

Histological changes in the digestive tract of striped murrel larvae during ontogeny

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In this study, the ontogeny of the digestive tract was studied histologically in striped murrel *Channa striatus* from hatching to 25 days post-hatching (DPH). At hatching, the digestive tract of striped murrel consisted of a straight tube dorsally attached to the yolk sac. At 2 DPH, the mouth opened, oral valves were visible and taste buds were detected between 2 and 3 DPH. During this period, intestine was differentiated into the anterior and posterior intestine, and the digestive accessory glands were also developed. Exogenous feeding started at 3DPH, and there was a 2-day mixed endogenous-exogenous feeding period. Most of the yolk sac reserves were consumed between 2 and 3 DPH, and by 5 DPH, the yolk sac was completely depleted and no longer visible in histological sections. At 10DPH, differentiation of gastric glands was noticed, and by 11-13DPH, there were abundant gastric tubular glands arranged along numerous longitudinal folds. From 15DPH to the end of the study at 25DPH, no noticeable histological modifications were observed. Hence, it is suggested that, striped murrel larvae have a morphologically complete digestive tract by 15DPH.

[**Keywords:** *Ontogeny, Larvae, Digestive tract, Histology, Striped murrel*]

Introduction

Striped murrel, *Channa striatus* is indigenous to many tropical countries and a valuable fish throughout Asia Pacific region and probably the main food fish in Thailand, Indo-China and Malaysia because of its firm, white and practically boneless flesh which has an agreeable flavour¹. Fish larvae are characterized by an incomplete organogenesis resulting in different organ functions compared to juvenile and adult fishes². As a critical period of fish life history, the larval stage is very important because it involves morphological and functional changes in the internal organs, especially the digestive system³. A successful development of the digestive system equips larval fish to digest and absorb food, increasing the survival rate during the larval and juvenile periods. Knowledge of gastrointestinal tract (GIT) developmental changes associated with the process of food assimilation is essential for understanding the nutritional physiology of larval fish⁴. Larval stages constitute a critical period during

life history of fish, where important structural and functional changes in body tissues and organs take place. For this reason both the success and progress of larviculture depends on the knowledge of the development of such elements, in order to adjust culture conditions to the ontogenic status and health of the larvae. Successful development of the digestive system is crucial for the survival and growth in fish larvae because an efficient digestive system enables fish to capture, ingest, digest and absorb food⁵. Although fish larvae may be morphologically capable of capturing food items at first feeding⁶, their digestive system needs a series of developmental changes before being fully functional³. The basic mechanisms of organ and system development are similar in all teleosts, but there are considerable inter-specific differences regarding the relative timing of differentiation, development and functionality during early ontogeny⁷. Hence, there is a need to conduct specific studies on the ontogenesis of fish digestive system for each species to better understand their nutritional physiology. The assessment of condition

by means of microscopic methods is probably the most accurate indicator of nutritional status of fish larvae⁸. This knowledge may help in identifying limiting factors during larval rearing, reducing bottlenecks in the weaning process and optimizing the rearing technology and feeding practices with the developmental stage of the fish.

Studies on the ontogenesis of the digestive system in some commercially important fish species have been carried out in recent years⁹. Most of these studies have been focused on marine fish species, but very little research has been done on freshwater aquaculture species¹⁰. To date, no detailed study has been documented on the ontogeny of the digestive system in striped murrel, one of the most interesting and promising species for diversification in freshwater aquaculture in the Indian subcontinent. However, the success and development of an aquaculture activity devoted to striped murrel culture still demands some rearing improvements, especially those affecting larval rearing practices at early stages of development such as the partial or complete replacement of live prey with a micro diet. Thus, in order to enhance the success of larval rearing of this air breathing fish species and facilitate overcoming one of the major bottlenecks of fish hatcheries, the description of the ontogeny of the larval digestive system is a necessary tool. This information will be of value for synchronizing the larval stage of development and maturation of their digestive organs with the feeding protocol and rearing practices. The present study aimed to describe the morphological and histological structure of digestive tract and accessory digestive organs during the ontogeny of striped murrel, from hatching to 25 days post-hatching (DPH), (Fig.1). This information is expected to provide fundamental knowledge for improving actual larval rearing practices for this murrel species.

