسم المعدة الى منطقتين القلبي والبوابى. فى الأمعاء، حدود الخلايا الطلائيه اظهرت نشاط حامضى للمواد المخاطيه. تحتوى الخلايا الطلائيه بالامعاء على مواد مخاطيه كبريتيه. نجد أن الجهاز الهضمي لآسماك السوليا فولجارس تتكون من خمس أجزاء: التجويف الفمى البلعومى، المرئ، المعدة القلبيه والبوابيه والأمعاء. بعد مرور 30 يوم من الفقس، فإن الجهاز الهضمي يظهر نفس التركيب النسيجي الذى يظهر عند الأسماك الناضجة.