

Histomorphological study of the gut developmental pattern in early life history stages of featherback, *Chitala chitala* (Hamilton)

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Abstract. The present study was conducted to observe morphological and histological changes in the developing digestive system during the ontogeny of featherback, *Chitala chitala* (Hamilton), from hatching to 30 days post hatch (dph). Three significant stages were identified during digestive tract development in featherback: (1) endotrophic (0-8 dph), (2) endoexotrophic (8-12 dph), and (3) exotrophic (12-30 dph). At hatching, the mouth was closed and the digestive tract was a straight tube. At 8 dph, the mouth was opened and the appearance of the esophagus was observed. Between 6-8 dph, the primordial liver and exocrine pancreas were observed. Intestinal enterocyte activity was observed before stomach development. The esophageal goblet cells, teeth, and taste buds developed 2 days after the opening of the mouth, at the time of exogenous feeding. The development of the stomach, gastric glands, and pyloric caeca took place between the 7 and 12 dph. Except the increase in size and complexity of the structures, no noticeable changes were observed after 12 dph in *C. chitala* during the experiment. Our findings in the current work provide valuable information which might be useful for improving current larval rearing techniques of this promising new candidate species for freshwater aquaculture diversification.

Keywords: endangered fish, fish larvae, digestive system

Introduction

Comprehensive knowledge on the structure and function of the larval and juvenile digestive system is a key factor in optimizing rearing strategies for a particular species. From practical and economical points of view, the appropriate moment for beginning exogenous feeding must be established. Thus, detailed observations on the changes of larval digestive tracts, including the development of digestion and nutrient absorption, is of principal importance. This knowledge can help to identify limiting factors during larval rearing, reduce bottlenecks in the weaning process, and optimize feeding technology practices (Zambonino-Infante et al. 2008). Numerous studies have been conducted in commercially important foodfish species, such as summer flounder, *Paralichthys dentatus* (L.) (Bisbal and Bengston 1995), gilthead bream, *Sparus aurata* (L.) (Elbal et al. 2004), haddock, *Melanogrammus aeglefinus* (L.) (Hamlin et al. 2000), cobia, *Rachycentron canadum* (L.) (Faulk et al. 2007), common dentex, *Dentex dentex* (L.) (Santamaría et al. 2004), and common pandora, *Pagellus erythrinus* (L.) (Micale et al. 2006) with the aim of describing histomorphology during early development under specific culture conditions.

However, to date there is no information available on the development of the digestive tract in featherback, *Chitala chitala* (Hamilton), despite its

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being the most commercially important fishery resource in different parts of southeast Asia (Khan et al. 2000) and particularly in India. Due to the over-exploitation of natural populations and subsequent declines in number, this species was categorized as endangered (EN) in the Conservation Assessment and Management Plan, 1998 (Ayyappan et al. 2001). Therefore, large scale farming of this new candidate species would ensure effective resource utilization, biodiversity conservation, and broadened consumer choice in the diversification of freshwater Indian aquaculture (Mitra et al. 2014). Thus, the present study was undertaken to focus on the digestive system development with descriptions of morphological and histological changes during the ontogeny of featherback from hatching to 30 days post hatch (dph).

Materials and methods

Egg incubation and larval rearing

Fertilized featherback eggs were obtained from induced spawning of two males and one female (weight = 2.31 ± 0.6 kg, total length = 86 ± 5.5 cm) at a local fish farm in West Bengal (India). The breeders were stocked in a pond with a surface area of 0.09 to 0.13 ha and a water depth of 150 to 170 cm. A wooden country boat was placed on the the pond bottom to provide artificial substratum. An injection of Ovaprim 1.5 ml kg^{-1} body weight was administered to the broodstock to induce spawning (Sarkar et al. 2006), and 14 to 18 h after injection courtship and spawning occurred. The spawned adhesive eggs were removed from the pond and placed in a 100 l circular recirculating tank until hatching (168–192 h) at $27\text{--}28^\circ\text{C}$, pH 7.81 ± 0.12 and water flow 0.3 l s^{-1} . After hatching, the larvae were reared in three tanks at a density of $45,000 \text{ larvae m}^{-3}$ (450 l). The photoperiod was maintained on a 12 L: 12 D cycle. Temperature and pH ranged from $30 \pm 2.2^\circ\text{C}$ and 7.4 ± 0.6 , respectively, throughout the experiment. Dissolved oxygen was maintained above 8.0 ± 2.5

mg l^{-1} by constant aeration. The larvae were fed *ad libitum* with *Artemia* nauplii three times daily. Throughout the study, daily water exchanges (10–15% of total volume) and bottom siphoning was performed to prevent the accumulation of dead *Artemia* and other organic matter. No mortality of the *C. chitala* larvae was observed during the experimental period.