Materials and Methods

Fertilized eggs from the same batch of brood striped murrel were collected from Centre for Aquaculture Research and Extension (CARE) Aquafarm (India). The eggs were produced through induced spawning under captive conditions. Female striped murrel (690–770 g) and male striped murrel (640–680 g) were injected with Luteinizing hormone releasing hormone analogue (LHRHa) at a dosage of 70 µg kg⁻¹ body weight (BW). Injections were administered intramuscularly in the dorso lateral

region of the body.

After hatching (23 ± 1 h post-fertilization), the larvae were stocked in three fibre glass tanks (20 l) at the density of 5 larvae l⁻¹. Tanks were connected to a flow-through water system with an exchange rate of 1.4–2.0 min⁻¹, where fish were reared until the juvenile stage (25 DPH). Two air stones were used in each tank to maintain dissolved oxygen at saturation and also to promote a homogeneous distribution of live feed. Larvae were fed to apparent satiation four times per day with non-enriched *Artemia nauplii* (O.S.I. PRO 80TM, Ocean Star International, Inc. USA) from mouth opening (3 DPH) until 9 DPH. From 7 DPH onwards, zooplankton collected from nearby ponds, which consisted mainly of copepods (Cyclopoida), were also added to fish rearing tanks. After 9 DPH, only zooplankton was given. Excess feed and faeces were daily removed before feeding. During the rearing period, water temperature, dissolved oxygen and pH values were maintained at (26.5 to 28°C), (5.9–7.3 mg/l) and (6.5 -7) respectively. Fish were held under natural photoperiod (13:11 h light and dark).

A random sample of 10 larvae was daily collected from hatching (0 DPH) to 12th DPH and thereafter on 14th, 16th, 20th and 25th DPH from the rearing tanks and immediately fixed in Bouin's solution for subsequent histological analysis. Within

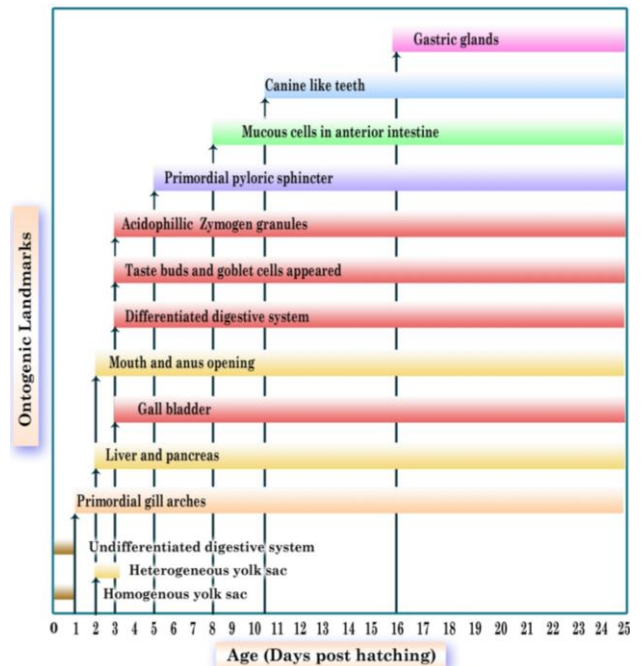


Fig. 1— Main histological ontogenic landmarks during the larval development of *C. striatus*.

48 hrs, the larvae were processed for histological features by following standard procedures¹¹. Larvae were processed, embedded in the paraffin wax as blocks. The paraffin wax block with the embedded larvae was trimmed and sections of 8 μm tissue thickness were taken using an Erma Rotatory microtome. Tissue sections along with the wax were then kept in the glass slide and floated on a hot water using distilled water. The flattened sections were collected on clean glass slides coated with Gelatine/Glycerol and dried overnight. For optimal adhesion the slides were placed in an oven at 60°C for 1 hr.

Hematoxylin and eosin staining was done by following the standard procedures¹². The sections were washed using xylene and then treated with 100%, 90% and 70% grades of alcohol and then dipped in water. Slides were stained with hematoxylin for 10 min followed by washing with running tap water until the section became blue. Further slides were stained in 1% eosin for 1 min followed by washing with running tap water for 4-5 min. The sections were dehydrated in ascending grades of alcohol, cleared in xylene and the sections were mounted in DPX mountant. The sections were examined and photographed using Nikon microscope (U III E-400 Eclipse) at different magnifications.