Sample preparation

A random sample of 50 larvae was collected on a daily basis from 0 dph to 30 dph. The sampled larvae were anesthetized with MS-222 (35 mg l^{-1}), rinsed in distilled water, and fixed in neutral-buffered formaldehyde. A subsample of 15 larvae was individually measured to the nearest 0.01 mm using a light microscope, and after this the larvae were weighed to the nearest 0.001 g (wet weight) with an analytical microbalance. For histomorphological analysis, the remaining 35 larvae were fixed in Bouin's solution, dehydrated through a series of alcohol concentrations, cleared in xylene, and submerged in paraffin wax, and cut into $5 \mu\text{m}$ sections. Serial, sagittal, and transverse sections were stained following Harris's Haematoxylin and Eosin (HE) procedure for general histomorphological observations (Pearse 1985) and viewed under a light microscope (Olympus BX 41, Tokyo, Japan) to describe the development of the digestive tract.

Results

Featherback larval growth (total length and weight) during the experimental period is described in Fig. 1. The main histomorphological changes of the digestive tract of the featherback larvae during the early developmental stages are summarized in Fig. 2. Three main stages were identified during larval development: the first stage, or exclusively lecithotrophic (endotrophic) period, was from 0–8 dph; the second stage, or lecithoexotrophic (endoexotrophic) period, was from 8–12 dph; the third stage, or exclusively exotrophic period, was from 12–30 dph.

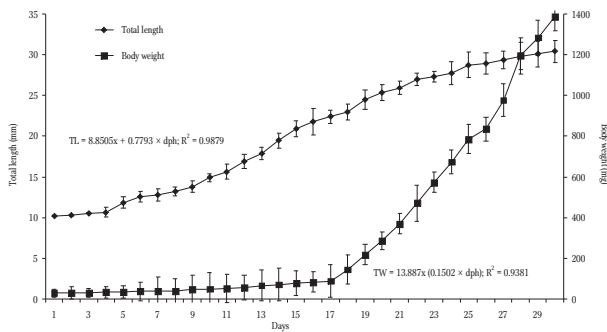


Figure 1. Growth in total length and weight of *C. chitala* larvae from hatching until 30 dph. Each point represents the mean of fifteen measurements \pm SE.

Intense larval histomorphogenesis was observed mostly during the first two stages, while during stage 3 only the size and the complexity of pre-existing organs increased. The histomorphological changes during the larval development are described below.

Endogenous reserve

At hatching, featherback larvae had a large yolk sac lined by a vitelline envelope and attached beneath the anterior half of the body. The undifferentiated primordial digestive tract lay dorsally to the yolk-sac and did not communicate with the exterior because the mouth and anus were not yet opened (Fig. 3a). The yolk sac contained several acidophilic yolk globules surrounded by a syncytial epithelium (Fig. 3b). There were many blood vessels around the yolk. The mouth and anus opened on 8 dph synchronously

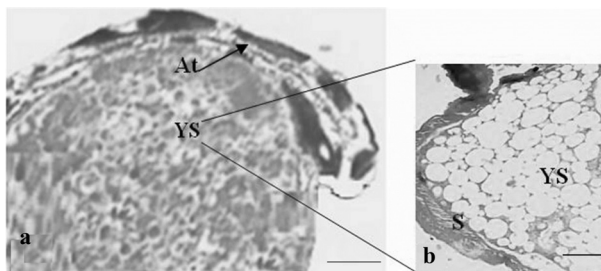


Figure 3. Histological sections of yolk sac larvae of *C. chitala*. (a) Sagittal section of *C. chitala* larva at 1 dph. The alimentary tract is undifferentiated, and the mouth is closed. At - alimentary tract; Ys - yolk sac. Scale bar 200 μ m. (b) Histological sections of the yolk sac in *C. chitala* larvae. Yolk globules surrounded by the yolk sac syncytium (S). Scale bar 50 μ m.

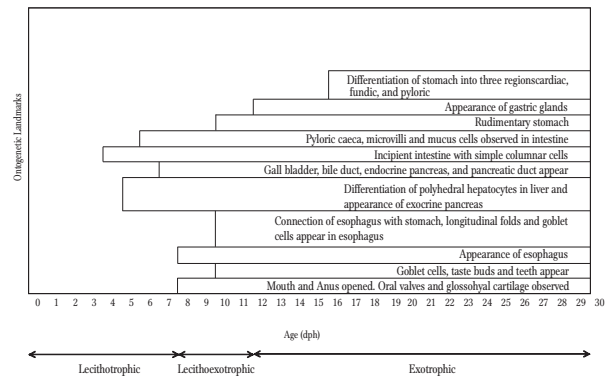


Figure 2. Diagram showing the main histological ontogenetic landmarks during larval development of *C. chitala*.

with the partial absorption of the maternal reserves. The yolk sac volume depleted gradually until complete absorption by the end of 12 dph.

Mouth, oral cavity, and pharynx

At hatching (TL= 10.21 \pm 0.03 mm), the mouth was closed by a covering membrane, and the jaw cartilage was not formed. The mouth opened on 8 dph, and two oral valves were evident in the larvae (Fig. 4a). Cement glands were obvious and located anteriodorsally to the head. At this age, the medial glossohyal cartilage, which would constitute the future tongue, was also evident on the ventral oral valve. The buccopharyngeal epithelium was

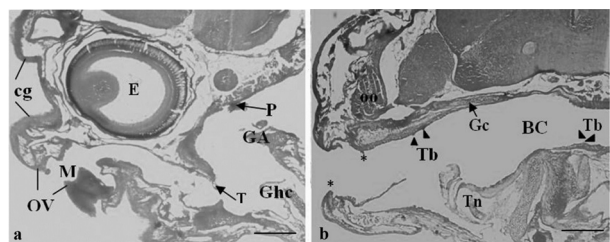


Figure 4. Histological sections of the buccopharynx at different developmental stages of *C. chitala*. (a) Sagittal section of *C. chitala* larvae at 8 dph. The mouth is open, and oral valves are present. (b) Sagittal section of the buccopharyngeal cavity during the development of *C. chitala* larvae at 10 dph. The buccopharyngeal cavity shows the presence of jaw teeth (*), taste buds, olfactory organ, and goblet cells in the buccopharynx. Abbreviations: cg - cement glands; E - eye; ga - gill arches; M - mouth; OV - oral valves; P - pseudobranch; T - teeth; Tn - tongue; Ghe - glossohyal cartilage; BC - buccopharyngeal cavity; Tb - Taste bud; GA - gill arch; Gc - goblet cell; OO - olfactory organ. Scale bar 200 μ m.

composed of a single layer of squamous cells with scattered round mucous cells, mostly found in both oral valves. The buccal cavity and pharynx were separated by the primordial gill arches, which is supported by cartilage and was also appreciated at this age. The first goblet-shaped mucous cells appeared at 10 dph (TL = 14.94 ± 0.5 mm) dispersed within the stratified squamous epithelium. The oral cavity was short, and canine-like teeth were detected on the oral valves protruding into the buccopharyngeal lumen. A few scattered taste buds were also visible in the buccal cavity epithelium at this age (Fig. 4b). With the larval development the size of the oral cavity increased, the number of teeth, mucous cells, and taste buds increased substantially. Four gill arches with pseudobranchia were visible at 8 dph proliferating toward the pharyngeal cavity. The gills were completely formed at 12 dph with filaments of increased length. The primordial lamellae increased in number and size throughout larval development. No noticeable histological changes were observed after 12 dph until the end of the study at 30 dph (TL = 30.45 ± 0.07 mm), except the increase in abundance of buccal mucous cells.

Esophagus

At the mouth opening (8 dph, TL = 12.85 ± 0.03 mm.), the buccopharynx communicated with the anterior intestine through a shorter and narrower esophagus, located directly posterior to the last gill-arch present in the pharynx. The anterior section of the incipient esophagus was filled with yolk and pigment granules until 10 dph (Fig. 5a). With the gradual disappearance of yolk material between 10 and 12 dph, the esophagus became completely pervious with the two different regions. The anterior esophageal region was lined by pseudostratified columnar epithelium cells, while the dilated posterior region was lined by a simple, cuboidal epithelium with abundant goblet mucosal cells and ciliated cells, from which the future stomach originates. On 10 dph, several longitudinal folds were present at the junction between the posterior region of the

esophagus and the stomach (Fig. 5a). These folds increased in complexity and length with larval age. Four tissue layers of the esophagus, namely mucosa (M), submucosa (S), muscularis (MU), and serosa (SE) also appeared on 12 dph (Fig. 5b). From 12 dph, the histological organization of the esophagus did not change except that the number and size of the goblet cells increased (Fig. 5c) in the posterior region of the esophagus and the longitudinal folds increased with a thicker muscular layer to accommodate the passage of large food items.