Results and Discussion

At hatching (3.2 ± 0.2 mm TL), the digestive tract of striped murrel appeared as a straight undifferentiated tube dorsally attached to the yolk sac that occupied most of the abdominal cavity. Larvae had a large ovoid yolk sac lined by a syncytial epithelium (Fig.2). The yolk sac of *C. striatus* larva was ovoid in shape occupying about 45% of the total length. During the yolk resorption, yolk sac matrix showed appearance of the resorption granules at the periphery. By the end of 2 DPH (5.4 ± 0.3 mm TL), fragmentation of the yolk was seen, as resorption preceded the yolk appeared granular and began to particularise in heterogeneous drops, until yolk sac reserves were completely exhausted at 3 DPH (5.8 ± 0.5 mm TL). At 3 DPH hatchling's buccal opening became functional and larva commences exogenous feeding. Lecithotrophic period of the yolk sac stage started at 3 DPH with the moment of first feeding, when mouth and anus were opened, and this period was characterized by the coexistence of endogenous (yolk sac) and exogenous (zooplankton) food sources.

At 4 DPH, the yolk sac was completely depleted and no longer visible in histological sections (Fig. 3d-f).

At 1 DPH posterior part of the digestive tract was bent ventrally and mouth and anus were closed (Fig. 3c-d). Digestive tract lumen was narrow with a tendency to widen at posterior end. Mouth was formed at 2 DPH with well-developed lower jaw (Fig. 3e-f). At 3 DPH lumen in the hindgut region became wider, the anal pore was opened, and mouth and pharynx started to be opened and were fully opened by 4 DPH (Fig.3 a-f). The formation of the intestinal valve at 4 DPH divided the incipient intestine into mid-gut and hindgut. Esophagus and stomach were also distinguished.

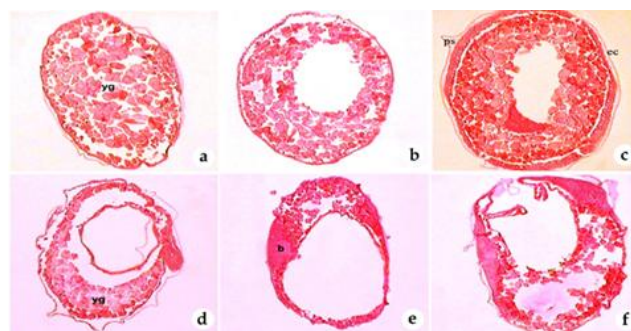


Fig. 2— Histology of egg showing active cytoplasm and yolk (yo-yolk granules; ps-previtelline space; ec-egg capsule; b-blastodisc).

At 0 DPH, buccopharyngeal cavity was not well formed (Fig. 3a-b). However, at 1 DPH the buccal cavity was lined by a layer of squamous epithelium, while the number of layers increased with age (Fig. 3c-d). At 3 DPH, a well developed gill arch was noticed (Fig. 5). Buccal cavity lined by stratified squamous epithelium led into the pharynx. Ventrolaterally pharynx communicated with the branchial chamber through four pairs of gill slits at 6 DPH. Taste buds and goblet cells were visible at 3 DPH when larvae started exogenous feeding. Between 10 and 11 DPH, goblet cells increased in number. At this age, canine like teeth were observed protruding in both oral valves and in the posterior region of the buccopharyngeal cavity and increased in number and size according to larval development. At the end of the 15-17 DPH, goblet cells and taste buds were visible throughout the buccopharyngeal epithelium, being more abundant in the anterior region of the buccal cavity. No noticeable histological changes were observed after 11 DPH until the end of the study at 25 DPH (26.69 ± 0.71 mm TL).