Stomach

At the beginning of hatching, the stomach was undifferentiated. At 10 dph, the future stomach appeared as a little pocket with an esophagus-stomach sphincter. The developing stomach was initially filled

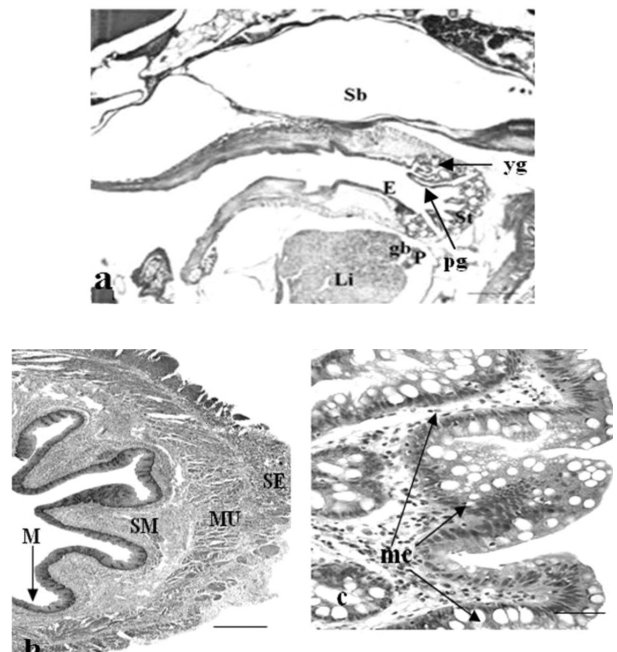


Figure 5. Histological section of esophagus at different developmental stages of *C. chitala*. (a) General view of the transition between the esophagus and the gastric stomach showing longitudinal folds surrounded by the yolk globule (yg) and pigmented granules (pg) at 10 dph. Scale bar 200 μ m. (b) Details of larval esophagus at 12 dph, showing four layers, Serosa (SE); Muscularis (MU); Submucosa (SM); Mucosa (M). Scale bar 100 μ m. (c) Abundance of mucus cells in the posterior esophagus. Abbreviations: E – Esophagus; Li – liver; I – intestine; St – stomach; P – pancreas; gb – gallbladder; Sb – swim bladder; mc – mucus cells. Scale bar 50 μ m.

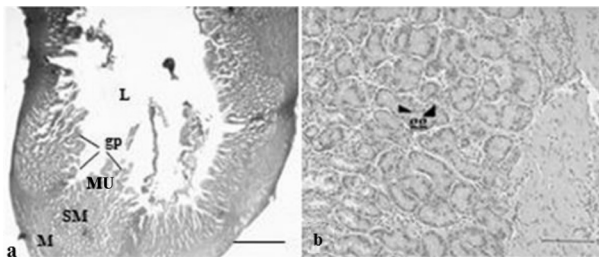


Figure 6. Histological section of stomachs at different developmental stages of *C. chitala*. (a) General histological view of the stomach wall at 15 dph *C. chitala* larvae showing L – lumen; gp – gastric pits; M – mucosa; SM – submucosa; MU – muscularis. Scale bar 200 μ m. (b) Histological section of the glandular fundic stomach of 25 dph *C. chitala* larvae, showing numerous gastric glands (gg). Scale bar 50 μ m.

with yolk and pigment granules that transformed into the cylindrical epithelium, and the yolk material gradually disappeared. The first gastric glands became visible on 12 dph, and their number increased through larval development. At 15 dph (TL = 20.94 \pm 0.06 mm.), the stomach exhibited a pouch shape and began to differentiate morphologically and histologically into three regions – the cardiac stomach, the stomach fundus, and the pyloric stomach. The wall of the stomach was very thin, and there were fewer mucosal folds in comparison with the esophagus and the intestine. In cross section, mucosae, submucosa, a muscular layer, and a serosa formed the stomach wall (Fig. 6a). Until 30 dph, the stomach did not show any further noticeable modification except for the increase in the folds and goblet cells. The anterior non-glandular cardiac region, which continued at the end of the esophagus, was lined with a pseudostratified ciliated columnar epithelium with central nuclei, and it exhibited several mucosal folds and goblet cells. The mucosa of the stomach fundus was formed by a single layered columnar epithelium. The submucosa was formed by loose connective tissue containing blood vessels. The muscle consisted of smooth muscle fibers arranged in a circular inner layer and a thinner longitudinal outer layer. The epithelium of the glandular fundic region was similar to the cardiac region, but the lumen in this part appeared wider. The mucosa contained a large number of gastric glands on the dorsal and ventral walls, surrounded by the connective tissue of the lamina

propria. The gastric glands were well established in the epithelium of this region. The eosinophilic particles of the gastric glands indicated pepsinogen secretion between 25 and 30 dph (Fig. 6b). The wall of the pyloric portion comprised four layers, similarly to the stomach fundus, and it was marked by the sudden disappearance of gastric glands. With larval developmental advancement, the number and size of the mucosal folds increased.