Esophagus in striped murrel larvae appeared

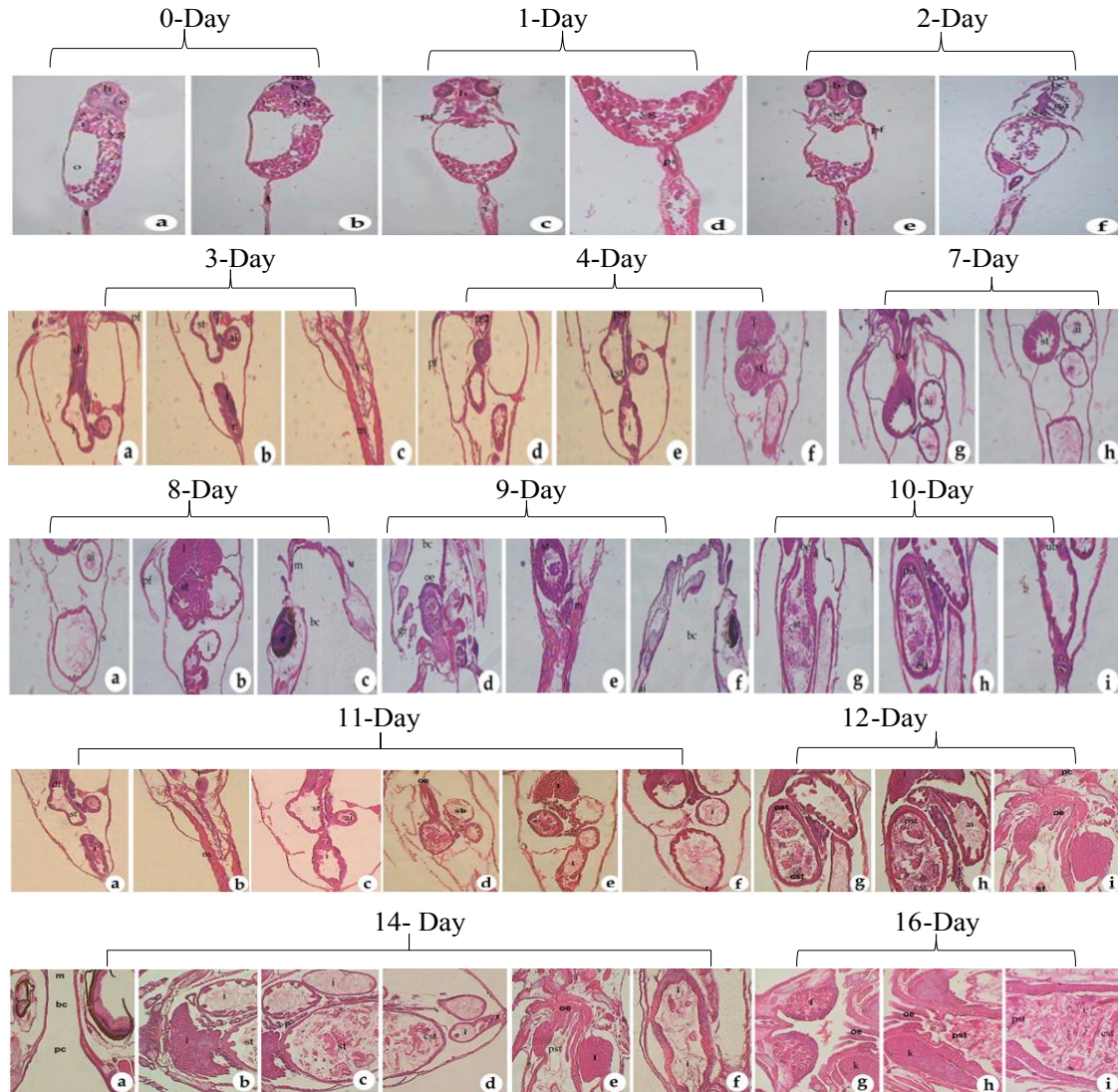
as narrowing of the pharynx at 2 DPH. Two different regions were distinguished in the esophagus, based on the histological features. The anterior region lined by a pseudostratified ciliated epithelium and the posterior region dilated and lined by a simple cuboidal epithelium. No remarkable histological changes were detected until 7–8 DPH, when the oesophagus longitudinal folds developed to accommodate the passage of large food items, as well as the muscular layers surrounding it that became thicker (Fig. 3g). Goblet cells appeared from 6 DPH and increased in number in the anterior region of esophagus mucosa, and longitudinal folds increased both in length and number as larvae grew. At later stages of development, the histological organization of the oesophagus did not change with the exception of the increase in the number and size of mucous cells and the posterior region of the oesophagus, which connected it with the glandular stomach.

Stomach was the last organ of the digestive system to differentiate. Incipient stomach appeared as a bulge in the middle of the digestive tract at 3 DPH (Fig. 3a). The narrow posterior part of the esophagus opened into the muscular stomach. Abrupt change from a stratified epithelium to a simple cubic epithelium made a distinction between the esophagus and the incipient stomach. At 5 DPH, the primordial pyloric sphincter was developed and divided the incipient stomach from the anterior intestine. At 8 DPH, the stomach had increased in size and several epithelial folds had appeared, and the mucosa was surrounded by an external circular layer of muscle cells (Fig. 3a-c). Stomach showed three different regions according to histological observations, viz cardiac (anterior), fundic (median) and pyloric (posterior). Around 14 DPH (14.6 ± 0.3 mm), clusters of cubic cells on the ventral side of the gastric submucosa were observed that would later develop into gastric glands. Gastric glands were fully developed and surrounded by a thin layer of connective tissue at 16 DPH (15.1 ± 0.2 mm) as shown in Fig. 3 (g-i). Around 20 DPH (22.9 ± 0.5 mm), the gastric glands proliferated actively and fully developed at 25 DPH. With the growth of the larvae, the number of gastric glands were found increased in the fundic region and to lesser extent in the cardiac stomach, however they were not observed in the pyloric part of the stomach. The pyloric region was folded and lined by a short ciliated columnar

epithelium.