Intestine

The intestine was the longest portion of the digestive tract and one of the first organs of the digestive system to differentiate in featherback larvae. At 4 dph (TL = 10.65 \pm 0.01 mm), the incipient intestine appeared as a straight translucent tubular segment laying dorsally to the yolk sac, and it was lined by a simple columnar epithelium. The intestinal segments showed a single epithelial layer composed of columnar cells with numerous microvilli at the luminal surface forming the brush border on 6 dph (Fig. 7a). At this age, the intestinal cells, or enterocytes, were also arranged in a single layer, and contained median to basally located nuclei and evident apical vacuoles. With the coincidence of exogenous feeding commencement from 12 dph, the lumen of the anterior intestine dilated and small, non-stained vacuoles could be seen in the cytoplasm of the intestinal enterocytes. As the larvae grew, the mucosal folds became deeper and more abundant, with an increase in number of intestinal mucus secreting goblet cells (Fig. 7b and 7c). The increased thickness of the enterocyte brush border indicated an increase in digestion and absorption area. Eosinophilic supranuclear inclusion vesicles (SIV) in the posterior gut were first observed on 12 dph (Fig. 7d). SIV increased in frequency and size up to 15 dph, but decreases in both the size and frequency of them occurred as development continued. The intestinal mucosa was surrounded by a thin musculature that comprised two muscular tissue layers: one circular internal and another longitudinal external separated by a very thin connective tissue layer. The

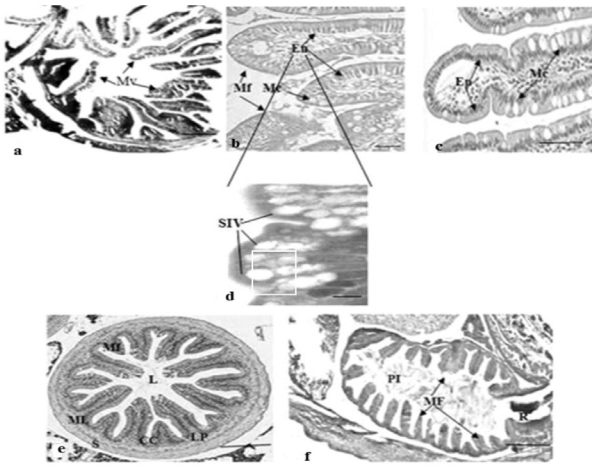


Figure 7. Histological sections of intestine at different development stages of *C. chitala*. (a) Histological section of intestine of *C. chitala* larvae on 6 dph showing the microvilli. Scale bar 200 μ m. (b) and (c) Details of the mucosal folds (Mf) with enterocytes (En) and mucous cell (Mc) on 12 and 20 dph. Note the increase in mucus cells on 20 dph as compared with 12 dph. Scale bar 100 μ m. (d) Enterocytes showing numerous SIV in 12 dph *C. chitala* larvae. Scale bar 50 μ m. (e) Transverse sections of pyloric caeca in 12 dph *C. chitala* larva showing the lumen (L); mucosal fold (MF); columnar cell (CC); lamina propria (LP); muscularis layer (ML); serosa (S). Scale bar 200 μ m. (f) Detail of posterior intestine and rectum in 12 dph *C. chitala* larvae showing mucosal folds (MF). Scale bar 200 μ m.

intestinal mucosa showed a pronounced folding on 6 dph, indicating the development of caecum, and the pyloric caeca joining the anterior intestine. The pyloric caeca became pronounced between 8-12 dph and were different from the intestinal ones with their larger amounts of submucosa, and shorter folds. The lumen of the pyloric caeca was starlike in cross section (Fig. 7e). The histological structure of the pyloric caeca was similar to that of the anterior intestine. In the distal portion, the folds containing large numbers of goblet cells were fewer and shorter, and they became progressively thicker. Intestinal mucosa developed longitudinal folds with the longest fold in the anterior section, which was shorter in the anterior-central part, and the shortest in the posterior intestine. The rectum was discernible as a short, flattened intestinal segment of the intestine devoid of mucosal folds (Fig. 7f). Except for increases in the length of the intestine, as well as the size and number of mucosal folds, no relevant modifications were