At hatching, the incipient intestine appeared as a straight translucent tubular segment laying dorsally to the yolk sac. Lumen of intestine appeared at 1 DPH. It was narrow with a tendency to widen at posterior end at 2 DPH (5.4 ± 0.3 mm). Mucosa was deprived of folds and goblet cells were lined by a single layer of columnar cells with basal nuclei, and acidophilic striated border. At 3 DPH, the posterior region of the intestine was noticed bent and a constriction of the mucosa (intestine valve) divided the intestine into two regions, the anterior intestine and posterior intestine (Fig. 3a-c). Terminal section of the posterior intestine was a short rectal duct named rectum. From 4 DPH, the intestinal region studded with mucosal folds and spread over the entire intestinal mucosa. From 5 DPH (7.4 ± 0.3 mm), the number of goblet cells increased with development particularly in the proximal portion of the anterior intestine. Mucous cells were observed in the intestine after 8 DPH (9.7 ± 0.3 mm) and their number increased with the development (Fig. 3a-c). Intestine continued to increase in length during the development and became highly convoluted over the time. At 20 DPH, rectal zone (the later region of the posterior intestine) was lined by a pseudostratified epithelium formed by cubic cells. At 25 DPH, homogenously distributed microvilli layer became dense and striated which constituted to form brush border membranes (data not shown).

When the larva was in the exo and endogenous feeding stage, accessory glands of digestive system were formed as cluster of hepatic and pancreatic cells. At hatching, the liver and pancreas were not differentiated. At 2 DPH, the incipient liver and pancreas were visible as two clusters of round basophilic cells situated dorsally to the yolk sac and ventrally to the developing digestive tract. At 4–6 DPH, the liver was bilobulated, started to differentiate and hepatocytes substantially increased in number. Hepatocytes were highly basophilic and appeared as bright pink in colour with haematoxylin and eosin. At 8 DPH, the size of liver increased due to hepatocyte differentiation and lipid vacuoles became more numerous (Fig. 3b-c). At 20 DPH, the liver appeared with very distinct and elongated lobes (Fig. 4c).



h-head; e-eye; b-brain; pf-pectoral fin; mo-mouth; ai-anterior intestine; bc-buccal cavity; oe-oesophagus; yg-yolk granules; ga-gill arch; pi-presumptive; o-oil globule; db-digestive system; st-stomach; pst-pyloric stomach; cst-cardiac stomach; l-liver; in-intestine; m-muscle; vc-vertebral column; oe-oesophagus; pst-pyloric stomach; bc-buccal cavity; r-rectum; s-skin; gr-gill racker; ub-uninary blader; dt-digestive tract; sb-swim bladder; pc-pharyngeal cavity; p-pancrease; k-kidney.

Fig. 3— Development of digestive system. (a-b) Sections of 0-day post hatch (DPH) larvae distinct with undifferentiated digestive tube, yolk mass and oil globule, (c-d) 1-day post hatch larvae showing digestive tract slightly bent in posterior portion and formation of mouth, (e-f) 2-day post hatch larvae with depleted yolk sac and undifferentiated digestive tract. (a-c) 3-day post hatch larvae with the distinct digestive tract, primordial liver and pancreas located at posterior portion of the oil globule, the anterior and posterior intestine, (d-f) 4-day post hatch larvae with primitive stomach and coiled intestine, (g-h) 7-day post hatch larvae with increased esophagus folds. (a-c) 8-day post hatch larvae showed intestinal mucous cells and stomach increased in size, (d-f) 9-day post hatch larvae with taste buds in buccopharynx region, (g-i) 10-day post hatch larvae showing well developed pancreas, liver, stomach, anterior intestine, middle intestine. (a-f) Development at 11-day post hatch with intestinal mucous folds, (g-i) 12-day post hatch larvae showing food mass within the intestinal epithelial mucosa. (a-f) 14-day post hatch larvae with increase in the buccopharynx cavity, thick mucous folds in intestinal and rectal segment, (g-i) 16-day post hatch larvae with proliferation of gastric glands in stomach and showing well developed liver, kidney.