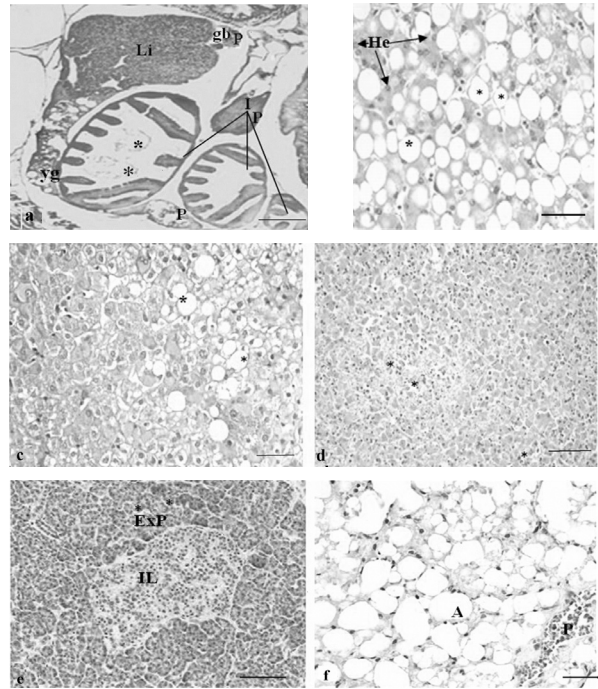


Figure 8. Histological sections of the liver and pancreatic tissue of *C. chitala* at different stages of development. (a) Sagittal section of *C. chitala* larvae at 8 dph showing developing liver and pancreas with gall bladder, ingested food material in gut (*). Scale bar 200 μ m. (b) liver in a 6 dph larvae filled with large yolk globules (*) within pigmented hepatocytes (He); (c) Reduction in fat deposition within hepatocytes at 10 dph; note the reduction of fat deposits between both ages with regard to 6 dph; (d) Regular arrangements of hepatocytes on 12 dph showing eosinophilic granules indicating glycogen deposits (*). Scale bar 50 μ m. (e) pancreatic tissue section at 8 dph *C. chitala* larvae, showing exocrine pancreas (ExP) and islets of Langerhans (IL). Note polyhedral basophilic pancreocytes arranged in acini containing zymogen granules (*). Scale bar 50 μ m. (f) adipose tissue (mesenteric fat deposits) surrounding the pancreas at 25 dph *C. chitala* larvae. Scale bar 100 μ m. Abbreviations: A – adipose tissue; IL – endocrine pancreas (islet of Langerhans); P – pancreatic tissue; Li – liver; I – intestine; P – pancreas; gb – gallbladder; yb – yolk globule; mc – mucus cells.

observed regarding the histological organization of the intestine until the end of the study.

Liver and pancreas

Accessory glands (liver and pancreas) were not differentiated at hatch (Fig. 8a). The primordial liver situated in the ventral region of the yolk sac was observed on 6 dph (TL = 11.98 \pm 0.05 mm), and it

appeared histologically as a mass of polyhedral hepatocytes. These hepatic cells were filled with yolk, pigment granules, and large lipid vacuoles (Fig. 8b) that were loosely organized around a central vein and hepatic sinusoids. On 8 dph, liver size increased because of hepatocyte differentiation and the lipid vacuoles becoming more numerous between 6 to 8 dph. On 10 dph, a decrease in the lipid vacuoles was observed with a concurrent increase in eosinophilic granules (Fig. 8c). On the 8 dph gallbladder and bile duct became visible but not fully pervious. They were lined with simple squamous epithelium, but later, on 10 dph, three layers became notable in the gallbladder with a simple epithelium of columnar inner cell layer surrounded by a layer of connective tissue and smooth muscle fibers towards the outside part of the wall. At the same time (10 dph), the hepatic yolk almost completely disappeared. Distinct histological changes took place after the onset of larval exogenous feeding. During this time, hepatocytes showed no more lipid vacuoles, and their shape became regular (Fig. 8d). Numerous blood vessels filled with blood cells and appeared among the hepatocytes. They were tightly packed between sinusoids, often around a central vein. Hepatocyte nuclei were located peripherally, and cytoplasm was almost completely filled with lipids. As the larvae grew, the livers continued to differentiate and liver size increased. Morphologically, it became a large organ that appeared ventral to the developing gut and formed a central anterior mass just behind the transverse septum, which separates the pericardial and the abdominal cavities.

Between 6 and 8 dph, the pancreas was first observed as small patches (diffused) of undifferentiated cells. With larval development, the pancreas started to move dorsally and compress around the stomach and the anterior intestine. On 8 dph, exocrine polyhedral cells were visible with round basal nuclei arranged around a central lumen to form acini interspersed with blood vessels, containing dense eosinophilic zymogen granules at the apical portion of cytoplasm toward the lumen. The endocrine cells at 8 dph could be distinguished as islets (islets of Langerhans) inside the exocrine pancreas (Fig. 8e).

Pancreatic ducts were formed at this age, and the opening of the main pancreatic duct into the anterior intestine, just behind the pyloric sphincter, could be clearly seen at this age. The pancreatic duct was lined with squamous epithelium, which appeared flattened and thin. The histological organization of the pancreas did not show major changes from 12 dph until the end of the study except in the increase in the size and number of pancreatic acini and the islets of Langerhans. With developmental advancement, mesenteric fat deposits observed surrounding the pancreatic tissue (Fig. 8f).