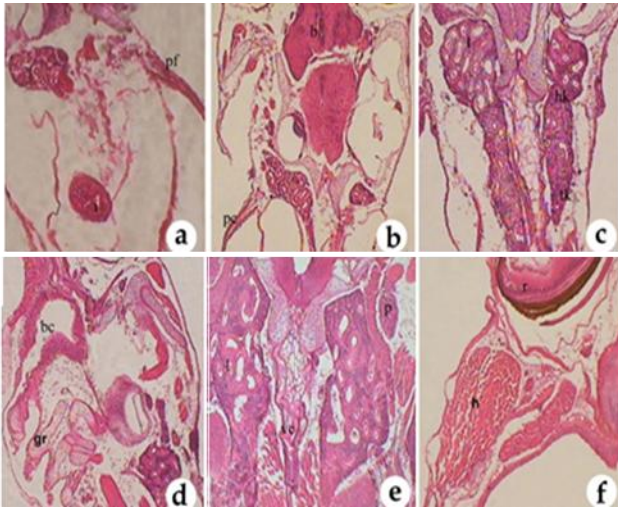


Fig. 4— Development of accessory glands. (a-f) *C. striatus* larvae showing development of liver, pancreas, kidney and heart (pf-pectoral fin; L-liver; i-intestine; hk-head kidney; tk-trunk kidney; h-heart; gr-gill rakers; bc-brachial cavity; vc-vertebral column; r-retina; b-brain; p-pancreas).

Numerous zymogen granules were visible in the centre of the acini after first feeding at 3 DPH. The pancreas was situated above the liver and elongated to the intermediate intestine at 5 DPH. During further development of the larva, exocrine and endocrine regions were differentiated in the pancreas. Pancreatic cells also were basophilic in nature with a prominent nucleus. Pancreas increased in size with more vacuoles during 14 to 25 DPH (Fig. 4e). Oval shaped gall bladder was first observed at 3 DPH larvae and it was situated between the liver and pancreas with simple cubic cells surrounded by a layer of connective tissue. At 15 DPH (14.8±0.2 mm), epithelium of the gall bladder appeared thickened with cuboidal and cylindrical cells.

The present study provides integrated information about the morphological and histological characteristics of the organogenesis of *C. striatus* larvae (from hatching to 25 DPH). Ontogeny of the digestive tract of *C. striatus* followed the general pattern that most fish species described to date. Although the basic mechanisms of larval development do not differ greatly among teleosts, the timing of developmental events and the duration of stages are variable^{13,14}.

As the most teleosts, the newly hatched larva of *C. striatus* presented the yolk surrounded by the periblast or syncytial layer of squamous cells^{14,15}. By endocytosis of the yolk sac through a syncytial layer

endogenous nutrition in fish larvae occurred¹⁶. Yolk sac of *C. striatus* larva was ovoid in shape occupying about 45% of the total length and was resorbed at 3 DPH (5.8 mm length) as reported by El Hag *et al.*, (2012) in catfish *Mystus nemurus*. This process took 8 DPH in gilthead seabream *Sparus aurata*¹⁷ and 9 DPH in Atlantic cod *Gadus morhua*¹⁸.