Discussion

The larvae of many fish species have three feeding phases: endogenous (lecithotrophic), mixed (lecithoexotrophic), and exclusively exogenous (exotrophic) (Ostaszewska et al. 2003). The present investigation showed that featherback larvae also depend exclusively on an endogenous source of nutrition during its first phase of life, i.e., from hatching until 8 dph, when the yolk and oil globule reserves are metabolized. Between 8-12 dph, the presence of yolk in the glandular stomach and anterior intestine indicated the mixed feeding period in *C. chitala*. From 12 dph, with the complete exhaustion of the yolk, exogenous feeding commenced. The majority of histomorphological changes in the digestive system of *C. chitala* larvae occurred before the start of exogenous feeding. The development of the liver, pancreas, and ossified gill arches occurred before hatching. The mouth opens and absorptive and digestive capabilities are developed (i.e., enterocytes with microvilli, a pancreas with zymogen granules) develop before the start of exogenous feeding. These results concur with other altricial teleost larvae developing from demersal eggs which can be fed more easily on artificial diets very soon after hatching (Govoni et al. 1986).

The lumen of the pharynx and esophagus of *C. chitala* was lined with numerous mucous cells secreting mucosubstances and ciliated cells which are

adaptable for rapid and consistent lubrication during ingestion of small fishes, worms, crustaceans, and other small aquatic animals (Yang et al. 1997). The striated muscle fibers in the pharynx and esophagus allow for expansion during the ingestion of food (Albrecht et al. 2001). The development of teeth, taste buds, and goblet cells in the buccopharynx of *C. chitala* was similar to the pattern noted in other teleosts (Baglole et al. 1997, Papandroulakis et al. 2004), and all of these histological features play important roles in gestation and pregastric digestion (Sánchez-Amaya et al. 2007). In the present study, the appearance of taste buds on the surface of the buccopharyngeal cavity at 10 dph confirms the ability of *C. chitala* to forage for and recognize food (Sánchez-Amaya et al. 2007). It is remarkable that the pharynx of featherback larvae contains four gill arches with primordial gill filaments at 12 dph, which is similar to observations of cod, *Gadus morhua* (L.), halibut, *Hippoglossus hippoglossus* (L.), and wolffish, *Anarhichas lupus* (L.) larvae, which also have gills filaments only in the advanced larval stage (Falk-Petersen 2005).

The development of gastric glands in fish larvae has been shown to indicate stomach differentiation (Verreth et al. 1992). Gastric gland development in altricial species like senegal sole, *Solea senegalensis* (Kaup), sea bass, *Dicentrarchus labrax* (L.) (Zambonino-Infante and Cahu 2001), and haddock (Hamlin et al. 2000) larvae occurred at 22, 25, and 33 dph, respectively, whereas in *C. chitala* larvae it occurred on 12 dph. It has been proposed that once the larvae complete metamorphosis, secretions of the gastric glands together with pepsin and HCl, facilitate the hydrolysis of proteins to peptides and amino acids (Govoni et al. 1986). Thus, the development of gastric glands helps larvae to digest a variety of prey items. This is particularly important in larval culture, as it indicates a potential dietary shift from live food organisms to artificial diets that can, consequently, reduce production costs. The location of gastric glands within the stomach is species-specific; an entirely glandular stomach, such as that found in the featherback larvae, suggests that this species could be capable of consuming large prey (Ortíz-Delgado et

al. 2003). On approximately 6 dph, pyloric caeca were seen first as slight evaginations of the anterior intestinal epithelium and the folding became distinct in the intestinal junction between 8 and 12 dph, indicating improved digestion and absorption ability with the help of residing gut microflora and increases in the surface area (Baglole et al. 1997).

The main function of the intestine, even in larvae, is the absorption of fat, protein, and carbohydrates. Possible functional capabilities can be inferred from the structural features of the differentiated cells composing the epithelium of the intestine (Ozaki 1965). Numerous microvilli were observed at the intestinal luminal surface of *C. chitala* forming the brushborder. Welsch and Storch (1976) proposed that these structures are particularly very rich in enzymes to facilitate the digestive process. The presence of supranuclear inclusion vesicles (SIV) in the posterior gut of *C. chitala* are thought to be associated with pinocytotic absorption of protein macromolecules from the intestinal lumen into the enterocytes. It has been postulated that pinocytosis is an adaptation to the lack of a glandular stomach producing proteolytic enzymes during early development (Govoni et al. 1986, Hamlin et al. 2000). The SIV in the intestinal mucosa of *C. chitala* larvae were first observed at 12 dph, i.e., at the onset of exogenous feeding, and then decreased in size and number around 15 dph eventually disappearing with larval growth. In contrast, the SIV in the enterocytes of African catfish, *Clarias gariepinus* (Burchell) (Verreth et al. 1992), and yellow catfish, *Pelteobagrus fulvidraco* (Richardson) (Yang et al. 2010), were detected after the onset of exogenous feeding. In the present study, the decrease in the size of SIV coincided with the proliferation of gastric glands at 15 dph. Santamaría et al. (2004) correlated the disappearance of SIVs with increased enzymatic activity. However, SIVs were found to be absent in starved gilthead sea bream larvae (Yúfera et al. 1996). The size and number of vacuoles decrease in consequence to the increased capacity of the enterocytes to synthesize lipoprotein (Deplano et al. 1991). The coexistence of pinocytosis and extracellular digestion in fish larvae has been described earlier and is considered to be an adaptive