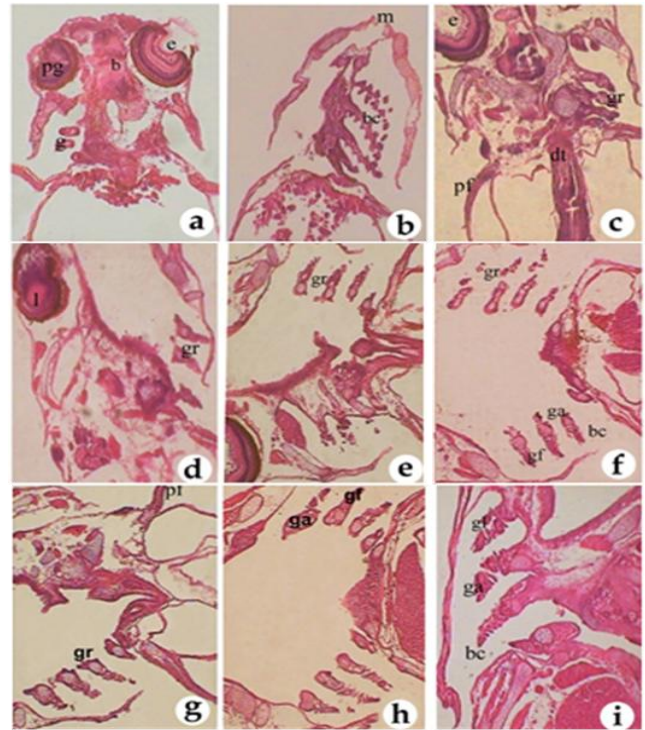


Fig. 5— Development of gills. (a) 1-day old larvae showing brachial cavity and primordial gill arches, (b-d) 3-day old larvae with brachial cavity showing gill arches and gill filaments, (e-f) 7-day old larvae showing well developed gill arches, (g-i) 16-days old larva showing fully developed gills (Pg-pigments; g-gills; b-brain; e-eye; m-mouth; pf-pectoral fin; dt-digestive tract; gf-gill filaments; gr-gill raker; ga-gill arch; bc-brachial cavity).

During hatching the digestive tract of *C. striatus* larvae was an undifferentiated straight tube, as reported in other teleost species like redbanded seabream *P. auriga*¹⁵ and brill *S. rhombus*¹⁹. The differentiation of the digestive tract in *C. striatus* larvae started before the onset of exogenous feeding since the buccopharyngeal cavity, oesophagus, primordial stomach and the intestine were distinguished at 4 DPH. These main developmental events are in agreement with previous reports of other species^{17,20,21}. All these changes were important for adaptation to exogenous feeding⁴. During this period *C. striatus* larvae advanced the digestive system in

structure and function for successful ingestion, digestion and assimilation on exogenous food before the depletion of endogenous reserves.

At 0 DPH, buccopharyngeal cavity was not well formed. However, at 1 DPH the buccal cavity was lined by a layer of squamous epithelium, while the number of layers increased with age. Similar reports of closed buccopharynx were reported in 1 DPH larvae of Siberian sturgeon *Acipenser baeri*²². Importance of taste buds in palatability evaluation of the feed to swallow or to reject has been discussed by many researchers in several fish species. Taste buds and goblet cells in *C. striatus* larvae were visible at 3 DPH when larvae started exogenous feeding. It is a chemosensory organ that consisted of modified epithelial cells, which are implied in the selection of the food and play a crucial role in gustation: foraging and food-recognition¹⁵.

An early development of esophagus may be important at the onset of the first feeding and is essential for other functions such as osmoregulation. Two differentiated regions of esophagus could be distinguished clearly through the histology. It was similar to yellowtail flounder *Limanda ferruginea*²³. Stratified squamous epithelium in the anterior region of esophagus can endure the stimulation from swallowing live preys such as Cladocerans and Copepods which have hard shell and spine. The developmental changes of esophagus described in the present study correspond well with that observed in other fish species. Esophagus in striped murrel larvae was not very distinct at hatching, but appeared as narrowing of the pharynx at 2 DPH. The development of esophagus folds increased from 7 DPH with the larval growth. Similar findings were reported in a catfish *Mystus nemurus*²⁴.

In most examined freshwater teleost larvae, a completely differentiated stomach appeared from 5 DPH to 25 DPH. Stomach plays a vital role in digestion of the food consumed by the fish larvae. However, fish larvae lack both morphological and functional (secretory) stomach, since it appears as gut^{25, 26}. Incipient stomach appeared as a bulge in the middle of the digestive tract following the yolk sac resorption at 3-5 DPH in *C. striatus* larvae. Similar observations were seen in catfish *Mystus nemurus* where stomach appeared following the complete yolk sac resorption 4-5 DPH²⁴. The appearance of the gastric glands at 7 DPH indicated the presence of gastric enzymes similar to that of catfish *M.*

*nemurus*²⁴.

The development of gastric glands in the stomach is the prerequisite for extracellular digestion, as they secrete hydrochloric acid and digestive enzymes. Gastric glands in striped murrel *C. striatus* were fully developed and surrounded by a thin layer of connective tissue at 16 DPH. With the growth of the larvae, the number of gastric glands was found increased in the fundic region and to a lesser extent in the cardiac stomach, but they were not observed in the pyloric part of the stomach. In the spotted sand bass, *Paralabrax maculatofasciatus* larvae gastric glands were reported in the anterior part of the stomach²⁷. Pyloric region was folded and lined by a short ciliated columnar epithelium. Appearance of gastric glands is an indicator of transition from larval to juvenile stage.