mechanism that compensates for the incomplete digestion of macromolecules until the proliferation of gastric glands (Govoni et al. 1986, Elbal et al. 2004). In the present study, no relevant differences were observed in the histological organization of the anterior and posterior regions of the *C. chitala* larvae intestine, except in the number and size of mucosal folds and the degree of fat accumulation within the enterocytes. The intestinal goblet cells were the abundant cell type and were scattered along the intestinal epithelium of *C. chitala* larvae. These cells are well known for the production of mucins to establish a mucopolysaccharide barrier between the epithelium and the content of the lumen (Gisbert et al. 1998). In the present investigation, the occurrence of goblet cells in the intestine of *C. chitala* larvae during the first feeding concurred with observations of yellowtail flounder, *Pleuronectes ferruginea* (Storer) (Baglolle et al. 1997), Dover sole, *Solea solea* (L.) (Bouhlic and Gabaudan 1992), common dentex (Santamaría et al. 2004), and Cuban gar, *Atractosteus tristoechus* (Bloch and Schneider) (Comabella et al. 2013), in which goblet cells appear in the intestinal mucosa before the first feeding and/or coincide with the onset of exogenous feeding. In other species, such as gilthead sea bream, California halibut, *Paralichthys californicus* (Ayres), senegal sole, common pandora, kelp grouper, *Epinephelus bruneus* (Bloch), cod, or haddock, goblet cells differentiate at latter stages of development (Zambonino-Infante et al. 2008). Intestinal coiling and mucosal folding began at an early stage (10 dph and 12 dph, respectively) in *C. chitala* larvae and appeared very pronounced in the anterior intestine by 12 dph, which indicated better gut functionality by increased intestinal length and absorption surface.

The timing of liver and pancreas differentiation varies among species, and is mainly related to their general life history traits (Hoehne-Reitan and Kjørsvik 2004). The early differentiation of the liver and pancreas in featherback larvae is similar to that reported in studies of several teleost species (Bisbal and Bengtson 1995, Guyot et al. 1995, Kurokawa and Suzuki 1996). Boulhlic and Gabaudan (1992) consider glycogen storage, as eosinophilic granule in the

liver, as a sign of the onset of hepatocyte functionality that is indicative of the synthesis and storage of macromolecules, which remain throughout the larval and juvenile stages. In the present study, lipid accumulation appeared in the liver between 6 to 8 dph. Thereafter, the level of fat deposits decreased and glycogen deposits were observed in hepatocyte cytoplasm. Similar results have been observed in European catfish, *Silurus glanis* (L.) (Kozarić et al. 2008), and yellow catfish (Yang et al. 2010), which tend to store glycogen and lipid reserves in the liver as their digestive systems gradually develop and the pancreatic and intestinal functions are established (Zambonino-Infante et al. 2008). The histological development of the liver and bile transport system in *C. chitala* is thought to be concurrent with the gradual maturation of hepatocytes, as well as their functionality to synthesize, store, and mobilize carbohydrates and lipids (Hoehne-Reitan and Kjørsvik 2004). The presence of the pancreas in *C. chitala* on 6 dph prior to exogenous feeding confers the priority to accumulate and synthesize the pancreatic digestive enzymes for food digestion at the beginning of exogenous feeding (Sarasquete et al. 2001). In altricial fish larvae, such as Senegal sole (Sarasquete et al. 2001), common pandora (Micale et al. 2006), and sea bass (Diplano et al. 1991), acidophilic zymogen granules appear within 2 to 3 dph just before or soon after the initiation of exogenous feeding. In the present study, the appearance of these pancreatic enzymatic precursors on 8 dph prior to exogenous feeding supports these observations and suggests that the appearance of zymogen granules before feeding is an indication of a genetically programmed developmental process rather than a nutritionally induced event (Zambonino-Infante and Cahu 2001, Micale et al. 2006).

Conclusion

This current work provides the first report on the digestive morphogenesis of *C. chitala* larvae which could be useful in the development of effective culture techniques to support the preservation and

restoration of natural populations of this endangered species. The digestive organogenesis of *C. chitala* was exhibited mainly within the first 12 dph of development and showed increases in tissue structure, number, and size until 30 dph. Less efficient intracellular, alkaline digestion was substituted by more efficient acidic digestion inside the gastric glands; this occurred at 12 dph in *C. chitala* and it improved food assimilation during larval development. These results should be taken into consideration when designing diets, and feeding schedules should be modified in order to start weaning this species at 12 dph.

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