Intestine, which starts from the pyloric sphincter, is the longest single part of an alimentary canal. In *C. striatus* larvae, the intestine is a simple columnar epithelium and was lined by brushborder of microvilli, which is a typical of absorptive tissues. The appearance of brushborder which is particularly rich in enzymes facilitates the digestive process²⁸. Epithelium of the intestine differed from that of the stomach and the histological characteristics of intestinal epithelial cells were very similar to many other fish larvae²⁹. Microvilli play a very important role by substantially increasing the surface area of the cells. Incipient intestine appeared as a straight translucent tubular segment laying dorsally to the yolk sac at 1 DPH in striped murrel larvae and was lined by a single layer of columnar cells with basal nuclei, and with acidophilic striated border. Bisbal and Bengston have reported that at the time of mouth opening in the larvae of summer flounder *Paralichthys dentatus*, the epithelium of the luminal surface of the intestine consisted of a single layer of columnar cells with a striated border of microvilli³⁰.

At hatching, the liver and pancreatic cells were visible as groups of relatively undifferentiated cells in striped murrel *C. striatus*. Appearance of liver and pancreatic cells was also reported at the early stages in many species. One day after hatching, the liver was appeared as undifferentiated basophilic cells in Sea bream *Pargus aurica*¹⁵. At 2 DPH, the incipient liver was visible as two clusters of round basophilic cells lying between the digestive tract and the yolk sac in *C. striatus* larvae. By 4-6 DPH, the liver was bilobulated, started to differentiate and hepatocytes substantially increased in number. The liver became

bilobulated and the right lobule was much longer and larger than the left at 5 DPH in Yellow croaker *Pseudosciaea crocea*³¹. In the present study it was observed that at 9DPH, the size of liver increased due to hepatocyte differentiation and lipid vacuoles became more numerous and at 20 DPH, the liver appeared with very distinct and elongated lobes in *C. striatus* larvae. The liver was more elongated between 9-33 DPH and was deeply lobed at 42 DPH in Haddock *Melanogrammus aeglefinus*³².

The pancreas was not differentiated at hatching in striped murrel *C. striatus*. In some fishes like summer flounder, the exocrine pancreas was differentiated and well developed at the time of hatching³⁰. Pancreas was absent at the time of hatching in *Pagellus erythrinus*²⁰ but started to differentiate between 1 and 2 DPH. In *C. striatus* larvae, pancreatic cells are basophilic in nature with a prominent nucleus. Pancreas was situated above the liver and elongated to the intermediate intestine at 5 DPH. The pancreas increased in size with more vacuoles during 15 to 25 DPH. At hatching pancreas was positioned in the dorsal of the intestine in Haddock *Melanogrammus aeglefinus*³². In the present study numerous zymogen granules were visible in the centre of the acini after first feeding at 3 DPH. During further development of larva, exocrine and endocrine regions were differentiated in the pancreas. Incipient pancreatic cells containing dense eosinophilic zymogen granules were observed at 3 DPH in *Pagellus erythrinus*²⁰. At 3 DPH, the exocrine pancreatic cells concentrated in acini as pancreatic ducts appeared and acidophilic zymogen granules were also apparent in Yellowtail kingfish *Seriola lalandi*³³. In Green sturgeon *Acipenser medirostris* zymogen granules were detected at 4-5 DPH and before the onset of first feeding³⁴.

Oval shaped gall bladder was first observed at 3 DPH larvae in the present study and it was noticed between the liver and pancreas with simple cubic cells surrounded by a layer of connective tissue. Similarly, the gall bladder was first detected at 3 DPH in summer flounder³⁰ and California halibut *Paralichthys californicus*³⁵.

The weaning of larvae from live to formulated feeds is the bottleneck of commercial aquaculture of many species. Knowledge of digestive tract developmental changes associated with food assimilation process may help to identify limiting factors during larval rearing and reduce bottlenecks in

the weaning process³². Given the current trend towards the development and formulation of formulated feeds, surprisingly little information is available focusing on the development of the digestive system of larval fish. Progress in the intensive culture of fish for commercial production warrants development of suitable dry diets for larval fish. A thorough evaluation of the factors associated with digestion and assimilation is critical to understand the success or failure of a particular diet. This information would enhance our understanding of the limiting factors associated with critical stages such as weaning. These findings on the development of the digestive system in striped murrel may lead to a better understanding of the ontogeny and would be useful to improve the larval rearing techniques of this promising air breathing species for freshwater aquaculture diversification.

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